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XVI GREMPA Meeting on Almonds and Pistachios

Edited by:
O. Kodad, A. López-Francos, M. Rovira, R. Socias i Company

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XVI GREMPA Meeting on Almonds and Pistachios

Editors: O. Kodad, A. López-Francos, M. Rovira, R. Socias i Company

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Meknès, Morocco, 12-14 May 2015

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Foreword

Almonds (1267 Mha) and pistachios (385 Mha) are important nut tree crops due to their economic and environmental interest in the Mediterranean and Middle East countries. These regions represent around 75% of the world crop surface for both species. Several scientific research programmes were undertaken by different universities and research centres to improve the quality and quantity of production and adaptation of almonds and pistachios to different growing conditions. Both plant material and orchard management have evolved considerably in the last 40 years in the Mediterranean area and during this time research and development have addressed both species according to the relevance of their respective surface area and production.

It is almost 40 years since the GREMPA (Mediterranean Research Group for Almond and Pistachio) working group was established in the 1970s, with CIHEAM support. The work carried out by this group was fundamentally based on the evaluation and improvement of genetic resources of Mediterranean origin. Later, the group evolved by promoting debate on research in all aspects of the cultivation and industrialisation of these two species, and was integrated into the FAO-CIHEAM Research Network on Nuts during the 1990s. GREMPA has been holding meetings approximately every 4 years, where scientists have exchanged research methods and results and debated the main challenges of almond and pistachio research and development.

The aim of this XVI GREMPA, held in Meknès (Morocco) on 12-14 May 2015, was not to be a Congress, but rather a meeting to discuss the issues challenging almond and pistachio research and to promote the active participation of scientists from all Mediterranean countries. Researchers from other Mediterranean climate areas of the world where those species are important were also invited to participate. More than 90 researchers, students and professionals from 10 different countries (Algeria, Australia, Belgium, France, Hungary, Morocco, Spain, Tunisia, Turkey, and USA) attended the Meeting and contributed by presenting their work and sharing their ideas and experiences.

The Meeting was structured in five sessions to deal with Genetic Resources and Breeding-Cultivars, Genetic Resources and Breeding-Rootstocks, Pests and Diseases, Orchard Management, and Quality, Industrialisation and Commercialisation. Each session had one keynote lecture and 3-4 short oral presentations, followed by substantial time for moderated debates to exchange knowledge and opinions among all participants; those exchanges continued intensively during the poster sessions and coffees offered in the gardens of the Ecole National d'Agriculture de Meknès (ENA) , the venue of the XVI GREMPA.

Two field visits were programmed; a visit to the collection orchard of ENA, where the Department of Fruit production evaluates local selections and crosses with commercial varieties. The morning of the second day was devoted to a field trip to visit modern commercial almond orchards around Meknès, where the participants had the opportunity to learn about and discuss the current projects to introduce self-compatible cultivars and modern management techniques in an area where almond production is expanding. In parallel, a workshop titled "The almond and pistachio crops in Morocco in a changing international context", supported by the Moroccan Ministry of Agriculture and Fisheries was addressed to the Moroccan almond and pistachio growers and industry, aiming to generate a discussion on the current situation and perspectives of the two crops in Morocco, profiting from the presence of the GREMPA meeting international experts who presented their experiences in their own countries.

The exchanges were not limited to the aspects of tree and nut production, but there was also time for visiting the splendid Medina of Meknès to learn about the history of this old city, as well as for sharing a typical Moroccan dinner seasoned with traditional music. The close of the XVI GREMPA meeting paid homage to Dr Rafael Socias i Company, who retired in 2014 following a professional career with important achievements and contributions to the development of the almond.

This volume compiles 60 articles of the contributions presented at the Meeting, a good representation of the research currently carried out on the different aspects of breeding, managing and industrialisation of almonds and pistachios in the Mediterranean basin countries and beyond.

The organisers of the XVI GREMPA, École Nationale d'Agriculture de Meknès and Mediterranean Agronomic Institute of Zaragoza (IAMZ-CIHEAM), express their acknowledgement to the Moroccan Ministry of Agriculture and Fisheries of the Kingdom of Morocco for its collaboration, and thanks the authors and the scientific reviewers for their commitment to this publication, as well as to the sponsoring institutions that contributed to the success of the Meeting.

Dr Ossama Kodad (ENA Meknès, convener)
Javier Sierra (Director, IAMZ-CIHEAM)
Mercè Rovira (Coordinator of the FAO-CIHEAM Network on Nuts)

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(Meknes, Morocco, 12-14 may 2015)

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Session 1

Genetic Resources. Cultivars

GREMPA: A useful initiative for cooperation in almond research

R. Socias i Company

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Abstract. The first GREMPA Colloquium was held at the Mediterranean Agronomic Institute of Zaragoza in February 1974, but was preceded by several initiatives mainly driven by Dr. Jacques Souty, Director of the Station of the Grande Ferrade of INRA in Bordeaux. Many of these previous actions are not well known, but were essential for the launching of such a useful group. In this review I have attempted to follow up these initiatives, as well as the first aims proposed for the success of GREMPA. Many of these objectives have been achieved, but the initial spirit of dialogue and open discussion has changed towards a more formal, congress-style meeting. Additionally, some conflictive moments of the history of GREMPA are recalled and homage is paid to the three great researchers who invested all their energy in this endeavour: A.J. Felipe, C. Grasselly and F. Monastra, whose enthusiasm and spirit we have not managed to maintain.

Keywords. Almond – Research – History – Mediterranean.

GREMPA: une initiative utile pour la coopération dans la recherche de l'amandier

Résumé. *Le premier Colloque du GREMPA avait eu lieu à l'Institut Agronomique Méditerranéen de Zaragoza en février 1974, mais il était précédé de plusieurs initiatives impulsées notamment par le Dr Jacques Souty, Directeur de la Station de la Grande Ferrade de l'INRA à Bordeaux. Beaucoup de ces actions antérieures ne sont pas très bien connues, mais elles furent essentielles pour le lancement d'un groupe si utile. Dans cette révision, j'ai voulu suivre ces initiatives, ainsi que les premiers objectifs proposés pour le succès du GREMPA. Une grande partie de ces objectifs ont été accomplis, mais l'esprit initial de dialogue et de discussion ouverte a dérivé vers un type de réunion plus formelle de style congrès. De plus, quelques moments conflictuels de l'histoire du GREMPA sont rappelés, tandis qu'hommage est rendu aux trois grands chercheurs qui ont investi toute leur énergie dans cet effort : A.J. Felipe, C. Grasselly et F. Monastra, dont nous n'avons cependant pas réussi à maintenir l'enthousiasme et l'esprit.*

Mots-clés. *Amandier – Recherche – Histoire – Méditerranéen.*

I – Introduction

Vargas (2014) clearly and concisely described the evolution of almond research in the Mediterranean over the last 40 years, showing how it had been unquestionably effective in the modernisation of almond growing in this region. It began with the creation of GREMPA (Group de Recherches et d'Études Méditerranéen pour l'Amandier) at the Mediterranean Agronomic Institute of Zaragoza (IAMZ) in February 1974, with the purpose of setting up a forum of exchange and discussion for all almond researchers of the Mediterranean region, which is why the GREMPA meetings were referred to at the beginning as Colloquia, rather than Symposia or Congresses. The frequent constructive discussions that arose about the most diverse aspects of almond growing and research probably provided the best basis for modernisation.

The GREMPA kick-off meeting in 1974 was preceded by a series of preliminary steps to found this working group. Unbeknown to many, it was M. Jacques Souty, Chargé de Mission of INRA (France), who performed this task personally. Consequently, my objective has been to look back over the groundwork leading up to the creation of GREMPA, as well the first steps of its fruitful path.

II – Background

A meeting of experts on cooperation in agricultural teaching and technical research took place in Zaragoza in May 1971 under the aegis of the OECD and the organisation of CIHEAM. The launching of a working group on almond breeding, both on cultivars and rootstocks, was considered most interesting (Souty, 1973). The main reason for launching this proposal was the low productivity of the Mediterranean almond orchards, most of them on non-irrigated and poor soils, unlike the irrigated and fertile Californian orchards. The increase of almond consumption all over the world at that time, a trend which continues today, was an incentive to seek greater productivity for the Mediterranean orchards, considering innovations such as the release of new, productive and high-quality cultivars from the French breeding programme and the introduction of new growing techniques, such as mechanical harvesting. Some of these ideas had already been discussed in February 1970 in Athens during a meeting on horticultural production and marketing in the Mediterranean Basin (Crossa-Raynaud, 1975).

Therefore, further coordination was needed to offer growers the newly selected plant material to ensure them a higher quality and more profitable production to satisfy market requirements. Unsurprisingly, this initiative followed the releases of the first cultivars, mainly 'Ferragnès' and 'Ferraduel', from the French breeding programme, led by the INRA researcher Charles Grasselly (Grasselly and Crossa-Raynaud, 1980). Despite several research studies conducted on almonds in the different Mediterranean countries, these activities were often carried out in isolation, with limited knowledge of the plant material involved and with a restricted diffusion of results. These activities were not very efficient and furthermore there were no reference cultivars for the different growing regions. The insistence of Jacques Souty on this project was highly significant, taking into account that from his position as Director of the Station of the Grande Ferrade in Bordeaux in the 1950s he became the promoter of the first studies on the almond (Hugard, 1974), which lead to the successful releases of Charles Grasselly's programme.

The objectives suggested for the working group were:

1. To facilitate cooperative research work to solve complex problems, difficult for a single country to tackle.
2. To harmonise research projects through knowledge of the work carried out in other centres, to standardise working techniques and to adopt common reference controls, etc.
3. To propose a distribution of tasks according to objectives, available resources in each centre, environmental conditions, etc.
4. To exploit the results of each group to benefit participants as a whole.

The preliminary activities to form this group started immediately and a provisional secretariat was set up in July 1971 at the Station de Recherches d'Arboriculture Fruitière de la Grande Ferrade, which sent a survey to the centres possibly interested in this initiative, including stations in Spain, France, Italy, Greece, Portugal, Romania, Tunisia, Turkey and even Iran. The results of the survey were published in January 1972 (Anonymous, 1972) in a document that included all the centres involved in almond research work:

1. Research on plant material, such as collections of local and foreign cultivars, breeding by crosses, etc.
2. Works on rootstock selection.
3. Physiological studies related with breeding.

Considering that some centres, possibly interested in taking part in the working group did not answer the survey, this document included centres of seven countries with their research lines, as well as the list of cultivars present in their collections. These centres were:

Spain:

- INIA CRIDA 03 (Estación Experimental de Aula Dei, Zaragoza), with A.J. Felipe, J. Herrero and M.C. Tabuenca.
- INIA CRIDA 04 (Estación de Viticultura, Enología y Fruticultura of Reus), with R. Vidal-Barrraquer.
- INIA CRIDA 08 (Extremadura), with B. Ramos.

France:

- INRA (Station de Recherches d'Arboriculture Fruitière de La Grande Ferrade, Bordeaux, with the joined farm of Manduel, Nîmes), with R. Bernhard, C. Grasselly and H. Gall.

Greece:

- Institute of Pomology, Naoussa, with D. Stylianides.

Italy:

- Instituto Sperimentale per la Frutticoltura (Roma), with F. Monastra.
- Instituto Sperimentale Agronomico (Bari), with F. Lanza.
- Instituto di Coltivazione Arboree (Università de Bari), with G. Donno.
- Instituto di Coltivazione Arboree (Università de Sassari), with A. Milella.

Romania:

- Institutul de Cercetari, Piteşti, with V. Cociu.

Tunisia:

- NRAT – Laboratoire d'Arboriculture Fruitière (Ariana), with A. Jaouani and P. Crossa-Raynaud.

Yugoslavia:

- Institut zu Jadranske Culture (Split), with A. Vlasić.

This document not only established the lines of research of each centre, often with the names of cultivars and rootstocks under study, but also the other points of interest, such as breeding perspectives, by crosses and irradiation, studies on frost and disease resistance, such as *Monilia* in humid environments, and selection of local populations.

All this information facilitated the proposal of a working programme (Souty, 1973).

III – Initial working programme

In order for the programme to be efficient, the consensuated coordination of all participants was necessary, therefore an initial informative meeting was imperative to provide better information on all the work performed and create an atmosphere of trust among the members.

This first meeting could have been held either in Spain or France, but the Manduel farm was the first to be suggested. This farm, near Nîmes (France), belonged to the Grande Ferrade. It had a Mediterranean climate, important trial plots, cultivar collections, parental genotypes, different progeny from breeding crosses and rootstock trials. Therefore, it was considered an ideal place for participants to gather information and discuss various activities to be undertaken and facilitate understanding of the problems and adoption of a joint programme of concerted activities. From the outset this programme contemplated important issues such as:

1. Standardisation of the methods for establishing and evaluating the cultivar collections.
2. Prospecting among local populations of interesting genotypes for their specific traits (late bloom, kernel quality, resistance to *Monilia*, leaf-hole, frost...).
3. Establishment of botanical collections of wild species of the subgenus *Amygdalus* in a small number of locations, but available to all participants.
4. Exchange of parental genotypes.
5. Study of the possibilities of obtaining F_1 cultivars vigorous enough to establish seedling orchards without grafting, mainly for arid regions.
6. The complex problem of drought resistance, both in the rootstocks (wild species, forms to be prospected in arid regions) and in the cultivars (because of their anatomical and physiological traits), seeking practical selection methods for adaptation to drought.
7. The search for genotypes with progeny showing better resistance to nematodes or to saline conditions.

The first meeting was to focus on establishing personal relationships and drawing up a programme, whereas subsequent meetings would be held every 2-3 years in the different participant countries, keeping in mind the funding resources required to implement this programme.

IV – GREMPA kick-off meeting

This meeting took place on 19-20 February 1974 at the IAM of Zaragoza. Only four countries were present, since for visa or agenda problems several potential participants could not attend and sent their apologies: S. Serafimov (Bulgaria), I. Saraiva (Portugal), V. Cociu (Romania) and A. Vlasic (Yugoslavia). Despite not being able to attend, other researchers, namely D. Stylianides (Greece), V. Grigorian (Iran) and P. Spiegel-Roy (Israel) sent their reports which were included in the final report of the meeting.

The participants from Spain were: M. Vidal-Hospital (Director of IAMZ), E. Prieto (representing INIA) and the researchers A.J. Felipe (INIA-CRIDA 03), R. Vidal-Barraquer (INIA-CRIDA 04) and F.J. Vargas (Tarragona Provincial Council); from France: J. Souty, R. Bernhard, C. Grasselly, M. Lansac and H. Gall, all from INRA-La Grande Ferrade; from Italy: F. Monastra (ISF of Rome) and Dionigi (IA of Bari); from Tunisia: P. Crossa-Raynaud and A. Jouani from INRAT-Ariana.

J. Souty, promoter of GREMPA, chaired the meeting. Unfortunately, he died several months later, on 13 October 1974, without being able to see the progress and success of GREMPA. During the opening session he recalled the fundamental objectives of the working group: to facilitate cooperative research work to solve complex problems difficult for a single country to undertake; to harmonise the research projects in the Mediterranean Basin by sharing information between the groups, standardising working techniques, adopting common controls, sharing responsibilities in relation to the objectives, available means and environment...; to make use of the synergies created between the participants.

The documents already prepared by the participants as well as those sent to the meeting were commented on and eight activities were chosen in order to reach the objectives set:

1. Bibliographical documentation, commissioned to G. Donno.
2. A list of reference cultivars to serve as controls in the different national collections. C. Grasselly and P. Crossa-Raynaud were commissioned to draw up this list, which would then be discussed and approved by all participants. Thus, the observation methods and recording data would be standardised by a working group formed by A.J. Felipe, C. Grasselly, A. Jouani, F. Monastra and D. Stylianides. If necessary, INRA-France would provide the buds for grafting the reference cultivars to complete the national collections. The definition of the blooming stages was one of the first accomplishments of this line of work (Felipe, 1977).
3. A list of suitable parents for their exceptional traits was drawn up by A.J. Felipe. It was already mentioned that the self-compatible cultivars would be included in this list, probably because of the recent studies already under way on almond self-compatibility (Grasselly and Olivier, 1976; Socias i Company, 1974).
4. Mutual information on the crosses made by the different breeding programmes, drawing up a list by the INRA-Bordeaux to be distributed and completed by all participants.
5. Collection and maintenance of a botanical collection of the subgenus *Amygdalus* including the species considered relevant by a group formed by A.J. Felipe, C. Grasselly and V. Grigorian, suggesting that the collection could be established in Spain.
6. Studying the possibilities of almond growing without grafting directly from seed, by P. Crossa-Raynaud and R. Bernhard, although this possibility could involve some risks.
7. Cooperation in rootstock study and selection, considering the different possibilities of using seedling almonds (INRAT-Ariana of Tunisia or INIA-Badajoz of Span), almond x peach and peach x plum hybrids (INRA-Bordeaux, France), several plum types (INIA-Zaragoza of Spain). The utilisation of peach as a rootstock for almond was not considered interesting.
8. Establishing a common reservoir of almond cultivars, suggesting the station of Bari.

At the same time it was intended to widen contacts with other researchers possibly interested in this almond network, and the names of L. Egea and E. García (CEBAS of Murcia), H. Andreu (INIA-Murcia) and B. Ramos (INIA-Badajoz) from Spain; Damigella (Univ. Catania), Amedda (Consorzio Provinciale di Frutticoltura di Cagliari) and Refatti (Fitopatologia Vegetale di Catania) from Italy; V. Vassilev (Station of Plovdiv) from Bulgaria; M. Dokuzoguz (Univ. Izmir) from Turkey; H. Al-Rais (Ministry of Agriculture) from Syria were put forward, as well as a representative from the Ministry of Agriculture of Cyprus.

Following discussion, the date of the following meeting was set for September 1975 in Montpellier, France. This was rather short term but would ensure a larger presence of participants. Finally the discussion focused on a very dissimilar trait between the Californian and the Mediterranean cultivars such as shell hardness, and a field visit to the trials under way in Zaragoza by A.J. Felipe.

V – First activities and II Colloquium

Several activities were undertaken in order to prepare the II Colloquium, establishing continuous contact between INIA-Zaragoza and INRA-Bordeaux, with activities such as the visit of A.J. Felipe to Bordeaux in December 1974. Another activity was the prospecting mission in June 1975 to Afghanistan by C. Grasselly and A.J. Felipe to oversee the wild species related to almond. Thus, a botanical collection was set up in Zaragoza (Felipe, 1984) and furthermore, with the support of

INIA and CIHEAM it was decided that the almond collection of Zaragoza would be considered as the reference collection for GREMPA.

The II Colloquium took place in Montpellier-Nîmes, France, on 8-11 September 1975, with the presence of a high number of researchers of the different Mediterranean countries: Algeria (2), Bulgaria (1), France (5), Iran (1), Italy (2), Romania (1), Spain (7), Tunisia (1), Turkey (3) and Yugoslavia (1). Researchers from Israel, Greece and the Soviet Union excused their absence, as did some researchers whose countries were already represented at the Colloquium. For the first time there was a researcher from beyond the Mediterranean Basin, Dr. Kester, from the University of California, who was in Europe on sabbatical leave.

After this Colloquium a publication was prepared with the different communications discussed during the meeting, mainly the reports on the work carried out in the different centres. Consequently, this publication has an introduction (Crossa-Raynaud, 1975) summarising the discussions on the broad working lines established at the beginning of GREMPA activities, maintaining the basic aim of these colloquia, i.e. to be the appropriate forum for exchanging ideas.

Some of the main points to be mentioned are the establishment of a list of 14 reference cultivars by C. Grasselly and P. Crossa-Raynaud, selected because of certain relevant traits, such as blooming time, ripening time, resistances, etc... The way of recording blooming time was also discussed as well as how to distinguish shell hardness. The general awareness regarding cultivar characterisation and avoiding confusions between research centres led to the creation of almond descriptors after the IV Colloquium in Izmir (Gülcan, 1981), published by the IBPGR (now Bioversity International). These descriptors were later revised in order to improve the definition of some traits (Gülcan, 1985).

In addition a list of appropriate parents for crosses in the breeding programmes was drawn up, mainly based on a very interesting trait, including the self-compatibility of 'Tuono', 'Genco' and 'Mazzetto' (not yet identified as 'Tuono'). The exchange of information on the crosses performed in the different centres was also decided. The expedition of C. Grasselly and A.J. Felipe to Afghanistan in the previous June was also commented on, as well as the studies on rootstocks and the other aspects included in the initial objectives of GREMPA.

VI – III Colloquium onwards

The III Colloquium took place in Bari (Italy) on 3-7 October, 1977. The number of participants and contributions had increased significantly. This Colloquium has been defined by Vargas (2014) as that of almond self-compatibility since during this Colloquium there were two very significant contributions: that of the wide presence of this trait in the almond population of the Puglia (Godini, 1977) and its transmission through the crosses of the breeding programme of Zaragoza (Socias i Company and Felipe, 1977). This Colloquium may be considered as the consolidation of GREMPA, as seen by the success of the three following Colloquia: those of Izmir (Turkey) in 1980, Sfax (Tunisia) in 1982 and Thessaloniki (Greece) in 1985.

In my opinion, the success achieved by GREMPA in the 1980s contributed to its development since this free association of researchers interested in the almond attracted the interest of other organisms whose interest lay not only in collaboration in the Mediterranean region but also in the exchange of almond-related ideas and materials.

The VII Colloquium in Tarragona and Reus (17-19 June, 1987) was preceded by some tension and also introduced two significant changes. The first was scientific, due to the introduction of another species, the pistachio, to the discussion of the GREMPA colloquia. Consequently, although maintaining the same acronym, pistachio was included in the name of Group de Recherches et d'Études Méditerranéen pour le Pistachier e l'Amandier The second change was the assumption of the

GREMPA activity by the Agrimed Research Programme of the Commission of the European Community, present at this colloquium by G. Rossetto.

The tensions had risen because in the past P. Crossa-Raynaud had unilaterally adopted the position of GREMPA coordinator, an act of selfishness only accepted by the GREMPA members to avoid further tension. In face of his coming retirement, P. Crossa-Raynaud in another act of selfishness unilaterally appointed a new GREMPA coordinator, a nomination that no members had accepted and which ended in an evening of heated discussion in the hall of the Tarragona hotel where the participants were staying. This tension came close to spoiling the friendly atmosphere among the GREMPA members, jeopardising the previous good relationship based on mutual confidence.

The introduction of pistachio into the GREMPA activities was justified by the frequent presence of the two species in the same research centres, often under the responsibility of the same researchers, since both species grew under Mediterranean climates and in the same or very close regions. This incorporation could also have been influenced by the second change, namely when Agrimed took over GREMPA. Undeniably this change gave rise to additional tensions during the Colloquium and interrupted another line of action in the basic activities of GREMPA: the publication of the Colloquium proceedings by IAMZ. From a personal point of view, I remember that I questioned this change, considering that the publication by IAMZ was a long-standing tradition. M. Rosetto answered me that "*Les traditions sont pour être interrompues*".

The efforts to maintain the initial spirit of GREMPA led to a specific meeting on almond rootstocks (Felipe and Socias i Company, 1989) the following year at IAMZ (29-30 November, 1988) with the aim of restoring the original interest in almond rootstocks in the first colloquia, far overtaken by the interest in cultivars due to the great progress achieved in cultivar breeding and in the knowledge on self-compatibility.

The VIII Colloquium (Nîmes, France, 26-27 June 1990) was organised by C. Grasselly, also marking his departure as he was close to retirement. His fruitful activity, however, continued to be highly appreciated by the GREMPA members and he was still present in other meetings, even once he had retired. This Colloquium was also witness to another tension between some members as just before this colloquium the kick-off meeting of the FAO Network on Nuts in the Mediterranean region had already been held. This meeting had taken place in Yalova (Turkey) on June 19-22, unaware to most of the GREMPA members, whereas some had assigned themselves a prominent position in the network. This one-sided behaviour, similar to P. Crossa-Raynaud's in the past, aroused the suspicion of the other GREMPA members.

Following this Colloquium, all the subsequent meetings were arranged by the FAO Network, originally motivated by similar objectives to GREMPA, but widening its interest to all nuts, including the pinion pine. Its functioning, however, did not follow the established rules (Anonymous, 1996), mainly when appointing those responsible for the different working groups, since they were designated without previous consensus, including the network coordinator, equally designated without consensus for a period of four years, but extended indefinitely without discussion.

The IX Colloquium was atypical, since the two species were separated once again contrary to previous decisions and the colloquium was held exclusively on pistachios (Bronte, Sicily, 20-22 May 2003), whereas the meeting on almond had been held just before and had become the I International Congress on Almond of the ISHS (Agrigento, Sicily, 16-19 May 1993), confirming once again that the success of GREMPA had attracted the attention of different institutes and international bodies which, to a certain extent, intended to take advantage of GREMPA's success to their own benefit forgetting the real GREMPA role. This step by the ISHS was conclusive, since the original idea of a colloquium aiming at discussions and collaboration, of maximum scientific rigour, became a congress of formal communications and less discussion.

The original idea of GREMPA was in some way recovered at the X Colloquium (Meknès, Morocco, 14-17 October 1996). In addition to the sessions devoted to the presentation of research results, there were other discussion sessions. One of them was specially important, devoted to the revision of the almond descriptors, mainly related to the advances in delaying blooming time. As a result of these advances, the scale established by Gülcan (1981) was no longer operative, since a score of 9 was attributed to 'Tardy Nonpareil', and at this time there were selections already blooming much later than this cultivar and which could not be adequately rated. Despite the contact with Bioversity International it has been impossible to publish a revision of the almond descriptors including the changes discussed in this Colloquium.

The X Colloquim also marked the occasion of another discussion on the dependence of the GREMPA activity on the FAO-CIHEAM network on nuts, following the tensions of previous colloquia because of the involvement of different organisms in GREMPA activity. I keep a card where R. Viti asked me a question after my communication on the genetics of late blooming in almond, where I took notes from the discussion of the independence of GREMPA. I. Batlle said that without the support of the FAO-CIHEAM network, GREMPA would disappear in three years; similarly F. Vargas said that CIHEAM would not support any GREMPA activity outside the network. Furthermore, E. Germain forecasted that GREMPA would disappear as such if it was a part of the FAO network, as did F. Monastra, who always showed a keen interest in maintaining the originality of GREMPA.

The IX Colloquium was held in Sanliurfa (Turkey), on 1-4 September 1999, after the II International Congress in Davis in 1997, a Congress where almond and pistachio were united once again. In this Colloquium the Mediterranean aspect of GREMPA was weakened in favour of an international scope as a consequence of the new functioning system, a trend which has been maintained in the following meetings, becoming an international meeting rather than a colloquium. This aspect was completely clear in the XII Colloquium, which was also the III International Congress of Almond and Pistachio (Zaragoza, 20-24 May 2001), as well as in the following GREMPA Colloquia, held independently of the ISHS: the XIII in Mirandela, Portugal (1-5 June 2004) and the XIV in Athens, Greece (30 March-4 April 2008). Subsequently there have not been any more GREMPA Colloquia, with the exception of the GREMPA session during the V International Congress on Almond and Pistachio (Murcia, Spain, 27-31 May 2013).

VII – Publications

The papers on the constitutive Colloquium of GREMPA were put together in a typeset document of limited circulation. For the II Colloquium the document was bound previously in order to facilitate its consultation, as for the III Colloquium, although the volume was significantly larger. The Italian conveners also published a book with the Italian translation of the communications as well as the transcription of the discussions following each presentation.

IAMZ undertook the publication of the three subsequent colloquia (IV-VI) as single volumes of the "Série Études" of "Options Méditerranéennes". The two following proceedings were published by the Agrimed Research Programme of the European Communities. The dual meeting of almond and pistachio in 1993 led to an independent pistachio GREMPA publication and resulted in a volume of Acta Horticulturae for the ISHS Congress on Almond. Consequently, all the following ISHS Congresses had their own volume of Acta Horticulturae, whereas the proceedings of the GREMPA colloquia X, XI and XIV were published by IAMZ-CIHEAM in the series of "Cahiers Options Méditerrenéennes". The latest colloquium published so far (XIV) is that of Athens with the format change in the "Options Méditerranéennes. Series A: Mediterranean Seminars".

VIII – Conclusions

From the outset, GREMPA activity has received the support of CIHEAM through IAMZ, a fact to be recognised and acknowledged. Only with the formation of the FAO network has this support been partially discontinued.

At the beginning, the GREMPA meetings provided a forum for the exchange of opinions, a true colloquium, not a congress at which to present research results. As a consequence, a real exchange of ideas was possible, in a relaxed atmosphere without rivalry or suspicion, genuine meetings of friends. Furthermore, the true satisfaction lay in the discussion of work results between friends, and supposed no further interest, such as the publication in a journal of high impact index.

However, problems of individualism appeared right from the beginning, worsened by the appropriation of GREMPA by other institutions. Probably the success of the first GREMPA colloquia and the spirit of collaboration provoked these successive appropriations, first by the Agrimed programme of the CE, later by FAO, and finally by ISHS in the Agrigento meeting and the problems related to this internationalisation and loss of the Mediterranean background. GREMPA was born with the idea of defending the Mediterranean production of almond against the production of California, that was increasing at that time and is predominant today, also exacerbated by the increasing production of Australia.

Homage

The author would like to pay tribute to the driving force of GREMPA, J. Souty, as well as to the three great researchers who invested all their energy in this endeavour: A.J. Felipe, C. Grasselly and F. Monastra, whose enthusiasm and spirit we have not managed to maintain.

Special thanks are due to the IAMZ and its librarians for the search of the old documents cited in this review.

Finally I would like to recall three symbols of GREMPA, highly appreciated by all and rose by Charles Graseelly: the green flag, embroidered by Mrs. Monique Grasselly and later by my wife, flown for the last time in Athens in 2008; the anthem, pointing to the importance of almond self-compatibility, sang for the last time in Zaragoza in 2003; and the investiture of the GREMPA knights, done also for the last time in Zaragoza in 2003.

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Almond Breeding: important issues and challenges for research

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Abstract. Due to the recent sharp increase in prices, the almond has become a very profitable culture. This has changed the landscape, not only for the productive sector, but also for breeders that have to satisfy the new demands of nurseries, growers, industry and consumers. The traditional goals of almond breeding in the Mediterranean basin, like late flowering and self-compatibility, currently allow the profitable almond cultivation in cold areas. The new extra-late flowering cultivars are allowing its cultivation in extremely cold areas, in which almonds had never been cultivated. In these areas, early maturation will be an essential trait of new cultivars. In this new environment, resistance to pests and diseases will be a new challenge for breeders, to reduce production costs, develop an agriculture friendly with the environment and produce healthier almonds. Due to the huge demand of almonds, the prices of different cultivars have approached and so, productivity will be the main trait over any other characteristic. However, almond quality will be a significant commitment in the future by designing cultivars for each industrial use. Although modern irrigated plantations, as Californian or Australian, are being imposed in many areas, in the Mediterranean basin there are thousands of rainfed hectares, for which we will have to bred suitable cultivars and rootstocks, and apply appropriate techniques of cultivation. Biotechnological advances will be very useful, facilitating breeders' efforts by means of Marker Assisted Selection (MAS) for the main characteristics (flowering time, self-compatibility, bitter taste) or molecular identification of cultivars. Breeders have the responsibility to obtain new good cultivars, which must be seriously evaluated in different growing areas and culture conditions before offering them to nurseries and growers.

Keywords. *Prunus dulcis* – Cultivars – Breeding – Flowering time – Self-compatibility.

Amélioration génétique de l'amandier: important enjeux et défis pour la recherche

Résumé. *En raison de la forte et récente augmentation des prix, l'amande est devenue une culture très rentable. Cela a changé le paysage, non seulement pour le secteur de la production, mais aussi pour les améliorateurs qui doivent satisfaire les nouvelles exigences des pépiniéristes, des producteurs, de l'industrie et des consommateurs. Les objectifs traditionnels de l'amélioration de l'amandier dans le bassin méditerranéen, comme la floraison tardive et l'auto-compatibilité, permettent actuellement la culture de l'amandier rentable dans des zones froides. Les nouveaux cultivars à floraison extra-tardive permettent sa culture dans des zones extrêmement froides, dans lesquelles cette espèce n'a été jamais cultivée. Dans ces zones, la maturation précoce sera une caractéristique essentielle pour les nouveaux cultivars. Dans ce nouvel environnement, la résistance aux ravageurs et aux maladies sera un nouveau défi pour les améliorateurs afin de réduire les coûts de production, de développer une agriculture respectueuse de l'environnement et de produire des amandes saines. En raison de l'énorme demande d'amandes, les prix des différents cultivars ont approché et ainsi, la productivité sera le trait principal sur toute autre caractéristique. Cependant, la qualité de l'amande sera un engagement important à l'avenir en concevant des cultivars pour chaque usage industriel. Bien que les plantations irriguées modernes, comme les californiennes ou australiennes, sont imposées dans de nombreux domaines, dans le bassin méditerranéen, il y a des milliers d'hectares non irriguées pour lesquelles nous aurons besoin des cultivars et des porte-greffes appropriés et d'appliquer des techniques appropriées de culture. Les progrès de la biotechnologie seront très utiles, ce qui facilite les efforts des améliorateurs au moyen de la sélection assistée par marqueurs (MAS) pour les principales caractéristiques (date de floraison, l'auto-compatibilité, le goût amer) ou identification moléculaire des cultivars. Les améliorateurs ont la responsabilité d'obtenir de nouveaux cultivars, qui doivent être sérieusement évalués dans des différentes zones de culture et des conditions de culture avant de les offrir aux pépinières et aux producteurs.*

Mots-clés. Prunus dulcis – *Cultivars – Amélioration génétique – Période de floraison – Auto-compatibilité.*

Not one man in a thousand has accuracy of eye and judgment sufficient to become an eminent breeder. If gifted with these qualities, and he studies his subject for years, and devotes his lifetime to it with indomitable perseverance, he will succeed, and may make great improvements; if he lacks any of these qualities, he will assuredly fail.

Charles Darwin 1859, The Origin of Species.

I – Almond breeding and new cultivars

The almond breeding is aimed at solving the problems of the culture by obtaining new cultivars. Looking at the high almond yields produced in the USA or Australia (more than 2000 kg of kernel per hectare), we might think that the growers in these countries have no problems, or at least not as important as in other countries, and therefore they do not need much to improve their varieties. In the other producing countries (such as the Mediterranean or the Middle East countries), although there are modern plantations with high yields (similar to American and Australian), the general situation is of low yields (about 100 kg of kernel per hectare), mainly due to a culture under dry conditions or with deficient irrigation, and with frequent problems of pollination and spring frosts.

As a result of the age of the crop and the heterogeneity in the producer regions, the varietal situation is different in both environments. In the USA and Australia, the predominant cultivar is 'Nonpareil', which represents 40% and 50% respectively. 'Nonpareil' cultivar is highly adapted to environmental, cultural and commercial conditions of these countries. Other important cultivars grown in these countries are 'Carmel', 'Butte', 'Prize' and 'Monterrey'. In Mediterranean and near Eastern countries many local cultivars adapted to each area, along with new cultivars recently obtained in breeding programs, mainly from French and Spanish, are nowadays cultivated.

These new cultivars are gradually displacing traditional cultivars for their self-compatibility and late flowering time, which is a great advantage in colder areas. The more important breeding programs have been the French of INRA (with cultivars like 'Ferragnès', 'Ferraduel', 'Lauranne' and 'Mandaline'), the Spanish of IRTA-Constantí ('Francolí', 'Masbovera', 'Glorieta', 'Vayro', 'Constantí', 'Marinada' and 'Tarraco'), CITA-Zaragoza ('Ayles', 'Guara', 'Moncayo', 'Blanquerna', 'Cambra', 'Felisia', 'Soleta', 'Belona', 'Mardía' and 'Vialfás') and CEBAS-CSIC ('Antoñeta', 'Marta', 'Penta' and 'Tardona'). We must emphasize the great diffusion of French cultivars 'Ferragnès' and 'Ferraduel' in several European countries, and of 'Guara' specifically in Spain. Despite the held predominance of 'Nonpareil' in the USA, there are important public and private breeding programs, and several new cultivars have been released ('Avalon', 'Carmel', 'Butte', 'Father', 'Price', 'Sonora', 'Sweetheart', 'Winter', 'Independence' and 'Folsom'). Independence cultivar is the first American cultivar fully self-compatible obtained to date. The Australian breeding program is more recent, but nevertheless, it has obtained very interesting advanced selections under evaluation.

II – Genetic control and inheritance of traits

But these new cultivars are not the only interesting results of the breeding programs. The study of the obtained progenies for their selection has allowed the researchers to advance in the knowledge of the genetic control of quantitative traits, such as flowering and ripening time, productivity and fruit characteristics (Dicenta *et al.,* 1993a and b; Sánchez-Pérez *et al*., 2007; 2012; Martínez-García *et al*., 2014) and qualitative, as seed bitterness and self-compatibility (Dicenta and García, 1993b; Dicenta *et al*., 2007). This information now allows, depending on the selected progenitors, to predict the value of the seedlings for each characteristic, since heritability of the main traits is usually high.

Furthermore, the knowledge of the *S*-genotype for floral incompatibility of the parents to be used in crosses allows the breeders to design different strategies for obtaining 100% of self-compatible seedlings in the new offspring (Ortega and Dicenta, 2003). Similarly, knowing the genotype of the parents for the seed bitterness, we can avoid the appearance of bitter descendants in the offspring (Dicenta and García 1993b; Dicenta *et al.*, 2007).

There have also been significant advances in the field of floral biology, which have established the factors that affect the flowering time (Egea *et al.*, 2003; Sánchez-Pérez *et al.*, 2012; 2014), pollination and fruit set (Dicenta *et al.*, 2002, Egea *et al.,* 2002; Ortega *et al.*, 2004) more favourable for abundant and stable yields. The analysis of the *S*-genotype currently allows the early selection of self-compatible seedlings after germination of obtained seeds, and the design of new plantations to ensure the cross-compatibility between cultivars (Ortega and Dicenta, 2003; 2004). The demonstration that self-fertilization is as efficient as the cross-fertilization regarding fruit set and fruit characteristics, today enables profitable solid plantations of self-compatible cultivars, as it occurs in other fruit species (Dicenta *et al.*, 2002; Ortega *et al.*, 2006; Martínez-García *et al.*, 2011).

Furthermore, the development of molecular markers type SSR is allowing the unequivocal molecular identification of cultivars (Sánchez-Pérez *et al.*, 2006), which is revealing cases in which a given cultivar was known by different names or, opposite, the use of the same name for different cultivars. Some examples include the existence of several 'Marcona' and 'Desmayo Largueta' types in Spain (Martínez-García *et al.*, 2011), the coincidence of the cultivar known in Spain as 'Felipa' with the traditional Italian 'Filippo Ceo' or that the released 'Supernova' was actually the ancient Italian 'Tuono' (Marchese *et al.*, 2008). More recently, analysing 35 SSRs in samples of 'Tuono' and 'Guara' cultivars from four research centres (IRTA-Constantí, INRA-Avignon, University of Bari and CEBAS-CSIC of Murcia) researchers have shown that 'Guara', the most cultivated cultivar in Spain in recent years, it is actually the Italian cultivar 'Tuono' (Dicenta *et al.*, 2015). For their high performance, SSRs are nowadays an indispensable tool in the fight against fraudulent multiplication of protected cultivars.

On the other hand, genetic mapping together with the study of the offspring has allowed the identification of different QTLs for flowering, ripening and fruit traits and it is facilitating the development of molecular markers for these characteristics, which could be used in the early selection medium term (Sánchez-Pérez *et al.*, 2007; 2010; 2012; 2014), together with other tools such as the correlation between traits (Dicenta *et al.*, 2005).

All these biotechnology tools are improving the efficiency of breeding programs, enabling a more efficient selection of the most interesting genotypes. In spite of this progress, the breeder always will have to continue the creation of new genotypes and their selection based on their agronomic behaviour in the field.

III – New goals for almond breeding

Until now, the breeding objectives, mainly in the Mediterranean basin, have aimed to obtain self-compatible and late-flowering cultivars to increase the fruit set and to avoid the spring frosts damages. Fruit quality and ease of management of the trees have also been important traits.

But what really matters for the growers, is the productivity of the plantation in terms of net income per hectare. This means incomes (kilograms of kernel multiplied by the prize of the kernel) minus expenses (including cultivation and all aspects related to the cultivar that affect to production costs). This obvious objective is especially important now, where the high demand for almonds in Russia, India, China and South Korea have pushed up prices, for example to triple in Spain. Result of this high demand, the important traditional difference of prices between different cultivars has approached, giving prominence to the quantity over quality, with some exceptions. Despite this fact, it is likely that in the medium-long term the market will demand breeders for almonds with different characteristics depending on their industrial use (different chemical composition, size or shape for the manufacture of nougat, marzipan, chocolates, snacks, etc.). But in short term, as we have indicated, the main objective will remain the high productivity.

Moreover, at present, new opportunities for almond breeding, which should play a meaningful role in the economic performance of plantations, are open. Early maturation has always been an interesting feature in almond but has not been paid sufficient attention. In warmer areas, it allows harvesting of the crop before the high summer temperatures, facilitating the preparation of the tree for the adequate development for the following year. In cold areas also prevents excessive delay in ripening. Some late ripening cultivars, in cold areas, can be harvested in October or November, when low temperatures and rain slow the ripening process and increase the risk of development of fungi, such as *Aspergillus*, producer of aflatoxins (Dicenta *et al*., 2003). We should note that in other *Prunus*, such as apricot or peach, development of fruits (from flowering to ripening) can become very short (70-90 days), while in some cultivars of almond like Desmayo Largueta (early flowering and late ripening), it reaches up to 220 days. Penta is the almond cultivar with the shortest cycle (130 days), due to its extra-late flowering and early maturation.

One of the great challenges we will face in almond breeding will be the resistance to pests and diseases. Navel orange worm, *Capnodis, Monilinia, Polystigma, Fusicoccum, Pseudomonas or Verticillium* will be among the most important. Resistance to pests and diseases has many advantages for producers and consumers. First of all, it reduces the production costs by eliminating or reducing phytosanitary applications, with a continuous and perpetual effect. In addition, cultivation of resistant cultivars is more environmentally-friendly and it generates healthier almonds for consumers. However, breeding for resistance presents numerous difficulties. First of all, for most of diseases we have not already identified good sources of resistance within the species, requiring the breeder to use wild related species, doing more complex and lengthy the breeding process (Gradziel *et al*., 2001). Secondly, we have no information on genetic control and transmission of resistance, so the most practical strategy would be to use resistant progenitors, assuming that the resistance is transmitted to the progeny with a high heritability. Furthermore, evaluation of resistance can be a more or less complex process depending on the pathogen. We will have to develop new methods to isolate, maintain and multiply the pathogen, as well as methods of inoculation and detection of pathogen in the plants being evaluated. Finally, we know the importance of environmental conditions on pathogen development (especially in the case of fungus), which requires the breeder to multiply their trials in different environmental conditions.

Although the breeder traditionally thought of obtaining a good cultivar, in a general way, more and more we will have to think in the creation of cultivars for each type of culture. We have already mentioned the differences between the American or Australian culture systems and most of the Mediterranean ones. These large differences show that the breeder must think if the new cultivar will be suitable for cultivation in rainfed or irrigated conditions. Under dry conditions, usually associated with a deficient culture, the resistance to drought and diseases, and the self-compatibility will be of primary importance. The less vigorous cultivars are not recommended for drought or low irrigation, as trees take longer to reach the commercial size and therefore its maximum production. This is the opposite in the new high-density plantations, where less vigorous rootstocks and cultivars are advisable, easier to control in these small frames and adapted for special harvesting machinery. These cultivars should come into bearing soon, be very productive and have an abundant branching adapted to the hedge structure. On the other hand, modern plantations, like American and Australian, where water resources and technical management of the plantation are insured, including the presence of pollinators, the main goal will remain the high productivity as a result of numerous factors related to the cultivar (abundant and stable flowering, high vigour, good performance for pruning and harvesting) and culture (water, fertilizers and diseases control).

And finally, and very important, breeders have to be aware of the consequences of the release of cultivars with poor performance or not adapted to different growing conditions. The cultivars behave differently in different regions as consequence of the environmental and culture conditions or the technical preparation of the growers. We know that several new almond cultivars have not had a good behaviour and they have been removed in a few years with the resultant loss of time and money for growers. The best cultivar is not that broadly planted, but that one that is not pulled off.

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Paternal effects on fruit characteristics of some almond cultivars

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Abstract. The paternal effect in fruit trees is exhibited by differences among fruits borne on the same cultivar but developed from different source of pollen. The effect of pollinator on nut and kernel characteristics of almond was studied by measuring the nuts and kernels obtained from 12 crosses between self-incompatible and self-compatible almond cultivars. Three domestic self-incompatible cultivars ('Gulcan 1', 'Gulcan 2' and 'Akbadem') were hand-pollinated with four male self-compatible cultivars: 'Guara', 'Lauranne', 'Felisia' and 'Moncayo'. Quality characteristics were determined as nut weight (g), kernel weight (g), shelling percentage, double kernels, and nut and kernel dimensions (length, width and thickness). Nut and kernel characteristics showed differences according to the pollen sources.

Keywords. Almond – Paternal effect – Nut – Kernel characteristics.

Effets paternels de certains cultivars d'amandier sur les caractéristiques du fruit

Résumé. *Chez les arbres fruitiers l'effet paternel a donné lieu à des différences entre fruits portés par le même cultivar mais développés à partir de différentes sources de pollen. Chez l'amandier, l'effet du pollinisateur sur les caractéristiques du fruit et de l'amandon a été étudié en mesurant les fruits et les amandons obtenus à partir de 12 croisements entre cultivars d'amandiers auto-incompatibles et auto-compatibles. Trois cultivars domestiques auto-incompatibles ('Gulcan 1', 'Gulcan 2' et 'Akbadem') ont été pollinisés à la main avec quatre cultivars mâles auto-compatibles: 'Guara', 'Lauranne', 'Felisia' et 'Moncayo'. Les caractéristiques de qualité ont été déterminées comme poids du fruit (g), poids de l'amandon (g), rendement au cassage en pourcentage, amandons doubles, et dimensions du fruit et de l'amandon (longueur, largeur et épaisseur). Les caractéristiques du fruit et de l'amandon ont montré des différences selon les sources de pollen.*

Mots-clés. *Amandier – Effet paternel – Fruit – Amandon – Caractéristiques.*

I – Introduction

Although Turkey has a considerable potential in terms of almond production, it is an almond importing country. Almost all domestic almond cultivars are stone class and they are self-incompatible. According to average almond production over the last three years, Turkey's almond production is 77,650 tons and 7th in the world (FAO, 2013). The Mediterranean, Southeast Anatolia and Aegean regions have potential for modern almond growing in Turkey. The Southeast Anatolian Project (GAP) is the largest irrigation and development project of Turkey covering about two million ha of cultivated land. Recently, modern almond orchards with foreign cultivars are being established in the GAP region.

The "xenia" term covers all direct pollen effects in seeds and fruits, whether discerned in embryo, endosperm, or maternal tissues, in the period from fertilization to germination (Denney, 1992). The paternal effects on seeds may describe as "xenia".

There were different results from different researchers about the pollen source effects on fruit characteristics of almond. According to Dicenta *et al*. (2002), there is no difference between self- and cross-pollination for several fruit traits of almond, including fruit weight, kernel weight, shelling percentage, double kernels, empty nuts, and split kernels. The artificial selfing influenced markedly the production of double kernels in almond (Palasciano *et al*., 1994). Fruits from open pollination of self-compatible seedlings had higher values of weight in-shell, kernel weight, nut volume and kernel volume than those from self-pollination after bagging (Vargas *et al*., 2005). Nut quality characteristics of almonds showed differences according to the years and the pollinators (Eti *et al*., 1994).

In this study, we pollinated 'Gulcan 1', 'Gulcan 2' and 'Akbadem' almond cultivars with pollen of 'Felisia', 'Moncayo', 'Lauranne' and 'Guara', and we used the fruits to determine the paternal effects of cultivars on physical nut and kernel characteristics in almond.

II – Materials and methods

Three domestic and self-incompatible almond cultivars ('Gulcan 1', 'Gulcan 2' and 'Akbadem') were hand-pollinated with 4 self-compatible and late flowering foreign cultivars ('Guara', 'Lauranne', 'Felisia' and 'Moncayo') in the study. Twelve controlled crosses were performed between the domestic and foreign cultivars as below:

'Gulcan 1' × 'Felisia', 'Gulcan 1' × 'Moncayo', 'Gulcan 1' × 'Lauranne', 'Gulcan 1' × 'Guara'; 'Gulcan 2' × 'Felisia', 'Gulcan 2' × 'Moncayo', 'Gulcan 2' × 'Lauranne', 'Gulcan 2' × 'Guara'; 'Akbadem' × 'Felisia', 'Akbadem' × 'Moncayo', 'Akbadem' × 'Lauranne' and 'Akbadem' × 'Guara'.

Controlled crosses were made in the Sekamer Station of Sutcuimam University in Kahramanmaras. Fruits were harvested at maturity, and fruit samples collected from each cultivar pollinated with four different cultivars to determine the effect of each type of pollen on expression of the fruit characteristics.

Fruit weight, kernel weight, shelling percentage, double kernels and dimensions of fruit and kernel were determined. Length, width, and thickness of fruits were measured with a precision of 0.01 mm with a digital caliper. After measurements, nuts were cracked to obtain the kernel and determine the shelling percentage by weight using an electronic balance. Length, width, and thickness were measured in all kernels.

The results are means of three replicate samples containing 30 fruits for each replicate. The replicated values on fruit and kernel traits were analyzed using the one-way ANOVA procedure. Mean values were compared by Fisher's least-significant difference (LSD) multiple-range test at $p \leq 0.05$.

III – Results and discussion

1. Paternal effects on fruit features of 'Gulcan 1' cultivar

All examined physical features of 'Gulcan 1' fruits were statistically affected by the pollen of 'Felisia', 'Moncayo', 'Lauranne' and 'Guara' cultivars. There was a general increasing at nut and kernel characteristics of 'Gulcan 1' almond when pollinated with 'Moncayo' and 'Lauranne'. Double kernels were observed 10% at 'Gulcan 1' x 'Felisia' fruits, while that value was 0% in the other 'Gulcan 1' crosses. 'Felisia' had a negative effect on nut and kernel traits of 'Gulcan 1' (Table 1). 'Moncayo' and 'Lauranne' can be used as pollinator for 'Gulcan 1' because of their positive effects on physical fruit traits.

Atli *et al*. (2011) had found nut weight, shelling percentage and double kernels of 'Gulcan 1' cultivar as 2.03 g, 36.4% and 10.0%, respectively. The nut weight of 'Gulcan 1' was higher in our experiment (Table 1). According to Oukabli *et al*. (2002), there was no pollination effect on the production of double kernels in 'Tuono' almond.

Table 1. Paternal effects on physical nut and kernel characteristics of 'Gulcan 1' almond

Crosses	Nut weight g	Kernel weight g	Shelling percentage %	Double kernels %	Nut dimensions mm			Kernel dimensions mm		
					Length	Width	Thick.	Length	Width	Thick.
'Gulcan 1' x 'Felisia'	2.35 d	0.72 d	30.40 b	10.00 a	1.45 c	0.89 b	0.63 b	1.09 c	0.51 d	0.33 d
'Gulcan 1' x 'Moncayo'	3.50 b	1.26 a	36.04 a	0.00 b	1.73 a	1.06 a	0.75 a	1.30 ab	0.58 b	0.41 a
'Gulcan 1' x 'Lauranne'	3.89 a	1.20 b	30.84 b	0.00 b	1.77 a	1.07 a	0.78 a	1.36 a	0.60 a	0.39 b
'Gulcan 1' x 'Guara'	2.86 c	0.87 c	30.34 b	0.00 b	1.67 b	0.89 b	0.66 b	1.21 bc	0.54 c	0.35 c
LSD ($p \leq 0.05$)	0.11	0.001	1.43	0.99	0.06	0.13	0.06	0.13	0.002	0.001

Letters next to numbers indicate different groups determined by LSD test ($p \leq 0.05$).

2. Paternal effects on fruit features of 'Gulcan 2' cultivar

Significant differences existed for examined fruit characteristics of 'Gulcan 2' cultivar within pollination treatments. 'Gulcan 2' fruits were affected by the same pollinators. The highest physical values were obtained from the 'Guara' pollinated fruits, and followed by 'Moncayo' and 'Lauranne' pollinated, respectively. The lowest values were obtained from the 'Felisia' pollination. Similarly, double kernels were observed 5% at 'Gulcan 2' x 'Felisia' fruits, while that value was 0% in the other 'Gulcan 2' crosses. 'Felisia' had also a negative effect on fruit features of 'Gulcan 2' (Table 2).

Atli *et al*. (2011) reported that, nut weight, shelling percentage and double kernels of 'Gulcan 2' cultivar were 2.80 g, 27.7% and 3.3%, respectively. The nut weight of 'Gulcan 2' was higher in this experiment (Table 2).

Table 2. Paternal effects on physical nut and kernel characteristics of 'Gulcan 2' almond

Crosses	Nut weight g	Kernel weight g	Shelling percentage %	Double kernels %	Nut dimensions mm			Kernel dimensions mm		
					Length	Width	Thick.	Length	Width	Thick.
'Gulcan 2' x 'Felisia'	4.72 d	1.16 d	24.60 a	5.00 a	1.71 b	1.11 b	0.75	1.29	0.69 b	0.36 a
'Gulcan 2' x 'Moncayo'	5.74 b	1.31 b	22.77 b	0.00 b	1.87 a	1.26 a	0.78	1.37	0.79 a	0.30 d
'Gulcan 2' x 'Lauranne'	5.28 c	1.30 c	24.64 a	0.00 b	1.81 ab	1.27 a	0.78	1.30	0.80 a	0.34 b
'Gulcan 2' x 'Guara'	6.12 a	1.32 a	21.56 b	0.00 b	1.88 a	1.30 a	0.79	1.33	0.84 a	0.33 c
LSD ($p \leq 0.05$)	0.24	0.002	1.25	0.99	0.11	0.11	N.S.	N.S.	0.06	0.002

Letters next to numbers indicate different groups determined by LSD test ($p \leq 0.05$).

3. Paternal effects on fruit features of 'Akbadem' cultivar

Domestic 'Akbadem' cultivar was pollinated with same almond cultivars. Significant differences existed for fruit and kernel weight, shelling percentage, double kernels, nut length and nut width of 'Akbadem' almond within pollination treatments. There was a positive effect on nut and kernel characteristics of 'Akbadem' when pollinated with 'Moncayo' and 'Lauranne'. Double kernels of 'Akbadem' were higher than the other cultivars, and had high value when pollinated with 'Felisia' and 'Moncayo'. 'Felisia' and 'Guara' had negative effects on nut and kernel characteristics of 'Akbadem' (Table 3).

According to Atli *et al*. (2011), nut weight, shelling percentage and double kernels of 'Akbadem' cultivar were 2.65 g, 45.9% and 65.0%, respectively. Kuden and Kuden (2000) were reported double kernels of 'Akbadem' as 26.6%. Our results were between those of Atli *et al*. (2011), and Kuden and Kuden (2000).

Dicenta *et al*. (2002) found no difference between self- and cross-pollination for several fruit traits, including fruit weight, kernel weight, shelling percentage, double kernels, empty nuts, and split kernels. According to Vargas *et al*. (2005), fruits from open pollination of 34 self- compatible seedlings had higher values of weight in-shell, kernel weight, nut volume and kernel volume than those from self-pollination after bagging. Eti *et al*. (1994) reported that, nut quality characteristics of 4 selected almond types showed differences according to the years and the pollinators. Our results agreed with those of Vargas *et al*. (2005), and Eti *et al*. (1994).

Table 3. Paternal effects on physical nut and kernel characteristics of 'Akbadem' almond

Crosses	Nut weight g	Kernel weight g	Shelling percentage %	Double kernels %	Nut dimensions mm			Kernel dimensions mm		
					Length	Width	Thick.	Length	Width	Thick.
'Akbadem' x 'Felisia'	4.41 b	1.99 d	45.41 c	40.00 a	2.36 a	1.16 b	0.77	1.62	0.78	0.39
'Akbadem' x 'Moncayo'	4.32 c	2.28 a	52.79 a	40.00 a	2.30 ab	1.18 ab	0.79	1.56	0.84	0.41
'Akbadem' x 'Lauranne'	4.96 a	2.22 b	44.77 d	19.33 b	2.44 a	1.24 a	0.87	1.68	0.85	0.38
'Akbadem' x 'Guara'	4.02 d	2.02 c	49.83 b	20.00 b	2.15 b	1.14 b	0.78	1.61	0.79	0.38
LSD ($p \leq 0.05$)	0.006	0.006	0.35	2.88	0.15	0.06	N.S.	N.S.	N.S.	N.S.

Letters next to numbers indicate different groups determined by LSD test ($p \leq 0.05$), NS: Not significant.

IV – Conclusions

The paternal effect in fruit trees is exhibited by differences among fruits borne on the same cultivar but developed from different source of pollen. We conclude that pollen of different almond cultivars has effect on fruit characteristics of 'Gulcan 1', 'Gulcan 2' and 'Akbadem' cultivars.

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Performance of the CITA almond releases and some elite breeding selections

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Abstract. Almond growing is lately increasing in Spain due to higher kernel prices and better orchard management techniques, making this crop more profitable and attractive. In addition, the release of more productive, extra-late blooming and fully autogamous cultivars, is allowing the shift of almond growing to inland areas with colder climates. The release of new cultivars must be based on their agronomic behavior under different climatic conditions. Consequently, the performance of the last commercial CITA almond cultivars ('Guara', 'Soleta', 'Isabelona', 'Diamar' and 'Vialfas') and some breeding selections ('G-3-3', 'G-3-4', 'G-5-25', 'G-2-22' and 'I-3-67') was studied in a trial at the "AFRUCCAS Experimental Fruit Farm" near Caspe (Zaragoza, Spain). The trial was established in 2005 at a distance of 6 x 7m on the peach x almond INRA GF-677 rootstock. Scions were grafted in 2006 and the trees were trained as free open vase and managed under usual commercial requirements and drip irrigation. Phenology, vigor, production, fruit and orchard parameters were controlled to ascertain the agronomical performance of each cultivar or selection. The success of any cultivar not only depends of the accuracy of the breeder's selection, but also its adaptability must be ascertained to be recommended in a specific region.

Keywords. *Prunus amygdalus* – Plant material – Production – Shelling percentage – Vigor – Adaptability.

Comportement des variétés d'amandier et de quelques sélections élite d'amélioration génétique du CITA

Résumé. *La production d'amandier ha récemment augmentée en Espagne. Les prix plus élevés de l'amande et l'amélioration des techniques de culture rendent cette production plus attractive et rentable. En plus, l'obtention de nouvelles variétés de fleuraison très tardive, complètement autogames et très productives ont permis l'introduction de cette culture aux zones d'intérieur de climat plus froid. L'enregistrement d'une nouvelle variété doit être basée sur son comportement agronomique dans différentes conditions climatiques. Par conséquent, le comportement agronomique des dernières variétés commerciales d'amandier du CITA ('Guara', 'Soleta', 'Isabelona', 'Diamar' and 'Vialfas') et de quelques sélections élite ('G-3-3', 'G-3-4', 'G-5-25', 'G-2-22' et 'I-3-67') a été étudié sur une parcelle expérimentale d'AFRUCCAS, près de Caspe (Saragosse, Espagne). La plantation des porte-greffe hybrides amandier x pêcher INRA GF-677 a été fate à une distance de 6 x 7 m en 2005. Les variétés ont été greffées en 2006, les arbres ont été formés comme vase libre ouvert et maintenus selon les pratiques commerciales normales à la région avec un système d'irrigation goutte-à-goutte. On a contrôlé la phénologie, le vigueur, la production, la productivité et les paramètres du fruit pour déterminer le comportement agronomique de chaque variété. Le succès d'une variété ne dépend pas seulement de la précision de la sélection de l'améliorateur, mais aussi son adaptabilité à une zone spécifique doit être évaluée pour déterminer sa convenance.*

Mots-clés. Prunus amygdalus – *Matériel végétal – Production – Rendement – Vigueur – Adaptabilité.*

I – Introduction

Since the beginning of almond research in Zaragoza in 1966, the most important problem detected in Spanish almond (*Prunus amygdalus* Batsch) growing was its low productivity attributable to the occurrence of frosts at blooming time or later, to deficient pollination, and to drought, because almond was mostly grown in rainfed conditions (Felipe, 2000). As a consequence, the main objective the CITA almond breeding program was the development of late-blooming and self-compatible cultivars. For more than 30 years, crosses between Spanish and foreign varieties were made with the aim of incorporating these and other interesting traits. 'Tuono' and 'Genco' were used as donors for self-compatibility, 'Titan' for late blooming, 'Desmayo Largueta', 'Marcona' and 'Belle d' Aurons' for fruit quality, 'Bertina' for fruit size and late blooming, 'Forastero' for disease resistance, etc. (Socias i Company *et al*., 2010). As a result, a series of successful cultivars have been released, including 'Guara', probably the plant cultivar of the highest success in Spanish agriculture, and, more recently, 'Belona', 'Soleta', 'Mardía', and 'Vialfas', now under evaluation for the Community Plant Variety Office.

Worldwide almond demand has been increasing during the last years. Additionally, some works reported that a regular almond consumption may protect against risk of heart disease and diabetes (Spiller *et al*., 1992; Hyson *et al*., 2002). The rise of the almond kernel price, as well as the possibility of high crop mechanization, has turned almond growing into a very attractive activity. New plantings have caused the almond plant stock-out at the nurseries. A high percentage of the new Spanish plantings is made with the new releases from the Spanish almond breeding programs (Socias i Company *et al*., 2011). The introduction of these new varieties has probably influenced the increase of the Spanish almond yield. On the other hand, the significant advancement in the delay of the blooming date, in addition to the current high crop profitability, is allowing an important shift of almond culture towards inner cereal areas of Spain, of colder climate, where traditional almond cultivars are not productive due to continuous crop losses by the high incidence of spring frosts.

The perfect cultivar does not exist. As a consequence, the release of a new cultivar is a great responsibility due to the high investment required for a new orchard. Any breeding program stresses the objectives of autogamy and late blooming, but also includes fruit quality, kernel composition in nutritious and healthy compounds, as well as resistance to pests and diseases. However, the final selection has to be based on the results of agronomic trials under different climatic conditions to ascertain the adaptability and agronomic performance of the candidate selection.

Consequently, this work represents an advancement of the results of an evaluation trial with the last commercial CITA almond cultivars and elite selections. This trial was proposed by a farmer association, with the technical advice of the Aragon Government, in order to assess the performance of the new almond plant material in its specific region.

II – Materials and methods

A trial-collection of the CITA almond recent releases and new elite breeding selections was established at the AFRUCCAS (Association of fruit-tree farmers of Caspe) experimental farm in Caspe, NE Spain (41.16°N, 0.01°W). This area is characterized by cold winters with high chill accumulation, warm springs and very hot summers, resulting in early ripening for most fruit tree cultivars. The objective of this orchard was the assessment of the performance of the last commercial CITA almond cultivars ['Guara', 'Soleta', 'Isabelona' (syn. 'Belona'), 'Diamar' (syn. 'Mardía') and 'Vialfas'] and some breeding selections (G-2-22, G-3-3, G-3-4, G-5-25. and I-3-67) (Table 1) in order to obtain direct information about the adaptability of these genotypes to this growing area. The trial was established in 2005, planting the hybrid peach x almond INRA GF-677 rootstocks at a distance of 6 x 7m. In the spring of 2006 the rootstocks were randomly grafted with the almond scions having finally six trees by genotype. The trees were trained as free open vase and managed under drip irrigation. The soil of the plot has an average depth of 1.5 m and is of sandy-loam type, being classified as calcic haploxerept, fine loamy, mixed, thermic (Soil Survey Staff, 2006).

Table 1. Origin, blooming date and harvest date of the studied plant material

Genotype	Pedigree	Blooming date	Harvest date
'Guara'	Unknown	4 March ± 7days	24 August ± 8 days
'Soleta'	'Blanquerna' x 'Belle d'Aurons'	1 March ± 7days	13 September ± 6 days
'Isabelona'	'Blanquerna' x 'Belle d'Aurons'	28 February ± 8 days	28 August ± 6 days
'Diamar'	'Felisia' x 'Bertina'	18 March ± 9 days	4 September ± 5 days
'Vialfas'	'Felisia' x 'Bertina'	15 March ± 7 days	2 September ± 8 days
G-2-22	'Felisia' x 'Bertina'	10 March ± 13 days	30 August ± 10 days
G-3-3	'Felisia' x 'Bertina'	15 March ± 6 days	7 September ± 7 days
G-3-4	'Felisia' x 'Bertina'	14 March ± 7 days	3 September ± 4 days
G-5-25	'Felisia' x 'Bertina'	14 March ± 8 days	10 September ± 5 days
I-3-67	'Felisia' x 'Moncayo'	26 February ± 9 days	5 September ± 5 days

The orchard was managed according to the normal cultural practices of the region: Irrigation was applied daily with an automated drip system with two laterals per tree row, located at 0.5 m from the rows, with self compensating emitters of 4 L h^{-1} spaced at 1 m. Irrigation water was pumped directly from the Mequinenza reservoir of the Ebro River. Soluble fertilizers were applied with the irrigation system along the whole season. The fertilizer amount per year included 80 kg ha^{-1} of N, 30 kg ha^{-1} of P_2O_5 and 120 kg ha^{-1} of K_2O according to Espada (2009).

Each tree was individually harvested when the genotype had more than the 70% of open mesocarps. For the seasons 2009-2014, fresh yield was weighted in the orchard, and a sample of 200 whole fresh fruits per tree was collected for further analysis. Once in the laboratory, mesocarps (hulls), endocarps (shells) and seeds (kernels) were separated and keep in a heater at 45ºC till constant weight. Fresh and dry weights of whole and shelled nuts and kernels were obtained for every year of study. Percentages of kernel from whole and from shelled nuts were calculated from these weights (Table 2).

Vigor was determined as the trunk cross sectional area (TCSA, cm^2). TCSA was calculated after harvest from the perimeter value measured at 30 cm above the soil surface. TCSA was calculated assuming a circular cross sectional area of the trunk. Tree productivity was expressed as the accumulated weight of the kernel yield (g of dry matter) divided by TCSA (g cm^{-2}) at harvest time.

Statistical analysis was performed using Analysis of Variance (ANOVA) and General Linear Model (GLM) procedure of the SAS 9.1 software (SAS Institute, 2004). Comparisons among genotypes were performed using Duncan test at $P = 0.05$.

III – Results and discussion

All almond genotypes studied showed kernel percentages (in relation to the whole fruit weight) from 19 to 28%, and shelling percentages from 25 to 35% (Table 2). 'Guara' showed the highest percentages for both cases. A high kernel percentage contributes to improve the crop input transformation, such as water and fertilizers (Alonso *et al.*, 2012).

Vigor measured has ranged between 326 and 166 cm^2. 'Soleta' was the most vigorous genotype whereas 'Vialfas' was the less vigorous, with half of the 'Soleta' TCSA. Presumably, vigorous and productive genotypes are more suitable for wide planting distances in high potentially growing conditions, whereas less vigorous and productive genotypes show better adaptation to reduced planting distances, even in high density plantings, or in more limiting growing conditions.

'Guara' and selection I-3-67 showed the highest accumulated yields and, with 'Vialfas', the highest productivities. These data corroborate that selection I-3-67 could be a firm candidate to be registered as suitable for growing areas with low risk of spring frosts, and that 'Vialfas', the new ultra late blooming cultivar, showed an excellent adaptability not only for high late frost risk areas, but also for very low frost risk areas as is the case of this trial.

The success of any cultivar not only depends on the accuracy of the breeder's selection, but also its adaptability must be ascertained to be recommended with success in a specific region, as shown with the results obtained in this trial.

Table 2. Vigor, kernel percentage, accumulated yield and productivity showed by the different CITA releases and elite selections

Genotype	Vigor TCSA (cm^2)	Kernel percentage: Whole nut	Kernel percentage: Shell almond	Accumulated yield (2009-2014): Unshelled nut kg/tree	Unshelled nut kg/ha	Kernel kg/tree	Kernel kg/ha	Productivity (g/cm^2)
'Vialfas'	166.2 e	20.4 b	25.3 c	56.6	13,479 bc	14.3	3,397 b	85.9 a
G-3-4	199.4 de	19.4 b	24.9 c	26.9	6,407 g	6.7	1,594 e	33.6 d
G-3-3	203.1 de	20.9 b	26.5 bc	35.5	8,444 efg	9.4	2,243 de	46.4 c
'Mardía'	215.9 cde	21.0 b	24.9 c	40.2	9,575 def	10.0	2,376 cd	46.2 c
G-5-25	219.6 cde	20.4 b	25.0 c	59.2	14,098 bc	14.8	3,517 b	67.3 b
G-2-22	255.9 bcd	19.6 b	24.9 c	33.7	8,030 gf	8.4	2,001 de	32.9 d
I-3-67	258.7 bce	23.2 b	29.3 bc	76.7	18,249 a	22.5	5,350 a	86.9 a
'Guara'	275.8 abc	28.4 a	35.8 a	66.4	15,792 b	23.8	5,654 a	86.1 a
'Belona'	303.0 ab	22.4 b	28.4 bc	45.4	10,810 de	12.9	3,060 bc	42.4 cd
'Soleta'	326.3 a	22.5 b	30.0 b	54.3	12,919 cd	16.3	3,877 b	49.9 cd

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Morphometric features of local and foreign female pistachio cultivars and ecotypes in northeast Tunisian conditions

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Abstract. In the framework of the Tunisian pistachio research program, a number of orchards were created in different bio-climatic areas of the country. Local and foreign cultivars and ecotypes were planted to study their environmental adaptation, agronomic performance and nut quality. This study was performed in one of these orchards at the INRAT Unity of Agricultural Experimentations of Mornag. Local (7) and foreign (5) cultivars and ecotypes were installed since 1989 under rainfed conditions. Local accessions are 'Mateur', 'Meknassy', 'Guermazi', 'El Guettar', "6/6", "8/7" and "28/4", and foreign accessions are 'Amiri', 'Jawad', 'Lassen', 'Ohadi' and 'Mumtaz'. Growth habit, vigor, yield and nut weight and size were evaluated during 2014. The growth habit of studied accessions was semi-erected or spreading. 'El Guettar', 'Guermazi', and the "8/7" ecotypes were the most vigorous whereas 'Ohadi' and 'Amiri' exhibited the lowest tree vigor. The fresh nut yield per tree varied between 0.43 and 23.4 kg. Foreign cultivars exhibited the lowest nut yields. It seems to be the consequence of their low adaptation. The "28/4" accession had the longest in-shell nut size while 'Jawad' nuts were the widest.

Key words. *Pistacia vera* – Cultivars – Ecotypes – Yield – Nut weight – Nut size.

Caractéristiques morphométriques de variétés et écotypes locaux et étrangers de pistachier femelle dans les conditions du nord-est tunisien

Résumé. *Dans le cadre du programme de recherche sur le pistachier en Tunisie, plusieurs vergers ont été créés dans le pays. Des variétés et écotypes locaux et étrangers ont été plantés pour étudier leur adaptation, leur performance agronomique et la qualité de leurs fruits. Cette étude a été réalisée dans l'un de ces vergers à l'Unité d'Expérimentations Agricoles de l'INRAT à Mornag. Sept accessions locales ('Mateur', 'Meknassy', 'Guermazi', 'El Guettar', "6/6" , "8/7" et "28/4") et cinq introduites ('Amiri', 'Jawad', 'Lassen', 'Ohadi' et 'Mumtaz') ont été installées depuis 1989 dans des conditions pluviales. Le port de l'arbre, la vigueur, le rendement ainsi que le poids et la taille du fruit ont été évalués au cours de l'année 2014. Les accessions étudiées avaient un port semi-érigé ou étalé. 'El Guettar', 'Guermazi' et "8/7" étaient les plus vigoureux alors que 'Ohadi' et 'Amiri' étaient les moins vigoureux. Le rendement en fruits frais par arbre a varié de 0,43 à 23,4 kg. Les plus faibles rendements ont été enregistrés chez les variétés étrangères ce qui semble être la conséquence de leur faible adaptation. Les fruits en coque l'accession " 28/4" étaient les plus longs alors que ceux de 'Jawad' étaient les plus larges.*

Mots-clés. Pistacia vera *– Cultivars – Ecotypes – Production – Poids du fruit – Taille du fruit.*

I – Introduction

The heterozygosis and dioecy of *Pistacia vera* resulted in great genetic diversity. Many producing countries have studied these inter and intra-specific variability (Ghrab *et al*., 2010 and 2012, Kaska *et al*., 2006) to select suitable cultivars for specific growing conditions (Aktug Tahtaci *et al*. 2011, Vargas *et al*., 1997). The Tunisian agricultural services and the INRAT fruit tree Laboratory have encouraged, since 1935 and later in 1950, the farmers to grow pistachio (Jacquy, 1973). Tunisian pistachio research gained more interest in the 1960's and 1970's years in the framework of the NUDP-FAO projects and the official agricultural services. Being aware of cultivars diversification impact on their adaptation, productivity and nut quality, local and foreign cultivars and rootstocks were planted in several geographic areas of the country to study their behaviors in these growing conditions. The most popular Tunisian cultivar and its two pollinators 25A and 40A were selected in 1974 and 1979, respectively. However, these cultivars have variable performances throughout cultivation areas (Chelli-Chaabouni *et al*. 2014). The aim of this study was to assess the performance and fruit quality of local and foreign (Iranian) *Pistacia vera* accessions growing in the North-east of Tunisia to select potential suitable cultivars for these environmental conditions.

II – Materials and methods

This study was carried out using 7 local and 5 foreign pistachio cultivars and ecotypes growing in the pistachio orchard of the Agricultural Experimentation Unity of the INRAT (National Institute of Agricultural Research of Tunisia) at Mornag (northern Tunisian area). This location has semi-arid Mediterranean climatic conditions with annual rainfall average of 450 mm. Twenty-five year old trees, grafted onto *P. vera* rootstock were growing under rainfed conditions with a spacing of 7 x 8 m. Visual evaluation was adopted to determine growth habit and tree vigor. Growth habit was defined according to the IPGRI Descriptors for *Pistacia vera* L. (IPGRI, 1997). Tree vigor was ranged in three classes: High, Medium and Low. Trees were harvested at the end of August 2014 at the maturity of the reference 'Mateur' cultivar and yield/tree was measured. The weight of in-hull and hulled fresh nuts, in-shell dry nuts and dried kernels were determined. The size (length (L), width (W) and thickness (T) of in-hull and in– shell nuts, and kernels was measured using a digital caliper. The geometric mean diameter and sphericity of nuts and kernels were calculated according to Mohsenin (1980) equations. Data were submitted to one-way ANOVA using SPSS software for Windows version 20. The multiple range test of Duncan was used for mean comparison. Cluster analysis was used to display the hierarchical classification of the studied accessions.

III – Results and discussions

Growth habit is an important trait to be considered in orchard management. It influences many physiological processes that have an impact on yield and nut quality. In this study, table 1 showed that all Tunisian pistachio accessions as well as 'Amiri' and 'Jabberi' Iranian accessions were spreading while 'Lassen', 'Jawad', 'Ohadi', and both 'Mumtaz' accessions "19/4" and "22/3" were semi-erect. Local accessions were characterized by high and medium vigor. 'El Guettar', 'Guermazi' and the "8/7" accession were particularly vigorous while 'Mateur', 'Meknassy', and "6/6" and "28/4" accessions had medium vigor. Among Iranian accessions, 'Jawad' was the most vigorous while 'Lassen', 'Ohadi' and 'Amiri' exhibited lower vigor. This evaluation is in part different from that of Rouskas (2002) in Greece environmental conditions who found that 'Mateur' accession was highly vigorous.

The local accessions showed a significantly higher yield per tree (Table 2). The average yields of all harvested trees were ranged between 0.43 and 23.4 kg. The local accession "8/7" gave the highest production followed by 'El Guettar' and 'Guermazi'. Foreign accessions yields did not exceed 2.4 kg/tree ('Amiri'). These results are not consistent with those found on 'Mateur' and 'Ohadi' in south-east conditions (Ghrab *et al*., 2005). Overall, in-hull and in-shell nut fresh weight of local accessions were low in comparison with foreign accessions. However, the in-shell/in-hull fresh weight ratio values of 'Mateur' and "6/6" accessions were significantly higher than 'Amiri' and 'Mumtaz' "19/4" accessions despite of the highest in-hull fresh weights of these latter. 'Amiri', 'Jawad' and 'Mateur' exhibited the highest in-shell dry weights among all studied accessions.

Table 1. Origin, growth habit, and vigor of accessions

Acs	N°	Origin	GH	Vigor	Acs	N°	Origin	GH	Vigor
'Mateur'	–	Tunisia	Spreading	Medium	Lassen	25/1	Iran	Semi-erect	Low
'El Guettar'	"3/5"	Tunisia	Spreading	High	Jawad	24/1	Iran	Semi-erect	High
'Meknassy'	–	Tunisia	Spreading	Medium	Ohadi	14/7	Iran	Semi-erect	Low
'Guermazi'	–	Tunisia	Spreading	High	Amiri	15/6	Iran	Spreading	Low
Local accession	"6/6"	Tunisia	Spreading	Medium	Mumtaz	19/4	Iran	Semi-erect	Medium
Local accession	"8/7"	Tunisia	Spreading	High	Mumtaz	22/3	Iran	Semi-erect	Medium
Local accession	"28/4"	Tunisia	Spreading	Medium					

Acs: Accessions, GH: Growth habit.

Table 2. Yield average per tree (kg) and fruit weight (g): in-hull and in-shell fresh weight (FW), and In-shell dry weight (DW)

Accessions	Yield/tree	In-hull FW	In-shell FW	In-shell/in-hull FW	In-shell DW
'Mateur'	5.5^{c}	2^{bc}	1.2^{cd}	0.6^{ab}	0.9^{bc}
'El Guettar'	13.7^{b}	1.6^{d}	0.9^{ef}	0.6^{cd}	0.7^{ef}
'Meknassy'	6.1^{c}	2.1^{b}	1.2^{cd}	0.6^{b-d}	0.9^{b-d}
'Guermazi'	12.8^{b}	1.5^{d}	0.9^{f}	0.6^{b-d}	0.7^{ef}
'LA 6/6'	10.5^{b}	1.8^{cd}	1.1^{cd}	0.7^{a}	0.9^{b-d}
'LA 8/7'	23.4^{a}	1.8^{cd}	1.1^{de}	0.6^{bc}	0.8^{cd}
'LA 28/4'	11.5^{b}	2.1^{bc}	1.2^{cd}	0.6^{bc}	0.8^{cd}
'Lassen'	0.5^{d}	2.2^{b}	1.3^{bc}	0.6^{bc}	0.8^{de}
'Jawad'	0.8^{d}	2.7^{a}	1.6^{a}	0.6^{bc}	1^{ab}
'Ohadi'	2.2^{cd}	2.2^{b}	1.3^{cd}	0.6^{b-d}	0.9^{b-d}
'Amiri'	2.4^{cd}	2.8^{a}	1.6^{a}	0.6^{cd}	1.1^{a}
'Mumtaz 19/4'	2^{cd}	2.7^{a}	1.5^{ab}	0.6^{d}	0.9^{b-d}
'Mumtaz 22/3'	0.4^{d}	2^{bc}	1.1^{cd}	0.6^{b-d}	0.6^{f}

LA: Local accession. For the same parameter, different subscript letters mean significant differences (Duncan test; $p<0.05$) among accessions.

The size measurements data of in-hull fruits showed that 'Lassen' and 'Mumtaz' "19/4" had the longest and widest fruits while 'Guermazi' and "6/6" local accessions had the lowest fruit lengths. The "8/7" fruit accession had the lowest width and thickness (Table 3). Among the studied in-shell nuts, those of the "28/4" accession were the longest while 'Jawad' nuts were the widest. 'Guermazi' had the lowest nut length and width and 'Meknassy' nuts had the lowest thickness. 'Amiri' kernels were the longest and thickest. The "28/4"accession and 'Meknassy' nuts were ranged between the most long and wide. Those parameters could be interesting in fresh fruit market. The "8/7" accession nuts were the widest. 'Mumtaz' "22/3" accession had the smallest kernels in all size parameters (Table 3). Except of this latter accession, Iranian accessions had, generally, bigger nuts than Tunisian ones. These results are similar to those of Karaca and Nizamoglu (1995) describing Iranian varieties as having bigger sizes than Turkish ones.

Hierarchical classification of all accessions was established according to fruit characteristics described in tables 2 and 3 in addition to fruit mean diameter and sphericity values (data not shown). Apart from the "8/7" local accession that was the most distant from all other accessions, hierarchical cluster analysis subdivided the 12 remaining accessions into two major groups. The first group included foreign accessions 'Jawad', 'Amiri', 'Ohadi', 'Lassen', and 'Mumtaz' "19/4" and "22/3". The second one was

Table 3. In-hull, In-shell and kernel size in mm

Accessions	In-hull			In-shell			Kernel		
	FL	FW	FT	FL	FW	FT	K L	K W	K T
'Mateur'	24.1^{bc}	13.2^{d}	11.9^{cd}	19.8^{cd}	10.6^{c}	9.5^{cd}	15.1^{b}	7.8^{a-c}	7.7^{b-d}
'El Guettar'	22.7^{d}	13.1^{de}	11.4^{de}	18.3^{f}	10.6^{cd}	8.8^{de}	15.5^{ab}	8.5^{a}	7^{c-e}
'Meknassy'	23.1^{c}	13.6^{cd}	11.6^{cd}	20^{b-d}	10.9^{c}	8.7^{e}	16.2^{ab}	8.6^{a}	7.6^{b-d}
'Guermazi'	21.1^{d}	12.8^{de}	11.2^{d-f}	16.9^{g}	9.6^{e}	7.6^{f}	15.2^{a-c}	8.3^{ab}	6.7^{de}
'LA 6/6'	21.4^{d}	11.4^{f}	10.8^{ef}	18.3^{f}	10de	9.3^{c-e}	14.9^{bc}	7.4^{cd}	7.5^{cd}
'LA 8/7'	21.4^{d}	12.3^{e}	10.7^{f}	18.6^{ef}	10.7^{cd}	8.9^{de}	15.6^{a-c}	8.7^{a}	7^{c-e}
'LA 28/4'	23.2^{c}	13.5^{cd}	11.8^{cd}	21.4^{a}	11.6^{b}	9.8^{c}	16.4^{ab}	8.6^{a}	7.6^{b-d}
'Lassen'	25.8^{a}	15^{a}	13.5^{ab}	21.3^{a}	12.9^{a}	11.2^{ab}	15^{bc}	7.5^{b-d}	7.1^{c-e}
'Jawad'	23.6^{bc}	14.3^{a-c}	13.7^{ab}	20.8^{a-c}	12.3^{ab}	11.6^{a}	16.1^{ab}	7.9^{a-c}	7.9^{a-c}
'Ohadi'	21.8^{d}	13.4^{d}	13.1^{b}	19.4^{de}	11.7^{b}	11.1^{ab}	15.8^{ab}	8.1^{a-c}	8.6^{ab}
'Amiri'	24.4^{b}	14.5^{ab}	14.1^{a}	21.3^{a}	12.9^{a}	11.4^{ab}	17.3^{a}	8.4^{a}	8.8^{a}
'Mumtaz 19/4'	25.6^{a}	14.8^{a}	13.8^{ab}	21.0^{ab}	12^{b}	10.8^{ab}	16^{ab}	7.5^{bc}	7.2^{c-e}
'Mumtaz 22/3'	23^{c}	13.7^{b-d}	12.2^{c}	19.7^{d}	12.2^{ab}	10.7^{b}	13.6^{c}	6.6^{d}	6.2^{e}

For the same parameter, different subscript letters mean significant differences (Duncan test; $p<0.05$) among accessions. LA: Local accession, FL: Fruit length; FW: Fruit width; FT: Fruit thickness; KL: Kernel length; KW: Kernel width; KT: Kernel thickness.

composed by the local accessions 'Mateur', 'Meknassy', 'El Guettar', 'Guermazi', and both accessions "6/6" and "28/4". The most closely related accessions were, in pairs, 'Jawad' and 'Amiri'; 'Lassen' and 'Mumtaz' "19/4"; 'Mateur' and 'Meknassy'; and 'El Guettar' and 'Guermazi'. The 'Mateur' reference accession was located at mid-distance between foreign and local accessions. On the opposite sides, 'Jawad' and "28/4" were the most distant accessions from 'Mateur' reference cultivar.

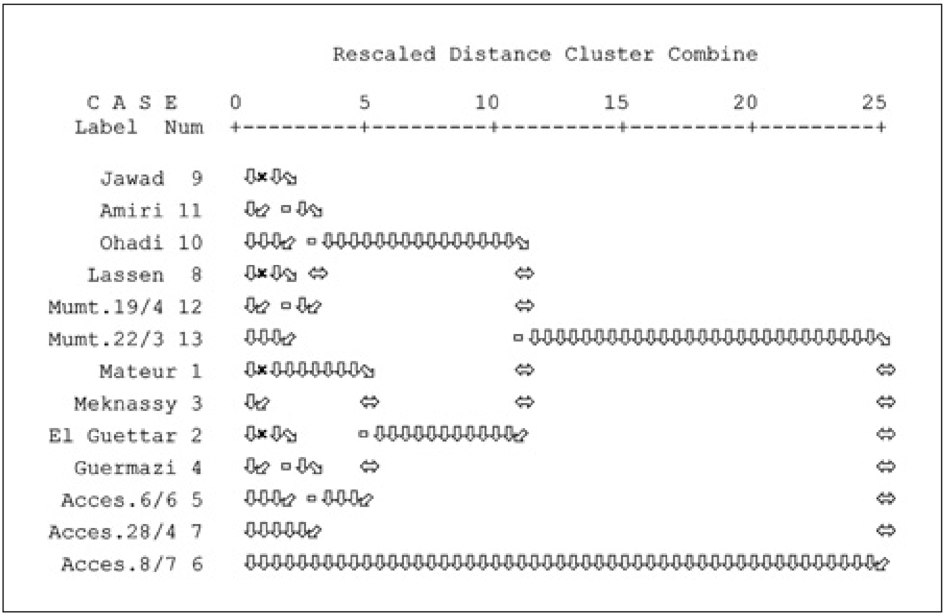

Fig. 1. Dendrogram using average linkage between groups according to fruit characteristics.

These preliminary results revealed the great adaptation of local accessions to the north-east Tunisian conditions in comparison with Iranian accessions. It should be pointed out that nut size of the studied foreign accessions showed good characteristics despite having been harvested before full maturity. This experiment is scheduled to continue for more few years for better evaluation results.

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Self-compatibility sources as sources of variation in advanced almond introgression lines

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Abstract. Breeding for self-fruitfulness in almond requires the combination of pollen-pistil self-compatibility which is controlled by a single-major *S* gene and self-pollination or self-fertility which is controlled by multiple genes. Important sources for self-compatibility include *Prunus webbii* and its derivatives such as *cv. Tuono* which are pistil based, and peach and its wild relatives which are pollen based. The loci controlling the pistil and pollen self-compatibility genes are tightly linked which frustrates accurate Marker Assisted Selection. Methods of Marker Assisted Breeding complemented by Pedigree Based Analysis have facilitated the introgression of self-compatibility alleles from multiple independent sources including *P. webbii,* cv. 'Tuono', *P. mira,* and peach *(P. persica).* The final assessment of self-fruitfulness is determined by selfed-seed set in orchard trees where individual branches are enclosed to prohibit insect cross-pollination. The degree of self-fruitfulness under these field conditions has been found to be controlled by a major *S*-gene as well as modifier genes, often with significant environment interactions. In certain introgression lines, seedling tree age affects the expression of self-fruitfulness. Genetic background also strongly influences the level of self-fruitfulness as well as differences in branch architecture and so bearing habit. Some self-incompatible genotypes have also been found to express relatively high but inconsistent levels of self-fruitfulness. High levels of both expression and stability of self-fruitfulness is being pursued in advanced almond introgression lines by combining pistil-based and pollen-based genes with appropriate genetic backgrounds

Keywords. Self-fruitfulness – Introgression – S_f locus – Bearing – Architecture.

Les sources de auto-compatibilité comme source de variation dans des lignes avancés d'introgression d'amandier

Résumé. *L'amélioration pour l'auto-fructification chez l'amandier nécessite la combinaison d'auto-compatibilité pollen-pistil, contrôlée par un seul S-gène majeur, et d'auto-pollinisation ou auto-fertilité, contrôlée par des gènes multiples. Parmi les sources importantes pour l'auto-compatibilité figurent* Prunus webbii *et ses dérivés tels que* cv. Tuono*, qui sont basés sur le pistil, et la pêche et ses apparentés sauvages, qui sont basés sur le pollen. Les loci qui contrôlent les gènes d'auto-compatibilité du pistil et du pollen sont étroitement liés, ce qui entrave la précision d'une sélection assistée par marqueurs. Les méthodes d'amélioration assistées par marqueurs couplées à l'analyse généalogique ont facilité l'introgression d'allèles d'auto-compatibilité provenant de multiples sources indépendantes y compris* P. webbii, *cv. 'Tuono',* P. mira*, et la pêche* (P. persica). *L'évaluation finale des graines auto-fécondées est déterminée par la fructification auto-pollinisée chez des arbres du verger où des branches individuelles sont ensachées pour empêcher la pollinisation croisée par les insectes. Le degré d'auto-fructification sous ces conditions de champs s'est avéré contrôlé par un S-gène majeur ainsi que par des gènes modificateurs, ayant souvent des interactions significatives avec l'environnement. Dans certaines lignées d'introgression, l'âge du plant d'arbre affecte l'expression de l'auto-fructification. Le fond génétique influence aussi fortement le niveau d'auto-fructification ainsi que les différences d'architecture des branches et donc le mode de fructification. Certains génotypes auto-incompatibles ont révélé exprimer des niveaux relativement élevés mais inconsistants d'auto-fructification. De hauts niveaux à la fois d'expression et de stabilité de l'auto-fructification sont recherchés dans les lignées avancées d'introgression d'amandier en combinant des gènes basés sur le pistil et des gènes basés sur le pollen avec des fonds génétiques appropriés.*

Mots-clés. *Auto-fructification – Introgression – Locus S_f – Architecture fruitière.*

I – Introduction

Almonds are naturally self-incompatible, requiring the inter-planting of often inferior pollinizer cultivars in orchards in order to set a commercial crop. Poor weather conditions and/or scarcity of required insect cross-pollinators during the short almond bloom period in early spring frequently contribute to crop reductions. Self-fruitfulness in almond is thus an important breeding objective as it could increase production uniformity while decreasing orchard inputs. Self-compatibility is controlled by a single-major *S* gene, while the capacity for self-pollination or autogamy is controlled by multiple genes affecting flower structure and development (López *et al*., 2006). Because the self-compatible allele *Sf* is not native to almond, it has to be introgressed from related self-fertile species either directly through interspecific hybridization and subsequent backcrossing or through intraspecific hybridization with self-compatible almond land races such as 'Tuono' where S_f introgression has occurred naturally. Both the inter-and intra-species origin as well as the mode-of-action of these different S_f gene sources vary. In peach species, self-fertility is determined by the pollen portion of the S_f gene, while in almond species, determination is within the pistil (Tao *et al*., 2007) though the relative performance of different S_f sources has rarely been compared. As part of a long-term cultivar development project, the almond breeding program at the University of California at Davis (UCD) has developed advanced introgression lines transferring self-fruitfulness from multiple independent sources including *P. webbii,* cv. 'Tuono', *P. mira,* and peach *(P. persica*. Field performance of some of the more promising advanced introgression breeding lines have been evaluated to assess both the magnitude and stability of different sources.

II – Materials and methods

Twelve advanced introgression lineages were evaluated, including breeding lines derived from *Prunus mira, Prunus fenzliana, P. persica,* cv. 'Tuono', and *P. webbii*. Trees were selected from seedling populations grown on their own roots under high orchard densities with standard commercial fertilizer and water inputs. Self-fruitfulness was evaluated from the 3rd or 4th year after planting up to the 10th year in the field. Self-fruitfulness was evaluated by enclosing approximately 80 cm of a flowering branch with an insect-proof mesh bag prior to flower opening. Bagged branches were self-pollinated every 3 days during bloom using an air-brush which caused self-pollen from adjacent dehisced anthers to be transferred to the stigma. Final fruit set was recorded 2 months after petal fall as the percentage of fruit that had set relative to the number of flowers estimated to be present at the time of bagging. Normal fruit-set on adjacent branches which had not been bagged were also used as controls.

III – Results and discussion

All advanced introgression lines evaluated possessed promising levels of self-fruitfulness as well as good tree productivity and kernel quality demonstrating the efficacy of simultaneous selection for these traits. More intensive selection was required for recovering commercially acceptable kernel sizes and shapes due both to the small initial sizes of most donor species as well as a greater tolerance for differences in tree architecture in commercial production (Kester and Gradziel, 1996). A consequence of the intensive effort required for foreign gene introgression, particularly the large number of recurrent backcrosses required to achieve commercially acceptable phenotypes, is that relatively few of the S_f alleles available in related germplasm is actually utilized. The current UCD almond breeding germplasm is dominated by only one source for *P. mira*, one source for *P. fenzliana*, 4 sources for *P. webbii* (including cv. 'Tuono' and 2 sources for *P. persica*. Both the magnitude and stability of self-fruitfulness varied among introgression sources within the limited breeding lines evaluated in this study (Fig. 1). The most consistent performance was observed in breeding lines derived from *P. mira*, particularly selection 2004,8-160. Good performance was also

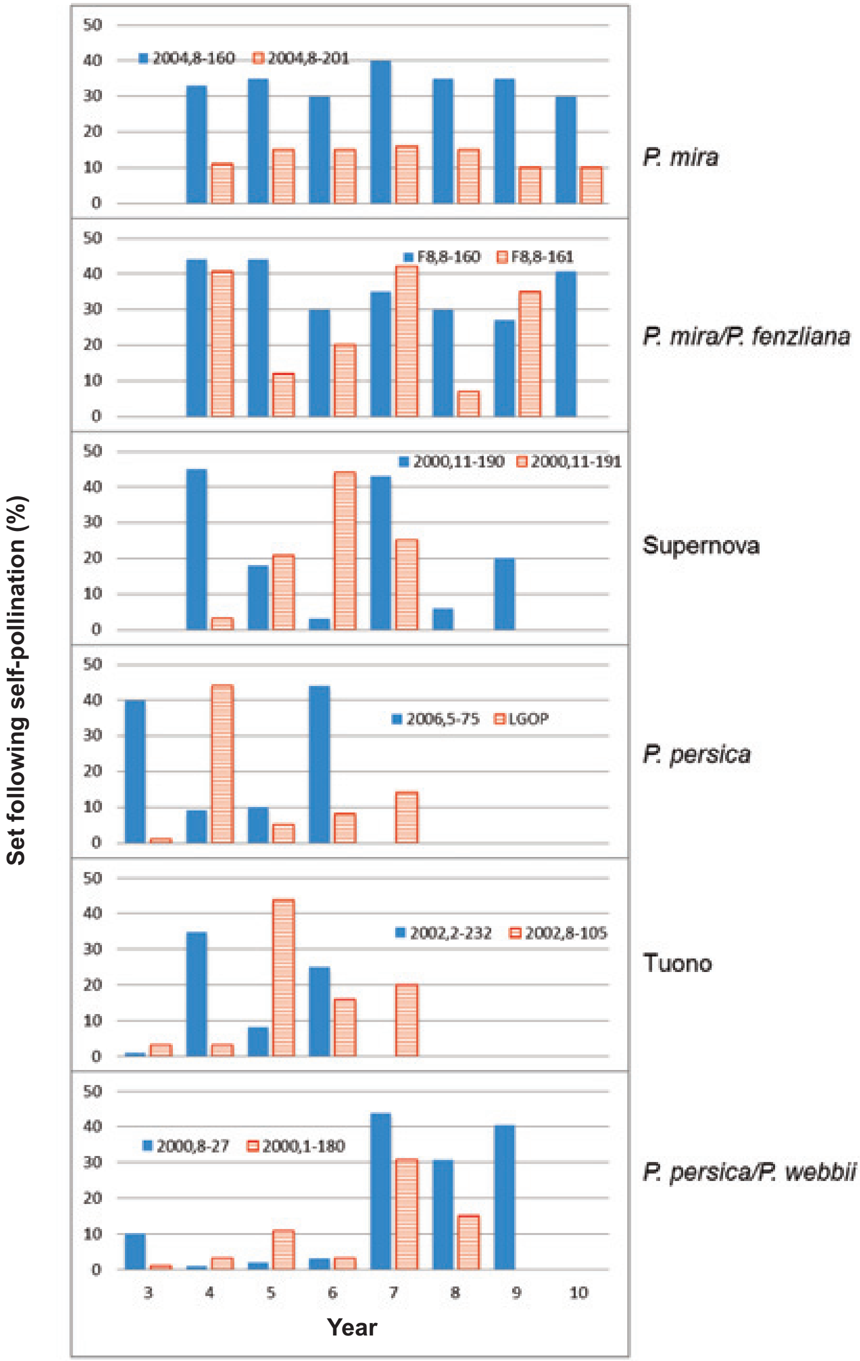

Fig. 1. Levels of self-fertility in 2 selected advanced almond selections from each of 6 separate introgression lines, with donor identity listed to the right of each plot.

observed in introgression lines derived from both *P. mira* and *P. fenzliana*. *P. fenzliana* was utilized as a source for improved tree architecture rather than self-fruitfulness since it is self-incompatible. Greater year-to-year variation was observed in breeding lines derived from *P. persica* and *P. webbii* including the cultivar's *Tuono* which appear to be derived from *P. webbii*. Of particular interest was the finding that in the breeding lines evaluated which combined sources of both *P. persica* and *P. webbii* promising levels of self-fruitfulness were only detected in mature seedling trees which were 7 years or older. Mature clonal trees, propagated on standard commercial rootstocks express more stable levels of self-fruitfulness, indicating that the variability observed is more a function of the early years of development and growth of own-rooted seedling trees. While the variability demonstrated may have implications concerning the best breeding sources for commercial production, it has more direct consequences for the initial seedling selection process since both expressivity and penetrance of the trait may vary considerably and so would confound effective response to selection. Although most traditional self-incompatible almond cultivars consistently express very low levels of self-fruitfulness (Kodad *et al*., 2008, Kodad *et al*., 2010, Martinez-Garcia *et al*., 2011), some self-incompatible genotypes such as S_1S_{14} as found in the cultivars Winters and Sweetheart can produce relatively high levels of self-seed set under conducive conditions (Gradziel *et al*., 2013) which would further confound accurate selection. In addition, the relatively late expression of self-fruitfulness in some of the introgression lines evaluated would frustrate a more efficient selection at an earlier tree age. Variations in self-fruitfulness among the different introgression lines may be as much a consequence of differences in tree growth and development as differences in Sf allele potential. Many almond seedling trees are notoriously shy-bearing in the early years even though profuse flowering may occur which is partly a result of the need to develop sustainable bearing-wood such as perennial spurs (Socias i Company *et al*., 2011). While interspecific breeding lines targeting the introgression of self-fruitfulness have the added benefits of enriching the breeding germplasm for both novel disease/pest resistance and tree architecture/productivity, modified fruit bearing habits such as shown in Fig. 2 would be expected to influence early tree cropping performance independent of self-fruitfulness, because of the differences in potential productivity as well as in bearing-wood renewal.

Fig. 2. An example of the more radial crop bearing-habit in an advanced almond introgression line derived from an initial interspecific hybridization with *Prunus webbii.*

IV – Conclusions

In the seedling selections evaluated, the degree and stability of self-fruitfulness varied among the different species sources for this trait as well as among different possible sources within species. The presence of multiple alleles for self-fruitfulness has been documented in many of the species sources including *P. persica, P. mira* and *P. webbii* (Hanada *et al.,* 2014, Tao *et al*., 2007), however the tedious and time-consuming backcrossing required for successful trait introgression has resulted in only a very small number of such alleles being transferred and tested within an almond genetic background. The more intensive selection encouraged by these more demanding recurrent backcrossing strategies also tend to limit the transfer of flower and branch development/architecture traits which could act to complement both intensity and stability of self-fruitfulness in new almond cultivars.

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Genetic characterization of almond (*Prunus dulcis*) cultivars and natural resources

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Abstract. This study was conducted to analyse the structure of molecular variation among Hungarian almond genotypes and to estimate the level of genetic diversity. A total of eighty-six almond samples originated from different geographical regions from Central Asia to California were evaluated using a set of 8 SSRs and 5 EST-SSR primer pairs. Amplification of DNA was successful for 11 of the 13 SSR loci. A mean value of 15.45 alleles per locus was found. Our data revealed a high level of genetic variability in most groups of samples. The highest number of alleles was detected in the Moroccan genotypes while the lowest number of alleles occurred in the group of self-compatible cultivars. All Hungarian cultivars and accessions were characterized by an average allele number of above 5. The correspondences between the genetic diversity and mating strategy highlight the role of the self-(in)compatibility system in shaping the genome of a fruit tree species.

Keywords. Almond – *Prunus dulcis* – Genetic diversity – SSR – Microsatellites.

***Caractérisation génétique de cultivars et de ressources naturelles d'amandier* (Prunus dulcis)**

Résumé. *Cette étude a été menée pour analyser la structure de la variation moléculaire entre génotypes hongrois d'amandier et pour estimer le niveau de diversité génétique. Un total de quatre-vingt-six échantillons d'amandiers provenant de différentes régions géographiques, depuis l'Asie centrale jusqu'en Californie, ont été évalués à l'aide d'un ensemble de 8 SSR et 5 primer pairs EST-SSR. L'amplification de l'ADN a été réussie pour 11 des 13 loci SSR. On a trouvé une valeur moyenne de 15,45 allèles par locus. Nos données révèlent un fort niveau de variabilité génétique dans la plupart des groupes d'échantillons. On a détecté le plus grand nombre d'allèles dans les génotypes marocains tandis que le plus petit nombre d'allèles se trouvait dans le groupe de cultivars autocompatibles. Tous les cultivars et accessions hongrois étaient caractérisés par un nombre moyen d'allèles supérieur à 5. Les correspondances entre la diversité génétique et la stratégie de fécondation mettent en relief le rôle du système d'auto-(in)compatibilité pour la configuration du génome d'une espèce fruitière arboricole.*

Mots-clés. *Amandier* – Prunus dulcis – *Diversité génétique – SSR – Microsatellites.*

I – Introduction

The cultivated almond [*Prunus dulcis* (Mill.) D.A. Webb] is thought to have originated in the arid mountainous regions of Central Asia (Grasselly 1976). Molecular results evidenced dissemination of the cultivated almond from Iran to the Eastern Mediterranean and subsequently to the Western Mediterranean regions, to North America and finally to the southern hemisphere including South America and Australia. Almond is a self-incompatible species which is governed by the highly polymorphic, multiallelic *S*-locus (Dicenta and García, 1993). Due to the genetically controlled self-incompatibility system, almond is one of the most polymorphic cultivated fruit species.

Simple sequence repeats (SSR) or microsatellites have been widely used to study tree species (Höhn *et al*., 2010; Lendvay *et al*., 2014). Since first SSR markers were described in peach (Cipriani *et al*., 1999), they have been developed in many other *Rosaceae* species, such as apricot, Japanese plum and cherry (Dirlewanger *et al*., 2002; Messina *et al*., 2004; Mnejja *et al*., 2004). Transferability (being able to use an SSR developed in one species in other species) has been frequently reported, particularly for peach SSRs. No significant differences were detected in transferability and the ability to detect variability between microsatellites of EST and genomic origin (Mnejja *et al.*, 2010). The first set of almond SSRs was published by Testolin *et al*. (2004).

Up to date, several studies were carried out to characterize the SSR diversity of almond cultivars and genotypes originated in specific geographical regions. Xu *et al*. (2004) developed SSR markers for the phylogenetic analysis of almond trees from China and the Mediterranean region. Genetic diversity of the Spanish national almond collection was characterized by Fernández i Martí *et al*. (2009). Twelve highly polymorphic SSR loci were selected to uniquely identify cultivars commonly grown in California, and to allow an accurate assessment of parent/offspring relationships among them (Dangl *et al*., 2009). Zeinalabedini *et al*. (2010) characterized Spanish, French, Italian, American, Iranian, Tunisian, Australian, Ukrainian, Portuguese and Slovakian almond cultivars by chloroplast and nuclear SSRs. Their results established the value of SSR markers for distinguishing different genetic lineages and characterize an extensive gene pool available to almond genetic improvement.

However, until now, there is no molecular information about the genetic diversity and relationship of the Hungarian almonds. Hence, this study was conducted to analyse, for the first time, the structure of molecular variation among Hungarian genotypes and to estimate the level of genetic diversity. In addition, the data are evaluated in comparison with a range of genotypes coming from different geographical areas, from Central Asia to California.

II – Materials and methods

A total of eighty-six almond samples originated from different geographical regions were evaluated. The Hungarian, Ukrainian and Italian cultivars are kept in the collection of the Corvinus University of Budapest, Department of Genetics and Plant Breeding. Other Hungarian samples were collected from abandoned orchards in Gellérthegy, Tétényi-fennsík, Cegléd and Monor. Leaf samples of the Moroccan accessions were collected in wild growing populations. Turkish wild almond genotypes originated in Bademli region and Akdamar Island (Lake Van). Some accessions were collected in Kyrgyzstan (near the city Osh). Californian cultivars and one accession of wild almonds (*Prunus tenella*, *P. arabica* and *P. webbii*) were sampled in the experimental orchard of Agricultural Research Service (United States Department of Agriculture, Fresno, CA).

Genomic DNA was extracted from fully expanded young leaves using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). A set of 8 SSRs and 5 EST-SSR primer pairs were selected on the basis of previous reports on different *Prunus* species, and included 6 for peach, 6 for almond and 1 for plum (Table 1), covering eight linkage groups (G1 to G8). The forward primers were labelled with 6-FAM fluorescent dye for detection in a capillary genetic analyzer. PCR reactions were carried out in a PTC 200 thermocycler (MJ Research, Budapest, Hungary) using the program described for the primers Approximately 20-80 ng of genomic DNA was used for PCR amplification in a 25 µl reaction volume containing 10 × Dream*Taq*™ Green buffer (Fermentas, Szeged, Hungary) as well as KCl and $(NH_4)_2SO_4$ at a ratio optimized for robust performance of Dream*Taq*™ DNA Polymerase in PCR with final concentrations of 4.5 mM $MgCl_2$, 0.2 mM of dNTPs, 0.2 µM of the adequate primers and 0.75 U of Dream*Taq*™ DNA polymerase (Fermentas).

Fragment lengths were estimated by comparison with the 1-kb DNA ladder (Promega, Madison, USA). To determine the exact size of the fragments, the fluorescently labelled products were run on an automated sequencer ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Budapest, Hungary).

For determination of fragment sizes (genotyping), GENOTYPER 3.7 software and the GS500 LIZ size standard (Applied Biosystems) were used. PopGene 1.32 (http://www.ualberta.ca/_fyeh/) software also was used for calculation of observed heterozygosity (*Ho*), expected heterozygosity (*He*), observed number of alleles (*Na*), Shannon's information index (*I*) and gene flow (*Nm* = 0.25(1 – FST)/FST.

III – Results and discussion

In the 86 almond genotypes, amplification of genomic DNA was successful for 11 of the 13 SSR loci including 7 genomic and four EST-SSR primers developed from different *Prunus* species (peach, almond and plum). The primer BPPCT 001 designed from peach genomic sequences provided unclear amplification; while the ASSR27 locus proved to be monomorphic and hence neither of them was included in the analysis. Altogether, 11 primer pairs produced a total of 170 alleles ranging from 4 to 21 per locus (Table 1). All primer pairs produced a maximum of two alleles per genotype in accordance with the diploid state of the species. Genotypes showing a single band were considered homozygous for that particular locus. A mean value of 15.45 alleles per locus was found. SSR primers derived from the non-coding DNA region had more alleles (an average of 17.85 per locus) than EST-SSR primers, originating from coding DNA regions (an average of 11.25 alleles per locus). Among EST-SSR loci, EPDCU 3083 had the highest number of alleles (20), while EPDCU5100 amplified the lowest number of alleles, only four. Observed heterozygosity ranged between 0.62 for CPPCT 044 and 0.81 for BPPCT 025, with an average of 0.73 across the SSR markers, while in case of EST-SSRs the average was much lower (0.53) since one of the loci (the EPDCU 5100) revealed an exceptionally low level of heterozygosity (0.19).

The highest number of alleles for most of the analysed markers (Table 1) was detected in the Moroccan genotypes (7.09). This huge genetic variability might be explained by previous studies based on nuclear DNA markers and showing that Moroccan genotypes are genetically different from the tested commercial cultivars (El Hamzaoui *et al.*, 2013). The lowest number of alleles occurred in the group of self-compatible cultivars (3.63). All Hungarian cultivars and accessions were characterized by an average allele number of above 5. The highest observed heterozygosity was detected in the Turkish accessions from Akdamar Island (0.76), while self-compatible cultivars showed the lowest value (0.58). The four groups of Hungarian genotypes (cultivars, accessions from Gellért-hegy, accessions from the Tétényi-fennsík and old genotypes from Cegléd and Monor) revealed very similar data for all parameters including Na, I, He, Ho, and N_m; however, two Turkish groups (accessions from Akdamar Island and Bademli region) showed marked differences. It was also reflected by the fact that approximately the same proportion of unique alleles (approx. 70%) was found in those groups as shown for groups more distantly related groups (e.g. Californian cultivars and self-compatible almonds).

Table 1. Number of alleles, Shannon's Information index (I), observed heterozygosity (*Ho*), expected heterozygosity (*He*), and gene flow (N_m) in the tested geographic groups of almond

Geographic groups	Number of alleles (*Na*)	Shannon's Information index (*I*)	Observed (*Ho*) heterozygosity	Expected (*He*) heterozygosity	Gene flow (N_m)
Hungarian cultivars	5.27	1.37	0.67	0.71	0.25
Gellért-hegy, Hungary	5.18	1.41	0.67	0.75	0.23
Tétényi-fennsík, Hungary	5.45	1.45	0.67	0.75	0.23
Old Hungarian genotypes	5.09	1.35	0.61	0.71	0.21
Californian cultivars	4.18	1.12	0.63	0.63	0.30
Akdamar Island, Turkey	4.45	1.16	0.76	0.64	0.41
Bademli region, Turkey	5.09	1.28	0.61	0.66	0.23
Morocco	7.09	1.60	0.66	0.75	0.20
Self-compatible cultivars	3.63	1.06	0.58	0.64	0.25

Our data revealed a high level of genetic variability in most groups of samples, which was very similar to the trend emerging from other studies on almond genetic variability (Szikriszt *et al*., 2011). Many of the genetic diversity parameters were almost identical for most groups with only few exceptions. Californian cultivars, accessions from Akdamar island and self-compatible cultivars showed decreased extent of variability.

Akdamar is a small island in Lake Van, while Bademli is a region where almond trees have been long known to grow around the region. Several trees in Akdamar Island share common or almost identical genotypes pointing to the consequences of a founder effect. It is supposed that only some trees have been brought to the island and all extent accessions are the offspring of a small number of individuals originally reaching the island. However, observed heterozygosity remained high since almond is self-incompatible. In turn, Nm is high, indicating only a moderate genetic differentiation of the population. In contrast, almonds in Bademli show higher genetic diversity since they do not face with such a geographic isolation. However, it is interesting to realize that trees from Bademli village and those from Akdamar Island showed as low genetic relatedness as trees originated in different regions of the world. We have determined self-incompatibility RNase alleles in Bademli almonds and found several *Prunus webbii* alleles in their *S*-locus. It suggests introgressive hybridization between *P. dulcis* and naturally occurring *P. webbi* accessions. This was also confirmed with other species like *P. orientalis* (Delplancke *et al*., 2012; Zeinalabedini *et al*., 2010) and indicates that differences in specific geographic regions might be formed by introgressive hybridization.

The number of alleles and Shannon index also indicated decreased genetic variability for Californian commercial cultivars. The use of a small number of accessions in breeding programs will eventually lead to a marked decrease in genetic variability as was experienced in our study, as well. Self-compatibility was described in some almond cultivars, and a slight decrease in variability parameters could have been shown in the present study. Self-compatibility in almond is a relatively new trait (spanning only some decades in time) and hence it has not been enough time to induce genetic erosion. The correspondences between the genetic diversity and mating strategy highlight the role of the self-(in)compatibility system in shaping the genome of a fruit tree species.

IV – Conclusions

There is no indication of major decrease in genetic variability in almond germplasm from Asia to Europe. Slight losses of genetic diversity are attributable to different reasons including geographic isolation, human selection and especially the relatively recent occurrence of self-compatibility. This information might be taken as a warning signal by almond breeders.

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Expression of the S_{fa}-allele in homozygote genotypes ($S_{fa}S_{fi}$) indicates a mutation in the stylar part of the Sf haplotype as origin of self-compatibility in almond

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Abstract. The S_f allele shows two different expressions: an active form (S_{fa}) inducing self-incompatibility and an inactive form (S_{fi}) inducing self-compatibility. Their interaction was studied in several hetero/homozygous genotypes ($S_{fi}S_{fa}$) in order to establish if self-compatibility was dominant as previously suggested. The seedling genotype was determined by PCR amplification of genomic DNA with universal and specific primers and the phenotype by pollen tube growth. The results showed full self-incompatibility of the $S_{fi}S_{fa}$ genotypes as a result of the recognition of any kind of S_f pollen (S_{fi} or S_{fa}) by the style, where S_f-RNase was produced due to the presence of the S_{fa} allele. These results confirm the allelism of the S_f allele with the series of *S* alleles of self-incompatibility and that a mutation in the stylar part of the S_{fa} haplotype has led to the self-compatibility of the S_{fi} form. The recognition of the S_{fi} pollen by the S_{fa} style confirms that the presence of the S_{fi} haplotype does not ensure self-compatibility, and that in these hetero/homozygous genotypes the expression of S_{fa} is dominant over that of S_{fi}.

Keywords. *Prunus amygdalus* Batsch – Self-compatibility – Breeding – S_f allele – Allele expression – Allele recognition.

L'expression de l'allèle S_{fa} dans les génotypes homozygotes $S_{fi}S_{fa}$ indique une mutation dans la partie stylaire du haplotype S_f comme l'origine de l'auto-compatibilité chez l'amandier

Résumé. *L'allèle S_f montre deux formes d'expressions différentes: une forme active (S_{fa}) qui confère l'auto-incompatibilité et une forme inactive (S_{fi}) qui induit l'auto-compatibilité florale. Leur interaction a été étudiée chez quelques génotypes hétéro/homozygotes ($S_{fi}S_{fa}$) pour établir si l'auto-compatibilité est dominante comme a été suggéré antérieurement. Le génotype des semis a été déterminé par l'amplification PCR de l'ADN génomique avec des amorces universelles et spécifiques et le phénotype a été évalué par l'étude de la croissance du tube pollinique dans le style. Les résultats ont montré que les génotypes $S_{fi}S_{fa}$ sont complètement auto-incompatibles due à la reconnaissance des deux formes de pollen S_f (S_{fi} ou S_{fa}) au niveau du style, où S_f-RNase est produite par la présence de l'allèle S_{fa}. Ces résultats confirment l'allelisme de l'allèle S_f avec la série d'allèles S de l'auto-incompatibilité florale chez l'amandier et indiquent qu'une mutation dans le style de l'haplotype S_{fa} a généré l'auto-compatibilité de la forme S_{fi}. La reconnaissance du pollen S_{fi} dans le style S_{fa} confirme que la présence du haplotype S_{fi} n'assure pas l'auto-compatibilité, et que l'expression du S_{fa} chez ces hétéro/homozygotes est dominante sur le S_{fi}.*

Mots-clés. Prunus amygdalus *Batsch – Auto-compatibilité – Amélioration – S_f allèle – Expression d'allèle – Reconnaissance d'allèle.*

I – Introduction

Self-compatibility (SC) has been considered a priority objective in almond (*Prunus amygdalus* Batsch) breeding (Socias i Company, 1990). After confirming that SC was a transmissible trait (Socias i Company and Felipe 1977) it was attributed to the presence of the S_f allele, allelic to the series of *S* alleles of self-incompatibility (SI) (Socias i Company 1984), being inherited as a Mendelian trait (Socias i Company and Felipe 1988). In almond, it was firstly established that SC is due to the lack of RNase activity of the S_f allele (Bošković *et al*., 1999). However, Kodad *et al.* (2009; 2010) reported that three local Spanish cultivars with the S_f allele were self-incompatible (SI), denominating as S_{fa} the active form of the S_f allele, showing a SI expression, whereas the denomination S_{fi} has been suggested for the inactive S_f allele showing a SC expression (Fernández i Martí *et al*., 2009). The two forms of the S_f allele are not only identical for the coding region sequence (C1 to C5) (Kodad *et al*., 2009; Fernández i Martí *et al*., 2009), but also at the alignment of their 5'-flanking regions, as shown by the construction of a fosmid library (Fernández i Martí 2010). Later Kodad *et al*. (2010) reported that some Spanish almond cultivars sharing similar *S*-genotype, including the S_{fa}-allele, are cross-incompatible. In almond, some cases of cross-incompatibility have been reported in combinations sharing identical *S*-genotypes (Bošković *et al*., 2007; Fernández i Martí *et al*., 2009; Socias i Company *et al*., 2012). Thus, in this situation a question has arisen: what will be the expression of homozygote genotypes sharing the two forms of the *Sf*-allele? Consequently, our objective was to study the possible interaction between the two forms of the S_f allele when present in the same genotype.

II – Materials and methods

Three almond cultivars with identical *S*-genotype were included as parents to obtain seedlings for analysis. 'Belona' and 'Soleta' (SC, $S_{fi}S_{23}$) were used as female parents and crossed with 'Vivot' (SI, $S_{fa}S_{23}$) pollen to obtain $S_{fi}S_{fa}$ heterozygotes. The crosses were made in the spring of 2009, nuts were collected in the following fall, seeds were stratified and the germinated seedlings were placed in growing plots and later transferred to the open field for flowering.

Genomic DNA was extracted from leaves following the CTAB extraction method based on Doyle and Doyle (1987). The consensus primers AS1II (forward) and AmyC5R (reverse), designed from conserved coding regions flanking the second intron of almond S-RNases, were used, as well as specific primers for the identification of the S_{23}- and S_f-alleles. PCR products were separated in 1.5% (w/v) agarose gels. Band scoring was carried out using a standard 1 kbp DNA ladder (Invitrogen).

The phenotype of the seedlings was determined by pollen tube growth as described by Socias i Company *et al*. (2013), since this method has been shown to be an efficient method for SC evaluation (Socias i Company *et al*., 2014). The pistils were rated according to the level where pollen tubes were observed as defined by Socias i Company *et al*. (2013). Finally, each genotype was classified according to the average rate of all the pistils observed, pooling the data of the two years of observation in order to obtain the SC classification for the genotype.

III – Results and discussion

PCR amplification of genomic DNA of the progeny resulting from the two crosses studied was performed with specific primers to detect the presence of the S_{23} and S_f alleles as shown in Fig. 1. As expected, only two genotypes were observed in the offspring of the two crosses (Table 1). Only the 'Vivot' pollen carrying the S_{fa} allele could grow trough the pistils of the 'Soleta and Belona' cultivars, despite the genetic identity of this S_{fa} allele with the S_{fi} allele of the pistils, reaching the ovule and accomplishing its fertilisation giving progeny with two possible combinations: $S_{fi}S_{fa}$ and $S_{23}S_{fa}$.

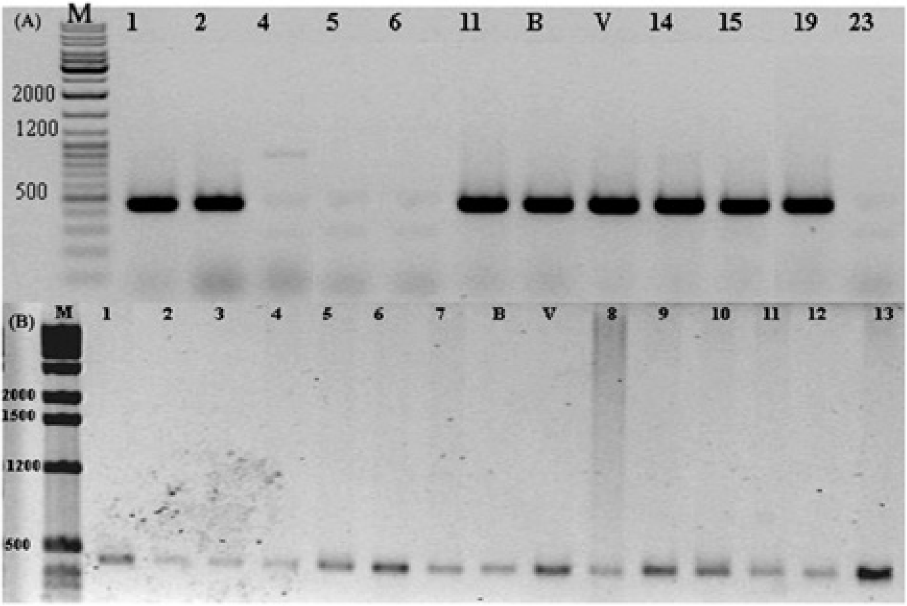

Fig. 1. Agarose gel showing *S*-allele fragments amplified with the *Sf*-specific primers (a) or with the S_{23}-specific primers (b) of some genotypes from the family 'Belona' × 'Vivot', compared to 'Vivot' (V) and 'Belona' (B). Lanes M, 1 kbp marker ladder.

Table 1. Distribution of the *S* genotypes in the offspring of the two almond crosses studied, 'Belona' × 'Vivot' and 'Soleta' × 'Vivot'

Cross	Genotype		χ^2	P
	$S_{fi}S_{fa}$	$S_{23}S_{fa}$		
'Belona' ($S_{fi}S_{23}$) × 'Vivot' ($S_{fa}S_{23}$)	15	10	0.64	0.01
'Soleta' ($S_{fi}S_{23}$) × 'Vivot' ($S_{fa}S_{23}$)	8	11	0.05	0.01
Total	23	21		

The results of pollen tube growth allowed the phenotypic characterisation of the seedlings. The seedlings of genotype $S_{fi}S_{fa}$ were fully SI and a single one was rated as only SI. These results show that the presence of the S_{fi} allele in these cases cannot be related to SC, but that the mechanisms of SI are fully active in this genotype. The homogeneity of results among all seedlings of $S_{fi}S_{fa}$ genotype may explain the interaction between both forms of the S_f allele. In previous studies it has been reported that the presence of the S_{fa} allele in some local almond cultivars showed a self-incompatible phenotype (Kodad *et al*., 2009; 2010; Fernández i Martí 2009) and cross-incompatibility when the two cultivars share the S_{fa}-allele (Kodad *et al*., 2010).

The full SI of the seedlings of $S_{fi}S_{fa}$ genotype indicates that a complete recognition of both alleles takes place in the pistils of this genotype. Taking into account that the S_{fi} allele does not code for any *S*-RNase and that the S_{fa} allele codes for the S_f-RNase (Kodad *et al*., 2009), only a *S*-RNase may be present in the pistils controlling the compatibility of the incoming pollen. The incompatibility of the self-pollination of these seedlings is explained by the recognition by the S_f-RNase of both kinds of pollen, those of S_{fi} and of S_{fa} genotypes. S_{fi} pollen, characterized by SC, was able to grow in S_{fi} pistils, but not in S_{fa} pistils, where the RNase produced by the S_{fa} pistils is able to recognize the S_{fi} pollen, thus stopping its growth and resulting in an incompatible pollination (Fernández i Martí *et al*., 2009). These observations, however, were in heterozygous S_fS_x genotypes, not in homozygous $S_{fi}S_{fa}$ genotypes. Our results show that the inactivation induced by the S_{fi} genotype only takes places in the pistil part, avoiding the production of the S_f-RNase, whereas the pollen part remains completely active, as shown by the recognition of the S_{fi} pollen by the S_f-RNase, probably due to the full genetic identity of both forms. Consequently, in the $S_{fi}S_{fa}$ genotypes, the presence of the S_{fi} allele is not a clue for SC, as generally accepted. These results confirm the hypothesis that S_f is allelic to the *S* alleles of SI (Socias i Company 1984) and suggest that S_{fa} is probably the original allele, being another allele of the *S* locus in a predominantly SI species such as almond. Consequently, S_{fi} expression may have resulted from a mutation, as first suggested by Grasselly and Olivier (1976). This mutation could have been an epigenetic change taking place in the upstream region of the S_f-RNase (Fernández i Martí *et al*., 2014).

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Do almonds (*Prunus amygdalus* Mill.) alpha-amylase germinating seedlings have a beta/alpha fold?

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Abstract. Alpha-amylases have been well characterized in microorganisms and mammals. Very little is known about plant amylases. A previous study has allowed us to demonstrate the presence of alpha-amylase in germinated almond seedling (*Prunus amygdalus* Mill.) var 'Tuono Mazzetto' (Rosaceae family). The enzyme activity was optimal at the 4^{th} day of germination with a Vmax = 2.5 UI and Km = 6.32 mM. In the current study, our aim was to propose *in silico*, by comparative modelling, a putative homologous model of three-dimensional (3D) structure of *Prunus persica* alpha-amylase, a species from the same family. The *Prunus persica* alpha-amyalse structure shows a beta/alpha fold with small deviation from the template 3D coordinates even in the loop regions. The geometry of the catalytic site is preserved. The Ramachandran plot confirmed that the proposed model might be of a good quality. Alpha-amylase structure from *Prunus Persica* was also compared to a porcine (*Sus scrofa*) one, indicating the structural homology of these two different species. Combination of bioinformatics and biochemistry would allow us to better understand almond alpha amylase function.

Keywords. Almonds – *Prunus Amygdalus* Mill. – Alpha-amylase – *Prunus Persica* – Beta/alpha fold – Structural homology.

L'alpha-amylase des graines d'amandier* (Prunus amygdalus *Mill.*) *en germination présente-t-elle un repliement bêta / alpha?

Résumé. *Les alpha-amylases ont été bien caractérisées chez les micro-organismes et les mammifères. Peu d'informations concernent les amylases végétales. Une étude précédente nous a permis de mettre en évidence la présence d'alpha-amylase dans des graines d'amandier en germination* (Prunus amygdalus *Mill.*) *var. 'Tuono Mazzetto' (famille des Rosacées). Cette activité de l'enzyme est optimale au $4^{ème}$ jour de germination avec une Vmax = 2,5 UI et km = 6.32 mM. Dans l'étude actuelle, notre objectif est de proposer in silico, par modélisation comparative, un modèle putatif d'homologie à trois dimensions (3D) de la structure d'une alpha-amylase de* Prunus persica*, une espèce de la même famille. La structure de l'alpha-amylase de* Prunus persica *présente un repliement bêta/alpha avec une déviation par rapport au modèle des coordonnées en 3D, même dans les régions en boucle. La géométrie du site catalytique est bien conservée. Le diagramme de Ramachandran montre que le modèle proposé est de bonne qualité. La structure de l'alpha-amylase de* Prunus persica *est également comparée à celle d'une espèce porcine* (Sus scrofa) *mettant en évidence l'homologie structurale de ces espèces différentes. La combinaison de la bioinformatique et de la biochimie nous permettrait de mieux comprendre le fonctionnement de l'alpha amylase d'amandier.*

Mots-clés. *Amandes –* Prunus Amygdalus *Mill. – Alpha-amylase –* Prunus Persica *– Repliement bêta/alpha – Homologie structurale.*

I – Introduction

In Tunisia, almond (Family: *Rosaceae*; Genus: *Prunus*) holds a very important place in agriculture after the olive tree. Almond plantations are spread across all the countrie and they are characterized by a relatively high genetic diversity (Gouta *et al.*, 2008, 2010). Moreover, almond studies concerned mainly its ecology as well as its physiology. Little work has concerned the metabolism of almond seeds and their germination. We are interested by enzymes involved in germination (Bahri, 2012) and in particular in the study of alpha amylase. Alpha amylases (EC 3.2.1.1.) are α- (1-4) D-glucan glucanohydrolase which catalyze α- (1-4) linkages in starch and any related oligosaccharides to produce D-glucose, D-maltose and a small amount of maltodextrins (Mercier, 1985; Graber and Combes, 1989). They have been classified in the family of glycosyl hydrolases 13: GH13 (Davies and Henrissat, 1995). Amylases from microorganisms have been extensively studied (Ben Abdelmalek *et al.*, 2009; Jay Kant, 2009). Plant, animal and microbial amylases show significant differences in the primary, secondary and tertiary structure as well as the catalytic mechanism (Tripathi *et al.*, 2007; Ben El Arbi *et al.*, 2009). It is well known in the literature, that the arrangement of the major elements of secondary structures and the topology of the connections between them corresponds to a protein fold (Chothia *et al.*, 1997). Three dimensional structures of proteins give valuable insights into the molecular organization and function (Messaoudi, 2011). Protein structure homology modeling has become a routine technique to generate 3D model for proteins when experimental structure are not available (Biassini *et al.,* 2014). Plant amylases are generally considered to be involved in the metabolism of germinating seedling and Biotechnology (Ben El Arbi *et al*., 2009; Khady *et al.,* 2013). Another species, *Prunus persica*, of the same genus and in the same family as the almond tree (Dirlewanger *et al*., 2002) is also much studied and has an importance in traditional medicine and pharmacology (Han *et al.*, 2015). To understand almond alpha-amylase mechanism, we combined bioinformatics and biochemistry. In a first step and in the absence of an almond alpha-amylase sequence in the databases, our aim is to propose, for the first time, a *Prunus persica* alpha amylase fold by homology modeling with Phyre 2 server (Kelley and Sternberg, 2009).

II – Materials and methods

1. Plant material

Samples of Italian almond seeds introduced in Tunisia (*Prunus amygdalus* Mill., var. 'Tuono'), were collected on 2012 and kindly provided by the (Institut de l'Olivier, Sfax-Tunisia). The Almond seeds were germinated at 26 ± 1°C in the darkness for different stages of germination. The alpha-amylase was extracted and identified by both the Somogyi-Nelson and the glucose oxydase methods (Trinder, 1969; Dingeon, 1975; Lott, 1975; Somogyi, 1952).

2. Homology modelling

The procedure for homology modeling involves 4 steps-template selection, target template alignment, model building and evaluation. We firstly selected the primary structure of α-amylase from *Prunus persica (*Uniprot database, code: M5VVU6). Many servers and algorithms are able to predict 3D structures with a good accuracy we used the Phyre 2 (Protein Homology/analogY Recognition Engine) (Kelley and Sternberg, 2009) server as a fully automated based method which can reliably detect up to twice as many distant homologies. This method represents one of the many alternatives available from the structural bioinformatics. The primary structure of our target protein was submitted to the the program server (Phyre 2) which returned a list of candidate 3D structures along the alignment of the target sequences with the corresponding template sequence. The 3D model was selected based on the quality of the alignment. The stereochemical assessment was then achieved by constructing the Ramachandran Plot (Sheik *et al.*, 2002; Kelley and Sternberg, 2009).

III – Results and discussion

A comparative modeling method was used to construct the 3D model of α-amylase from *Prunus persica* which α-amylase sequence contains 401 residues (Fig. 1). The template used for the construction of the model is an orthologous protein sequence of *Hordeum vulgare* alpha amylase (PDB code: 2QPU), a plant member of the Poaceae family. The template selection by Phyre 2 is highly entrusted as judged by the confidence level score (100) which shows that both the target and the template sequences are homologous with an identity value of 65%.

The structure shows a beta/alpha fold with small deviation from the template 3D coordinates even in the loop regions.The model presents 9 alpha helices surrounding a hydrophobic core, consisting mainly on a beta sheet layer. The enzyme also presents two other exposed beta sheets on the protein surface with 5 and 2 strands.

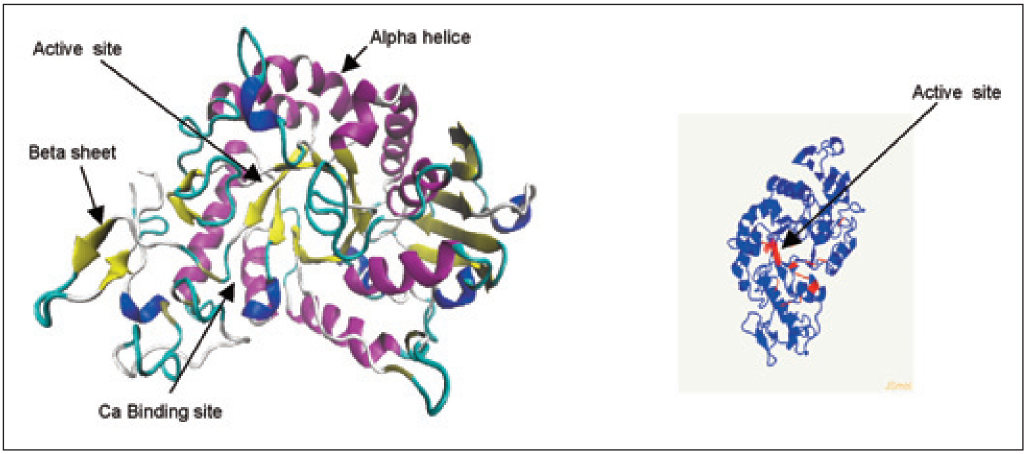

Fig. 1. Three dimensional structure of *Prunus persica*: (a) Ribbon representation of three dimensional structure of *Prunus persica* alpha amylase: strands are shown in light and helices in dark colour. (b) Active site of *Prunus persica* three dimensional structure.

The Ramachandran Plot indicates also a good stereochemical quality of the model. It's a significant result because 96% of all φ/Ψ angles residues are located in the favored regions and 4% in the allowed regions while no residue is located in the outlier zone of the Ramachandran Plot (Fig. 2).

Ramachandran Plot reveals a model of good quality which suggested that alpha amylase is well preserved between these two species *Prunus persica* and *Hordeum vulgare* which belong to two different families: respectively Rosaceae and Poaceae.

We also compared the proposed model to *Sus scrofa alpha-amylase* (Q7M328). Despite the low sequence identity of 14%, we observed that this later, revealed a backbone RMSD value of 3.4 angstrom suggesting the presence of a preserved structure between two different species. This result is in agreement with that described by Svensson (1994) in which diverse alpha amylases contains a characteristic catalytic $(\beta/\alpha)_8$-barrel domain.

In a previous biochemical study (unshown data) we have identified the presence of an alpha amylase during the germination of almond (*Prunus amygdalus* Mill.) seedlings. The enzyme showed an optimal activity at the 4^{th} step of germination and has kinetic parameters Vmax = 2.5 UI and Km = 6.32 mM.

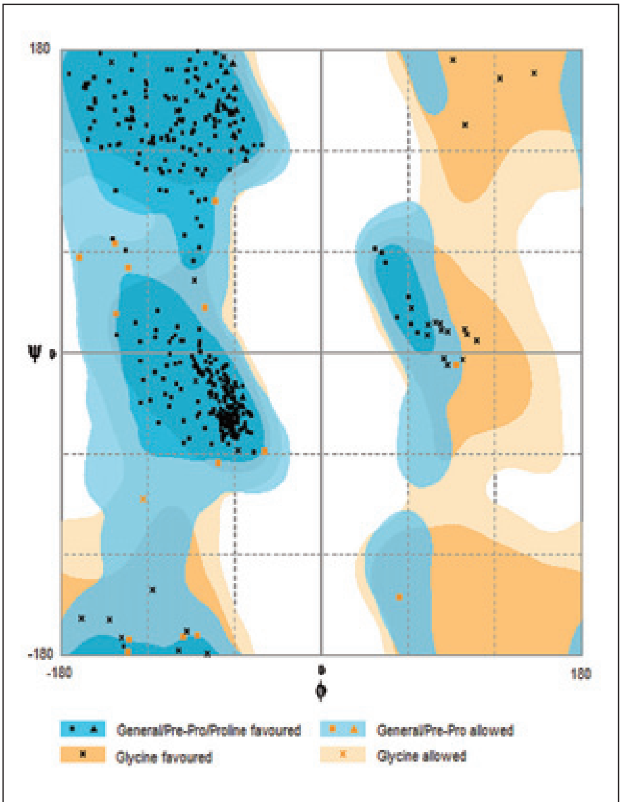

Fig. 2. ***Prunus persica*** **Ramachandran Diagram.**

IV – Conclusions

For the first time, folding of alpha amylase from peach has been proposed. The model shows a beta / alpha fold, as described for microrganisms with small deviation from the template 3D coordinates even in the loop regions. It is well known in literature that the 3D structures of proteins are more highly conserved than their sequences.

The involvment of alpha-amylase in oligosaccharides hydrolysis is well demonstrated among different species. Alpha-amylase structure seems to be preserved between two diverse species (*Prunus persica* and *Hordeum vulgare*). This folding was also observed in an animal species (*Sus scrofa*). This leads to suggest that the enzyme stucture is probably conserved during evolution.

In order to preserve oligosaccharides hydrolysis function, could almond alpha-amylase from germinating seedlings (*Prunus amygdalus* Mill.), has a conserved beta/alpha fold like *Prunus persica* and as described in microorganisms? (Ben Abdelmalek *et al.*, 2009; Tayyaba *et al.*, 2014). Further investigations would allow us to determine it.

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Pistachio industry in Tunisia: Opportunities of improvement

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Abstract. Pistachio is an important nut crop well extended in the centre and the south of Tunisia. An important increase of growing area occurred during the last forty years using almost only one variety 'Mateur', selected in the North of the country. However, the productivity is still very weak under low annual precipitation, warm climate and deficient cultural practices. These limiting factors for pistachio yield increased also the alternate bearing phenomena. Consequently, efforts are being made to improve the productivity of pistachio. The effect of main endogenous and environmental factors and their interactions on yield and alternate bearing was analyzed. The chilling and heat requirement of the main cultivar 'Mateur' were estimated to determine the best growing zone for its cultivation. This paper presents the main endogenous and environmental factors associated to alternate bearing and discuss possible approaches to be used to improve productivity and reduce alternate bearing of pistachio.

Keywords. *Pistacia vera* – Warm climate – Productivity – Alternate bearing – Chilling requirement.

Industrie du pistachier en Tunisie : Opportunités d'amélioration de la productivité

Résumé. *Le pistachier est une culture très importante, répartie essentiellement au centre et au sud de la Tunisie. Au cours des quarante dernières années, la superficie a subit une nette augmentation avec une nette dominance de la variété 'Mateur' qui a été sélectionnée au nord du pays. Cependant, la productivité demeure toujours très faible due aux basses précipitations annuelles, à un climat relativement chaud et aux déficiences des techniques culturales. Ces facteurs, limitant pour le rendement, ont aussi accentué le phénomène d'alternance. Des efforts sont fournis pour l'amélioration de la productivité à travers l'étude de l'effet des principaux facteurs endogènes et exogènes et de leurs interactions sur le rendement et l'alternance de production. Les besoins en froid et en chaleur de la principale variété cultivée 'Mateur' ont été estimés pour déterminer la meilleure zone de croissance pour sa culture. Ce papier présente les principaux facteurs endogènes et exogènes et associés à l'alternance de la production et discute les approches qui pourraient être utilisées pour améliorer la productivité et minimiser le phénomène d'alternance chez le pistachier.*

Mots-clés. Pistacia atlantica *– Hiver doux – Productivité – Alternance – Besoin en froid.*

I – Introduction

Pistachio (*Pistacia vera* L.) is a dioecious species, widely cultivated in the Mediterranean basin. Pistachio is wide spread in Tunisian arid land, and is considered as the most interesting drought tolerant species. During the period of the seventies, some development projects, supported by international organizations like FAO, were carried out. The growing area, localized mainly in the center and the south of the country, has been extended from less than 50 ha sixty years ago to more than 35,000 ha nowadays. During the period between 1985 and 1995, many technical problems related to crop multiplication were resolved and the pistachio production has increased greatly. This is probably due to the entering of young plantations to productive cycle and the increased attention given to orchard management (pruning, pollination technique, fertilization…). Despite these efforts and the relatively large land occupation, productivity was low and highly variable under harmful climatic conditions and rainfall scarcity with an average yield of 2.5 kg tree^{-1} (Ghrab *et al*., 2008).

The female trees strongly alternate bears (Wolpert and Ferguson, 1990). The fundamental hypothesis underlying alternate bearing is that yield in the current year affects yield in the following year (Rosenstock *et al*., 2010). Two competing hypotheses for the irregular production are suggested: endogenous and environmental factors. Endogenous factors suggest that reproduction is carbon-limited and the individual plant alternates carbohydrate allocation between reproductive and vegetative structures (Nzima *et al*., 1997; Spann *et al*., 2008). In contrast, environmental factor hypothesis contends that annual variability in nut production is produced by the climatic variations of precipitation and temperature (Elloumi *et al*., 2013). The objective of this work was to present the main endogenous and environmental factors and their interactions associated to alternate bearing and to review approaches that may be used to attenuate this phenomenon.

II – Methodology

The responses of pistachio female trees cv. 'Mateur' to severe climatic conditions were investigated in the centre of Tunisia (34°94', 10°60'). Nut yield was monitored for a long period and related to precipitation and winter chill. Winter chill was estimated as chilling hours (CH) according to Crossa-Raynaud model (Elloumi *et al*., 2013).

Under warm climate, fruit trees are affected by lack of chilling which amplifies the alternate bearing. An experiment was conducted to mitigate the harmful effect of warm winter on flowering and fruiting of pistachio trees. The hydrogen cyanamide (Dormex) was applied at concentrations of 2 and 4% and sprayed 40 days before the estimated bud break. The effect of these applications on bud break, shoot growth and yield of pistachio trees was evaluated. The main endogenous factors involved in pistachio alternate bearing were studied. Disbudding treatments consisting of flower buds removal were applied. In reference to untreated trees as control (T0), three treatments were applied as (i) removal of all flower buds for one year (T1); (ii) removal of all flower buds for two successive years (T2); and (iii) removal of 50% of flower buds for each year (T3). The storage and the mobilization of starch, dry matter and nutrients were evaluated in fruiting and non-fruiting branches.

III – Results and discussion

Flowering and yield of pistachio trees cv. 'Mateur' were analyzed under semi-arid climate during 1997-2014. This long period was characterized by strongly variable annual winter chill accumulation and precipitation (Table 1). Yield correlated poorly with precipitation and showed a moderate alternate bearing index of 0.63. However, flowering and nut yield of pistachio trees was a function of chill accumulation computed as chilling hours (CH) as previously reported (Elloumi *et al*., 2013). 'Mateur' cultivar was suggested to have low chilling requirements. Previous reports indicated that this cultivar vegetated and grew under warmer conditions in the south of Tunisia (Jacquy, 1973). However, Salhi *et al*. (2014) found that this cultivar had a minimum of 600 CH of chilling requirement. The obtained result explains in part the low productivity of this cultivar in the centre and the south of Tunisia, where the average of chilling accumulation is less than 300 CH.

With the climate change, warm winters are projected to become more frequent in Tunisia. The lack of chilling resulted in abnormal patterns of bud-break and development of pistachio trees cv. 'Mateur' with delayed flowering date and an extended flowering period. For this reason, hydrogen cyanamide was evaluated on pistachio trees to compensate for the lack of chilling. Results showed that hydrogen cyanamide treatments at 2-4% advanced floral bud break and the flowering period and increased the percentage of floral bud break compared to the untreated control trees (Ghrab and Ben Mimoun, 2013; Elloumi *et al*., 2013). If it is economically feasible, winter oil application could be a solution to improve pistachio production in low winter chill areas. This practice is commonly used in most fruit tree orchards but growers need a prediction of chill accumulation to decide whether to apply oil or not.

Table 1. Winter chill, precipitation, flowering date and yield monitored during the experimental period (1997-2014)

	Winter chill (CH)	Precipitation (mm)	Flowering date (DOY†)	Yield (kg tree^{-1})	Flowering *vs* CH (*r*††)	Yield *vs* CH (*r*)
Min	72	79	84	0.0	–	–
Max	346	405	118	26.4	–	–
Mean	221	202	99	6.5	0.77	0.65

† DOY: day of year; †† *r*: correlation coefficient.

Pistachio trees are an assemblage of carbohydrate sinks including fruit, stems, leaves and floral buds. In arid zone, under scarce rainfall conditions, the processes of partitioning among these different organs are of interest. The balance of vegetative and reproductive growth has important consequences for tree morphology and productivity. Results showed that once pistachio nuts start developing in July, they become major sinks that draw resources from individual vegetative organs (Elloumi *et al*., 2014a; Elloumi *et al*., 2014b). Disbudded trees permitted an estimation of vegetative growth potential of cv. 'Mateur' pistachio tree. These findings revealed the importance of individual one-year-old wood in source-sink relationships and in the regulation of the carbohydrates distribution within isolated branches. Pistachio appears to have a specific pattern of annual carbohydrate storage and mobilization. In fact, results showed a mobilization of carbohydrates from current-season and one-year old stem wood of On-trees during kernel fill period that corresponded with the period of flower bud abscission. Thus the alternate bearing of Mateur pistachio cultivar may be associated to mid-season mobilization of stored carbohydrates in current season stems.

Besides, alternate bearing influences directly the nitrogen, phosphorus, and potassium dynamics of the pistachio isolated branches (Elloumi *et al*., 2014c). In Off year, different sink organs store an important quantity of macronutrients, and mobilize it during the subsequent On-year to support initial spring growth. However, mobilized quantity does not seem to be enough to satisfy the nutrients demand of the tree. Macronutrients, accumulate in the same season must be also mobilized. Leaf K concentration explained 75% of yield variation (Fig. 1). Leaf K concentration decreased with the increase of nut production.

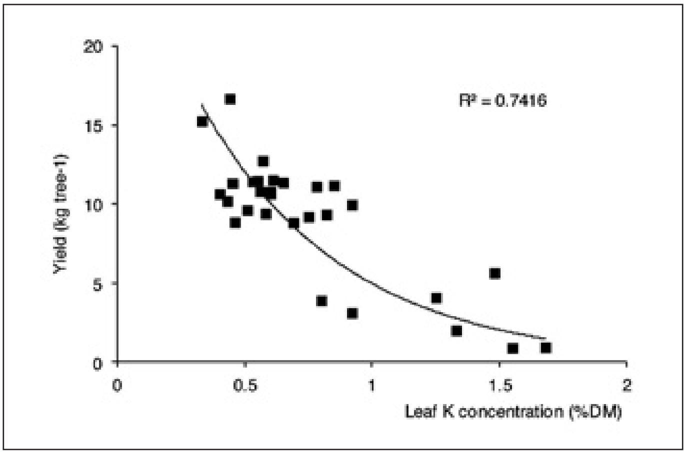

Fig. 1. Relationship nut yield (kg tree^{-1}) *vs* leaf K concentrations (%DM) determined in late July.

Yearly removal of 50% of flower buds showed a beneficial result. It leads to an earlier and regular accumulation of dry matter and starch in fruiting and non-fruiting branches. It induces less variation of leaf K concentrations with regular nut yield and a significant improvement of the cumulated production compared to bi-annual On-Off cycle. The induced better and stable inter-annual tree K status could be helpful for applying adequate potassium fertilization to improve the nutritional status of pistachio trees. Moreover, under rain-fed conditions, annual hand-pruning could be used to prevent or to minimize alternate bearing of pistachios.

IV – Conclusions

According to this study, the productivity of pistachio may be improved and alternate bearing may be reduced in dry and warm area Tunisian climate. Results showed that: (i) the annual pruning could be applied to improve the tree carbon status, to regularize the nut production and to significantly increase the cumulated production; (ii) the application of hydrogen cyanamide could be used to improve pistachio production in low winter chill regions. It will be interesting to consider the important genetic diversity of pistachio species to mitigate the harmful effect of environmental factors and to valorise tolerant wild species as rootstocks.

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Fine-mapping of the bitterness locus in almond

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Abstract. During domestication, almond kernels have been selected for low content of amygdalin, a cyanogenic glucoside responsible for bitterness. The taste of the almond kernel is under monogenic control, with the dominant sweet kernel (*Sk*) allele associated to sweetness and the recessive *sk* allele associated to bitterness. Since most of the cultivated almonds are heterozygous, new bitter almond seedlings are usually obtained during breeding programs and it is valuable to develop molecular markers enabling assisted selection. Although its biochemical function remains unidentified, the *SK* gene has been localised on linkage group five (G5) in the almond genetic linkage map obtained from the cross between the two heterozygous genotypes 'R1000' and 'Desmayo Largueta' (RxD). We report here the identification of several SNPs over a 3.7 Mb peach (*Prunus persica* L.) physical region, which is synthenic to the one containing the *Sk* locus in almond. Some of them were converted into cleaved amplified polymorphic DNA (CAPS) markers suitable for assisted selection. Further studies are in progress aiming to the functional characterization of candidate genes for controlling the phenotype.

Keywords. Almond – Fine mapping – Bitterness – Amygdalin – SNPs – HCN.

Cartographie fine du locus de l'amertume dans l'amande

Résumé. *Au cours de la domestication, les amandes ont été sélectionnées pour une faible teneur en amygdaline, un glucoside cyanogène responsable de l'amertume. Le goût de l'amande est sous un contrôle monogénique, avec l'allèle dominant (Sk) associé à la douceur du noyau et l'allèle récessif de (sk) associée à l'amertume. Comme la plupart des amandes cultivées sont hétérozygotes, de nouveaux plants d'amandes amères sont habituellement obtenus au cours des programmes de sélection et il est utile de développer des marqueurs moléculaires permettant la sélection assistée. Bien que sa fonction biochimique reste non identifié, le gène SK a été localisé sur le groupe de liaison cinq (G5) dans la carte d'amande obtenue à partir d'un croisement entre les deux génotypes hétérozygotes 'R1000' et 'Desmayo Largueta' (RxD). Nous rapportons ici l'identification de plusieurs SNP plus de 3,7 Mb de pêche* (Prunus persica *L.) de région physique, qui est synthenic à celui contenant le locus Sk en amande. Certains d'entre eux ont été convertis en (CAPS) (cleaved amplified polymorphic DNA) marqueurs appropriés pour la sélection assistée. Des études supplémentaires sont en cours visant à la caractérisation fonctionnelle des gènes candidats pour commander le phénotype.*

Mots-clés. *Amandier – Cartographie génetique – Amertume – Amygdaline – SNPs – HCN.*

I – Introduction

Bitterness is one of the most studied traits in almond (*Prunus dulcis* Miller D.A. Webb syn. *Prunus amygdalus* Batsch). This is due to the degradation of amygdalin, a cyanogenic diglucoside, which also releases cyanide, toxic for animals and humans (McCarty *et al*., 1952; Conn, 1980; Poulton, 1990; Swain *et al*., 1992 and Sánchez-Pérez *et al*., 2008).

The bitter or sweet taste has a monogenic control in almond, the *sweet kernel* (*Sk*) allele being dominant over the bitter kernel (*sk*) allele (Heppner, 1923; Dicenta and García, 1993 and Sánchez-Pérez *et al*., 2010). Bitter is the original taste of kernels of wild almond species. Due to an unknown mutation and human domestication, most of the cultivated almonds are sweet and heterozygous at the *Sk* locus. The inheritance of bitterness in almond is controlled by the seed mother genotype (Heppner, 1923; Kester and Assay, 1975; Kester and Gradziel, 1996; Dicenta and Garcia, 1993; Dicenta *et al*., 2000 and 2007; and Sánchez-Pérez *et al*., 2010). Therefore, in practical breeding programs, in which two heterozygous varieties are crossed, 25% of the seedlings will be bitter and therefore need to be removed (Grasselly and Crossa-Raynaud, 1980; Dicenta and Garcia, 1993).

The selection process is complicated by the almond long juvenile period, as breeders have to wait three to four years to analyze traits related to reproductive organs. For this reason, it would very useful to develop molecular markers to distinguish between sweet and bitter kernelled seedlings in the nursery during the first year, just after the germination of seedlings (Sánchez-Pérez *et al.,* 2010). Previous studies have shown that the *Sk* locus is localised in almond linkage group five (G5) (Joobeur *et al*., 1998, Sánchez-Pérez *et al*., 2008). Almond G5 was saturated with SSRs and six of them (UDA-045, EPDCU2584, CPDCT028, BPPCT037, PceGA025 and CPDCT016) were found very close to the *Sk* locus.

In this work we try to shorten this region with new CAPS molecular markers in order to fine mapping the G5.

II – Materials and methods

Aiming to find a *Sk*-ortholog region in the peach genome, a BLAST analysis against the Genomic Database of Rosaceae (www.rosaceae.org) (Jung *et al*., 2008) was carried out, using sequences of *Sk*-linked SSR markers as query. Peach genes included in this region were sequenced on the two genotypes ‘R1000’ (R) and ‘Desmayo Largueta’ (D), aiming to identify single nucleotide polymorphisms (SNPs).

Afterwards, five new cleaved amplified polymorphic sequence (CAPS) markers linked to the *Sk* locus were designed by means of the CAPS designer tool available at the SolGenomics website (http://solgenomics.net/).

DNA of a large F_1 population (550 individuals), originating from the RxD cross, was isolated according to a CTAB method (Doyle and Doyle, 1990), and used as template for marker analysis with the CAPS above mentioned and the *Sk*-linked SSRs UDA-045, EPDCU2584, BPPCT037 and CPDCT028 (Sánchez-Pérez *et al*., 2010). Marker scoring was performed for CAPS using Metaphor® agarose gel electrophoresis (Lonza), and for SSR with the ABI Prism 3500 Genetic Analyzer (AB HITACHI).

Mapping was performed through the JoinMap 4.1 software (Van Ooijen, 2001), using the ML algorithm and a LOD score threshold value of 3.0.

III – Results and discussion

BLAST search, using sequences of *Sk*-linked almond markers, resulted in hits all localizing on the peach LG5 chromosomal region, suggesting the identification of a synthenic region containing the *Sk* ortholog.

Two SSR markers previously shown to flank the *Sk* locus (EPDCU2584 and CPDCT028) were found to delimit a peach genomic region of about 800 Kb. We used five genes included in this region to identify SNP polymorphisms between the R and D genotypes and develop CAPS markers saturating the *Sk* region. An example of CAPS, requiring simple gel electrophoresis is shown in Fig. 1.

Notably, mapping of newly developed CAPS markers and four previously reported SSR markers revealed full overlap with the peach physical map (Fig. 2), thus substantiating the notion that *Prunus* genomes are colinear (Dirlewanger *et al*., 2004).

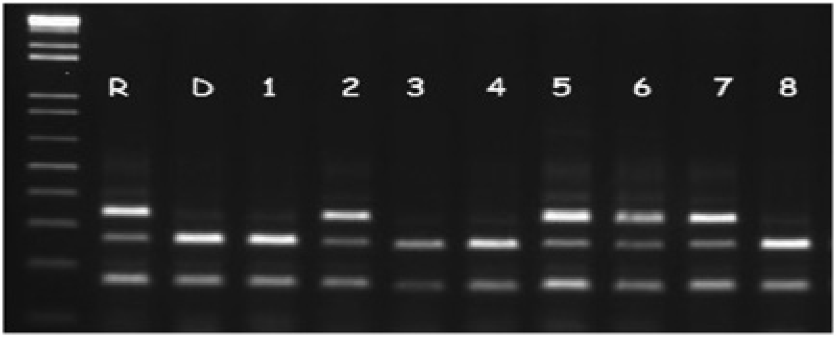

Fig. 1. Segregation analysis of R1000 (R), Desmayo Largueta (D) and 8 segregant F_1 individuals by means of one of the newly developed CAPS markers.

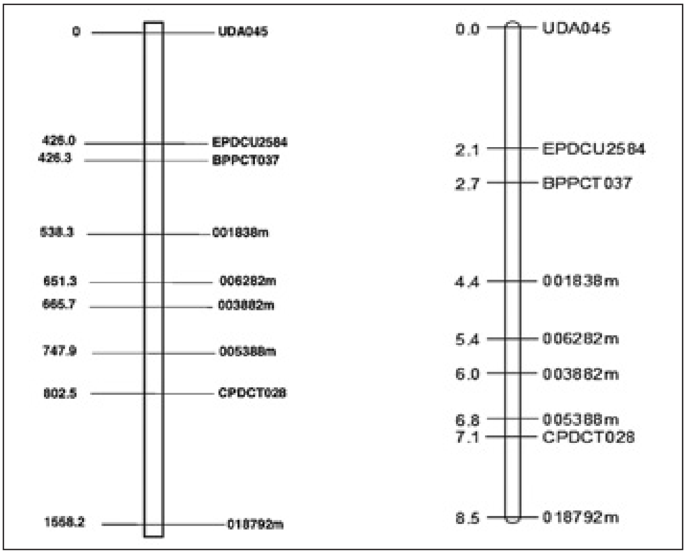

Fig. 2. Co-linearity between the physical map of peach GL5 (left) and the genetic map of almond (right). Markers distances are indicated in Kb and cM, respectively.

At the moment, we are assaying the phenotyping of the F_1 RxD population, aiming to fine-map the *Sk* locus. We expect that our work will be of significant value for marker-assisted selection of bitterness in almond, thus by-passing limitations due to phenotypic screenings. Moreover, this is expected to provide useful information for the positional isolation of the *Sk* gene and reveal molecular mechanisms underlying bitterness in almond.

IV – Perspectives

Currently, we are characterizing the F1 population with respect to the kernel taste. In the meantime, we are carrying out a de novo sequencing of the almond *Sk* genomic region and transcriptome analysis of the tegument in sweet and bitter almonds.

Our results, based on syntheny between peach and almond, pave the way to the positional cloning of the *sk* gene. Moreover, they could be useful to assist the selection of sweet or bitter almonds in practical breeding programs.

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Cyanogenic glucosides from dormancy to flowering time in early and late almonds

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Abstract. Cyanogenic glucosides are well-known defense compounds produced as a protection against herbivores. In a process called cyanogenesis, a toxic gas hydrogen cyanide (HCN) is released from cyanogenic glucosides upon tissue disruption. Our research, however, indicates that cyanogenic glucosides could develop new functions in other plant physiological processes, such as dormancy release in flower buds. In order to investigate this hypothesis, flower buds and individual parts of the fully-developed flower of five almond cultivars (early flowering time: 'Achaak' and 'Desmayo'; late flowering time: 'S3067' and 'Lauranne'; extra late flowering time: 'Penta') were collected in the experimental orchard of CEBAS-CSIC, in Santomera (Murcia, South-East Spain), to analyze the content of cyanogenic glucosides by LC-MS/MS. The two main cyanogenic glucosides, prunasin and amygdalin, were found in all the varieties, with the concentrations being highest in the bitter variety ('S3067'). Interestingly prunasin was observed in the flower buds from dormancy having its highest concentration right before flowering took place for all the five cultivars, suggesting that this compound could play an important role in flower development. Moreover, new derivatives of cyanogenic glucosides, namely prunasin amide, acid, anitrile, anitrile apioside and apioside, were also found in the tissues analysed, albeit in much lower concentration. The elevated levels of prunasin found in tissues as pollen or the presence of the new derivates in different parts of almond flower are also discussed.

Keywords. Prunasin – Amygdalin – Dormancy – Flowering time – LC-MS/MS.

Les glucosides cyanogènes, de la dormance à la floraison dans les amandes précoces et tardives

Résumé. *Les glucosides cyanogènes sont des composés de défense bien connus comme protection contre les herbivores. Dans un processus appelé cyanogenèse, un gaz toxique, le cyanure d'hydrogène (HCN) est libéré à partir de glucosides cyanogènes lors de la rupture du tissu. Notre recherche, cependant, indique que les glucosides cyanogènes pourraient développer de nouvelles fonctions dans d'autres processus physiologiques des plantes, comme la levée de dormance des bourgeons floraux. Afin de vérifier cette hypothèse, des bourgeons floraux et des parties individuelles de fleurs entièrement développées de cinq cultivars d'amandier (floraison précoce: 'Achaak' et 'Desmayo', floraison tardive: 'S3067' et 'Lauranne', extra tardive: 'Penta') ont été recueillis, auprès du verger expérimental de CEBAS-CSIC, à Santomera (Murcia, sud-est de l'Espagne), pour analyser la teneur en glucosides cyanogènes par LC-MS/MS. Les deux principaux glucosides cyanogènes, prunasine et amygdaline, ont été trouvés dans toutes les variétés, avec des concentrations plus élevées dans la variété amère ('S3067'). Il est intéressant de noter que pour les cinq cultivars, la prunasine a été observée dans les bourgeons floraux dès la dormance, la plus haute concentration étant trouvée juste avant la floraison pour les cinq cultivars, ce qui suggère que ce composé pourrait jouer un rôle important dans le développement des fleurs. En outre, de nouveaux dérivés de glucosides cyanogènes, tels que prunasine amide, prunasine acide, prunasine anitrile, prunasine anitrile apioside et prunasine apioside, ont également été trouvés dans les tissus analysés, mais avec une concentration beaucoup plus faible. Les niveaux élevés de prunasine trouvés dans des tissus comme le pollen ou la présence des nouveaux dérivés dans différentes parties de la fleur d'amandier sont également discutés.*

Mots-clés. *Prunasine – Amygdaline – Dormance – Temps de floraison – LC-MS / MS.*

I – Introduction

Cyanogenic glucosides are defense compounds of the plants presents in more than 3000 species including economically important crops such as almond (*Prunus dulcis* Miller D.A. Webb syn. *Prunus amygdalus* Batsch). Upon tissue disruption, in a process called cyanogenesis, the toxic gas hydrogen cyanide (HCN) is released from cyanogenic glucosides (Poulton, 1990). However, our research indicates that cyanogenic glucosides could develop new functions in other plant physiological processes, such as breaking dormancy or flower development. There are two cyanogenic glucosides in almond: prunasin and amygdalin, both derived from the amino acid phenlyalanine. Prunasin is the precursor of amygdalin. When both are degraded, glucose, benzaldehyde (bitter flavour) and hydrogen cyanide (toxic) are liberated (Sánchez-Pérez *et al*., 2008. When this last one is released, detoxification pathway is activated forming ammonia, aspartate and asparagines which could be a nitrogen supply for the plant (Swain and Poulton, 1994). Although the main function described of the cyanogenic glucosides is such as first chemical defense against pathogens and other predators, also they have other functions such as transport and storage of nitrogen and sugar for the kernel, being a precursor for protein synthesis or like metabolites source (Swain *et al*., 1992 and Sánchez-Pérez *et al*., 2008). On the other hand, breaking dormancy is the ability of the tree to start floral or vegetative budbreak. Consequently, flowering will only happen when the dormancy is broken. In almond, flowering time is one of the most important agronomic traits studied in breeding programs, because a late flowering variety avoids the lost of the yield because of the late or spring frosts. It is clear that cyanogenic glucosides have an important role in the plant and especially in the kernel as defense, for this the bitter taste of the kernel. Other functions as nitrogen supply and metabolites transport have been already detected but this is the first time that levels of cyanogenic glucosides have been measured from dormancy to flowering time, suggesting that these compounds could be involved in one of the two processes just mentioned. In relation with this, we try to answer the next questions: Could cyanogenic glucosides develop new functions in other plant physiological processes, such as dormancy release in flowerbuds and the flower development? How do cyanogenic glucosides evolve during the flower development? Is the content similar in all the tissues? Are there more cyanogenic glucosides derivates involve?

II – Materials and methods

Flowerbuds and individual parts of the fully-developed flower (petals, sepals, pistils, and pollen) of five almond cultivars (early flowering time: 'Achaak' and 'Desmayo'; intermediate flowering time: 'S3067'; late flowering time: 'Lauranne'; and extra late flowering time: 'Penta') were collected from November 2013 to March 2014 every two weeks in the experimental orchard of CEBAS-CSIC, in Santomera (Murcia, South-East Spain). Three branches for each cultivar were collected every two weeks from the field in Santomera and placed in a growth chamber in controlled conditions (25ºC during the photoperiod of 16h and 20ºC during 8h of darkness, with a constant relative humidity of 60% during the night and 40% during the day). Almond branches were placed in a 5% saccharose and 1% Aluminum Sulfate solution. After five days the solution was changed. After 10 days the development state of the flower buds was measured. The date of dormancy breakage was established when 50% of the flower buds were in the b-c state of Fleckinger (Felipe, 1977). The flowering time date was determined when the 50% of the flowers in the tree were completely opened. To extract cyanogenic glucosides and their derivates, once the samples were collected and keep at -80ºC, they were grinded with a mortar and liquid nitrogen and weighed frozen. Then between 50 and 100 mg of the samples were added to 400 µL methanol 85% in a threaded tube of 1,5 ml. The samples with methanol were boiled 5 min in a bath and they were put in ice. After this were centrifuged 5 min 2000 g and the supernatant was collected and taken out to a HPLC tube and keep at -20ºC. 20 µL of this supernatant was filtered with 70 µL of water and 10 µL Linamarin (internal standard) 500 µM (final concentration 50 µM) in a ELISA filter (5x dilution). The order was lid + ELISA plate + ELISA filter. The mix from the filtering was centrifuged 5 min 3000 rpm and 60 µL were transferred to a HPLC vial in a HPLC tube and sent to analyze by LC-MS.

III – Results and discussion

The two main cyanogenic glucosides, prunasin and amygdalin, were detected in the flowerbuds of all the varieties (Figs. 1A and B), both early and late flowering cultivars, during the flower development. There were not significant differences among early and late flowering cultivars. The bitter cultivar (S3067) had the highest concentration of prunasin and amygdalin respectively. Whereas the prunasin peak was 1.727 µmoles/100 g, the amygdalin peak was 0.023 µmoles/100 g, what means that prunasin concentration was around 75 times more than amygdalin in the almond flowerbuds (Figs. 1A and B). According to Nahrstedt *et al*. (1972) only prunasin was detected in flowers of

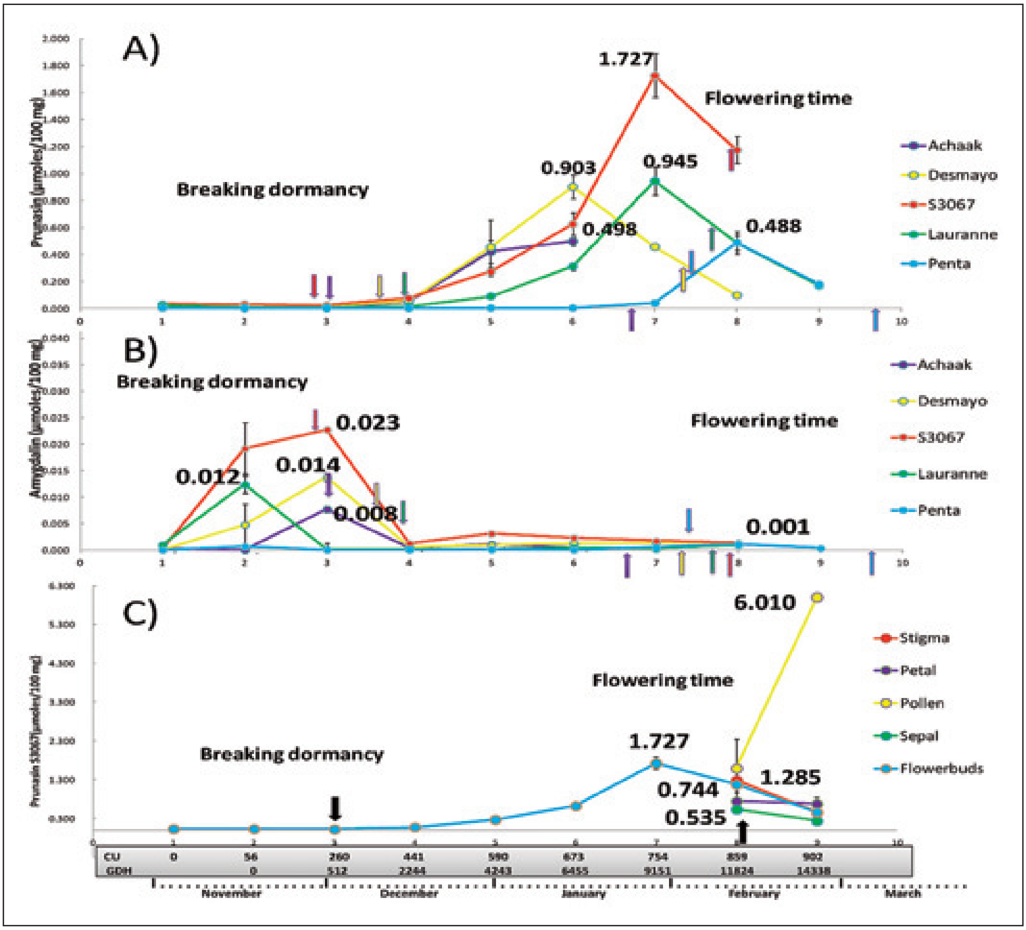

Fig. 1. Cyanogenic monoglucoside prunasin in flowerbuds during their development (A). Cyanogenic diglucoside amygdalin in flowerbuds during their development (B). Cyanogenic monoglucoside prunasin in flowerbuds, pollen, sepals, petals and pistils of S3067 variety during flower development (C). Down arrows indicate Breaking dormancy and up arrows indicate Flowering time.

Prunus avium, whereas amygdalin was not found. The highest concentration of prunasin was right before flowering took place for all the five cultivars (Fig. 1A), suggesting that this compound could play an important role in flower development. The highest concentration of amygdalin was when the break dormancy was taking place (Fig. 1B). So the tendency of the prunasin was to increase from break dormancy until flowering time whereas the amygdalin tended to decrease from break dormancy until flowering time. This could suggest that amygdalin could be used to break the flower-bud dormancy and prunasin to help the development of the the flowerbud to a flower. Lieberei *et al*. (1985), Selmar *et al*. (1988), Swain *et al*. (1992) and Sánchez-Pérez *et al*. (2008) suggested that cyanogenic compounds could have a nitrogen supply function. In relation with this, prunasin and amygdlain were detected in the almond kernel and during its development too (Swain *et al*., 1992 and Sánchez-Pérez *et al*., 2008) and in other parts of the almond tree like roots, stems and leaves (Dicenta *et al*., 2002). But not only flowerbuds had cyanogenic glucosides. Also other parts of the flower like petals, sepals, pistils and pollen contained prunasin and amygdalin and the derivates (Fig. 1C). In this sense, pollen was the tissue with the highest prunasin level compared with the other tissues, even with flowerbuds. London-Shafir *et al*. (2003) got the same results in almond pollen amygdalin, according to this author the amygdalin would inhibit inefficient pollinators allowing a more efficient pollinization by honeybees, which are able to tolerate the amygdalin toxicity up to a certain level. Regards to the others, petals, sepals and pistils had a tendency similar to the flowerbuds, so decreased after flowering until almost zero. Abarrategui (2010) detected prunasin by LC-MS in sepals, petals, pistils and pollen of bitter and sweet varieties of almond. Amygdalin level was almost zero in all the varieties except in the pollen of the bitter one. Moreover, new derivatives of cyanogenic glucosides namely prunasin anitrile, amide, acid, apioside and apioside anitrile were also found in the tissues analyzed (data not shown), although the concentration of these compounds was much more lower than prunasin. Only prunasin anitrile apioside had very high values. Pičmanová *et al*. (2015) suggested that these derivates could have a role in an alternative turnover pathway in which cyanogenic glucosides are converted to non-cyanogenic glucosides without any release of HCN.

IV – Conclusions

For the first time prunasin and amygdalin were detected in flowerbuds during the flower development in almonds. The bitter genotype was the highest concentrated. In the flowerbuds, prunasin concentration was around 75 times more than amygdalin. Prunasin had its highest concentration right before flowering took place, suggesting that this compound could play an important role in flower development. Amygdalin was the main compound detected during breaking dormancy while prunasin was the main compound during flowering time. Pollen was the tissue with the highest level of cyanogenic glucosides, even more than flowerbuds, especially in the bitter genotype. Other parts of the flower like petals, sepals and pistils contained prunasin and amygdalin in lower concentrations. Moreover, new derivatives of prunasin, namely prunasin amide, prunasin acid, prunasin anitrile, prunasin anitrile apioside and prunasin apioside, were also found in the tissues analyzed, although in much lower concentration.

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Comparative proteomics of pistils and anthers from self-incompatible and self-compatible almonds by iTRAQ and 2D-nano-LC ESI-MSMS

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Abstract. It is known that the cultivated almond [*Prunus dulcis* (Miller) D.A. Webb] exhibits gametophytic self-incompatibility controlled by the *S* locus. This locus contains two genes that codify for the S-RNase and SFB proteins, which seem to determine the specificity of incompatibility. However, recent studies support the involvement of other unidentified proteins in the incompatibility system of this species. A few proteomic studies have tried to identify candidates to modifier factors of the incompatibility system in *Prunus* sp. using comparative proteomics of pollinated pistils. However, there are no studies in which pollen and un-pollinated pistils from self-incompatible and self-compatible individuals are compared. To identify proteins differentially expressed in the pistils and anthers of almonds with an identical *S*-haplotype but different incompatibility phenotypes, iTRAQ and 2D-nano-LC ESI-MSMS were carried out. Seventeen and 23 proteins were identified as differentially expressed in anthers and pistils, respectively. Most of these proteins had a metabolic or stress resistance and defence function, and some of them had been associated to pollen development, pollen dynamics or to pollen-pistil interactions. These results provide proteomic profiles of differential expression in mature pistils and pollen, and could also serve as a reference for other comparative proteomic studies in almond and in other species with the same incompatibility system.

Keywords. *Prunus dulcis* – Self-incompatibility – iTRAQ – 2D-nano-LC ESI-MSMS.

Protéomique comparative des pistils et anthères d'amandes auto-incompatibles et auto-compatibles par iTRAQ et 2D-nano-LC-ESI MSMS

Résumé. *Il est connu que l'amande cultivé [*Prunus dulcis *(Miller) D.A. Webb] présente une auto-incompatibilité gamétophytique contrôlée par le locus S. Ce locus contient deux gènes qui codifient les protéines S-RNase et SFB, qui semblent déterminer la spécificité d'incompatibilité. Cependant, des récentes études soutiennent l'implication d'autres protéines non-identifiés dans le système d'incompatibilité de cette espèce. Certaines études protéomiques ont essayé d'identifier des candidats facteurs-modificateur du système d'incompatibilité dans* Prunus *sp. en utilisant la protéomique comparatives de pistils pollinisés. Cependant, il n'existe aucune étude dans laquelle le pollen et les pistils non pollinisés, appartenant à des auto-incompatibles et auto-compatibles, sont comparés. Pour identifier les protéines différentiellement exprimées dans les pistils et les anthères d'amandiers avec un S-haplotype identiques mais ayant différents phénotypes d'incompatibilité, iTRAQ et 2D-nano-LC-ESI MSMS ont été réalisées. Dix-sept et 23 protéines, exprimées de manière différentielle, ont été respectivement identifiés dans les anthères et les pistils. La plupart de ces protéines avaient une fonction métabolique ou de résistance au stress et un rôle de défense. Certaines d'entre elles avaient été associées au développement du pollen, de la dynamique de pollen ou aux interactions de pollen pistil. Ces résultats fournissent des profils protéomiques d'expression différentielle dans les pistils matures et le pollen, et pourraient également servir de référence pour d'autres études protéomiques comparatives pour l'amande et d'autres espèces avec le même système d'incompatibilité.*

Mots-clés. Prunus dulcis *– Auto-incompatibilité – iTRAQ – 2D-nano-LC ESI-MSMS.*

I – Introduction

The cultivated almond [*Prunus dulcis* (Miller) D.A. Webb] exhibits gametophytic self-incompatibility (GSI) controlled by the *S*-locus (Tao and Iezzoni, 2010). However, some almond cultivars from Apulia (Italy) were found to be self-compatible, which have been used in breeding programmes to obtain other self-compatible cultivars. Newly characterised almond accessions with the S_f haplotype, traditionally associated with self-compatibility, have been found to be phenotypically self-incompatible (Martínez-García *et al*., 2011). This finding supports the involvement of modifier factors coded outside the *S*-locus in the GSI system. Several studies intended to identify these factors in *Prunus* species using different variants of 2D electrophoresis after self-incompatible and self-compatible pollen-pistil interactions (Martínez-García *et al.*, 2015). However, comparative proteomic analyses of pollen or un-pollinated pistils have not yet been reported in *Prunus*.

The aim of this work was to identify proteins differentially expressed in pistils and in anthers of self-compatible and self-incompatible almonds with the S_f-haplotype. To achieve this goal, the differential protein expression in mature anthers and pistils was analysed using iTRAQ and 2D-nano-LC ESI-MSMS.

II – Materials and methods

Flower buds from the almond selections A2-198 (homozygous self-compatible, S_fS_f), and ITAP-1 (heterozygous self-incompatible, $S_{11}S_f$) were collected at 'D-E' developmental stage. Pistils and anthers samples were taken from the buds and frozen separately at -80ºC.

Proteins were extracted from 0.5 g of pistils and anthers samples following the protocol described in Martínez-García *et al.* (2015) with some modifications. A total of 40 µg of protein from each condition was precipitated for digestion by the methanol/chloroform method. Digested pistil and anther samples were labelled with an iTRAQ Reagents Multi-plex kit, using a 2-plex design for each studied condition. A 2.5 µg aliquot of the resulting mixture was subjected to LC ESI-MSMS analysis using a nano liquid chromatography coupled to a high speed Triple TOF 5600 mass spectrometer with a duo spray ionization source. Mass spectrometry and MS/MS data obtained were processed using Analyst® TF 1.5.1 Software (AB SCIEX). Raw data file conversion tools generated mgf files which were also searched against the UniProtKB/SwissProt database from *Prunus persica* (taxon identifier: 3,760).

The confidence interval for protein identification was set to ≥ 95%, and only peptides with an individual ion score above the 5% False Discovery Rates (FDR) threshold were considered as correctly identified. Only proteins having at least two quantitated peptides were considered in the quantitation. Finally, the identity of each protein was assessed after a BLAST search in the UniProtKB/SwissProt database.

III – Results and discussion

A total of 1,667 proteins were identified in pistils and 1,391 in anthers by iTRAQ and mass spectrometry. Of these proteins, 945 in pistils and 844 in anthers had at least two quantified peptides and were thus considered in protein quantitation. Seventeen and 23 proteins could be identified as differentially expressed in anthers and pistils, respectively (Table 1). Two of the down-regulated proteins and three of the up-regulated proteins in pistils were found to be differentially expressed in the same way in anthers (Table 1).

Table 1. Differentially expressed proteins in the pistils and anthers of ITAP-1 (self-incompatible, SI) and of A2-198 (self-compatible, SC) almonds

Pistils			Anthers		
UniProt number	Protein name	Ratio SI/SC	UniProt number	Protein name	Ratio SI/SC
Down-regulated in A2-198					
M5W1Q5	Pathogenesis-related protein PR-4	6.67	M5VQU4	Glucan endo-1.3-beta-glucosidase	4.20
M5VQU4	Glucan endo-1,3-beta-glucosidase	2.15	M5X1U2	Annexin	3.11
M5WI14	Mitochondrial fission 1 protein	2.13	M5VGZ1	GDSL esterase/lipase	2.24
M5Y240	Polygalacturonase	2.07	I2BF37	Chalcone synthase (CHS)	2.21
M5XB01	Uncharacterised protein	2.04	M5W1Q5	Pathogenesis-related protein PR-4	1.92
M5XD05	DEAD box RNA helicase RH2a	1.93	M5X5T2	Quinone oxidoreductase	1.73
M5XNQ1	Ribulose bisphosphate carboxylase	1.89			
M5Y9J3	Glycine-rich RNA-binding protein	1.84			
M5WDV5	Uncharacterised protein	1.79			
M5XMY7	Glutathione S-Transferase	1.76			
Up-regulated in A2-198					
M5WT96	PR thaumatin-like protein	0.36	M5XDU4	(R)-mandelonitrile lyase 2	0.14
M5XJV8	Amine oxidase	0.38	M5XCQ5	Class IV chitinase	0.38
M5XDU4	(R)-mandelonitrile lyase 2	0.40	M5XJJ3	Enolase	0.39
M5WWB9	Epoxide hydrolase 3	0.47	M5WGH9	Plastid lipid-associated protein	0.43
M5W7R0	Uncharacterised protein	0.51	M5XF62	GTP-binding nuclear protein	0.46
M5Y9F2	Pleckstrin homology domain	0.53	M5WD64	Patatin	0.49
M5VPU2	Class V chitinase	0.53	M5WC10	40S Ribosomal protein S7	0.52
M5XLQ7	Periplasmic beta-glucosidase	0.54	M5XS55	Serine carboxypeptidase	0.54
M5WXM6	Uncharacterised protein	0.55	M5WGA1	Uncharacterised protein	0.56
M5XJJ3	Enolase	0.55	M5WRJ6	Pollen coat-like protein	0.57
M5W0M7	Histone H2B	0.57	M5XQ02	Uncharacterised protein	0.61
M5Y2Y6	Mandelonitrile glucosyltransferase	0.58	M5WX95	Uncharacterised protein	0.61
M5XIU5	Uncharacterised protein	0.58	M5X4I0	Malate dehydrogenase	0.61
M5W248	Endo-1.4-beta-glucanase	0.58	M5W0M7	Histone H2B	0.64
I1U4K7	Polyphenol oxidase II	0.59			
M5W266	Prunasin hydrolase	0.60			
M5WP60	Ribosomal protein L2	0.61			
M5VMV1	Putative p23 co-chaperone	0.61			

Functional classification of differentially expressed proteins according to their role in cellular pathways indicated that metabolism and stress resistance and defence were the predominant categories in both conditions. Similar results were obtained in Chalivendra *et al*. (2012), who indicated that lipid metabolism and cell-wall loosening and defence proteins are characteristic of pistil mature stages.

Among all the proteins indicated in Table 1, thaumatine-like protein, glucan endo-1,3-beta-glucosidase and glutathione S-transferase deserve a special mention because they have been linked to pollen-pistil interactions (Wang *et al*., 2014). In the case of proteins differentially expressed in anthers annexin, chalcone synthase, GDSL esterase/lipase and serine carboxypeptidase should be highlighted since they have been linked to pollen development, pollen dynamics or to pollen-pistil interactions (Taylor and Jorgensen, 1992; Dai *et al*., 2006; Updegraff *et al*., 2009).

These results provide proteomic profiles of differential expression in mature pistils and anthers, and could also serve as a reference for other comparative proteomic studies of pollinated pistils in almond and in other species with GSI.

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Significance of *S*-genotype determination in the conservation of genetic resources and breeding of almond

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Abstract. Most almonds are self-incompatible, and they are also cross-incompatible with those having an identical incompatibility (*S*) genotype. In recent years, several almonds have been *S*-genotyped using different approaches. This information has been included in different tables of cross-incompatibility groups, which are an update of the previously proposed one. However, no attempt has been made to reconcile the information of all these tables neither to correct possible inconsistencies. In this work we have determined the *S*-genotype of 15 Spanish almond cultivars previously un-genotyped using consensus primers for the first and second introns of *Prunus* *S*-RNases, and also allele-specific primers here designed. We could identify a new *S*-RNase allele numbered as S_{51}, which was cloned and sequenced. With the information obtained in this work and in previous studies, we have established eight new cross-incompatibility groups. Some of the already proposed groups need to be re-numbered to avoid the gaps left by those no longer existing. The determination of the *S*-genotype of almond genetic resources to be preserved as part of the agricultural biodiversity will facilitate the use of this material in research and breeding, and also provides useful information about phylogenetic relationships.

Keywords. *Prunus dulcis* – Genetic resources – Cross-incompatibility – *S*-genotyping.

Importance de la détermination du génotype S dans la conservation des ressources génétiques et la sélection d'amande

Résumé. *La plupart des amandes sont auto-incompatibles, ils sont aussi cross-incompatibles avec celles ayant un génotype (S) d'incompatibilité identiques. Au cours des dernières années, plusieurs amandes ont été S-génotypés en utilisant différentes approches. Cette information a été incluse dans différents tables des groupes cross-incompatibilité, qui sont une mise à jour de ceux proposés précédemment. Cependant, aucune tentative n'a été faite pour concilier les informations de toutes ces tables ni de corriger les éventuelles incohérences. Dans ce travail, nous avons déterminé le S-génotype de 15 cultivars d'amandier espagnols précédemment non-génotypés en utilisant des amorces consensus pour les premier et seconde introns de S-RNases de* Prunus*, mais aussi des amorces allèle-spécifiques ont été conçus. Nous avons pu identifier un nouvel allèle S-RNase numéroté comme* S_{51}*, qui a été cloné et séquencé. Avec les informations obtenues dans ce travail et dans les études précédentes, nous avons établi huit nouveaux groupes cross-incompatibilité. Certains des groupes déjà proposés doivent être re-numéroté pour éviter les lacunes laissés par ceux qui n'existe plus. Le S-génotypage des ressources génétiques d'amande préservée dans le cadre de la biodiversité agricole facilitera l'utilisation de ce matériau dans la recherche et la sélection, et fournira également des informations utiles sur les relations phylogénétiques.*

Mots-clés. Prunus dulcis *– Ressources génétiques – Cross-incompatibilité – S-génotypage.*

I – Introduction

Most almond cultivars [*Prunus dulcis* (Mill.) D.A. Webb] are self-incompatible, and they are cross-incompatible with those cultivars with the same incompatibility genotype. Self-incompatibility in almond is of the gametophytic type and it is controlled by the *S* locus, which has two genes with expression in pistil (S-RNases) and in pollen (SFB) (Tao and Iezzoni, 2010). The *S*-genotype of several almond cultivars has been determined initially by controlled crosses and later by isoelectric focusing and PCR (Kester *et al*., 1994; Bošković *et al.*, 2003; Ortega *et al.*, 2005; Halász *et al.*, 2010; Kodad *et al*., 2010). The information obtained with the different methods indicated has been included in different versions of a table of cross-incompatibility groups (CIGs) (Bošković *et al.*, 2003; López *et al*., 2006; Ortega *et al*., 2006; Valizadeh *et al.*, 2009; Halász *et al.,* 2010; Kodad *et al*., 2010; Mousavi *et al*., 2011; Hafizi *et al*., 2013). Each of these tables is essentially an update of the previously proposed one. However, no attempt has been made to reconcile the information of all these tables neither to correct possible inconsistencies. Knowledge of the CIGs is very useful in breeding programs and it makes easier the design of plantations.

As part of a complete agronomical characterization of almond genetic resources grown in an open field genebank, we aimed to determine the *S*-genotype of 15 cultivars. We also aimed to establish cross-incompatibility groups with the information of this and previous studies.

II – Materials and methods

Young leaves were collected from trees of 15 Spanish almond cultivars previously un-genotyped for self-incompatibility and cultivated at the collection of IRTA (Tarragona, Spain). Leaf samples were also collected from 15 almond cultivars and one selection grown at the experimental field of CEBAS-CSIC (Murcia, Spain), which were used as reference for the almond *S*-alleles S_1-S_{29} and S_f described in Ortega *et al.* (2005).

Genomic DNA was extracted from leaf samples following the CTAB protocol described in Sonneveld *et al*. (2001). *S*-genotypes were identified by PCR of *S*-RNases using the consensus primers EM-PC2consFD + EM-PC3consRD and PaConsI-F + EM-PC1consRD as indicated in Ortega *et al.* (2005). Moreover, in a few particular cases, PCR with allele specific primers for S_3 or S_{10} designed in the present study was performed. When the PCR products generated using the sets of primers above indicated differed in size from those of the reference *S*-RNase alleles they were considered to correspond to potentially new *S*-RNase alleles, and they were therefore cloned and sequenced to ensure their identity. For this, the region between the signal peptide and the conserved region C5 was amplified from genomic DNA using the PCR reaction and cycling parameters indicated in Ortega *et al.* (2006). Purification of PCR products, cloning, transformation and selection of positive clones were as detailed in Ortega *et al.* (2006). For each *S*-RNase allele, three plasmids were sent for sequencing to STAB VIDA (Caparica, Portugal) using the M13 primers. The identity of the sequences obtained was ascertained by comparison with those available in the European Bioinformatics Institute web site (http://www.ebi.ac.uk).

III – Results and discussion

1. *S*-genotyping and new *S*-RNase

Using the different sets of primers above indicated, most of the 15 almond cultivars could be *S*-genotyped (Table 1). However, in two of the cultivars cloning and sequencing of the *S*-RNase alleles were necessary to complete the characterisation. In this manner, the presence of S_{35}, and of S_{24} and a new *S*-RNase allele named S_{51} was determined in 'Parque Samá' and 'Mollar de la Princesa', respectively. As observed in Table 1, the most frequent alleles were S_{10}, S_{12} and S_{27}.

2. Establishment of cross-incompatibility groups

Eight new CIGs were established after drawing together the results of this work and the information included in the previously proposed tables of CIGs (Bošković *et al.*, 2003; López *et al.*, 2006; Ortega *et al.*, 2006; Valizadeh *et al.*, 2009; Halász *et al.*, 2010; Kodad *et al.*, 2010; Mousavi *et al.*, 2011; Hafizi *et al.*, 2013). The new CIGs XLI and XLII are formed by cultivars with S_4S_{13} and S_3S_9 genotype, respectively. The other six CIGs, to which the cultivars *S*-genotyped in this work belong to, are indicated in Table 1. In addition, some of the already proposed groups were re-numbered to avoid the gaps left by those no longer existing. It is noteworthy that, in the literature, accessions with the same name had a different *S*-genotype, what needs to be checked to ascertain which one is the correct.

Table 1. PCR product sizes with second and first intron consensus primers, allele-specific PCR scores, *S*-genotype and cross-incompatibility group (CIG) assessed to 15 Spanish almond cultivars

Cultivar	Second intron product size (bp)	First intron product size (bp)[a]	Allele-specific PCR		*S*-genotype	CIG
			S_3	S_{10}		
'Angones'	300, 1130	n.a., 400		+	$S_{10}S_{22}$	O
'Asperilla'	300, 1360	n.a., 380		+	$S_{10}S_{27}$	O
'Belardino'	450, 400				S_2S_{11}	O
'Caima'	350, 1300	n.a., 200		–	S_5S_{12}	XLVIII**
'Carreró'	1300, 1360	200, 380			$S_{12}S_{27}$	XLVII**
'Esperanza Forta'	1300, 1130	200, 400			$S_{12}S_{22}$	XLIII**
'Mollar de la Princesa'*	875, 570		–		$S_{24}S_{51}$	O
'Mollar de Tarragona'	750, 1300	590/700/870, 200			S_1S_{12}	XLVI**
'Nano'	1300, 340	200, 300			$S_{12}S_{28}$	XLIV**
'Parque Samá'*	750, 1280	590/700/870, 380			S_1S_{35}	O
'Pauet'	570, 300	350, n.a.		+	S_6S_{10}	XLV**
'Pep de Juneda'	750, 300	590/700/870, n.a.		+	S_1S_{10}	XIII
'Rof'	330, 690	n.a., 375	–		S_5S_{23}	O
'Tardaneta'	330, 1300	n.a., 200	–		S_5S_{12}	XLVIII**
'Verd'	1300, 1360	200, 380			$S_{12}S_{27}$	XLVII**

[a] n.a.: no amplification.
* *S*-genotype determined after sequencing.
** CIG established in this work.

The determination of the *S*-genotype of almond genetic resources to be preserved as part of the agricultural biodiversity will facilitate the use of this material in research and breeding. Moreover, S-genotyping allows detecting the frequency of the *S* alleles in local populations, what provides useful information about phylogenetic relationships.

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Early selection for flowering time in almond breeding programs

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Abstract. Late flowering to reduce the risk of late frosts is one of the main objectives of the almond breeding programs. Breeders have to wait at least three years to know the flowering time of the seedlings, so it would be interesting to develop early selection strategies for this trait. Although some trials have been conducted correlating chilling requirements of seeds to germinate, leafing and flowering, the methodologies employed did not find a close correlation between these characteristics. In this work we studied the relationships between germination, sprouting and flowering in four families created in 2009 between very early and very late genitors that segregate well for flowering time. In winter, the seeds obtained were placed in imbibition for three days, removed the shell and stratified at 10°C in vermiculite for germination. Weekly the emergence of the root was noted as the date of germination. The plants obtained were taken to greenhouse in pots where they grew up till summer. Then they were taken to a cold room at 10°C, where weekly the number of buds sprouted was noted as the leafing time in controlled conditions. Finally, the plants were taken to the definitive plots and in 2013, 2014 and 2015 the leafing and flowering time were scored. Correlations between the requirements of seed to germinate and flowering time were no significant. Correlations between leafing time in the cold chamber and flowering time in the field several years later were intermediate, questioning the use of this correlation as early selection criterion for flowering time in almond.

Keywords. *Prunus dulcis* – Germination time – Leafing time – Flowering time – Early selection.

Séléction précoce pour la date de floraison dans les programmes d'amélioration de l'amandier

Résumé. *La floraison tardive pour réduire le risque de gelées est l'un des principaux objectifs des programmes de sélection d'amandiers. Les sélectionneurs doivent attendre au moins trois ans pour connaître le temps de floraison des plants, de sorte qu'il serait intéressant de développer des stratégies de sélection précoce pour ce caractère. Bien que des essais aient été menés pour la corrélation entre les besoins en froid des graines pour la germination, la feuillaison et la floraison, les méthodologies employées n'ont pas trouvé de corrélation étroite entre ces caractéristiques. Dans ce travail, nous avons étudié les relations entre la germination, le bourgeonnement et la floraison chez quatre familles créées en 2009 entre des géniteurs très précoces et très tardifs qui se ségrègent bien pour la date de floraison. En hiver, les graines obtenues ont été placées pour imbibition pendant trois jours, dépouillées de leur coque et stratifiées à 10°C sur vermiculite pour assurer la germination. L'émergence de la racine a été notée chaque semaine en tant que date de germination. Les plantes obtenues ont été placées sous serre dans des pots où elles ont grandi jusqu'à l'été. Ensuite elles ont été emmenées dans une chambre froide à 10°C, où hebdomadairement le nombre de bourgeons ayant poussé a été noté comme temps de feuillaison en conditions contrôlées. Enfin, les plantes ont été plantées dans les parcelles définitives et, en 2013, 2014 et 2015, les temps de feuillaison et de floraison ont été évalués. Les corrélations entre les exigences de la graine pour germer et le temps de floraison n'étaient pas significatives. Les corrélations entre la date de feuillaison en chambre froide et la date de floraison aux champs quelques années plus tard ont été intermédiaires, remettant en question l'utilisation de cette corrélation comme critère de sélection précoce pour la date de floraison chez l'amandier.*

Mots-clés. Prunus dulcis *– Temps de germination – Temps de feuillaison – Temps de floraison – Sélection précoce.*

I – Introduction

Late flowering is one of the main objectives of breeding programs, since it determines the vulnerability of production to spring frosts (Dicenta *et al*., 1993; 2005, García-Gusano *et al*., 2010). Almond breeding for late flowering is a laborious task that implies the production and study of numerous seedlings. These descendants cannot be selected until the third year after planting, when the seedlings have their first flowering. For this reason, it would be interesting to use a method for early selection of late-flowering individuals, which will be the only ones planted in the orchard for a later selection for other characteristics.

Some authors studied the correlation between the flowering time and the stratification requirements of seeds for germination (Kester *et al*., 1977; Dicenta *et al*., 2005; García-Gusano *et al*., 2010) or the leafing time (Kester *et al*., 1977; Vargas and Romero, 1984; Dicenta *et al*., 2005; García-Gusano *et al*., 2010). These correlations were more or less important depending of the work but they were always affected by environmental factors that could have hidden the genetic relationship between these traits.

The objective of this work was to study the correlations between seed germination, first leafing time under controlled conditions and flowering time several years later by using a new methodology, in order to determine the accuracy of using these characteristics as criteria for early selection of late flowering in almond breeding programs.

II – Plant material and methodology

Plant material assayed included 128 almond seeds and seedlings from 4 families created in 2009 (Table 1).

Table 1. Mean number of days for germination (2009) and leafing (first to fifth bud sprouted) in cold room (2010) by families. Leafing time and Flowering time mean values (Julian days) for each family in the orchard in 2013, 2014 and 2015

			2009	2010					2013		2014		2015	
Female	Male	N	Germination	Sprouted buds					Leafing	Flowering	Leafing	Flowering	Leafing	Flowering
				1st	2nd	3rd	4th	5th						
Desmayo	Achaak	31	38	87	100	100	102	108	28	18	31	27	41	33
Desmayo	Tardona	85	38	121	136	139	141	141	46	43	50	48	62	60
Tardona	Desmayo	8	57	122	153	175	202	202	46	46	51	48	62	61
Tardona	D00-078	4	51	121	127	127	127	136	60	66	55	58	68	71
TOTAL		128	42	110	122	125	125	127	42	36	46	43	58	54

During the winter 2009-2010, the nuts (with endocarp) were placed in imbibition for 3 days. Then the endocarp was removed and seeds (with tegument) were stratified at 10°C individually in cell trays covered by a permeable bag with wet vermiculite inside, without contact with the seeds. Weekly the emergence of the root (0.5 cm) was noted as the date of germination. The plants obtained in 2010 were cultivated in a greenhouse in 3 liter pots, where they grew up till summer. Then they were taken to a cold chamber at 10°C and pruned at 40 cm high removing all lateral branches. Weekly, the number of buds sprouted was noted as the leafing time in controlled conditions. Finally, the plants were taken to plots where they remained till today. In 2013, 2014 and 2015, the time for full leafing (50% of vegetative buds sprouted) and the time for full flowering (50% of floral buds opened) were recorded as Julian days (number of days after January 1st).

Pearson´s correlation coefficient was calculated between the number of days in stratification required for seed germination (2009), the time of leafing in the cool chamber (2010) for 1, 2, 3, 4 and 5 buds sprouted, the time of leafing in the field (2013, 2014 and 2015), and the time flowering in the field (2013, 2014 and 2015).

III – Results and discussion

Table 1 shows the families studied and the mean values for the studied traits during seven years (2009-2015). We observe the maternal effect on the germination, being the female genitor the responsible for the germination, regardless the male. The families of 'Desmayo' as female were the earliest and those of Tardona the latest. In the case of leafing and flowering of seedlings, we observe a clear effect of both progenitors on the offspring, being early flowering the offspring of 'Desmayo' × 'Achaak', intermediate 'Desmayo' × 'Tardona' (direct and reciprocal) and late flowering 'Tardona' × 'D0-078'.

The weather of the three years was very different delaying the flowering time 7 days (in 2014) and 11 more in (2015), on average. In general, leafing was some 3-7 years later than flowering.

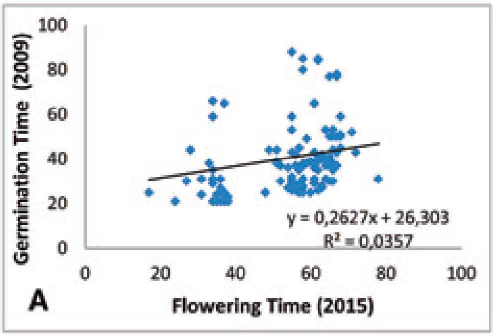
There is no correlation between the chilling requirements of seeds to germinate and the buds to sprout or flowering, any year (Table 2, Fig. 1A). In agreement with our results, Kester *et al*. (1977) and Dicenta *et al*. (2005) obtained a low correlation between the stratification requirements of seeds for germination and the time of flowering of seedlings. García-Gusano *et al*. (2010) obtained a correlation intermediate (0.5). Considering all these results, we conclude that the stratification requirement of seed is not an efficient criterion for the early selection of late-flowering cultivars.

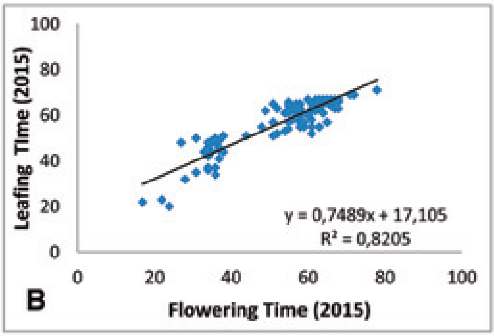
Table 2. Pearson correlation coefficients between germination (2009), leafing time in cold chamber (2010), and leafing time and flowering time in the orchard (2013, 2014, 2015), with individual values of seedlings

	2010	2013		2014		2015	
	Leafing (chamber)	Leafing	Flowering	Leafing	Flowering	Leafing	Flowering
Germination		0.12[a]	0.13[a]	0.11[a]	0.14[a]	0.08[a]	0.19[a]
Leafing (cold chamber)	1st sprout	0.56	0.59	0.63	0.57	0.55	0.60
	2nd sprout	0.59	0.63	0.67	0.59	0.61	0.63
	3rd sprout	0.57	0.60	0.59	0.49	0.56	0.54
	4th sprout	0.56	0.59	0.72	0.66	0.66	0.68
	5th sprout	0.55	0.58	0.61	0.60	0.57	0.58
Leafing time (same year that flowering)			0.86		0.92		0.91

All values are significant at 0.001 level except for those of germination[a] (non-significant at 0.05 level).

On the other hand, as expected, a narrow correlation (0.86-0.92) between leafing and flowering time in the same year was observed (Table 2, Fig. 1B). The relationship between chilling requirements for leafing and flowering times has been shown (Kester *et al*., 1977; Vargas and Romero, 1984; Dicenta *et al*., 2005; García-Gusano *et al*., 2010). Different correlations were observed when flowering and leafing of the same year were considered or when mean values of families were studied (Kester *et al*., 1977, Vargas and Romero 1984; Dicenta *et al*., 2005). However the only efficient tool for early selection is the individual correlation between different years. Correlations between progenitors and family means or between data of the same year are not interesting for this purpose and could be predicted because of the high heritability of this trait (Dicenta *et al*., 1993).





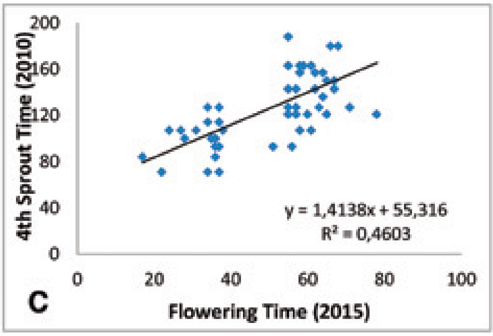


Fig. 1. (A) Relationship between germination (number of days of stratification in 2009) and flowering time (Julian days in 2015) of seeds and the seedlings coming from them, belonging to four families of almond. (B) Relationship between leafing time (Julian days) and flowering time (Julian days) in 2015 of seedlings belonging to four families of almond. (C) Relationship between leafing (number of days in cold chamber for sprouting four buds in 2010) and flowering time (Julian days in 2015) of seedlings belonging to four families of almond.

When individual data of first leafing in the field was compared with flowering some years later, the only useful for early selection (Dicenta *et al*., 2005), the correlation was lower. This low correlation was explained because, even if they are related, they are different traits (Sánchez Pérez *et al.*, 2012). In fact, there are almonds which flower before leafing, leaf before flowering or flower and leaf simultaneously. Furthermore, there is certain interaction genotype x environment, which brought about slight differences in the leafing and flowering times, depending on the climatic conditions of the year and the age of the tree.

Significant intermediate correlations were observed between leafing in the cold chamber in 2010 and leafing and flowering in the field in 2013, 2014 and 2015 (Table 2, Fig. 1C). These correlations varied from 0.49 (2^{nd} sprouts in 2014) to 0.68 (4^{th} sprouts in 2015, Table 2) between the first leafing time in cold chamber and flowering time in the field several years later, using this original methodology, that could be applied for early selection in the breeding programs, but assuming certain error.

Improvement of this interesting method could increase this correlation or perhaps we will have to assume that the first leafing (in cold chamber or field) and the first flowering some years later in the field are related traits but different enough to question their use in early selection of late almonds. Correlations between leafing time in the cold chamber and flowering time in the field several years later were intermediate, questioning the use of this correlation as early selection criterion for flowering time in almond.

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Contribution in tracing the origin and evolutional history of almond around the Mediterranean basin

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Abstract. Results regarding the origin and domestication steps of almond and its dissemination around the Mediterranean basin are very controversial. The general belief is that the domestication was in the eastern side and then its dissemination to the western part was through the different trade routes. A recent study has proved using a combination of nuclear and chloroplast microsatellites a westward dispersal of almond and a long-standing presence of domesticated almond in northern Africa. In order to contribute to this discussion, new interpretation of the previous study using 10 SSRs to analyse 82 almond accessions from different origins was undertaken. The dendogram based on the similarity matrix presented four main clusters. In group A were present the majority of the local genotypes that originate from the centre and the south. Contrarily, all cultivars from the north were in group C and clustered with the European and American genotypes. In fact, the Bayesian-based analysis endorses a two divergent population structure. Accordingly, our study supports the presence of two genetically distinct groups. One located in the north and a second in the central and southern part that may be issued from mutational processes. Thus, our work supports the hypothesis of the French team regarding a native existence of almond in North Africa and a possible westward dispersal.

Keywords. *Prunus dulcis* L. – Domestication – Gene pools – Bayesian analysis.

Contribution au traçage de l'origine et de l'histoire évolutive de l'amandier aux alentours du bassin méditerranéen

Résumé. *Les résultats relatives à l'origine et aux différentes étapes de domestication et de dissémination de l'amandier au alentour du bassin méditerranée sont très controversées. Les hypothèses avancées à ce jour évoquent une domestication dans la zone Est suivie d'une dissémination au coté Ouest à travers les différentes voies de commerce terrestres et maritimes. Des études récentes ont montré par combinaison de marqueurs microsatellites nucléaires et chloroplastiques l'existence plausible d'une étape de dissémination West-est et d'une présence très ancienne de l'amandier dans le nord de l'Afrique. Ainsi et dans le but de contribuer à cette discussion une nouvelle interprétation des résultats relatifs à l'utilisation des dix marqueurs microsatellites utilisées pour la caractérisation moléculaire de 82 génotypes d'amandier issus de différentes origines a été entrepris. Le dendrogramme basé sur la matrice de similarité montre la présence de 4 groupes distincts. Dans le groupe A on note la présence de toutes les variétés et écotypes locaux issus du centre et du sud tunisien alors que dans le groupe C existent toutes les variétés locales du nord regroupées avec celles originaires de l'Europe et de l'Amérique. De plus, l'analyse bayesienne approuve la présence en Tunisie de deux pools génétiques d'amandier très distincts. Ainsi, ce travail soutient l'hypothèse émise par l'équipe française quand à l'ancienne existence de l'amandier en l'Afrique du nord ainsi que la possibilité d'une dissémination west-est.*

Mots-clés. Prunus dulcis *L. – Domestication – Pools de gènes – Analyse bayesienne.*

I – Introduction

Almond trees belong to the subgenus *Amygdalus* (L.) Focke, an Irano-Turanian complex of *Prunus* including more than 30 species (Browicz and Zohary 1996) but only *P. dulcis* was domesticated to produce sweet almonds. The introduction of cultivated almond in the eastern Mediterranean area took place by the second millennium BCE and there is evidence of extensive of almond trade in the fourth century BC (Cerda, 1973). Kester *et al.* (1991) suggested the wide dissemination of modern almond and its cultivation in four separated phases: Asiatic, Mediterranean, Californian and Southern Hemisphere. In Tunisia the origin of the existing almond genotypes is very controversial being the granary of the Roman Empire it is considered as the main routes along witch almond was spread along the Mediterranean basin. It may be also a secondary centre of diversity for almond. In fact, Tunisian almond plantations are located throughout all the country in different climatic conditions. In the north with a rainfall more than 700 mm/year are located cultivars that are adapted to sub humid climate and fungus resistant. The prospecting effort that was done last few years in the central and southern part of the country permitted the discovery of large diverse ecotypes.

A previous study (Gouta, 2012) has supposed using microsatellites the presence in Tunisia of two genetic pools. The first in the north resulting from plant material exchange with the northern shore of the Mediterranean and the second pool existing in the central and southern part that is probably the result of the dissemination of almond from its origin to north Africa via one of the ancient silk road caravan routes crossing north-central Africa through Timbuktu as it was advanced by Gradziel (2011). Recent study dealing with chloroplast and nuclear genotyping (Delplancke *et al.*, 2012) sheds light on a long standing presence of domesticated almond in North Africa and considered that this area is outstanding with a divergent haplotype and a phylogeographic cluster differentiated from the others mostly by mutational processes. The aims of this work are to ensure the hypothesis evocated by previous studies, to analyse the population structure of the almond genotypes from different origins and to contribute to this discussion about the origin of the existent germplasm in North Africa and particularly in Tunisia.

II – Materials and methods

Eighty-two almond accessions from different origins were analyzed in this study. Most of them originated from Tunisia (50), the others included in the National Collection were from France (9), Italy (7), Morocco (1), Spain (8), USA (3), or were of unknown origin (4).

DNA extraction: Total DNA was extracted from young and healthy leaf tissue following the protocol of Doyle and Doyle (1987).

DNA amplification: DNA was amplified by PCR using ten primer pairs of microsatellite, nine pairs derived from a library enriched for AG/TC motifs, constructed with the almond cultivar 'Texas' (Mnejja *et al.,* 2005) and one pair previously cited by Joobeur *et al.* (2000).

PCR amplification reactions were carried out according to Gouta *et al.* (2010).

Data analysis: Data were recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. A similarity matrix was generated with NTSYS software version 2.1 (Rohlf, 2000). Genetic similarity between pairs was estimated by the Dice coefficient (SD). Cluster analysis was done using the Sequential Agglomerative Hierarical Nested cluster analysis (SAHN) procedure of NTSYS. Bootstrap support values were obtained from 2000 replicates using TREECON 1.3b (Van de Peer and De Wachter, 1994).

The genetic population structures were accessed by using the admixture model of STRUCTURE 2.3.4 software (Pritchard *et al.,* 2000).

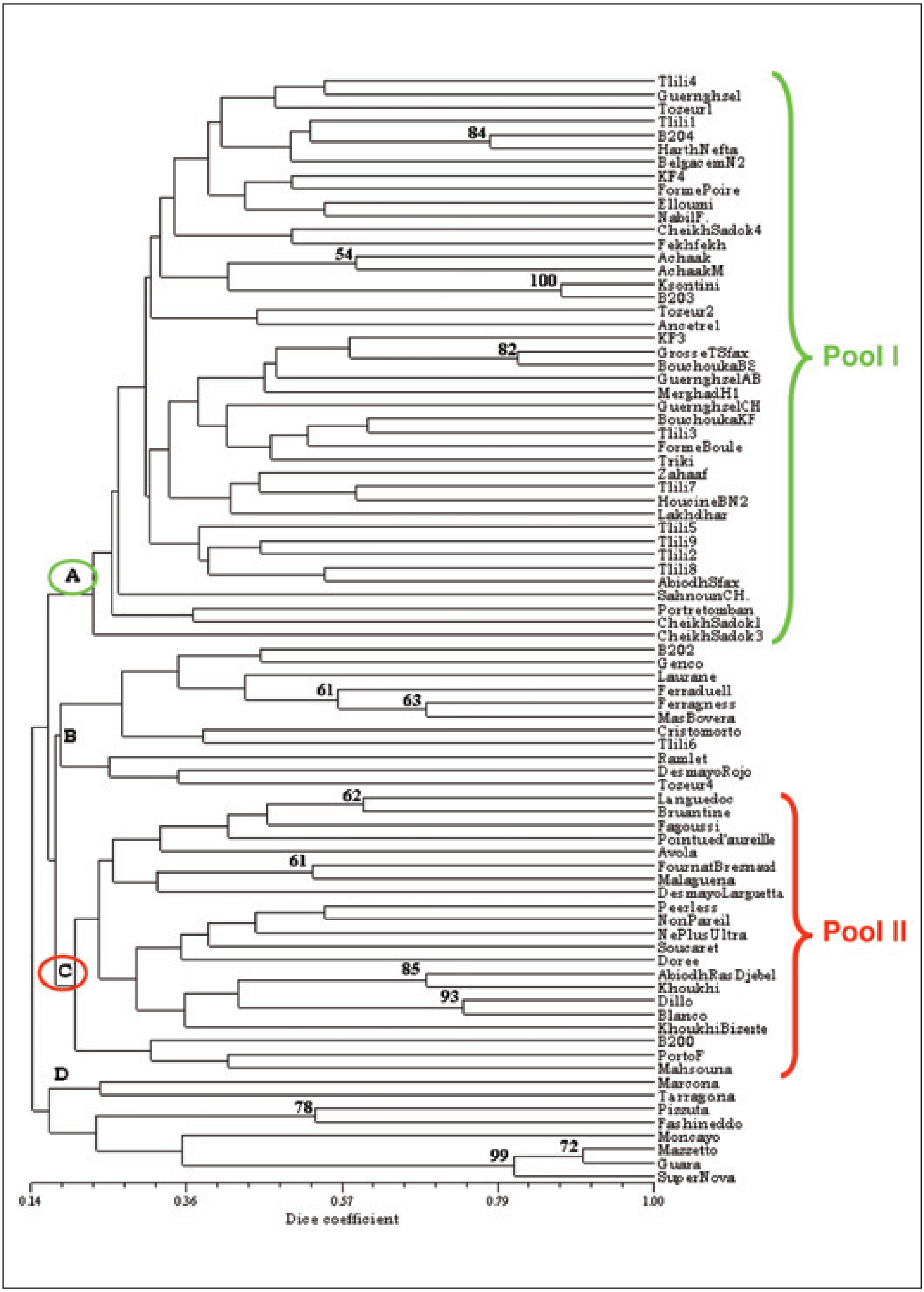

Fig. 1. Dendogram based on Dice coefficient illustrating the genetic similarities among 82 almond genotypes obtained by 10 SSR primers data.

III – Results and discussion

The dendrogram based on dice coefficient illustrating the genetic similarities among eighty two almond genotypes accessed by 10 genomic microsatellites (Fig. 1) showed that the majority of the Tunisian genotypes issued from the central and southern part (pool 1) were clustered together but they showed several minor groups, which revealed their high heterogeneity (cluster A, Fig. 1). This is probably due to the traditional method of propagation of this species all over the country which was mainly done by seeds (open-pollinated), until the more extensively use of grafting in the Mediterranean at the beginning of the 20th century (Grasselly and Crossa Raynaud 1980).

In contrast to what has been observed in group A (Fig. 1), the local cultivars from Bizerte (north of Tunisia), were clustered in the group C (pool 2) with some European and all the North American cultivars. These last originated from material of the Languedoc region of France (Kester, 1994). In fact, the position of this area in the extreme north of Tunisia probably favored the exchange of genotypes between both shores of the Mediterranean Sea (Fig. 1). The presence in group C of the two cultivars: 'Porto Farina' as was the old name of Ghar El Melh (a city in Bizerte) and 'Faggoussi' could be another fact in favor of this hypothesis. Moreover, the high bootstrap values observed in the sub cluster grouping cultivars from Bizerte (85% for 'Abiodh Ras Djebel' and 'Khoukhi' and 93% for 'Dillou' and 'Blanco') support the specificity of this site.

Furthermore and in order to strengthen the hypothesis regarding the genetic structure of the populations a Bayesian model based clustering was used. Results have moreover showed (Fig. 2) the clear existence of two distinguished populations that are geographically distinct. In fact it was clearly elucidated that a part of the local cultivars of the north (region of Bizerte) and another part of the local ecotypes were in the same cluster as the foreigner group (black colour). In this last were grouped all of the French, Spanish, Italian and American cultivars. The presence of the Californian cultivars in the same cluster with the French corroborates with results of Fernández i Martí *et al.* (2015) assuming that they belong all to the Mediterranean almond pool.

Thus, and tacking into account the results evocated by Delplancke *et al.* (2012) we can confirm that in Tunisia prevails two almond genetic pools: one in the northern part of the country that is historically recent issued from exchange of genotypes between both shores of the Mediterranean, and a second natively existing in the central and southern part that may be issued from mutational processes.

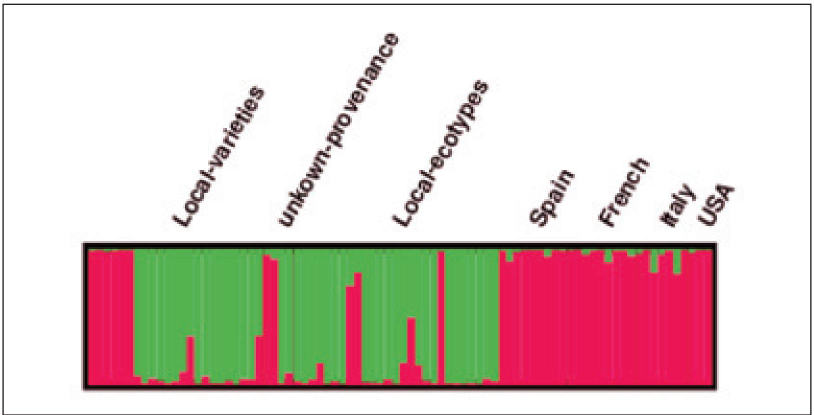

Fig. 2. Genetic structure of almond cultivars inferred from the admixture model.

IV – Conclusions

This study reveals the high diversity and the distinct origin of the Tunisian almond germplasm and can be considered as a first step forwards the understanding the origin of local and existing cultivars. The great diversity found in the almond germplasm supports the idea that Tunisia has a valuable source of almond genotypes that is tolerant to many biotic and abiotic stresses to be exploited in further breeding programs.

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Flower description and fertility of the main Tunisian almond cultivars

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Abstract. The almond *Prunus dulcis* (Mill.) D.A. Webb is an old edible nut that is present in Tunisia with a large phenotypic and genetic diversity of cultivars and landraces. However, no much investigation has treated the characterization of the local cultivars regarding flower description and fertility parameters. This study is dealing with the evaluation of the almond genetic diversity on the basis of some parameters related to flower characteristics. Also, the quality of pollen as well as the fructification rates for self and cross-pollination were studied in order to differentiate among genotypes according to their degree of self compatibility or incompatibility. Results showed a great intraspecific genetic diversity. Analysis and observations allowed us to distinguish some particular genotypes such as 'Zahaf' which is characterized by its largest flower size. Also it was concluded that 'Achaak 2' has the highest potential of fertility (80%) and a considerable fruit bearing and productive capacities. 'Mahsouna' and 'Tlili 8' presented the highest rates of fruit set when self-pollinated and as a consequence showed a tendency for self-compatibility.

Keywords. *Prunus dulcis* L. – Flower descriptors – Pollen fertility – Self pollination.

Description florale et niveau de fertilité des principales variétés tunisienne d'amandier

Résumé. *L'amandier* Prunus dulcis *(Mill.) D.A. Webb est une espèce fruitière de culture très ancienne en Tunisie. Elle compte de nombreux variétés et écotypes ayant une diversité phénotypique et génétique importante. Toutefois, peu de travaux se sont intéressés à la caractérisation de cette richesse en se référant aux caractéristiques morphologiques de la fleur en relation avec la biologie de fructification. Cette étude se propose la caractérisation de la diversité génétique locale d'amandier en se basant sur de nouveaux descripteurs morphologiques pour les différentes pièces florales. Aussi, on s'est intéressé à l'évaluation de la qualité du pollen et des niveaux d'auto-fertilité et/ou d'autostérilité des principaux génotypes d'amandier. Les résultats sont très encourageants et nous ont permis de prouver d'une part la richesse de notre patrimoine génétique en amandier ainsi que sa diversité et de faire ressortir d'autre part certaines spécificités variétales. D'ailleurs, les deux variétés 'Zahaf' et 'Achaak' se sont bien distinguées, la première par la taille de la fleur et la deuxième par le niveau de fertilité élevé et par conséquent des potentialités de fructification assez remarquables. Finalement, il s'est avéré que les variétés 'Mahsouna', 'Porto' et les écotypes 'Tlili 8', 'Amara4' présentent des taux d'autopollinisation satisfaisant et donc des tendances vers l'auto-compatibilité.*

Mots-clés. Prunus dulcis *L. – Fertilité pollinique – Descripteurs floraux – Autopollinisation.*

I – Introduction

Almond [*Prunus dulcis* (Mill.) D.A. Webb] belongs to the genus *Prunus* and the large family of the Rosaceae. The world production is estimated to 2.00 million tons per year (FAO, 2011). For this species flowering, flower quality and fruit set are of great economical importance while yields depend on them. Moreover, it is largely self-incompatible and necessitates cross-pollination, with overlapping bloom time to ensure good yield (Sharafi *et al.*, 2010). Nevertheless, few studies were dealing with flower biology for almond. The almond descriptors established by the former International Board for Plant Genetic Resources and edited by Gülcan (1985) present only very few parameters such as self compatibility, colour of petals and number of pistils.

The aims of this study are to present new almond descriptors related to flower components, to demonstrate the large diversity existent in the local germplasm expressed by flower descriptors and to estimate the level of self compatibility for the main local cultivars.

II – Materials and methods

Twenty-eight local genotypes and four European cultivars were characterised using 14 descriptors related to flower anatomy. They were preserved in the national collection of Sidi Bouzid (35.117 N, 9.567 E; 369 m above sea level.). Trees were drip irrigated grafted on 'Garnem' rootstock and receive conventional technical practices. Descriptors used in this study were related to petals (colour, number, length and width), stamens (number, length of the filament, colour of the filament), pistils (number, length and colour), calyx diameter, the position of the pistil regarding the stamens, the colour of the attachment point of petals and to the ratio length/width of petals.

The pollen viability tests were done individually. Acetic carmine was used for pollen coloration. Viable pollens were dyed in red and light red; dead pollens were not dyed. The pollen rate viability (PRV %) was calculated as the ratio PVR % = (number of dyed pollen / total pollen number)*100.

Pollen germination rates were analyzed on agar sucrose based media described by Hedhly *et al*. (2004) an in Petri dishes at 20°C according to Pinney and Polito, (1990). Observations were done after 2, 4, 6 and 24 hours and the germination was stopped by adding formaldehyde to the Petri dish. Observations were done using a light microscope (Leica, Germany) with 10 x ocular and 40 x objectives.

For self pollination trial three branches per tree were chosen and the number of flowering buds was registered. Self-compatibility levels were estimated by the number of fruit set. For that three branches/tree were covered before blooming by insect-proof cheesecloth cages. For each branch the number of flowers was noted. After petal fall the bags were removed. Data presented in this paper reported two dates of fruit sets. The first after removing the bags (D1) and the second (D2) two months after the end of physical fruit drop in almond (Kester and Griggs, 1959).

For open pollination three branches/tree were marked, flower buds and fruit set consequently were recorded. Fruit set was calculated for different pollination treatments and dates by dividing the number of fruit set by total number of flowers present on the shoot.

For statistical analysis an ANOVA was done using SPSS 17.0 and the differences between means were compared using Duncan test at $p \leq 0.05$.

III – Results and discussion

The principal component analysis (PCA) realized for the 33 genotypes with the 14 flower descriptors showed that the two first principal components explained 42% of the total variability observed (Fig. 1). The first axis was formed by petal length (PL), petal width (PW), pistil length (PSL) and calyx diameter (CD). The second axis was associated with the petal colour (PC), the stamen filament colour (FC) and the colour of the point of petal attachment (CAPP). Consequently, it is assumed that the local cultivar 'Zahaaf' was distinguished by the large size of its flower. In the opposite side it was demonstrated that even though the cultivar 'Achaak' is genetically distinct from 'Mazetto' they seemed to have close flower sizes. In the upper left quarter part of Fig. 1 is clearly noticeable the ecotype 'Amara 4' presenting special colours for petals, stamen filament and petal attachment point.

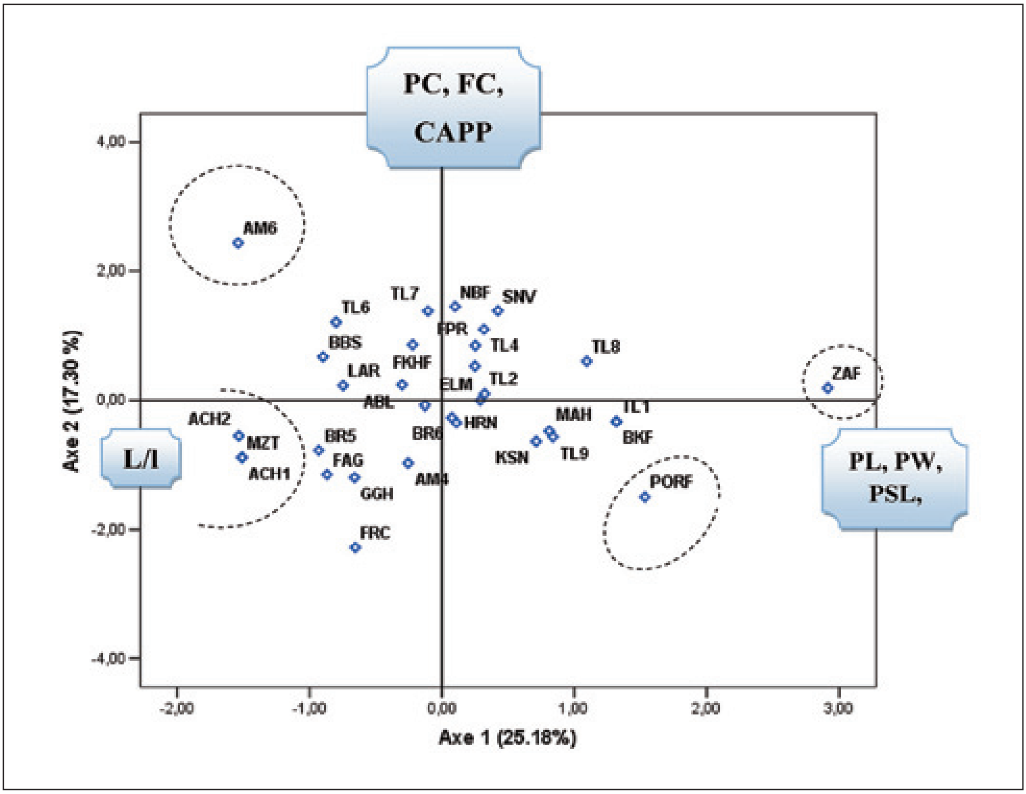

Fig. 1. Principal component analysis for 33 almond genotypes based on 14 flower descriptors.

Pollen viability test was very expressive while all the self-compatible cultivars presented values higher than 60% (Table 1). For the local genotypes values ranged from 37.5% for **'**RasBouma' to 79.9% for 'Tlili8'. High values were also observed for 'Porto' (76.2%), 'Achaak' (72.1%), 'Amara 4' (67.9%), 'Elloumi' (62.4%). The high fertility of the ecotype 'Tlili 8' was also expressed by the germination rates presented after 2, 4, 6 and 24 hours (Table 1). In fact 57.6% of the pollen germinates after only two hours. Similar value (53.9%) was reached for 'Porto' after four hours and for 'Lauranne' after 24 hours. At this last date a clear superiority was observed for many local genotypes in comparison to the introduced ones. That was clear for 'Amara4' (57.5%), 'Porto' (70.6%) and 'Elloumi' (56.9%). This may be due to the fact that they need a relatively high temperature similar to that prevails during the month of March when these cultivars bloom. Differences in pollen performances have been also noted, in different genotypes of almond (Martínez-Gómez *et al*., 2002) and apricot (Egea *et al*., 1992).

Results regarding self pollination treatment for the 10 local almond genotypes and the 4 introduced cultivars revealed that fruit set rate means after petal fall were highly variable for all locals (Table 2). In fact, they varied from 0% for 'Achaak' to 37.6% for 'Mahsouna'. This confirms previous annotations that the more famous Tunisian almond cultivar 'Achaak' is strictly self-incompatible. High values were noted for 'Tlili8' (31.3%), 'Porto' (28.5%), 'Bouchouka KF.' (18.7%) and 'Tlili9' (17.3%). Nevertheless, for the open pollination treatment and at the same date fruit set values were even higher. They varied from 26.4% for 'Achaak' to 68.3% for 'Bouchouka KF'.

Table 1. Pollen viability (VR%) and germination rates after 2, 4, 6 and 24 hours for some local and introduced almond genotypes

	Cultivars	VR (%)	GR 2 h (%)	GR 4 h (%)	GR 6 h (%)	GR 24 h (%)
Local Cultivars	‘Achaak’	72.1	3.7	6.6	3.7	39.2
	‘Amara4’	67.9	17.7	17.4	24.5	57.5
	‘Bouchouka B.S.’	48.4	9.2	9.1	17.5	28.9
	‘Bouchouka K.F.’	37.7	17.3	25	34	31.5
	‘Elloumi’	62.4	7.8	35.6	40.7	56.9
	‘Mahsouna’	56.3	7	6.3	16.8	20.7
	‘Porto F.’	76.2	34	53.9	71.5	70.6
	‘RasBouma’	37.6	14.6	20.1	27.8	30.9
	‘Tlili8’	79.9	57.6	63.8	63.3	72.7
	‘Tlili9’	51.2	2	8.1	9.1	42.5
Introduced Cultivars	‘Francoli’	66.8	6	12.6	18.6	40.8
	‘Lauranne’	71.5	30.1	15.6	20.6	57.9
	‘Mazetto’	74.9	17.5	14.5	19	50.6
	‘Supernova’	58.4	14.3	17.1	15.1	45.1
Significance P ≤ 0.05	**	**	**	**	**	**

Table 2. Fruit set of auto and open pollination treatments for some local and introduced almond genotypes

	Cultivar	Fruit set % (D1) auto-pollination	Fruit set % (D1) open-pollination	Fruit set % (D2) auto-pollination	Fruit set % (D2) open-pollination
Local Cultivars	‘Achaak’	0f	26.4f	0b	9f
	‘Amara4’	16.8cde	44.8de	3.4b	16de
	‘Bouchouka BS’	13de	64.1ab	0b	14.6de
	‘Bouchouka K.F’	18.7e	68.3a	0b	29.7ab
	‘Elloumi’	8.1e	52.2bcde	0b	16.4de
	‘Mahsouna’	37.6c	49.1cde	4.4b	12.1e
	‘Porto F.’	28.5cde	45.5de	6.0b	12.6e
	‘Rasbouma’	6.5e	65.7ab	0.6b	30ab
	‘Tlili8’	31.3cde	29.2h	1.6b	11.2e
	‘Tlili9’	17.3e	58.2bcde	0.8b	18.8cde
Introduced Cultivars	‘Francoli’	69.3a	64.5 abcd	17.3a	23.5abcd
	‘Lauranne’	70.1a	51.6 bcde	20.6a	30.3a
	‘Mazetto’	50.1b	45.1de	22.4a	20.4cde
	‘Supernova’	69.05a	65.1acde	17.3a	17.6cde

a,b,c,d,e,f,g,h: Signicant difference at Duncan’s multiple Range Test (5%).

The tendency for both treatments after two months of fruit sets (D2) was completely different. In fact, compared to D1 fruit set values have sharply decreased for both treatments and for almost all the local genotypes. Except, ‘Porto’, ‘Mahsouna’, ‘Amara4’ and ‘Tlili8’ presenting respectively 6%, 4.4%, 3.4% and 1.6% of fruit set, all the others were almost null in auto-pollination treatment. This indicates that almost all the local cultivars studied are self-incompatible and therefore need external pollinator with overlapping bloom periods. Since the cultivar ‘Achaak’ presented the lower value of fruit set for open pollination it is clear the need for this high quality kernel cultivar to select a good pollinator for it. The existence of cross-incompatibility groups in the Tunisian germplasm may be a first hypothesis for the understanding of this result. In fact it is well known that this cultivar is the first to bloom in Tunisia and our results added that it has high fertile pollen. An important increase of the final fruit set values for open pollination versus self pollination was very clear for the local genotypes 'RasBouma' (from 0.6% to 30%) and 'Bouchouka KF.' (from 0% to 29.7%). These values above 25% confirmed potentialities of these cultivars to give good yields.

For the introduced cultivars it was observed that the self pollination has increased fruit set recorded after petals fall but this was not the case at the final fruit set date (Date 2). In fact, for this last date fruit set was even improved for some cultivars by open pollination. Fruit set values increased from 17.3% to 23.5% for 'Francoli', from 20.6% to 30.3% for 'Lauranne' and from 17.3% to 17.6% for 'Supernova'. The few comparative studies of self-pollination and cross-pollination in self-compatible almond carried out with the varieties 'Guara' (Socias i Company and Felipe, 1992) and 'Lauranne' (Legave *et al.*, 1997) encourage the mono-varietal almond orchards. Even though it is true, for self-compatible genotypes we agree with the idea advanced by Dicenta *et al.* (2001) regarding the importance of cross pollination and mixing even for auto-fertile cultivars. Our work finally confirmed previous reports regarding the self-compatibility of the Spanish cultivar 'Francoli' (López *et al.,* 2004).

IV – Conclusions

The large diversity of the Tunisian gemplasm was clearly elucidated with the new flower descriptors presented in this work. High levels of pollen viability and germination for many ecotypes approve the importance of the prospecting effort and the need to preserve diversity. The dominance of fruit set values for open pollination treatments versus self pollination support the add value of multivariate plantations for both auto-compatible and incompatible genotypes with the necessity of booming overlapping combinations.

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Pomological, phenotypical diversity and biochemical characterization of fortheen almond morphotypes from Morocco

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Abstract. This study was focused on almond germplasm characterization of 14 genotypes from the experimental Ain Taoujadate collection based on morphological, pomological and biochemical traits. Results revealed that Commercial and local (*Prunus dulcis* L.) cultivars vary considerably in their fruit and kernel characteristics. The majority of important correlations were determined among the fruit traits (length, width, thickness, and weight) and the kernel traits (length, width, thickness, and weight). Morphological dendrogram clustered the genotypes into two main groups. The first group consists of only local accessions. The second group includes, in addition to local genotypes, a mixture of foreign genotypes. The heterogeneous cluster obtained, maybe the cause of an exchange of plant material as seeds between Morocco and other countries.

Keywords: Almond – *Prunus dulcis* – Phenotypic characters – Correlation.

Diversité pomologique, phénotypique et caractérisation biochimique de quatorze morphotypes d'amandier du Maroc

Résumé. *Cette étude s'est centrée sur la caractérisation du germoplasme de 14 génotypes d'amandier provenant de la collection expérimentale d'Ain Taoujadate basée sur des caractères morphologiques, pomologiques et biochimiques. Les résultats ont révélé que les cultivars commerciaux et locaux* (Prunus dulcis *L.) variaient considérablement pour les caractéristiques du fruit et de l'amandon. La majorité des corrélations importantes furent trouvées parmi les caractères du fruit (longueur, largeur, épaisseur, et poids) et les caractères de l'amandon (longueur, largeur, épaisseur, et poids). Le dendrogramme morphologique séparait les génotypes en deux groupes principaux. Le premier groupe consistait seulement en accessions locales. Le deuxième groupe comportait, en plus de génotypes locaux, un mélange de génotypes étrangers. Le cluster hétérogène obtenu pouvait être causé par un échange de matériel végétal comme des semences, entre le Maroc et d'autres pays.*

Mots-clés. *Amandier* – Prunus dulcis – *Caractères phénotypiques – Corrélation.*

I – Introduction

Almond is one of the oldest nut crops [*Prunus dulcis* (Mill.) D.A.Webb; syn. *P. amygdalus* Batsch]. It is mentioned far back in history, even in the Bible. The almond tree is thought to have originated in the mountainous regions of Central Asia (Grasselly, 1976). It was spread from its origin through the Mediterranean by the Phoenicians, Greeks and Romans. World almond production is concentrated in Asia, Mediterranean basin, California (Kester and Horel, 1980). Morocco ranks fifth among the nations of the world in almond production, with many zones where climatic conditions are ideal for this species. The variability in the environment and climate has led to an extensive diversity of almond genotypes in each productive region (Lansari *et al.,* 1994). In previous works, phomological and productive traits in some Moroccan almond cultivars have been already reported

(Lansari *et al*., 1994). The extent of the diversity of the Moroccan almond collection has not been sufficiently described, so there is a need to identify the best cultivars for almond cultivation development (Lansari *et al*., 1994). To maintain and exploit crop germoplasm efficiently, analysis of phenological and pomological traits is required.

Recent research findings are associated with characterization and identification of almond hull, shell and skin phenolic compounds to use them as natural antioxidants and antiradicals in foods and oxidative damage (Esfahlan *et al*., 2012, Jahanban *et al*., 2012; Hanine *et al*., 2014, 2015).

The objective of this study was to evaluate the morphological and pomological characteristics, determine the correlation among traits, and the physicochemical composition of 14 almond genotypes from the experimental Ain Taoujadate collection. Furthermore, an evaluation of economically valuable traits was performed to identify useful genotypes for almond producers and breeding programs.

II – Material and methods

The plant material examined was collected from the experimental Ain Taoujadate of the National Agronomic Research Institute (Meknes, Morocco). The name and geographic origins of cultivars are reported in Table 1. A total of 14 samples were included in this study. The fourteen cultivars included eight major commercial cultivars and six local Moroccan cultivars. Quantitative traits were investigated using the IPGRI almond (Gulcan, 1985). The pomological research was conducted on 30 fruits per cultivar. Data were recorded on fruit and kernel weight (g), width (mm), length (mm), and thickness (mm), and on percentage of kernel (%). The percentages of empty fruits and double kernels were also recorded. Almond blanching and skin removal was carried out according to Bolling *et al*. (2009). Total polyphenols analysis was performed colorimetrically by the Folin-Ciocalteu method as modified by Singleton *et al*. (1965). Total flavonoid content was determined spectrophotometrically according to Zhishen *et al*. (1999) with some modifications. Oil content was measured on dried kernels (30-40 g) using nuclear magnetic resonance (NMR) (Oxford 4000, Oxford Analytical Instruments Ltd.) according to AOCS (1998). All the assays were carried out in triplicate. Significant variables were calculated, subjecting results to a linear regression, using SPSS statistical program version 10.0 (SPSS Inc., Chicago, Illinois). Only variables with a confidence level superior to 95% ($P<0.05$) were considered as significant.

III – Results and discussion

Physical characteristics of the almond fruit and kernels differed significantly among cultivars (Table 1). Kernel length and width ranged from 16.74mm to 27.49mm and 10.72 mm to 16.1 mm, respectively. The kernels weights was found to be from 0.55 g for Tahala to 2.12 g for Tizin'addi 2, and fruit weight 1.41 g for Rizlane 1 to 4.66 g for Texas and Ferragnès.

Regarding width and length, Ferragnes and Fournat de Breznoud showed the longest fruits and kernel (over 36 and 26 mm, respectively) while Tahala produced the smallest (23 mm and 16 mm).The percentage of empty fruits of all genotypes was null. Only Toundout, Amekdouch, Rizlane1, Lauranne, and Texas showed double kernels. The mean values of the double kernels varied from 3 to 27%. Oil content varied significantly among genotypes, ranging from 35% to 57% of the kernel dry mass (Table 1). The oil content of Rizlane 2 and Fournat de Brezenaud was consistently low (between 35.2 and 41.74%), while that of Toundout and Texas was consistently high 56% with the remaining genotype being intermediate. The ranges of variability for this parameter were similar to those already reported (Abdallah *et al.* 1998; Askin *et al*., 2007; Kodad *et al*., 2014).

However the values obtained were remarkably lower than those registered in some Spanish, Italian, French and North American commercial cultivars (Sathe *et al*., 2008). Almond oil content and com-

Table 1. Mean values of examined quantitative traits observed in 14 almond cultivars.

Cultivar	Origin	Fruit weight (g)	Fruit length (mm)	Fruit width (mm)	Fruit thickness (mm)	Kernel weight (g)	Kernel length (mm)	Kernel width (mm)	Kernel thickness (mm)	Empty fruit %	Double kernels %	Kernel %	Flavonoids (mg Eq Catechin/ g DW)	Oil cointent %	Total phenol cont. of oil (mg Eq gallic/g oil)	Phenols content of oil (mg GAE/g DW)
'Toundout/1R'	Morocco (Errachidia)	2.46^{f}	27.84^{e}	23.04^{dc}	16.47^{cd}	1.16^{c}	21.46^{c}	14.35^{b}	8.74^{b}	0	3.3	47.1^{a}	16.4^{ed}	56.8^{a}	1.5^{bc}	16.1±0.47
'Amekchoud/3J'	Morocco (Errachidia)	3.35^{ed}	33.3^{c}	22.69^{ed}	17.19^{abc}	1.39^{ab}	24.02^{b}	13.83^{b}	9.63^{a}	0	26.7	41.5^{b}	14.5^{e}	53.2^{d}	0.3^{f}	15±0.76
'Rizlane 1'	Morocco (Oujda)	1.41^{g}	24.07^{g}	14.42^{j}	11.28^{g}	0.63^{e}	17.9^{e}	15.24^{a}	6.67^{g}	0	3.3	47.4^{a}	17.9^{cd}	46.9^{j}	1.6^{b}	7.8±0.65
'Rizlane 2'	Morocco (Oujda)	3.37^{ed}	25.75^{f}	20.84^{f}	16.1^{d}	0.63^{ef}	19.43^{d}	11.37^{d}	5.78^{h}	0	0	18.7^{f}	18.0^{cd}	35.2^{m}	1.3^{bc}	20.3±1.32
'Tizin'addi 2'	Morocco (Tiznit)	2.36^{f}	2411^{g}	15.65^{j}	11.27^{g}	2.12^{f}	17.91^{e}	10.72^{d}	3.74^{j}	0	0	22.8^{ef}	18.0^{cd}	45.7^{k}	1.5^{bc}	12.7±0.96
'Tahala'	Morocco (Tiznit)	2.42^{f}	23.16^{g}	17.96^{h}	12.68^{f}	0.55^{ef}	16.74^{f}	15.98^{a}	6.26^{gh}	0	0	23.1^{ef}	18.1^{cd}	46.4^{j}	0.8^{e}	7.5±0.50
'Marcona'	Spain	4.28^{b}	29.23^{d}	25.03^{a}	17.62^{a}	1.05^{d}	19.97^{d}	15.59^{a}	7.45^{ef}	0	0	24.5^{ef}	20.3^{bcd}	50.3^{h}	2.0^{a}	8.2±0.03
'Ferraduel'	France	4.43^{ab}	35.02^{b}	24.11^{b}	16.28^{d}	1.21^{c}	24.6^{b}	14.38^{b}	7.54^{def}	0	0	27.4^{cde}	17.7^{cd}	50.8^{g}	0.9^{de}	11.6±0.19
'Tuono'	Italy	3.16^{e}	28.46^{ed}	22.01^{e}	17.27^{ab}	0.97^{d}	19.93^{d}	12.97^{c}	8.55^{bc}	0	0	30.8^{cd}	18.0^{cd}	51.7^{e}	0.8^{e}	13.5±0.57
'Ferragnès'	France	4.6^{a}	37.74^{a}	23.68^{cb}	16.63^{bcd}	1.43^{a}	27.49^{a}	14.39^{b}	8.17^{bcd}	0	0	31.1^{cd}	22.1^{b}	52.4^{d}	0.9^{de}	16.1±0.85
'Fournat de Brezenaud'	France	4.48^{ab}	36.69^{a}	25.42^{a}	15.07^{e}	1.48^{a}	26.69^{a}	16.1^{a}	6.94^{fg}	0	0	33.2^{c}	14.2^{e}	41.7^{l}	1.2^{cde}	7,1±0,81
'Desmayo Rojo'	Spain	3.71^{c}	29.16^{d}	23.24^{cbd}	16.73^{bcd}	0.95^{d}	20^{d}	12.95^{c}	8.37^{bc}	0	0	25.8^{de}	21.5^{bc}	51.1^{f}	1.2^{cd}	5.2±0.84
'Lauranne'	France	3.47^{cd}	35.15^{b}	25.8^{a}	17.51^{a}	1.3^{b}	24.97^{b}	15.36^{a}	7.98^{cde}	0	6.9	42.3^{ab}	21.1^{bc}	46.5^{j}	1.5^{bc}	16.1±0.44
'Texas'	USA	4.66^{a}	27.37^{e}	19.84^{g}	17.25^{abc}	1.18^{c}	20.14^{d}	12.79^{c}	9.94^{a}	0	3.3	25.7^{de}	19.2^{cd}	56.4^{b}	1.3^{bc}	22.9±2.00

Values with different letters show significant differences according to Duncan's Multiple Range Test (95%).

position depend primarily on the genotype but may be affected by factors such as the year and the specific environmental conditions of the growi*ng* region (Yada *et al.,* 2011). The high oil content, comparable to commonly used oil seeds like rapeseed or sunflower seeds, makes kernels from genus *Prunus* highly suitable for commercial oil production.

Total flavonoids levels were significantly determined by cultivar. Rizlane-2 and Ferragnès exhibited the highest contents of these compounds (25.7 and 22.1 mg/g), while Fournat de Breznaud, Toundout and Amekchoud/3J showed the lowest concentrations (between 14.1 and 16.3 mg/g) (Table 1).

The most significant content of phenolic compounds is observed in the variety Marcona, followed by the genotype Rizlane1, the values fluctuate between 20 and 16 mg / g. These values are much higher than those obtained by the cultivars Amekchoud/3d and Tahala which recorded the lowest values and whose contents are respectively 3.1 and 8.1 mg / g, lowest oil content (3.1 and 8.1 mg/g, respectively).

The morphological dendrogram (Fig. 1) clustered the genotypes into two main groups. The first group consists of only local accessions. The second group includes, in addition to local genotypes, a mixture of foreign genotypes. The heterogeneous cluster obtained, maybe the cause of an exchange of plant material as seeds between Morocco and other countries.

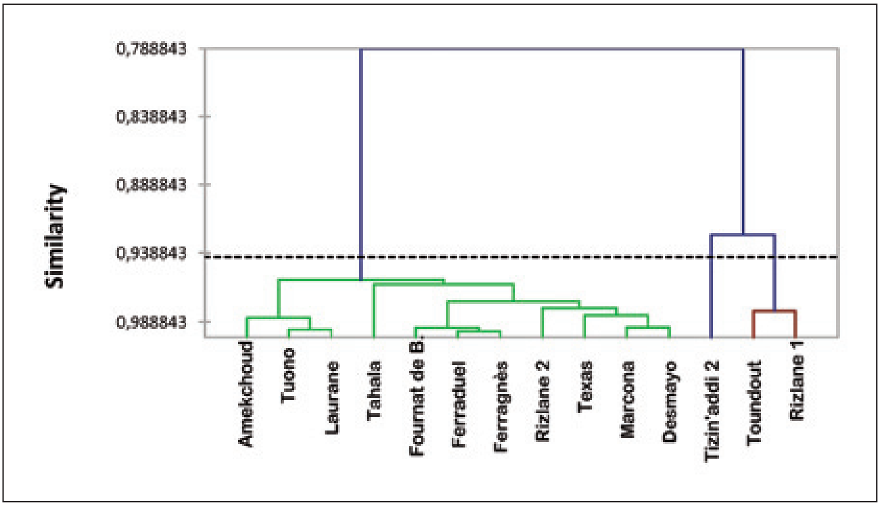

Fig. 1. Dendrogram of similarity with 14 almonds using morphological descriptors.

All examined genotypes are highly adapted to the environmental conditions in Morocco and could be a very interesting source of genetic diversity as indicated in the correlation of some characters of kernel (Table 1). High levels of variation in fruit and kernels were reported by other authors (Čolić, 2012). These results can be explained by self-incompatibilty of almond trees. This high phenotypic variability corresponds with previous reports on molecular characterization using different markers as nuclear and chloroplast simple sequence repeats (Zeinalabedini, 2012) or amplified fragment length polymorphisms (Sorkheh *et al*., 2007). The results of the empty kernels agree with the previous ones generated by other authors (Dicenta and García,1993; Sánchez-Pérez *et al*., 2007). In addition, the influence of the environment on the production of double kernels is also well known (Kester and Asay, 1975).

Correlation coefficients between the fruit traits show a strong correlations ($p<0.01$) were observed between most of the studied traits. In addition to the high correlations between fruit traits (length, width, thickness, and weight) (r = 0.48-0.77), kernel traits (length, width, thickness, and weight) were also correlated with each other (r = 0.16-0.70) and with fruit traits. However, no correlation (r = 0.086; p>0.05) was found between the fruit weight and kernel width. Similar findings were reported in 32 almond cultivars investigated by Zeinalabedini (2012). Fruit weight had significant positive correlation with kernel weight (r = 0.53; p<0.01), fruit length (r = 0.60; p<0.01) and the fruit width (r = 0.58; p<0.01).Talhouk *et al*. (2000), Ledbetter (2008), Tavassolian (2008) and Sorkheh *et al*. (2010) established significant correlations between fruit weight and kernel weight. A negative correlation was determined between the fruit weight and the kernel percentage (r = -0.37; p<0.01). These findings are in harmony with the results obtained on almond (Sánchez-Pérez *et al*., 2007).

The results of correlation analysis between polyphenolic and flavonoids content exhibited significant correlations among the total phenolic content, the flavonoid content. Statistically significant (P<0.05) correlation was found between the total phenolics in both almond skin and blanched almonds (r = 0.29 and r = 0.33, P<0.05).

IV – Conclusion

In this study, morphological, pomological and biochimical traits of 14 cultivar from morocco were evaluated. According to our research, genetic diversity was observed regarding morphological traits, indicating that there are rich and valuable plant materials. The research will be continued with the aim of improving production rates for further selection programmes in almond culture breeding. A potential source of markers is available for characterizing almond and for testing species differentiation at the molecular level.

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Molecular characterization of Spanish autochthonous almond breeding collections using SSRs

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Abstract. Despite the new almond cultivars released from breeding programs are displacing the autochthonous cultivars in each area, there are a lot of local cultivars that still have a great interest and they are cultivated in the different areas. Some of them are difficult to identify and frequently the same name is given to different cultivars or a genotype is known with different names. These varieties were selected over the years for its adaptation to warm Mediterranean climate and they are a valuable plant genetic resource that must be protected. The objective of this work is the molecular characterization of Spanish autochthonous almond breeding collections using SSRs. Forty two different early flowering and self-incompatible cultivars were assayed using 5 SSRs of reference in almond: BPPCT007, CPPCT005, MA27a, MA40a and UDP96005. Results have showed the great diversity of this autochthonous germplasm and the effectiveness of SSRs by the correct identification, detecting some synonymies and suggesting the origin of some autochthonous cultivars.

Keywords. Almond – Germplasm – Characterization – Simple sequence repeat – SSR.

Caractérisation moléculaire des collections espagnoles autochtones d'amandier utilisant SSR

Résumé. *Bien que les nouveaux cultivars d´amandier issus des programmes d´amélioration remplacent les cultivars autochtones dans chaque région, il y a beaucoup de cultivars locaux qui ont encore un grand intérêt et ils sont cultivés dans les différents domaines. Certains d'entre eux sont difficiles à identifier et fréquemment, le même nom est donné à différents cultivars ou un génotype est connu avec des noms différents. Ces variétés ont été sélectionnées au fil des ans pour son adaptation au climat méditerranéen chaud et ils sont une ressource phytogénétique précieuse qui doit être protégée. L'objectif de ce travail est la caractérisation moléculaire des collections espagnoles autochtones d'amandier utilisant SSR. Quarante-deux différents cultivars à floraison précoce et auto-incompatibles ont été analysés en utilisant cinq SSR de référence pour l´amandier: BPPCT007, CPPCT005, MA27a, MA40a y UDP96005. Les résultats ont montré la grande diversité de ce matériel génétique autochtone et l'efficacité de la SSR dans l'identification correcte, la détection de certaines synonymies et pour suggérer l'origine de certains cultivars autochtones.*

Mots-clés. *Amandier –Germoplasme – Caractérisation – Simple sequence repeat – SSR.*

I – Introduction

Simple sequence repeats (SSR markers or microsatellites) characterized by their high polymorphism, abundance, and codominant inheritance, are becoming the markers of choice for fingerprinting studies for a wide range of plants including almond (Martínez-Gómez *et al*., 2003; Fernández i Martí *et al*., 2009). These markers are very useful in the development of DNA fingerprinting used in the identification of cultivars (Sánchez-Pérez *et al*., 2005). In this context the right characterization of autochthonous cultivars used in breeding programs is of especial interest. Some of these cultivars are difficult to identify and frequently the same name is given to different cultivars or a genotype is known with different names. These varieties were selected over the years for its adaptation to warm Mediterranean climate and they are a valuable plant genetic resource that must be protected.

The objective of this work was the development of DNA fingerprinting using SSR markers for the molecular characterization of Spanish autochthonous almond cultivars from collections of CEBAS-CSIC of Murcia and IRTA of Constantí.

II – Materials and methods

Almond genotypes assayed included the 42 early flowering and self-incompatible local cultivars from the CEBAS-CSIC of Murcia and the IRTA of Constantí breeding programs (Table 1).

Total DNA was isolated using the procedure described by Doyle and Doyle (1987) and PCR-amplified using five pair of primers (BPPCT007, CPPCT005, MA27a, MA40a and UDP96005) flanking nuclear SSR sequences cloned in peach (Table 1). PCR reactions were performed according to the protocol optimized by Sánchez-Pérez *et al*., (2006). Amplified PCR products were analyzed by Automated Sequencer Capillary Electrophoresis using ABI PRISM® Genetic Analyzer (Applied Biosystems, Foster City, California, USA). The size standard used in the sequencer was Gene ScanTM 500 RoxTM using in the analysis the software GeneScan 3.7 (Applied Biosystems).

III – Results and discussion

Amplification of SSR loci was obtained for the five primer pairs developed in peach (BPPCT007, CPPCT005, MA27a, MA40a and UDP96005), all producing polymorphic amplification (Table 1). The number of alleles revealed by the SSR analysis ranged from 12 to 16 with a total number of polymorphic bands of 72. The mean number of alleles per locus was of 14.4 with heterozygosity between 0.80 and 0.89, and a discrimination power between 0.80 and 0.87.

The results leave no doubt that SSR markers are very suitable for identification of almond releases as indicated by different authors (Martínez-Gómez *et al*., 2003; Sánchez-Pérez *et al*., 2006; Fernández i Martí *et al*., 2009).

Results have showed the great diversity of this autochthonous germplasm and the effectiveness of SSRs by the correct identification, detecting some synonymies and suggesting the origin of some autochthonous cultivars. We should highlight the same fingerprinting found between ‘Desmayo AD’ and ‘Desmayo Lorca’, ‘Marcona AD’ and ‘Marcona’, ‘Planeta Fina’ and ‘Planeta Roja’, and between ‘Carreró’ and ‘Verd’, so they may be the same cultivars. Because of these markers present a great polymorphic nature, the fact of presenting the same profile is important evidence on these synonyms (Table 1).

On the other hand, these results showed the origin of the selection CEBAS-1 as a descendant of Garrigues and Carretas (Table 1).

Table 1. Spanish autochthonous almond cultivars assayed from the CEBAS-CSIC of Murcia and the IRTA of Constantí breeding programs and the SSR fingerprinting obtained with the analysis of five SSR markers

		SSR marker				
Cultivar	Centre	BPPCT7	CPPCT5	MA27	MA40	UDP96-5
Atascada	CEBAS	134	125/131	118/126	243/253	131/161
Atocha	CEBAS	134/160	125/151	128	195/253	156
Avellanera	CEBAS	134/150	131/133	126/128	219/253	161
Bonita	CEBAS	134/146	125	118/126	229/281	126/142
Carretas	CEBAS	134/142	125/142	107/153	248/277	156
CEBAS-1	CEBAS	142/146	125/146	126/153	221/277	126/156
Colorada	CEBAS	126/134	142/166	114/126	229/261	140/142
Del Cid	CEBAS	134/142	133/146	126/140	245/261	140/145
Desmayo AD	CEBAS	146/155	144/146	138/147	227/229	126/142
Desmayo Lorca	CEBAS	146/155	144/146	138/147	227/229	126/142
Fina del Alto	CEBAS	134/166	125/144	107/114	225/229	142/156
Fournat B.	CEBAS	160	133/159	138	221	126/156
Garrigues	CEBAS	134/146	144/146	126	221/255	126
J. Salazar	CEBAS	142/160	133	120/140	227/235	135/142
Jordi	CEBAS	142/166	144/157	130/138	221/225	140/156
La Mona	CEBAS	134	146/149	122/126	221/253	142/156
Malagueña	CEBAS	142/160	133/142	118/120	225/235	156/165
Marcona	CEBAS	134/162	133/149	120/126	263	142
Marcona AD	CEBAS	134/162	133/149	120/126	263	142
Marcona San Joy	CEBAS	134/153	149/151	116/140	221/253	175
Marcona Flota	CEBAS	134	146/149	120/126	227/261	142
Pajarera	CEBAS	144/162	125/151	118/126	225/261	142/175
Peraleja	CEBAS	142/150	133/144	112/118	221/225	140/159
Planeta Fina	CEBAS	134	146/162	118/126	225/253	126/142
Planeta Roja	CEBAS	134	146/162	118/126	225/253	126/142
Ramillete	CEBAS	134	146/162	118/140	213/225	126/142
Rumbeta	CEBAS	134	149/162	118/120	253/263	142/175
Tío Martín	CEBAS	158/172	133/151	118/147	221/225	126/156
Verruga	CEBAS	158	144/151	140/149	197/229	142/165
Angones	IRTA	142/146	131/144	114/138	221/227	126/142
Aspirilla	IRTA	146/153	144/146	120/138	227	129/142
Belardino	IRTA	134/155	133/142	126/138	225/263	142/156
Caima	IRTA	153/160	146	130	211	137/161
Carrero	IRTA	162/168	149/157	120/126	225/261	142/161
Esperanza Forta	IRTA	144	133/159	116/138	211/251	123/161
Gabaix	IRTA	153/158	146	134/138	207/223	137/161
Mollar de la Princesa	IRTA	160	133/159	138/140	221/225	126/161
Mollar de Tarragona	IRTA	155/160	131/144	116/138	211/245	156/161
Nano	IRTA	142/162	133/144	120/126	225/261	142
Parque Samá	IRTA	146/158	144/153	126/147	229	123/142
Pauet	IRTA	146/160	144	120/138	227	123/142
Pep de Juneda	IRTA	146/155	144/146	138/147	227/231	126/142
Rof	IRTA	153/162	144/146	130	211/213	156/161
Tardaneta	IRTA	153/160	144/146	116/130	211	137/161
Verd	IRTA	162/168	149/157	120/126	225/261	142/161

IV – Conclusions

Results showed the great diversity of this autochthonous germplasm and the effectiveness of SSRs by the correct identification, detecting some synonymies and suggesting the origin of some autochthonous cultivars.

Acknowledgements

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Self-compatibility and floral morphology of almond cultivars

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Abstract. Self-compatibility is an important almond trait aimed in new cultivars of most breeding programs. Nevertheless, different levels of self-fertility occur in almond self-compatible cultivars. Some hypotheses have been postulated about this phenomenon, and it seems that different aspects could be involved. One of them is the position of the stigmata in relation to the anthers in the flowers. In this work the morphology of almond flowers has been studied in 27 almond self-compatible genotypes (12 cultivars and 15 advanced selections from IRTA's almond breeding program). Data have been collected in 100 flowers/genotypes measuring the pistil and higher stamen length. According to these results, flowers were classified as "hypostigmate" (stigmata below the anthers) or "epistigmate" (stigma above the anthers). On the other hand, self-fertility percentages were studied in these self-compatible genotypes for several years, by counting the number of flowers present in bagged branches and the final fruit set obtained. No correlation was found between floral morphology and levels of self-fertility. Other aspects involved in the levels of almond self-fertility, apart from the morphology of the flower, are yet to be evaluated.

Keywords. Almond – Self-fertility – Epistigmatic flowers – Hypostigmatic flowers.

Auto-compatibilité et morphologie florale des cultivars d'amandier

Résumé. *L'auto-compatibilité est un caractère important chez l'amandier, qui est ciblé chez les nouveaux cultivars pour la plupart des programmes d'amélioration. Cependant, différents niveaux d'auto-fertilité existent chez les cultivars auto-compatibles. Certaines hypothèses ont été émises concernant ce phénomène, et il semble que différents aspects puissent être impliqués. L'un d'eux est la position du stigmate par rapport aux anthères chez les fleurs. Dans ce travail, la morphologie des fleurs d'amandier a été étudiée chez 27 génotypes auto-compatibles (12 cultivars et 15 sélections avancées du programme d'amélioration de l'amandier de l'IRTA). Les données ont été collectées sur 100 fleurs/génotypes en mesurant la longueur du pistil et de la partie supérieure de l'étamine. Selon les résultats, les fleurs ont été classées comme "hypostigmatique" (stigmate en dessous des anthères) ou "épistigmatique" (stigmate au-dessus des anthères). Par ailleurs, les pourcentages d'auto-fertilité ont été étudiés chez ces génotypes auto-compatibles sur plusieurs années, en comptant le nombre de fleurs présentes dans les branches ensachées et la mise à fruit finale obtenue. Aucune corrélation n'a été trouvée entre la morphologie florale et les niveaux d'auto-fertilité. D'autres aspects intervenant sur les niveaux d'auto-fertilité de l'amandier, outre la morphologie de la fleur, restent encore à évaluer.*

Mots-clés. *Amandier – Auto-fertilité – Fleurs épistigmatiques – Fleurs hypostigmatiques.*

I – Introduction

Self-compatibility is defined as the capacity of the pollen to set the female flower of the same cultivar. In almond, several works showed that floral architecture could play an important role in the different levels of self-fertility expressed by different cultivars. According to Vasilakakis and Porlingis (1984), Socias i Company and Felipe (1992 and 1993) and Bernard and Socias i Company (1994 and 1995), flowers with stigma length at the same level or below the anthers (peristigmatic or hypostigmatic flowers), should be considered as a feature easing self-pollination in almond self-fertile cultivars. However, studies carried out with Apulian self-compatible almond cultivars showed that the position stigma/anthers has not any influence in almond fruit-set (Godini *et al*., 1992; De Palma and Godini, 1994; De Palma, 1996).

Given the difference results of the cited studies, further work involving a wider range of self-compatible almond cultivars is necessary. The aim of this work was to study the possible correlation between flower morphology and self-fertility in some self-compatible genotypes.

II – Materials and methods

Twenty-seven self-compatible almond genotypes (12 cultivars and 15 advanced selections) were used in this work. Cultivars used are from different Spanish almond breeding programs: IRTA, Catalonia (‘Constantí’, ‘Francolí’, ‘Marinada’ and ‘Vairo’); CITA, Aragón (‘Belona’, ‘Guara’, ‘Mardía’ and ‘Soleta’); CEBAS-CSIC, Murcia (‘Antoñeta’ and ‘Penta’), and other cultivars like ‘Lauranne’ (France) and ‘Tuono’ (Italy). The 15 advanced selections are from the IRTA’s breeding program. Material is growing at IRTA’s almond plots, in Mas de Bover (Tarragona) and Les Borges Blanques (Lleida).

One hundred flowers per almond genotype were randomly collected around the tree in the field in “F” state (Felipe, 1977) and they were taken to the laboratory. Pistil and longer stamen length, from pistil base, were measured. Distances between both flower parts were calculated for each genotype. Flowers presenting positive values were classified as epistigmatic flowers (pistil above anthers); flowers with negative values were classified as hypostigmatic flowers (pistil below anthers) and flowers with values around (± 0,5 mm) were classified as peristigmatic (pistil and anthers at the same level). Data were recorded for two years (2011 and 2012). Other aspects of floral morphology as pistil shape (straight or curved in the upper part) were also recorded.

Records of self-fertility levels of the same almond genotypes, in almond IRTA’s collection, were collected from different years. Around 100 flowers from 2-5 branches were counted in each tree. Before anthesis, these branches were bagged to prevent cross pollination. Fruit set in each branch was scored two months later (Vargas *et al*., 1997).

Correlation between floral morphology and self-fertility levels was studied (Pearson’s correlation coefficient).

III – Results and discussion

Data of flower morphology (distance between pistil and the largest stamen) of the 27 almond genotypes studied are shown in Table 1. All types of floral morphology have been found in these self-compatible almonds, with predominance of epistigmatic and peri- epistigmatic flowers (stigma above or at the same level of the anthers) (63% of material studied), a floral architecture not favourable for autogamy according to Socias i Company (1995). De Palma and Godini (1994) presented similar results in the study of 15 Apulian self-compatible almond cultivars, suggesting that the reciprocal stigma/anthers position within the same flower should be considered as a morphological trait of almond cultivars, susceptible to variations, not necessary related to biological behaviour. Socias *et al*. (2004) found in some self-compatible almond selections that the stigma was above the level of the upper anthers, but the upper position of the pistil was coiled and may allow stigma-anther contact.

Referring to specific cultivars studied, the stigma/anthers reciprocal position of our data (Table 1) agree with those defined by ‘Guara’ (Felipe and Socias i Company, 1987) and with ‘Tuono’ (synonym of ‘Truoito’) (Godini *et al*., 1992), and disagree with Vasilakis and Porlingis (1984) for this last cultivar. These authors presented data of stigma/anthers position of -2,3 mm (hypostigmatic flower) for ‘Truoito’ cv.

Table 1. Distance between pistil and longer stamen length (mm) (2011 and 2012), and levels of self-fertility observed in the field (different years)

Almond genotypes studied	Distance pistil-longer stamen length (mm) (standard deviation)	Flower classification ♠	Self-fertility (%) (years of study) ♦ (cvs. in Les Borges Blanques) • (cvs. in Mas de Bover)
Cultivars			
'Antoñeta'	0.84 (±0.14)	epistigmatic	♦ 15.65 (2011-2012-2014)
'Belona'	2.10* (±0.1)	epistigmatic	♦ 25.47(2011-2012-2014)
'Constantí'	0.45* (±0.08)	peri-epistigmatic	• 26.37*
'Francolí'	0.96* (±0.13)	epistigmatic	• 28.89*
'Guara'	-0.01* (±0.11)	peri-hypostigmatic	♦ 33.6 (2011-2012-2014)
'Lauranne'	2.15 (±0.09)	epistigmatic	♦ 36.87 (2011-2012-2014)
'Mardía'	2.22 (±0.15)	epistigmatic	♦ 5.4 (2012-2014)
'Marinada'	-1.09* (±0.18)	hypostigmatic	• 25.30*
'Penta'	1.44 (±0.23)	epistigmatic	♦ 17.95 (2012-2014)
'Soleta'	-0.28* (±0.08)	peri-hypostigmatic	♦ 19.03 (2012-2014)
'Tuono'	0.18 (±0.12)	peri-epistigmatic	♦ 8.7 (2011-2012-2014)
'Vairo'	-0.21* (±0.12)	peri-hypostigmatic	• 19.98*
Advanced Selections			
12-350	-1.55 (±0.1)	hypostigmatic	• 7.2 (2008-2013)
13-531	2.13 (±0.16)	epistigmatic	• 19.1 (2008-2013)
23-160	1.75 (±0.16)	epistigmatic	• 17.7 (2008-2013)
23-173	0.59 (±0.12)	epistigmatic	• 29.52*
24-53	-0.44 (±0.15)	peri-hypostigmatic	• 11.4 (2009-2013)
26-258	0.04 (±0.12)	peri-epistigmatic	• 2.8 (2009-2013)
26-408	0.79 (±0.13)	epistigmatic	• 7.9 (2008-2013)
27-103	1.29 (±0.16)	epistigmatic	• 34.1 (2009-2013)
28-105	-0.90 (±0.10)	hypostigmatic	• 31 (2009-2013)
28-117	1.63 (±0.16)	epistigmatic	• 40.3 (2008-2013)
29-59	0.60 (±0.12)	epistigmatic	• 9.3 (2009-2013)
29-143	-1.07 (±0.08)	hypostigmatic	• 13.5 (2010-2013)
29-147	-1.39 (±0.08)	hypostigmatic	• 5.9 (2010-2013)
29-148	-1.50 (±0.11)	hypostigmatic	• 22.06*
30-297	1.09 (±0.12)	epistigmatic	• 25.33*

*Two years data (2011-2012), ♠ epistigmatic: flowers with a positive distance pistil-anthers, hypostigmatic: flowers with a negative (-) distance pistil-anthers, peristigmatic: flowers with pistil-anthers at the same level (± 0,5 mm); *more than 8 years of observation.

In our work no relationship was found between the position of stigma in relation to the anthers and fruit set data (r = 0,27) for the 27 autocompatible genotypes studied (Fig. 1). Our results agree with those reported by Godini *et al*. (1992) and De Palma (1996), and disagree with those observed by Vasilakakis and Porlingis (1984) and Socias i Company and Felipe (1992 and 1993).

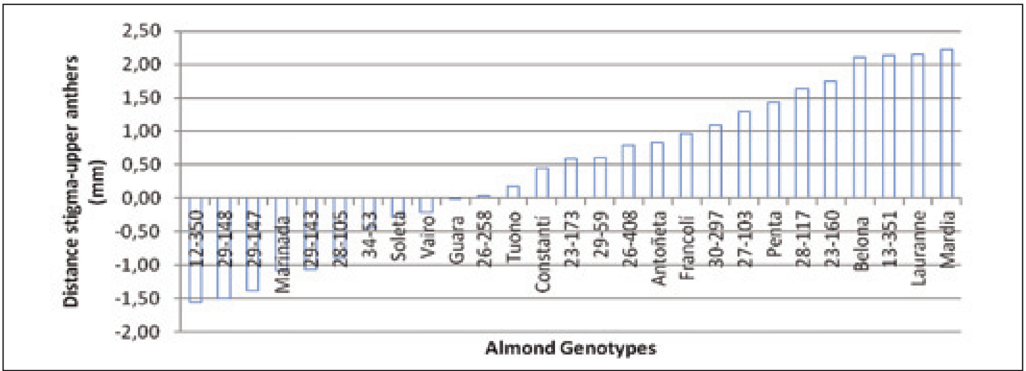

Fig. 1. Distance between stigma and the upper anthers (mm) of different almond genotypes.

IV – Conclusions

No relationship has been found between floral morphology and fruit set in twenty-seven self-compatible almond genotypes. So, the capability of these cultivars to set fruits after flower bagging was independent of the relative position between the stigma and the anthers within the same flower.

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Effect of different concentrations of $ZnSO_4$ on *Pistacia atlantica* plantlets growth under semi-controlled conditions

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Abstract. The effect of $ZnSO_4$ stress on some morphological, physiological and biochemical parameters of *Pistacia atlantica* plantlets was studied under semi-controlled conditions. The plants were grown in pots and irrigated with distilled water for 30 days. Then, the plantlets were treated with 0, 0.01, 0.1 and 1 mmol of $ZnSO_4$ for 18 days. The application of these concentrations caused stress on the young *Pistacia atlantica* plantlets by reducing the growth of morphological parameters. The amount of free proline in shoots and roots grows significantly with the concentration in the medium of $ZnSO_4$ for all the treatments. On the other hand, no significant difference in relative water content was noted. These results are indicating that *P. atlantica* can be used in phytoremediation because of its rusticity and resistance to stress caused by Zinc.

Keywords. Water – Soil – Heavy metals – Phytoremediation – *Pistacia atlantica*.

Effet des différentes concentrations de $ZnSO_4$ sur la croissance de semis de **Pistacia atlantica** ***Desf.), cultivés en conditions semi-contrôlées***

Résumé. *L'effet du stress causé par $ZnSO_4$ sur quelques paramètres morphologiques, physiologiques et biochimiques des plantules de Pistacia atlantica a été étudié sous conditions semi contrôlées. Les plantules ont été cultivées dans des pots et irriguées avec de l'eau distillée durant 30 jours. Ensuite, les plantules ont été traitées à 0- 0,01- 0,1 et 1 mmol de $ZnSO_4$ pendant 18 jours. Il a été établi que l'application de ces concentrations de $ZnSO_4$ cause un stress non significatif aux plantules de pistachier qui s'exprime par une faible réduction de la croissance au niveau des caractères morphologiques. Après 18 jours de stress, le taux de proline chez* P. atlantica *est très élevé, dans les parties aérienne et racinaire et croît significativement en fonction de la concentration du milieu en $ZnSO_4$ pour tous les traitements. Par ailleurs, une différence non significative pour la teneur en eau a été enregistrée. Les résultats obtenus au terme de cette étude suggèrent que* P. atlantica *pourrait être utilisé en phytoremédiation, du fait de sa rusticité et de sa résistance au stress causé par le Zinc.*

Mots-clés. *Eau – Sol – Métaux lourds – Phytoremédiation –* Pistacia atlantica.

I – Introduction

Phytoextraction is related to plants that are able of absorbing heavy metals through their rooting system to the aerial parts (Zheng *et al.,* 2011). *Pistacia atlantica*, which has a huge rooting system and is adapted to degraded environments with various climates. The tree forms mycorhizal associations with many species of fungi which play a role in the restoration of polluted soils by heavy metals, by reducing their amounts (Fortas and Dib, 2003). This work aims to study the mechanism of tolerance of *Pistacia atlantica*, under metal stress, in order to use this tree in phytoremediation.

II – Material and methods

The seeds are collected from fruits of *P. atlantica*, at the maturation stage in Messaad region. They were stored in the refrigerator until their use. The seeds of *P. atlantica* may need stratification for better and uniform germination (Belhadj, 2007). After stratification, sprouted seeds are sown directly in a peat-sand sterilized mixture. Growth assay is performed under semi-controlled conditions. The seedlings are irrigated daily, once a day with 20 ml (per pot), firstly with distilled water for 30 days and then with $ZnSO_4$ solutions at different concentrations (0.01, 0.1, 1 mmol and control). Forty pots were used for each test.

Morphological (stem height (cm), number of nodes, total number of leaves, number of compound leaves, root length (cm), number of secondary roots, shoot and root biomass ratio of the aerial/root part), physiological [water content, calculated by the formula of Heller (1998)] and biochemical parameters [proline contents in the aerial and the root parts of the seedlings, as determined by the method described by Troll and Lindsley (1955)] were measured. All data were subjected to statistical analysis using Excel software. The analysis of variance (ANOVA) and Newman-Keuls test were performed.

III – Results and discussion

The effect of $ZnSO_4$ was not significant on the growth of the seedlings, for the duration of our test, despite a clear reduction in stem height, number of nodes, number of leaves, root length and number of secondary roots, as well as biomass of both aerial and root parts (Fig. 1). Similar results were obtained by Benhassaini *et al.* (2011) for the length of the stems and the roots, on of *P.atlantica* salt stressed seedlings.

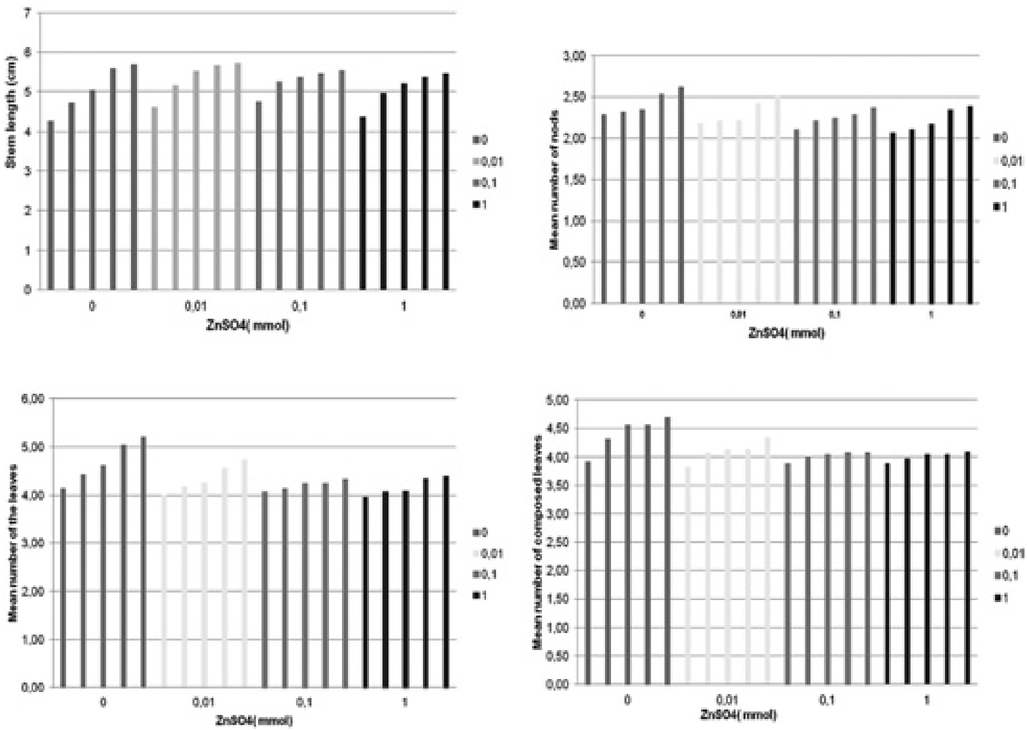

Fig. 1. Evolution of morphological characters of *P.atlantica* plantlets cultivated in different concentrations of $ZnSO_4$.

Plants exposed to $ZnSO_4$ shows a insignificant variation in water content (Fig. 2 and Table 1) which means a good tissue hydration despite the stresses status. According to Brunet *et al.* (2008), low variations in the water content for *Lathyrus sativus* plants treated with lead were observed. However, the applied stress levels induced a very highly significant increase (P <0.001) in proline contents for the shoot and root portions of the seedlings (Fig. 3 and Table 2). The more the concentration of $ZnSO_4$ increases, the more the proline contents become marked. This result is in line with those of Monneveux and Nemmar (1986). The highest levels of proline are recorded in the aerial parts. This is consistent with the work of Benhassaini *et al.* (2011).

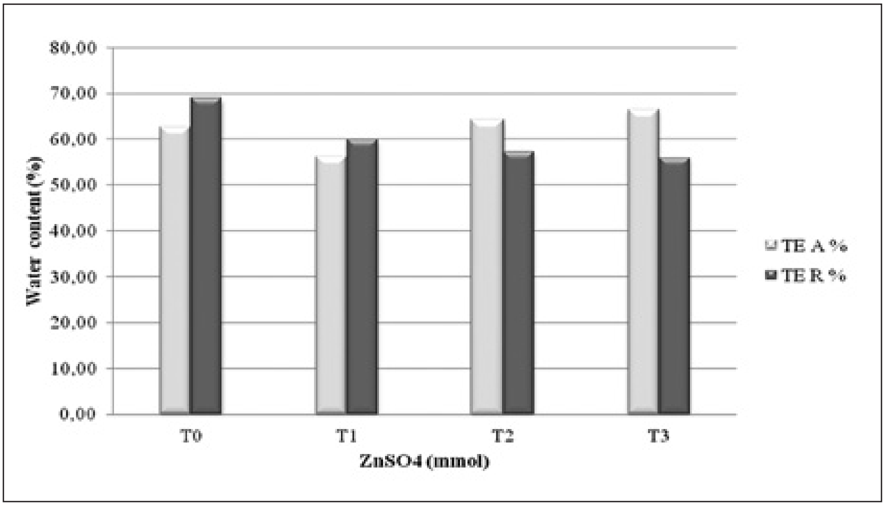

Fig. 2. Water content (TE) variation of the aerial parts (A) and root parts (R) of *P. atlantica* plantlets cultivated in different concentrations of $ZnSO_4$ (T0,T1,T2,T3).

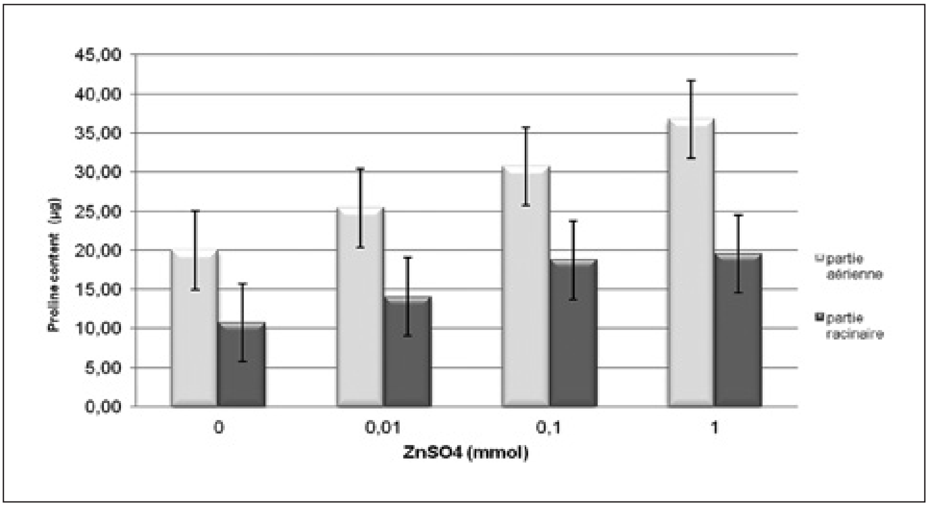

Fig. 3. Effect of $ZnSO_4$ concentrations on the proline contents of *P. atlantica* plantlets.

Table 1. Newman-Keuls Test for the water content (α < 5%)

	$ZnSO_4$ (mmol)	0	0.01	0.1	1
Aerial part	Water content ± S.D.	62.75 ± 16.40	56.26 ± 10.39	63.95 ± 16.75	66.56 ± 18.55
	Homogenous groups	A	A	A	A
Root part	Proline content ± S.D.	65.88 ± 15.40	59.91 ± 11.49	57.23 ± 18.75	
	Homogenous groups	A	A	A	A

Table 2. Newman-Keuls test for the proline contents (µg) in the aerial and the root parts of *P. atlantica* plantlets cultivated in different concentrations of $ZnSO_4$

$ZnSO_4$ (mmol)	Aerial part Mean (µg) ± S.D.	Root part Mean (µg) ± S.D.	Mean (µg) ± S.D.
0	20.000 ± 1.039 **d**	10.733 ± 0.306 **c**	15.367 ± 0.672
0.01	25.400 ± 4.000 **c**	14.067 ± 1.155 **b**	19.733 ± 2.577
0.1	30.733 ± 2.309 **b**	18.733 ± 0.306 **a**	24.733 ± 1.307
1	36.733 ± 3.055 **a**	19.533 ± 0.808 **a**	28.133 ± 1.932

IV – Conclusions

Our results showed that *Pistacia atlantica* can withstand stressing status caused by zinc in soil solution. Thus this tree can be used in soil and water remediation in contemned areas by this metal.

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‘Vialfas’, a new late blooming almond cultivar

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Abstract. The almond breeding programme of the CITA of Aragón aims at late-blooming cultivars with good horticultural behaviour, self-compatible, and with good kernel quality. After previous releases, ‘Vialfas’ responds to most of these objectives. This new release (selection I-3-27, clone 546) comes from the cross ‘Felisia’ × ‘Bertina’. Its blooming time is very late, three days before ‘Mardía’ on the average, with similar chilling requirements than ‘Mardía’ but with slightly lower heat requirements which could explain the difference in blooming time. Flowers are of mean size, white, with peristigmatic style. Bloom density is high and consistent. It is self-compatible (genotype $S_{fi}S_{11}$), of high fruit set and resistance to frosts. Nuts are of mean size (4.7 g), as well as kernels (1.2 g), with a low shelling percentage (25%) and hard shell, and early ripening. Kernel composition is average for protein (18.84% of dry matter), oil (56.72%), and tocopherols (251.8 g/kg oil), and phytosterols (2589 mg/kg oil), but very high for oleic acid (78% of total oil). The tree is slightly upright, with relative tolerance to disease and highly productive.

Keywords. Almond – Cultivar – Breeding – Late blooming – Self-compatibility.

Vialfas’, une nouvelle cultivar d’amandier à floraison tardive

Résumé. *Le programme d’amélioration génétique de l’amandier du CITA d’Aragón a comme objective l’obtention de cultivars de floraison tardive, bon comportement agronomique, auto-compatibles, et avec amandons de bonne qualité. Après les obtentions antérieures, ‘Vialfas’ répond à la plus part de ces objectives. Cette nouvelle obtention (sélection I-3-27, clone 546) vient du croisement ‘Felisia’ × ‘Bertina’. L’époque de floraison est très tardive, trois jours avant ‘Mardía’ comme moyenne, avec similaires besoins en froid que ‘Mardía’, mais avec besoins en chaleur un peu moindres, ce que pourrait expliquer la différence en date de floraison. Les fleurs son de largeur moyenne, blanches, avec un style peristigmatique. La densité florale est élevée et consistent. Le cultivar est auto-compatible (genotype* $S_{fi}S_{11}$*), avec une nouaison élevée et résistance aux gelées. Les fruits sont de largeur moyenne (4,7 g), ainsi que l’amandon (1,2 g), avec un bas rendement au cassage (25%), coque dure et maturation précoce. La composition de l’amandon est moyenne pour la protéine (18,84% de matière sèche), huile (56,72%), et tocophéroles (251,8 g/kg huile), et phytostéroles (2589 mg/kg huile), mais très haute pour l’acide oléïque (78% de l’huile total). L’arbre est légèrement érigé, avec tolérance relative aux maladies et très productive.*

Mots-clés. *Amandier – Cultivar – Amélioration – Floraison tardive – Auto-compatibilité.*

I – Origin

The almond breeding programme of the CITA of Aragón aims at late-blooming cultivars with good horticultural behaviour, self-compatible, and with good kernel quality. After previous releases, ‘Vialfas’ responds to most of these objectives. This new release (selection I-3-27, clone 546) comes from the same cross than ‘Mardía’ (Socias i Company *et al*., 2008): ‘Felisia’, a self-compatible and late-blooming cultivar from the same breeding programme, but with a small-sized kernel, by ‘Bertina’, a local self-incompatible and late-blooming selection, with a large-sized kernel.

II – Description

Blooming time is very late, three days before 'Mardía' on the average (Fig. 1), with similar chilling requirements than 'Mardía' (354 CU), but with slightly lower heat requirements (10,066 *vs* 10,663 GDH), which could explain the difference in blooming time. Flowers are of mean size, white, with peristigmatic style. Bloom density is high and consistent. It is self-compatible (genotype $S_{fi}S_{11}$), of high fruit set and resistance to frosts.

Fruit are of mean size (4.7 g), as well as kernel (1.2 g), with a low shelling percentage (25%) and hard shell (Fig. 2). However, the kernel percentage over the total fruit DW is 22.1%, quite high when compared with other cultivars: 23.1% for 'Guara', with the highest kernel percentage, whereas for 'Marcona', 'Desmayo Largueta' and 'Nonpareil' it is slightly lower than 10% (Alonso *et al*., 2012). Ripening is early, slightly later than 'Guara'.

Kernel composition is average for protein (18.84% of DM), oil (56.72% of DM), tocopherols (251.8 g/kg oil), and phytosterols (2589 mg/kg oil), but very high for oleic acid (78% of total oil).

The tree is slightly upright (Fig. 3), with relative tolerance to diseases and highly productive (Alonso *et al*., 2015).

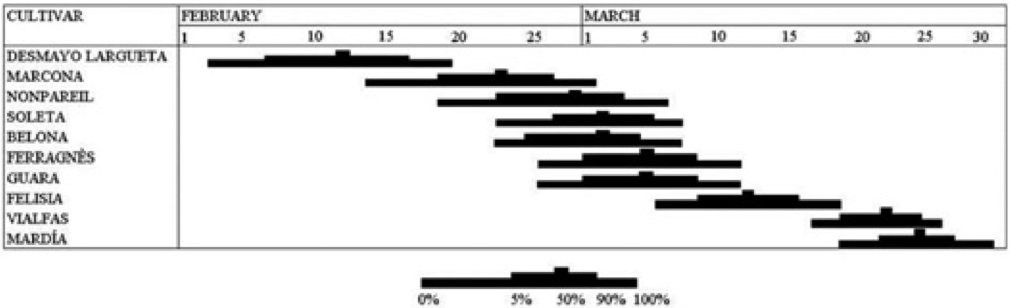

Fig. 1. Blooming time of 'Vialfas' as compared to other cultivars (average of 7 years). Percentages refer to the amount of open flowers).

Fig. 2. Nuts and kernels of 'Vialfas'.

Fig. 3. 'Vialfas' tree in full production.

Acknowledgments

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Methylation of the S_f locus in almond is associated with *S*-RNase loss of function

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Abstract. Self-compatibility in almond (*Prunus amygdalus* Batsch) is attributed to the presence of the S_f haplotype, allelic to and dominant over the series of *S*-alleles controlling self-incompatibility. Some forms of the S_f haplotype, however, are phenotypically self-incompatible even though their nucleotide sequences are identical. DNA from leaves and styles from genetically diverse almond samples was cloned and sequenced and then analyzed for changes affecting S_f variants. Epigenetic changes in several cytosine residues were detected in a fragment of 4700 bp of the *5'* upstream region of all self-compatible samples of the S_f allele, differentiating them from all self-incompatible samples of S_f analyzed. This is the first report of DNA methylation in a Rosaceae species and appears to be strongly associated with inactivation of the S_f allele.

Keywords. Almond – Self-compatibility – DNA methylation – Epigenetics – *S*-RNase upstream region.

La méthylation du locus* S$_f$ *chez l'amandier est associée avec la perte de fonction de la* S-*RNase

Résumé. *L'auto-compatibilité chez l'amandier (*Prunus amygdalus *Batsch) est attribuée à la présence du haplotype S_f, allélique avec et dominant sur la série d'allèles S qui contrôlent l'auto-incompatibilité. Néanmoins, quelques formes du haplotype S_f sont phénotypiquement auto-incompatibles malgré l'identité des séquences de nucléotides. Le DNA de feuilles et de styles de différents génotypes d'amandier a été cloné, séquencé et après analysé pour changes pouvant affecter les variantes de l'allèle S_f. Changes epigénétiques pour quelques résidus de cytosine ont été détectés dans un fragment de 4700 bp de la région 5' upstream de tous les génotypes auto-compatibles de l'allèle S_f, ce qui les différencie de tous les génotypes auto-incompatibles de l'allèle S_f analysés. Ce résultat est le premier rapport sur la méthylation du DNA dans une espèce des Rosacées et semble être fermement associé avec l'inactivation de l'allèle S_f.*

Mots-clés. *Amandier – Auto-compatibilité– Métilation du DNA – Épigénétique – Région upstream de la* S-*RNase.*

I – Introduction

Almond (*Prunus amygdalus* Batsch) shows gametophytic self-incompatibility, controlled by a single polymorphic locus. Most almond cultivars are self-incompatible and the few exceptions that have been identified as self-compatible have been shown to carry a mutation at the *S*-locus referred to as S_f (Socias i Company, 1990). Bošković *et al*. (1997) showed that in almond the *S* alleles code for stylar proteins with RNase activity whereas S_f does not. Quantitative real-time PCR analysis has revealed transcripts of S_f-RNase in the style tissue of a SI cultivar ('Vivot', $S_{23}S_f$) as well as the absence of the S_f-RNase transcripts in a SC cultivar ('Blanquerna', S_8S_f). Because of this differential transcription of the S_f-RNase, the S_f allele conferring SI has been designated S_{fa} (active), whereas the same S_f allele conferring SC has been designated S_{fi} (inactive) (Kodad *et al.,* 2009; Fernández i Martí *et al*., 2010). Sequencing confirmed that S_{fa} and S_{fi} were identical in the coding region and in their 5'-flanking regions (Fernández i Martí *et al*., 2010). Similarly, the TATA-Box and IB-like motifs did not reveal any difference in the *cis*-element in the active relative to inactive S_f types (Fernández i Martí *et al*., 2010).

The absence of S_f nucleotide sequence differences in SC and SI phenotypes, suggests that either modifier genes can strongly affect the expression or that epigenetic changes are involved. Consequently, our objective was to determine if DNA methylation of the S_f allele was associated with its differential phenotypic expression in almond.

II – Material and methods

1. Plant material

Six almond genotypes, containing both homozygotes and heterozygotes for the S_f haplotype, were included for analysis (Table 1). Genotypes were selected based on previous genetic and phenotypic characterization of the S_f allele established by pollen tube growth and field pollination studies. The S_f alleles analyzed could be traced back to two different origins: the Italian region of Puglia for the standard self-compatible form exemplified by the heirloom cultivar 'Tuono', and the island of Majorca where S_f types showing self-incompatible phenotypes have been identified (Fernández i Martí *et al*., 2010).

Table 1. Almond genotypes included in the methylation analysis

Genotype	S-genotype	Phenotype	Origin
'Blanquerna'	$S_8 S_f$	SC	Genco × AS-1
'Vivot'	$S_{23} S_f$	SI	Local of Majorca, Spain
'Soleta'	$S_{23} S_f$	SC	'Blanquerna' × 'Belle d'Aurons'
'Ponç'	$S_{23} S_f$	SI	Local of Majorca, Spain
A2-199	$S_f S_f$	SC	C-1322 ['Tuono' × 'Genco'] ⊗
M-2-16	$S_f S_f$	SC	M-2-2 ['Tuono' × 'Ferragnès'] ⊗

SC: self-compatible; SI: self-incompatible.

2. DNA methylation

Total DNA was extracted from leaves and styles at stage D (Felipe, 1977) using the procedure of Doyle and Doyle (1991). Once extracted, DNA was was submitted to the DNA bisulphite modification treatment 'MethylEasy' (Human Genetic Signatures, Brisbane, Australia). This kit has been designed to efficiently convert cytosine to uracil and to reduce DNA degradation and loss without decreasing the conversion of C to U residues. The converted DNA was then used for PCR amplification, cloning and sequencing.

For PCR amplification, specific primers were designed in this work. The thermal cycling program was as follows: an initial denaturation at 95ºC for 1 min followed by 35 cycles of 94ºC for 30 s, annealing for 30 s of 53ºC and extension at 72ºC for 1 min, ending with a 5 min extension at 72ºC. The PCR products were cloned using the pGEM-T-Easy Vector System (Promega, Madison, WI, USA). Cloning and sequencing of the whole region of 4700 bp in the 5' upstream were initially performed for 'Blanquerna' and 'Vivot' and methylation changes over the whole sequence in both cultivars were carefully analyzed. Subsequently, only those samples showing a methylation change were used for cloning and sequencing the remaining genotypes.

III – Results and discussion

Cloning and sequencing of the PCR products obtained confirmed that the bisulphite treatment worked properly in all DNA tested. In the partial sequence shown in Fig. 1, the presence of several cytosine residues can be observed in the upstream region of genomic DNA of 'Blanquerna' obtained from pistil before the bisulphite treatment. However, after the treatment, sequencing of all clones from ‘Blanquerna’ showed that almost all cytosines were converted to thymines with a single exception (large arrow).

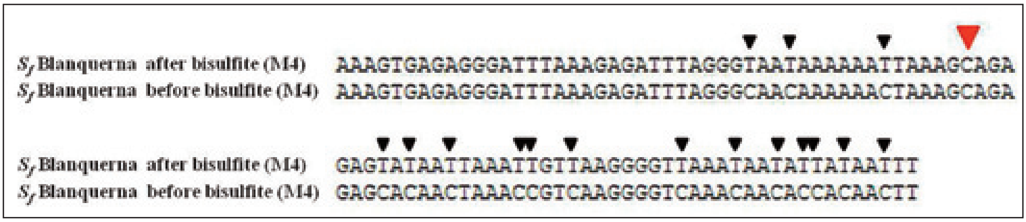

Fig. 1. Nucleotide sequence using the primer pair CIPABF4/CIPABR4 of ‘Blanquerna’ showing that the sequence from pistil DNA with bisulphite treatment is the same as that of genomic DNA from pistil without bisulphitetreatment, with the single exception of the conversion of cytosines to thymines (black arrows). The large (red) arrow shows where the methylation of the cytosine residue appeared.

After confirming the efficacy of the treatment, every nucleotide was carefully compared between the two sequences for a length of 4700 bp in the upstream region, as well as in the first and second intron of the S_f haplotype (Fig. 2). Among all sequences obtained, no changes were produced in the introns of the S_f gene; however four cytosine residues were not able to be converted to thymine in the upstream region of the SC cultivars indicating the presence of methylation. Fig. 2 shows the whole sequence of the unconverted S_f upstream region where the four methylation points have been identified, as well as the four primer pairs overlapping these methylated cytosines.

The first methylated nucleotide was located approximately 4275 bp upstream to the start codon in all samples from leaf as well as pistil tissue. This methylated region was stable in all five clones obtained from the SC cultivar ‘Blanquerna’. The other three cytosine epi-mutations were located 3200, 2400 and 650 bp upstream of the start codon. Analysis of the DNA samples of the SI cultivars ‘Vivot’ and ‘Ponç’ showed that all cytosine residues were converted to thymine in all the clones obtained.

The differential expression of the two S_f haplotypes was initially attributed to a possible mutation (Kodad *et al*., 2009) and the present results suggest that this mutation could be an epigenetic change. Thus, when the S_f-RNase sequence is methylated, its expression is inhibited resulting in an SC phenotype, as occurs in ‘Blanquerna’, ‘Soleta’ and the two homozygous SC selections. When S_f-RNase sequences do not show methylated cytosines, the RNase remains active, resulting in a SI genotype and phenotype, as in ‘Vivot’ and ‘Ponç’. The original S_f haplotype would have been in an active form, thus conferring SI, as with other *S* haplotypes. Epigenetic change through DNA methylation resulted in loss of RNase function and so a novel SC phenotype in a in an otherwise SI species such as almond.

While the S_f alleles examined were derived from two distinct Mediterranean regions, they may be identical by descent from the original pool of Mediterranean germplasm. The epigenetic change appears to have taken place in the Italian region of Puglia, where most SC cultivars have been described (Socias i Company, 1990). Additionally, all SC almond genotypes identified so far have shown an identical S_f allele (Fernández i Martí *et al*., 2010) and are of Mediterranean origin and so probably related to the Puglia almond population.

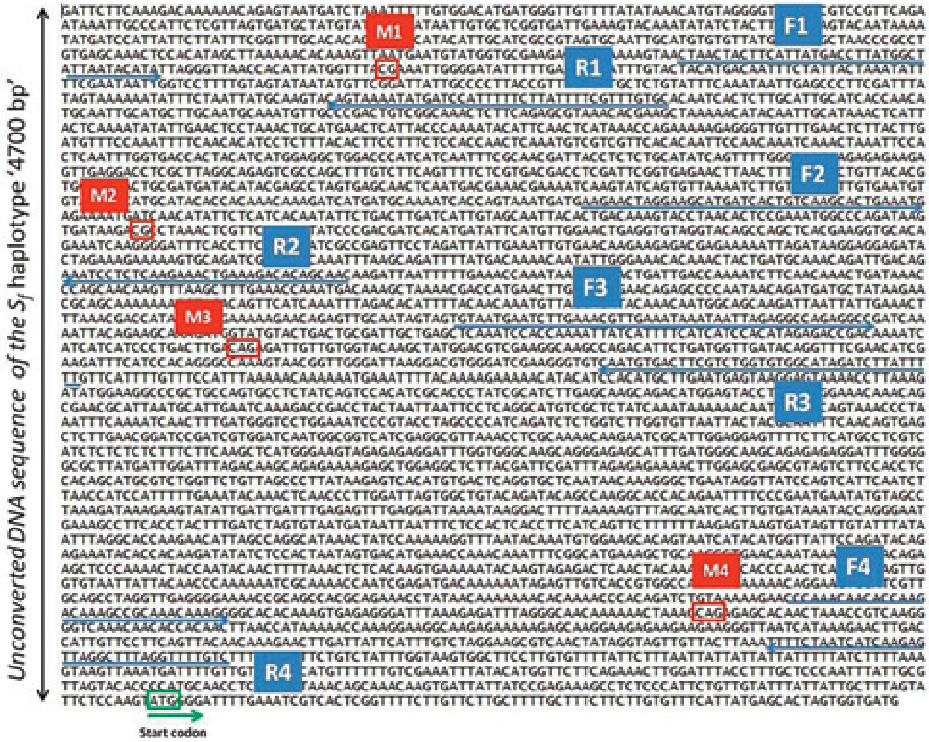

Fig. 2. Detection of methylated sites in the 5' untranslational region of S_{fi}-RNase. Primer positions overlapping the cytosine residues are indicated by blue arrows. These primers identify the best primer pair for limiting the region where the cytosine base is non-methylated.

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Session 2

Genetic Resources. Rootstocks

Almond Rootstocks: Overview

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Abstract. Almond growing has significantly changed over the last decades in the. Production has decreased in some countries such as but in others, including, an important change has taken place. The possibilities for choosing a rootstock have also significantly increased. The use of almond seedlings has always been linked to rainfed conditions and peach seedlings to irrigated conditions. The peach x almond hybrid 'GF- has been the rootstock most utilized in the past years, either for rainfed or irrigated conditions. Nowadays, however, with the high demand for these crops and the new areas planted in substitution of other fruit crops, new rootstock releases are being used more often, mainly newly-bred Spanish rootstocks. The rootstock choice must respond to better management, adaptability to different soil types, and resistance to nematodes. The studies of compatibility with plum species, especially the selection and use of some clonal almond x peach hybrid rootstocks have increased the selection possibilities considerably. In this situation, the red-leaved and root-knot nematode-resistant almond x peach hybrids (GxN) that are well adapted to Mediterranean conditions due to their 'Garfi' parentage, in both irrigated and non-irrigated conditions (mainly 'Garnem'), have become the predominant rootstocks. Particularly noteworthy among the recent releases has been the incorporation of root-knot nematode (RKN) resistance. Special emphasis has also been placed on developing dwarfing rootstocks for high-density orchard systems. New hybrid rootstocks of complex origin are now under experimentation and initial results indicate that they could improve almond production if they fulfil the requirements of modern fruit growing, as discussed in this revision.

Keywords. Nematodes – Rootstocks – Vigour control – *Prunus dulcis* – Drought – Intensive.

Les porte-greffes d'amandier

Résumé. *La culture de l'amandier a évolué au cours des dernières décennies dans le bassin méditerranéen. Bien que dans certains pays, comme en Italie, la production ait diminué, dans d'autres, comme l'Espagne, a eu lieu un changement rapide. Les possibilités de choix des porte-greffes ont également connu une avancée significative. L'utilisation de semis d'amandier a toujours été liée à des conditions non irriguées et le semis de pêchers à des conditions irriguées. Le pêcher*amandier 'GF-677' a été le porte-greffe le plus utilisé dans les dernières années, que ce soit pour les cultures irriguées ou non. Cependant de nos jours, suite à la forte demande de cette culture et au nombre de surfaces plantées en substitution d'autres cultures fruitières, il y a une utilisation croissante des nouvelles obtentions, notamment des nouveaux porte-greffes espagnols. Le choix des porte-greffes doit répondre à une bonne adaptation aux différents types de sol, au système de culture et à la résistance aux nématodes. Les études de compatibilité avec des espèces de pruniers et spécialement la sélection et l'utilisation de certains porte-greffes hybrides clonaux entre amandier x pêcher ont notamment accru les possibilités de choix du matériel végétal. Dans cette situation, les hybrides amandier x pêcher à feuilles rouges et résistants aux nématodes à galles (GxN) bien adaptés aux conditions méditerranéennes en raison de leur parent amandier 'Garfi', à la fois dans des conditions irriguées et non irriguées spécialement 'Garnem', sont devenus les porte-greffes prédominants. Particulièrement remarquable parmi les obtentions récentes a été l'incorporation de la résistance aux nématodes à galles (RKN). Uun accent particulier a été mis aussi sur le développement des porte-greffes de nanisme pour les systèmes de vergers à haute densité. De nouveaux porte-greffes hybrides complexes sont maintenant en expérimentation au champ et les premiers résultats indiquent qu'ils pourraient améliorer la production d'amandes s'ils remplissent les nouvelles exigences des cultures fruitières, comme il l'est montré dans cette révision.*

Mots-clés. *Nématodes – Porte-greffes – Contrôle de vigueur* – Prunus dulcis – *Sécheresse – Culture intensive*.

I – Introduction

Almond growing in the Mediterranean area has undergone significant changes in recent decades. Whilst in some countries production has substantially decreased, in others a renewal of the concept of almond growing is taking place. In such a situation, not only new cultivars but also new rootstocks are essential tools to achieve success. For cultivars, 'Nonpareil' has been, and continues to be, the essential cultivar in, while in the Mediterranean area the outlook has dramatically changed. In relation to rootstocks, in California the changes have also been smaller, since new releases represent only a small percentage of the new plantings (Socias i Company *et al*., 2009), and peach seedlings, mainly 'Lovell' and 'Nemaguard', remain the most utilized rootstocks, whereas in the Mediterranean area sharp changes have been taking place, with a shift from the utilization of different almond seedlings to the wide implantation of peach x almond hybrids.

Due to the non-irrigated conditions of most Mediterranean orchards, almond seedlings were the dominant rootstock for centuries, because of their deep growth and associated efficiency for mining nutrients and water. However, they are susceptible to root asphyxia and nematodes. Unselected rootstocks, often bitter almonds, were used for producing seedling rootstocks, although some efforts were subsequently directed toward some seedling lines because of their homogeneity (Felipe, 1989) or resistance to nematodes in (Kochba and Spiegel-Roy, 1976).

More recently, peach x almond hybrids are showing promising performance under non-irrigation, due in part to the loss of the deep-mining almond tap-root when transplanting (Felipe, 2000). 'GF– has been the rootstock most utilized for decades worldwide, probably being the almond x peach hybrid clonal rootstock most planted at present, both in irrigated and non-irrigated conditions. Later, several new releases have been increasingly utilized, especially the Spanish rootstocks from the CITA programme ('Garfi' x 'Nemared' series), mainly 'Garnem', rather than the Italian rootstocks from the University of Pisa, probably due to their better management, adaptability and resistance to nematodes (Socias i Company *et al*., 2009).

Other almond x peach hybrids adapted to alkaline soils have been selected in Spain from spontaneous hybrids: 'Adafuel' (Cambra, 1990) in EEAD-Zaragoza and 'Mayor' in CIDA-Murcia (Rodriguez and Carrillo, 2002). However, they suffer several shortcomings, such as difficult propagation and susceptibility to pests and diseases, mainly nematodes (Felipe, 2000). Several diploid plum clones have been selected from the local population of 'Pollizo de Murcia', with three releases: 'Adesoto (Moreno *et al*., 1995) from EEAD-Zaragoza, and 'Monpol' and 'Montizo', from CITA-Zaragoza (Felipe *et al*., 1990, 1997). The main selection objective of these rootstocks was the introduction of waterlogging tolerance and vigour reduction.

Root-knot nematode (RKN) resistance has been the main goal for *Prunus* rootstock breeding for decades and hence for almond. Partial RKN resistance was introduced from an open pollinated 'GF- resistant to *M. arenaria* and *M. incognita* (Esmenjaud *et al*., 1997). In, 'Sirio' (Loreti and Massai, 1998) from the IS series also showed good performance in calcareous soils. Progressively, more resistance was introduced with two interspecific hybrids from *P. davidiana*, resistant to the four main *Meloidogyne* species: 'Barrier' (Roselli, 1998) from, and 'Cadaman' (Edin and Garcin, 1996) from. Partial resistance not only to *Meloydogyne* spp., but also to the ring nematodes (*Mesocrinema* spp.) was introduced through the peach seedling 'Guardian', released as tolerant to Peach Tree Short Life, an important syndrome in the southern United States (Reighard and Loreti, 2008). Several interspecific hybrids were introduced from (Eremin and Eremin, 2002), showing cold hardiness and dwarfing as their main characteristics. In the last 20 years, the interspecific almond x peach hybrids 'Garfi' x 'Nemared' have become increasingly popular. They aim to benefit from 'Nemared' resistance to RKN (Ramming and Tanner, 1983) and from adaptation to Mediterranean conditions from 'Garfi': Three of the selected rootstocks, 'Garnem' 'Felinem' and 'Monegro' (Felipe *et al*., 1997, Felipe, 2009), also provide good vigour and performance in replanting conditions (Gómez-Aparisi *et al*., 2000).

Other different almond rootstocks are also under evaluation, such as Replant-PAC (Pinochet, 2010), Root-PAC-40 and Root-PAC-20 (Gasic and Preece, 2014), with 40 and 20% vigour reduction in comparison to 'Garnem'. In USA peach seedlings have recently been described as almond rootstocks 'Controler- (Gasic and Preece, 2014), derived from an open pollinated *P. persica* HBOK series (Harrow Blood x Okinawa) both unpatented, with 60% vigour control respectively, compared to 'Nemaguard' (DeJong *et al*., 2004).

II – Breeding objectives

The most important innovation over recent years is the introduction of 'in vitro' propagation in the nurseries (Loreti and Massai, 2006). At present, in almost all clonal peach rootstocks are produced by micropropagation and most laboratories have been set up for tissue culture of fruit tree rootstocks. The technique allows production of large quantities of materials within a short time in a restricted space. So nowadays propagation for large quantities of plants is not an objective in itself but an inherent need in all breeding programmes, in order to conduct further studies to select other desirable traits in the breeding objectives. Propagation ability is linked to genetics and almond genetics apparently are more difficult than those from peach parentage. Repeated attempts to select a clonal almond rootstock have failed due to the difficulty of vegetative propagation in this species (Felipe and Pascual, 1990). Modern laboratories are able to overcome this issue and several advances have been made focusing on hormone combinations.

Priority objectives reported 25 years ago in stone fruit rootstocks were nematode resistance ranking first place, followed by other adaptational traits associated to abiotic stresses, drought, chlorosis and waterlogging (Rubio Cabetas, 2012). However, observing all the breeding programmes after the survey conducted by Reighard (2002), it can clearly be seen that nematode resistance (root knot, ring and lesion) was still reported as top priority. Adding the number of breeding programmes that are still working with adaptational traits, we can consider the following traits by order of importance: nematodes, waterlogging, vigour control, adaptation to calcareous soils, drought, soil diseases (fungal and bacterial) and cold hardiness. At the moment, 5 years later, minor priorities may have emerged.

Significant progress has been made in the development of broadly resistant rootstocks, more specifically in RKN. Screening methodologies are somewhat laborious, involving either field tank or greenhouse assays. Therefore this is one of the most mature areas, where several genes have already been located on genetic maps (Lecouls *et al*., 2004, Duval *et al*. 2014). However, this recent knowledge has not been the same for other soil-borne diseases caused by bacteria or fungi, which would still be the first priority (Rubio-Cabetas, 2012). Even though RKN resistance could prevent some bacterial damage and help develop resistance to *Agrobacterium* (Rubio-Cabetas *et al*., 2001) in stone fruit rootstocks.

In recent plant material releases calcareous tolerance and some advances in waterlogging traits can be observed, incorporated by almond x peach hybrids and plum selections respectively (Rubio-Cabetas *et al*., 2005).

In the main breeding programmes aim to improve drought tolerance and vigour, and also to increase climate adaptability with low hardiness to adapt early ripening varieties. Almond size control through rootstocks of other *Prunus sp*. has not been achieved satisfactorily in the past. Two commercial almond rootstocks Root-PAC 40 and Root-PAC 20 rarely reduce scion vigour by more than 40 and 20%, which has progressively become a main concern in breeding programmes. Although dwarfing rootstocks are in demand, cold hardiness and dwarfing, characteristics of the Russian hybrids (Eremin and Eremin, 2002) seem to be linked, thus this concern has to be solved for Mediterranean conditions. Such is the case of Root-Pac 20 that would not be appropriate for all Mediterranean conditions.

Size-controlling rootstocks for almond could increase orchard productivity via intensive training systems but they are difficult to achieve. New dwarfing rootstocks for almond must reduce vigour, be graft compatible, and provide good fruit production without reduction of kernel size and quality. Vigour requisites may not necessarily be the same in all almond production countries, since they must be adapted to the mechanical harvesting equipment so medium vigour seems to be acceptable at the moment. However, an agronomical aspect, such as replanting conditions, must acquire more relevance in the near future, since even in the most traditional almond industries, replanting is acquiring more importance because other fruits such as grapes and citrus are now been replaced by almond and an adequate vigour for good performance in replanting conditions on low fertility sites must also become a priority when choosing the almond rootstock.

III – Almond rootstock types

1. Almond seedling

Traditionally, the bitter almond seedling has been used without paying attention to its origin, since seed orchards were not intended for seed production. Often seeds were collected from wild almond populations that were cross pollinated by the nearest orchards. This is the origin of seeds collected from their area of origin in East Asia and also in a population of *P. webbii* in, central (Felipe, 1989). Classical Spanish cultivars described as best for rootstocks are 'Atocha', 'Desmayo Largueta', 'Garrigues', 'Marcona', and 'Ramillete' (Ramos, 1976). In Spain the cultivar 'Garrigues' has been the most common, where seedlings are uniform with a strong, deep, root system, although there are some feathers in the graft union area, whereas in the cultivar 'Atocha' we find less feathers. Other cultivars used as seedling rootstocks are 'Marcona' in Spain, 'Mission' in California, 'Chellaston' in Australia and 'Don Carlo' in Italy (Fideghelli and Loreti, 2009). Almond seedlings are generally sensitive to nematodes, fungi, bacteria and capnode.

The most remarkable feature of almond seedlings is their hardiness and ability to grow in poor, high limestone content soils with little natural rainfall. In extreme situations, such austerity allows them to survive better than other crops, although growing almonds under these conditions has little or no economic interest today. Another characteristic is a deep root system; however, observations on different soil types indicate that root growth depth is limited by available oxygen in the soil pores. Therefore, in porous soils roots reach deeper than in more compact soils. A negative attribute of seedlings is that they are not homogeneous in growth development and behaviour and are sensitive to handling and transplanting from the nursery to the field, often with poor survival after planting. They are also susceptible to soil pathogens such as nematodes, *Agrobacterium*, *Phytophthora*, *Armillaria*, etc. and sensitive to neck and root asphyxia and as such they are unsuitable for cultivation under irrigated conditions, except when the irrigation system is localized (drippers, micro, etc.) and the soil has good drainage. As a general rule the almond seedlings are suitable for growing rainfed almonds (Felipe, 1989).

Nowadays the cultivar more often used in as the almond seedling is 'Garrigues' and 'Atocha'. In California the 'Texas' (= 'Mission') cultivar is normally used and in Israel some bitter almond cultivars such as 'Alnem 1' 'Alnem y 'Alnem are resistant to RK nematodes.

2. Peach seedlings

Peach seedlings were selected for irrigated crop production since they are less sensitive to problems affecting almond seedlings, though they are not suitable for cultivation in rainfed soils. Peach is very compatible with almond, and almond cultivars on peach grow rapidly in the first years after planting. Peach seedlings have been selected explicitly for seed production since they typically give more homogeneous plants. Some good seedling producing cultivars are INRA's 'GF-305', 'Montclar', and the U.S. cultivars 'Lovell', 'Nemaguard', 'Nemared', which have been widely described (Felipe, 1989) and have been used for a long time for both peach and almond cultivars in different countries.

The positive features of peach seedlings are that they are best adapted to cultivation in irrigated soils and the knowledge of their known agronomic performance against some stresses, such as tolerance to certain species of nematodes. In general, peach seedlings cannot be regarded as a definitive solution for the cultivation of almonds, but they represent an improvement for irrigated almond production. In contrast, they remain highly sensitive to some of the common pathogens: *Agrobacterium*, *Armillaria, Phytophthora*, etc. They induce a shorter tree life than almond rootstocks. Plants produced from specific cultivars (e.g., 'Nemaguard' and 'Nemared') are resistant to RKN Meloidogyne spp, and others (e.g., 'Rutgers Redleaf') tolerate heavy soil conditions better. Some cultivars have been used in crosses aimed to introduce resistance to RKN as well as other interesting characters such as red leaf colour.

3. Clonal rootstocks

Clonal propagation, though more expensive than grafting to seedlings, offers the advantage that the behaviour of the material produced is very homogeneous and growth characteristics are more consistent and better known. In the cultivar 'Garfi', a descendant of 'Garrigues', was selected because it was easier to propagate. Research trials of propagation via rooting hardwood cuttings gave satisfactory results for 'Garfi' when compared with other almond cultivars ('Garrigues' among them), peach, and almond x peach hybrids (Felipe, 1984; Felipe 1992).

4. Plum as almond rootstock

The plum root system has a shallow development and generally the roots are smaller in number and thickness than for peach or almond. Plums are usually more tolerant to certain pathogens and more resistant to the waterlogging conditions in heavy soil. They perform better in heavy soils than almond and peach roots. Therefore, these rootstocks are used when soils are not sufficiently healthy, coarse textured, and aerated. Graft compatibility with almond is highly variable and thus, previous trials are required before a particular combination of almond can be used on plum.

Low vigour plums. The plums corresponding to the species *P. domestica* L. and *P. insititia* L., (i.e. European plums, 'Sanjulián', 'Damas', etc.) are included in this group. In many cases the compatibility is good, but varieties to be grafted on some of the clones have localized incompatibility, and the graft union eventually deteriorates. The well known "pollizos" originating from are fully compatible if they are true "pollizos" and are free of infectious agents such as viruses. It is recommended to use selections that are currently in an advanced commercial stage such as 'Adesoto', and 'Montizo'. The compatibility tests conducted over several years with these rootstocks showed that very few of them had incompatibility problems when grafted with almond varieties. The selected pollizo clones are plums that offer the best prospects for use as almond rootstocks for specific soil conditions or vigour reduction since these offer greater compatibility. Other European plums 'Penta' and 'Tetra' are also being used in with somewhat delayed flowering compared to 'GF-677'. The advantages of these slow-growing plums are that this group is more resistant than almond and peach seedling trees to root asphyxia, as well as root parasites, such as the nematodes *Phytophthora*, *Armillaria*, *Agrobacterium*, etc. Trees are smaller, allowing for semi-intensive cultivation of irrigated almond and also provide good anchorage. Because of low drought resistance, plum rootstocks should only be considered for irrigated crops. The good compatibility of the clones in this group with almond is not universal, hence the risk of localized graft incompatibility with certain varieties of almond. They tend to produce suckers, which are a problem for cultivation.

Vigorous plums. This group includes plums belonging to *P. cerasifera* Ehrh diploid species (Myrobalan), *P. salicina* Lindl. (Japanese plums), some hybrids like 'Marianna (*P. cerasifera x P. munsoniana*) and other diploid plums from different backgrounds**.** This group of plums generally has poor compatibility with almond varieties. Usually it is translocated incompatibility, although in some combinations incompatibility is found at the graft union. Some clonal selections grafted with certain varieties of almond show apparently good compatibility, as in the case of 'Marianna 2624' grafted with some California cultivars and some clones of Myrobalan with certain European cultivars, where there have been some combinations of either almond / Myrobalan or almond / Marianna showing some compatibility (Felipe, personal communication). The advantages of these fast-growing plums are usually rootstocks that provide good vigour and development when grafted with compatible varieties of other species. In addition, they adapt well to different types of soil, and tolerate a certain amount of humidity. They also have some tolerance to *Phytophthora* and *Agrobacterium*. Some selections are resistant to RKN *Meloidogyne sp*. In general, they are easy to propagate vegetatively, provide good anchorage and do not normally sucker. These rootstocks should be used with irrigation only, since their behaviour on dry land is not satisfactory. Their compatibility problems with almond cultivars make these plums virtually impossible to use without prior experience with each particular almond scion / cultivar combination.

5. Interspecific hybrids

The Mediterranean area has a long-standing tradition of almond tree cultivation. Among the interspecific hybrids between *Prunus*, almond and peach are the best known and most widespread. There are several commercially propagated selections: 'INRA-GF-677', 'Adafuel' and controlled crosses such as ''Garnem' 'Monegro' 'Felinem', especially 'Garnem are the most well-known and widespread to date. In recent years some other peach x almond hybrid clones are under study with both species and with several species of plum, which may be of interest when the compatibility has been studied and they show a satisfactory behaviour at agronomic level. Those recently propagated are 'Replantpac' a Myrobalan x almond hybrid whose compatibility with almond has also been studied (Pinochet, 2010), Root-Pac 40 (complex almond x peach hybrid) and Root-Pac 20 (P.besseyi x P. cerasifera).

6. The positive characteristics of hybrid between almond and peach

One of the features that attracted attention from the beginning was the remarkable vigour of the plants that were used. This aspect is interesting for the cultivation of almonds for good growth in either rainfed or irrigated conditions, which in turn results in an earlier onset of production. Experience to date shows that there are differences in development that achieve the same variety when grafted on different clones of this type of hybrid. The clones used so far acceptably adapt to different soil types and better support the almond seedling cultivation in irrigated conditions. Also better performance than the almond seedling has been observed in rainfed cultivations in both growth and production.

7. Negative characteristics of hybrids between almond and peach

The selected clones that are already in use accumulate many good qualities, but also have negative aspects that can and should still be improved. A common feature of almost all of them is that propagation is not as easy as one would wish in a good rootstock. Some techniques have been developed for multiplication and with acceptable results (Pascual and Felipe, 1988), but the normal nursery still finds it difficult to multiply their own plants. Most of the clones used are still sensitive to *Agrobacterium* and *Armillaria* and they are, in varying degrees, susceptible to root asphyxia. One negative aspect is high vigour. In the last five years some progeny from a private breeding programme between different interspecific *Prunus* has been used as rootstocks to reduce vigour in some intensive orchard trials: Replant–R, Root-PAC-40 Root-PAC-20. However there is insufficient available data to predict the behaviour of those rootstocks in the forthcoming years and trials with more cultivars are needed. These hybrids with medium vigour, equivalent to the peach seedlings or less, are commercially available for use in intensive plantations, etc. and in USA the most recent 'Controler- are also being studied.

IV – Clones of peach × almond hybrids in use

'INRA-GF677' is a natural hybrid of peach and almond found in Lot et Garonne (France), introduced in 1939 and selected in the French resort of La Grande Ferrade (INRA, Bordeaux), (Bernhard and Grasselly, 1981). The leaves are intermediate in appearance and size between almond and peach. The appearance of the branches is closer in winter peach for its thick and downy buds. The flower is rosacea pink. Regarding growth habit, it is open and branched, producing many feathers. The root system consists of numerous strong roots. As almond rootstock is very vigorous, it enters fruition rapidly and produces a large yield. Its behaviour with rainfed almond rootstocks is very good, the almond almost always overcoming the almond seedling almost always almond. The resistance to asphyxia is similar to the common peach seedling and it is more resistant to chlorosis. It is also sensitive to *Agrobacterium* and nematodes. The propagation of 'INRA-GF-677' is not easy, although progress has been made in the development of the right to obtain acceptable commercial returns techniques, therefore between cuttings with leaves in summer and by hardwood cuttings in autumn; the latter procedure should be followed in the early autumn in highly permeable soils. Numerous prospective hybrids producing branches in hedge planting system and make their preparation a slow and expensive operation in the nursery for subsequent grafting, as vegetative growth ceases in favour of branch growth. The 'INRA-GF-677' is a standard rootstock for difficult soils (calcareous, arid, exhausted). It also tolerates moderate salinity levels and has a good tree growth and higher productivity with the traditional cultivars. However it does not perform well in replanting conditions and branching in the nursery is common. It is highly susceptible to *Phythophtora*, *Armillaria*, *Agrobacterium* and *RKN*, and may also be inappropriate with modern varieties that are very productive at least under irrigated conditions. It is also as sensitive to waterlogging as all the almond x peach hybrids. However it is an excellent rootstock for the cultivation of almonds, in rainfed and irrigated conditions whenever the soil is free from RKN and not very compact. It supports all varieties of almond and transplants well, however we must ensure good tree formation with green pruning and pinching and winter pruning should be lenient in the early years. This clone represented more than 15% of clonal rootstocks in (Arquero *et al*., 2002)

'Adafuel' is a clone selected in the Aula Dei Experimental Station (Spain) (Cambra and Iturrioz, 1986), from a collection of over sixty spontaneous hybrids from various Spanish regions. It comes from a hotbed of 'Marcona' almonds. The leaves are deep, narrow, green and slightly curved lengthwise. Although aspects between peach and almond resemble the 'Marcona', the almond leaf has left its mark on 'Adafuel'. It is erect, as a tree nursery, branches in winter are dark, resembling 'Marcona' almond, althought not produce many feathers as 'INRA-GF-677', it is also still abundant in this clone. The flower is rosacea bright pink. The root system consists of abundant and strong roots. It is a vigorous rootstock, compared to 'INRA-GF-677' and as an almond rootstock, is stronger in irrigated conditions than in rainfed conditions. The resistance to asphyxia is the same as that of peach seedlings. It is more resistant to chlorosis that the 'INRA-GF-677'. At the moment, it appears to be quite resistant to Phytophthora and does not seem as sensitive to *Agrobacterium* as 'INRA-GF-677'. However it has shown sensitivity to nematodes. With slight differences, it has the same capacity and the same problems for propagation as the 'INRA-GF-677'. Production of woody cuttings in mother plants is good though with many feathers. In the nursery, rooted plants are upright which facilitates grafting. It is an interesting rootstock for cultivation in replanting and in calcareous and poor soil conditions. For the experience gained so far in reference plantations, 'Adafuel' is proving to be an excellent rootstock for almond, provided the soil is free of nematodes. It supports all almond varieties.

'Felinem' 'Garnem' and 'Monegro'. (Felipe, 2009). These three clones were selected from the progeny obtained in the cross between the Spanish almond 'Garfi' (*Prunus amygdalus* Batsch.) as the female parent and the North American peach 'Nemared' [*P. persica* (L.) Batsch] as the pollen donor. 'Garfi' almond had been previously selected because of its good morphological characteristics and easy clonal propagation (Felipe, 1989; Felipe *et al*, 1997). 'Nemared' was chosen mainly as a source for root-knot nematode resistance (Ramming and Tanner, 1983). This progeny was selected at the Servicio de Investigación Agraria de la Diputación General de Aragón (Spain), now Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). These rootstocks have strong, thick, root systems. This aspect is interesting for the cultivation of almonds with a strong growth either under rainfed or irrigated conditions and also results in an earlier onset of production. Unbudded trees of 'Garnem', 'Felinem' and 'Monegro' are vigorous and no differences in size are noticeable between them. The vigour induced in grafted cultivars is comparable to that induced by 'GF- or 'Hansen, with a similar productivity. Non-grafted plants are vigorous and in the nursery exhibit erect growth with little or no feathering during the first season (Felipe, 1989). Fruits are green with reddish tones. The mesocarp is thin and non-edible, leaving a free stone. Despite their similar morphology and performance, the three clones may be distinguished by molecular markers (Serrano *et al*., 2002).

Their level of tolerance to iron chlorosis is similar to that of 'GF- and 'Adafuel' (Felipe, 1989). They also tolerate drought conditions well, with higher resistance to water stress in 'Monegro'. Adaptation to poor soils is good if the soils are well drained. Incorporation of root-knot nematode resistance was the primary breeding objective in order to replace several widespread nematode susceptible peach x almond hybrids used for peach production in, especially in replanting situations (Pinochet, 1997). They are very resistant to the main root-knot nematode species attacking *Prunus* showing a broad spectrum of resistance (Marull *et al*., 1994; Esmenjaud *et al*., 1997). Like most almonds and almond x peach hybrids, they exhibit low tolerance to root asphyxia caused by waterlogging. They are also susceptible to the root lesion nematode *Pratylenchus vulnus* (Pinochet *et al*., 1996) and to crown gall caused by *Agrobacterium* tumefaciens.

The GN series clones used so far are acceptably adapted to different soil types and are a better support than the seedling almond for irrigated cultivation. Good performance has also been observed of many rainfed cultivations, having overcome the seedling rootstocks in growth and production. Transplantation is easier than the seedling almond. 'Garnem', 'Felinem' and 'Monegro' propagate well by hardwood and herbaceous cuttings in aerated and well drained soils. Best results for hardwood cuttings are obtained in autumn. Cuttings are easily obtained thanks to the lower level of shoot branching. They also propagate very well in vitro. Nursery operations are facilitated by the low presence of feathers and red leaves, as well as by the long vegetative period of the plants, allowing the production of nursery plants in a shorter period. The percentage of bud uptake is high for all known peach, nectarine and almond cultivars (personal communication by different nurserymen). These rootstocks have been selected primarily for almond showing good graft compatibility with numerous almonds. Tests made with almond varieties grafted on various clones of hybrids between almond and peach have shown that even within this group of rootstocks, there are differences in agronomic performance.

V – Current rootstock trials

The main remarks on the choice of the rootstock are fruit tree growing intensification and quality, with the need to control vigour and management costs. Another consideration is high water and fertilizer use efficiency and specific soil and climatic conditions with soil borne pathogens. Additionally the choice of rootstocks for almond growing in hot-arid climates must be based on the scarce availability or poor quality of water or strict irrigation schedules imposed by the irrigation boards. Therefore rootstocks must be tolerant to water stress with deep and extended root systems together and adapt to heavy soils with poor drainage and highly saline soils are need.

Recently in the last five years some trials for almonds have been conducted on different types of rootstock. From the traditional almond x peach rootstock 'GF-, 'Garnem', 'Felinem' y 'Monegro' other older hybrid rootstocks such as 'Barrier' and 'Isthara' were used as well as some plum selections such as 'Adesoto' and 'Montizo' with some new released interspecific hybrids of different origins such as Root-PAC-40 and Root-PAC-20 to try to elucidate in the short medium term which are the best rootstocks for each specific situation to respond to the almond growth expansion in the Mediterranean area. In Table 1 we review the most recent trials established mainly in but also in where almond growing is expanding and they will give responses for the almond growing in the near future.

Table 1. Rootstocks involved in recent almond trials established mainly in Spain but also in USA and Australia

Country	SPAIN	SPAIN	SPAIN	SPAIN	SPAIN	SPAIN	AUSTRALIA	USA
REGION	Aragón	Aragón	Cataluña	Cataluña	Cataluña	Andalucia	Victoria	California
YEAR	2011	2011	2009	2010	2012	2015	2014	2012
	Rootpac-40*	R-20**	GF-677*	Cadaman		Garrigues	Nemaguard	Lovell
				Garnem		Garnem	GF557	Nemaguard
				GF677		GF-677	GF677	Empyream 1 = Barrier 1
				Ishtara		Nemaguard	GF749	Avimag = Cadaman
				MB 1-37		R90	Adafuel	HBOK50
				(PxA) x Myrobalan		RR	Garnem	Hansen
				Puebla de soto	MB-1-37	IRTA-1	Felinem	Bright's
				Rootpac-20		MONTIZO	Monegro	BB106
				Rootpac-40		TETRA	Brights Hybrid	Paramount = GF677
				Rootpac-R		R-20	Hansen 536	Flordaguard x Alnem
						R-40	Cornerstone	PAC 9908-02
						REPLANT	Nickels	HM2 +
						R-20	Krymsk 86	Viking
						R-40	Atlas	Atlas
							Cadaman	Krymsk 86
							Nemaguard	Rootpac R

* Training System.

** Graft Compatibility.

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Impact of osmotic drought stress on carbon isotope discrimination and growth parameters in three pistachio rootstocks (*Pistacia* spp., Anacardiaceae)

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Abstract. Pistachio (*Pistacia vera* L., Anacardiaceae) is one of the major horticultural crops in Iran with high plantation surface areas, and production and export rates. However, growing water deficiency has become a main limiting factor for growth and development, and especially for maintaining yield quality and volume of this crop. In this study, the responses of carbon isotope discrimination ($\Delta^{13}C$) and some growth parameters of three pistachio rootstocks (*P. vera* cv. Badami, *P. vera* cv. Sarakhs and *P. terebinthus*) to different osmotic drought stress levels (-0.1, -0.50, -1.0 and -1.5 MPa) were investigated in a greenhouse experiment carried out at the Faculty of Bio-science Engineering, Ghent University, Belgium. The impact of different rootstocks on leaf $\Delta^{13}C$ varied significantly. Results show that *P. vera* cv. Sarakhs had the highest discrimination for carbon isotopes, whereas *P. vera* cv. Badami had the middle and *P. terebinthus* the minimum discrimination values, respectively. An increase in drought stress intensity caused a decrease in leaf carbon isotope discrimination values in all three rootstocks, although these differences were not significant. Drought stress significantly decreased both plant fresh and dry weight, shoot dry weight, root dry weight, leaf area and stem elongation. There also was a significant rootstock effect of treatments on these growth parameters. Under osmotic stress treatments, root/shoot ratio increased significantly. Control plants showed normal elongation growth, but growth was stopped for all drought stress treatments, and differences were significant for all three rootstocks. There were no significant differences between pistachio rootstocks and drought stress treatments for leaf shedding.

Keywords. Biomass – Water deficiency – Plant growth parameters.

Impact du stress osmotique dû à la sécheresse sur la discrimination isotopique du carbone et les paramètres de croissance chez trois porte-greffes de pistachier* (Pistacia *spp., Anacardiaceae)

Résumé. *Le pistachier (*Pistacia vera *L., Anacardiaceae) est une des espèces majeures de l'horticulture en Iran avec de vastes surfaces plantées, et de forts taux de production et d'exportation. Toutefois, le manque d'eau croissant est devenu un principal facteur limitant pour la croissance et le développement, et en particulier pour le maintien de la qualité et du volume de production. Dans cette étude, les réponses à la discrimination isotopique du carbone ($\Delta^{13}C$) ainsi que certains paramètres de croissance de trois porte-greffes de pistachiier (*P. vera *cv. Badami,* P. vera *cv. Sarakhs et* P. terebinthus*) à différents niveaux de stress osmotique dus à la sécheresse (-0,1, -0,50, -1,0 et -1.5 MPa) ont été testés dans une expérimentation en serre menée en Belgique, Faculty of Bio-science Engineering, Ghent University. L'impact des différents porte-greffes sur $\Delta^{13}C$ des feuilles a varié significativement. Les résultats montrent que, respectivement,* P. vera *cv. Sarakhs a la plus forte valeur de discrimination isotopique du carbone, tandis que* P. vera *cv. Badami a la valeur moyenne et* P. terebinthus *a la plus petite valeur. Une augmentation de l'intensité du stress de sécheresse a causé une baisse des valeurs de discrimination isotopique du carbone des feuilles chez les trois porte-greffes, bien que ces différences ne soient pas significtives. Le stress de sécheresse a fait baisser significativement le poids frais et poids sec des plantes, le poids sec des pousses, le poids sec des racines, la surface foliaire et l'élongation des tiges. Il y a eu également un effet significatif des traitements des porte-greffes sur ces paramètres de croissance. Sous traitements de stress osmotique, le ratio racines/pousses a augmenté significativement. Les plantes témoins ont montré une croissance d'élongation normale, mais la croissance a été stoppée pour tous* *les traitements de stress de sécheresse, et les différences ont été significatives pour les trois porte-greffes. Il n'y a pas eu de différences significatives entre porte-greffes de pistachiers et traitements de stress de sécheresse pour la chute des feuilles.*

Mots-clés. *Biomasse – Manque d'eau – Paramètres de croissance de la plante*.

I – Introduction

Pistachio belongs to the Anacardiaceae family. Only *P. vera* L., i. e. cultivated pistachio, has sizeable economic importance. Iran, as the region of origin of pistachio has always had the largest cultivation area in the world. In Iran, pistachios are usually cultivated under dry and saline conditions (Sheibani, 1995), as they have a high tolerance to drought and soil and water salinity. Still, water deficit and salinity can cause a reduction in plant growth, and eventually yield and nut quality.

Drought stress adversely affects growth, dry mass accumulation, and productivity of plants (Anjum *et al*., 2011) and causes a higher rate of impairment than any other environmental factor (Shao *et al*., 2009). Higher drought tolerance of wild pistachio species could be related to a deep taproot, high water conservation ability by stomatal adjustment, stomatal features, leaf characteristics, and leaf shedding. Therefore, these wild species are very often used as rootstock. It's in this context that drought stress was evaluated for *P. vera* Kerman grafted onto three different pistachio rootstocks. Grafting onto hybrid rootstock (UCB#1) and *P. terebinthus* resulted in a higher growth reduction compared with *P. atlantica* under drought stress (Gijón *et al*., 2010).

Carbon isotope discrimination (δ) decreases with increasing salinity in leaves, stems and roots of pistachio seedlings. However, there were no significant difference in carbon isotope discrimination between three *P. vera* (Sarakhs, Badami-zarand, and Ghazvini) rootstocks was evidenced by Hokmabadi *et al*. (2005).

There are three pistachio species in Iran (Esmaeilpour and Khezri, 2006) which are grown under different environmental conditions (altitude 900-2000 m; latitude 24-37 N; temperatures ranging between -10°C in winter and 48°C in summer, low to moderate humidity, and long, hot summers). Responses of two of the three species have been investigated to osmotic drought stress treatments by Fardooei (2001). *Pistacia vera* L. is the country's most common rootstock. Yet, physiological responses of this pistachio rootstock to drought stress and comparison to other recommended rootstocks have not been studied enough. The aim of this study was to evaluate the effects of osmotic drought stress on carbon isotope discrimination and plant biomass, leaf area, elongation rate and root/shoot ratio of *P. vera* cv. Badami, *P. vera* cv.Sarakhs (native), and *P. terebinthus rootstocks (used in Turkey)*.

II – Materials and methods

This study was carried out in a greenhouse at Ghent University (51°3' N, 3°42' E). Certified seeds of two pistachio rootstocks, *P. vera* L. cv. Badami (Badami) and *P. vera* L. cv. Sarakhs (Sarakhs) were collected from Rafsanjan, Iran and *P. terebinthus* (Terbinthus) seeds were obtained from the pistachio production area in Turkey. Transplanted 1-year-old seedlings were grown hydroponically in a glasshouse environment using Hoagland solution (Picchioni *et al*., 1991). Osmotic drought treatments were control (Ψ_s = -0.10) and three drought stress levels ($\Psi_s = -0.5$; $\Psi_s = -1.0$, $\Psi_s = -1.5$ MPa) using PEG 6000. Stress levels were maintained for two weeks; then all solutions were replaced by the control treatment, and this level was maintained for two recovery weeks.

Dried leaf samples were ground by a grinder. Five mg subsamples of ground plant material were packed in tin capsules and analyzed for natural abundance of C ^{13} by combustion to CO_2 in the present of O_2 by an elemental analyzer (EA) coupled to an isotope ratio mass spectrometer (IRMS) to measure $\delta\,^{13}C$ (Staelens *et al*., 2012).

At the end of the second recovery week, seedlings were harvested. Plant fresh weight (PFW) of leaves, shoots, and roots was determined. Plant dry weight (PDW) of the respective plant fractions was determined after drying at 85°C for 72 hours. Plant height was measured with a ruler (± 0.1 cm) at the beginning and end of the experiment. The experiment was designed as a randomized complete design (RCD). Each experimental unit contained 5 plants and a total of 20 plants were used for four treatments in every rootstock. A two-way analysis of variance was used to test for effects of drought treatments and rootstocks. Means were compared using Tukey's HSD test (P = 0.05). All analyses were performed in SPSS 22.

III – Results and discussion

1. Carbon isotope discrimination

The impact of drought stress treatments on $\Delta^{13}C$ varied significantly in the different rootstocks. Leaf carbon isotope discrimination was highest in Sarakhs (23.00 ‰), average in Badami (22.19 ‰) and lowest in Terebinthus (22.07 ‰) rootstocks. Increasing drought stress intensity decreased the values of leaf carbon isotope discrimination, although differences were not significant (Fig. 1 A and B). Findings of this study on leaf $\delta^{13}C$ for drought stress treatments are in agreement with the results of Hokmabadi *et al*. (2005). However, latter authors reported there was no significant difference in carbon isotope discrimination among pistachio rootstocks, whereas in this experiment we observed significant differences among evaluated rootstocks.

There were negative significant relationships between carbon isotope discrimination ($\delta^{13}C$) with PFW and PDW values (data not shown). Based on the correlations, we evidenced carbon isotope discrimination would seem to be a good indicator of drought stress effects that significantly influence biomass factors of pistachio plants in water deficit conditions.

2. Biomass characterization

Drought stress treatments significantly decreased PFW, PDW, shoot dry weight (SDW) and leaf dry weight (LDW) compared with control plants. Although root dry weight was not affected by the osmotic stress treatments, root/shoot ratio increased significantly (Table 1, figure 1). Plant leaf area was not affected by treatments, although overall control plants had a higher leaf area than drought-exposed plants. Control plants showed elongation growth during the experimental period, but a growth arrest was noted under drought stress treatments. There were no significant differences among drought treatments in shedding rates, although the latter increased with drought stress intensity (Table 1). Effects of rootstock were significant on all growth parameters. Terbinthus had the highest values for biomass parameters, leaf area and stem elongation, while Badami had the highest value for leaf shedding compared to the other rootstocks (Table 1).

Dry biomass is considered as an appropriate parameter for stress tolerance evaluation in many crops (Munns, 2002). In our experiment, when plants were subjected to osmotic drought stress treatments, we observed dry biomass reduction values were found by more than 30.7%, 42.8% and 42.3% in mild (-0.5 MPa), moderate (-1.0 MPa) and severe (-1.5 MPa) drought stress levels compared to control respectively. The significant decline in total plant biomass of pistachio root-

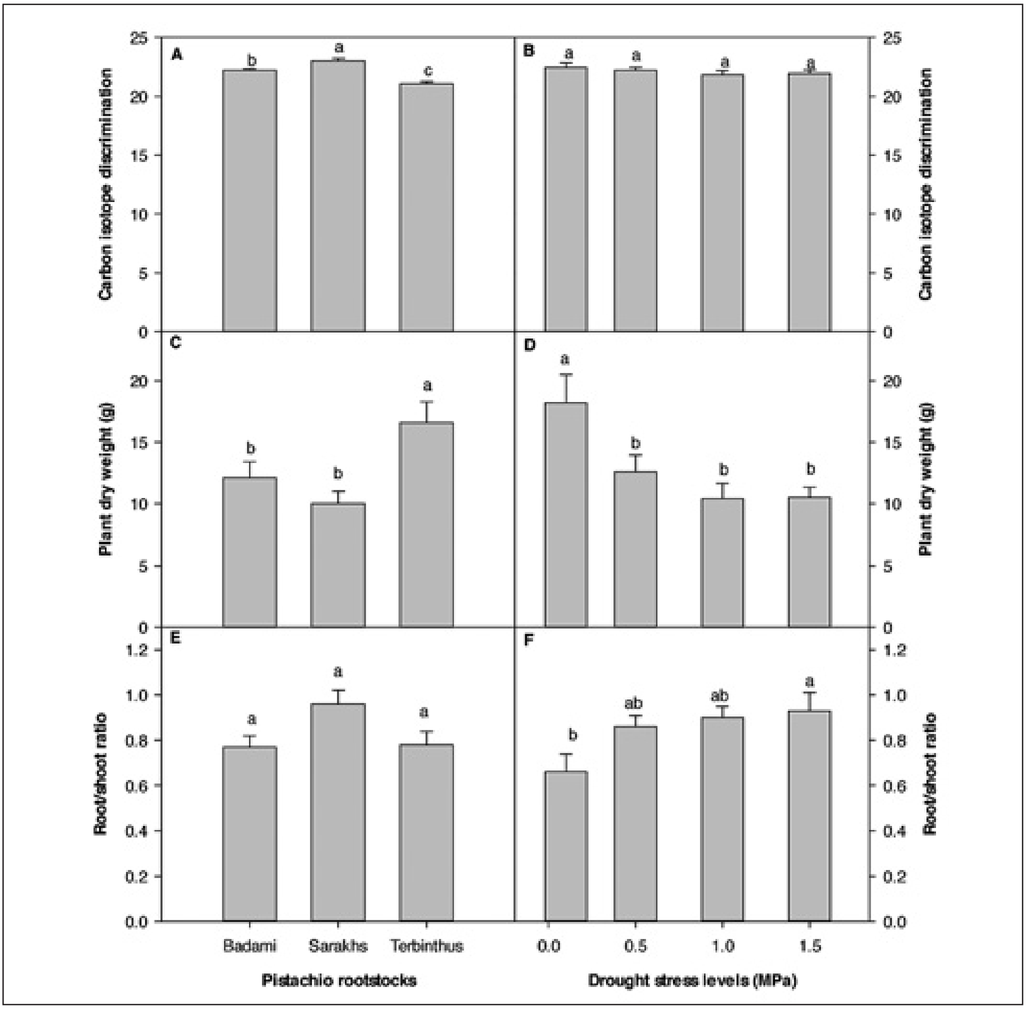

Fig. 1. Changes in carbon isotope discrimination in rootstocks (A) and treatments (B); plant dry weight in rootstocks (C) and treatments (D); and root/ shoot ratio in rootstocks (E) and treatments (F) in three pistachio rootstocks. For each column, different letters indicate significant differences (*P* ≤ 0.05) according to Tukey's range test.

stocks with increasing drought is in line with results reported by Abbaspour *et al*. (2012), Habibi and Hajiboland (2013) and Ranjbarfordoei *et al*. (2000) in pistachio species, Rouhi (2007) in almond species, and Maraghni *et al*. (2011) in *Ziziphus lotus* plants.

Osmotic stress resulted in growth arrest for all rootstocks, and no regrowth was observed after 2 weeks of recovery. Root growth is generally less sensitive to drought stress compared to other biomass components (Hsiao and Xu, 2000). An increase in root/shoot ratio has been proposed as one of the mechanisms involved in the adaptation of plants to drought stress (Turner, 1997). In our study, increasing root/shoot ratios under osmotic stress are only related to a decrease in aboveground biomass (Table 1).

Table 1. **Effects of osmotic stress treatments (T, MPa) on plant fresh weight (PFW, g), shoot dry weight (SDW, g), leaf dry weight (LDW, g), root dry weight (RDW, g), leaf area (LA, cm), elongation length (EL, cm) and shedding (Shed, g) after two weeks of recovery for pistachio rootstocks (C)**

		PFW	SDW	LDW	RDW	LA	El	Shed
Treatments	-0.1	52.9 a	11.7 a	6.1 a	6.6 a	22.8 a	16.2 a	0.020 a
	-0.5	29.7 b	6.9 b	3.2 b	5.7 a	18.7 a	1.6 b	0.167 a
	-1	25.9 b	5.6 b	2.5 b	4.8 a	17.1 a	0.9 b	0.174 a
	-1.5	24.5 b	5.5 b	2.5 b	5.0 a	16.7 a	2.3 b	0.150 a
Rootstocks	Badami	32.2 b	7.1 b	2.7 b	5.1 ab	12.1 b	5.7 ab	0.27 a
	Sarakhs	22.6 b	5.2 b	2.1 b	4.9 b	12.9 b	2.3 b	0.05 b
	Terbinhus	45.0 a	10.0 a	5.9 a	6.7 a	31.4 a	7.7 a	0.06 b
Anova	Rootstock	**	**	**	*	**	*	**
	Treatment	**	**	**	ns	ns	**	ns
	Rootstock*treatment	ns	ns	ns	ns	ns	**	ns

Within each column in every rootstock, means superscripted with different letters are significantly different [** - (P< 0.0 1), * - (*P*< *0.05*) and ns- none significant].

Under control conditions, Terbinthus maintained the highest PDW compared to both other rootstocks (Fig. 1C) but this rootstock showed the largest decrease for this parameter in reaction to severe drought stress. Reduction rates were 42.4%, 33.1% and 47.1% for Badami, Sarakhs and Terbinthus, respectively. Therefore, Sarakhs kept the lowest PDW reduction values among the other rootstocks in this study.

IV – Conclusion

Pistachio trees are considered drought tolerant, yet the applied osmotic drought stress induced significant reductions in leaf carbon isotope discrimination. In conclusion, Sarakhs better tolerated the applied drought stress as shown by the growth reduction performance in the severest drought condition as compared with Badami and Terbinthus. However, further research in field conditions is needed to confirm this survey's research results.

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Assessment of control pollinated progenies as almond rootstocks

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Abstract. In order to select almond seedling rootstocks, many trials were conducted, at the Agricultural Experimental Unity located at the north east of Tunisia, to study the value as rootstocks of open pollinated almond genotypes. More vigorous and more developed seedlings with branched root system progenies were obtained. This study aimed to assess growth characteristics of almond seedlings obtained from controlled pollination. Thus, eight local genotypes: 'G12-2', 'Tozeur 3', 'G12-4', 'G24-12', 'G24-10', 'G23-15', 'G25-6', 'G25-8' and one foreign almond variety 'Fasciuneddu', were used as parentages in several controlled crosses. The fruit set was followed, but only two out of seven crosses carried out yielded fruits. Seeds harvested from the two crosses and a mixture of bitter almond seeds, the most popular rootstock in Tunisian nursery, used as control, were germinated and sown in plastic bags. Plant height, stem diameter at the ground level, number of shoots and distance up to the first shoot, were determined during vegetative dormancy. Statistical analysis showed significant differences between progenies derived from cross pollination and those from bitter almond seeds. A great vegetative growth was recorded on the progenies of 'G25-6' x 'G25-8'. The average plant height and stem diameter at the ground level was 103.88cm and 8.95mm respectively. Moreover, these progenies showed the higher distance up to the first shoot, which facilitate the grafting. The cross made between 'G12-2' and 'Tozeur 3' has also provided homogeneous and vigorous seedlings. So these results showed that cross pollination may be used to select almond rootstock seedlings.

Keywords. Rootstocks – Almond – Progenies – Crosses – Vigorous – Pollination.

Evaluation de la valeur comme porte-greffe de descendants d'amandiers issus de pollinisation contrôlée

Résumé. *Plusieurs études de la valeur comme porte-greffe de semis d'amandiers ont été menées à l'Unité d'Expérimentation Agricole située au nord est de la Tunisie. Des descendants issus de pollinisation libre très vigoureux et à système radiculaire très développé et ramifié ont été obtenus. Cette étude a pour objectif l'évaluation des caractéristiques de croissance de descendants issus de pollinisation contrôlée. Ainsi, 8 génotypes locaux: 'G12-2', 'Tozeur 3', 'G12-4', 'G24-12', 'G24-10', 'G23-15', 'G25-6', 'G25-8' et une variété introduite d'Italie 'Fasciuneddu' ont été utilisés comme parents pour la réalisation de 7 croisements. Le taux de nouaison a été suivi et seul deux croisements ont donné des fruits. Après stratification, le semis des amandes obtenues et d'un mélange d'amandes amères, principal porte-greffe utilisé en Tunisie pour l'amandier, a été réalisé en sachets. La hauteur, le diamètre de la tige au niveau du sol, le nombre d'anticipés et la distance d'insertion du premier anticipé ont été déterminés au cours de la dormance végétative. Des différences significatives ont été obtenues entre les descendants issus de pollinisation contrôlée et ceux issus du mélange d'amandes amères. Ceux issus du croisement 'G25-6' x 'G25-8' se sont distingués par leur grande vigueur. Leur hauteur moyenne a été de 103.88cm et le diamètre moyen au sol de la tige a été de 8.95mm. De plus, ils se sont caractérisés par la plus grande distance d'insertion du premier anticipé. Le croisement 'G12-2' x 'Tozeur 3' a également fourni des descendants vigoureux et homogènes. Ces résultats montent que la pollinisation contrôlée peut être utilisée pour la sélection de porte – greffes francs d'amandier.*

Mots-clés. *Porte-greffe – Amandier – Descendants – Croisement – Vigoureux – Pollinisation.*

I – Introduction

Almond is the second stone fruit specie cultivated in Tunisia, for that reason a research for new almond seedling rootstock was undertaken by the National Institute of Agronomic Research since the seventies. A large phenotypic diversity of local and foreign almond varieties was collected (Dumont *et al.*, 1970). Collections were established in the north, center and the south of the country, with a project supported by FAO, the Ministry of Agriculture and GREMPA coordination.

The observation along 2002 until 2005 of the *Prunus* rootstock collection located at the Agricultural Experiment Unity, allowed the selection of several local and foreign almond genotypes. The behavior of their open pollinated progenies as almond seedling rootstocks seems to be promising in comparison with those derived from bitter almond seed mixtures of unknown origin. Vigorous seedlings with expanded and ramified root system were obtained in pots in 2002 (Mselmi *et al.*, 2014).

Thereafter, the possibility to produce progenies from concurring flowering period in almond varieties proposed by Bernhard and Grasselly (1969) and applied by Simard *et al*. (1997), became our main objective since 2006. Thus, the aim of this study was first to cheek pollinators for five selected mother almond genotypes and to evaluate their control pollinated offspring as almond rootstocks. Almond progenies arising from artificial pollination were assessed for growth characteristics. Seedlings derived from mixture of bitter almond seeds, were used as control.

II – Materials and methods

This study was carried out at the Agricultural Experimental Unity situated at the northeast Tunisia at an altitude of 44 meters during 2006-2008. Eight local genotypes and one foreign almond variety: ‘Fasciuneddu’ (Table 1), selected in *Prunus* rootstock collection for their good production in a rainfed orchard, were used as parents.

Table 1. List of selected almond genotypes

Genotypes	Origin	Peak production kg almond shell /tree
‘Fasciuneddu’	Italy	6
‘G12-2’	Unknown origin	6
‘Tozeur 3’	Oasis Tozeur Tunisia	8
‘G23-15’	Chance cultivar North (Tunisia)	5
‘G24-10’	Chance cultivar North (Tunisia)	9.8
‘G24-12’	Chance cultivar North (Tunisia)	13
‘G12-4’	Unknown origin	5
‘G25-8’	Chance cultivar North (Tunisia)	4
‘G25-6’	Chance cultivar North (Tunisia)	6

Five selected genotypes (‘Tozeur 3’, ‘G12-2’, ‘G24-12’, ‘G25-6’ and ‘Fasciuneddu’) were used as mother parents and seven crosses were carried out in 2006 between mid – February and early March. Hand pollination was done on emasculated flower buds, at stage D (Felipe, 1977). The fruit setting was observed two months after pollination and fruit set rate was determined on July.

At the end of 2006, all harvested seeds together with a mixture of bitter almond seeds, were stratified in a moist perlite in plastic bags and kept at 7°C in a fridge. The germinated seeds were sown in February 2007, in a 3:1 (v/v) mixture of peat and perlite in ten liter plastic bags and kept in shaded greenhouse. Heights, diameters at the ground level, number of shoots of the progenies and the distance up to the first shoot were recorded at vegetative dormancy. One way analysis of variance was done using SAS-98 (version 2008) and means values were compared using Duncan’s test.

III – Results and discussion

1. Fruit set percentage

In total we have pollinated 874 flower buds; a total fruit setting rate of 13% was obtained. Similar fruit rates setting ranging from 4% to 11% were reported on 64 crosses made two years at INRA Avignon (French) (Simard *et al*., 1997), although a higher number of flowers were pollinated in this last trial.

The fruit setting obtained two months after pollination showed that in four crosses we did not observed fruit setting (Table 2). A large number of flowers should be carried out to verify their incompatibility. The fruit set rate ranged from 2.2% to 60% and the two crosses: 'G12-2'x'Tozeur 3' and 'G25-6'x'G25-8' gave 16.7% and 60% respectively. Almonds obtained from these two crosses were harvested respectively at mid – July and late August to assess their agronomic trait as an almond rootstock.

Table 2. Fruit set of seven crosses carried between eight almond genotypes

Crosses	No. of pollinated flowers	No. of fruits two months after pollination	Fruit set rate (%)	No. of fruits harvested
'Tozeur 3'x'G12-4'	95	0	0	0
'G12-2'x'Tozeur 3'	280	47	16.7	47
'G24-12'x'G24-10'	77	0	0	0
'Fasciuneddu'x'G12-2'	150	0	0	0
'G12-2'x'Fasciuneddu'	72	0	0	0
'G12-2'x'G23-15'	90	2	2.2	2
'G25-6'x'G25-8'	110	66	60	47

No: Number.

2. Growth characteristics

A high emergence rate, at about 88% was recorded one month after planting germinated seeds, which also showed high percentage. The height, the ground diameter of the stem, the number of shoot and the distance up to the first shoot of the progenies recorded at vegetative dormancy are reported on Table 3.

Table 3. Growth characteristics of controlled and open mixture bitter almond progenies

Crosses	No. of seedlings	Height (cm)	Stem diameter (mm)	No. of shoots	Distance up to the first shoot (cm)
'G25-6'x'G25-8'	34	103.88a† ± 26.99	8.95a ± 1.0	24.5a ± 8.47	15.69a ± 3.23
'G12-2'x'Tozeur 3'	32	94.28a ± 18.7	8.14b ± 0.97	20.5a ± 8.20	9.92b ± 3.12
Control: bitter almond	15	69.32b ± 11.54	6.60c ± 0.64	21.9a ± 3.01	5.72c ± 2.46

†: Mean values followed by the same letters within a column are not significantly different at the 0.05 level according to Duncan's test.

The progenies derived from control pollinated seeds were significantly ($P \leq 0.05$) more vigorous than those obtained from an unknown mixture of bitter almond. The height ranged from 94.2cm on the progenies of 'G12-2'x'Tozeur 3' cross to 103.8cm on those obtained from the crossing of 'G25-6' by 'G25-8'. Statistical analysis showed high significantly differences ($P \leq 0.01$) among stem diameters at the ground, showing the highest value by the crossing 'G25-6'x'G25-8'. All seedlings were sufficiently branched; the mean number of shoots ranged from 20.5 to 24.5 and was significantly similar. Similar rate of branching in stem seedlings, were obtained in open pollinated progenies of ‘Garrigues’ and ‘Tozeur 3’ almond varieties (Simard *et al*., 1997). An average shoot number of 17.7, 19.7, 20.1 and 22.9, was also recorded on respectively open pollinated seedlings of ’G25-8’, ‘G25-6’, ‘Tozeur 3’ and ‘G12-2’ almond genotypes planted in nursery in 2004 (Unpublished data). The mean distance up to the first shoot was significantly ($P \leq 0.01$) higher on the offspring of ‘G25-6’x ‘G25-8’ cross (15.69cm). The highest distance is the better; since it facilitates the grafting. Homogeneity was observed on progenies of ‘G12-2’ crossed by the local almond ‘Tozeur 3’. This genotype might be a good pollinator for ‘G12-2’ particularly as their open pollinated offspring showed good graft compatibility in orchard with ‘Tuono’ variety (unpublished data).

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Endophytic fungi of leaf of Atlas pistachio of Dayate Aiat (Laghouat, Algeria)

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Abstract. Atlas Pistachio, *Pistacia atlantica* Desf. is an Anacardiaceae, ubiquitous North Africa and the Middle East. In Algeria, this species is widespread in arid regions where difficult natural conditions the push to form symbioses to counter the water deficit. Its leaves are deciduous. They are present on the tree when the rains decrease and temperatures rise. To mitigate its constraints symbiotic associations with mutualistic fungal endophytes are possible. Our sampling was done at a daya of the wilaya of Laghouat. Harvested in April 2013 and ten normal individuals subjectively selected leaves are stained by the method of Hayman and Phillips (1970) and observed under an optical microscope. Observations have shown the presence of several differently colored structures. Different ranges of colors are distinct along the limbus at all compartments of the leaflets. Endophytic fungi are noted in stomata, glandular hairs and between certain epidermal cells within parenchymal cells, but also in the primary and secondary veins (xylem and phloem). A variety of fungal endophytes is noted in the leaves of pistachio Atlas. These are probably able to maintain the eco-physiological and adaptive balance of this species under the restrictive conditions of the natural environment in which it lives.

Keywords. *Pistacia atlantica* Desf. – Laghouat – Algeria – Foliar mycoendophytes.

Champignons endophytes foliaires du pistachier de l'Atlas de dayate Aïat (Laghouat, Algérie)

Résumé. *Le pistachier de l'Atlas,* Pistacia atlantica *L. est une Anacardiacée, ubiquiste du Nord de l'Afrique et du Proche Orient. En Algérie, cette essence est répandue dans les régions arides où les conditions naturelles difficiles la poussent à former des symbioses pour contrer le déficit hydrique. Ses feuilles sont caduques. Elles sont présentes sur l'arbre lorsque les pluies diminuent et les températures augmentent. Pour pallier à ses contraintes, des associations symbiotiques avec des champignons endophytes mutualistes sont possibles. Notre échantillonnage a été fait au niveau d'une daya de la wilaya de Laghouat. Les feuilles récoltées en avril 2013 sur dix individus sains et choisis de manière subjective, sont colorées selon la méthode de Phillips et Hayman (1970) et observées au microscope optique. Les observations ont montré la présence de plusieurs structures colorées différemment. Des plages de différentes couleurs sont bien distinctes tout le long du limbe, au niveau de tous les compartiments des folioles. Des champignons endophytes sont notés au niveau des stomates, des poils glandulaires et entre certaines cellules épidermiques, à l'intérieur de cellules parenchymateuses, mais aussi au niveau des nervures principale et secondaires (xylème et phloème). Une diversité de champignons endophytes est notée au niveau des feuilles du pistachier de l'Atlas. Ces derniers sont probablement capables de maintenir l'équilibre écophysiologique et adaptatif de cette espèce sous les conditions contraignantes du milieu naturel dans lequel elle vit.*

Mots-clés. Pistacia atlantica *Desf. – Laghouat – Algérie – Mycoendophytes foliaires.*

I – Introduction

The Atlas pistachio is the most ubiquitous tree in Northern Africa and the Middle East (Monjauze, 1980). It presents an ecological amplitude and a remarkable plasticity. It is found in the heart of the Sahara to the edges moist bioclimate (Quézel and Médail, 2003). These plants adapted to arid ecosystems have developed several mechanisms, such as symbiotic associations that reduce this stress, improve nutrition and survival (Barrow Aaltonen, 2001). These symbioses are often contract with fungi and can concern the roots, but also the leaves. They are an integral part of plant microbiome. The mycoendophytes penetrate plant tissues without causing disease symptoms (Li *et al*., 2012). They confer benefits to their hosts through improved nutrient absorption (Mandaym and Jumpponen, 2005) and increased resistance to pathogens (Ghimire *et al*., 2010).

This mycoendophytes diversity is not known at the Atlas pistachio. We are interested in this study to its highlighted and its distribution in the different compartments of the leaves of this species.

II – Material and methods

The leaves of pistachio Atlas were collected in April 2013 from dayate Aïat (area of Timzerth, Laghouat), of ten subjects chosen subjectively. The subjects were in good health status. For each selected tree, ten leaves are harvested around the canopy. For the coloring of the leaves, it was made according to the protocol of Phillips and Hayman (1970). Observations were made by an optical microscope. Photos are taken at different magnifications.

III – Results and discussion

Observations under an optical microscope reveals the presence of hyphae at the inter– and intracellular spaces of epidermis of the leaf of *Pistacia atlantica* (Fig. 1).

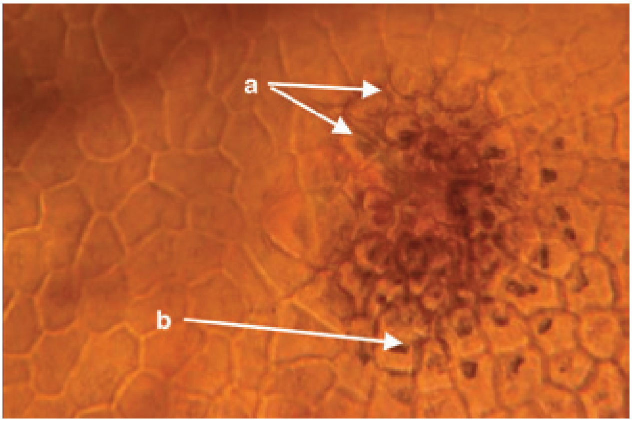

Fig. 1. Microscopic observation of inter (a) and intracellular (b) colonization of the epidermal cells of the leaf of Atlas pistachio (X 400).

These results are agree with those of Bernadi-Wenzel *et al*. (2010) and Orlandelli *et al*. (2012), which showed that endophytic fungi can form associations from an inter and intracellular colonization of the host plant tissue. It should be noted that the intercellular space is rich in substances needed to support the growth of fungal endophytes (Kuldan and Bacon, 2008).

The presence of mycoendophytes seems as important in stomata (ostioles, guard cells and the cells that surround the stomata) (Fig. 2). Fungal endophytes can alter hormone levels that control the stomatal behavior and osmotic adjustment (Mandyam and Jumpponen, 2005 ; Bezzerra *et al*., 2013).

Glandular hairs are also affected by the presence of endophytic fungi (Fig. 3). Indeed, they are responsible for a significant portion of the secondary chemistry of a plant (Glas *et al*., 2012).

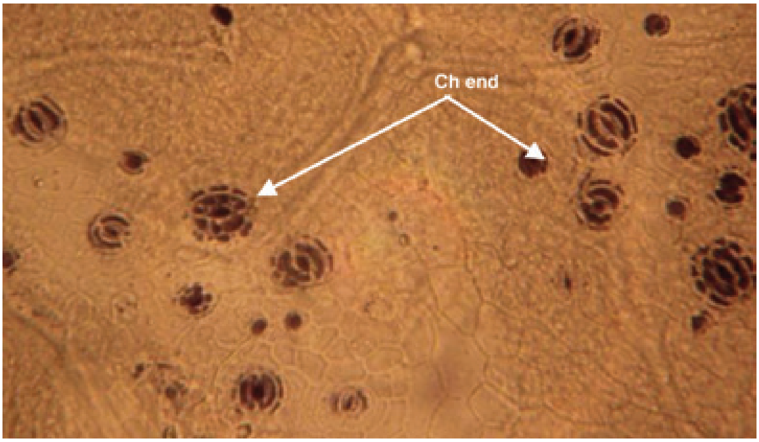

Fig. 2. Microscopic observation of endophytic fungi colored in blue (ch end) in the stomata of the leaf of Atlas pistachio (X 400).

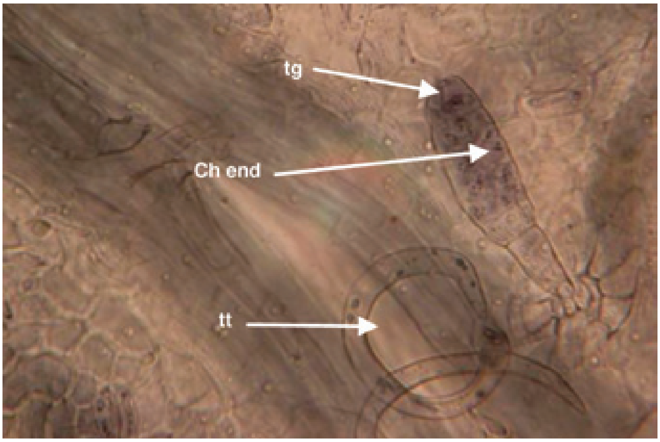

Fig. 3. Microscopic observation of a glandular hair (tg) colonized by mycoendophytes (Ch end) and a tector trichome (tt) not colonized by them at the leaf of Atlas pistachio (X 400).

It should be noted, however, the absence of endophytic fungi at the tector trichomes (Fig. 3). This can be explained by the absence of synthetic defense metabolites in these structures. In fact, the tector trichomes provide more mechanical role against abiotic and biotic factors (Szyndler *et al*., 2013).

Microscopic observations of leaves show the existence of endophytic fungi within and between the cells of the parenchyma, but also at the level of conductive tissues (xylem and phloem) (Fig. 4). These results confirm those of Sanchez-Azofeira *et al*. (2012).

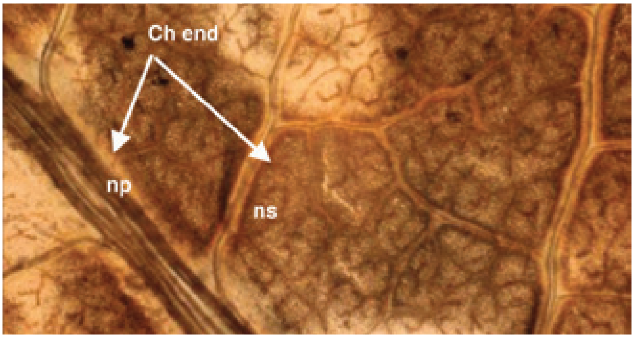

Fig. 4. Microscopic observation of endophytic fungi (Ch end) at the midrib (np) and secondary veins (ns) of the leaf of Atlas pistachio (X 40).

IV – Conclusion

Microscopic observation leaves *Pistacia atlantica* Desf. stained with trypan blue showed the presence of endophytic fungi in almost all compartments of the leaf (epidermal cells, stomata, glandular hairs, parenchyma, xylem and phloem) from all sampled subjects.

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Rate of endomycorrhizal colonization in *Pistacia vera* L.

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Abstract. In arid areas, plants develop several strategies to adapt to bad conditions of survival especially drought. To resist, they develop associations with symbiotic fungi: mycorrhizae. The object of our study is to know the influence of seasonal variation (spring and winter) on root colonization rates by these fungi. For this purpose we chose *Pistacia vera* L., a fruit species cultivated in the orchard of Ross-Loayone located in the province of Djelfa in Algeria. The soil analysis have revealed that the soil is not salty, has high total calcium and is rich in organic matter. Microscopic observations showed the presence of endomycorrhizae-type fungal structures belonging to the class of Glomyromycètes. These fungal structures are vesicles, arbuscules and siphoned hyphae. T-test shows that there is a highly significant difference at p <0.001 between two seasons for the number of vesicles and arbuscules per fragment, that they are higher in the spring and that there is a good positive correlation between them. The hyphae are siphoned intra and extra –roots, and they are present at high rate in the winter.

Keywords. Arid areas – Endomycorrhizae – *Pistacia vera* L. – Djelfa.

***Évaluation du taux de colonisation endomycorhizienne chez* Pistacia vera L.**

Résumé. *En milieu aride, les végétaux développent plusieurs stratégies pour s'adapter aux conditions difficiles de survie surtout pour la sécheresse. Pour résister, elles développent des associations avec les champignons symbiotiques : la mycorhize. L'objectif de notre étude est de connaitre l'influence de la variation saisonnière (printemps et hiver) sur le taux de colonisation par ces champignons. Pour cela on a choisi le Pistacia vera qui est une espèce fruitière cultivé dans le verger de Ross-Loyoune situé dans la province de Djelfa en Algérie. Les résultats obtenus ont révélé que le sol est non salé, a un taux élevé de calcaire total et est riche en matière organique. Les observations microscopiques ont montré la présence des structures fongiques de type endomycorhize appartenant à la classe des Glomyromycètes. Ces structures fongiques sont les vésicules, les arbuscules et les hyphes siphonnés. Le test- t montre qu'il y a une différence hautement significative à p<0.001entre les deux saisons pour le nombre de vésicules et le nombre des arbuscules par fragment. Ils sont élevés au printemps et ont une corrélation positive élevée entre eux. Les hyphes siphonnés se trouvent au niveau intra et extra–racinaire et, sont plus élevés en hiver.*

Mots-clés. *Milieu aride – Endomycorhize –* Pistacia vera L. *– Djelfa.*

I – Introduction

Terrestrial plants have developed numerous strategies to cope with diverse edapho-climatic conditions. One of the most successful strategies is the ability of root systems to establish symbiotic relationships with mycorrhizal fungi (Gianinazzi-Pearson, 1996). The plant typically provides fungus carbohydrates while the latter provides a water intake or elements by increasing the absorption surface (Dalpé, 1997).

This study was interested to determine the type of mycorhizal association and to know the effects of seasonal variations (spring and winter) on arbuscular mycorrhizae (AM) colonization, for understanding more about this dynamic life process. For this we chose *Pistacia vera* L., a fruit species belonging to the family of Anacardiaceae. The development of this culture is interesting for many arid and semi-arid regions in Algeria while the study of these symbiotic fungi is the one of the best solutions to improve its quantitative and qualitative production.

II – Materials and methods

The study area is located in Ross-Loyoune the province of Djelfa which is 300 km south of Algiers, at latitude 34°38' North and longitude 3°15' East. This site is located at 1153 m in altitude, it is characterized by a semi-arid climate with cool winters, average temperatures of 16.78°C and rainfall of 312.76 mm/year. Soils under *Pistacia vera* trees are alcaline (pH: 8.90) and unsalty (Electrical conductivity: 0.163 mmhos/cm) with 43.04%) in humidity. The organic matter content is about 4.35%. These soils are highly calcareous (TC ≥ 34.05) with a high amount of active calcareous (AC = 12.5).

Sampling was conducted in 2014. The roots were collected simultaneously with soils under 10 trees of *Pistacia vera* L., at one level (20 cm) and in two seasons (spring and winter). Roots with diameter less 0.5 mm were selected from the soil samples, cut (≈1 cm) and preserved in ethanol at 70°.

The observation and detection of AM fungi and their structures (vesicles, hypheas and arbuscules) is only possible after the treatment of the samples adopting the Phillips and Hayman staining technique (1970). Roots fragments are mounted between microscope slides and cover slips then observed under light microscopy. A total of 1000 observations are performed. Brundrett *et al*. (1996) method is used to count the number of vesicles, arbuscules and hyphae per fragment, where an observer simply provide a visual estimate of the degree of mycorhizal colonisation.

Descriptive statistics, correlation matrix and t-test of student were performed using Excel software.

III – Results and discussion

Our observation about colorful roots of *Pistacia vara* shows colonization by AM in both spring and winter seasons. These AM are characterized by the presence of three structures: the vesicles, the arbuscules and the siphoned hyphae (Table 1). The vesicles are present in a large number, having different sizes and shapes (oval or round, large and small). These structures are formed in the intracellular cell wall where they appear much larger than in the intracellular spaces of the cortical parenchyma. Arbuscules were observed within some root cells (structure of exchange between partners). Siphoned hyphae are intracellular or intercellular. The involved fungi belong to the monophyletic group of Glomeromycetes (Balzergue, 2012).

The number of vesicles and arbuscules per fragment is important in spring (exceeds 2) (Table 1). While for hyphae, their number was higher in winter (0.6 hyphae per fragment). The important number of symbiotic structures (arbuscules and vesicles) in spring time may be due to the increase in symbiotic exchange between plants and AM. The arbuscules are the functional organ between the two partners (plant and fungus), while the vesicles are the preserved organ in root cells (Harly and Smith, 1983).

The t-test shows a highly significant difference ($p < 0.001$) between the two seasons regarding the number of vesicles and arbuscules per fragment, while no significant difference is registered for the number of hyphae (Table 1). In spring time, fungus activity is increased, showing higher values for the vesicles and arbuscules; this can be explained by the symbiotic relationship between the two partners, as the leaves of this species synthesized sugars (source of energy for the fungus) in spring time trough photosynthesis. Correlations between the fungal structures are not significant except between vesicles and arbuscules, the value is moderately significant ($r = 0.62$). After Hampp and Wingler (1997) sugars (sucrose and glucose), are transformed by fungus into specific sugars (trehalose or mannitol) and consumed for growth and respiration. This flow of carbon is also carried out in the form of amino acids and vitamins (biotin and thiamine) (Lassac, 1992).

Table 1. Number of vesicles, arbuscules and hypheas per 1 cm root fragment of *Pistacia vera* in spring and winter time

	Spring Mean ± S.D (min-max)	Winter Mean ± S.D (min-max)	T test
Number of vesicles (cm^{-1} root)	2.16 ± 2.53 (0-9)	0.78 ± 1.34 (0-6)	***
Number of arbuscules (cm^{-1} root)	2.78 ± 2.53 (0-7)	0.92 ± 1.55 (0-7)	***
Number of hyphae (cm^{-1} root)	0.38 ± 0.95 (0-4)	0.6 ± 0.93 (0-4)	NS

S.D.: Standard deviation; *** P < 0.001; NS, not significant.

IV – Conclusions

According to our results, there is a close relationship between the two partners, translated by the number of the fungal structures which may change depending on the vegetative state of the plant, the latter being influenced by the climatic conditions. In spring time (which matches with higher arbuscules and vesicles rates in the rooting system), in presence of solar radiation, plants photosynthesize better, this requires water which is extracted by fungus from the soil particles and solution. In return, the fungus get photosynthesized sugars which are stored or used for their development. In general, the use of the mycorhizae in agriculture must be regarded as a biological strategy for the increase and the improvement of the production. Hence, the tolerance and adaptation of *Pistacia vera* to a large range of soil types is due to symbiotic fungi such as endomycorrhizae which play several roles such as resistance to drought, protection against disease and an increase in production. Studying symbiotic fungi is one of the solutions to improve pistachio culture. It has been suggested that AM symbiotic association can be used as a biofertilizer to improve nutrient cycling and crop productivity by reducing the fertilizer inputs, thereby conserving soil fertility and reducing production and environmental costs. Mycorrhizal colonization, by helping plants to become established in eroded and degraded habitats, for example in soils with high calcareous rate in arid and semi-arid areas, can be regarded as an important alternative strategy for a more rational and sustainable agriculture. This work is a first contribution to the knowledge of behavioral response of *Pistacia vera* and its corresponding mycorrhizal fungus. Further trials must be conducted in different sites and during different seasons in order to improve our knowledge in this field.

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Genetic diversity of almond rootstocks. The INRA *Prunus* rootstock breeding program

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Abstract. Almond and peach seedlings and peach*almond hybrids are still the most grown almond rootstocks in the Mediterranean countries. However a lot of new almond rootstocks were released in the last years, with different vigour, disease and nematode resistance, tolerance to abiotic stress. The almond rootstock choose is become more important, but there are opportunities for new rootstocks adapted to certain soil types like heavy soils or adapted to new high density systems. It could be possible to create them by interspecific crosses between several Prunus species, checking first the graft-compatibility with almond varieties. The main objective of the Breeding rootstock program is to pyramide three root-knot nematode resistance genes by interspecific crosses almond*peach*myrobalan.

Keywords. Almond rootstock – Root-knot nematodes – Resistance gene – *Prunus* species.

Diversité génétique des porte-greffes de l'amandier. Le programme d'amélioration des porte-greffes Prunus à l'INRA

Résumé. *Les semis d'amandier et pêcher, ainsi que les hybrides pêcher*amandier sont toujours les porte-greffes les plus utilisés dans les pays méditerranéens. Cependant beaucoup de nouveaux porte-greffes ont été inscrits dans les dernières années avec différentes vigueurs, avec des résistances aux maladies et aux nématodes, tolérants aux stress abiotiques comme l'asphyxie racinaire ou des porte-greffes adaptés à des systèmes haute-densité. Il est possible de créer de nouveaux porte-greffes par croisements interspécifiques entre les différentes espèces Prunus, mais en vérifiant l'incompatibilité au greffage avec les variétés d'amandier. Le principal objectif du programme porte-greffe INRA est de pyramider 3 gènes de résistance aux nématodes à gales par croisement interspécifique amandier x pêcher x myrobolan.*

Mots-clés. *Intensité de floraison – Persistance – Germoplasme –* Prunus*.*

I – The place of the of almond rootstocks in the Redher *Prunus* classification

Rootstock allows a good adaptation of the almond trees to the soil and can be prevent the tree decline against biotic and abiotic stress. Many *Prunus* species are used as rootstocks for apricot, peach or almond crops. The wide genetic variability within the *Prunus* genus allows to breed a high diversity of rootstocks for acumulating the characteristics by intraspecific or interspecific crosses. In the Redher *Prunus* classification, Fruit Prunus species are grouped in the three sections *Cerasus*, *Prunophora* and *Amygdalus* (Figure 1). For almond rootstocks. most of them belong to the section *Amygdalus* because the species of this section have good graft-compatibility with almond varieties. For calcareous soils, peach*almond hybrids are preferred to peach seedlings because they have better tolerance and a higher vigour. 'GF677' is still widely used, but the peach*almond hybrids like 'Hansen' and 'Garnem', resistant to root-knot nematodes replace 'GF677' in the infected soils. In the heavy soils, plum rootstock or amygdalus*prunophora hybrids are the best good alternative. They are more tolerant to root diseases such as *Armillaria* than peach and almond. In California, the prunus Marianna clone 'M2624' is the most planted, but now there are new Amygdalus*Prunophora hybrids clones like 'Krymsk, 'Atlas','Viking' that would be replaced 'M2624'. The INRA hybrids 'Myran' and 'Ishtara' are not very planted, but have shown good results in heavy soils, like also for some slow growing plums from domestica and insititia species that are graft-compatible with almond.

Microcerasus species like *Prunus besseyi* are interesting genitors to obtain dwarfing rootstocks. The new rootstock Rootpac®20 is well adapted for the super high density orchards. The horticultural characteristics of the main commercial almond rootstocks are summarized in the Table 1.

II – The INRA *Prunus* Rootstock breeding program

A lot of *Prunus* rootstock are available to cover all soil types (cf Table 1) and several of them are resistant to RKN nematodes (Reighard and Loretti 2008), however in prevision of the climatic changes, in all rootstock breeding programs, the RKN resistance is a required trait to introduce and face the risk of breaking resistance, it is important to build temperature-stable resistances. Three RKN resistance genes have been identified in three *Prunus* species, *Ma* in the myrobalan plum (*Prunus cerasifera,* clone P2980 and P2175), *RMia* in the peach rootstock 'Nemared' and *RMja* in the almond 'Alnem' variety. So pyramiding these three genes by interspecific crosses almond* peach*myrobalan is the main objective of the breeding rootstock program.

However, to ensure the presence of the three genes in a same rootstock, it has been necessary to develop molecular markers to detect genes in the 3 way-hybrids. The identification of intra-gene markers for the two nematodes resistance genes *Ma* and *RMia* has allowed to apply marker assisted selection for these two genes (Claverie *et al.*, 2011, Duval *et al.*, 2014). A new F2 almond population of Lauranne x Alnem, with more 1000 hybrids has been created in order to clone the *RMja* resistance gene of 'Alnem' and to find intragenic molecular markers for this gene. The *RMja* gene is localized in the linkage group 7 of the *Prunus* genome in the same region than the *Ma* gene (Van Ghelder *et al.*, 2010). After that, it will be possible to select in the hybrid population' (Alnem*Nemared)*myrobalan', the pyramided hybrids with the three nematode resistance genes.

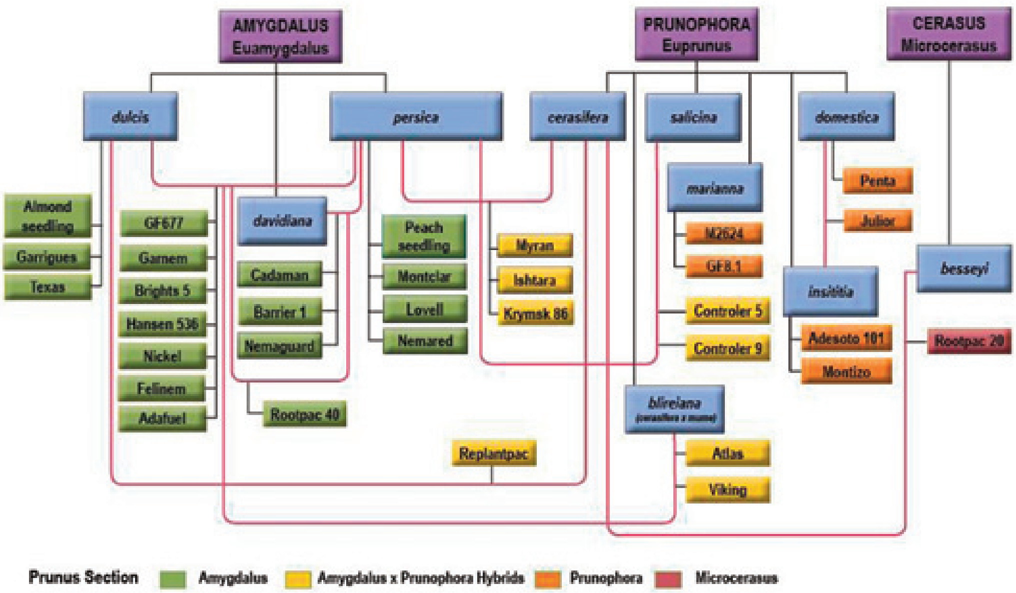

Fig. 1. Main almond rootstocks positioned in the Redher *Prunus* classification.

Table 1. Horticultural characteristics of commercial almond

Name Rootstock	Vigor	Heavy soil	Chlorosis	Nematode				Armillaria
				RKN -Mi	RKN -Ma	RKN -Mj	Lesion	
Almond seedling	standard	poor	high	S	S	S	S	S
Lovell	standard	fair	poor	S	S	S	S	S
Nemared	standard	poor	poor	R	R	MS	S	S
GF677	high	fair	high	S	S	S	MR	S
Hansen 536	high	fair	high	R	R	MR	MR	S
Brights 5	standard	fair	high	R	R	MR	MR	S
Garnem (GN15)	high	fair	high	R	R	MS	MR	S
Nemaguard	standard	fair	poor	R	R	MR	S	S
Cadaman®avimag	high	fair	fair	R	R	MR	S	MS
Barrier I	standard	fair	fair	R	R	MR	S	S
Krymsk 86	semi-dwarf	good	poor	S	S	S	S	S
Rootpac® R	standard	good	good	MR	MR	MR	S	
Ishtara®Ferciana	semi-dwarf	good	good	R	R	R	MS	MR
Myran®Yumir	semi-dwarf	good	poor	R	R	R		MR
Atlas	standard	poor	fair	R	R	R	S	
Viking	high	fair	fair	R	R	R	S	
Marianna 2624	semi-dwarf	good	good	R	R	R	S	R
Penta	semi-dwarf	good	good	R	R	MR	S	
Julior®	semi-dwarf	good	fair	R	R	R	S	
Rootpac®20	dwarf	good	fair	R	R	R	R	

RKN : Root-Knot Nematode (Mi: Meloidogyne incognita, Ma: M arenaria, Mj: M javanica).

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Effectiveness of histological techniques for early identification of scion rootstock incompatibility

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Abstract. Traditionally almond was cultivated in Tunisia extensively under rainfed conditions with local almond seedlings as rootstock. The new tendency for modernization of almond orchards has implemented the use of some non native cultivars that are compatible with the existing rootstocks. In order to evaluate the behaviour of some local cultivars for high density orchard some trials were established using the cultivars 'Achaak' and 'Porto' with 'GF-677' and 'Garnem' as rootstocks. Early observations revealed a plausible incompatibility of the cultivar 'Achaak' with 'Garnem'. In order to contribute to the understanding of this phenomenon and to detect other cases of scion-rootstocks incompatibility histological techniques were used as an early detection practice. Preliminary results for the combination 'Achaak'/'Garnem' showed some discontinuity of cells in the intermediate zone between scion and rootstocks in addition to an accumulation of black points of starch. Moreover, observations in field showed low circumferences values and weak growth of both parts. Our work reveals for the first time this scion-rootstock incompatibility and underlines the necessity of biochemical tools for a better understanding of this phenomenon.

Keywords. Local cultivars – Intensification – Interface scion/rootstock – *Cambium* cells.

Efficience des Techniques histologiques pour la détection précoce de l'incompatibilité greffon/porte-greffe chez l'amandier

Résumé. *Traditionnellement, l'amandier a été conduit en Tunisie en extensif avec les semis d'amande amer comme porte-greffes. La nouvelle tendance vers la modernisation des vergers d'amandier a entrainé l'utilisation de variétés introduites avec des porte-greffes bien adaptés à ce système de conduite. Dans le but d'étudier le comportement de quelques variétés locales d'amandier conduites en vergers à haute densité un essai a été réalisé pour la variété 'Achaak' greffée sur les deux porte-greffes 'GF-677' et 'Garnem'. Les premières observations ont montré l'existence de quelques symptômes d'incompatibilité avec ce dernier. Dans le but d'approfondir nos connaissances sur le sujet on s'est intéressé à l'application de quelques techniques histologiques pour une éventuelle détection précoce de cas semblables d'incompatibilité. Les premiers résultats ont montré chez la combinaison 'Achaak'/'Garnem' une discontinuité au niveau des cellules cambiales existantes dans la zone d'interface et une accumulation de polyphenols. Ces résultats viennent appuyer des observations sur terrain relatives à un rétrécissement de la circonférence des individus étudiés de part et d'autre du point de greffe et de confirmer l'incompatibilité de la variété 'Achaak' avec le porte-greffe 'Garnem'. Finalement ce travail souligne l'efficacité des techniques histologiques pour une détection précoce et approuve l'apport de l'outil biochimique.*

Mots-clés. *Variétés locales – Intensification – Interface greffon/porte – Cellules du* cambium.

I – Introduction

In Tunisia, Almond [*Prunus dulcis* (Miller) D.A. Webb, syn. *Prunus. amygdalus* Batsch] has been known since the Carthaginian era. About 90% of the almond production is in the semi-arid central and southern part of the country and conducted under rainfed conditions. Traditional almond plantations are dominated by old selected cultivars such as 'Achaak', 'Fekhfekh', 'Ksontini', 'Zahaaf', selected by farmers for fruit quality, good production and high adaptation to specific agro-ecosystems (Gouta *et al*, 2012) and grafted on bitter almond seedlings.

Three rootstocks 'GF-677', 'Cadaman' and 'Garnem' are being used when almond trees are conducted under irrigation conditions. Each one has advantages and disadvantages associated with its use. Although 'GF-677' is not resistant to nematodes, it is still used by nurseries while it has not shown any compatibility problems with any of the local cultivars. 'Cadaman' was used for few years but due to many phytosanitary problems it was replaced by 'Garnem'. In fact, this last was supposed to be tolerant to irrigated soil conditions and resistant to root knot nematodes. With the recent tendency for almond planting under irrigation conditions and in order to overcome the excessive use of introduced almond cultivars in modern plantations, 'Garnem' was used as a rootstock for local cultivars such as 'Achaak', 'Fekhfekh', 'Ksantini' and 'Zahaaf'. Field observations and discussions with many responsible of nurseries have incited some researcher to evocate a dwarfing effect and an eventual incompatibility of this rootstock when used with the most important local cultivar 'Achaak'. For that, and in order to contribute to a deep study of this phenomenon an investigation was started since last year. The aim of this study was to prospect plantations where 'Garnem' was used as a main rootstock and to establish a preventive method based on histological techniques to an early detection of scion-rootstock incompatibility for local almond cultivars.

II – Materials and methods

Two rootstocks 'Garnem' and 'GF-677' showing different graft compatibility performance with almond cultivar 'Achaak' were used. The two rootstocks were propagated in a seed plot and transferred in a nursery. Budwood of the cultivar 'Achaak' were collected from the Experimental Station of Ettaous (Sfax, Tunisia) and grafted in situ on the two rootstocks on June 2013. Two types of combinations were carried out (GF-677/Achaak and Garnem/Achaak) for a deep understanding of the phenomena. Scion/rootstock interface zones were collected and fixed for further histological analyses, after five and eight months of grafting.

Three graft unions per combination were collected and each stem piece (3-5 mm in diameter) was fixed in 2.5% glutaraldehyde, containing 0.1% caffeine in 0.1 M phosphate buffer (pH 7.4), for 4 h at 4°C according to Ermel *et al*., (1999). A free hand section is the simplest method of preparing sections for microscopic viewing. Stem pieces were cutting several sections at a time. Sections will certainly vary in thickness. The thinnest sections were selected and transferred onto a glass slide. Samples were swabbed in 50% (v/v) sodium hypochlorite for 20 min. and rinsed at least three-times with sterile distilled water. The last step two drops of acetic acid were added. Sections were stained with iodine green and observed under a bright field in a light microscope (Ernst Leitz, Wetzlar, Germany) equipped with a digital camera (Sony, Japan).

III – Results and discussion

Field observations for 'Achaak' grafted on the rootstock 'Garnem' showed no sign of incompatibility at the nursery level. In fact the scion is normally vigorous with green leaves. Nevertheless, after two to three years of plantation the first clear symptoms are a tendency for dwarfing. In fact both parts remain weak with small trunk diameters and no expanding branches and shoots with large cracklings at both sides of the grafting point (Fig. 1A, 1B, 1C and 1D). This corroborate with earlier observations of Kester and Hansen (1964) who reported that the incompatibility symptoms advance, trees show signs of ill-health even in the early spring: poor growth, dieback of shoots, small leaves and small trunk diameters and general decline sometimes results in death.

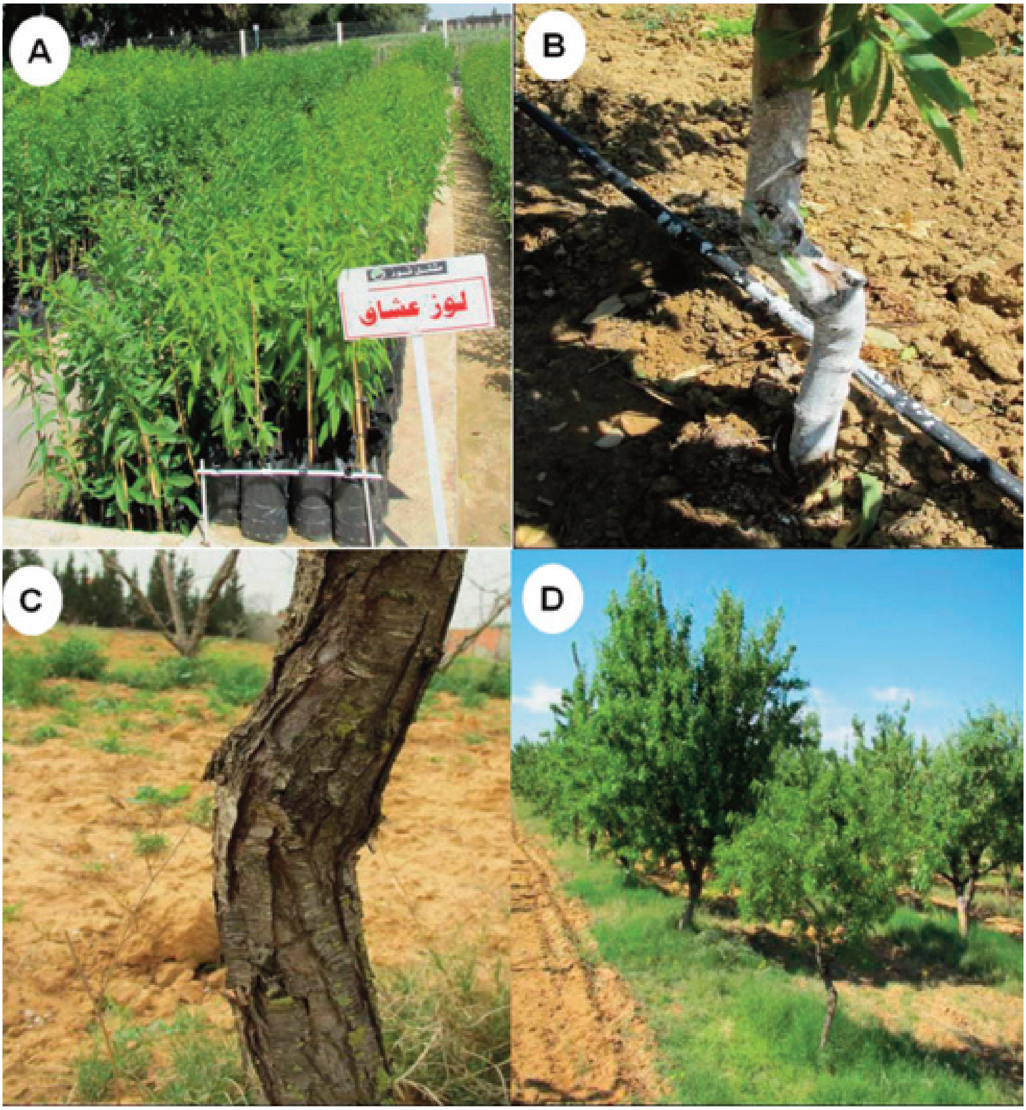

Fig. 1. Field observations of incompatibility between scion cv. 'Achaak' and rootstock 'Garnem'. (A) Nursery plants with normal behavior. (B) Three years old 'Achaak' tree grafted on 'Garnem' (C) Symptoms of large cracklings at both sides of the grafting. (D) Difference in vigor between 'Achaak' and 'Fekhfekh' cultivars grafted on 'Garnem' rootstock.

Figure 2 shows the major differences found in the histological study at the interface level, related to cambium and vascular organization. In fact, the interface scion/stock showed a separation between the bark tissues of the two components at the graft union. Also, a distinct brown line at the union delimiting scion from rootstock tissues (Fig. 2B) was strongly stained with iodine green and was continuous for the incompatible combination 'Achaak'/'Garnem (Fig. 2C). The compatible combination 'Achaak'/'GF-677' showed organized and homogenous arrangement of cambium cells in the contact interface with a complete differentiation of wood rays and scion phloem (Fig. 2D). In contrast, cambium cells in the incompatible interface showed a disorganized arrangement (Fig. 2A).

Differences exclusively located at the scion/rootstock interface mainly in cambium and vascular components, have already been mentioned by other authors (Zarrouk *et al*., 2010) for some peach/plum cultivars and are in line with reports for woody (Ermel *et al*., 1999) and herbaceous species (Wang and Kollmann, 1996). Moreover, the fact that the arrangement of the cambium cells was less organized in graft interface of the incompatible combinations corroborates previous reports regarding apricot (Errea *et al*., 2001) and pear (Ermel *et al*., 1997). Moreover, as it was already reported in pear (Espen *et al*., 2005), incompatible grafts showed less tracheary elements (Fig. 2B). Finally, this supports, that cambium cell disorganization is an early indicator of graft incompatibility.

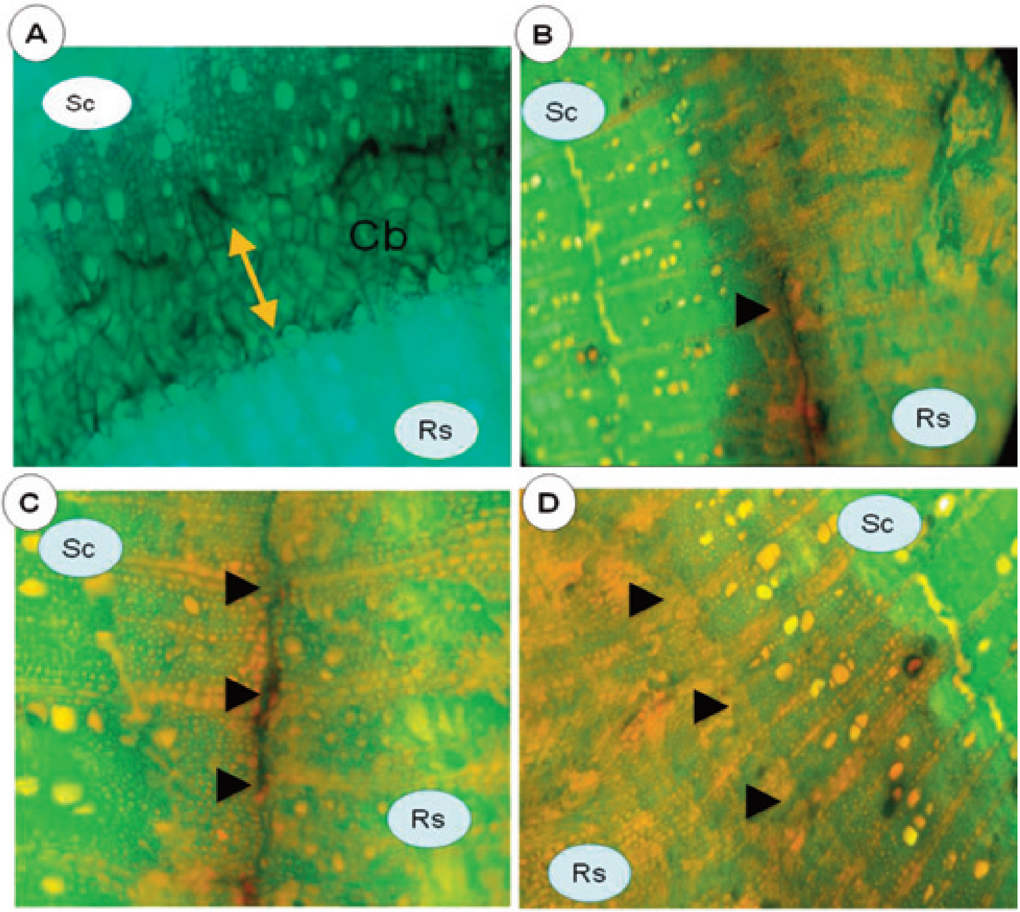

Fig. 2. Transversal sections of graft interface structure of 'Achaak'/'Garnem' and 'Achaak'/ 'GF-677' combinations. (A) Cell cambium disorganization. (B) Incompatible overstraining line delaminated part of stock from scion. (C) Continuous line of the interface zone. (D) Compatible line delimited stock 'GF-677' from scion 'Achaak'.

IV – Conclusions

Field observations regarding the scion/rootstock incompatibility between ‘Achaak’ and ‘Garnem’ were confirmed by histological techniques. At a first level the heterogeneous cambium cell arrangement can be considered as a first indicator followed by the presence of the brown line at the union delimiting scion from rootstock tissues. Thus, our study confirmed the efficiency of this technique for early detection of scion/rootstock incompatibility for almond.

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Budding success and growth aptitude under rainfed conditions during the first year of plantation: Comparison between *Pistacia terebinthus* L. and UCB-I

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Abstract. In Spain, pistachio area was negligible in the 1990's, nowadays, it is estimated to 6,000 hectares. However, one of the most important issues that created doubt among farmers for its introduction has been the choice of rootstock. The most used rootstocks in the world are *Pistacia atlantica, Pistacia integerrima, Pistacia terebinthus* and *UCB-I. Pistacia terebinthus* is a native Spanish forests rootstock known for its adaptation to the lands of the Iberian Peninsula. However, it is also known for its small vigor and its low tolerance to some diseases like *Verticilium dahliae*. In contrast, UCB-I, a hybrid between *Pistacia integerrima* and *Pistacia atlantica* is a rootstock known for its high vigor, its tolerance to *Verticillium dahliae* and its high yields, however, its behavior is unknown under rain-fed conditions. For this purpose, as a first step, a Split-plot trial comparing these two rootstocks with different cultivars under rain-fed conditions was implemented in Castilla-La-Mancha (Spain). The primary objective was to assess the aptitude of budding success and the difference in the vegetative growth of these two rootstocks under rain-fed conditions. Rootstock diameter, scion diameter, the percentage of budding success and the length of resulted scions were measured. The results showed that UCB-I had a higher percentage of budding success (70% vs. 54%), a significantly higher rootstock and scion diameters (25 mm vs 15 mm for rootstock and 22 mm vs 15 mm for scion). However there was no difference between lengths of sprouted buds. Also, there was no influence of the variety in relation to these parameters.

Keywords. *Pistacia* – Rootstock – Adaptation – Budding – Vegetative growth – Scion.

Succès du bourgeonnement et aptitude à la croissance en conditions pluviales durant la première année de plantation : Comparaison entre* Pistacia terebinthus *L. et UCB-I

Résumé. *En Espagne, la surface plantée en pistachiers était négligeable dans les années 1990, mais elle est estimée aujourd'hui à 6 000 hectares. Cependant, l'une des plus importantes problématiques qui rendait les agriculteurs réticents à l'introduire était le choix du porte-greffe. Les porte-greffes les plus utilisés à l'échelle mondiale sont* Pistacia atlantica, Pistacia integerrima, Pistacia terebinthus *et* UCB-I. *Pistacia terebinthus est un porte-greffe originaire des forêts espagnoles connu pour son adaptation aux terres de la Péninsule Ibérique. Toutefois, il est aussi connu pour son peu de vigueur et sa faible tolérance à certaines maladies comme* Verticilium dahliae*. Par contre, UCB-I, un hybride entre* Pistacia integerrima *et* Pistacia atlantica, *est un porte-greffe connu pour sa forte vigueur, sa tolérance à* Verticillium dahliae *et ses bons rendements, mais son comportement est inconnu en conditions pluviales. Dans ce but, comme premier pas, un essai split-plot comparant ces deux porte-greffes portant différents cultivars en conditions pluviales a été mis en place en Castilla-La-Mancha (Espagne). L'objectif premier était d'évaluer l'aptitude à un bon bourgeonnement et la différence de croissance végétative de ces deux porte-greffes en conditions pluviales. Le diamètre des porte-greffes, le diamètre des scions, le pourcentage de réussite du bourgeonnement et la longueur des scions résultants ont été mesurés. Les résultats ont montré que UCB-I a un plus grand pourcentage de réussite du bourgeonnement (70% vs 54%), des diamètres significativement plus grands pour le porte-greffe et le scion (25 mm vs 15 mm pour le porte-greffe et 22 mm vs 15 mm pour le scion). Cependant il n'y avait pas de différence entre les longueurs des bourgeons qui avaient poussé. Il n'y avait pas non plus d'influence de la variété pour ces paramètres.*

Mots-clés. Pistacia *– Porte-greffe – Adaptation – Bourgeonnement croissance végétative – Scion.*

I – Introduction

In Spain, pistachio area was negligible in the 1990's, nowadays, it is estimated to about 6,000 hectares (Couceiro *et al*., 2013). However, one of the most important issues that created doubt among farmers for its introduction has been the choice of rootstock since drought is the most limiting abiotic factor in the Mediterranean area and the majority of orchards are grown under rainfed conditions or with limited water inputs. Increasing crop resistance to this stress would be the most economical approach to improve productivity (Wang *et al*., 2012) and grafting is a widespread technique used in arboriculture to achieve this objective. Omitting *Pistacia vera*, the most used rootstocks in the world are *Pistacia atlantica*, *Pistacia integerrima*, *Pistacia terebinthus* and UCB-I. *Pistacia terebinthus* is a native Spanish forests rootstock known for its adaptation to the lands of the Iberian Peninsula. However, it is also known for its small vigor and its low tolerance to some diseases like *Verticilium dahliae*. In contrast, UCB-I, a hybrid between *Pistacia integerrima* and *Pistacia atlantica* is a rootstock known for its high vigor, its tolerance to *Verticillium dahliae* and its high yields, however, its behavior is unknown under rain-fed conditions.

The objective of this work was to start early the monitoring of the behavior of these two rootstocks in order to detect any difference that could prevent their use in this area.

II – Material and methods

The study was conducted during the month of June one year after the budding of an orchard located in "La Entresierra" research station, Ciudad Real (Centre of Spain) (L 3º56´W; L 39º0´N; altitude 640 m). One budding event was done in the subsequent summer after plantation. The plantation was established in the month of February and due to reasons of plants availability, UCB-I has been planted with 6 month old and *P. terebinthus* (PT) with 2 year old and was previously maintained in pots under greenhouse conditions. Weeds were controlled with a tillage management and irrigation was applied only at the moment of plantation. The climate in this area is Mediterranean with an average annual rainfall of 397 mm, mostly distributed outside a four-month summer drought period. The soil at the experimental site is an alkaline (pH 8.1) shallow soil with a discontinuous petrocalcic horizon located at 0.50 m (Petrocalcic Palexeralfs), with a clay loam texture, low electrical conductivity (0.2 dS m^{-1}), 1.05% of organic matter, 0.12% of nitrogen, 17 × 10^{-4} mol kg^{-1} potassium levels and high cationic exchange capacity (0.186 mol kg^{-1}). The volumetric water content of the soil for the first 0.3 m of depth was 22.8% at field capacity (soil matric potential -0.03 MPa) and 12.1% at permanent wilting point (soil matric potential -1.5 MPa); from 0.3 m to 0.5 m it is 43.0% and 21.1%, respectively.

The experimental design was a split plot design with 3 blocks. Each experimental block was composed by two rootstocks and three cultivars for each rootstock. The main factor was rootstock: *Pistacia terebinthus* and UCB-I. The secondary factor was cultivars: Kerman, Sirora and Larnaka. Tree spacing was set at 8 x 6 m (208 trees ha^{-1}) and male cultivars were distributed at a rate of 11% equally divided between subplots.

Rootstock diameter was measured at soil level and scion diameter at 5 cm above budding point. The percentage of budding success was counted and the length of resulted scions was also determined.

III – Results and discussion

The diameter of the rootstock was highly correlated with the diameter of the scion (Fig. 1) being at both levels significantly higher in UCB-I than PT (Table 1). The rootstock diameter was 25.48 mm for UCB-I and 14.98 mm for PT. The diameter of the scion was 22 mm for UCB-I and 15 mm for PT. These

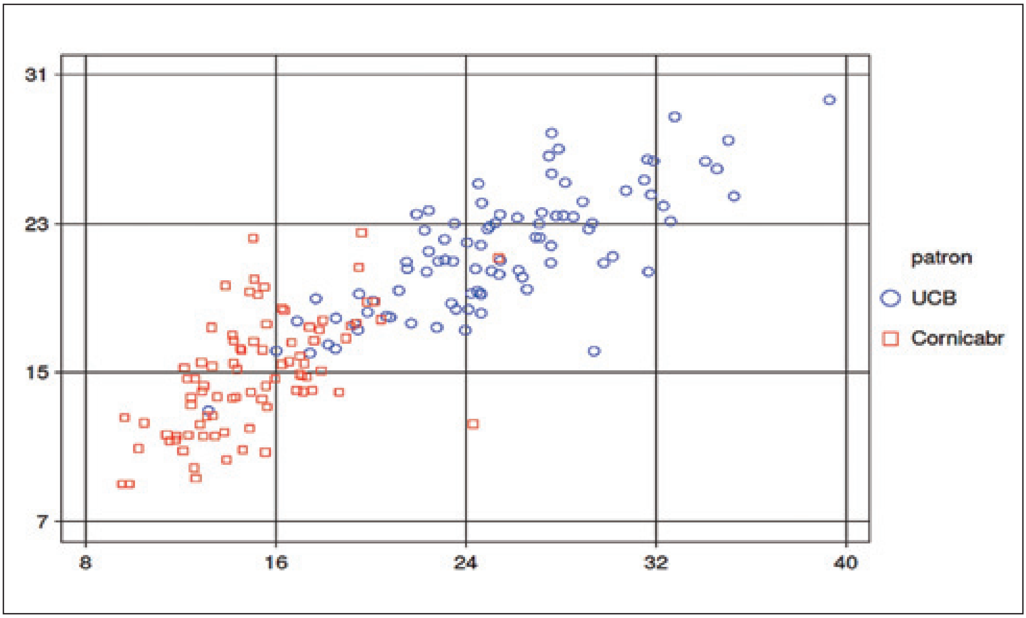

Fig. 1. Scatter graph plotting rootstock diameter in cm (X axis) vs scion diameter in cm (Y axis) of UCB-I (circles) and *Pistacia terebinthus* (squares).

Table 1. Comparison of budding success, rootstock diameter, scion diameter and shoot length between UCB-I and *Pistacia terebinthus*

Rootstock	Budding success (%)	Rootstock diameter (mm)	Scion diameter (mm)	Shoot length (cm)
UCB-I	70.41a	25.48a	22a	58.38a
P. terebinthus	54.33b	14.98b	15b	51.18a

results would have been expected under watered and deep soil conditions since UCB-I is considered the best commercial rootstock under irrigation and is widely characterized to be much more vigorous than PT (Ferguson *et al*., 2005). However, under rainfed conditions and shallow soils, it was expected that UCB-I will endure worst these conditions and would slow substantially its growth compared to PT. Nevertheless, UCB-I showed a higher growth than PT in the first year of plantation.

This behavior could be related to the age of each rootstock at the moment of plantation since the conservation of PT in pots for a longer time could generate a punctual stopping of growth at the moment of transplantation.

The length of scions was 58.38 cm for UCB-I and 51.18 cm for PT with no significant difference between both. Also, the same amplitude of shoots length variation between trees of the different rootstocks was observed meaning that there was no difference in the homogeneity of plots between the two rootstocks.

The fact that no difference was observed in the length of the resulted shoots weaken the importance of these differences in diameter growth, if the same rate of growth is followed in the next years, since the objective to reach faster a formed tree would be lost.

Regarding the budding success, UCB-I showed a significantly higher percentage than PT being respectively 70.41 and 54.33%. Guerrero *et al.* (2007) mentioned that rootstock diameter is the most important factor for budding success which could explain the obtained results.

Regarding cultivars, no influence was observed of the effect of the variety in relation to these parameters.

IV – Conclusion

UCB-I showed a very promising behavior under the conditions of Castilla-La-Mancha contrasting with the assumptions advancing its poor adaptation. However, no decisive validation could be obtained in a first year of study. These results are preliminaries and should be confirmed in the following years to have conclusive findings.

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Effect of two rootstocks on growth, yield and nut characteristics of 'Mateur' and 'Achouri' pistachio varieties

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Abstract. This experiment aimed to study the effect of rootstock on 25-year-old pistachio trees in the Tunisian northeast rainfed conditions. 'Mateur' and 'Achouri' female varieties were grafted onto *Pistacia vera* and *Pistacia atlantica* rootstocks. The vegetative and flower bud breaks have been followed from April to June. The diameter growth of current year shoot was measured. The architecture of flower clusters was studied. Yield and fruit weight, size and dehiscence rate were determined. Results showed no effect of rootstocks on the date of vegetative bud break for both cultivars. 'Achouri' developed flower clusters earlier on *P. atlantica* rootstock and showed a significantly higher shoot diameter growth on *P. vera* rootstock. The nut size characteristics showed no significant differences between both combinations cultivar/rootstock for 'Mateur'. These parameters were significantly higher on *P. atlantica* rootstock for 'Achouri'. According to these preliminary results, rootstock seemed not to affect growth and nut quality of 'Mateur' cultivar while *P. atlantica* rootstock appeared to have some beneficial effects on 'Achouri'.

Key words. Rootstock – *Pistacia* – Growth – Yield – Nut characteristics.

Caractéristiques morphométriques de variétés et écotypes locaux et étrangers de pistachier femelle dans les conditions du nord-est tunisien

Résumé. *L'effet de deux porte-greffes* (Pistacia vera *et* Pistacia atlantica) *sur les cultivars de pistachier 'Mateur' et 'Achouri' a été étudié dans les conditions pluviales du nord-est tunisien. Le débourrement végétatif et floral a été suivi d'avril à juin. La croissance du diamètre de la pousse de l'année a été mesurée. L'architecture des grappes fruitières a été étudiée. Le rendement, le poids, la taille et le taux de déhiscence des fruits ont été déterminés. Le porte-greffe n'a pas eu d'effet sur la date de débourrement végétatif chez les deux cultivars. Le débourrement floral était plus précoce sur* P. atlantica *chez 'Achouri'. Ce cultivar a eu une croissance du diamètre des pousses significativement plus élevée sur* P. vera*. Les paramètres relatifs à la dimension du fruit étaient significativement plus élevés sur* P. atlantica *chez 'Achouri' mais n'était pas affectés par le porte-greffe chez 'Mateur'. Selon ces résultats préliminaires, le porte-greffe ne semble pas avoir un effet sur la croissance et la qualité du fruit de la variété 'Mateur' tandis que le porte-greffe* P. atlantica *aurait quelques effets bénéfiques sur 'Achouri'.*

Mots-clés. *Porte-greffe –* Pistacia *– Croissance – Production – Caractéristiques du fruit.*

I – Introduction

Pistachio was used in to develop and valorise arid and semi arid regions subjected to drought. Currently pistachio plantations covers around (DGPA, 2014) concentrated in the center and the south of the country. is ranked 9th in terms of pistachio world production (FAO, 2015) with 3000 t in 2014 (DGPA, 2014). The low diversity of rootstocks as well as less adaptation of some cultivars seems to be the main problems affecting the cultivation of such crop in. Rootstock choice plays one of the most important roles in orchard management and has economical importance. Selection of the most suitable rootstock/scion combination may have positive effect on tree productivity, fruit quality and resistance to biotic and abiotic stresses. *Pistacia vera* is the main species used as rootstock in. Despite the well adaption of the native *Pistacia atlantica* species to arid conditions, its use as rootstock by farmers is still poor due to the low availability of seeds. The use of this endangered species as rootstock for pistachio varieties contributes certainly in the diversification of rootstocks and more adaptation of pistachio plantations. This study aimed to evaluate the effects of *P. vera* and *P. atlantica* rootstocks on 'Mateur' and 'Achouri' female varieties in terms of yield, growth and fruit cluster and nuts characteristics.

II – Materials and methods

This research was carried out at the INRAT Unity of Agricultural Experimentation at Mornag (North-east of). Twenty-year-old trees were conducted under rainfed conditions. The Tunisian 'Mateur' and the Syrian 'Achouri' cultivars were grafted on *Pistacia atlantica* and *Pistacia vera* rootstocks. For each treatment (rootstocks/cultivars combination), five trees of comparable size and vigor were selected for the study. The current shoot diameter was measured on Mai 14th and November 19th 2014 to determine the annual diameter growth. From April to June, vegetative and reproductive bud breaks were weekly monitored. Fruit clusters were sampled on July, before full maturity, and the total number of fruits per cluster and per each cluster branch order was counted. Fruits were harvested on August 25th at their maturity. The yield per tree was measured at harvesting on 2013 and 2014 years. The in-hull and in-shell fruit fresh weights, in-shell dry weight and nut dehiscence were measured for three replicates of 100 fruits per treatment. The nut and kernel sizes were measured with a digital caliper for 25 fruits per treatment.

III – Results and discussion

During 2014 growth season, vegetative bud break started by the 1st of April for all cultivar/rootstock combinations. Flowering of 'Achouri' cultivar started 5 to 7 days earlier (8th April) on *P. atlantica* rootstock than on *P. vera* one. No rootstock effect on phenology was observed on 'Mateur' cultivar. The rootstock had no effect on shoot diameter growth of 'Mateur' cultivar (Fig. 1). However, shoot diameter growth of 'Achouri' was significantly higher on *P. vera* than on *P. atlantica* rootstock. These results were not consistent with those of Kaska *et al*. (2002) on different cultivars that reported high or invariable shoot diameter on *P. atlantica* rootstock. In the Tunisian south-east conditions, Chelli Chaabouni *et al*. (2009) described enhancing or no effects of *P.atlantica* rootstock on shoot diameter growth of four year-old irrigated 'Mateur' trees depending on the season of growth.

Statistical data analysis related to fruit cluster showed no significant effect of rootstock on cluster ramification and on the number of fruits per cluster (Table 1). These results corroborate those of Tajabadipour *et al*. (2006). However, some differences were noted on cluster organization. Great variability was noted on the number of tertiary ramifications and the number of fruits per secondary and tertiary ramifications. This is particularly true for 'Mateur' grafted on *P. atlantica* with a standard deviation higher than mean values. 'Achouri' recorded the greatest number of fruits per infructescence independently of the rootstock. The number of fruits per primary branching was significantly higher on 'Achouri' grafted on *P. vera* than on 'Mateur' having *P. atlantica* as rootstock.

At harvest time, the yield per tree was no significantly different neither between cultivars nor between rootstocks (Table 2). Rootstocks had no effect on endocarp dehiscence rate of nuts that was significantly higher on 'Achouri' than on 'Mateur'. These results were clearly below the dehiscence rate (76%) of 'Mateur' cultivar but were comparable to the rate obtained for 'Achouri' (86%) recorded in the same orchard in 2013. The year to year variation of nut splitting noted on 'Mateur' cultivar in this study was reported by Loudyi (2001) as a common characteristic of *P. vera* cultivars. The dehiscence rate was reported to be of 70-90% for 'Mateur' (Anonymous, 1972) and 55-99% for 'Achouri' (Oukabli, 1995; Hadj-Hassan, 2001).

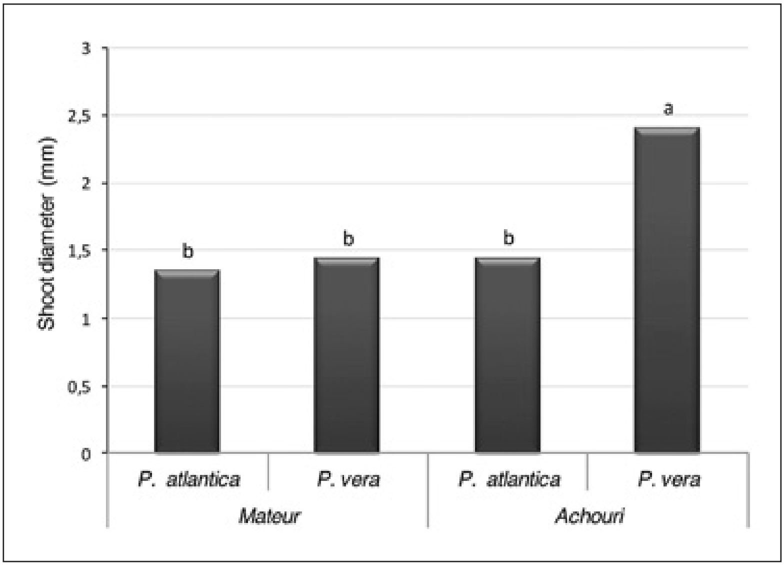

Fig. 1. Effect of Rootstock on diameter growth of current year shoot.

Table 1. Number of fruit cluster ramification and number of flowers per cluster and on each branching order

Cultivar	Rootstock	PR	SR	TR	FR	FPB	FSB	FTB	TF
'Mateur'	*P. vera*	11.1[a]	8[a]	0.8[a]	8[a]	44.4[ab]	14.8[a]	1.8[a]	68.9[a]
	P. atlantica	8.9[a]	5.8[a]	1.4[a]	6.1[a]	32.7[b]	12.7[a]	1.9[a]	53.3[a]
'Achouri'	*P. vera*	11.5[a]	9.6[a]	1.9[a]	7.5[a]	54.6[a]	19.1[a]	3.8[a]	85[a]
	P. atlantica	12.3[a]	11.7[a]	2.8[a]	6.3[a]	47.3[ab]	21.3[a]	3.7[a]	78.7[a]
SD		*3.4*	*6.5*	*2.1*	*2.9*	*21.1*	*14.3*	*3.2*	*36*

In the same column, values followed by same letters are not significantly different (Duncan; p<0.5).

PR: Number of primary ramification; SR: Number of secondary ramification; TR: Number of tertiary ramification FR: Number of fruits on rachis; FPB: Number of fruits on primary branching; FSB: Number of fruits on secondary branching; FTB: Number of fruits on tertiary branching; TF: Total number of fruits per infructescence; SD: standard deviation.

Table 2. Average yield per tree and nut dehiscence rate

Cultivar	Rootstock	Yield (kg)	Nut dehiscence rate (%)
'Mateur'	*P. vera*	8.4 ± 4[a]	32.5 ± 23.2[b]
	P. atlantica	9.1 ± 7.6[a]	31.0 ± 17.9[b]
'Achouri'	*P. vera*	7.5 ± 2.8[a]	83.8 ± 10[a]
	P. atlantica	7.2 ± 3.6[a]	76.5 ± 23.4[a]

The rootstock had no significant effect on in-hull and de-hulled fresh fruit weight of 'Mateur' cultivar. In contrary, 'Achouri' showed significantly lower values on *P. vera* rootstock. On another hand, the rootstock/cultivar combination had no effect on in-shell nut dry weight. 'Mateur' and 'Achouri' grafted on *P. vera* and *P. atlantica* rootstocks produced nuts having a dry weight ranged between 82 and 89 g (Table 3). In these experimental conditions, rootstocks seem to more affect hull than the endocarp and kernel of the fruit.

Results related to fruit size are presented in table 4. For 'Mateur' cultivar, all fruit size parameters were not affected by the type of rootstock. For 'Achouri', the lengths of in-hull and de-hulled fruit as well as the length and width of kernel were significantly higher for trees grafted on *P. atlantica* rootstock. This effect was particularly notable on kernel length whose value passed, according to rootstock, from being significantly lower to significantly higher than 'Mateur' value.

Overall, 'Mateur' and 'Achouri' cultivars showed similar fruit characteristics independently of rootstock.

Table 3. In-hull and de-hulled fruit fresh weight and in-shell nut dry weight (average of 100 unities)

Cultivar	Rootstok	Ih FW (g)	Dh FW (g)	Is DW (g)
'Mateur'	*P. vera*	219.9 ± 31.5^{ab}	127 ± 10.5^{a}	88.8 ± 7.7^{a}
	P. atlantica	229.1 ± 23.6^{a}	124.6 ± 11.7^{a}	86.2 ± 9.2^{a}
'Achouri'	*P. vera*	203.7 ± 12.3^{b}	110.6 ± 6.3^{b}	81.9 ± 4.8^{a}
	P. atlantica	217.3 ± 42.6^{ab}	123.6 ± 29.8^{a}	87.7 ± 18.7^{a}

Ih FW: In-hull fresh weight; Dh FW: De-hulled fresh weight; Is DW: In-shell dry weight.

Table 4. In-hull fruit, de-hulled nut and kernel sizes (mm)

Cultivar	Rootstok	In-hull fruit			De-hulled nut			Kernel		
		Length	Width	Thickness	Length	Width	Thickness	Length	Width	Thickness
'Mateur'	*P. vera*	24^{a}	11.8^{a}	10.7^{a}	20.5^{a}	11.1^{b}	9.8^{ab}	16^{b}	7.8^{ab}	7.6^{b}
	P. atlantica	24.3^{a}	12.1^{a}	11^{a}	20.6^{a}	11.1^{b}	9.8^{ab}	16^{b}	7.8^{ab}	7.4^{b}
'Achouri'	*P. vera*	22.3^{c}	10.9^{b}	10^{b}	19.5^{b}	10.6^{b}	9.5^{b}	15.4^{c}	7.6^{b}	7.5^{b}
	P. atlantica	23.2^{b}	10.7^{b}	10^{b}	20.6^{a}	10.9^{ab}	9.9^{b}	16.5^{a}	8^{a}	7.9^{b}

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Rootstock trial of eight GxN interespecific hybrids in almond

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Abstract. Almond growing is increasingly extending to areas where the species is not truly suited to, despite favourable climate. The most common problems in many Mediterranean countries include replanting, limestone chlorosis, and soilborne pests and diseases such as *Meloidogyne* root-knot nematodes and *Armillaria mellea* root-knot. Several almond x peach hybrids have shown good performance as rootstocks for different stone fruit species, including almond. The first commercially known hybrid was 'GF- and it is still used worldwide and demanded at present for almond growing. Eight almond x peach hybrids from the CITA breeding programme were evaluated in a rootstock trial located at CITA. They were grafted with the almond selection 'B-2- in 1994 and planted in 1995 at a distance of 5 ×, using 'GF- and 'Nemared' as controls. The trunk cross sectional area (TCSA) and productivity were evaluated. The results showed a differential performance for production and vigour between the 'GF- and 'Nemared' as compared to the GxN series. Some differences were also observed for vigour among the three already commercially propagated 'Garnem' 'Felinem' and 'Monegro'.

Keywords. Agronomic behaviour – Breeding – *Prunus amygdalus* – Vigour – Production.

Essai de huit porte-greffe interespecific hybrides GxN en amandier

Résumé. *Le culture de l'amandier est de plus en plus étendue aux zones où il n'est pas vraiment adapté, malgré leur climat favorable. Les problèmes les plus communs dans de nombreux pays méditerranéens comprennent replantation, chlorose ferrique, et les ravageurs et les maladies du sol telles que* Meloidogyne, *nématodes à galles et* Armillaria mellea *root-knot. Plusieurs hybrides amandier x pêcher ont montré une bonne performance en tant que porte-greffe pour les différentes espèces de fruits à noyau, y compris l'amandier. Le premier hybride commercialisé était 'GF-677' et il est encore utilisé dans le monde entier pour le culture de l´amandier. Huit hybrides amandier x pêcher du programme d'amélioration génétique du CITA ont été évaluées dans un essai de porte-greffe situé au CITA, Saragosse, Espagne. Ils ont été greffés avec la sélection d'amandier 'B-2-5 'en 1994 et plantés en 1995 à une distance de 5 x, en utilisant 'GF- et 'Nemared' en tant que témoins. La superficie de coupe transversale du tronc et la productivité ont été évalués. Les résultats ont montré une performance différentielle pour la production et la vigueur entre le 'GF- et 'Nemared' par rapport à la série GxN. Quelques différences ont également été observées pour la vigueur parmi les porte-greffes GxN les plus utilisés, 'Garnem' 'Felinem' et 'Monegro'.*

Mots-clés. *Comportement agronomique – Amélioration génétique –* Prunus amygdalus *– Vigueur – Production.*

I – Introduction

Almond [*Prunus amygdalus* Batsch; syn. *P. dulcis* (Mill.) D.A. Webb] is the most important tree nut crop in terms of production in the Mediterranean countries (FAOSTAT, 2015). This crop, although well adapted to the Mediterranean climate, is sometimes limited by many soil-related problems: replanting, limestone chlorosis, and soilborne pests and diseases such as *Meloidogyne* root-knot nematodes and *Armillaria mellea* root rot, which are common in this region. For these reasons, traditional rootstocks should be replaced by new ones in order to provide a good adaptation to each soil condition, thus ensuring a commercial production. Peach x almond hybrid rootstocks have been widely utilized in during the last decades. Among the interspecific hybrids, the mostly used is 'GF– due to its good adaptation to calcareous soils. However, this hybrid is sensitive to root knot nematodes (RKN). Other interspecific hybrids from the CITA breeding program are already commercially propagated, such as Felinem', 'Garnem' and 'Monegro', from the 'Garfi' x 'Nemared' cross, aimed to introduce RKN resistance from 'Nemared'. These interspecific hybrids were characterized by a good adaptability to poor soils and easy propagation by hardwood cuttings and in vitro (Felipe, 2009*).* These three hybrids are red-leafed and have shown a better performance in peach replanting conditions than 'GF- (Gomez-Aparisi *et al.*, 2000). Thus, in order to evaluate the agronomic performance of other genotypes of the GxN hybrids with almond, we studied eight different rootstocks grafted with an almond selection in a trial at CITA, during ten years.

II – Materials and methods

The experiment was carried out in a plot located at CITA. Six almond x peach hybrids ('GN1', 'GN2', 'GN14', 'Garnem', 'Felinem' and 'Monegro' from the CITA breeding program) were grafted with the almond selection 'B-5- in 1994 and planted in 1995 at a planting distance of 5x5m, and evaluated from 1998 to 2006. 'GF- and 'Nemared' rootstocks were used as controls.

The experimental design was a randomized complete block with 9 to 7 single tree replications for each rootstock.

The data collected yearly per tree were trunk diameter growth measured above the graft union (cm) and production per tree (kg/tree). Trunk cross sectional area, TSCA (cm^2), was also calculated.

The data were subjected to a one-way analysis of variance (ANOVA) with SPSS software. The means were separated using 's post-hoc test ($p \leq 0.05$).

III – Results and discussion

The vegetative growth expressed as TSCA showed that the GxN hybrids had a significantly higher vigour than 'GF- and 'Nemared' (Fig. 1). All of them induced a positive lineal growth along the years (Fig. 1). Comparing the TCSA values among the GxN series, there were no differences until 2005, when 'GN- showed the highest area, followed by 'GN-, 'Garnem' and 'Monegro'. In 2006, 'GN- maintained the highest vigour in comparison to the others, followed by 'Garnem'.

During 2000, 2002 and 2005, significant differences were observed in production among all rootstocks. In 2003, production was low due to spring temperatures and in 2004 all genotypes were chilling injured (Fig. 2). When the production of 'GF- and 'Nemared' were compared to the GxN hybrids, no significant differences were observed, except for 'GN- which showed the highest production in 2005, however, this hybrid showed the lowest production, despite its vigorous vegetative growth during that year (Fig. 1). 'Garnem', 'Felimen' and 'Monegro' showed similar values among them, but higher than 'Nemared' along the years (Fig. 2). The general trend followed by the GxN clones from 2002 to 2006 demonstrates these three hybrids are a good choice for re-planting instead 'GF-677' due to their high vigour and root knot nematodes resistance for Mediterranean conditions as well as the high productive efficiency of these genotypes.

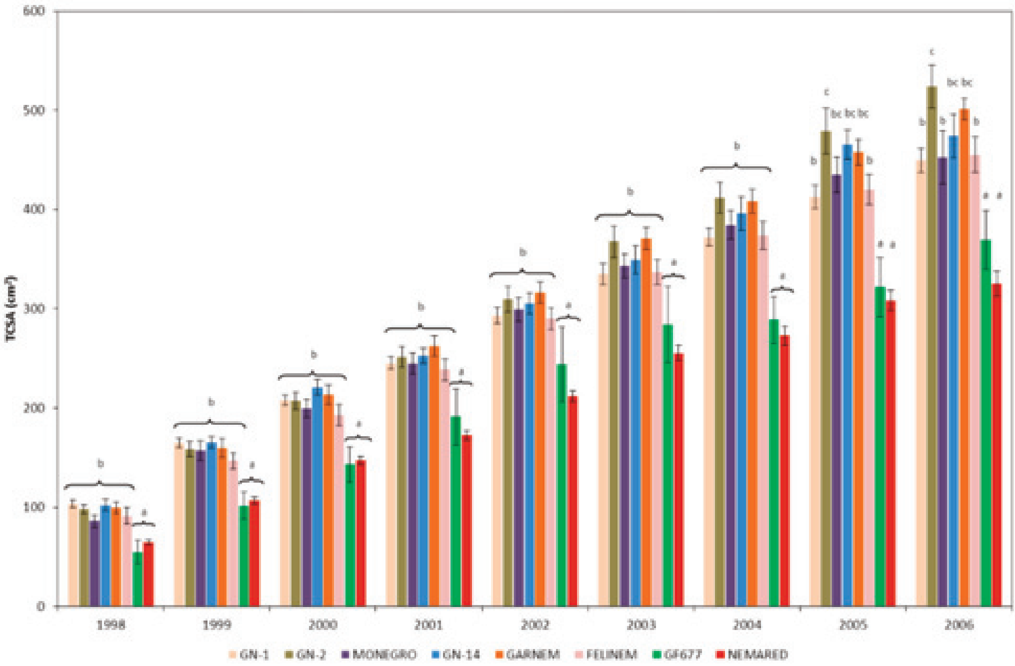

Fig. 1. Trunk cross-sectional area (TCSA) of almond selection 'B-5-2' grafted onto eight rootstocks along the years. Same letter values indicate a no significant difference (p ≤ 0.05) following Duncan's post hoc test. Error bars means the standard error from the mean.

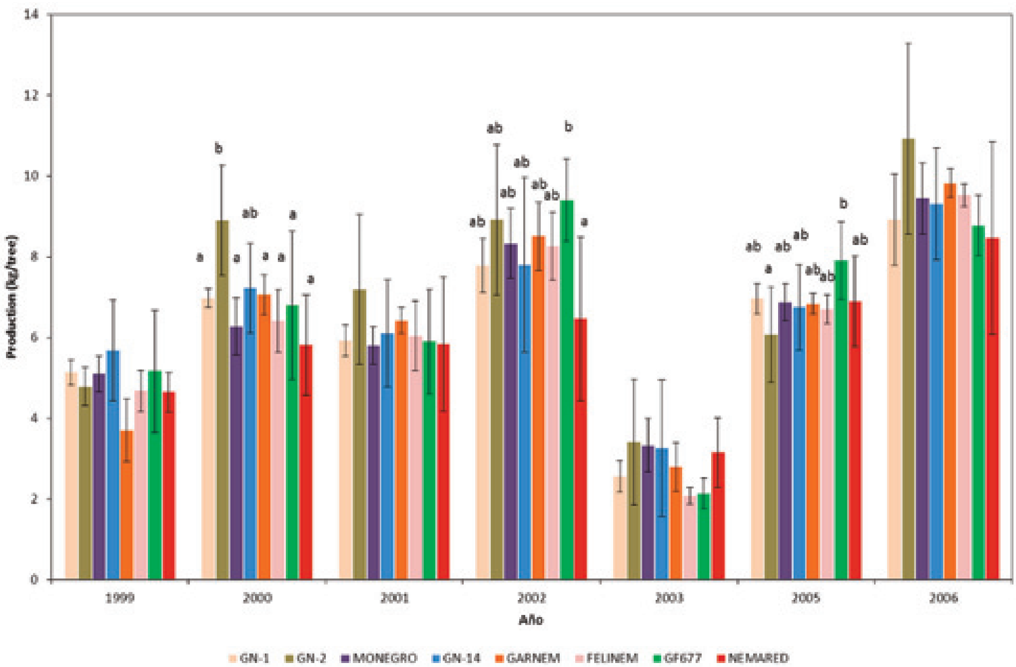

Fig. 2. Production per tree (kg/tree) of almond selection 'B-5-2' grafted onto eight rootstocks along the years. Same letter values and years without letter indicate a no significant difference (p ≤ 0.05) following Duncan's post hoc test. Error bars means the standard error from the mean.

Acknowledgments

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Pistacia atlantica rhizosphere characterization under arid climate

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Abstract. The root environment is characteristic for each species, soil type and environmental condition. Interactions may be established between the roots and its environment, which influences the mineral nutrition and the relationship soil / plant. The importance of environmental characterization of Atlas pistachio root rhizosphere under arid climate is our primary objective in this work. The knowledge of the arid climates and their water and soil resources, that lead to vegetation in severe conditions, suggests the implementation of serious measures in plantations development in drylands. Our results confirmed these assumptions and revealed the presence and the extent of poor soils in these regions likely with arido-soils type, unsalty, limestone with very low organic matter content and low phosphorus rates. The richness of these soils in nitrogen, especially their rhizospheric fraction, may be explained by the root activity and the microbial biomass in the soil / root interface.

Keywords. *Pistacia atlantica* – Rhizosphere – Chemical analysis – Soil type.

Caractérisation de la rhizosphère de Pistacia atlantica sous climat aride

Résumé. *L'environnement de la racine est caractéristique pour chaque espèce, le type de sol et de l'environnement. Les interactions peuvent s'établir entre les racines et leur environnement, ce qui influe sur la nutrition minérale et la relation sol / plante. L'importance de la caractérisation environnementale de la rhizosphere de la racine du pistachier de l'Atlas sous climat aride est notre objectif principal dans ce travail. La connaissance des climats arides et de leurs ressources en eau et des sols qui mènent à la végétation dans des conditions sévères suggère des mesures sérieuses dans le développement des plantations dans les zones arides. Nos résultats confirment ces hypothèses et révèlent la présence et l'étendue de sols pauvres de cette région, de type arido-sols, non salés, calcaires à très faible teneur en matière organique et des taux de faible teneur en phosphore. Leur richesse en azote, en particulier la zone rhizosphérique, est dû essentiellement à l'activité des racines et de la biomasse microbienne dans l'interface sol / racine.*

Mots-clés. Pistacia atlantica – Rhizosphere – Analyses chimiques – Type de sols.

I – Introduction

Intense germplasm erosion is underway in the semiarid, arid and Saharian areas due to human activities (Quezel and Santa, 1963). Initiatives have been taken to conserve and propagate pre-Saharian and Saharian genetic resources. The Atlas pistachio (*Pistacia atlantica* Desf.) is one of the species considered in this initiative, but very little information is available about the ecological adaptation of this species. Due to the lack of information, intraspecific variation is poorly understood and there is also limited knowledge about distribution of characters within the wide-ranging populations (Belhadj *et al*., 2007). *Pistacia atlantica* (Anacardiaceae) is a dioecious tree, widely distributed in Algeria from the Mitidja plains to the Saharian regions (Monjauze, 1980). This drought-tolerant tree, with an extensive root system, has been the subject of several studies aimed at selecting ecotypes best-adapted to present weather and soil conditions (Belhadj, 2007).

Rhizosphere is the most important chemically, biologically and ecologically microactive location in the soil (Toal *et al*., 2000). The rhizosphere is also defined as the volume of the soil under the influence of living roots (Lucas, 2002). It is the obligatory pathway of all minerals from the soil to plants, and a place of strong interactions between plants and soil microorganisms (Walter *et al*., 2005). The root environment is characteristic for each species, soil type and environmental condition. Interactions may establish between the roots and their environment, which influences the mineral nutrition and the relationship soil/plant. The importance of environmental characterization of *Pistacia atlantica* root environment under arid climate is our primary objective in this work. The knowledge of the arid climates and their water and soil resources, that lead to vegetation in severe conditions, suggests the implementation of serious measures in plantations development in drylands and areas.

II – Material and methods

Soils were collected randomly under twenty individuals from a natural *Pistacia atlantica* orchard located at Messaad site in Djelfa district (Algeria) which is characterized by an arid climate with a fresh winter (Fig. 1). Five soil samples were collected from each individual, three under soil vegetation (SSV) and two from soil without soil vegetation far from the rooting system (SHC) (Fig. 2). Physical and chemical analyses (Tables 1 and 2) were then performed and the data were statistically analysed using Stat-Box software.

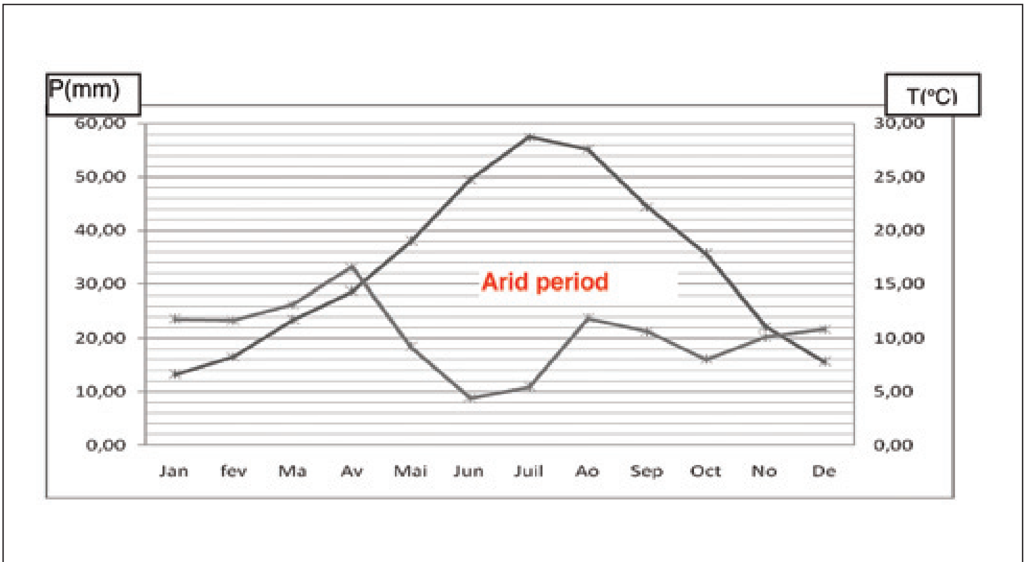

Fig. 1. Ombrothermic diagramm of Messad site.

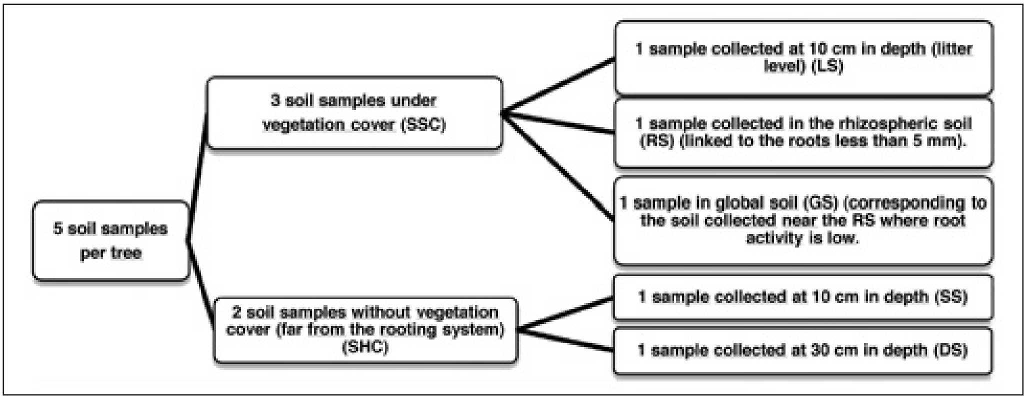

Fig. 2. Sampling scheme and soil collection.

III – Results and discussion

Atlas Pistachio root environmental characterization under arid climate revealed that the soils in this area are aridosoils type mainly with a sandy to sandy-silty texture (Tables 1 and 2). Their pH is slightly alcaline to alcaline in the samples without vegetation. The soils are limestony (calcareaous), with very low rate of organic matter. Under the trees, the amount of limestone in global soils is higher than in the rizospheric fraction. More organic matter is registered in soils under tree cover than in nude soils. The soil samples are non salty. Concerning the phosphorus, the amount is lower whenever we get closer to the vegetation layer.

ANOVA showed significant differences in pH and organic matter rate between the different soil samples, while significant differences were noticed among individuals for organic matter and phosphorus amounts (Table 3).

Table 1. Physical properties of sampled soils in *P. atlantica* orchad in Messad area

Soil samples	Soil fraction (%)					Soil texture
	Clay (%)	Thin silty (limons) (%)	Large silty (limons) (%)	Thin sand (%)	Large sand (%)	
SSCRS	1.60	7.20	34.34	1.85	55.01	Sandy-silty
SSCGS	0.23	3.43	46.10	9.36	40.88	Silty-sandy
SSCLS	1.03	9.40	59.19	1.67	28.71	Silty-sandy
SHCSS	1.90	3.23	61.70	9.44	18.72	Silty-sandy
SHCDS	2.13	0.27	35.05	2.79	59.76	Sandy-silty

Table 2. Chemical properties of sampled soils in *P. atlantica* orchad in Messaad area

Soil samples	pH	$CaCO_3$ (%)	Organic matter (C) (%)	Total Phosphorus (ppm)	Olsen Phosphorus (ppm)	†Electrical conductivity (Ms/cm)	†Total Nitrogen (%)
SSCLS	7.57	11.67	0.36	117.92	11.80	0.22	0.07
SSCRS	7.74	10.98	0.38	137.52	12.28	0.20	0.08
SSCGS	7.73	12.08	0.38	104.53	11.56	0.21	0.06
SHCSS	7.91	10.83	0.32	164.94	13.72	0.26	0.05
SHCDS	7.76	10.00	0.30	132.35	12.04	0.26	0.05

†: Measures performed under one tree.

Table 3. ANOVA for the chemical data of sampled soils in *P. atlantica* orchad in Messaad area

		S.C.E	DDL	M. S	TEST F	PROBA	Sig.
pH	TOTAL VAR.	2.62	59	0.04			
	VAR. individuals (1)	0.002	1	0.002	0.07	0.79516	NS
	VAR. soil (2)	0.99	4	0.25	9.45	0.00001	***
	VAR. 1*2	0.33	4	0.08	3.11	0.02311	*
	Residual VAR.	1.31	50	0.03			
$CaCO_3$ (%)	TOTAL. VAR.	832.68	59	14.11			
	VAR. individuals (1)	1.35	1	1.35	0.09	0.76701	NS
	VAR. soil (2)	22.42	4	5.60	0.36	0.83657	NS
	VAR. 1*2	32.07	4	8.07	0.52	0.72704	NS
	Residual VAR.	776.85	50	15.54			
Organic matter (C) (%)	TOTAL VAR.	11.52	59	0.19			
	VAR. individuals (1)	1.13	1	1.13	6.81	0.0115	*
	VAR. soil (2)	1.88	4	0.47	2.82	0.0342	*
	VAR. 1*2	0.2	4	0.05	0.30	0.87606	NS
	Residual VAR.	8.31	50	0.17			
Total Phosphorus (ppm)	TOTAL VAR.	98742.67	59	1673.61			
	VAR. individuals (1)	10820.84	1	10820.84	7.58	0.00803	**
	VAR. soil (2)	11941.36	4	2985.34	2.09	0.09483	NS
	VAR. 1*2	4616.21	4	1154.05	0.81	0.52775	NS
	Residual VAR.	71364.26	50	1427.29			
Olsen Phosphorus (ppm)	TOTAL VAR.	387.24	59	6.56			
	VAR. individuals (1)	211.56	1	211.56	72.08	0.....	***
	VAR. soil (2)	12.03	4	3.01	1.03	0.40442	NS
	VAR. 1*2	16.90	4	4.23	1.44	0.23387	NS
	Residual VAR.	146.74	50	2.94			

This work shows the effect of *Pistacia atlantica* rooting system on the soil properties. After Dambrine and Tessier (2001), alcaline soils are common under arid climates with very low rainfall and high evapotranspiration, such as our sampling site, which is characterized by a very long arid period (more than 7 months) (Fig. 1).

IV – Conclusions

Our results revealed the presence of poor soils in the steppic area, likely with arid soils type with very low organic matter content and low phosphorus rates. The soils are unsalty and calcareous (limestone). Their richness in nitrogen, especially the rhizospheric area, is the result of the root activity and the microbial biomass in the soil/root interface.

Atlas pistachio remains, potentially, a very interesting species for the development and the conservation of such arid areas hence, the protection and the preservation of this tree, in its natural ecosystems, is a sinequanone condition.

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Effect of gender on the leaf morphological diversity in *Pistacia lentiscus*

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Abstract. *Pistacia lentiscus* (mastic tree) is very common in the Mediterranean region. In Algeria, Mastic tree is distributed in a wide range of habitats along the climatic gradient. We studied the factors that may shape its morphological, physiological, and genetic differentiation. For this purpose, we analyzed the morphological variability intra-population between male and female individuals of *Pistacia lentiscus*, by using leaf characters. In total, seven quantitative morphological variables were measured for the leaves. The statistical analysis (PCA), showed significant differences for the measured variables between male and female plants of *Pistacia lentiscus*.

Keywords. Algeria – *Pistacia lentiscus* – Variability – Leaves – Morphology – Gender.

Effet du genre sur la diversité morphologique foliaire chez **Pistacia lentiscus**

Résumé. Pistacia lentiscus *(arbre au mastic) est très commun dans la région méditerranéenne. En Algérie, l'arbre au mastic est distribué dans une grande variété d'habitats le long du gradient climatique. Nous avons étudié les facteurs susceptibles de configurer sa différenciation morphologique, physiologique et génétique. Dans ce sens, nous avons analysé la variabilité morphologique intra-population entre individus mâles et femelles de* Pistacia lentiscus, *en utilisant les caractères de la feuille. Au total, sept variables morphologiques quantitatives ont été mesurées chez les feuilles. L'analyse statistique (PCA) a montré des différences significatives pour les variables mesurées entre plantes mâles et femelles de* Pistacia lentiscus.

Mots-clés. *Algérie –* Pistacia lentiscus *– Variabilité – Feuilles – Morphologie – Genre.*

I – Introduction

Protection of the forest ecosystems passes necessarily by the knowledge of the ecophysiological requirements of the genus and species widely distributed in within. Among those, *Pistacia lentiscus* L. (lentisk). is a Mediterranean and wild evergreen shrub, belonging to the Anacardiaceae family that consists of more than eleven species (Zohary, 1952). Common within the Mediterranean basin, and having typical attributes that characterize this common life form of Mediterranean plants (Mulas *et al*., 1999), it has a large geographical and bioclimatical distribution, extending from the humid to the arid areas (Lo Presti *et al*., 2008). The tree is widespread in forest alone or in association with other tree species such as pistachio terebinth, olive and carob trees (Yildirim, 2012). In Algeria, this species occupies a wide climatic range, as well, which provides to the plant a very high morphological diversity, noticed especially at leaf level. Lentisc pistachio is used in traditional medicine for several purposes in all the Mediterranean countries. Besides that the shrub plays a plethora of ecological roles. Unfortunately, lentisk pistachio faces severe genetic erosion especially in Northern Algeria, caused by forest fires and global warming (Belhadj, 2007).

The aim of this study is to identify and to gain a better knowledge of this species, which can help conserving the genetic diversity of this native species which remains unknown and therefore rarely used in the preservation of forest and pre-forest ecosystems despite its ecological and economic interests.

II – Material and methods

The leaves of *Pistacia lentiscus* L. were harvested in December 2011 from spontaneous plants in Berrouaghia (Medea), located in the North-central part of Algeria. From this location, ten female and male trees were selected and thirty leaves were harvested per tree (in total 20 trees and 600 leaves). Once harvested, these leaves were carefully dried and kept in herbarium prior to biometric measurements (leaf length (lng feu) and width (lrg feu), petiole length (L petiole) and the number of leaflets (Nbr fol)). The PCA analysis was then performed in order to show differences between the female (f) and male (m) trees and which variables were best discriminating the two groups.

III – Results and discussions

Significant differences were recorded for leaf size and petiole length while no significant differences were recorded for the remaining variables, among, both the male and female individuals (Table 1). The PCA analysis showed a strong correlation for the number of leaflets (Nbr fol) and the total length of the leaves (lng feu) on the circle of correlation (Fig. 1), but this difference is not enough to segregate the different individuals, even though several of them are clearly separated (Fig. 2).

Table 1. Morphological data measured for male and female individuals of *P. lentiscus*

	Mean ± Std. Dev./Min-Max (C.V.)	
Character	**Male individuals**	**Female individuals**
Leaf length (cm)	7.04*** ± 1.28	7.46*** ± 1.05
	3.2 – 11 (18.21)	4.5 – 12 (14.15)
Leaf width (cm)	4.61*** ± 0.87	4.90*** ± 0.97
	2 – 7.1 (19.05)	2 – 7.3 (19.89)
Number of leaflet	8.35 ± 1.92	8.14 ± 1.66
	4 – 14 (23.03)	4 – 12 (20.47)
Terminal leaflet length (cm)	2.44 ± 0.50	2.62 ± 0.53
	1.4 – 4.1(20.77)	1.2 – 4.1 (20.44)
Terminal leaflet width (cm)	0.86 ± 0.34	0.88 ± 0.53
	0.2 – 2.3 (39.27)	0.3 – 1.5 (29.19)
Length/width of terminal leaflet ratio	3.13 ± 1.06	3.10 ± 0.79
	1.3 – 7.5 (33.94)	1.6 – 5 (25.55)
Petiole length (cm)	1.27*** ± 0.25	1.42*** ± 0.3
	0.7 – 2.2 (19.94)	0.8 – 2.5 (21.62)

*** Significantly different.

Similar results were reported in kiwifruit leaves of both male and female plants, grown on the vegetative and generative shoot. The plants showed different leaf area and shape (Olah *et al*., 1997). Compared with males, females had higher values of leaf surface and stomata density and lower values of shoot height, in *Hippophae rhamnoides* (Li *et al*., 2007).

After Hoffman and Alliende (1984) and Vasiliauskas and Aarssen (1992), reported by Li *et al*. (2007), differences in growth characteristics between males and females of dioecious species have been documented previously, with most studies showing females to be smaller than males and to grow more slowly. These differences between male and female plants, may be due to the energy requirements to reproduction (female trees need more energy than male trees, or to the variations of the age and genetic diversity of some individuals of this species.

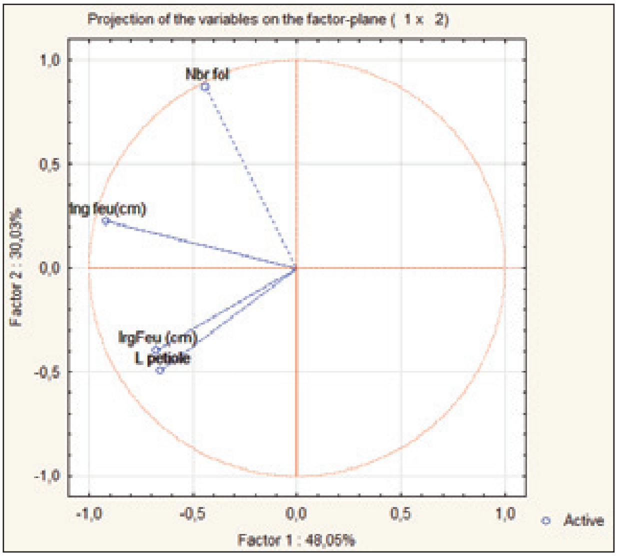

Fig. 1. Bifactorial projection of P.C.A. for the measured morphological variables for *Pistacia lentiscus* leaves.

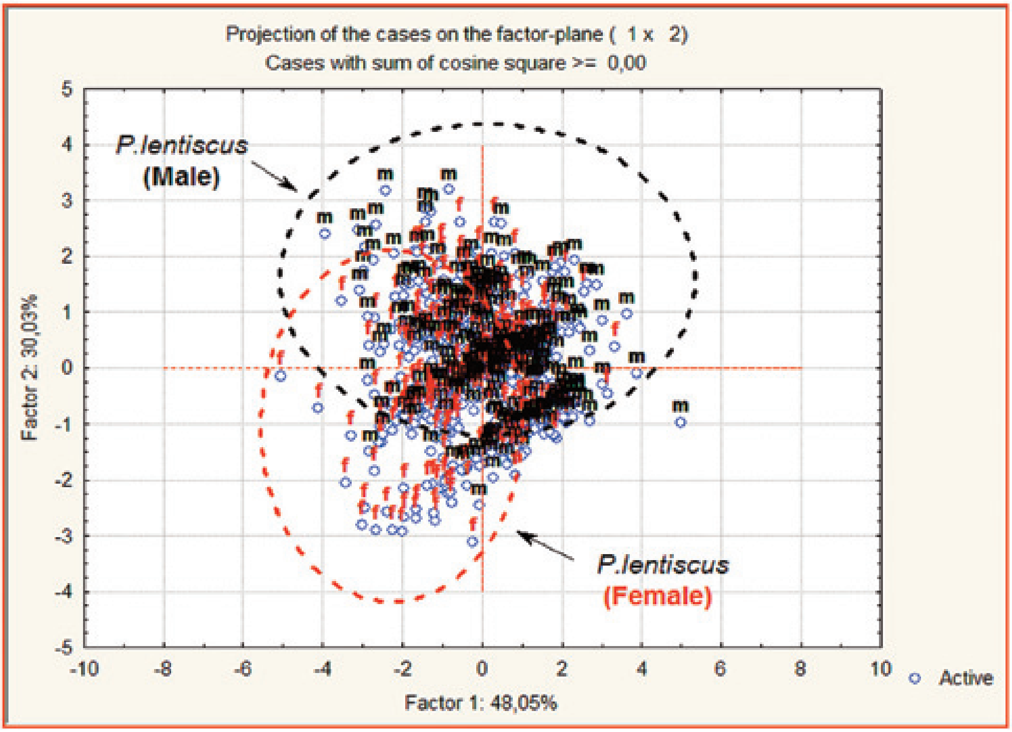

Fig. 2. Bifactorial projection of P.C.A. for the male and female individuals of *Pistacia lentiscus* trees.

IV – Conclusion

At this point, the best discriminating traits recorded in this study, for some individuals, were leaf length and petiole length as shown in Fig. 1. But more leaf samples as well as more sampling sites are needed to perform a more complete study in order to get suitable conclusions. This may provide more data that could be useful to refine the gender relationship in *Pistacia lentiscus*.

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Session 3

Pest and diseases

A review of research on diseases and pests of almonds in Morocco

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Abstract. In Morocco, almonds cover 151 Kha producing 19.8 Kt of shelled nuts. This makes almonds rank 2nd after the olives in terms of area covered. Due to its rusticity, the almond tree is found in various ecological zones throughout Morocco, generating additional income to small farmers operating on marginal lands. The major diseases attacking almond orchards in Morocco are Monilia blossom blight (*Monilia laxa*), leaf curl (*Taphrina deformans*), anthracnose (*Gleosporium amygdalynum*) and shothole (*Coryneum beijerinkii*) while crown gall (*Agrobacterium tumefasciens*), nematodes (*Meloïdogyne* spp.) and *Phytophthora* root rot may occur in almond nurseries. The most prevalent pests on almonds remain the mites (*Tetranychus* spp., *Briobia rubrioculus*), the aphids (*Myzus persicae, Brachycaudus amygdalinus, Hyaloperus pruni*) that attack the foliage of almond, older and stressed almonds trees are subject to the attack of the Flat-headed Root Borer (*Capnodis tenebrionis*) and the bark beetles (*Scolytus amygdali*). While fungal diseases can be easily controlled through appropriate fungicidal sprays during critical periods, the flat-headed root borer and the bark beetle remain uncontrolled because of lack of registered appropriate pesticides that are cost effective to be used by the farmers. The Green Morocco Plan (Plan Maroc Vert) initiated in 2008 to enhance agricultural production in Morocco and ensure food security of the country, has opted for extending almond plantations to valorize marginal lands, thereby increasing farmers income while preserving soils from erosion in these areas. To ensure the success of the new plantations, certified disease and pest-free almond nursery stocks are promoted within the Green Morocco Plan.

Keywords. Almond – *Prunus amygdalus* – Morocco – Pests – Diseases – IPM – Breeding.

Révision des recherches menées sur les maladies et les ravageurs de l'amandier au Maroc

Résumé. *Au Maroc, les amandiers sont cultivés sur 151 Kha et produisent 19,8 Kt de fruits décortiqués. Ceci place les amandes au deuxième rang après les olives en termes de surface. En raison de sa rusticité, on rencontre l'amandier dans diverses zones écologiques à travers le Maroc, permettant un revenu additionnel aux petits agriculteurs exploitant des terres marginales. Les principales maladies qui attaquent les vergers d'amandiers au Maroc sont la moniliose* (Monilia laxa)*, la cloque de l'amandier* (Taphrina deformans)*, l'anthracnose* (Gleosporium amygdalynum) *et la criblure* (Coryneum beijerinkii) *tandis que la galle du collet* (Agrobacterium tumefasciens)*, les nématodes* (Meloïdogyne *spp.*) *et la pourriture des racines due à* Phytophthora *peuvent survenir en pépinières. Les ravageurs prévalents de l'amandier sont les acariens* (Tetranychus *spp.,* Briobya rubrioculus)*, les pucerons* (Myzus persicae, Brachycaudus amygdalinus, Hyaloperus pruni) *qui attaquent le feuillage de l'amandier ; les amandiers plus vieux et stressés font l'objet d'attaques du capnode* (Capnodis tenebrionis) *et du scolyte* (Scolytus amygdali). *Tandis que les maladies fongiques peuvent être facilement contrôlées par des nébulisations fongicides appropriées pendant les périodes critiques, les capnodes et scolytes restent incontrôlés en l'absence de pesticides enregistrés appropriés qui soient d'un bon rapport coût-efficacité et puissent être utilisés par les agriculteurs. Le Plan Maroc Vert, démarré en 2008 pour encourager la production agricole au Maroc et assurer la sécurité alimentaire du pays, a opté pour accroître les plantations d'amandier afin de mettre en valeur les terres marginales, augmentant ainsi le revenu des agriculteurs tout en protégeant les sols de l'érosion dans ces zones. Pour s'assurer du succès des nouvelles plantations, des porte-greffes d'amandier de pépinières, certifiés indemnes de maladies et de ravageurs, sont favorisés dans le cadre du Plan Maroc Vert.*

Mots-clés. *Amandier* – Prunus amygdalus – *Maroc – Ravageurs – Maladies – IPM – Amélioration.*

I – Introduction

Due to their rusticity, requiring little care as compared to other fruit crops, their drought tolerance and adaptation to diverse environments, the almonds conquered large areas in Morocco, ranking 2nd after the olive tree (Anonymous, 2012). Historically, the almond cultivation has know 2 main expansions after independence in 1956: the first one was in the 1960's when the Government of Morocco decided to expand almond plantations along those of olives, to protect land from erosion especially in hilly and mountainous areas, and the 2nd since the advent of the Green Morocco Plan (Plan Maroc Vert, GMP) in 2008, with objective of diversifying framers' income. At present, the almond insures 1 millions work days and generates a commercial value of 750 Million Dh.

In Morocco, the Almond sector is structured in 2 sub-sectors:

1. The Traditional sub-sector where trees propagated through seeds, are grown in uneven patterns on shallow soils, limited water and nutrients supply, non optimal crop husbandry practices, mostly intercropped with other plants especially in small-holding farms.
2. The Modern sub-sector where almonds are planted in rows, in more fertile soils with adequate crop husbandry techniques including irrigation, fertilizing and plant protection programs. Even though the modern sector covers only 50% of the national almond area, it contributes by 80% to the total national production.

As can be expected, almonds in Morocco are subject to the attacks of a number of diseases and pests some of which are known worldwide. The importance of these diseases and pests and their impact on almond production and quality vary according to the varieties used, the local environment and the crops husbandry techniques used.

It goes without saying, that the traditional almond sub-sector using no plant protection means harbors a diversity of diseases and pests that cause great damage to the almond orchard. In fact, under these condition land owners are practicing a "gathering" type production system; they harvest whatever is left after the pests and diseases have taken their shares. In this respect we can say that the traditional almond production in Morocco is organic.

In the Modern sub-sector, where almonds are grown in rows of more performing varieties, generally introduced in accordance with modern markets' objectives, pests and diseases can cause heavy losses too when the environment is favorable to the pests and diseases and no control is practiced.

II – Major diseases of almond in Morocco

Research and observations carried out during the past 40 years showed that blossom blight (*Monilia laxa*), anthracnose (*Gleosporium amygdalinum*), leaf cul (*Taphrina deformas*), shot hole (*Coryneum Beijerinkii*) and crown gall (*Agrobacterium tumefaciens*) are very common in almond orchards in Morocco. Several other studies have reported the occurrence of virus diseases on almonds in Morocco.

1. Fungal diseases

While fungal diseases go unnoticed in extensive almond plantations, in mountainous and semi-arid areas, they were very importance in the 1970's in intensive almond production orchards using more performing varieties originating from California, southern Europe and Tunisia, like Abiod, Nec + Ultra and others. In a monitoring study conducted in 5 modern almond farms in the Meknes area in the 1970s, Lamnouni (1976) found that blossom and twig blight (*Monilia laxa*) and anthracnose (*Gleosporium amygdalynum*) were the most devastating fungal diseases on selected varieties, while leaf curl (*Taphrina deformans*) and Shot hole (*Coryneum bejerinkii*) were of lesser importance. Because of the gradual occurrence of the diseases during the growing season, farmers applied from 5 to 7 fungicidal sprays to protect their orchards.

In a subsequent study focusing on the 2 most susceptible varieties Abiod and Nec+Ultra, Aber (1977) found that 3 to 4 sprays of the associations Methylthiopanate + maneb and Captan + mancozeb, applied at bud break, flowering, fruit set and fruit development, greatly reduced the incidence of both Monilia blossom blight and fruit anthracnose in the treated orchards.

2. Crown gall

Even though it has been detected in several localities in the country, Crown Gall is a serious threat in the Meknes area where most of the stone fruit nurseries are located. The disease is especially important in nurseries where it can reduce the quality of young trees produced. Older trees seem to tolerate the disease.

Crow gall was intensively investigated by BENJAMA (1997) who studied the pathotyping of Moroccan isolates of *A. tumefaciens* and tested their sensitivity to the antagonistic strain K84. He showed that Moroccan strains were sensitive to pure extracts of stain K84 cultures suggesting that this possibility may be used in the field. Even though the method proved to be successful in other countries in North America and Europe, it did not gain wide use in Morocco where prophylactic measures are preferred.

3. Viral diseases

Fisher and Baumann (1977) have already indicated the wide occurrence of virus infection in zones of intense cultivation of almond, but almost un-existing in areas of extensive almond production. In graft transmission experiments all examined virus containing samples revealed to be infected by various strains of Prunus necrotic ringspot virus (PNRV). For intensive almond production, Fisher and Baumann recommended the indexing of varietal collections on imported certified virus-free rootstocks before the distribution of healthy bud wood to authorized private nurseries for further multiplication. For areas of extensive almond production they recommended the indexing of the varietal clones adapted to the specific ecological zones and their propagation on virus-free rootstocks.

In a more recent study, Koutou (2000) examined 156 samples originating from 24 almond orchards in the Meknès-Fes area, using the ELISA technique. He detected PNRSV in all almond orchards surveyed. The incidence of PNRSP varied from 17 to 30% among orchards and localities. With the aim of refining the technique of viral detection, EL JADD (2011) compared ELISA vs. RT-PCR techniques for detecting and extracting of RNA viruses (PNRSV, ACLSV, PDV) infecting stone fruits in Morocco. Both techniques detected PNRSV infection in 12 samples out of the 304 samples of almond leaves tested. Concerning PDV and ACLSV, samples with doubtful viral infections using ELISA technique were found to be virus-free by the RT-PCR technique, suggestion the joint usage of these 2 techniques for reliable viral detection in almond samples.

III – Major pests of almond in Morocco

Of the pests found on almonds, the flat-headed root borer (*Capnodis tenebrionis*) became a serious threat to all stone fruits during the droughty years in the early 1980s, especially in the Meknes region where almonds were heavily attacked along with apricots and prunes. This led to the pulling out of many devastated orchards. The larvae of this beetle attack the roots and crowns of trees that have been weakened by drought or disease. Hmimina (1989) has described the life cycle of this insect. Once eggs have been laid, control becomes difficult because of the subterranean development of the larvae. Thus, baits containing hexachlorohexane (HCH) were applied to the soil 50cm around the almond stem base during the oviposition period. With the banning of this product in the 1990 due to environmental concerns, growers were urging for a substitute product that was as effective as HCH but less expensive (Moussaoui, 1995). Subsequently, new products came to be experimented, but their markets did not develop accordingly.

The bark beetle (*Scolytus amygdali*) was intensively investigated by Benazoun (1984) in two locations: Tafrout and Beni Mella. Although some natural predators were found at that time, none was effective in controlling the bark beetle. A single spray of delta-methrin was partially effective when applied during spring emergence of adults (Benazoun and Schvester, 1989). Beyond the cost and environmental issues, the farmers were reluctant to apply pesticides fearing to damage crops grown in association with almonds. With the bark beetle becoming a serious threat not only in stressed orchards, but also in orchards that are well cared for, and considering the specificity of its biology, other methods including mass trapping using pheromones, will contribute to the integrated pest management of this insect.

Aside from the 2 major pests cited above, almonds may be attacked by various species of mites and aphids during the growing season (Benazoun, 2001; Boutaleb-Joutei, 2011).

IV – Integrated pest and disease management in almond in Morocco

1. Prevention through regulatory methods

In order to ensure the success of new plantations, Morocco has promulgated in 2004 a decree approving standards for the production, control, packaging, storage and certification of seeds and nursery stocks of rosaceous stone fruit trees including almonds and their rootstocks. The diseases and pest concerned are Crown gall, Verticillium, neamtodes (*Meloidogyne, Xiphinema, Longidorus and Trichodorus*) and the flat-headed root borer *Capnodis tenebrionis.* Nursery stocks found or suspected to harbor one of the pre-mentioned diseases and/or pests will not be certified. Heavily attacked lots of plants or seeds are destroyed by incineration. Table 1 presents the standards required for virus diseases.

Lots found to harbor viruses and/or other pathogens or pests are simply incinerated.

With the advent of the GMP in 2008, institutional reforms of the Ministry of Agriculture and Marine Fisheries led to the creation of the National Office of Sanitary Safety of Food products (ONSSA) in 2010. This Office invested with the mission of controlling nursery and seed stocks among other things works diligently on updating regulatory texts to make them match the international standards.

Table 1. Standards required by ONSSA before nursery stocks and seeds stocks can be certified

Virus	Category		
	Pre-base	Base	Certified
Prunus Necrotic Ring Spot Virus (PNRSV)	0%	0%	1%
Prune dwarf virus (PDV)	0%	0%	1%
Apple Chlorotic Leaf Spot Virus (CLSV)	0%	0%	1%
Total	0%	0%	2%†

† Cumulative total of virus-infected plants by species.

2. Pesticides

Active ingredients presently registered for use on almonds are *Ziram*, *Thiram* and *Maneb* as fungicides. *Propargit* is registered for mite control (AMPP, 2015; Anonymous, 2015). No pesticide is labeled for aphid control on almond. While the use of chemical pesticides almost absent in the traditional almond sub-sector, it is a necessity in semi-intensive and intensive almond production. However, the list of pesticides registered for almonds is progressively shrinking. This will undoubtedly stimulate research to develop alternative methods for controlling pests and diseases.

3. Cultural practices

It goes without saying that appropriate crop husbandry practices such as adequate irrigation and fertilization of almond orchards is of paramount importance in preserving the good health of trees and therefore their productivity. Furthermore, the prevention of early development of diseases and pests relies heavily on sanitation practices, including pruning of affected branches and eliminating dead wood and fruit mummies. Attacked trees and colonized wood should be incinerated on the spot.

4. Breeding

During the last 40 years, researchers of INRA (National Institute for Agricultural Research) have surveyed the local almond populations and collected germplasm of importance to the almond breeding program. Foreign germplasm was also introduced from California, Europe and Tunisia. In total, the current almond collection consists of 245 accessions that are maintained in INRA's experiment stations where active breeding efforts led to the selection late flowering almond varieties to avoid the risk of spring frost along with other qualities required by almond markets. The selection of late flowering varieties will undoubtedly contribute to reduce the risk of Monilia infection by avoiding the coincidence of flowering with the wet winter periods. This explains why the association 'Ferragnes'-'Ferraduel' composed of the two late flowering varieties that are auto-incompatible has become popular since the advent of the 21st century. The number of nursery stocks produced of these 2 varieties increased from 1.45 million plants in 2008 to 3.2 million in 2012, thereby accounting for almost half of the Moroccan almond orchard. More recently, continuous breeding efforts have led to the selection of 2 almond varieties that are late flowering but self-fertile, 'Lauranne' and 'Mandaline'. Nursery stocks of these 2 varieties increased from 78,000 plants sold in 2010 to 110,000 in 2012, representing à 41% increase in a 2 year laps time (Anonymous, 2012).

It is hoped that the study of the genetic diversity of Moroccan almond populations will help identify almond varieties with highly desirable characteristics both agronomically and technologically. It is essential that plant protection specialists work hand in hand with breeders, agronomists and industry professionals to breed future almond cultivars that are resistant or tolerant to pests and diseases to dispense the framers from using chemical pesticides and help preserve consumer's health and the environment.

V – Conclusion

This paper gives a brief review of the research carried out during the last 40 years in Morocco on diseases and pests of almond and their control. It appears as if almond diseases and pests were not as intensively investigated as those of other fruit crops such as apple. This may be explained by the fact that almonds are still grown more extensively in the country. With the more recent option taken by the government to expand plantations and improve almond production in the frame work of the Green Morocco Plan, research on almond protection from pest and diseases will receive inevitably more attention. It is only hoped that the research to be conducted will help develop alternative methods for controlling almond pests and diseases while protecting consumer's health and the environment.

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Contribution to a better understanding of flight pattern and egg-laying duration of the Pistachio seed wasp

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Abstract. In Tunisia, the pistachio seed wasp, *Eurytoma plotnikovi* Nikolskaya (Hymenoptera, Eurytomidae) is a pest of economic importance attacking the edible part of the pistachio nut. The damage can be important seriously affecting harvest. To control the insect, a better knowledge of its biological features is needed. This paper aims to contribute to the understanding of adult flight activity, its extent and the duration of egg-laying period in order to establish better control methods. In the field, the emergence of adults from infested pistachio nuts of the previous year was monitored as was the progress of the development of diapausing larvae through pupae and adults by collecting and splitting old nuts at regular intervals from April to June in 2005, 2006 and 2011. The characterization of the egg-laying period was evaluated from April to July in 2005, 2006 and 2011 by sampling freshly nuts and dissecting. Results show that in 2005, the period of egg-laying was positioned between May 4 and June 4; in 2006, this period was concentrated in May and in 2011 between 7 May and 31 May. The splitting of aged nuts revels that in 2005, pupation started from the third decade of April with a presence of pupae inside aged nuts on April 27. Adult emergence began on May, 10 and ended by the end of month. In 2006, pupae were present in old nuts from April 18, the adults inside old nuts from May 26. Old larvae were detected in the nuts on June 28. With regard to 2011, overwintering larvae pupate in late April while adults begin their emergence from May 7, 2011. The results of this study will better positioning control operations against this pest.

Keywords. *Eurytoma plotnikovi* – *Pistacia vera* – Flight duration – Oviposition – Adult emergence.

Contribution à une meilleure connaissance de la période d'envol et de la durée d'oviposition du ver des pistaches

***Résumé**. En Tunisie, le ver des pistaches,* Eurytoma plotnikovi *Nikolskaya (Hymenoptera : Eurytomidae) est un ravageur d'importance économique attaquant les drupes (fruits) du pistachier (Pistacia vera L.). Les dégâts peuvent être très importants affectant sérieusement la production. Par conséquent, une meilleure connaissance du cycle biologique de l'insecte est primordiale afin de définir une stratégie de lutte adéquate. Ainsi, le développement des larves diapausantes en nymphes et adultes et l'émergence des imagos d'E. plotnikovi ont été évalués par la collecte et la dissection des fruits anciens à intervalle régulier. La période d'oviposition dans la nature a été évaluée par la collecte et la dissection des jeunes drupes durant la période allant du mois d'avril au mois de Juillet des années 2005, 2006 et 2011. Les résultats indiquent qu'en 2005, la nymphose a débuté à partir de la 3^{ème} décade d'avril avec une présence des nymphes dans les anciennes drupes le 27 avril 2005. L'émergence des adultes a débuté le 10 mai pour se terminer durant la quatrième semaine du mois de mai 2005. La période de ponte a été concentrée entre le 4 mai et le 4 juin. En 2006, les nymphes sont présentes dans les anciennes drupes à partir du 18 avril, les adultes dans les fruits à partir du 26 mai ; alors que les œufs ont été détectés dans les jeunes drupes durant le mois de mai 2006. Les larves hivernantes sont présentes dans le fruit à partir du 28 juin 2006. En ce qui concerne l'année 2011, la période de présence des œufs dans les jeunes fruits s'étale du 7 au 31 mai. Concernant les drupes anciennes, les larves hivernantes se transforment en nymphes vers la fin du mois d'avril alors que les adultes entament leur émergence à partir du 7 mai 2011. Les résultats obtenus de cette étude permettront un meilleur positionnement des opérations de lutte contre ce déprédateur.*

Mots-clés. Eurytoma plotnikovi – Pistacia vera – *Envol imaginal – Oviposition – Émergence des adultes.*

I – Introduction

Pistachio tree (*Pistacia vera* L.), has been grown in almost all regions of Tunisia occupying large lands which are less appropriate or even completely inappropriate for other crops such as olive or almond trees. Pistachio cultivation is considered to be of great economic importance and improvements have been permanently introduced affecting agricultural techniques. The local production of pistachio nut in Tunisia is currently increasing to reach the increase of the need (from 200 tons in 1988 to 2100 tons in 2011) testified by the dramatic increase in acreages ranging from 4400 ha in 1980 to 43,000 ha in 2002 (Onagri, 2015). Nevertheless the yield is still very low (averaging 28 kg per ha in 2002) mainly due to technical problems such as the lack of synchronization of flowering periods for male and female trees and the attack of insects and pathogens.

The pistachio seed wasp, *Eurytoma plotnikovi* Nikolskaya (Hymenoptera, Eurytomidae) has been reported as a primary pest of pistachio in a number of countries of the Mediterranean and Asia such as Tunisia, Iran, Turkey, Syria and recently Italy (Sicily) (Jerraya, 1977; Davatchi, 1958, Mehrnejad, 2001; Basirat and Seyedoleslami 2000; Longo and Suma, 2011). The larva destroys up to 90% of the crop by damaging the edible part of the pistachio nut (Mourikis *et al.,* 1998). Since contact insecticides targeting adults were usually effective, the control of the insect relays on the proper identification of adult flight and its duration. Recently, farmers complained that the control of the pest became difficult and sprays were not effective. So, the objectives of the present work were to contribute to the knowledge of the biology of Pistachio seed wasp as well as to determine the period of oviposition activity of females in order to establish better control methods.

II – Materials and methods

All field observations were made in an experimental pistachio orchard planted in the late seventies belonging to the Higher Institute of Agricultural Sciences of Chott-Mariem located in the Center-East (Sousse, Tunisia 35.8° North ; 10.6° East) at about two kilometers far from the sea and characterized by a semi-arid climate with hot summers and mild winters. Pistachio trees (21 females and 9 males of the cultivar 'Mateur'), conducted under rain fed conditions were planted at 7 meters interval apart.

From late April to early July, old as well as newly formed pistachio nuts were sampled. Fifty, one-year old nuts and 50 newly formed nuts were collected from at least 5 trees, put in plastic bags and brought to the laboratory. Old nuts were dissected and classified as un-pollinated, healthy or infested by *E. plotnikovi* larvae, pupae or adults. Nuts having insect exit holes were considered to be attacked by the pistachio seed wasp and adults had emerged. Newly formed nuts were dissected; eggs and larvae were recorded. The identity of the species, *E. plotnikovi* was confirmed to us in May 2006 by Pr Jean-Yves Rasplus (INRA, Montpellier, France).

III – Results and discussion

1. Monitoring the infestation and adult emergence in the orchard

Year 2005. The collection and splitting of pistachio nuts belonging to the yield of 2004 begin on April 15, showing 98% of old diapausing larvae and 2% of white pupae. Colored pupae as well as nuts with exit holes were detected on early May, 2005. So, the flight period of adults emerging from infested nuts begins early May and finishes at the end of the month testified by a percentage of 100% of nuts with exit holes beginning on May 27, 2005 (Fig. 1).

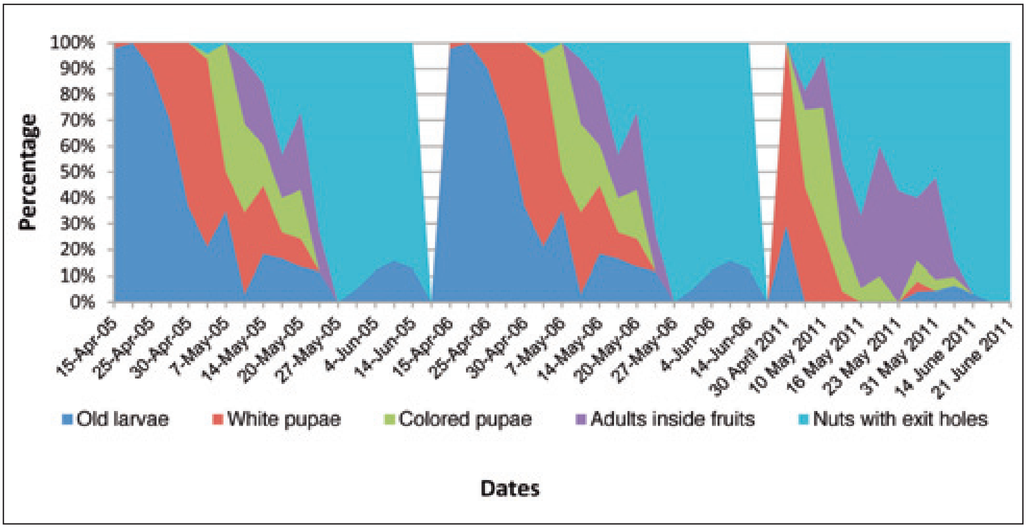

Fig. 1. Features of *E. plotnikovi* observed by splitting nuts from the previous seasons.

Year 2006. Pistachio nuts of the previous year were collected twice a week from April, 15, 2006 to June 14, 2006. During the second half of April only old larvae (79%) and white pupae were present (21%; Fig. 1). Colored pupae and nuts with exit holes were detected since May 4, 2006. From May 14 to May 24, old larvae, white and colored pupae, adults inside nuts and nuts with adult exit holes were present. During June, only old larvae (11.5%) and nuts with exit holes were detected (88.5%, Fig. 1). These results confirm that the period of adult flight was concentrated in May. Old larvae detected in June were probably in diapause. Indeed, Jerraya (1977) suggested that a small percentage of *E. plotnikovi* old larvae may stay in diapauses for up to 5 years.

Year 2011. The sampling of pistachio nuts (from 30 April to 21 June 2011) belonging to the previous year and left un-harvested on the trees shows as years 2005 and 2006 that the flight pattern of the adults were concentrated in May (Fig. 1). A small proportion of old larvae may remain in diapause. During the three year of the study, in the field, the first adults of *E. plotnikovi* appear in the beginning of May until the end of the month; these data agree with results reported by Braham (2005) in experiments conducted in another pistachio orchard located in the centre of Tunisia. However, in Greece, Lykouressis *et al.,* (2007) reported a concentrated period of *E. plotnikovi* adult emergence (from May 13 to May 23) peaking in May 19. The differences in time emergence may be due to local climatic conditions and/or pistachio cultivar.

2. Egg laying period characterization in the orchard

In 2005, the oviposition commenced on May 4 to take end on June 4. Although, eggs were detected in nuts on July 9, but in very small numbers (Table 1). The maximum of eggs were deposited at around 10 May 2005 (Table 1). In 2006, the egg laying period commenced on May 4 to finish at the end of the moth (27 May) with a maximum from 4 May to 14 May 2006 (Table 1). In 2011, the egg laying period was concentrated in May with a maximum occurring on 16 May, 2011 (Table 1).

Table 1. Number of eggs deposited per 50 newly formed pistachio nuts in 2005, 2006 and 2011

Dates	Number of eggs	Dates	Number of eggs	Dates	Number of eggs
27/04/2005	0	23/04/2006	0	30/04/2011	0
30/04/2005	0	25/04/2006	0	07/05/2011	19
04/05/2005	3	27/04/2006	0	10/05/2011	7
07/05/2005	8	30/04/2006	0	12/05/2011	5
10/05/2005	24	04/05/2006	17	16/05/2011	17
14/05/2005	3	07/05/2006	11	19/05/2011	2
17/05/2005	7	10/05/2006	16	23/05/2011	6
20/05/2005	8	14/05/2006	13	26/05/2011	2
24/05/2005	6	17/05/2006	5	31/05/2011	2
27/05/2005	7	20/05/2006	5	06/06/2011	0
01/06/2005	4	24/05/2006	4	10/06/2011	0
04/06/2005	2	27/05/2006	7	14/06/2011	0
09/06/2005	0	01/06/2006	0	17/06/2011	0
14/06/2005	0	04/06/2006	0	21/06/2011	0
23/06/2005	0	09/06/2006	0	24/06/2011	0
28/06/2005	0	14/06/2006	0		
02/07/2005	0				
05/07/2005	0				
09/07/2005	2				
12/07/2005	0				

IV – Conclusion

The determination of the onset of *E. plotnikovi* adult emergence and its duration as well as the characterization of egg-laying period are considered to be of prime importance for the control of this pest using contact insecticides targeting adults. Our data for the years 2005, 2006 and 2011 suggested that the adult flight begins in late April, early-May and lasted to late May, early-June. Indeed, egg-laying period was concentrated during the same period. Thus, sprayings have to be undertaken during this period. Nevertheless pistachio growers have to be trained to detect the first adult emergence with field cages in order to apply sprays at the optimal time.

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Shoot feeding period characterization of the pistachio twig borer

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Abstract. In Tunisia, The pistachio twig borer, *Chaetoptelius vestitus* Mulsant & Rey (Coleoptera, Curculionidae, Scolytinae) is a serious pest of pistachio tree, *Pistacia vera*. The insect has two dispersal phases: in the first, mature adults seek recently cut pistachio branches, or live tree branches weakened by drought or physiologically stressed to lay eggs and initiate reproduction; in the second, new emerged adults fly toward healthy trees where they feed on the buds and excavate feeding galleries just beneath the buds toward the center of the twig. This paper deals with a study over two successive years conducted in the field in the Centre-East of Tunisia on shoot feeding, overwintering and the presence of *C. vestitus* inside pistachio twigs. The duration of these processes including age, diameter, length, of the burrowed tunnels as well as degree of shoot infestation were reported. The sampled twigs varied from 1 to 53 cm in length and between 0.2 and 1.1 cm in section. Results indicated that the percentage of the shoots damaged by *C. vestitus* ranged from 70 to 100% all year round except during the period from late March to late April where the percentage of infestation varied from 15 to 25%. This period could be attributed to the swarming of adults emerged from reproduction sites. Adults are present in feeding galleries all year round. However their densities dramatically decrease from late October to late March from an average of 16 adults per meter of twigs to 4 adults. This period could be a sign of adult migration as demonstrated the low number of adults per gallery; an average of 0.2 adult per gallery compared with an average of 0.8 adult per gallery throughout the other period. Most adults were found in feeding tunnels in current-year shoots. The length of feeding galley varied from 0.03 cm to 2.75 cm and diameter varied from 0.2 cm to 1.1 cm. The mean number of adults per meter of twigs varied from 0 to 68.18 depending on sampling dates. Consequently, the insect overwinters in two forms (1) a portion of the population hibernate as adult stage inside feeding galleries of pistachio shoots and (2) the other fraction leaves feeding galleries in autumn to initiate reproduction phase. The knowledge of shoot-feeding phase is one of the main elements when developing integrated management strategies.

Keywords. *Chaetoptelius vestitus* – *Pistacia vera* – *S*hoot feeding – Tunisia.

Essais de caractérisation de la phase de nutrition du scolyte du pistachier

Résumé. *Le scolyte du pistachier,* Chaetoptelius vestitus *Muls & Rey (Coleoptera, Curculionidae, Scolytinae) est un ravageur clé du pistachier, Pistacia vera L. L'insecte évolue en deux phases bien distinctes : dans la première, les adultes matures recherchent des branches cassées du pistachier, des arbres stressés ou dans un état physiologique médiocre pour pondre des œufs et se reproduire ; dans la seconde l'insecte attaque les bourgeons puis mine des galeries profondes dans le cœur du rameau. Les rameaux minés par les galeries de nutrition flétrissent très rapidement puis se dessèchent. Dans ce travail d'une durée de deux ans, réalisé dans le centre Est de la Tunisie, on a essayé de caractériser la phase de nutrition de l'insecte, la durée de présence de l'insecte dans les galeries nutritionnelles, son mode d'hibernation ainsi que la longueur, le diamètre des galeries et le taux correspondant d'infestation des rameaux. Vingt rameaux (dont la longueur varie de 1 à 53 cm et le diamètre de 0,2 à 1,1 cm) ont été prélevés au hasard, avec une fréquence généralement hebdomadaire sur au moins 5 arbres et examinés au laboratoire en distinguant les rameaux de l'année, des rameaux d'un an d'âge puis disséqués et examinés sous loupe binoculaire. Les résultats indiquent un fort pourcentage d'infestation des rameaux variant de 70 à 100% durant les deux années d'étude à l'exception de la période allant de la fin mars à la fin avril où ce taux variait de 15 to 25%, période caractérisée par le début de débourrement des arbres avec faible présence de nouvelles pousses et où les adultes issus de la phase de reproduction ont débuté leur émergence. Les adultes sont présents dans les galeries de nutrition l'année du-*

rant toutefois leur densités diminuent fortement (de 16 adultes par mètre linéaire de rameau à 4 adultes par mètre) durant la période de la fin octobre à la fin mars témoignant de la migration des imagos vers les sites de reproduction. La plupart des adultes sont localisés dans les pousses de l'année. La longueur moyenne de la galerie varie de 0,03 cm à 2,75 cm alors que le diamètre entre 0,2 cm et 1,1 cm. Le nombre moyen d'adultes par mètre linéaire de rameau varie de 0 to 68.18 et ce en fonction des dates d'échantillonnage. De ces résultats il ressort que l'insecte hiverne sous deux formes (1) une fraction de la population passe l'hiver sous forme d'adultes dans les galeries nutritionnelles, pour les quitter au débourrement de l'arbre et s'attaquer aux nouvelles pousses (2) une fraction quitte sa logette pour amorcer une phase de reproduction en automne. La connaissance de la période de nutrition du scolyte du pistachier, C. vestitus *est un élément important à prendre en considération dans une stratégie de lutte intégrée contre ce déprédateur.*

Mots-clés. Chaetoptelius vestitus – Pistacia vera – *Galeries nutritionnelles – Tunisie.*

I – Introduction

The pistachio bark beetle, *Chaetoptelius vestitus* Mulsant & Rey (Coleoptera, Curculionidae, Scolytinae), has been reported as a pest of pistachio tree throughout the Mediterranean as well as in Asia (Davatchi, 1958, Balachowsky 1963, Mehrnejad, 2001). In Tunisia, *C. vestitus* is considered to be an important pest of Pistachio tree. The insect has two biological periods (1) a reproduction phase which takes place in stumps, pruned pistachio logs left over from annual pruning or occasionally in live tree branches, weakened by drought, physiological stress, mechanical damage or unsatisfactory pruning and (2) a feeding phase on healthy pistachio shoots and buds (Braham and Jardak, 2012). Generally, the new generation of beetle emerges in spring. Adults fly to the crown and nearby pistachio trees where they feed on the buds and excavating feeding galleries inside twigs seriously affecting the growth of the tree and its flowering. This paper deals with the characterization of the duration of feeding phase, its importance, the presence of *C. vestitus* inside shoots and the length of feeding galleries. The knowledge if these processes contribute to the best management of the insect.

II – Materials and methods

The study was undertaken in a private pistachio orchard in the Sfax region (34°44'N; and 10°45' East). Sixty one female pistachio trees and 9 males were planted in 1979 under rain fed conditions. The orchard, unsprayed was known to be infested by the pistachio twig borer. Twenty pistachio shoots were randomly sampled at weekly interval from a least 5 different trees during two consecutive years (from 21 June 2001 to 23 August 2003). Twigs were categorized in the laboratory as newly shoots (current shoots) or one-year old shoots and then dissected under stereomicroscope to record the number of feeding galleries, the length of each gallery, the number of alive or dead insects. The percentage of infested twigs was calculated as the ratio of infested twig divided by the total number of sampled twigs.

III – Results

1. Twig section

The diameter of the sampled twig varied from 0.2 to 1.1 cm with an average of 0.51 cm (Fig. 1).

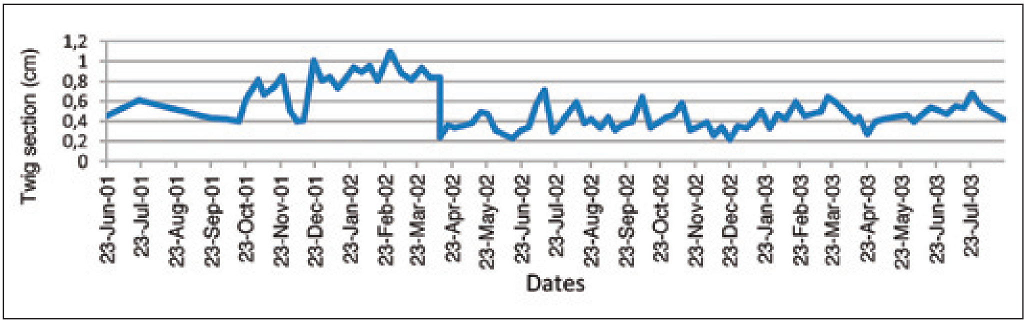

Fig. 1. The average measures of twig sampled during the study.

2. Shoot and twig infestation

Twigs were highly infested showing high percentage of buds damaged by adults. This percentage varied from 70 to 100% all year round except from late March to late April where the percentage of infestation varied from 15 to 25% (Fig. 2). During April newly formed shoots were sampled following bud and leaves appearing. Likely, this period could be attributed to the swarming of adults emerged from reproduction sites.

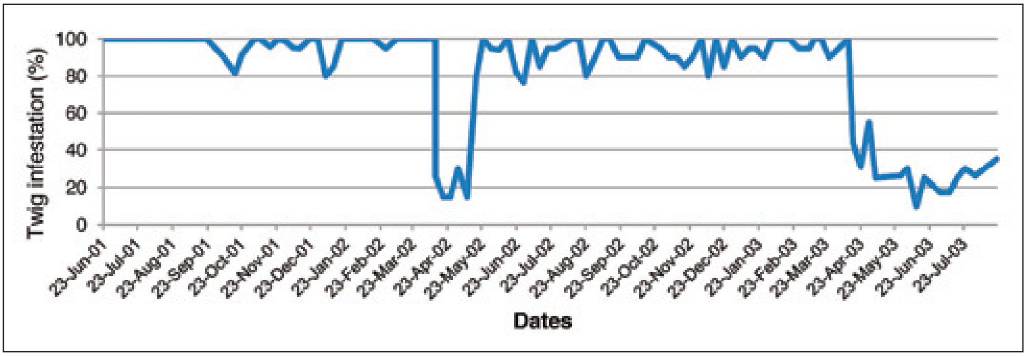

Fig. 2. Percentages of infested twigs during the study period.

3. Average number of adults per feeding gallery

The average number of adult per gallery varied from zero to 1 according to season. The mean number varied between 0.8 in summer and autumn to 0.13 in winter-early spring. In spring this percentage approaching 0.6 adults per gallery. We only found at the most 1 adult per gallery (Fig. 3). It is possible that the adult inside gallery emits pheromone to prevent congeners for colonizing twigs. Most adults were found in feeding tunnels in current-year shoots.

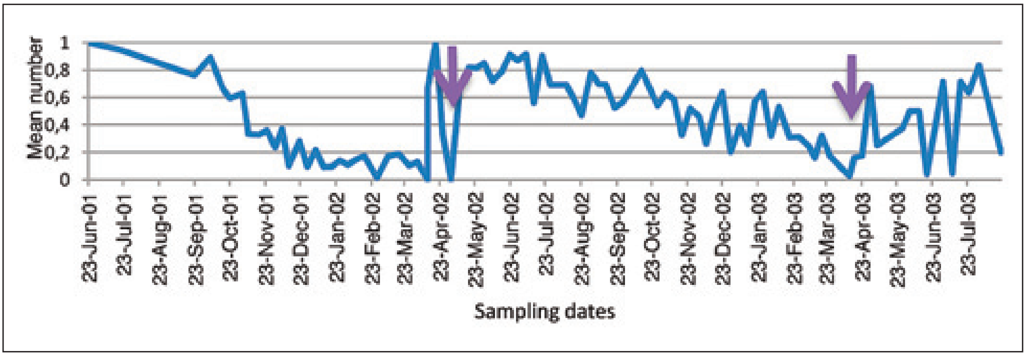

Fig. 3. Number of *C. vestitus* adult per feeding gallery (arrows indicate new flushing).

4. Mean gallery length

During the study period, the mean length of feeding gallery was 1.23 ± 0.66 cm (Min = 0.03 cm; Max = 2.75 cm) and diameter varied from 0.2 cm to 1.1 cm. The gallery length is low following bloom then increases thereafter (Fig. 4).

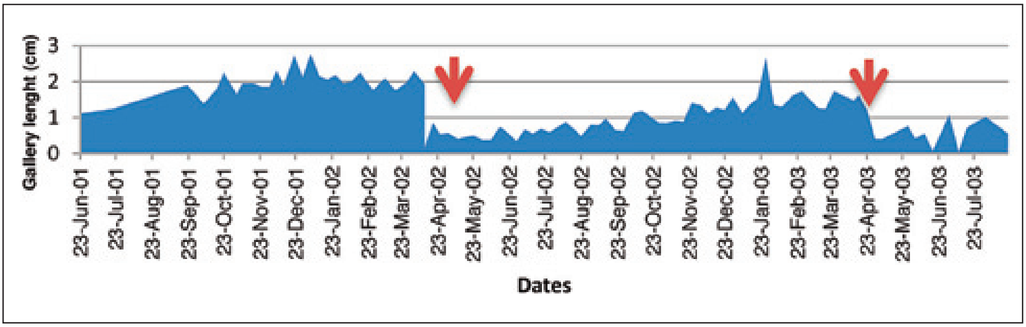

Fig. 4. Mean gallery length (arrows indicate new flushing).

5. The mean number of adults per meter of twig

For the two years of study, adults are present in feeding galleries all year round with an average of 8 insects per meter of twig (minimum 0; maximum 68.18 insects depending on sampling dates). However their densities dramatically decrease from late October to late March (from an average of 16 adults per meter of twigs to 4 adults). This period could be a sign of adult migration as demonstrated the low number of adults per gallery; an average of 0.2 adult per gallery compared with an average of 0.8 adult per gallery throughout the other period.

IV – Discussion and conclusions

According to our Knowledge there is no published study reporting the characterization of *C. vestitus* feeding period. Indeed, In Algeria, Chebouti-Meziou *et al*. (2011) studied mainly the reproductive period of the insect in Tlemecen. They reported that the insect lays eggs and makes the reproductive tunnels in the young shoots of the year. Their results were in contradiction with ours, since we have never found eggs or reproduction galleries in young shoots. Indeed Braham and Jardak

(2012) studying the life cycle of the pistachio twig borer reported two different ecological cycles (1) a reproduction phase which takes place in stumps, pruned pistachio logs left over from annual pruning, weakened by drought, physiological stress, mechanical damage or unsatisfactory pruning and (2) a feeding phase on healthy pistachio shoots and buds. The insect begins attacking new shoots few days following bud and leaves initiation (late April-early May). Our data are in agreement with results reported by Mehrenjed (2001) in Iran who indicated that *C. vestitus* infestation commenced in May. The small difference in time emergence between Tunisia and Iran may be attributed to local climatic conditions. The insect overwinters in two forms (1) a portion of the population hibernate as adult stage inside feeding galleries of pistachio shoots and (2) the other fraction leaves feeding galleries in autumn to initiate reproduction phase. The knowledge of shoot-feeding phase is one of the main elements when developing integrated management strategies.

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Determination of certain biological characteristics of the pistachio seed wasp, *Eurytoma plotnikovi* Nik. (Hymenoptera, Eurytomidae), for its natural enemies in South East Anatolia, Turkey

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Abstract. The project was conducted in pistachio orchards in Gaziantep in 2011-2013. The purpose of the study was to determine biological characteristics of pistachio seed wasp, *Eurytoma plotnikovi* Nik. (Hymenoptera, Eurytomidae). Fruits infested with the pest were placed in cages in January in orchard. The cages were checked weekly starting in the first week of April. After the first adult emergence in the orchard in Gaziantep, daily monitoring was initiated. First and maximum adult emergence times and adult emergence period were determined in orchard. According to examination of the fruits in the orchard in Gaziantep in July in 2011 and 2013, it was determined that the pest pupates within fruits and later leaves the fruit as an adult. During the study, pupa period, adult lifespan, male/female ratio and host range of the pest in Southeastern Anatolia were determined.

Keywords. Pistachio – Pistachio seed wasp – *Eurytoma plotnikovi* Nik. (Hymenoptera, E urytomidae) – Naturel enemies.

Détermination de certaines caractéristiques biologiques de la guêpe des semences du pistachier,* Eurytoma plotnikovi *Nik. (Hymenoptera, Eurytomidae), pour ses ennemis naturels dans le sud-est de l'Anatolie, Turquie

Résumé. *Le projet a été mené dans des vergers de pistachiers à Gaziantep en 2011-2013. Le propos de l'étude était de déterminer les caractéristiques biologiques de la guêpe des semences du pistachier,* Eurytoma plotnikovi *Nik. (Hymenoptera, Eurytomidae). Les fruits infestés par le ravageur furent placés en janvier dans des cages dans le verger. Les cages étaient inspectées toutes les semaines à partir de la première semaine d'avril. Après la première émergence d'adultes dans le verger à Gaziantep, une surveillance journalière fut mise en place. Le moment de la première émergence d'adultes, de l'émergence maximale d'adultes, ainsi que la période d'émergence d'adultes, furent déterminés au verger. Selon l'examen des fruits du verger à Gaziantep en juillet 2011 et 2013, on a établi que le ravageur se nymphose à l'intérieur des fruits et quitte ensuite le fruit ayant atteint l'âge adulte. Durant l'étude, on a déterminé la période de nymphose, espérance de vie à l'âge adulte, le rapport mâle/femelle et spectre d'hôtes du ravageur dans le sud-est de l'Anatolie.*

Mots-clés. *Pistachier – Guêpe des semences du pistachier –* Eurytoma plotnikovi *Nik. (Hymenoptera, Eurytomidae) – Ennemis naturels.*

I – Introduction

Pistacia species exist and can grow within 30-45^o latitude both in North and South hemisphere and in microclimates resembling them (Bilgen, 1973). According to Ülkümen and Özbek (1950), the origin of pistachio is Turkey, Iran and Afghanistan. Turkey is in the North hemisphere and within the area of its genetic origin. Southeastern Anatolia has an important role in pistachio production. Besides being the place of its first cultivation, due to its own ecological characteristics, the region provides opportunities for its cultivation and dispersal. Pistachio is an undemanding plant in all respects. Therefore, it can grow in places which are stony, insufficient in nutrients and rich with lime. Furthermore, because pistachio cultivation is possible on areas where irrigation water is restricted, amount of rainfall is low (300-500 mm/m^2) and on areas where cultivation of any other crop is not economically feasible, pistachio production has considerable economic value for both growers and the country (Tekin *et al.,* 2001).

Three pest insect species were determined on pistachio fruits in Turkey. These are *Megastigmus pistaciae* Wal., *Shneidereria (= Recurvaria) pistaciicola* Danil. and *Eurytoma plotnikovi* Nik. (Hymenoptera: Eurytomidae) (Doğanlar & Karadağ, 2008). Difficulties on the control of, *Eurytoma plotnikovi* entailed this study to elucidate the biology of the pest in the region.

II – Materials and methods

Materials: The main materials of the study were infested pistachio orchards by the pest, fruits in these orchards, insect culturing cages, branch cages, and meteorological data.

Some morphological features of *Eurytoma plotnikovi* Nik: Morphological measurements were taken from 30 males, females, larvae and pupa.

Adult lifespan: To find the adult lifespan, 47 individuals were taken into culture in natural conditions. As nutrition, cotton wetted with sugar solution was placed in each cage and renewed daily. Dead individuals were recorded daily and removed from the cages.

Overwintering status: Wintering pistachio orchards in order to detect the status of the pest from the fruit samples were collected. A portion of the collected samples individually opened and wintering pest status is determined.

Adult emergence: In order to determine the time of adult emergence, cages containing damaged fruits were placed in orchards in Gaziantep and şanlıurfa in the first week of January. Adult emergence was monitored weekly starting from the first week of April. The data was evaluated to determine first and maximum adult emergence times and adult emergence period. During adult emergence period, maximum and minimum temperatures and average relative humidity were recorded daily.

Pupa period: Larvae removed from injured fruits were placed in culture cages and left in natural conditions. Time between pupation and adult emergence was determined by periodical.

Host plants: Samples were collected from *Pistacia* species in order to find whether or not the pest has other hosts.

III – Results and discussion

Certain morphological features of *Eurytoma plotnikovi* Nik: Female body length (excluding ovipositor) is 2.1-2.5 mm while male body is mm long. The body is generally rust-red in color, head, black base with metal hose and show pronotum the middle of the segment; antenna and legs rust color. Male body black; legs rust-colored; black antennae; wing veins brown; abdomen short ball-shaped. The male antenna is tall. The larvae mature in the period 5-6 mm long, white-colored, legless. The eggs are oval. Pupa first color is white and the dark color is close to adult emergence. Pupa is free stern type.

Adult lifespan: 20 individuals have been released into the cage from individuals living 5 days with the shortest adult individuals who lived the longest 20 days. Adult life continued an average 13.5 days. Male individuals live up to 10 days with 2 adults and the average male individual life lasts 6 days. Female individuals are living up to the 2 to 13 days. The average adult female individual’s life lasts 8 days.

Overwintering status: It was determined that the pest larvae during winter.

Adult emergence: Adult emergence was found that first week of May. However, depending on the phenology can be output in mid May. The average duration of 32.5 days for the emergence Gaziantep, Sanliurfa province has been identified as 27 days.

Pupa period: First pupa was observed on the second week of April. The time between the first adult emergence and the date of first pupae in nature is 15-16 days.

Host plant: Besides *Pistacia vera*, the pest was also found in the fruits of *P. khinjuk* and *P. terebinthus.*

IV – Conclusions

As a result E.plotnikovi is an important species to be considered in the areas of pistachios. it is especially important in determining the time of adult emergence to monitor for that struggle has concluded that there is need to.

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Morphology of healthy leaves and galling leaves in *Pistacia terebinthus* L.

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Abstract. The pistachio is one of the genetic resources under threat of extinction in Algeria, given the combination of stresses of biotic and abiotic nature. Among these biotic factors, the aphids can induce galls on the leaves and hence, change their morphology. The study of morphological changes occurred on the leaves of *Pistacia terebinthus,* L. following gall aphids attack was analyzed in two different sites in the province of Djelfa (Gottiya and Senalba) in Algeria. Ten healthy leaves and ten galling leaves were sampled from ten trees of *P. terebinthus*. Quantitative analysis of morphological variables of healthy and infected leaves has been conducted. T-test showed highly significant differences between infected and healthy leaves of *P. terebinthus* regarding the length, the width of the leaves, the terminal leaflet and the petiole length.

Keywords. Galls – *Pistacia terebinthus,* L. – Quantitative variables – T-test.

***Morphologie des feuilles saines et des feuilles infectées de galles chez* Pistacia terebinthus**

Résumé. *Le pistachier est l'une des ressources génétiques menacée d'extinction en Algérie, vu la combinaison de stress de nature biotique et abiotique. Parmi ces facteurs biotiques, on peut citer les aphides comme insectes ravageurs induisant la formation de galles. L'étude des modifications morphologiques produites sur les feuilles de* Pistacia terebinthus *suite à l'attaque de pucerons gallicoles, a été analysée à l'échelle du peuplement, au niveau de deux différentes stations dans la wilaya de Djelfa. Dix feuilles saines et dix feuilles portant des galles ont été échantillonnées sur dix arbres de* P. terebinthus*, au niveau de deux forêts (*Gottiya et *Senalba) dans la wilaya de Djelfa en Algérie, une analyse quantitative des variables morphologiques des feuilles saines et infectées a été conduite. Le test t montre qu'il existe des différences hautement significatives entre les feuilles infectées et saines de* P. terebinthus *concernant la longueur, la largeur de la feuille et de la foliole terminale ainsi que la longueur du pétiole.*

Mots-clés. *Galles* – Pistacia terebinthus – *Variables quantitatives – Test t.*

I – Introduction

Pistachios, family Anacardiaceae, is a genus of remarkable morphological and genetic biodiversity (Gaussen *et al*., 1982). It is rare and it is endangered in Algeria. This is the result of a chain of interrelated factors such as abiotic and biotic stresses (Belhadj, 2001). The plant-insect relationships have been the subject of extensive studies. If insects are particularly useful for plants, many pests that feed on the plant are sometimes causing irreparable damage (Djazouli, 2010). Aphids attack the pistachio producing galls of different shapes and sizes, causing morphological, anatomical or physiological changes. In this work, a morphological study of infected leaf galls and healthy uninfected leaves of *Pistacia terebinthus* was conducted in order to find out the influence of galling formations on the morphology and size of the leaves.

II – Materials and methods

1. Plant material

Healthy and infected leaves of *P. terebinthus* were collected from two sites within the province of Djelfa in Algeria: (1) Senalba forest (36°42' N, 3°12' W), located about 6 km from the town of Djelfa whith an area of 13,700 ha (Fig. 1), characterized by a continental Mediterranean climate going from cold semi-arid to arid fresh with an average altitude of 1200 m; and (2) Gottiya forest (34°33 'N and 2°48' W) located about 3 km south east of Charef (located about 41 km from town of Djelfa) and extending over an area of 2770 ha at 1320 m mean altitude (Fig. 1), under a semi-arid climate with a cold winter.

The main objective of this study is to investigate the effect of the development of galls on the morphology of *P. terebinthus* leaves. Seven morphological characters (length and width of the leaf, number of leaves, length and width of the terminal leaflet, length/width terminal leaflet ratio and petiole length) were measured (IPGRI, 1998) (Table 1), in ten healthy and ten infected leaves (bearing galls) that were taken randomly on 5 trees from each site.

The data obtained were subjected to statistical analysis using the "Statistica 9 and 10" software. Descriptive and t test analyses were performed.

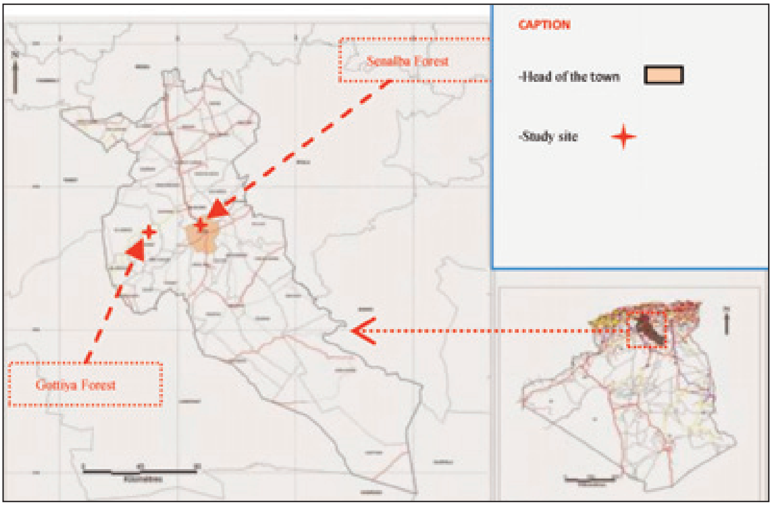

Fig. 1. Geographical location of the sampling sites (Gottiya and Senelba) in the province of Djelfa, Algeria (DPAT, 2012, modified).

III – Results

1. Gottiya Site

The leaf: Leaf length varies between 4.00 to 14.90 cm for healthy leaves and from 3.80 to 16.10 cm for infected leaves. For the width, healthy leaves have an average of 7.15 cm against 7.35 cm for infected ones. The number of leaflets varies between 5 to 11 in healthy leaves and infected leaves (Table 1).

The terminal leaflet: Healthy leaves have a terminal leaflet with an average of 3.75 cm in length and 1.28 cm in width against 3.91 cm in length and 1.29 cm in width for the infected leaves. Infected leaves have a terminal leaflet length / width ratio higher (3,06 cm) than the infected ones (2,96 cm).

The petiole: It is longer for the healthy leaves (2.84 cm) as compared to the infected ones (2.73 cm) (Table 1).

T-test revealed no significant difference between healthy and infected leaves of *P.terebinthus* in Gottiya site for the following characters, length and width of the leaves, the terminal leaflet length/ width ratio and length of the petiole, while a significant difference in the number of leaflets between healthy and infected leaves is registered (Table 1).

Table 1. Characteristics of quantitative variables measured for healthy and infected leaves of *P. terebithus* Mean ± SD ; Min-Max (C.V.%)

Sampling site	Gouttaya			Senalba		
Variables	**Healthy leaves**	**Infected leaves**	**Sig.**	**Healthy leaves**	**Infected leaves**	**Sig.**
1-Leaf length (cm)	10.49 ± 2.35 4.00-14.90 (22.45)	9.77 ± 2.35 3.80-16.10 (24.08)	NS	12.25 ± 3.26 7.10-18.90 (26.61)	8.70 ± 2.07 4.60-12.20 (23.78)	***
2-Leaf width (cm).	7.15 ± 1.57 4.7-10.30 (21.95)	7.35 ± 1.55 4.2-11.20 (21.16)	NS	11.38 ± 2.33 6.80-15.40 (20.50)	9.14 ± 1.51 6.20-13.40 (16.58)	***
3-Number of leaflets	8.40 ± 1.53 5-11 (18.31)	7.64 ± 1.67 5-11 (21.92)	*	7.36 ± 1.56 5-11 (21.21)	7.10 ± 1.32 4-11 (18.71)	NS
4-Terminal leaflet Length (cm)	3.75 ± 0.83 1.90-5.1 (22.14)	3.91 ± 1.12 2.10-6.7 (28.82)	NS	6.18 ± 1.90 3.10-13.80 (30.85)	3.92 ± 1.48 1.50-6.60 (37.73)	***
5-Terminal leaflet width (cm)	1.28 ± 0.27 0.80-1.90 (21.09)	1.29 ± 0.31 0.5-1.8 (24.43)	NS	1.75 ± 0.45 0.70-2.30 (26.03)	1.24 ± 0.39 0.60-1.80 (31.84)	***
6-length/ width of the terminal leaflet ratio	2.96 ± 0.53 1.52-4.36 (18.15)	3.06 ± 0.56 1.90-4.60 (18.50)	NS	3.60 ± 0.82 2.27-6.27 (22.79)	3.12 ± 0.51 2.10-4.00 (16.60)	***
7-Petiole length (cm)	2.84 ± 0.79 2.84-0.79 (27.83)	2.73 ± 0.76 1.20-4.70 (28.10)	NS	3.35 ± 0.94 1.90-5.60 (28.23)	2.39 ± 0.47 1.50-3.30 (20.00)	

**** Significant at the 0.05 level; ** Significant at 0.01 level; *** Significant at 0.001 level.

2. Senalba site

The leaf: Healthy leaves dimensions vary between 7.10 to 18.90 cm in length (an average of 12.25 cm), and from 6.80 to 15.40 cm for the width (an average of 11.38 cm). The infected leaves are shorter (8.70 cm) and narrower (9.14 cm). The number of leaflets varies between 5 to 11 in healthy leaves and between 4 to 11 for the infected leaves (Table 1).

The terminal leaflet: A mean value of 6.16 cm is recorded for the length of the terminal leaflet in healthy leaves against 3.92 cm for the infected ones. Terminal leaflets in healthy leaves have a higher length/width ratio (3.60) than in the infected ones (3.12) (Table 1).

The petiole: It is longer for healthy leaves (2.39cm) as compared to the infected ones (2.73 cm).

The t-test revealed a highly significant difference between healthy and infected leaves of *P.terebinthus* for all the studied characters excepted for the number of leaflets (no significant difference) (Table 1).

IV – Discussion

To provide information on the morphological changes due to the aphids (galls) on the leaves of *P. terebinthus* in Algeria, two sampling sites were selected and a total of seven morphological quantitative characters were measured in this study. The *t-test* allowed to distinguish the existence of discriminating characteristics between healthy and infected leaves in *P.terebinthus.* A noticeable decrease in size of the infected leaves is recorded specially for the length and the width of the leaves, for the terminal leaflet length/width ratio and for the length of the petiole. Aphids cause physiological and metabolic disturbances which produce changes in normal tissue which grows and changes in gall tissue (Wool, 2004). Also according to Shorthouse *et al.* (2005), the galling insects are among the most fascinating herbivores due to their ability to control and redirect the growth and physiology of the attacked body. In our study, the Senalba site showed significant differences for most of the studied characters between healthy and infected leaves, while Gouttaya site did not show significant differences for most of the characters, this difference in the frequency attack can be attributed either to the difference in morphological appearance of *P. terebinthus* trees between the two sites. In fact the foliage of the trees is not similar between the two sites which consequently generates sunshine and a different light and then a different attractiveness groups of aphids.

V – Conclusion

The size of the leaves, the terminal leaflets and the petiole as well as the number of leaflets were all influenced by the galling structures which caused dysfunction and reorientation of the normal development of *P. terebinthus* leaves morphology. This study revealed new insights into the aphid-pistachio relationship as no morphological studies have been conducted previously in Algeria. There are still other aspects to be investigated about this complex relationship, which will allow a better understanding of this species, for its preservation from one hand and for its use in reforestation or improvement programs on the other hand.

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Transferring resistance to *Plum pox virus* (PPV, sharka) from almond to peach by crossing and grafting

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Abstract. *Plum pox virus* (PPV) is a limiting factor for peach and many other stone fruit production in the areas that are affected. Although no natural sources of resistance have been identified in peach, intespecific crosses with almond can transmit this resistance. In addition, recent studies have demonstrated that grafting almond cultivar 'Garrigues' onto 'GF305' (a very PPV susceptible indicator) peach seedlings heavily infected with PPV, can progressively reduce disease symptoms and virus accumulation. This response appears to be specific between almond and peach. Furthermore, grafting 'Garrigues' onto 'GF305' before PPV inoculation completely prevented virus infection, showing that resistance is constitutive and not induced by the virus. This study aims to analyze the transmission of PPV resistance from almond to peach by grafting from a breeding point of view. In addition, the regulation of gene expression of the resistance to PPV transmitted by grafting among *Prunus* species will be discussed.

Keywords. Almond – Peach – Sharka – *Plum pox virus* – Resistance.

Transfert de la résistance au virus de la sharka (PPV) de l'amandier au pêcher par croisement et greffage

Résumé. Le Plum pox virus *(PPV) est un facteur limitant pour la pêche, et pour beaucoup d'autres productions de fruits à noyau de la zone, qui sont touchées. Bien qu'aucune source naturelle de résistance n'ait été identifiée chez le pêcher, les croisements interspécifiques avec l'amandier peuvent transmettre cette résistance. En outre, des études récentes ont démontré que le greffage du cultivar d'amandier 'Garrigues' sur plants de pêchers 'GF305' (un indicateur très sensible au PPV) fortement infectés par PPV, peut progressivement réduire les symptômes de la maladie et l'accumulation de virus. Cette réponse semble être spécifique entre l'amandier et le pêcher. En outre, le greffage de 'Garrigues' sur 'GF305' avant inoculation du PPV a complètement empêché l'infection par le virus, montrant que la résistance est constitutive et non induite par le virus. Cette étude vise à analyser la transmission de la résistance au PPV de l'amandier au pêcher par greffage du point de vue de l'amélioration. En outre, la régulation de l'expression du gène de résistance au PPV transmis par greffage entre espèces de Prunus sera discutée.*

Mots-clés. *Amandier – Pêcher – Sharka –* Plum pox virus *– Résistance.*

I – Introduction

Sharka, caused by *Plum pox virus* (PPV), is the most important viral disease affecting stone fruit species from the genus *Prunus*, in which varietal susceptibility is widespread. The importance of this disease is due to the strong symptoms it produces on fruits, making them unmarketable, and the reduced yield of infected trees. Moreover, transmission of PPV by both aphids and vegetative propagation of infected plant material has in many cases undermined efforts to contain the disease. These features make PPV one of the ten most important plant viruses in the world, and one of the most studied viral diseases (Scholthof *et al*., 2011). Within the genus *Prunus*, peach [*Prunus per-*

sica (L.) Batsch] is the most economically important species, with a world production of more than 20 million tons in 2010. For breeders and producers, the lack of widely studied sources of resistance to PPV in peach (Rubio *et al*., 2012) is the most significant factor limiting the release of new resistant varieties.

The incorporation of resistance genes from other related wild species has been an alternative. For instance, *P. ferganensis* (Kostov and Rjabov) Kovalev and Kostov and particularly *P. davidiana* (Carrière) Franch were the first species described as potential sources of resistance to PPV in peach (Pascal *et al*., 2002). However, the ability of some clones of *P. davidiana* to transmit PPV resistance to descendants has been questioned, and the importance of the genetic background in the expression of this resistance has been highlighted (Rubio *et al*., 2010), significantly limiting the use of this species as a source of resistance. Another alternative is the use of the closely related species almond (*P. dulcis* Mill, DA Webb).

In this work, transferring resistance to PPV from almond to peach by crossing and grafting is presented as an alternative to the lack of resistance in peach cultivars.

II – Transferring PPV resistance from almond to peach by crossing

Rubio *et al*. (2003) evaluated the resistance to PPV of ten almond varieties on peach 'GF305' rootstock infected with PPV-D and observed that no almond variety showed symptoms. Martínez-Gómez *et al.* (2004) subsequently demonstrated the usefulness of almond as a donor of resistance to peach through interspecific crosses (Fig. 1). Finally, Rubio *et al*. (2003) observed that sharka symptoms on 'GF305' rootstocks on which 'Garrigues' was grafted tended to disappear.

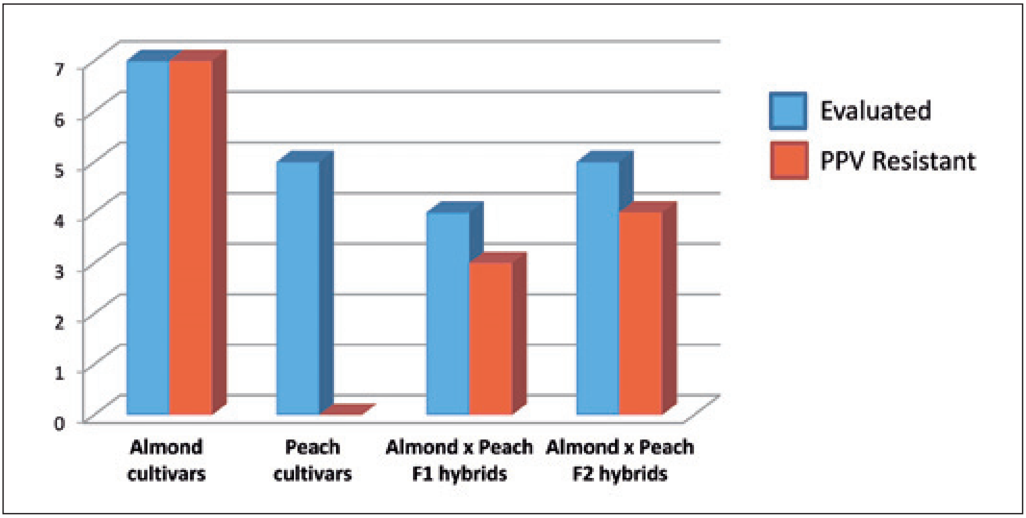

Fig. 1. Evaluation of resistance to PPV in almond and peach cultivars and in F1 and F2 interspecific crosses. Adapted from Martínez-Gómez *et al*. (2004).

III – Transferring PPV resistance from almond to peach by grafting

Recent studies, however, have also demonstrated that grafting the almond cultivar ‘Garrigues’ onto ‘GF305’ (a very PPV susceptible indicator) peach seedlings heavily infected with PPV, can progressively reduce disease symptoms and virus accumulation (Rubio *et al*., 2013). This response appears to be specific between almond and peach. The ability to induce resistance to PPV in ‘GF305’ was transmitted to the sexual descendants of Garrigues.

Furthermore, grafting ‘Garrigues’ onto ‘GF305’ before PPV inoculation completely prevented virus infection, showing that resistance is constitutive and not induced by the virus (Fig. 2). This fact suggests that resistance may be due to the transfer of a defense factor from ‘Garrigues’ almond through the graft union and its interaction with specific factors of ‘GF305’ peach to produce the antiviral response.

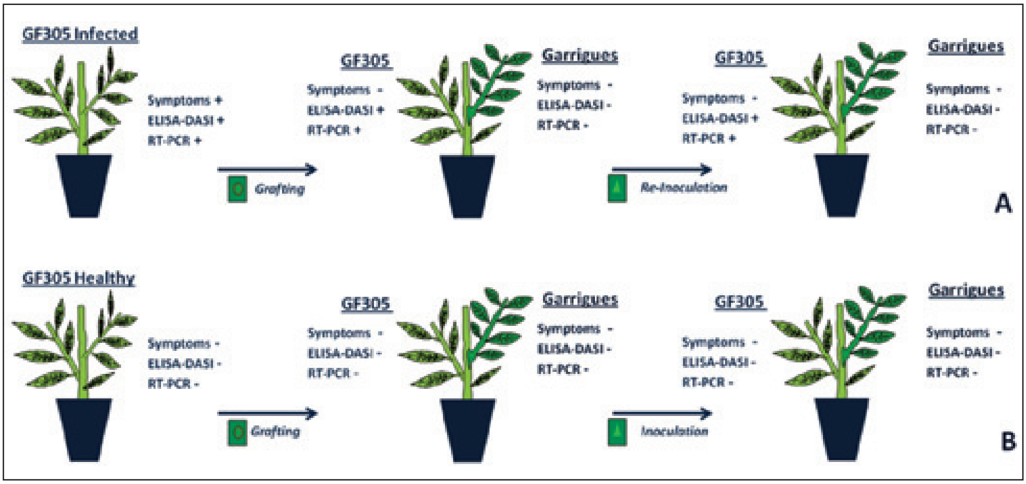

Fig. 2. Transmission of PPV resistance from almond to peach by grafting. Adapted from Rubio *et al.* (2013). Schematic representation of the assay processes infected GF305 peach showing strong sharka symptoms grafted with Garrigues almond (A) and healthy GF305 grafted with Garrigues and later inoculated with PPV (B). GF305 peach seedling inoculated with PPV and showing strong sharka symptoms.

IV – Conclusions

Results have shown the successful transmission of PPV resistance from almond to peach by crossing and grafting. These results open new avenues to potential protection against PPV in peach, the most economically important species among stone fruits.

Acknowledgements

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Comparison between phyllosphere components of the leaves of *Pistacia lentiscus* in two stations of the Wilaya of Medea (Cherrata and Tamezguida) in Algeria

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Abstract. The aerial part of plants, phyllosphere, is colonized by complex communities of microorganisms. The microorganisms of the phyllosphere influence many processes in ecosystems and play a non-negligible role in agronomic and environmental field but its origin remains unclear. Bacteria are numerically more important and can modify plant growth and suppress or stimulate tissue colonization and infection by pathogens. In this study we aimed to describe the differences between the fungal and bacterial communities, their compositions and their structures on the leaf surface area of *Pistacia lentiscus* so called mastic pistachio. The biodiversity indices were performed to determine the organization of microorganisms in their communities after their characterization and taxonomy. Our results showed that the composition and the number of microorganisms are correlated positively with the leaf area.

Keywords. Phyllosphere – *Pistacia lentiscus* – Leaf area – Biodiversity.

Comparaison entre composantes de la phyllosphère pour les feuilles de* Pistacia lentiscus *dans deux stations de la Wilaya de Médéa (Cherrata et Tamezguida) en Algérie

Résumé. *La partie aérienne des plantes, la phyllosphère, est colonisée par des communautés complexes de micro-organismes. Les micro-organismes de la phyllosphère influencent de nombreux processus dans les écosystèmes et jouent un rôle non négligeable dans le domaine agronomique et environnemental mais leur origine reste floue. Les bactéries sont numériquement plus importantes et peuvent modifier la croissance de la plante et supprimer ou stimuler la colonisation des tissus et l'infection par les pathogènes. Dans cette étude nous nous proposons de décrire les différences entre les communautés fongiques et bactériennes, leurs compositions et leurs structures sur la surface de la feuille de Pistacia lentiscus, appelé aussi arbre au mastic. Les indices de biodiversité ont été calculés pour déterminer l'organisation des micro-organismes dans leurs communautés après leur caractérisation et taxonomie. Nos résultats montrent que la composition et le nombre de micro-organismes sont positivement corrélés à la surface foliaire.*

Mots-clés. *Phyllosphère* – Pistacia lentiscus – *Surface foliaire – Biodiversité.*

I – Introduction

Phyllosphere is the habitat provided by the leaf of the plant for many microbial species such as pathogens, saprophytes or mutualistic microorganisms. So this microbial compartment influences the dynamics and structure of plant communities. Although the phyllosphere composition was determined in some species, its origin remains unclear (Compant *et al.*, 2010), it may be linked to genetics of the species (Cordier, 2012) everything that defines the phenotype such as essential oils and phenols (Yadav *et al*., 2008). Also, climate variation could change the structure and assembly of these microorganisms. In this structure, Bacteria are numerical majority along with the Archaea and Fungi. These microorganisms influence many processes in ecosystems and play an important role in agronomical and environmental perspectives (Compant *et al*., 2010).

The mastic tree, also called lentisc or *Pistacia lentiscus*, from Anacardiaceae family, is a sclerophyllous evergreen shrub, with strong odor of resin and a very slow growing (Munné-Bosch et Peñuelas, 2003 ; Belhadj, 2007). This species belongs to the *Eu-Lentiscus* section along with *P. weinmannifolia* and *P. saportae*. The leaves, 1.5-3 cm long, may last 2 years on the tree (Ain-Lhout *et al*., 2004), with winged rachis, paripinnate, 2-3 pair of leaflets. This shrub is widely distributed in the Mediterranean basin (Zohary, 1952). In Algeria, this species is widely present in the thermo-Mediterranean bioclimatic stage. The shrub, about 2 m in height, may reach higher size (Belhadj, 2007) when growing in protected and humid places (Munné-Bosch et Peñuelas, 2003).

The bacterial communities of lentisc phyllosphere have been studied, particularly in relation to the composition of the leaves on essential oils (Yadav *et al*., 2008), as well as the interaction of chemical factors with the microorganisms and their opposite reaction to the change of the ecosystem (Yadav *et al*., 2005). The size of epiphytic populations is defined by the profile and the chemical structure of the mastic leave; many bacterial colonies have adapted to the antimicrobial activity of essential oils, which are used as a source of carbon (Yadav *et al*., 2008). In this study, our interest has focused on the study of the phylloplane microorganisms of the lentisc tree, to find out the relationship between these communities and their environment (leaf).

II – Material and methods

The composition of the phyllosphere is compared according to the leaf area. Leaves (3 leaves from nine trees, nine trees per site) were sampled in two sites in the area of Medea (Cherrata and Tamezguida, called the Platrière) (Fig. 1). First of all, measurement of the leaf area of 27 leaflets, per site, was carried out through a Mesurim software.Secondly, we carried out the cultivation of microorganisms of the phylloplane of the 27 measured leaflets, in Petri dishes. Counting of the number of bacterial and fungal colonies was then performed. The data were then analyzed using the PAST software. Simpson and Shanon biodiversity indices were calculated as well as the correlations between the leaf area and the number of microorganisms to find out whether their organization within their communities were similar or not.

Fig. 1. Location of the study area.

III – Results and discussion

The bacteria species are numerically higher. The number of species for both bacterial and fungal is well correlated to the leaf surface, since the leaves were ranked from the largest to the smallest area. The number of microorganisms increases with the leaf surface (Fig. 2).

Concerning the biodiversity indices (Simpson and Shannon), both populations are well balanced on the leaf surface, communities are distributed homogeneously (Fig. 3 and Table 1).

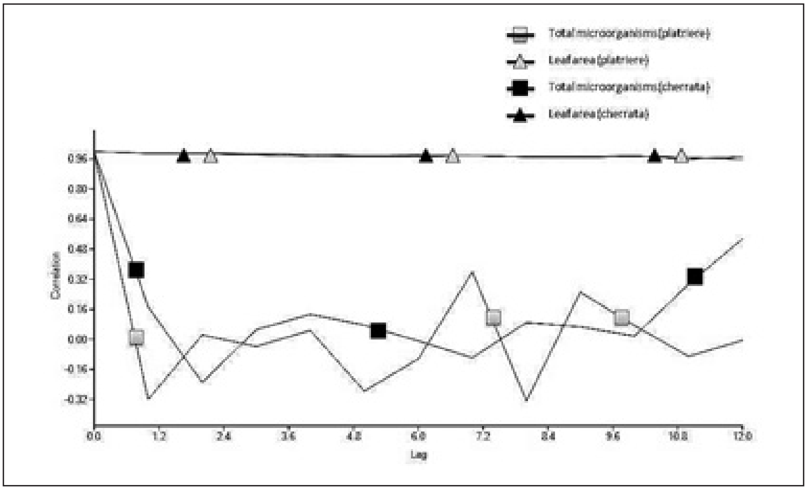

Fig. 2. Correlation between leaf area and total microorganisms from both sites.

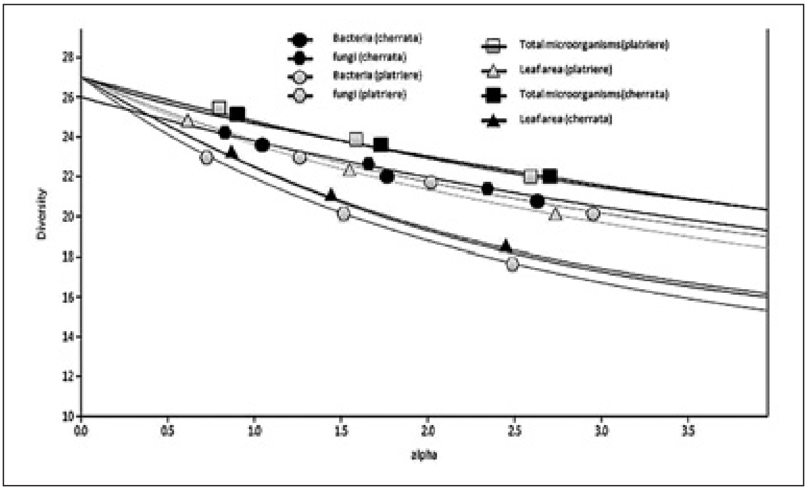

Fig. 3. Comparison of the diversity between the two sites over the leaf surface.

Table 1. Biodiversity indices calculated for both sites

	Bacterial colonies	Fungal colonies
Taxa_S	27	26
Individuals	193	112
Dominance_D	0.04771	0.04624
Simpson indice_1-D	0.9523	0.9538
Shanonindice_H	3.149	3.16
Evenness_e^H/S	0.8631	0.9068

IV – Conclusions

The organization of the phyllospheric components in *Pistacia lentiscus* leaves, their distribution and density is homogeneous. Bacteria are numerically higher than the fungi. The number of the colonies is correlated to the leaf surface.

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Sanitary status of Spanish autochthonous almond cultivars and its implication in breeding strategies

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Abstract. Traditional almond cultivars are an interesting source of different traits for breeders. These old cultivars have been multiplied by grafting for a long time and usually have been accumulating different viruses. The objective of this work is to determine the presence of different virus in these cultivars by multiplex RT-PCR. Virus analysed included: *Apple chlorotic leaf spot virus* (ACLSV), *Plum pox virus* (PPV), *Apple mosaic virus* (APMV), *Prunus necrotic ring spot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apricot latent virus* (APLV), *Plum bark necrosis and stem pitting-associated virus* (PBNSPaV), and *American plum line pattern virus* (APLPV). The obtained results showed the generalized presence of PNRSV and PDV in the analyzed collections with a preesence of 90% and 68% respectively. Despite of these virus do not show noticeable symptoms in the tree nor fruits, these poor phytosanitary state urge to the adoption of new estrategies for elimination of virus and cleaning these materials.

Keywords. Almond – Germplasm – Virus – RT-PCR – Sanitary status.

Statut sanitaire des collections espagnoles autochtones d'amandier et son implication dans les stratégies de sélection

Résumé. *Les cultivars d'amandier traditionnels sont une source intéressante de différents traits pour les éleveurs. Ces anciens cultivars ont été multipliés par greffage depuis longtemps et généralement ils ont accumulé virus différents. L'objectif de ce travail est de déterminer la présence du virus dans ces différents cultivars par RT-PCR multiplex. Les virus analysés on été: Apple chlorotic leaf spot virus (ACLSV), Plum pox virus (PPV), Apple mosaic virus (APMV), Prunus necrotic ring spot virus (PNRSV), Prune dwarf virus (PDV), Apricot latent virus (APLV), Plum bark necrosis and stem pitting-associated virus (PBNSPaV), et American plum line pattern virus (APLPV). Les résultats obtenus montrent la présence généralisée de PDV et PNRSV dans les collections analysés avec un présence de 90% et 68% respectivement. Malgré que ces virus ne présentent pas de symptômes visibles dans l'arbre, ni dans les fruits, le pauvre état phytosanitaire exhorte à l'adoption de nouvelles stratégies pour l'élimination de virus et de nettoyage de ces matériaux.*

Mots-clés. *Amandier – Germoplasme – Virus – RT-PCR – SSR.*

I – Introduction

Traditional almond cultivars are an interesting source of different traits for breeders. These old cultivars have been multiplied by grafting for a long time and usually have been accumulating different viruses.

Although no data is available in almond, *Prunus* orchards are affected by numerous viruses including *Plum pox virus* (PPV), causing sharka disease, and *Apple chlorotic leaf spot virus* (ACLSV). These are the most widespread viruses, followed by ilarviruses like *Prunus necrotic ring spot virus*

(PNRSV), *Apple mosaic virus* (ApMV) and *Prune dwarf virus* (PDV) (Dominguez *et al*., 1998; Myrta *et al*., 2003; García-Ibarra *et al*., 2012). The following are other new viruses described in Spain: *Plum bark necrosis and stem pitting-associated virus* (PBNSPaV), *American plum line pattern virus* (APLPV) and *Apricot latent virus* (APLV) (García-Ibarra *et al*., 2010).

The objective of this work was to determine the presence of different viruses in the Spanish autochthonous almond collections from the breeding programs of CEBAS-CSIC of Murcia and IRTA of Reus by multiplex RT-PCR. Virus analysed included ACLSV, PPV, APMV, PNRSV, PDV, APLV, PBNSPaV and APLPV.

II – Materials and methods

Almond genotypes assayed included 44 early flowering and self-incompatible local cultivars from the almond collections of CEBAS-CSIC of Murcia and IRTA of Reus (Table 1).

Leaf samples were analysed by multiplex RT-PCR to detect eight viruses, including *American plum line pattern virus* (APLPV), *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), *Apricot latent virus* (ApLV), *Plum bark necrosis and stem pitting-associated virus* (PBNSPaV), *Prune dwarf virus* (PDV), *Prunus necrotic ringspot virus* (PNRSV) and *Plum pox virus* (PPV) (Sánchez-Navarro *et al*., 2005).

III – Results and discussion

The results of this work show the high rate of infections in the Spanish autochthonous almond collections from CEBAS-CSIC of Murcia and IRTA of Reus. These results showed the generalized presence of PNRSV and PDV in the analyzed collections with a preesence of 90% and 68%, respectively. However no presence of ACLSV, PPV, APMV, APLV, PBNSPaV or APLPV was detected (Table 1).

These levels of infection are much higher than previously detected by serological techniques in almond (48% infection with PNRSV and PDV 23%) (Myrta *et al*., 2003). PNRSV is distributed worldwide in *Rosa* and *Prunus* spp. Generally, symptoms of PNRSV appear in the first year after infection, showing as asymptomatic in subsequent years, except for some isolates causing recurrent symptoms each year. The second multiplex RT-PCR carried out on CEBAS-CSIC collections showed the same results.

The potential yield loss caused by PNRSV in almond is usually low. PDV is similar to PNRSV in worldwide distribution. It is one of the most important viruses of stone fruit trees, predominantly sour and sweet cherry. PDV causes yellowing of leaves in these species and the dwarf peach alone or in mixed infections with PNRSV. In almonds were not described symptoms. Although these viruses are not a major threat to the almond tree, this species may play an important role in the spread of these viruses to other fruit, its control in nurseries, plantations and genebanks still needed.

IV – Conclusions

The obtained results showed the generalized presence of PNRSV and PDV in the analyzed collections with a presence of 90% and 68%, respectively. Despite the fact that these viruses do not show noticeable symptoms on the tree or fruits, this poor phytosanitary state calls for the urgent adoption of new strategies for the eliminating the viruses and cleaning up these materials.

Table 1. Spanish autochthonous almond cultivars assayed from the CEBAS-CSIC of Murcia and the IRTA of Reus collections

Variety	Origin	Virus							
		ACLSV	ApLV	APLPV	ApMV	PBNSPaV	PDV	PNRSV	PPV
CEBAS									
Atascada	Murcia	–	–	–	–	–	+	+	–
Atocha	Murcia	–	–	–	–	–	–	+	–
Avellanera	Murcia	–	–	–	–	–	+	+	–
Bonita	Baleares	–	–	–	–	–	+	+	–
Carretas	Murcia	–	–	–	–	–	+	+	–
CEBAS–1	Murcia	–	–	–	–	–	+	+	–
Colorada	Murcia	–	–	–	–	–	–	+	–
Del Cid	Desconocido	–	–	–	–	–	+	+	–
Desmayo AD	Incierto	–	–	–	–	–	+	+	–
Desmayo Lorca	Murcia	–	–	–	–	–	+	+	–
Fina del Alto	Murcia	–	–	–	–	–	–	+	–
Fournat	Francia	–	–	–	–	–	–	+	–
Garrigues	Murcia	–	–	–	–	–	+	+	–
J. Salazar	Murcia	–	–	–	–	–	+	+	–
Jordi	Mallorca	–	–	–	–	–	–	+	–
La Mona	Murcia	–	–	–	–	–	+	+	–
Malagueña	Desconocido	–	–	–	–	–	–	+	–
Marcona	Alicante	–	–	–	–	–	+	+	–
Marcona AD	Murcia	–	–	–	–	–	–	+	–
Marcona de San Joy	Murcia	–	–	–	–	–	+	+	–
Marcona Flota	Murcia	–	–	–	–	–	+	+	–
Pajarera	Murcia	–	–	–	–	–	+	+	–
Peraleja	Murcia	–	–	–	–	–	+	+	–
Planeta Fina	Alicante	–	–	–	–	–	+	+	–
Planeta Roja	Alicante	–	–	–	–	–	–	+	–
Ramillete	Murcia	–	–	–	–	–	–	+	–
Rumbeta	Alicante	–	–	–	–	–	+	+	–
Verruga	Murcia	–	–	–	–	–	+	+	–
IRTA									
Angones	Lleida	–	–	–	–	–	+	+	–
Asperilla	Huesca	–	–	–	–	–	+	+	–
Belardino	Castellón	–	–	–	–	–	+	–	–
Caima	Lleida	–	–	–	–	–	–	+	–
Carreró	Castellón	–	–	–	–	–	+	+	–
Esperanza forta	Tarragona	–	–	–	–	–	+	–	–
Gabaix	Tarragona	–	–	–	–	–	+	+	–
Mollar de la Princesa	Francia	–	–	–	–	–	+	–	–
Mollar de Tarragona	Tarragona	–	–	–	–	–	+	+	–
Nano	Castellón	–	–	–	–	–	+	+	–
Parque Samà	Tarragona	–	–	–	–	–	+	–	–
Pauet	Lleida	–	–	–	–	–	–	+	–
Pep de Juneda	Lleida	–	–	–	–	–	+	+	–
Rof	Tarragona	–	–	–	–	–	–	+	–
Tardaneta	Tarragona	–	–	–	–	–	–	+	–
Verd	Castellón	–	–	–	–	–	+	–	–

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Effect of *Chaetoptelius vestitus* on the chemical compostion of pistachio seed (*Pistacia vera*) harvested Bechloul (Algeria)

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Abstract. It is noteworthy that in the fruit pistachio orchard Bechloul two types of pistachio fruits are available namely the following healthy tree seed and the second type comes from tree attacked by wood-boring. For this purpose, a comparative study that will focus on the nutritional value of each type of seed on the water content, dry matter content, the determination of crude protein, the rate of crude fat, and the determination of sugars .The total analysis results pistachio seeds infested *Chaetoptelius vestitus* not reveal solids content with an average of 3.60 ± 0.45%. Regarding the dry matter content of seeds from trees infested by xylophagous, it is 3.85 ± 0.12%. There was no significant difference between the dry matter after pistachio control seeds and trees infested with wood-boring because the probability Pr 0.05 is greater than 0.228 (Pr > 0.05) for a 95% confidence interval. The water rate control seeds is 7.21 ± 0.36%. For pistachio seeds taken from trees infested the percentage of water reached 7.63 ± 0. 8%. The analysis of variance on the water content contained in the control trees and pistachio seeds infested by xylophagous shows that there is no significant difference, the probability (Pr) is 0.57, greater than 0.05 (Pr > 0.05) for a confidence interval equal to 95% .. in this study the average fat content of 47.1 ± 1.21% for controls pistachio seeds. It is 45.4 ± 2.09% for the seeds of pistachio trees from infested. In this study the analysis of variance of the fat content of the seeds from witnesses pistachio fruit trees and those infested Scolytidae, shows that there is a significant difference because the probability is 0.04 or less than 0.05 (Pr < 0.05) for a 95% confidence interval. As for the average rate of sugars, it is 3.9 ± 0.49% for the fruit pistachio seed witness. The sugar rate is 5.4 ± 0.88% in fruit pistachio seeds harvested from infested trees. The analysis of variance of total sugars between the seeds of the fruit pistachio witness and those of infested trees bud borer shows that there is no significant difference since the probability is therefore 0.50 greater than 0.05 (Pr > 0.05) (95% confidence interval). Proteins rate reached 39.5 ± 2.88% in the control seeds. One of infested seeds is 21.1 ± 6.48%. Analysis of variance between proteins pistachios seeds harvested from control trees and those from trees infested seeds shows that there is a significant difference considering the fact that the probability 0.007 is less than 0.05 (Pr < 0.05) for a 95% confidence interval. The total nitrogenous matter is 6.2 ± 0.99% in the control pistachio seed. The percentage of total nitrogen matter, it is 4.8 ± 0.21% in seeds from trees infested *Chaetoptelius vestitus.*

Keywords. *Chaetoptelius vestitus* – Chemical composition – *Pistacia vera* – Algeria.

Effet de* Chaetoptelius vestitus *sur la compostion chimique des graines de pistache* (Pistacia vera) *récolté à Bechloul (Algérie)

Résumé. *Il est à noter que dans le verger de pistachier fruitier à Bechloul deux types de graines de pistaches à savoir la graine saine issue d'arbre sain et le second type vient de l'arbre attaqué par le xylophage. A cet effet, une étude comparative qui mettra l'accent sur la valeur nutritionnelle de chaque type de semence sur la teneur en eau, teneur en matière sèche, la détermination de protéine brute, le taux de matières grasses brutes, et la détermination des sucres . En ce qui concerne la teneur en matière sèche des graines des arbres infestés par les xylophages, il est de 3,85 ± 0,12%. Il n'y avait pas de différence significative entre la matière sèche des graines saines de pistache et les graines issues d'arbres infestés par le xylophage parce que la probabilité est supérieur à 0,228 (Pr > 0,05) pour un intervalle de confiance de 95%. Les graines saines ont un taux d'eau de 7,21 ± 0,36%. Pour la graine de pistache provenant d'arbres infestés le pourcentage d'eau atteint 7,63 ± 0. 8%. L'analyse de la variance sur la teneur en eau contenue des graines de pistaches infestées par xylophages montre qu'il n'y a pas de différence significative, la probabilité (Pr) est de 0,57, supérieur à 0,05 (Pr >*

0,05) pour un intervalle de confiance à 95..% dans cette étude la teneur moyenne en matière grasse est de 47,1 ± 1,21% pour les graines saines. Il est de 45,4 ± 2,09% pour les graines d'arbres de pistaches infestée.. Dans cette étude, l'analyse de la variance de la teneur en matières grasses des graines saines et ceux infestées de Scolytidae, montre qu'il ya une différence significative parce que la probabilité est de 0,04 ou inférieur à 0,05 (Pr <0,05) pour un intervalle de confiance de 95%. Comme pour le taux moyen de sucres, il est de 3,9 ± 0,49% pour graines de pistache issues d'arbres sains. Le taux de sucre est de 5,4 ± 0,88% dans les graines de pistaches de fruits récoltés à partir arbres infestés. L'analyse de variance de sucres totaux entre les graines saines de pistache et ceux des arbres infestés par le foreur des bourgeons montre qu'il n'y a pas de différence significative puisque la probabilité est donc 0,50 supérieur à 0,05 (Pr > 0,05) (intervalle de confiance de 95%). Le taux de Protéines a atteint 39,5 ± 2,88% dans les graines saines. Des semences infestées est de 21,1 ± 6,48%. Analyse de la variance entre les protéines de graines de pistaches récoltées sur des arbres sains et les graines issues d'arbres infestées montre qu'il existe une différence significative compte tenu du fait que la probabilité est inférieure à 0,007 0,05 (Pr < 0,05) pendant un intervalle de confiance de 95%. La matière azotée totale est de 6,2 ± 0,99% dans la graine issue d'arbre sain.

Mots-clés. Chaetopterus vestitus – *Composition chimique* – Pistacia vera – *Algérie.*

I – Introduction

Generally pistachios are characteristic species of the Mediterranean region (Boudy, 1952). Yet in the field of pistachio production in Algeria certainly remains far behind the other Mediterranean countries. It should be recalled that Aleta *et al.* (1997) insist that gender *Pistacia* brings together a large number of species that have no agronomic interest appart their possible use as rootstocks. Similarly in Algeria, Boudy (1952) mentions several endemic species, as the pistachio atlas (*Pistacia atlantica*), the terebinth (*Pistacia terebinthus*) and the mastic tree (*Pistacia lentiscus* L.). In Algeria, pests of pistachio fruit (*Pistacia vera* L.) are little studied, despite their harmful effects and their economic importance. Benmenni (1995) in Batna, Abdesselem (1999) in Djelfa and Boukeroui (2006) in Blida have established inventories on insect fauna in fruit pistachio orchards. In the same context, Messaoudene (2006) in the region of Ain Oussara near Djelfa examined fluctuations of aphids on the pistachio of the Atlas. Similarly in Batna, Bouira and Tlemcen, Chebouti-Meziou *et al.* (2006), Chebouti-Meziou *et al.* (2007) Chebouti-Meziou *et al.* (2009a), Chebouti-Meziou *et al.* (2009b) and Chebouti-Meziou e*t al.* (2009c) report the presence of a driller buds *Chaetoptelius vestitus* (Mulsant and Rey) on the young shoots of the fruit pistachio inducing a significant loss of production.

The beetle pistachio *Chaetoptelius vestitus* is a species found in the Mediterranean. It is subservient to wild Pistacia such as *P. Terebinthus, Lentiscus P. and P. atlantica)* and grown Pistachio *(P. vera*). It belongs to the Scolytidae family. It measures 2.5 to 3.5 mm long. The husks are dark, black or very dark brown with a pronotum almost entirely denuded above. The elytra are covered with white and brown spiniform squamules among which emerges a row of spaced stiff bristles (Balachowsky, 1949).

It is noteworthy that in the pistachio orchard Bechloul, two types of pistachio fruits are available, some from healthy trees and other from trees infested with *Chaetoptelius vestitus*. A comparative study of the chemical composition of two kinds of seed was made.

II – Material and methods

Bechloul station is located at an altitude of 449 meters (36° 18' 44" N, 03° 4' 42" E). This pistachio orchard was planted as part of a cooperation program with FAO between 1972 and 1975. It consists of a homogeneous population, aged about 36 years and not maintained. Tree height is between 3 and 5 meters and occupies an area of 40 hectares. Adjacent rows are spaced 6 meters.

The total number of trees is 1059. It should be noted the presence of a row of male plants from the northeast side of the prevailing wind. Inside the orchard, the male feet are arranged in a random manner. The orchard does not benefit from cultural operations such as control of the size of tree branches and irrigation.

The dry matter content of various foods was conventionally determined by the weight of the food after drying in an oven. The crude protein was assayed by the Kjeldahl method. The crude fat correspond to substances extracted under reflux with a solvent. The determination of total sugars was carried out by the method of Dubois.

The data were subject to an analysis of variance. This test consisted of comparing the means of several populations from data of random samples, simple and independent (Dagnelie, 1970). Performing the test was done either by comparing the value of Fobs with the theoretical value F1 corresponding α, extracted from the Fisher F table for a significance level α = 0.05 or 0.01 or 0.001 with k1 and k2 degrees of freedom, or by comparing the value of the probability p with always different values α = 5% or 1% or 0.1%.

III – Results

The results of analysis of pistachio seeds non infested with *Chaetoptelius vestitus* (Table 1) reveal a water content is 7.21 ± 0.36%. Fat contents are 46.00 ± 0.90%. As for the average sugar content is 4.02 ± 0.47%. The protein reaches 29.88% ± 0.76. The crude protein was 6.53 + 0.55%.

Table 1. Results of chemical analysis of seeds from fruit pistachio infested and non-infested (control) by the bark beetle

Control	Infested									
Repetition	Water %	Fat %	Sugar %	Protein %	T.N.M. %	Water %	Fat %	Sugar %	Protein %	T.N.M. %
1	6.99	47.39	4	29.01	7.01	7.67	43.59	4.04	29.37	4.63
2	7.61	45.12	3.39	30.13	6.99	7.91	43.72	5.97	13.75	4.68
3	6.74	46.19	4.2	31	6.12	7.25	47.79	5.56	19.37	5.1
4	7.5	45.3	4.5	29.4	5.99	7.69	46.55	5.78	21.9	4.87
Average	7.21	46	4.02	29.88	6.53	7.63	45.41	5.34	21.10	4.82
S.D.	0.36	0.90	0.47	0.76	0.55	0.28	2.09	0.88	6.48	0.21

	F (Fisher test)	Pr > F
Water	0.361	0.574
Fat	7.368	0.042
Soluble sugar	0.521	0.503
Protein	19.713	0.007

T.N.M.: total nitrogenous matter; Pr: probability; F: fisher test.

Regarding the dry matter content of seeds from trees infested by *Chaetoptelius vestitus*, it is 3.85 ± 0.12%. The percentage of water is 7.6 ± 0.28%. The fat is 45.4 ± 2.09%. Sugar rate is 5.3 ± 0.88%, the protein of 21.1 ± 6.48%. The percentage of the total nitrogenous matter was 4.8 ± 0.21.

There was no significant difference between the water and soluble sugar contents found in the seeds of control and pistachio tree seeds infested by the xylophagous insect.

It should be noted that the analysis of variance of the fat and the protein contents of the seeds from the fruit pistachio witnesses and those of infested trees, shows that there are significant differences at Pr < 0.05 for a confidence interval of 95% (Table 1).

IV – Conclusions

The objective of this study is to provide an overview of the insect fauna effect on the fruit pistachio in Algeria, which is unknown. Statistical analysis of the biochemical composition of seeds harvested from pistachio *Chaetoptelius vestitus* uninfested and infested trees shows significant differences in lipid and protein levels. Thus, the infestation of fruit pistachio by *Chaetoptelius vestitus* not only causes reduced yields but it also causes a decrease in the value of the nutritional quality of the seed pistachio. However, further studies are required on the parasites and pest insects used in the context of a biological control against the enemies of the fruit pistachio. And technical improvement and selection of the genus *Pistaci*a are to be considered in order to obtain genetically resistant varieties.

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Session 4

Orchard management

Challenges in orchard management for almond and pistachio

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Abstract. Almond and pistachio are the two main fruit nuts in the Mediterranean region and the USA. According to acreage importance and history in producing countries, USA is considered the latest country that introduced these two crops, starting by the beginning of the 20th century. Yet, the country has evolved tremendously in orchard management beside the other factors to assure high production to become the first almond and the second pistachio world producer. Mediterranean countries, where most of the acreage of these two species is located, developed good research and development in orchard management approaches and techniques and reached excellent production and fruit quality levels. However, their advances are uneven, slowed by the historical load of the crop and its management in their respective countries. At present, new issues have raised that will impact the future of orchard management. Per se, water resources shortage and fertilizers use in relation to climatic changes and soil contaminations, pesticides use in relation to environment pollution and health concerns, and the new developing trends such as bio, labeled and natural products. Thus the challenge in orchard management in pistachio and almond is greater since it should in the mean time aim to guarantee good and profitable economical yields and respect all the environmental and climatic changes concern.

Keywords. Orchard Management – Almond – Pistachio – Global Warming – Climatic Change – Greenhouse effect – Fertilizers – Irrigation – Pesticides.

Les défis dans la conduite technique des vergers d'amandier et de pistachier

Résumé. *L'amandier et le pistachier sont les deux principales espèces de fruits secs dans le bassin méditerranéen et en Amérique du Nord. Les Etats Unis sont considérés le dernier pays à avoir introduits ces deux espèces au début du 20ème siècle. Toutefois, le pays a réalisé une telle avancement en techniques de conduite en verger, associée à d'autres techniques de production, qu'il détient actuellement la première place mondiale en production de ces deux espèces. Les pays du bassin méditerranéen qui détiennent les plus grandes superficies de ces deux espèces ont réalisés de très bons niveaux de recherche et développement en techniques de production en verger. Toutefois, les résultats inégaux auxquels les différents pays méditerranéens sont arrivés sont reflétés par le poids de l'histoire de production de ces espèces dans leurs pays respectifs. Par ailleurs, de nouveaux thèmes agricoles sont d'actualité telle la réduction des ressources hydriques et l'utilisation des fertilisants minéraux en relation avec les changements climatiques et la contamination des sols, l'utilisation des pesticides en relation avec la pollution de l'environnement et la santé humaine et animale, les tendances vers les produits bio, labellés et naturels. Ainsi, le challenge en techniques de conduite des vergers d'amandier et de pistachier se reflète dans la garantie de la rentabilité économique des productions tout en s'alignant avec les chartes du changement climatique et du respect de l'environnement.*

Mots-clés. *Amandier – Pistachier – Techniques culturales – Changement Climatique – Effet serre – Irrigation – Fertilisants – Pesticides.*

I – Introduction

Hunter-gathers would live off the land forging berry and edible plants, as well as hunting wild animals. These types of people lived in smaller groups because they had to be mobile to find more food. It was not until man began to plant and harvest crops that large permanent settlements could be established (Crisp, 1993). The crucial trap for the development of agriculture is the availability of wild edible plant species suitable for domestication. Farming arose early in the Fertile Crescent since the area had an abundance of wild wheat and pulse species that were nutritious and easy to domesticate (Diamond, 1997). Agriculture involving domestication of plants was developed around 11,500 years ago mostly in the Fertile Crescent (Hillman & al., 2001). Historically the fruit trees have evolved from simply as seasonal food gathered by the migratory nomadic, to a place of prominence in agricultural civilizations, often greatly sought after by those designing and developing cultivated gardens of the wealthy elite and emperors. Domestication of fruit trees seems to have started with olives 5 to 6000 years ago (Vossen, 2007). Domesticated almonds appear in the Early Bronze Age (3000-2000 BC) (Ladizinsky, 1999). The modern pistachio *P. vera* was first cultivated in Bronze Age Central Asia 2000 BC where the earliest example is from Djarkutan, modern Uzbekistan (Potts).

Agricultural techniques such as irrigation, crop rotation and the application of fertilizers were developed soon after the Neolithic Revolution 3000 BC, and farming changed very little until about 1700. In the 1700's, an agriculture revolution took place which led to a large increase in the production of crops, and in the 1850's, the industrial revolution spilled over to the farm with new mechanized methods which increased production rates (Grigg, 1974). Over the years man has invented new machines and techniques to increase the amount and variety of crop production and it appears that along the development of new varieties, production and management process seems to become difficult.

Advances in orchard management allowed fruit growers to produce larger crops of higher quality fruit year after year, generating surplus for wholesale in unprecedented volume. But with greater ease in production came chemical quality, food quality, and chemical residues concerns. As other fruit crops, orchard management on almonds and pistachios deals mostly with planting design and planting techniques, pruning, irrigation, fertilizers application, pest control, soil management and weed control, and harvest. The manner this package is adopted in the orchard management program will affect the final yield and the final quality of the nuts. In comparison to the Mediterranean almond and pistachio producing countries, high yields of US almonds and pistachio orchards are mainly explained by plantings on good soils and intensive use of water, fertilizers and pesticides. Almond and pistachio on most of the Mediterranean basin are of two types: 1) Scattered plantings of numerous local varieties or seedlings with low yielding potential, planted on marginal lands under rain fed conditions, characterized by low or no input. Most of these orchards generate low yields and poor fruit quality. This situation makes improvement through orchard management techniques difficult to reach; and 2) Modern farms with high yielding varieties, regular and intensive planting designs and adequate orchard management program. These orchards represent between 20 to 25% of the Mediterranean countries total acreage. In these countries, research and technology are gaining ground and higher yield and fruit quality are being obtained through improvement of orchard management.

As a conclusion, the difference in orchard management among almond and pistachio producing countries stands on variety performance, planting designs, level of mechanization, and use of water, fertilizers and pesticides.

In all the almond and pistachio producing countries, most of the improvement of yields and fruit quality is mainly bound to three orchard management techniques: water use, fertilizers and pesticides, beside soil fertility and planting design and spacing. The best example is the California almond history. The first almond orchards in California were primarily planted as done for centuries in Europe on hillsides without irrigation. As the industry grew in the first half of the 20th century, a revolution in California almond farming took place when growers discovered that planting orchards in fertile, well-drained soils with irrigation and fertilization could double or triple yields (Sonk *et al.*, 2010). Rising global demand has spurred growers to plant almond and pistachio trees on the dry and salty soils of the west side of the San Joaquin valley, creating more water supply demand.

But as an Arabic proverb says: "overuse of resources will oppose expectations". As a matter of fact, California almond and pistachio industries are actually facing serious water shortage problems, just as the Mediterranean almond and pistachio culture have faced at all time. Within the Global Warming and climatic change debates, water resources shortage is a main issue. These facts lead to adoption of appropriate irrigation systems and accurate monitoring in orchard management.

On the other hand, pesticides have led to preservation of the trees, keep the soil weed free, guarantee superior yield and obtain high quality fruits. However, pest management became the epitome of intensive agriculture, observed in the chemical dependency of our management programs. The excessive and inappropriate use of pesticides conducted to the raise of resistant pathogen and insects strains, to insect unbalanced population dynamics, and to residues problems. Integrated pest management (IPM) should be the rule in any almond and pistachio orchard management program. Pesticide residues and health concerns opened the way to organic, labeled and natural almond and pistachio commodities. In developing countries, there is serious environment and health concern about pesticides banished elsewhere, misuse of pesticides and inappropriate handling of empty containers.

The third component in orchard management programs that should be watched closely is the use of fertilizers. Yet, fertilizers have surely played and still play an important role in improving almond and pistachio yields, but overuse of certain fertilizers such as nitrates led to serious soil and underground water contaminations and are finger pointed as a severe cause to the greenhouse effect. Nitrogen fertilizers applied to soils and soil management are estimated to be the major sources of N_2O production in agriculture. N_2O is 296 fold more harmful than CO_2.

II – Conclusions

Orchard management practices on almond and pistachios should be optimized to reach and maintain profitable economical yields with excellent commercial quality nuts. While some more advanced countries tripled yields and obtained excellent quality nuts at the expense of water resources, underground water and soils contaminations by excessive irrigation fertilizers and pesticides, other developing countries counter low yields and poor fruit quality due to lack in orchard management practices, either through low or no inputs at all or through absence of some orchard management practices. These orchards, usually under rain fed environment and on sloppy and/or poor quality soils, are at risk of being abandoned by farmers, thus contributing to rural exodus and climatic changes.

Orchard management is definitely highly needed to secure profits to almond and pistachio farmers. However, consequence of the actual management of these 2 crops in the different producing countries suggests a better implementation of the almond and pistachio management practices to reach and maintain profitable income returns in conjunction to Global Warming Solutions Acts and residues norms. Yet, USA is leading in finding solutions to water shortage and nitrate contaminations through actions such as the California almond sustainability program.

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Climate change and vulnerability of the pistachio and almond crops in the Mediterranean arid areas

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Abstract. Almond and Pistachio are the most important nut crops in Tunisia. The main production areas are located in the central and southern part of the country, characterized by scarce precipitations and harsh environments. Low and highly variable yields are commonly observed under those conditions. Nut crops are reported to be vulnerable to temperature fluctuations and decreasing water availability. In this study, yield response of almond and pistachio to global warming is evaluated under the arid conditions of Tunisia. The approach is focussing on chilling and its incidence on yield, using various chilling accumulation models. On the basis of 35 years of daily climatic data (1980-2014), results show a declining trend in winter chill units. Pistachio yields varied between 0 and 35 kg/tree and were affected by chill accumulation. Yields for three almond cvs varied within similar ranges but seemed to be less affected by warm winters, whereas one variety seemed to be non responsive to chill conditions, probably because of high sensitivity to rainfall variations. With the prospects of global warming, increasing temperatures are expected to be detrimental to nut cultivation in arid Tunisia; unless efforts are deployed to grow the appropriate cultivars.

Keywords. Almond – Pistachio – Dry area – Winter chill – Precipitation.

Changement climatique et vulnérabilité des cultures d'amandier et du pistachier dans la région aride Méditerranéenne

Résumé. *Les cultures d'amandier et du pistachier occupent une place importante dans le système de production du centre et du sud tunisiens. Elles sont conduites en pluvial sous climat aride. Des rendements faibles et très variables ont été réalisés sous ces conditions sévères. Ces cultures ont été signalés être vulnérables aux fluctuations de température et à la diminution de la disponibilité en eau. L'évaluation des risques imposés par le changement climatique en particulier pour les cultures des régions marginales de la Tunisie centrale est d'un grand intérêt. Les rendements de l'amandier et du pistachier ont été évaluées par rapport aux variations de température et de précipitation. L'impact du froid hivernal a été analysé et différent modèles d'estimation du froid ont été utilisés. Les résultats d'analyses des données climatiques sur 35 ans (1980-2014) ont révélé une tendance décroissante de l'accumulation du froid. Les rendements du pistachier ont varié entre 0 et 35 kg/arbre et ont été affectés par le manque du froid. Les performances des variétés d'amandier ont varié dans les mêmes ordres de grandeur et semblaient peu affectées par les hivers doux, alors qu'une variété paraissait plutôt sensible aux variations des précipitations que des accumulations du froid. Avec les perspectives du réchauffement climatique, la hausse des températures pourrait limiter le développement des cultures des fruits secs; moins que des efforts seront déployés pour valoriser les ressources génétiques appropriées.*

Mots-clés. *Amandier – Pistachier – Région aride – Froid hivernal – Précipitation.*

I – Introduction

Observed increases in temperature and in precipitation variability, induced by global warming, are expected to have harmful effects on fruit trees, particularly in rain-fed agro-systems. Nut crops such as almond and pistachio are among the most important fruit species used to make important parts of dry lands productive. For instance these species are largely grown in the central and southern areas of Tunisia, where climate variability, drought and heat spells represent major constraints to developing intensive cropping systems.

Drought is known to be a significant environmental stress in agriculture, and many efforts are deployed to improve crop productivity under water scarcity. However, impacts of warm winter temperatures on fruit species in arid areas are less documented. Lack of chilling affects flowering and fruiting of many species (Campoy *et al*., 2011). Various models were developed to quantify the chilling accumulation and requirements (Weinberger, 1950; Crossa-Raynaud, 1955; Richardson *et al*., 1974; Fishman *et al*., 1987). Chilling hours (CH) under +7.2°C method was conventionally used to measure chilling for fruit trees (Weinberger, 1950; Crossa-Raynaud, 1955). Recently, the Dynamic model is considered to be suitable for warm areas (Elloumi *et al*., 2013; Ghrab *et al*., 2014).

In this study, attempts are made to assess risks imposed by global warming on the main nut crops grown in central part of Tunisia. The analysis concerns primarily the actual trends in winter chill accumulation and the impact on the yield of almond and pistachio cultivars.

II – Materials and methods

The almond and pistachio orchards used in the study belong to the experimental station of the Olive Institute, Taous (34°94'11", 10°60'82"). Located in Central Tunisia, the production area is characterized by deep sandy soils; annual precipitations of 204 mm and reference evapotranspiration (ET_o) of 1340 mm. The experimental orchards have been conducted during the experimental period (1980-2014) under rain-fed conditions without any supply of irrigation water or fertilizers. For pistachio, the most common local cultivar Mateur grafted on *Pistacia vera* rootstock and planted on a wide spacing (12 m x 12 m) is used. Whereas for almond, the local genotype Fekhfekh and three introduced cultivars 'Ferragnes', 'Ferraduel' and 'Tuono' are included in this work. Measurements of flowering and nut yield per individual trees were carried out routinely on all cultivars.

Climatic data, in terms of daily records of air temperature and precipitation, were obtained from a local weather station. Our calculations of chilling accumulation used an hourly interpolation function applied to daily maximum-minimum temperature data (Darbyshire *et al*., 2011). The Crossa-Raynaud's method (Crossa-Raynaud, 1955), the Utah model (Richardson *et al*., 1974) and Dynamic model (Fishman *et al*., 1987) were used to estimate chilling accumulation as chilling hours (CH_{CR}), chill unit (CU) and chilling portions (CP), respectively. The summation periods correspond to October 1st – March 31st for pistachio and October 1st to January 31st for almond. Annual precipitations were computed from September to August.

III – Results and discussion

Based on 35 years of climatic data, the amount of winter chill occurring in central Tunisia shows an important decline over the period 1980-2014 (Fig. 1). This trend is expected to continue since most climate change scenarios are forecasting major increases of winter temperature in the Mediterranean areas. All chill accumulation models used in the analysis are concordant in showing the decline in winter chill over the last three decades and bring additional evidence to statements on how decline in winter chill became apparent in different parts of the globe (Baldocchi and Wong, 2008; Luedeling *et al*., 2011; Darbyshire *et al*., 2011). Similarly, high variability in annual precipitations was observed over the same period, but the decreasing tendency occurred with less evidence.

The analysis also revealed that almond and pistachio trees in the centre of Tunisia are increasingly subjected to lack of chilling, since warm winters are becoming more frequent. During the last period, a winter with severe lack of chilling occurred every three-four years (1997, 2001, 2007 and 2010). Such conditions are known to impact on the adaptability of deciduous fruit crops (Darbyshire *et al*., 2011). In a previous work, the beginning of flowering of pistachio was found to be highly related to winter thermal regimes (Elloumi *et al*., 2013). The correlation indicated that the severe lack of chilling delays the flowering of pistachio cv. Mateur by about one month. Almond cultivar Fekhfefk was similarly affected with a flowering delay of 15 days.

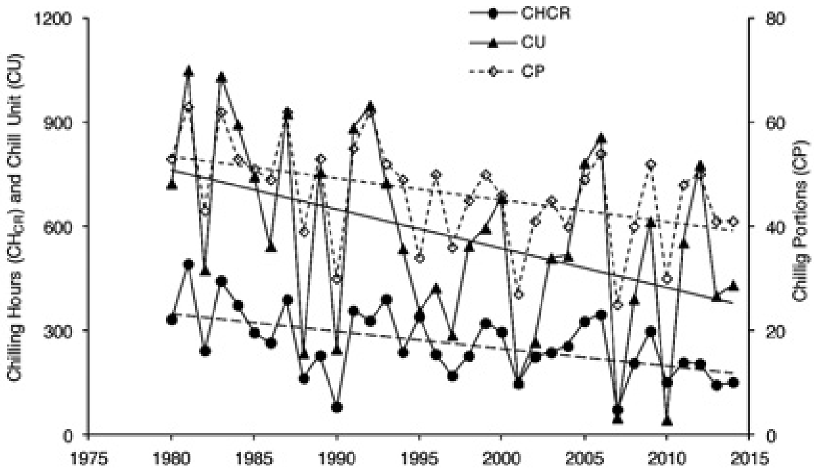

Fig. 1. Declining of chilling accumulation measured as chilling hours (CH_{CR}), chill units (CU) and chilling portions (CP) over the period of 1980-2014 in a warm production area.

The yearly variations of pistachio yields are closely related to climatic conditions (Fig. 3). Results show a positive correlation between nut yield and chilling accumulation. It appears that an exponential increase of nut yield occurred with winter chill accumulation until reaching the chilling requirement. After that nut yield seemed to be unaffected by chilling accumulation but rather by other factors such as precipitation.

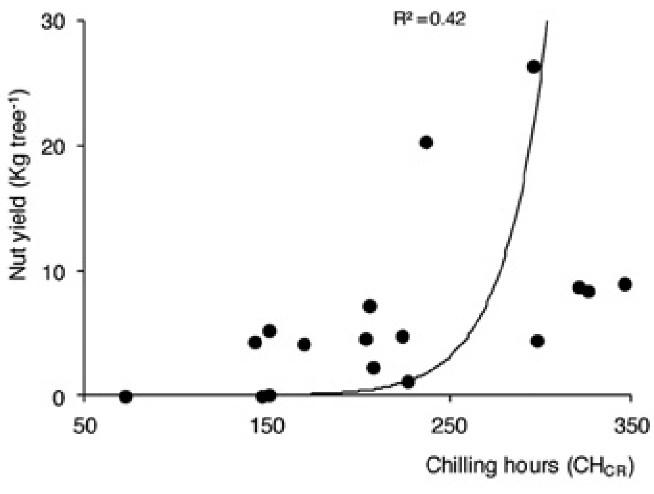

Fig. 2. Relationship between nut yield (kg $tree^{-1}$) of pistachio trees cv. Mateur and chilling accumulation as chilling hours (CH_{CR}) for data collected over the period 1997-2013.

Chilling had a lower impact on almond varieties and there is no strong relationship between chilling trends and nut yield (Fig. 3). There may have been sufficient chilling during much of the analysis period ensuring little yield influence. However, some of the recent reductions in yields of almond were attributed to a decline in winter chill and/or drought, depending on the cultivar. Hence, nut yield of Fekhfekh, Ferragnes and Ferraduel seemed to be related to chilling accumulation, whereas Tuono appeared to be not as affected.

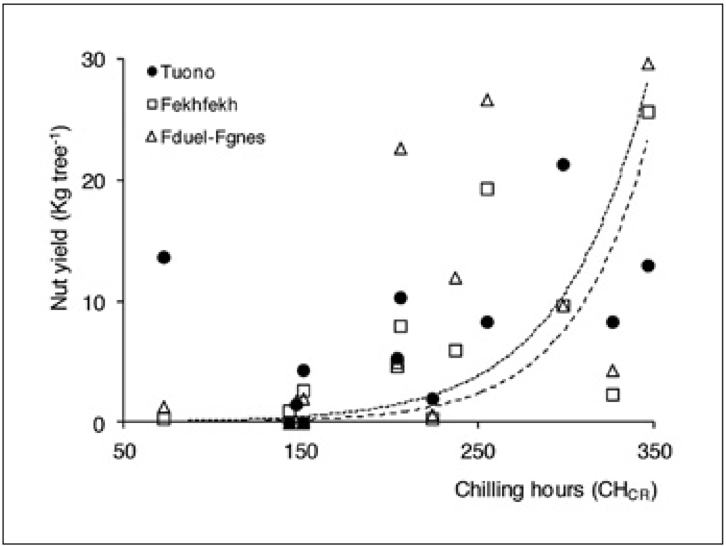

Fig. 3. Relationship between nut yield (kg tree^{-1}) of almond cultivars and chilling hours (CH_{CR}).

IV – Conclusions

Analysis of daily climatic data revealed important decline in winter chill over the 1980-2014 period in Central Tunisia. Chilling accumulation models were used effectively to study the impact of increasing winter temperature on fruit production. Warm winters impacted strongly yields of the pistachio crop cv. Mateur but only within some limits those of three almond cultivars 'Fekhfekh', 'Ferragnes' and 'Ferraduel', whereas cv. Tuono appeared to be more sensitive to drought. In the future, it will be interesting to consider the important genetic diversity of the almond and pistachio species to mitigate the harmful effect of global warming.

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Determination of yield and some quality traits of Pistachio cv. 'Siirt' trained different systems

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Abstract. Pistachio trees grow under sunny and hot areas, for preventing those damages and circulation, it is needed good shaped tree crown. This just can be done after planting with each year regular pruning. There is not much more studies on this matter. It is unknown how to effect training systems to yield and quality. Via a good training system and pruning that is made for physicological base of tree, yield can be seperated in each year production. This study was started in 1995 to determine the best training system to get high yield and having more quality. Three (The Open-Center System, Open center system closed with lateral branches and Modified Central Leader system) training systems were applied to the experimental trees. Yield and quality in cultivar 'Siirt' was recorded.

Keywords. Pistachio – Training– Pruning – Yield – Quality.

Détermination du rendement et de certains caractères de qualité selon différents systèmes de conduite architecturale chez le pistachier cv. 'Siirt'

Résumé. *Les pistachiers poussent en zones ensoleillées et chaudes, et pour prévenir les dommages et favoriser la circulation, la couronne de l'arbre doit avoir une bonne forme. Ceci peut être fait simplement après la plantation par une taille régulière à chaque année. Il n'y a pas beaucoup d'études sur cette question. On ne connaît pas bien comment les systèmes de conduite architecturale affectent le rendement et la qualité. Par un bon système de conduite architecturale et de taille en fonction de la base physiologique de l'arbre, le rendement peut être séparé pour la production de chaque année. Cette étude a été entamée en 1995 pour déterminer le meilleur système de conduite architecturale afin d'obtenir plus de rendement et de qualité. Trois systèmes d'architecture (Système ouvert au centre, système ouvert au centre et fermé par branches latérales, et système leader central modifié) ont été appliqués aux arbres expérimentaux. Le rendement et la qualité ont été enregistrés chez le cultivar 'Siirt'.*

Mots-clés. *Pistachier – Conduite architecturale de l'arbre – Taille – Rendement – Qualité.*

I – Introduction

Turkey has big pistachio production potential, and third pistachio producer country after Iran and USA in the world. Pistachio has mainly cultivated in Southeast Anatolian part of Turkey (95% of total production). These areas are arid and hot climated, and no rain from June to October during the year. Kernel of pistachio fruits have been developed during the summer and matured in September in Turkey. For this reason, the pruning and training systems are very important to save the water balance in the tree canopy. Crane and Maranto, (1988) reported that, pistachio young tree should be trained during the first 3 years with 3 main branches and 1 leader branch.

Arpaci *et al*. (2001) reported that, open center training system mainly used at pistachio orchards in Southeast Anatolia, and there was some sunburn problems on the main branches.The pruning studies were neglected for pistachio trees in Turkey. We know that, pruning affects the yield 15-17% in pistachio. This is the first training experiment in pistachio orchard of Turkey.

Pruning is a general term which refers to selective removal of plant parts to obtain a desired growth or developmental response. However, for fruit trees, pruning usually refers to mature, bearing trees and is done primarily to increase production of high quality fruit and limit tree height and spread. Pruning is necessary to maintain tree health, vigor and productivity throughout its life cycle. Proper pruning and training of fruit trees is necessary to obtain maximum yields of high quality fruit.

Proper tree training also opens up the tree canopy to optimize light penetration. For most deciduous tree fruit, flower buds for the current season's crop are formed the previous summer. Light penetration is essential for strong flower bud development and optimal fruit set, flavor, and quality. Although a mature tree may be growing in full sun, a very dense canopy may not allow adequate light inside the canopy. Opening the tree canopy also permits air movement through the tree, which promotes rapid drying to minimize disease infection and allows thorough spray penetration. Additionally, a well-shaped fruit tree is aesthetically pleasing, whether in a landscaped yard, garden, or commercial orchard (Parker, 2010).

Pruning is the removal of a portion of a tree and is used to correct or maintain tree structure. Training is a practice that allows tree growth to be directed into a desired shape and form. Training young fruit trees is essential for proper tree development. It is more efficient to direct tree growth with training than to correct it with pruning (Parker, 2010).

This study is carried out in order to determine the effects of different training systems on yield and quality of pistachio cv Siirt.

II – Materials and methods

This work is carrying out at Pistachio Research Station in Gaziantep-Turkey. The orchard was established in 1995 with 'Siirt' cultivar, 4 m by 7 m intervals, with randomized parcel design (5 tree per treatment). *Pistacia khinjuk* is used as rootstock.

Three training systems were practiced after plantation. These are: the Open-center system, Open center system closed with lateral branches and Modified Central Leader system.

Yield and some pomological traits were evaluated in the experiment. Yield per ha (kg), 100 nut weight (g), shelling percentage (%) and split nuts percentage (%). Data were evaluated by analysis of variance When the F- test was significant, means were separated by the least significant (LSD) test at a 5% level.

III – Results and discussion

Yield averages per tree are given in Table 1. The results were not statistically important. However the highest average yield (1021 kg/ha) was obtained from Open center system closed with lateral branches (Table 2).

The data about the one hundred nut weight are given in Table 3. As it was seen Table 3, The 100 nut weight of Siirt cultivar effected by training systems statistically. According to average value the highest 100 nut weight was obtained from Open center system closed with lateral branches.

The harvested fruits were analysed to observe the effect of training systems on Shelling percentage. The data are given in Table 4. According to Table 4, there is no effect of training systems on shelling percentage in dehulled fruits.

The effect of training systems on splitting rate is given in Table 5. There is no effect as statistically. But according to average modified central Leader was affected the splitting rate.

Table 1. Effect of training systems on yield per tree (kg)

Training system	Years			Average
	2011	2012	2014	
Open center system	1,42	1,69	2,84	1,98
Open center system closed with lateral branches	2,09	2,66	4,00	2,92
Modified central leader	1,72	2,61	2,87	2,40
LSD ≤ 5%	ns	ns	ns	ns

Table 2. Effect of training systems on yield per ha (kg)

Training system	Years			Average
	2011	2012	2014	
Open center system	497	592	994	694
Open center system closed with lateral branches	732	931	1400	1021
Modified central leader	602	914	1005	840

Table 3. Effect of training systems on 100 nut weight (g)

Training system	Years			Average
	2011	2012	2014	
Open center system	116 b	116 b	131 a	121
Open center system closed with lateral branches	117 ab	123 a	125 b	122
Modified central leader	124 a	109 b	121 b	118
LSD ≤ 5%	5.8	7.0	5.9	ns

Table 4. Effect of training systems on shelling percentage (%)

Training system	Years			Average
	2011	2012	2014	
Open center system	51.5	50.4	51.1	51.0
Open center system closed with lateral branches	51.6	51.7	50.4	51.2
Modified central leader	48.4	50.7	49.8	49.6
LSD ≤ 5%	ns	ns	ns	ns

Table 5. Effect of training systems on split nuts (%)

Training system	Years			Average
	2011	2012	2014	
Open center system	93	96	97	95.4
Open center system closed with lateral branches	96	94	97	95.8
Modified central leader	95	98	99	97.2
LSD ≤ 5%	ns	ns	ns	ns

IV – Conclusions

Pruning of mature deciduous trees has several objectives. One is to confine the tree to its allotted space. This, for reasons already described, is of major importance in pistachio. Secondly, pruning is performed to renew fruitwood and distribute light throughout the canopy. The invigoration effect from pruning also helps maintain tree health and longevity. Pruning can improve nut removal at harvest and equipment damage from low branches. Finally, pruning can also be used to mitigate strong alternate bearing (Ferguson *et al*.,1991). A seven-year trial involving severe mechanical hedging and topping combined with normal pruning showed that cumulative split nut yield for the severely pruned trees was not different from the handpruned control. Further, severe hedging and topping, followed by normal hand-pruning in subsequent years strongly and persistently mitigated alternate bearing (Beed and Ferguson, 2005).

Pruning on pistachio tree that grown under dry and poor soil conditions should be done carefully. Otherwise tree can be damaged seriously. The pruning application in Turkey and Syria in two stages, after harvest and before flowering time. First stage after harvest dried shoots must be cutted out. Second stage, normally pruning the tree general situation. In all pistachio producer countries pruning is done by hand using labor. But in U.S.A pruning is done mechanically due to very good vegetative growth by irrigation (Ak, 2015).

The shape of tree canopy is more important for the arid areas. Yield and quality characteristics of pistachio cv Siirt affected by training systems. It is concluded that, Open center system closed with lateral branches is suitable for pistachio trees in arid areas. Main Pistachio growing areas have high sunshine and dry climate conditions. Because of this ecological system very high hot temperature in summer time. The tree shape or pruning system is very important. The branches and fruits should be protected from direct exposure of sun. In this point training or pruning system prevents the tree. But we need to have high yielding and quality. The trunk system for all kind of fruit trees are important as well as pistachio tree. In this study training system were investigated. According to obtained results, quality is not effected but yielding. The yield was found higher in closed center system. But such experiment should be going on. In these conditions the plant are very young. The next years the results may be changed year after year.

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Macro- and microelement concentrations in different parts of "on"- and "off"-year 'Siirt' Pistachio trees

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Abstract. This study was conducted to determine the mineral element concentrations in different parts of 'Siirt' pistachio trees. This research was carried on 35-year old, fruitful or unfruitful (showing alternate bearing) trees that were grafted on *Pistacia vera* rootstock at the Ceylanpinar State Farm near Sanliurfa, Turkey. The results of the analysis of leaf samples taken from fruitful or unfruitful trees as follow; nitrogen (N), phosphor (P), potassium (K), magnesium (Mg), calcium (Ca) contents were not sufficient; iron (Fe), copper (Cu) and manganese (Mn) contents of the samples were sufficient. Also it was determined that zinc (Zn) level is insufficient in both kind of trees (fruitful and unfruitful trees). In this conditions the pistachio trees could not utilize the elements in the soil because of high level of Ca, low soil moisture content and high air temperature.

Keywords: *Pistacia* – Mineral nutrition – Fertilization – Alternate bearing – Tree nut.

Concentrations en macro- et microéléments dans différentes parties en année productive et non productive pour les pistachiers 'Siirt'

Résumé. *Cette étude a été menée pour déterminer les concentrations en éléments minéraux dans différentes parties des pistachiers 'Siirt'. Cette recherche a été menée sur des arbres de 35 ans d'âge, fructifères ou non fructifères (montrant une alternance de production) qui avaient été greffés sur porte-greffe* Pistacia vera *à la Ferme d'État de Ceylanpinar près de Sanliurfa, Turquie. Les résultats de l'analyse d'échantillons de feuilles prélevées sur des arbres fructifères ou non fructifères sont comme suit; les teneurs en azote (N), phosphore (P), potassium (K), magnésium (Mg), calcium (Ca) n'étaient pas suffisantes; les teneurs en fer (Fe), cuivre (Cu) et manganèse (Mn) étaient suffisantes. Il a aussi été déterminé que le niveau de zinc (Zn) était insuffisant dans les deux types d'arbres (fructifères et non fructifères). Dans ces conditions les pistachiers ne pouvaient pas utiliser les éléments du sol à cause du haut niveau de Ca, de la faible teneur en humidité du sol et de la haute température de l'air.*

Mots-clés. Pistacia *– Nutrition minérale – Fertilisation – Alternance de production – Fruit sec.*

I – Introduction

Pistachio is grown most intensively in Iran, Syria, Turkey, and USA. The other pistachio-producing countries are in the Near East, North Africa and Southern Europe. Based on nut production data for 2009-2012, Iran is responsible for over 459,000 tons, about half (53.2%) of world production. The second highest-yielding country, USA, produces 207,000 tons, about 22.3% of world production, while Turkey is third with a yield of 117,000 tons and 12.7% of total world production.

Pistachio trees can be grown under very poor soil conditions. *Pistacia* spp. tolerate very high lime contents in soil and can be grown on calcareous soils that would limit production of other fruit crops. Fertilization is a very important factor to ensure high nut quality and yield. Pistachio trees do suffer from excessive alkalinity. Nutrient availability and soil fertilization strategies depend on irrigation practices and soil pH. Some soil nutrients are poorly available because of high pH and competitive uptake caused by soil nutrient imbalances (Ak, 1992; Ak, *et al*., 2002; Gursoz, *et al*., 2010).

In Turkey, pistachios are grown on soils that typically contain inadequate supplies of N, P, K, and organic matter. In a survey conducted in 30 pistachio orchards in southeast Anatolia, Tekin *et al*. (1985) found that the trees in many orchards were markedly deficient in P and Zn and slightly deficient in N, Fe and Mn. The level of K was found adequate in many orchards though there were some districts where the trees showed slight deficiencies. Soil pH in the pistachio-growing region of Turkey varied between 7.5 and 9.3 while the organic matter content was very low. Zinc deficiency appears to substantially limit fruit set.

Crane and Maranto (1988) concluded that pistachio is not a luxury N consumer when N is abundantly available in the soil. The chemical form of nitrogenous fertilizer should be chosen depending on soil pH. For instance, in the GAP (Southeast Anatolia Region) area, only ammonium sulfate is recommended as a source of N because of the alkaline soils. Nitrogenous fertilizers should be applied at the end of February or the beginning of March at rates of 1.5 to 4 kg per tree (Kaska, 1995).

The current experiment was conducted to determine (1) the macro– and microelemental status of pistachios grown at Ceylanpinar State Farm in Sanliurfa, Turkey; (2) whether pistachio trees are getting enough nutrients from the soil; and (3) the distribution of nutrients among the various components of the pistachio fruits.

II – Materials and methods

This research was carried on 35-year-old fruitful or unfruitful ‘Siirt’ pistachio trees grafted on *P. vera* rootstock at the Ceylanpınar State Farm in Sanliurfa. This cultivar is very prone to alternate bearing. The trees were grown under non-irrigated conditions. Rainfall and relative humidity are very low at this farm while temperature is very high (Table 1).

The six trees are selected for testing. Three of the trees were bearing, or “on”-year trees. The remaining three were non-bearing, or “off”-year, trees. Any flower clusters developing on the non-bearing trees were removed manually so that no flower clusters were present at all. The leaves were sampled in late July from middle portion of the current year’s shoots (Tekin *et al*., 1990). The fruits were sampled when they have reached harvesting maturity. Hull, endocarp, and kernel portions of the fruit, and the remaining stem portion of the clusters, were separated and dried. Soil samples were collected and analyzed (Tables 2 and 3). Analyses were conducted following procedures in Kacar (1972). All analyses were done at the Laboratory of the Gaziantep Pistachio Research Institute.

Table 1. Climatical data at the Ceylanpınar State Farm

Months	Minimum Temp. (°C)	Maximum Temp. (°C)	Average Temp. (°C)	Relative Humidity	(%) Rainfall (mm)
January	-3.4	17.0	7.1	73.3	52.8
February	-2.6	16.4	6.3	73.0	88.2
March	-3.0	21.6	9.6	63.4	52.0
April	1.6	28.0	15.9	60.5	27.6
May	7.0	37.0	23.8	36.9	16.4
June	14.2	41.0	29.2	27.6	1.0
July	16.8	46.0	31.8	32.4	–
August	14.9	45.4	32.0	33.1	–
September	8.3	42.2	24.8	34.0	–
October	0.4	37.0	20.3	43.2	20.1
November	-1.7	27.3	11.3	64.4	45.8
December	-4.2	16.8	6.6	74.1	35.8

Source: State Farm Records.

Table 2. Soil physical traits and organic matter content of the experimental orchard

Soil depth (cm)	Soil attribute				
	pH (Soluble in Water)	Salt (%)	Lime (%)	Texture (%)	Organic matter (%)
0-20	8.07†	0.03	50.14	loam	1.54
20-40	8.20	0.04	51.37	loam	0.80
40-60	8.00	0.04	46.90	loam	1.07
Average	8.09	0.03	49.47	loam	1.14

† Each number is average of three different part of orchard of soil samples.

Table 3. Macro- and microelemental content of the soil in the experimental orchard

Soil depth	Nutritional content of the soil							
	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Mn (ppm)
0-20	5.27	303.40	15455.50	414.37	4.17	5.60	0.02	20.30
20-40	1.97	152.84	15324.80	534.60	4.27	5.80	0.07	15.53
40-60	3.20	150.24	17215.20	550.34	5.34	5.07	0.10	19.23
Average	3.48	202.16	15998.5	499.77	4.59	5.49	0.06	18.35

III – Results and discussion

1. Macro- and micronutrient concentrations in leaves

The results of the leaf analyses appear in Table 4, along with the critical levels published by Brown (1995) and Tekin (2002). There are some differences in nutrient status between "on"-year and "off"-year pistachio leaves. Some elements were higher in "off-year" than in "on-year" trees. With the exception of N, K, Fe and Cu, leaf nutrient concentrations were low compared to the recommended critical ranges, suggesting that tree nutrient status is generally inadequate. Because the soil contains adequate amounts of nutrients, the poor nutrient phytoavailability may be due to indequate soil moisture, high soil pH, or competitive nutrient uptake. The orchard was established under rainfall conditions. That means the trees are not irrigating during summer.

Table 4. Effect of alternate bearing on leaf macro- and micronutrient concentrations of the experimental 'Siirt' pistachio trees, and comparison with suggested desirable ranges

Some elements	"On" year	"Off" year	Average	Critical levels† (Brown, 1995)	Critical levels†† (Tekin, 2002)
N (%)	1.00	1.03	1.02	2.50-2.90	1.80-2.40
P (%)	0.07	0.05	0.06	0.14-0.17	0.06-0.14
K (%)	0.51	0.52	0.52	1.00-2.00	0.80-1.20
Mg (%)	0.51	0.47	0.49	0.60-1.20	0.50-0.90
Ca (%)	0.91	1.21	1.06	1.30-4.00	2.30-3.00
Fe (ppm)	167.17	181.07	174.12		43-170
Cu (ppm)	12.20	17.87	15.04		6-90
Zn (ppm)	6.93	3.57	5.25		10-25
Mn (ppm)	51.30	49.07	50.19		25-50

† Suggested range under irrigated conditions for pistachio.
†† Suggested range under dryland conditions.

2. Some macro and micro nutrient contents in different fruit organs

Distribution of macro– and microelements in the various components of the pistachio fruit were given Table 5. There was differential preferential accumulation of elements in the different organs. Nitrogen and Mn concentrations were highest in the kernel. Manganese and Iron concentration also was high in the cluster stems. Phosphorus concentration was appreciably higher in the endocarp. The hull contains higher concentrations of K, Mg, and Zn. Calcium, Fe and Cu concentrations were highest in the hull and cluster stems.

Table 5. Nutrient concentrations in different organs of the 'Siirt' pistachio cultivar

Some Elements	Cluster	Hull (Red Skin)	Endocarp	Kernel	LSD (% 5)
N (%)	0.93 b	0.73 b	0.02 c	2.16 a	0.218
P (%)	0.05 b	0.03 b	34.30 a	0.26 b	6.319
K (%)	1.02 b	5.61 a	0.08 b	0.79 b	3.152
Mg (%)	0.06 c	0.15 a	0.01 d	0.10 b	0.021
Ca (%)	0.88 a	0.70 ab	0.19 b	0.22 b	0.510
Fe (ppm)	138.37 a	150.47 a	74.98 b	72.30 b	56.950
Cu (ppm)	11.32	18.36	19.93	21.21	Ö.D.
Zn (ppm)	3.30	22.73	0.00	2.20	52.360
Mn (ppm)	8.71 ab	2.21 b	5.83 b	16.06 a	8.440

IV – Conclusions

Turkey is the only country in the world that pistachio nuts are grown on such marginal lands with a dry climate and poor, rocky and calcareous soils. Alternate bearing is one of the important features of pistachio. Alternate bearing cultivars produce heavy crops in "on" years and little or no crop in the "off" years (Ak and Kaska, 1992). The tendency for alternate bearing varies among the different pistachio cultivars. This can be decreased by irrigation and fertilization. Research has shown that management practices can dramatically influence yield of pistachio trees, with irrigation enhancing yields by up to 70%, fertilization 50%, choice of cultivar 45%, and light pruning 17% (Ak and Agackesen, 2005). Resolution of such limitations would substantially enhance the importance of pistachio as a world food source.

Our study showed that the Turkish soil on which the experimental pistachio trees were grown contains a very high calcium content and high pH. These soil factors may interfere with nutrient supply, restricting uptake from the soil into the trees. This effect may be exacerbated by the low soil moisture status of the nonirrigated orchard soils, which reduces root activity as well as soil nutrient solubility and translocation.

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Determination of carbohydrates contents pruned and unpruned almond cultivars

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Abstract. The effect of pruning on carbohydrates content of 'Nonpareil' and '101-9' almond cultivars was investigated. In this experiment, summer and winter (dormant) pruning was applied at different times. A randomized complete block design with 3 single tree replications for each pruning treatment was used for each cultivar. The following pruning treatments were each applied to one tree in each block: (a) unpruned (control); (b) pruned in June; (c) pruned in July; (d) pruned in August; (e) pruned in September; and (f) only winter pruning (WP). Water-soluble reducing sugars (SRS), total sugars, hydrolyzed starch and sucrose contents were analyzed. The samples were taken after pruning. Dormant and summer pruning treatments had different effects on carbohydrate contents of almond. Generally, control and dormant pruned trees had higher carbohydrate content than summer pruned trees. Earlier summer pruning (June or July) lowered carbohydrate content more than late summer pruning.

Keywords. Almond – Pruning – Dormant pruning – Summer pruning – Carbohydratre content.

Détermination de la teneur en hydrates de carbone dans des cultivars d'amandier taillés et non taillés

Résumé. *Les effets de la taille sur la teneur en hydrates de carbone pour les cultivars d'amandier 'Nonpareil' et '101-9' ont été étudiés. Dans cette expérience, la taille d'été et d'hiver (dormance) a été appliquée à des moments différents. Un dispositif en blocs aléatoires complets avec 3 répétitions pour les arbres individuels pour chaque traitement de taille a été utilisé pour chaque cultivar. Les traitements de taille suivants ont été appliqués à un arbre dans chaque bloc: (a) non taillé (témoin); (b) taillé en juin; (c) taillé en juillet; (d) taillé en août; (e) taillé en septembre; et (f) seulement taille d'hiver (WP). Les teneurs en sucres réducteurs solubles dans l'eau (SRS), sucres totaux, amidon hydrolysé et sucrose ont été analysées. Les échantillons ont été prélevés après la taille. Généralement, les arbres taillés témoins et dormants avaient une plus forte teneur en hydrates de carbone que les arbres taillés en été. Une taille d'été plus précoce (juin ou juillet) a abaissé davantage la teneur en hydrates de carbone qu'une taille en fin d'été.*

Mots-clés. *Amandier – Taille – Taille en dormance – Taille d'été – Teneur en hydrates de carbone.*

I – Introduction

Almond, which is a hot climate fruit, is raised on the latitudes 30-44^{o} in the Northern Hemisphere and on the latitudes 20-40^{o} in the Southern Hemisphere. Almond is among the substantial hard shell fruits adapted to Turkey's climate conditions. In Turkey, almond trees are used for afforestation of the infertile, rocky and calcic regions where annual precipitation rate is low.

Hard shelled fruits consist of 6.4% of total fruit production of Turkey. Almond consists of the 1.2% of the number of trees and 4.3% of the output amongst the hard shell fruit species. Turkey is the 6th of the world with its 75 055 ton almond productions (FAOSTAT, 2012). Turkey imported 34,626 tons of almond while exporting 19 537 tons of almond in 2011 and 2012. Consumption amount of almond in Turkey is 81,997 tons and self-sufficiency rate in production amount of almond is at 82%.

As almond is cultivated using seeds up until 20-30 years ago in Turkey, there is a wide genetic diversity of almonds. In the last years, with the importing of standard species, available genetic potential has been enriched. In the last years a lot of commercial almond orchards, which have broad production areas, have been established in the Southeastern Anatolia Region which has the most appropriate ecology for almond cultivation. Southeastern Anatolia Region supplies 17% of almond production of Turkey. In the near future, Turkey will be one of the self-sufficient countries in almond production with the help of obtaining efficiency of new almond orchards which have been established and will be built in this region.

It is necessary that several cultural operations have to be carried out in full in the large orchards with commercial almond production purpose. Shape pruning of young trees and yield pruning of almond trees are fundamental applications which are necessary.

Fruit trees are pruned for several purposes such as limiting the height of the tree, controlling the canopy of the tree, maintaining the balance between vegetative and generative growth, producing good quality and highly productive fruits every year and increasing production and size of the fruit.

Summer pruning has long been used as a management method for fruit trees. It was shown to be a value method of controlling tree growth (Íkinci, 1999, Hossain *et al*., 2006; Demirtaş *et al*., 2010a; Bayazit *et al*., 2012), increasing flower bud formation (Miller, 1982), increasing fruit color (Taylor and Ferree, 1984, Íkinci, 1999; Hossain and Mizutani, 2008; Bayazit *et al*., 2012), increasing soluble solids concentration (SSC) (Íkinci, 1999; Hossain *et al*., 2006; Demirtaş *et al*., 2010a), and decreasing titratable acid content (TA) (Íkinci, 1999; Hossain and Mizutani, 2008).

This study is carried out in order to determine the effects of summer pruning in different periods on carbohydrate accumulation of almond trees in addition to compulsory winter pruning in the almond cultivation.

II – Materials and methods

This research was conducted on 8 year-old trees of 'Nonpareil' and '101-9' almond cultivars on GF 677 (*Prunus persica* X *Prunus amygdalus* Batsch.) rootstock, growing at the Koruklu Research Station (37º08' N; 38º46' E; 460 m above sea level) (Sanliurfa, Turkey). During the experiment, the air temperatures were in average 29.6ºC in summer and 6.4ºC in winter, while annual precipitation ranged between 360-423 mm, mainly concentrated between the months of November and April. The average relative humidity is at the level of 55%. Relative humidity is the highest (69%) ratio in January, in July is the lowest (33%) level. Trees were planted at a 6x6 m spacing (277 trees ha^{-1}) trained to a central leader system, mini-sprinkle-irrigated. Standard orchard management practices (irrigation, fertilization, pest control) were followed in all years. The soil in the orchard (0-) is loamy with 44.5% clay, 31.3% silt, 19.8% sand, low in organic matter (1.3%), rich in calcium carbonate contents (24%), and has a high pH (8.1).

Trees were selected for uniformity based on tree size and trunk circumference. All trees had been uniformly pruned during previous dormant season. A randomized complete block design with three single tree replications of pruning treatment was used for each cultivar. The following treatments were applied to trees: (a) unpruned (control); (b) summer pruned (SP) in early June; (c) summer pruned in early July; (d) summer pruned in early August; (e) summer pruned in early September; and (f) only winter pruning (WP). Winter pruning was performed in January and consisted of heading vertical shoots to maintain tree height 3.5 m, thinning cuts, and removal of vigorous watersprouts.

A random sample of 12 mid terminal shoot was collected from each tree (at the beginning of rest period/in Dec.). Phloem with cambium was used and prepared for analyses. Barks with a knife peeled branches dried at 70ºC for at least 72 hr then frozen at -18ºC, lyophilized, and stored in a desiccators at -18ºC for carbohydrate analysis. Total sugar and starch analyses were carried out by Kaplankiran (1984) with the modified anthrone method. The reducing sugar was determined by Kaplankiran (1984) through a modified dinitrophenol method and sucrose content was calculated by (Total sugar % – Reducing sugar %) x 0.95 formula.

Data were evaluated by analysis of variance with Minitab 16.1.0 *Statistics software* package. When the F- test was significant, means were separated by the least significant (LSD) test at a 5% level. An arcsin square-root transformation was performed on percent data.

III – Results and discussion

Summer pruning plus dormant (winter) pruning treatments had a significant effect on all carbohydrate contents of two almond cultivars (Table 1).

Table 1. Effect of pruning treatments on the content of water-soluble reducing sugars, total sugars, hydrolyzed starch, sucrose in dormant shoots of 'Nonpareil' and '101-9' almond cultivars on GF 677 rootstock

Pruning treatments	Water soluble reducing sugars (%)		Total sugars (%)		Hydrolyzed starch (%)		Sucrose (%)	
	'Nonpareil'	'101-9'	'Nonpareil'	'101-9'	'Nonpareil'	'101-9'	'Nonpareil'	'101-9'
Control	1.36 a†	1.80 a	3.32 c	3.87 c	9.26 a	7.99 a	1.86 c	1.97 b
WP	1.38 a	1.71 ab	5.05 b	5.17 a	8.26 ab	6.89 ab	3.49 b	3.28 ab
SP-June + WP	1.22 ab	1.40 c	3.94 c	4.65 ab	5.93 c	6.05 b	2.58 bc	3.09 ab
SP-July + WP	1.16 b	1.35 c	3.36 c	5.31 a	6.68 b	5.59 b	2.09 c	3.76 a
SP-Aug. + WP	1.12 b	1.37 c	6.02 a	4.75 ab	5.26 c	5.55 b	4.66 ab	3.22 ab
SP-Sept. + WP	1.12 b	1.66 ab	6.15 a	3.86 c	5.73 c	6.04 b	4.78 a	2.09 b
LSD (0.01)	*0.21*	*0.31*	*0.74*	*0.59*	*1.95*	*1.41*	*1.01*	*0.93*

† Mean separation within columns by LSD test at 1% level.

According to two year average values, WP (1.38%) and control (1.36%) trees for 'Nonpareil' and control (1.80%) trees for '101-9' had the highest water-soluble reducing sugars content. In 'Nonpareil' variety, the highest total sugars content was found in trees with SP-Sept. + WP (6.15%) and SP-Aug. + WP treatment (6.02%). On the other hand, in '101-9' variety, the highest starch value was obtained from trees with SP-July + WP (5.31%) and in WP (5.17%) treatments. Unpruned (control) 'Nonpareil' (9.26%) and '101-9' (7.99%) trees had the highest hydrolyzed starch content. According to pruning treatments, the highest sucrose content was found in SP-September + WP treatments (4.78%) for 'Nonpareil' and SP-July + WP treatments (3.76%) for '101-9' cultivar.

Average values of two years, unpruned and dormant pruned of 'Nonpareil' and '101-9' almond cultivars were higher in carbohydrate concentration than all of summer pruned trees. Generally, early summer pruning treatments (June or July) had the lowest carbohydrate concentrations, whereas carbohydrate fractions had relatively great increases pruning at August or September.

Carbohydrates are an essential source of reserve energy in temperate zone trees. They can be mobilised for metabolism or translocated to other plant organs. The concentration and localisation of carbohydrates, such as sugars and starches, within tissues are affected by many factors, such as temperature, moisture, light, pruning and time of planting (Daie, 1985).

In summer pruning treatments conducted on plenty of fruit varieties, many researchers reported that shoot re-enlargement was observed in trees with early summer pruning so as to compensate decreasing leaf areas. Due to the shoot re-enlargement on trees, decreases were observed in stored carbohydrates of trees. Greene and Lord (1983) suggested that although summer pruning may reduce carbohydrate levels enough to restrict the increase in trunk circumference, they may still be above that critical level required to reduce terminal growth.

Lang (2005) reported that summer pruning to be conducted until harvest results in the decrease in storage reserves of trees to be used in following periods. Demirtaş *et al*. (2010b), conducted 5 different summer and winter pruning treatments on 'Hacıhaliloğlu' apricot trees and found that post-harvest summer pruning treatment has the highest increasing effect on average total sugar, reducing sugar and starch contents. In sweet cherry, one year after summer pruning, the level of carbohydrate in trunk was lower compared to unpruned trees (Clair-Maczulajtys *et al*., 1994). Previous studies have also shown that pruning results in quantitative changes in carbohydrate reserves.

The results that we obtained about almond varieties related to carbohydrate contents are completely compatible with the findings of Stutte *et al*. (1994), Danielle *et al*. (1994) and Ikinci (1999) who studied apple, cherry, apricot and peach varieties and of abovementioned researchers.

IV – Conclusions

The influence of pruning on the trees and quality of fruits is only seen in the following season. In this study, it is determined that carbohydrate content of almond trees pruned in summer decreases prominently in comparison to the trees which are controlled and only pruned in winter. Yet it is found that this decrease occurred mostly in trees that are summer pruned in pre-harvest (june or july) season.

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Pistacia atlantica, a spontaneous hypermycotrophic phanerophyte: could be a natural tool to enhance the potential of mycorrhizal infectivity (PMI) of soils in arid regions?

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Abstract. In our drylands, spontaneous perennials species should be used to play the role of facilitator species in the success of plurispecific agro-ecosystems. It's the case of Atlas pistachio (the main spontaneous phanerophyte in Algerian pastoral steppe). The mycorrhizal status of this species could potentially increase the potential of mycorrhizal infectivity (PMI) of soils in drylands and benefit these poor soils by its rhizospheric effect. In Algeria, we have chosen for this study, two populations of *Pistacia atlantica*. The first one is located in semi-arid region (province of Médéa) and the other in hyper arid region (province of Béchar). We found that all the roots of the studied samples were infected by Arbuscular Mycorrhizal Fungi (AMF). Identification of their spores showed that Atlas pistachio is a hypermycotrophic species. At least we found 5 different species of Glomeromycetes in the first population (Médéa) and 3 different species in the second one (Béchar). It may be a reservoir of AMF propagules which will potentially infect cultivated species and thus will enhance their yields.

Key words. Arid soils – PMI – *Pistacia atlantica* – Rhizospheric effect – AMF – Glomeromycetes – Propagules – Algeria.

Pistacia atlantica, phanérophyte spontanée hypermycotrophe : peut-être un outil naturel pour améliorer le potentiel infectieux mycorhizogène des sols des régions arides ?

Résumé. *Dans nos zones arides, les espèces pérennes spontanées doivent être utilisées pour jouer le rôle d'espèces facilitatrices dans le succès des agroécosystèmes plurispécifiques. C'est le cas du pistachier de l'Atlas, espèce par excellence de la steppe pastorale algérienne. Le statut mycorhizien de cette essence est potentiellement prometteur pour augmenter le potentiel infectieux mycorhizogène (PIM) des sols arides et bénéficier de l'effet rhizosphérique sur ces sols indigents. En Algérie, nous avons choisi pour étude, deux populations situées, l'une en milieu semi-aride (Médéa) et l'autre en milieu hyperaride (Béchar). Nous avons constaté que toutes les racines des échantillons étudiées sont endomycorhizées par des Glomeromycètes. L'identification de leurs spores a montré que le pistachier de l'Atlas est une espèce hypermycotrophe (au moins : 5 espèces différentes chez la population en semi-aride et 3 espèces différentes chez celle en milieu hyperaride). Il pourra être un réservoir à propagules d'AMF qui s'associera potentiellement aux espèces cultivées et améliorerai ainsi leurs rendements.*

Mots-clés. *Sols arides – PIM –* Pistacia atlantica *– Effet rhizosphérique – AMF – Glomeromycetes – Propagules – Algérie.*

I – Introduction

Among the microbial components involved in soil biofunctioning, mycorrhizal fungi are considered as major elements in the soil / plant interface (Duponnois *et al,*. 2012). In fact, their key roles are the mobilization of soil nutrients that have low mobility, especially phosphorus (Duponnois *et al*., 2005a; Lambers *et al*., 2008); improving plant hydration (Augé, 2001); and the reduction or even total inhibition of the negative effects of some pathogenic agents (Smith and Read, 2008).The colonization of the soil by extramatrical mycelium and the production of a glycoprotein (glomalin) by mycorrhizal hyphae generate better soil structure by forming more stable aggregates (Lovelock *et al*., 2004; Rillig and Mummey, 2006).These fungi promote coexistence between different plant species, improving productivity and plant biodiversity in the ecosystems where they are present (van der Heijden *et al*., 1998 a,b; Sanon *et al*., 2006; Kisa *et al*., 2007).The presence of mycorrhizal plants can act as a reservoir of mycorrhizal propagules, and thus should be a very effective means of ensuring the establishment of young regeneration by facilitating the infection of seedlings, and thus their survival in these often-hostile environments (Newman, 1988; Simard and Durall, 2004).

In the arid regions of Algeria, there exists one of the rare spontaneous phanerophytes which could allow a natural approach to increasing the potential of mycorrhizal infectivity (PMI) of those soils: it is the Atlas pistachio (*Pistacia atlantica* subsp. *atlantica*). This phanerophyte colonizes disparate habitats, constituting an important metapopulation which ranges from the Mediterranean coast to the heart of the Hoggar (in the extreme south of the Algerian Sahara), where some old individuals are regarded almost as relics (Monjauze, 1967).

The aim of this work is to try to establish for the first time (to our knowledge), the mycorrhizal status (root colonization and spores) of Atlas pistachios belonging to two spontaneous populations: one located in a semi-arid environment, and another located in a hyper-arid environment.

II – Materials and methods

We sampled two spontaneous populations of Atlas pistachio, one located in Sidi Naamane (SN) in the province of Medea and the other in Beni Ounif (BO) in the province of Béchar. These two populations are situated on a gradient of increasing climatic and edaphic aridity (Table 1).

We took samples of roots with diameter less than 1 cm from the 0-20 cm soil level, along with their ramifications and their rhizospheric soil. We sampled six individuals from the SN population and six individuals from the BO population. In the laboratory, we gently released the roots from their rhizospheric soil. Using a digital caliper, we selected fine roots of less than 1 mm of diameter. The rhizospheric soil collected was admixed to form two composite samples: one for SN and one for BO.

Table 1. Climatic data of the two stations

Stations	P mm/year	T (°C) (average annual)	PET mm/year	AI	Ecoclimatic zonation (UNEP, 1992)	LDS (Bagnouls and Gaussen, 1953)
Sidi Naamane (Médéa)	628	15	1597	0.39	Semiarid	4.5
Béni Ounif (Béchar)	76	22	2366	0.03	Hyper arid	12

P: Precipitations; T: Temperatures; PET: Potential Evapotranspirational; AI: Aridity Indices; LDS: Longer of Dry Saison.

The roots of the Atlas pistachio are very dark because of their high tannin content. Therefore, we have adapted the protocol of Brundrett *et al*. (1996) by increasing the bleaching time to as much as 8 days for the darkest samples. After rinsing roots with tap water to remove the fixative solution (formalin), we separately placed root samples of each station in heat-resistant containers. The selected roots were completely immersed in a solution of KOH (10% w / v) and baked at 90°C for one hour. After that, we left the roots immersed in the KOH overnight. The next day, we replaced the now-brown KOH with fresh solution, and began a new cycle of bleaching. This process was repeated until the roots became decoloured. The bleached roots were immersed in a 2% HCl solution for 30 minutes to neutralize the KOH. After rinsing with tap water, we put the roots in a trypan blue solution (0.05% w / v in lactoglycerol) and baked at 90°C for 4 hours. The roots were then preserved in a solution of 50% glycerol. The prepared slides were observed using an optical microscope.

The spores were separated from the rhizospheric soil by a wet-sieving and decanting technique (Gerdemann and Nicolson, 1963). Under a stereomicroscope, the spores were manually extracted using fine forceps and micropipettes, and sorted by morphotypes. They were then transferred to microscope slides and covered with PVLG. The same steps were performed for the mounting of spores in the PVLG-Melzer (1: 1). Under the microscope, we measured their diameters, examined their shapes and when possible, their walls, suspending hyphae, and ornamentation, using the identification keys from Blaszkowski (2012).

III – Results and discussion

No ectomycorrhizae were detected in either sample population. However, all of the roots presented abundant endomycorrhizae (Fig. 1).

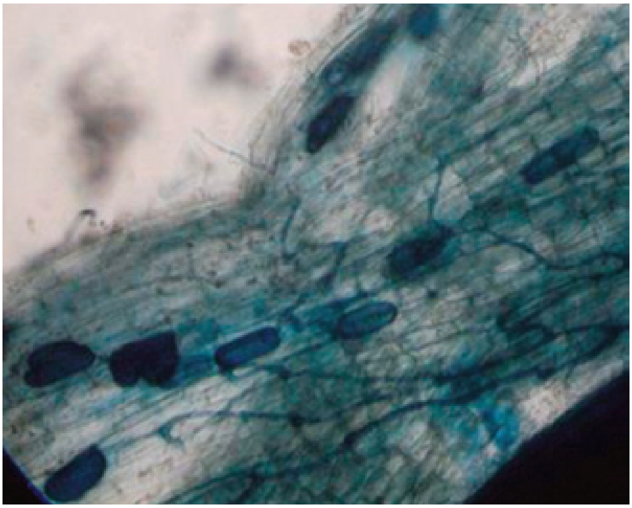

Fig. 1. *Pistacia atlantica* root colonisation by AM fungi (x 100).

The examination of spores allowed the identification of 4 species belonging to *Glomus* genus and one belonging to Scutellospora genus in the rhizospheric soil of the SN sample. In the rhizospheric soil of the BO sample, we identified 3 species from *Glomus* genus and 2 unidentified morphotypes (Figs. 2, 3, 4 and 5).

Despite the disparity between the sampled habitats (semi-arid for SN and hyper-arid for BO), these results show that the Atlas pistachio is a hypermycotrophic species (a plant associated with abundant and diverse mycorrhizal fungi). Therefore, it has the ability to promote the growth of fungal symbionts and may constitute an AMF propagules reservoir likely to be associated with cultivated plants, as well as improved crop yields (Duponnois and *al*., 2012).

The Atlas pistachio is naturally adapted to these arid environments due in part to its highly flexible and efficient root system (Limane *et al*., 2014). Its roots can reach 6 meters deep (Monjauze, 1968), and can expand horizontally to more than 12 meters (personal data). With such a large volume of soil influenced, it is able to increase the PMI of these soils. It should thus be considered as a native facilitative tool for integration in agro-ecosystems, especially in arid environments.

Fig. 2. Spore of *Glomus* sp. 1 mounted in PVLG.

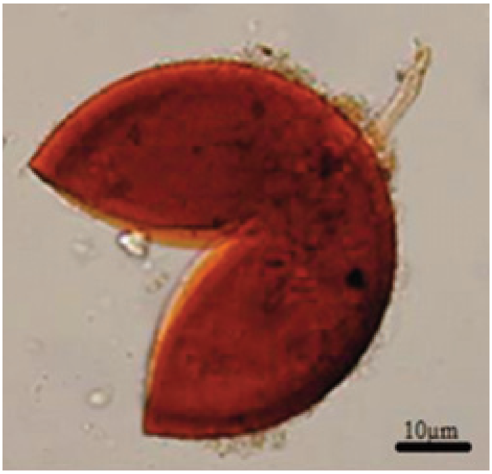

Fig. 3. Spore of *Glomus* sp. 2 mounted in PVLG+Melzer.

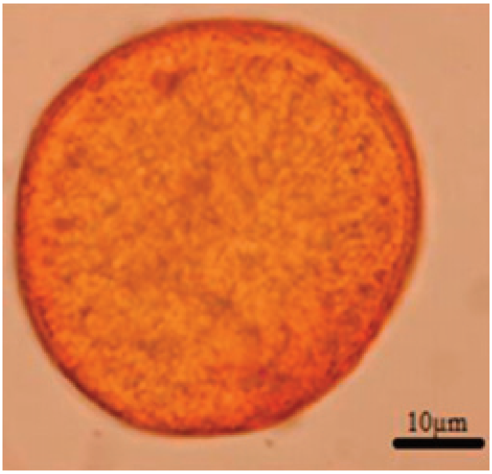

Fig. 4. Spore of *Scutellospora* sp. mounted in PVLG.

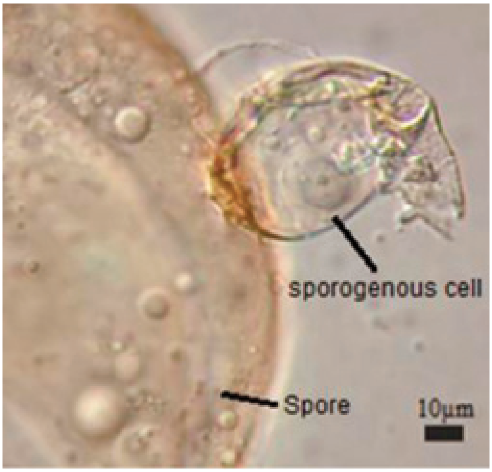

Fig. 5. Spore of *Glomus* sp. 3 mounted in PVLG.

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Session 5

Quality, industrialisation and marketing

Chemical changes in the composition of roasted kernels of Moroccan almonds

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Abstract. The present work aims at determining the changes in the chemical components of almond kernels after roasting in pan with sand at during 25 min, as done in Northern Morocco. After roasting, oil content varied between 56% and 58.8%, protein content between 18.24% and 23.27%, and sugar content between 5.47% and 6.84%. These results show that the kernels maintain their nutritional value after roasting. Fatty acid composition was affected, with significant decreases in the percentage of palmitoleic, oleic and linoleic acids. The ratio of oleic to linoleic acids, a good index of resistance to rancidity, also decreased after roasting, although it was still similar to that of raw kernels of some commercial cultivars. The amount of the different tocopherol homologues was altered after roasting: α-tocopherol decreased, mainly in 'Beldi', characterised by low values before roasting, whereas in 'Marcona', with higher tocopherol concentration, the decrease was not so drastic. On the other side, γ-tocopherol increased in all samples after roasting. These results clearly show that the fatty acid profile and the tocopherol concentration are affected, but not drastically, by this roasting method. As a consequence, the quality of these roasted kernels is not significantly altered as compared to the raw kernels.

Keywords. *Prunus amygdalus* Batsch – Almond – Roasting – Fatty acids – Tocopherol.

Changement de la composition chimique des amandons grillés d'amandier au Maroc

Résumé. *L'objective de ce travail est la détermination des changements dans la composition chimique des amandons après grillage dans des poêles avec sable à une température de 80°C pendant 25 min, technique adoptée au Nord du Maroc. Après le grillage, le content en huile a varié entre 56% et 58,8%, le contenu en protéine entre 18,24% et 23,27%, et le taux de sucre entre 5,47% et 6,84%. Ces résultats montrent que les amandons maintiennent leur valeur nutritionnelle après le grillage. La composition en acides gras a été affectée, avec une diminution significative dans les pourcentages des acides palmitoléïque, oléïque et linoléïque. La relation entre les acides oléïque et linoléïque, un indice utilisé pour évaluer la résistance de l'amandon à la rancidité, a aussi diminué après le grillage, même si il reste comparable à l'indice trouvé dans les amandons de quelques cultivars commerciaux. Le grillage a aussi altéré la quantité des différents homologues de tocophérol: l'α-tocophérol a été diminué, surtout pour le type 'Beldi', caractérisé par des valeurs faibles avant le grillage, tandis que pour 'Marcona', avec des concentrations de tocophérol plus élevées, la diminution n'a pas été si drastique. En outre,le γ-tocophérol a augmenté dans tous les échantillons après le grillage. Ces résultats montrent clairement que le profile des acides gras et la concentration des tocophérols est affecté par l'opération du grillage utilisée au Nord du Maroc, mais pas drastiquement. Par conséquent, la qualité de ces amandons grillés n'est pas significativement affectée en comparaison aux amandons non grillés*

Mots-clés. Prunus amygdalus *Batsch – Amandier – Grillage – Acides gras – Tocophérol.*

I – Introduction

In Morocco, almond is grown in several regions from north to south, under different environmental conditions, mostly on non-irrigated areas of poor soils and receiving little attention from farmers. Almost all almond production in Morocco is provided by a traditional production sector, characterized by a dominance of almond trees propagated by seed, located primarily in the north and the south. Almost all production is commercialized directly by farmers in local markets at low prices. Kernels produced by almond seedling in the North of Morocco is characterized by high protein content, ranging from 21 to 27% of kernel DM, and oil content from 55 to 58% of kernel DM (Kodad *et al*., 2011). Almonds are marketed in different commercial forms: in-shell almonds, shelled kernels, blanched, roasted or not. In Northern Morocco, the local farmers roast almond in a pan with sand. This method could alter the flavor and cause product rancidity. Roasting promotes different changes in the kernels, mainly for the volatile compounds (Gou *et al*., 2000). Consequently, our aim was to study the chemical composition of raw and roasted almond kernels as done in Northern Morocco in order to evaluate the potential changes during the roasting process.

II – Materials and methods

This study was carried out in the Rif Mountains (North of Morocco). The samples were collected from local genotypes named 'Beldi' and from cultivars 'Marcona' and 'Ferragnès'. The nuts were collected in summer of 2012. Before processing, the mesocarp was removed and the nuts were dried and stored at ambient temperature during three weeks. A sample of 30 kernels was collected and the remaining kernels were roasted traditionally, without any additives, in pan with sand at 80°C during 25 min. After roasting, two independent samples, two replicates for each sample, were randomly taken for analysis. Kernels were ground in an electrical grinder.

For kernel composition, protein, oil and total sugar content, as well as fatty acid and tocopherol composition were determined as already described (Kodad *et al*., 2011).

III – Results and discussion

The oil, protein and sugar contents of roasted kernel varied significantly among samples (Table 1). Oil content varied from 59.8% DM in 'Marcona'" to 56.2% DM in 'Beldi', whereas protein content was higher in 'Beldi' (23.7% DM) and lower in 'Ferragnès'. Sugar content was higher in 'Ferragnès' and lower in 'Beldi'. These results do not differ from those reported for 'Marcona' and 'Ferragnès' raw kernel (Kodad *et al*., 2011), clearly showing that roasted kernels maintain their nutritional quality after roasting.

Table 1. Chemical components of roasted almond kernels

Sample	Sugar content (% Glucose)	Protein content (% DM)	Oil content (% DM)
'Beldi'	5.47 b	23.27 a	56.2 b
'Marcona'	5.99 b	21.31 a	59.39 a
'Ferragnès'	6.84 a	18.24 b	58.84 a

Mean values of each component followed by different letters are significantly different between samples at $P < 0.05$.

Almost all studied variables showed significant differences among samples, probably due to their different genetic origin. Since unsaturated fatty acids are prone to oxidation during heat processing, the study of the fatty acid composition and their changes during roasting is relevant. The treatment effect was significant for all fatty acids with the exception of palmitoleic acid (Table 2). Oleic acid percentage decreased significantly after roasting whereas palmitic, stearic and linoleic acids increased (Table 3).

Table 2. Mean value of oleic and linoleic fatty acids of raw and roasted almond kernels

	Raw kernels		Roasted kernels	
Sample	**Oleic ac.**	**Linoleic ac.**	**Oleic ac.**	**Linoleic ac.**
'Beldi'	74.22 a	15,89 a	73.3 a	16.14 a
'Ferragnès'	73.97 a	16.40 b	68.01 b	22.2 a
'Marcona'	73.05 a	17.01 b	69.86 b	20.48 a

Mean values of each component followed by different letters are significantly different between treatments at $P < 0.05$.

Table 3. Mean values of fatty acids and tocopherol homologues of raw and roasted kernels

	Treatment	
Component	**Raw kernel**	**Roasted kernel**
Palmitic acid (% of total oil)	6.30 a	6.74 b
Palmitoleic acid (% of total oil)	0.65 a	0.62a
Stearic acid (% of total oil)	1.54 a	2.03 b
Oleic acid (% of total oil)	73.7 a	70.39 b
Linoleic acid (% of total oil)	16.43 a	19.60 b
Oleic acid/Linoleic acid ratio	4.49 a	3.59 b
Linolenic acid (% of total oil)	0.019 a	0.03 a
α-tocopherol (mg/kg oil)	398.8 a	277.78 b
γ-tocopherol (mg/kg oil)	9.64 b	17.41 a
δ-tocopherol (mg/kg oil)	0.52 b	3.17 a

Mean values of each component followed by different letters are significantly different between treatments at $P < 0.05$.

The ratio of oleic to linoleic acid (O/L) is used as an index of resistance to oil rancidity (Kester *et al*., 1993), with high ratios being preferable (Socias i Company *et al*., 2008). This ratio decreased after roasting (Table 3), although its value is still similar to some commercial cultivars (Kodad *et al*., 2011). The percentage of linolenic acid is considered low, even if it increased slightly after roasting. Linoleic acid, an essential fatty acid, is reported to be useful for reducing the risk of diabetes and cardiovascular disease (Venkatchalam *et al*., 2004).

The percentage of oleic acid decreased significantly after roasting in 'Ferragnès' and 'Marcona' (Table 2), whereas in 'Beldi' the differences between raw and roasted kernels were not significant, not detecting changes in the flavour of the kernels before and after roasting. Therefore, the roasting method used in this traditional processing does not drastically affect the fatty acid composition and quality of the almond kernels.

Table 4. Means values of tocopherol homologues in raw and roasted kernels

	δ-tocopherol (mg/kg oil)		γ-tocopherol (mg/kg oil)		α-tocopherol (mg/kg oil)	
	Raw kernel	**Roasted kernel**	**Raw kernel**	**Roasted kernel**	**Raw kernel**	**Roasted kernel**
'Beldi'	0.74 b	3.60 a	10.58 b	13.23 a	358.42 a	161.33 b
'Ferragnès'	0.41 b	1.65 a	6.08 b	17.74 a	413.11 a	302.01 b
'Marcona'	0.44 b	3.76 a	8.28 b	21.22 a	485.02 a	363.88 b

Mean values of each component followed by different letters are significantly different between treatment at $P < 0.05$.

The tocopherol concentration was significantly affected by the roasting process, with a significant decrease for α-tocopherol (Table 3). Garcia-Pascual *et al.* (2003) studied the effect of storage time of raw and roasted kernels of four almond cultivars reporting that α-tocopherol decreased significantly in roasted kernels and with storage time. On the contrary, γ-tocopherol increased after roasting (Table 4). Lane *et al*. (1997) suggested that a significant amount of the vitamin E homologues are bound to proteins or linked to phosphate or phospholipids. Heat breaks these bonds, resulting in an increase of extractable tocopherols. This phenomenon could explain the increase of this homologue in the roasted almond kernels.

The analysis of variance showed that the sample effect was significant for all tocopherol homologues (Table 3). 'Marcona' showed the highest concentration of α-tocopherol and 'Beldi' the lowest values (Table 4). Moreover, the decrease of α-tocopherol was drastic in 'Beldi', as opposite to 'Marcona'. It has been reported that α-tocopherol has ten times higher biological activity than γ-tocopherol in almond (Zacheo *et al*., 2000). Thus, the presence of a high concentration of α-tocopherol in almond oil is of primary importance because its levels declined slightly two years after harvest, to approx. 90% of the initial concentration (Zacheo *et al*., 2000). Our results confirm that α-tocopherol plays an important role in the protection of almond kernels against oxidation. A high level of this isomer is suitable not only to prolong the storage period without quality losses (Senesi *et al*., 1996), but also to maintain high nutritional value and to maintain the quality of the kernel after roasting.

As a conclusion, the roasting treatment affected the fatty acid composition and the tocopherol concentration of almond kernels. However, since the decrease of α-tocopherol was lower in 'Marcona', this cultivar is mostly suitable to roasting with this traditional method because it maintains relatively high levels of tocopherol homologues, which protect almond kernel against oxidation.

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Soluble sugars and mineral nutrient contents in kernels of three Tunisian almond cultivars

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Abstract. Almond is an important food crop grown mainly in the Mediterranean region and the USA. The kernel is the edible part of the nut and is considered a rich source of many nutrients. In the present study, total soluble sugars and mineral nutrient contents were quantified in the kernel of three local *Prunus dulcis* (Mill.) D.A. Webb cultivars selected from Tunisia. Cultivars are from three different origins. 'Tozeur 1' is from the south (Tozeur region), 'Achaak' is from the centre (Sfax region) while 'G25-5' is from the north (Mornag region). Mineral composition was analyzed by atomic absorption spectrometer and the amount of Mg, K, Na, Ca, Fe, Zn, Cu and Mn was determined. Among mineral nutrients, K, Ca and Mg were the predominant elements in kernels. Moreover, potassium (K) was the major mineral element for which amounts up to 683 mg/100 g (dry matter). 'Achaak' had higher Ca level (234.6 mg/100 g), however, there was no significant difference in K and Mg contents between three studied cultivars. Total soluble sugar contents showed difference among cultivars and the highest value (5.7 g/100 g) was found in 'G25-5' kernels. So, the obtained results showed that almond kernels are being potential sources of mineral elements which might be used as useful dietary supplements. Additionally, results of this study may provide relevant data for future studies on the chemical composition and will be useful to know about the nutritional properties of the local almond kernels.

Keywords. Almond – Kernel – Minerals – Total sugars.

Détermination de la teneur en sucres solubles et en éléments minéraux des amandons de trois cultivars tunisiens d'amandiers

Résumé. *La culture de l'amandier est très répandue dans le bassin méditerranéen et aux Etats Unis d'Amérique. L'amandon est la partie comestible; il est considéré comme une source riche en nutriments. Dans la présente étude, les sucres solubles totaux et les teneurs en éléments minéraux ont été quantifiés dans l'amandon de trois cultivars tunisiens d'amandiers* Prunus dulcis (Mill.) D.A. Webb. *Ces cultivars proviennent de trois différentes régions: la variété 'Tozeur 1' provient des oasis de Tozeur au sud, la variété 'Achaak' provient de la région de Sfax au centre et la variété 'G25-5' provient de la région de Mornag située au nord de la Tunisie. Les teneurs en Mg, K, Na, Ca, Fe, Zn, Cu et Mn ont été déterminées par spectrométrie d'absorption atomique. Nos résultats ont montré que le potassium (K), le calcium (Ca) et le magnésium (Mg) sont les éléments prédominants dans les amandons et que le potassium est l'élément minéral majeur (683 mg/100 g en matière sèche). La variété 'Achaak' est la plus riche en Ca (234,6 mg/100 g), alors qu'il n'y a aucune différence significative pour le K et Mg entre les trois variétés étudiées. La teneur en sucre solubles totaux diffère significativement entre les cultivars. La variété 'G25-5' étant la plus riche: 5,7 g/100 g. Dans l'ensemble, les résultats obtenus montrent que les amandes sont des sources potentielles d'éléments minéraux qui pourraient être utilisées comme suppléments alimentaires. En outre, ces résultats peuvent fournir des données pertinentes pour de futures études sur la composition minérale et les propriétés nutritionnelles des amandiers locales.*

Mots-clés. *Amandier – Amandon – Composition minérale – Sucres solubles totaux.*

I – Introduction

The cultivated almond [*Prunus dulcis* (Mill.) D.A. Webb; syn. *P. amygdalus* (L.) Batsch] is considered as one of the oldest nut crops. This crop is cultivated mainly in the Mediterranean region and the USA. The almond species is well adapted to Mediterranean climate. In Tunisia, almond is the second agricultural product after the olive tree. Almond plantations are located throughout all the country in different climatic conditions and are characterized by an important genetic diversity (Gouta *et al.*, 2010).

Almond kernels have an economic value where they are mostly consumed as fresh fruit, raw or toasted and salted, as snack. Moreover, kernels are widely used in cooking as ingredients for both savory and sweet dishes. The demand for almond fruits is increasing, partly because of their associated health benefits. Indeed, almond fruits exert health-promoting effects that have been attributed to their supply of wide variety of nutritional components such as fiber, vitamin, mineral elements, proteins, fats; especially high level of monosaturated acid (Karatay *et al.,* 2014). Hence, almonds are nutritious and delicious fruits.

Although there are several reports of evaluating almond kernels composition worldwide, there are few studies regarding to the nutritional properties of Tunisian almond kernels. Therefore, the aims of this work were to evaluate the total soluble sugars as well as mineral nutrient contents in kernels of two autochthonous almonds cultivars 'Tozeur 1' and 'G25-5'. They are originated from two different regions, but, they are cultivated and grown in the same open field. Results were compared to those of 'Achaak', which is much appreciated by consumers for the quality of its fruit in local market.

II – Materials and methods

Fruits of two cultivars of almond 'Tozeur 1' and 'G25-5' were collected by hand in late July and mid-July 2014, respectively, from seven years old trees growing in the Experimental Station of the Tunisian National Agricultural Research Institute (INRAT), located in the city of Mornag (the north of Tunisia). Cultivars were originated from two different regions. 'Tozeur 1' is from the south (Tozeur) and obtained by seedling selection while 'G25-5' is from the north (Mornag) and obtained by chance seedling. The third cultivar used in this study is an old local cultivar 'Achaak' which originated from the centre (Sfax region). Dried fruits were transferred to laboratory in polypropylene bags under cool conditions. Kernels were obtained from shells by hand processing and stored at 6°C until analyzed.

Kernels were milled by a grinder (Sunbeam Osterizer blender, Boca Raton, USA) just before analysis. To avoid overheating, the sample was flaked for 10 s, then grinding was halted and the sample was shaken for another 10 s, and the milling process was continued. For ion analyses, 1g of dry ground kernels was extracted with 20 ml of 0.1 M HNO_3. After filtration, Mg, K, Na, Ca, Fe, Zn, Cu and Mn contents were determined with an atomic absorption spectrometer (Avanta, GBC, Australia).Total soluble sugars were determined according to the method of Robyt and White (1987).

The data were subjected to an analysis of variance (ANOVA) using SAS statistical software version 6.12 (SAS Institute, Cary, NC, USA). A completely randomized design with three replicates was used. Where applicable, means were separated by Duncan's multiple range test with the level of significance $P \leq 0.05$. Results are reported as average of at least six repetitions.

III – Results and discussion

It was reported that the individual mineral elements found in almonds are calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), potassium (K), phosphorus (P), selenium (Se), sodium (Na) and zinc (Zn) (USDA, 2010). The mineral nutrient contents of almond kernels belonging to the three studied cultivars are reported in Table 1. Our results revealed that K, Mg and Ca were the most abundant minerals (Table 1). Similar findings were reported by Piscopo *et al*., (2010). Potassium was the most abundant element and levels ranging from 645 (Tozeur 1) to 683 mg/100 g (G25-5). These values were lower than those observed in kernels of some almond varieties originating from Turkish orchards where values ranged between 1314 to 1510 mg/100g (Ozcan *et al*., 2011). However, such values were close to those reported by previous works (Ayadi *et al*., 2006 and Piscopo *et al*., 2010). K and Mg did not varied significantly among cultivars. The variety 'Achaak' showed the highest level in Ca (234.54 mg/100 g) in comparison to others two varieties.

The obtained results of the three almond cultivars showed that the almond kernels showed high levels of magnesium, potassium and calcium and, in contrast, very low levels of sodium, zinc, copper, manganese and iron. Accordingly, such composition confirmed the nutritional interest of almond kernel and allows us to consider the almond an excellent source of bioelements. Indeed, magnesium and potassium may improve blood pressure and it is important to maintain the proper proportion of calcium to potassium. Moreover, their high magnesium content makes them a very suitable food to offset deficiencies in this mineral (Tapia *et al*., 2013).

Table 1. Mineral content in almond kernels. Data are expressed as mg/100 g of dry matter. Mean separation within columns by Duncan's Multiple Range Test (P ≤ 0.05). Different letters indicate significant differences between cultivars for a given element

Cultivar	Ca	Cu	Fe	K	Mg	Mn	Na	Zn
'Tozeur 1'	193.59b	0.6a	2.16ab	645.09a	150.36a	1.73a	0.93b	2.60b
'Achaak'	234.54a	0.34c	1.93b	661.37a	143.42a	1.85b	0.86a	2.91a
'G25-5'	204.8b	0.43b	2.52a	683.39a	144.95a	1.68ab	0.96ab	2.65b

The mean values and standard deviations of total soluble sugar contents for the kernels of the three studied cultivars are presented in Fig 1. The higher value of total soluble sugar contents was detected in 'G25-5' cultivar (5.7 g/100 g); however, 'Achaak' and 'Tozeur 1' showed similar content which was

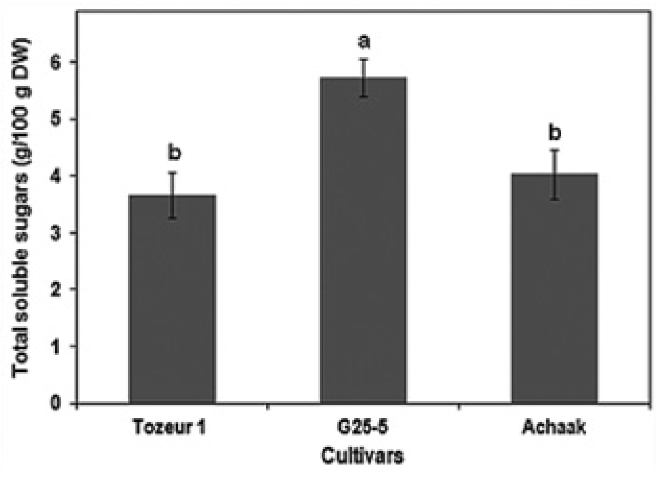

Fig. 1. Soluble sugars content in kernels of three studied cultivars. Each point represents the mean (±SE) of six replicates. Mean separation by Duncan's Multiple Range Test. Different letters indicate significant differences between treatments (P ≤ 0.05).

about two-thirds of that observed in 'G25-5'. A wide range of soluble sugar contents in almond kernels is reported in the literature. Indeed, published values for sugar were comprised between 3.3 to 7.1 g/100 g (Socias i Company *et al*., 2008), with sucrose representing the predominant sugar in kernel (Schirra, 1997). According to the USDA SR database, the value provided for total sugars (3.89 g/100 g natural almonds) is the sum of determinations of individual mono– and disaccharides that include sucrose, glucose, fructose, galactose and maltose (USDA, 2010). Based on this standard, 'G25-5' had higher total sugar contents, while the other two cultivars were in a similar range.

IV – Conclusions

Our results prove that the three studied almond cultivars constitute a natural source of sugars and many minerals, mainly, potassium, calcium and magnesium that may prevent many diseases. Although the two varieties 'G25-5' and 'Tozeur 1' were not well known in market as 'Achaak', mineral nutrient contents were almost similar for three studied cultivars. Therefore, such data shows the potential of 'G25-5' and 'Tozeur 1' that could be interesting cultivars. Also, it is very interesting to accomplish this study by other investigation to know the nutritional properties of our local almond cultivars.

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Pistacia vera: chemical composition and pharmacological activities (Pistachio nuts)

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Abstract. The pistachio is a nutrient-dense nut with a heart healthy fatty-acid profile as well as protein, dietary fiber, potassium, magnesium, vitamin K, g-tocopherol, and a number of phytochemicals.this particular composition makes this dry fruit a fabulous way to prevent cardiovascular disease. The details of these pharmacological activities will be discussed in this review of the literature.

Keywords. Pistaciavera – Nuts – Antioxydative activity – *Review*.

Pistacia vera *: composition chimique et activités pharmacologiques (Pistaches)*

Résumé. *Les noix de pistaches sont riches en nutriments avec un profil d'acides gras insaturé ainsi que des protéines, fibres alimentaires, de potassium, de magnésium, de la vitamine K, g-tocophérol, et un certain nombre de phytoconstituants. Cette composition particulière rend ce fruit sec un fabuleux nutriment pour prévenir les maladies cardiovasculaires. Les détails de ces activités pharmacologiques seront discutés dans cette revue de la littérature.*

Mots-clés. *Pistaciavera – Noix – Activité antioxydante –* Revue.

I – Introduction

The pistachio is a dry fruit produced by a mediterranean shrub, the real (*Pistacia vera* L.) belongs to the family Anacardiaceae. Is native of aride zones of Central and west Asia and distributed throughout the Mediterranean basin (US Department of Agriculture, 2012). For several years, special attention was given to food consumption of nuts (almonds, pistachios, etc.) because of their high content of unsaturated fatty acids and thus their l beneficial effects on cardiovascular function and lipidic profile in individuals with high risk of coronary heart disease (Seeram *et al.,* 2006).

It contains about 50% of fat products, 83% composed of unsaturated acids, and about 23% of proteins and 13% of carbohydrates. It is a source of potassium, copper, magnesium and iron.Various pharmacological activities very recently have been studied in experimental works and in clinical trials to evaluate these effects on human health (F.B. Hu, W.C. Willett, 2002). Numerous studies have demonstrated beneficial effects of regular consumption of pistachios on glycaemic profile, lipid and oxidative stress parameters.

II – Materials and methods

A bibliographic investigation was carried out by analysing recognized peer-reviewed papers, consulting worldwide accepted scientific databases from the last decade (Scopus, Embase, MEDLINE/PubMed, Springerlink and Scholar databases) using medical subject heading terms and the words: 'Pistacia vera','Pistacio nuts','anticancer','antiinflammatory','antidiabetic','antiproliferative','antioxydative', and 'cytotoxic', to identify relevant articles.

We read the titles and abstracts of all articles in an initial screen, obtaining full text unless there was clear evidence that the article would not be eligible. In the present study, interest is focused on experimental research and clinical trials.

III – Results and discussion

Tomaino *et al.* (2010) have demonstrated the excellent antioxidant activity of pistachio skins that could be explained by the high content of antioxidant compounds (Table 1). The pistachio skin could be primarily responsible for the high antioxidant activity of whole pistachio nuts. Briefly, due to their better nutritional and health profile, unpeeled pistachios should be preferred to the peeled ones in the human diet both if consumed as whole and if used in processed foods.

Table 1. Key nutrients and phytochemicals of pistachio nuts (per ounce = 28.35 g). Taken from Dreher, 2011

Nutrient	Pistachios (dry roasted/salted)
Energy (kcal)	160
Total lipid content (g)	12.7
Monounsaturated fat (g)	6.7
Polyunsaturated fat (g)	3.8
Saturated fat (g)	1.5
Protein (g)	5.9
Dietary fiber (g)	2.8
K (mg)	285
Mg (mg)	31-34
Vit K (mg)	3.7
Vit E (α-tocopherol) (mg)	0.7
Total phenols (mg)	470
γ-Tocopherol (mg)	6.7
Total phytosterols (mg)	61-82

Exploratory clinical studies suggest that pistachios help maintain healthy antioxidant and anti-inflammatory activity, glycaemic control, and endothelial function (Sari I *et al*., 2010). When consumed in moderation, pistachios may help control body weight because of their satiety and satiation effects and their reduced net metabolizable energy content. One study with subjects in a weight-loss program demonstrated lower body mass index and triglyceride levels in individuals who consumed pistachios compared with those who consumed an isocaloric pretzel snack (Dreher M.L. 2011, Kocyigit A. *et al*., 2006).

IV – Conclusions

The Pistachios are nutrient-dense nuts that contain a heart-healthy fatty-acid profile, protein, dietary fiber, potassium, magnesium, vitamin K,g-tocopherol, and a number of phytochemicals, including phytosterols, phenolic acids, and xanthophyll carotenoids. Among nuts, pistachios contain the highest levels of potassium, phytosterols, vitamin K,g-tocopherol, and lutein.A growing number of clinical studies suggest potential health benefits of pistachio nuts. Five published randomized clinical studies have shown that pistachios have a beneficial effect on blood lipid profiles. Furthermore, emerging clinical evidence suggests that pistachios may help reduce oxidative and inflammatory stress and promote vascular health, glycaemic control, appetite management, and weight control.

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Extraction methods of seed oil and oil quality of *Pistacia atlantica* grown in dry land

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Abstract. Wild natural species could offer an important issue for the sustainable development and environmental protection. *Pistacia atlantica* is an interesting wild species wide spreading in arid land in Tunisia. Recent works focused on the use of this species as rootstock for pistachio cultivation and the investigation of oil quality of seeds. This study aims to evaluate three methods of oil extraction from *Pistacia atlantica* seeds. Seed oil was extracted by pressing, supercritical CO_2 and organic solvent and respective oil qualities were determined. Results showed that the supercritical CO_2 method is the most efficient with an extraction rate of 25% compared to extraction by pressing (5.3%) or by solvent (7.9%). The extracted oil was rich in unsaturated fatty acids with similar acid composition between the three extraction methods. However, the supercritical CO_2 method produced better oil quality with higher antioxidant activity and polyphenols and tocopherols contents.

Keywords. *Pistacia atlantica* – Seed oil – Extraction methods – Characterisation.

Méthodes d'extraction et qualité d'huile du* Pistacia atlantica *planté en région aride

Résumé. *Les espèces naturelles sauvages pourraient contribuer au développement durable et à la protection de l'environnement.* Pistacia atlantica *est une espèce sauvage largement répandue dans les régions arides de Tunisie. Des travaux récents ont porté sur l'utilisation de cette espèce comme porte-greffe et l'investigation de la qualité de l'huile extraite des graines. Cette étude vise à évaluer trois méthodes d'extraction d'huile de graines de* P. atlantica. *Il s'agit de l'extraction par pression, par CO_2 supercritique et par solvant organique. La qualité des huiles respectives a été déterminée. Les résultats obtenus ont révélé que le procédé au CO_2 supercritique était le plus efficace avec un taux d'extraction de 25% par rapport à l'extraction par pression (5,3%) ou par solvant (7,9%). L'huile extraite a été riche en acides gras non saturés mais avec une composition en acides similaire entre les trois méthodes d'extraction. Toutefois, le procédé au CO_2 supercritique a produit une meilleure qualité d'huile ayant une activité anti-oxydante élevée et une plus grande richesse en poly-phénols et en tocophérols.*

Mots-clés. Pistacia atlantica *– Huile de graine – Méthodes d'extraction – Caractérisation.*

I – Introduction

Three *Pistacia* species are widely spread in the Mediterranean region. *Pistacia atlantica* is the most characteristic plant species of the pre-Saharian regions in the North Africa (Benabid, 2000; Yousfi *et al*., 2002). Its seed oil has a good nutritive quality because of its content in unsaturated fatty acids and saturated fatty acids (Yousfi *et al*., 2002).

Several studies have been reported on the chemical composition of commercial pistachio species. However, few dealt with wild pistachio species. Previous works on *P. atlantica* reported the fruit composition of this species in flavonoids (Mosharrafa *et al*., 1999), fatty acids and triglycerides (Yousfi *et al.*, 2005; Benhassaini *et al*., 2007; Farhoosh *et al*., 2008). Other reports focused on the chemical composition of oleoresin (Delazar *et al*., 2004), and essential oil (Barrero *et al*., 2005; Tzakou *et al*., 2007).

This study investigated the effect of the extraction method on seed oil properties of *P. atlantica* grown in arid land of Tunisia.

II – Materials and methods

Pistacia atlantica fruits were collected from Sidi Bouzid location in the center of Tunisia. The seeds of wild pistachio *(Pistacia atlantica)* were sampled in August. The seed oil was extracted using three extraction methods: cold pressing, organic solvent (hexane) and supercritical CO_2 technique. For each method, a sample of 0.5 kilograms of seeds was used.

The oil extracted by these methods was analyzed. Oil acidity, UV absorption characteristics at 232 and 270 nm (K_{232}, K_{270}), chlorophyll and carotenoid contents, total phenols, and fatty acid composition were performed in accordance to the standard method established by IUPAC (1979) and to the European Official Methods (UE 1989/2003 modifying ECC 2568/91).

A sample of 0.05g of oil was used to separate the fatty acid methyl esters (FAMEs) according to the method of Arena *et al.* (2007). FAMEs were analyzed by gas chromatography using a Shimadzu 17A chromatograph equipped with detector flame ionization and a capillary column. Total phenol contents were determined using Folin-Ciocalteu's colorimetric analysis method and were expressed as parts per million (ppm) of gallic acid.

The antioxidant activity of seeds oil of *P. atlantica* was evaluated in comparison to the 4-hydroxyanisole (BHA).

III – Results and discussion

Table 1 shows the fatty acid composition of *Pistacia atlantica* seed oils. The acidity of oil is expressed as the percentage of total free fatty acids present in the oil and gives information about the alteration level of the product. The supercritical extraction technique reduced alteration through hydrolysis reaction and gave less altered oil with lower acidity level. The main fatty acids from the studied samples were oleic, linoleic and palmitic acids. Oleic acid was the most abundant with a percentage higher than 55% followed by linoleic acid (about 28%). The main saturated fatty acids were palmitic and stearic acids. Similar acidic composition was previously reported by Givianrad et *al*. (2013) for this wild pistachio species. The acidic composition of oils extracted from *P. atlantica* was very close to that of argan oil. Extraction methods seemed to not affect acidic composition.

The colour and taste of oil are in part determined by chlorophyll pigments. These compounds are easily degraded under the action of light. Extraction by organic solvent lead to high chlorophyll content oil in comparison with the other tested methods. It may be the result of the solubility of these compounds in hexane. Moreover, seeds oil of *P. atlantica* was not enough rich with food colorants. The extraction method did not affect the oxidation state of the extracted oils as the oil absorbance at 232 and 270 nm (K_{232}, K_{270}) did not change significantly with the used technique (Table 1).

Polyphenols and tocopherols are among the most natural antioxidants present in vegetable oils in significant quantities. Few studies mentioned the analysis of phenolic compounds in *P. atlantica.* It has been shown that the Anacardiaceae family is characterized by the occurrence of both gallic acid and myricetin derivatives (Umadevi *et al*., 1988). This study revealed that the levels of tocopherols were not affected by the extraction technique, while the polyphenol content decreased significantly when organic solvent extraction was used (Table 1).

Table 1. Oil quality of *P. atlantica* extracted using three extraction methods

	Supercritical CO_2	Pressure	Hexane
Acidity (%)	1.00	3.80	4.45
C16 :0	11.58	11.42	11.16
C16 :1	0.14	0.13	0.23
C17 :0	0.03	0.04	0.04
C17 :1	0.02	0.03	0.03
C18 :0	2.27	2.39	2.42
C18 :1	55.35	56.40	56.35
C18 :2	29.63	28.50	28.74
C18 :3	0.34	0.41	0.35
C20 :0	0.13	0.15	0.14
C20 :1	0.4	0.46	0.46
Chlorophyll (ppm)	1.67	4.99	5.78
Carotenoid (ppm)	2.65	2.21	4.22
K_{232}	2.15	2.17	2.16
K_{270}	0.84	0.93	0.95
Tocopherols (ppm)	446.32	440.36	419.73
Polyphenols (ppm)	2100	1800	500

The seeds of *P. atlantica* had an antioxidant activity much higher than the BHA (results not shown). With regard to oil extraction method, supercritical CO_2 provided oils which had a greater activity than that of the antioxidant BHA. Previous works used BHA to prevent oxidative rancidity of fats (Safer and Nughamish, 1999). Several researches reported the extensive use of these additives in agro alimentary industry.

IV – Conclusions

The oil extracted from *P. atlantica* seeds had interesting physicochemical characteristics. This oil may be used in the fields of cosmetic and phytotherapy industry. Extraction with hexane greatly reduced the quality mainly the content of these oils in polyphenols and subsequently its antioxidant activity.

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Valorisation of local almond genotypes regarding their biochemical and mineral compositions

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Abstract. The new tendency for intensification of almond plantations has induced a clear neglect of local ecotypes and cultivars that have proved high performances and good adaptation to many biotic and abiotic stresses. Prospecting the main producing regions in Tunisia has demonstrated a high genetic diversity. In addition, all of the pomological, biochemical and mineral characterizations have confirmed the potentialities of some local genotypes in comparison to the newly introduced cultivars. The analysis of their content in some biochemical compounds such as antioxidant has furthermore shown that some landraces are highly rich in α, γ and δ-tocophérols. Our result reported that the mineral composition of this nut is dominated by phosphor, calcium, iron, sodium, zinc and copper. In comparison to some introduced cultivars such as 'Mazzetto', 'Lauranne' and 'Supernova' many local ecotypes presented high performances. In fact the ecotype 'BF2', was highly rich in potassium and phosphor while 'TL7' in calcium. Additionally, the dominance of 'khoukhi', 'Dillou' and 'Blanco' for the iron element composition is another statement in favour of existent possibilities for a better valorisation of the local germplasm and consequently for its better preservation.

Keywords. *Prunus dulcis* L. – Local landraces – Antioxydants – Fat acids.

Valorisation des génotypes locaux d'amandier par analyse de leurs compositions biochimiques et minérale

Résumé. *La tendance actuelle vers l'intensification des plantations d'amandier a induit une nette négligence des variétés et des écotypes locaux. Ceux-ci ont montré des potentialités agronomiques très intéressantes ainsi que des niveaux satisfaisant de tolérance à divers stress biotiques et abiotiques. Les travaux de caractérisation pomologique, biochimique et minérale ont prouvé les performances de ces individus vis a vis des variétés introduites comme 'Mazzetto', 'Lauranne' et 'Supernova'. En effet, l'analyse du contenu des fruits en anti-oxydants a montré la richesse de certains écotypes en* α, γ *and* δ*-tocophérols. D'autre part, nos résultats ont confirmé que la composition minérale des fruits d'amandier est dominé par les éléments phosphore, calcium, fer, sodium, zinc et cuivre. D'ailleurs, les fruits de l'écotype 'BF2' sont remarquablement riche en potassium et en phosphore. De plus, la richesse en élément fer des écotypes du nord comme 'khoukhi', 'Dillou' et 'Blanco' sont tous des arguments en faveur de la possibilité d'une meilleure valorisation des ressources génétique d'amandier en cosmétique et dans l'industrie alimentaire ainsi que leurs conservations.*

Mots-clés. Prunus dulcis *L. – Écotypes locaux – Antioxydants – Acides gras.*

I – Introduction

Almond (*Prunus dulcis* L.) is a major nut tree grown around the Mediterranean area. In Tunisia it occupies the second position after olive tree with more than 250,000 ha. It is spread all over the country under different bioclimatic stages and it represents a principal income for many farmers living in extreme climate condition regions. Fruits are generally kept in the tree until their hulls are almost dry for an easier removal at harvesting time. In the local market quality and consumer choice is based on the presence of the main cultivars traditionally known as good products for consumption and culinary preparations. In fact, the two cultivars ‘Achaak’ and ‘Porto’ are the more desirable and quoted in the almond market. Unknown ecotypes are generally mixed altogether and sold at very low prices. The prospecting effort started few years ago has demonstrated the presence of a large almond diversity with two distinguished genetic pools one in the north and a second in the central and southern part of the country (Gouta *et al.*, 2010). Unfortunately, the Tunisian almond germplasm is actually threatened by lost and genetic erosion.

As the kernel is the edible part of the nut and the main fraction of it are lipids, many researchers have evocated that this fraction constitutes an important caloric source. It does not contribute to cholesterol formation in humans due to the high level of unsaturated fatty acids which are negatively correlated with serum lipid profiles and cholesterol status associated with a lower risk of cardiovascular diseases (Sabate and Hook, 1996; Jenkins *et al.*, 2008). Tocopherols with the four different homologues acting as antioxidants are believed to be involved in a diversity of physiological, biological and biochemical functions (Reische *et al.*, 1998) and are considered as a valuable added compound in almond (Marwede *et al.*, 2004). Moreover, sweet almonds are considered to be as an important source of macro and micro-elements especially potassium, calcium, magnesium and manganese (Souty *et al.* 1971; Saura Calixto and Canellas, 1982; Schirra, 1997).

The aim of this study is the evaluation of the biochemical and mineral compositions of the main almond cultivars in order to find an added value for the local almond genotypes for a better valorisation and consequently a better preservation.

II – Materials and methods

Fruits were harvested at maturity stages from 10 years old almond trees grafted on ‘Garnem’ rootstock and preserved at the national collection of Sidi Bouzid (35.117 N, 9.567 E; 369 m above sea level.). Trees were drip irrigated and conventional technical practices were applied. For each genotype, three replicates of 50 fruits were randomly collected. After cracking, seed coats were removed and kernels were dried at room temperature for 2 days and ground in an electrical grinder. Oil was extracted from 4-5 g of ground almond kernels in a commercial fat extractor (Selecta, Barcelona, Spain) for 2 h with petroleum ether as solvent. The fat content was determined as the difference in weight of the dried kernel sample before and after extraction. The oil sample was utilized to prepare the methyl esters of the corresponding fatty acids (FAME) according to the EU official method (EEC Regulation 2568/91). These methyl esters were separated by use of a flame ionization detector (FID) gas chromatograph. The identification of the FAMEs was achieved by comparison with relative retention times in a reference sample that contained standard methyl esters. The individual tocopherol isomers were analyzed using a reversed phase by high performance liquid chromatography, model 360 (Kontron, Eching, Germany) (Kodad *et al.*, 2006).

For mineral analyses K, Na and Ca were analysed using a Spectrophotometric method while Fe and Cu were analysed by using an Atomic Absorption Spectrophotometer according to Pauwels *et al.*, 1992.

Statistical analysis: The one factor ANOVA and the principal component analysis were done with the software SPSS, 17.0. The mean separation was done with the Duncan test at a probability of 0.05.

III – Results and discussion

The concentrations of most of the mineral elements in the kernel for the fifteen almond genotypes of this study revealed significantly differences (Table 1). Potassium (K) was the most predominant mineral element with values ranging from 595.12 mg/100g for 'Supernova' to 1381.87 mg/100g for the local ecotype 'BF2'. In the second position we found the phosphorus (P) and as for potassium the ecotype 'BF2' has the highest content (768.33 mg/100g) compared to the lowest (367.71 mg/100g) presented by 'KF1'. This last value does not differ significantly with those presented by the introduced cultivars 'Supernova' (396.30 mg/100g), 'Mazzetto' (436.67 mg/100g) and 'Lauranne' (470.83 mg/100g). Values observed for the well known local cultivars 'Achaak'; 'Fekhfekh', 'Ksantini' and 'Zahaaf' for K and P were important but not the highest. In fact, they were respectively 897.56, 1151.22, 1146.34 and 1073.17 mg/100g for K and 478.33, 467.29, 646,04 and 472.29 mg/100g for P. The dominance of these two macroelements (K and P) in almond was also confirmed by previous reports (Prats-Moya *et al.*, 1997; Saura Calixto *et al.*, 1981; Schirra, 1997). Almond kernels of the genotypes studied are also an important source of calcium. Values were 367.67 mg/100g for the ecotype 'TL7', 346.67 mg/100g for 'TL6' and 316.67 mg/100g for 'BF2'. Relatively low values were observed for 'Mazzetto' (191.67 mg/100g), 'Lauranne' (200.00 mg/100g) and 'Supernova' (137.5 mg/100g).

Regarding microelements our results showed a clear superiority of cultivars from the north of Tunisia for iron (Fe). This was clear for the cultivars 'Khoukhi' (133.7 mg/100g) and 'Blanco' (103.3) and for the ecotype 'Dillou' (112.8 mg/100g). For the remaining elements copper (Cu) and zinc (Zn) the performances of the ecotype 'BF2' were also confirmed by the high values of 3.63 mg/100g and 5.52 mg/100g, respectively.

The significant differences in the contents of individual mineral elements can have different origins starting by genetic to ecological, culture (soil, water availability, rootstocks) or climate conditions (Aslantas *et al.*, 2001). Socias *et al.* (2008) showed that genetic variability coefficients for calcium, magnesium and potassium tend to be less significant among years and suggested opportunities for genetic manipulation.

The high content of macro and microelements observed for some ecotypes was clearly confirmed by the principal component analysis (Fig. 1). The two first components explained 60% of the variability observed. The first component was correlated with P, Cu, K and Zn contents, while the second one was correlated with Ca, Fe and Na contents. The presence of the ecotypes 'BF5' on the extreme left and 'TL7', 'TL6' and 'Dillou' on the upper party reflects their potentialities regarding mineral contents. These can be valorised as important dietary source for these essential elements. Also with the actual development of processing of almond these nuts can be used as natural additives and sources of iron, calcium or potassium. Moreover, almond milk is considered as a vegetable milk substitute recommended in cases of intolerance to cow's milk (Cotta Ramusino *et al.*, 1961).

In a second step we were interested in the biochemical analyses of the main Tunisian almond cultivars (Table 2). Although, the local cultivars 'Mahsouna' and 'Faggoussi' showed the highest lipid content (respectively 59.2 and 59.3%) no large range was observed for the ten genotypes concerned by this study. In fact the lowest value was noted for 'Elloumi' but this value does not differ significantly from the contents of the foreigner cultivars: 'Mazzetto' (54.3%), 'Lauranne' (55.5%) and 'Supernova' (55.5%). These values are relatively higher then those reported by Ahrens *et al.* (2005) for the Californian commercial cultivars (from 35 to 54%).

Regarding fatty acid composition we confirmed as reported in the literature (Yada *et al.*, 2011) the dominance of five major fatty acids (FA). They were in decreasing order (Table 2), oleic (18:1) with values ranging from 78.3% for 'Elloumi' to 64% for 'Porto'. The second is linoleic (18:2) with a highest value of 25.9% for 'Porto' followed by palmitic (16:0) with two superior values for 'Elloumi' and 'Ksontini'. These two local cultivars dominate for the stearic (18:0) with a value of 3.3%. The last is the palmitoleic (16:1) and is generally presented as traces (<1%). Our study revealed an exception with the cultivar 'Elloumi' that present a small content (3.9%) of gadoleic (20:1). According to many authors these differences are genetically and independent of growth conditions. Prats-Moya *et al.* (1999) showed that triacylglycerol composition could be used to distinguish among almond genotypes.

Table 1. Kernel mineral composition of the main Tunisian almond cultivar in comparison to some foreigner cultivars

Cultivar	Cu (mg/100 g)	Fe (mg/100 g)	Zn (mg/100 g)	K (mg/100 g)	P (mg/ 100 g)	Ca (mg/100 g)	Na (mg/100 g)
'BF2'	3.63 a	24.97 gh	5.52 a	1381.87 a	768.33 a	316.67 cb	83.33 ab
'Achaak'	1.16 ef	84.53 de	3.90 bc	897.56 cd	478.33 cdef	225.00 efg	76.7 abc
'Fekhfekh'	1.70 cb	28.77 g	4.60 ab	1151.22 b	467.29 def	191.67 fgh	82.5 ab
'Ksantini'	1.49 cdef	72.05 e	3.14 cd	1146.34 b	646.04 ab	291.67 bcd	75.0 abc
'Zahaaf'	1.19 ef	85.50 de	4.14 cb	1073.17 bc	472.29 cdef	175.00 gh	70.0 bcd
'TL7'	1.22 def	86.13 de	2.66 d	790.24 d	404.38 ef	376.67 a	78.3 abc
'KF1'	1.60 bcde	11.05 h	2.62 d	1151.22 b	367.71 f	183.33 gh	68.3 cd
'TL6'	1.97 b	93.97 cd	3.74 bcd	926.83 cd	572.08 bcd	346.67 ab	80.3 abc
'TL8'	1.21 de	86.13 de	4.16 bc	1180.49 b	624.38 abc	160.00 h	79.3 abc
'Blanco'	0.79 f	103.30 bc	4.84 ab	1195.12 b	608.44 bc	255.00 def	51.3 e
'Dillou'	1.63 bde	112.80 b	4.24 bc	1056.91 cb	555.83 bcde	253.33 def	85.0 a
'Khoukhi'	1.96 b	133.70 a	3.81 bcd	1089.43 bc	433.54 def	282.67 cde	77.6 abc
'Mazzetto'	1.66 bcd	34.87 g	4.04 bc	1087.80 bc	436.67 def	191.67 fgh	67.5 cd
'Lauranne'	1.78 bc	52.40 f	4.36 abc	892.68 cd	470.83 cdef	200.00 fgh	58.3 de
'Supernova'	1.10 f	49.15 f	3.31 cd	595.12 e	396.30 ef	137.50 h	77.5 abc

a,b,c,d,e,f,g,h Significant difference at Duncan's multiple Range Test (5%).

Table 2. Oil content, fatty acid and α, γ and δ- tocophérol compositions of the main Tunisian almond cultivars

Cultivar	Oil content (%)	C16:0 (%)	C16:1 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	C20:1 (%)	α-tocophérol (mg/kg)	γ-tocophérol (mg/kg)	δ-tocophérol (mg/kg)
'Zahaaf'	54.0 d	6.8 b	0.6 ab	1.6 e	73.9 bc	16.9 bc	–	344.4 c	5.0 e	.09 e
'Ksontini'	54.3 cd	9.0 a	0.5 cde	3.3 ab	76.2 ab	10.0 d	–	296.8 d	7.0 c	.15 cd
'Mahsouna'	59.2 a	6.6 b	0.6 b	2.7 c	75.9 bc	14.0 bc	–	257.0 e	10.2 b	.57 a
'Faggoussi'	59.3 a	6.2 b	0.5 bc	1.8 de	71.5 c	19.7 b	–	269.5 de	6.1 d	.087 e
'Elloumi'	53.3 d	10.0 a	0.4 e	3.3 a	78.3 a	3.5 e	3.9	208.4 f	3.41 f	.02 f
'Abiodh Sfax'	58.1 ab	6.2 b	0.5 cd	2.8 ab	73.3 bc	16.9 bc	–	277.6 de	5.41 de	.14 de
'Porto'.	57.0 abc	7.1 b	0.5 cd	2.2 cd	64.0 d	25.9 a	–	340.1 c	6.0 d	.19 c
'Mazzetto'	54.3 dc	6.6 b	0.5 cd	2.6 c	73.5 d	16.5 a	–	495.9 a	12.5 a	.37 b
'Lauranne'	55.5 bcd	6.5 b	0.7 a	1.7 de	74.4 abc	16.4 bc	–	289.81 d	3.1 f	.08 e
'Supernova'	55.5 bcd	6.4 b	0.5 de	2.5 bc	74.5 abc	15.9 bc	–	403.6 b	6.9 c	.017 f

a,b,c,d,e,f,g,h Significant difference at Duncan's multiple Range Test (5%).

Finally, it is actually well known that almond kernel is a good source of tocopherols with a domination of α-tocophérol followed by γ and δ isomers (Kodad *et al.* 2006).

In our study (Table 2), the concentration of α-tocophérol ranges from 495.9 mg/kg of oil for 'Mazzetto' to 208.6 mg/kg for 'Elloumi' but many other local cultivars presented high values. In fact we registered also the values of 344.4 mg/kg for 'Zahaaf' and 340.1 mg/kg for 'Porto'. For the other isomers high values of γ tocopherol were obtained for 'Mazzetto' (12.5 mg/kg) and 'Mahsouna' (10.2 mg/kg) while only traces (<1 mg/kg) were observed for the δ tocopherol.

While α-tocophérol is the form of vitamin E efficiently used by the human body and is yet often deficient in modern diet local (Pongracz *et al.*, 1995; Krings and Berger, 2001) genotypes with high content could be used in almond confectioneries, with chocolates or to prepare sweets and syrup.

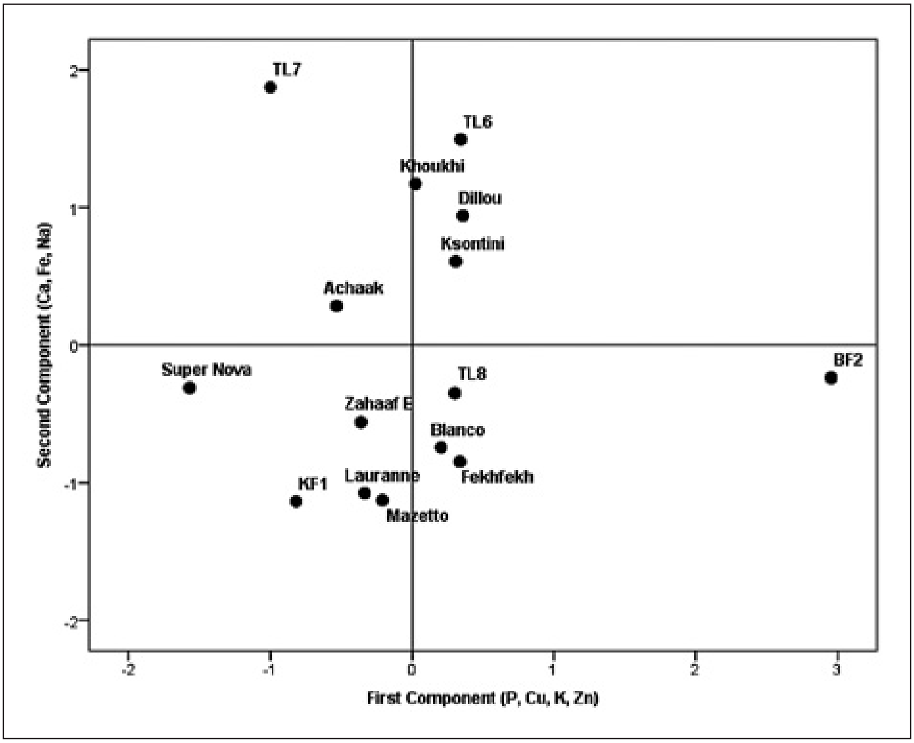

Fig. 1. PCA on the mineral composition of some local almond genotypes.

IV – Conclusions

The biochemical and mineral composition analyses carried out in this study have demonstrated that many local cultivars and unknown ecotypes showed high performances and can be easily valorised and, therefore, preserved from genetic erosion. This fruit consumption either roasted, crude, as natural food additives or as supplements for some deficiencies such as anaemia or bones calcium degradation can incite a national effort to preserve an important heritage in degradation.

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Some physical properties in nut and kernel of two almond varieties (‘Marcona’ and ‘Tuono’) grown in Northern Morocco

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Abstract. Cultivated almonds (*Prunus dulcis* [Mill.] D.A. Webb) are the second fruit culture in importance after olive trees in Morocco. The present work was carried out to evaluate some physical properties in nut and kernel of two almond varieties (‘Marcona’ and ‘Tuono’) widely grown in northern Morocco. 30 fruits were collected in three marked almond trees randomly around the canopy between August, 15 and September, 10 of the 2014 season in three different sites namely Aknoul (Taza), Bni Hadifa (Al Hoceima) and Tahar Souk (Taounate). ANOVA analyses indicated that environmental effect accounted for low variation percentages for most of studied traits except nut length and kernel thickness where it explained half of variation. Genotypic effect was more important in describing variation in the majority of characters. Mean comparison revealed that no significant differences were encountered among sites for nut thickness, spherecity and kernel weight and volume. For the others traits, Tahar Souk showed the lowest values. Between varieties, ‘Marcona’ presented higher values for most of characters except for length and shelling percentage.

Keywords. *Prunus dulcis* – Nuts – Kernels – Physical properties – Northern Morocco.

Quelques caractères physiques de l’amande et l’amandon chez deux variétés d’amandier (‘Marcona’ et ‘Tuono’) cultivées au nord du Maroc

Résumé. *L’amandier (Prunus dulcis [Mill.] D.A. Webb) est le deuxième arbre fruitier le plus cultivé au Maroc après l’olivier. Le présent travail a été mené dans le but d’évaluer quelques propriétés physiques de l’amande et l’amandon chez deux variétés d’amandier (‘Marcona’ et ‘Tuono’) largement cultivées dans le nord du Maroc. 30 fruits ont été collectés sur trois arbres sélectionnés au hasard dans le champ entre le 15 Août et le 10 Septembre 2014 dans trois sites différents à savoir : Aknoul (Taza), Bni Hadifa (Al Hoceima) et Tahar Souk (Taounate). Les analyses de la variance ont indiqué que l’effet du milieu a contribué faiblement à la variabilité de la plupart des caractères étudiés à l’exception de la longueu de l’amande et l’épaisseur de l’amandon, chez lesquels il a expliqué la moitié de la variabilité obtenue. Effet génotypique a contribué fortement dans la variabilité de tous les caractères. La comparaison des moyennes n’a révélé aucune différence significative entre les sites pour l’épaisseur de l’amande et la sphéricité, le poids et le volume de l’amandon. Pour les autres caractères, Tahar Souk a montré les valeurs les plus basses. Entres les variétés, ‘Marcona’ s’est révélée supérieure à ‘Tuono’ pour la majorité des caractères à l’exception de la longueur et du rendement au cassage.*

Mots-clés. Prunus dulcis – *Amandes – Amandons – Caractères physiques – Nord du Maroc.*

I – Introduction

The almond (*Prunus dulcis* [Miller] DA Webb) is a widely grown fruit tree throughout the world. It is a major tree nut grown in areas of Mediterranean climate. In Morocco, almond is the second fruit culture in importance after olive trees in Morocco. It is grown in several regions from north to south, under different environmental conditions, mostly on non-irrigated lands (Kodad *et al*., 2015). The total almond national acreage is about 146.100 ha. Taza-Al Hoceima-Taounate region accounted for more than 37% of total cultivated area and for more than 18% of total production (Ministry of Agriculture, 2013). In this region, almond trees are conducted following the traditional system where one or more environmental requirements are limiting including water during the growing season, soil depth, and nutrient availability (Mahhou and Dennis, 1992). At present, harvesting and handling of almond fruits carried out manually in the region. For optimum performance of threshing, conveying, sorting, storing and other processes of almond nuts and kernels, their physical and mechanical properties must be known (Mirzabe *et al*., 2013). Previous studies on physical traits in almonds were undertaken in Turkey (Kalyoncu, 1990; Aydin, 2003), Iran (Mirzabe *et al*., 2013), Australia (Zheng and Fielke, 2014) and Spain (Valverde *et al*., 2005). Kodad *et al*. (2015) studied physical fruit traits in almond local populations in northern and central Morocco. This work describes a comparative study of some physical properties of nuts and kernels in two almond varieties ('Marcona' and 'Tuono') from three representative sites of Taza-Al Hoceima-Taounate region (northern Morocco).

II – Materials and methods

This study was carried out in three sites of northern Morocco (Fig. 1), namely Aknoul (60 km from Taza, 34°39′0″ N, 3°52′0″ W), Bni Hdifa (50 km from Al Hoceima, 35°1′22″ N, 4°8′27″ W), and Tahar Souk (50 km from Taounate, 35°1′22″ N, 4°8′27″ W). Plant material consisted on two almond varieties ('Marcona' and 'Tuono') widely grown in northern Morocco.

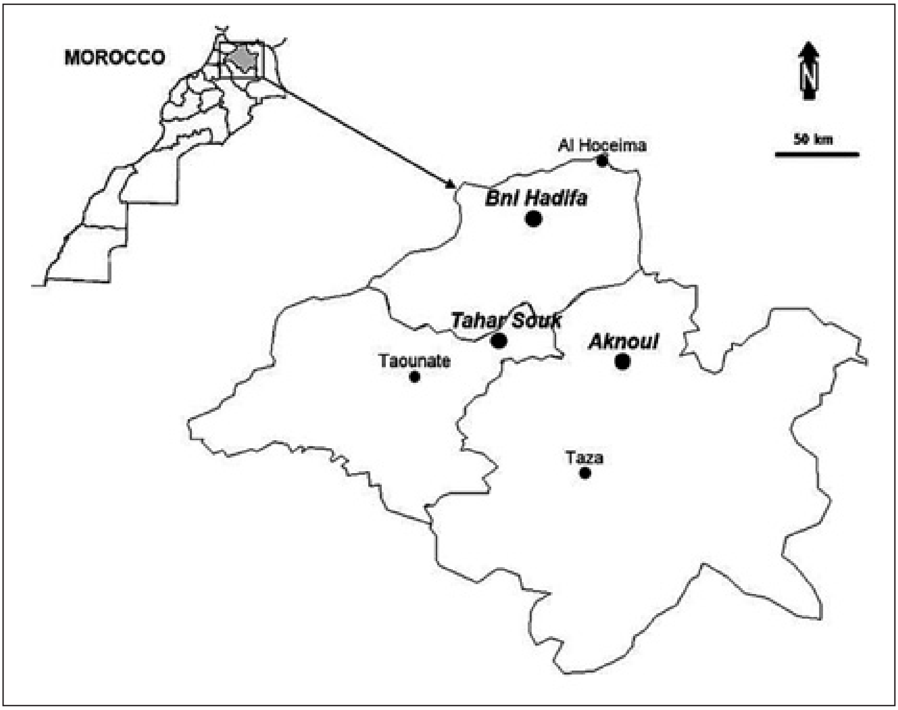

Fig. 1. Geographic localisation of the studied sites (Aknoul, Bni Hadifa and Tahar Souk).

Before harvest, three individual trees were selected in each site between August, 15 and September, 10 of the 2014 season. A sample of 30 fruits was collected randomly from the marked plants at maturity, when fruit mesocarp was fully dried and split along the fruit suture and peduncle abscission was complete.

After harvesting, all nuts were cleaned to remove foreign matter such as soil and stones, as well as immature fruit. For each individual almond nut, three main dimensions (Fig. 2), namely length (L), width (W), and thickness (T) were measured. For measuring principal dimensions of the kernel, almond nut was broken; then length (L), width (W) and thickness (T) of the kernel were measured. For all measurements, a digital caliper with accuracy of 0.01 mm was used. Nut and kernel mass was measured with an electronic balance of 0.001 g sensitivity.

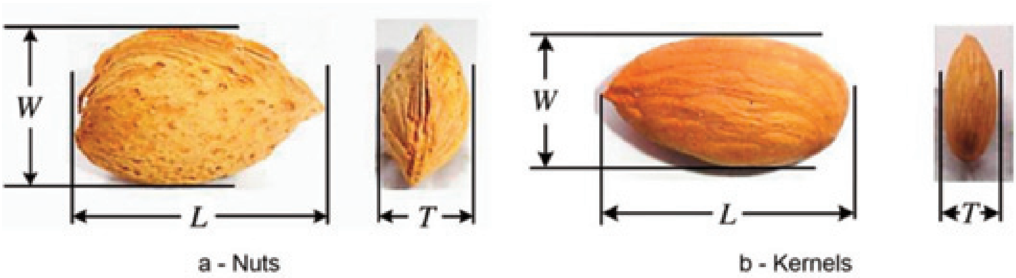

Fig. 2. Almond size parameters: L = length; W = width; T = thickness (Zheng and Fielke, 2014)

The geometric mean diameter (Dg) and sphericity (Φ) were calculated by using the following equations (Mohsenin, 1970): $Dg = (LWT)^{1/3}$ and $\Phi = (LWT)^{1/3} \times 100/L$, respectiveley. The volume (V) were calculated as follow (Mirzabe *et al.*, 2013): $V = (\pi \times Dg^3) / 6$. Combined analyses of variance and Duncan's test were performed for measured and calculated parameters by using the SAS program (SAS, 2004).

III – Results and discussion

Results from the combined analyses of variance for almond nuts (Table 1) showed that site affected at a lesser extent the variability for the majority of characters except for length where it explained the half and it was not significant for some parameters such as thickness and sphericity. In contrast, variety effect was very significant and predominant accounting for more 75% of variability for most characters. Concerning almond kernels (Table 1), the same picture was reflected. In fact, site effect had a lower influence except for thickness and shelling percentage; while variety effect was significant for all traits and explained more than 70% of total variation. Site by variety interaction was of a lower extent and only significant in kernel characters.

Mean comparison among sites (Table 2) revealed that, for almond nuts, Aknoul and Bni Hadifa showed higher values for unit mass (3.88 and 4.40 g, respectively), length (30.61 and 32.04 mm, respectively), width (23.58 and 24.02 mm, respectively), geometric mean diameter (22.06 and 22.85 mm, respectively) and volume (5314 and 6258 mm^3, respectively). For the rest of traits, no significant differences were obtained. For almond kernels, Tahar Souk presented the highest value for thickness (7.38 mm), sphericity (61.61%) and shelling percentage (30.18%) while it had a lower score for length and width. For the remaining traits, no significant differences were encountered.

Mean comparison between varieties (Table 3) demonstrated the superiority of 'Marcona' for the majority of characters in nuts and kernels except for length where 'Tuono' presented higher values (31.33 mm in nut and 22.34 in kernel). 'Tuono' was also superior to 'Marcona' in shelling percentage (28.69%).

Table 2. Mean values of sites for unit mass, length (L), width (W), thickness (T), geometric mean diameter (Dg), sphericity (F), volume (V) and shelling percentage (SP) of almond nuts and kernels. Means for each character followed by the same letter are not significantly different at P < 0.05

Sites	Mass (g)	L (mm)	W (mm)	T (mm)	Dg (mm)	F (%)	Φ (mm^3)	SP (%)
Nuts								
Aknoul	3.88 b	30.61 b	23.58 a	14.96 a	22.06 a	72.13 a	5314 a	
Bni Hadifa	4.40 a	32.04 a	24.02 a	15.60 a	22.85 a	71.70 a	6258 a	
Tahar Souk	3.13 c	28.43 c	20.91 b	15.13 a	20.76 b	73.14 a	4740 b	
Kernels								
Aknoul	0.87 a	21.89 a	14.19 a	5.95 c	12.25 b	55.91 c	984 a	22.39 b
Bni Hadifa	1.01 a	22.34 a	14.21 a	6.87 b	12.93 a	58.45 b	1132 a	23.39 b
Tahar Souk	0.87 a	20.18 b	12.85 b	7.38 a	12.39 ab	61.61 a	1000 a	30.18 a

Table 3. Mean values of varieties for unit mass, length (L), width (W), thickness (T), geometric mean diameter (Dg), sphericity (F), volume (V) and shelling percentage (SP) of almond nuts and kernels. Means for each character followed by the same letter are not significantly different at P < 0.05

Sites	Mass (g)	L (mm)	W (mm)	T (mm)	Dg (mm)	F (%)	Φ (mm^3)	SP (%)
Nuts								
'Marcona'	4.63 a	29.39 b	24.88 a	16.66 a	23.01 a	78.28 a	6412 a	
'Tuono'	2.97 b	31.33 a	20.79 b	13.80 b	20.77 b	66.37 b	4729 b	
Kernels								
'Marcona'	1.01 a	20.60 b	15.28 a	7.16 a	13.09 a	63.68 a	1174 a	21.95 b
'Tuono'	0.83 b	22.34 a	12.22 b	6.31 b	11.96 b	53.64 b	903 b	28.69 a

Our results are in concordance with those obtained by El-Amrani *et al*. (2012) in Al Hoceima region. In fact, most of the studied characters were under genetic dependency. It has been demonstrated that nut mass and dimensions were controlled genetically (Kodad *et al*., 2011). Kernel mass is determined by genetic additive effects (Spiegel-Roy *et al*., 1981), with a heritability of 0.64 (Kester *et al*. 1977). Almond shells are generally characterized by their hardness and shelling percentage. Hard shells can reduce the proportion of nut meats recovered after shelling if adequate equipment is not utilized (Socias i Company *et al*., 2008). In our work, 'Marcona' presented a very hard shell in comparison to 'Tuono'. In a study with local populations of almonds carried out in northern and central Morocco, Kodad *et al*. (2015) reported that all genotypes produced a hard to very hard shells. It has been noticed that with this kernel protection the nuts can be stored for a long time if not exposed to sunlight due to the fact that intact hard shells protect kernels from both insect damage and deterioration from molds. Kernel size is commercially important, as larger sizes are generally better valued (Socias I Company *et al*., 2008). Kernel size depends on kernel mass and in our work it ranged, upon Gülcan classification (Gülcan, 1985), from very small (0.83 g for 'Tuono') to small (1.01 g for 'Marcona'). Kernel mass may be reduced by severe drought conditions (Goldhamer and Viveros, 2000. In the three sites of our study, almonds are grown under no-irrigation and may suffer from late drought resulting in small kernel mass and size. For kernel thickness, and according to Gülcan descriptors, 'Marcona' produced medium kernels while 'Tuono' presented thin kernels. These low values could be explained by the fact that kernel thickness is more dependent on final seed filling, which is more vulnerable to late-season environmental stresses, mainly drought and diseases (Kester and Gradziel, 1996).

IV – Conclusions

It can be concluded that because of narrow distances between the three sites of this study, climatic conditions may not vary for a large degree resulting in a lesser effect on physical properties of almond nuts and kernels in our region. In contrast, the origin of the two studied varieties 'Marcona' (Spain) and 'Tuono' (Italy), which affect strongly their genetic pool, could be the reason for major differences between these two varieties for all studied traits. Furthermore, 'Marcona' was clearly superior for most of parameters confirming its large use in traditional and modern almond systems in Morocco.

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Industrial characteristics of Spanish native almond cultivars and their interest for breeding programs

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Abstract. The displacement of almond orchards towards inland regions of Spain with high risk of spring frost fostered the use of self-compatible late-flowering varieties, adapted to these new areas. This fact is causing a progressive reduction of Spanish native, early flowering and self-incompatible local cultivars, many of them at risk of extinction or already extinct. Native varieties were selected over the years for their adaptation to the warm Mediterranean climate conditions and their high organoleptic quality. In addition, traditional confectionery products are based on particular characteristics of these cultivars. The first results on the industrial aptitude of these native cultivars are reported. It is outstanding the absence of cultivars with a high toasting aptitude and those with a broad shape.

Keywords. Almond – Germplasm – Characterization – Quality.

Caractérisation industrielle des collections espagnoles autochtones d'amandier et son intérêt pour les programmes d'améliorèrent génétique

Résumé. *Le déplacement de l'amandier jusqu'à zones intériences, avec le risque de gelées de printemps, ont favorisé l'utilisation des variétés auto-compatibles et de floraison tardive, plus adaptées a ces zones. C'est la raison de réduction progressive des cultivars autochtones qui sont en risque d'extinction. Ces cultivars ont été sélectionnes au fil des ans par les agriculteurs pour son adaptation au climat méditerranéen chaud et par raison d'une bonne qualité organoleptique. Ils sont un ressource génétique a protéger pour les futures programmes d'améliorement. En plus, l'industrie traditionnelle est bien adaptée aux caractéristiques de ces variétés. Ont présente des résultats sur l'aptitude industrielle des cultivars espagnoles autochtones. Ont peut remarquer l'absence de variétés de bonne aptitude au grillage et aussi des variétés de forma arrondie.*

Mots-clés. *Amandier – Germoplasme – Caractérisation – Qualité.*

I – Introduction

Few decades ago, there were many almond cultivars in Spain (Felipe *et al*., 1984; Egea *et al*., 1985; Rivera *et al*., 1997; Ramos Carmona 1983; Salazar and Melgarejo, 2002; Vargas,, 1975). This was due to the traditional propagation system by seed. Those native cultivars share some characteristics like early blooming, floral self-incompatibility or drought tolerance, and many of them taste better than American cultivars. The displacement of new orchards towards inland regions of Spain with high risk of spring frost fostered the use of self-compatible late-flowering varieties, adapted to these new areas. This fact is causing a progressive reduction of Spanish native early flowering and self-incompatible local cultivars, many of them at risk of extinction or already extinct. The progressive reduction of Spanish native local almond cultivars is a handicap for the traditional industry. In fact, traditional confectionery products are based on particular characteristics of the kernel of these cultivars that are few common in the new almond cultivars. Toasting aptitude, sugar coating performance or round shapes are unusual in many cultivars from ongoing Spanish breeding programs. These aptitudes could be recovered from old native cultivars and used in future crosses.

Industrial aptitude is related to some physical and chemical traits that are responsible for processing problems, like splitting, breakage, browning or irregular coating (Socias i Company *et al*., 2008; Romero *et al*., 2011). The European project SAFENUT (AGRI GEN RES action 068) was focused on the characterization of hazelnut and almond native cultivars. This project succeeded on recovering some cultivars from Greece, Slovenia and Italy (Socias i Company *et al*., 2011). Regarding Spain, this project focused only in a small set of cultivars from the Reference Germplasm Bank from CITA-Zaragoza which is not including many of the most relevant native Spanish cultivars, due to the inland placement of this collection. The aim of this work was the evaluation of physical traits and industrial aptitude of Spanish native almond cultivars from collections of CEBAS-CSIC of Murcia and IRTA of Constantí.

II – Materials and methods

Almond genotypes studied included 44 early flowering and self-incompatible native cultivars from CEBAS-CSIC Murcia and IRTA-Constantí cultivar collections (Table 1). Almond samples were taken in 2013 and 2014 harvests.

Physical parameters were: kernel weight, weight loses after skin removal, dimensions, shape, kernel strength and elasticity (INSTRON texture analyzer using 34 mm penetration probe at 1 mm. sec^{-1} rate), raw kernel taste, kernel defects and skin color (MINOLTA spectrophotometer Model M3500, using CIELAB color space). These parameters were used to score industrial aptitude by using IRTA's predictive models (not published). Industrial aptitudes were: raw almonds, blanched, sugar coated, toasted, slices and thin tablet products. Mean values and least significant differences were computed by using SAS/STAT software (r.6.03, Cary).

III – Results and discussion

Physical traits for each almond cultivar are summarized in Table 1. Industrial aptitudes, derived from physical traits, are described in Table 2. Prevailing Kernel shape was elongated. Only few cultivars are broad in shape (width-to-length ratio higher than 0.75), as 'Marcona' and it's group, 'Verd', 'Carreró' and 'Nano'. 'Marcona San Joy' is clearly different from Marcona's group. Almond skin aspect (light color and smooth surface) is adequate for raw consumption only in 'Atocha', 'Malagueña' and 'Nano', while most of the cultivars are less valued for this purpose. Regarding blanching aptitude, this is good for almost all the cultivars, except for 'Gabaix' and 'Asperilla' that show weight loss after blanching higher than 11% (Fig. 1).

Kernel strength range is quite broad, from 70 to 170 N. This parameter is highly correlated to splitting trend that is a negative attribute for hull kernel manipulation (Romero *et al*., 2011). 'Fournat', 'Marcona', 'Tio Martín', 'Garrigues' and 'Planeta Roja' can be considered as good cultivars for industrial manipulation, with less than 20% of splits on average. Regarding sugar coating aptitude, this is related to kernel integrity and smooth surface, that are higher in 'Atascada', 'Bonita', 'Fina del Alto', 'Fournat', Marcona's group, 'Planeta Roja', 'Rumbeta' and 'Verruga' (although this is too small for this purpose).

Toasting aptitude is only high in 'Desmayo Largueta' and its group. This is particularly relevant, since new almond releases from breeding programs are also few adapted to this purpose (Romero *et al*., 2011).

Table 1. Physical traits of Spanish native almond cultivars (years 2013 and 2014)

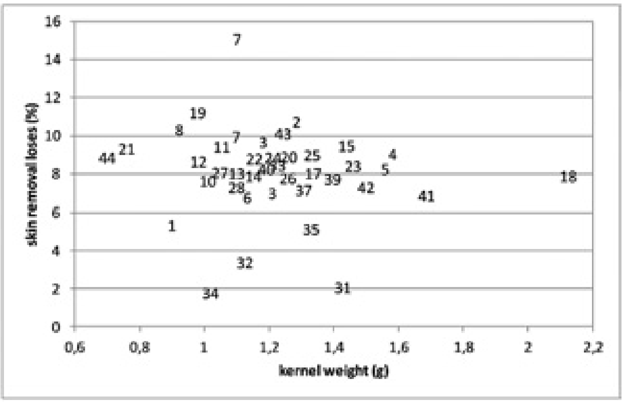
Cultivar	Length (mm)	Width (mm)	Thick (mm)	Width/ length	Creasing??? (%)	Toasting (%[c])	Skin color			Sweet
							L*	a*	b*	
1 Angones[a]	19.7	11.2	7.6	0.57	0.0	30.0	26.3	10.5	16.8	sweet
2 Asperilla[a]	24.1	13.0	7.1	0.54	17.0	30.0	26.0	9.1	16.3	sweet
3 Atascada[b]	24.6	12.9	8.3	0.52	81.3	6.7	29.0	10.3	17.6	slight
4 Atocha[ab]	27.8	13.6	8.2	0.49	5.5	2.5	32.9	10.1	19.6	slight
5 Avellanera[b]	25.9	15.3	8.2	0.59	0.0	5.0	26.6	9.0	15.0	sweet
6 Bonita[b]	22.3	12.7	7.8	0.57	0.0	0.0	30.2	9.4	18.5	sweet
7 Carreró[a]	19.1	14.3	6.8	0.75	27.8	5.0	25.1	7.9	13.6	sweet
8 Carretas[b]	21.4	11.9	8.5	0.56	0.0	10.0	25.7	8.1	13.6	sweet
9 Carriset[a]	–	–	–	–	–	0.0	29.7	9.8	19.6	–
10 CEBAS-1[b]	22.9	11.2	8.0	0.49	3.8	15.0	25.3	9.6	15.0	sweet
11 Colorada[b]	22.9	12.7	8.0	0.55	5.0	45.0	29.0	10.2	16.7	sweet
12 Del Cid[b]	20.3	13.5	7.9	0.66	10.0	30.0	27.1	9.2	14.9	sweet
13 DesmayoAD[b]	24.3	13.3	7.9	0.55	0.0	95.0	29.4	9.8	17.1	sweet
14 Desmayo Lorca[b]	23.6	13.6	8.0	0.58	0.0	100.0	27.8	9.4	15.0	sweet
15 Desmayo Largueta[ab]	25.2	14.0	8.3	0.55	0.0	90.0	26.3	9.0	14.4	sweet
16 Esperança Forta[a]	21.1	13.6	10.5	0.65	0.0	25.0	26.6	10.5	16.8	sweet
17 Fina del Alto[b]	23.9	13.8	8.4	0.57	7.5	5.0	28.6	10.5	17.2	sweet
18 Fournat[b]	30.4	17.6	8.7	0.58	27.9	40.0	27.7	11.0	16.0	sweet
19 Gabaix[a]	21.1	11.6	7.5	0.55	21.3	0.0	28.9	10.2	19.3	sweet
20 Garrigues[ab]	24.3	14.5	7.4	0.60	3.2	15.0	27.7	9.8	15.5	slight
21 J.Salazar[b]	17.9	12.2	7.3	0.68	5.0	5.0	29.8	9.5	18.2	slight
22 Jordi[b]	23.1	12.5	8.8	0.54	0.0	20.0	29.3	12.0	19.6	sweet
23 La Mona[b]	24.0	15.5	9.0	0.64	30.5	0.0	31.6	10.3	20.0	slight
24 Malagueña[b]	27.9	11.4	8.3	0.41	26.4	0.0	30.7	8.8	17.0	sweet
25 Marcona[ab]	19.7	15.5	9.0	0.79	0.0	2.5	29.0	9.1	15.7	sweet
26 Marcona AD[b]	20.3	15.2	9.2	0.75	0.0	5.0	27.7	9.1	14.9	sweet
27 Marcona Flota[b]	18.9	14.2	8.5	0.75	2.1	5.0	25.8	8.8	12.9	slight
28 Marcona San Joy[b]	20.7	13.6	8.9	0.65	0.0	20.0	28.8	9.8	17.4	sweet
29 Marquet[a]	22.6	15.6	10.7	0.69	0.0	0.0	26.4	9.7	14.9	–
30 Mena d'en Musté[a]	24.3	14.4	9.5	0.59	0.0	30.0	30.8	11.3	20.0	–
31 Mollar de Tarragona[a]	22.0	13.8	8.9	0.63	39.2	5.0	27.6	9.0	14.3	slight
32 Nano[a]	18.5	13.9	7.7	0.75	0.0	0.0	32.0	9.6	19.8	sweet
33 Pajarera[b]	22.8	16.2	7.9	0.71	96.6	0.0	31.3	9.1	18.1	slight
34 Pauet[a]	22.6	12.9	6.9	0.57	13.3	50.0	27.9	10.1	17.4	slight
35 Pep Juneda[a]	24.2	13.3	7.6	0.55	0.0	60.0	27.2	11.5	17.9	sweet
36 Peraleja[b]	25.7	12.2	7.7	0.47	24.6	0.0	28.1	10.8	18.1	sweet
37 Planeta Fina[b]	24.5	15.5	7.4	0.63	5.0	0.0	30.9	10.5	18.2	sweet
38 Planeta Roja[b]	24.5	14.5	7.4	0.59	0.0	0.0	30.7	10.4	18.1	sweet
39 Ramillete[ab]	28.1	13.2	7.3	0.47	4.7	12.5	28.2	9.5	16.0	sweet
40 Rof[a]	20.9	14.9	8.4	0.71	100.0	0.0	29.3	9.8	18.4	slight
41 Rumbeta[b]	26.9	16.1	7.9	0.60	30.7	0.0	33.7	10.3	21.8	slight
42 Tío Martín[b]	24.8	14.5	9.3	0.58	100.0	5.0	27.0	9.0	15.0	sweet
43 Verd[a]	18.8	14.9	7.8	0.79	29.5	5.0	23.6	9.5	14.2	slight
44 Verruga[b]	18.5	10.9	7.6	0.59	0.0	0.0	26.3	10.4	15.9	sweet
LSD[d]	5.03	2.74	1.86	0.009	17.5	12.0	4.54	2.10	5.59	–
Significance level	0.041	0.061	NS	0.007	<.0001	0.0005	0.001	0.006	0.010	–

[a]: IRTA's collection; [b]: CEBAS-MURCIA collection; [c]: full peeled % after toasting; [d]: least significant difference.

Table 2. Industrial aptitude of Spanish native almond cultivars (years 2013 and 2014)

Cultivar	Raw almonds	Blanched almonds	Sugar coating	Toasted almonds	Sliced almonds	Thin tablets
1 Angones[a]	low	mean	mean	mean	mean	high
2 Asperilla[a]	low	mean	mean	mean	low	mean
3 Atascada[b]	low	low	high	low	low	mean
4 Atocha[ab]	high	mean	mean	low	mean	low
5 Avellanera[b]	low	mean	mean	low	low	mean
6 Bonita[b]	low	high	high	low	high	high
7 Carreró[a]	low	mean	mean	low	low	high
8 Carretas[b]	low	mean	low	low	mean	high
9 Carriset[a]	low	–	–	low	–	–
10 CEBAS-1[b]	low	low	high	low	high	high
11 Colorada[b]	low	mean	mean	mean	mean	mean
12 Del Cid[b]	low	mean	mean	mean	low	high
13 DesmayoAD[b]	low	mean	mean	high	mean	mean
14 Desmayo Lorca[b]	low	mean	mean	high	mean	mean
15 Desmayo Largueta[ab]	low	mean	mean	high	mean	mean
16 Esperança Forta[a]	low	–	–	mean	low	–
17 Fina del Alto[b]	low	high	high	low	low	mean
18 Fournat[b]	low	high	high	mean	low	low
19 Gabaix[a]	low	mean	low	low	low	high
20 Garrigues[ab]	low	low	mean	low	low	mean
21 J.Salazar[b]	low	mean	low	low	low	high
22 Jordi[b]	low	low	low	low	mean	mean
23 La Mona[b]	mean	mean	mean	low	low	mean
24 Malagueña[b]	high	low	low	low	low	low
25 Marcona[ab]	low	high	high	low	mean	high
26 Marcona AD[b]	low	high	high	low	mean	high
27 Marcona Flota[b]	low	high	high	low	mean	high
28 Marcona San Joy[b]	low	high	high	low	mean	high
29 Marquet[a]	low	low	low	low	low	low
30 Mena d'en Musté[a]	mean	–	–	mean	–	–
31 Mollar de Tarragona[a]	low	low	low	low	low	mean
32 Nano[a]	high	mean	mean	low	mean	mean
33 Pajarera[b]	mean	mean	mean	mean	low	mean
34 Pauet[a]	low	low	mean	mean	low	high
35 Pep Juneda[a]	low	mean	mean	low	mean	mean
36 Peraleja[b]	low	mean	mean	low	low	mean
37 Planeta Fina[b]	mean	high	mean	low	low	mean
38 Planeta Roja[b]	mean	low	high	low	low	mean
39 Ramillete[ab]	low	mean	low	low	low	low
40 Rof[a]	low	low	low	low	low	low
41 Rumbeta[b]	mean	high	high	low	low	low
42 Tío Martín[b]	low	low	mean	low	low	mean
43 Verd[a]	low	mean	low	low	low	mean
44 Verruga[b]	low	mean	high	low	mean	high

[a]: IRTA's collection; [b]: CEBAS-MURCIA collection.



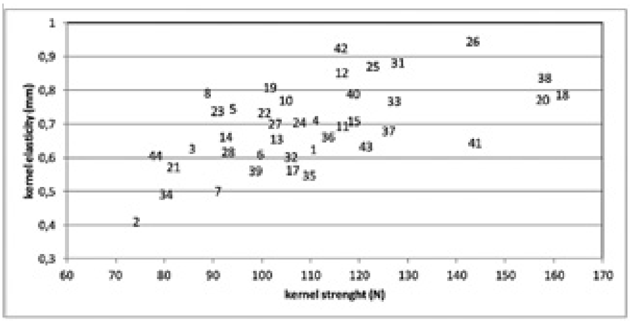


Fig. 1. Left: Kernel weight and loses after skin removing. Right: Textural properties of the almond cultivars (coded by numbers from table 1).

IV – Conclusions

Results showed the wide diversity of this almond native germplasm for industrial purposes. Some cultivars have interesting industrial traits that can be included in future breeding programs. Interestingly, none of the studied cultivars showed a kernel shape wider than 'Marcona' and only 'Desmayo Largueta' shows a high aptitude for toasting. This means that confectionery industry will demand these characteristics in future releases from future breeding programs, in order to reduce dependence on such traditional cultivars. More studies are required on this set of native cultivars, including nutritional characteristics that are related to complementary industrial properties.

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Influence of region and variety on fatty acid and tocopherol concentration of almond

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Abstract. This paper describes an investigation into the fatty acid and tocopherol concentration of several varieties of almonds grown in different almond-producing regions of Australia, as well as almonds grown in Spain and California. Considerable variation was observed not only in linoleic acid (15.7% – 29.9% of total lipids) and Vitamin E (8.2 mg – 21.5 mg/100 g) content, but also lipid (46.1 – 63.5 g/100 g), oleic acid (58.5% – 71.3% of total lipids), palmitic acid (5.9% – 7.5% of total lipids) and stearic acid (1.0% – 2.4% of total lipids) content. Understanding the influence of region and variety on almond composition will enable industry to make informed decisions with regards to almond breeding programs and the suitability of almond cultivars to particular growing regions.

Keywords. Almond – Fatty acid – Linoleic acid – Lipophilic antioxidant – Vitamin E – Tocopherol.

Influence de la région et de la variété sur la concentration en acides gras et tocophérol des amandiers

Résumé. *Ce document décrit une enquête sur la concentration en acides gras et tocophérol pour plusieurs variétés d'amandiers cultivés dans différentes régions productrices d'amandes de l'Australie, ainsi que pour les amandiers cultivés en Espagne et en Californie. Une variation considérable a été observée non seulement pour la teneur en acide linoléique (15,7% – 29,9% des lipides totaux) et en vitamine E (8,2 mg – 21,5 mg/100 g), mais aussi en lipides (46,1 – 63,5 g/100 g), acide oléique (58,5% – 71,3% des lipides totaux), acide palmitique (5,9% – 7,5% des lipides totaux) et acide stéarique (1,0% – 2,4% des lipides totaux). La compréhension de l'influence de la région et de la variété sur la composition des amandes permettra à l'industrie de prendre des décisions éclairées en ce qui concerne les programmes d'amélioration de l'amandier et l'adéquation des cultivars d'amandier à des régions particulières de culture.*

Mots-clés. *Amandier – Acides gras – Acide linoléique – Antioxydant lipophile – Vitamine E – Tocophérol.*

I – Introduction

Almonds are a nutrient-rich source of lipids, protein, dietary fibre, minerals, vitamins and polyphenols (Esfahlan *et al*., 2010). The health benefits afforded by almonds have been largely attributed to lipids and vitamins, with numerous clinical and pre-clinical trials and epidemiologic studies showing that regular consumption of almonds can: significantly reduce low density lipoprotein (LDL), cholesterol, postprandial glycaemia and insulinaemia; improve body weight control; and reduce the risk of obesity-related diseases such as coronary heart disease (CHD) and type II diabetes (Jenkins *et al*., 2008, Rajaram *et al*., 2010, Wien *et al*., 2010, Damasceno *et al*., 2011). All of these health benefits have been largely attributed to high proportions of the unsaturated fatty acids (USFA) oleic acid, linoleic acid and Vitamin E.

Linoleic acid, an omega-6 fatty acid, is an essential fatty acid for humans and is involved in children's growth and the prevention of cardiovascular disease (Jenskin *et al*., 2008, Wien *et al*., 2010). However, high levels of linoleic acid have been considered as markers of almond spoilage, since linoleic acid's double bonds are susceptible to oxidation, thus, high levels of oleic acid and low levels of linoleic acid have been associated with prolonged shelf-life of almonds and are often advocated (Zaplin *et al*., 2013). However, another property in lipids, Vitamin E, can improve almond shelf-life (Socias i Company *et al*., 2010). Of the various tree nuts, almonds typically contain the most Vitamin E; with two handfuls of almonds providing the average daily-recommended dose of Vitamin E, being 15 mg per day (Institute of Medicine, 2000). Previous studies have shown almond Vitamin E largely comprises α-tocopherol and γ-tocopherol, with lesser amounts of β-tocopherol, δ-tocopherol and α-tocotrienol also present; the relative proportions of which are thought to be influenced by genotype and region of origin (Yildirim *et al*., 2010; Kodad *et al*., 2011a).

Very little is known about the Vitamin E and fatty acid composition of almonds grown in different Australian regions. This study aimed to investigate the influence of environmental conditions (i.e. regionality) and genetics (i.e. variety) on almond fatty acids in particular linoleic acid and Vitamin E. Improved knowledge of the regional and/or varietal differences in almond composition may enable industry to make more informed decisions with respect to varietal selection.

II – Materials and methods

Australian almond samples were collected from Willunga, Adelaide, Riverland (South Australia) and Sunraysia (Victoria) during the 2012 season. Spanish almonds were sourced from Zaragoza and North American almonds were sourced from Merced, (California). The cultivars selected were: 'Nonpareil' (from Sunraysia, Adelaide, Willunga, Riverland, Zaragoza and Merced), 'Johnston' (from Adelaide and Willunga), 'Somerton' (Willunga), 'Carmel' (Riverland and Merced) and 'Guara' (Riverland and Zaragoza). Samples (100 kernels randomly harvested per tree per cultivar) were harvested when the mesocarps had naturally split, indicating ripening. For each cultivar, 25 trees from growers' orchard were harvested. Kernels were dried at 50°C for 48 hours and the moisture content (approximately 2%) confirmed by the gravimetric technique. Dried kernels were ground using a coffee grinder to a fine powder, sieved through a 1000 μM mesh and then stored under nitrogen prior to analysis.

Lipid extraction and fatty acid determinations were performed (in triplicate) using chloroform-methanol extraction and methanol-sulphuric acid FAME formation (fatty acid methylation), based on methodology reported by Makrides and colleagues (Makrides *et al*., 1996). Fatty acid composition was determined using an HP 6890 Gas Chromatograph (Hewlett Packard, Palo Alto, CA. USA) equipped with a flame ionization detector (FID), split/splitless injection, HP 7683 autosampler and HP Chemstation. A capillary GC column SGE BPX 70 (50 m, 0.32 mm ID, 0.25 μm) was used (SGE Analytical Science Pty. Ltd. RingWood, VIC. Australia). Helium was the carrier gas and the split-ratio was 20:1, the injector temperature was set at 250°C and the detector temperature at 300°C, the initial oven temperature was 140°C increasing to 220°C at 5°C/min, and then held at this temperature for 3 min. FAMEs were identified based on the retention time of the internal standard free fatty acid C17.

Tocol extraction was based on the alkaline saponification and hexane extraction method used previously for cereals and nuts (Xu, 2002). HPLC analysis protocol referred to Lampi and colleagues (Lamp *et al*., 2008; Lampi, 2011), specifically, the isocratic mobile phase was hexane (with 2% 1,4-dioxane), flow rate 1.0 mL/min, injection volume 20 μL, column temperature 25°C. HPLC analysis was performed using an Agilent 1200 HPLC (Agilent Technologies, Deutschland, Germany) coupled with a diode array detector (DAD), fluorescence detector (FLD), autosampler, quaternary pump, Agilent Chemstation and Grace Alltime HP silica column (150 mm, 3 mm, 3 μm; Grace Discovery Sciences, Deerfield, IL. USA). α, β, γ, δ-Tocopherol and α-tocotrienol were quantified using calibration curves prepared from external standards. α-Tocopherol was measured by DAD at a signal wavelength of 292 nm, while β, γ, δ-tocopherol and α-tocotrienol were analysed by FLD at signal wavelengths of 292 nm (excitation) and 325 nm (emission).

Chemical data were analysed by ANOVA using GenStat (14th Edition, VSN International Limited, Herts, UK) and GraphPad Prism 5 (Version 5.01 GraphPad Software, Inc. La Jolla, CA, USA). Mean comparisons were performed by least significant difference (LSD) multiple-comparison test at $p < 0.05$. Pearson's co-efficient was used for correlation analysis.

III – Results and discussion

1. Influence of region and genotype on almond lipid content

The lipid content of the almonds studied ranged from 46.1 to 63.5 g/100 g (Table 1). The lowest lipid content (46.1 g/100 g) was observed in 'Carmel' almonds from California, while 'Nonpareil' almonds from Willunga (Australia) contained the highest lipid level (63.5 g/100 g). The lipid concentrations of Australian grown almond samples were 53.1 to 63.5 g/ 100 g. This was considered representative of the major commercial varieties grown in the four key regions of Australia. In this study environment appeared to have a significant effect on the lipid content of 'Nonpareil', 'Carmel' and 'Guara' almonds, with the northern hemisphere grown almonds containing lower lipid levels (between 46.1-51% of kernel weight) than almonds grown in the southern hemisphere (between 53.7-63.5% of kernel weight) (Table 1). For example, Californian 'Nonpareil' lipid content (47.1 g/100 g) was lower than the levels reported in an earlier study (Sathe *et al*., 2008), but similar with a recent study (Yada *et al.*, 2013). However, 'Somerton' and 'Johnston' varieties showed no compositional differences between sites (Table 1). In contrast, genotype had a greater influence on almond lipid content. In the Riverland, lipid content varied significantly between genotypes ($P < 0.05$).

2. Influence of region and genotype on almond fatty acid composition

Unsaturated fatty acids comprised oleic acid (58.1-71.3% of total lipids), linoleic acid (15.7-29.9% of total lipids), palmitoleic acid (0.20-0.62% of total lipids) and vaccenic acid (0.77-2.17% of total lipids), which made up more than 90% of the total lipids. Saturated fatty acids, including palmitic acid (5.9-7.5% of total lipids), stearic acid (1.0-2.4% of total lipids), arachidic acid (0.07-0.10% of total lipids) and myristic acid (0.02-0.05% of total lipids, data not shown in the table) accounted for the remaining 10%. These results demonstrate the influence of environment and genotype on almond fatty acid composition (Table 1). Oleic acid and linoleic acid were the most abundant fatty acids, in agreement with previous studies on almonds grown around the world, i.e. in Turkey, Iran, Spain, Italy, China, India, California (Kodad *et al*., 2010; Moayedi *et al*., 2011; Tian *et al*., 2011). A negative correlation was observed between oleic acid and linoleic acid concentrations (Pearson r -0.993, p value (two tails) < 0.0001) as in other studies (Sathe *et al*., 2008; Kodad *et al*., 2010; Kodad *et al*., 2011b,). No correlation was observed between any lipid fractions and the total lipid content not even with the major fatty acid, oleic acid, which was also consistent with previous reports (Sathe *et al*., 2008; Kodad *et al*., 2011b).

Significant differences were observed in the oleic acid levels of almonds harvested from different genotypes and from different regions. The highest oleic acid level (68.1% of total lipids) was observed in 'Nonpareil' almonds from Spain, followed by 'Nonpareil' almonds (65.6% of total lipids) from California (Table 1). 'Nonpareil' almonds produced in Australia generally contained lower oleic acid levels, albeit 'Nonpareil' almonds from Adelaide plains were similar to almonds from California, with 64.2% of total lipids. Likewise, 'Guara' almonds from Spain contained oleic acid levels greater than 'Guara' almonds from Australian Riverland, but the oleic acid content of 'Carmel' almonds showed no significant difference when grown in California or Australia (Table 1). On the other hand, genotype had a significant impact on oleic acid levels for almonds grown at the same sites; i.e. in Willunga, 'Somerton' almonds had the highest oleic acid levels, followed by 'Johnston' and 'Nonpareil' almonds (Table 1). In Adelaide Plains, the local variety 'Johnston' yielded almonds that

Table 1. Lipid and fatty acid compositions of almonds from different genotypes

Sample		Concentration†							
Genotype	Region	Lipids (g/100g)	Palmitic (C16:0)	Palmitoleic (C16:1n-7)	Stearic (C18:0)	Vaccenic (C18:1n-7)	Oleic (C18:1n-9)	Linoleic (C18:2n-6)	Ratio O/L
'Nonpareil'	Willunga	63.5 ± 0.7 a	6.8 ± 0.0 c	0.41 ± 0.01 d	1.4 ± 0.1 a	1.2 ± 0.0 d	58.5 ± 0.4 d	29.9 ± 0.6 a	1.96 ± 0.04 d
	Adelaide	60.0 ± 1.1 ab	6.9 ± 0.1 bc	0.49 ± 0.01 c	1.4 ± 0.0 a	1.3 ± 0.0 c	64.2 ± 0.4 bc	23.9 ± 0.5 c	2.69 ± 0.04 c
	Riverland	56.5 ± 2.8 b	6.7 ± 0.1 c	0.45 ± 0.02 d	1.3 ± 0.0 b	1.3 ± 0.0 c	63.6 ± 0.7 c	24.8 ± 0.9 b	2.57 ± 0.08 c
	Sunraysia	57.7 ± 1.3 ab	6.9 ± 0.0 b	0.52 ± 0.01 b	1.1 ± 0.0 c	1.5 ± 0.0 b	63.8 ± 0.3 c	24.4 ± 0.4 bc	2.61 ± 0.03 c
	Spain	51.0 ± 2.0 bc	7.5 ± 0.0 a	0.62 ± 0.00 a	1.4 ± 0.0 ab	2.2 ± 0.0 a	68.1 ± 0.1 a	19.0 ± 0.0 e	3.58 ± 0.00 a
	California	47.1 ± 3.2 c	6.6 ± 0.0 c	0.48 ± 0.01 cd	1.2 ± 0.0 b	1.5 ± 0.0 b	65.6 ± 0.1 b	22.6 ± 0.0 d	2.90 ± 0.01 b
	P	*0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
'Somerton'	Willunga	60.5 ± 1.5	6.1 ± 0.0 a	0.41 ± 0.00	2.4 ± 0.0 a	1.0 ± 0.0 b	67.0 ± 0.1 a	21.4 ± 0.2 a	3.13 ± 0.02 b
	Riverland	59.4 ± 0.8	5.9 ± 0.0 b	0.40 ± 0.00	1.7 ± 0.0 b	1.2 ± 0.0 a	70.1 ± 0.2 a	18.9 ± 0.2 b	3.71 ± 0.03 a
	P	*ns*	*< 0.01*	*ns*	*< 0.001*	*< 0.001*	*ns*	*< 0.001*	*< 0.001*
'Johnston'	Willunga	60.8 ± 0.8	6.9 ± 0.0	0.44 ± 0.00 b	2.0 ± 0.0 a	1.1 ± 0.0 b	63.0 ± 0.1 b	25.0 ± 0.1 a	2.52 ± 0.01 b
	Adelaide	58.2 ± 0.7	6.7 ± 0.1	0.56 ± 0.01 a	1.8 ± 0.1 a	1.3 ± 0.0 a	67.0 ± 0.4 a	21.0 ± 0.6 b	3.19 ± 0.07 a
	P	*ns*	*ns*	*< 0.001*	*< 0.05*	*< 0.001*	*< 0.001*	*< 0.001*	*< 0.001*
'Carmel'	Riverland	53.7 ± 1.0 a	6.8 ± 0.0 b	0.39 ± 0.01 b	1.2 ± 0.0	1.2 ± 0.0 b	58.6 ± 0.4	29.8 ± 0.5	1.96 ± 0.03
	California	46.1 ± 1.5 b	7.2 ± 0.0 a	0.44 ± 0.00 a	1.2 ± 0.0	1.4 ± 0.0 a	58.1 ± 0.1	29.7 ± 0.1	1.96 ± 0.00
	P	< 0.05	< 0.001	< 0.05	ns	< 0.001	ns	ns	ns
'Guara'	Riverland	60.4 ± 2.0 a	5.9 ± 0.0 b	0.23 ± 0.00 b	2.4 ± 0.0 a	0.8 ± 0.0 b	66.4 ± 0.0 b	22.3 ± 0.1 a	2.97 ± 0.01 b
	Spain	50.7 ± 2.1 b	6.9 ± 0.0 a	0.45 ± 0.00 a	2.3 ± 0.0 b	1.2 ± 0.0 a	71.3 ± 0.1 a	15.7 ± 0.0 b	4.53 ± 0.01 a
	P	< 0.05	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Values are means of three replicates ± standard error. Values followed by different letters within a column are significantly different ($P < 0.05$); ns = not significant.
† Fatty acid content as a percentage of lipids.

also contained more oleic acid than 'Nonpareil' almonds. In the Riverland, 'Somerton' almonds had the highest oleic acid levels (Table 1). The differentiation of oleic acid and linoleic acid concentrations led to variation in the O/L ratio (oleic/linoleic) for almonds of different varieties and from different regions. We found that the O/L ratio largely depended on linoleic acid rather than oleic acid levels, due to a highly negative relationship between the O/L ratio and linoleic acid (Pearson r 0.9984, 95% CI 0.9974-0.9991, *p* value (two tailed) <0.0001) which is greater than the positive relationship between the O/L ratio and oleic acid (Pearson r 0.9892, 95% CI 0.9819-0.9936, *p* value (two tailed) <0.0001). The O/L ratio typically represents almond kernel shelf-life: with higher O/L ratio indicating greater storage capacity (Piscopo *et al*., 2010; Kodad *et al*., 2011b). However, a higher O/L ratio means a lower proportion of linoleic acid, i.e. the healthiest fatty acid present in almond lipids. Therefore, consideration of high O/L ratio for long storage and high linoleic acid for more nutrition has opened space to further research.

Linoleic acid concentrations were significantly affected by both environment and genotype (Table 1). Willunga grown almonds contained more linoleic acid than almonds from other sites. For example, 'Nonpareil' almonds grown in Willunga contained significantly higher linoleic acid levels than 'Nonpareil' almonds grown in the Riverland, Sunraysia and Adelaide plains, by 17%, 19% and 20%, respectively. Similarly, 'Somerton' and 'Johnston' almonds grown in Willunga contained 12% and 16% more linoleic acid than Riverland and Adelaide plains almonds, respectively. Considering the environmental influence, solar radiation and temperature (data not shown) varied between sites during the 2012 growing season. However, differences observed in rainfall would likely be negated by irrigation. Willunga experienced less solar radiation than the other sites, which may lead to higher linoleic acid content. Willunga also experienced comparatively lower temperatures than the other sites, which could influence linoleic acid concentrations, i.e. high temperatures have been shown to negatively impact linoleic acid synthesis in sunflower seeds (Harris *et al*., 1978).

Australian grown almonds also had higher linoleic acid levels between 18.1-29.9% than Spanish almonds (Table 1). According to the literature, Spanish almonds contain linoleic acid levels ranging from 12.6-27.1% while Mediterranean almonds contained between 12.9-25.9% linoleic acid (Kodad *et al*., 2011b). Californian almonds were reported to contain 21.5-31.1% linoleic acid (Sathe *et al*., 2008). Noticeably, the regions producing almonds with lower linoleic acid are not irrigated, whereas Californian and Australian regions routinely apply irrigation to their orchards. Irrigation could therefore be a reason for linoleic acid differentiation. Nanos and colleagues found irrigation resulted in lower linoleic acid in Texas almond variety, but not in 'Ferragnès' almond variety (Nanos *et al*., 2002). Apparently, environmental factors such as irrigation, as well as genotype, can influence almond linoleic acid levels.

3. Influence of variety/region on almond tocols

Almond storage time not only depends on the O/L ratio, but also the relative concentration of Vitamin E compared to almond lipids; since higher Vitamin E content can improve lipid resistance to rancidity (Socias i Company *et al*., 2010). Therefore, breeding programs aim to improve the O/L ratio and Vitamin E content of almonds. As such, the Vitamin E content of different varieties and selections grown in particular regions was investigated. Vitamin E comprises eight tocopherol homologues: α, β, γ and δ-tocopherol and α, β, γ and δ-tocotrienol. In this study, four homologues α-tocopherol, β-tocopherol, γ-tocopherol and α-tocotrienol, were quantified in each of the different samples (Table 2). α-Tocopherol was the major component and accounted for more than 90% of the total tocols, followed by γ-tocopherol, in agreement with previous studies (Matthäus and Özcan, 2009; Kodad *et al*., 2011a). β-Tocopherol was the third most abundant tocol in Australian grown almonds, but not in Spanish or Californian almonds, which instead, contained higher concentrations of α-tocotrienol. δ-Tocopherol was only observed in trace amounts, as reported elsewhere (Kornsteiner *et al*., 2006; Matthäus and Özcan, 2009). α-Tocotrienol was detected in all samples, but considerable variation was observed between Australian almonds and almonds grown overseas (Table 2). Previous studies by Kodad and colleagues (Kodad *et al*., 2011a) reported high concentrations of δ-tocopherol, while, Zacheo and colleagues (Zacheo *et al*., 2000) detected β-tocopherol at concentrations greater than γ-tocopherol in almonds from an Italian growing region. The variation in tocol concentrations observed in almonds from different origins indicated that both genotype and environment likely affect almond tocol composition.

Table 2. Tocol composition of almonds from different genotypes

Sample		Concentration (mg/100 g)				
Genotype	**Site**	**Total tocols**	**α-tocopherol**	**γ-tocopherol**	**β-tocopherol**	**α-tocotrienol**
'Nonpareil'	Willunga	14.4 ± 0.5 b	13.7 ± 0.5 b	0.6 ± 0.0 a	0.09 ± 0.01a	0.05 ± 0.01b
	Adelaide	14.4 ± 1.3 b	13.9 ± 1.2 b	0.3 ± 0.0 b	0.08 ± 0.00 b	0.04 ± 0.00 b
	Riverland	11.0 ± 0.4 b	10.7 ± 0.4 b	0.2 ± 0.0 cd	0.06 ± 0.01 b	0.02 ± 0.00 b
	Sunraysia	11.0 ± 0.8 b	10.7 ± 0.8 b	0.2 ± 0.0 d	0.07 ± 0.00 b	0.03 ± 0.00 b
	Spain	18.5 ± 1.7 a	17.9 ± 1.7 a	0.3 ± 0.0 c	0.09 ± 0.01 a	0.26 ± 0.02 a
	California	18.3 ± 1.6 a	17.9 ± 1.6 a	0.1 ± 0.0 e	0.01 ± 0.00 c	0.30 ± 0.03 a
	P	< 0.05	< 0.05	< 0.001	< 0.001	< 0.001
'Somerton'	Willunga	9.5 ± 0.1	9.3 ± 0.1	0.2 ± 0.0	0.04 ± 0.00 b	0.04 ± 0.00
	Riverland	12.1 ± 1.3	11.8 ± 1.3	0.2 ± 0.0	0.06 ± 0.00 a	0.03 ± 0.00
	P	*ns*	*ns*	*ns*	*< 0.05*	*ns*
'Johnston'	Willunga	11.8 ± 1.8	11.5 ± 1.7	0.3 ± 0.0 a	0.04 ± 0.00 b	0.01 ± 0.00
	Adelaide	10.1 ± 0.2	9.8 ± 0.2	0.2 ± 0.0 b	0.07 ± 0.00 a	0.01 ± 0.00
	P	*ns*	*ns*	*< 0.05*	*< 0.001*	*ns*
'Carmel'	Riverland	14.7 ± 0.8	14.4 ± 0.8	0.2 ± 0.0 a	0.07 ± 0.00 a	0.02 ± 0.00 b
	California	14.9 ± 1.7	14.6 ± 1.7	0.1 ± 0.0 b	0.02 ± 0.00 b	0.20 ± 0.02 a
	P	*ns*	*ns*	*< 0.001*	*< 0.001*	*< 0.001*
'Guara'	Riverland	18.9 ± 1.2	18.4 ± 1.1	0.4 ± 0.0 a	0.08 ± 0.00	0.09 ± 0.01 b
	Spain	21.5 ± 0.9	20.9 ± 0.9	0.3 ± 0.0 b	0.12 ± 0.01	0.24 ± 0.02 a
	P	*ns*	*ns*	*< 0.05*	*ns*	*< 0.05*

Values are means of three replicates ± standard error. Values followed by different letters within a column are significantly different ($P < 0.05$); ns = not significant.

In this study, we found environment significantly influenced α-tocopherol levels in 'Nonpareil' almonds (Table 2), but not in almonds of other varieties. Genotype affected α-tocopherol concentrations in Riverland almonds (p <0.001). We observed that 'Guara' had the highest α-tocopherol concentration (18.4 mg per 100 g dry weight), followed by 'Carmel' (14.4 mg per 100 g dry weight). In contrast, γ-tocopherol concentration was more strongly affected by environment and genotype than α-tocopherol concentration; an environmental effect on γ-tocopherol levels occurred in 'Nonpareil', 'Johnston', 'Carmel' and 'Guara' almonds, but not in 'Somerton' almonds (Table 2). Variation due to genotype was observed at all three sites, i.e. Adelaide plains, Willunga and Riverland. β-Tocopherol was influenced by environment and genotype, with significant differences observed in all cases (Table 2). Noticeably, α-tocotrienol showed little difference among Australian almonds from different growing regions, but varied widely between southern and northern regions: i.e. Spanish and Californian almonds contained higher concentrations of α-tocotrienol than Australian almonds (Table 2). The levels of α-tocotrienol observed equaled or surpassed the γ-tocopherol levels of Spanish and Californian almonds. This finding has not been reported in previous research. One possible explanation is due to geographical origin, but the exact factors influencing γ-tocopherol remain unclear. Further research is required to investigate the factors affecting tocol composition of almonds from different growing regions.

Intriguingly, we found an environmental influence on almond tocol homologues that may also depend with genotype. For example, a large difference was seen in the α-tocopherol concentration of 'Nonpareil' almonds, with 23% higher levels corresponding to Willunga and Adelaide plains 'Nonpareil' than Riverland and Sunraysia 'Nonpareil'. This could be due to the lower solar radiation experienced in Willunga, compared to the Riverland and Sunraysia. However, differentiation did not occur in other genotypes. In addition, α-tocopherol was quite stable in 'Carmel' almonds grown in distinct regions; Australian Riverland 'Carmel' and Californian 'Carmel' contained 14.4 mg/100 g and 14.6 mg/100 g, respectively. Likewise, 'Guara' almonds had similar β-tocopherol levels when grown in the Australian Riverland or Spain, i.e. 0.08 mg/100 g and 0.10 mg/100 g, respectively (Table 2). These results therefore suggest that the environmental influence on almond tocol homologues also depends on the genotype involved.

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Characterization of almond kernel oils of five almonds varieties cultivated in Eastern Morocco

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Abstract. This study focuses on characterization of almond kernel oils extracted mechanically from five sweet almond varieties: 'Marcona', 'Fournat', 'Ferragnes', 'Ferraduel' and 'Beldi'), cultivated in eastern Morocco. Oil content, physicochemical parameters, triacylglycerol and fatty acid compositions were determined. Analyzed oils showed low acidity values that range between 0.77-0.88%, peroxide values range between 6.43-16.39 meq/kg and Iodine values range between 98.42-103.90%. The principal fatty acid of almond kernel oils is oleic acid (C18:1); oils of 'Ferragnes-Ferraduel' and 'Beldi' varieties show higher values of C18:1 respectively of 72.87 and 71.62%, however 'Fournat' almond kernel oil shows the lowest content of C18:1 (63.54%). HPLC analysis of triglycerides was carried out, and results show that analyzed almond kernel oils are characterized by the dominance of trioleylglycerol (OOO) that contents range between a minimum of 31.48% for Fournat's oil and 43.82% for Ferragnes-Ferraduel's oil. The oxidative stability of almond kernel oils was determined by rancimat tests as the induction period (IP, h recorded by a 743 Rancimat apparatus Metrohm, Switzerland). Results show that stability, of almond kernel oils is clearly influenced by the almond variety; Oxidative stability of tested almond kernel oils ranged between an IP = 20.28 h for 'Marcona' oil and an IP = 27.55 h for 'Ferragnes-Ferraduel'.

Keywords. Almond kernel oil – Fatty acid – Triglycerides – Oxidative stability.

Caractérisation des huiles de cinq variétés amandes cultivées au Maroc oriental

Résumé. *Cette étude porte sur la caractérisation physico-chimique d'huiles de 5 variétés d'amande douce ('Marcoma', 'Fournat', 'Ferragnes', 'Ferraduel' et 'Beldi'), cultivées dans la région orientale du Maroc. Les huiles d'amandes sont extraites mécaniquement, le rendement en huile, les paramètres de qualité (les indices d'acidité, de peroxyde, d'iode et l'absorbance en UV) ont été déterminés, et également la composition en acide gras et le profil de triglycérides, ont été analysés. L'huile extraite possède un faible indice d'acidité qui s'oscille entre 0,77-0,88% d'acide Oleic, l'indice de peroxyde varie entre 6,43-16,39 meq/kg et l'indice d'iode varie entre 98,42-103,90%* I_2*. Les profils d'acides gras des huiles d'amandes analysées montrent une dominance de l'acide oléique (C18:1) dont les teneurs varient respectivement entre une teneur minimale de 63,54% pour 'Fournat' et un maximum de 72,87% pour le mélange 'Ferragnes-Ferraduel'. L'analyse des triglycérides a été réalisée par HPLC et se caractérise par la dominance de trioleylglycerol (OOO) dont les teneurs varient entre un minimum de 31,48% et un maximum de 43,82% respectivement pour 'Fournat' et le couple 'Ferragnes-Ferraduel'. Les résultats de test Rancimat pour l'analyse de stabilité oxydative des huiles d'amandes étudiées montrent que ces huiles sont relativement stables avec des temps d'induction qui varient entre 20,28 h et 27,55 h.*

Mots-clés. *Huile d'amande – Acide gras – Triglycérides – Stabilité oxydative.*

I – Introduction

The almond tree (*Prunus amygdalus L.*), is considered as a drought tolerant crop, its ability to endure high water deficits is related to its efficiency in valorizing marginal soils.In morocco, cultivation of almond tree constitutes the second most important plantation of fruit trees after olive growing; it is mostly cultivated in two regions, « Taza-Al Houceima-Taounate » in the north and « Souss-Massa Draa » in the south. In Eastern Morocco plantations of almond trees cover 9% andproduce 14% of Moroccan production of almond kernels (MAPM, 2014). According to (Giove and Abis, 2007), (FAOStat, 2012) and the Moroccan Agriculture Ministry's Report (MAPM, 2014), Morocco is generally classified as the sixth producer in the world. Many times, swe*et al*monds are simply eaten raw or toasted. However, there are numerous uses of almonds as an ingredient in manufactured food products or to extract odorlessedible oil that's largely used in cosmetic for external applications for the skin and hair. Thus this study aims to characterize swe*et al*mond oils extracted mechanically from five most important varieties ('Marcona', 'Fournat', 'Ferragnes', 'Ferraduel' and 'Beldi') cultivated in Eastern Morocco.

II – Materials and methods

Samples of sweet almonds (Crop year 2013) of five varieties ('Marcona' (Mr), 'Fournat' (Fn), 'Ferragnes-Ferraduel' mixture(F/F) and 'Beldi' (Be)) were provided from a cooperative of almond producers "AMANDES-SidiBouhria" located nearby Oujda in eastern region of Morocco. The extraction of almond oil (AO), by screw press was realized in a company of seed oils (PRODIGIA, Casablanca). Pressing was carried out at room temperature with a Komet screw press (model DD85G, Germany), with a 5mm restriction die and a screw speed of 20 RPM. Oilswere conserved at 4°C.

Free acidity,peroxide values and UV absorption indices (K_{232}, K_{270}) were determined according to commercial standard methods for olive oil(IOOC, 2001).

Fatty acid (FA) composition: FAs were converted to FA methyl esters and were analyzed by a HP 5880 A series GC System chromatograph, equipped with a capillary column (25 m x 0.25 mm x 0.26 μm) and a FID detector. The carrier gas was nitrogen, at a flow of 1.7 ml/min. the temperatures of the injector and detector were set at 150 and 250°C respectively and the oven temperature was set at 250°C. The injection volume was 1 μl.

Iodine values (I_2N) were calculated from fatty acid percentages (Torres and Maestri, 2006, Maestri *et al*., 2014) by using the following formula:

$$\mathbf{I_2N} = (\%\ \text{Palmitoleic acid x } 1{,}001) + (\%\ \text{oleic acid x } 0{,}899) + (\%\ \text{linoleic acid x } 1{,}814).$$

Triacylglycerol (TAG) analysis was determined by HPLC system.The mobile phase consisted of acetone/acetonitile (60/40; V/V)). HPLC analyses were conducted using C18 reversed- phase column (ODS C18: 250 × 5 mm, 5 μm). Almond oils were dissolved in acetone (9%) and filtered through 0.45 μm membranes. The injection volume is 20 μl.

Oxidative stability indices (OSI) of AO were evaluated by Rancimat test (Rancimat model 743, Metrohm, Switzerland) with an air flow rate of 15L/h and temperature of the heating block maintained at 100°C, OSI of oils was reported as their equivalent induction times (h).

III – Results and discussion

1. Oil Content and oils analysis

The oil content from studied almond varieties (Table 1) varies from 48.62% for 'Fournat' to a maximum of 61.62% for 'Marcona' and they are comparable to that reported previously by other authors(Kornsteiner *et al*., 2006, Kodad and Socias i Company, 2008, Moayedi *et al*., 2011, Martínez *et al*., 2013, Maestri *et al.*, 2014). Acid values (AV) ranged from 0.077 to 0.088 mg KOH/g oil and are much lower than the maximum values established for non-refined vegetable oils (Commission of Codex Alimentarius, 2009). High peroxide values (PV) were recorded only for AO's from 'Marcona' and 'Beldi' but, high iodine values (I_2V) were observed for all analyzed AOs (Table 1), indicating that AOs studied here are highly unsaturated and therefore susceptible to oxidative degradation. In relation to this and according to varieties, significant differences were found ($p<0.05$) for PV and UV values.

Table 1. Oil contents and physicochemical characteristics of analyzed almond oils

Parameters	Almond varieties			
	Mr	**Fn**	**F/F**	**Be**
Oil (%)†	61.62 ± 1.4	48.62 ± 2.1	58.99 ± 1.1	61.00 ± 1.7
AV	0.81 ± 0.008	0.88 ± 0.005	0.88 ± 0.004	0.77 ± 0.009
PV	14.32 ± 0.41	6.43 ± 2.73	8.13 ± 2.81	16.39 ± 2.95
K_{232}	1.54 ± 0.2a	3.53 ± 0.11c	1.33 ± 0.14a	2.29 ± 0.03b
K_{270}	0.073 ± 0.004 ab	0.129 ± 0.005c	0.053 ± 0.006a	0,088 ± 0.003b
I_2N	102.25 ± 0.13	103.90 ± 0.16	98.42 ± 0.11	98.97 ± 0.25
OSI (h)	20.28 ± 0.45a	21.22 ± 3.31a	27.55 ± 0.714b	23.5 ± 0.62a

† Oil content (%dry basis), AV acid value (mg KOH/g oil), PV peroxide value (meq.O_2 /kg oil), IV iodine value (g I_2% g oil), OSI: Oxidative Stability (Induction time, h)Significant differences in the same row are shown by different letters (a-c) varieties ($p<0.05$).

2. Fatty acid and Triacylglycerol compositions

Fatty acid (FA) and Triacylglycerol (TAG) compositions of oils of the studied almond species are presented in Table 2 and Fig. 1 respectively. Low contents of saturated fatty acids and high contents of monounsaturated oleic acid are highly favorable for human nutrition. For examined AOs the main FAs are oleic, linoleic and palmitic acids and fatty acid compositions shown a typical characteristic of a low concentration of SFAs (SSFA,8-10%), intermediate for PUFAs (SPUFA, 17-25%), and high for MUFAs (SMUFA, 64-73%). Similarities between the studied AOs permit to class them in two categories (Fn, Mr); and (B, F/F). According to I_2N and OSI (Table 1), AOs of Be& F/F shown the best stability.

Table 2. Fatty acid compositions (%) of analyzed almond oils

Fatty acid %	Almond varieties			
	Mr	**Fn**	**F/F**	**Be**
Palmitic (C16:0)	7.15 ± 0.14c	7.91 ± 0.05d	6.53 ± 0.12a	6.79 ± 0.09b
Palmitoleic (C16:1)	0.70 ± 0.03c	0.61 ± 0.01b	0.55 ± 0.02a	0.59 ± 0.02b
Margaric (C17:0)	0,102 ± 0.002	0.101 ± 0.001	0.104 ± 0.003	0.100 ± 0.002
Stearic (C18:0)	2.20 ± 0.05b	2.15 ± 0.04b	2.01 ± 0.04a	1.98 ± 0.09a
Oleic (C18:1)	66.87 ± 0.66b	63.54 ± 0.32a	72.87 ± 0.42d	71.62 ± 0.45c
Linoleic (C18:2)	22.84 ± 0.38c	25.45 ± 0.31d	17.84 ± 0.38a	18.74 ± 0.34b
Oleic / Linoleic (O/L)	2.92 ± 0.007	2.49 ± 0.006	4.082 ± 0.005	3.82 ± 0.002
SSFA	9,35 ± 0.09c	10.06 ± 0.01d	8.54 ± 0.09a	8.78 ± 0.10b
SMUFA	67.56 ± 0.64b	64.15 ± 0.31a	73.42 ± 0.41d	72.21 ± 0.43c
SPUFA	22.84 ± 0.38c	25.453 ± 0.31d	17.843 ± 0.38a	18.737 ± 0.34b

'Marcona' (Mr), 'Fournat' (Fn), 'Ferragnes / Ferraduel' mixture (F/F), 'Beldi' (Be) Values are means of four different almond oil samples (n = 3) ± SD (standard deviation) Significant differences ($p<0.05$) in the same row are shown by different letters (a-d). SSFA: Sum of saturated fatty acids; SMUFA: Sum of mono-unsaturated fatty acids; SPUFA: Sum of polyunsaturated FA.

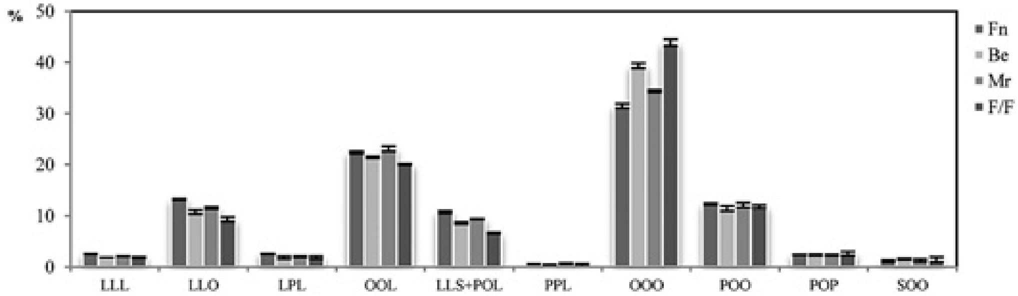

Fig. 1. Triacylglycerol composition of almond oils analyzed by HPLC, TAGs are abbreviated using L, O, P, S respectively for Linoleoyl, Oleoyl, Palmitoyl, and Stearoyl FA radicals.

TAG profiles (Fig. 1) show that contents of TAGs in analyzed AOs, decrease in the following order: OOO > OOL > POO >LLO> POL+ LLS>LLL > LPL > POP > SOO >PPL. Two predominant TAGs are OOO (31-44%) and OOL (20-23%) which altogether represent more than 50% of the total TAGs, they are followed by POO and LLO, with quantities around 10%.Significant differences are found when analyzed oils are compared pairwise and particularlyfor their contents in OOO, OOL, and LLO.

IV – Conclusion

Oil content, FA composition, TAG profile and the physicochemical characteristics of sweet AOs of five varieties cultivated in eastern Morocco were studied for the first time. Significant variations have been observed in FA among studied varieties and similarities between the studied AOs permit to class them in two groups (Fn, Mr); and (Be, F/F). Oils, of group “Be, F / F”, are distinguished by their richness in C18:1 and low content of C18:2, which confer them a better oxidative stability, and therefore they could be recommended as varieties for planting program in the eastern region of Morocco.

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Pistachio production and industry in Turkey: current status and future perspective

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Abstract. The pistachio nut belongs to the genus *Pistacia* of the family Anacardiacea. *Pistacia* genus have 11 species. Some of the species play an important role in vegetation at the Mediterranean and Asian regions and most of them have proved successful as rootstocks for top working the cultivated pistachio nut. Except *Pistacia vera* the other species have no economical significance. They are called wild pistachios. In Afganistan, Iran, Pakistan, Turkey and all the Mediterranean countries there are millions of wild pistachio trees or bushes which belong to different *Pistacia* species. Pistacia species are grown in 30º-40º latitude and are suited for different climate areas in the world. In Turkey, during last decades new plantations are being established and pistachio farmers conscious of the importance of male tree and irrigation. Nowadays due to governmental support the farmers are applying good agricultural practices rules for their orchards

Keywords. Pistachio – Rootstock – Cultivar – Irrigation – Fertilization.

Production et industrie des pistaches en Turquie : situation actuelle et perspectives futures

Résumé. *Les pistaches appartiennent au genre* Pistacia *de la famille Anacardiaceae. Le genre* Pistacia *possède 11 espèces. Certaines espèces jouent un rôle important pour la végétation dans les régions méditerranéenne et asiatique et la plupart se sont avérées performantes comme porte-greffes pour les pistaches cultivées. Excepté* Pistacia vera*, les autres espèces sont économiquement insignifiantes. Elles sont dénommées pistachiers sauvages. En Afghanistan, Iran, Pakistan, Turquie et dans tous les pays méditerranéens, il y a des millions de pistachiers sauvages, arbres ou buissons, qui appartiennent aux différentes espèces de* Pistacia*. Les espèces de* Pistacia *grandissent à une latitude de 30º-40º et sont adaptées aux différentes zones climatiques du monde. En Turquie, durant les dernières décennies, de nouvelles plantations sont mises en place et les cultivateurs de pistaches sont conscients de l'importance de l'arbre mâle et de l'irrigation. De nos jours, en raison de l'appui du gouvernement, les producteurs appliquent les principes des bonnes pratiques agricoles dans leurs vergers.*

Mots-clés. *Pistachier – Porte-greffe – Cultivar – Irrigation – Fertilisation.*

I – Introduction

The pistachio nut belongs to the genus *Pistacia* of the family Anacardiacea. The *Pistacia* genus has 11 species. The edible pistachio for commerce is the species *Pistacia vera* L. In addition to many named cultivars, significant populations of wild germplasm exist, primarily in central Asia, from Turkey to Afghanistan.

Pistacia vera is grown at 30º-40º latitude and suited for different climate areas in the world. The area of pistachio plantation in the last 11 years has increased from 407,900 ha in 1994, to 526,600 ha in 2004. The pistachio plantation area of the main producing country, Iran, has increased (53%) to 280,000 ha in 2004 (Razavi, 2006). Turkey has a big plantation area but its yield is very low when compared to the production area (Arpaci *et al*., 2005).

Pistachio is grown most intensively in Iran, Syria, Turkey and USA. The other pistachio-producing countries are in the Near East, North Africa and Southern Europe.Based on nut production data for 2009-2012, Iran is responsible for over 459,000 tons, about half (53.2%) of world production. The second highest-yielding country, USA, produces 207,000 tons, about 22.3% of world production, while Turkey is third with a yield of 117,000 tons and 12.7 % of total world production. The most important exporters in the world are Iran, USA, Greece, Italy, Syria and Turkey. Iran is considered as one of the greatest exporters, with a share of about 62.7% in the world (Razavi, 2006).

Pistachio trees are frost hardy and can normally withstand temperatures as low as -14 to . In Iran, pistachios can tolerate temperatures down to -20°C, and in Turkey pistachios can withstand winter temperatures of down to -30°C without any injury.

P. vera usually blooms during the first week of April which prevents the flowers from being damaged by late spring frost. However, plantations established in frost pockets may be at risk. Flowers may also be damaged by burn caused by dry and hot winds during the spring. Like other fruit trees pistachio requires chilling during the winter period. Under mild climatic conditions, inadequate chilling causes problems such as very low yield, unsuitable timing and low percentage of budbreak, late flowering, death of stigma, very late vegetative and generative development, late ripening, less leaf and pollen production and irregular sprouting of the buds (Kuden *et al*., 1995). Studies have shown that the cultivar 'Kerman' needs about 1000 hours chilling (Crane and Takeda, 1979) whereas the cultivar 'Uzun' needs 600 hours and 'Ohadi' needs more than 1000 hours (Kuden *et al*., 1995). Kuden *et al*. (1995) also reported that male trees require less chilling than females.

Summer temperatures are another very important climatic factor influencing pistachios. If the summer temperatures are insufficient then the kernel does not develop properly which prevents splitting and dehulling.

II – Pollination of pistachio

Pistachio trees are dioecious and it has been recognized since 1697 (Whitehouse and Stone, 1941) that pollination and fertilization are necessary to obtain seeded fruits. Trees are wind pollinated as pistachio flowers have no petals with which to attract insects. Therefore pistachio orchards must contain both male and female trees at suggested ratios from 1:8 to 1:11 (male to female) (Kaska, 1990; Ak, 1992). It is necessary to have enough male trees to ensure adequate pollination as insufficient pollen is a primary cause of crop yield failure. Alternatively, pollen from wild pistachios can be used to artificially pollinate *P. vera*. Protandry is common in pistachios.

Male tree should have the following traits: strong and upright growth; flowering period must be synchronized with that of female trees; flower clusters must be large and numerous; the amount of pollen produced in each cluster must be high; yield potential must be high; germination rate of pollen must be high; pollen viability *in vivo* must be long, and trees must not show alternate bearing (Ak et al., 1998; Ak, 2001). Last decades farmers are realized the importance of male trees. Nowadays the new orchards are established using suitable males for female cultivars in enough number for the orchards.

III – Rootstocks

Anatolia is a major centre of diversity for the genus *Pistacia*; species are naturally distributed throughout the region, with the exception of very cold and humid areas. Pistachios are present in the form of bushes or trees of different sizes dependent upon genetic factors and soil types and climatic conditions that characterize the region. In the Mediterranean and Aegean region, *P. atlantica* trees reach gigantic dimensions. In the transitional zone between the Mediterranean and central Anatolia where the rainfall is lower, a gradual reduction in height of these trees is observed. Smaller specimens of *P. terebinthus* are found in southeast Anatolia compared to the relatively big bushes found in the Mediterranean. Due to its wide adaptability to a variety of soils and climate, *P. terebinthus* thrives well from the coastal zone at sea level to up to in Turkey, where it is the most widespread of all *Pistacia* species (Kaska and Bilgen, 1998).

Mainly *P. vera*, *P terebinthus*, *P. atlantica*, *P. khinjuk*, *P. palaestine, P. mutica* are distributed in Turkey. Except *P. mutica* the others are using as rootstock for *Pistacia vera* cultivars. The best rootstocks seem to be *P. khinjuk* for the all types or cultivars of pistachio nut. The weak or dwarf rootstock is *Pistacia terebinthus* for pistachio cultivars when it is budded on it. There are some research results on pistachios budded or used as rootstock some *Pistacia* spp. The incompatibility problem can occur between *Pistacia vera* seedlings and cultivars. This problem is solving using *Pistacia khinjuk* as interstocks. . Pistachio nuts are affected by rootstocks on different traits of nut quality (; Turker and Ak, 2010; Atli *et al*., 2011).

IV – Cultivars

The most important characteristics of the pistachio nuts desired by the markets are the following: large size, high percentage of shell splitting, low percentage of blank nuts, high oil and protein content, regular bearing and high percentage of green kernels. Pistachio green kernel is one of the most desired characteristics, green kernel nuts are always at a premium. Though the green kernel is a varietal characteristic it is also related to altitude and harvest time. Generally the nuts of early harvested trees and plants grown on high plateaus produce greener kernels than those of late harvested and low land grown plants. On the other hand greenish is affected by pollen source. According to observations *Pistacia terebinthus* pollens causes metaxenia and xenia (Ak, 1992). In Turkey, 'Kirmizi', 'Uzun', 'Halebi' and 'Siirt' are the major pistachio cultivars.

Alternate bearing is one of the important features of pistachio. Alternate bearer cultivars produce heavy crops in "on" years and little or no crop in the "off" years. In contrast to other fruit species such as olive, apple etc (Ak and kaska 1992). This situation can be changed from one cultivar to another one; for example 'Siirt' variety is less alternating (Ak, 1998). Alternate bearing can be decreased by irrigation and fertilization. The farmers are aware of the importance of production of pistachio because of the high profits.

In Turkey, two female ('Barak Yildizi' and 'Tekin') and four male ('Kaska', 'Ozturk', 'Uygur' and 'Atli') were registrated as cultivars. These cultivars were released by Gaziantep Pistachio Research Institute-Turkey. 'Barak Yildizi' is in need of less total heat accumulation. So it can be recommended for inland and transitional areas. This cultivar matures earlier (25-30 days before) than other standard Turkish cultivars. Chilling requirement is also low. It is suitable to consume fresh because of its earliness. 'Tekin' is suitable for the international market because of its good traits. It is high yielding, has a high splitting rate and has large fruit in size. The endocarp color is light and it bears every year regularly. That means no alternate bearing habit. 'Tekin' cultivar is better than 'Siirt' cultivar (Ak, 2014).

V – Husbandry

Pistachio orchards have been established in dry and non-irrigated lands in Turkey and Syria. The soil is ploughed in autumn in order to preserve water from winter and spring rain and sometimes snow. The soil is also cultivated by chisel ploughs in the spring and summer months to prevent evaporation. This working of the soil destroys weeds which are in competition with pistachios and are also host plants for many diseases and insects (Kaska, 1995).

Pistachio trees are irrigated in Iran and the USA and it is well known that irrigation is a very important factor in obtaining high yields and good quality. There are plans to expand Turkey's pistachio producing areas with new and irrigated orchards in the southeast Anatolia region, which is expected to double Turkey's pistachio production. Irrigation experiments with different rootstocks have already been started in Sanliurfa.

The use of irrigation increases leaf size, number of current year's shoots and length of shoot. Irrigation also increases yield, nut size, splitting percentage and decreases blank nut rate and degree of alternate bearing.

Irrigation system was drip irrigation which the pipeline on the surface of the soil. But in some areas birds damaged the pipeline, and to avoid this lines can be lay underground. This was very advantageous application. Irrigation by underground drip systems is already being used on olive plantations in Spain. This system has been starting to use for pistachio and almond orchard in Turkey, nevertheless farmers has some suspicious about this method. The advantages of underground drip irrigation system are: 1. lower water consumption, 2. better water distribution, 3. greater uniformity, 4. use of waste water, 5. less evaporation, 6. greater transpiration, 7. location of fertilizer, 8. less clacification, 9. fewer diseases, 10. possibility of working the ground, 11. reduction of labor, 12. longer lasting, 13. no vandalism (Ak, 2004).

All above advantages are valid for all kind of fruit trees. In fact this system now in experimentally using at Ceylanpinar State Farm for pistachio orchard. The yield seems to be regulated, because there was alternate bearing habit of especially 'Kirmizi' variety. This underground irrigation system was established in a 20 da area where the adult tress (30 years old) using 'Siirt' and 'Kirmizi' cultivars. The depth of lineis approximately 20-25 cm deep from soil surface. After this experiments of governmental orchard, farmers are practiced in their pistachio and almond orchards.This irrigation system is using in Iran because of lack of water.

Fertilization is a very important factor in obtaining high quality yield from fruit trees. The degree of fertilization largely depends on irrigation and pH of soil. The pH of soils in the main production areas of Iran is between 7.2 and 8.5, with an average of 7.9. Pistachios are tolerant to salinity, and studies have shown that they can tolerate an electrical conductivity (EC) of 8.0 dS/m without considerable decrease of in yield (Sheibani, 1995).

Soils of pistachio orchards often have inadequate levels of nitrogen, phosphorus, potassium and organic matter. In a survey conducted in 30 pistachio orchards in southeast Anatolia, Tekin *et al.* (1985) found that in many orchards the trees were markedly deficient in phosphorus and zinc and slightly deficient in nitrogen, iron and manganese. The level of potassium was found to be adequate in many orchards though there were some districts where trees showed slight deficiencies. In such regions the soil pH varied between 7.5 and 9.3 and the organic matter content was very low.

Crane and Maranto (1988) claimed that pistachio is not an excessive nitrogen consumer when it is abundantly available in the soil. Nitrogenous fertilizers should be chosen depending on the soil pH. For example, in the southeast Anatolia region only ammonium sulphate is recommended as a source of nitrogen because of the alkaline soils. Nitrogenous fertilizers should be given at the end of February or the beginning of March at the rate of 1.5 to per tree (Kaska, 1995). In the USA, boron plays a unique role in pistachio production. Responses to foliar boron application include increased pollen viability and germination rate, increased fruit set and yield, decreased blanking percentage and increased leaf boron concentration (Brown, 1995).

Fertilizers are applied regularly and produce better vegetative growth. Pruning should also be carried out although care must be taken with trees growing on poor and dry soils. In Turkey and Syria, pruning is carried out once after harvesting and once again before flowering. Pruning is carried out by hand in most countries apart from the USA where mechanical pruning is necessary due to such good vegetative growth resulting from irrigation.

VI – Harvesting

Pistachios are harvested from late August to late October depending on cultivar. During maturation the colour of the exocarp changes from light green to pale cream or white. The hulls can be removed from the fruits by being squeezed between the fingers. Another sign of maturity is splitting of the shell. Kernel dry weight and crude fat content also increase (Ak, 1998).

In Turkey and Syria, pistachio nuts are harvested manually as clusters which can easily be separated from branches by bending them back. Nuts drop on to canvas spread out under the trees. The clusters are put in sacks and brought to drying yards where they are laid out and left to dry in the sun. The in-shell nuts are separated from their clusters and left again in the sun for further drying. In Turkey, all the cultivars except Siirt and Ohadi are stored with their hulls. The nuts are kept in the sun until they are completely dry and then put in sacks and stored (Kaska, 1995).

In Iran, harvesting is carried out in a similar manner to that in Turkey. However, in the USA machines are using to harvest the pistachios. Post-harvest practices such as dehulling, drying, separation of blank nuts and separation of split nuts are mechanized.

Turkey has the advantage of producing nuts free from aflatoxins, due to low humidity and high temperature experienced during growing and harvesting. Processing systems are also undergoing modernization. Turkish cultivars are also preferred in many European and American markets due to their good taste and uniformly green kernels. The pistachio nut is considered good for human health due to its nutritive content.

VII – Pest and diseases

The main pistachio pests are: Psylla (*Agonoscena pistaciae*), the pistachio twig borer (*Kermania pistaciella*, Ams.), pistachio fruit moth (Recurvaria pistaciicolla, Danil.), pistachio leaf hopper (*Idiocerus stali* Fieb.), pistachio root beetle (*Capnodis cariosa*) etc.

Pistachio psylla (*Agonoscena pistaciae* Licht): This is the most destructive pest of pistachio grown in Turkey and Iran. The damage caused by this insect includes direct injury as a result of sucking plant sap and stunting the tree, shedding of leaves, fruits and the buds, increasing the rate of nut blanks, lowering the quality of nut, hindering transpiration and photosynthesis due to the closure of stomata by honeydew secreted from the insect larvae and/or sooty mould growing on leaves if conditions become favourable.

The main diseases: Septoria leaf spot, Verticillium wilt, *Phytophtora parasitica,* Root and crown rot, stigmatomycosis, etc. Septoria leaf spot caused by Septoria pistaciae. It is controlled with preventative fungicide sprays such as dithiocarbamates (e.g. Zineb, Mancozeb). Copper fungicides are also effective, but they should only be applied after the fruit has reached size, since they might be phytotoxic to very young fruit. Applications should begin after the first leaves have unfolded and if necessary be repeated at monthly intervals up to the beginning of June (Michailides and Young, 1989; Michailides, 1998).

Integrated Pest management application is very important for the Good agricultural Practices applicant farmers (Ak, 2015). Nowadays Turkish government is supporting the Good agricultural Practices orchards through consultants of special firms.

VIII – Conclusions

Pistachio nut culture is centuries old in Iran, Turkey and Syria. In these countries the area under pistachio nut is the largest and the number of trees is the highest in the world. In spite of these facts the production is very low in Turkey and Syria. The kernel taste is good but the nuts are small and their splitting percentage is low. However, one should bear in mind that Turkey is the only country in the world that pistachio nuts are grown in such marginal lands with dry climate and poor, rocky and calcareous soils. The latest development is the processing system is changed at Ceylanpinar state farm in Sanliurfa. Pistachio orchards and production development will be seen in some European Countries and some west Asian countries. New technologies have been started to be used in Turkey. The government of Turkey is supporting, running experiments to solve different problems.

Worldwide supply of and demand for pistachios are generally in equilibrium and the producer countries usually are the consumers at the same time. While Iran, Turkey, United States, and Syria are the top consumers, the highest consumption among the European countries is in Italy. The majority of pistachios grown globally (60%-70%) is consumed as saltily roasted snacks, and 30-40% consumed in the confectionary industry as an ingredient in chocolate, cakes, ice cream and other sweets. In the United States and Europe, however, 90% of pistachios are consumed as salty nuts.

In order to increase the competition power of Turkish pistachio sector, production costs have to be reduced and new cultivars oriented toward international markets must be developed or adopted as well as issues in farming and marketing have to be resolved. Agricultural practices in pistachio production on irrigated land help maintain both the demand-supply equilibrium and the stability in international markets and so, the Southeastern Anatolia Project is an opportunity in this respect. Nevertheless, Turkey as being the consumer of what is produced domestically looks nothing but a closed economy.

Varietal choice, production and price stability are essentially important factors in pistachio export. First we need to find out which varieties are most wanted at the international level: roasted nut varieties or varieties used in confectionary industry? In other words, what are the criteria of countries on this matter? It is important to determine correctly what the best answer is to these questions. Problems often arise when it comes to storing of pistachios after the harvest. For example in recent years, Iran is reportedly having export problems arising from defects in pistachio storage. The Southeastern region of Turkey where pistachios are grown widely has an advantage in this respect, since the dry and hot climate prevailing in the region provides for safe storage conditions free of aflatoxin, which is suspected to cause liver cancer in humans and other animals. Iranian pistachios, illegally entered Turkey, are perceived as Turkish pistachios, leading to problems when they are consumed either domestically or internationally upon imported from Turkey. Other important point is the problem of unstable price policies. Prices set by different policies each year have negative impacts on both domestic and international markets. While it is possible to solve existing problems pertaining to an agricultural commodity, like pistachios native to Turkey, by applying serious and stable policies, it feels quite frustrating not to be dominant in pistachio markets globally. Hazelnut is the first nut for Turkish economy, but pistachio is also very important for South East Anatolia Region. Because of this reason governmental support has been provided last decades. This support is mainly for irrigation of non- irrigated pistachio orchards (Isgin and Ak, 2011). Nowadays government of Turkey is supporting Good Agricultural Practices in pistachio orchards. Farmers also follow the advisors about these subjects to produce high yield and to get high profit.

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Programme and List of participants

XVI GREMPA MEETING
(Meknes, Morocco, 12-14 may 2015)

PROGRAMME

12 MAY	
9:00 – 10:00	Registration
10:00 – 11:00	**Opening ceremony** Mohammed Sadiki. Secretary General, Ministry of Agriculture and Fisheries, Morocco Taoufik Benziane. Director of ENA Meknès Ignacio Romagosa. Director of IAMZ-CIHEAM Mercè Rovira. Coordinator of FAO-CIHEAM Network on Nuts Meeting Convener – Ossama Kodad (ENA Meknès, Morocco)
11:00 – 11.30	*Coffee break*
11:30 – 12:00	**Introductory presentation:** GREMPA: A useful initiative for cooperation in almond research. *Rafael Socías i Company (CITA, Spain)*
12:00 – 13:00	Visit to the almond germplasm collection of ENA Meknès
13:00 – 15:00	*Visit to posters and lunch*
15:00 – 18:00	**Session 1. Genetic Resources. Cultivars** *Moderator: Federico Dicenta (CEBAS-CSIC, Spain)* *Secretary: Azza Chelli Chaabouni (INRAT, Tunisia)*
15:00 – 15:20	Introductory presentation by the Session moderator
15:20 – 16:20	Oral presentations (10 min. each) 1. Paternal effects on nut characteristics of some almond cultivars. *Izzet Acar (Harran Univ., Turkey)* 2. Performance of the CITA almond releases and some elite selections. *José Manuel Alonso (CITA, Spain)* 3. Morphometric features of local and foreign female pistachio varieties and ecotypes in the North-eastern Tunisia conditions. *Azza Chelli Chaabouni (INRAT, Tunisia)* 4. Self-compatibility sources and sources of variation in advanced almond introgression lines. *Thomas Gradziel (University of California Davis,USA)* 5. Genetic characterization of almond (*Prunus dulcis*) cultivars and natural resources. *Julia Halasz (Corvinus Univ. of Budapest, Hungary)* 6. The expression of the *Sfa*-allele in homozygote *SfaSfi* genotypes indicates a stylar mutation as the origin of self-compatibility in almond. *Ossama Kodad (ENA, Morocco)*
16:20 – 16:50	*Coffee break*
16:50 – 18:00	Discussion on Session 1
20:30	*Social dinner*

13 MAY	
9:00 – 13:00	**Parallel activities (participants may choose one of the following activities)**
	Technical trip. Almond plantation and visit to "Chateau Rhozlane" near Meknes
	Workshop: "The almond and pistachio crops in Morocco in a changing international context". This workshop, addressed to the Moroccan almond and pistachio industry, aims to generate a discussion on the current situation and perspectives of the two crops in Morocco, profiting from the presence of international experts who will present their experiences in their own countries **9:00 – 9:20. Introductory presentation.** Bahaji J (Direction de l'Enseignement, de la Formation et de la Recherche, Ministère de l'Agriculture et de la Pêche Maritime). **9:20 – 11:30. Oral presentations** **1.** The almond crop in the USA. Current situation and challenges for the future. *Thomas Gradziel (University of California Davis, USA)* **2.** Pistachio production and industry in Turkey: current status and future perspectives. *Beckir Erol Ak and Serpil Karadag (Turkey)* **3.** The almond sector in Spain. Synergies between the public and private sectors for the development of the crop. *Rafael Socias i Company (CITA, Spain)* **4.** Pistachio culture and industry in Tunisia. Current situation and future axes of improvement. *Mohamed Ghrab (Institut de l'Olivier, Tunisia)* **5.** Challenges for almond cropping in Morocco. *Ali Lansari (consultant, Morocco)* ***11:00 – 12:00. Coffee break*** **12:00 – 13:00. Discussion and recommendations**
13:00 – 14:00	*Lunch*
14:00 – 17:00	**Session 2. Genetic Resources. Rootstocks** *Moderator: María José Rubio (CITA, Spain)* *Secretary: Henry Duval (INRA, France)*
14:00 – 14:20	Introductory presentation by the Session moderator
14:20 – 15:00	Oral presentations (10 min. each) 1. Impact of osmotic drought stress on carbon isotope discrimination and growth parameters in three pistachio rootstocks. *Ali Esmaeilpour (Ghent University, Belgium)* 2. Field evaluation of new rootstocks for almond. *Xavier Miarnau (IRTA, Spain)* 3. Assessment of control pollinated progenies as almond rootstocks. *Saloua Mselmi Taoueb (INRAT, Tunisia)* 4. Morphological variation in the Pistachio terebenth tree (*Pistacia terebinthus* L.; Anacardiaceae). *Safia Belhadj (Univ. of Djelfa, Algeria)*
15:00 – 16:00	Discussion on Session 2
16:00 – 17:00	*Coffee break and visit to posters*
17:00 – 19:00	**Session 3. Pest and diseases** *Moderator: Mohammed Boulif (ENA Meknès, Morocco)* *Secretary: Safia Belhadj (Univ. of Djelfa, Algeria)*
17:00 – 17:20	Introductory presentation by the Session moderator
17:20 – 17:50	Oral presentations (10 min. each) 1. Pistachio seed wasp, pistachio twig borer. *Mohamed Braham (Centre régional de recherche en horticulture et en agriculture biologique, Tunisia)* 2. Alternaria blight of *Pistacia* species in Turkey. *Hilal Ozkilinc (Çanakkale Onsekiz Mart Univ., Turkey)* 3. Susceptibility to the major almond diseases red leaf blotch and almond canker in the main almond Spanish cultivars. *Laura Torguet (IRTA, Spain)*
17:50 – 19:00	Discussion on Session 3

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9:00 – 12:00	**Session 4. Orchard management** *Moderator: Ali Lansari (Consultant, Morocco)* *Secretary: Bekir Erol Ak (Harran Univ., Turkey)*
9:00 – 9:20	Introductory presentation by the Session moderator
9:20 – 9:50	Oral presentations (10 min. each) 1. Climate change and nut crops vulnerability in the Mediterranean area. *Mohamed Ghrab (Institut de l'Olivier, Tunisia)* 2. Update in super high density almond orchards. *Xavier Rius (Agromillora, Spain)* 3. Determination of yield and some quality traits of Pistachio cv. Siirt trained with different systems. *Hatice Gözel (Pistachio Research Station, Turkey)*
9:50 – 11:00	Discussion on Session 4
11:00 – 12:00	*Coffee break and visit to posters*
12:00 – 14:00	**Session 5. Quality, industrialisation and marketing** *Moderator: Michelle Wirthensohn (Univ. of Adelaide, Australia)* *Secretary: Agustí Romero (IRTA, Spain)*
12:00 – 12:20	Introductory presentation by the Session moderator
12:20 – 13:00	Oral presentations (10 min. each) 1. Chemical composition of roasted kernels of Moroccan almond seedlings. *Ossama Kodad (ENA Meknès, Morocco)* 2. BIOFOS: Micro-ring resonator-based biophotonic system for food analysis. *Agustí Romero (IRTA, Spain)* 3. Relations between the yield and some quality traits on pistachios. *Ebru Sakar (Univ. of Harran, Turkey)* 4. Almond processing and industrialisation. *Rafel Socias I Company (CITA, Spain)*
13:00 – 14:00	Discussion on Session 5
14:00 – 15:00	*Lunch*
15:00 – 16:00	**Meeting conclusions, final debate and close**
16:00 -	***Guided tour of Meknes ancient city (optional)***

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XVI GREMPA Meeting on Almonds and Pistachios

Edited by:
O. Kodad, A. López-Francos, M. Rovira, R. Socias i Company

Almonds (1267 Mha) and pistachios (385 Mha) are important nut tree crops in the Mediterranean area, including Iran country, and representing both around the 75% of the world surface. Universities and research centres have done and continue to do much research in these two species, resulting in a considerable evolution on both plant material and orchard management. During more than 40 years, the GREMPA (Mediterranean Research Group for Almond and Pistachio), established with CIHEAM support in the 1970s, has carried out work on the evaluation and improvement of genetic resources of Mediterranean origin, and has promoted debate on research in all aspects of the cultivation and industrialisation of these two species. The group is currently in integrated into the FAO-CIHEAM Research Network on Nuts and meets approximately every 4 years.

The **XVI GREMPA Meeting** was held in Meknès, Morocco from 12 to 14 May 2015, organized by the École Nationale d'Agriculture de Meknès and the Mediterranean Agronomic Institute of Zaragoza (IAMZ-CIHEAM), with the collaboration of the Moroccan Ministry of Agriculture and Fisheries of Morocco. The XVI GREMPA was a forum to discuss the issues challenging almonds and pistachios and to promote the active participation of researchers from all Mediterranean countries as well as from other parts of the world. More than ninety researchers, students and professionals from 10 different countries (Algeria, Australia, Belgium, France, Hungary, Morocco, Spain, Tunisia, Turkey, and USA) attended the Meeting and contributed by presenting their works and sharing their ideas and experiences.

This publication compiles 60 full articles of the contributions presented at the Meeting, that represent a good sample of the research currently carried out on the different aspects of breeding and genetic resources evaluation, crop managing and industrialization of almonds and pistachios in the Mediterranean basin countries and beyond.

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Nut Network

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