LEGUME PERSPECTIVES

Imagine all the legumes studied just as one

Integrating research on various legume species and crops

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Office and subscriptions
CSIC, Institute for Sustainable Agriculture
International Legume Society
Apdo. 4084, 14080 Córdoba, Spain
Phone: +34957499215 • Fax: +34957499252
diego.rubiales@ias.csic.es

Publishing Director
Diego Rubiales
CSIC, Institute for Sustainable Agriculture
Córdoba, Spain
diego.rubiales@ias.csic.es

Editor-in-Chief
Carlota Vaz Patto
Instituto de Tecnologia Química e Biológica António Xavier
(Universidade Nova de Lisboa)
Oeiras, Portugal
cpatto@itqb.unl.pt

Technical Editor
Aleksandar Mikić
Institute of Field and Vegetable Crops
Novi Sad, Serbia
aleksandar.mikic@nsseme.com

Front cover art:
O, leguminosae! O, varietas!
by Aleksandar Mikić

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EDITORIAL

The contents of the Legume Perspectives issue that is before you is remembering us all about one of the main permanent goals of the body standing behind it, the International Legume Society (ILS): to identify, link and integrate the research on various legume species and crops. The times when soybean, grain legumes, forage legumes and other groups of legumes were studied separately from each other served every of these research communities well, but have definitely passed away, transforming into something novel, meet to answer the challenges of the moment and working for the benefit of all. The following pages will bring you an air of the ILS first meeting, in Novi Sad, in 2013, filled with a sense of being united in differences, exchange of thoughts and ideas and a feeling how comforting and powerful is to know that none of us is alone or isolated in his own hard scientific work. May it remain so forever!

Aleksandar Mikić and
Vuk Đorđević
Managing Editors of
Legume Perspectives *Issue 6*

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Carte blanche to…

…M. Carlota Vaz Patto & Diego Rubiales

Current and past Editors-in-Chief of *Legume Perspectives*

For a "legumefied" greater World!

2016 ***is the United Nations' International Year of Pulses Grain Legumes.*** *Legumes are attractive crops offering wonderful opportunities for food and feed together with invaluable ecological services. In this context, the* **Legume Society** *will hold its next International Conference in Portugal. A thorough analysis of the production, utilization and trade of legumes is anticipated, with the expected goal of promoting leguminous crops in modern agrifood and feed production systems.*

Presently legumes play a critical role in crop rotations and their products are still much appreciated for food and feed. Indeed, the cultivation of warm-season legumes (soybean, cowpea, common bean, groundnut, pigeonpea) is continuously increasing at global level. However, the world cultivated area of most temperate grain legumes (pea, faba bean, lupin) has declined in past decades, although with promising increases in Canada and Australia. Other regional tendencies are also particularly notorious. In Europe we assist to a remarkable general decrease of most legumes production, including common bean, in spite of the political efforts made by the European Union and national governments to promote legume cultivation through farmers' subsidies. Soybean is the only legume whose acreage is increasing in Europe this decade. In spite of this regressive pattern, producers, manufactures and consumers are demanding more legumes, particularly in recent years, willing to grow more environmentally friendly crops, as soon as proper cultivars are available, and conscientiously selecting more health promoting food products for their grocery lists, even though at higher prices. Indeed, most of the blames on legumes are just "urban legends". They are blamed of providing unreliable and instable yields; however, the success histories of Canada and Australia show that legumes can be cash crops even at world-prize with no subsidies. Legumes are also blamed of being unpopular with consumers. This contrast with reality: legumes are attractive food products, very much appreciated by better educated consumers performing wiser food choices (see the Mediterranean diet food lovers), but not only. Indeed, legumes are gaining visibility as healthy, popular food choices, very rich in fiber or proteins, low in fat and packed with essential nutrients. They are recommended by health professionals to maintain good health and prevent chronic diseases, such diabetes or cardiovascular diseases, and assist weight management. Their gluten-free status makes them an excellent choice for people with celiac disease. Legumes are also crucial components of vegetarian diets, and this is not only important by ethnic or regional issues, but is becoming trended, with a remarkable potential within vegan people. In fact, all Mediterranean countries are heavy importers of food legumes from world market. Also, feed compounders rely heavily on legumes. Today the leader is soybean, but peas or faba beans have also huge potential for feed and manufactures are ready to use them, provided there is a constant and homogeneous supply.

Therefore, the problem of legumes is not that they are not demanded, but that we are unable to produce them in sufficient quantity, at the right price. Returning legume crops to our rotations and market shelves can only be achieved through an integrative approach leading to the adjustment of cropping practises and breeding more adapted, attractive and productive cultivars. Present consumers are pressing research to develop an environmentally friendly production and distribution chain, able to deliver safer, healthier and more varied food to people.

We hope that Legume Perspectives *can effectively contribute to legume revalorization. Let the legumes make the difference!*

Soybean yield improvement: Achievements and challenges

by Joseph W. BURTON[1]* and Lilian MIRANDA[2]

Abstract: Soybean (*Glycine max*) is a major source of vegetable protein and oil in the world. In the United States, average yield increase from 1972 to 1998 was 31.2 kg ha^{-1} each year. It is estimated that about 80% of that yield increase has been due to genetic improvement. Possibly the greatest challenge to increased productivity is global warming and climate change. While increases in CO_2 might be expected to increase productivity, other effects of climate change may counteract, especially drought. Some public breeding programs have placed an emphasis on increasing the genetic diversity by developing germplasm with increased productivity that is at least partially derived from exotic sources.

Key words: breeding, germplasm, soybean, yield improvement

Soybean (*Glycine max* (L.) Merr.) is a major source of vegetable protein and oil in the world. Worldwide demand continues to be high and production has more than doubled in the past 20 years to a total of 264.2 million t in 2011 (9). Much of this increase has been due to increased planting in Argentina and Brazil. But, there have been genetic gains as well.

In the United States, average yield increase from 1972 to 1998 was 31.2 kg ha^{-1} each year (12). Beginning in the early 1990s, many of the smaller soybean breeding seed companies were purchased by what has now become the big three - Monsanto, Du Pont Pioneer, and Syngenta. Recently Bayer Crop Science has joined this group and has begun soybean breeding research. Since then, the average gains have been slightly lower at 29.9 kg ha^{-1}. Based on public and proprietary plant breeding data, it is estimated that about 80% of that yield increase has been due to genetic improvement (12). A recent study of cultivars in maturity groups II, III, and IV estimated yield gains between 1923 and 2008 at 22.8 kg ha^{-1} year^{-1} (5).

These achievements in soybean production are impressive, but there are challenges ahead which may slow the rate of improvement. Possibly the greatest challenge to increased productivity is global warming and climate change. In 2012, a severe drought in the United States corn-belt caused an 8.6% decrease in soybean yields from the previous two years. In addition, some water resources are being depleted. For example, the Ogallala aquifer which is used to irrigate crops in the western states is being used up (1). Now a large number of wells into this aquifer no longer provide water. While increases in CO_2 might be expected to increase productivity, other effects of climate change may counteract those increases. The severe drought in the U.S. corn-belt in 2012 is an example of what the future may hold. In the U.S., it is doubtful that significantly more land will be used for soybean production. At least in the foreseeable future, it is unlikely that land used for maize production will be converted to soybean production and other land resources suitable for soybean production are not available. It may be possible to bring more hectares into soybean production in South America, but that use must be weighed against the need to preserve natural vegetation and water resources, as well as a need to produce other food crops.

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[1] North Carolina State University, Department of Crop Sciences, Raleigh, USA (ltta@ncsu.edu)
[2] USDA-ARS, North Carolina State University, Raleigh, USA

Another challenge to increased productivity is a lack of genetic diversity. In North America, using pedigree relationships, it was shown that 35 ancestors contributed to 95% of genes in the cultivars released between 1947 and 1988 (6). Furthermore, 16 of those 35 contributed 85% of the genes. More recently, a study (7) compared sequence diversity within and between four populations representing elite North American soybean cultivars, Asian landrace founders of those elite cultivars, Asian landraces with no known relationship to the founding stock and wild soybean (*G. soja* Siebold & Zucc.) accessions. This research showed that 79% of rare alleles found in Asian landraces had been lost in modern cultivars. This narrow genetic base would be duplicated in South American soybean production to the extent that South American cultivars derive from North American germplasm. Plant introductions from the U.S.D.A. germplasm collection have been used in breeding programs. But, they have been used primarily as a source of genes for disease and pest resistance in backcrossing programs, and therefore, have contributed little to overall diversity. Another factor which may result in less genetic diversity is the consolidation of smaller seed companies into the three large corporations previously mentioned and the use of utility patents to protect released cultivars (8). This has resulted in reduced germplasm exchange. Overtime, the germplasm pool within each corporation may become narrower. In addition, emphasis in private breeding companies is on adding traits such as disease resistance and herbicide tolerance to already successful cultivars (C. Tinius, pers. comm.). This will also contribute to a narrower gene pool as it reduces genetic recombination and research on genetic diversity.

Some public breeding programs have placed an emphasis on increasing the genetic diversity by developing germplasm with increased productivity that is at least partially derived from exotic sources. Chinese cultivars have been identified which yield from 80% to 88% as much as elite U.S. cultivars (2). High yielding cultivars and germplasm have been developed using these and other introductions from the U.S.D.A. germplasm collection (3, 4, 10, 11). Standard plant breeding practices have been used for this, e.g. hybridization between adapted high yielding cultivars, followed by inbreeding and selection, sometimes with backcrossing.

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Root words relating to grain legumes in the proto-languages of Asia, Europe and North Africa

by Aleksandar MIKIĆ

Abstract: Grain legumes have been known to man long before their domestication. Palaeolinguistics joins the efforts by anthropology, archaeobotany and palaeogenetics in casting more light onto the grain legume use history by attesting the root-words in the proto-languages of Asia, Europe and North Africa. Its results demonstrate a remarkable morphological and semantic similarity between them and their modern descendants, witnessing the significance grain legumes have had in the everyday diets of mankind from its very beginnings.

Key words: crop history, ethnolinguistic families, grain legumes, palaeolinguistics

Introduction

Grain legumes, such as chickpea (*Cicer arietinum* L.), grass pea (*Lathyrus sativus* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), bitter vetch (*Vicia ervilia* (L.) Willd.) and faba bean (*Vicia faba* L.), have been used by humans long before they were domesticated. Their presence was determined in the food remains of the Neanderthal man, dated back to more than 40,000 years ago and from present Iraq (12), in the macroremains of the diets of the Iberian Paleolithic hunter-gatherers 10,000 BC (1) and at the first permanent settlements of the earliest world farmers in modern Syria 8,000 BC (27).

Borean

It is estimated that Near East, Europe and North Africa are the home to more than 300 living and extinct languages classified into numerous ethnolinguistic families (19). The mainstream linguistics usually claims there is no solid basis to reconstruct more distant timely links among them than at a level of the proto-languages of individual families, such as Proto-Indo-European.

On the other hand, several linguistic schools, mostly in Russia and USA, consider assessing the relationships among the attested ethnolinguistic families feasible as far back as more than 10,000 years. In their opinion, only one human community, descending from those who migrated out of Africa via Sinai to Near East, approximately 46,000 years ago (11), survived the peak of the last Ice Age, between 26,500 and 19,000 years ago (6). They bore the Kebaran and the Zarzian cultures (28) and spoke a hypothetical language called Borean (9), that might have been a single ultimate predecessor of all the ethnolinguistic families in most of Asia, Europe and North Africa, between 18,000 BC and 15,000 BC (8).

An increasing number of recent studies in anthropology, archaeobotany, historical linguistics and palaeogenetics move towards a mutual integration (2, 7), that, apart from other issues, opens a possibility of reconstructing the proto-words relating to the plants used by the Borean community. The still unattested Borean word denoting pea or grain legumes in general could be morphologically similar to the roots **KVCV* ('to scratch') and **KVNKV* ('shell'), both being of a description of removing grains from pods by hand (13).

The Borean ethnolinguistic group splitted into two main macrofamilies (25). One of them, Dené-Daic, was spoken by a Borean group that left Near East, from 15,000 BC to 10,000 BC (20). It inhabited Eurasia from Pyrenees to Kamchatka and crossed into North America, bearing the hunter-gatherers culture and being the ultimate precursor of the modern language families and isolates such as Basque, Caucasian, Burushaski, Yenisseian, Na-Dené, Sino-Tibetan and Austric (21). Palaeogentics confirms that the supposed point of diversifying the initially uniform Dené-Daic population may be West Asia (10).

The Borean speakers who remained in Near East evolved into the first world farmers when the last Ice Age finished (5). Their language, Nostratic, spoken between 15,000 BC and 12,000 BC, also had a complex evolution: from 8,000 BC, it gave forth Proto-Afroasiatic, the forefather of modern Semitic languages, Proto-Kartvelian, evolving into Georgian, Proto-Elamo-Darvidian, Paleo-Siberian and the Eurasiatic languages, that finally split into Proto-Altaic, Proto-Indo-European and Proto-Uralic by 5,000 BC (4).

Dené-Daic

Proto-Austric. **ʔbaj* ('a kind of grain legume') > Thai *pi* (a kind of *Vigna* species); **tVk* ('a kind of grain legume') > Khmer *s∂nțe :k* (a kind of *Vigna* species) (18).

Proto-Dené-Caucasian. **xqŏrʔā* (*~-rħ-*) ('a kind of cereal') > Chechen *qö* ('faba bean'), Hunza *γarás* ('pea'), Old Chinese *krās* ('grain') (17); **hVwłV* ('lentil'; 'faba bean') > Avar *holó* ('faba bean'), Basque *ilar* ('pea') (3).

Nostratic

Proto-Afroasiatic. **ʕadas-* ('faba bean') > Arabic *ʕadas-* ('lentil'); **pal-* ('faba bean') > Hebrew *pōl* ('faba bean'); *ŝV(m)bar* ('chickpea') > Gollango *sumburo* ('pea'), Oromo *šumbur-ā* ('chickpea') (15).

Proto-Altaic. **bŭkrV* ('pea') > Kazakh *burşaq* ('pea') (Fig. 1); **ʒi̯ăbsa* ('lentil') > Tatar *jasmyq* ('lentil') (26).

Proto-Dravidian. **parup-* ('grain legume'; 'pea') > Tamil *paruppu* ('pea') (22).

Proto-Indo-European. **bhabh-* ('faba bean' > Russian *bob* ('faba bean'); **er∂g^{w}[h]-* ('the kernel of a leguminous plant'; 'pea') > German *Erbse* ('pea'); **ghArs* ('a leguminous plant') > Serbian *grašak*; **kek-* ('pea') > French *pois chiche* ('chickpea') (Fig. 2); **lent-* ('lentil') > Spanish *lenteja* (16).

The roots in other Nostratic proto-languages relating to grain legumes have not been attested yet, although Proto-Kartvelian **kurć-* ('husk') and **ķaķa* ('grain') (23) and Proto-Uralic **kača* ('cavity'; 'hole') (24) may be among the candidates (13).

Institute of Field and Vegetable Crops, Novi Sad, Serbia (aleksandar.mikic@ifvcns.ns.ac.rs)

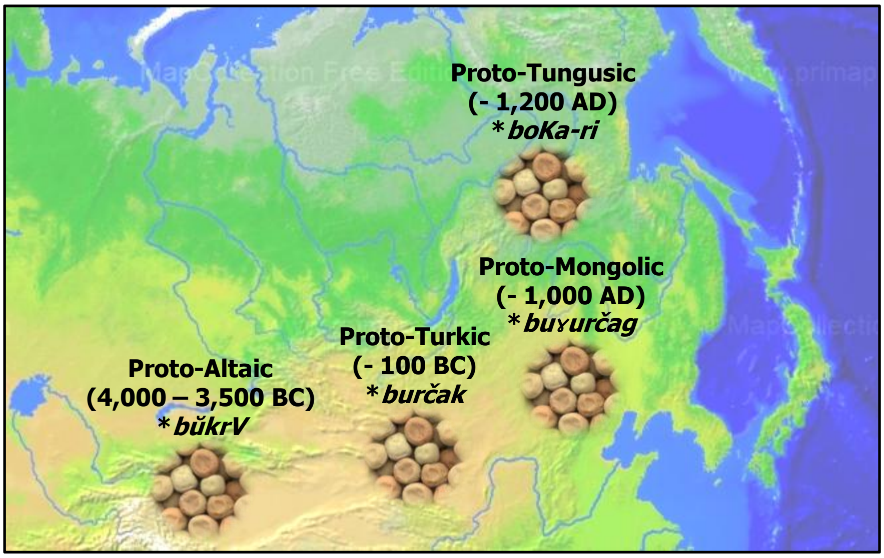

Figure 1. Initial evolution of the Proto-Altaic root ****bukrV*

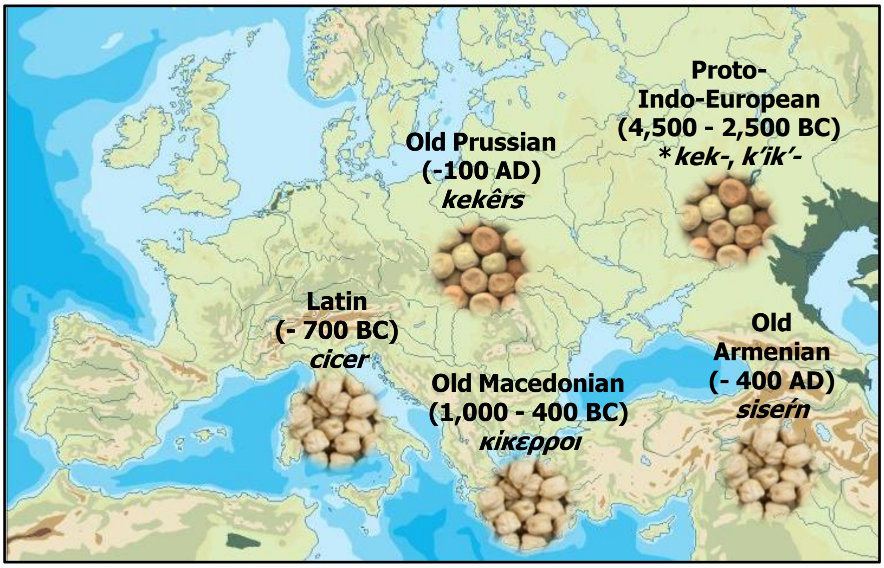

Figure 2. Initial evolution of the Proto-Indo-European root ****kek-*

Conclusions

The palaeolinguistic analysis have brought forth numeorus root words relating to grain legumes in the proto-languages of both Dené-Daic and Nostratic ethnolinguistic macrofamilies, from their direct and indirect derivatives to the modern descendants.

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Minor forage legume crop genetic resources

by Tomáš VYMYSLICKÝ

Abstract: Genetic resources have nowadays increasing importance as a source of wide biodiversity. They are used in breeding programmes in order to overcome problems in crops. Even pure non-bred forage species are popular in agriculture. Successful introduction of these minor crops to seed market is in the most cases limited by low seed yield – caused by pod dehiscence, common seed predation and lack of pollinators in the agro-ecosystems. Modern breeding methods, like marker-assisted selection (MAS), significantly speed up the development of modern cultivars even in minor forages.

Key words: breeding, genetic resources, genebanks, minor legume forages, varieties

The study of genetic resources has nowadays increasing importance because of both genetic erosion in agriculture and global climatic change. Wide spectrum of genetic resources stored in gene banks is essential for breeders, researchers and farmers - all users of this conserved diversity.

The use of plant genetic resources in crop improvement is one of the most sustainable ways to conserve valuable genetic resources for the future while simultaneously increasing agricultural production and food security. The key to successful crop improvement is a continued supply of genetic diversity in breeding programs, including new or improved variability for target traits. Collectively, around one million of samples of grain legume genetic resources are preserved in ex-situ genebanks globally. Managing and utilizing such large diversity in germplasm collections are great challenges to germplasm curators and crop breeders (6).

Concerning the minor forage crops, in the past much more cultivars were used, there were many landraces and local populations that have never been bred and mostly natural forces and positive selection of the farmers have kept the best plants for future generations. In the first half of the 20th century wide spectrum of landraces and cultivars based on landraces were used. Since the fifties, process of significant genetic erosion both in the east and in the west started and only few modern intensive cultivars prevailed. Minor forages were shifted only to marginal areas or enthusiastic and organic farmers.

Since the nineties of the last century, diversity of cultivated crops is increasing. The same situation started in minor forages. New species and varieties have been introduced to cultivation. Nowadays farmers have large spectrum of minor crops and species that could be used even in marginal conditions. But there are still some limitations both in cultivation and in *ex situ* conservation of minor forages.

Successful introduction of these minor crops to seed market is in the most cases limited by low seed yield - caused by their specific agronomic needs and tendency for pod dehiscence, seed dormancy, seed shattering, increased level of seed predation, high variability in blooming and lack of pollinators in the agro-ecosystems (6).

Although modern agriculture feeds more people on less land than ever before, it also results in high genetic uniformity by planting large areas of the same species with genetically similar cultivars, making entire crops highly vulnerable to pest and diseases and for abiotic stresses (5). Thus uniform high-yielding cultivars are displacing traditional local cultivars, a process known as genetic erosion (3).

Legumes, together with cereals, have been fundamental to the development of modern agriculture. Legumes are second only to grasses in importance for human and animal dietary needs. The major crop grain legumes include chickpea (*Cicer arietinum* L.), common beans (*Phaseolus* spp.), cowpea (*Vigna unguiculata* (L.) Walp.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.) and pigeonpea (*Cajanus cajan* (L.) Huth) (6). In forages it is common to observe crosses between the wild relatives, ecotypes, landraces and cultivars from the same location. Landraces developed semi-naturally with local adaptation and local identity (7).

Current situation in perennial clovers breeding, mainly *Trifolium pratense* L. and *T. repens* L. is described in (1). Current achievements and challenges are given in detailed review focused on perennial forages (2). The importance of well described genebank material is stressed in (4). Breeders working with grasses and forage legumes for sustainable agriculture are fortunate in the wealth of the genetic variation available both within the primary species of interest and among related species. The author stressed also concerning the genome analyses that apart from alfalfa significant programmes were developed in the USA and Japan on *Medicago truncatula* Gaertn., *Lotus corniculatus* L. var. *japonicus* Regel and nowadays a genetic map is developed in *T. repens*. The hybrids of *T. repens* with *T. nigrescens* Viv. and *T. ambiguum* M. Bieb. are developed.

Concerning the Common catalogue of varieties of crops (Table 1) in the group of forages only first four species (*Medicago sativa* L., *T. pratense*, *T. repens* and *Vicia sativa* L.) could be considered as major forage crop, based on the number of varieties. All other species belong to minor forages. More than hundred crop wild relatives of leguminous species, which are not listed in the Common catalogue, belong also to minor forage legumes.

Agricultural Research Ltd., Troubsko, Czech Republic (vymyslicky@vupt.cz)

Table 1: The survey of forage crops varieties based on the Common catalogue of the EU; EUR-Lex, 33rd complete edition of the Common catalogue released on November 16, 2014 (4)

Medicago sativa	385
Trifolium pratense	218
Trifolium repens	139
Vicia sativa	132
Trifolium incarnatum	36
Trifolium alexandrinum	35
Lotus corniculatus	30
Trifolium resupinatum	25
Vicia villosa	23
Onobrychis viciifolia	20
Trifolium hybridum	17
Hedysarum coronarium	9
Medicago × *varia*	8
Vicia pannonica	4
Medicago lupulina	3
Galega orientalis	2
Trigonella foenum-graecum	2

Figure 1. *Trifolium pannonicum* Jacq. is perspective minor perennial forage crop for warm and dry regions. It is very attractive for pollinators

In the future tendency of increasing both the number of cultivated species and also varieties of cultivated species will be essential (Fig. 1, 2 and 3). The importance of minor forages will increase mainly in marginal conditions, biodiversity conservation areas and in organic farming. Promising will be their use in anti-erosion measures and as components of species-rich seed mixtures. Increasing importance will have *in situ* conservation.

Figure 2. An interspecific hybrid of *Trifolium pratense* L. and *Trifolium medium* L.: *Trifolium* × *permixtum* Neuman, combining good traits of both parental species; a product of interspecific hybridisation *in vitro*

Figure 3. *Trifolium fragiferum* L. naturally grown on salty soils, with high content of nutrients and nitrogen. Typical are inflated infructescences, resembling hairy brownish raspberry

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Hay quality of some native clover (*Trifolium* sp.) species from Asia Minor

by Ozlem ONAL ASCI[1]*, Metin DEVECI[1] and Zeki ACAR[2]

Abstract: Some clover species (*Trifolium* spp.) grow naturally in cool and moist regions of Turkey. In this study, crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and mineral content of *T. angustifolium* L., *T. arvense* L., *T. aureum* Poll., *T. hybridum* L., *T. meneghinianum* Clem and *T. scabrum* L. collected from natural range areas were determined. When harvested in the flowering stage, CP ranged from 17.3% to 21.3%, while ADF varied between 32.5% and 40.2% and NDF from 38.9% to 55.9%. The ratios of Ca : P and K : (Ca + Mg) ratios were 4.03-5.58 and 0.80-1.52, respectively.

Key words: acid detergent fibre, mineral nutrients, neutral detergent fibre, *Trifolium* spp.

Introduction

The clovers (*Trifolium* spp.) are used for forage, pasture, soil improvement and silage (8). Turkey is the richest Mediterranean country in clover species, with over 100 species in its natural flora (10). Moreover, there are 53 species in the natural flora, especially range and meadows, of the Black Sea Region (4). For this reason, the nutrient content of *T. angustifolium* L., *T. arvense* L., *T. aureum* Poll., *T. hybridum* L., *T. meneghinianum* Clem and *T. scabrum* L. accessions, grown naturally in the Ordu province, was determined.

Material and methods

The accessions of *T. angustifolium, T. arvense*, *T. aureum*, *T. hybridum*, *T. meneghinianum* and *T. scabrum*, growing wild in the coastal districts Fatsa, Gulyalı, Merkez, Persembe and Unye of the Ordu, Turkey, were collected in 2010. In the sampling areas, the soil texture changed from silty clay to sandy clay loam. While the soil organic matter content changed from low to high (1.18% - 3.61%), the phosphorus (P) content varied from 80 kg P_2O_5 ha^{-1} to 1958 kg P_2O_5 ha^{-1}. The potassium (K) content was high, from 346 kg K_2O ha^{-1} to 1620 kg K_2O ha^{-1}, in all the rangelands. The pH values varied from 5.8 to 7.65. The long-term (1970-2011) annual rainfall and mean temperature in Ordu are 1042.7 mm and 14.3 ^{0}C, respectively.

The plant samples, cut in the flowering stage, were dried at 60 ˚C until the constant mass. The contents of dry matter (DM), crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), calcium (Ca), K, magnesium (Mg) and P were determined by using Near Reflectance Spectroscopy (NIRS, *Foss 6500*) with the software package program IC-0904FE. The relative feed value (RFV) was calculated as follows:

DMI = 120 / NDF, % DM basis,
DDM = 88.9 - 0.779 × ADF, % DM basis,
RFV = DDM % × DMI % × 0.775 (3).

All the data were presented as a mean and its standard error. The results were reported on a DM basis.

Results and discussion

It is widely known that CP, ADF and NDF contents in hay are the important properties for evaluating quality (2). In this study, the CP content varied from 17.34 % in *T. arvense* to 21.25% in *T. meneghinianum* (Table 1). In other words, CP in these species is twice or thrice as much as the minimum limit needed for ruminants, which is approximately 7% (5). The ADF and NDF contents of the analysed clover species ranged from 30.52% to 40.24% and from 38.92% to 55.90%, respectively (Table 1). *T. hybridum* and *T. meneghinianum* produced a premium-quality hay, while *T. arvense*, *T. aureum* and *T. scabrum* were classified as having a good-quality forage, according to their RFV values (7).

The mineral compounds have different roles in animal metabolism, making their individual quantity and mutual balance important. While the contents of Ca, K and Mg in the tested clover species were higher than recommended for feeding ruminants, the P content was optimal in *T. arvense* and high in the others (6) (Table 2). All the species in this study had a lower K : (Ca + Mg) ratio than 2.2, causing tetany (4), but also had a higher Ca : P ratio (2.0) probably causing lesser milk production potential (1).

Conclusions

All the analysed wild clover species showed a potential for supplying an optimum or higher crude protein and mineral compunds in the voluminous animal feeds. The species *T. meneghinianum* provided the best hay quality, followed by *T. hybridum*.

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[1] Agricultural Faculty of Ordu University, Ordu, Turkey (onalozlem@hotmail.com, oasci@odu.edu.tr)

[2] Agricultural Faculty of Ondokuz Mayis University, Samsun, Turkey

Table 1. The average contents (%) of crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF) and relative feed value (RFV) and its standard error (SE) in the *Trifolium* species from the wild flora of Ordu, Turkey

Species	CP	SE	ADF	SE	NDF	SE	RFV	SE
T. angustifolium	19.47	0.15	38.37	0.96	55.90	0.34	98.22	1.84
T. arvense	17.34	1.28	40.24	0.68	48.71	1.32	110.11	3.76
T. aureum	19.17	1.15	37.95	0.77	52.56	0.66	105.06	2.41
T. hybridum	19.93	0.94	32.47	1.40	44.81	0.94	132.35	4.88
T. meneghinianum	21.25	1.38	30.52	1.43	38.92	2.13	157.61	11.82
T. scabrum	18.12	1.08	39.11	1.74	48.65	1.38	111.89	5.75

Table 2. The average contents (%) of the mineral compounds and their ratios in the *Trifolium* species from the wild flora of Ordu, Turkey

Species	Ca	SE	Mg	SE	K	SE	P	SE	Ca: P	SE	K : (Ca + Mg)	SE
T. angustifolium	1.91	0.05	0.25	0.04	1.72	0.11	0.34	0.01	5.59	0.13	0.80	0.08
T. arvense	1.61	0.09	0.36	0.02	1.61	0.19	0.31	0.02	5.20	0.06	0.81	0.06
T. aureum	1.64	0.05	0.28	0.01	1.31	0.05	0.36	0.02	4.49	0.16	0.68	0.02
T. hybridum	1.82	0.06	0.43	0.01	2.37	0.12	0.41	0.01	4.43	0.17	1.06	0.08
T. meneghinianum	1.71	0.13	0.34	0.02	3.00	0.41	0.43	0.02	4.03	0.43	1.52	0.29
T. scabrum	1.71	0.01	0.39	0.01	1.89	0.10	0.35	0.04	5.06	0.48	0.90	0.05

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Mutations of determinate growth and their application in legume breeding

by Andrey A. SINJUSHIN

Abstract: Mutations which cause determinate growth pattern in different leguminous species are briefly reviewed. Both their molecular basis and breeding value are in scope. With rare exceptions, the most part of cultivated legumes are characterized with an indeterminate growth pattern, when annual shoot proliferates unlimitedly producing new leaves and axillary racemose inflorescences. The presented short survey on mutations causing determinate growth type (DT) in few legume species may lead to conclusion that genetic basis for different DT types persists through evolution of the family. *TFL1*-dependent control of inflorescence structure seems conservative among Fabaceae, as mutations in *TFL1* orthologs cause similar phenotype.

Key words: inflorescence, Fabaceae, growth pattern

With rare exceptions, the most part of cultivated legumes, such as pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medik.), vetches (*Vicia* spp.), vetchlings (*Lathyrus* spp.), chickpea (*Cicer arietinum* L.), pigeon pea (*Cajanus cajan* (L.) Huth), beans (*Phaseolus* spp.), soybean (*Glycne max* (L.) Merr.), fenugreek (*Trigonella foenum-graecum* L.) and many others, are characterized with an indeterminate growth pattern, when annual shoot proliferates unlimitedly producing new leaves and axillary racemose inflorescences. As a result, crop is usually harvested when lots of floral buds and pods remain of no practical value. A difference between potential productivity and actual yield is therefore striking.

To overcome such disproportion and make seed ripening more synchronous, different monogenous mutations altering growth habit are being introduced into genotypes of cultivated legumes. Different cases of so-called determinate growth type (DT) are briefly described below.

Mutations in *TFL1*-like genes

Studies on a model plant species, *Arabidopsis thaliana* L. (Heynh.) (cruciferous family), uncovered a gene *TERMINAL FLOWER1* (*TFL1*) which maintains shoot apical meristem indeterminate. A terminal flower is formed in *tfl1* mutants. Three orthologs of *TFL1* were initially described in a garden pea (5). One of these orthologs, *PsTFL1a*, was found identical to already known gene, *DETERMINATE* (*DET*). Mutation *det* causes conversion of shoot apical meristem into racemose inflorescence, so the shoot ends with few-flowered raceme and no longer proliferates. Gene *DET* is tightly linked to gene *RUGOSUS* (*R*) which defines seed shape (rounded or wrinkled) and hence assignment of cultivar – grain (*R*) or vegetable (*r*). In Russia, two independently obtained mutants (*det r* and *det* R) were used for breeding new determinate vegetable and grain cultivars, respectively (6).

Combining recessive mutations *det* and *fa* results in production of weakly fasciated determinate peas which bear many-flowered apical raceme, somewhat similar to one of lupines (10, Fig. 1A). These forms were called "lupinoid" and are of potential value for breeding.

Using candidate gene approach, it was later demonstrated that mutations in orthologous *TFL1*-like loci cause DT in few other leguminous species: *Vicia faba* (1), *Phaseolus vulgaris* (8), *G. max* (11), and, recently, in *C. cajan* (9). Data on molecular basis for such mutations provides possibility of marker-assisted selection in breeding of new cultivars. Phenotypically similar forms with anomalous terminalized inflorescence were recorded in different alfalfa species, such as *Medicago sativa* (4) or *M. lupulina*. Most probably, these cases have the same molecular basis as in aforementioned leguminous taxa.

In some species, genetic control of shoot determinacy is more complicated than monogenous. For example, two non-allelic mutations, *dt1* and *dt2* (one of them disrupting a *TFL1*-dependent pathway), predispose to DT in *P. vulgaris* (3). In pea, different lines exist which have either 2 or 3-5 lateral racemes on *det* background (6). The latter phenomenon still awaits exploration.

Other types of DT

One more type of shoot determinacy is known in pea dealing with apex dying back rather than with production of ectopic terminal raceme. This DT is predisposed by mutation *determinate habit* (*deh*). Although possessing variable expression and possibly semidominant inheritance (2), this mutation is introduced into some Russian cultivars (e.g. Flagman 7).

M.V. Lomonosov Moscow State University,
Moscow, Russia (asinjushin@mail.ru)

Although species of the genus *Lupinus* L. normally have determinate shoots with multiflorous terminal racemes, a problem of breeding new cultivars with reduced lateral branching nevertheless exists. A genetic control of lateral branching is complex, but some mutations cause suppression of lateral shoots growth via conversion of axillary branch meristems into floral ones (7). As a result, single flowers are borne in axils of vegetative leaves (Fig. 1B). Similar phenotypes were discovered in *L. angustifolius* (narrow-leafed lupin) and *L. luteus* (yellow lupin) and such phenotype is called determinate in this genus.

Mutant with DT was induced in chickpea but, in addition to inflorescence determinacy, it also had floral abnormalities, sterility and reduced leaflet number (12). It has no practical value and its phenotype resembles pea mutants *unifoliata* (*uni*) more than *det*. The *uni* mutants also have flower and inflorescence distortions together with reduction of leaf complexity.

The presented short survey on mutations causing DT in few legume species may lead to conclusion that genetic basis for different DT types persists through evolution of the family. *TFL1*-dependent control of inflorescence structure seems conservative among Fabaceae, as mutations in *TFL1* orthologs cause similar phenotype. Possibly screening germplasm collections for mutations in certain loci or site-directed mutagenesis can result in breeding novel cultivars with desired inflorescence architecture.

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Figure 1. Determinate habit in pea (A) and yellow lupin (B): pea plant is also characterized with weak fasciation ("lupinoid" phenotype); single flowers in leaf axils are marked with arrows; photos were generously provided by Nina A. Vykhodova (A) and Vera S. Anokhina (B)

Leaves leave when flowers flower

by Julie HOFER[1]*, Nathan GREENAWAY[1], Mike AMBROSE[2], Eric FERRÉ[2], Francisco MADUEÑO[3], Cristina FERRÁNDIZ[3] and Noel ELLIS[3]

Abstract: In this mini-review we describe some of the molecular processes known to underlie inflorescence and flower development in arabidopsis (*Arabidopsis thaliana*) and consider them in the context of legumes. We pay special attention to what molecular mechanisms might be involved in the partitioning of leaf and flower development in pea (*Pisum sativum*) and barrel medic (*Medicago truncatula*), whereby leaf development is shut down on lateral I2 inflorescences and flowers, but leaves continue to be produced on the main shoot inflorescence, known as the I1.

Key words: *AGAMOUS*, compound leaf, flower development, *Pisum*, *Medicago*

What happens when a plant flowers? Existing models are based on a scheme whereby incoming molecular signals, triggered by cues such as photoperiod and temperature, are integrated at the plant shoot apex. Growth continues, but developmental gene networks in the shoot are now modified to produce inflorescences. These produce meristems within which floral developmental programs have been activated (9). Here we focus in particular on relationships between flower and leaf formation.

Legumes such as pea (*Pisum sativum* L.)have a compound inflorescence, consisting of the main shoot inflorescence, I1, plus lateral inflorescences, I2s. In contrast, arabidopsis (*Arabidopsis thaliana* (L.) Heynh.) has only a simple inflorescence, the I1. In pea, transcription factors defining the identity of the I1, I2 and floral meristems are encoded by the genes *DETERMINATE* (*DET*), *VEGETATIVE1* (*VEG1*) and *PROLIFERATING INFLORESCENCE MERISTEM* (*PIM*), respectively (3). *PIM* and *VEG1* are both MADS-box transcription factors. When pea transitions from the vegetative phase of growth to the flowering phase, the main shoot becomes an I1, though it continues to produce compound leaves at each node, just as it did during the vegetative phase. When Arabidopsis flowers, it gradually suppresses leaf development on the I1: early lateral inflorescences are subtended by leaf-like bracts, but later flowers are produced in the axils of very reduced, rudimentary bracts (1). An equivalent process of suppression of leaf development seems to occur on the I2 in pea, where flowers can be subtended by bracts (11), but compound leaves are absent. Leaf development is fully suppressed on the floral meristems of both species. What molecular mechanisms might be involved in this developmental partitioning in legumes, whereby leaf development is shut down on the I2 and flowers, but continues on the I1?

More than two decades ago it was hypothesised that certain combinations of MADS box genes expressed in cells of the floral meristem act to confer floral organ identity: when those gene functions were disabled in arabidopsis, leaf-like structures formed instead of floral organs (4). It took another ten years to confirm those findings and further demonstrate that the encoded MADS box proteins act together in transcription factor complexes: floral organs formed in place of leaves when MADS box genes were co-expressed throughout the plant (5, 8).

Recent studies of MADS box protein complexes in flowers have revealed that they are large agglomerations of transcriptional machinery, including other types of transcription factors, such as homeodomain proteins (12) and these complexes directly target hundreds of other genes for activation or repression (6, 15). In so doing, are MADS box proteins actively repressing leaf developmental programs in flowers, or simply over-riding them by activating new floral organ development programs? Analysis of 225 targets of the MADS box gene *AGAMOUS* (*AG*) showed that many are regulatory genes, which in turn directly repress genes known for their roles in leaf development (7). As a demonstration, these authors used an artificial microRNA to knock down *AG* expression during flower development. This resulted in flowers with carpels that exhibited branched trichomes, typically found on leaves. Therefore, at least one leaf development process, branched trichome formation, is actively suppressed by *AG* during normal flower development in arabidopsis.

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[1]Aberystwyth University, Institute of Biological, Environmental & Rural Sciences, Aberystwyth UK (J.Hofer@cgiar.org)
[2]John Innes Centre, Norwich, UK
[3]Instituto de Biología Molecular y Celular de Plantas, UPV-CSIC, Valencia, Spain

What would an *ag* mutant look like in pea or barrel medic (*Medicago truncatula* Gaertn.)? Loss of AG function in arabidopsis flowers results in petals replacing stamens and additional flowers replacing carpels (4). Intruigingly, there are two *AG* genes in pea and barrel medic (Fig. 1, 10) in contrast to Arabidopsis, which has only one. It has been proposed that these legume gene paralogues share the *AG* role in flowers because an *ag*-like floral mutant phenotype was observed only when both genes were knocked out by virus-induced gene silencing (10). Recently an existing spontaneous pea mutant with *ag*-like flowers (Fig. 2) was investigated as a possible pea *ag* mutant. Preliminary work showed that this *ag*-like mutation segregated as a single Mendelian recessive gene, loosely linked to the *a* locus on linkage group II (unpublished data). The known map locations of *PsAGa*(*PsPM7*), on linkage group VII and *PsAGb*(*PsPM12*), on linkage group VI, means that neither of these *AG* paralogues is likely to correspond to the *ag*-like mutation.

Leafy bracts are present in the flowers of a pea *pim* mutant, which lacks an *AP1*-like MADS box gene (14) capable of conferring *AP1* functions (2). The inappropriate presence of leafy bracts in *pim* mutants may be explained because the lack of just one MADS box component can perturb the functioning of the whole transcription complex. Since *AG* and *AP1* are known to participate together in MADS-box protein complexes (5, 12), it follows that many leaf development genes which are no longer repressed in *ag* mutants (7) may also be de-repressed in *ap1* (or *pim*) mutants.

The *VEG1* MADS box gene of pea confers I2 inflorescence identity (3). This I2 grows out from the flanks of the I1 in an additional developmental step compared to arabidopsis. It would seem likely that MADS-domain protein complexes with different compositions are competing for interacting partners and DNA-binding sites within legume I2 meristems, just as they are known to do in floral meristems. It also seems likely that as a consequence of those interactions, compound leaf development in the I2 is suppressed, just as leaf development in the floral meristem is suppressed, but this remains to be demonstrated.

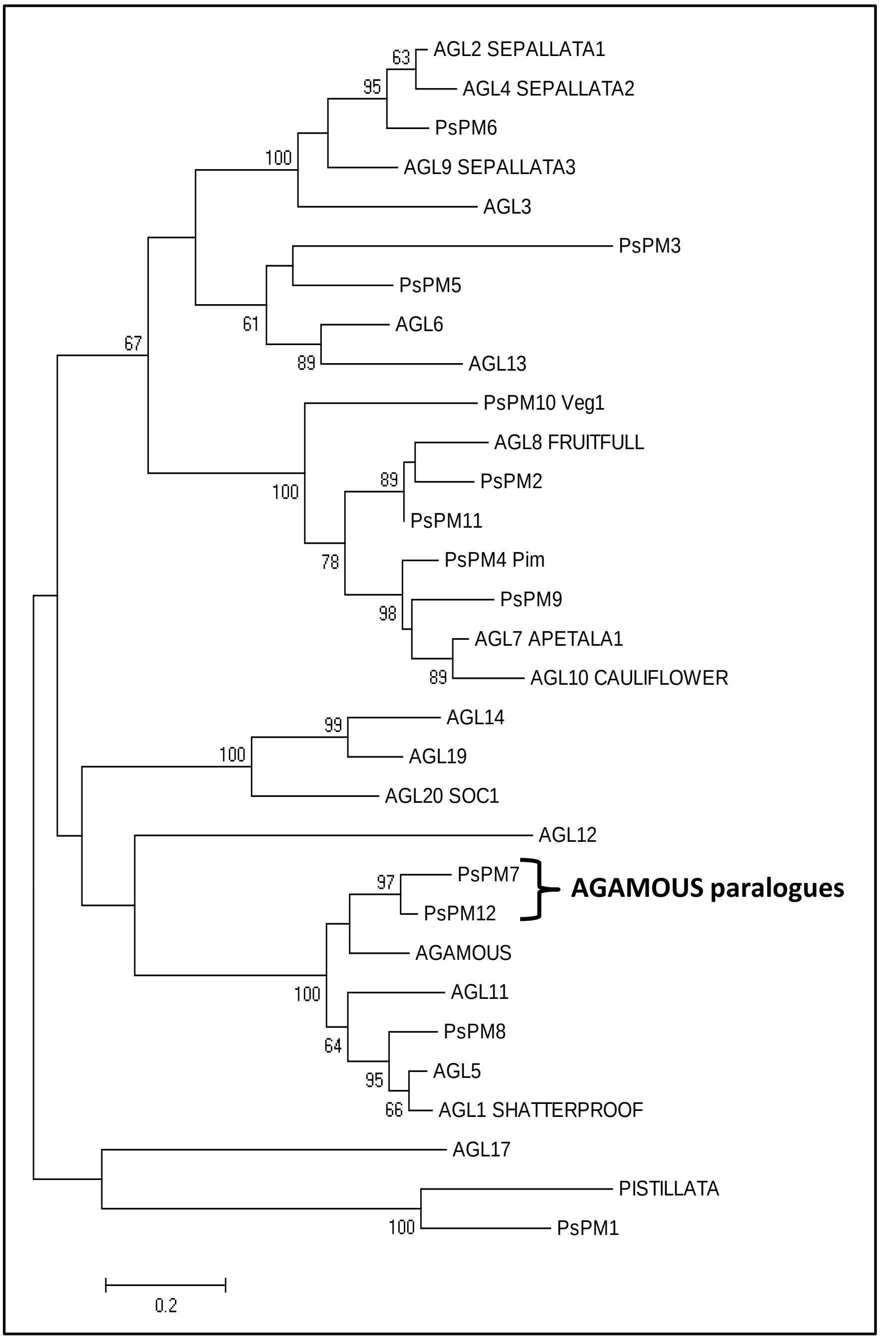

Figure 1. Molecular phylogenetic analysis of pea and arabidopsis MADS-box protein sequences: MADS and K domains were aligned with ClustalOmega; phylogeny was inferred in MEGA6 (13) using the Maximum Likelihood method based on the JTT matrix-based model; a total of 147 positions was included; the percentage of trees in which the associated taxa clustered together is shown next to the branches, with support < 50% not shown; the tree (log likelihood = -5210) is drawn to scale, with branch lengths measured in the number of substitutions per site; pea sequences are annotated Ps, all others are arabidopsis

Figure 2. Wild-type pea (left) and *agamous*-like mutant (right)

As genome sequence information from legumes increases and additional mutants are characterised, we can expect the protein participants regulating organ production at each developmental stage to be identified. Could suppression of leaves in the I2 and floral meristems of legumes turn out to be a running battle of the bulges? Has either of the legume *AG* paralogues acquired additional roles in the I2 of legumes? These and many further questions remain for future research.

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Improving the health benefits of pea

by Thomas D. WARKENTIN

Abstract: Increasing the nutrient density of field pea is a current breeding objective. Efforts are underway to increase the concentration and bioavailability of micronutrients including iron and carotenoids. Variability for these traits useful for breeding has been identified. The low phytate trait provides a substantial benefit in terms of iron bioavailability.

Key words: bioavailability, breeding, iron, pea, phytate

Field pea seeds, like those of other pulse crops, are rich in protein, slowly digestible carbohydrates, and fiber. Recently, the British Journal of Nutrition published a supplement on “The nutritional value and health benefits of pulses for obesity, heart disease and cancer” emphasizing the value of pulse consumption on key health characteristics (12).

One in three people in the world suffer from a lack of vitamins and minerals in their diet, particularly vitamin A, zinc, and iron. HarvestPlus, a program of the Consultative Group on International Agricultural Research (http://www.cgiar.org/our-research/challenge-programs/harvestplus/), has the objective of breeding staple foods with greater concentrations of these key nutrients, a process called biofortification, and this is also a goal of the pulse crop breeding program at the University of Saskatchewan.

All measured values for heavy metals including cadmium, arsenic, lead and mercury in pea samples harvested in western Canada were below the Maximum Residue Levels established by the FAO and WHO in the Codex Alimentarius (3). Field pea, common bean, chickpea, and lentil grown at several locations in Saskatchewan contained significant proportions of the recommended daily intake (RDA) for magnesium, potassium, iron, zinc, manganese, copper, and selenium (Se) (6). In many cases a 100 g (dry weight) portion provided over 50% of the RDA (Table 1). The effect of location was highly significant in most instances, that of year and cultivar generally less so. Total Se concentrations of 17 field pea cultivars evaluated over six locations × two years in Saskatchewan ranged from 373 μg kg^{-1} to 519 μg kg^{-1}, corresponding to 68% - 94% of the RDA (11). In an evaluation of cultivar, year and location on Se concentration in pea, 25% of the variability was due to soil edaphic factors, particularly organic C and pH, and this increased to 39% with inclusion of great soil group classification (2). The remaining variability was due to growing season weather conditions, with hotter drier summers leading to higher Se concentrations.

Phytate is often considered an antinutrient in crop seeds; although it is the major storage form of phosphorus, it is relatively indigestible by humans and chelates minerals including iron. Field pea lines were identified with a 60% reduction in phytate-phosphorus concentration in seeds, while the inorganic (available) phosphorus concentration increased by a similar amount (12). These lines were relatively sound agronomically, but with a 15% yield penalty compared to their progenitor, and breeding efforts are in progress to overcome this deficit. A single recessive gene controls the low phytate trait (7), and it has been mapped on the pea genome (8, 9). Iron bioavailability of seed of the low phytate lines was 30% - 100% greater than those of their progenitor depending on their location of production (5, Fig. 1). In broiler chickens, the total tract apparent availability (TTAA) of phosphorus was higher ($P < 0.02$) for broilers fed a low-phytate pea diet than for birds fed the normal pea diets (10). Increasing the availability of the phosphorus in peas could mean that less inorganic phosphorus would be required in order to meet the nutritional requirements of broilers. Since inorganic phosphorus sources tend to be expensive, a reduction in their use would lower ration costs. In addition, increased availability of phosphorus would reduce the amount of phosphorus excreted, thus reducing the amount of phosphorus that can potentially pollute the environment.

University of Saskatchewan, Crop Development Centre, Department of Plant Sciences, Saskatoon, Canada (tom.warkentin@usask.ca)

In terms of carotenoids in mature seeds, mean lutein concentration ranging from 7.2 µg g-1 to 17.6 µg g-1 and 6.3 µg g-1 to 11.0 µg g-1 in pea and chickpea, respectively (4). Violaxanthin, zeaxanthin, and β-carotene were also present in harvested seeds of both crops. Green cotyledon pea cultivars had approximately double the total carotenoid concentration (16 µg g^{-1} - 21 µg g^{-1}) compared to yellow cotyledon pea cultivars (7 µg g^{-1} - 12 µg g^{-1}).

Cultivar had a greater effect than environment on carotenoid concentration in both crops. The potential synergistic effects of low phytate concentration and green cotyledon on iron bioavailability in pea are being investigated.

Table 1. Percentage of Recommended Daily Allowance (RDA) provided by 100 g (dry weight) of common bean, chickpea, field pea and lentil (6)

Component	Male	RDA female	Units	% of RDA in 100 g dry weight							
				Bean		Chickpea		Field pea		Lentil	
				Male	Female	Male	Female	Male	Female	Male	Female
K	4700	4700	mg	44	44	n/a	n/a	22	22	20	20
Mg	420	320	mg	54	70	40	53	28	36	24	31
Ca	1000	1000	mg	n/a	n/a	5	5	n/a	n/a	3	3
Zn	11	8	mg	27	38	23	31	27	38	42	58
Fe	8	18	mg	87	39	65	29	67	30	109	48
Mn	2.3	1.8	mg	56	72	104	133	54	69	60	76
Cu	0.9	0.9	mg	113	113	78	78	64	64	90	90
Ni†	1	1	mg	60	60	n/a	n/a	27	27	15	15
Se	55	55	µg	81	81	133	133	85	85	215	215

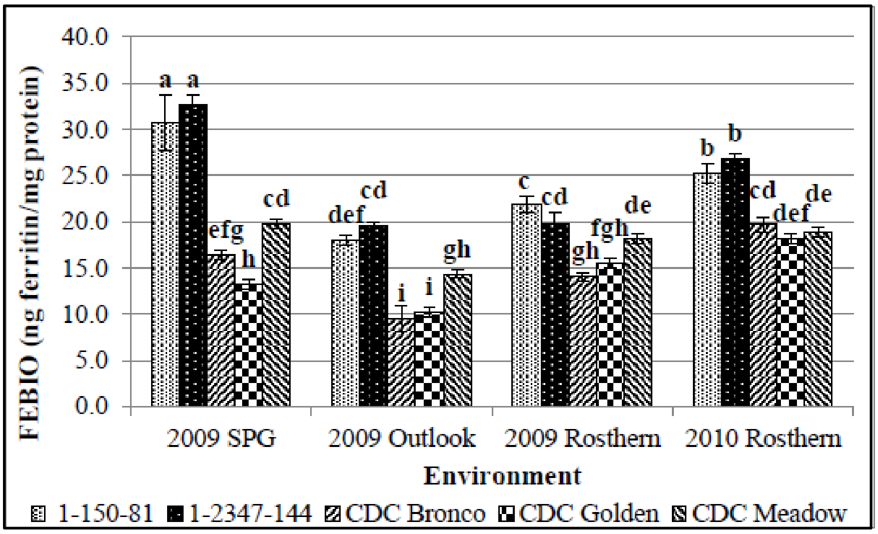

Figure 1. FEBIO (iron bioavailability) of two low phytate lines (1-150-81, 1-2347-144) and three normal phytate varieties (CDC Bronco, CDC Golden and CDC Meadow) at 2009 SPG, 2009 Outlook, 2009 Rosthern and 2010 Rosthern environments; letter grouping by Tukey's Mean Comparison of the interaction (variety × environment) above bars indicates significant differences ($P \leq 0.05$); error bar shows the standard error of the means (5)

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Forage legumes in ruminant production systems

by Padraig O'KIELY*, Edward O'RIORDAN, Aidan MOLONEY and Paul PHELAN

Abstract: Forage legumes can make an important contribution to the nutrient requirements of ruminants and with a lesser environmental challenge than some alternative systems. In addition to reducing or eliminating the requirement for purchased inorganic fertiliser nitrogen, forage legumes can support the efficient production of quality meat and milk. However, the extent of their use has declined in recent decades. This reflects the perception of commercial farmers that the balance of advantages vs. disadvantages for forage legumes is inferior to other feed provision options. Farmers need to see the successful use of forage legumes demonstrated within competitively profitable meat and milk production systems to convince them to incorporate these crops in their ruminant production systems.

Key words: forage, grazing, hay, legume, ruminant, silage

Many forage-based ruminant production systems have members of *Poaceae* as the primary feed source. These crops range from highly productive, recently sown monocultures to permanent pastures of diverse botanical composition. Forage legumes are also grown in monocultures or are included in some of the graminoid-based crops on farms. Their most immediately compelling benefit is if their presence increases the overall profit of the livestock enterprise. This is most likely to derive from an increase in herbage yield or nutritive value, or a reduction in the cost of providing livestock with feed. In some circumstances, profit may be enhanced by a premium price for the animal product sold.

The Food and Agriculture Organisation of the United Nations lists 153 different legumes used as forages (www.feedipedia.org), but those that are most widely used in agriculture are lucerne (*Medicago sativa* L.), white clover (*Trifolium repens* L.), red clover (*Trifolium pratense* L.), subterranean clover *(Trifolium subterraneum* L.) and birdsfoot trefoil *(Lotus corniculatus* L.). Their primary agronomic benefits are (i) their contribution to the nitrogen (N) economy of agricultural land due to their association with N fixing bacteria and (ii) their ability to increase herbage production, herbage feed value and ultimately meat and milk production by ruminants, particularly in areas of low fertiliser N input.

Forage legumes provide a diverse suite of opportunities for ruminant production systems. Some legumes are most productive when managed under a grazing regime (e.g. clone-formers such as white clover and kura clover (*Trifolium ambiguum* M. Bieb.)) while others perform best under a conservation regime (e.g. upright crown-formers such as red clover and lucerne). Some are typically grown in grassland (e.g. clovers) while others (e.g. vetches (*Vicia* spp.), forage pea (*Pisums ativum* L.)) tend to be sown in mixtures with annual small-grain cereals. Some can be grown successfully under conditions of low soil fertility (e.g. birdsfoot trefoil, crown vetch (*Securigera varia* (L.) Lassen), sainfoin (*Onobrychis viciïfolia*Scop.), cicer milkvetch (*Astragalus cicer* L.)), soil acidity (e.g. birdsfoot trefoil), drought (e.g. lucerne, sainfoin), poor drainage (e.g. alsike clover (*Trifolium hybridum* L.)) or cold winters (e.g. lucerne, kura clover, cicer milkvetch) (3). Some lead to the risk of bloat when grazed by ruminants (e.g. clovers, lucerne) while others do not (e.g. sainfoin, birdsfoot trefoil, crown vetch, cicer milkvetch) (3).

Forage legumes present some unique advantages and disadvantages for ruminant production. When compared to grasses or cereals their main advantages are generally a low reliance on fertiliser nitrogen (N) inputs, a high voluntary intake and animal production when feed supply is non-limiting, and a high protein content. They also tend to have less negative environmental impacts on biodiversity, N losses to water and greenhouse gas emissions. The main disadvantages of forage legumes are generally a lower persistence than grass under grazing, a risk of livestock bloat and difficulties in their conservation as silage or hay. In comparison to grass or legume monocultures, grass + legume mixtures can provide increased herbage production, more balanced rations and improved resource use efficiency. However, maintaining the optimum legume content (40% - 60% of herbage dry matter) to achieve these benefits remains a major challenge on farms. In addition, forage legume-based swards struggle to achieve the herbage yields feasible with graminoid crops receiving maximal inputs of inorganic fertiliser N. This can result in a lower intensity of animal production per ha of land.

Despite the benefits to farmers and wider society of forage legumes being used in ruminant production systems, their use remains low or is declining relative to other forages (1, 5, 6). This is most likely a result of their disadvantages being perceived to outweigh their advantages on farms (and the disadvantages may be under-reported in scientific publications (4)). However, this may change if the price ratio of fertiliser N to product (meat/milk) continues to increase as it has done in some regions in recent years (2). A continuation of this progression is expected to greatly increase the use of forage legumes relative to grass produced using

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Teagasc, Grange, Dunsany, Ireland
(padraig.okiely@teagasc.ie)

inorganic N fertiliser. A different outcome transpires when forage legumes are compared with maize silage plus soyabean meal for providing ruminants with much of their energy and protein needs. This reflects the potentially high yields achievable with the latter two crops compared to swards with forage legumes, the reality that soyabean also fixes atmospheric N, and the fact that much of the inorganic N requirement of forage maize can be provided as recycled manure produced by housed cattle. It is also influenced by the relative ease of ensiling maize compared with forage legumes, and the predictably high energy and protein value of the maize plus soyabean meal ration.

The integration of forage legumes into ruminant production systems by farmers has been low despite empirical studies suggesting potential benefits to the farm business. Agricultural knowledge transfer activities need to include the farm-scale demonstration of profitable meat and milk production systems in which forage legumes make a long-term and meaningful contribution to their economic, environmental and social sustainability. In addition, farmers require an enlarged skill-set if they adopt systems that include forage legumes. However, the adoption of forage legume technologies will primarily hinge on farmers being convinced of their full economic merit.

Figure 1. Field plots aimed at assessing the role of legumes in ruminant feeding at Grange, Ireland

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Schemes for varietal mixtures of pea with different leaf types

by Aleksandar MIKIĆ[1]*, Svetlana ANTANASOVIĆ[2], Branko ĆUPINA[2]* and Vojislav MIHAILOVIĆ[2]

Abstract: The Faculty of Agriculture and Institute of Field and Vegetable Crops, both from Novi Sad, Serbia, developed the basic principles for both mutual intercrops and varietal mixtures of legumes. A specific form of intercropping the 'short' cool season annual legumes are varietal mixtures, with the research on pea varieties with different leaf types as the most advanced so far. The sole crops of the varieties with the three tested pea leaf types have their own advantages and shortcomings. The mixtures of wild-type and *afila* varieties proved as the optimum balance between yield and weed control and thus are recommended for using in a wide commercial production.

Key words: intercropping, land equivalent ratio, leaf types, pea, varietal mixtures, yield

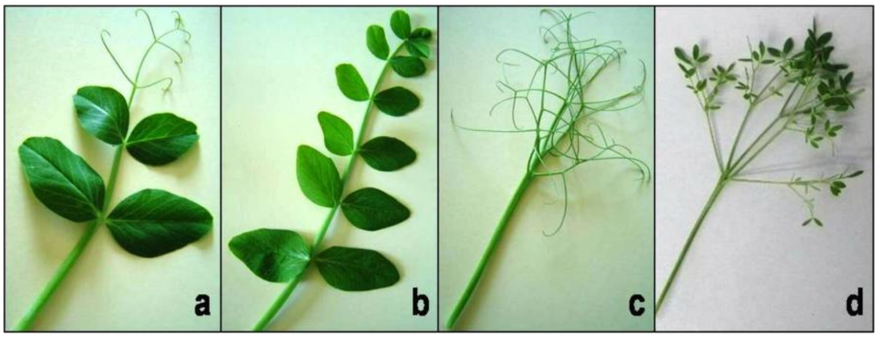

Figure 1. Four basic types of the pea leaf type, regarding leaflets and tendrils (8): (a) wild type, *AFAF TLTL*; (b) *tendril-less, AFAF tltl*; (c) *afila, afaf TLTL*; (d) *afila-tendril-less, afaf tltl*

The agronomists worldwide consider the term *intercropping* a simultaneous cultivation of at least two field crops at the same location (12), without obligatory sowing or harvesting them together. This is globally one of the most ancient cropping systems. Intercropping was widely used by the first farmers in Near East at the beginning of Neolithic agricultural revolution, about 10,000 years ago, where the first domesticated plant species, such as cereals and legumes, were sown, grown, harvested and used together, mostly for human consumption (1).

Annual legumes, such as grass pea (*Lathyrus sativus* L.), white lupin (*Lupinus albus* L.), pea (*Pisum sativum* L.), vetches (*Vicia* spp.) and faba bean (*Vicia faba* L.), are traditionally intercropped with cereals (6), especially common wheat (*Triticum aestivum* L.), and brassicas (3) for both forage and grain production and many beneficial effects for both components (11).

Since the available results of the research on intercropping legumes with each other are rather scarce and without a clearly defined methodology, Faculty of Agriculture and Institute of Field and Vegetable Crops, both from Novi Sad, Serbia, developed the basic principles for both mutual intercrops and varietal mixtures of legumes.

The first of these schemes describes the use of annual legume, usually pea, as a companion and bioherbicide crop in establishing perennial forage crops, such as lucerne (*Medicago sativa* L.), sainfoin (*Onobrychis viciifolia* Scop.) or red clover (*Trifolium pratense* L.) (4).

Another scheme postulates the principles for intercropping annual legumes and mixing varieties of individual species with each other (9). Two components in a mixture should have 1) the same time of sowing, 2) the similar growing habit, 3) the similar time of maturing for cutting (for forage production) or harvest (for grain production) and 4) one component has good standing ability (supporting crop) and another is susceptible to lodging (supported crop). Thus the mutual legume intercrops of annual legumes may be classified into 1) 'tall' cool season, 2) 'short' cool season and 3) warm season ones (10). There is a solid basis for establishing breeding for intercropping (5).

A specific form of intercropping the 'short' cool season annual legumes are varietal mixtures, with the research on pea varieties with different leaf types (Fig. 1) as the most advanced so far in comparison to the other annual legume crops.

The sole crops of the varieties with the three tested pea leaf types have their own advantages and shortcomings (8). The wild-type varieties satisfactorily fights with weeds (Fig, 2), but suffers from lodging (Fig. 2, left top); the *afila*-leafed varieties has an excellent standing ability, but are easily infested with weeds (Fig. 2, left middle); the *tendril-less* varieties often produce abundant aboveground biomass and totally suppress weeds (7), but severely lodge and loose a considerable proportion of forage yield since lower leaves are in a total shade and rather early and fully wither (Fig. 2, left bottom).

The mixtures of wild-type and *afila* varieties proved as the optimum balance between yield and weed control and thus are recommended for using in a wide commercial production (Fig. 2, right top, and Fig. 3) (2). On the other hand, mixing the *tendril-less* varieties with both wild-type and *afila* ones has more disadvantages than benefits, mostly due to a great physical pressure on the plants of the other two leaf types, eventually resulting in lower yield.

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[1] Institute of Field and Vegetable Crops, Novi Sad, Serbia (aleksandar.mikic@ifvcns.ns.ac.rs)
University of Novi Sad, Faculty of Agriculture, Department of Field and Vegetable Crops, Novi Sad, Serbia (cupinab@polj.uns.ac.rs)

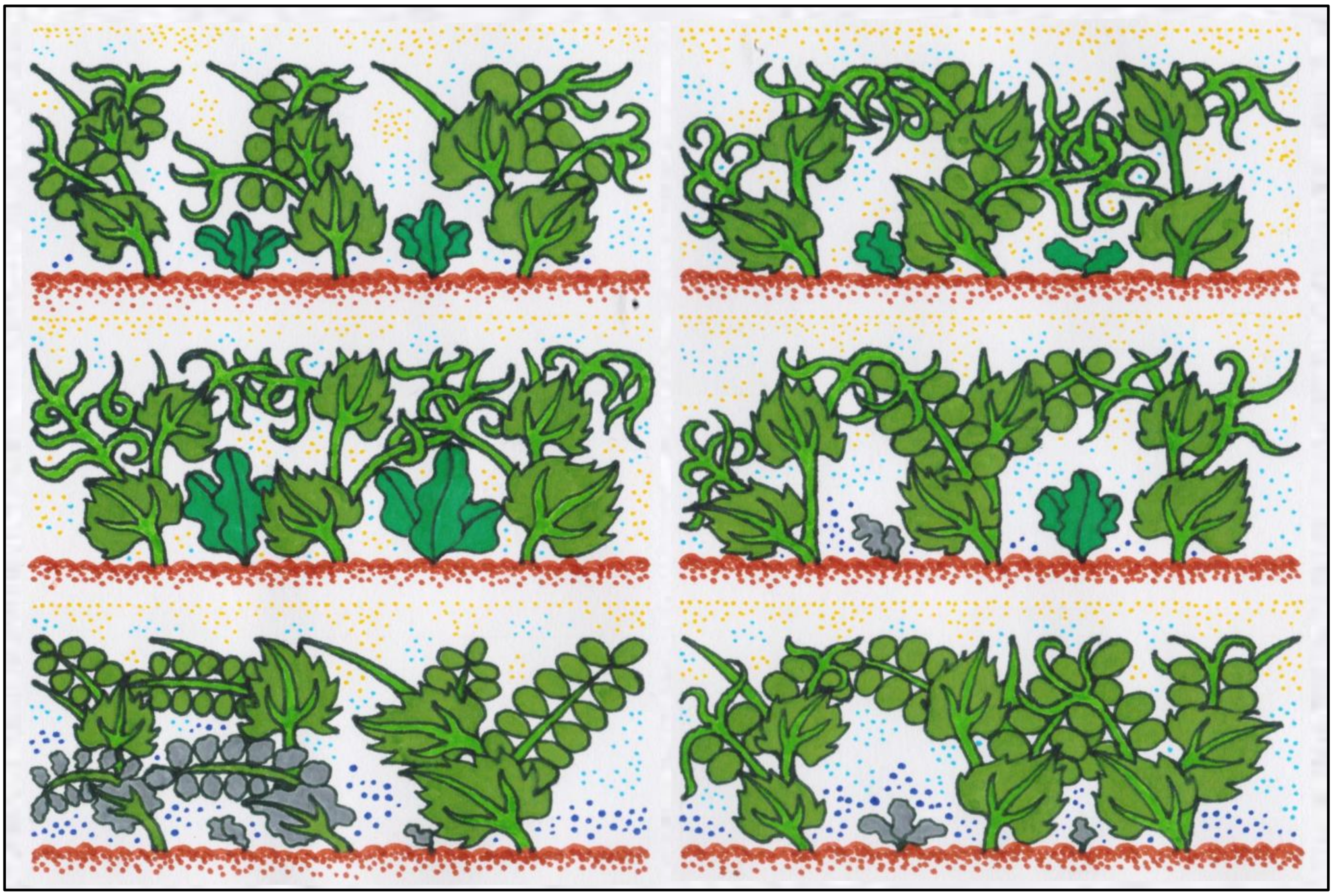

Figure 2. Sole crops of wild type (left top), *afila* (left middle) and *tendril-less* (left bottom) varieties and mixtures of wild-type and *afila* (right top), *afila* and *tendril-less* (right middle) and wild-type and *tendril-less* varieties (right, bottom)

Figure 3. A mixture of spring-sown wild-type and *afila* pea varieties

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Modern biology analysis of the legume tree *Pongamia pinnata* as a sustainable biofuel source

by Peter M. GRESSHOFF

Abstract: The legume tree *Pongamia pinnata* (also called *Millettia pinnata*) has potential as a biological feed stock for biofuel synthesis through a high capacity of plant-oil rich seeds, nitrogen fixation and salinity tolerance. It also has additional biological products such a seed protein cake and biochar derived from waste plant material. Pongamia oil is suitable for direct energy use in the electrical industry, or transesterification or hydrogenation for biodiesel and aviation jet fuel. We combine modern biological analyses with phenotypic analyses in the glasshouse and various field environments to quantify traits of commercial significance and develop the capacity to advance the genetic capabilities of the tree through targeted selection.

Key words: bioenergy, life cycle, molecular biology, seed oil, silviculture

Pongamia biology

Pongamia pinnata (L.) Pierre (also called *Millettia pinnata* (L.) Panigrahi) is a legume tree characterised by fast vegetative growth (Fig.1A, 1B and 1C), dark-green foliage (Fig 1E), beautifully clustered flowers of long life span for ornamental purposes (Fig. 1D), and abundant oil-rich seed production, with 10,000 seeds tree^{-1} - 40,000 seeds tree^{-1} have been observed; Scott et al, 2008 (3, 11). The seeds are rich in non-edible vegetable oil (Fig. 3A) comprised of 50-55% mono-unsaturated oleic (C18:1, Fig. 3A), optimal for renewable biodiesel production (11). On average, seed dry weight for superior trees (called 'elite trees') is about 2.0 gm seed^{-1} - 2.5 gm seed^{-1} (Fig. 1E and 1F), suggesting a potential oil yield of 3 t ha^{-1} year^{-1} - 5 t ha^{-1} year^{-1}. Anticipated oil cost are about 500 USD t^{-1} - 600 USD t^{-1}, competitive with crude oil prices at present. Pongamia oil, itself a biofuel for electricity generation, can also be converted by hydrogenation, as compared to transesterification for biodiesel production, to aviation A1 jet fuel (8).

Pongamia distribution

Pongamia is native to India, South-East Asia and Northern Australia, is salinity-tolerant (upto an electric conductivity of 20 dS m^{-1}) and drought-tolerant (4 months rain-less period at high temperatures have been tolerated in Australia and India), once established with deep roots (2, 3). Relatives are also found in Indochina, Southern China and islands north of Australia. The true source of diversity is unknown. Trees have been found in Madagascar, Hawaii and Florida, but there most likely due to human distribution.

Pongamia nodulation

As a legume it has the ability to nodulate with coralloid nodules and fix atmospheric nitrogen (Fig. 1G, 10), thus eleviating extentive fertiliser supplementation commonly found with non-legume feedstock species for biofuels, such as canola, oil palm, switchgrass, corn, jatropha, and others (3, 5, 9). Nodules are initially spherical but develop new nodes, taking on a coralloid shape. Acetylene reduction and plant growth benefits were verified.

Pongamia genetics

Pongamia is a true diploid ($2n = 22$). The chromosomes are small and relatively uniform. The nuclear genome is estimated by flow cytometry to be around 1300 Mb per haploid genome, making it somewhat equivalent to that of soybean. The assembly and annotation of both the chloroplastic and mitochondrial genomes after DNA deep sequencing (Fig. 3D) was published (7). The tree can flower one year after seed germination, but only for selected germplasms. Major flower development is by year 3 with major first harvest of seeds by year 4. To further evaluate variation in circadian rhythm we cloned four Pongamia genes and evaluated their expression (Fig. 3B, 12).

Our research effort is looking not only at basic biological and genetic properties, but also at field environmental analysis. Seed-derived trees and clonal plants derived either by rooted cuttings or cotyledon-regenerants (note, the later are themselves identical but not to the mother plant) were planted in a large variety of environments including the wet and dry tropics of northern Queensland, the dry outback of central Queensland (near Roma), the Stanwell Meandu coal mine (near Kingaroy) and the University of Queensland Agricultural Campus at Gatton (Fig. 2E, 2F, 2G and 2H).

The University of Queensland, Centre for Integrative Legume Research (CILR) and School of Agriculture and Food Sciences
St Lucia, Brisbane, Australia
(p.gresshoff@uq.edu.au)

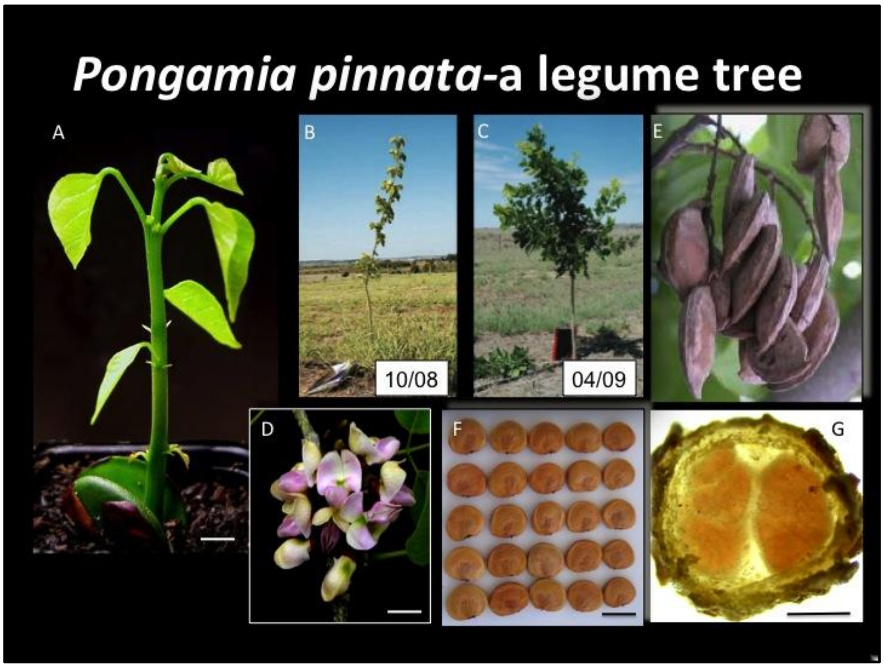

Figure 1. Pongamia biology: A) germinating seedling illustrating fast growth and dark green foliage; seeds are best germinated at warm temperature with frequent exchange of water; soil-planted seeds are low in plantlet-establishment, making seedling establishment in nursery conditions valuable; usually 3 to 6 months old seedlings are planted in the field; B) young tree at Spring Gully property of Origin Energy, near Roma, Central Queensland, October 2008); C) same tree in April 2009; 6 months growth - note identical notebook next to trunk as control reference; D) pongamia flowers; E) resultant pongamia seed pods (usually one seed per pod); F) Pongamia seeds from one tree; G) young pongamia root nodule illustrating red-brown coloured infected zone needed for nitrogen fixation

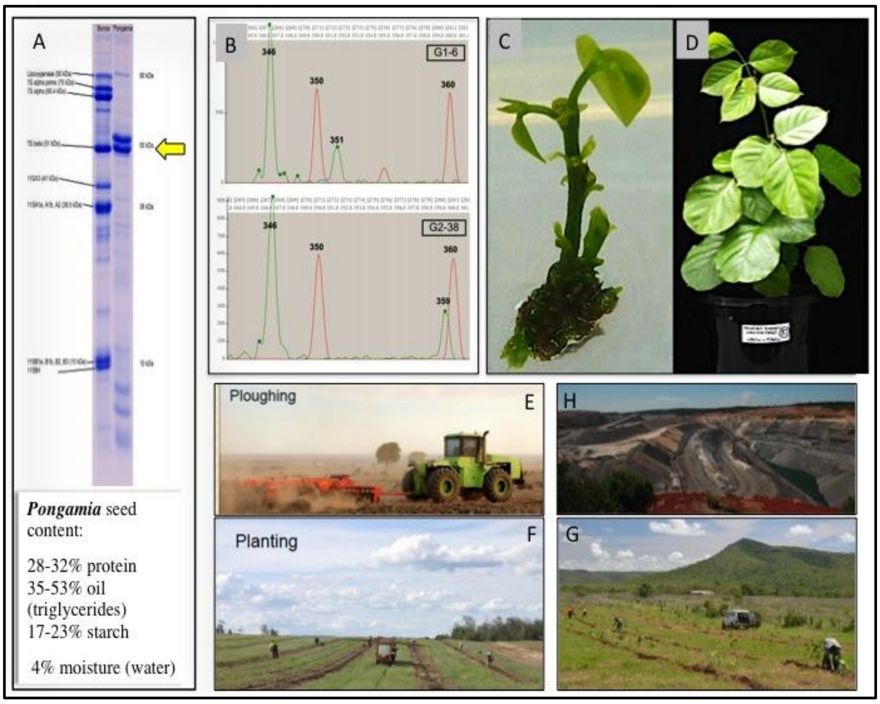

Figure 2. Spectrum of pongamia analysis: A) pongamia and soybean seed proteins separated by one dimensional electrophoresis and Coumassie Blue staining; B) capillary gel electrophoresis print-out for two separate trees illustrating common and diverse DNA bands; C) regenerating tissues from immature pongamia seed explant; note the emerging plantlet; roots are induced afterwards; D) vigorously growing pongamia clonal plant in pot; such clones are now field grown awaiting yield evaluation in different climatic zones; E) and F) major pongamia planting at Spring Gully using modern technology; 300 ha were installed in 2011, now yielding its first pre-commercialisation crop; G) Meandu coal mine near Kingaroy (Queensland), exploring pongamia utility in rehabilitation, stabilisation and biofuel production on minimal mining spoil soil; H) pongamia plantation at Hope Vale (400 km north of Cairns, Queensland) in typical tropical environment (aboriginal project for effective land use and localised energy production)

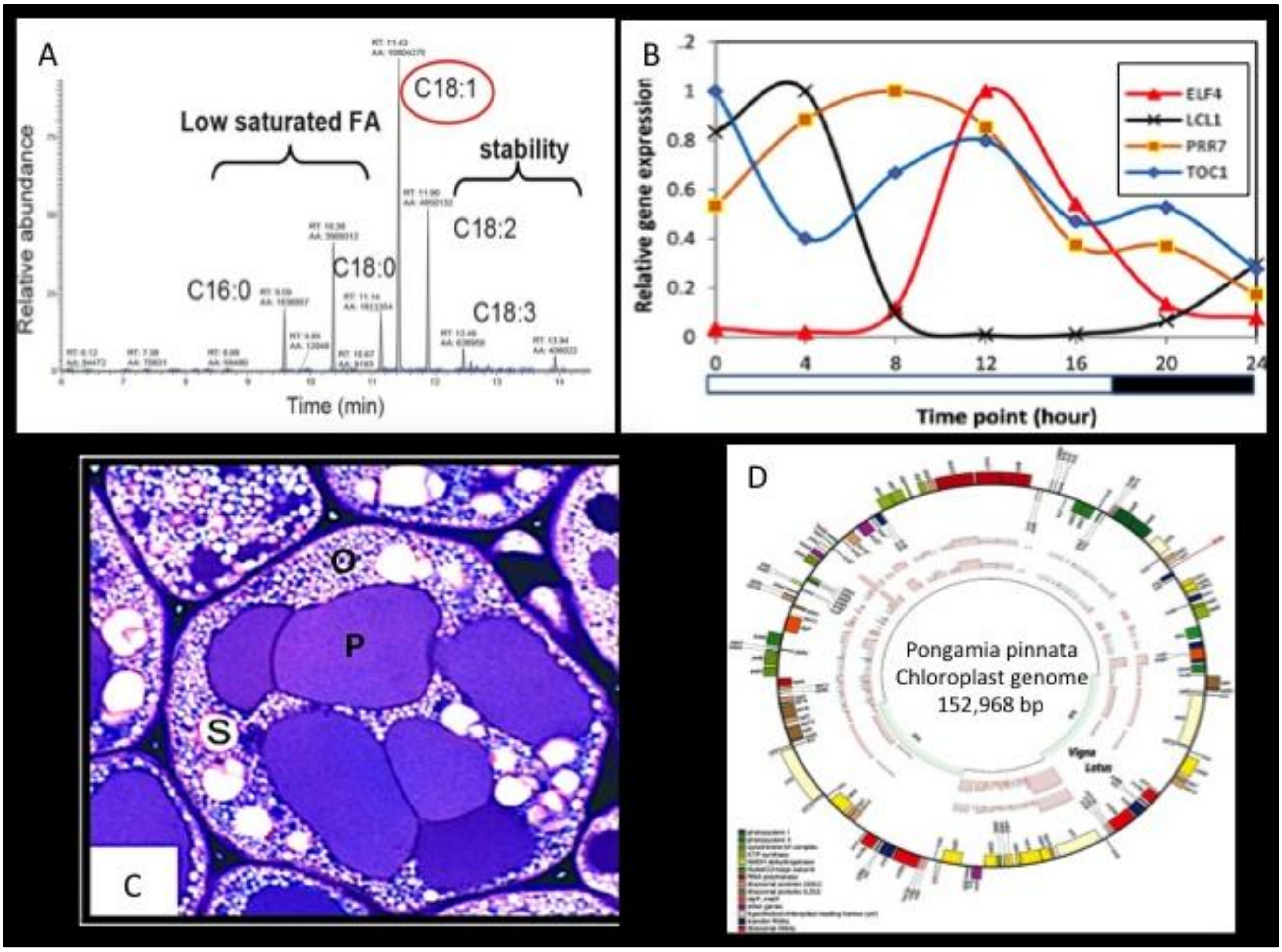

Figure 3. Pongamia scientific development: A) Pongamia oil (fatty acid) composition (11); B) Pongamia gene discovery (12) and expression during circadian cycle. C) Pongamia seed tissue illustrating protein bodies (P), oil vesicles (O) encased by the protein oleosin (also cloned for pongamia) and starch grains (S); D) Pongamia chloroplast genome illustrating gene density and similarity to other legumes like *Lotus* spp. and *Vigna* spp. (7)

However, Pongamia is outcrossing, meaning that every seed on a single tree is genetically different, because the mother is heterogenous to start out with, and the fertilising pollen came from unknown male parent flowers of proper gametic development. Thus just 6% of observed trees, derived from only two parents now grown at Gatton, Queensland, flowered after one year after germination. By year 5, 100% of the trees flowered and set seed; however the annual pod yield varied from a few kg per tree to 20 kg per tree (P. Scott, unpublished data). Jiang et al (6) developed DNA marker technology (ISSR) to visualise the diversity (Fig. 2B) and found that individual tree samples, collected from around the city of Brisbane (Queensland; planted for horticultural and shade purposes, not biofuel) were more diverse than progeny seeds from one single mother. The diversity is also seen in morphological characteristics, such as tree growth habit, leaf and pod shape, seed size, abundance of annual seed formation, flowering time, and growth rate.

Whilst such genetic variability is of value to the geneticist, it is undesired by the producer who seeks uniformity for harvesting and maintenance. The reality means that both aspects, variability and uniformity, can be utilised. It was possible to visually select elite trees from an estimated street population of 20,000 trees in Brisbane and surroundings. The elite trees serve as the foundation for clonal propagation using various established techniques of rooted cuttings and grafts (Fig. 2C). Additionally advances were achieved in tissue culture propagation using callus and plantlet regeneration from immature cotyledons from elite parent trees (3). Whilst such clonal trees, arising from a single cotyledon (Fig. 2D) are clonal relative to each other, they are not genetically identical to the parent plant. Further characterisation for growth and seed yield is needed after field trials.

Diversity also exists for seed traits such as oil content / oil composition and seed storage protein level, all related to yield and commercial profit. These properties allow transesterification of pongamia oil to form biodiesel called FAME (Fatty Acid Methyl Ester) with valuable chemical characteristics, such as a Cetane Number of 55.8, a pour point of 2.1°C, viscosity at 40°C of about 4.3 mm^2 s^{-1}, and an Iodine Value of 80.9. These are well within the limits set by the North American and European Industry Standards (11).

Pongamia seed biology

The average oil content per seed (w/w) was between 35% to 40% with some minor variability observed. Seed protein content (Fig 2A; around 25-30% of dry seed) is another commercially important trait, as pongamia seed cake, obtained after oil extraction. It is envisaged to be used as supplemental animal feed or field fertiliser. This potential depends on the nutritional quality, such as essential amino acids, and toxicity (remnant oils) of the seed cake. With solvent extraction of the oil, the resultant seed cake is literally free of oil. Hexane and oil are easily separated and hexane recycled for renewed oil extraction. In contrast, for seed crushed by rotary force to release the oil at moderately elevated temperature, seed cake is still containing oil and usually is re-extracted. Remnant seed cake of the crushed pathway is less suitable as supplemental animal feed.

Most of the seed storage protein (SSP) of pongamia is comprised of a 7S-beta-conglycinin (50,000 and 52,000 daltons in size). These two proteins appear to be similar but vary in glycosylation. Both have a low content of essential amino acids, making the cake even less attractive as a supplementary feed source. Possibly seed cake may be used as a fertiliser for pongamia or adjacent plantations.

Discussion

The genetics and genomics of pongamia are less advanced than crop legumes; at the same time the tree species, just being one step into modern domestication, displays a huge range of biodiversity. This is of value as selected trait-bearing plants can be cloned by either grafting or rooted cuttings. Such uniform clones serve the industrial needs of the emerging industry, but also provide a suitable host for broad pathogen attacks. Intelligent selection of diverse germplasm, and planting of large-scale patterned mosaics of clonal plant genotypes, will be part of the future expansion of the pongamia biofuel industry (4).

The benefit of analysing diversity, whether existing or induced, is the resultant ability to select suitable germplasm for specific environmental conditions. Academically it also allows the elucidation of complex biochemical and developmental pathways as limited but precise variation (point mutants, or knock-out transgenics) allow comparative analysis. Molecular biology alone by determining transcript differences by RNAseq, Northern or microchip analysis only defines structures and abundance, but function is derived from mutant (molecular genetic) analyses. This specialisation provides increased productivity required in this modern world agriculture.

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Faba bean breeding for disease resistance

by Diego RUBIALES[1]*, Angel M. VILLEGAS-FERNÁNDEZ[1] and Josefina C. SILLERO[2]

Abstract: Faba bean (*Vicia faba*) is a major grain legume with ancient tradition of cultivation for human food in Mediterranean countries. In other areas it is mainly used for animal feed. However, faba bean acreage is continuously decreasing at a world level, from 6 million ha in 1960 to 2.4 million ha in 2012, largely due to low and unstable yields. The deployment of cultivars resistant to most important diseases is a major need in low input farming systems. In this paper we will review the state of the art and future strategies on breeding faba bean for disease resistance.

Key words: ascochyta blight, broomrape, chocolate spot, rust, *Vicia faba*

Faba bean (*Vicia faba* L.) is a traditional legume highly valued for food and feed that has however followed a general trend for a decline in acreage from 6 million ha in 1960 to 2.4 million ha in 2012. Many reasons have been given for this decline but low and unstable yields as well as susceptibility to biotic and abiotic stresses are chiefly to blame. Some sources of resistance to most of these constraints have already been identified in faba bean germplasm but they usually provide only incomplete resistance, controlled quantitatively by multiple genes and are therefore difficult to manipulate by breeding (12).

Broomrapes are weedy root parasitic plants that severely constraint faba bean production in Mediterranean countries. The most damaging and widespread species is *Orobanche crenata* Forssk. (Fig. 1) although other species such as *O. foetida* Poir. and *O. aegyptiaca* Pers. can be of local importance (7). Only moderately resistant cultivars are available being resistance of complex nature complicating breeding (4, 6). A new type of resistance has recently been identified based in low induction of germination of *Orobanche* seeds. The fact that it is similarly operative against *O. crenata*, *O. foetida* and *O. aegyptiaca* reinforces the value of this resistance (3).

Rust incited by *Uromyces viciae-fabae* (Pers.) J. Schröt. is an airborne disease of worldwide importance (Fig. 2). Incomplete resistance has frequently been reported (10). The most frequently identified type of incomplete is non-hypersensitive resistance, seen microscopically as reduced hyphal growth that hampers the formation of haustoria, resulting fewer and smaller pustules that appear later (9). Late acting hypersensitive resistance has also been identified, resulting in a reduction of the infection type rather than complete resistance (10). It is controlled by genes with major effects and is race-specific. The existence of races of *U. viciae-fabae* reinforces the need to look for additional genes and to implement strategies to increase durability of the resistance. Genetic analysis has so far been performed only with one of these accessions (1). Identification of additional genes in other accessions and the associated markers will contribute to an efficient pyramidization programme.

Ascochyta blight, incited by *Ascochyta fabae* Speg. (teleomorph *Didymella fabae* G.J. Jellis & Punith.) is another major disease (Fig. 3) for which only incomplete resistance is available (8, 11). Both polygenic and major gene inheritance has been suggested (2, 5).

Similarly, only incomplete resistance to chocolate spot (*Botrytis fabae* Sardiña) (Fig. 4) has been reported (13). No QTLs or associated molecular markers have been reported so far, although molecular characterization of the resistance is in progress. A recent study of using the model *Medicago truncatula* Geartn. (15) has allowed the identification of transcription factors involved resistance that will help in breeding programs of faba bean for resistance to chocolate spot.

With this wide array of challengers faba bean crops encounter, if new cultivars are to succeed they should include resistances to different diseases. Hence, integrating genes from different sources is necessary, which makes breeding even more complicated and costly. The identification of material possessing simultaneous resistance to different stresses may result greatly useful (14). In any case, effectiveness of faba bean breeding will certainly increase soon with the adoption of the new improvements in marker technology together with the integration of comparative mapping and functional genomics. In contrast, insufficient efforts are being paid in the understanding of the biology of the causal agents and on the faba bean / disease interactions. Comprehensive studies on host status and virulence of the causal agents are often scarce or even completely absent, which is a major limitation for any breeding programme. Only after significant input to improve existing knowledge on the biology of the causal agents as well as on the plant, resistance breeding will be efficiently accelerated.

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[1] CSIC, Institute for Sustainable Agriculture, Córdoba, Spain (diego.rubiales@ias.csic.es)
[2] IFAPA, Centro "Alameda del Obispo", Córdoba, Spain

Figure 1. Crenate broomrape (*Orobanche crenata*) infecting faba bean

Figure 2. Rust (*Uromyces viciae-fabae*) lesions on susceptible (left) and partially resistant faba bean accessions

Figure 3. ***Asochyta fabae*** **lesions on faba bean stem and leaves**

Figure 4. Chocolate spot (*Botrytis fabae*) infection on whole faba bean plants

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Phytotoxins produced by fungal pathogens of legume crops

by Alessio CIMMINO*, Marco EVIDENTE, Marco MASI and Antonio EVIDENTE*

Abstract: The phytotoxins produced by necrotrophic fungal pathogens of legume crops, including essentially *Ascochyta* (*Didymella*) and *Botrytis* spp., belong to different classes of natural compounds as anthraquinones, macrolides, naphthalenones, isocoumarins, cytochalasans and some substituted phenols and salycilic aldehyde derivatives. Although they play an important role in the disease symptoms induction, they could be a very useful tool to select new biomarkers of resistance to the pathogens in the host plants.

Key words: legumes, phytopathogenic fungi, phytotoxins

Despite their historical importance for the agriculture and the environment, the production of food legumes is decreasing in most of the Mediterranean farming systems. A major cause for this is the low and irregular yield as a consequence of biotic and abiotic stresses. Necrotrophic fungi, including essentially *Ascochyta* and *Botrytis* species, are among the main biotic constraints. They produce phytotoxins (necrotrophic effectors), belonging to different classes of natural compounds, involved in the development of disease symptoms. In order to improve productivity and sustainable exploitation of agricultural lands in the Mediterranean countries, as well as people's quality of life. a multidisciplinary and integrated research including plant breeding, plant physiology and organic chemistry is in progress in order to identify the best food legume genotypes that can resist to disease infection and to propose appropriate agronomic practices that may help different grain legumes to resist better to this limiting factors (10).

Ascochyta pinodes L. K. Jones, recently re-classified as *Didyimella pinodes* (Berk. & A. Bloxam) Petr., isolated from pea (*Pisum sativum* L.), and responsible for the ascochyta blight produced several phytotoxins *in vitro*. When the fungus was firstly grown on solid wheat (*Triticum aestivum* L.) culture it showed to produce for the first time phytotoxic nonenolides the main of which, named pinolidoxin (1, Fig. 1) was characterized as 2-(2,4-hexadienoyloxy)-7,8-dihydroxy-9-propyl-5-nonen-9-olide (6). Pinolidoxin, was isolated together with three minor analogs namely 7-epi-, 5,6-epoxy- and 5,6-dihydro-pinolidoxins (4), which showed a lower phytotoxicity on host and non host plants suggesting that the diol system at C(7)-C(8), the stereochemistry at C-7 and the conformational freedom of the lactone ring are important features for the toxicity. Recently, a more aggressive isolate of the same fungus collected in Cordoba field, and grown on liquid culture, produced again pinolidoxin as the main phytotoxins but also other nonenolides. In fact, a new nonenolide, named pinolide was isolated and characterized as (2S*,7R*,8S*,5E,9R*)-2,7,8-trihydroxy-9-propyl-5-nonen-9-olide together to the well known herbarumin II and 2-*epi*-herbarumin II previously isolated from the fungi *Phoma herbarum* sensu Cooke fide Saccardo and *Paraphaeosphaeria recurvifoliae* H.B. Lee, K.M. Kim & H.S. Jung, respectively. When tested on leaves of the host plant and other legumes and weeds, pinolidoxin showed phytotoxicity in all the plant species, while the other three nonenolides did not produce any symptoms (3). Pinolidoxin was reported to inhibit the activity of phenylalanine ammonia-lyase (PAL), an enzyme involved in the biosynthesis of phytoalexins, a mechanism of plant defense (11). Extensive studies were carried out on pinolidoxin to realize its partial or total enantioselective synthesis. This goal was full reached (6), assigning the absolute stereochemistry to this nonenolide and reported its ability to inhibit the actin cytoskeleton organization.

A. pisi Lib. is another causal agent of pea ascochyta blight but it showed to produce different phytotoxins the main of which named ascosalitoxin (2, Fig. 1) was isolated and characterized as a new trisubstituted derivative of salicylic aldehyde, namely 2,4-dihydroxy-3-methyl-6-(1,3-dimethyl-2-oxopentyl) benzaldehyde (5, 7).

Botrytis fabae Sardiña isolated from faba bean (*Vicia faba* L.) plants displaying clear chocolate spot disease symptoms, produced phytotoxic metabolites *in vitro*. The first disease symptoms are discrete dark-brown spots surrounded by an orange-brown ring on leaves, flowers and stems. The phytotoxins isolated from the culture filtrates appeared to be new naphthalenone pentaketide, named botrytone and characterized as (4*R*)-3,4-dihydro-4,5,8-trihydroxy-1(2*H*)naphthalenone together with other well-known closely related naphthalenones such as regiolone (3, Fig. 1), and *cis*- and *trans*-3,4-dihydro-2,4,8-trihydroxynaphthalen-1(2*H*)-ones. When tested on leaves of the host plant, regiolone demonstrated the highest level of phytotoxicity together with *cis*- and *trans*-3,4-dihydro-2,4,8-trihydroxynaphthalen-1(2*H*)-ones, while botrytone showed moderate phytotoxic activity (2).

(-)-Regiolone was frequently isolated, as well as its enantiomer (+)-isosclerone, as metabolite from plants and other phytophatogenic and not phytotpathogenic fungi. However, despite so many studies on the isolation and bioactivity of regiolone/isosclerone, some contradictory results on their absolute configurations (ACs) have been reported in the literature with no certain or reliable assignment. The (*R*) and (*S*) absolute configurations of regiolone and isosclerone have been unambiguously assigned by *ab initio* computational prediction of their theoretical optical rotatory powers and electronic circular dichroism spectra. Isosclerone is produced, among the other fungi, by the plant pathogen *B. cinerea* (De Bary) Whetzel, whereas regiolone is produced by *B. fabae*. The phytotoxic activities of the two

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Università di Napoli Federico II, Dipartimento di Scienze Chimiche, Napoli, Italy
(alessio.cimmino@unina.it, evidente@unina.it)

compounds were tested for comparison on faba bean (host of both pathogens) and vine plants (host of only *B. cinerea*). The (*R*) configuration at C-4 was found to be a fundamental structural feature for bioactivity (8).

Ascochyta fabae showed to produce different metabolites the main of which was named ascochitine (4, Fig. 1) with antibiotic and phytotoxic properties (5).

Among the several biotic stresses lentil (*Lens culinaris* Medik.) can encounter, Ascochyta blight stands out as one of the most destructive. The pathogen responsible is the necrotrophic fungus *A. lentis* Vassiljevsky which is present in nearly all lentil cultivation areas. Its symptoms are necrotic lesions on leaflets, stems, pods and seeds, and when these lesions coalesce, they may cause serious damage. *A. lentis* produced in culture filtrates different phytotoxic metabolites. A new phytotoxic anthraquinone, named lentisone (5, Fig. 1), was isolated and characterized as (1*S**,2*S**,3*S**)-1,2,3,8-tetrahydroxy-1,2,3,4-tetrahydro-6-methylanthraquinone together with the well known pachybasin (1-hydroxy-3-methylanthraquinone), tyrosol and pseurotin A. Lentisone, tyrosol and pseurotin A were phytotoxic to lentil, with lentisone the most toxic of all. The toxicity of these compounds is light-dependent. Finally, lentisone was also found to be phytotoxic to chickpea, pea and faba bean, with toxicity in the latter higher than in any other tested legumes, including lentil (1).

A. lathyri Trail is the pathogen of *Lathyrus* spp. CBS strain collection grown on wheat showed to produce phytotoxic metabolites. The main toxins isolated from the corresponding organic extract were identified as the well known cytochalasin A and cytochalasin B (6, Fig. 1), both belonging to the class of 24-oxa[14]cytochalasan (12), whose biological activity was extensively studied (7, 13).

In conclusion, the different fungi belonging to *Ascochyta* and *Botrytis* species, pathogens of legumes as pea, faba bean, lentil and lathyrus showed to produce phytotoxic metabolites when grown in solid and or liquid cultures. The phytotoxins belong to different classes of natural occurring compounds as anthraquinones, macrolides, naphhtalenones, isocoumarins, cytochalasans and some substituted phenols and salycilic aldehyde derivatives and when tested on the host plants resembled the symptoms induced by the pathogens.

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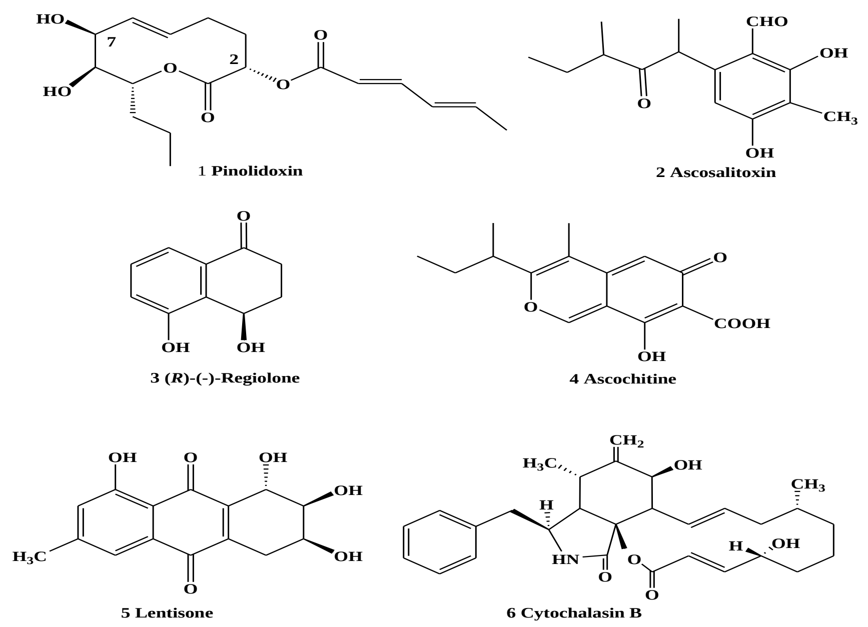

Figure 1. Chemical structures of pinolidoxin (1), isolated from *Dydimella pinodes*, ascosalitoxin (2), isolated from *Ascochyta pisi*, regiolone (3), isolated from *Botrytis fabae*, ascochitine (4), isolated from *Ascochyta fabae*, lentisone (5), isolated from *Ascochyta lentis*, and cytochalasin B (6), isolated from *Ascochyta lathyri*

Second International Legume Society Conference (ILS2) *2016: Legumes for a Sustainable World*

Tróia, Portugal, 12-14 October 2016

The International Legume Society and the Instituto de Tecnologia Química e Biológica of the Universidade Nova de Lisboa cordially invite you to join us at the Second International Legume Society Conference, scheduled from 12-14 October, 2016 at Tróia resort, in the vicinity of Lisbon, Portugal.

In a world urgently requiring more sustainable agriculture, food security and healthier diets the demand for legume crops is on the rise. This growth is fostered by the increasing need for plant protein and for sound agricultural practices that are more adaptable and environmentally sensitive. Food, feed, fiber and even fuel are all products that come from legumes – plants that grow with low nitrogen inputs and in harsh environmental conditions. The Second Legume Society Conference will be held during 2016 - the United Nations' International Year of Pulses. The goals of this UN International Year include: the encouragement of connections throughout the food chain that would better utilize pulse based proteins; increase global production of pulses; better utilization of crop rotations; and to address challenges in the trade of pulses.

The conference will address the following themes: Legume Quality and Nutrition; Farming Systems/Agronomy; Abiotic and Biotic Stress Responses and Breeding; Legume Genetic Resources; and New "Omics" Resources for Legumes. The health and environment benefits, as well as, the marketing of legumes will be transversal topics throughout the conference. Special attention will be given to foster the interaction of researchers and research programs with different stakeholders including farmers and farmer associations, seed/feed and food industries, and consumers. For this, the conference will also be the site of the Final Meeting of the EU-FP7 ABSTRESS project, the Annual Meeting of EU-FP7 LEGATO project; and final dissemination events of EU-FP7-ERANets MEDILEG and REFORMA. The results and conclusions from these four important research programs will be shared with conference attendees.

Please join us in beautiful Tróia, Portugal from 12-14 October, 2016! Plan now to include the Second ILS Conference in your busy agenda. Kindly share this information with any colleagues dealing with legumes.

Diego Rubiales, on behalf of the Scientific Committee
Pedro Fevereiro, Carlota Vaz Patto and Susana Araújo, on behalf of the Organizing Committee

Local Organizers

The Instituto de Tecnologia Química e Biológica / Universidade Nova de Lisboa (ITQB/UNL) will be responsible for the organization of the Conference, in cooperation with the International Legume Society. The official language of the Conference will be the English.

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Carlota Vaz Patto - cpatto@itqb.unl.pt
Susana Araújo - saraujo@itqb.unl.pt

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Venue

The conference will be held in Tróia in the vicinity of Lisbon, Portugal. Tróia is a beautiful sand peninsula dividing the Sado River from the Atlantic Ocean.

The nearest airport is the Lisbon International Airport, about 50 Km away. Shuttles will be made available from and to Lisbon International Airport.

During the period of Roman occupation, date from the 1st century to the 6th century AD, Tróia was an island of Sado delta, called Ácala Island.

Sado Estuary Nature Reserve, where dolphins swim, and the Serra da Arrábida Natural Park, where a full developed Mediterranean forest can be seen, are two of the main natural attractions nearby Tróia peninsula.

The Tróia Golf Championship Course is considered the best course in Portugal in the categories of difficulty and variety. It also stands in 20th place in the list of the best golf courses in Europe drawn up by the Golf World magazine.

Tentative Programme

October 11th, 2016
Morning-Afternoon: Satellite projects meetings
Evening: Conference Registration

October 12th, 2016
08:00 Registration; 09:00 Welcome addresses;
09:45 Session 1 (Opening plenary)
11:15 Coffee break
11:45 Sessions 2 & 3
12:45 Lunch
14:30 Sessions 2 & 3
16:30 - 19:00 Sessions 4 & 5
20:45 Third International Legume Football Cup

October 13th, 2016
9:00 Session 6
11:15 Coffee break
11:45 Sessions 7 & 8
12:45 Lunch
14:30 Sessions 7 & 8
16:00 Coffee break
16:30 International Legume Society Assembly
20:45 Third International Legume Football Cup

October 14th, 2016
09:00 Session 9
11:15 Coffee break
11:45 Sessions 10 & 11
12:45 Lunch
14:30 Sessions 10 & 11
16:00 Coffee break
16:30 Session 12 (Closing plenary)
20:00 Farewell Dinner

October 15th, 2016
Satellite projects meetings

Bem vindos a Tróia, amigos das leguminosas!

EUCARPIA
European Association for Research on Plant Breeding
Europäische Gesellschaft für Züchtungsforschung
Association Européenne pour l'Amélioration des Plantes

Symposium of the Protein Crops Working Group, Protein Crops Section, and Spanish Association for Legumes

Plant Proteins for the Future

Pontevedra, Spain, 4-7 May 2015
http://www.symposiumproteincrops.org/

Symposium of the Fodder Crops and Amenity Grasses Section

Breeding in a World of Scarcity

Ghent, Belgium, 14-17 September 2015
http://www.eucarpia-fcag2015.be/

18th Symposium of the European Grassland Federation

Grassland and Forages in High Output Dairy Farming Systems

Wageningen, the Netherlands, 15-18 June 2015
http://www.europeangrassland.org/events.html

8th International Herbage Seed Group Conference

Lanzhou, Gansu, China, 21-30 June 2015
http://www.ihsg.org/content/8th-international-herbage-seed-group-ihsg-conference

XIV International Lupin Conference

Developing Lupin Crop into a Modern and Sustainable Food and Feed Source

Milan, Italy, 21-26 June 2015
http://users.unimi.it/ILC_2015/Home.html

Legume Perspectives is an international peer-reviewed journal aiming to interest and inform a worldwide multidisciplinary readership on the most diverse aspects of various research topics and use of all kinds of legume plants and crops.

The scope of *Legume Perspectives* comprises a vast number of disciplines, including biodiversity, plant evolution, crop history, genetics, genomics, breeding, human nutrition, animal feeding, non-food uses, health, agroecology, beneficial legume-microorganism interactions, agronomy, abiotic and biotic stresses, agroeconomy, sociology, scientometrics and networking.

The issues of *Legume Perspectives* are usually thematic and devoted to specific legume species or crop, research topic or some other issue. They are defined by the Editorial Board, led by the Editor-in-Chief with the help from Assistant Editors, who select and invite one or more Managing Editors for each issue. Having accepted the invitation, the Managing Editor agrees with the Editorial Board the details, such as the deadline for collecting the articles and a list of the tentative contributors, from whom he, according to his own and free choice, solicit the articles fitting into the defined theme of an issue. A possibility that every member of the global legume research community, with a preference of the International Legume Society members or established authorities in their field of interest, may apply to the Editorial Board to be a Managing Editor and suggest a theme for his issue is permanently open and can be done simply by contacting the Editor-in-Chief by e-mail, with a clearly presented idea, structure and authors of the potential issue.

Since one of the main missions of *Legume Perspectives* is to provide as wide global readership with the insight into the most recent and comprehensive achievements in legume science and use, the articles published in *Legume Perspectives* are usually concise, clear and up-to-date reviews on the topic solicited by the Managing Editor from each author. Managing Editor is solely responsible for collecting the articles from the authors, anonymous peer-review, communicating with the Technical Editor and providing the authors with the proofs of their manuscript prior to the publication.

Apart from review articles, *Legume Perspectives* is keen on publishing original research articles, especially if they present some preliminary results of an outstanding significance for legume research and before they are published in their full volume, as well as brief reports on already held and announcements about the forthcoming national and international events relating to legumes, descriptions of the projects on legumes, book reviews, short articles on legumes in popular culture or everyday life, fiction stories on legumes and obituaries. The authors of such contributions are advised to contact the Editor-in-Chief first, in order to present the draft of their idea first and receive a recommendation if it is appropriate.

Regardless of the article category, *Legume Perspectives* prefers a clear, simple and comprehensive writing style that would make its articles interesting and useful for both academic and amateur audience. Your article is expected to assist in the exchange of information among the experts in various fields of legume research.

Legume Perspectives welcomes either longer (900-1,100 words + up to 3 tables, figures or photos + up to 10 references) or shorter (400-500 words + 1 table, figure, photograph or drawing + up to 4 references) manuscripts. The Editor-in-Chief, depending on the opinion of the Managing Editor, may allow any variation in length or structure, from case to case.

The manuscripts for *Legume Perspectives* should be prepared in Microsoft Office Word, using Times New Roman font, 12 points size and single spacing. Please provide each manuscript with a 100-word abstract and 4-6 key words listed alphabetically. The references should follow the style of the published papers in this issue, be given in full and listed alphabetically. The tables may be incorporated in the manuscript, while figures, photographs or drawings should be submitted separately as jpg files with a resolution of at least 600 dpi. The authors whose native language is not English are strongly advised to have their manuscripts checked by a native English speaker prior to submission and be persistent in following only one of all the variants of English they themselves prefer.

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