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CONTENTS

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20(2013): 3 – 11

GLANDULAR HAIRS, NON-GLANDULAR HAIRS, AND ESSENTIAL OILS IN THE WINTER AND SUMMER LEAVES OF THE SEASONALLY DIMORPHIC *THYMUS SIBTHORPII* (LAMIACEAE)

BOSABALIDIS ARTEMIOS Michael[1]

Abstract: The structure and function of the glandular and non-glandular hairs, and also the yield and chemical composition of the essential oils in the winter and summer leaves of the seasonally dimorphic plant *Thymus sibthorpii* were studied. Glandular hairs comprise peltate hairs only (capitate hairs are missing). Peltate hairs are the sites of essential oil biosynthesis. They are more numerous in the winter leaves than in the summer leaves and consist of a 12-celled secretory head, a unicellular stalk, and an also unicellular epidermal foot. The essential oil of the winter leaves is mainly composed of linalool (42.4%), thymol (7.0%), p-cymene (5.8%), β-caryophyllene (5.7%), borneol (5.6%), and terpinen-4-ol (4.8%). The oil of the summer leaves is principally constituted of p-cymene (25.0%), linalool (19.1%), terpinen-4-ol (8.5%) and borneol (8.3%). Non-glandular hairs proliferate in the summer leaves. They are conical in shape and consist of one basal epidermal cell and one apical pointed cell. Glandular and non-glandular hairs are implicated in the chemical and mechanical defense of the plant, respectively.

Keywords: anatomy, leaf hairs, *Thymus sibthorpii*.

Introduction

The aerial organs of many plants are covered with hairs which exhibit a great diversity in shape, size, structure and function. According to WEISS's (1867) definition, plant hairs are structures which owe their origin to outgrowths of single epidermal cells, eventually accompanied by divisions. Similarly, UPHOF (1962) considers that the name "trichome" should be applied to all outgrowths of the epidermis of leaves, shoots and roots, no matter whether they are unicellular or pluricellular. The distinction of plant hairs into "glandular" and "non-glandular" which is largely used today, has its origination to SOLEREDER (1908).

The functional role of plant hairs is multifarious. Non-glandular hairs were found to have an implication in reduction of transpiration and leaf overheating, and also in protection from UV-B radiation [MANETAS, 1999]. Their principal role, however, is mechanical protection against various predators, and particularly the insects (obstacles in insect movement, feeding, and oviposition on leaves) [GOERTZEN & SMALL, 1993]. Glandular hairs, on the other hand, exert chemical protection by secreting different kinds of secondary metabolites which may be repellent and lethal to insects, skin irritant and deleterious to mammals, and toxic to microorganisms [ROSENTHAL & BERENBAUM, 1991; AZAZ & al. 2004].

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The objectives of the present work comprised determination of the types of glandular and non-glandular hairs in *Thymus sibthorpii* and examination of their anatomy, in order to compare them with other *Thymus* species. Moreover, the size, density distribution, and morphometry of the hairs, as well as the chemical constitution of the secreted essential oils were studied in winter and summer leaves in an attempt to find out whether low and high temperatures have any possible effects on these parameters. The obtained data would provide useful information to taxonomists dealing with plant hairs, and also to ecologists working on plant adaptation.

Materials and methods

Plant material and sampling

Native plants of *Thymus sibthorpii* Bentham (Lamiaceae) were studied in the region of Ormylia, Chalkidiki, N. Greece (N 40°16'53", E 23°31'43", altitude 51m a.s.l.). In this region, the meteorological data in the three years of study (2009-2011) showed that during the winter months the average daily air temperature was 7.3 °C, the average daily relative air humidity 78.0%, and the average daily rainfall 1.9 mm. During the summer months, the climatic conditions were mild (not hot and dry) with an average daily air temperature of 24.3 °C, an average daily relative air humidity of 63.3%, and an average daily rainfall of 1.0 mm. Meteorological data were provided by the Regional Center for Plant Protection and Quality Control, Thermi, Thessaloniki, Greece. Winter sampling was performed in January and summer sampling in July. Fully-expanded leaves of annual shoots were used (3rd node from the shoot basis).

Microscopy

From a sample of 18 leaves (3 leaves x 6 plants), 5 leaves were randomly selected for light microscopy (LM), and another 5 leaves for scanning electron microscopy (SEM). Leaves for LM were cut into small pieces which were subsequently fixed for 3h with 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2). After washing in buffer, the specimens were post-fixed for 4h with 2% osmium tetroxide, similarly buffered. Samples were then dehydrated in an ethanol series (50-100%) and finally embedded in Spurr's resin. Semithin sections (1 μm thick) of plastid embedded tissue were obtained with a Reichert Om U_2 microtome (Reichert Optische Werke AG, Vienna, Austria), stained with Toluidine Blue O and photographed on a Nikon Eclipse ι80 light microscope (Nikon Instruments, Amstelvee, The Netherlands). For SEM, the specimens, after fixation and dehydration, were critical-point dried in a Balzers CPD 030 device (Balzers Union AG, Liechtenstein) and then carbon-coated in a Jeol JEE-4X vacuum evaporator. Observations were made with a Jeol JSM 840-A scanning electron microscope.

Morphometry

The densities (No/mm^2) of the glandular and non-glandular hairs on both surfaces of the winter and summer leaves were determined using 36 SEM micrographs. These micrographs were also used for conducting morphometric assessments on the hairs.

Essential oils

Leaf material was air-dried at room temperature and then grossly pulverized and subjected to hydrodistillation for 2h using a modified Clevenger-type apparatus. The oil content was expressed in ml/100 g leaf dry weight. Essential oil analyses were performed on a Shimadzu GC-2010-GCMS-QP 2010 system operating at 70eV. This was equipped with a split/splitless injector (230 °C) and a fused silica HP-5MS capillary column (30 m x

0.25 mm i.d., film thickness 0.25 μm). The temperature program ranged from 50 °C to 290 °C, at a rate of 4 °C/min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Injection volume of each sample was 1 μl. Arithmetic indices for all compounds were determined using n-alkanes as standards [VAN DEN DOOL & KRATZ, 1963]. Relative percentage of separated compounds was calculated from total ion chromatogram by a computerized integrator. The identification of the components was based on comparison of their mass spectra with those of NIST21 and NIST107 [MASSADA, 1976], and on comparison of their arithmetic indices with literature data [ADAMS, 2007]. Essential oils were often subjected to co-chromatography with authentic compounds (Fluka, Sigma).

Statistics

Statistical analysis was performed with the SPSS package (SPSS Inc. Chicago, USA) using ANOVA for comparison of means between treatments. Significance was determined at $p \leq 0.05$ probability level.

Results

Thymus sibthorpii plants have an entirely different appearance in winter and summer (Fig. 1A, B), a fact principally due to the different environmental conditions prevailing in each of these seasons. Thus, winter plants (Fig. 1A) compared to summer plants (Fig. 1B) are greatly smaller, with shorter densely-arranged shoots and shorter, dark-green leaves. Scanning electron microscopy disclosed that both the winter and summer leaves bear on their surfaces glandular and non-glandular hairs (Fig. 1C). Glandular hairs consist of essential oil-secreting peltate hairs only (Fig. 1C, large asterisks) with no presence of capitate hairs. Peltate hairs are more numerous in the winter leaves (Tab. 1) and they are constructed of a 12-celled secretory head (Fig. 2B), a unicellular stalk and an also unicellular epidermal foot. The 12 head cells are arranged in such a manner that 4 small cells are located in the centre of the head and 8 large ones in the periphery. The foot cell is radially surrounded by 13-15 elongated epidermal cells (Fig. 2C). Non-glandular hairs proliferate in the summer leaves and particularly on their upper side (Tab. 1). They are of one type only, i.e. short conical structures covered with granula (Fig. 1C, small asterisks; Fig. 2D). Their size (height, thickness) does not appear to differ between the winter and summer leaves. Anatomically, non-glandular hairs are composed of one large basal cell located at the level of the epidermis and one pointed cell sited above it (Fig. 2E).

Comparative quantitative analyses of the leaf essential oils in *T. sibthorpii* showed that the winter leaves have a higher essential oil yield (1.20%, i.e. 1.20 ml /100 g leaf d.w.) compared to the summer leaves (1.11%) (Tab. 2). Qualitative analyses of the winter and summer oils disclosed the existence of 49 compounds accounting for 97.6-99.3% of the total oils (Tab. 2). Both types of oils contain as principal components p-cymene, linalool, borneol, terpinen-4-ol, thymol, and β-caryophyllene. The major constituent of the winter oil is linalool (42.4%), followed by thymol (7.0%), p-cymene (5.8%), β-caryophyllene (5.7%), borneol (5.6%) and terpinen-4-ol (4.8%). The major constituent of the summer oil is p-cymene (25.0%), followed by linalool (19.1%), terpinen-4-ol (8.5%) and borneol (8.3%). Thymol and β-caryophyllene occur at a low percentage (about 1%).

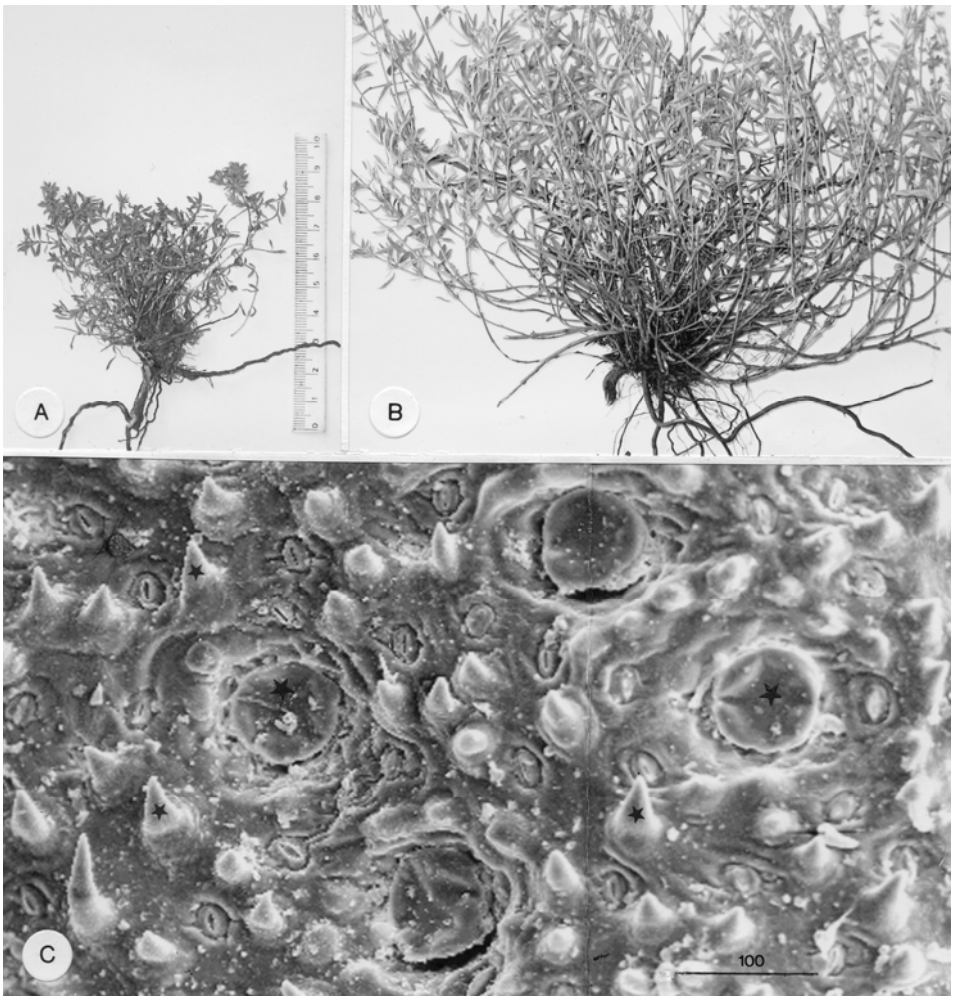

Fig. 1. *Thymus sibthorpii*. Lateral view of herbarium material of winter plant (A) and summer plant (B). Compare the size and density of shoots and leaves. C. SEM view of the leaf surface with peltate glandular hairs (large asterisks) and conical non-glandular hairs (small asterisks). Bar in μm.

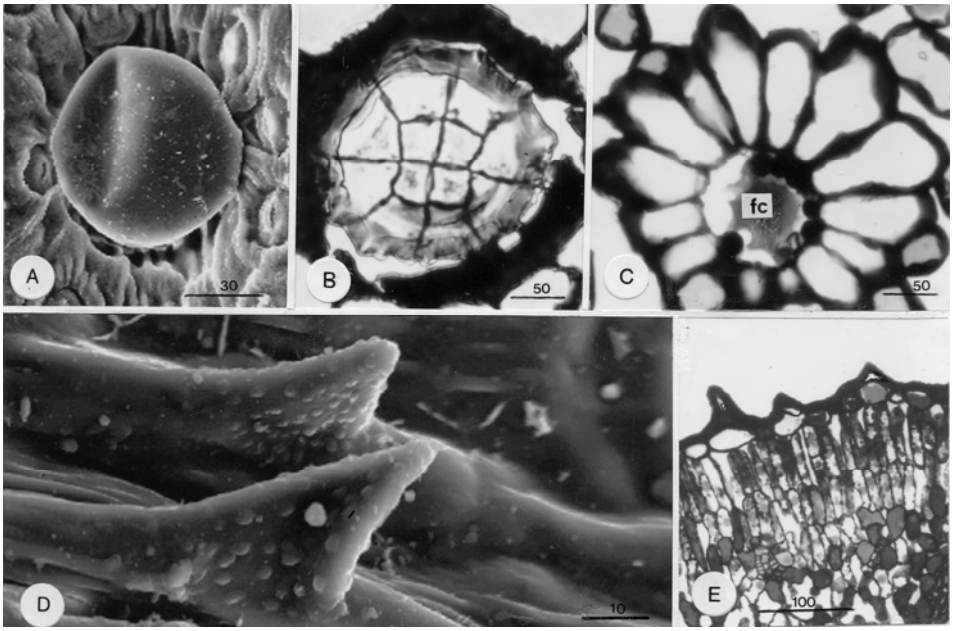

Fig. 2. A. SEM view of a peltate hair. B. Paradermal section of the head of a peltate hair. The head consists of 12 cells. C. Paradermal section of the foot cell (fc) which appears radially surrounded by 13-15 elongated epidermal cells. D. Non-glandular hairs as they appear in SEM. E. Longitudinal section of non-glandular hairs composed of a large basal epidermal cell and a small apical pointed cell. Bars in μm.

Discussions

SEM observations on the blade surfaces of the winter and summer leaves of *Thymus sibthorpii* revealed the presence of numerous peltate glandular hairs and the absence of capitate glandular hairs. SATIL & al. (2005) also reported a rare presence of capitate hairs in the leaves of *T. migricus.* However, studies on *T. malyi, T. vulgaris* and *T. capitatus* mentioned the existence of capitate glandular hairs with a unicellular head [WERKER & al. 1985; MARIN & al. 2008; BOZ & al. 2009]. The peltate hairs in *T. sibthorpii* appeared to consist of a secretory head with 12 cells. The number of head cells in the peltate hairs of other *Thymus* species was found to fluctuate, as in *T. capitatus* where the head consisted of 14 cells [WERKER & al. 1985], in *T. serpyllum* of 8 cells [UPHOF, 1962], in *T. vulgaris* of 8-12 cells [YAMAURA & al. 1992] and also of 10-14 cells [BRUNI & MODENESI, 1983], and in *T. malyi* of 8 cells [MARIN & al. 2008]. The peltate hairs and specifically their head cells are the only cells from all leaf tissues which possess the necessary enzymic equipment for essential oil biosynthesis [McCASKILL & al. 1992]. Thus, the higher the number of peltate hairs on leaves is, the higher the amount of the essential oil derived by distillation. This is in accordance with the morphometric assessments on *T. sibthorpii* in which the higher number of peltate hairs on winter leaves compared to summer leaves resulted in a higher essential oil yield.

The elongated epidermal cells which radially surround the foot cell of each peltate hair (peribasal cells) are considered as a fundamental accessory of the hair. Thus, the large surface area defined by the radial arrangement of the peribasal cells, their convergence towards the foot cell, and their location at the border with the mesophyllic photosynthetic parenchyma, favour the interpretation that peribasal cells collect from the mesophyll photosynthates which become centripetally transferred first to the foot cell (large central vacuole) and then to the apical head cells. There, they will constitute the precursors for the biosynthesis of the essential oil.

Qualitative analysis of the essential oils from *T. sibthorpii* winter and summer leaves showed that in both types of oils the major components are linalool and p-cymene. Linalool dominated in the winter leaves and p-cymene in the summer leaves. The high content in linalool of the thyme oil is ascribed by VERNET & al. (1986) to the low environmental temperatures (winter conditions). In accordance to our results, high contents of p-cymene, γ-terpinene, borneol, and terpinen-4-ol were also recorded by VOKOU (1993) in the oil of the summer leaves of *T. capitatus.*

Tab. 1. *Thymus sibthorpii.* Morphometric assessments of peltate glandular hairs and conical non-glandular hairs in fully expanded leaves of winter and summer (±SD, n=36). Means between columns with different letters are significantly different at 0.05 level

	Winter leaves	Summer leaves
Density of peltate glandular hairs on the upper leaf side (No/mm^2)	20.7±2.9 a	12.8±2.1 b
Density of peltate glandular hairs on the lower leaf side (No/mm^2)	8.8±0.3 a	7.7±0.4 b
Head diameter of peltate glandular hairs in surface view on the upper leaf side (μm)	75.9±6.5 a	85.4±9.1 b
Head diameter of peltate glandular hairs in surface view on the lower leaf side (μm)	71.7±5.7 a	82.6±8.5 b
Density of conical non-glandular hairs on the upper leaf side (No/mm^2)	149.6±17.6 a	164.5±19.3 b
Density of conical non-glandular hairs on the lower leaf side (No/mm^2)	*	62.6±5.3
Thickness of the base of conical non-glandular hairs on the upper leaf side (μm)	26.5±2.3 a	27.1 ±3.0 a

Thickness of the base of conical non-glandular hairs on the lower leaf side (μm)	*	10.1±2.2
Height of conical non-glandular hairs on the upper leaf side (μm)	39.8 ±5.7 a	40.2±8.6 a
Height of conical non-glandular hairs on the lower leaf side (μm)	*	18.3±7.8

*There are no non-glandular hairs on the lower leaf side of the winter leaves

Tab. 2. *Thymus sibthorpii*. Qualitative and quantitative compositions (%) of the essential oils from winter and summer leaves

Components [a]	AI [b]	Winter leaves	Summer leaves	Identification [c]
α-Thujene	926	n.d.	0.4	AI, MS
α-Pinene	932	n.d.	1.0	AI, MS, Co-GC
Camphene	946	0.2	1.7	AI, MS
Sabinene	972	n.d.	0.2	AI, MS
β-Pinene	974	n.d.	0.3	AI, MS, Co-GC
1-Octen-3-ol	980	1.0	0.3	AI, MS
3-Octanone	988	0.5	0.4	AI, MS
β-Myrcene	991	0.1	0.2	AI, MS, Co-GC
3-Octanol	997	0.6	0.2	AI, MS
α-Phellandrene	1002	n.d.	0.3	AI, MS
α-Terpinene	1017	0.5	1.7	AI, MS
***p*-Cymene**	**1024**	**5.8**	**25.0**	**AI, MS, Co-GC**
Limonene	1028	0.5	4.5	AI, MS, Co-GC
1,8-Cineole	1029	1.1	4.4	AI, MS, Co-GC
γ-Terpinene	1058	2.4	3.6	AI, MS, Co-GC
cis-Sabinene hydrate	1067	0.5	3.2	AI, MS
cis-Linalool oxide (furanoid)	1072	0.3	0.5	AI, MS
Terpinolene	1087	n.d.	0.8	AI, MS
trans-Linalool oxide (furanoid)	1087	0.6	0.5	AI, MS
trans-Sabinene hydrate	1095	n.d.	0.5	AI, MS
Linalool	**1100**	**42.4**	**19.1**	**AI, MS, Co-GC**
cis-Verbenol	1138	0.1	n.d.	AI, MS
trans-p-Menth-2-en-1-ol	1138	0.1	n.d.	AI, MS
Camphor	1142	0.5	1.0	AI, MS, Co-GC
Borneol	**1165**	**5.6**	**8.3**	**AI, MS, Co-GC**
Terpinen-4-ol	**1176**	**4.8**	**8.5**	**AI, MS, Co-GC**
p-Cymen-8-ol	1184	0.2	0.4	AI, MS

α-Terpineol	1190	0.3	0.8	AI, MS
cis-Dihydro carvone	1196	0.1	0.3	AI, MS
trans-Dihydro carvone	1203	0.2	0.3	AI, MS
trans-Carveol	1219	0.1	0.3	AI, MS
Cumin aldehyde	1240	n.d.	0.1	AI, MS
Carvone	1244	n.d.	0.1	AI, MS, Co-GC
Carvacrol methyl ether	1244	n.d.	0.1	AI, MS
Bornyl acetate	1288	0.2	0.2	AI, MS, Co-GC
Thymol	**1292**	**7.0**	**1.0**	**AI, MS, Co-GC**
Carvacrol	1301	3.9	2.3	AI, MS
Thymol acetate	1355	0.2	n.d.	AI, MS
Carvacrol acetate	1374	0.1	n.d.	AI, MS
β-Bourbonene	1384	0.7	0.5	AI, MS
β-Caryophyllene	**1421**	**5.7**	**1.4**	**AI, MS, Co-GC**
cis-Cadina-1(6),4-diene	1462	0.2	n.d.	AI, MS
9-epi-(E)-Caryophyllene	1462	0.2	n.d.	AI, MS
Germacrene D	1482	2.1	n.d.	AI, MS
Bicyclogermacrene	1497	2.6	n.d.	RI, MS
β-Bisabolene	1509	3.8	0.7	AI, MS
Spathulenol	1578	0.7	0.4	AI, MS
Caryophyllene oxide	1583	3.3	2.1	AI, MS, Co-GC
Caryophylla-4(12), 8(13)-dien-5-ol [d]	1639	0.1	n.d.	AI, MS
TOTAL		**99.3**	**97.6**	
Essential oil yield (%)		**1.20**	**1.11**	

[a] Compounds listed in order of elution from an HP-5 MS capillary column; [b] AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C25); [c] Identification method: AI= Arithmetic index. MS=mass spectrum. Co-GC=coinjection with authentic compound. [d] Correct isomer not identified. n.d.: not detected

Conclusions

Thymus sibthorpii plants bear on their leaves glandular and non-glandular hairs which differ in anatomy from other *Thymus* species. The structural model of the glandular hairs (peribasal cells, basal cell, head cells) is functionally related to the uptake of photosynthates from the mesophyll and their transportation to the head cells where they constitute precursors for the biosynthesis of the essential oil. In winter leaves, glandular hairs are more numerous and secrete a higher amount of essential oil, compared to summer leaves. Due to the antioxidant properties of the essential oils, the plant can thus tolerate winter low temperatures which stimulate oxidative stress.

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20(2013): 13 – 24

FLORAL ANATOMY OF *ALPINIA SPECIOSA* AND *HEDYCHIUM CORONARIUM* (ZINGIBERACEAE) WITH PARTICULAR REFERENCE TO THE NATURE OF LABELLUM AND EPIGYNOUS GLANDS

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Abstract: The floral anatomy of *Alpinia speciosa* Schum. and *Hedychium coronarium* Koenig. (Zingiberaceae) was investigated for an understanding of the structure and nature of the labellum and epigynous glands. The observation presented in this study supported the classical view of the labellum as a double structure rather than a triple or five-parted structure, as were proposed previously. The dorsal carpellary bundles in the studied species only continue into the style, fade out in the stigmatic tissue without feeding sepals or labellum as suggested in the previous studies. The glands in both studied species are found with very prominent masses of vascular tissue. The presence of vascular tissue seems to be connected with the more organized nature of the gland. Furthermore, the present study appears that the glands are not merely epidermal emergence of the ovary and similar to nectarines which may be vascularized.

Keywords: Zingiberaceae, *Alpini*a, *Hedychium*, floral anatomy, labellum, epigyny glands.

Introduction

Anatomical features are widely used in systematics, identification, placing anomalous groups in a satisfactory position in classification and explanation of the origin, position and nature of every plant organ. The vascular system of the flower has been studied extensively with regard to taxonomic and phyletic relationships among the angiosperms and to the morphological nature of the flower and its parts [MURTY, 1958; SUBRAMANYAM, 1960; EL-SHAFEY & al. 1966; AL-NOWAIHI & KHALIFA, 1973; ESAU, 1976; BARABE, 1981; ALVAREZ, 1988; KUMAR & MANILAL, 1988, 1992; MATTHEWS & ENDRESS, 2002; SAJO & al. 2004; SHARAWY & KARAKISH, 2005]. The use of the vascularization pattern in the interpretations of various phenomena in the flower is more conservative because it is less subjected to alteration by the habitat factor than the organs themselves [PURI, 1951]. Since, the classical research of van TIEGHEM (1871), the floral anatomy has been used as a reliable source of information in floral studies. Subsequent work by the same author and that of his followers, especially HENSLOW (1891), placed this research on a sound footing. He stated that the importance of this line of research lied in the fact that the origin, position and union of every organ were bounded to the particular cord or trace in the axis which subsequently enters it. SAUNDERS (1925) revived more interest in this field of study but her extensive and detailed work was mainly directed towards the application of floral morphology to her

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theories on carpel polymorphism. Further progress in floral anatomical studies took place, particularly in India [PURI, 1961] and the USA (e.g. EAMES, 1961). In addition, the internal anatomical structure of the flower provides many useful characters which can be used in taxonomic and phylogenetic studies. SUBRAMANYAN (1960) mentioned that the internal characters of flowers may be more useful in certain cases than the external because of the frequent persistence of the vascular supply of the lost organs after all the external evidence has disappeared.

Zingiberaceae flowers are bisexual, irregular, commonly in bracted spikes, heads, or panicles, one or more under each bract. Perianth of 6 parts in a calyx series and corolla series. Calyx tubular or somewhat spathe like, generally 3-toothed. Corolla tubular, unequally 3-lobed. Fertile stamen 1, the 2-anther-cells separated by connective. Labellum, one adnate at the base to the corolla tube and showy. Lateral staminodes petaloid free from the labellum. Ovary inferior, 1-celled, or sometimes 2-3 celled many ovules. Epigynous glands called stylodes form 2 erect outgrowths on top of the ovary. Style linear, held between the anther-thecae.

More than any other part of the Zingiberaceae flower, the labellum received a deal of great attention. PANDY (1989) stated that the labellum is two- or three- lobed and is produced by the fusion of the two laterals staminodes from the inner whorl of the stamens. In contrast, HEYWOOD (2001) stated that the labellum is produced from the fusion of two staminodes from the outer whorl of the stamens. Since the nineteenth century two theories about the nature of the labellum have been presented that it either has a double or a triple nature [PAYER, 1857; Van TIEGHEM, 1868, 1871]. GREGORY (1936) and ZHANG & al. (2009) supported that the labellum is triple in constitution from external morphology and flower vasculature. Other evidences are obtained from previous studies may very well be adduced in support of the classical conception, that the labellum is double nature [PAYER 1857; Van TIEGHEM 1868, 1871; PAI, 1963]. On the other hand, LIAO & al. (2008) indicated in *Alpinia hainanensis* that the labellum incorporates elements of five androecial members rather than two or three, as suggested by previous authors for Zingiberaceae flowers.

In addition, the epigynous glands in most Zingiberaceous flower are simply epidermal appendages of the ovary [VALETON, 1918; GREGORY, 1936; RAO, 1963]. According to their opinions the epigynous glands do not contain any vascular tissues. The presence of vascular tissues seems to be connected with a more organized nature of the glands [PURI, 1951; ESAU, 1953]. Furthermore, it appears that the glands are not merely epidermal emergences of the ovary. Comparative observation in the variation of their form, structure, development and vasculature seem to suggest strongly that they are more deeply connected with organs of the ovary [PAI, 1961, 1965; BHAT, 1993; KIRCHOFF, 1997; LIAO & al. 2008; ZHANG & al. 2009].

The flower of Zingiberaceae has a higher degree of organ specialization especially in the nature of labellum and the epigynous glands [BORAH & SHARMA, 2012; NGAMRIABSAKUL & al. 2000]. However, the origin and course of the floral vasculature may provide credible evidence to understand the nature of these floral structures. So, the present work deals with the study of floral anatomy of *Alpinia speciosa* and *Hedychium coronarium* (Zingiberaceae) to interpret the nature of the labellum and the epigynous glands in the studied taxa.

Materials and methods

Flower buds were collected during (2010) from the Botanical Garden of Faculty of Science, Ain Shams University, Cairo, Egypt. Five to eight flower buds of the studied taxa were fixed and preserved in F.A.A. and embedded in paraffin wax, then serially sectioned at 10-15µ according to the conventional method [JOHANSEN, 1940]. Sections stained in crystal violet-erythrosine (saturated in clove oil) combination. Drawings were made at bench level by the aid of a "Ken a vision Microprojector" Model x 1000. The magnification is given by Beck stage micrometer scaled to 0.1 and 0.01 mm.

Descriptive anatomical terms related to the floral vascularization are (i) the vascular supply to the floral organ is termed "trace" when it is still in the receptacular tissue and joined with the central stele and (ii) the same is termed "bundle" when it enters the organ.

Results

In *Alpinia speciosa***,** at the pedicel level, the vasculature bundles are arranged in two rings; the outer ring consists of numerous bundles whereas the inner ring is composed of more or less fused ones (Fig. 1a, b). At the floral axis beneath the ovary, the outer ring is further branched forming numerous vascular traces whereas the inner ring contains three large bundles and three small radially bundles alternating with each other (Fig. 1c).

At the base of the ovary, the three large bundles of the inner ring diverge outwards and become oriented higher in the ovary wall as the three carpellary dorsal bundles which eventually enter the style. Meanwhile, the remaining three small bundles are divided radially to form six bundles; three outer and three inner on the same radii (Fig. 1d). The former outer three bundles (septal carpellary bundles) also travel out exhibiting extensive branching during their outward transit. The latter inner three vascular bundles fuse to certain distance and then separate again to form three united masses whereas, at higher level divided into six ventral carpellary bundles two for each carpel and then fade out gradually while progressing upwards till the top of the ovary (Fig. 1e-h).

At the top of the ovary, the three septal carpellary bundles increase in size, divide into two or three bundles and bear outer and lateral branches, adjacent ones of which fuse to form an anastomosing vascular plexus. Some bundles of the outer ring are associated with this plexus but the median and marginal bundles of the sepals remain unaffected (Fig. 1h-k). Many vascular bundles are derived from this anastomosing plexus to supply petals, glands, androecium and style.

A calyx tube starts to separate and is supplied by three large bundles (sepal median bundles) alternating with many small bundles from the outer ring of the vascular bundles. Meanwhile, each of the sepal median bundles divides on each side once and then supply sepal marginal (Fig. 1l).

The glands are initially attached to the inner column of tissue (androcorolla tube) for a short length and become free at little higher within the tube. Two masses of vascular bundles which are derived from the vascular plexus enter the two glands and extend in an antero-posterior direction (Fig. 1m).

At higher level, after the complete separation of the calyx tube, the corolla tube is detected. Higher up, three ridges of the corolla tube are developed on its outer face, one

posterior and the other two antero-laterals. Some of the bundles in the corolla tube divide and some of them travel out into the ridges. The latter subsequently separates from an inner cylinder and represents the three petals. The larger, posterior petal embraces the two other smaller, antero-laterals (Fig. 1n).

Thus, the outer three stamens are morphologically absent; the vasculature of the outer three stamens is not detected. The remaining inner cylinder represents the fused bases of the two lateral staminodes (labellum) and the posterior fertile stamen. This cylinder splits into two segments, a flat posterior one and thick crescentric anterior one. The flat part contains five bundles; one median and four laterals traces. In the upper part of the anther, the connective is very much reduced in width and appears as a narrow plate connecting the large anther lobes on either side. The median bundles split into two small bundles and each of the resultant bundles travels towards the laterals. The connective itself, splits in the middle and separates from the anthers a little later. The bundles in the connective run in the split crest of the anther and then disappear (Fig. 1n-p).

The thick crescentric anterior segment is the base of labellum. One of the bundles is on the mid-anterior line and develops a shallow groove on its inner face. This bundle divides higher up to two small bundles. The daughter bundles also shift laterally; these lateral bundles soon undergo further splitting forming the marginal supplies of the labellum (Fig. 1o).

The filament of the fertile stamen is mostly slender and deeply grooved. In such groove, the style extends between the anther cells and the capitate stigma is protruding. This style contains a flattened canal and three bundles which extended to stigma (Fig. 1p).

In *Hedychium coronarium***,** two rings of bundles, each with variable numbers of strands, run in the floral axis beneath the ovary. The outer ring is composed of 16 vascular bundles, while the inner ring consists of six vascular bundles (Fig. 2a).

At the base of the ovary, the vascular bundles of the outer ring travel out exhibiting extensive branching giving rise to three sepal median bundles alternating with the numerous sepal marginal bundles and three large vascular complexes that are alternating with the loculi of the ovary (Fig. 2b, c). At a little higher, the six large bundles of the inner ring separate into three septal carpellary bundles and three ventral carpellary bundles. At the beginning of the ovuliferous zone, the latter bundles split to six small bundles that send traces to the ovules. They bear no branches in the non-ovuliferous zone (Fig. 2d, e). At the upper portion of the ovary, the ventral carpellary bundles fade out gradually till they are lost completely at the base of the stylar tissue (Fig. 2f-h).

On the top of the ovary, at the beginning of separation of calyx tube, the remaining vascular bundles of the outer vascular ring and the three carpellary septal bundles coalesce and form temporary anastomosing vascular plexus (Fig. 2g-j). At a little higher, the anastomosing vascular plexus is differentiated into three large bundles and many small bundles which later supplied petals, glands and androecium.

The former large bundles are recurved downwards from the top of the ovary as dorsal carpellary bundles then bent up again to feed the style. The three dorsal carpellary bundles continue into the style and gradually fade out in the stigmatic tissue (Fig. 2k).

The glands are initially attached to the androcorolla tube for a short length and become free at little higher. Two masses of vascular bundles enter the two glands that extend in an antero-lateral direction (Fig. 2k, l).

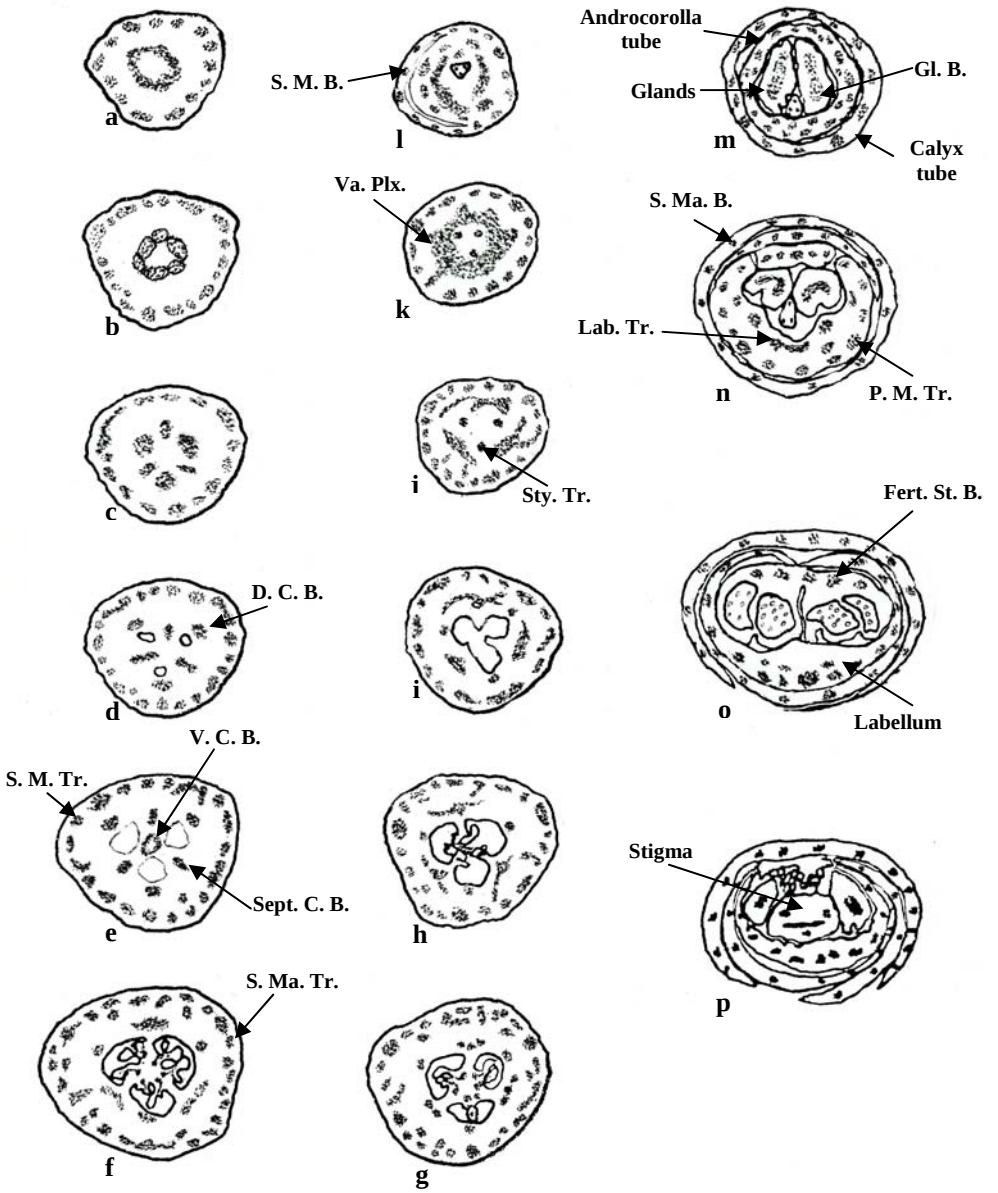

Fig. 1 a-p. Serial transverse sections from the pedicel upwards through the flower of *Alpinia speciosa*. D. C. B. = Dorsal Carpellary Bundles; Gl. B. = Glandular Bundles; Fert. St. B. = Fertile Staminal Bundle; Lab. Tr. = Labellum Trace; P. M. Tr. = Petal Median Trace; S. M. B. = Sepal Median Bundle; S. M. Tr. = Sepal Median Trace; S. Ma. B. = Sepal Marginal Bundle; S. Ma. Tr. = Sepal Marginal Trace; Sept. C. B. = Septal Carpellary Bundle; Sty. Tr. = Stylar Trace; V. C. B. = Ventral Carpellary Bundle; Va. Plx. = Vascular plexus.

At a higher level, the outer whorl of the perianth leaves (calyx) disappears whereas the inner whorl of the perianth leaves (corolla tube) starts to separate from the androcorolla (hypanthium) tube. At a little higher, the corolla tube develops three petals, one posterior and two in antero-lateral position. The three median petal bundles in the corolla tube divide and some of them travel out into the petals as marginal petal bundles. The larger posterior petal embraces the two other smaller, antero-laterals petals (Fig. 2m, n).

After the separation of the inner perianth leaves (corolla), the outer petaloid staminodes separate from the inner three stamens. The former outer stamens are consisting of two staminodes fused together forming a large compound labellum (Fig. 2n). The inner whorl of stamens is represented by one posterior fertile stamen and two lateral staminodes.

The remaining tissue of the vascular plexus is differentiated into the six staminal bundles mass in two rings; three in each ring (Fig. 2m, n). The outer three bundles represented by two large postero-laterals bundles and one anterior small bundle. The former two bundles supply the labellum (posterior-lateral staminodes); each one divides into two and the resultant bundles travel laterally in the labellum. The latter anterior small bundle gradually decreases in size and completely disappears (Fig. 2o).

The inner three staminal bundles divided into numerous small bundles; five of them supply the filament of the fertile stamen, while the remaining supplies the two lateral staminodes. The five vascular bundles of the fertile filament are represented by one median and four laterals. These five bundles exhibiting extensive branched into small traces. In the upper part of the anther the connective tissue appears as a narrow plate connecting the two large anther lobes. At the top of the anther, the connective tissue itself splits in the middle and separates from the anther. The vascular bundles in the connective tissue run in the split part of the anther and fades out (Fig. 2n, o).

The style is filiform enveloped within a channel extending between the anther lobes. The capitate stigma protrudes from the top of the anther. The stigma contains a small triangular canal and three strands flanking the arms of the triangular canal (Fig. 2p).

Discussion

In earlier floral anatomical studies of members of the family Zingiberaceae, GREGORY (1936) and RAO & al. (1954) describe the presence of twelve bundles; nine peripherally disposed in groups of three each and three forming a central group in the pedicel. PAI (1965) interprets the median bundles of the peripheral three sets as derived from the inner rings which initially, contain six bundles. He suggested that the presence of the three median bundles of the peripheral sets in the outer ring occurs only as a secondary feature. Evidently, he said that GREGORY (1936) and RAO & al. (1954) based their description from sections taken a few microns beneath the ovarian loculi and not from lower levels. In the present study, the pedicel vasculature consists of two rings of vascular bundles; the outer ring consists of numerous vascular bundles and the inner ring is composed of six vascular bundles.

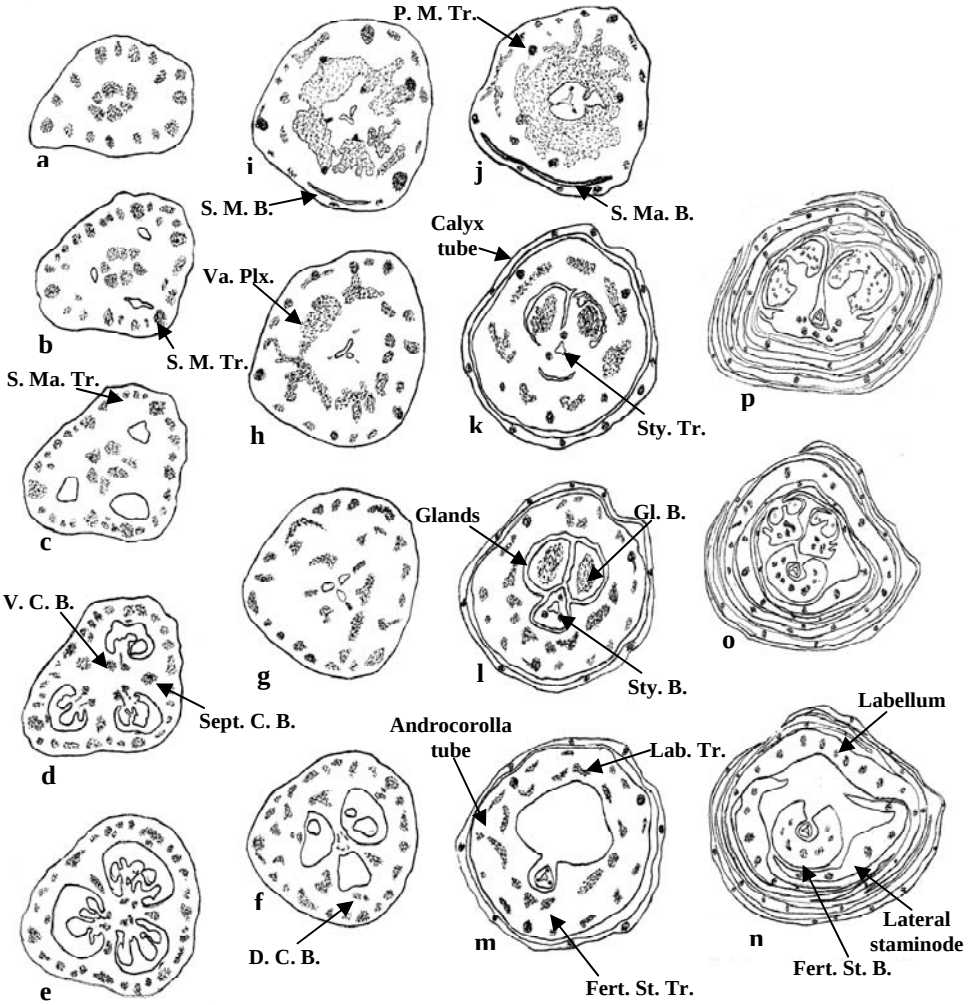

Fig. 2 a-p. Serial transverse sections from the pedicel upwards through the flower of *Hedychium coronarium*. D. C. B. = Dorsal Carpellary Bundles; Gl. B. = Glandular Bundles; Fert. St. B. = Fertile Staminal Bundle; Fert. St. Tr. = Fertile Staminal Trace; Lab. Tr. = Labellum Trace; P. M. Tr. = Petal Median Trace; S. M. B. = Sepal Median Bundle; S. M. Tr. = Sepal Median Trace; S. Ma. B. = Sepal Marginal Bundle; S. Ma. Tr. = Sepal Marginal Trace; Sept. C. B. = Septal Carpellary Bundle; Sty. B. = Stylar Bundle; Sty. Tr. = Stylar Trace; V. C. B. = Ventral Carpellary Bundle; Va. Plx. = Vascular plexus.

According to GREGORY (1936), the three peripheral bundles, after supplying the sepals, precede upwards and are divided laterally; terminated in a number of outer stamens (staminodes) and the original median one is continued into the style. In the present investigation, the pedicel vasculature of *Alpinia speciosa* and *Hedychium coronarium* consists of two rings; outer and inner vascular bundles. In these two species, each sepal is supplied by median sepal bundles and numerous marginal sepal bundles that originate from the outer vascular ring. It is pertinent to note here that the median bundles of the peripheral vascular groups run as the median bundles of sepals, leaving no vascular tissue at all at the top of the ovary. PAI (1965), also in his study on *Elettaria cardamomum*, observed that the median of the peripheral sets which run opposite the loculi represent the median traces of the sepals.

After the separation of the calyx tube, three daughter bundles which were derived from the vascular plexus are in turn derived from the inner ring of the pedicel vasculature, function as median traces of the petals. Later on, these three median petal traces start to ramify into numerous marginal petal traces.

In the earlier studies on Zingiberaceous flowers the presence of the carpellary ventral bundles in the axile region of the ovary is not mentioned. These have been observed in the specimens studied here. Such vascular bundles are also present in some species of the Zingiberaceae but referred to as the placental bundles concerned with the bearing of branches to the ovules. According to GREGORY (1936), PAI (1961) and LIAO & al. (2008), ovular traces are borne by the three parietal bundles (septal bundles) running opposite the septa which constitute the inner of the two rings in the pedicel of the *Cardamom* and *Alpinia* flowers. It may be pointed out here that the three bundles to which GREGORY (1936), PAI (1961) and VAN TIEGHEM (1968) refer are compound cords, being the fusion product of the carpellary septal (parietal) and the carpellary ventral (placental) bundles. In this study, just below the base of the ovarian loculi, these cords split into the constituent strands; the inner carpellary ventral traces and the outer carpellary septal bundles. The former provide traces to the ovules, but this was not mentioned in Gregory's investigation. The later bundles (carpellary septal bundles) are completely absent in *A. speciosa*, while in *H. coronarium* they coalesce at the top of the ovary with other bundles and form anastomosing vascular plexus. In *A. hainanensis* the three parietal bundles divide into five strands; of which the outer strands enter into the petals and the remaining enter into the functional stamen and in the labellum [LIAO & al. 2008].

In the studied species, the dorsal carpellary bundles in *A. speciosa* are derived from the inner vascular ring at the base of the ovary and just below the locules of the ovary. While in *H. coronarium*, at the top of the ovary, three bundles are derived from the vascular plexus. These bundles are recurved downwards as dorsal carpellary bundles and bent up again to the style. In both studied species the three dorsal carpellary bundles continue into the style and fade out in the stigmatic tissue. Unfortunately, few references mentioned the dorsal carpellary bundles in previous studies on Zingiberaceous flower. These carpellary dorsal bundles in *A. hainanensis* divided into five traces, of which the outer strand becomes the median bundle for each sepal and the inner strand runs into the style [LIAO & al. 2008]. While in *H. forrestii* the carpellary dorsal bundles continue to feed the petaloid staminodes [ZHANG & al. 2009].

GREGORY (1936), regards the glands of some members of Zingiberaceae as simply epidermal appendages of the ovary, since according to him, they do not contain any vascular tissue. RAO (1963) argued that the Gregory explanation needs confirmation. In the

present study, the glands in both studied species are found with very prominent masses of vascular tissue. The presence of vascular tissue seems to be connected with the more organized nature of the gland and similar to nectarines which may be vascularized [ESAU, 1953]. Furthermore, the present study appears that the glands are not merely epidermal emergences of the ovary. Comparative observations on the variation in their form, structure, development and vasculature, seem to suggest strongly that they are more deeply connected with ovary [PAI, 1961; KIRCHOFF, 1997; ZHANG & al. 2009]. However, it must be mentioned that Gregory (1936) was correct in as much as he associated the glands with the ovary and, at the same time regarded them as of no particular significance in morphological considerations of the Zingiberaceous flower.

In *A. speciosa*, the outer three stamens are morphologically absent and the vasculature of these three outer stamens is not detected. While the inner stamens are represented by one posterior fertile stamen and two staminodes united to form the labellum (lip). The absence of the three outer stamens in this species was also detected in *Curcuma amada* [PAI, 1962], *Kaempferia scaposa* [RAO & PAI, 1959] and in *A. hainanensis* [LIAO & al. 2008]. The mid-anterior bundle in the floral tube of *A. speciosa* is derived from the vascular plexus and showed an earlier splitting into two small traces at the base of the labellum. PAI (1965) explained that the mid-anterior bundle is divided into two strands in the labellum of *Elettaria cardamomum*.

GREGORY (1936) and ZHANG & al. (2009), in the study of the labellum in *E. cardamomum* and *H. forrestii* assumed that the labellum is a triple structure. While LIAO & al. (2008) indicate that the labellum of *A. hainanensis* incorporates elements of five members rather than two or three as suggested by previous authors for Zingiberaceae flowers. Data of the present study may be added in support of the classical conception suggested by PAYER (1957) and VAN TIEGHEM (1868, 1871), that the labellum is a double structure rather than triple or five. This mid-anterior bundle may conveniently be interpreted as a composite bundle being the fusion product of the marginal bundles of the two component member of the inner staminal whorl. The variation seen in the course of this bundle in some of the plants studied bears testimony to this contention. In *Zingiber macrostachyum*, there are no mid-anterior bundles, but laterally on either side of the mid-anterior line, the marginal bundles of the two constituents are present and they continue upwards into the two segments of the labellum [RAO & PAI, 1959]. The same condition occurs in some other species e.g. *Curcuma decipiens* [PAI, 1962], *Kaempferia scaposa* [RAO & PAI, 1959] and *Curcuma amada* [PAI, 1962]. The resultant traces also continue for the rest of the length of the labellum and into the two segments.

Gregory supports from the external morphology his contention that the labellum is triple in constitution. He found it more or less three-lobed which he considers significant from the point of view of its morphological nature. In this study, the flower of *A. speciosa* shows a median apical split in the labellum. PAI (1965) suggested that when the apical notch of the labellum is absent, the median trace in the labellum continue for some length and then clearly exhibits a bifurcation. This might once again be used in support of the contention that the development of the mid-anterior traces in the labellum is related to the degree of connation of its two components. Although, the connation has progressed in some flowers removing all external indications of the constitution of the labellum, floral anatomy

helps us to understand its morphological nature. In this connection, the remarks of WILLIS (1948) are significant. According to him, the labellum in Zingiberaceae might be two- or three lobed but it still comprises only the two antero-lateral members of the inner androecial whorl. The evidence provided by THOMPSON (1933, 1936), KIRCHOFF (1997) and BARAH & SHARMA (2012) on the morphological, anatomical and organogenesis studies of family Zingiberaceae may well be agreed with the present study and the classical conception that the labellum is of a double nature rather than triple or five-parted.

In *Hedychium coronarium* the flower has a single pollen-bearing stamen and four petaloid staminodes that are united in various ways. In tribe *Hedychieae* the most common interpretation of these fusions is that of EICHLER (1884). According to this interpretation, two staminodes are fused to form a large compound labellum (or lip), two other stamens form the lateral staminodes [KIRCHOFF, 1977]. In the present study, at the top of the ovary, the outer stamens are represented by three vascular bundles; two bundles supply the two postero-lateral staminodes that unite to form the posterior labellum, the third bundle (anterior bundle) fades out and disappear. Like the vasculature of labellum of *A. speciosa*, the labellum of *H. coronarium* well is adduced in support the classical theory, that the labellum is double in its nature.

The anterior outer staminal bundle is fading out. Thus, this stamen (anterior outer stamen) is morphologically absent. The evidence obtained from the floral ontogeny of *H. coronarium* carried by THOMPSON (1933, 1936) and KIRCHOFF (1977) supported the presence of three outer stamens but the primordium of the anterior one ceases growth soon after its initiation.

Conclusions

The present study supports the old classical concept, that the labellum is a double structure rather than triple or five-parted that was suggested by previous authors. Also, confirmed the ontogenetically evidence for the origin of the outer stamens in *A. speciosa* and *H. coronarium*. The fertile stamen of the inner whorl in the both studied species (*A. speciosa* and *H. coronarium)* is supplied by five vascular traces. These five bundles exhibiting extensive branched into small traces to feed the two large anther lobes. In addition, the investigation indicates that the ovary glands of the two studied species are vascularized.

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EFFECT OF 2,6-DICHLOROBENZONITRILE (DCB) ON SECONDARY WALL DEPOSITION AND LIGNIFICATION IN THE STEM OF *HIBISCUS CANNABINUS* L.

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Abstract: Light and electron microscopic studies were carried out on the secondary xylem of actively growing shoots of *Hibiscus cannabinus* treated with cellulose synthesis inhibitor 2,6-dichlorobenzonitrile (DCB). Treatment with 20μM DCB induced differentiation of xylem fibres with thin secondary walls and parenchyma cells with abnormal wall thickening and lignification. At concentration above 50 μM resulted in the disappearance of cambial zone, inhibition of secondary wall deposition, lignification of primary walls, deformed vessel walls and dispersed lignin distribution in secondary walls. Transmission electron microscopic study revealed the initiation and formation of large intercellular spaces between the walls of differentiating xylem elements. Abnormal pattern of wall deposition and inhomogeneous lignin distribution was evident in fibres and vessel. The length and width of both fibres and vessel elements were reduced significantly even with lower concentrations of DCB.

Keywords: 2,6-dichlorobenzonitrile, secondary wall deposition, lignification, *Hibiscus cannabinus.*

Introduction

Dichlobenil is an herbicide commonly used to control weeds in gardens, lawns, near ornamental trees etc. It is a well-known inhibitor of seed germination and growth in plant roots and shoots. The growth inhibition in actively growing plants has been demonstrated to be mainly through hindering cellulose biosynthesis which affects the cell wall formation. In most of the studies, cell culture method has been adopted to elucidate the DCB effect on plant growth. However, the effect of DCB on cell wall formation in mature intact plants is not yet studied in detail. Since wood formation represent one of most complex system of cell wall formation which has directly related to cellulose biosynthesis, present study aimed to study the effect of DCB on secondary wall deposition during wood formation in *Hibiscus cannabinus*.

The presence of a rigid cell wall is a characteristic feature of plant cells which determines the size and shape of the cell and its structure directly related to cell function [EVERT, 2006]. The wall is secreted and assembled as a complex structure in a rhythmic manner. Therefore, the secondary growth of woody stem is considered as a dynamic process that integrates multiple developmental mechanisms [GROOVER & ROBISCHON, 2006]. Structural models have been proposed for the cell wall that emphasizes a defined organization of cellulose fibrils, hemicellulose, pectins and random intervening of cell wall polymers such as lignin [ALBETSHEIM & al. 1973]. Cellulose microfibrils are synthesized by cellulose synthesizing complexes embedded in the plasma membrane [DELMOR & ARMOR, 1995], whereas other polysaccharides of cell wall matrix are assembled in the Golgi apparatus and transported in secretary vesicles to the cell surface [DRIOUICH & al.

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1993; ISABELLAE & al. 2001]. From the developmental point of view, cellulose is considered as the principal component of plant cell wall and cellulose microfibril deposition has been controlled by cortical microtubules [BASKIN, 2005]. It is widely believed that the extent of primary wall radial expansion in cambial derivatives controls how much secondary wall deposition and lignification occurs subsequently [SAVIDGE, 2000]. Many studies have been carried out to understand the role of cellulose in development of cell wall through the inhibition of cellulose biosynthesis using 2, 6-Dichlorobenzonitrile (DCB). DCB is demonstrated as inhibitor of many processes involved in cell wall development such as formation of cellulose microfibrils [MIZUTA & BROWN, 1992], cell plate formation [BURON & GARCIA-HERDUGO, 1983; VOUGHN & al. 1996] and regeneration of cell walls of protoplast [ARAD & al. 1994] and cell cycle progression [ALVIN & JOSEPH, 2003]. The first indication for the existence of feedback mechanism regulating the polysaccharide composition of cell walls came from the study of tomato and tobacco cells adapted to DCB. There was a drastic reduction of cellulose and significant enrichment in pectin [SHEDLETSKY & al. 1992]. In contrast, cell walls from DCB adapted monocot cells did not show increased pectin contents, but normal to elevated amount of other non-cellulosic materials [SHEDLETSKY & al. 1992] showing fundamental difference between dicot and monocot cell walls, including the way they compensate for the reduction of their cellulose content. Though much information is available on the role of DCB on development and biochemistry of cell wall formation particularly from cell culture experiments, it is not know how the alternations in cellulose biosynthesis affect secondary wall structure and histochemistry in woody stems. Therefore, in the present study, we examine the effect of cellulose biosynthesis inhibitor DCB on secondary growth and cell wall structure of *Hibiscus cannabinus* (Kenaf), an important fibre yielding plant belongs to Malvaceae.

Materials and methods

Plant Materials. Kenaf, a fibrous plant native to east-central Africa, belongs to family Malvaceae. It is a common wild plant of tropical and subtropical Africa and Asia. It has been a source of textile fibre for products such as rope, twins, bagging etc. As a promising source of raw material for pulp, paper and other fibre products, Kenaf has been introduced and cultivated in several countries like China, Russia, Thailand, South Africa, Egypt, Mexico and Cuba. It is woody to herbaceous annual plant, mostly unbranched, fast growing with prickly stem. It was selected for the present study as it is grows rapidly with a straight stem having distinct and sufficient amount of secondary vascular tissue. Four month old plants having 4-5 feet height growing in the Botanical Garden of Sardar Patel University were used for the experiments.

Preparation of DCB solution. A stock solution of DCB (Fluka, Gemany) was prepared in DMSO. A final experimental concentration of 20, 50, 80 and 100 µM DCB prepared by dissolving stock solution in DMSO.

Treatments. DCB treatment was carried out at the 10^{th} to 11^{th} internodes of the main stem where secondary growth was prominent. A set of 6 plants were used for each treatment. To the cut end of stem, microtip was fixed by using stripe of parafilm. Then it was filled with DCB solution through the pointed tip using a syringe. For controls, microtip was filled with DMSO for one set and distilled water for another set of plants. At an interval of every 4 days, microtip was replaced after removing 2-3 mm tissue from the cut surface and the DCB solution was applied. Segments of stems measuring 3 cm below the

site of application were collected and fixed in 3% glutaraldehyde in phosphate buffer (*p*H 7.4) after 15 days of treatment.

Sectioning and staining. Samples were subjected to hand sectioning using double edge razor blades. The sections were stained with 0.05% Toluidine blue 'O' (Sigma, Germany) for general histology [BERLYN & MIKSCHE, 1976], Phloroglucinol (Sigma, Germany)/HCl method for lignin localization [GAHAN, 1984], 0.02% aqueous Ruthenium red (Sigma, Germany) for pectic polysaccharides [JOHANSEN, 1940] and 1% Congo red for cellulose [PRAT, 1993].

Measurements. The length and width of vessel elements and fibres were measured with an ocular micrometer scale mounted in a research microscope. Ray dimension and density were measured from tangential longitudinal sections using ocular micrometer scales, 1 mm and 1 mm^2 respectively. The fibre wall thickness and vessel density were also recorded from transverse sections using ocular micrometer scales. For each parameter 100 reading were taken from randomly selected elements from six replicants. Student t-test was carried out to determine statistically significant differences of anatomical parameters at a 0.05 confidence level using Sigmastat software (Version 3.5, San Jose, CA, USA).

Electron microscopy. For ultrastructural studies the samples were immediately fixed in 2.5% glutaraldehyde in phosphate buffer (*p*H 7.2) followed by 2% osmium tetroxide. After the routine dehydration and infiltration the samples were embedded in Spurr's resin [SPURR, 1969]. Semithin sections on glass slides were stained in 1% toluidin blue and photographed using a Zeiss microscope with Carl Zeiss (KS 300) Image Analyzer. For transmission electron microscopy (TEM), ultrathin sections on nickel and gold grids were subjected to potassium permanganate and periodic acid- thiocarbohydrazide-silver proteinate (PATAg) [THIERY, 1967] staining respectively and observed under TEM (Philips, Tecnai). Micrographs were taken using Keenview soft Imaging system.

Results

Control plants treated with either distilled water or DMSO showed as active cambium with 4-6 layers of differentiating xylem elements (Fig. 1A). Towards phloem, along with sieve elements, differentiation of parenchyma and phloem fibres were noticed. Phloem fibre bundles showed 2-3 groups of cells undergoing secondary wall deposition and were rich in crystalline polysaccharides (Fig. 1C). Centripetally cambial zone was followed by 3-4 layers of cells showing secondary wall deposition (Fig. 1C). Cell walls of majority of vessels completed secondary wall deposition and underwent lignification. The vessels were round to oval in shape in control plants (Fig. 1D). Acidic polysaccharides were observed in the walls of cambial cells, dividing and differentiating elements and its distribution was more prominent towards the cell corners. DCB treatment, even in low concentration resulted in changes in the structure of cells in the cambial zone and its derivatives towards xylem and phloem. At a concentration of 20 µM, cambial cell layers reduced to 1-2 and the cells close to cambial zone showed elongation of radial walls. Cambial derivatives towards phloem were characterized by the absence of phloem fibres and abnormal wall thickening and lignification of parenchyma cells (Fig. 1E). The phloroglucinol-HCl reaction of xylem derivatives showed fibres with thin lignified secondary walls underwent lignification. Vessel walls became deformed and wavy (Fig. 1E). Treatment with 50, 80 and 100 µM DCB resulted in disappearance of cambial initials by elongation of their radial walls followed by differentiation into parenchyma cells (Fig. 1F). Parenchyma cells with primary wall showed lignification. In those cells which showed a thin secondary wall, inhomogeneous lignin distribution was noticed (Fig. 1G).

Lignin distribution was more in the cell corners and primary wall whereas secondary wall showed dispersed pattern of lignin distribution. Thin walled vessels underwent lignification without much expansion (Fig. 1G). In deformed vessels, the walls bulged into the lumen (Fig. 1H). At a concentration of 80 and 100 µm thin walled xylem fibres showed large intercellular spaces (Fig. 1I).

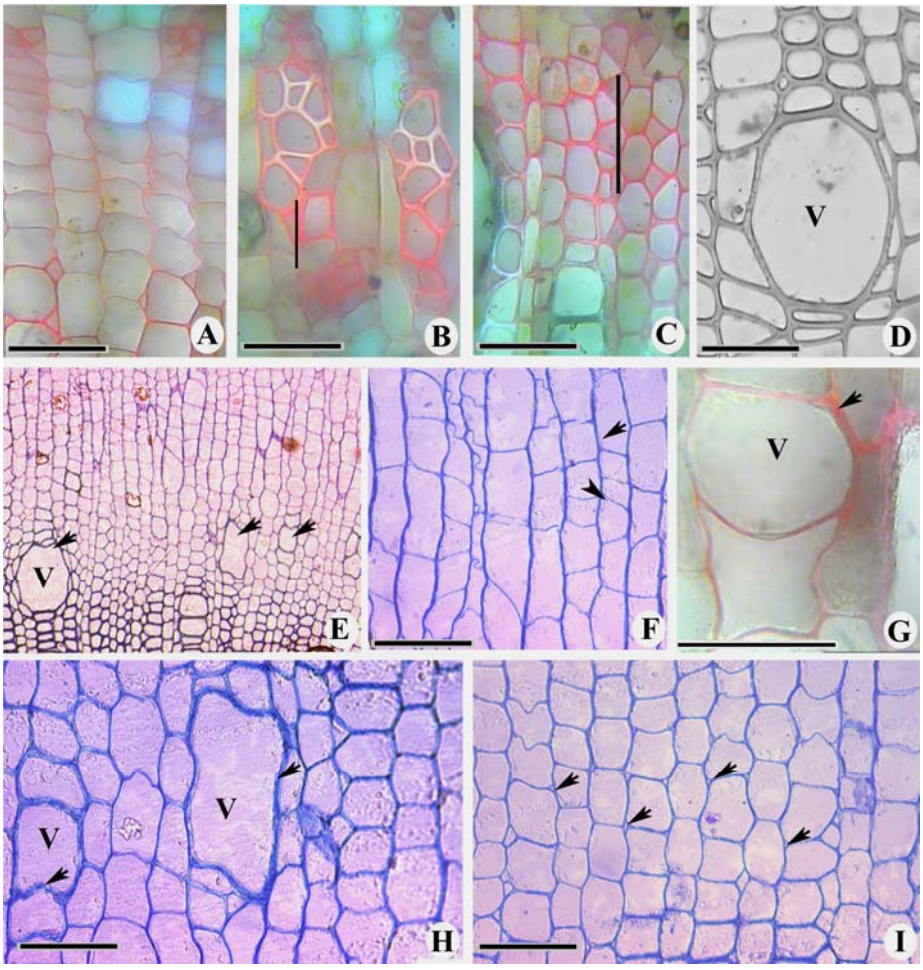

Fig. 1. (A-J) Transverse sections of stems of Kenaf treated with distilled water and DMSO (A-D) and with DCB (E-I): **A.** Cambial zone showing initial cells and differentiating elements. **B.** Phloem fibre bundles phloem fibres undergoing secondary wall deposition (vertical line). **C.** Xylem derivatives showing secondary wall deposition (vertical line) in the fibres. **D.** Stem showing thick walled vessel with a large lumen (V). **E.** cambial zone and differentiating xylem showing elongation of radial walls and deformed vessels (arrows) in the 50µm DCB treated stem. **F.** Cambial zone of 80 µm DCB treated stem showing oblique divisions (arrow head) and expansion of radial walls (arrow). **G.** Stem treated with 50 µm DCB showing partially lignified, thin secondary walls of vessels. **H.** Deformed vessel (V) in the 50 µm DCB treated stems showing poorly lignified wavy wall bulged into the lumen (arrow). **I.** Xylem derivatives of 80 µm DCB treated stem showing cells with intercellular spaces (arrows). Scale Bar = 50 µm

Transmission electron microscopic study revealed intact cell wall with relatively more lignin distribution in middle lamellae and S3 wall layers of fibres in control plants (Fig. 2A). Fibres in the DCB treated plants showed cell wall separation between adjacent cells and formation of large inter cellular spaces among differentiating xylem derivatives. Cell wall separation initiates at the cell corner middle lamellae and extends along the radial walls (Fig. 2B*).* Inhomogeneous distribution of lignin was observed in the middle lamellae region of fibres during early stages of lignification (Fig. 2C). The deformed vessel walls showed abnormal pattern of secondary wall deposition and lignification (Fig. 2D). The S1 layer became thicker and became more or less similar thickness of S2 layer while S3 layer remain thinner among three layers. The S2 layer showed more lignin distribution than S1 and S3 layer. This shows that the rhythm of secondary wall deposition and lignification were altered following DCB treatment.

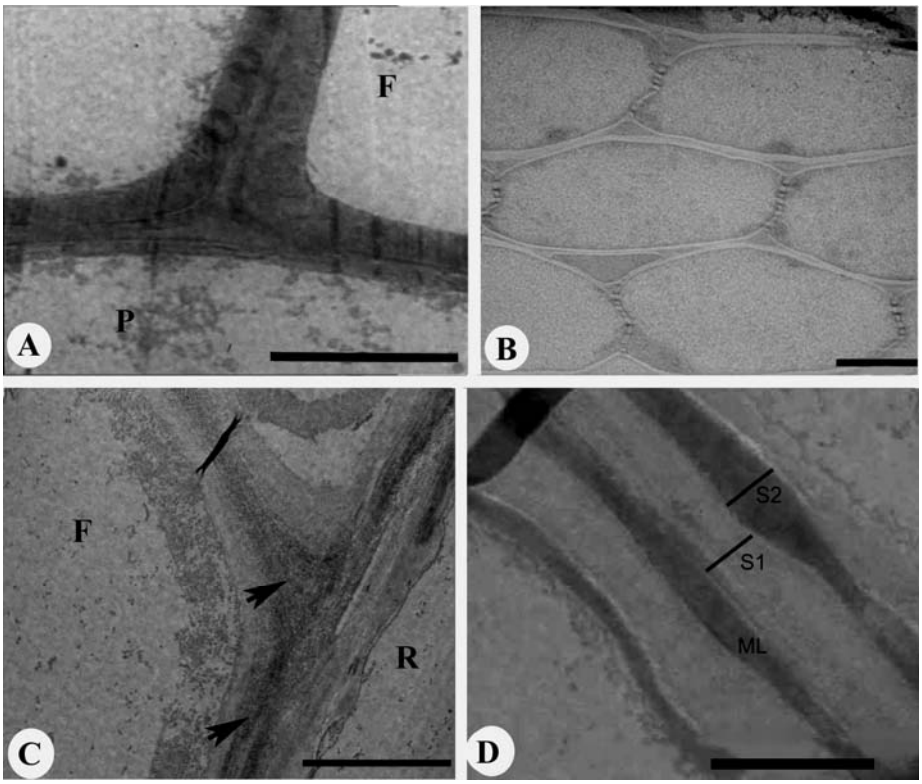

Fig. 2. (A-D) Transmission electron microscopic images from the secondary xylem of 50µm DCB treated stems of Kenaf showing intercellular space formation (A, B) and lignin distribution in xylem fibre wall (C) and deformed vessel wall (D).
A. The fibre and axial parenchyma of control stem showing the lignification pattern in cell wall ($KMnO_4$ staining) Bar = 5 µm. **B.** Radially elongated xylem derivatives showing separation of walls at cell corners (PATAg Staining) Bar = 2 µm. **C.** Fibre wall showing inhomogeneous lignin distribution during its early lignification stage ($KMnO_4$ staining) Bar = 2 µm. **D.** A magnified view of deformed vessel wall showing secondary wall layers and pattern of lignin distribution ($KMnO_4$ staining) Bar = 4 µm

Dimensions of fibre and vessel elements

There was no significant difference observed in fibre and vessel elements dimensions between plants treated with distilled water and DMSO (Tab. 1). DCB treated plants showed significant reduction in fibre length and width. Fibres became shorter and narrow with increasing concentration of DCB (Tab. 1).

DCB treatment also resulted in the reduction in length and width of vessel element. Vessel elements exhibited considerable variations in their dimensions from 20 µM concentration of DCB treatment and they became shorter and narrow with higher concentrations of DCB (Tab. 1).

Tab. 1. Dimensional characteristics of vessel element and fibres in control and DCB treated plants of Kenaf

Characte-ristics	Control				DCB Treatment							
	DW		DMSO		20µm		50µm		80µm		100µm	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Fibre dimensions												
Length	1170	140	1192	205	1014	121	1008	114	1003	153	971	219
Width	26	4	26	4	25	3	24.5	3.4	24.2	3.2	21	3.6
Vessel element dimensions												
Length	617	105	612	117	499	129	469	128	462	95	402	150
Width	93.5	16	92	12	77	13	74	14	70.6	13.7	68	10.7

Discussion

Biosynthesis and deposition of cell wall polysaccharides are believed to be the fundamental building blocks for development of plant cells. The present study reveals that the inhibition of cellulose biosynthesis by DCB has significant impact on secondary growth by altering the activity and cell wall structure of cambial cells. The division and differentiation of vascular cambial cells are the key factors determining secondary growth in higher plants [EVERT, 2006]. The expansion of radial walls and abnormally oriented tangential walls in the cambial zone cells indicates that the deposition of cellulose microfibrils has been altered after DCB treatment. Primary cell walls of plants are known to be composed of α-cellulose with high degree of crystallinity and microfibrils that are parallel to the elongation axis which determines the characteristic shape of the cell [KATAOKA & KONDO, 1999]. The orientation and crystallinity of cellulose microfibrils may have been changed by the action of DCB. There are various reports on the effect of DCB on microtubules and orientation of cellulose microfibrils. The microtubules are proposed to be guide the deposition of the cellulose synthase complex in the plasma membrane during secondary wall synthesis [GARDINER & al. 2003; WIGHTMAN & TURNOR, 2008; RAJANGAN & al. 2008]. On the other hand, HIMELSPACH (2003) reported that, DCB can deviate and disrupts microtubule orientation in root tips; however, the cellulose synthesizing machinery can re-establish well-ordered microfibril deposition indicating a self-assembly mechanism that have little reliance on cortical microtubule orientation. The altered cellulose biosynthesis by DCB or by genetic modification [PAGANT & al. 2002; SUGIMOTO & al. 2003] also shows that microfibril lose its parallel orientation indicates the importance of the certain rate of cellulose production possibly related to synthase complex density. In the presence of DCB, the primary wall cellulose synthase complexes (CSCs) appeared aggregates at sites within the plasma membrane and

sites may represent insertion site of CSC from intercellular compartment [DEBOLT & al. 2007]. The expansion and abnormal wall thickening of radial walls sites of phloem parenchyma can be such aggregation sites of CSCs.

The structural changes in the secondary wall of xylem elements are a further evidence for the inhibition of cellulose biosynthesis by DCB. Xylem fibre with thin secondary walls shows that cellulose deposition has been greatly reduced following DCB treatment. RAYMOND & al. (2009) reported that DCB has a significant impact on secondary wall deposition by slowing down the movement of CSCs beneath the regions of secondary wall formation. Reduction of secondary wall formation has been followed by the early lignification of cell walls. Though there is a significant reduction in wall thickness, the fibre walls remain rigid after lignification. However, the vessel walls became wavy and deformed. The proportion of cell wall layers altered and S2 layer showed relatively more lignin deposition than S1 and S3 layers. Earlier reports have also shown that the cell walls from dichlobenil habituated cells possess a multi-lamellate structure lacking a proper middle lamellae and were thicker and more irregular [ENCINA & al. 2001, 2002; GARCIA-ANGYULO & al. 2009]. SABBA & al. (1999) reported that long term DCB habituation results in the replacement of normal cellulosic wall with one enriched in both unesterified and highly esterified pectins. The pectins in the habituated cells appear to be arranged in prominent strands or lamellae and occur across the entire expense of the wall giving multi-lamellate structure to the cell wall and are mainly composed of polygalactouronic acid [SABBA & al. 1999; ENCINA & al. 2001]. Therefore, the abnormal wall thickening and lignification pattern might be associated with the altered chemical composition induced by DCB. Compared to fibres, the process of secondary wall deposition and lignification is faster in vessel elements. Therefore, DCB induced inhibition of secondary wall deposition followed by early lignification of thin walled vessels which already underwent expansion results in destabilized walls with poor mechanical strength. Cellulose is considered as a principal component of plant cell walls as it plays dynamic role in maintaining cell size and shape through controlling cell expansion and elongation [BASKIN, 2005]. The present study also reveals a significant reduction in length and width of fibres and vessel elements treated with DCB. This confirms that there is a positive correlation exists between cellulose microfibrils and elongation and expansion of cells.

The present study demonstrates that cellulose inhibition may induce changes in composition of other cell wall polysaccharides and polyphenols. Earlier studies reported that there is a considerable increase in pectic polysaccharides in tobacco cells adapted to DCB [SHEDLEZSK & al. 1990]. On the other hand, there was no change in pectic polysaccharides in DCB adapted monocots [SHEDLEZSK & al. 1992]. Therefore, ISABELLA & al. (2001) suggested that there is fundamental difference that occurs between dicots and monocot cell walls in feedback mechanism following inhibition of cellulose biosynthesis. Our results also indicate altered chemical composition particularly in pectic polysaccharides resulting in the formation of wide intercellular spaces in differentiating xylem elements. This demonstrates a further complex relationship between biosynthesis of crystalline and acidic polysaccharides. In addition, the high lignification even in primary walls of cells in cambial zone and phloem shows that there may be a close association that exists between flux of carbohydrates for biosynthesis of cellulose and polyphenols. The increased carbohydrate reserves following inhibition of cellulose biosynthesis may have diverted to the phenolic pathway which leads to the lignification of cell walls. However, the inhomogeneous distribution of lignin in the cell walls indicates that the changes in the

orientation of cellulose microfibrils may affect the linkage of cellulose-lignin monomers within cell walls.

Conclusions

The present study suggests that inhibition of cellulose biosynthesis has a significant effect on secondary growth through changes in the cell wall structure, histochemistry, shape and dimensions of cells in the stem of DCB treated *Hibiscus cannabinus*. The major changes induced by DCB are decrease in secondary wall thickness, abnormal distribution of lignin in cell wall layers, formation of intercellular spaces among differentiating xylem and reduction in dimensions of vessel elements and fibres. The study demonstrates that DCB influences the structure of secondary xylem through its impact on distribution pattern of major cell wall polymers cellulose and lignin.

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CONTRIBUTIONS TO THE SEEDS' STUDY OF SOME SPECIES OF THE *PLANTAGO* L. GENUS

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Abstract: *Plantago* genus includes many species, some of them known to be used in traditional and modern medicine. The most numerous information about the *Plantago* species usage in our country refers to the leaves, while information about seeds usage is sporadically reminded. Lately, there was a particular interest in the consumption of psyllium, the trade name used for the product from seeds of *Plantago ovata*, *P. psyllium* (*P. afra*) or *P. arenaria*. A special economic interest presents the seeds of these species as they are a cheap source of gelling agent for micro-propagation techniques.
The morphological study of the seeds from populations of different areas has been focused on issues of biometrics, testa micro-morphology and myxospermy. Observations have shown differences between species, and also between different populations of the same species. The myxospermy phenomenon (formation of mucilage) emphasizes individual characteristics for several taxa which may have practical uses. The achieved results have both theoretical (in order to clarify some taxonomic issues) and practical value (by capitalization in pharmaceutical or other similar domain).

Key words: *Plantago*, seeds morphology, fructification, myxospermy, seed mucilage.

Introduction

The *Plantago* genus is represented in the Romanian flora by 16 species, among which we can mention *P. lanceolata* and *P. major*, which are known for a long time [WERYSZKO-CHMIELEWSKA & al. 2012] and used by the modern and traditional medicine, with major use of their leaves. The use of seeds from *Plantago lanceolata* and *P. major*, although less known, are numerous and diverse. Outside their common usage as laxative, due to their emollient and diuretically properties, they are also used in the temperate regions but also in some areas such as Africa, in the treatment of several diseases such as gastritis, gastro-enteritis and salmonellosis, as in the cases of different respiratory illnesses. The dry seed infusion is used in the treatment of intestinal parasites in children, against diarrhea and dysentery, or as eye soothing lotion.

The seeds' mucilage is an excellent thickening agent used in cosmetics (e.g. in different hair lotions and hair sprays) but also as a stabilizer in the ice-cream industry. It is also used for different chocolate products. The seeds can be used as a source of jellifying agent in the tissue cultures. Its quality is comparable with the one of the agar, but the cost is 10 times smaller [GURIB-FAKIM, 2008]. Recent studies [SAEEDI & al. 2013] have shown that the seeds' mucilage of *P. major* can be used as excipient which allows the controlled release of the active substance.

In the last two decades a specific interest was manifested on the Romanian market for the consumption of psyllium; under this name it is sold the product obtained from the seeds of the species *Plantago ovata*, *P. psyllium* (*P. afra*) or *P. arenaria*. The latest is a

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species which, in the studies for Romanian ethno-botanic was remembered as a laxative [BUTURĂ, 1979], week-known in the traditional medicine, although in some works published around the '50 it was mentioned to be the source of some active, yet unidentified, principles. The specie is widely used in the Mediterranean areas and in China is cultivated for its medicinal seeds.

The laxative action of the seeds is due to their capacity of forming mucilage when in contact with water. The phenomena called myxospermy, is characteristic to several botanical families among which we can mention *Plantaginaceae*. From an ecological point of view the seeds' mucilage is beneficial for their dispersion, for the germination, the plants' development and the seeds' protection, which is considered as an adaptation of the plants to the specific conditions of the deserts and the sandy areas.

Materials and method

The studied material is represented by seeds collected from 5 species of the genus *Plantago* [CHATER & CARTIER, 1976], each one taken from two different areas (Tab. 1).

Tab. 1. The Provenance of the collected material (GBI – Botanical Garden Iassy)

Species	**Years**	**Place**	**Distribution**
Plantago arenaria Waldst. & Kit. (a)	2011	GBI, Iaşi	Eurasia
Plantago arenaria Waldst. & Kit. (b)	2011	Letea Forest, Tulcea	
Plantago lanceolata L. (c)	2011	Poiana Stampei, Suceava	Eurasia
Plantago lanceolata L. (d)	2012	Natural Reserve Repedea, Iaşi	
Plantago major L. (e)	2011	Aluniş, Buzău	Eurasia
Plantago major L. (f)	2011	Gheorgheni, Harghita	
Plantago media L. (g)	2012	Natural Park Vânători Neamț, Neamț	Eurasia
Plantago media L. (h)	2011	GBI, Iaşi	
Plantago schwarzenbergiana Schur. (i)	2011	GBI, Iaşi	Hungary, Romania, Serbia, S Ukraine
Plantago schwarzenbergiana Schur. (j)	2011	Reserve Valea Ilenei, Iaşi	

The species *P. arenaria*, *P. lanceolata*, *P. major* and *P. media* are known as medicinal plants, used especially in the modern and traditional medicine. *P. schwarzenbergiana* is an endemic species in the alkaline sylvosteppe from the Eastern area of the Carpathian Basin, with a specific bio-geographical importance, present only in Hungary, Romania, Serbia and the South of Ukraine. In the Red List of the superior plants from Romania published in 1994 [OLTEAN & al. 1994], it is considered a rare sub-endemic taxon in the Romanian flora. Recent pharmacognostic studies [BEARA & al. 2011] have suggested that *P. schwarzenbergiana* is a natural source of antioxidants and anti-inflammatory agents which can be exploited in the future.

The characteristics parameters of the seeds, as shape, color, dimensions and aspect were observed under the binocular microscope type Optika. The photos which are illustrating the observations were done with a Canon A540 camera type.

For underlining the myxospermy phenomena, the nutlets were humidified in distilled water and maintained for a period of approx. 30 minutes, immersed in a solution of rhutenium red, and then analyzed by means of the binocular microscope type Optika. There

were followed some aspects concerning the presence or absence of the mucilage, its aspect and consistency, the proportion as compared to the seed's volume.

The study was conducted in the Laboratory of micropropagation and germplasm preservation of the Botanical Garden, University "Alexandru Ioan Cuza" Iasi.

Results and discussions

The descriptive information of the seeds of the studied species is not homogeneous in the specific literature [GLEASON & CRONQUIST, 1991; GRIGORIEV, 1958; ZHENYU, 2002; PAUCĂ & NYÁRÁDY, 1961], and in most cases is incomplete. Characteristics such as the number of seeds in the pyxis, the dimensions or the colour are different in the works published in different periods of time and with reference to species from very diverse areas (Tab. 2). Most probably these characteristics are mostly influenced by the ecological conditions, which is in connection to the very wide distribution area of the mentioned species (with the exception of *Plantago schwarzenbergiana*).

The taxa we have analyzed are presenting an obvious morphological diversity (dimensions, color), both between the individuals from different areas as well as within the collected material from the same area. The biometrical measurements have shown obvious differences to the species *P. lanceolata* and *P. media*, originated from areas where the ecological conditions are very different (mountain area compared with hilly area, with different pluviometric regimes). As well, all data obtained by us for the species *P. arenaria* and *P. lanceolata* are slightly different from those mentioned in the literature. We have observed that the width of the seed is a variable character within a much restrained interval as compared to the length (Tab. 3).

The morphological types of seeds (Fig. 1, 2) belonging to the *Plantago* genus mentioned by different authors are different. LIU & al. (1992) are mentioning four types: multi-angular, in species with 6-30 seeds in the fruit; navicular, in species with two seeds in the fruit; ovoid; recti-circular, in species with 1-2 or 4-5 seeds in the fruit, while SHEHATA & LOUTFY (2006) is underlining a wider morphological diversity (oblong, cymbiform, ovoid, fusiform, lenticular, circular, angular, ellipsoidal and reniform).

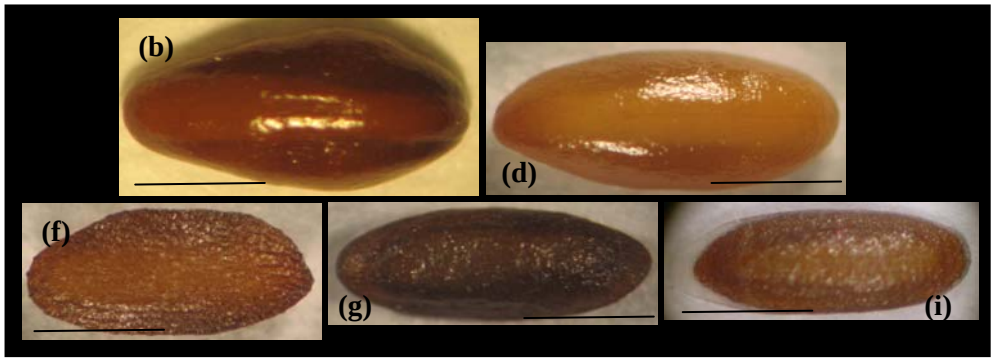

Fig. 1. Morphological aspects of the seeds of *Plantago* – dorsal (scale bar – 1 mm)

Tab. 2. Bibliographical information concerning the seeds of the *Plantago* species

Species	Flora RSR (Paucă, A., Nyárády E. I., 1961)	Flora URSS (Grigoriev Yu., 1958)	Flora of China (Zhenyu L., 2002)	Manual of Vascular … (Gleason H. A. & Cronquist A., 1991)	PROTA (Gurib-Fakim, A., 2008)
P. arenaria	Seeds 2, 2-2.5 mm, navicular shape, with a broad groove on a side	Seeds oblong- ellipsoid, dark brown, shiny, 2,5 mm long; one side convex, other canaliculate	Seeds 2, brown to blackish brown, ovoid-ellipsoid to ellipsoid, 2.5-2.8 mm, shiny, with a broad groove on ventral face	Seeds 1 or 2, brown, 2-3 mm	
P. lanceolata	Seeds 2, 2 mm long, blackish	Seeds oblong or oblong-ellipsoid, one side convex, other canaliculate	Seeds (1 or) 2, brown to dark brown, narrowly ellipsoid to oblong, 2-2.6 mm, shiny, with a broad groove on ventral face	Seeds (1) 2, shiny, blackish, 2 mm, deep concave on adaxial face	Seeds (1–)2–3, oblong-ellipsoid, 2.5–3 mm long, yellow-brown to dark brown, mucilaginous when wet
P. major	Seeds 6-30, dark brown, moderate verrucous	Seeds 1 mm long, horizontal, ± angulate	Seeds (8-)12-24(-34); yellowish brown, ovoid, ellipsoid, or rhomboid, 0.8-1.2 mm, angled, ventral face prominent to slightly flat	Seeds 6-30, 1 mm long, strongly reticulate	Seeds (4–)6–34, ellipsoid or ellipsoid-trigonous, 1–1.5 mm long, dark brown to dull black, mucilaginous when wet
P. media	Seeds 4 or more, moderate verrucous, black	Seeds 2-5(6)	Seeds 2-4, yellowish brown to brown, ellipsoid, 1.5-2 mm, shiny, ventral face prominent	Seeds 2-4, 2 mm	
P. schw.	Seeds 1, 2.5 mm long, irregular, ellipsoid or ± oblique truncate, moderate fleshy, convexe on dorsal face and minutely dotted				

Tab. 3. Comparison between personal biometrical data and the specific literature regarding the seeds of *Plantago* species

Species	Personal results (mm) min. – max.		Flora RSR (Paucă, A., Nyárády E. I., 1961)	Flora URSS (Grigoriev, Yu. 1958)	Flora of China (Zhenyu L., 2002)	Manual of Vascular … (Gleason H. A. & Cronquist A., 1991)	PROTA (2008) (Gurib-Fakim, A., 2008)
	Long	Width					
P. arenaria (a)	1.2 – 2.3	0.6 – 1.3	2-2.5 mm	2.5 mm	2.5 – 2.8 mm	2 – 3 mm	
P. arenaria (b)	1.7 – 2.5	0.5 – 1.2					
P. lanceolata (c)	2 – 2.8	0.9 – 1.1	2 mm		2 – 2.6 mm	2 mm	2.5 – 3 mm
P. lanceolata (d)	1.5 – 2.4	0.7 – 1.1					
P. major (e)	0.9 – 1.6	0.5 – 0.9		1 mm	0.8 – 1.2 mm	1 mm	1 – 1.5 mm
P. major (f)	0.7 – 1.6	0.4 – 0.9					
P. media (g)	1 – 1.9	0.4 – 1			1.5 – 2 mm	2 mm	
P. media (h)	1.2 – 2.1	1.1 – 1.8					
P. schw. (i)	1 – 1.6	0.4 – 0.6	1.25 mm				
P. schw. (j)	1 – 1.6	0.3 – 0.6					

According to the typology established by LIU (1992), the studied seeds are: multi-angular - *P. major*, the navicular type - *P. asiatica*, *P. lanceolata* and *P. media*, ovoid – P. *schwarzenbergiana*.

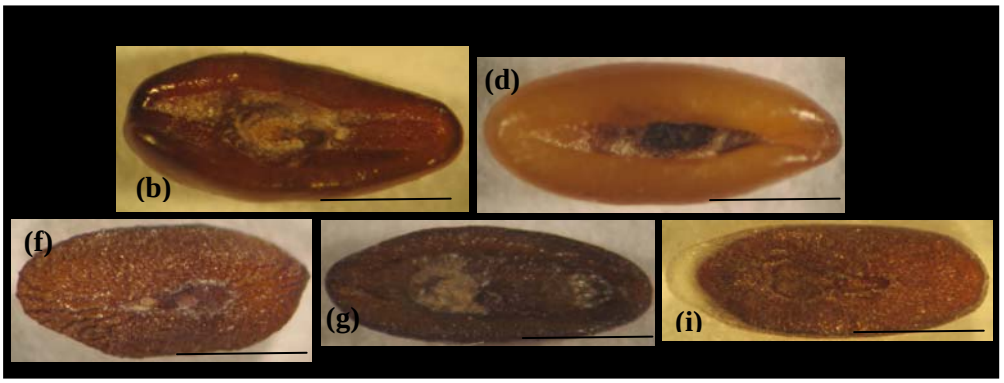

Fig. 2. Morphological aspects of the seeds of *Plantago* – ventral (scale bar – 1 mm)

The diversity of the seeds shape in *Plantago major*, which has a wide number of seeds in the pyxis belonging to the multi-angular type, is determined by their disposal in fructification (Fig. 3) and by the asymmetrical shape of the pyxis. Thus the seeds from the top of the pyxis are following its semi-spherical shape, and during the development the seeds are occupying all available spaces, which are shaping their angular, irregular characteristic. Another feature influenced by the simultaneous development of the seeds is the placement of the hilum on the median axes (Fig. 3), either in the central position, or in the lower third part.

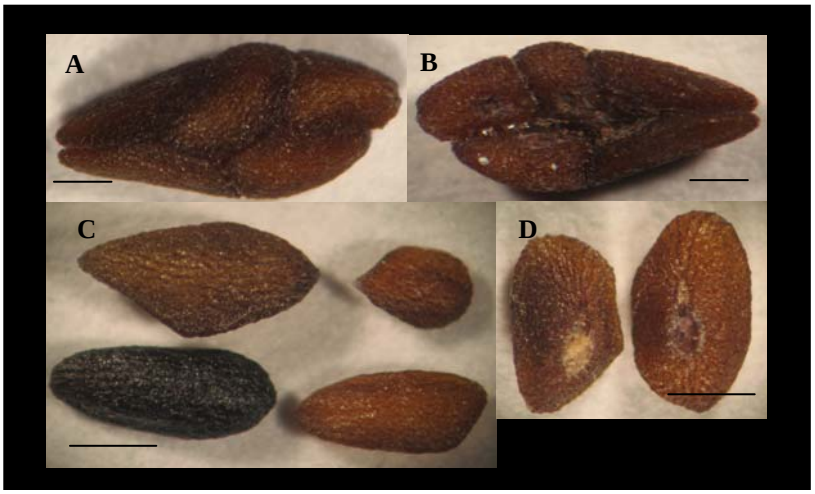

Fig. 3. Morphological aspects of the seeds of *Plantago major*. A. Fructification – dorsal view; B. Fructification – ventral view; C. Dorsal view of the seeds for highlighting the shape; D. Ventral view of the seeds for highlighting the hilum (scale bar – 1 mm)

At *Plantago arenaria* we can observe (Fig. 1) on the dorsal surface a light brown longitudinal area, an aspect mentioned only in the official monographs of the medicinal plants [WHO, 1999]; although here it is specified that the area is widening in the median area, and in the case of our studied material, the width of the band is quite variable, but always present.

Plantago schwarzenbergiana, due to its restrained area, is the species for which we can find less morphological descriptions. This taxa presents seeds of 1-1.6 mm long and 0.3-0.6 mm width, are brown-yellowish to black, dull, with an ellipsoidal shape or diagonally truncate, the surface has a reticular aspect, on the back they are convex, ventral almost flat, with a narrow and deep excavation in the center which is corresponding to the hilum (Fig. 2).

The myxospermy phenomenon is manifesting through the dispersion of the mucilaginous seeds, mainly with the help of the rain drops. The seeds are adhering through the mucilaginous layer to the moist surface of the soil together with the dry mother plant (atelechory) or they can adhere to the bird legs, being thus spread to large distances (zoochory). This feature can also have a defensive character, as it can annul or prevent the collection of the seeds by ants.

From a pharmacological point of view the presence of this phenomenon is valued by using seeds belonging to species like *Plantago* as laxative agents, but the information concerning the particularities of this phenomenon are very few. We know that the mucilage contains hydrophilic polysaccharides [KAR, 2003], present especially in the seeds testa, and in the case of *Plantago major* it has been recently shown [MAJID SAEEDI & al. 2013] that this mucilage is not essentially modifying the accompanied active substances, thus it can be used as excipient. But we do not have enough data upon the quantity and the composition of polysaccharides and it is very probable that these vary a lot, an aspect suggested by our results upon the studied species.

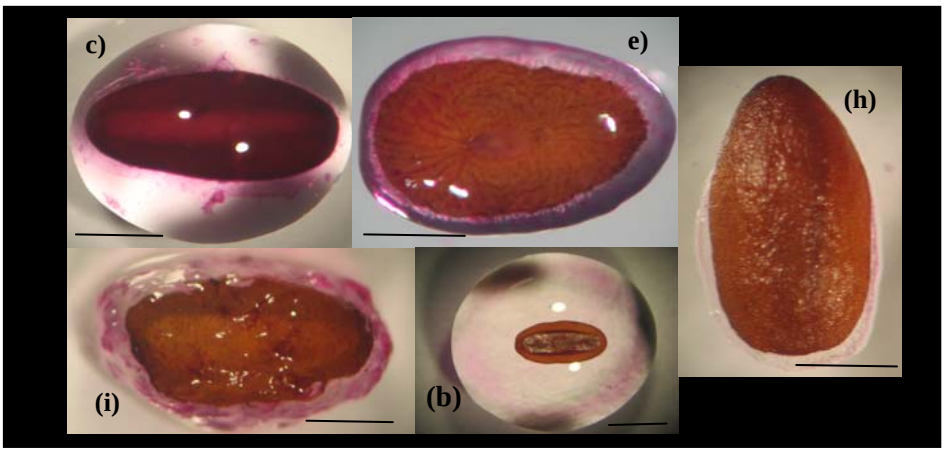

Fig. 4. Highlighting the myxospermy phenomenon to *Plantago* species (scale bar – 1 mm)

After soaking the seeds with water, we have observed the presence of the mucilage in all taxa, but the aspect and especially the resulted quantity are presenting obvious differences. Thus, the aspect (Fig. 4) is hyaline and even to all taxa, with the exception of *Plantago schwarzenbergiana* seeds; its form is circular to *P. arenaria* and *P. lanceolata* and it follows the same form of the seed to the others taxa. The mucilage quantity is smaller (less comparative with the volume of the seed) in *P. major*, *P. media* and *P. schwarzenbergiana*, while in *P. arenaria* and *P. lanceolata* is bigger (bigger or equal to the volume of the seed).

We can notice that there is no linear correlation between the xerofitism degree and the mucilage percentage emitted by seeds. Thus *P. arenaria* and *P. lanceolata*, where the quantity of mucilage is bigger are xerophilous species, respectively euryhygrous, with totally contrasting ecological requests. The quantitative differences between the species with similar ecological requests (*P. arenaria* – xerophilous and *P. media* – xeromesophilous) relating to the emitted mucilage are quite large.

Conclusions

The morphological study of the seeds is underlying their ecological variability, which explains the relatively wide range of descriptions from the classical textbooks. The data resulted from our observations can help completing the information of the specific literature and also to clarify some aspects relating to the genre taxonomy.

The study of the myxospermy phenomenon can supply useful data from a taxonomic, ecologic but also from a pharmacological point of view (aiming to obtain new pharmaceuticals). The diversity of the forms is that a more thorough study can provide more clear explanations upon the importance of this phenomenon in the process of plant adaptation to the environment.

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A UNIQUE TYPE OF ENDOSPERM IN *PANAX WANGIANUS* S. C. SUN

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Abstract: *Panax wangianus* S. C. Sun (Araliaceae) is a critically endangered, medicinal plant of north-east India. The objective of this research was to study post-fertilization changes in embryo-sac of *P. wangianus.* A characteristic feature has been observed in the endosperm in *P. wangianus* in which both the nuclear and cellular endosperm remains together in the mature seeds. The embryo is present in the nuclear part of endosperm so that it can draw the nutrition easily. The embryo probably exerts some physical and physiological forces that prevent the nuclear endosperm to become cellular.

Keywords: *Panax wangianus* S. C. Sun, medicinal plant, North-East India, post-fertilization changes, endosperm.

Introduction

The genus *Panax* L. (Araliaceae) consists of 18 species worldwide, of which two species grow in eastern North America and the other species in eastern central Asia. [REUNOV & al. 2008]. The generic name *Panax* is derived from the Greek term meaning "cure all" for its reputed medicinal use in China [ANDERSON & al. 2002]. The Chinese have been using ginseng for over 2000 years as a tonic, a stimulant and a fatigue-resistance medicine [WEN & NOWICKE, 1999].

P. wangianus (syn. *Panax pseudoginseng*) S. C. Sun is a critically endangered [PUSHPANGADAN & NAIR, 2005] herb located in the dense wet forests and bamboo forests of southwestern China, altitude 800-1350 m AMSL [WEN, 2001]. In India, it is native to sub-tropical wet forests of North-East Himalayan regions particularly in Meghalaya sacred groves such as Law Lyngdoh, Smit (Nongkrem), Law Lyngdoh (Mawphlang) and Shillong peak [VENUGOPAL & AHUJA, 2011]. Out of 18 species of the genus *Panax*, the 17 species are monoecious and *P. trifolius* is a dioecious plant [WEN, 2001].

Most of the seeds have persistent endosperm which acts as the repository of reserve food materials in the form of proteins, carbohydrates, fats and vitamins [KRISHNAMURTHY, 1998]. Apart from the importance of endosperm for the plants themselves, they are most important in the food chains of both the man and animals. Probably, endosperm is more valuable to man than any other plant part [VIJAYARAGHAVAN & PRABHAKAR, 1984]. During the course of study of post-fertilization changes in embryo-sac the authors came across a new type of endosperm in *P. wangianus*.

Materials and methods

The bisexual flowers of *P. wangianus* at various stages were collected from the field during the growing season April to September from 2007-2009 and fixed in FAA

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(formalin : acetic acid : 50% ethyl alcohol = 5 : 5 : 90 v/v). The customary methods of dehydration, infiltration, and embedding in paraffin were used [O'BRIEN & McCULLY, 1981]. Samples were sectioned at a thickness of 7-10µm by using Leitz rotatory microtome. The sections were stained with Safranin fast green [JOHANSEN, 1940] and observed at 40x under Olympus BX-51.

Results and discussion

P. wangianus grows in a colony of a few plants under shady forest floor with rich leaf litter in Nongkrem sacred grove. *P. wangianus* had a whorl of digitately compound leaf at the summit of aerial shoot; the leaf (prong) consists of three to eight palmately compound leaflets with a petiole, of which the terminal three to five leaflets are larger than the lateral two to three leaflets. Leaves are exstipulate or occasionally with persistent stipules; leaflets are bristly along veins and veinlets on both surfaces, narrowly lanceolate to broadly linear. Rhizome is creeping and elongated with thick internodes [VENUGOPAL & AHUJA, 2011].

P. wangianus is a monoecious plant. The flowers are on umbellate inflorescence (Fig. 1). Flowers are actinomorphic, bracteate, small, often greenish-yellow, perianth biseriate, pentamerous, the calyx inconspicuous, adnate to ovary, petals 5 broad at base, arising from disc and usually valvate. Nectariferous epigynous disc present, covering the ovary top. Ovary is inferior, three to five carpellate with uniovulate. The ovules are unitegmic, pendulous, anatropous, with an enlarged obturator derived from the funiculus.

Fig. 1. An enlarged view of umbel inflorescence of *Panax wangianus*. Bar = 2.0 cm.

The fusion product of two polar nuclei with the second male gamete or sperm cell constitutes the primary endosperm nucleus (PEN) which is triploid in nature located just below the zygote. The PEN shows precocious development than the zygote. It undergoes several mitotic divisions resulting into a nuclear condition; all nuclei are suspended in a cytoplasmic strand around the central vacuole and contain more than one nucleolus (Fig. 2). Nucleolar fragmentation is of common occurrence [GOPINATH, 1944]. Initially the

endosperm is nuclear with two distinct highly folded haustoria when the ovule is young (Fig. 3). Subsequently, centripetal wall formation sets in and the endosperm becomes cellular (Fig. 4). The cells are large, polyhedral, thin-walled and vacuolated with prominent nuclei. At maturity they become packed with reserve food materials mainly starch and proteins in nature as similar in *P. ginseng* [YU & al. 1992]. The starch was localized with IKI (Iodine potassium iodide) and PAS reaction while the protein bodies were stained with methyl green bromophenol blue, which gave positive reaction [O'BRIEN & McCULLY, 1981]. As the ovule enlarges in size, the haustoria are unfolded and absorb nutrients from the integument (testa). The endosperm is very large and occupied entire portion of the seed.

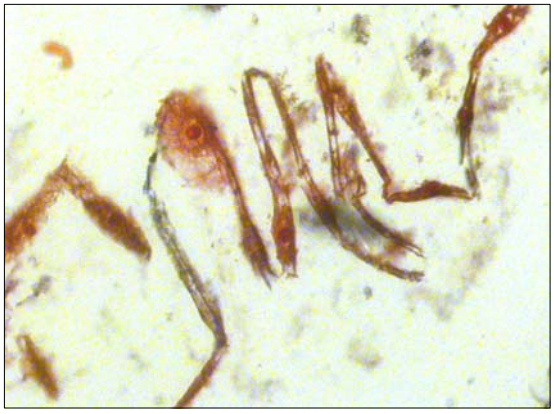

Fig. 2. An enlarged view of haustorium with several nuclei suspended within the haustorium. Bar = 40 µm.

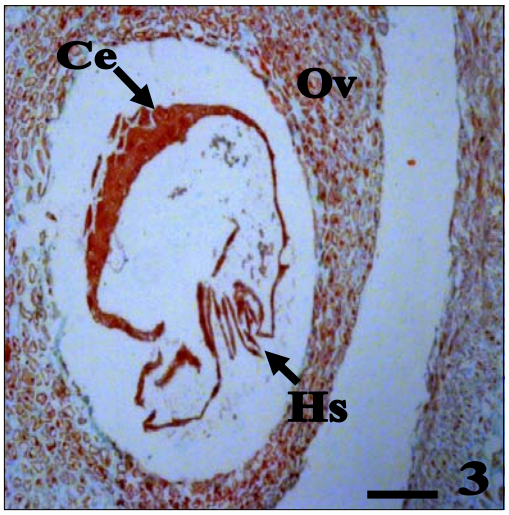

Fig. 3. Longitudinal section of ovule showing the differentiating endosperm proper with two highly folded haustoria. Ov = ovule; Ce = cellular endosperm; Hs = haustoria. Bar = 50 µm.

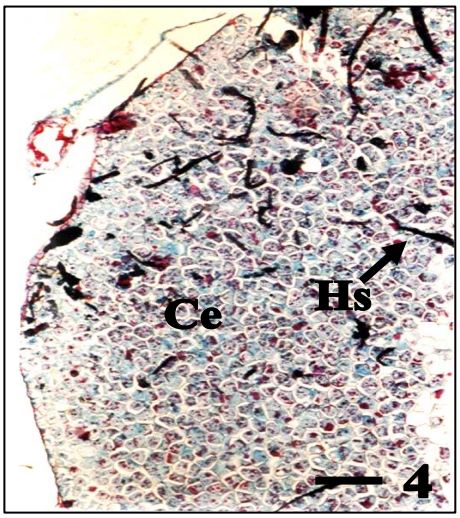

Fig. 4. A portion of cellular endosperm showing the haustoria in the cellular region. Hs = haustoria; Ce = cellular endosperm. Bar = 45 µm.

In Araliaceae, the endosperm is first nuclear and later wall formation sets in and it becomes completely cellular e.g. *Panax fruticosa, Polyscias pinnata, Hedera australiana, Heptapleuram venulosum, Brassaia actinophylla, Tieghemopanax sambucifolius, Eleutherococcus senticosus* and *Panax ginseng* [GOPINATH, 1944; MOHANA RAO, 1972; YU & KIM, 1991; ZHURAVLEV & al. 2008]. In the majority of the angiospermic families, the endosperm becomes cellular as seed mature. However, in *Limnanthes* and *Oxyspora paniculata*, free-nuclear condition persists until the endosperm is almost completely consumed by the developing embryo [MATHUR, 1956; SUBRAMANYAM, 1951].

In some families like Brassicaceae, Cucurbitaceae, Fabaceae and Proteaceae, wall formation is no doubt initiated but does not proceed beyond the central region. Thus, the central chalazal region remains free-nuclear. Since the cellular region grows further by cell divisions and the nuclear region by free nuclear divisions, the distinction between the two regions is maintained for some time [VENKATA RAO, 1967]. Cell formation in the nuclear region commences after the cellular part has attained the maximum dimensions [VIJAYARAGHAVAN & PRABHAKAR, 1984]. A characteristic feature has been observed in the endosperm in *P. wangianus* in which both the nuclear and cellular endosperm remains together in the mature seeds. The embryo is present in the nuclear part of endosperm so that it can draw the nutrition easily (Fig. 5). The embryo probably exerts some physical and physiological forces that prevent the nuclear endosperm to become cellular.

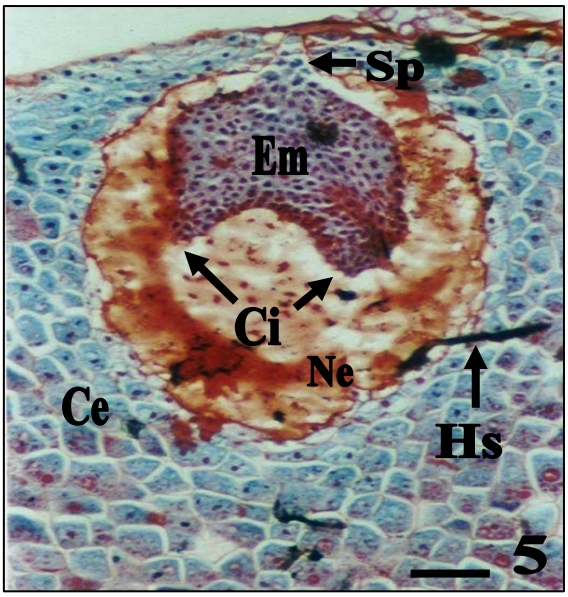

Fig. 5. Longitudinal section of seed showing endosperm with both the nuclear and cellular nature. Note the embryo is present in the nuclear part of endosperm. Ne = nuclear endosperm; Ce = cellular endosperm; Hs = haustoria; Em = embryo; Sp = suspensor; Ci = cotyledonary initials. Bar = 70 µm.

In *P. wangianus*, the cellular region of endosperm proper produces several haustoria to absorb nutrients (Fig. 4). The integuments (testa) are totally absorbed by the endosperm haustoria. Therefore, in *P. wangianus*, the seed coat is derived by the innermost layers of the locule by undergoing lignifications to protect the embryo and endosperm which is also a significant feature in *P. wangianus.*

Conclusions

During the course of study of post-fertilization changes in embryo-sac we came across anew type of endosperm in *P. wangianus.* The co-existence of nuclear and cellular type of endosperm in the mature seeds is a peculiar feature in *P. wangianus*. The heart-shaped embryo is present in the nuclear part of endosperm so that it can draw easily the nutrition.

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IN VITRO RHIZOGENESIS IN PAPAYA (*CARICA PAPAYA* L.)

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Abstract: The seeds of two papaya (*Carica papaya* L.) cultivars ('Rainbow' and 'Sunrise Solo') were germinated on Murashige and Skoog (MS) medium with 3% sucrose, and free of plant growth regulators. Papaya contains some important secondary metabolites such as papain, and there would be interest in the *in vitro* mass production of papaya tissue of uniform origin. The most obvious form would be through the induction of somatic embryos, but rhizogenesis, an unexplored method, could provide as-yet unknown advantages. In this study, with the objective of artificaially inducing rhizogenesis *in vitro*, young leaves of both cultivars were placed on MS basal medium exposed to 5 concentrations (0, 1, 2, 4 or 8 mg/l) of auxins (2,4,5-trichlorophenoxyacetic acid, 2,4,5-T; indole-3-acetic acid, IAA; indole-3-butyric acid, IBA; α-naphthaleneacetic acid, NAA; β-naphthoxyacetic acid, BNOA) or phloroglucinol. All auxins could induce adventitious roots. Most roots (23/explant) formed with 2 mg/l NAA. The ability to induce only roots without any other intermediary organs such as callus or shoots provides an exclusive system for possible root-specific secondary metabolite production without the need for transgenic technologies such as *Agrobacterium rhizogenes*, or could provide a model protocol for more in-depth developmental studies on root development in papaya, an unexplored topic for this tropical plant.

Key words: leaf, Murashige and Skoog, paw-paw, roots, seeds.

Introduction

Papaya (*Carica papaya* L.; Caricaceae) is most frequently propagated by seed (reviewed in TEIXEIRA DA SILVA & al. 2007; JIMÉNEZ & al. 2014). Papaya seeds and other organs contain papain (fruit) and other secondary metabolites such as flavonoids and coumarin compounds in leaves [CANINI & al. 2007]. An *in vitro* protocol that would allow for the mass production of papaya tissue could benefit the commercialization of plants for products other than just the fruit. Several protocols for somatic embryogenesis in papaya exist (reviewed in TEIXEIRA DA SILVA & al. 2007; ANANDAN & al. 2012), as does a protocol for the photoautotrophic micropropagation of this tropical crop [TEIXEIRA DA SILVA, unpublished data]. The objective of this study was to induce rhizogenesis from young leaf tissue of seed-derived seedlings, which are vigorous and highly receptive *in vitro*. To date, no study has yet examined rhizogenesis in papaya.

Materials and methods

All auxins (PGRs) were purchased from Sigma-Aldrich (St. Louis, USA) and were of tissue culture grade. All other chemicals and reagents were of the highest analytical grade available and were purchased from Wako or Nacalai Tesque (Osaka, Japan), unless specified otherwise. Seeds of two hybrid papaya (*Carica papaya* L. cv. 'Rainbow' and

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'Sunrise Solo') cultivars were purchased from a local supermarket with guaranteed import quality and with no (or few) apparent surface infection or markings and were surface sterilized and germinated using the protocol of GIANG & al. (2011). Briefly, seeds were removed from ripe fruits, soaked for 48 h, washed in running tap water to remove the sarcotesta. Only sinking seeds following a floatation test (i.e., viable seeds) were used. Seeds were surface sterilized in 0.1% mercuric chloride ($HgCl_2$) + 2-3 drops of Tween-20 for 5 min, rinsed 3 times in sterilized distilled water (SDW), sprayed with 80% ethanol for 1 min then rinsed 3 times in SDW. Surface-sterilized seeds were slightly embedded (5/Petri dish) in autoclaved (100 KPa; 21 min) full-strength (macro- and micronutrients) MURASHIGE & SKOOG (1962) (MS) medium (pH 5.8) containing 3% sucrose and 2 g/l gellan gum (Gelrite®, Merck, USA). Petri dishes were sealed with Parafilm® and incubated at 25 °C under a 16-h photoperiod with a light intensity of 45 µmol/m^2/s provided by plant growth fluorescent lamps (Plant Lux, Toshiba Co., Japan). Young leaves of 10-day-old seedlings were cut at the junction with the stem, pooled, and exposed to 5 concentrations (0, 1, 2, 4 or 8 mg/l) of auxins (2,4,5-trichlorophenoxyacetic acid, 2,4,5-T; indole-3-acetic acid, IAA; indole-3-butyric acid, IBA; α-naphthaleneacetic acid, NAA; β-naphthoxyacetic acid, BNOA). The effect of phloroglucinol, which has strong auxin-like activity [TEIXEIRA DA SILVA & al. 2013], was also tested. After 30 days, the number of adventitious roots that formed from seed-derived seedling epicotyls was assessed. Experiments were organized in a randomized complete block design (RCBD) at 10 explants/treatment. All treatments and experiments repeated in triplicate. Data was subjected to analysis of variance (ANOVA) with mean separation by Tukey's multiple range test ($P \leq 0.05$; IRRISTAT version 3.0).

Results and discussions

Using the GIANG & al. (2011) protocol, the seeds of both papaya cultivars could be successfully sterilized. 100% germination was possible (Fig. 1A). Even though protocols for somatic embryogenesis in papaya exist (reviewed in TEIXEIRA DA SILVA & al. 2007a; ANANDAN & al. 2012), no study has yet examined rhizogenesis.

In this study, all auxins could induce roots (Fig. 1B-F). However, exposure to 2 mg/l NAA resulted in highest root formation (23.6/explant and 21.8/explant for 'Rainbow' and 'Sunrise Solo', respectively) (Tab. 1). For both cultivars, in the absence of any auxin, no roots formed. Except for IBA, in which rhizogenesis peaked at 2 mg/l for both cultivars, the other auxins showed a decreasing trend in terms of number of roots/explant and explant weight as the concentration of auxin increased from 1 to 8 mg/l (Tab. 1). IAA, IBA and 2,4,5-T induced more root hairs than the other auxins (Fig. 1D, 1E, 1F, respectively). PG, a known auxin-like compound [TEIXEIRA DA SILVA & al. 2013], produced few roots on explants (Tab. 1), but could increase the amount of adventitious roots on plantlets when cultured on Hyponex® medium (data not shown). The ability to induce only roots without any intermittent organs such as callus or shoots could be a simple yet effective way to mass produce root-specific secondary metabolites without using transgenic agents such as *Agrobacterium rhizogenes*, even though the transgenic route remains an important tool for other applications, such as the introduction of virus resistance to papaya [TENNANT, 2010]. This rhizogenic model could also allow for in-depth developmental analyses of root development in papaya. Auxin-induced rhizogenesis through the application of single doses of auxins, usually singly, was also possible in chrysanthemum thin cell layers [TEIXEIRA DA SILVA, 2003]. Thin cell layers provide an effective system for regeneration in many model plant systems [TEIXEIRA DA SILVA & DOBRÁNSZKI, 2013].

Tab. 1. Rhizogenic response of two papaya (*Carica papaya* L.) cultivars to auxins and PG.

Treatment	Conc. (mg/l)	Cultivar	No. roots/explant	Explant weight (mg)*
Control (no auxins)		Rainbow	0 h	44 f
		Sunrise Solo	0 h	47 f
NAA	1	Rainbow	14.2 b	224 b
	2		23.6 a	316 a
	4		8.7 cd	109 d
	8		0 h	39 f
	1	Sunrise Solo	11.2 bc	203 b
	2		21.8 a	286 ab
	4		7.9 cd	104 d
	8		0 h	42 f
IAA**	1	Rainbow	4.8 e	106 d
	2		2.6 fg	85 de
	4		1.1 gh	73 e
	8		0 h	38 f
	1	Sunrise Solo	4.1 ef	97 d
	2		1.8 g	78 e
	4		0.3 h	51 f
	8		0 h	40 f
BNOA	1	Rainbow	2.7 fg	91 d
	2		3.1 f	103 d
	4		1.3 gh	77 e
	8		0 h	40 f
	1	Sunrise Solo	2.1 g	88 de
	2		2.8 f	99 d
	4		0.4 h	56 f
	8		0 h	43 f
IBA**	1	Rainbow	6.7 d	93 d
	2		8.4 cd	114 cd
	4		2.6 fg	74 e
	8		0 h	44 f
	1	Sunrise Solo	7.1 d	104 d
	2		9.4 c	136 c
	4		4.2 ef	86 de
	8		0 h	44 f
PG	1	Rainbow	1.4 gh	76 e
	2		3.2 f	108 d
	4		2.1 g	74 e
	8		0 h	46 f
	1	Sunrise Solo	1.9 g	78 e
	2		3.6 f	132 c
	4		2.4 fg	91 d
	8		0 h	46 f
2,4,5-T**	1	Rainbow	8.2 cd	144 c
	2		4.1 ef	116 cd
	4		0.8 gh	72 e
	8		0 h	41 f
	1	Sunrise Solo	6.8 d	141 c
	2		2.3 g	108 d
	4		0.4 h	76 e
	8		0 h	40 f

Data presented as means (n = 30/treatment); different letters within a column across treatments and cultivars indicate significant differences ($P \leq 0.05$; Tukey's multiple range test). Auxin abbreviations: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; BNOA, β-naphthoxyacetic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, α-naphthaleneacetic acid. PG, phloroglucinol. * The basal weight of a cotyledonary explant = 36 mg (n = 30). ** Many root hairs formed on the roots induced by these auxins (see Fig. 1D, 1E, 1F)

Fig. 1. The qualitative response of papaya (*Carica papaya* L. cv. 'Sunrise Solo') to different auxins. (A) Sterilized seed germination and 10-day-old young leaves used for the study. Rhizogenesis in response to 1 mg/l of (B) NAA, (C) BNOA, (D) IAA, (E) IBA or (F) 2,4,5-T.

Conclusions

Rhizogenesis, defined in this study as the artificial induction of roots *in vitro*, was possible from young papaya leaves in response to several auxins, but not to phloroglucinol.

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Abbreviations: 2,4,5-T, 2,4,5-trichlorophenoxyacetic acid; BNOA, β-naphthoxyacetic acid; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; NAA, α-naphthaleneacetic acid; PG, phloroglucinol; PGR, plant growth regulator.

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PRELIMINARY CONSERVATION EFFORT ON *RHIZOPHORA ANNAMALAYANA* KATHIR., THE ONLY ENDEMIC MANGROVE TO INDIA, THROUGH *IN VITRO* METHOD

KATHIRESAN Kandasamy[1], RAVINDER Singh Chinnappan[1*]

Abstract: An efficient protocol was established for *in vitro* clonal propagation for *Rhizophora annamalayana* Kathir. the only endemic mangrove species to India. Initially the explants were surface sterilized appropriately with 0.1% mercuric chloride for one minute, 70% ethanol for 30 seconds and 3% hydrogen peroxide for one minute. Then the explants were treated with three different antioxidants for reduction of phenol browning of the explants. Among the antioxidants, 10 mg/L ascorbic acid was found to be the best. Among the five different tissue culture media - B5, WPM, MS, SH and Y3- tested, MS medium was chosen to be the best for meristem culture. Among the growth regulators, cytokinins (benzyl adinine, kinetin and zeatin) used alone and in combination with auxins (naphthalene acetic acid, indole acetic acid, indole butyric acid), the shoot growth was better observed after 20 days when MS medium was incorporated with 3.0 mg/L of benzyl adinine and 3.0 mg/L of kinetin with coconut milk. This is the initial step of tissue culture for the recovery of the fast disappearing *Rhizophora annamalayana* Kathir. Further research is progressing on mass multiplication and field transfer.

Keywords: mangroves, *in vitro* propagation, growth regulators, *Rhizophora annamalayana*.

Introduction

Rhizophora annamalayana Kathir. is fast disappearing mangrove species and is the only species endemic to India (Fig. 1a), restricted to Pichavaram mangrove forests of Southeast coast of India. This species faces serious problem of poor flowering, fall of flower buds and extremely poor seed setting making natural regeneration of the species very difficult [KATHIRESAN, 2000]. Since the rate of species recovery by conventional method is less effective, the present work attempted micropropagation of the species through tissue culture, which is a universally accepted alternative method to save and recover the species. The initiative has been taken by the author, one who has identified the species (Fig. 1b). Such attempts in mangroves have already been made, but with little favorable effect: callus initiation from the leaf explants of *Bruguiera sexangula* (Lour.) Poir. on MS medium supplemented with amino acid, 2 µM 2,4-dichlorophenoxy acetic acid and 2 µM N- (2-chloro-4-pyridyl)-N-phenyl urea [MIMURA & al. 1997; VANDER VELDE & VANDER VELDE, 2005]. However, an attempt was made here in *Rhizophora annamalayana* Kathir. for the first time. For the flourishing tissue culture practice, standardization of several aspects is required for culture media, hormones, prevention of phenolic browning of explants and microbial contaminations. Hence, the present

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investigation was made to optimize such requirements for successful tissue culture operations.

Materials and methods

Sterilization of Glasswares

All the glasswares were washed thoroughly with detergent (Teepol) in tap water and rinsed with double distilled water twice and dried in an oven at 30 ºC. The distilled water and other accessories such as forceps, blade holder, cotton, etc., were autoclaved at 121 °C for 15-20 minutes. After autoclaving, they were kept in an oven until use. And aseptic transfer of tissue was done in laminar air-flow hood (Airtech, India) and its interior was swabbed with 95% ethanol before inoculation. The autoclaved instruments were flame sterilized thrice in 95% ethanol before using them for tissue transfer.

Sample collection and surface sterilization

Fresh shoot tip and leaf explants of *Rhizophora annamalayana* Kathir., were collected from the Pichavaram, Southeast coast of India. The explants were washed thoroughly in water immediately after collection and treated with 10 mg/L of three antioxidants - citric acid, polyvinyl pyrolidone and activated charcoal separately for prevention of phenolic browning of explants. Then the explants were washed in 0.1% mercuric chloride followed by 3.0% hydrogen peroxide and 70% ethanol. Thereafter the explants were disinfected with a detergent solution (2% Teepol, Reckitt and Colman, India) for 10 min.

Stock solution preparation

Among the five different tissue culture media (B5, WPM, MS, SH and Y3) tested, MS medium was chosen for meristem culture. For the preparation of MS basal salt medium [MURASHIGE & SKOOG, 1962], separate stock solution of macronutrients, micronutrients, iron supplements, vitamins and amino acid were prepared by dissolving required amounts of chemicals in double distilled water and were stored at 4 ± 1 °C in a refrigerator. Individual growth regulators such as benzyl adenine, kinetin and zeatin alone or in combinations with different auxins like naphthalene acetic acid, indole acetic acid, indole butyric acid were also prepared and kept at 4 ± 1 °C.

Media preparation

For preparing the MS medium, all the stock solutions were taken in appropriate proportions and the final volume was made up to required quantity by adding double distilled water. Sucrose was added at 3% (w/v) to the medium as a source of carbon. Various concentrations and combinations of growth regulators were added to the medium before adjusting the pH to 5.8 using 1.0 N NaOH or 1.0 N HCl and solidified with 0.8 % agar. The culture media with all necessary ingredients were dispensed into culture tubes, conical flasks and phyta jar and sterilized. The autoclaved media were kept in inoculation room until use.

Aseptic transfer of explants

After sterilization, all the explants were cut into small pieces (1.0 to 1.5 cm long) and were individually placed on 25 x 150 mm culture tubes (Borosil, India) containing 15 ml of MS medium supplemented with various concentrations and combinations of cytokinins and auxins along with 3.0 % sucrose and 0.8 % agar (Himedia, Mumbai, India).

Micropropagation

The MS medium was prepared and to it different antioxidants {ascorbic acid (w/v) [5-15mg/L]; polyvinyl pyrolidone (w/v) [5-15mg/L] and activated charcoal (w/v) [5-15mg/L]} were added separately, solidified with 0.8% agar (Himedia, India) and the pH was adjusted to 5.8 prior to autoclaving at 121 °C for 15 min at 15 lb. The medium was poured in to phyta jar (25 ml/jar). After the aseptic transfer of explants, the phyta jars were incubated at 25 ± 2 °C for a 16:00 h photoperiod and observations on browning and percentage survival of explants were recorded.

Effect of media on shoot induction

Shoot-tip and leaf explants were cultured on test tube containing 15 ml of culture media of B5, WPM, MS, SH and Y3 (Annexure-1). All the cultures were incubated at 25 ± 2 °C under continuous irradiation with a white fluorescent tube (30 µmol $m^{-2}s^{-1}$) for a photoperiod of 16:00 h light per day. A total of 40 explants were used for the experiment and was repeated thrice. The culture conditions remained the same for all experiments unless and otherwise specified. Data on per cent response with number of shoot formation per explants was recorded after 25 days of culture.

Effect of cytokinin and auxin combinations on shoot induction

The leaf and shoot-tip explants were cultured on MS basal medium supplemented with 0.5 to 5.0 mg/L of individual cytokinins such as benzyl adenine/zeatin/ kinetin in combinations with 0.1–0.7 mg/L of naphthalene acetic acid or indole acetic acid in addition with coconut milk. Sub-culturing was done after two week interval. The culture conditions stated above remained the same for all experiments unless and otherwise specified. A total of 20 explants were taken for each experiment and were repeated thrice. Results were recorded on number of shoot formed per explants after 21 days of culture.

Results and discussion

The present investigation was aimed at *in vitro* shoot multiplication using shoot tip explants of *Rhizophora annamalayana* for its micropropagation as the first attempt. A major recurring problem which confronts tissue culture especially of tree species is the exudation of phenolic substances from the cut surface of the explants. As a result, the medium turns dark brown in colour due to oxidation of phenolics which becomes toxic to the explant tissue leading to its death under tissue culture conditions. Naturally phenolic compounds are abundant in plants, particularly in woody species, playing an important role in hormone balance, disease resistance and protection of injured tissue from infection [COMPTON & PREECE, 1986]. In the present study, MS medium without coconut milk showed the high phenolics in shoot explant and this problem was totally overcome when the medium was incorporated with 1000 µl/L of coconut milk and 10 mg/L of activated charcoal. However, other antioxidants such as ascorbic acid and polyvinyl pyrolidone showed the poor response and high phenolic exudation.

Prevention of microbial contamination in plant tissue culture is critical for effective micropropagation. Epiphytic and endophytic microorganisms can cause severe losses to micropropagated plants at each stage of growth [CASSELLS, 1991; DEBERGH & VANDERSCHAEGHE, 1988; LEIFERT & al. 1991]. In order to arrest the microbial growth, the time duration of surface sterilization using different chemicals such as mercuric

chloride, ethanol and hydrogen peroxide were optimized to prevent microbial contamination in the explants. The surface sterilization was optimized to arrest the growth of microbial contamination for the explants when treated with 0.1% for one minute in mercuric chloride, 30 seconds in 70% ethanol and one minute in 3% hydrogen peroxide.

The addition of growth regulators in MS medium induced microshoot formation in *Rhizophora annamalayana* from the shoot-tip explants (Fig. 1d) cultured on MS basal salts, 3.0% sucrose and 1000 µl/L of coconut milk and 0.8% agar medium supplemented with individual hormones such as benzyl adenine, zeatin and kinetin. The explants responded to bud sprouting after 7 days (Fig. 1e).The microshoot was observed from 3.0 mg/L of benzyl adenine and 3.0 mg/L of kinetin and 1000 µl of coconut milk. Shoot-tip explants responded well with high frequency (70%) of shoot induction (Fig. 1f). Leaf (Fig. 1c) explants did not show any microshoot induction in all the treatments of growth regulators.

Conclusions

In order to recover the mangrove species *Rhizophora annamalayana* Kathir, the present work was attempted through tissue culture. Microshoot formation was achieved in the species by 70% using shoot tip as explant when the MS medium incorporated with 3.0 mg/L of BA and 3.0 mg/L of kinetin with 1000 µl of coconut milk. The culture techniques were standardized for surface sterilization and prevention of phenolic browning and microbial contaminations of the explants. The results are promising for micropropagation of the rare mangrove species.

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Fig. 1. a. *Rhizophora annamalayana* from the habitat, b. a rare propagule of *Rhizophora annamalayana* Kathir., shown by the author (Prof. K. Kathiresan), c. leaf explant on MS medium, d. meristem tissue on MS medium, e. bud sprouting from meristem tissue, f. shoot bud sprouting after 20 days.

J. Plant Develop.
20(2013): 63 – 70

SMOKE-SATURATED WATER FROM FIVE GRASSES GROWING IN JAPAN INHIBITS *IN VITRO* PROTOCORM-LIKE BODY FORMATION IN HYBRID *CYMBIDIUM*

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Abstract: Smoke derived from the burning of plant material has been shown to stimulate seed growth of several species. In addition, several studies have reported that when smoke is condensed with water, smoke-saturated water (SSW) can also stimulate the germination of orchid seeds. In this study, SSW was derived from burning the aerial part of five grasses growing in the wild in Shikoku, Japan (*Arundinella hirta* (Thunb.) C. Tanaka var. *hirta*, *Microstegium japonicum* (Miquel) Koidzumi, *Miscanthus sinensis* Andersson, *Paspalum thunbergii* Kunth ex Steud., *Themeda triandra* Forssk. var. *japonica* (Willd.) Makino), all of which flower between August and October. SSW was added at three concentrations (1, 5, 10%, v/w) to solid, agarized Teixeira *Cymbidium* (TC) medium to assess the impact on *in vitro* organogenesis of hybrid *Cymbidium*, specifically on new protocorm-like body (*neo*-PLB) formation. The SSW of all five species strongly inhibited the formation of *neo*-PLBs at all concentrations relative to the control (no SSW added). Since PLBs are considered to be the equivalent of somatic embryos in orchids, and since SSW is able to stimulate the germination of zygotic embryos in other plant families, the mechanism of action is clearly different between zygotic and somatic embryos.

Key words: karrakinins, orchid, PLB, smoke-saturated water, Teixeira *Cymbidium* (TC) medium.

Introduction

DE LANGE & BOUCHER (1990) were the first to note, in the South African Cape fynbos, how fynbos plant-derived smoke or its aqueous extract, i.e., smoke-saturated water (SSW), could stimulate seed germination of *Audouinia capitata* (L.) Brongn., a threatened monotypic fynbos species. Burning fynbos (aerial parts) could also stimulate seed germination of fire-climax grass *Themeda triandra* Forssk. (syn. *Triandra australis* (R.Br.) Stapf) [BAXTER & al. 1994]. Since these studies, several other studies have emerged showing how burning plant material, particularly in South Africa and in Australia, has led to seed effective germination of many species. This may be an ecological adaptation since these dry, Mediterranean-type climates receive little rainfall and are often prone to bush or wild fires, therefore the ability to use elements of both fire and water would enhance survival. BROWN & al. (2003) noted, however, that the seeds of not all fynbos species are stimulated to germinate in response to burning fynbos smoke: seed germination of members of the Asteraceae, Bruniaceae, Crassulaceae, Ericaceae, Geraniaceae, Mesembryanthemaceae, Proteaceae and Restionaceae was stimulated, but not of members of the Amaryllidaceae, Hyacinthaceae or Iridaceae. Similarly, ROCHE & al. (1994) could only germinate seed of 45 out of 94 species of native Western Australian plants that are

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normally hard to germinate by exposing dormant seed to cold smoke derived from burnt native vegetation. Dormant seeds of *Emmenanthe penduliflora* Benth., an annual from the California chaparral, were induced to germinate by smoke or vapors emitted from smoke-treated sand or paper [KEELEY & FOTHERINGHAM, 1997]. Seedlings of two South African indigenous medicinal plants, *Albuca pachychlamys* Baker and *Tulbaghia violacea* Harv., germinated with smoke solutions, showed improved plant growth characteristics *in vitro*, but a third plant, showed no improvement relative to non-smoke treatments [SPARG & al. 2005]. Only one (*Aristolochia debilis* Sieb. & Zucc.) out of 13 species growing in China showed improved germination in the presence of a commercial Australian-derived SSW product [ZHOU & al. 2013].

The germination-promoting compounds present in plant- and cellulose-derived smoke include a butenolide, 3-methyl-2*H*-furo [2,3-*c*] pyran-2-one [FLEMATTI & al. 2004], a non-toxic compound [VERSCHAEVE & al. 2006], renamed karrikinolide 1 (KAR_1) [LONG & al. 2010], or alkyl-substituted 2H-furo[2,3-c]pyran-2-ones [FLEMATTI & al. 2009]. KAR_1 improved somatic embryogenesis of an important, bamboo-like commercial horticultural species, *Baloskion tetraphyllum* (Labill.) L.A.S. Johnson & B.G. Briggs APNI [MA & al. 2006] and SSW enhanced secondary somatic embryogenesis in *Brassica napus* L. [ABDOLLAHI & al. 2012]. Other KARs have since been identified (KAR_2, KAR_3, KAR_4; NELSON & al. 2009).

Since KARs are relatively non-volatile in nature (m.p. 118–119 °C) and soluble in water [FLEMATTI & al. 2004], they can be concentrated in the aerosol component of smoke created from burning fresh plant material and transported by steam distillation. Using this method to create SSW, SSW was shown to enhance seed germination *in vitro* of several orchid species: *Vanda parviflora* Lindl. [MALABADI & al. 2008], *Xenikophyton smeeanum* (Rchb.f.) Garay [MALABADI & al. 2011], *Pholidota pallida* Lindl. [MULGUND & al. 2012], and *Oberonia ensiformis* (Rees) Lindl. [MALABADI & al. 2012]. It was also the method used in this study.

There is no English literature pertaining to the use of Japanese vegetation-derived SSW for improving seed germination. Based on this gap in the literature, and based on the assumption that a protocorm-like body (PLB) is a somatic embryo in orchids [TEIXEIRA DA SILVA & TANAKA, 2006], this study was conducted to assess the impact of SSW derived from five grasses growing wild in Shikoku, Western Japan, on the induction of new or *neo*-PLBs and *in vitro* organogenesis of hybrid *Cymbidium*. No such study exists for *Cymbidium* Swartz [HOSSAIN & al. 2013; TEIXEIRA DA SILVA, 2013a]. Even though many media can support the induction and development of *Cymbidium* PLBs *in vitro* [TEIXEIRA DA SILVA & al. 2005], Teixeira *Cymbidium* (TC) No. 1 medium [TEIXEIRA DA SILVA, 2012] was used in this study.

Materials and methods

All protocols (experimental design, chemicals, reagents, explant preparation, treatment analysis and advice) strictly follow TEIXEIRA DA SILVA & al. (2005, 2006a, 2006b), TEIXEIRA DA SILVA (2013b), and TEIXEIRA DA SILVA & DOBRÁNSZKI (2013). All chemicals and reagents, including plant growth regulators (PGRs), were of the highest analytical grade available and were purchased from either Sigma-Aldrich (St. Louis, USA), Wako Chemical Co. (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan), the cheapest choice at the highest tissue-culture grade, unless specified otherwise.

Plant material and culture conditions

PLBs of hybrid *Cymbidium* Twilight Moon 'Day Light' (Bio-U, Tokushima, Japan) originally developed from shoot-tip culture on VACIN & WENT (VW, 1949) agar medium without PGRs, were induced and subcultured (PLB induction and proliferation medium) every two months on Teixeira *Cymbidium* (TC) No. 1 medium [TEIXEIRA DA SILVA, 2012], which contains 0.1 mg/l α-naphthaleneacetic acid (NAA) and 0.1 mg/l kinetin (Kin), 2 g/l tryptone and 20 g/l sucrose, and solidified with 8 g/l Bacto agar (Difco Labs., USA). All media were adjusted to pH 5.3 with 1 N NaOH or HCl prior to autoclaving at 100 KPa for 17 min. Cultures were kept on 40 ml medium in 100-ml Erlenmeyer flasks, double-capped with aluminium foil, at 25 °C, under a 16-h photoperiod with a light intensity of 45 μmol/m^2/s provided by 40-W plant growth fluorescent lamps (Homo Lux, Matsushita Electric Industrial Co., Japan). Longitudinally dissected as two pieces of PLB (3-4 mm in diameter) segments [TEIXEIRA DA SILVA, 2013b], 10/flask, were used as explants for *neo*-PLB induction and proliferation.

Response of Cymbidium to smoke-saturated water

The effect of SSW from five grass (Poaceae) species growing in Japan (*Arundinella hirta* (Thunb.) C. Tanaka var. *hirta*, *Microstegium japonicum* (Miquel) Koidzumi, *Miscanthus sinensis* Andersson, *Paspalum thunbergii* Kunth ex Steud., *Themeda triandra* Forssk. var. *japonica* (Willd.) Makino), all of which flower between August and October, on *neo*-PLB formation from half-PLBs was assessed by adding 0 (control), 1%, 5% and 10% (v/w) SSW of each grass to solid TC medium (without PGRs) using the experimental design of TEIXEIRA DA SILVA (2012). All five species, collected from Kagawa, Shikoku, were identified by comparison with internet sites and Japanese field guides such as OHWI (1984). No specimens were deposited in a herbarium. The logic behind removing PGRs was to assess whether SSW could effectively induce *neo*-PLBs in the absence of PGRs and thus test their PGR-like ability. Plant material was collected between August and October when plants were in full flowering. Several hundred flowering stems, weighing a few kilograms each, were harvested from the base, just above the soil line, to allow for future restoration and resprouting. Aerial parts were bundled and placed in cooled conditions and transported to a shaded area with a net screen. Using a Clevenger-type apparatus (self-designed) in Fig. 1, material was set alight, 100 ml of tap water was evenly sprinkled over the plant material (100 ml/kg), each species separately, and the SSW that condensed was collected into separate 500-ml Erlenmeyer flasks. Flasks were placed on ice, covered with a single layer of aluminium foil, transported to the laboratory, kept at 4 °C and used within 24 h. A single stock for each grass species was used for all dilutions and for all replications. Prior to adding to TC medium at the desired concentration, each SSW was passed through a 22 μm Millipore filter, and pH was adjusted to 5.8 prior to adding Difco agar and autoclaving. Explants were photographed using a stereo light microscope and/or a digital camera. Chemical analysis of the SSW was not conducted.

Statistical analyses

Experiments were organized in a randomized complete block design (RCBD) with three blocks of 10 replicates per treatment (i.e., SSW concentration). All experiments were repeated in triplicate (n = 30, total sample size per treatment). The resulting organogenic outcome (*neo*-PLB or root response) was scored visually after 60 days. Data was subjected to analysis of variance (ANOVA) with mean separation by Duncan's multiple range test (DMRT) using SAS® version 6.12 (SAS Institute, Cary, NC, USA). Significant differences between means were assumed at $P \leq 0.05$.

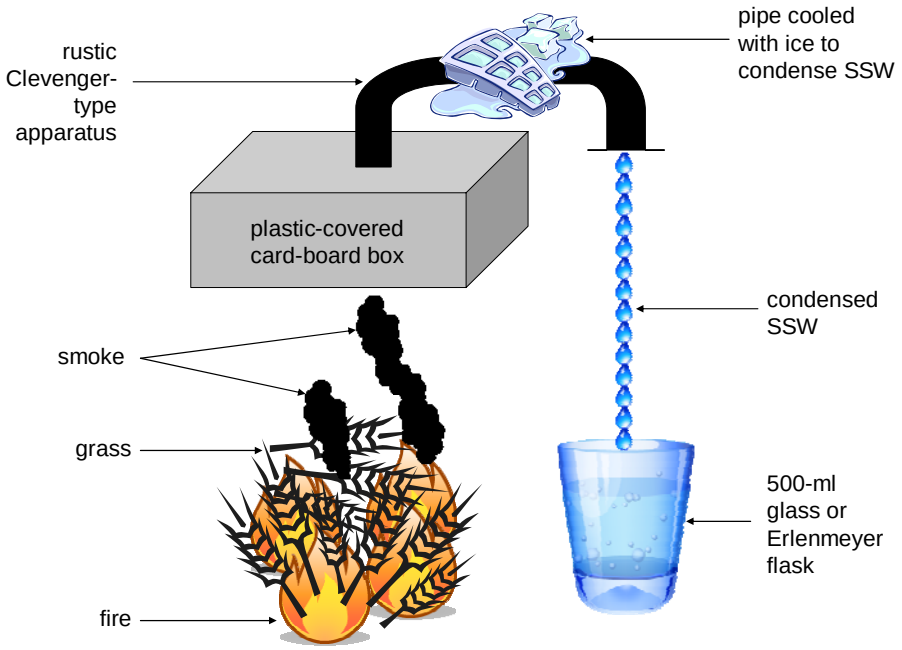

Fig. 1. Schematic of the experimental system for deriving smoke-saturated water (SSW) from grasses growing in Japan for *in vitro* experiments. Each grass is burned separately. To 1 kg of plant material (aerial, flowering parts), 100 ml of tap water is sprayed to increase the level of steam. As smoke rises and is captured by a plastic-covered card-board box, it travels up a pipe 10 cm in diameter. As the smoke reaches the U-turn in the pipe, which is cooled externally by packets holding blocks of ice or crushed ice, the hot steam condenses and runs down into a glass vessel to capture the SSW. This is the "concentrate" that is then diluted down to 1, 5 or 10% (v/w) in TC medium.

Results and discussion

The most notable finding of this study is that SSW could not stimulate *neo*-PLB formation more than the control treatment containing PGRs (standard TC medium), and performed as poorly as (with 1% SSW) or worse than (5 or 10% SSW) the control treatment without PGRs (Fig. 2). The latter concentrations tended to "bleach" explants (Fig. 3C), suggesting some negative impact on the photosynthetic apparatus. In this study, unlike other studies in which SSW or smoke has improved seed germination, SSW has had an inhibitory effect. This is not altogether a bizarre result since the seed germination of many species has not been stimulated by SSW (e.g., BROWN & al. 2003; SPARG & al. 2005; ZHOU & al. 2013), in the latter study only one out of 13 species in the southern tropical belt of China (Guangzhou) being stimulated by a commercial Australian-derived SSW. Even though PLBs are analogous to somatic embryos, and even though somatic embryos are analogous to zygotic embryos (i.e., within seed), it is evident that either a) SSW would also not stimulate hybrid *Cymbidium* seed, or that b) the mechanism by which SSW impacts

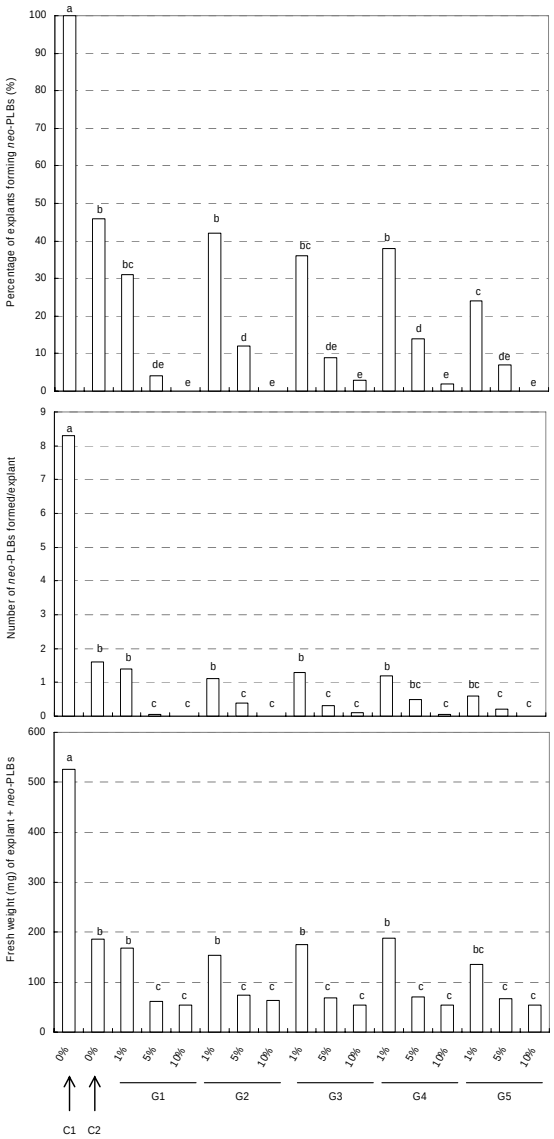

Fig. 2. Impact of smoke-saturated water (SSW) from five grasses growing in Japan on *neo*-PLB formation from hybrid *Cymbidium* Twilight Moon 'Day Light' half-PLBs after 60 days in culture [TEIXEIRA DA SILVA & DOBRÁNSZKI, 2013]. Three parameters were assessed: (Top) Percentage of half-PLBs forming *neo*-PLBs (%)*; (Center) Number of *neo*-PLBs formed per half-PLB; (Bottom) Fresh weight (mg) of half-PLB + *neo*-PLBs**. Notes: * All percentage data was arc-sine transformed prior to analysis. ** average fresh weight of initial half-PLB explants is 54 mg (n = 10). Treatment notes: Control 1 = TC + PGRs (0.1 mg/l NAA + 0.1 mg/l Kin); Control 2 = TC – PGRs; G1 = *Arundinella hirta* (Thunb.) C. Tanaka var. *hirta*; G2 = *Microstegium japonicum* (Miquel) Koidzumi; G3 = *Miscanthus sinensis* Andersson; G4 = *Paspalum thunbergii* Kunth ex Steud.; G5 = *Themeda triandra* Forssk. var. *japonica* (Willd.) Makino. Mean values with by the same letter are not significantly different based on DMRT (P = 0.05). n = 90 (10 × 3 × 3). C = control; G = grass species; Kin, kinetin; NAA, α-naphthaleneacetic acid; PGR, plant growth regulator; PLB = protocorm-like body; TC = Teixeira *Cymbidium* medium No. 1 [TEIXEIRA DA SILVA 2012].

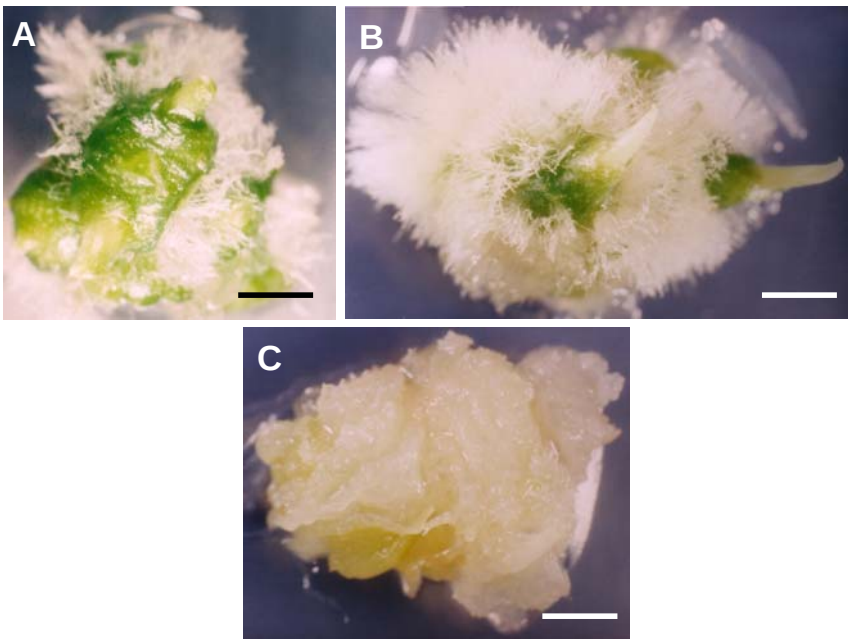

Fig. 3. Growth and development of hybrid *Cymbidium* Twilight Moon 'Day Light' *neo*-PLBs under control (no SSW) (A), 1% (v/w) *Themeda triandra* Forssk. var. *japonica* (Willd.) Makino SSW (B), or 5% *Microstegium japonicum* (Miquel) Koidzumi SSW (C) in solid basal TC [TEIXEIRA DA SILVA 2012] medium. Bars = 1 mm.

seed germination and *neo*-PLB formation is different. Most likely the latter option is more plausible since a component of the mechanism underlying smoke-induced germination is gibberellin synthesis, which results in a decrease in abscisic acid pools [GARDNER & al. 2001; SCHWACHTJE & BALDWIN, 2004].

A search of the literature did not reveal any study on the use of SSW derived from plants in Japan (endemic, or not) on seed germination, or on other plant-based responses. One of the possible reasons may be that the climate is tropical to sub-tropical in the south and south-west, becoming increasingly temperate as one moves north. The incidence of bush fires in Japan is rare and rainfall is generally abundant, so one would imagine that vegetation – specifically seed germination – has evolved to survive without the need for fire, unlike in Mediterranean-style climates like in Western Australia [ROCHE & al. 1994], the South African Cape fynbos [BAXTER & al. 1994], or the US California chaparral [KEELEY & FOTHERINGHAM, 1997]. Even so, the seed of not all plants are sensitive to smoke or SSW, displaying family-wide divergence [BROWN & al. 2003; SPARG & al. 2005; ZHOU & al. 2013]. Hybrid *Cymbidium* could generally be referred to as broadly tropical, requiring high humidity to survive and propagate effectively, and fire would not be a natural form of asymbiotic seed germination. Even though SSW has been shown to effectively stimulate seed germination in some orchids (*Vanda parviflora*, *Xenikophyton smeeanum*, *Pholidota pallida*, *Oberonia ensiformis*) [MALABADI & al. 2008, 2011, 2012; MULGUND & al. 2012], to date no study has examined the use of SSW on *in vitro* organ development of other orchids. However, two other studies have shown that SSW stimulates

somatic embryogenesis in *Baloskion tetraphyllum* [MA & al. 2006] and *Brassica napus* [ABDOLLAHI & al. 2012]. No other studies on the use of SSW for assessing *in vitro* plant organogenesis appear to have been conducted. This area of research is at a nascent phase of development and many more trials would be required on more plant species, including horticultural, medicinal and agronomic, to assess the broad range of effects *in vitro* and under greenhouse and field trials. Key questions that still need to be answered: A) What is the toxicity of SSW, as assessed by toxicity assays? B) What is the mechanism by which a plant takes up and responds to SSW? C) To what level and in what organelles and parts of the plant are SSW or components of SSW accumulated, or used? The fact that each batch of grass that is burnt would likely yield a different concentration of active compounds in the SSW is a weakness of the protocol. Yet, by using only one compound such as butenolide or KAR would most likely not be a viable solution to this problem since SSW may contain multiple organogenesis-influencing factors or PGR-like substances other than the KAR family.

Conclusions

Smoke-saturated water (SSW) can provide a valuable alternative to conventional plant growth regulators for stimulating *in vitro* or *in situ* growth. In this study, SSW derived from five grass species growing in Japan inhibited protocorm-like body formation in hybrid *Cymbidium*, which would not be unusual considering that fire or smoke are not aspects related to this genus under natural/wild conditions.

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THE BIOLOGICAL CYCLE OF SUNFLOWER BROOMRAPE

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Abstract: *Orobanchaceae* is a dicot family, which consists of annual and perennial plants distributing from tropical to subarctic regions, predominately in temperate regions. Broomrape (*Orobanche cumana* Wallr. = *Orobanche cernua* Loefl.) is a parasitic angiosperm that has been causing a great deal of damage to sunflower production in many countries, including Republic of Moldova. This parasitic angiosperm depends entirely on the host for its supply of water and nutrients. A thorough understanding of its biology, including detailed knowledge of the specific mechanisms of parasitism, is needed in order to develop novel control methods. Some main developmental steps are described for the root parasites: seed conditioning and germination, haustorium formation, penetration into host tissues, maturation of the parasite plant, and seed production. All these stages were studied in artificial and natural conditions.

Key words: *Orobanche cumana* Wallr., holoparasite, host, exudate, appressorium, haustorium, attachments, tubercles, shoots, maturation, seeds.

Introduction

Broomrapes are holoparasitic members of the *Orobanchaceae* family. Of the 100 species belonging to the genus *Orobanche*, only a few parasitize crop plants. Amongst these, *Orobanche cumana* Wallr., *O. ramosa* L., *O. aegyptiaca* L. and *O. crenata* Forssk. are of economic importance since they cause severe yield losses in numerous commercial crops [LINKE & al. 1989]. This is particularly true of *O. cumana* whose hosts include sunflower (*Helianthus annuus* L.), tomato (*Lycopersicon esculentum* Mill.), aubergine (*Solanum melongena* L.) and tobacco (*Nicotiana tabacum* L.). This parasitic weed has caused heavy yield losses in sunflower grain, oil and proteins in many countries such as Turkey, Romania, Ukraine, Bulgaria, China, the Black Sea countries and ex-URSS countries [GARCIA-TORRES, 1994; PARKER, 2009; MELERO, 2000; KAYA & al. 2004; SHINDROVA, 2006; MASIREVIC & MALIDZA, 2006; FERNANDEZ-ESCOBAR & al. 2009; GLIJIN, 2012].

Root parasites like broomrape are difficult to control, partly due to their complex life cycle which has been summarized conclusively by PAKER & RICHES (1993). The growth of this pathogen includes a series of developmental and metabolic steps, each crucial for the establishment of a direct connection with the host and for the survival of the parasite. Some developments, like the formation of a haustorium, are common only to parasitic plants.

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The *Orobanche cumana* Wallr. biological cycle comprises well-defined steps, separated both spatially and temporally, that are potential targets for host defense strategies. Individual seed in the soil require a conditioning period of 1 to 2 weeks to become imbibed, and temperature is important during that stage (15 °C to 20 °C). Following the conditioning process, each individual seed must receive a chemical stimulant from the host root, to alert the seed that host is in close proximity [PARKER & RICHES, 1993; KROSCHEL, 2001].

The first stage of the infection process is the germination and chemical guidance of the seedling (chemotropism) towards the host root. Because *Orobanche* species require the presence of germination stimulants exuded from the host root to germinate and locate the host root [WORSHAM, 1987], low stimulant producing plants could be a suitable option to reduce *O. cumana* infection. Most of these substances belong to the strigolactones and are active in extremely low concentrations (1 ppb to 1 ppm).

Upon germination, stimulated by exogenous chemical signals, broomrape seed develops a small seedling that attaches to the host root and differentiates in the attachment organ – the appressorium. On contact with the host root, the radical adheres to the surface by sticky papillae and penetration is facilitated by separation of the host cells, caused by enzymatic activity. Subsequently a connective organ, the haustorium, develops between host and parasite, with cells from each species playing part in the junction [STEWART & PRESS, 1990].

Once the haustorium has reached the host stele, haustorial cells at the interface penetrate host vessel members through their pits. These cells then open at their tips and lose their cytoplasm [DÖRR, 1997]. Adjacent cortical cells progressively differentiate into xylem elements until a continuous water conducting system is established linking the host and parasite vascular systems [MUSSELMAN & DICKISON, 1975]. The development of the xylem bridge is absolutely dependent upon direct contact of the haustorium with the host stele [RIOPEL & MUSSELMAN, 1979; HASSAN, 2004].

The nature of the host signal(s) that triggers xylem differentiation is currently uncharacterized but the phytohormones auxin and cytokinin are good candidates since these are known to trigger vascular regeneration in wounded tissues [ALONI, 1995]. After the parasite has developed a haustorium and established vascular connections with the host, it becomes one with the plant, acting as a sink for water and nutrients. This connective structure swells and forms a nodule that after one to two weeks differentiates into a tubercle with shoot bud, and eventually a flowering shoot.

After four to five weeks the flowering spike emerges from the soil, and grows to heights between 10 and 65 cm with slender stems slightly thickened at the base [PUJADAS-SALVA & VELASCO, 2000]. Stem color is variable, and it has been reported to be whitish [PUJADAS-SALVA & VELASCO, 2000], yellowish or brownish and bluish-violet. Stem color in *Orobanche* spp. is mainly determined by the accumulation of anthocyanins, which are more conspicuous because of the absence of chlorophyll [WHELDALE, 1916]. The bisexual flowers [MOLAU, 1995] are insect pollinated and the resulting seeds are produced in capsules with 1000 to 10000 seed per capsule. The seeds can remain viable in the soil for more than 10 years [LINKE &. al. 1989]. This ability to produce a prodigious number of seed per plant is the forte of these and similar parasitic agricultural weeds. Depending on environmental conditions the underground phase of the life-cycle of *Orobanche cumana* ranges from 30 to over 100 days. The whole life-cycle from seed germination to seed production lasts about 3-5 months [KROSCHEL, 2001].

This study is focused on observation and understanding of *O. cumana* stages of development in dynamics on the sunflower roots.

Materials and methods

Host and parasitic plant material

The seeds of genotype FS-6 of *Helianthus annuus* L., which is susceptible to broomrape, were used as a host plant. The seeds of *O. cumana* were collected in 2011 from inflorescences of broomrape that was parasitizing sunflower fields in Singera (municipality Chisinau). The inflorescences were dried for 60 days at temperatures ranging from 20 to 34 °C, after which the seeds were separated with 300-mm sieves and were stored in darkness at 4 °C. Seeds of sunflower and *O. cumana* were surface sterilized by soaking them in sodium hypochlorite (1%) for 15 minutes and washed twice with sterilized water before use.

Subterranean stages on development of *Orobanche*

Conditioning of broomrape seeds

Batches of 30 seeds of broomrape were placed on 5 discs of 2 cm diameter glass fiber filter paper moistened with 250 µl of sterile distilled water and incubated in the dark at 24 °C for 12 days in order to promote the necessary conditioning for germination [FERNANDEZ-APARICIO & al. 2008].

Germination bioassay

The broomrape seeds germination potential was tested in the presence of sunflower root exudates from susceptible genotype FS-6. Sunflower seeds were sown on glass balls soaked in sterile distilled water. Between first to the fifth week after sunflower germination, to collect root exudate, two seedlings were placed in 40 ml sterile distilled water for 2 days. This solution, which contained root exudates with germination stimulants, was then sterilized by filtration and could was stored at -20 °C. Preconditioned seeds of *O. cumana* on glass fiber filter paper were transferred to a small Petri dish containing 250 µl of root exudate solution and incubated at 25 °C. Germination was determined after radicle emergence.

Root chamber technique. The device (Fig. 1) was used to evaluate the underground development of root parasitic weeds such as appressorium and haustorium formation and further growth stages since such evaluation is impossible in the field.

The technique was described by KROSCHEL (2001) and slightly modified during this study. It is based on the use of a 3 sides-wood frame (30 x 15 x 2 cm) covered by glass covers on its two faces. Space between the two transparent covers was filled with sterilized sand. In the frontal face, white filter paper was put between sand and the glass cover. *O. cumana* seeds were conditioned with sterilized distilled water for 14 days in a growth chamber at 23 °C. Ten-days-old sunflower seedlings were transplanted to chamber and put in contact with *Orobanche* seeds (Fig. 1). The germination of broomrape seeds and the tubercles setting were determined at the different levels under optic microscope at different times.

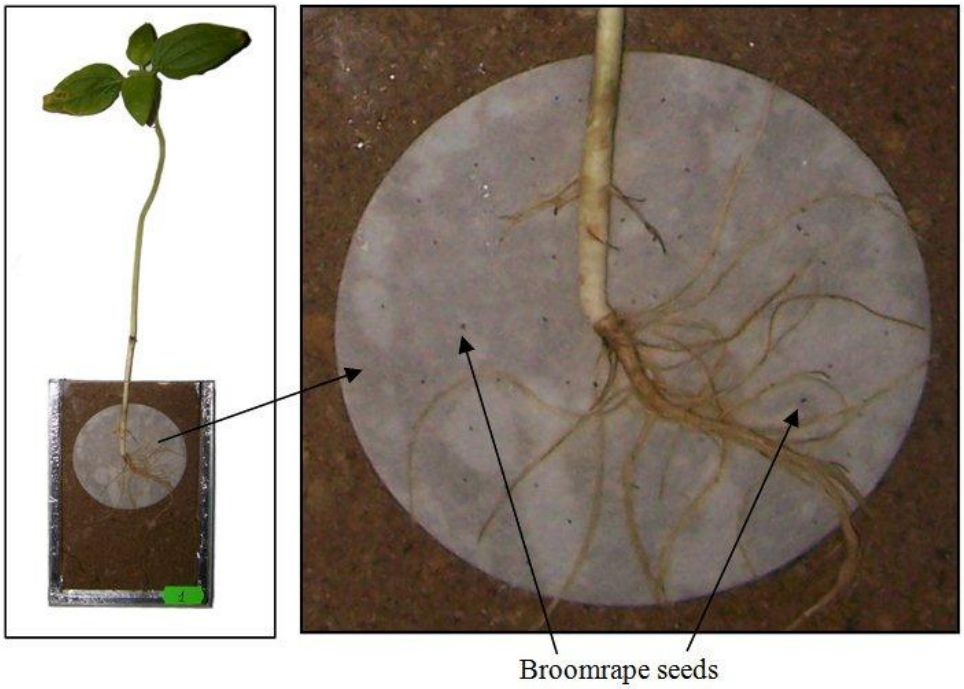

Fig. 1. The root chamber used in the assay. The root chambers were maintained in a growth chamber adjusted at 25 °C and 12 hours of photoperiod, and placed side by side to protect the roots from light. When necessary, plants were watered.

Aerial stages of *Orobanche* development

The evaluation of broomrape emergence, flowering and seed dispersal was made in a separate experiment, using small pots 5.5 x 6 x 16 cm were filled with a mixture of sand and peat (1 : 1 by wt). The soil mixture was carefully mixed with 25 mg of *O. cumana* seed (equivalent to around 5000 seeds) to obtain a homogeneously infested substrate. Sunflower seeds of the genotype FS-6 were germinated on moistened filter paper in Petri dishes and 2-day-old seedlings were planted in the pots. The plants were maintained in a growth chamber for 60 days at 25 °C / 20 °C (day / night) with a 16-h photoperiod for incubation.

Results and discussion

Results of these investigations showed a high affinity of *O. cumana* to host plant and sunflower tolerance to that agent. The analysis of ontogenetic development of host - parasite, made within sixty days, with periodic evaluation of 10 in 10 days and thoroughly study of the root system at the end of experience, revealed the several developmental stages

of sunflower broomrape: appressorium and haustorium formation, attachments, tubercles, underground and aerial shoots, maturation and seed production (Fig. 2).

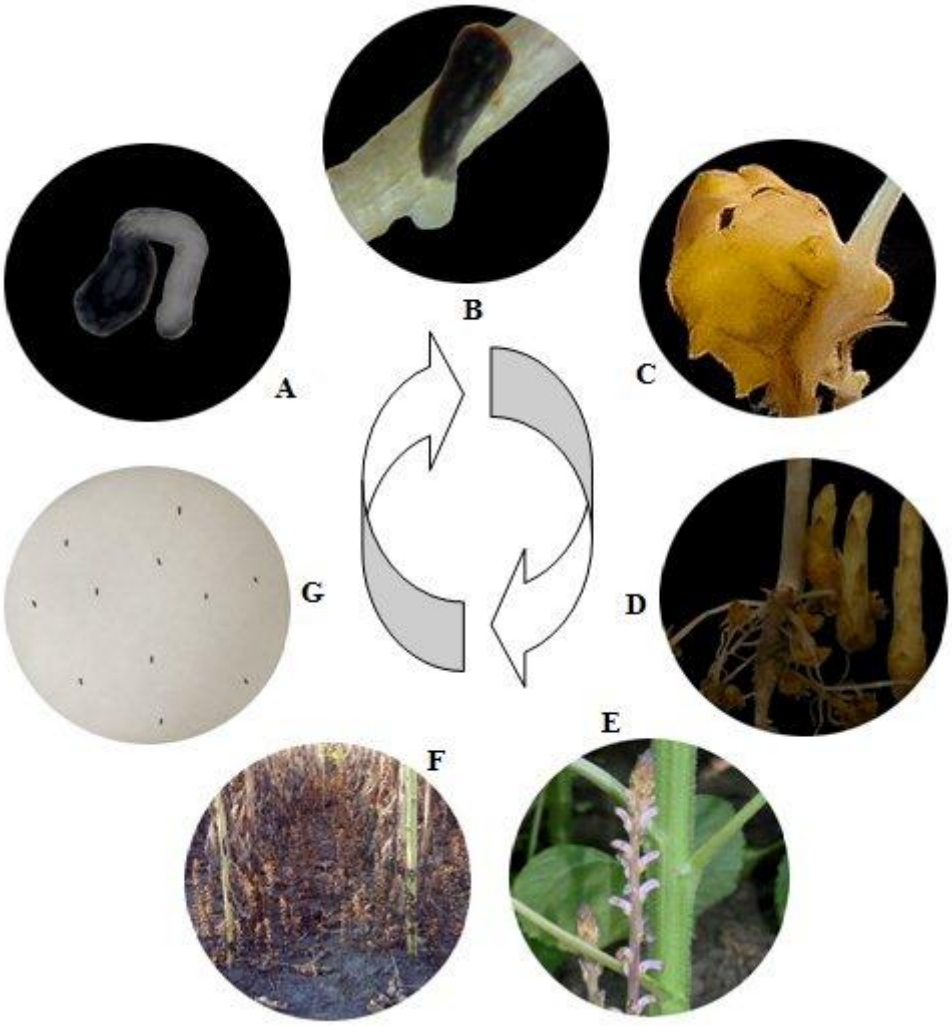

Fig. 2. *Orobanche cumana* life cycle
(A – seed germination and appressorium development; B – haustorium development;
C – nodule development; D – nodule differentiation into tubercles;
E – broomrape flowering; F – broomrape seed maturation; G – broomrape seeds)

Root exudates obtained from 1- to 5-week-old plantlets triggered *germination* of broomrape seed; germination reached approx. 90% in the second week (Fig. 2 A). Conditioned *Orobanche* seeds respond to very low concentrations of germination stimulants that are normally released from host roots [WIGCHERT & al. 1999]. Stimulant

concentrations that are higher than the optimal, inhibit *Orobanche* seed germination [JOEL & al. 1995]. An understanding of the biochemical basis of germination stimulation in root parasites may lead to the development of new practical control measures. These can be based either on the promotion of suicidal germination in the absence of a host [OSWALD & al. 2002], depleting the soil of parasitic weed seeds, or on inhibiting germination.

The germination of *O. cumana* seeds, induced by host-root exudates, leads to the development of a root-like organ, known as the germ tube or appresorium (Fig. 2 A).

The root chamber method proved useful also for studying the dynamics of the first ontogenetic stages of *Orobanche* attachments and haustorium development (Fig. 2 B). Attachment of the parasite to host root surface takes place as soon as the parasite meets a host root. This is facilitated by the secretion of an adhesive substance by the parasite [BAIRD & RIOPEL, 1985; JOEL & LOSNER-GOSHEN, 1994]. Potentially, this step may be vulnerable for control, but so far nothing is known about its control.

The next parasitism stage, the penetration into the host tissue, occurred 21 days after planting (DAP). Penetration is the first stage of intimate contact between cells of host and parasite. This is also the beginning of the true parasitic phase in which the parasite takes nutrients and water from the host. Therefore, it is crucial to any further development of the parasite. After the establishment of a conductive connection between host and parasite, at 35 DAP the parasite developed a tubercle (Fig. 2 C, D).This tubercle is the juvenile parasite. From tubercles, inside of host plant was formed a specialized structure known as haustorium, which is a connective tissue that active the junction between host and parasite [ECHEVARRÍA-ZOMEÑO & al. 2006]. At this stage one can physiologically regard the parasite as an integral part of the host, competing for host resources like a host organ. The growth of the parasite occurs at the expense of water, mineral and organic compounds from the host. The tubercle and underground shoots accumulate carbohydrates and thereby become a strong sink for all plant nutrients.

Beginning with 55 DAP on the explored sunflower roots were observed aerial shoots emerging above the soil surface (Fig. 1 D) and starts to flower (Fig. 2 E) and to produce seeds after another short period of time -70 DAP (Fig. 2 F, G).

Starting from obtained results, we can affirm that in the period 15 - 70 days from the moment of sunflower planting have been attested all *Orobanche cumana* ontogenetic stages. We can therefore conclude that after seed maturation *Orobanche* exhibits two main life phases: (a) the independent life phase, (b) the parasitic life phase. The independent phase begins with seed conditioning. It has been widely reported that for germination under chemical stimulation *Orobanchaceae* seeds required conditioning for several days in a wet environment and at suitable temperatures [PIETERSE, 1979; PRESS & al. 1990; CHAE & al. 2003]. However, the recent results of PLAKHINE & al. 2009 and PLAKHINE & JOEL, 2010, showed that non-conditioned seeds of both *Orobanche cumana* Wallr. and *O. aegyptiaca* Pers. were able to germinate in response to chemical stimulation by GR24 even without prior conditioning. Germination lasts a few days until the parasite finds a host, and attaches to it. This life phase is facilitated by the consumption of material stored in the seed. According to OKONKWO & NWOKE (1978), the parasitic life phase starts as soon as a haustorium has developed. At this point the parasite becomes dependent on nutrients derived from the host. JOEL & PORTNOY (1998) demonstrated that intrusive cells of the haustorium penetrate the host root, eventually forming a physiological bridge between the

vascular system of the host and that of the parasite. Subsequently, the parasite develops a shoot that emerges from the soil, flowers and sets seeds.

The understanding of metabolic and developmental aspects of root parasites is essential for any effort to develop effective control measures that will specifically prevent the damage it causes in agricultural fields. This is true because each developmental stage is crucial for the growth and dispersal of these root parasites. Thus, each step can potentially serve as a target for their control.

Conclusions

According to the present results, using the root chambers enables detection of *Orobanche* seeds, observation of the parasitism stages of germination, penetration into the host root tissue, establishment, tubercle production and apex production without disturbance, under natural conditions. These conditions are essential for studying major aspects of the host–parasite relationship to gain knowledge for use in modeling the parasitism process, optimizing chemical control and studying the resistance mechanism of resistant cultivars.

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VARIATION OF MACROMYCETES SPECIES COMPOSITION IN TWO FOREST HABITATS FROM GIUMALĂU MASSIF (EASTERN CARPATHIANS, ROMANIA)

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Abstract: Norway spruce (*Picea abies*) is the most common species with a large spreading in forests from Giumalău Massif. In this study the authors investigated the macromycetes species composition in two forest communities from Giumalău Massif: *Hieracio transsilvanico-Piceetum* and *Leucanthemo waldsteinii-Fagetum*. A total of 243 macromycetes species in 30 sampling areas have been identified. Changes in macromycetes species composition have been related to environmental factors (altitude) and vegetation (canopy cover, plant species diversity). The results suggest that variation in macromycetes species composition in the two forests types from Giumalău Massif is directly related to abiotic factors (altitude), woody species composition and plants communities' structure.

Keywords: altitude, fungi, diversity, tree species, vegetation, Giumalău Massif.

Introduction

Fungi represent a vast group of heterotrophic organisms as their global diversity is estimated at over than 1.5 million species, presenting very different structural organization and adapted to almost all ecosystems types [HAWKSWORTH, 1991]. Among all these species, the fungi from forests are considered to have the highest diversity within all ecosystems. Also, the fungi have major roles in forest ecosystem including: nutrient cycles, forming and keeping soil structure, food source in trophic chain for detritivores, mycorrhizal symbiosis [WIENSCZYK & al. 2002] etc.

In temperate zones, several studies have found that abundance and macromycetes diversity are related to plants species and microenvironment [GOMEZ-HERNANDEZ & WILLIAMS-LINERA, 2011]. Also, the spatial distribution of saprophytic fungi is associated with substratum and it is usually more uniform than mycorrhizal fungi distribution [LAGANÀ & al. 1999]. Spatial distribution of saprophytic fungi is associated with the substratum and usually is more uniform than mycorrhizal fungi. However, many saprophytic fungi prefer a particular tree or shrub species [ROBERTS & al. 2004]. Fungal communities are strongly influenced by vegetal community composition, structure and age because of its closed relationship with trees and soil nutrients [KÜFFER & SENN-IRLET, 2005].

From this reason, there is an interest in studying of macromycetes distribution depending on forest species composition. Vegetation structure and plant species composition varies depending on the environmental conditions and their fluctuation

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influences macromycetes community. The relationship between trees and fungal communities depend on host-tree species which influence fungi specialization and provide unique habitats [LAGANÀ & al. 1999]. All these are depending on environmental factors [GOMEZ-HERNANDEZ & WILLIAMS-LINERA, 2011]. Geomorphological features (as slope, aspect and altitude) seem to influence macromycetes communities. On the other way, the climatic and microclimatic conditions depend on altitude; anyway, changes of macromycetes species composition and diversity due to altitude have been barely investigated. Recent researches concluded that rainfall, humidity and temperature are the main factors influencing the appearance of sporocarps [GOMEZ-HERNANDEZ & WILLIAMS-LINERA, 2011]. In addition, richness and abundance of macromycetes species are related to microclimatic conditions. The results suggest that variations of humidity, precipitations and temperature are the most important factors influencing sporocarps production and macromycetes diversity [LAGANÀ & al. 2002].

In this study we investigated macromycetes species composition in two forests communities from Giumalău Massif: *Hieracio transsilvanico-Piceetum* Pawlowski et Br.-Bl. 1939 where the trees layer is almost totally edified by Norway spruce (*Picea abies*) and *Leucanthemo waldsteinii-Fagetum* (Soó, 1964) Taüber 1987 where the trees layer is co-dominated by spruce (*Picea abies*) and beech (*Fagus sylvatica*) with sporadically appearences of fir (*Abies alba*), birch (*Betula pendula*) and maple-mountain (*Acer pseudoplatanus*). Also we tried to detect if altitude, slope, canopy cover and plants species diversity could influence the macromycetes species composition.

Materials and methods

The studied area represents a small region of the Eastern Carpathians from Romania situated at the intersection of the following geographical coordinates: 47°25' N and 25°25' E. It has a total area of about 213 km^2 and is rhomboidal shaped (Fig. 1). The mountain relief presents medium altitudes varying between 1100-1200 m. Geological substratum belongs to the crystalline block and pedological coating is included into the following classes: cambisoils, spodosoils, litomorphic and undeveloped soils. The Giumalau Massif is characterized by a continental climate presenting excessive nuances, with differentiations determined by altitude, mountainous depressions and corridors [LESENCIUC, 2006].

The investigations in sampling areas (which had 1000 m^2 in size) have been realized from May to October during three consecutive years. A total of 30 observations have been performed as follows: relevés R01 - R15 for surfaces of *Hieracio transsilvanico-Piceetum* association and relevés F16 - F30 for surfaces of *Leucanthemo waldsteinii-Fagetum* association. The macromycetes species from inside of investigated areas have been identified „*in situ*", if that has been possible. Sporocarps of unidentified species were investigated through laboratory specific methods based on micromorphological and macromorphological characters according to keys and reference guides [BORGARINO & HURTADO, 2001; BREITENBACH & KRÄNZLIN, 1984, 1986, 1991; COURTECUISSE & DUHEM, 1994; ROUX, 2006; SĂLĂGEANU & SĂLĂGEANU, 1985; ŞESAN & TĂNASE, 2006; TĂNASE & ŞESAN, 2006]. We defined macromycetes as visible fungi which produce sporocarps with a diameter larger than 5 mm. The scientific names (current names) have been updated according to *The Index Fungorum* database. The collected macromycetes species have been classified into functional groups based on their primary

nutrition mode as following: saprophytic, mycorrhizal and parasitic. Functional groups used are slightly arbitrary because it is well known that many fungi can switch between these functional groups, depending on environmental conditions. Some saprophytic decomposing species can also function as a weak parasite species (e.g. *Armillaria mellea*). However, analysis of diversity for the functional groups is still an interesting subject because it may highlight some differences between macrofungal communities from different types of forests.

For vegetation analysis, a set of 30 relevés (1000 m^2) with phytosociological data collected in the same time with those referring to macromycetes has been realized. Vegetation sampling has been made according to the standard Central European Phytosociological Method [BRAUN-BLANQUET, 1964]. The hierarchical agglomerative clustering has been realized using the GINKGO software [DE CÁCERES, 2003]. In this application we created a rectangular matrix where the mid-percentages values of the 6 degrees Braun-Blanquet scale have been inserted. These mid-percentages values were square-root transformed and used to create a similarity matrix using Jaccard index as resemblance measure. Agglomerative hierarchical clustering has been realized using the UPGMA algorithm. The phytosociological nomenclature, classification [COLDEA, 1991; CHIFU & al. 2006] and cormophytes nomenclature [CIOCÂRLAN, 2000] followed some prestigious works in this domain. For each vegetal association, chorology, floristic and phytosociological composition are presented.

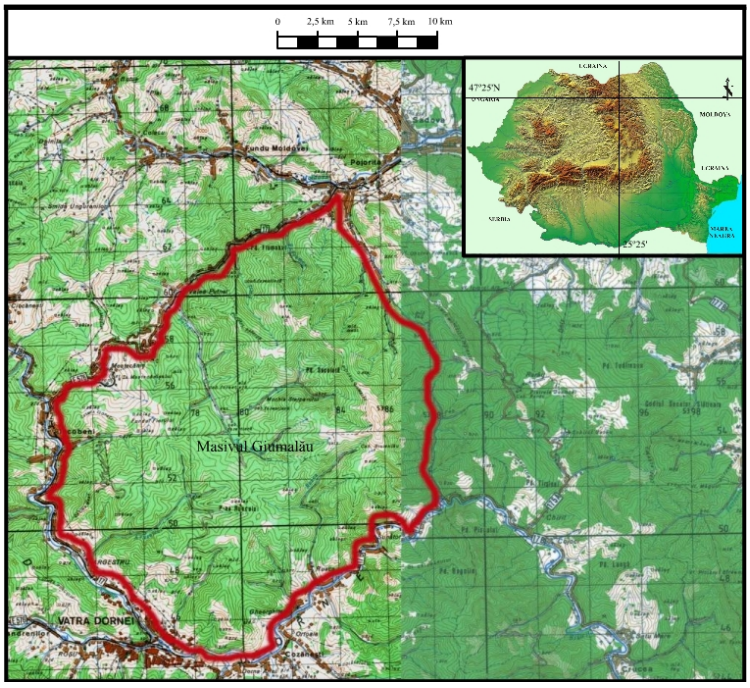

Fig. 1. Geographical position of Giumalău Massif

Macrofungal similarity of investigated areas was estimated based on Jaccard Index which evaluates similarity and matching between species [VARVARA & al. 2001] in different sites. Their hierarchical agglomerative clustering of has been also realized using the GINKGO software [DE CÁCERES, 2003], on presence-absence data using Jaccard Index as resemblance measure and the UPGMA clustering algorithm. For each sample area, geographical coordinates and altitude were recorded using a geographic positioning device (GPS II Plus Garmin Ltd.). The slope was measured with a clinometer and the exhibition was determined using a compass.

Detrended Correspondence Analysis (DCA) has been realized in order to distinguish the main gradients in macromycetes species composition and to characterize them from an ecological perspective. Detrending by segments and non weighted average values of altitudes, slopes, trees canopy covering and diversity (as Shannon indices) for each relevés were used (as passive projected variables). DCA has been realized in CANOCO 4.5 [TER BRAAK & ŠMILAUER, 2002].

Results and discussion

In the forest communities of Giumalău Massif have been identified 243 species of macromycetes from 30 sample areas. In *Hieracio transsilvanico-Piceetum* association have been identified 162 macromycetes species. They were identified on the following substrate types: soil, wood, animal excrements, and Norway spruce cones. In *Leucanthemo waldsteinii-Fagetum* association 120 macromycetes species have been identified. Approximately 16% of macromycetes species were common taxa. It was also observed that maximum development of sporocarps collected within investigated areas is clearly registered in August and September with more than 50% of identified species.

Phytocoenosis from *Hieracio transsilvanico-Piceetum* Pawlowski et Br.-Bl. 1939 association are frequently found all around in Giumalău Massif areas (Mestecăniş, Giumalău Secular Forest, Bâtca Neagră, Rusca, etc.), where they are populating more or less accentuated slopes (10-50°), with various aspects, on acid soils, poor in nutrients. Trees layer is dominated by Norway spruce (*Picea abies*) realizing a cover percentage between 75% and 95%, with lower proportions of *Sorbus aucuparia*, *Abies alba*, *Fagus sylvatica*, *Betula pendula* and *Acer pseudoplatanus*. Shrub and regeneration layer presents a low covering between 3% and 15%. In its composition have been identified more frequently the following species: *Vaccinium myrtillus*, *Vitis vitis-idaea*, *Daphne mezereum*, *Rubus idaeus*, *Sambucus racemosa*, *Corylus avellana*, *Lonicera xylosteum*, together with *Picea abies*, *Abies alba* and *Sorbus aucuparia* seedlings. Herbaceous layer is the most diverse, it presents different coverages between 10% and 70% and includes characteristic species for *Piceion excelsae* alliance, *Piceetalia excelsae* order (*Melampyrum sylvaticum*, *Luzula luzuloides*, *Gymnocarpium dryopteris*, *Calamagrostis villosa*, *Deschampsia flexuosa* etc.), *Vaccinio-Piceetea* class (*Oxalis acetosella*, *Campanula abietina*, *Homogyne alpina*, *Orthilia secunda* etc.) and also species characteristic for other vegetation classes interferring with the spruce stands: species of deciduous or mixed forests typical for *Querco-Fagetea* class (*Pulmonaria rubra*, *Euphorbia amygdaloides*, *Lilium martagon*, *Athyrium filix-femina*, *Mycelis muralis* etc.), characteristic species for forest clearings from *Epilobietea angustifolii* class (*Senecio ovatus*, *Fragaria vesca*, *Galeopsis speciosa* etc.), or for *Mulgedio-Aconitetea* class (*Polygonatum verticillatum*, *Hypericum maculatum* etc.). According to the classification from The Habitats Directive, these phytocoenoses belong to **9410** type - Acidophilous spruce forests (*Picea*) from mountain to alpine zones.

Analysis of ecological categories of *Hieracio transsilvanico-Piceetum* association revealed predominance of mycorrhizal species of following genera: *Amanita, Boletus, Cantharellus*, *Chalciporus*, *Cortinarius*, *Elaphomyces*, *Gomphidius*, *Hydnum*, *Hygrophorus*, *Inocybe*, *Lactarius*, *Leccinum*, *Neolecta*, *Paxillus*, *Porphyrellus*, *Russula*, *Sarcodon*, *Thelephora* and *Tricholoma*. Among these 68 mycorrhizal species, many of them are characteristic for coniferous forests, and they are associated mainly with spruce: *Amanita regalis*, *Amanita spissa*, *Boletus badius*, *Cortinarius caperatus*, *Cortinarius sanguineus*, *Cortinarius semisanguineus*, *Elaphomyces granulatus*, *Gomphidius glutinosus*, *Hygrophorus agathosmus*, *Hygrophorus olivaceoalbus*, *Hygrophorus persicolor*, *Lactarius deterrimus*, *Lactarius picinus*, *Lactarius salmonicolor*, *Lactarius scrobiculatus*, *Leccinum piceinum*, *Neolecta vitellina*, *Porphyrellus porphyrosporus*, *Russula badia*, *Russula integra*, *Russula queleti*, *Sarcodon imbricatus*, *Tricholoma subannulatum*, and *Tricholoma vaccinum*. Besides these species, there have been identified other species that have not specificity for this habitat type, but they prefer coniferous forests. In this category, it should be mentioned species from *Amanita* genus (*Amanita battarrae*, *Amanita muscaria), Boletus* genus (*Boletus calopus*, *Boletus chrysenteron*, *Boletus edulis*, *Boletus erythropus*, *Boletus luridus*, *Boletus pulverulentus*, and *Boletus subtomentosus*) and *Russula* genus (*Russula aeruginosa*, *Russula delica*, *Russula foetens*, *Russula fragilis* var. *fragilis*, *Russula nigricans*, and *Russula ochroleuca*).

In the epixylous synusiae have been identified 43 species: saprophytes (32 species), saproparasites (10 species) and parasite (1 species). Of these some colonize exclusively coniferous wood, especially spruce: *Amylostereum areolatum*, *Calocera viscosa*, *Clitocybula lacerata*, *Dacrymyces stillatus*, *Gloeophyllum abietinum*, *Gloeophyllum odoratum*, *Gloeophyllum sepiarium*, *Gymnopilus penetrans*, *Hericium alpestre*, *Heterobasidion annosum*, *Hydnellum geogenium*, *Hypholoma capnoides*, *Mycena epipterygia*, *Pholiota astragalina*, *Pseudohydnum gelatinosum*, *Sparassis crispa*, *Spongipellis borealis*, *Tremella encephala*, *Trichaptum abietinum*, *Tricholomopsis decora*, *Tricholomopsis rutilans, and Xeromphalina campanella*. In the spruce woods, there was registered several species which frequently occurred in deciduous forests. Among them, we mention: *Armillaria ostoyae*, *Fomitopsis pinicola*, *Ganoderma applanatum*, *Hypholoma fasciculare* and *Pleurotus dryinus* (Tab. 1). Sporadic presence of deciduous species (*Sorbus aucuparia*, *Fagus sylvatica*, *Sambucus racemosa*, *Acer pseudoplatanus* and *Lonicera xylosteum*) is a support for lignicolous macromycetes with affinity for this kind of wood. Thus, on decidous wood debris (stumps, fallen branches) were identified following species: *Calocera cornea*, *Chlorociboria aeruginascens*, *Micromphale foetidum*, *Pholiota adiposa*, *Piptoporus betulinus*, *Stereum gausapatum*, *Stereum hirsutum*, *Trametes hirsuta*, *Tremella foliacea* and *Tubaria furfuracea*. Compared to the total number of macromycetes species of *Hieracio transsilvanico-Piceetum* association, lignicolous macrofungi represent 26.5%. This is explained by the higher amount of dead wood, especially in protected area from Giumalău Secular Forest (Fig. 4).

On fallen spruce cones, which are partially buried in soil, sporocarps from three macromycete species have been found: *Baeospora myosura*, *Rutstroemia bulgarioides* and *Strobilurus esculentus*. Foliicolous macromycetes are represented by three species identified on spruce needles: *Gymnopus perforans*, *Marasmius androsaceus* and *Mycena rosella*.

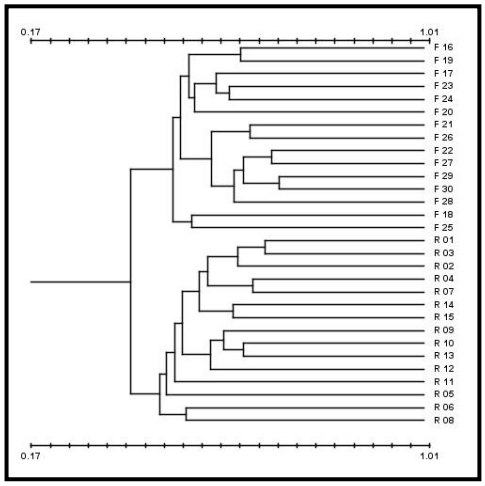

Fig. 2. Dendrogram of vegetation agglomerative hierarchical clustering (*Hieracio transsilvanico* and *Leucanthemo waldsteinii-Fagetum*)

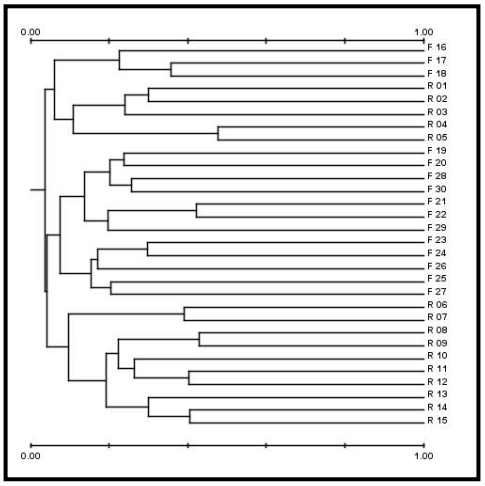

Fig. 3. Dendrogram of agglomerative hierarchical clustering of macromycetes from *Hieracio transsilvanico-Piceetum* and *Leucanthemo waldsteinii-Fagetum* associations

In Giumalău Massif, beech and spruce mixed forests (sometimes, they could be mixed with fir, but in reduced ratios) are included within *Leucanthemo waldsteinii-Fagetum* (Soó, 1964) Taüber 1987 association, from a phytosociological point of view. They are found at altitudes varying between 897 and 1200 m, on inclined slopes (15-45^{o}), with different aspects, on acid soils, richer in humus and nutrients than spruce fir forests. The trees layer has a good coverage, consisting of *Fagus sylvatica* (beech) and *Picea abies* (spruce), that are found in relatively equal proportions (co-dominant). In some phytocoenoses, they are accompanied by *Abies alba*, but with a relatively low proportion. Besides co-dominant species, within tree layer can also be found the following species: *Sorbus aucuparia*, *Betula pendula*, *Acer pseudoplatanus*, *Tilia cordata* etc. Shrub layer is relatively species poor with coverage between 3% and 5%. In this area, most common are following species: *Sambucus racemosa*, *Rosa pendulina*, *Rubus idaeus*, *Corylus avellana*, *Vaccinium myrtillus* and juveniles of *Fagus sylvatica*, *Picea abies*, *Abies alba* or *Sorbus aucuparia*. The herbaceous layer has a high diversity, presents coverages between 10% and 40% and includes characteristic species for *Symphyto cordati-Fagion* alliance (*Symphytum cordatum*, *Aconitum moldavicum* etc.), *Fagetalia sylvaticae* order (*Lamium galeobdolon*, *Actaea spicata*, *Epilobium montanum*, *Salvia glutinosa* etc.) and *Querco-Fagetea* class (*Dryopteris filix-mas*, *Viola reichenbachiana*, *Poa nemoralis* etc.). In the floristic composition there are also species characteristic for other vegetation classes: for coniferous forest species typical for *Vaccinio-Piceetea* class (*Dryopteris dilatata*, *Hieracium transsilvanicum* etc.) or species characteristic for forest clearings from *Epilobietea angustifolii* class (*Stachys sylvatica*, *Fragaria vesca* etc.). According to classification from

The Habitats Directive, these belong to habitat type **91V0** – Dacian Beech forests (*Symphyto-Fagion*).

In *Leucanthemo waldsteinii-Fagetum* association, 120 macromycetes species have been identified. Depending on ecological categories analysis, there where observed a predominance of lignicolous species with 59 species as follows: saprophytic (42 species), saproparasites (15 species) and parasitic (2 species). Saprophytic species were observed mainly on beech wood (stumps, fallen trunks, dry or rotten branches). Regarding to specificity depending on wood type where they are growing, most of identified species are oligophagous (they have not a strictly affinity based on substrate type – they were been identified on various kind of deciduous trees: beech, birch, alder and maple). Among monophagous species, they were observed only *Piptoporus betulinus* (on birch wood) and *Cytidia salicina* (on willow wood). Also, among polyphagous species that develops both on hardwood and softwood within this association, there have been identified: *Armillaria ostoyae*, *Crucibulum laeve*, *Fomitopsis pinicola*, *Hypholoma fasciculare*, *Merulius tremellosus and Pluteus cervinus*. Investigations on studied areas concerning to this macromycete category revealed a numerical superiority for species from *Trametes* and *Xylaria* genera (with 3 species); *Armillaria*, *Auricularia*, *Hypholoma*, *Polyporus* genera (with 2 species). Among genera with one single species identified in this association we mentioned: *Ascocoryne*, *Bisporella*, *Bjerkandera*, *Bulgaria*, *Chlorociboria*, *Chondrostereum*, *Crucibulum*, *Cyathus*, *Cytidia*, *Daldinia*, *Exidia*, *Flammulina*, *Fomes*, *Fomitopsis*, *Hericium*, *Hypoxylon*, *Kretzschmaria*, *Merulius*, *Mycena*, *Nectria*, *Oudemansiella*, *Panellus*, *Phlebia*, *Pholiota*, *Lycoperdon*, *Piptoporus*, *Pleurotus*, *Plicaturopsis*, *Pluteus*, *Pseudoclitocybe*, *Pycnoporus*, *Sarcoscypha*, *Schizophyllum*, *Stereum*, *Tremella*, and *Xerula*. Compared with total number of macromycetes species from the *Leucanthemo waldsteinii-Fagetum* association, lignicolous macromycetes percentage is 49% (Fig. 4). Significant amount of dead wood in the forest phytocoenosis favored development of this macromycetes ecological category.

Among of these 19 mycorrhizal species, the highest proportion holds characteristic species of deciduous forest, where they are associated particularly with beech trees: *Amanita crocea, Craterellus cornucopioides*, *Lactarius vellereus*, *Russula cyanoxantha* and *Russula virescens* (Tab. 1). Also, there were identified some species that occur in both deciduous and coniferous forests which have no specificity only to one of these two habitats. This category includes species of the following genera: *Amanita* (*Amanita pantherina*, *Amanita rubescens* var. *rubescens*), *Boletus* (*Boletus edulis*, *Boletus chrysenteron*), *Russula* (*Russula aurea*, *Russula delica*, *Russula foetens*, *Russula nigricans*, *Russula ochroleuca*, and *Russula vesca*).

The humicolous macromycetes of this association belong to following genera: *Bovista*, *Clitocybe*, *Coprinopsis*, *Coprinus*, *Helvella*, *Laccaria*, *Lycoperdon*, *Macrolepiota*, *Marasmius*, *Mycena*, *Stropharia* and they have a relatively low sharing percentage (18.68%) compared with total number of identified species.

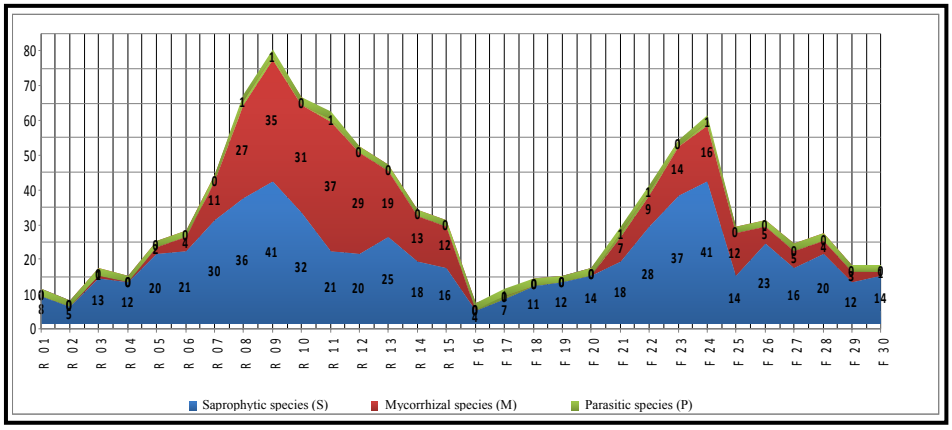

Fig. 4. Ecological spectrum for colected macromycetes of *Hieracio transsilvanico- Piceetum* and *Leucanthemo waldsteinii-Fagetum* associations

After that, we realized comparative analysis between results from those two investigated forest types. Comparative chart of similarity based on Jaccard index values between adjacent sample surfaces were in concordance with cluster analysis and it indicating two different fungal communities: those of *Hieracio transsilvanico-Piceetum* association and those of *Leucanthemo waldsteinii-Fagetum* association (Fig. 5). This index is based on probability that two individuals chosen at random, one from each of those two samples belong to species common to both samples. Estimations for this index take into account its contribution to the real value of this probability actually made on species presence within both sites, but which were not detected in one or both samples [CHAO & al. 2005]. Out of those 243 identified species, 39 species were common to both associations. Analysis of similarity dendrogram indicated that macromycetes species were distributed in two communities, according to Jaccard index values. Limits of species distribution ranges along environmental gradients are an interesting aspect used to identify different communities and determine species assemblages in numbers over many years of study. In the analysis of the similarity dendrogram (Fig. 3**)** it can be observed that, at the first hierarchical level, a separation among phenophases appears, distinguishing, first, macromycetes in vernal season, and, second, the macromycetes in summer and autumnal seasons. Then, in the second hierarchical level, macromycetes within each phenophase are differentiated depending on the forest community. The results suggest that macromycetes species composition is influenced by period of sporocarps occurrence and the environmental factors according to the functional group to which they belong and structure of forest communities. Sample surfaces with high similarity were observed in summer season in July - R06 and R07 (in *Hieracio transsilvanico-Piceetum* association), F21 and F22 (*Leucanthemo waldsteinii-Fagetum* association), in August - R08 and R09 respectively F23 and F24. For autumnal season R14 and R15 respectively F28 and F30 showed a high similarity.

Detrended Correspondence Analysis

Detrended Correspondence Analysis (Fig. 5) shows that, taking into account the macrofungal composition, two groups can be separated within the studied area. The first group located at the left, includes the macromycetes from *Hieracio transsilvanico-Piceetum* association and, the second one includes macromycetes from the *Leucanthemo waldsteinii-Fagetum* at the right.

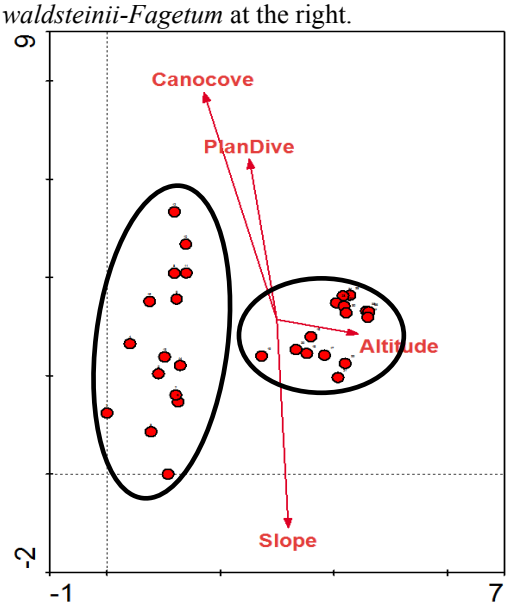

Fig. 5. DCA ordination diagram of the 30 samples using trees canopy covering, plants diversity, slope and altitude as passive variables first two axes presented. Eigenvalues: 1st axis: 0.716, 2nd axis: 0.430, total inertia: 6.974.

The first DCA axis is strongly correlated with altitude (Fig. 5). This suggests that altitude represent the main factor influencing the macromycetes composition in the vegetal communities from Giumalău Massif. This ecological factor generates a differentiation among the vegetal communities from increased altitudes, which are species poorer compared to the communities from lower altitudes including a higher number of (macromycetes) species. The second axis was correlated with plants species diversity, slope and canopy cover, indicating a gradient from relatively open forests stands, on accentuated slopes and richer in plants species to closed forests stands on less inclined terrains and poorer in plants species. Together, the first two axes of DCA explain 16.4% from total variance of macromycetes species and 13.0 of total species-environment relation.

Axes	**1**	**2**	**3**	**4**	**Total inertia**
Eigenvalues	0.716	0.430	0.251	0.174	6.974
Lenghts of gradient	4.615	5.334	2.872	3.137	
Species-environment correlations	0.183	0.493	0.236	0.471	
Cumulative percentage variance					
of species data	10.3	16.4	20.0	22.5	
of species-environment relation	2.5	13.0	0	0	
Sum of al eigenvalues					6.974
Sum of all canonical eigenvalues					*1.075*

In conclusion, along the first axis, which explains a higher percentage of variance (10.3%), we can identify an altitudinal gradient, which means that inside of studied area two altitudinal zones for macromycetes distribution have been identified: a) under 1000 m (lower mountain level) where 59% out of total species have been identified; b) upper 1000 m (superior mountain level) where 41% out of total species have been identified. The

influence of altitude on macromycetes species composition is consistent with other studies [GOMEZ-HERNANDEZ & WILLIAMS-LINERA, 2011].

The second axis show that trees canopy covering, plants species diversity and slope presents influence also the macromycetes communities composition as it was indicated in other studies [BONET & al. 2004; KÜFFER & SENN-IRLET, 2005; LAGANA & al. 1991]. Obtained results show that canopy cover has an important role in spreading of macrofungi species. Thus, within samples R08, R09 and R10 with 90-95% canopy covering a large number of mycorrhizal fungi has been determined. Moreover, different trophic groups had different responses to the influence of canopy covering. A possible explanation is that canopy is less important for saprophytic fungi than the existence of a given substratum. Our results suggest that canopy cover presents positive influence on mycorrhizal species as long as their number increases in a direct proportion with trees covering degree. Usually, mycorrhizal fungi are located in the upper layers of soil and, often, closely associated with roots of big trees. Regarding to saprophytic macrofungi, their distribution is strongly linked to a certain type of substratum and less dependent on canopy cover [SANTOS-SILVA & al. 2011]. Also, plants species diversity differed between the analyzed communities. But, within the same forest type, trees species composition was comparable for sites located at similar altitudes and, consequently, fungal species composition was relatively similar in all samples made in the same season. Comparing the 2 forests types, macromycetes composition changed, depending on tree species composition of forests and their abundance-dominance.

In conclusion, results of DCA analysis showed us clearly a separation in two different macromycetes groups for investigated areas. Macromycetes species composition is influenced primarily by altitude, and secondarily by plant diversity and canopy covering (Fig. 5). Also, out of the entire number of macromycetes, mycorrhizal macromycetes had a positive correlation with trees canopy covering.

Conclusions

Our results suggest that variation of macromycetes species composition in the two forests types from Giumalău Massif is directly related more to abiotic factors (altitude with influences on the climate) than woody species composition and plants communities diversity. Only complementary, changes in canopy covering, species composition of arboretum, together with some other factors as slope, can influence diversity and abundance of macromycetes.

Macromycetes species composition presents a high degree of similarity between surfaces situated at same altitudes, investigated at the same time (for harvesting of sporocarps). Among all investigated (passive) variables, it seems that altitude is the most important factor influencing the macromycetes species composition in Giumalău Massif. Vegetation diversity (which depends also on altitude) determines changes of macromycetes composition. Slope and canopy cover are less important factors for macromycetes species from vegetal communities in Giumalău Massif. From another perspective, the high diversity of macromycetes from habitats of Giumalau forests enforces us to conserve forests for several reasons. Thus, spruce forests are an important source of mycorrhizal species diversity (*Amanita*, *Boletus*, *Cantharellus*, *Chalciporus*, *Cortinarius*, *Elaphomyces*, *Gomphidius*, *Hydnum*, *Hygrophorus*, *Inocybe*, *Lactarius*, *Leccinum*, *Neolecta*, *Paxillus*, *Porphyrellus*, *Russula*, *Sarcodon*, *Thelephora and Tricholoma*). These fungi are key

species in development, balance and preservation of forest communities. The most frequent macromycetes in the spruce forests of Giumalău Massif are common species for coniferous forests, but the most valuable importance is given by some rare species which have been found in few areas: *Elaphomyces granulatus*, *Guepinia helvelloides*, *Helvella acetabulum*, *Hericium alpestre*, *Neolecta vitellina*, *Pterula subulata*, *Rutstroemia bulgarioides*, *Tremella foliacea* and *Tremella encephala*. The presence of large amounts of dead wood and a large diversity in wood species that populate wood substrate clearly favors lignicolous macromycete populations. Conservative importance of habitats in *Leucanthemo waldsteinii-Fagetum* association of Giumalău is emphasized by the presence of few rare species as *Cytidia salicina*, *Hericium coralloides* and *Tremella foliacea*.

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Tab. 1. Macromycetes identified within *Hieracio transsilvanico – Piceetum* and *Leucanthemo waldsteinii – Fagetum* associations

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
1	Sh	*Agaricus sylvaticus* Schaeff.																							1	1						
2	Sh	*Agaricus sylvicola* (Vittad.) Peck																								1	1					
3	Sh	*Albatrellus confluens* (Alb. & Schwein.) Kotl. & Pouzar										1	1	1																		
4	Sh	*Albatrellus ovinus* (Schaeff.) Kotl. & Pouzar									1	1																				
5	Sh	*Aleuria aurantia* (Pers.) Fuckel																							1	1						
6	M	*Amanita battarrae* (Boud.) Bon					1	1		1																						
7	M	*Amanita crocea* (Quél.) Singer																						1								
8	M	*Amanita fulva* Fr.									1	1														1	1					
9	M	*Amanita muscaria* (L.) Lam.								1	1	1	1	1																		
10	M	*Amanita muscaria* var. *aureola* (Kalchbr.) Quél.										1																				
11	M	*Amanita pantherina* (DC.) Krombh.																							1	1	1					
12	M	*Amanita regalis* (Fr.) Michael								1	1	1		1																		
13	M	*Amanita rubescens* var. *rubescens* Pers.						1	1		1		1	1									1	1		1						
14	M	*Amanita excelsa* var. *spissa* (Fr.) Neville & Poumarat								1			1	1	1																	
15	M	*Amanita vaginata* (Bull.) Lam.																								1	1					

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
16	Sl	*Amylostereum areolatum* (Chaillet ex Fr.) Boidin					1	1		1												1										
17	SPl	*Armillaria mellea* (Vahl) P. Kumm.																									1	1	1	1		
18	SPl	*Armillaria ostoyae* (Romagn.) Herink											1	1	1	1	1											1	1	1		
19	Sl	*Ascocoryne sarcoides* (Jacq.) J.W. Groves & D.E. Wilson								1	1			1		1	1						1			1		1				
20	SPl	*Auricularia auricula-judae* (Bull.) Quél.																		1			1	1	1							
21	SPl	*Auricularia mesenterica* (Dicks.) Pers.																			1	1								1		1
22	Scp	*Baeospora myosura* (Fr.) Singer								1	1			1		1	1															
23	Sl	*Bisporella citrina* (Batsch) Korf & S.E. Carp.																						1		1		1				
24	Sl	*Bjerkandera adusta* (Willd.) P. Karst.																					1		1	1		1			1	
25	Sc	*Bolbitius titubans* var. *titubans* (Bull.) Fr.	1		1				1												1											
26	M	*Boletus badius* (Fr.) Fr.							1		1		1			1	1															
27	M	*Boletus calopus* Pers.								1	1	1																				
28	M	*Xerocomellus chrysenteron* (Bull.) Šutara							1		1		1										1	1			1		1			
29	M	*Boletus edulis* Bull.								1	1	1	1		1										1	1		1				
30	M	*Boletus erythropus* var. *erythropus* Pers.								1	1		1	1	1	1	1															
31	M	*Boletus luridus* var. *luridus* Schaeff.									1		1																			
32	M	*Boletus pulverulentus* Opat.											1	1		1	1															
33	M	*Boletus queletii* Schulzer								1		1	1																			
34	M	*Boletus subtomentosus* L.							1			1																				
35	Sh	*Bovista nigrescens* Pers.																							1							
36	Sl	*Bulgaria inquinans* (Pers.) Fr.																							1			1				

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
37	Sl	*Calocera cornea* (Batsch) Fr.					1			1			1																			
38	Sl	*Calocera viscosa* (Pers.) Fr.			1			1	1			1				1	1															
39	M	*Cantharellus cibarius* Fr.								1	1	1		1	1	1	1								1	1	1		1			
40	M	*Craterellus lutescens* (Fr.) Fr.										1	1	1	1																	
41	M	*Craterellus tubaeformis* (Fr.) Quél.							1	1	1	1	1																			
42	M	*Chalciporus piperatus* (Bull.) Bataille							1		1		1	1	1		1															
43	Sl	*Chlorociboria aeruginascens*							1													1		1								
44	Sl	*Chondrostereum purpureum* (Pers.) Pouzar																								1		1	1			
45	M	*Chroogomphus helveticus* (Singer) M.M. Moser										1	1																			
46	Sh	*Clitocybe clavipes* (Pers.) P. Kumm.								1		1			1	1																
47	Sh	*Clitocybe nebularis* (Batsch) P. Kumm.																							1	1		1	1			
48	Sh	*Clitocybe odora* (Bull.) P. Kumm.						1	1		1				1					1				1	1						
49	Sl	*Clitocybula lacerata* (Scop.) Métrod					1		1																							
50	Sc	*Conocybe pubescens* (Gillet) Kühner				1	1																									
51	Sh	*Coprinopsis atramentaria* (Bull.) Redhead																						1								
52	Sh	*Coprinus comatus* (O.F. Müll.) Pers.																							1	1						1
53	M	*Cortinarius caperatus* (Pers.) Fr.								1	1		1	1		1	1															
54	M	*Cortinarius cinnamomeus* (L.) Fr.										1	1																			
55	M	*Cortinarius collinitus* Pers. Fr.										1	1																			
56	M	*Cortinarius croceus* (Schaeff.) Gray									1	1		1																		
57	M	*Cortinarius flexipes* var. *flexipes* Pers. Fr.										1	1																			

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
58	M	*Cortinarius sanguineus* (Wulfen) Fr.								1		1			1		1															
59	M	*Cortinarius semisanguineus* (Fr.) Gillet										1	1			1																
60	M	*Cortinarius trivialis* J.E. Lange																											1			
61	M	*Craterellus cornucopioides* (L.) Pers.																							1	1		1	1			
62	Sl	*Crepidotus applanatus* var. *applanatus* (Pers.) P. Kumm.																						1	1	1						
63	Sl	*Crucibulum laeve* (Huds.) Kambly	1			1	1																1			1				1		1
64	Sl	*Cyathus striatus* (Huds.) Willd.																	1					1				1				
65	Sh	*Cystodermella granulosa* (Batsch) Harmaja						1	1		1				1	1																
66	Sl	*Cytidia salicina* (Fr.) Burt																							1							
67	Sl	*Dacrymyces stillatus* Nees					1			1			1	1																		
68	Sl	*Daldinia concentrica* (Bolton) Ces. & De Not.																				1	1			1				1		
69	P	*Dumontinia tuberosa* (Bull.) L.M. Kohn																	1													
70	M	*Elaphomyces granulatus* Fr.					1		1																							
71	Sh	*Entoloma incanum* (Fr.) Hesler								1		1	1																			
72	Sl	*Exidia glandulosa* (Bull.) Fr.																			1			1	1							1
73	SPl	*Flammulina velutipes* var. *velutipes* (Curtis) Singer																1														
74	SPl	*Fomes fomentarius* (L.) Fr.																1	1	1			1	1		1				1	1	
75	SPl	*Fomitopsis pinicola* (Sw.) P. Karst.	1		1	1	1		1			1		1	1		1					1		1			1					
76	SPl	*Ganoderma applanatum* (Pers.) Pat.			1			1				1					1															
77	Sh	*Geastrum triplex* Jungh.						1		1																						

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
78	Sl	*Gloeophyllum abietinum* (Bull.) P. Karst.						1	1				1	1																		
79	Sl	*Gloeophyllum odoratum* (Wulfen) Imazeki	1	1				1	1			1	1		1		1															
80	Sl	*Gloeophyllum sepiarium* (Wulfen) P. Karst.	1		1			1	1			1			1																	
81	M	*Gomphidius glutinosus* (Schaeff.) Fr.									1		1		1																	
82	Sh	*Guepinia helvelloides* (DC.) Fr.								1		1																				
83	Sl	*Gymnopilus penetrans* (Fr.) Murrill						1	1			1	1	1																		
84	Sh	*Gymnopus dryophilus* (Bull.) Murrill						1	1																							
85	Sf	*Gymnopus perforans* (Hoffm.) Antonín & Noordel.						1	1		1																					
86	Sf	*Gymnopus peronatus* (Bolton) Gray																							1							
87	Sh	*Gyromitra gigas* (Krombh.) Cooke																		1												
88	Sh	*Gyromitra infula* (Schaeff.) Quél.			1	1																										
89	Sh	*Helvella acetabulum* (L.) Quél.			1															1												
90	Sh	*Helvella crispa* (Scop.) Fr.																	1	1	1											
91	Sh	*Helvella elastica* Bull.																	1	1												
92	SPl	*Hericium alpestre* Pers.									1				1																	
93	SPl	*Hericium coralloides* (Scop.) Pers.																							1			1				
94	SPl	*Heterobasidion annosum* (Fr.) Bref.	1	1						1		1		1																		
95	Sh	*Hydnellum ferrugineum* (Fr.) P. Karst.										1	1																			
96	Sl	*Hydnellum geogenium* (Fr.) Banker								1	1																					
97	Sh	*Hydnellum suaveolens* (Scop.) P. Karst.								1	1																					
98	M	*Hydnum repandum* L.								1	1				1	1									1	1					1	

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
99	Sl	*Hygrophoropsis aurantiaca* (Wulfen) Maire												1	1																	
100	M	*Hygrophorus agathosmus* (Fr.) Fr.									1		1	1		1	1															
101	M	*Hygrophorus chrysodon* (Batsch) Fr.																									1	1				
102	M	*Hygrophorus cossus* (Sowerby) Fr.																									1					
103	M	*Hygrophorus discoxanthus* (Fr.) Rea												1	1																	
104	M	*Hygrophorus olivaceoalbus* (Fr.) Fr.							1			1	1		1	1																
105	M	*Hygrophorus persicolor* Ricek							1			1																				
106	Sl	*Hypholoma capnoides* (Fr.) P. Kumm.								1	1		1	1																		
107	Sl	*Hypholoma fasciculare* (Huds.) P. Kumm.						1	1														1	1		1		1			1	
108	Sl	*Hypholoma lateritium* (Schaeff.) P. Kumm.																						1		1	1		1			
109	Sl	*Hypoxylon fragiforme* (Pers.) J. Kickx f.																			1	1			1	1				1		1
110	Sh	*Infundibulicybe geotropa* (Bull.) Harmaja																											1			
111	M	*Inocybe assimilata* Britzelm																								1	1					
112	M	*Inocybe geophylla* var. *geophylla* (Fr.) P. Kumm.								1	1				1										1			1				
113	M	*Inocybe geophylla* var. *lilacina* Gillet																							1	1						
114	M	*Inocybe rimosa* (Bull.) P. Kumm.																								1						
115	Sl	*Kretzschmaria deusta* (Hoffm.) P.M.D. Martin								1	1				1					1		1		1						1		1
116	Sh	*Laccaria amethystina* Cooke								1	1														1	1			1			
117	Sh	*Laccaria bicolor* (Maire) P.D. Orton									1	1																				
118	Sh	*Laccaria laccata* (Scop.) Cooke				1	1		1			1	1	1		1	1						1	1		1					1	1
119	Sh	*Laccaria proxima* (Boud.) Pat.								1	1	1													1	1						

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
120	Sh	*Laccaria tortilis* (Bolton) Cooke						1	1	1																						
121	M	*Lactarius deterrimus* Gröger								1	1	1	1	1																		
122	M	*Lactarius lignyotus* var. *lignyotus* Fr.										1	1																			
123	M	*Lactarius picinus* Fr.										1		1																		
124	M	*Lactarius rufus* (Scop.) Fr.								1	1																					
125	M	*Lactarius salmonicolor* R. Heim & Leclair									1		1	1																		
126	M	*Lactarius scrobiculatus*(Scop.) Fr.											1	1		1																
127	M	*Lactarius trivialis* (Fr.) Fr.								1	1																					
128	M	*Lactarius vellereus* var. *vellereus* (Fr.) Fr.																							1	1						
129	M	*Lactarius volemus* (Fr.) Fr.									1														1		1		1			
130	M	*Leccinum piceinum* Pilát & Dermek.									1																					
131	M	*Leccinum scabrum* (Bull.) Gray																					1	1	1		1					
132	Sh	*Lepiota castanea* Quél.								1	1																					
133	Sh	*Lepiota clypeolaria* (Bull.) P. Kumm.										1																1				
134	Sh	*Lepista flaccida* (Sowerby) Pat.				1	1		1			1		1																		
135	Sh	*Lepista nuda* (Bull.) Cooke								1	1				1	1																
136	M	*Leucocortinarius bulbiger* (Alb. & Schwein.) Singer												1																		
137	Sh	*Lichenomphalia umbellifera* (L.) Redhead, Lutzoni, Moncalvo & Vilgalys						1	1																							
138	Sh	*Lycoperdon echinatum* Pers.																								1	1					
139	Sh	*Lycoperdon perlatum* Pers.									1														1				1			
140	Sl	*Lycoperdon pyriforme* Schaeff.																							1	1			1			

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
141	Sh	*Lycoperdon umbrinum* Pers.								1	1		1	1																		
142	Sh	*Lyophyllum connatum* (Schumach.) Singer								1	1				1	1	1															
143	Sh	*Lyophyllum decastes* (Fr.) Singer										1		1																		
144	Sh	*Macrolepiota procera* var. *procera* (Scop.) Singer									1	1	1		1	1									1	1		1				
145	Sh	*Macrolepiota rachodes* var. *bohemica* (Wichanský) Bellù & Lanzoni								1		1																				
146	Sh	*Marasmius alliaceus* (Jacq.) Fr.						1	1		1														1	1					1	1
147	Sf	*Marasmius androsaceus* (L.) Fr.						1	1		1																					
148	P	*Marasmius oreades* (Bolton) Fr.																					1	1		1						
149	Sl	*Marasmius rotula* (Scop.) Fr.																				1	1	1								
150	Sl	*Megacollybia platyphylla* (Pers.) Kotl. & Pouzar																					1	1								
151	Sl	*Merulius tremellosus* Schrad.								1		1			1										1				1			
152	Sl	*Gymnopus foetidus* (Sowerby) J. L. Mata & R. H. Petersen						1	1																							
153	Sh	*Mycena aetites* (Fr.) Quél.								1																						
154	Sh	*Mycena aurantiomarginata* (Fr.) Quél.							1			1																				
155	Sh	*Mycena crocata* (Schrad.) P. Kumm.																						1				1				
156	Sl	*Mycena epipterygia* (Scop.) Gray							1		1				1	1	1															
157	Sh	*Mycena galopus* var. *candida* J. E. Lange			1		1	1																								
158	Sl	*Mycena haematopus* (Pers.) P. Kumm.																							1		1		1		1	1
159	Sh	*Mycena pelianthina* (Fr.) Quél.																							1	1				1	1	
160	Sh	*Mycena pura* (Pers.) P. Kumm.								1	1		1	1	1	1							1	1			1	1		1		

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
161	Sh	*Mycena rosella* (Fr.) P. Kumm.								1		1	1		1	1	1							1	1							
162	Sl	*Mycena silvae nigrae* Maas Geest. & Schwöbel								1						1																
163	SPl	*Nectria cinnabarina* (Tode) Fr.																							1					1		
164	M	*Neolecta vitellina* (Bres.) Korf & J. K. Rogers			1																											
165	Sh	*Omphalina demissa* (Fr.) Quél.					1																									
166	Sl	*Oudemansiella mucida* (Schrad.) Höhn.																							1			1	1			
167	Sc	*Panaeolus semiovatus* var. *semiovatus* (Sowerby) S. Lundell & Nannf.					1		1						1		1															
168	Sl	*Panellus stipticus* (Bull.) P. Karst.																							1	1					1	
169	M	*Paxillus involutus* (Batsch) Fr.								1	1		1	1																		
170	Sh	*Peziza badiofusca* (Boud.) Dennis							1		1																					
171	Sl	*Phlebia radiata* Fr.																				1		1		1						
172	SPl	*Pholiota adiposa* (Batsch) P. Kumm.										1			1																	
173	Sl	*Pholiota astragalina* (Fr.) Singer											1	1																		
174	SPl	*Pholiota squarrosa* (Vahl) P. Kumm.																							1		1	1	1			
175	SPl	*Piptoporus betulinus* (Bull.) P. Karst.				1	1			1		1	1										1	1		1				1		1
176	Pl	*Pleurotus dryinus* (Pers.) P. Kumm.								1	1		1																			
177	SPl	*Pleurotus ostreatus* (Jacq.) P. Kumm.																							1			1				1
178	Sl	*Plicaturopsis crispa* (Pers.) D. A. Reid																								1	1					
179	Sl	*Pluteus cervinus* (Schaeff.) P. Kumm.																				1			1	1						
180	Sl	*Pluteus salicinus* (Pers.) P. Kumm.																									1	1				
181	Sl	*Pluteus thomsonii* (Berk. & Broome) Dennis																								1						

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
182	Sl	*Polyporus brumalis* (Pers.) Fr.																							1	1						
183	Sl	*Polyporus varius* (Pers.) Fr.																								1		1	1			
184	M	*Tylopilus porphyrosporus* (Fr. & Hök) A. H. Sm. & Thiers								1	1		1		1		1															
185	Sl	*Pseudoclitocybe cyathiformis* (Bull.) Singer																								1	1		1			1
186	Sl	*Pseudohydnum gelatinosum* (Scop.) P. Karst.								1	1		1	1	1		1															
187	Sh	*Pterula subulata* Fr.										1																				
188	Sl	*Pycnoporus cinnabarinus* (Jacq.) P. Karst.																			1	1			1		1	1				
189	Sh	*Ramaria botrytis* (Pers.) Ricken								1	1															1	1					
190	Sh	*Ramaria flava* (Schaeff.) Quél.								1	1														1		1					
191	Sh	*Ramaria pallida* (Schaeff.) Ricken								1	1																					
192	Sh	*Rhodocollybia butyracea* (Bull.) Lennox							1		1																					
193	Sh	*Rhodocollybia maculata* (Alb. & Schwein.) Singer									1	1				1	1															
194	M	*Russula aeruginosa* Massee								1	1		1																			
195	M	*Russula aurea* Pers.																							1					1		1
196	M	*Russula badia* Quél.								1	1																					
197	M	*Russula cyanoxantha* (Schaeff.) Fr.																					1	1		1				1	1	
198	M	*Russula delica* Fr.										1			1	1	1								1		1					
199	M	*Russula foetens* Pers.						1	1				1	1		1							1	1				1				
200	M	*Russula fragilis* var. *fragilis* Fr.								1	1	1	1	1																		
201	M	*Russula heterophylla* (Fr.) Fr.										1	1																			

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
202	M	*Russula integra* var. *integra* (L.) Fr.										1	1	1																		
203	M	*Russula nigricans* Fr.								1	1	1	1	1		1	1							1		1					1	
204	M	*Russula ochroleuca* Fr.										1		1	1										1	1						
205	M	*Russula queletii* Fr.										1		1																		
206	M	*Russula vesca* Fr.																					1	1						1		
207	M	*Russula virescens* (Schaeff.) Fr.																					1	1	1							
208	Scp	*Rutstroemia bulgarioides* P. Karst.	1	1	1																											
209	M	*Sarcodon imbricatus* (L.) P. Karst.								1		1	1	1																		
210	Sl	*Sarcoscypha coccinea* (Gray) Boud.																	1	1												
211	SPl	*Schizophyllum commune* Fr.																			1	1	1	1	1					1		1
212	Sl	*Scutellinia scutellata* (L.) Lambotte						1	1			1		1																		
213	Sl	*Sparassis crispa* (Wulfen) Fr.								1		1																				
214	SPl	*Climacocystis borealis* (Fr.) Kotl. & Pouzar									1				1	1																
215	SPl	*Stereum gausapatum* (Fr.) Fr.				1	1					1																				
216	SPl	*Stereum hirsutum* (Willd.) Pers.				1	1			1					1		1	1	1	1			1	1		1				1	1	
217	Scp	*Strobilurus esculentus* (Wulfen) Singer	1		1		1																									
218	Sh	*Stropharia aeruginosa* (Curtis) Quél.																					1	1		1		1				
219	Sh	*Stropharia coronilla* (Bull. ex DC.) Quél.																						1								
220	Sl	*Tapinella atrotomentosa* (Batsch) Šutara								1	1																					
221	M	*Thelephora palmata* (Scop.) Fr.													1																	

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
222	M	*Thelephora terrestris* Ehrh.						1	1		1				1																	
223	Sl	*Trametes hirsuta* (Wulfen) Lloyd				1	1	1											1		1		1		1					1	1	
224	SPl	*Trametes pubescens* (Schumach.) Pilát																			1			1	1					1		1
225	Sl	*Trametes versicolor* (L.) Lloyd																			1			1		1						
226	Sl	*Tremella encephala* Willd.							1		1			1																		
227	Sl	*Tremella foliacea* Pers.									1		1													1		1				
228	Sl	*Tremella mesenterica* Retz.																											1	1		
229	Sl	*Trichaptum abietinum* (Dicks.) Ryvarden				1	1		1	1	1		1		1	1																
230	M	*Tricholoma terreum* (Schaeff.) P. Kumm.											1	1																		
231	M	*Tricholoma saponaceum* Fr. P. Kumm.												1	1															1		
232	M	*Tricholoma batschii* Gulden								1																						
233	M	*Tricholoma sulphureum* var. *sulphureum* (Bull.) P. Kumm.									1															1	1					
234	M	*Tricholoma vaccinum* (Schaeff.) P. Kumm.								1	1		1	1	1		1															
235	Sl	*Tricholomopsis decora* (Fr.) Singer							1		1				1	1																
236	Sl	*Tricholomopsis rutilans* (Schaeff.) Singer								1	1	1	1		1		1															
237	Sl	*Tubaria furfuracea* (Pers.) Gillet			1																											
238	M	*Tylopilus felleus* (Bull.) P. Karst.								1	1																					
239	Sl	*Xeromphalina campanella* (Batsch) Maire		1	1		1				1																					

No.	Ecological category	Species	R 01	R 02	R 03	R 04	R 05	R 06	R 07	R 08	R 09	R 10	R 11	R 12	R 13	R 14	R 15	F 16	F 17	F 18	F 19	F 20	F 21	F 22	F 23	F 24	F 25	F 26	F 27	F 28	F 29	F 30
240	Sl	*Xerula radicata* (Relhan) Dörfelt																			1	1	1			1				1	1	
241	Sl	*Xylaria hypoxylon* (L.) Grev.																			1			1		1						
242	Sl	*Xylaria longipes* Nitschke																				1	1							1		
243	Sl	*Xylaria polymorpha* (Pers.) Grev.		1	1	1	1				1							1		1				1						1	1	
TOTAL			**8**	**5**	**14**	**12**	**22**	**25**	**41**	**64**	**77**	**63**	**59**	**49**	**44**	**31**	**28**	**4**	**8**	**11**	**12**	**14**	**26**	**38**	**51**	**58**	**26**	**28**	**21**	**24**	**15**	**15**

Mychorrizal species	**84**
Saprophyte species on cones	**3**
Coprophilous saprophyte species	**3**
Folicolous saprophyte species	**3**
Humicolous saprophyte species	**59**
Lignicolous saprophyte species	**67**
Lignicolous saproparasite species	**21**
Lignicolous parasite species	**1**
Parasite species	**2**

LEGEND:

macromycetes identified in *Hieracio transsilvanico – Piceetum* association

macromycetes identified in *Leucanthemo waldsteinii – Fagetum* association

macromycetes identified in both associations

May
June
July
August
September
October

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DESCRIPTION OF THE CULTURE CHARACTERISTICS OF SOME LIGNICOLOUS BASIDIOMYCETES SPECIES GROWN ON THREE SYNTHETIC MEDIA

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Abstract: A number of 12 species of lignicolous basidiomycetes were cultivated on potato dextrose agar and malt extract agar, incubated at 25 °C and carefully analyzed for a period of 5 weeks. Lignicolous basidiomycetes are fungi that produce potent enzymes and bioactive secondary metabolites which are successfully used in various industries: bioremediation of polluted environments, biodegradation of toxic substances, pharmacology or agriculture. The objective of this study was the description of the main characteristics of *in vitro* cultures of some lignicolous basidiomycetes species grown on synthetic media. The main characteristics followed were: the growth rate of the colonies, the general features of the mycelium: shape, color, surface aspect, reverse, the presence of fruiting bodies and exudates and the particular odor.

Key words: lignicolous basidiomycetes, *in vitro* cultures, culture characteristics.

Introduction

Lignicolous basidiomycetes are fungi that play very important ecological roles within an ecosystem.

Through their complex enzymatic system, the lignicolous basidiomycetes are the main decomposer of wood in natural and anthropogenic habitats, being involved from this point of view in the natural cycles of some essential elements, such as carbon and nitrogen.

Because of this particular capacity, showed by very few organisms, researchers began testing with impressive results the great potential of the lignolytic enzymes for degrading several manmade substances and materials: pesticides, synthetic dyes, polychlorinated biphenyls, synthetic polymers.

Besides the highly reactive enzymes the lignicolous basidiomycetes produce during their metabolism several metabolites which are bioactive molecules with antibiotic and antimicrobial [KELLER & al. 2005], antioxidant [KARAMAN & al. 2009], cytotoxic and hallucinogenic properties [ANKE, 1989].

Referring to the characterization of fungal *in vitro* cultures the international literature offers some, but not sufficient information, describing especially species of wood decaying fungi found in North America [FRITZ, 1923; NOBLES, 1948, 1964; STALPERS, 1978, ADASKAVEG & GILBERTSON, 1989; FLOTT & GILBERTSON, 1991; BIGELOW & al. 1998].

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In Romania the study of lignicolous basidiomycetes *in vitro* cultures is still a new direction of research, this being the reason the literature offers very few data [BALAEŞ & TĂNASE, 2012a, 2012b].

The aim of this study is the description of the main characteristics of *in vitro* cultures of some lignicolous basidiomycetes species grown on various synthetic media, in order to observe the potential differences between the colonies that use a standard substrate and the colonies from a more economic adapted media.

Materials and methods

Fungal strains

Several fruiting bodies of lignicolous basidiomycetes species were collected from wood in different stages of decay from various forest habitats from Romania. Only the fresh and healthy fruiting bodies were collected which were suitable for correct identification.

The species of fungi were identified after their macroscopic and microscopic characteristics using literature data from different authors [ERIKSSON & RYVARDEN, 1973, 1976; ERIKSSON & al. 1978, 1981; HJORTSTAM & al. 1987; HANSEN & KNUDSEN, 1992, 1997; BERNICCHIA, 2005; TĂNASE & al. 2009]. The scientific names used for the isolated fungal species were according to the nomenclature presented in the Index Fungorum Database [www.indexfungorum.org – accessed from 1st August 2013 – 31th October 2013] and Dictionary of Fungi [KIRK & al. 2008].

Part of the fruiting bodies was used in the isolation process and the rest was preserved in order to be stored within the Faculty of Biology Herbarium. The preservation was done by hot air dehydration (using an Ezidri Ultra model 1000 FD) and lyophilization (using a UNICRYO MIC 4 L model, Planegg, Germany).

Isolation process

The fungal strains were cultivated on two standard synthetic media MEA (20 mg malt extract, 15 mg agar, 1000 ml distilled water) and PDA (200 g washed, sliced and peeled potatoes, 20 mg dextrose, 20 mg agar, 1000 ml distilled water) and one adapted media with potato flakes and bean flakes (10 g potato flakes, 10 g bean flakes, 5 g glucose, 15 mg agar, 1000 ml distilled water).

All media were sterilized by autoclaving at 121 °C for 15 minutes. Subsequently, 25 ml of each media were poured into different 9 mm diameter Petri dishes.

In a sterile environment, small fragments of dikaryotic mycelium taken from the context of fresh and healthy fruiting bodies were inoculated approximately 2 cm from the edge of the plate.

The plates were incubated in the dark at 25 °C in an automatic aeration incubator (Microbiotest, Gent, Belgium), for 5 weeks. Every species was cultivated in duplicate on all three media.

Analysis of the *in vitro* cultures

The analysis of *in vitro* cultures implied the careful observation of several features: the growth rate of the colonies, the general characteristics of the mycelium: shape, color, surface aspect, reverse, the presence of fruiting bodies and exudates and the particular odor which were followed starting from the first week of incubation.

Some of these characteristics were observed using an Optika B-353 PLi trinocular microscope and an Optika SZM-2 stereomicroscope.

Results and disscutions

A number of 12 species of lignicolous basidiomycetes were isolated in pure culture. The species belong to 7 families and 3 orders (Tab. 1) and are part of Subclass Agaricomycotina, Class Agaricomycetes, Phyllum Basidiomycota.

After the cultivation of the lignicolous basidiomycetes species on all thee synthetic media we were able to observe a better growth rate on the adapted media in comparison with the standard media for every one of the 12 species (Tab. 2). Also in some cases the reverse of the colony was different from the standard media (Tab. 2).

Moreover, 6 species formed fruiting bodies starting from the fourth week of incubation and only 2 species produced exudates (Tab. 2).

For every *in vitro* culture a specific odor was identified which is an indicator of the presence of the volatile organic compounds synthetized by the mycelium (Tab. 2).

Depending on the synthetic media on which the colonies were grown we noticed some differences in what concerns the developing of the hyphae, the general aspect of the mycelium and the formation of fruiting bodies in comparison to other studies [BALAEŞ & TĂNASE, 2012a, 2012b].

Culture characteristics of lignicolous basidiomycetes species isolated in pure culture

***Bjerkandera fumosa* (Pers.) P. Karst.** On all three media, the mycelium is white, relatively homogeneous, felty, adpressed, with aerial hyphae that have a radial development. The edges of the colony are regular and they cover the margins of the plate after two weeks of incubation. On the adapted media, after four weeks of incubation, the mycelium becomes very dense and several fruiting bodies develop between the two plates of the Petri dish, which reached the maturity after one week. The generative hyphae are branched, hyaline with clamp connections, 1.5 – 3.5 µm in diameter, while the skeletal hyphae are whitish, thick walled, rarely branched, 4 – 5 µm in diameter.

***Crepidotus applanatus* (Pers.) P. Kumm**. On all three media the mycelium is white, adpressed, heterogeneous, with felty areas, lax. The edges of the colony are regular and the hyphae have a radial development, covering the margins of the plate after two weeks of incubation. After four weeks of incubation near the edges of the plate the hyphae tend to crowd giving the colony a granular aspect and forming primordia of fruiting bodies that do not reach maturity. The aerial hyphae are branched, hyaline with clamp connections, 2.5 – 4 µm in diameter and the skeletal hyphae have thinner walls, 2 – 3.5 µm in diameter.

***Hypholoma lateritium* (Schaeff.) P. Kumm.** On all three media the mycelium is white to cream colored, downy, wavy and heterogeneous. Around the inoculum point the hyphae form a translucent ring where thick cords can be seen. The edge of the colony is regular and the hyphae have a radial development. No primordia or fruiting bodies were observed on the surface of the colony. The generative hyphae are branched, hyaline, with

clamp connections, 2.5 – 3.5 µm in diameter and the skeletal hyphae are cream – colored, thick walled and have 3.5 – 4 µm in diameter.

***Piptoporus betulinus* (Bull.) P. Karst.** On all three media the mycelium is white, relatively homogeneous, downy – felty. The edges on the colony are regular and the hyphae have a radial development. The only difference that we recorded when inoculated on various media was that when grown on the adapted media the hyphae were denser. No primordia or fruiting bodies were developed *in vitro* by this species. The generative hyphae are branched, hyaline, with clamp connections, 1.5 – 3.5 µm in diameter, while the skeletal hyphae are less branched and have thicker walls, 3 – 4 µm in diameter.

***Polyporus squamosus* (Huds.) Fr.** On all three media the mycelium is heterogeneous, with felty and adpressed areas alternating with translucent ones. The edges of the colony are irregular and the hyphae have a radial development. The young aerial hyphae are white, becoming brown with age. After five weeks of incubation on the surface of the colony, brown, crusty areas appeared near the margins of the plate. No primordia or fruiting bodies were developed *in vitro* by this species. The generative hyphae are tree – like branched, hyaline, with clamp connection, 2 – 3.5 µm in diameter and the skeletal hyphae are thick walled, rarely branched, 2.5 – 4 µm in diameter.

***Psathyrella candolleana* (Fr.) Maire.** On all three media the mycelium is cream colored or light brown, very dense, homogeneous, downy – fluffy. The edges of the colony are relatively regular and they cover the margins of the plate after one and a half week of incubation. No primordia or fruiting bodies were observed on the surface of the colony. The generative hyphae are branched, very curly, hyaline, with clamp connections, 1.5 – 3 µm in diameter and the skeletal hyphae are thin walled, rarely branched, 2.5 – 3 µm.

***Stereum hirsutum* (Willd.) Pers.** On MEA and PDA the colony is cream colored with light orange areas, heterogeneous, with downy areas where the hyphae tend to crowd, giving the colony a granular aspect, alternating with translucent areas where the submerged mycelium is seen. The edge of the colony is irregular. On the adapted media, the colony is cream colored with brown areas, relatively homogeneous, downy, with irregular edges which covered the margins of the plate after two weeks of incubation. After four weeks of incubation, the colony forms on the edges of the plate, near the upper part of the Petri dish primordia of fruiting bodies. The generative hyphae are rarely branched, hyaline, with clamp connection, 1.5 – 3 µm in diameter, while the skeletal hyphae are cream colored, thick walled and have 3 – 4.5 µm in diameter.

***Trametes hirsuta* (Wulfen) Lloyd.** On MEA and PDA, the mycelium is white, homogeneous, dense and downy. The edges of the colony are regular and the aerial hyphae tend to crowd forming in several areas small cottony clusters. Inoculated on the adapted media, the mycelium is on a radius of 2.5 cm around the inoculum point adpressed. Near the edges, the colony is downy with hyphae that tend to crowd forming cottony clusters. No primordia or fruiting bodies were developed *in vitro* by this species, unlike other authors reported [BALAEŞ & TĂNASE, 2012]. The generative hyphae are branched, hyaline, with clamp connections, 1.5 – 3.5 µm in diameter, while the skeletal hyphae are very rarely branched, with thin walls, 1.5 – 3 µm in diameter.

***Trametes ochracea* (Pers.) Gilb. & Ryvarden.** On all three media the mycelium is white to cream colored, relatively homogeneous and felty on PDA and MEA and downy on the adapted media. The edges of the colony are regular and they cover the margins of the plate after two weeks of incubation. On PDA and on the adapted media, after four weeks of incubation several primordia appeared on the surface of the colony but none reached the maturity. The generative hyphae are branched, hyaline, with clamp connections, 1.5 – 3 µm in diameter while the skeletal hyphae are unbranched, thick – walled and have 3 – 4.5 µm in diameter.

***Trametes pubescens* (Schumach.) Pilát.** On all three media, the mycelium is white to cream colored, homogeneous, downy and very dense with granular areas where the aerial hyphae tend to crowd, forming cottony clusters. The edges of the colony are regular and they cover the margins of the plate after two weeks of incubation. After four weeks of incubation on the surface of the colony several primordia appear but none reaches maturity. The generative hyphae are branched, curly, hyaline, with clamp connections, 3.5 – 5.5 µm in diameter and the skeletal hyphae are whitish, thin – walled, rarely branched and have 1.5 – 3 µm in diameter.

***Trametes trogii* Berk.** On MEA and PDA the mycelium is white to cream colored or yellowish and adpressed. On a radius of 2.5 cm around the inoculum point, the mycelium is homogeneous, felty to granular. After this area the mycelium becomes heterogeneous, with felty areas alternating with translucent ones with an obvious radial development of the hyphae. On the adapted media, the mycelium is white, homogeneous and downy. On all three media the edges of the colony are regular covering the margins of the plate after two weeks of incubation. No primordia or fruiting bodies were observed *in vitro* for this species. The generative hyphae are branched, hyaline with clamp connections, 2.5 – 3.5 µm in diameter and the skeletal hyphae are rarely branched, thick walled, 3.5 – 4.5 µm in diameter.

***Trametes versicolor* (L.) Lloyd.** Inoculated on the MEA and PDA, the mycelium is white, relatively homogeneous, adpressed, with granular areas near the inoculum point and translucent ones near the edges of the colony. The edges of the colony are irregular and easily cover the margins of the plate after two and a half weeks of incubation. Inoculated on the adapted media, the colony is white, with downy – felty areas alternating with translucent ones which form a circle that surrounds the inoculum point. In this case, the edges of the colony are regular and cover the margins of the plate after two weeks of incubation. After four weeks of incubation several primordia appear on the surface of the colony, but none reaches maturity. The generative hyphae are branched, hyaline, with clamp connections and 2 – 3.5 µm in diameter, while the skeletal hyphae are unbrached, hyaline and thin – walled, 2 – 3.5 µm in diameter.

Conclusions

A number of 12 species of lignicolous basidiomycetes belonging to 7 families and 3 orders were isolated in pure culture.

To determine the behavior of the colonies *in vitro* we tested three different synthetic media: two standard media and one adapted media. Concerning the growth rate and the fruiting bodies and primordia development the adapted media was proven to be the most efficient for all 12 species.

On the adapted media, the fastest growth rate was showed by *Psathyrella candolleana* and the slowest by *Polyporus squamosus*. On the same media, 6 species formed fruiting bodies starting from the fourth week of incubation: *Bjerkandera fumosa*, *Crepidotus applanatus*, *Stereum hirsutum*, *Trametes ochracea*, *Trametes pubescens*, *Trametes versicolo*r and only 2 species produced exudates: *Polyporus squamosus*, *Trametes ochracea*.

For all the 12 species of lignicolous basidiomycetes we were able to determine a characteristic odor, which indicates the presence of the volatile organic compounds synthesized by the *in vitro* colony.

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Tab. 1. Taxonomic distribution of the lignicolous basidiomycetes species isolated in pure cultures on families and orders

Order	**Family**	**Species**
Polyporales	Fomitopsidaceae	*Piptoporus betulinus*
	Meruliaeae	*Bjerkandera fumosa*
	Polyporaceae	*Polyporus squamosus*
		Trametes hirsuta
		Trametes ochracea
		Trametes pubescens
		Trametes trogii
		Trametes versicolor
Agaricales	Inocybaceae	*Crepidotus applanatus*
	Psathyrellaceae	*Psathyrella candolleana*
	Strophariaceae	*Hypholoma lateritium*
Russulales	Stereaceae	*Stereum hirsutum*

Tab. 2. Main features of the lignicolous basidiomycetes isolated in pure culture

Species	Growth rate*			Reverse**			Exudates	Odor
	PDA	MEA	A.M.	PDA	MEA	A.M.		
Bjerkandera fumosa	14	14	10	white	white	yellow	No exudates	Fruity, sweet
Crepidotus applanatus	14	14	12	white	white	white	No exudates	Woody
Hypholoma lateritium	14	16	12	cream	cream	light brown	No exudates	Moist soil
Piptoporus betulinus	16	16	14	white	white	white	No exudates	Easily astringent
Polyporus squamosus	22	24	18	white	white	cream	Light and dark brown	Moist soil with a special fragrance
Psathyrella candolleana	10	10	7	cream	cream	light brown	No exudates	Moist soil
Stereum hirsutum	14	16	12	light brown	brown	light brown	No exudates	Rotten – wood
Trametes hirsuta	12	14	10	white	white	yellowish	No exudates	Moist wood, faint odor
Trametes ochracea	16	14	10	cream	cream	cream	Cream colored	Rotten wood
Trametes pubescens	14	14	10	white	white	white	No exudates	Moist wood, faint odor
Trametes trogii	18	18	12	yellowish	yellowish	yellowish	No exudates	Rotten wood
Trametes versicolor	16	16	12	white	white	white	No exudates	Moist wood, faint odor

* The growth rate shows the number of days in which the colony covered the whole plate

** We considered only the reverse of the mature colony. The reverse of the young colony remained unchanged.

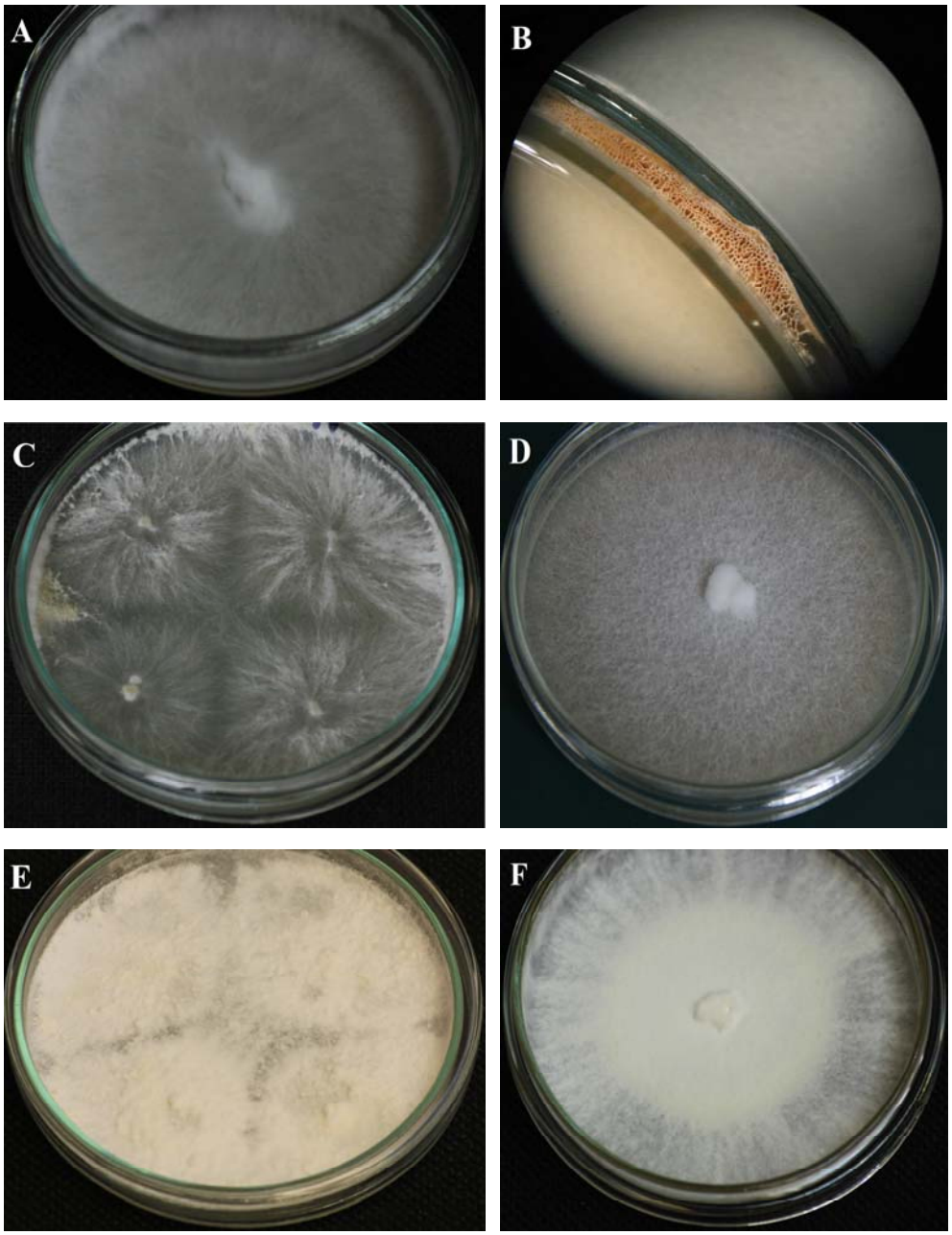

Fig. 1. General aspect of lignicolous basidiomycetes *in vitro* cultures on adapted media in 9 mm Petri dish: A – *Bjerkandera fumosa*; B – Fruiting body of *Bjerkandera fumosa*; C – *Crepidotus applanatus*; D – *Piptoporus betulinus*; E – *Trametes ochracea*; F – *Trametes trogii*.

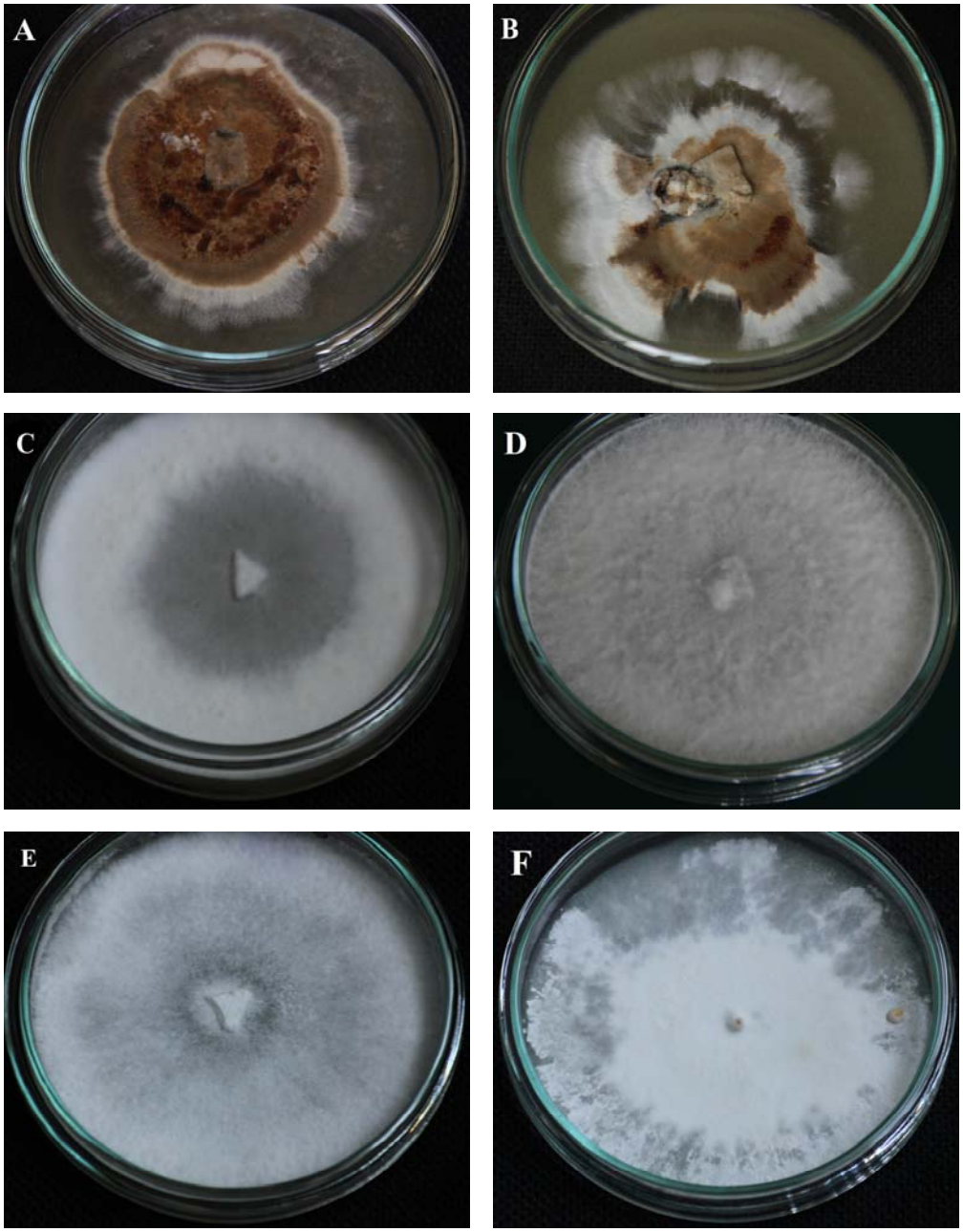

Fig. 2. General aspect of lignicolous basidiomycetes *in vitro* cultures grown on different media in 9 mm Petri dishes: A – *Polyporus squamosus* (on adapted media); B – *Polyporus squamosus* (on PDA); C – *Trametes hirsuta* (on adapted media); D – *Trametes hirsuta* (on PDA); E – *Trametes versicolor* (on adapted media); F – *Trametes versicolor* (on PDA).

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20(2013): 115 – 120

IN SITU AND *EX SITU* CONSERVATION OF RARE AND ENDANGERED GEOPHYTES OF THE HIRKAN NATIONAL PARK (AZERBAIJAN)

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Abstract: The Hirkan National Park consists of natural region of Talish Mountains characterized with their unique natural complex. This research was carried out from 2004 to 2007 in order to study the floristic and taxonomical composition of geophytes, elaborate optimal measures of biosafety and their sustainable use. According to floristic composition of the National Park it is a valuable forest which includes 150 endemic species of trees and bushes out of 435 species of trees and bushes. As a result of researches for the first time were found that more than 15 geophyte species are endemic plants of Caucasus or Azerbaijan. Some geophyte species are *Allium lenkoranicum* Miscz. ex Grossh., *A. talyschense* Miscz. ex Grossh., *Bellevalia fominii* Woronow, *Ornithogalum hyrcanum* Grossh., *Fritillaria grandiflora* Grossh., *Crocus caspius* Fisch. & C. A. Mey., *Iris helena* (C. Koch) C. Koch, *Himantoglossum formosum* (Stev.) C. Koch, *Ophrys oestrifera* M. Bieb., etc. among many others. Isolation of a geographical position of Talish, which vegetation differ a variety of life forms, allows considering geophytes as a group of independent bioecological value. 92 species of geophytes identified and registered in the Hirkan National Park is grouped into 21 families and 46 genera, including 33 rare and endangered species, of which 11 species are included into the "Red Data Book" of Azerbaijan.

Keywords: Hirkan National Park, plant conservation, rare species, geophytes, tuber, rhizome, Central Botanical Garden.

Introduction

The richness of Talish flora is a leader not only in Azerbaijan, as well as botanical and geographical regions of the Caucasus. It is located in the extreme South-eastern part of the country. In the West, Talish Mountains is bordered by Iran Republic, and in the East with the Caspian Sea. Flora of the region and its genetic fund has incorporated the remnants of the flora of various geological eras, especially the Tertiary period and emerged as a result of long historical development. Isolation of a geographical position of Talish, which vegetation differs in a variety of life forms, allows considering geophytes as a group of independent bioecological value. So, this study was carried out to determine the geophytic flora of Talish region in Azerbaijan, and to observe the conditions of the endemic and/or rare geophyte populations.

During the last decade man's impact on natural ecosystems started rapidly to grow and seriously to threat natural equilibrium in ecosystems. When habitats of a rare and/or endemic species are damaged and/or fragmented by mismanagement and various other human activities (intensive urbanization, over exploitation of natural resources,

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development of tourism), distribution ranges, population sizes, and genetic variability of the species will be reduced and its members will become vulnerable to extinction at a faster rate than others. Due to that, special attention should be paid on investigation of threatened taxa.

It was necessary to count the best way of storage of genetic resources of plants preservation *in situ.* Example of such activity is the organization of natural reserve Hirkan in Azerbaijan. The Hirkan National Park was established in 2004 on the basis of the Hirkan State Reserve which it superseded, on a surface area of 29,760 hectares (297.6 km^2), it was enlarged in 2008 to 42,797 hectares (427.97 km^2) (Fig. 1). The main purpose of establishment of the National Park is complex preservation of nature of this area, protection of relict and endemic plants of Tertiary period and characteristic flora and fauna types, which were not affected by Pliocene and Pleistocene glaciations and included into the *Red Book* of Azerbaijan Republic (1989), monitoring of environment, awareness of the public and also creation of favorable conditions for researches, tourism and recreation.

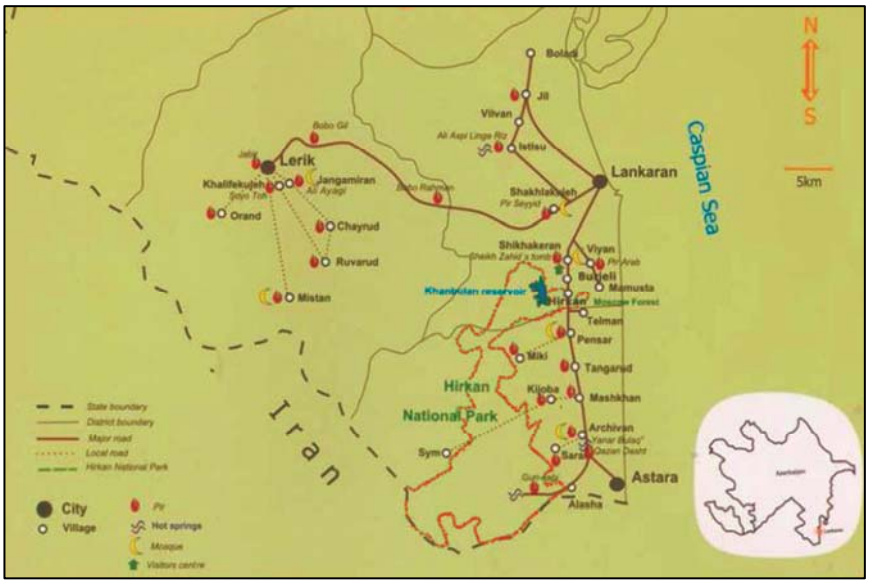

Fig. 1. The Talish region (Azerbaijan) map
The Hirkan National Park borders - indicated in red color

The ecosystem of the Hirkan National Park belongs to the Caspian Hyrcanian (Hirkan) mixed forests ecoregion, an area of lush deciduous broadleaved lowland and mountain forests (subtropical and temperate rainforests) that completely cover the Talish Mountains and partially cover the Lenkoran Lowland. One of the main characteristics of subtropical forests of Lenkoran zone where Hirkan National Park is located is well preservation of Hirkan type forests and wide spread of many endemic, rare trees, bushes and herbs here [HACIYEV & al. 1979]. Botanical expeditions during last 50 years and numerous herbariums collected by world botanists over 19-20 century, written by scientists cited in the list of literature especially “Flora of Azerbaijan” in 8 volumes [KARYAGIN,

1950-1961] and “Conspectus of Caucasian geophytes” [IBADLI, 2005] published in collaboration with assistants gave us the general view of region vegetation (Tab. 1) [YUSIFOV & HACIYEV, 2004; SAFAROV, 2010].

Tab. 1. The plant composition of Hirkan National Park (Azerbaijan)

Plant groups	Family	Genus	Species
Bryophyta and Pteridophyta	15	19	25
Pinophyta (Gymnospermae)	4	8	10
Magnoliphyta (Angiospermae)	113	536	1169
including Magnoliatae (dicots)	89	429	951
Liliatae (monocots)	24	107	218
Geophytes	21	46	92

Materials and methods

Our field investigation began in 2004. With the fulfillment of the work in connection with a theme, HACIYEV & al. (1979) and IBADLI (2004) researches were used. Nomenclature of taxa is according to KARYAGIN & al. (1950-1961), IBADLI (2004), IBADLI (2005) and CZEREPANOV (1995). The identification was also checked in herbaria of the Botanical Institute of Baku (BAK). The specimens were deposited in the Botanical Institute, Herbarium Fund. The list of taxa was arranged according to “The Flora of Azerbaijan” [KARYAGIN, 1950-1961] and IBADLI (2004, 2005), with the species name, locality, habitat, properties, and altitude identified.

On the basis of this information and the corresponding literature [IBADLI, 2005; KARYAGIN, 1950-1961], received as a result of the research, geophytes plants, on the basis of types of underground storage organs were grouped as: bulbous, tubers, roots, rhizomes and corms. The endemic species were determinate according to AHUNDOV (1973) and MUSAYEV (2005). For each species, a certain category is applied to, being accompanied by their threaten degree due to IUCN Red List Categories [Red Data Book, 1989].

The following category abbreviations are used in the text: BAK (Herbarium Fund of Botanical Institute of Azerbaijan National Academy of Sciences, Baku, Azerbaijan), IUCN (International Union for Conservation of Nature – Red List Categories and Criteria), CR (critically endangered), EN (endangered), VU (vulnerable), CBG (Central Botanical Garden).

Results and discussion

The study area covers the protected area called Hirkan. Herbarium specimen and also sowing materials those relating to the geophyte species of the different family, which were identified in the Hirkan National Park, were collected basically in the spring and the autumn during the period of 2004-2007, photographs were taken and areas of distribution are specified [FARZALIYEV & al. 2007; SALIMOV, 2008].

As a result of the field studies, 92 species of geophytes identified by us and registered in the Hirkan National Park is grouped into 21 families and 46 genera [SALIMOV, 2008], including 33 rare and endangered species, of which 11 species are entered in the “Red Data Book” of Azerbaijan (Fig. 2A, B & 3A, B) [Red Data Book,

1989]. The distribution of species according to families in the study area was categorized and listed. The families which include the largest number of species are as follows: Orchidaceae (26 spp.), Hyacinthaceae (11 spp.), Alliaceae (9 spp.), Iridaceae (8 spp.) Asparagaceae (5 spp.). Families which possess less than 5 species constitute 64.13% from the floristic fund of the Hirkan National Park [SALIMOV, 2008].

Fig. 2. Exposition of *Galanthus caspius* (Rupr.) Grossh. *in situ* (A) and in the CBG (B)

Fig. 3. Exposition of *Limodorum abortivum* (L.) Sw. *in situ* (A) and in the CBG (B)

In the research area more than 15 geophyte species are endemic plants of Caucasus or Azerbaijan. Some geophyte species are: *Allium lenkoranicum* Miscz. ex Grossh., *Allium talyschense* Miscz. ex Grossh., *Bellevalia fominii* Woronow, *Ornithogalum hyrcanum* Grossh., *Fritillaria grandiflora* Grossh., *Crocus caspius* Fisch. & C. A. Mey., *Iris helena*

(C. Koch) C. Koch, *Himantoglossum formosum* (Stev.) C. Koch, *Ophrys oestrifera* M. Bieb., etc. among many others.

In flora of the Hirkan National Park 19 rare geophyte species, representing 3.3% of floristic fund of named area, the following classification according IUCN categories was relieved [IUCN, 2003]:

– Critically endangered (CR): 3 species: *Ornithogalum hyrcanum* Grossh., *Fritillaria grandiflora* Grossh., *Cephalanthera longifolia* (L.) Fritsch.

– Endangered (EN): 5 species: *Crocus caspius* Fisch. & C. A. Mey., *Lilium ledebourii* (Baker) Boiss., *Cephalanthera rubra* (L.) Rich., *Himantoglossum formosum* (Stev.) C. Koch, *Limodorum abortivum* (L.) Sw.

– Vulnerable (VU): 11 species: *Bellevalia fominii* Woronow, *Allium paradoxum* (M. Bieb.) G. Don fil., *Galanthus caspius* (Rupr.) Grossh., *Sternbergia fischeriana* (Herb.) M. Roem., *Puschkinia scilloides* Adams, *Scilla caucasica* Miscz., *Iris helena* (C. Koch) C. Koch, *I. pseudacorus* L., *Crocus speciosus* M. Bieb., *Anacamptis pyramidalis* (L.) Rich., *Cyclamen elegans* Boiss. & Bushe.

As noted above, *in situ* conservation refers to conservation of biodiversity in populations growing in their place of origin. However, the organization of reserves is connected with significant, frequently insuperable difficulties in the present period. Besides even in working reserves are subject to extreme influences of natural factors and anthropogenic impacts therefore it is not excluded separate forms. All of theses cause necessity of preservation plants, including endemic and relict, rare and endangered geophyte species of Talish region registered in the Hirkan National Park *ex situ* – in collections. On the other hand, multilateral research activity frequently demands presence of a material easily accessible and in enough quantities that also can be provided only with its preservation in living collections. *Ex situ* conservation is the method predominately used in agriculture. Arboreta and botanical gardens are also *ex situ* collections, but generally have only few individuals to be useful for conserving rare and/or endemic plants.

One of the main tendencies of Central Botanical Garden of Azerbaijan are based on the selection of prospective species of plants, their introduction and study, aimed at gardening Baku City. It is also a way of conservation rare and endangered species, studying introduction and climate adaptation of decorative, medicinal, ether-oiled and other plants in order to enrich raw-material bases of plant resources. Among these groups geophytes play an important role [FARZALIYEV & al. 2006; IBADLI & al. 2006]. However, due to research data, geophytes consist of 4.25% of the flora of Azerbaijan. So, Talish floristic exposition makes a great importance in CBG [FARZALIYEV & al. 2007]. About 30 endemic and relict plants, especially trees, bushes and geophytes species have been planted here (Fig. 4).

Fig. 4. Exposition of tulips in the CBG

Culture conditions, as a rule, positively operate on the general habitus of plants, accelerating the ontogenesis. Thus, a comprehensive study of morphological and ecological and biological characteristics, economic-important signs, the development of methods and techniques of reproduction of protected geophytes in a culture will help address the issue of rational use and conservation of rare and endangered species as *in situ* and *ex situ* collections.

Conclusions

- Special and detailed floristic studies related with geophytes were never conducted in the Lenkoran-Lerik regions.
- Our researches were carried out within 2004-2007 years, as a result of the field studies.
- For the first time, 92 geophytes species were identified and registered in the Hirkan National Park and grouped into 21 families and 46 genera.
- 33 rare and endangered species, of which 11 geophyte species are entered in the "Red Data Book" of Azerbaijan.
- In the surveyed area, more than 15 geophyte species are endemics to Caucasus or Azerbaijan floras.
- On the basis of our researches, actions for protection and restoration were determined, and practical recommendations are offered.

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THE DWARF SHRUBS COMMUNITIES WITHIN *LOISELEURIO-VACCINIETEA* Eggler ex Schubert 1960 FROM ROMANIAN EASTERN CARPATHIANS

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MÂNZU Ciprian[2], BÎRSAN Ciprian[1]

Abstract: A numerical analysis of dwarf shrubs communities within *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960 class, based on 181 relevés assigned to *Loiseleurio-Vaccinion*, *Rhododendro-Vaccinion* and a part of *Pinion mugo* is presented in this paper. An agglomerative hierarchical clustering (Flexible beta algorithm with Bray-Curtis dissimilarity) was performed. Six vegetal associations were distinguished and characterized: *Cetrario islandicae-Loiseleurietum procumbentis*, *Empetro-Vaccinietum gaultherioidis*, *Rhododendro myrtifolii-Vaccinietum gaultherioidis*, *Campanulo abietinae-Juniperetum nanae*, *Campanulo abietinae-Vaccinietum myrtilli* and *Bruckenthalio-Vaccinietum myrtilli*. Detrended correspondence analysis complementarily confirmed the hierarchical clustering. Main environmental factors influencing the floristic composition of clusters were analyzed by canonical correspondence analysis using altitude and Ellenberg indicator values of plants species as variables. Canonical correspondence analysis confirmed that altitude, light and temperature are the main factors influencing the floristic composition of the vegetal communities from *Loiseleurio-Vaccinietea*.

Key words: vegetation, *Loiseleurio-Vaccinietea*, diagnostic species, numerical classification.

Introduction

Loiseleurio-Vaccinietea Eggler ex Schubert 1960 class groups together alpine and subalpine dwarf shrubs communities from boreal and arctic regions [MUCINA, 1997] edified by *Ericaceae* species (e.g. *Loiseleuria*, *Vaccinium* spp., *Rhododendron* spp., *Bruckenthalia* spp. etc.). This vegetation class include one phytosociological order, *Rhododendro-Vaccinietalia* Br.-Bl. in Br.-Bl. et Jenny 1926 (arctic-boreal and (sub-)alpine ericoid dwarf shrub heathlands), with two alliances (in the Romanian Carpathians): *Loiseleurio-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926 (cryophilous dwarf-shrub heathlands on wind-swept slopes and edges) and *Rhododendro-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926 (subalpine chionophilous wind-swept dwarf shrub heathlands) [CHIFU & al. 2006].

The syntaxonomic affiliation of the alliances of *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960 was questionable from the beginning. Both *Loiseleurio-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926 and *Rhododendro-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926 alliances were first classified, based on floristic and physiognomic criteria, in the *Rhododendro-Vaccinietalia* Br.-Bl. in Br.-Bl. et Jenny 1926 order within *Vaccinio-Piceetea* Br.-Bl. in Br.-Bl. et al. 1939 class. Later, on exclusively floristic considerents, Braun-Blanquet included *Loiseleurio-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926 alliance in the

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Piceetalia excelsae Pawłowski in Pawłowski et al. 1928 order [BRAUN-BLANQUET, 1964]. In an other classification system (of Krajina, 1933) [BOŞCAIU, 1971], the *Loiseleurio-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926 alliance is included in *Caricetalia curvulae* Br.-Bl. in Br.-Bl. et Jenny 1926 within *Juncetea trifidi* Hadač 1946 class, while the *Rhododendro-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926 is classified under *Vaccinio-Piceetea* Br.-Bl. in Br.-Bl. et al. 1939 class [BOŞCAIU, 1971]. Eggler unite all the communities edified by nanophanerophytes from the subalpine and alpine regions in one class, *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960, including one order (*Empetretalia hermaphroditae* Schubert 1960) and three alliances (*Cetrario-Loiseleurion* Br.-Bl. et Siss. 1939, *Rhododendro-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926, and *Juniperion nanae* Br.-Bl. et Siss. 1939) [BOŞCAIU, 1971]. Other studies using physiognomic, quantitative and qualitative parameters support also a delimitation among vegetal communities of *Juncetea trifidi* Hadač 1946 and *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960 [DÚBRAVCOVÁ & al. 2005]. Classification of the alpine and subalpine dwarf shrubs communities in one vegetation class is also retained in newer books and papers [GRABHERR, 1993; RIVAS-MARTINEZ & al. 1999; RODWELL & al. 2002; ŠIBÍK & al. 2006; KLIMENT & al. 2010].

Another problem which generated different opinions is the classification of the secondary pure communities edified by *Vaccinium myrtillus* in *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960. Initially, *Vaccinietum myrtilli* Szafer 1923 has been classified in *Calamagrostion villosae* Pawłowski in Pawłowski et al. 1928 (within *Juncetea trifidi* Hadač 1946) and later in *Vaccinion myrtilli* Krajina 1933 alongside dwarf pine communities and spruce forests (within *Vaccinio-Piceetea* Br.-Bl. in Br.-Bl. et al. 1939 class). An interesting approach [ŠIBÍK & al. 2006] is that to restrict the *Vaccinion myrtilli* Krajina 1933 alliance only to the acid mesophilous dwarf-shrub communities of the subalpine belt (of Western Carpahians and High Sudeten) and include it in *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960 class. Another perspective was to unite the phytocoenoses edified by *Vaccinium myrtillus* with those of *Bruckenthalia spiculifolia* and *Juniperus sibirica* from the subalpine areas (of Romanian Carpathians) in a particular alliance, namely *Junipero-Bruckenthalion* (Horvat 1949) Boşcaiu 1971, within *Junipero-Pinetalia mugi* Boşcaiu 1971 and *Vaccinio-Piceetea* Br.-Bl. in Br.-Bl. et al. 1939. In this context, the *Junipero-Bruckenthalion* alliance represents a vicariant syntaxon of the *Juniperion nanae* Br.-Bl. et Siss. 1939 from Alps and Pyrrenees [BOŞCAIU, 1971], which is integrated in the class of *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960 [GRABHERR, 1993; RODWELL & al. 2002]. Taking into consideration all these aspects, we also included in the analisys the communities of *Juniperus sibirica* and *Vaccinium myrtillus* from the (sub-) alpine belt of vegetation in the Romanian Eastern Carpathians.

Vegetal communities from *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960 have been well documented for Romanian Carpathians [BORZA, 1934; BELDIE, 1967; VICOL & al. 1967; RESMERIȚĂ, 1979; BOŞCAIU, 1971; MITITELU & al. 1986; COLDEA, 1990; CHIFU & al. 2006]. Also, in romanian phytosociological literature there are some synthesis papers in which different opinions on classification of (sub-) alpine dwarf-shrubs exists [COLDEA, 1991; SANDA & al. 1997; CHIFU & al. 2006; SANDA & al. 2008], each one with its particularities. Based on the altitudinal location and the floristic composition, rich in microtherm elements characteristic to the order *Caricetalia curvulae* Br.-Bl. in Br.-Bl. et Jenny 1926, Coldea (1991) include the *Loiseleurio-Vaccinon* Br.-Bl. in Br.-Bl. et Jenny 1926 alliance under the *Juncetea trifidi* Hadač 1946 vegetation class. Also,

Rhododendro-Vaccinion Br.-Bl. in Br.-Bl. et Jenny 1926 alliance representing dwarf shrubs from subalpine belt vegetation (edified by *Vaccinium myrtillus* and *Rhododendron myrtifolium*) has a suballiance status (*Rhododendro-Vaccinenion* Br.-Bl. 1926 em. Oberd. 1957) included under the *Pinion mugi* Pawłowski 1928 alliance and *Junipero-Pinetalia mugo* Boşcaiu 1971, from *Vaccinio-Piceetea* Br.-Bl. in Br.-Bl. et al. 1939 class. A similar opinion on the classification of *Loiseleurio-Vaccinon* Br.-Bl. in Br.-Bl. et Jenny 1926 alliance presents SANDA & al. 2008. Referring to *Rhododendro-Vaccinion* Br.-Bl. in Br.-Bl. et Jenny 1926 alliance, SANDA & al. (2008) include it under the *Athyrio-Piceetalia* Hadač 1962 order from *Vaccinio-Piceetea* class. CHIFU & al. (2006) choose to classify all the dwarf-shrub heathlands communities from subalpine and alpine belts in one class, namely *Loiseleurio-Vaccinietea* Eggler ex Schubert 1960.

The article's main task is to present a numerical classification (hierarchical, agglomerative), an ordination of these vegetal communities, to see if it is consistent with classifications realized in the spirit of Central European School made and published in time by numerous prestigious Romanian phytosociologists. Another objective is to detect the diagnostic species for the distinguished vegetation units and the main ecological factors with a significant influence on their floristic composition.

Material and method

Study area

Eastern Carpathians (Romania) represents the largest geographic unit of the all South-Eastern Carpathians (33,584 km²) and are situated between the northern border of the country and the Prahova valley (Fig. 1). They are relatively "young" mountains, presenting parallel ridges, fragmented by valleys and depressions. Average altitude varies around 1,025 m (the highest altitude – 2,303 m – the summit of Pietrosul Rodnei). Eastern Carpathians includes three geomorphological units, as: the *crystalline* (including Maramureşului, Rodnei, Suhardului and Bistriţei Mountains), the *flysch* (including Stânişoarei, Ceahlău, Tarcăului, Nemirei and Vrancei Mountains) and the *neo-volcanic* unit (including Gutâi, Ţibleş, Călimani-Harghita Mountains). The climate is temperate-continental moderate, characterized by average annual temperatures of 0-2 ºC and average annual precipitations of >1200 mm/m^2 in the highest areas, to 4-6 ºC and 700-800 mm/m^2 in the intra-mountain depressions [BADEA & al. 1987].

The main zonal vegetation units include the boreal forests (coniferous forests extending up to 1700-1750 m), the subalpine belt (of *Pinus mugo* communities, extending up to 1900-2000 m) and alpine belt (alpine dwarf shrubs and meadows, up to 2300 m) [CHIFU & al. 2006]. From a floristic perspective, the Eastern Carpathians are integrated in the Central European floristic region and Carpathian province, including six floristic districts [CIOCÂRLAN, 2000]. According to the Habitats Directive (1992) Eastern Carpathians belong to alpine biogeographic region.

Fig. 1. Geographical position of Eastern Carpathians reported to Romanian territory

Vegetation data

For vegetation analysis, an initial set of 256 relevés (including 242 species) was used. The selection process was based on their assignment to *Loiseleurio-Vaccinon*, *Rhododendro-Vaccinion* and a part of *Pinion mugo* by the original authors. There are more relevés in the phytosociological literature, but they are included in synoptic tables and consequently unusable for this analysis. All relevés were made using the standard method elaborated by the Central European phytosociologic school [BRAUN-BLANQUET, 1964], adapted for Romanian vegetation [BORZA & BOȘCAIU, 1965]. Provenience of relevés is presented in App. 1. Relevés have 2-200 m^2 in size. From the initial dataset, only relevés realized at altitudes exceeding 1400 m and relevés in which the sum of the covering percentages of the dwarf shrubs species was at least 50% were retained. Duplicates or highly-similar relevés (values 0.9-1) were identified using Sorensen similarity index (on presence-absence data) and one member of the relevés pair was randomly removed. Rare species (occurring in less than 5 relevés) were also removed. In these conditions, the final dataset, which was analyzed, included only 181 relevés (with 91 species).

Juvenile trees, bryophytes and lichens were included in the analysis, although they have not been recorded in all relevés. Nomenclature of plants species follows CIOCÂRLAN (2000), of bryophytes follows HILL & al. (2006) and of lichens follows *Index Fungorum*. The term of "diagnostic species" is used only in the context of the studied area (Eastern Carpathians, Romania).

Data analysis

Hierarchical agglomerative clustering has been realized using the GINKGO program from the VEGANA software package [de CÁCERES, 2003; BOUXIN, 2005]. For hierarchical clustering, the mid-percentages values corresponding to the 6 degrees Braun-Blanquet scale were used. These values were square-root transformed and used to create a dissimilarity matrix using the Bray-Curtis index. The flexible beta algorithm (ß = -0.25) was used in order to realize the hierarchical clustering. Afterthat, 19 partitions with 2-20 clusters were computed by pruning the output dendrogram at different hierarchical levels. Optimal number of clusters was determined using the *corrected Rand index* and the *average mean Silhouette index*, both implemented in GINGKO [de CÁCERES, 2003]. Determination of the diagnostic species was realized a posteriori, using the *indicator value* (**IndVal**) coefficient [DUFRÊNE & LEGENDRE, 1997] which is independent of the relative size of the target vegetation unit. Square-rooted values of the **IndVal** were the subject of a permutation test (999 iterations) in order to observe which are the species significantly associated with the clusters [DE CÁCERES & LEGENDRE, 2009]. The results are presented in a table in which the diagnostic species are ranked by decreasing permutation test values alongside its *P*-values ≤ 0.01.

Detrended Correspondence Analysis (DCA) was performed for two reasons: first, in order to validate the hierarchical agglomerative classification (exclusively from vegetation data) and second, as an indirect gradient analysis in order to relate the relevés ordination to environmental data. The square root transformation, detrending by segments and non weighted average values of the Ellenberg indices -EIVs- (as environmental variables, passive projected) for light (L), temperature (T), continentality (C), soil moisture (H), soil pH (R) and nutrients (N), alongside altitude were used [ELLENBERG & al. 1992]. Also, *Canonical Correspondence Analysis* (CCA) was realized in order to observe the effect of each variable (altitude and Ellenberg's indicator values) on the species

composition of each vegetal community within *Loiseleurio-Vaccinietea*. DCAs and CCAs were realized in CANOCO 4.5 [TER BRAAK & ŠMILAUER, 2002].

The box-plots with Ellenberg's indicator values made in order to compare the clusters' ecological features were realized in PAST software [HAMMER & al. 2001]. Environmental differences among the six associations were highlighted using the Kruskal-Wallis non-parametric test and the Mann-Whitney post-hoc test (Bonferroni corrected). Both tests were also carried out in PAST software [HAMMER & al. 2001].

Results and discussions

Univariate analysis of available data referring to *Loiseleurio-Vaccinietea* (Tab. 1) vegetation class as a whole reveals the fact that, in the studied area, these vegetal communities includes species growing in well lighted places, but also occurring in partial shaded places, still preferring alpine and subalpine climate conditions. Most of the specis from the floristic composition occurr mainly in Central Europe and preffer mainly acid and nitrogen deficient soils, with average humidity.

Tab. 1. Univariate analysis of relevés from *Loiseleurio-Vaccinietea* vegetation class

	L	T	C	H	R	N	Altitude
N	181	181	181	181	181	181	181
Min.	4.6	1.8	2.3	4.0	2.0	1.3	1450
Max.	8.3	4.3	4.5	6.2	4.0	4.5	2150
Mean	6.6812	2.6657	3.5662	5.0513	2.8519	2.3000	1805.448
Std. error	0.0544	0.0363	0.0251	0.0250	0.0258	0.0442	11.1811
Variance	0.5369	0.2394	0.1144	0.1135	0.1213	0.3550	22628.42
Stand. dev.	0.7327	0.4893	0.3383	0.3369	0.3494	0.5958	150.4274
Median	6.7	2.6	3.5	5.0	2.8	2.2	1800

Hierarchical agglomerative clustering (Fig. 2): Bray-Curtis dissimilarity and the Flexible beta algorithm generated an ultrametric matrix, from which 19 partitions with 2-20 clusters were extracted. In order to identify the partition with an optimum number of clusters (App. 2) the *corrected Rand index* which compares the probability that 2 randomly chosen clusters to be treated in the same manner in 2 different partitions was used. The matrix of Rand indices showed three local maxima (App. 2a): (0.955) between 5 and 6

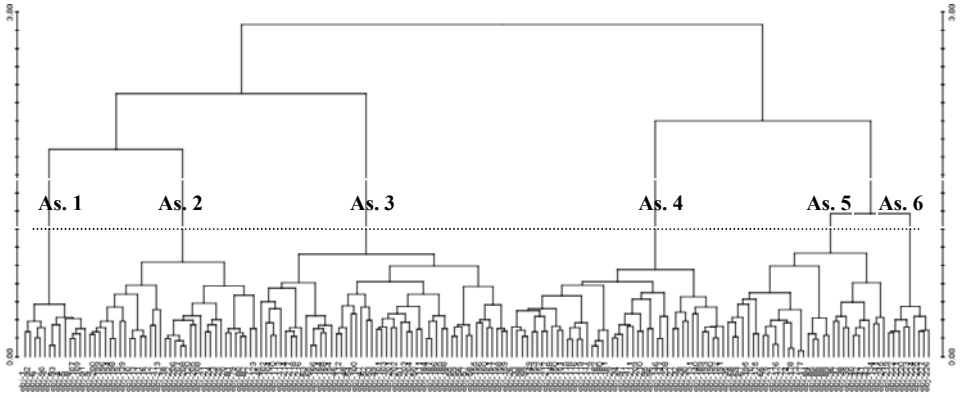

Fig. 2. Dendrogram of the numerical classification (Flexible beta + Bray-Curtis) of the dwarf shrubs communities within *Loiseleurio-Vaccinietea* class in Eastern Carpathians

clusters, (0.979) between 9 and 10 clusters very close to its maximum value (which is 1), indicating that the data clusters are exactly the same. Corroborated with *silhouette statistic* (App. 2b) showing local maxima for the partition with 6 clusters (0.291) and partition with 10 clusters (0.221), the 6 clusters partition was further analyzed at association level (Fig. 2).

Moreover, DCA, complementary used in order to confirm the hierarchical clustering, shows clearly a separation of the six plant communities (Fig. 3). In this case, the relevés are ordonated along the first two axes depending on their floristic similarity. Thus, as more closed are two relevés in the ordination space, the differences regarding their floristic composition are less important. The axes have no ecological meaning (in this case) and represent gradients of floristic similarity.

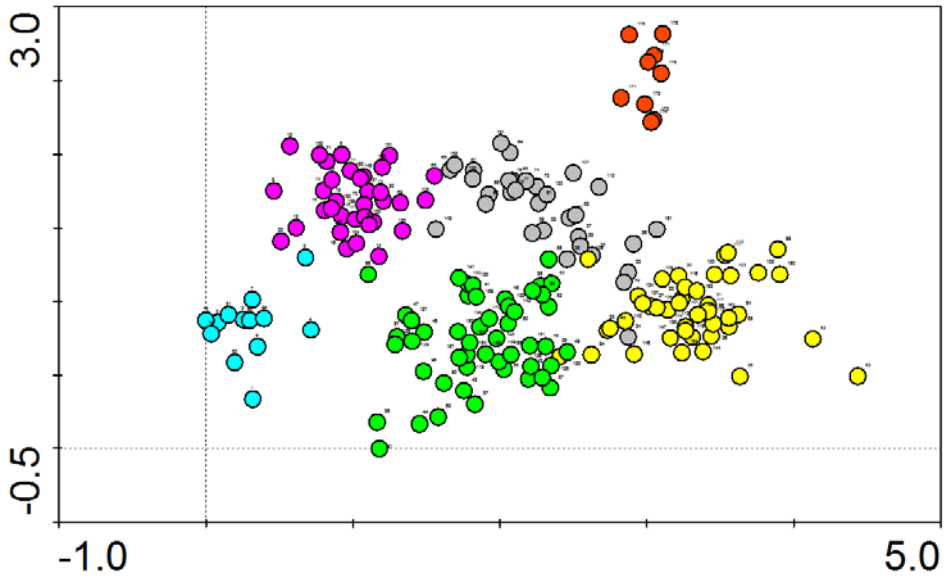

Fig. 3. DCA ordination diagram of the 181 relevés (with clusters generated by hierarchical classification colored as follows: *Cetrario-Loiseleurietum procumbentis*-blue circles, *Empetro-Vaccinietum gaultherioidis*-violet circles, *Rhododendro-Vaccinietum gaultherioidis*-green circles, *Campanulo abietinae-Juniperetum*-yellow circles, *Campanulo abietinae-Vaccinietum*-grey circles, *Bruckenthalio-Vaccinietum*-red circles); first two axes presented. Eigenvalues: 1st axis: 0.535, 2nd axis: 0.242, total inertia: 4.705.

Based on diagnostic species analysis (Tab. 2) we related the groups (clusters) generated by hierarchical clustering to vegetal associations described in literature, which were integrated in the next syntaxa conspectus within *Loiseleurio-Vaccinietea* class, in Eastern Carpathians (Romania):

LOISELEURIO-VACCINIETEA Eggler ex Schubert 1960
RHODODENDRO-VACCINIETALIA Br.-Bl. in Br.-Bl. et Jenny 1926
Loiseleurio-Vaccinion Br.-Bl. in Br.-Bl. et Jenny 1926
Cetrario-Loiseleurietum procumbentis Br.-Bl. 1926
Rhododendro-Vaccinion Br.-Bl. in Br.-Bl. et Jenny 1926
Empetro-Vaccinietum gaultherioidis Br.-Bl. 1926

Rhododendro-Vaccinietum gaultherioidis Borza 1959 em. Boşcaiu 1971
Junipero-Bruckenthalion (Horvat 1949) Boşcaiu 1971
Campanulo abietinae-Juniperetum nanae Simon 1966
Campanulo abietinae-Vaccinietum myrtilli (Buia et al. 1962) Boşcaiu 1971
Bruckenthalio-Vaccinietum Coldea et al. 2008

Tab. 2. Species significantly associated to the groups resulted from hierarchical clustering (with values of permutation test and P values ≤ 0.01)

Species name	Stat.	P-value
Group 1 Cetrario-Loiseleurietum procumbentis Br.-Bl. 1926		
Loiseleuria procumbens	0.996	0.001
Campanula alpina	0.539	0.003
Carex curvula	0.517	0.001
Group 2 Empetro-Vaccinietum gaultherioidis Br.-Bl. 1926		
Vaccinium gaultherioides	0.782	0.001
Empetrum nigrum subsp. *hermaphroditum*	0.645	0.001
Cetraria islandica	0.539	0.005
Group 3 Rhododendro-Vaccinietum gaultherioidis Borza 1959 em. Boşcaiu 1971		
Rhododendron myrtifolium	0.850	0.001
Ligusticum mutellina	0.646	0.001
Gentiana punctata	0.400	0.004
Group 4 Campanulo abietinae-Juniperetum nanae Simon 1966		
Juniperus sibirica	0.917	0.001
Picea abies	0.686	0.001
Campanula abietina	0.655	0.001
Calamagrostis villosa	0.518	0.005
Achillea distans	0.462	0.004
Oxalis acetosella	0.403	0.008
Poa chaixii	0.403	0.003
Rubus idaeus	0.403	0.01
Senecio ovatus	0.403	0.009
Group 5 Capanulo abietinae-Vaccinietum myrtilli (Buia et al. 1962) Boşcaiu 1971		
Cetraria cucullata	0.685	0.001
Hieracium alpinum	0.637	0.001
Deschampsia flexuosa	0.571	0.002
Hypericum richeri subsp. *grisebachii*	0.523	0.003
Melampyrum saxosum	0.516	0.009
Group 6 Bruckenthalio-Vaccinietum Coldea et al. 2008		
Bruckenthalia spiculifolia	0.876	0.001
Ranunculus montanus subsp. *pseudomontanus*	0.837	0.881
Hylocomium splendens	0.828	0.001
Vaccinium vitis-idaea	0.796	0.001
Nardus stricta	0.744	0.001
Pleurozium screberi	0.741	0.001
Polytrichum commune	0.668	0.001
Potentilla erecta	0.645	0.001
Potentilla ternata	0.612	0.001
Festuca supina	0.520	0.007

Group 1: ***Cetrario-Loiseleurietum procumbentis*** Br.-Bl. 1926

Diagnostic species: *Loiseleuria procumbens*, *Campanula alpina*, *Carex curvula*.

Vegetal communities identified only in Rodnei Mountains [COLDEA & al. 1981; COLDEA & PÎNZARU, 1986; COLDEA, 1990], at high altitudes, ranging between 1930 and 2150 m (mean 2027 m), on plane terrains or with low slopes (mean slope 6º) and

various aspects, exposed to cold winds. *Loiseleuria procumbens* is the dominant species (sometimes alongside *Vaccinium gaultherioides* and *Rhododendron myrtifolium*, with increased abundances), and rare specimens of *Juniperus sibirica* or *Pinus mugo* in the shrubs layer. In the herbs layer higher frequencies present *Juncus trifidus*, *Campanula alpina*, *Avenula versicolor*, etc. There are diagnostic species to the *Loiseleurio-Vaccinion* alliance (e.g. *Cetraria islandica*, *Thamnolia vermicularis*, *Primula minima* etc.) and to *Rhododendro-Vaccinietalia* order and *Loiseleurio-Vaccinietea* class (e.g. *Vaccinium gaultherioides*, *V. myrtillus*, *V. vitis-idaea*, *Juniperus sibirica* etc.) in the floristic composition. High constancy presents also some species from the alpine meadows of *Juncetea trifidi* class (e.g. *Festuca supina*, *Juncus trifidus*, *Pulsatilla alba* etc.). These are relative species-poor communities, including heliophyte plants (mean 7.79), indicators of cool conditions specific to subalpine or subalpine sites (mean 2.14). From the continentality perspective, most of the species are transgressive from oceanic (occurring in the western parts of Central Europe) to suboceanic (mean 3.21) species (occurring in the Central Europe), on soils with average humidity (mean 4.89), acid (mean 2.80) and very poor in available nitrogen (mean 1.67).

Group 2: ***Empetro-Vaccinietum gaultherioidis*** Br.-Bl. 1926

Diagnostic species: *Vaccinium gaultherioides*, *Empetrum nigrum* subsp. *hermaphroditum*, *Cetraria islandica*.

Dwarf-shrubs communities edified by *Empetrum nigrum* subsp. *hermaphroditum* and *Vaccinium gaultherioides* in various codominace repports, developed at altitudes varying between 1620 and 2100 m (mean 1845 m), on gentle inclined slopes (mean slope 10°), with various aspects, in Rodnei [RESMERIȚĂ, 1976; COLDEA, 1990], Suhard [COLDEA & PÎNZARU, 1986], Maramureşului [RESMERIȚĂ, 1976], Bistriței [SEGHEDIN, 1983], Hăşmaşul Mare [NECHITA, 2003] and Vrancei Mountains [VICOL & al. 1967; SÂRBU & al. 1999; ŞTEFAN & al. 1999]. The shrubs layer includes few other species, depending on altitude: *Pinus mugo*, *Juniperus sibirica*, *Vaccinium myrtillus*. The herbs layer has a pretty low diversity, *Carex atrata*, *Deschampsia flexuosa*, *Huperzia selago* etc., being among the most frequent species. The lichens layer is very well developed, *Cetraria islandica* presenting coverages up to 75% of the relevé surface. In the floristic composition there are diagnostic species to the *Loiseleurio-Vaccinion* alliance (e.g. *Cetraria islandica*, *Thamnolia vermicularis*, *Primula minima* etc.) and to *Rhododendro-Vaccinietalia* order and *Loiseleurio-Vaccinietea* class (e.g. *Rhododendron myrtifolium*, *Vaccinium gaultherioides*, *V. myrtillus*, *V. vitis-idaea* etc.). High constancy presents also some species from the alpine meadows of *Juncetea trifidi* class (e.g. *Juncus trifidus*, *Festuca supina*, *Campanula alpina* etc.). These phytocoenoses include, generally, species preferring well lighted places, but also occurring in partial shaded places (mean 7.05), and adapted to cool conditions of alpine and subalpine sites (mean 2.31) from Central Europe (mean 3.58). They preffer acid (mean 2.63), medium moisted (mean 4.77) and nitrogen deficient (mean 1.89) soils.

Group 3: ***Rhododendro-Vaccinietum gaultherioidis*** Borza 1959 em. Boşcaiu 1971

Diagnostic species: *Rhododendron myrtifolium*, *Ligusticum mutellina*, *Gentiana punctata*.

Rhododendron myrtifolium and *Vaccinium gaultherioides*, in various codominance repports, edifies vegetal communities from the upper limit of the mountain forests up to the

alpine belt, on an altitudinal range of 1600-2120 m, depending on the mountain massif (mean altitude 1890 m), on areas very variable in terms of slopes (5º-50º) and aspects. They were described from Rodnei [COLDEA & al. 1981; RESMERIȚĂ & RAȚIU, 1983; COLDEA & PÎNZARU, 1986; COLDEA, 1990], Călimani [HOREANU & VIȚALARIU, 1991], Suhard [COLDEA & PÎNZARU, 1986] and Maramureşului [RESMERIȚĂ, 1978] mountains and present a floristic composition characterized by a low number of species. The shrubs layer is dominated by the species characteristic for the association, alongside with sporadically species as *Vaccinium myrtillus*, *Juniperus sibirica*, or rare individuals of *Pinus mugo*. The herbs layer presents variable coverages and includes a low number of species (*Homogyne alpina*, *Calamagrostis villosa*, *Agrostis rupestris*, *Oreochloa disticha*, *Deschampsia flexuosa* etc.). High constancy present also diagnostic species for *Rhododendro-Vaccinion* and *Loiseleurio-Vaccinion* alliances (*Cetraria islandica*, *Primula minima*), for *Rhododendro-Vaccinietalia* order and *Loiseleurio-Vaccinietea* class (e.g. *Vaccinium myrtillus*, *V. vitis-idaea, Primula minima*, *Juniperus sibirica* etc.). Also, there are many species infiltrated from *Juncetea trifidi* (e.g. *Campanula alpina*, *Juncus trifidus*, *Pulsatilla alba* etc.) and *Vaccinio-Piceetea* (e.g. *Calamagrostis villosa*, *Soldanella hungarica* etc.). In the floristic composition, most of the species preffer generally well lighted places, but they can also occur in partial shaded places (mean 6.75) and are adapted to the cool conditions of the subalpine and alpine sites (mean 2.57) specific to Central European regions (mean 3.45). They also preffer average humid (mean 5.22), acid (mean 2.95), and deficient in available nitrogen (mean 2.38) soils.

Group 4: ***Campanulo abietinae-Juniperetum nanae*** Simon 1966

Diagnostic species: *Juniperus sibirica*, *Picea abies*, *Campanula abietina*, *Calamagrostis villosa*, *Achillea distans*, *Oxalis acetosella*, *Poa chaixii*, *Rubus idaeus*, *Senecio ovatus*.

Include shrubs communities edified by *Juniperus sibirica* described from Maramureşului [RESMERIȚĂ, 1978; RESMERIȚĂ, 1984], Rodnei [COLDEA & PÎNZARU, 1986; COLDEA, 1990], Suhardului [COLDEA & PÎNZARU, 1986], Hăşmaşul Mare [NECHITA, 2003], Bistriței [SEGHEDIN, 1983; OPREA, 2006] and Vrancei Mountains [ŞTEFAN & al. 1999] but probable much more frequent in Eastern Carpathians. These phytocoenoses are developed from the upper limit of the coniferous forests up to the alpine belt, in an altitudinal range between 1450 and 2000 m (mean 1711 m) on terrains with medium slopes (mean 15º) and various aspects. The shrubs layer is compact, including few species alongside the dominat one (e.g. *Pinus mugo*, *Vaccinium myrtillus* etc.). The herbs layer is the most diversified one, in some cases, some species can present higher cover degrees (as *Deschampsia flexuosa*, *Luzula luzuloides* etc.). The bryophytes and lichens layer is also well developed; among the species presenting higher coverages there are *Pleurozium schreberi*, *Hylocomium splendens* or *Cetraria islandica*. From a phytosociological perspective, high constancies presents diagnostic species to *Junipero-Bruckenthalion* (e.g. *Potentilla ternata*), *Rhododendro-Vaccinietalia* and *Loiseleurio-Vaccinietea* (e.g. *Rhododendron mytifolium*, *Vaccinium myrtillus*, *V. vitis-idaea*, *V. gaultherioides*, *Ligusticum mutellina* etc.). These communities includes a mix of half shade species and heliophytes (mean 6.0), indicators of the cool conditions (mean 3.19) from the montane or subalpine areas of the Central Europe (mean 3.83). Most of them prefer average dampness soils (mean 5.22), acid (mean 3.03) and nitrogen deficient (mean 2.86).

Group 5: ***Campanulo abietinae-Vaccinietum myrtilli*** (Buia et al. 1962) Boşcaiu 1971

Diagnostic species: *Cetraria cucullata*, *Hieracium alpinum*, *Deschampsia flexuosa*, *Hypericum richeri* subsp. *grisebachii*, *Melampyrum saxosum*

Vegetal communities from the upper limit of the coniferous forests described from Maramureşului [RESMERIȚĂ, 1976; RESMERIȚĂ, 1984], Rodnei [COLDEA, 1990], Călimani [MARDARI, 2010], Hăşmaşul Mare [NECHITA, 2003], Ciucaş [PAUCĂ & al. 1960] and Vrancei Mountains [SÂRBU & al. 1999; ŞTEFAN & al. 1999], developed at altitudes ranging between 1580 and 1950m (mean 1727 m) on terrains with various slopes and aspects. Besides the dominant species, in the shrubs layer, sporadically appear species as *Juniperus sibirica*, *Pinus mugo* or juvenile trees of *Picea abies*, and *Sorbus aucuparia*. In the herbaceous layer among the most frequent species there are *Luzula luzuloides*, *Hieracium alpinum*, *Hypericum richeri* subsp. *grisebachii*, *Homogyne alpina* etc. There is also a layer of bryophytes and lichens where *Polytrichum juniperinum*, *Dicranum scoparium* and *Cetraria islandica* have high abundancies. In the floristic composition there are diagnostic species for the superior syntaxa and other for the alpine meadows of *Juncetea trifidi* (e.g. *Festuca supina*, *Juncus trifidus*) or for the forests within *Vaccinio-Piceetea* class (e.g. *Soldanella major*, *Luzula sylvatica*). These are relative species-rich communities compared to other *Loiseleurio-Vaccinietea* associations, including plants generally growing in well lighted places, but also in partial shaded places (mean L 6.66), and in cool conditions specific to high montane and subalpine sites (mean 2.73). Most of them are suboceanic species (mean 3.65), occurring mainly in Central Europe, on soils with average humidity (mean 4.97), acid (mean 2.70) and very poor in available nitrogen (mean 2.17).

Group 6: ***Bruckenthalio-Vaccinietum*** Coldea et al. 2008

Diagnostic species: *Bruckenthalia spiculifolia*, *Ranunculus montanus* subsp. *pseudomontanus*, *Vaccinium vitis-idaea*, *Nardus stricta*, *Pleurozium schreberi*, *Polytrichum commune*, *Potentilla erecta*, *Potentilla ternata*, *Festuca supina*.

Classification of the phytocoenoses included by us in *Bruckenthalio-Vaccinietum* is problematic. In the latest classification [COLDEA & al. 2008] they were included in *Bruckenthalio-Vaccinietum* association within *Genistion pilosae* Duv. 1942 em. Schubert 1960 alliance, from *Vaccinio-Genistetalia* Schubert ex. Passarge 1964 order, and *Calluno-Ulicetea* Br.-Bl. et Tx. ex Klika et Hadac 1944 class. In this association were included also phytocoenoses (very similar to those analyzed by us) initially considered as subassociation with *Vaccinium myrtillus* (*vaccinietosum myrtilli*) within *Bruckenthalietum spiculifoliae* Buia et al. 1962 and classified in *Bruckenthalieto-Vaccinion* alliance within *Vaccinio-Piceetalia* order and *Vaccinio-Piceetea* class [BUIA & al. 1962]. Based on the presence of species characteristic to *Loiseleurio-Vaccinietea* (e.g. *Bruckenthalia spiculifolia, Cetraria islandica, Dicranum fuscescens, Vaccinium gaultherioides*) [MUCINA, 1997] and the presence of only two species characteristic to *Nardo-Callunetea* (*Nardus stricta* and *Potentilla erecta*) we consider that these phytocoenoses are more similar to the arctic-alpine dwarf shrubs vegetation and could be classified in *Junipero-Bruckenthalion* alliance from *Rhododendro-Vaccinietalia* order, within *Loiseleurio-Vaccinietea* class.

From another perspective, these phytoceonoses are not similar to those included in the subassociation *bruckenthalietosum* Coldea 1991 from the *Vaccinio-Callunetum vulgaris* Büker 1942 (*Genistion*, *Nardetalia* and *Nardo-Callunetea*), where there are many species characteristic to the above mentioned syntaxa.

We have chosen to classify the vegetal communities within *Bruckenthalio-Vaccinietum* Coldea et al. 1998 from the Eastern Carpathians of Romania in the *Loiseleurio-Vaccinietea microphylli* class because it groups together the alpine and subalpine dwarf-shrubs communities from the boreal and arctic regions of Europe in contrast with the *Calluno vulgaris-Ulicetea minoris* class which include temperate shrubs landes and associated meadows on nutrients deficient soils [MUCINA, 1997]. We believe that classification of such vegetal communities from Romanian Carpathians in *Calluno vulgaris-Ulicetea minoris* could be somehow forced in the absence of many of its diagnostic species in Romania's flora as: *Agrostis curtisii*, *Alchemilla wichurae*, *Anthyllis vulneraria* subsp. *corbierei*, *Campanula recta*, *Carex binervis*, *Centaurea nigra*, *Cladonia portentosa*, *Conopodium majus*, *Daboecia cantabrica*, *Dianthus seguieri*, *Dicranum spurium*, ***Erica ciliaris***, ***E. cinerea***, ***E. vagans***, *Euphorbia polygalifolia*, *Festuca rubra* subsp. *pruinosa*, *Genista anglica*, *Gentiana pannonica*, *Hypnum jutlandicum*, *Jasione laevis*, *Juncus squarrosus*, *Polygala serpyllifolia*, *Thesium pyrenaicum*, *Thymus arcticus* subsp. *drucei*, ***Ulex cantabricus***, ***U. europaeus***, ***U. gallii***, ***U. minor***, *Viola lutea*. Even if there are present some diagnosis species for *Calluno vulgaris-Ulicetea minoris* class (e.g. *Nardus stricta*, *Potentilla erecta*, *Pseudorchis albida*) [MUCINA, 1997] these are transgressive species in more than one vegetation class in Romania. Moreover, the physiognomy of vegetation within *Calluno vulgaris-Ulicetea minoris* is determined by the presence of some *Fabaceae* and *Ericaceae* species with relative tall habitus (from *Ulex* and *Erica* genera which are absent in Romanian flora) [BARDAT & al. 2001]. From another perspective, in the dwarf-shrubs communities within *Loiseleurio-Vaccinietea* from Romanian Eastern Carpathians there are some many diagnostic species for this vegetation class: *Alectoria ochroleuca*, *Bruckenthalia spiculifolia*, *Cetraria nivalis*, *Dicranum fuscescens*, *Empetrum nigrum subsp. hermaphroditum*, *Hieracium alpinum*, *Loiseleuria procumbens*, *Lycopodium clavatum* sensu lato, *Rhododendron myrtifolium*, *Thamnolia vermicularis, Vaccinium gaultherioides* [MUCINA & al. 1997]. Even in the Western Europe this vegetation class is represented by other alliances, the alliances from the Carpathians can be considered as their vicariants. In contrast, in the *Calluno vulgaris-Ulicetea minoris* there are grouped some alliances without correspondence in Romanian vegetation (e. g. *Cisto salviifolii-Ericion cinereae, Daboecion cantabricae, Dactylido oceanicae-Ulicion maritime, Ulicion minoris, Ulici minoris-Ericenion ciliaris, Genistion micrantho-anglicae* etc.).

These phytocoenoses were identified at increased altitudes (mean 1631 m) in Baiului Mountains [TODOR & CULICĂ, 1967], on relatively inclined terrains (mean 30°) with northern and north-eastern aspects. In the shrubs layer, *Vaccinium myrtillus* is the dominant species. *Bruckenthalia spiculifolia* is present in almost all relevés, with coverages up to 25%. Herbaceous layer includes few species, among which *Deschampsia flexuosa*, *Festuca rubra*, *F. supina*, *Potentilla ternata*, *Geum montanum*, *Ranunculus montanus* subsp. *pseudomontanus* and *Nardus stricta* are more frequent. The bryophytes and lichens are also well developed (e.g. *Pleurozium screberi, Hylocomium splendens, Polytrichum commune*, *Cetraria islandica*). In their floristic composition there are preponderantly species preferring lighted places, but also occurring in partial shaded places (mean 6.45), which are growing in the climate conditions specific to high montane to subalpine sites (mean 2.46). Most of them are suboceanic species (mean 3.43), occurring in the most parts of Central Europe, on soils with average humidity (mean 4.80), acid (mean 2.81), and poor in available nitrogen (mean 2.06).

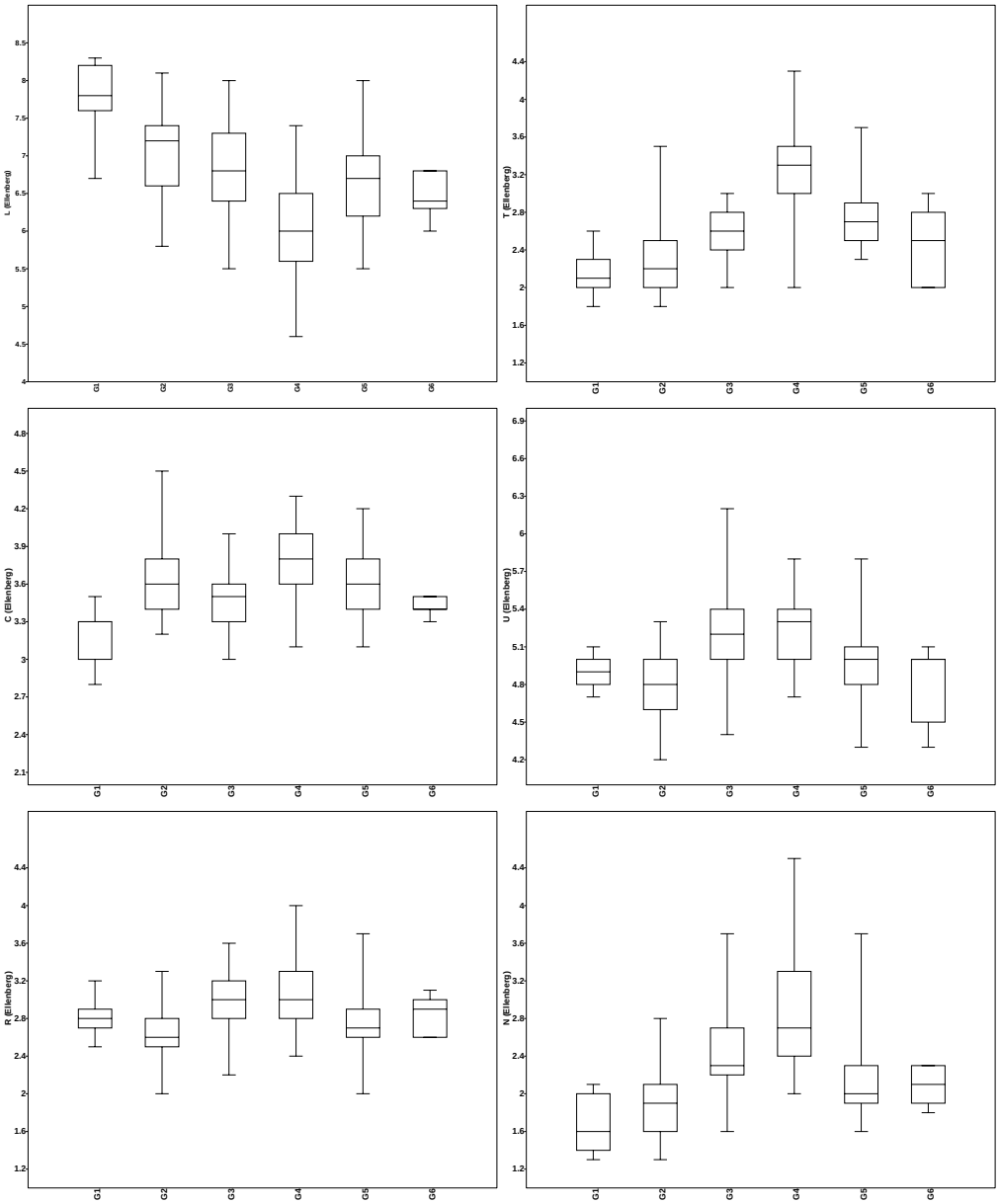

Fig. 4. Box (showing medians) with whisker plots (indicating the minimum and maximum values) of the average Ellenberg indicator values for relevés from the 6 clusters resulted in hierarchical clustering.

Detrended correspondence analysis (DCA)

Detrended correspondence analysis used in order to relate the relevés ordination to environmental data (altitude and means of Ellenberg indicator values) showed that the first

axis is the most important one (eigenvalue 0.535), explaining 11.5% of the cumulative percentage variance of species data and 47% of the cumulative percentage variance of species-environment relation. The second axis is less important (eigenvalue 0.242) and explains only 5.1% of the cumulative percentage variance of species data and 16.9% of the cumulative percentage variance of species-environment relation. Together, the first two axes explain 16.5% of the cumulative percentage variance of species data and 63.9% of the cumulative percentage variance of species-environment relation (Tab. 3).

The first DCA axis is strongly positively correlated with EIVs for temperature, for continentality and nutrients and strongly negatively correlated with altitude and EIVs for light and altitude (Fig. 5). This suggests that altitude, light, nutrients and temperature could represent the main factors influencing the floristic composition of the vegetal communities from *Loiseleurio-Vaccinietea*. These ecological factors generate a differentiation among the communities from increased altitudes, including heliophytes species, adapted to more extreme conditions of temperature and low nutrients availability (*Cetrario-Loiseleurietum procumbentis*) from the left side of the ordinogram compared to the communities from lower altitudes including species with the ecological optimum in a higher range of temperature, developed on soils richer in nutrients (*Campanulo abietinae-Juniperetum nanae*) situated in the right side of the ordinogram. The second DCA axis is mainly (negatively) correlated with EIVs for soil moisture and soil reaction (*p*H) indicating that floristic variation is affected also by soil characteristics but in a lower degree as temperature, nutrients, altitude and light. Thus, the more mesophylous vegetal communities developed of less acid substrata, from the inferior part of the ordinogram (*Rhododendro-Vaccinietum gaultherioidis*) can be differentiated from those more xerophylous and developed on more acid soils (*Bruckenthalio-Vaccinietum*) from the superior part of the ordinogram (Fig. 5).

Tab. 3. Summary of detrended correspondence analysis presenting eigenvalues, lengths of gradients and variances of species composition and species environment along axes

Axes	**1**	**2**	**3**	**4**	**Total inertia**
Eigenvalues	0.535	0.242	0.180	0.134	4.705
Lengths of gradient	4.435	2.818	2.367	1.908	
Cumulative percentage variance of species data	11.4	16.5	20.4	23.2	
Cumulative percentage variance of species-environment relation	47.0	63.9	0	0	
Sum of all eigenvalues					4.705

Tab. 4. Characteristics of the six dwarf shrubs communities from Eastern Carpathians (means and standard deviations). *P* values are derived from Kruskal-Wallis non parametric ANOVA.

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**	***P***
EIV L	7.79±0.47	7.05±0.58	6.75±0.59	6.01±0.57	6.65±0.57	6.45±0.50	< 0.001
EIV T	2.14±0.22	2.31±0.40	2.57±0.24	3.19±0.45	2.73±0.33	2.46±0.38	< 0.001
EIV C	3.21±0.21	3.58±0.37	3.44±0.25	3.83±0.30	3.55±0.29	3.43±0.07	< 0.001
EIV U	4.89±0.12	4.77±0.25	5.22±0.29	5.22±0.26	4.97±0.35	4.8±0.28	< 0.001
EIV R	2.80±0.19	2.63±0.30	2.95±0.31	3.03±0.37	2.70±0.29	2.81±0.20	< 0.001
EIV N	1.67±0.30	1.89±0.31	2.38±0.42	2.86±0.68	2.14±0.39	2.06±0.16	< 0.001
Altitude	2027±106	1845±110	1883±112	1711±143	1727±75	1631±57	< 0.001

Significant differences (Fig. 4; Tab. 4; App. 3) among the six vegetal communities have been also detected using Mann-Whitney post-hoc test (Bonferroni corrected). Thus, from the light perspective, *Cetrario-Loiseleurietum procumbentis* association, developed at highest elevations, present the highest exigencies which significantly differ from all the other communities. Other significant differences are between the phytocoenoses within *Campanulo abietinae-Juniperetum nanae*, developed at lower altitudes, including species occurring in more shaded places and those from *Empetro-Vaccinietum gaultherioidis*, *Rhododendro-Vaccinietum gaultherioidis* and *Campanulo abietinae-Vaccinietum myrtilli*. Excepting the *Cetrario-Loiseleurietum procumbentis*, taking into consideration the EIVs for light, between the *Bruckenthalio-Vaccinietum* and the rest of communities there are no significant differences. Temperature separates the communities from *Cetrario-Loiseleurietum procumbentis* and *Empetro-Vaccinietum gaultherioidis* (*Loiseleurio-Vaccinion*) and differentiates them from *Rhododendro-Vaccinietum gaultherioidis* (*Rhododendro-Vaccinion*), and from *Campanulo abietinae-Juniperetum nanae* and *Campanulo abietinae-Vaccinietum myrtilli* (Junipero-Bruckenthalion). Also, the dwarf juniper communities differ in EIVs for temperature from *Rhododendron* and *Vaccinium* communities. EIVs for continentality show that, among all communities, *Cetrario-Loiseleurietum procumbentis* presents the smallest values, due to the presence of more transgressive species from oceanic (mainly in the western parts of Central Europe) to suboceanic (mainly in the Central Europe) areas. Soil moisture also differentiates the communities within *Loiseleurio-Vaccinion* from those of *Rhododendro-Vaccinion* and *Junipero-Bruckenthalion*. Soil reaction shows no difference between *Cetrario-Loiseleurietum* and *Bruckenthalio-Vaccinietum* phytocoenoses and the rest of communities, but differentiate the *Empetrum* from *Rhododendron* edified communities and *Vaccinium myrtillus* from those edified by *Juniperus communis*. Soil available nitrogen differentiates *Cetrario-Loiseleurietum* and *Empetro-Vaccinietum* from *Rhododendro-Vaccinietum*, *Campanulo abietinae-Vaccinietum myrtilli* and *Campanulo abietinae-Juniperetum nanae*. Also EIVs for available nitrogen make the distinction among *Rhododendro-Vaccinietum* and *Campanulo abietinae-Vaccinietum myrtilli* or *Campanulo abietinae-Juniperetum nanae*. Finally, the altitude clearly separates the *Cetrario-Loiseleurietum* from all other vegetal communities, which can also be differentiated among each other on elevation criterion.

As Detrended Correspondence Analysis (DCA) used in order to explore the general variation of floristic composition generated a length of gradient of 4.435 SD units along the first axis, we used further CCA ordination method because it is the most appropriate for our data showing a unimodal response of species to variables [LEPŠ J. & ŠMILAUER, 1999]. Monte Carlo test with 999 permutations (Tab. 5) produced *F* values, measuring the strength of the effect of each variable on species composition, which demonstrated that light, altitude and temperature are the most important factors modeling the floristic composition of vegetal communities within *Loiseleurio-Vaccinietea*.

The influence of light on the floristic composition is significant at higher altitudes, where this ecological factor is more intense and richer in violet rays light and the effect is that the plants are shorter than in lower vegetation levels [ELLENBERG, 1988]. Corroborated with the low temperatures which is another factor responsible for the small growth of plants species, light and altitude differentiate the dwarf shrubs communities of higher altitudes, including almost exclusively heliophytes species, from those of lower altitudes and slightly increased temperatures including species with a taller growth which can generate enough shadow to permit the infiltration of another species normally occurring in more shaded places.

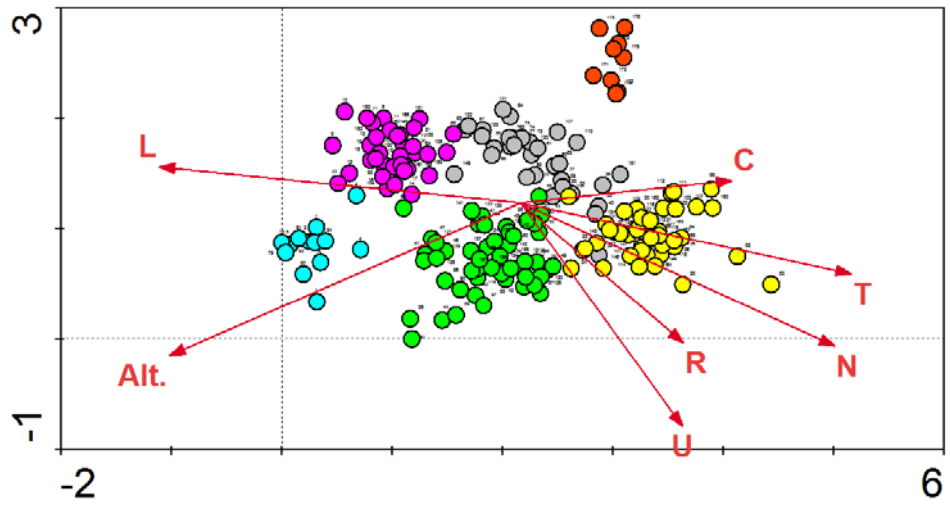

Fig. 5. DCA ordination diagram of the 181 relevés using Ellenberg's indicator values and altitude as passive variables (with clusters generated by hierarchical clustering colored as follows: *Cetrario-Loiseleurietum procumbentis* – blue circles, *Empetro-Vaccinietum gaultherioidis* – violet circles, *Rhododendro-Vaccinietum gaultherioidis* – green circles, *Campanulo abietinae-Juniperetum nanae* – yellow circles, *Campanulo abietinae-Vaccinietum* – grey circles, *Bruckenthalio-Vaccinietum* – red circles); first two axes presented. Eigenvalues: 1st axis: 0.535, 2nd axis: 0.242, total inertia: 4.705. Correlation of DCA axes with variables: L (1st axis: -0.7289, 2nd axis: 0.1127), T (1st axis: 0.6692, 2nd axis: -0.1900), C (1st axis: 0.4243, 2nd axis: 0.0346), H (1st axis: 0.3370, 2nd axis: -0.5281), R (1st axis: 0.3346, 2nd axis: -0.3353), N (1st axis: 0.6393, 2nd axis: -0.3549), altitude (1st axis: -0.6988, 2nd axis: -0.3228).

Tab. 5. Effect of each variable on *Loiseleurio-Vaccinietea* species composition - CCA analysis and Monte Carlo test with 999 permutations, $P = 0.001$. Variables are ranked by decreasing value of the F statistic, measuring the strength of the effect of each variable)

Variable	*F*-value
EIV light	12.949
altitude	12.442
EIV temperature	10.922
EIV nutrients	10.700
EIV humidity	6.384
EIV continentality	6.354
EIV soil pH	4.757

Conclusions

Multivariate analysis of the dwarf shrubs communities (within *Loiseleurio-Vaccinietea* class) from Eastern Carpathians (Romania) has as result their classification in 6 vegetation types. Based on the identified diagnostic species they can be assigned to the vegetal associations described in phytosociological literature. In their floristic composition

there are preponderantly heliophytes species, adapted to low temperatures from subalpine and alpine belts, occurring in the whole Central Europe and preferring moderate humid, acid, and very poor in nutrients soils. Light, altitude, temperature and nutrients are the main factors influencing the floristic composition of these plant communities. The investigated plant communities are all from the Eastern Carpathians and they are not necessarily representative for the whole range of Romanian Carpathians. It is necessary to conduct similar studies in the Southern and Western Carpathians, in order to improve the classification and ecological characterization of Romanian (sub-) alpine dwarf shrubs communities.

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Appendix 1

Provenience of relevés used in analysis

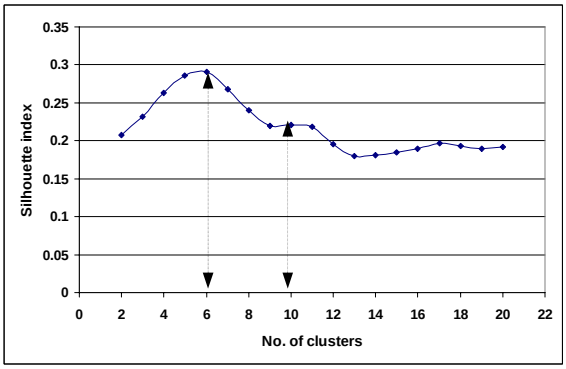
Author(s)	Year	No. of relevés	Mountain massif(s)
COLDEA GH. et al.	1981	10	Rodnei Mountains
COLDEA GH. & PÎNZARU G.	1986	15	Rodnei Mountains
COLDEA GH. & PÎNZARU G.	1987	12	Suhardului Mountains
COLDEA GH.	1990	54	Rodnei Mountains
DIHORU GH.	1975	1	Siriu Mountains
HOREANU CL. & VIŢALARIU GH.	1991	11	Călimani Mountains
MARDARI C.	2010	17	Călimani Mountains
NECHITA N.	2003	16	Hăşmaşul Mare Mountains
OPREA A.	2006-2007	2	Bistriţei Mountains
PAUCĂ et al.	1960	1	Ciucaş Mountains
RAŢIU O. & MOLDOVAN I.	1974	4	Gutâiului Mountains
RESMERIŢĂ I.	1976	24	Maramureşului Mountains
RESMERIŢĂ I.	1978	24	Maramureşului Mountains
RESMERIŢĂ I. & RAŢIU O.	1983	11	Rodnei Mountains
RESMERIŢĂ I.	1984	5	Maramureşului Mountains
RESMERIŢĂ I.	1984	10	Maramureşului Mountains
SÂRBU I. et al.	1999	6	Vrancei Mountains
SEGHEDIN T. G.	1983	10	Bistriţei Mountains
ŞTEFAN N. et al.	1999	8	Vrancei Mountains
TODOR I. & CULICĂ S.	1967	10	Baiului Mountains
VICOL et al.	1967	5	Vrancei, Rodnei Mountains

Appendix 2

a) Incidence matrix presenting the corrected Rand index between partitions with *k* number of clusters

	K=2	K=3	K=4	K=5	**K=6**	K=7	K=8	**K=9**	K=10	K=11	K=12	K=13	K=14	K=15	K=16	K=17	K=18	K=19	K=20
K=2	1,000																		
K=3	0,711	1,000																	
K=4	0,495	0,747	1,000																
K=5	0,441	0,677	0,924	1,000															
K=6	0,405	0,630	0,872	0,947	1,000														
K=7	0,377	0,592	0,828	0,902	**0,955**	1,000													
K=8	0,312	0,502	0,720	0,792	0,843	0,887	1,000												
K=9	0,277	0,452	0,657	0,726	0,776	0,819	0,930	1,000											
K=10	0,268	0,437	0,639	0,707	0,756	0,799	0,910	**0,979**	1,000										
K=11	0,234	0,387	0,574	0,638	0,685	0,726	0,834	0,902	0,923	1,000									
K=12	0,190	0,320	0,485	0,543	0,586	0,624	0,725	0,790	0,810	0,886	1,000								
K=13	0,161	0,274	0,420	0,473	0,512	0,547	0,642	0,704	0,723	0,796	0,908	1,000							
K=14	0,146	0,250	0,387	0,436	0,474	0,507	0,597	0,657	0,676	0,747	0,857	0,948	1,000						
K=15	0,140	0,239	0,372	0,420	0,456	0,488	0,577	0,636	0,654	0,724	0,832	0,923	0,975	1,000					
K=16	0,137	0,234	0,365	0,412	0,448	0,480	0,567	0,626	0,644	0,713	0,821	0,912	0,964	0,989	1,000				
K=17	0,129	0,222	0,346	0,392	0,426	0,457	0,542	0,599	0,616	0,684	0,791	0,880	0,932	0,957	0,968	1,000			
K=18	0,124	0,214	0,336	0,380	0,414	0,444	0,527	0,583	0,601	0,668	0,773	0,862	0,913	0,938	0,950	0,981	1,000		
K=19	0,114	0,197	0,311	0,352	0,384	0,413	0,492	0,546	0,563	0,627	0,730	0,817	0,868	0,892	0,904	0,935	0,954	1,000	
K=20	0,110	0,190	0,300	0,341	0,372	0,400	0,477	0,530	0,546	0,610	0,711	0,798	0,848	0,872	0,884	0,915	0,934	0,980	1,000

b) Determination of the optimum number of clusters using Silhouette index



Appendix 3

***P* values derived from Mann-Whitney post-hoc test (Bonferroni corrected) indicating significant differences between communities from EIV and altitude perspectives.**

a) EIVs light

	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
Group 1	0					
Group 2	0.003828	0				
Group 3	7.447E-05	n.s.	0			
Group 4	1.882E-06	5.64E-08	3.181E-06	0		
Group 5	0.000142	n.s.	n.s.	0.0002083	0	
Group 6	0.002662	n.s.	n.s.	n.s.	n.s.	0

b) EIVs temperature

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1	0					
Group 2	n.s	0				
Group 3	0.0002064	0.002431	0			
Group 4	5.284E-06	6.188E-09	3.591E-09	0		
Group 5	4.309E-05	0.0001648	n.s.	0.0001335	0	
Group 6	n.s.	n.s.	n.s.	0.003634	n.s.	0

c) EIVs continentality

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1	0					
Group 2	0.004227	0				
Group 3	0.04367	n.s.	0			
Group 4	1.671E-05	0.02067	1.203E-07	0		
Group 5	0.00774	n.s.	n.s.	0.002799	0	
Group 6	n.s.	n.s.	n.s.	0.001774	n.s.	0

d) EIVs soil moisture

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1	0					
Group 2	n.s.	0				
Group 3	0.0004753	2.128E-08	0			
Group 4	0.002606	1.833E-07	n.s.	0		
Group 5	n.s.	n.s.	0.007722	0.02524	0	
Group 6	n.s.	n.s.	0.007885	0.02207	n.s.	0

e) EIVs soil reaction

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1	0					
Group 2	n.s.	0				
Group 3	n.s.	0.0003705	0			
Group 4	n.s.	0.000267	n.s.	0		
Group 5	n.s.	n.s.	0.002204	0.003658	0	
Group 6	n.s.	n.s.	n.s.	n.s.	n.s.	0

f) EIVs soil available nitrogen

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1	0					
Group 2	n.s.	0				
Group 3	3.937E-05	2.222E-06	0			
Group 4	1.845E-06	3.131E-10	0.00553	0		
Group 5	0.00765	n.s.	0.02603	5.411E-07	0	
Group 6	n.s.	n.s.	n.s.	0.0008066	n.s.	0

g) altitude

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1	0					
Group 2	0.000696	0				
Group 3	0.00481	n.s.	0			
Group 4	6.312E-06	0.001345	1.162E-06	0		
Group 5	6.789E-06	0.0002977	1.016E-07	n.s.	0	
Group 6	0.00159	0.0006746	9.362E-05	n.s.	0.02358	0

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PHEMERANTHUS CONFERTIFLORUS: NEW ALIEN SPECIES TO EUROPE

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Abstract: *Phemeranthus confertiflorus* (Montiaceae) is reported as a new alien species to Europe. It is native to North America and used as decorative in rock gardens. The specimens of this plant were collected in Bucharest (Romania) and deposited in BUC. The mode of introduction of the plant is unknown, most likely escaped from cultivation. Data about habitat and population of the taxon are presented. One of the plants that accompany *Phemeranthus confertiflorus* is also an alien to Europe – *Portulaca pilosa* and this is the first record of this plant for Romania.

Kew words: alien species, Europe, invasive plants, *Phemeranthus*, *Portulaca*, Romania.

Introduction

Phemeranthus belongs to the family Montiaceae (fameflowers, rockpinks, sunbrights), part of the traditionally recognized Portulacaceae s.l. [NYFFELER & EGGLI, 2010]. *Phemeranthus* species are almost entirely North American, with one exception *P. punae* (Fries) Carolin found in northern Argentina. The centre of diversity of this genus is northern Mexico and the south-western United States [PRICE, 2012].

Phemeranthus has been included for a long time in the genus *Talinum* Adans. as *Talinum* sect. *Phemeranthus* [KIGER, 2001]. Morphological data such as the shape of the basis leaf, the capsule dehiscence and structure, the seed surface texture [CAROLIN, 1987, 1993; HERSHKOVITZ, 1993; FERGUSON, 2001] and molecular phylogenetic investigations [HERSHKOVITZ & ZIMMER, 1997, 2000; APPLEQUIST & WALLACE, 2001; NYFFELER & EGGLI, 2010] suggest that the two groups are not closely related.

Over time, new combinations have appeared through the transfer of the *Talinum* species to *Phemeranthus*. For this reason, the genus comprises a various number of species according to different authors: 25 [PRICE, 2012], 30 [NYFFELER & EGGLI, 2010], 25-30 [KIGER, 2003], 27 [OCAMPO, 2003] or 20 according to The Plant List [http://www.theplantlist.org].

The genus comprises species of succulent, herbaceous perennials with terete leaves and fleshy roots, most of them growing in xeric habitats.

No previous literature reference on the occurrence of *Phemeranthus* in Europe was found.

During our floristic investigations on flora from Bucharest (Romania), we recorded specimens of the genus *Phemeranthus*. The preliminary survey showed that the collected material belongs to *Phemeranthus confertiflorus* (Greene) Hershkovitz. A review

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of the literature data revealed that this taxon had not been yet recorded for the flora of Romania and Europe.

Material and methods

Plant material was photographed, collected and herborised. The herbarium specimens were deposited in the Herbarium of the Botanical Garden "D. Brandza", University of Bucharest (BUC). The location of the population was registered with a hand-held Garmin GPS model eTrex Legend C, using WGS84 system. The morphological description, ecological features and the plant species associated were based on field observations and on individuals collected and were compared with data from the literature [FERGUSON, 2001; KIGER, 2003; PRICE, 2012]. Based on detailed photographs, Taina Price from the Washington University in St. Louis helped us identify the species.

Results and discussion

***Phemeranthus confertiflorus* (Greene) Hershkovitz** belongs to Montiaceae family and is native to south-western United States and northern Mexico [PRICE, 2012].

Nomenclature:

***Phemeranthus confertiflorus* (Greene) Hershkovitz**, Taxon **46**(2): 222, 1997.
= *Talinum confertiflorum* Greene, Bulletin of the Torrey Botanical Club **8**(11): 121, 1881.
= *Talinum gracile* J. N. Rose & Standl., nom. illeg. hom. [non Colla, 1833], Contributions from the United States National Herbarium **13**(8): 285, 1911.
= *Talinum rosei* P. Wilson, North American Flora **21**(4): 287, 1932. nom. nov. for *Talinum gracile* J. N. Rose & Standl.
= *Talinum gooddingii* P. Wilson, North American Flora **21**(4): 287, 1932 [as "*Gooddingii*"].
= *Talinum fallax* Poelln., Berichte der Deutschen Botanischen Gesellschaft **51**(2): 113, 1933.

The species is closely related to *P. parviflorus* (Nutt.) and has generally been treated as synonym [PRICE, 2012; BAIR & al. 2006]. The key differences between the two taxa are:

1a Inflorescence white (rarely) to magenta; sepals early deciduous, obtuse; fruit split open at maturity, dark brown to black seeds ..... *Phemeranthus parviflorus*
1 b Inflorescence more congested, white to pink flowers; sepals usually persistent, often with dark purplish pigment apically; fruit persistent at maturity (but delicate), dark grey seeds ..... *Phemeranthus confertiflorus*

Voucher specimens: Romania, Bucharest: Balta Văcăreşti (44°24'16"N, 26°08'02"E), 65 m alt., 17.07.2013, leg. E. Nagodă, P. Anastasiu, P. Comănescu, G. Negrean; det. Taina Price [BUC 400625].

Description (Fig. 1): Perennial plants 5–36 cm tall in the observed field specimens [5-25 cm according to PRICE (2012)], erect. *Stems* short, slender, branching, arising from an elongated, fleshy taproot (Fig. 1C). *Leaves* alternate, sessile, tightly clustered, terete, fleshy, acute, 1-5 cm long (Fig. 1C). *Inflorescences* terminal, many flowered dichasia. Divisions of inflorescence subtended by scale-like bracts. Peduncles erect, wiry, straw-coloured, scape-like (8-20 cm long). *Pedicels* green, 3-5 mm long. *Flowers* slightly fragrant, ephemeral, opening in the afternoon, for about one hour (between 5 and 6 p.m.).

Sepals two, broadly ovate, acute to acuminate, purplish apically, persistent in fruit (Fig. 1E, F). *Petals* 5, 3.5–5 mm long [3.5-7 cm according to PRICE (2012)], pale pink, obovates, with rounded or mucronate apex, flower of about 10-12 mm diameter (Fig. 1B, D). *Stamens* 5, erect, equal to the length of the style (Fig. 1B). Filaments glabrous, 2-4 mm long, white. *Stigma* capitate. *Fruit* capsule ovoid, 3–4 mm long, dehiscing by three valves beginning at apex, persisting for a short time after dehiscence (Fig. 1 F). Each fruit contains around 15-30 small seeds. *Seeds* smooth, dark grey, cca. 0.7–1 mm wide (Fig. 1G).

Distribution: *Phemeranthus confertiflorus* seems to be the most widespread species of the genus [FERGUSON, 1995]. It is spread in North America, from central Chihuahua and north-eastern Sonora in Mexico to central Utah, Wyoming, and North Dakota, eastward into western Texas, Oklahoma, Kansas, and Nebraska [FERGUSON, 1995].

In Romania we recorded a single population of *Phemeranthus confertiflorus*, in Bucharest, on the north part of an area named "Balta Văcăreşti" (Fig. 2), with about 175 individuals (N44°24'16", E26°08'02").

"Balta Văcăreşti" was conceived as part of the complex development of the river Dâmbovița and remains an unfinished hydrologic project in South Bucharest. "Balta Văcăreşti" stretches over an area of 190 hectares and is surrounded by a concrete dam. The river bed of "Balta Văcăreşti" includes swampy areas with reedbeds, grassland and ponds fed by underground springs (Fig. 2).

Habitat: *Phemeranthus confertiflorus* can be found in a wide range of habitats from near desert to mountain forest [FERGUSON, 1995]. It grows in sunny places on shallow, sandy and rocky soils with the rhizome resting on bedrock.

We recorded the specimens of this species on shallow soil pockets (3-8 cm) at the boundary (the angle) between the inclined concrete edge of the dam and the horizontal vegetation layer (grassland). The soil, with little organic matter, provides by silt deposited on the damp along the time.

According to PRICE (2012), the plants are active during warm weather, growing, flowering, and bearing fruit in the hottest, driest part of the summer. The plants are highly resistant to drought and can remain in dormant state for prolonged periods.

The place where we identified the population of *Phemeranthus confertiflorus* receives plenty of sun, and is located in a marginal habitat. This is explained in the literature through the fact that the plant prefers harsh conditions (lack of water and nutrients) in order to exclude possible competitors [WARE, 1969; BASKIN & BASKIN, 1988; WARE, 1991; PRICE, 2012; FERGUSON, 2001].

The following taxa accompanied the species *Phemeranthus confertiflorus* in the identified location: *Portulaca pilosa* (Fig. 1A), *Tragus racemosus*, *Sedum acre*, *Setaria viridis*, *Lotus tenuis*, *Portulaca oleracea* subsp. *oleracea*, *Vulpia myuros*, *Eragrostis minor*, *Eragrostis pilosa*, *Echium vulgare*, *Erigeron annuus* s.l., *Digitaria sanguinalis*, *Galium humifusum*, *Cichorium intybus*, *Berteroa incana*, *Convolvulus arvensis*, *Petrorhagia prolifera*, *Plantago lanceolata*, *Bromus tectorum*.

We mention that *Portulaca pilosa*, native to Asia (Japan, China, Singapore) [PIER, 2005], is known as alien to Europe (DAISIE 2009), being reported from Hungary and Italy (http://www.europe-aliens.org/speciesFactsheet.do?speciesId=8004#). For Romania this is the first record. The plant is represented in "Balta Văcăreşti" by numerous flowering and fruiting individuals. In Romania there are two other species of *Portulaca*:

Portulaca oleracea L., as spontaneous, and *Portulaca grandiflora* Hook., cultivated and often escaped from cultivation. We provide an identification key for these three species:

1a Leaves oblong-lanceolate ..... *Portulaca oleracea*
1b Leaves linear 2
2a Flowers cca 4-5 cm diameter ..... *Portulaca grandiflora*
2b Flowers cca 1 cm diameter ..... *Portulaca pilosa*

Invasiveness: In "Balta Văcăreşti", *Phemeranthus confertiflorus* blooms, bears fruits and produces seeds and many of them have more than one above-ground stem. Even though it is found on a small area (approx. 200 m^2), the specimens are not distributed homogenously. They are grouped in four points and with a few sparse individuals.

According to Ware (1968), cited by PRICE (2012), this species usually does not flower their first year as seedlings in the field. Also, *Phemeranthus* plants grow very slowly in nature and young plants may have a linear, semi-erect rhizome and single above-ground stem for several years [WARE, 2011]. For these reasons we consider *Phemeranthus confertiflorus* as established in "Balta Văcăreşti", Bucharest.

In the context of the current climate changes, its high resistance to drought, the ability to remain dormant over long periods of time and to survive in shallow soils, and the small number of possible competitors suggest this plant could become invasive. In the future, the population will be monitored and analysed from this perspective.

As regards the way of introduction we have not certain data, but we suppose the plant is escaped from cultivation. Cacti and succulent plants collectors all over the world usually have this species in their collections. Further, as the plant is fairly easy to grow and has great decorative values, gardeners are seeking to introduce this plant in rock gardens and greenroofs [GETTER & al. 2009; DVORAK, 2010].

Conclusion

A new alien plant is reported from Europe – *Phemeranthus confertiflorus*, native in North America. Probably escaped from cultivation, the plant is established in a single location, "Balta Văcăreşti" from Bucharest. One of the species that accompany *Phemeranthus confertiflorus* is *Portulaca pilosa*, also an alien and new record for Romania.

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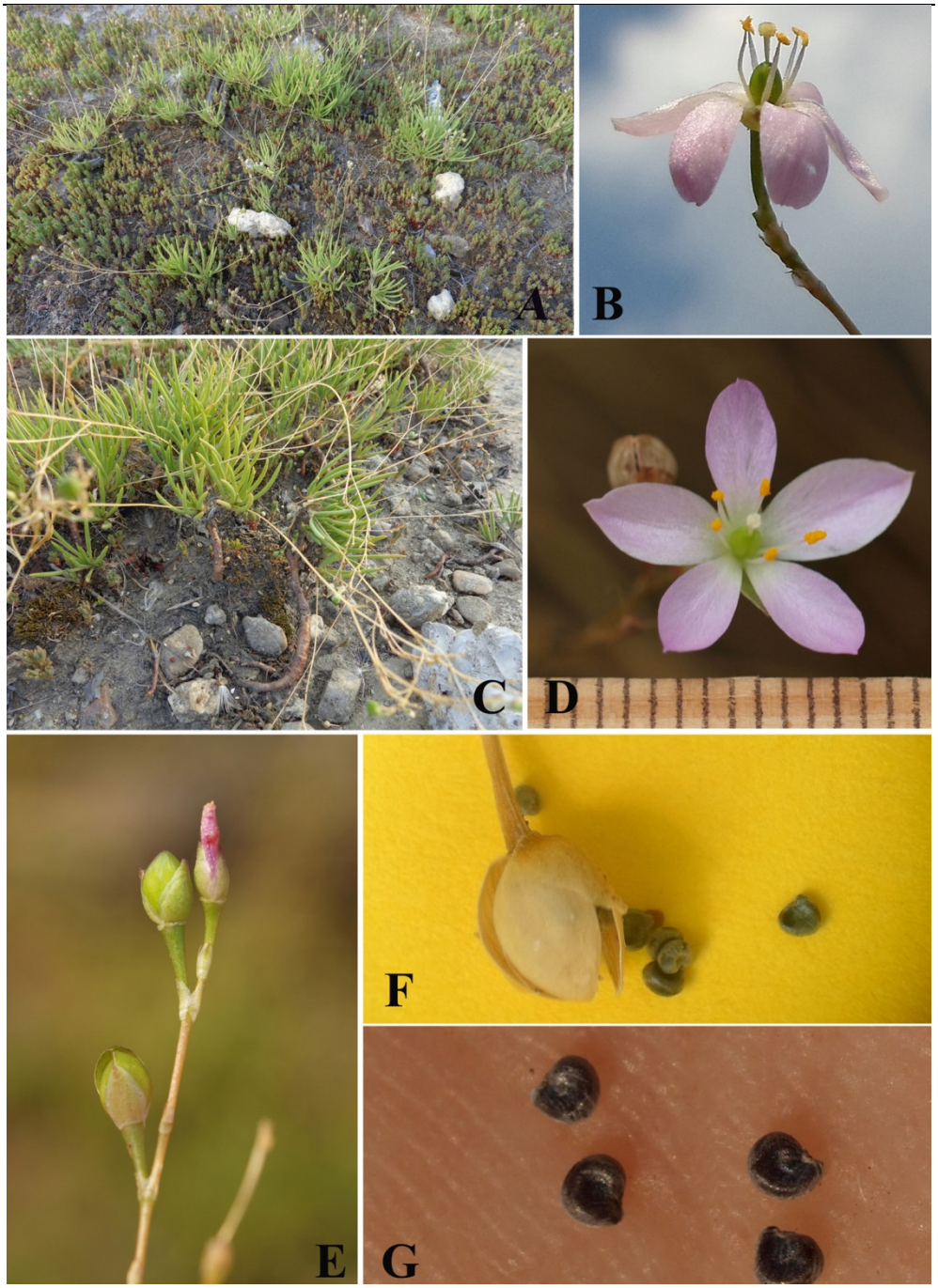

Fig. **1**. *P. confertiflorus*. A – habitat; B – flower, lateral view; C – habitus; D – fully open flower, frontal view; E – sepals persistent in fruit; F – fruit capsule dehiscing by three valves; G – seeds [Photos: Nagodă E. (Fig. 1A, C), Comănescu P. (Fig. 1B, F) & Anastasiu P. (Fig. 1 D, E, G)].

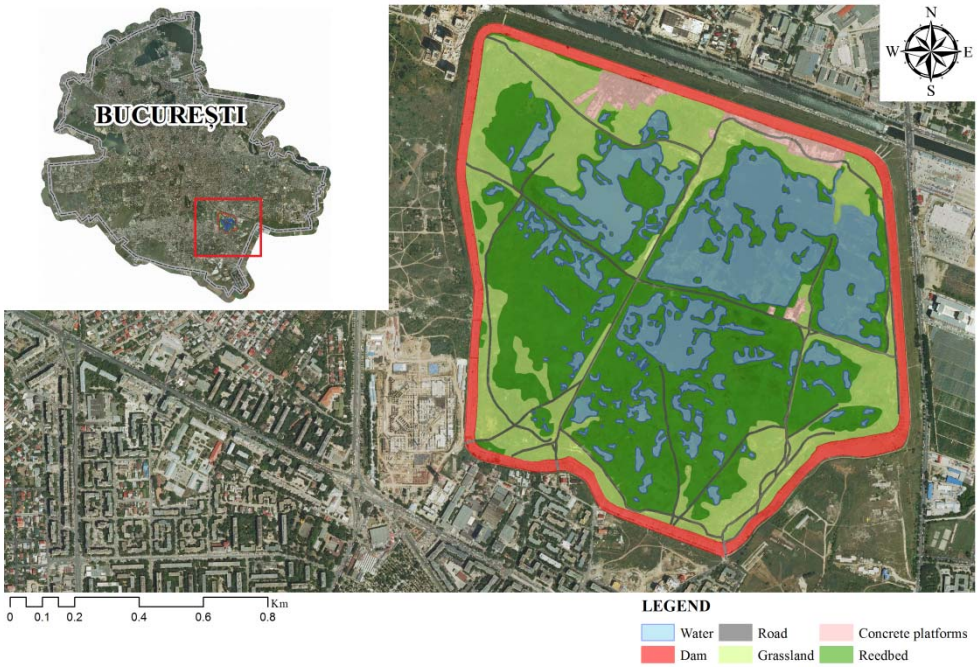

Fig. **2**. The location of "Balta Văcăreşti" in Bucharest – *Phemeranthus confertiflorus* was recorded in northern part of this area (map compiled by Tiberiu Săhlean)

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THE WOODY VEGETATION IN THE MIDDLE STREAM OF THE NIRAJ VALLEY (ROMANIA, MUREŞ COUNTY)

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Abstract: In this paperwork we presented a phytocoenologic study on the woody vegetation of the middle stream of the Niraj valley, part of Natura 2000 Special Protection Area from Romania. The territory is very important in terms of bird species conservation. The study provides a description of the woody vegetation on habitat types. Plant community of six associations was attributed to *Salicetum triandrae*, *Salici-Populetum*, *Carpino-Fagetum*, *Carpino-Quercetum petraeae*, *Genisto tinctoriae-Quercetum petraeae* subass. *melicetosum uniflorae* and *Pruno spinosae-Crataegetum*. One of the six habitat types are of Community Interests and requires designation of Special Areas of Conservation, according to the Habitats Directive.

Keywords: biocoenology, protected area, species conservation, syntaxonomy.

Introduction

The middle stream of the Niraj valley (N46.27117, E24.45289) is part of the Special Protection Area (from Natura 2000 network) named Târnavelor Hills-Niraj Valley and covers an area of 120 km^2. The territory is situated in the Transylvanian Plateau, in central part of Romania and includes the localities: Acățari, Văleni, Găieşti, Suveica, Murgeşti, Roteni, Gălățeni, Păsăreni, Bolintineni, Troița, Bedeni, Găleşti, Adrianu Mic and Adrianu Mare [ORBÁN, 1991] (Fig. 1). The landscape is specific to an intra-Carpathian depression with tectonic nature and plateau features. Altitude varies between 320 and 600 m. The average slope inclination is of 18°, but slow or moderate inclinations are dominating. The depression was filled with marl, argil, sand, intercalation of tuff and sandstone, all arranged in domes and wide cuvettes. The most common rocks are loam and argil. In the river meadow, at the base it can be found gravel and boulder trapped in a sandy mass while in the upper layer sand and mud. The soils are represented by various types. The predominating one is the luvosol, followed by regosol and faeziom. The meadow part of the Niraj valley presents aluviosol and hydromorphic soil [MAC, 1972, JOSAN, 1979, BLAGA & al. 2005]. The study area belongs to the temperate continental climate. The average annual temperature is 8.5 °C. The average annual rainfall is 600 mm. River Niraj rises from the altitude of 1300 m from the volcanic mountains of Gurghiu. Natural course length is 79 km. It flows into the River Mureş near the locality called Ungheni [ÚJVÁRI, 1972]. Settlements in the study area are located on the right side of the River Niraj, and in the valley of the Lucion and Dorman rivulets. Deciduous forests (about 2809 ha) are stationed on the left side of the Niraj river and belong to the Forest Departments from Târgu-Mureş and Ghindari. Floodplain vegetation, which was once represented by

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floodplain forests, is characterized by narrow strips of shrubs and coppices. On the shaded slopes there are present scrubs composed mainly of the formerly forests shrub layer [CSŰRÖS, 1963]. Meadows are used as pastures and hayfields.

The aim of the present study was the identification and characterization of the woody associations, for a better knowledge of the habitats that play an important role in sustaining a large number of endangered bird species. This is the first step in the long-term conservation of these species and their habitats. The study provides a description of the woody vegetation on habitat types, one of the objectives of our doctoral thesis.

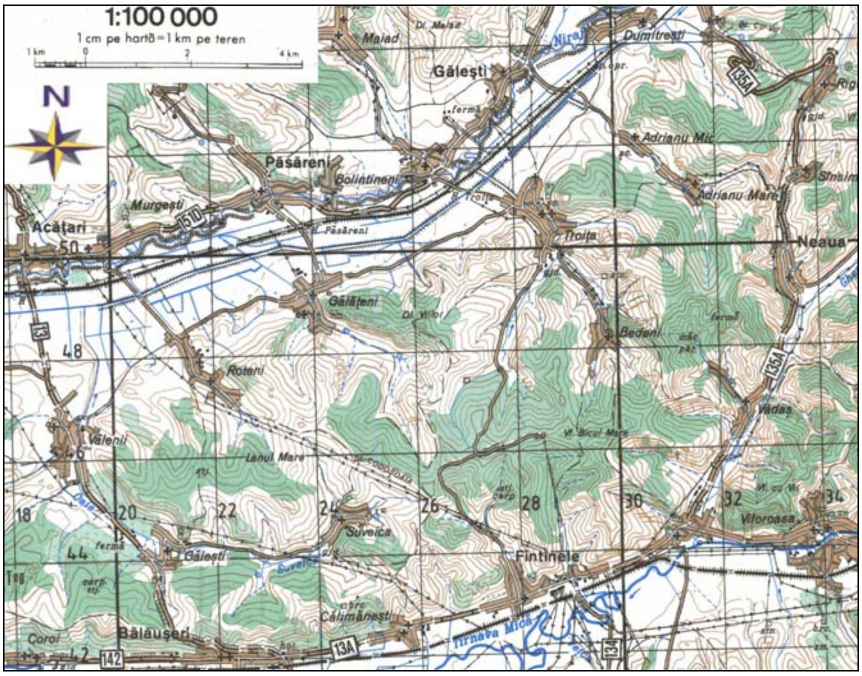

Fig. 1. The middle stream of the Niraj valley-Romania, Mureş County (Military Topographic Directorate, 1997).

Materials and methods

In this study of the woody vegetation, for describing plant communities, we used the phytosociological research method of Central European School, based on the principles and methodology developed by BRAUN-BLANQUET (1964) and adapted by BORZA & BOŞCAIU (1965) to the features of vegetation cover in our country. Phytocoenologic relevés including floristic and physiognomic homogeneous sample surfaces were chosen in the woody vegetation [CRISTEA & al. 2004]. In this sense we conducted field trips during 2011-2012. For the nomenclature of the taxa *Flora ilustrată a României-Pteridophyta et Spermatophyta* [CIOCÂRLAN, 2009] was used. Habitat classification was made according to DONIŢĂ & al. (2005) and GAFTA & MOUNTFORD (2008).

Results

The associations presented in this work have been included in the following phytocoeno-system according to SANDA (2002) and SANDA & al. (2008):
Salicetea purpureae Moor 1958
Salicetalia purpureae Moor 1958
Salicion triandrae Th. Müller et Görs 1958
Salicetum triandrae Malcuit 1929
Salicion albae Soó 1930 em. Th. Müller et Görs 1958
Salici-Populetum Meijer-Drees 1936
Querco-Fagetea Braun-Blanquet et Vlieger in Vlieger 1937 em. Borhidi 1996
Fagetalia sylvaticae Pawlowski in Pawlowski et al. 1928
Symphyto cordati-Fagion Vida 1959
Lathyro hallersteinii-Carpinenion Boşcaiu et al. 1982
Carpino-Fagetum Paucă 1941
Carpino-Quercetum petraeae Borza 1941
Quercetalia roboris R. Tüxen 1931
Genisto germanicae-Quercion Neuhäusl et Neuhäuslová-Novotná 1964
Genisto tinctoriae-Quercetum petraeae Klika 1932 subass. *melicetosum uniflorae* (Gergelý 1962) Sanda et Popescu 1999
Rhamno-Prunetea Rivas Goday et Borja Carbonell 1961
Prunetalia spinosae R. Tüxen 1952
Prunion spinosae Soó 1951
Pruno spinosae-Crataegetum (Soó 1927) Hueck 1931

Salicetum triandrae Malcuit 1929

The association grows in form of a continuous strip along the River Niraj, just above the flowing water or on higher places of the riverbanks. In the *Salicetum triandrae* association 50 vascular plant species were found (Tab. 1). The bush layer has a height of 2 to 8 m. The *Salicetum triandrae* association forms the habitat type: Thickets of willow (*Salix triandra*) (code: R4416), corresponding to Emerald-!44.1 Riparian willow formations, Corine-44.121 Almond willow-osier scrub, Palearctic Habitats 1999-44.121 Almond willow-osier scrub, Eunis-F9.121 Almond willow-osier scrub.

Salici-Populetum Meijer-Drees 1936

This association appears in the form of a narrow band accompanying the upper part of water courses. It forms a dense riverside coppice. Centuries ago vast floodplain forests were instead, edified by *Salix alba*, *S. fragilis*, *Alnus glutinosa*, *Populus alba* and *P. nigra* in the trees level. In the *Salici-Populetum* association 98 vascular plant species were identified (Tab. 1). Trees diameter vary between 30-80 cm and have a height of 18-23 m. The association *Salici-Populetum* Meijer-Drees 1936 (Syn.: *Salicetum albae* Issler 1924 s.l.=*Salicetum albae-fragilis* R. Tüxen 1937) forms the habitat type: Danube forests of White Willow (*Salix alba*) with *Rubus caesius* (code: R4407), corresponding to Natura 2000-92A0 *Salix alba* and *Populus alba* galleries Emerald-!44.66 Ponto-Sarmatic mixed poplar riverine forest, Palearctic Habitats-44162 Pontic willow galleries, Eunis-G1.1142 Ponto-sarmatic steppe willow galleries.

Carpino-Fagetum Paucă 1941

Hornbeam-beech forests from the study area inhabit restricted areas (less than 5% from the study area), the depths of streams and narrow valleys on luvosol. In the *Carpino-Fagetum* association 59 vascular plants were identified (Tab. 2). Age of these forests is between 65 and 110 years. Beech diameter is between 10 and 65 cm, height between 10 and 28 m. Hornbeam diameter is between 20 and 40 cm and height between 15 and 22 m. The association forms the following habitat type: Dacian forests of beech (*Fagus sylvatica*) and hornbeam (*Carpinus betulus*) with *Carex pilosa* (code-R4119). We noted the correspondence with other habitat classification systems: Natura 2000-9130 *Asperulo-Fagetum* beech forests, Emerald-!41.2 Beech forests, Corine-, Palearctic Habitats-41.1D22 Dacian hairy sedge beech-hornbeam forests, Eunis-G1.6D22 Dacian hairy sedge beech/hornbeam.

Carpino-Quercetum petraeae Borza 1941 (Syn.: *Querco petraeae-Carpinetum* Soó et Pócs 1957)

This association evolved from oak forests as a result of oak deforestation. Oak-hornbeam forests from the study area inhabit especially less or moderately inclined slopes on week acid luvosol. The associations occupy the largest area (about 80%) of the deciduous forest habitats in the middle stream of the Niraj valley. In the sloped valleys or at higher altitudes beech appears which abundance-dominance values can reach from 1 to 3. In the *Carpino-Quercetum petraeae* association 118 vascular plants were identified (Tab. 2). Oak diameter is between 15 and 100 cm and height between 15 and 30 m. Hornbeam diameter is between 5 and 40 cm and height between 5 and 25 m. Age of the oak and hornbeam forests varies between 20 and 135 years. The association *Querco petraeae-Carpinetum* Soó et Pócs 1957 forms the following habitat type: Dacian forests of sessile oak (*Quercus petraea*), beech (*Fagus sylvatica*) and hornbeam (*Carpinus betulus*) with *Lathyrus hallersteinii* (code: R4124), corresponding to Natura 2000-91 Y0 Dacian oak-hornbeam forests, Emerald-!41.2 Oak-hornbeam forests, Corine-, Palearctic Habitats-41.2C12 Dacian *Lathyrus hallersteinii* oak-hornbeam forests, Eunis-G1.A1C1 Dacian oak-hornbeam forests.

Genisto tinctoriae-Quercetum petraeae Klika 1932 subass. *melicetosum uniflorae* (Gergelý 1962) Sanda et Popescu 1999 (Syn.: *Melico uniflorae-Querceto petraeae* Gergelý 1962)

This subassociation is installed on the crest of the hills or on the upper part of the slopes on luvosol. It is representing about 8% of the deciduous forest habitats from the study are. In the sessile oak forests 75 vascular plants were identified (Tab. 3). Age of these forests is between 95 and 110 years. Sessile oak diameter is between 20 and 70 cm and height between 22 and 28 m. The association *Genisto tinctoriae-Quercetum petraeae* Klika 1932 (Syn.: *Luzulo albidae-Quercetum petraeae* (Hillitzer 1932) Passarge 1953 em. R. et Z. Neuhäusl 1967) forms the habitat type: Dacian forests of oak (*Quercus petraea*) and beech (*Fagus sylvatica*) with *Festuca drymeia* (code: R4129), corresponding to Emerald-!41.2 Oak-hornbeam forests, Palearctic Habitats-41.7A151. Getic pre-Carpathic Festuca drymeia oak forest, Eunis-G1.8713. Pre-Carpathian beech sessile oak forest.

***Pruno spinosae-Crataegetum* (Soó 1927) Hueck 1931**

Prunus spinosa and *Crataegus monogyna* bushes are encountered at the edge of woods, on cleared sites or sunny coasts. In the association 112 species were identified. According to DONIȚĂ & al. (2005) the association forms the habitat type: Ponto Pannonic scrubs of blackthorn (*Prunus spinosa*) and hawthorn (*Crataegus monogyna*) (Code: R3122), corresponding to Natura 2000-40A0* Subcontinental peri-Pannonic scrub, Emerald-31.8B1 Pannonic and sub-Pannonic thickets, Corine-31.8B3 South-eastern sub-Mediterranean deciduous thickets, Palearctic Habitats-31.8B131 Peri-Pannonic hawthorn-blackthorn scrub, Eunis-F3.241 Central European subcontinental thickets. According to other authors [GAFTA & MOUNTFORD, 2008] the association is not indicated in this habitat type, because the distribution of the association exceeds the Peri-Pannonic area.

Tab. 1. **A -** *Salicetum triandrae* Malcuit 1929; **B** - *Salici-Populetum* Meijer-Drees 1936

	A	**B**	
Number relevés	4	7	
Altitude (m)	320-330	330-350	
Cover tree layer (%)	-	30-60	
Cover shrub layer (%)	95-100	20-70	
Cover herb layer (%)	50-70	30-70	
Surface (m^2)	100	400	
	AD	**AD**	**K**
Salicion, Salicetalia et Salicetea purpureae			
Salix alba	+-1	1-3	V
Salix x *rubens*	-	+-1	V
Populus nigra	-	1-3	V
Salix fragilis	+	1-3	V
Salix triandra	1-5	+-1	IV
Salix viminalis	+-3	+-1	IV
Symphytum officinale	+	+	IV
Populus alba	-	+-1	III
Salix purpurea ssp. *lambertiana*	1	+-1	III
Calystegia sepium	+	+	III
Cucubalus baccifer	+	+	III
Saponaria officinalis	+	+	III
Polygonum hydropiper	+	+	I
Alnion glutinosae			
Alnus glutinosa	+	+-1	V
Humulus lupulus	+-1	1-2	V
Eupatorium cannabinum	+-1	+-1	IV
Prunion, Prunetalia et Rhamno-Prunetea			
Sambucus nigra	+	+	IV
Corylus avellana	-	+	III
Cornus sanguinea	+	+-1	III
Euonymus europaea	-	+	III
Prunus spinosa	-	+	III
Clematis vitalba	+	+-1	III
Ligustrum vulgare	-	+	II

Phragmiti-Magnocaricetea			
Phragmites australis	1-2	1-3	V
Lythrum salicaria	+	+	II
Arrhenatheretalia et Molinio-Arrhenatheretea			
Angelica sylvetris	+	+-1	IV
Achillea millefolium	+	+	III
Galium mollugo	-	+	III
Prunella vulgaris	-	+	II
Vicia cracca	+	+	II
Lotus corniculatus	-	+	II
Agrostis stolonifera ssp. *stolonifera*	+-1	-	-
Sonchus arvensis ssp. *uliginosus*	+	-	-
Festuco-Brometea			
Euphorbia salicifolia	+	+	V
Salvia pratensis	-	+	II
Bromus inermis	+	-	-
Stellarietea mediae			
Galeopsis tetrahit	-	+-1	III
Brassica nigra	+	+	III
Capsella bursa-pastoris	-	+	II
Galio-Urticetea			
Urtica dioica	1	+-1	IV
Galium aparine	+-1	+-1	III
Rubus caesius	1-3	2-3	III
Myosoton aquaticum	+	+	I
Artemisietea			
Tanacetum vulgare	+-1	+	III
Artemisia vulgaris	+-1	+	II
Silene latifolia ssp. *alba*	+	+	I
Variae syntaxa			
Petasites hybridus	1	+-2	V
Echynocystis lobata	+-3	1-2	V
Brachypodium sylvaticum	-	+	III
Erigeron acris	-	+	III
Parthenocissus quinquefolia	+	+	III
Fallopia baldschuanica	-	1	II
Carex hirta	-	+	II
Oenothera parviflora	-	+	II
Equisetum telmateia	+	+	II
Dactylis polygama	-	+	II
Aegopodium podagraria	-	+	II
Robinia pseudacacia	+	+	II
Echinochloa crus-galli	-	+-1	II
Lamium album	-	+	II
Chelidonium majus	-	+	II
Senecio ovatus	+	+	I
Galeopsis speciosa	+	-	-

Species found in a single relevé:
A. *Xanthium italicum* (4, +), *Mentha longifolia* (4, +), *Mentha arvensis* ssp. *arvensis* (4, +), *Rumex crispus* (4, +), *Elymus repens* (4, +), *Daucus carota* (1, +), *Erigeron annuus* (4, +), *Centaurea nigrescens* (4, +), *Polygonum amphybium* f. *terrestre* (4, +), *Sonchus arvensis* ssp. *arvensis* (4, +). **Place of relevés:** 1, 2: Păsăreni; 3, 4: Murgeşti.
B. *Crataegus monogyna* (4, +), *Acer negundo* (5, +), *Scrophularia scopolii* (1, +), *Actaea spicata* (4, +), *Stellaria nemorum* (4, +), *Ranunculus repens* (1, +), *Cnidium dubium* (4, +), *Carex riparia* (6, +), *Veronica chamaedrys* (1, +), *Taraxacum officinale* (1, +), *Cirsium palustre* (5, +), *Filipendula vulgaris* (5, +), *Lysimachia vulgaris* (5, +), *Arrhenatherum elatius* ssp. *elatius* (5, +), *Centaurea indurata* (5, +), *Senecio doria* (4, +), *Lysimachia nummularia* (7, +), *Mentha aquatica* (7, +), *Chenopodium album* (4, +), *Papaver rhoeas* (5, +), *Glechoma hederacea* (2, +), *Carduus crispus* (4, +), *Barbarea vulgaris* ssp. *vulgaris* (1, +), *Alliaria petiolata* (1, +), *Lapsana communis* (4, +), *Solidago canadensis* (4, +), *Chaerophyllum aromaticum* (5, +), *Solanum nigrum* (6, +), *Carduus acanthoides* (4, +), *Tussilago farfara* (4, +), *Conium maculatum* (4, +), *Ballota nigra* ssp. *nigra* (6, +), *Malva sylvestris* ssp. *sylvestris* (6, +), *Oxalis fontana* (5, +), *Sonchus oleraceus* (5, +), *Rudbeckia laciniata* (5, +), *Fallopia japonica* (5, +), *Rumex sanguineus* (5, +), *Agrimonia eupatoria* (5, +). **Place of relevés:** 1, 3: between Murgeşti and Păsăreni; 2, 4: between Păsăreni and Bolintineni; 5: near Găleşti; 6, 7: near Dumitreşti.

Tab. 2. A - *Carpino-Fagetum* Paucă 1941; **B** - *Carpino-Quercetum petraeae* Borza 1941

	A		**B**	
Number relevés	11		88	
Altitude (m)	400-480		420-600	
Exposition	SE; SV		N; NV; NE	
Slope (°)	5-20		5-30	
Cover tree layer (%)	80-90		60-90	
Cover herb layer (%)	5-80		5-90	
Surface (m^2)	400		400	
	AD	**K**	**AD**	**K**
Lathyro-Carpinion				
Carpinus betulus	+-4	V	+-4	V
Carex pilosa	+-2	V	+-3	III
Tilia cordata	-	-	+-1	II
Stellaria holostea	+-1	II	+-3	II
Cerasus avium	-	-	+-2	I
Erythronium dens-canis	+	II	+	I
Scilla bifolia	+	II	+-1	I
Dactylis polygama	+	I	+-2	I
Vinca minor	-	-	+-2	I
Dentaria glandulosa	-	-	+-2	I
Lathyrus laevigatus	-	-	+	I
Fagetalia				
Fagus sylvatica	3-5	V	+-3	II
Dentaria bulbifera	+-2	IV	+-4	IV
Galeobdolon luteum	+-2	V	+-3	IV
Pulmonaria officinalis	+	V	+-2	III
Euphorbia amygdaloides	+-1	IV	+-1	III
Galium odoratum	+-1	IV	+-3	III

Carex sylvatica	+-1	IV	+-1	II
Asarum europaeum	+-3	IV	+-2	II
Rubus caesius	+	III	+-1	II
Lathyrus vernus	+	III	+	I
Polygonatum multiflorum	+	II	+-1	II
Acer pseudoplatanus	+-1	II	+	I
Anemone nemorosa	+-1	I	+-3	IV
Dryopteris filix-mas	+	I	+-1	II
Athyrium filix-femina	+	I	+	II
Sanicula europaea	+	I	+	I
Maianthemum bifolium	+	I	+	I
Salvia glutinosa	+	I	+-1	I
Stachys sylvatica	+	I	+-1	I
Fragaria vesca	+	I	+	I
Geranium robertianum	+	I	+-1	I
Alliaria petiolata	+	I	+-1	I
Circaea lutetiana	-	-	+	II
Anemone ranunculoides	-	-	+-1	II
Scrophularia nodosa	-	-	+-1	II
Helleborus purpurascens	-	-	+	I
Isopyrum thalictroides	-	-	+	I
Lilium martagon	-	-	+	I
Carex divulsa	-	-	+	I
Rubus hirtus	-	-	+	I
Arum maculatum	-	-	+-1	I
Cystopteris fragilis	-	-	+	I
Querco-Fagetea				
Quercus petraea	+-1	IV	+-4	V
Viola reichenbachiana	+	V	+-2	V
Geum urbanum	+	III	+	II
Mycelis muralis	+	I	+	II
Brachypodium sylvaticum	+-1	III	+-1	II
Sambucus nigra	+	I	+	II
Rosa canina	+	III	+	II
Convallaria majalis	+	I	+-1	II
Populus tremula	-	-	+-2	I
Acer campestre	-	-	+-2	I
Quercus robur	-	-	+-1	I
Crataegus monogyna	-	-	+-1	I
Corylus avellana	+	I	+-1	I
Clematis vitalba	+	II	+	I
Ligustrum vulgare	-	-	+	I
Polygonatum latifolium	+-1	III	+-1	I
Ulmus minor	-	-	+	I
Galium schultesii	+	I	+	I
Hedera helix	+	I	+	I
Neottia nidus-avis	+	II	+	I

Lapsana communis	+	I	+	I
Euonymus europaea	-	-	+	I
Poa nemoralis	-	-	+	I
Hypericum perforatum	-	-	+	I
Aegopodium podagraria	+	II	+-1	I
Prunus spinosa	-	-	+	I
Melica nutans	-	-	+	I
Euonymus verrucosa	+	I	+	I
Ranunculus ficaria	-	-	+-3	I
Lathyrus niger	+	I	+	I
Vicia dumetorum	-	-	+	I
Ranunculus auricomus	+	I	+	I
Staphylea pinnata	+	I	+	I
Symphytum tuberosum	+	I	+	I
Campanula rapunculoides	+	I	+	I
Acer pseudoplatanus	1	I	-	-
Quercion, Quercetalia et Quercetea pubescentis				
Melica uniflora	+	I	+-2	II
Sorbus torminalis	-	-	+	I
Melittis melisophyllum	+	I	+	I
Cephalanthera damasonium	-	-	+	I
Polygonatum odoratum	-	-	+	I
Vincetoxicum hirundinaria	-	-	+	I
Tanacetum corymbosum	-	-	+	I
Carex montana	-	-	+	I
Arrhenatheretalia et Molinio-Arrhenatheretea				
Ajuga reptans	+-1	V	+-1	III
Prunella vulgaris	-	-	+	I
Lysimachia nummularia	+	I	+	I
Campanula patula	-	-	+	I
Veronica chamaedrys	+	I	+	I
Anthriscus sylvestris	-	-	+	I
Variae syntaxa				
Galeopsis bifida	-	-	+-2	II
Fallopia dumetorum	-	-	+	II
Juncus effusus	-	-	+	I
Erigeron acris	-	-	+	I
Urtica dioica	-	-	+	I
Galium aparine	-	-	+	I
Stellaria alsine	-	-	+	I
Chelidonium majus	-	-	+	I
Oxalis fontana	-	-	+	I
Torilis japonica	-	-	+	I
Veronica officinalis	-	-	+	I

Species found in a single relevé:

A. *Campanula trachelium* (11, +), *Holcus lanatus* (11, +). **Place of relevés:** 1-3, 5-11: Fântânele; 4-Capu Fagului-Fântânele.

B., *Lathyrus hallersteinii* (8, +), *Milium effusum* (10, +), *Crocus vernus* (16, +), *Mercurialis perennis* (11, 2), *Paris quadrifolia* (12, +), *Campanula persicifolia* (14, +), *Viola odorata* (16, +), *Platanthera bifolia* (17, +), *Atropa bella-donna* (18, +), *Myosoton aquaticum* (19, +), *Robinia pseudacacia* (20, +), *Chenopodium album* (21, +), *Galinsoga parviflora* (22, +), *Juglans regia* (23, +), *Leucanthemum vulgare* (24, +). **Place of relevés:** 1-3: Păsăreni; 4, 75-79: Bedeni; 5, 8, 10-18: Roteni; 6-9: Văleni; 19-39, 82-88: La Săgeata-Adrianu Mare; 40-45: Găieşti; 46, 47: Şanţ-Suveica; 48-59, 80, 81: Troiţa; 60-63: Dumitreştilor-Adrianu Mic; 64: Neaua; 65-74: Fântânele.

Tab. 3. **A** - *Genisto tinctoriae-Quercetum petraeae* Klika 1932 subass. *melicetosum uniflorae* (Gergelý 1962) Sanda et Popescu 1999; **B** - *Pruno spinosae-Crataegetum* (Soó 1927) Hueck 1931

	A		**B**	
Number relevés	12		14	
Altitude (m)	459-550		387-480	
Exposition	N; NE		N; NV	
Slope (°)	5-20		5-30	
Cover tree layer (%)	60-70		-	
Cover shrub layer (%)	0-0,5		60-90	
Cover herb layer (%)	40-90		5-40	
Surface (m^2)	400		50	
	AD	**K**	**AD**	**K**
Genisto germanicae-Quercion				
Lathyrus niger	+-1	V	-	-
Trifolium medium ssp. *medium*	+	II	+	II
Lathyro-Carpinion				
Carpinus betulus	+-1	V	+	I
Stellaria holostea	+-2	V	-	-
Carex pilosa	+-1	IV	+	II
Dactylis polygama	+	II		
Cerasus avium	+	I	+	II
Fagetalia				
Euphorbia amygdaloides	+	V	+	I
Galeobdolon luteum	+-2	V	-	-
Galium odoratum	+	IV	-	-
Ajuga reptans	+	IV	-	-
Alliaria petiolata	+	IV	+	I
Dentaria bulbifera	+-1	III	-	-
Lathyrus vernus	+	III	+	I
Pulmonaria officinalis	+	III	+	I
Carex sylvatica	+	III	-	-
Circaea lutetiana	+	II	-	-
Anemone nemorosa	+	II	-	-
Stachys sylvatica	+	II	-	-
Geranium robertianum	+	II	+	I

Querco-Fagetea				
Viola reichenbachiana	+-1	V	+	II
Convallaria majalis	+-1	IV	-	-
Acer campestre	+	III	+	II
Galium schultesii	+-2	III	-	-
Polygonatum latifolium	+	III	+	I
Brachypodium sylvaticum	+-1	III	+-2	IV
Mycelis muralis	+	III	-	-
Rosa canina	+	II	+-2	V
Geum urbanum	+	II	-	-
Campanula rapunculoides	+	I	+	II
Sedum maximum	+	I	-	-
Quercus robur	+	I	+	I
Quercetalia et Quercetea pubescentis				
Melica uniflora	2-4	V	-	-
Quercus petraea	4	V	+	I
Melittis melissophyllum	+	II	-	-
Vincetoxicum hirundinaceum	+	II	-	-
Pyrus pyraster	+	I	+	II
Coronilla varia	+	I	+	I
Prunion, Prunetalia, Rhamno-Prunetea				
Crataegus monogyna	+	I	2-4	V
Prunus spinosa	+	I	1-4	V
Ligustrum vulgare	+	II	+-1	IV
Cornus sanguinea	+	I	+-2	IV
Clematis vitalba	+	III	+-1	IV
Glechoma hederacea	-	-	+	III
Origanum vulgare	-	-	+-1	III
Rubus caesius	+-1	III	+-1	III
Fragaria vesca	+	I	+	III
Viburnum opulus	-	-	+	II
Sambucus nigra	+	I	+	II
Hypericum perforatum	+	I	+	II
Euonymus europaea	+	II	+	II
Euonymus verrucosa	-	-	+-2	II
Astragalus glycyphyllos	+	I	+	I
Festuco-Brometea				
Agrimonia eupatoria	+	I	+	IV
Euphorbia cyparissias	+	I	+-1	III
Dorycnium herbaceum	-	-	+-1	III
Galium verum	-	-	+-1	II
Filipendula vulgaris	-	-	+-1	II
Salvia pratensis	-	-	+	II
Scabiosa ochroleuca	-	-	+	II
Euphorbia salicifolia	-	-	+	II
Pimpinella saxifraga	-	-	+	I

Salvia verticillata	-	-	+	I
Arrhenatheretalia et Molinio-Arrhenatheretea				
Achillea millefolium	-	-	+-1	III
Prunella vulgaris	+	I	+	III
Agrostis capillaris	-	-	+	II
Mentha longifolia	-	-	+	II
Galium mollugo	-	-	+	II
Leucanthemum vulgare	-	-	+	II
Knautia arvensis	-	-	+	I
Juncus effusus	-	-	+	I
Stellaria graminea	-	-	+	I
Campanula patula	-	-	+	I
Briza media	-	-	+	I
Dactylis glomerata	-	-	+-1	I
Artemisietea vulgaris				
Dipsacus laciniatus	-	-	+-1	I
Tanacetum vulgare	-	-	+	I
Galium aparine	+	I	-	-
Stellarietea mediae				
Equisetum arvense	-	-	+	I
Mentha arvensis	-	-	+	I
Variae syntaxa				
Galeopsis bifida	+-1	IV	-	-
Fallopia dumetorum	+	IV	-	-
Torilis japonica	+	II	-	-
Erigeron acris	+	I	+	I
Aegopodium podagraria	-	-	+	II
Pulicaria dysenterica	-	-	+	I
Bupleurum falcatum var. *falcatum*	-	-	+	II
Centaurea stenolepis ssp. *stenolepis*	-	-	+	I

Species found in a single relevé:

A. *Tilia cordata* (4, +), *Polygonatum multiflorum* (6, +), *Salvia glutinosa* (5, +), *Rubus idaeus* (3, +), *Milium effusum* (8, +), *Dryopteris filix-mas* (4, +), *Carex divulsa* ssp. *chabertii* (6, +), *Lapsana communis* (4, +), *Acer pseudoplatanus* (4, +), *Sorbus torminalis* (3, +), *Scrophularia nodosa* (5, +), *Poa nemoralis* (6, +), *Polygonatum odoratum* (3, +), *Lithospermum purpurocaeruleum* (2, +), *Lathyrus laevigatus* (3, +), *Stellaria nemorum* (10, +), *Anthriscus sylvestris* (10, +), *Inula britannica* (9, +). **Place of relevés:** 1, 2, 5-7, 9, 10, 12: Troița; 3, 11: Dealu de Mijloc-Troița; 4, 8: Fântânele.

B. *Malus sylvestris* (2, +), *Acer platanoides* (11, +), *Fagus sylvatica* (8, +), *Ranunculus auricomus* (8, +), *Festuca rupicola* ssp. *rupicola* (8, +), *Eryngium campestre* (14, +), *Carlina vulgaris* (14, +), *Fragaria viridis* (3, +), *Medicago falcata* (11, +), *Potentilla recta* (2, +) *Potentilla argentea* ssp. *argentea* (2, +), *Thymus glabrescens* (2, 1), *Helianthemum canum* (2, +), *Hieracium pilosella* (2, +), *Bromus erectus* ssp. *erectus* (10, +), *Cerinthe minor* ssp. *minor* (3, +), *Cytisus nigricans* (8, +), *Poa pratensis* (14, +), *Ononis arvensis* (14, +), *Colchicum autumnale* (13, 1), *Leontodon hispidus* (14, +), *Pastinaca sativa* ssp. *sylvatica* (13, +), *Lychnis flos-cuculi* (2, +), *Veronica chamaedrys* (2, +), *Stachys officinalis* (3, +), *Lysimachia nummularia* (3, +), *Rumex crispus* (12, +), *Carex tomentosa* (8, +), *Holcus lanatus* (14, +), *Tragopogon orientalis* (7, +), *Calamagrostis epigejos* (8, +), *Carduus acanthoides* (2, +), *Elymus repens* (10, +), *Linaria vulgaris* (12, +), *Cirsium arvense* (13, +), *Adonis*

aestivalis (2, +), *Anagallis arvensis* (3, +), *Lathyrus aphaca* (3, +), *Sambucus ebulus* (11, +), *Daucus carota* (4, +), *Scabiosa argentea* (12, +), *Mentha pulegium* (14, +), *Myosotis arvensis* (3, +), *Eryngium planum* (8, +), *Verbascum nigrum* ssp. *nigrum* (8, +), *Clinopodium vulgare* (10, +), *Veronica officinalis* (10, +). **Place of relevés:** 1, 2, 13, 14: Dealul Tolugheț-Bedeni; 3-between Găieşti and Suveica; 4-Dealul Ou-Găieşti; 5-between Bălăuşeri and Suveica; 6-8: Dealul Gorjat-Gălățeni; 9-near Fântânele; 10-Capu Fagului-Fântânele.

Discussions

In the middle stream of the Niraj valley one of the six encountered habitat types is of Community Interest, the conservation of which requires the designation of Special Areas of Conservation, according to the Habitats Directive: R4407-Danube forests of white willow (*Salix alba*) with *Rubus caesius*. Regarding the capacity of sustaining the avifauna, the deciduous forest habitats are the most important from the studied habitats. Unfortunately forests had suffered serious damages in the past decades till 2008. Because of intensive grazing in the forest, deforestation and exploitation of timber, hornbeam proliferated in these forests excessively. The studied habitats give shelter for a considerably number of jeopardized, rare and vulnerable species: *Dentaria glandulosa* (Carpathian Endemism); species registered on the Romanian Red List [OLTEAN & al. 1994] – *Neottia nidus-avis*, *Platanthera bifolia* (rare), *Cephalanthera damasonium* (not threatened), *Lilium martagon* (rare); species with peculiar areal of distribution – *Lathyrus hallersteinii*, *Crocus vernus* (Carpathian-Balkan), *Helleborus purpurascens* (Daco-Balkan), *Centaurea indurata* (Dacic); species in the category of threat for entire Carpathians [WITKOWSKI & al. 2003] with vulnerable status-*Erythronium dens-canis*, *Adonis aestivalis*; IUCN Red List of Threatened Species – *Alnus glutinosa*. From this reason they must be protected from any exploitation. The blackthorn and hawthorn scrubs together with agricultural lands are important bird feeding habitats and nesting habitats. All of the studied associations contribute to habitat heterogeneity and promote species diversity.

Conclusions

In the middle stream of the Niraj valley six forestry associations were revealed: *Salicetum triandrae* Malcuit 1929, *Salici-Populetum* Meijer-Drees 1936, *Carpino-Fagetum* Paucă 1941, *Carpino-Quercetum petraeae* Borza 1941, *Genisto tinctoriae-Quercetum petraeae* subass. *melicetosum uniflorae* (Gergelý 1962) Sanda et Popescu 1999 and *Pruno spinosae-Crataegetum* (Soó 1927) Hueck 1931. The description of the forestry vegetation was made on habitat types, based on the latest international regulations. The studied habitats give shelter for a considerably number of jeopardized, rare and vulnerable plant species. The results provide us information in establishing a viable and applicable management plan for the existing Special Protection Area.

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We thank engineer Gabriel Darida from Romsilva, forester Siklódi Attila and forestry technician Aszalos Ottó Szabolcs for providing bibliography, guidance and information about the forest habitats. Special thanks are due to Daróczi J. Szilárd from the Milvus Group for giving help in surveying of the important habitats in term of ornithology from the study area.

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*** Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora.

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Happy Anniversary, botanist dr. Ion SÂRBU!

On September, 17th, 1933, was born the Romanian botanist Ion Sârbu, in Tudor Vladimirescu commune (Galați county). He followed the primary school classes in the same village (Suceveni today). The secondary and high school courses were attended at the Normal School "Costache Negri" in Galați (Pedagogic High School for now), which he graduated in 1952. Further on, he goes on the Faculty of Natural Science in Iași, at the speciality of Biology-Geography, and graduate it in 1963, with the final exam in 1964.

Between 1963 and 1967 he worked as a teacher of biology and geography and as a School Inspector, in District of Bujor, Galați Region. In 1967 he held through competition a biologist position in the Department of Botany, at the University "Alexandru Ioan Cuza" in Iași, where besides researches in flora and phytocenology, he took hours of practical work laboratory and field in Systematic Botany, Nature Protection, Biodiversity and aquatic environments and taught a graduate course on forest resources. In 1975 Mr. Ion Sârbu was accepted as a researcher at the Botanical Garden of the University, where he realized, on an area of 25 hectares, the "Romanian Flora and Vegetation" section. For many years he served as the Scientific Secretary of the Botanic Garden, supporting with his skills and competency the various community events in which the institution was involved.

He get his PhD title in Biology at the same University, in 1978, with a thesis called *Flora şi vegetația din Bazinul Chinejii şi al Prutului între Rogojeni-Mastacani* / *Flora and vegetation of Chinejii and Prut Basin between Rogojeni-Mastacani* / under the guidance of the Professor Constantin Burduja.

In 2006 he was granted with the special prize "EMANOIL TEODORESCU" of the Romanian Academy of Science, for the 4 volumes book called *Flora lemnoasă spontană şi cultivată din România* / *The spontaneous and cultivated lignaceous flora of Romania* (în collaboration).

The research carried out has resulted in the publication of a number of 121 papers, of which 5 in journals abroad (Germany, Italy, Sweden); he also published, in collaboration, a total of 10 books in Romania, and collaborated to other 4 books published abroad (Finland, Netherland, Germany, Sweden and Italy). The published papers relate to vascular plant taxonomy, chorology, phytocoenology, and environmental protection, being referred in all specialty synthetic works in the country.

The whole research activity resulted in the publication of many reference works, such us:

- an illustrated flora of vascular plants in eastern Romania, volumes I and II / 2001 (781 pages), in collaboration;
- the spontaneous woody flora cultivated in Romania, volumes I, II, III, IV / 1996-2004 (1407 pages), in collaboration;
- explanation to the vegetation map of the Romanian Danube Delta Biosphere Reserve/ 1993, in collaboration;

– vegetation of the Biosphere Reserve "Danube Delta" with transboundari vegetation map/ 2002, in collaboration;
– vascular plant species threatened in Moldova / 2005;
– red lists of species and animals in the Danube Delta Biosphere Reserve;
– a guide to the identification and inventory of seminatural grasslands in Romania / 2001, in collaboration;
– guidelines for identification and protection of Important Plants Areas in Romania / 2003, in collaboration.

He has an internationally recognition, attested by a continue working and collaboration to several great works of synthesis on the European level, as:
– *Atlas Florae Europaeae*, volumes 12, 13, and 14 (in Helsinki)
– *Euro+Med PlantBase* (a database on www for "Flora Europaea" – romanian contributions).

He participated in the development and implementation of the "Natura 2000" protected area network in Romania, with the selection and preparing standard formulars for 273 sites of European interest, being also a member of a team for validation of all proposed "Natura 2000" sites in Romania. He was also involved in 18 national projects, most of them on the biodiversity of the Danube Delta flora and vegetation, development of management plans, the study of alternative solutions to reduce human impact on ecosystems in the Danube Delta. He initiated and led projects on capacity assessment of conservative protected areas in Moldova or assessment of the current status of protected areas in eastern Romania.

Though he is retired at his 70 years old, he continue to work on botany field, coming weekly at the Botanic Garden "Anastasie Fătu" in Iaşi, where all his collaborators ask him to give many valuable advices on the romanian flora and vegetation, or on the garden history, checking-up a lot of sample seeds for the "Seeds Catalogue" every year, on the herbarium collection, and so on.

The whole experience of him on botany was materialized in a 6-year project, publishing thus a comprehensive field guide for the vascular flora of the whole Romania (Sârbu I., Ştefan N. & Oprea A. 2013. *Plante vasculare din România. Determinator ilustrat de teren / Vascular Plants of Romania. Illustrated Field Guide/,* Publishing House "Victor B Victor", Bucharest, 1320 pp.), with the ink-drawings and color photos for almost all the species included in this most up-dated book.

Through its scientific work and research projects he is a recognized personality among the Romanian botanists, and not only! Thus, in 2007, the European Conference *Planta Europa*, held in Cluj-Napoca city, awarded him and his activity on the realm of botany with a special prize of this international organization on plant conservation, namely the "LINNAEUS AWARD" and the silver medal "FOR EXCELLENT WORK IN PLANT TAXONOMY".

Botanist Ion Sârbu participated at several scientific meetings in Germany, Austria and Turkey. He also travelled, privately, to Germany, Canada and the Unitated States of America, where he made botanical trips along different regions. At his returns he presented to us some excellent slideshows, delighting us with many aspects on flora and vegetation of those more or less remote countries.

The 80th anniversary represents a special moment both for Botanist PhD Ion Sârbu's life and the entire academic community. All of us want to wish him "Happy anniversary and that all his wonderful dreams become true!"

Adrian OPREA

IN MEMORIAM
PROFESSOR DR. VASILE CIOCÂRLAN

On December, 2nd, 2013, Professor Dr. Vasile Ciocârlan passed away, at his 87 years old. He was born on December, 21st, 1926, in Grumăzeşti village (Neamț county). He followed the primary school classes in the same village; later on, he attend the so-called "Şcoala Normală Gheorghe Asachi" in Piatra Neamț, and graduate it in 1946. Further on, he goes on the Faculty of Natural Science in Iaşi, at the speciality of Biology-Botany, and graduate it in 1951, with the final exam in 1952, with a diploma of merit. Being one of the best students of his generation, he worked temporary as a preparator within the Departament of Botany in the same faculty.

After graduation he made a two-year stage of probation in the Agricultural Secondary School in Calafat, Dolj county. After it, on 20th of November, 1953, he was called as an assistant professor in the Department of Botany, Faculty of Horticulture, Agronomic Institute "Nicolae Bălcescu" in Bucharest. Over the times, he promoted all levels of university hierarchy: Lecturer in 1968, Associate Professor in 1978 and Professor in 1990, honoring with a special competence the agricultural botany field.

In 1968 he get his PhD in biology from the University of Bucharest, with a thesis entitled: *Flora and vegetation of the Subcarpathic Basin of Slănicul de Buzău*, under the guidance of Professor Dr. Traian Ştefureac, a work widely appreciated for remarkable contributions at flora and vegetation in eastern Romania.

In 1974 he get the Romanian Academy Award "Traian Săvulescu" for a notable work entitled *Weeds in agricultural crops and their control*. After 1990, due to the recognition of his professional competence and exigency in scientific research, Professor Vasile Ciocârlan gets right to drive PhD theses at the Faculty of Biology, University of Bucharest, specialty Botany, contributing thus to the formation of a plethora of young botanists. In those 53 years of teaching and scientific career, he led lot of practical laboratory and field undergraduate works. His activity resulted in the publication of many scientific papers, alone or in collaboration, of 18 books and about 90 scientific articles in various journals, all targeting the knowledge of the Romanian flora.

Many published works concern the study of weeds in crops and their control, identification books of weeds, an atlas of the main weeds in Romania, and so on. He published a monumental book of the identification of the vascular Romanian flora, published first of all in 2 illustrated volumes (vol. I in 1988, vol. II in 1990); after it, this book was published again in two succesive, enlarged, updated, and revised editions, in 2000, and 2009. This books was appreciated by all specialists, both in Romania and abroad. This latest edition is the quintessential of his concerns and experience as a teacher and scientist, placing the author in the gallery of the great botanists of Romania. This identification book was requested in England, France, Germany, Netherland, Serbia, Czech Republic, Slovenia, Poland, Ukraine, and Moldova.

Hailed as a specialist in Systematic Botany, he was requested and conducted the chapter no. 4 of the 1st volume called *Ampelografia Republicii Socialiste România – Sistematica familiei Vitaceae*. He was part of the team that studied the flora and vegetation of the "Iron Gates", coordinated by the Romanian Academy. Since 1990 he participated in many research projects, funded by the national authorities or the European Comission, concerning the biodiversity in the Biosphere Reserve Danube Delta, National Park Piatra Craiului, and so on.

He participated in several international scientific symposiums, in Bratislava, Halle, Sofia, where he held papers on taxonomy and chorology of some plant species in Romanian flora.

He was an avid and carefully documented observer of the plant world, publishing a total of 64 taxa new to Romania; also, he discovered and published five taxa new to science and published 19 new nomenclatural combinations. Through his studies of taxonomy, chorology, phytogeography, plus many others related topics in weeds and agricultural crops, Professor Dr. Vasile Ciocârlan was known and appreciated all over the country, by all who working in plant biology, as botanists, phytocenologists, agronomists, foresters and pharmacists, enjoying an unanimous appreciation.

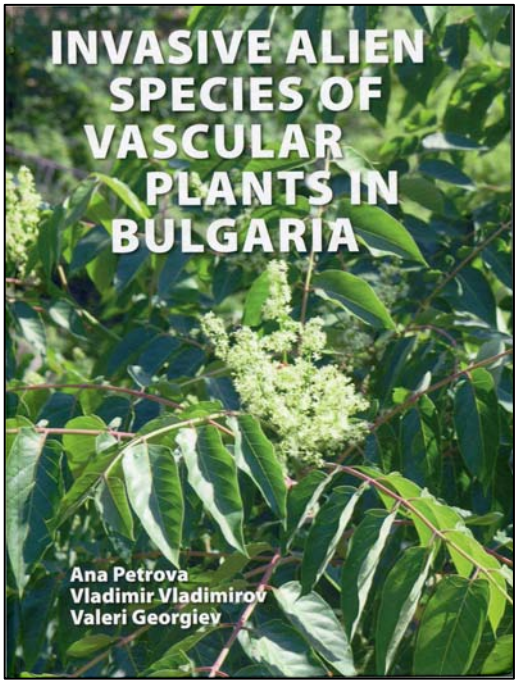
Being one of the greatest personalities of Romanian botany, he passed through different stages of the his life with verticality, facing many difficulties, but he did not made any compromises in the scientific truth and asked the same thing to its colleagues, or aspiring youngs to get into the scientific world. As a botanist he was a nature lover, crossing the whole country over the years, from the seashore to the highest peaks of the Carpathians, helped by a robust constitution and a good health, believing that nature is the best teacher and the most perfect botanical book. His entire life has proven reliability, depth competence and professionalism, ambition and perseverance, with a deep passion for floristic studies, all of these contributing to the settlement of Professor Vasile Ciocârlan among the greatest botanists of Romania.

We all are bringing a pious homage to Professor Dr. Vasile Ciocârlan, and we will bear our utter gratitude for what he has done for us, as romanian botanists.

Adrian OPREA
December, the 5th, 2013

BOOK REVIEW

ANA PETROVA, VLADIMIR VLADIMIROV, VALERI GEORGIEV, *Invasive alien species of vascular plants in Bulgaria*, Sofia, 2013, 319 p., 242 photos, 60 maps, and 434 references.



In 2013 a very valuable book was published at Sofia, namely *Invasive alien species of vascular plants in Bulgaria*, as an output of a national scientific project called "Biology, Ecology and Control of the Invasive Alien Species in the Bulgarian flora" (DO 02-194, 2009-2012), financed by the National Science Fund of the Ministry of Education, Youth and Science of the Republic of Bulgaria. The authors are well known Bulgarian botanists: Ana Petrova, Vladimir Vladimirov, and Valeri Georgiev.

The book has been published in very good technical conditions, with color, detailed photographs, and lot of references for every described plant species.

In the first part of the book, the authors presented a well documented analysis concerning the next principal issues: the impact of invasive species on natural biodiversity; ways and pathways for introduction of the invasive alien plants; international and Bulgarian legislation, organizations and documents related to the invasive alien species; the terminology related to alien plants used in the book; the current state of knowledge on invasive plant species in Europe, Balkan region and Bulgaria; the control of the invasive alien species.

Of the invasive alien species spread in Bulgaria, the authors separated a number of 10 species of the worst invasive ("Top 10"), including the following ones: *Acer negundo*,

Ailanthus altissima, *Ambrosia artemisiifolia*, *Bidens frondosus*, *Elodea nuttallii*, *Fallopia* × *bohemica*, *Opuntia humifusa*, *Paspalum distichum*, and *Robinia pseudacacia*.

We note that the authors consider as invasive those alien species whose introduction and / or spread threaten native biodiversity and natural ecosystems. This definition, which is in the spirit of the Convention on Biological Diversity, differs to some extent from the approach of Richardson et al. (2000), who proposed that the term 'invasive' should be used, without any implication to environmental or economic impact, in order to designate those naturalized species which have a great potential to spread over a considerable area (the cited authors consider the plants, not necessarily alien, that have harmful economic or environmental effects, as weeds and / or transformers).

In the main section of the book, the authors described a number of 60 alien vascular plants species, from 25 plant families, which are invasive and potentially invasive on the teritory of Bulgaria. These 60 species were chosen based on the available data in the botanical references, as well as on the author's experience. Among these, 44 species are of American origins, the others being originated in Asia, Australia, Africa, the Mediterranean region, etc. For each species the authors presented comprehensive information about its morphology, biology and ecology, origin and distribution, control, and finally, a list of main references. Each species is illustrated with high quality original photographs, and distribution maps in Bulgaria, using the UTM-grid network (10 × 10 km grid squares).

We consider this book as an exceptional editorial issue and recommend it warmly to anyone interested in botany, not only in the study of alien plants.

Adrian OPREA, Culiță SÎRBU

JOURNAL OF PLANT DEVELOPMENT
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Examples of papers quotation:

References for papers in periodicals:

CIOCÂRLAN V. 2008. *Lathyrus linifolius* (Reichard) Bässler in the Romanian flora. *J. Plant Develop.*, **15**: 3-6.
MEHREGAN I. & KADEREIT J. W. 2008. Taxonomic revision of *Cousinia* sect. *Cynaroideae* (*Asteraceae*, *Cardueae*). *Willdenowia*. **38**(2): 293-362.

References for books:

BOŞCAIU N. 1971. *Flora şi Vegetaţia Munţilor Ţarcu, Godeanu şi Cernei*. Bucureşti: Edit. Acad. Române, 494 pp.
HILLIER J. & COOMBES A. 2004. *The Hillier Manual of Trees & Shrubs*. Newton Abbot, Devon, England: David & Charles, 512 pp.

Serials:
JALAS J. SUOMINEN J. LAMPINEN R. & KURTTO A. (eds). 1999. *Atlas Florae Europaeae*. *Distribution of vascular plants in Europe*. Vol. **12**. *Resedaceae to Platanaceae*. Helsinki: Committee for Mapping the Flora of Europe and Societas Biologica Fennica Vanamo. Maps 2928-3270, 250 pp., ill (maps), ISBN 951-9108.
TUTIN T. G., BURGES N. A., CHATER A. O., EDMONDSON J. R., HEYWOOD V. H., MOORE D. M., VALENTINE D. H., WALTERS S. M. & WEBB D. A. (eds, assist. by J. R. AKEROYD & M. E. NEWTON; appendices ed. by R. R. MILL). 1996. *Flora Europaea*. 2nd ed., 1993, reprinted 1996. Vol. **1**. *Psilotaceae to Platanaceae.* Cambridge: Cambridge University Press, xlvi, 581 pp., illus. ISBN 0-521-41007-X (HB).
Chapters in books:
†TUTIN T. G. 1996. *Helleborus* L. Pp. 249-251. In: †T. G. TUTIN et al. (eds). *Flora Europaea*. 2nd ed., 1993, reprinted 1996. Vol. **1**. *Psilotaceae to Platanaceae.* Cambridge: Cambridge University Press, xlvi, 581 pp., illus. ISBN 0-521-41007-X (HB).

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CONTENTS

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