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Correspondence Address:
GRĂDINA BOTANICĂ "ANASTASIE FĂTU"
Str. Dumbrava Roşie nr. 7-9, 700487 - IAŞI
http://www.botanica.uaic.ro
E-mail: gbot.is@uaic.ro

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SESLERIA ULIGINOSA OPIZ – A COMPARATIVE STUDY OF LEAF ANATOMICAL TRAITS

KUZMANOVIĆ NEVENA[1], COMĂNESCU PETRONELA[2], LAKUŠIĆ DMITAR[1]

Abstract: The species *Sesleria uliginosa* is relatively common and widespread in Central (Hungary, Czech republic, Slovakia, Austria, Romania) and Northern Europe (Scandinavian peninsula), while in the Southeastern (Montenegro, Croatia, Bulgaria) and Southern Europe (Italy) it is a true natural rarity. Ecologically it is the typical species of calcareous swamps and by this feature it is an extreme quite singular within the whole genus. *S. uliginosa* can also grow on soils moist in spring but drying out later in summer, often at sunny stands, especially on clay soils.
According to Deyl, *S. uliginosa* belongs to Calcaria section, turma Uliginosa. The wax cover of the young leaves is typical for this species, but this pruining is nearly completely absent in the old leaves. It is closely related to *Sesleria heuflerana* Schur with which it shares some common characters – pruinos leaves, three floretted spikes and the occurrence in lower altitudes. But in the Balkan Peninsula it seems to have a far wider amplitude of its stands, so it can be found on the localities that reach up to the subalpine zone.
The aim of this study was to establish and describe the anatomical differentiation of populations of *S. uliginosa* from Romania, Hungary and Montenegro. The measurements were carried out on permanent handmade slides, prepared by the standard method for the light microscopy. To determine the significance of anatomical variation and differentiation, the following analysis were carried out: Principal component analysis (PCA), Canonical discriminant analysis (CDA) and cluster analysis by UPGMA method.

Keywords: *Sesleria uliginosa*, Poaceae, anatomy of leaves

Introduction

The taxon *S. uliginosa* was described by OPIZ (1836) in Flora of Bohemia, and the name was established for the populations of *S. caerulea* (L.) Ard. which are distributed in calcareous swamps. Over the time, the distinction between these two taxa became complicated, which led to many confusions [JANCHEN, 1960, 1965, 1966; PIGNATTI, 1982; ADLER, 1994].

A detailed analysis of the nomenclature conducted by FOGGI & al. [2001] confirmed the validity of *S. uliginosa* for the populations on wet habitats, and *S. caerulea* for dry grassland ones. These two species probably developed from common ancestor, but they represent independent evolutionary branches, and *S. uliginosa* is more related to *S. heuflerana* Schur [DEYL, 1946].

The species *S. uliginosa* belongs to the section *Calcariae*, turma *Uliginosa* [DEYL, 1946], and it is a European species with relatively limited and highly disjunctive areas. This species has a relatively continuous area in southern Scandinavia and the Baltic

[1]Institute of Botany and Botanical Garden Jevremovac, Takovska 43, Belgrade – Serbia, e-mail: nkuzmanovic@bio.bg.ac.rs, dlakusic@bio.bg.ac.rs
[2]Botanical Garden "Dimitrie Brandza", Şos. Cotroceni, no. 32, Bucureşti – Romania, e-mail: vpetronela@yahoo.com

countries. The incidence in the rest of the area is scattered on different, often restricted areas (Romania, Montenegro, Bulgaria, Czech Republic and Italy).

Ecologically, it is one of the major indicators of wet habitats [HÁJEK & al. 2005]. By this feature it is an extreme, even singular, within the whole genus. *S. uliginosa* grows on soils moist in spring but drying out later in summer, often at sunny stands, especially on clay soils.

In his monography on the genus *Sesleria*, DEYL (1946) recognized the population from Montenegro – Moračke planine (Habitat: Montenegro, Korytan Rovački, leg. Rohlena VII. 1903) as a different entity, and described the variety *S. uliginosa* var. *rohlenae*. He also assumed that the variability of the Balkan localities on which *S. uliginosa* was recorded is large and that this hangs together with the age of these localities that is probably Tertiary, while the northern localities are of post-glacial origin [DEYL, 1946].

The basic aim of the present study was to quantify anatomical variation within and between populations of *S. uliginosa* from Romania, Hungary and Montenegro on the basis of multivariate statistics, and to determine whether there are clear anatomical differences between these populations.

Material and methods

Three populations from distant parts of the area (Romania, Hungary, Montenegro) were sampled for anatomical analyses. The plant material was either fixed in 50% ethyl–alcohol solution or dried out and deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade [BEOU].

Voucher specimens:

Romania, Transilvanija, Brašov, Prejmer, alkaline wet grassland, 511 m, 25.7382 E, 45.73085 N, (*Kuzmanović N*., *Comanescu P*. *30336*, 27.04.2010, BEOU).

Hungary, Budapest, Soroksar botanic garden, wet grassland, 110 m, 19.154327 E, 47.400341 N, (*Barina Z*. *32879*, 26.04.2011, BEOU).

Montenegro, Durmitor, Crno Jezero, Seslerietum uliginosae, 1399 m, limestone, 19.096156 E, 43.147777 N (*Lakušić D*. *24439*, 31.05.2007, BEOU)

Anatomical analyses of the leaves were done on the permanent handmade slides, prepared by the standard method for the light microscopy. Cross-sections of the tiller leaves were cleared in Parazone and thoroughly washed before staining in safranin (1% w/v in 50% ethanol) and alcian blue (1% w/v, aqueous). The measurements were performed on the cross-section of 30 tiller leaves, each obtained from different individuals (10 per population). All measurements were performed using the software Leica Q Win. 17 characters were measured and subjected to statistical processing (Tab. 1).

Descriptive statistics (mean, minimum, maximum, standard deviation and coefficient of variation) were calculated for each character state. To determine the significance of anatomical variation and differentiation, the following statistical analyses were carried out: Principal component analysis (PCA), Cannonical discriminant analysis (CDA) and cluster analysis by UPGMA method based on Mahalanobis distances. Statistical analyses were performed using the package Statistica 5.1 [StatSoft 1996].

Results

MORPHOLOGICAL CHARACTERISTICS OF PLANTS

In its general habitus *S. uliginosa* is rather variable, but pruined young leaves are typical for all the individuals.

In his paper *Sesleria* studien, UJHELY (1938) gave a detail description of the leaf anatomy of *S. uliginosa*. He analyzed the differences between *S. uliginosa* and *S. caerulea* and found that the anatomical structure of the leaves is a very good distinguishing character between these two species, and that the anatomical differences are as great as between distant groups.

The leaves of the analyzed populations are in the cross section slightly rolled around the central nerve or unrolled and flat. The width of the leaves varies from 1176.7 to 2159.0 μm, with 7-13 vascular bundles. Sclerenchyma is discontinued and is present mostly in the form of sclerenchyma girders. In the zone of the central vascular bundle the sclerenchyma is organized exclusively in the form of a sclerenchyma girder. Bulliform cells are present in all the analyzed individuals. Small thinned out hairs were observed on the edge of some leaves (Fig. 1).

STATISTICAL DATA ANALYSIS

Coefficient of Variation

The analysis of variation of particular anatomical characters in populations of *S. uliginosa* was performed to establish that the highest number of characters show a moderate degree of variability (CV=10-30% – Tab. 1). Within the group of a highly variable characters, whose coefficient of variation (CV %) is higher than 30% is only surface of the sclerenchyma of tiller leaf (TL_ScS_Ar) – 33.2%. In the group of stable characters whose coefficient of variation is below 10% is only thickness of the tiller leaf blades in zone of the central rib (TL_To) – 8.8%.

Principal component analysis (PCA)

First three PCA axes account 72.06% of the total variability. Most of the variation was explained by the first axis (49.15%), 14.76% by the second and only 8.14% by the third. As it can be inferred from the component loadings, expressing character correlation with the axes, several characters are responsible for the differentiation along the first axes: width of the tiller leaf blades (TL_W), largest thickness of the tiller leaf blades (TL_T1), height of the central vascular bundle of tiller leaf (TL_VBC_H), width of the central vascular bundle of tiller leaf (TL_VBC_W), height of the largest lateral vascular bundle of tiller leaf (TL_VB1_H), width of the largest lateral vascular bundle of tiller leaf (TL_VB1_W), width of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScS1_W), surface of the sclerenchyma of tiller leaf (TL_ScS_Ar), surface of the tiller leaf blades (TL_B_Ar) (Tab. 1).

On the principal component analysis (PCA) diagram the individuals from Montenegro are mostly grouped in the bottom-right quadrant with two individuals in

upper and bottom-left quadrant. The individuals form Hungary are grouped in the upper-left quadrant with one individual in bottom-left and one in upper-right quadrant. The individuals from Romania are scattered in two quadrants – upper-right and bottom-left (Fig. 2).

Canonical discriminant analysis (CDA)

The canonical discriminant analysis (CDA) resulted in two differentiated groups, which are completely separated along the first axis – population from Montenegro as one group, and populations from Hungary and Romania as the other group (Fig. 3).

Along the second axis, the populations from Hungary and Romania are clearly separated also.

A clear differentiation between the population from Montenegro and populations from Hungary and Romania is shown also by the UPGMA cluster analysis, in which two completely separated clusters were formed (Fig. 4).

Discussion

The material for the analyses was collected from the habitats that are typical for *S. uliginosa*, from Romania – Braşov (alkaline wet grassland, altitude 510 m), Hungary – Budapest (alkaline wet grassland, altitude 110 m) and Montenegro – Durmitor (shore of Crno Jezero – Lake, altitude 1459 m). But the population from Durmitor is very particular because it is several months during a year under the water!

The most significant differences in leaf anatomy between the population from Durmitor and populations from Braşov and Budapest are observed in the height of the largest lateral and central vascular bundle, largest thickness of the tiller leaf blades, height of the sclerenchyma strand of central vascular bundle, surface of the sclerenchyma of tiller leaf and number of the minor vascular bundles (Tab. 2).

DEYL also noticed that the population from Montenegro (Moračke planine) is different from other populations of *S. uliginosa* that he had seen, and described a variety *S. uliginosa* var. *rohlenae*, pointing out that the populations of *S. uliginosa* on the Balkan peninsula are probably from Tertiary, while nothern ones are of post glacial origin [DEYL, 1946].

It is possible that all this together (specific life cycle and historical circumstances) led to the formation and expression of the differencies in leaf anatomy (maybe also genetical differencies) of the population from Durmitor in regard to the populations from Budapest and Braşov.

Finally, we can conclude that the populations of *S. uliginosa* from Montenegro, growing on the specific highmountain habitat, show a significant degree of anatomical differentiation from the lowland populations from Hungary and Romania. Furthermore, if we consider that the leaf anatomical characters have a significant diagnostic character within the genus *Sesleria* [KOLÁŘ, 1930; UJHELYI, 1938; UJHELYI & FELFOLDY, 1948; STRGAR, 1966, 1980, 1985; DI PIETRO, 2007; ALEGRO, 2007, KUZMANOVIĆ & al. 2011], the obtained results support Deyl's opinion that populations from Montenegro represent a separate taxon [DEYL, 1946]. Because only the leaf anatomical characters were analyzed in this paper**,** and sample of population was restricted**,** a question of a definite

taxonomic status of the Montengrine populations can be solved only following a detailed comparative morphological study of the reproductive organs, as well as a comprehensive molecular and phylogenetic study, which is in progress.

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Tab. 1. Basic and multivariate statistic parameters of all analyzed populations of *S. uliginosa*. All measures in µm (STD – standard deviation, CV% - coefficient of variation, Comp.1, 2, 3 – principal components, **bold** components with loadings >0.7)

Characters	Min	Mean	Max	STD	CV %	Comp.1	Comp.2	Comp.3
Width of the tiller leaf blades (TL_W)	1176.7	1695.5	2159	268.5	15.8	**0.86**	0.35	-0.17
Distance between the middle and largest leaf blade thickness point of tiller leaf (TL_T2)	636	888.5	1104.2	123.3	13.9	0.67	0.25	-0.37
Thickness of the tiller leaf blades in zone of the central rib (TL_To)	160.8	205.9	238.9	18.2	8.8	0.61	-0.21	-0.25
Largest thickness of the tiller leaf blades (TL_T1)	165.7	220.8	295.3	35.5	16.1	**0.82**	-0.28	0.32
Width of the central rib of tiller leaf (TL_Rc_W)	142.1	241	326.9	49.4	20.5	0.67	-0.01	-0.36
Height of the central vascular bundle of tiller leaf (TL_VBC_H)	60.4	82.4	101.4	11.0	13.4	**0.78**	-0.44	0.07
Width of the central vascular bundle of tiller leaf (TL_VBC_W)	47.9	66.8	87.3	11.0	16.5	**0.78**	-0.13	0.02
Height of the largest lateral vascular bundle of tiller leaf (TL_VB1_H)	64.6	89.8	120.7	15.5	17.3	**0.79**	-0.34	0.35
Width of the largest lateral vascular bundle of tiller leaf (TL_VB1_W)	49.4	70	95	12.1	17.2	**0.79**	-0.13	0.28

Height of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScSC_H)	27.2	57.6	90.2	16.1	28	0.66	-0.3	0.25
Width of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScS1_W)	75.3	117.7	154	23.0	19.6	**0.74**	-0.05	-0.46
Surface of the sclerenchyma of tiller leaf (TL_ScS_Ar)	12849.1	29123.6	49189.4	9674.6	33.2	**0.89**	0	0.22
Surface of the tiller leaf blades (TL_B_Ar)	163445	305938	475260	83901.9	27.4	**0.94**	0.21	0.03
Number of the major vascular bundles of tiller leaf (TL_VB2_No)	4	4.7	6	0.7	15.2	0.55	0.54	-0.16
Number of the minor vascular bundles of tiller leaf (TL_VB3_No)	3	5.3	8	1.1	21.7	0.19	**0.79**	0.32
Total number of the vascular bundles (TL_VB_No)	7	10	13	1.5	15	0.41	**0.86**	0.17
Dimension of the bulliform cells of tiller leaf (TL_BC_Ha)	28.5	41.3	64.6	9.1	22.1	0.24	-0.22	-0.49

Tab. 2. Anatomical differences between analyzed population of *S. uliginosa*. All measures in µm

	Budapest	Brašov	Durmitor
Width of the tiller leaf blades (TL_W)	(1177 -) 1243 - 1751 (- 1873)	(1497 -) 1579 - 1822 (- 1873)	(1514 -) 1629 - 2149 (- 2159)
Distance between the middle and largest leaf blade thickness point of tiller leaf (TL_T2)	(636 -) 660 - 912 (- 1034)	(730 -) 813 - 963 (- 969)	(931 -) 925 - 1056 (- 1104)
Thickness of the tiller leaf blades in zone of the central rib (TL_To)	(161 -) 175 - 217 (- 235)	(188 -) 193 - 226 (- 238)	(190 -) 199 - 225 (- 239)
Largest thickness of the tiller leaf blades (TL_T1)	(173 -) 172 - 218 (- 253)	(198 -) 211 - 282 (- 295)	(166 -) 193 - 249 (- 250)
Width of the central rib of tiller leaf (TL_Rc_W)	(171 -) 181 - 240 (- 273)	(142 -) 179 - 294 (- 311)	(223 -) 242 - 311 (- 327)
Height of the central vascular bundle of tiller leaf (TL_VBC_H)	(60 -) 62 - 78 (- 84)	(85 -) 87 - 98 (- 101)	(77 -) 81 - 89 (- 89)
Width of the central vascular bundle of tiller leaf (TL_VBC_W)	(48 -) 48 - 66 (- 79)	(59 -) 64 - 80 (- 87)	(55 -) 63 - 80 (- 83)
Height of the largest lateral vascular bundle of tiller leaf (TL_VB1_H)	(65 -) 66 - 86 (- 93)	(83 -) 92 - 115 (- 121)	(74 -) 79 - 102 (- 104)
Width of the largest lateral vascular bundle of tiller leaf (TL_VB1_W)	(49 -) 50 - 70 (- 82)	(66 -) 67 - 85 (- 95)	(58 -) 64 - 84 (- 91)
Height of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScSC_H)	(27 -) 34 - 62 (- 71)	(61 -) 63 - 83 (- 90)	(37 -) 41 - 64 (- 75)
Width of the sclerenchyma strand of central vascular bundle of tiller leaf (TL_ScS1_W)	(75 -) 79 - 118 (- 142)	(99 -) 104 - 134 (- 141)	(107 -) 117 - 154 (- 154)
Surface of the sclerenchyma of tiller leaf (TL_ScS_Ar) / 100	(134 -) 160 - 268 (- 295)	(273 -) 285 - 440 (- 492)	(128 -) 202 - 392 (- 394)
Surface of the tiller leaf blades (TL_B_Ar) / 100	(1724 -) 1819 - 3008 (- 3607)	(2645 -) 2826 - 3836 (- 4003)	(1634 -) 2454 - 4413 (- 4753)
Number of the major vascular bundles of tiller leaf (TL_VB2_No)	(4 -) 4 - 5 (- 6)	(4 -) 4 - 5 (- 6)	(4 -) 4 - 6 (- 6)
Number of the minor vascular bundles of tiller leaf (TL_VB3_No)	(4 -) 5 - 6 (- 7)	(4 -) 4 - 6 (- 6)	(3 -) 4 - 7 (- 8)
Total number of the vascular bundles (TL_VB_No)	(8 -) 9 - 11 (- 12)	(8 -) 9 - 10 (- 10)	(7 -) 8 - 13 (- 13)
Dimension of the bulliform cells of tiller leaf (TL_BC_Ha)	(31 -) 31 - 49 (- 65)	(31 -) 35 - 52 (- 60)	(29 -) 31 - 51 (- 58)

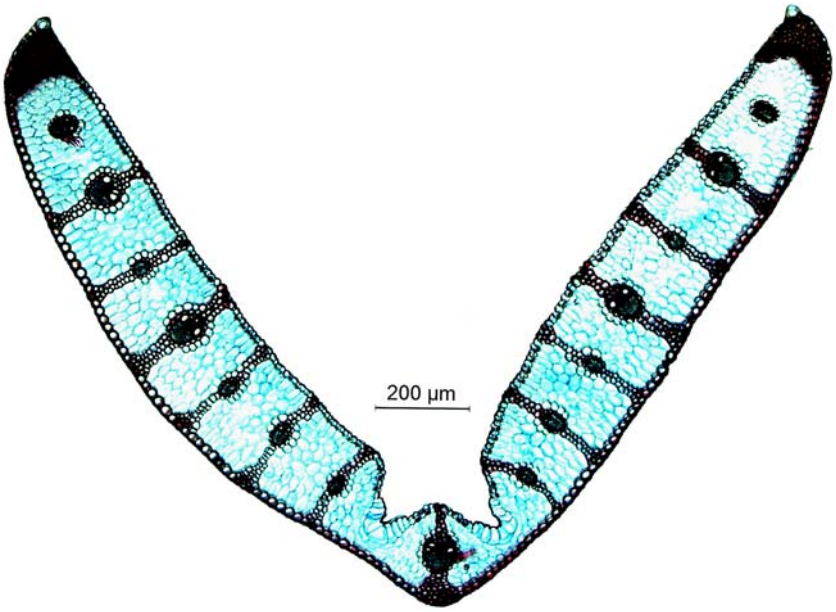

Fig. 1. Tiller leaf cross section of *S. uliginosa* from Montenegro

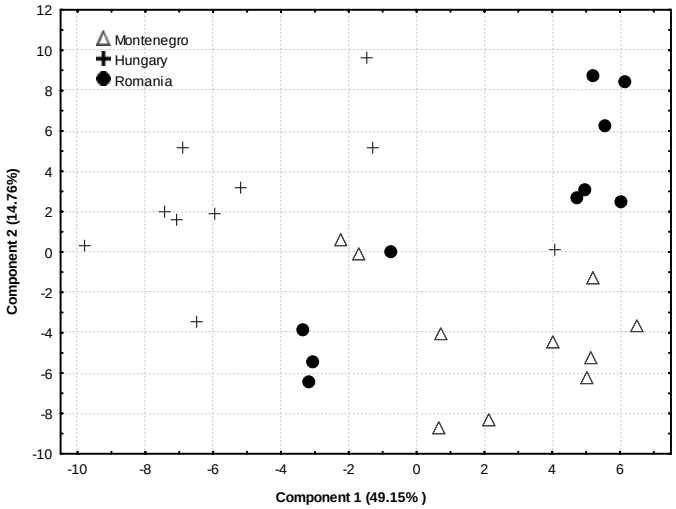

Fig. 2. Results of Principal Component Analysis (PCA) plotted along the first two discriminant axes

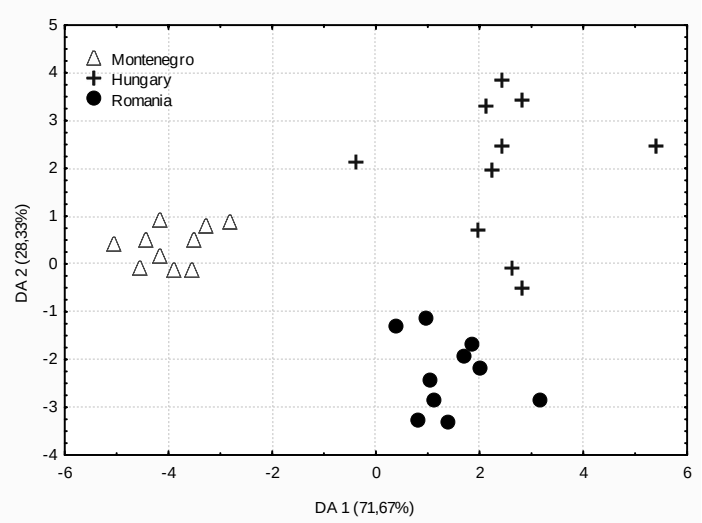

Fig. 3. Results of Canonical Discriminant Analysis (CDA) plotted along the first two discriminant axes

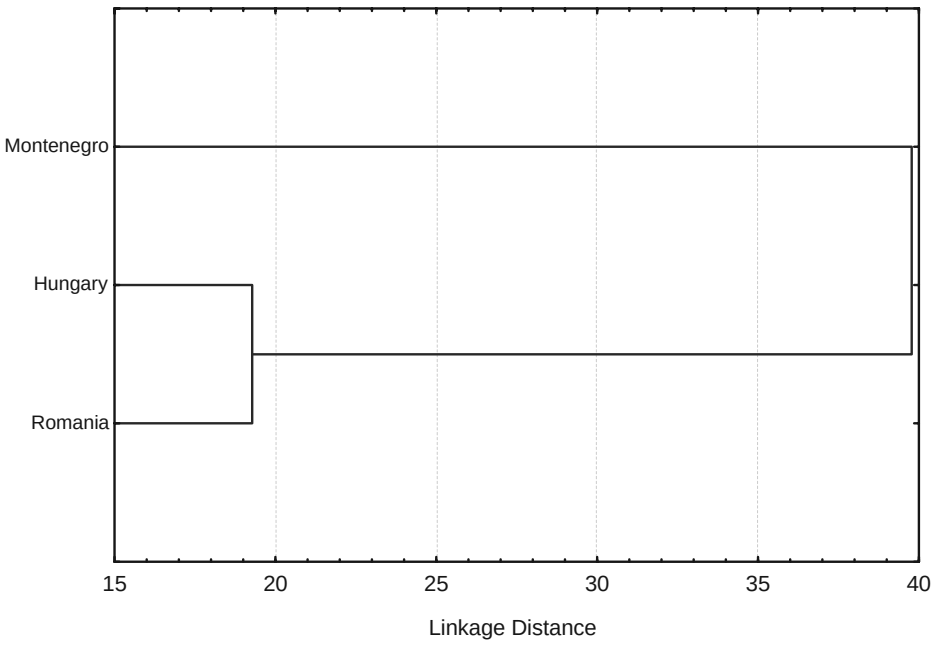

Fig. 4. Results of cluster analysis (UPGMA) based on Mahalanobis distances

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LEAF ANATOMICAL VARIATION IN RELATION TO STRESS TOLERANCE AMONG SOME WOODY SPECIES ON THE ACCRA PLAINS OF GHANA

DZOMEKU BELOVED MENSAH[1]

Abstract: Leaf anatomical study was conducted on some woody species on the Accra Plains of Ghana. Leaf epidermal strips and transverse sections were mounted in Canada balsam and studied. The anatomical studies revealed numerous stomata on the lower epidermis of *Azadirachta indica*. The anatomical studies revealed the presence of thick cuticles, double-layered palisade mesophyll in most species and the presence of epidermal hairs in some species. *Ficus capensis* showed the presence of cystolith in the lower epidermis whereas *Zanthoxylum zanthoxyloides* showed the presence of mucilage gland in the upper epidermis. Epidermal cell of *Chromolaena odorata* are very large with undulating cell walls. The species studied had various adaptive anatomical features. The stomatal frequency of *Azadirachta indica* was very high. With the exception of *Chromolaena odorata* the stomatal frequencies of the species were relatively high. The stomatal dimensions showed that most of the species maintained constant stomatal length during the study period except *Griffonia simplicifolia* that increased the stomatal width during the afternoon. Unlike *Morinda lucida, Griffonia simplicifolia* and *Chromolaena odorata*, that showed reduction in the breadth of stomata, the other species maintained constant stomatal width.

Key words: leaf anatomy, stomatal dimension, woody species, Accra Plains, drought stress

Introduction

The morphological features (both external and internal) and physiological responses are linked to adaptive characteristics of plants in stressed environments. There is a close relationship between the thickness of leaves and degree of cutinization of their outer epidermal wall on one hand and the extent to which the underlying tissues are required to be protected against excessive transpiration on the other hand [HABERLANDT, 1928]. In some plant species, the upper epidermis is often thicker-walled than the lower epidermis [YANNEY-WILSON, 1963]. It is obvious that the upper surface receives more light and heat hence requires more effective protection against evaporation [EHLERINGER & MOONEY, 1978]. The outer walls of epidermal cells may be coated with wax so as to avoid the capillary occlusion of stomata and in some species; each stoma is surrounded by a ring of wax which thus forms an external air-chamber [MULROY, 1979].

The presence of leaf pubescence has long been positively associated with arid climate conditions. It has been shown to increase light reflectance from the leaf surface [EHLERINGER & MOONEY, 1978]. The boundary layer also affects leaf temperature by modifying the rate of heat transfer from the leaf [EHLERINGER & MOONEY, 1978]. The hairy cover is also reported to impede surface-ventilation by producing a labyrinth of spaces filled with stationary air [HABERLANDT, 1928].

[1] CSIR – Crops Research Institute, P.O. Box 3785, Kumasi – Ghana, West Africa
e-mail: bmdzomeku@gmail.com, mdzomeku@yahoo.com

Many plants such as *Nauclea latifolia*, *Zanthoxylum zanthoxyloides*, *Ficus capensis* and *Azadirachta indica* are also reported to develop smooth and shinning upper leaf surfaces. Such polished surfaces protect the leaf against excessive insolation, which undoubtedly may prevent certain proportions of the incident light from penetrating into the leaf. However, smooth epidermal surfaces are often susceptible to wetting; but the deposition of wax protects the surfaces from wetting. Also the external resin coating of leaves conserve water by reducing non-stomatal transpiration [BAZZAZ & al. 1987].

The presence of thick cuticle and dense cell structure are inherently the modifications possessed by plant species for water conservation and also to withstand temperature stress [TRESHOW, 1970; MOONEY & GULMON, 1979]. The presence of hypodermis has been indicated as evolutionary solution to water stress [MOONEY & GULMON, 1979]. TRESHOW (1970) indicated that xerophytic conditions often reduce cells size, and intercellular spaces in leaves are less extensive. The increase in palisade mesophyll layer and reduction in the spongy mesophyll layer are modification to withstand drought stress. Drought stress has been indicated to increase the mechanical tissues for plants so as to be able to withstand the mechanical stress of desiccation.

The presence of mucilage in some cells and also in the cell wall of some species serves to conserve much water during water stress. The mucilage swells in water to form loose gels or slimy mass [GREEN & al. 1986]. The aim of this study is to determine the leaf anatomical variation in relation to stress tolerance among some woody species on the Accra Plains.

Study site. The Accra Plains is a triangular area in the southeastern part of Ghana. It covers an area of about 2,800 km^2 [JENIK & HALL, 1976] being bordered on the east by the lower reaches of the Volta river, the west by Winneba, the north by the Akwapim Scarp, and the south by the Gulf of Guinea. The plains is not a homogenous area, thus JENIK & HALL (1976) have divided it into seven (7) smaller geographical units based on the vegetation and soil types. The northern boundary is a hilly forest whereas the eastern and western boundaries are the Guinea savanna. Although the vegetation of the Accra plains is referred to as savanna, it does not fit into any of the main savanna types found in West Africa; hence might better be referred to as a kind of steppe since the grasses in this very dry area of less than 750mm rainfall rarely exceed 80 cm in height [LAWSON & JENIK, 1967; JENIK & HALL, 1976].

The plains is spatially isolated from the other savanna areas and its anomalous dry climate with a combination of low rainfall, moderate and rather high humidity has been designated the "Accra-Togo Dry Coastal Climate", rendering it of special ecological interest [BRAMMER & DE ENDREDY, 1962; HARRISON CHURCH, 1963; JENIK & HALL, 1976].

The Accra Plains is obviously under stress due to (i) the low rainfall [BENNEH & AGYEPONG, 1990] and (ii) the increase in population on the plains (in general) and also in the vicinity of the study site, Pinkwae. This study was conducted to examine the anatomical features of some woody species in relation to their adaptation to the environment.

Materials and methods

Leaf anatomical study was conducted on the following woody species: *Azadirachta indica* (*Meliaceae*), *Capparis erythrocarpa* (*Capparaceae*), *Millettia thonningii* (*Fabaceae*), *Chromolaena odorata* (*Asteraceae*), *Zanthoxylum zanthoxyloides* (*Rutaceae*), *Griffonia simplicifolia* (*Fabaceae*), *Lonchocarpus macrophyllus* (*Fabaceae*),

Ficus capensis (*Moraceae*), *Nauclea latifolia* (*Rubiaceae*) and *Morinda lucida* (*Rubiaceae*).

Anatomical Studies. The work was done in Accra in the coastal Savanna zone of Ghana from mid October to mid February 2001. Leaf epidermal strips of some of the species listed above, were obtained and mounted overnight in Canada balsam. Leaves of some of the species were collected and stored in Formal Acetic Alcohol (FAA) in sample tubes for subsequent anatomical sectioning in the laboratory using a microtome.

Thin Transverse sections (TS) of 3 µm thickness of each available leaf sample were made by holding the leaf in place in the correct orientation in a fine slit down a piece of carrot tuber and sectioning with the microtome. The thin sections of each sample obtained using the microtome, were then screened under a microscope, stained with safranin, washed through a series of ethanol of the following concentrations and sequence: 50% (1 min), 70% (1 min), 90% (1 min) and absolute ethanol (two changes 5 min each). The specimens were next stained in light green (½ - 1 min) and then cleared and washed in clove oil (5 min). Any over staining with light green was corrected by washing the specimens back through the series of alcohols before restaining in safranin. The sections were finally mounted in Canada balsam.

Results and discussions

Anatomical Studies. Anatomical studies presented here included camera lucida drawings of transverse section (TS) of lamina without the midvein and lower epidermal strips of leaves.

Azadirachta indica

Numerous stomata occurred on lower epidermis of *Azadirachta indica*. The epidermis is relatively thin. The palisade mesophyll composed of two layers of elongated closely packed cells. The spongy mesophyll is composed of loosely arranged cells with air spaces (Figs. 1a, 1b).

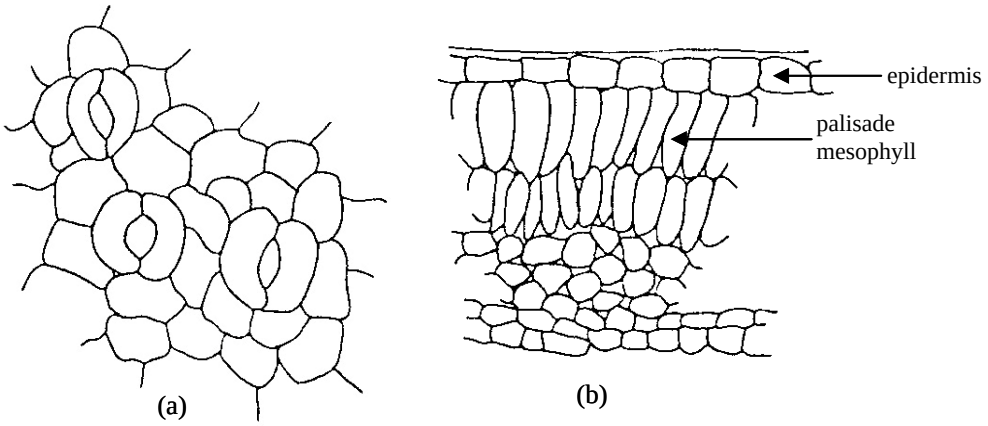

Fig. 1. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Azadirachta indica*

Millettia thonningii

Leaves are compound pinnate; pinnate entire with no hairs. Stomata confined to lower leaf surface. Epidermis is thick. Palisade mesophyll composed of two layers of longitudinal cells. Spongy mesophyll, loosely arranged with numerous air spaces.

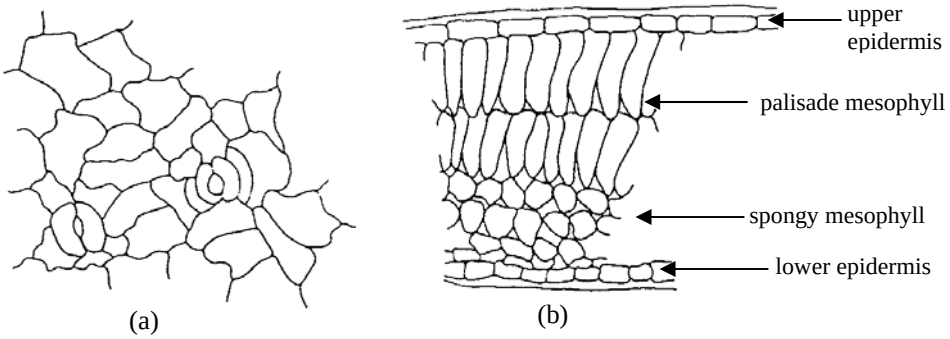

Fig. 2. (a) Camera Lucida drawing of lower epidermis and (b)TS of leaf lamina of *Millettia thonningii*

Capparis erythrocarpa

The leaf is simple, entire, leathery and evergreen. The plant is shrubby and thorny. Anatomically, there is a thick cuticle with no distinct epidermis (Fig. 3). The mesophyll is diffused with no clearly distinct palisade and spongy mesophylls. However, palisade mesophyll-like layer showed smaller cells than the spongy mesophyll-like layer. The spongy mesophyll-like layer had few air spaces (Fig. 3b). No distinct palisade layer evident. Cuticle appears to be prominent on both the upper and lower sides of leaf lamina.

Stomata appeared on only the abaxial leaf surface although YANNEY-WILSON (1963) recorded stomata on both surfaces (Fig. 3a).

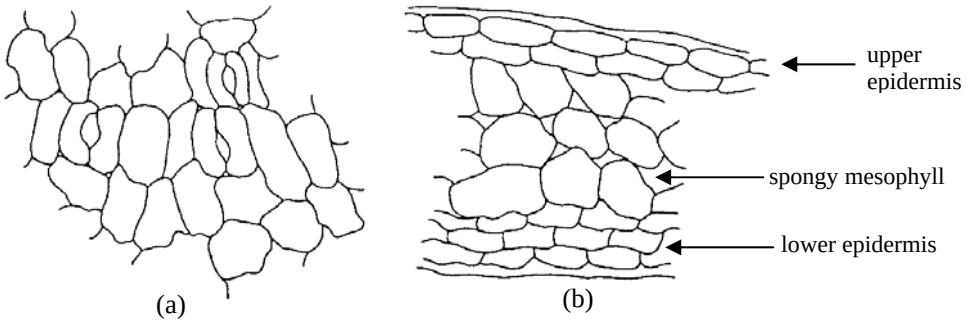

Fig. 3. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Capparis erythrocarpa*

Zanthoxylum zanthoxyloides

Leaves are compound pinnate, alternate with thorns on the lower surface of midvein and on the stem. The pinnae are entire and evergreen. Leaves are leathery with shiny upper surface. No hairs present. Mucilage gland occurred in the upper epidermis of the leaf (Figs. 4a, b).

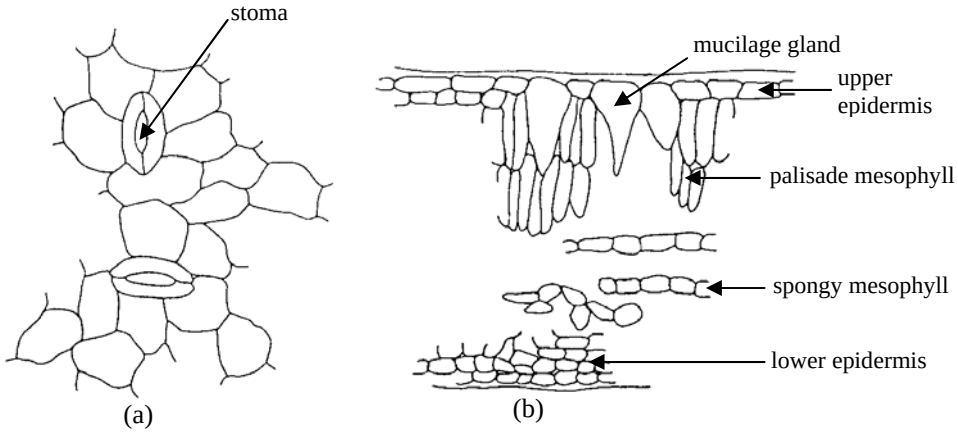

Fig. 4. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Zanthoxylum zanthoxyloides*

Griffonia simplicifolia

The leaves are simple and entire with folded margins. The leaves are leathery with shiny surface. There are no hair(s) present. Stomata appear on lower surface of leaf (Fig. 5a). Anatomy of the lamina showed a thick epidermis. Palisade mesophyll made up of two layers of short, closely packed palisade parenchyma cells; spongy mesophyll made up of loosely packed parenchyma cells with intercellular air spaces (Fig. 5b).

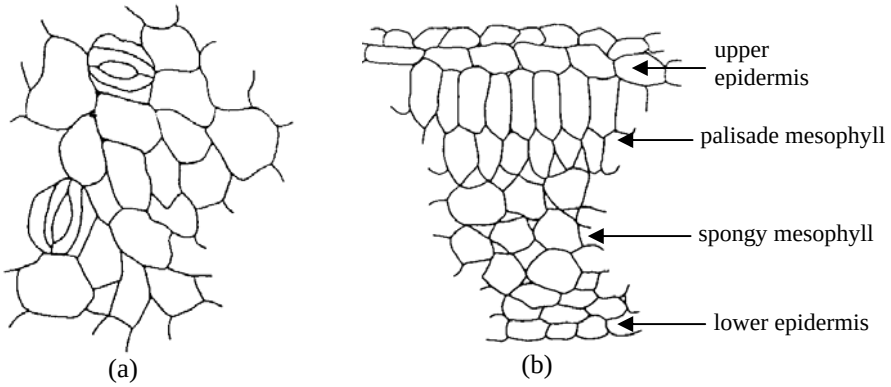

Fig. 5. (a) Camera Lucida drawing of lower epidermis and (b)TS of leaf lamina of *Griffonia simplicifolia* showing stomata (H.P)

Nauclea latifolia

The leaves are broad, entire, thick leathery with shiny adaxial surfaces. The surface is smooth.

Stomata occurred on the abaxial leaf surface. No stomata occurred on the adaxial leaf surface (Fig. 6a). The epidermis is thick with hypodermis. Palisade mesophyll cells are made up of two layers of elongated, closely packed cells (Fig. 6b).

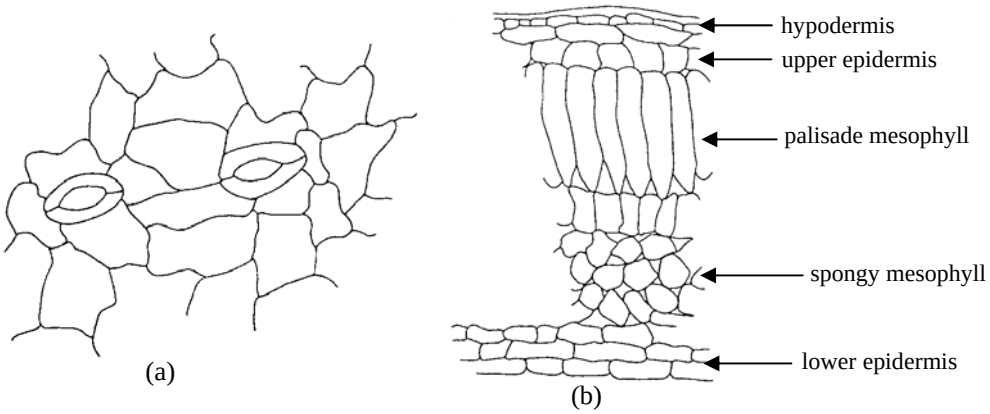

Fig. 6. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Nauclea latifolia* showing stomata (H.P.)

Ficus capensis

The Leaf is simple and entire, thick and pale green. There is presence of few unicellular hairs on the lower surface of leaf (Fig. 7b). Stomata confined to abaxial leaf surface (Fig. 7a). Epidermis contains numerous cystoliths (Fig.7a). Upper epidermis is thick. Palisade tissue composed of two layers of closely packed cells. Spongy mesophyll occupied larger portion of lamina with numerous intercellular air spaces (Fig. 7b).

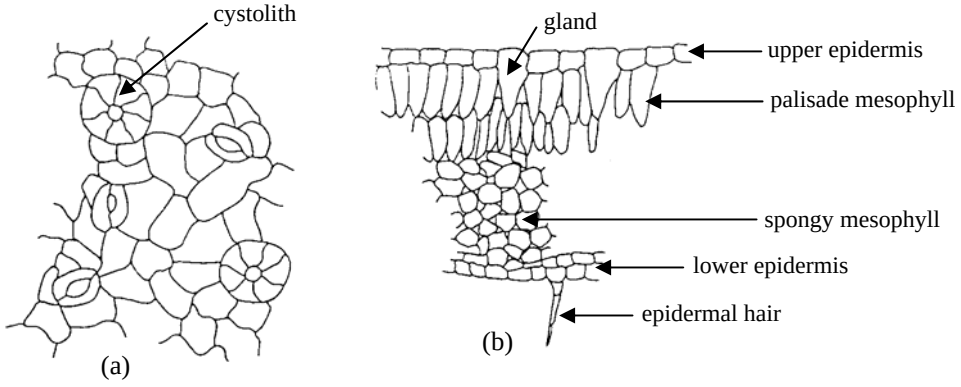

Fig. 7. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Ficus capensis* (H.P.)

Lonchocarpus macrophyllus

Epidermis composed of one layer of cell thick. Palisade mesophyll composed of two layers of longitudinal closely packed cells. The spongy mesophyll cells loosely arranged with numerous intercellular air spaces (Figs. 8a, b).

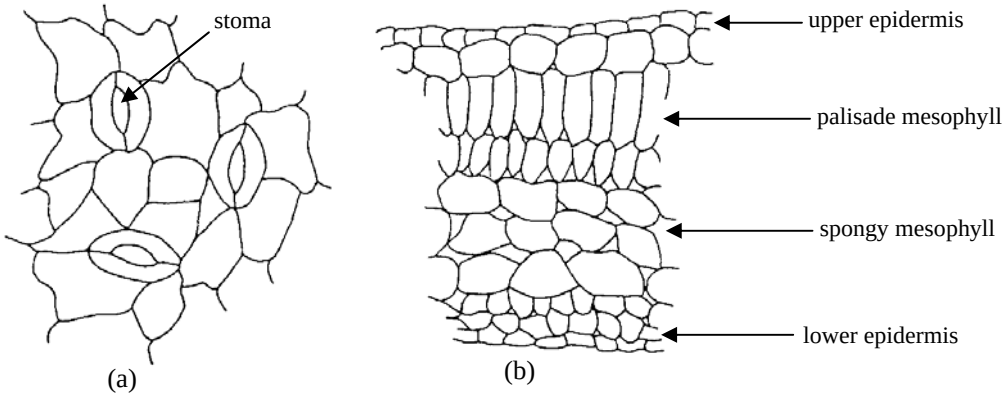

Fig. 8. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Lonchocarpus macrophyllus*

Morinda lucida

Leaves are pinnate; pinnae entire and leathery. Few epidermal hairs present on the lower epidermis (Fig. 9a). Stomata occurred on the lower epidermis. Epidermis composed of two layers of cells. Palisade mesophyll, one layer thick. Spongy mesophyll composed of several layers of cells (Fig. 9b).

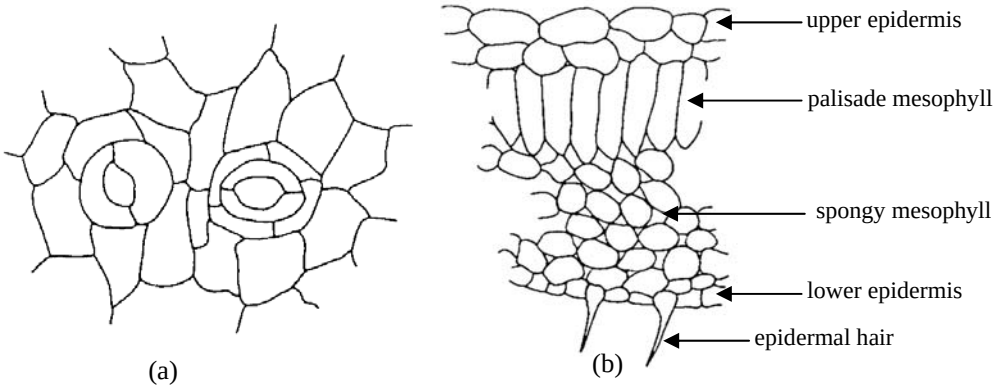

Fig. 9. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Morinda lucida*

Chromolaena odorata

Stomata confined to the lower epidermis. Epidermal cell are very large with undulating cell walls. Leaf anatomy showed two layers of short cells palisade mesophyll. Spongy mesophyll composed of loosely packed cells (Figs. 10a, b).

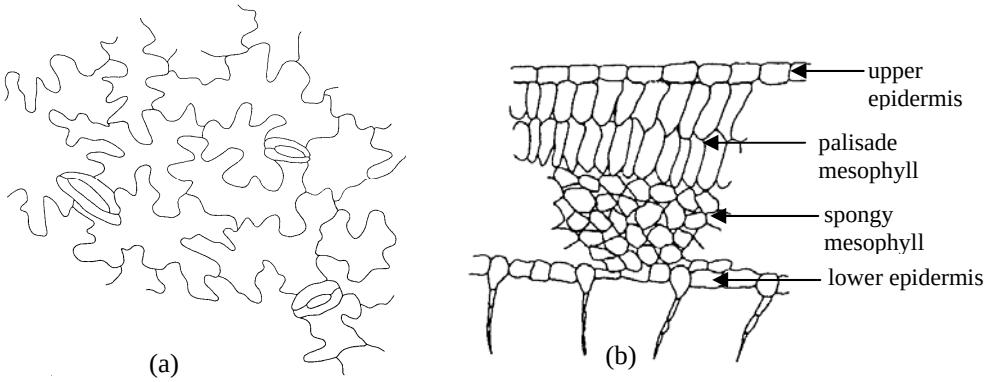

Fig. 10. (a) Camera Lucida drawing of lower epidermis and (b) TS of leaf lamina of *Chromolaena odorata* (H.P.)

Stomata Distribution/Frequency and Dimensions

The stomatal frequency, stomatal length and breadth (at the widest portion of stoma) are shown in Table 1.

Tab. 1. Diurnal Stomatal Dimensions of Some Species in Study Area at Pinkwae (Values are Means of Five Replicate Fields of View)

Species	**Stomatal frequency (L.P.)**	**Stomatal length (µm)**		**Stomatal width (µm)**	
		Morning	Afternoon	Morning	Afternoon
Azadirachta indica	1137 ± 27	0.028	0.028	0.014	0.014
Capparis erythrocarpa	484 ± 4	0.014	0.014	0.007	0.007
Chromolaena odorata	341 ± 0	0.028	0.028	0.014	0.007
Griffonia simplicifolia	486 ± 5	0.021	0.014	0.014	0.007
Millettia thonningii	512 ± 0	0.014	0.014	0.007	0.007
Morinda lucida	617 ± 0	0.028	0.028	0.014	0.007
Nauclea latifolia	607 ± 6	0.028	0.028	0.007	0.007
Zanthoxylum zanthoxyloides	452 ± 6	0.028	0.028	0.007	0.007

L.P. Low Power Magnification (10 x 10 x 10)

The stomatal frequency of *Azadirachta indica* was very high. With the exception of *Malachantta alnifolia* and *Chromolaena odorata* the stomatal frequencies of the species were relatively high.

The stomatal dimensions showed that most of the species maintained constant stomatal length during the study period except *Griffonia simplicifolia* that increased the

stomatal width during the afternoon. With the exception of *Morinda lucida*, *Griffonia simplicifolia* and *Chromolaena odorata*, which showed reduction in the breadth of stomata, the other species maintained constant stomatal width.

The anatomical features of *Azadirachta indica*, showed relatively thick cuticle, with a double layer of palisade mesophyll (Fig. 1), which may probably be the feature for its adaptation to withstand the drought stress. Also, the small pinnae area, high stomatal frequency (Tab. 1) coupled with open stomata may have contributed to the efficiency of the plant; as these features have been reported to influence high diffusion conductance [MOONEY & GULMON, 1979; BANNISTER, 1978]. In their reports it was indicated that high transpiration rates occur when leaf resistance were low; and that was dependent on stomatal depth, area and number. BANNISTER (1978) also indicated that leaf modification was associated with dry habitats; and that epidermal modification might serve to maintain epidermal turgidity and thus enable the stomata to remain open [BANNISTER, 1964]. The high transpiration rates may also be effective in cooling the leaves.

Morphological features have been reported to be features used by plants to withstand drought [EHLERINGER & MOONEY, 1978; MOONEY & al. 1977; MULROY, 1979; TURNER, 1986]. TURNER (1986) indicated leaf wilting and leaf rolling to increase water use efficiency in rice. These features were reported to increase the stomata depth. BANNISTER (1964) observed hairiness in *Calluna* to support the epidermal modification that enables the stomata to remain open over a wide range of water deficits. In *Nauclea latifolia* the cuticle was relatively thin. The species may probably be using the leaf pubescence and the peculiar anatomical features to withstand drought.

All the species studied showed relatively thick cuticle and this may probably have contributed to their ability to withstand drought stress. However, *Zanthoxylum zanthoxyloides* showed the presence of mucilaginous cells in the epidermis which may possibly have contributed to its ability to tolerate the drought stress. It is reported that mucilage can absorb water and hence form a loose gel to help plants withstand drought [GREEN & al. 1986]. *Capparis erythrocarpa* showed the cuticle penetrating between epidermal cells. The spongy mesophyll was also relatively closely packed which may also contribute adaptation to drought.

The features observed conform to those observed and reported by YANNEY-WILSON (1963). Hairiness has been reported to be more likely concerned with protection from excessive insolation than from high transpiration [YANNEY-WILSON, 1963; SCHULZE & al. 1987].

With the exception of *Chromolaena odorata*, *Griffonia simplicifolia* and *Morinda lucida* which or that reduced their stomatal breadth in the afternoon, all the other species showed constant breadth/width dimensions. Also *Griffonia simplicifolia* also showed reduction in stomatal length in the afternoon. It is believed that during stomatal movement (opening and closure), the stomatal length is fixed but there is variation in the width of the stoma [MOONEY & GULMON, 1979; GIFFORD & MUSGRAVE, 1973]. YANNEY-WILSON (1963) however, reported reduction in the stomatal aperture of *Capparis erythrocarpa* during the afternoon. *Capparis erythrocarpa* was reported in the work of YANNEY-WILSON (1963) as the species with the thickest cuticle, however, in this study *Nauclea latifolia* showed the thickest cuticle.

Conclusions

The results showed that *Azadirachta indica* was a good potential in plant-water economy. *Millettia thonningii* on the other hand is drought-resistant deciduous. The study revealed variation in the stomatal shapes. The leaf anatomy of the plant studied were varied and species specific. However, double layer of palisade mesophyll was very common. This could be a characteristic for adaptation to the stress environment. The stomatal dimensions of the species with time of day showed that all the plants had adaptation mechanisms the stress environment. *Azadirachta indica* with numerous stomatal compared to the leaf surface remained evergreen during the dry season. It could be using an innate ability to adapt to the drought stress. However, leaf anatomy, stomatal behavior coupled with external morphological features may be contributing to the efficiency of the species.

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INFLUENCE OF HIGH LIGHT INTENSITY ON THE CELLS OF CYANOBACTERIA *ANABAENA VARIABILIS* SP. ATCC 29413

OPRIŞ SANDA[1], SICORA COSMIN[1], RUSU TEODOR[2]

Abstract: In this article is presented the result of research regardind the effect of high light intensity on the cells of *Anabaena variabilis* sp. ATCC 29413, the main objective is to study the adaptation of photosynthetic apparatus to light stress. Samples were analyzed in the present of herbicide diuron (DCMU) which blocks electron flow from photosystem II and without diuron. During treatment maximum fluorescence and photosystems efficiency are significantly reduced, reaching very low values compared with the blank, as a result of photoinhibition installation. Also by this treatment is shown the importance of the mechanisms by which cells detect the presence of light stress and react accordingly.

Keywords: cyanobacteria, *Anabaena* sp., photosynthesis, high light, fluorescence

Introduction

Study cyanobacteria is of great importance because it can help to develop biofuels as biodiesel and biohydrogen or to better know photosynthesis mechanisms (cyanobacteria are model organisms for studying photosynthesis).

Cyanobacteria are prokaryotic oxygen-evolving photosynthetic organisms which had developed a sophisticated linear electron transport chain with two photochemical reaction systems, PSI and PSII, as early as a few billion years ago cyanobacteria. By endosymbiosis, oxygen-evolving photosynthetic eukaryotes are evolved and chloroplasts of the photosynthetic eukaryotes are derived from the ancestral cyanobacteria engulfed by the eukaryotic cells [GAULT & MARLER, 2009].

The aim of this paper is to study cyanobacterial photosynthesis, study in which chlorophyll fluorescence induced by "flash" is used to elucidate the effect of high light intensity on photosystem II.

Chlorophyll fluorescence may reflect photosynthetic activities in a complex manner. The method discussed in the experiment performed, based on chlorophyll fluorescence induced by "flash" is new in our country.

Research objectives are:

1. Characterization of the growth process based on specific parameters (growth curve, optical density, doubling time,) under the influence of high light;
2. Study of cyanobacterial photosynthesis based on chlorophyll fluorescence induced by "flash";
3. Evaluation the resistance of studied strain under light stress, in order to highlight the suitability of culture growth in open pond.

[1] Biological Research Center Botanical Garden "Vasile Fati" Jibou, 14 Parcului Street, Jibou – Romania, e-mail: sanda_opris@yahoo.com
[2] University of Agricultural Sciences and Veterinary Medicines, 3-5 Mănăştur Street, Cluj-Napoca – Romania

Material and method

Biological material which has been subject of this study is strain *Anabaena variabilis* sp. ATCC 29413, filamentous cyanobacteria that fix aerobic molecular nitrogen and does not requires special growth conditions.

Growth medium used is the BG-11 medium.

For 1000 ml of BG-11 medium were used 10 ml of macronutrients (100 x); 1 ml Trace metal solution (1000 x); 1 ml of each stock solution (1000 x): dipotasic phosphate (K_2HPO_4) 175 mM; sodium carbonate (Na_2CO_3) 189 mM and ferric ammonium citrate 6 mg / ml; 20 ml of buffer pH, Hepes-1M NaOH (pH 7.5) and double distilled water.

For 1000 ml of macronutrients (1000 x) were used 149.6 g of sodium nitrate ($NaNO_3$), 7.5 g of magnesium sulphate heptahydrate ($MgSO_4$ x $7H_2O$), 3.6 g calcium chloride dihydrate ($CaCl_2$ x $2H_2O$), 0.65 g citric acid and 0.1 g Na_2-EDTA.

For 1000 ml Trace metal solution (1000 x) were used: 2.86 g of boric acid (H_3BO_3), 1.81 g of manganese chloride tetrahydrate ($MnCl_2$ x $4H_2O$), 0.222 g of zinc sulphate heptahydrate ($ZnSO_4$ x $7H_2O$), 0.391 g of sodium molybdate dihydrate (Na_2MoO_4 x $2H_2O$), 0.079 g of copper ph. - sulfate pentahydrate ($CuSO_4$ x $5H_2O$) and 4.947 g of cobalt nitrate hexahydrate (Co (NO_3) 2 x $6H_2O$).

BG-11 medium is placed in containers and sterilized by autoclaving at 120˚ C, 20 minutes [ATLAS, 2004].

Treatment was performed in a tank of 10/10 cm, in which was placed a magnet and the tank was placed on a shaker. Culture density was approximately 6 mg chlorophyll, culture volume of 150 ml, 30˚ C temperature and light intensity of 600 µE.

For chlorophyll fluorescence measurements were used 3 ml of culture samples placed in cuvettes with all four sides transparent.

Samples were adjusted to the dark 5 minutes before fluorescence measurement.

Measurements were made in the presence and in absence of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea]. The amount of DCMU added was 1.5 µl.

Curves obtained were recorded using the FluorWin program and reported to a logarithmic time scale and exported to excel. DCMU is a herbicide commonly used in experiments that aims photosynthesis because of the ability to block electron transfer between QA and QB.

DCMU inhibits electron transfer because compete with the plastochinona for QB binding site, blocking chain irreversibly. So QA can not be reduced by the QB and recombine with redox components of the donor side electron of transport chain.

By adding DCMU to the sample can obtain information about the donor side integrity of the electron transport chain.

At normal light intensity, electron transfer from water to chlorophyll is efficiently done and does not requires artificial electron donors. Photosystem II efficiency is provided by the participation of a protein complex of membrane subunits, which transfer electrons from water across the tilacoid membrane to plastochinone [SIPPOLA, 2000].

Chlorophyll fluorescence decrease is due to increased energy dissipation rate and decreasing photochemical rate, because chlorophyll fluorescence is complementary with photochemistry and heat dissipation [BERCEA, 2008].

If photoinhibition installs photosynthetic capacity reduction occurs through a reduction in the proportion opened reaction centers of photosystem II and the state of tilacoid membranes is high energized [KOBLIŽEK & al. 2001].

Photoinhibition of photosystem II activity involves at least two levels of inactivation. The first level is considered reversible and occurs during approximately one hour without causing injury to photosystem II [LEITSCH & al. 1994].

The second level is supposed to be accompanied by degradation on the reaction centers of photosystem II, this level is oxygen dependent and is reversible by replacing the structural protein [JAHNS & MIEHE, 1996].

The graphic curve form shows the photosystem electron transfer. Samples without DCMU shows the acceptor side of photosystem, and by adding DCMU we can see the donor side of photosystem.

Results and discussion

The effect of high light on *Anabaena variabilis* sp. ATCC 29413 cells is presented in Fig. 1. Curves obtained are represented by colors corresponding to samples taken at different time intervals (control sample – dark blue, sample at 15 minutes – pink, sample at 30 minutes – yellow, sample at 60 minutes – light blue, sample at 120 minutes – cherry). Panels (a, b, c) give test results with DCMU and panels (d, e, f) give the results of samples without DCMU. Panels (a, d) represent curves without normalization and panels (b, e) represent curves normalized to the same initial amplitude. Panels (c, f) are schematic representation of the evolution of maximum fluorescence values (Fig. 1).

Panel (a) shows the relative fluorescence intensity (with DCMU). Diuron (DCMU) added to the sample increased the amplitude of chlorophyll fluorescence. Maximum amplitude recorded at control sample. Depending of exposure time to high light chlorophyll fluorescence amplitude decreases significantly during the 120 minutes of exposure, compared with control sample.

PARK & al. (1995) said there is reciprocity between light intensity and duration of illumination for photosystem II functionality, indicating that inactivation of photosystem II depends on the total number of absorbed photons rather than the photons absorption rate. Photoinhibition of photosystem II is due to amino acid changes in protein D1 [SCHULZE & CALDWELL, 1995]. Continue photodegradation and resynthesis of D1 protein is called rapid turnover of the D1 protein of photosystem II [MATTO & al. 1984].

Panel (b) shows the normalized fluorescence intensity (with DCMU). Normalization helps to observe the differences between curves. In this case the curves are relatively close re-oxidation rates from the QA samples are similar, which indicates slow recombination of QA with the water oxidation complex.

Panel (c) shows the variation of maximum fluorescence values (fmax) under the action of high light (with DCMU). During treatment there is an inhibition of active reaction centers at a rate of 56%. Decreases of maximum fluorescence leads to decreases in the proportion of active reaction centers. The initial curve slope is steeper in the fact that the first 30 minutes of exposure to high light degradation of the photosystem II is more pronounced. After the 30 minutes of exposure the slope curve is smoother as a result to the initiation of defense mechanisms to accommodate the new conditions. This are mechanisms of regulation of D1 protein synthesis cycle by phosphorylation and dephosphorylation of D1 protein.

Panel (d) shows the relative fluorescence intensity (without DCMU). It can be seen rapid growth in the number of active reaction centers of photosystem II (able to reduce QA) at control sample but after the first 15 minutes of treatment the proportion of active centers decreases reaching values below the control sample.

The maximum amplitude it has been registered at control sample. During the 120 minutes of treatment occurs reduction of photosynthetic activity. This fact results from reduced amplitude of chlorophyll fluorescence. It can be observed a decrease in chlorophyll fluorescence amplitude from sample to which was added DCMU.

Panel (e) shows the normalized fluorescence intensity (without DCMU). Control sample indicates the steeper slope therefore electron transport between QA and QB performs faster compared with samples exposed different times to high light. The intensity of electron transfer is performed in the following order: control sample, sample exposed to 120 minutes (purple curve), the sample exposed to 15 minutes (pink), the sample exposed to 30 minutes (yellow) and the sample exposed to 60 minutes (blue).

Panel (f) shows the variation of maximum fluorescence values (fmax) under the action of high light treatment (without DCMU). In this case, at measuring time the maximum proportion of active centers was recorded at cotrol sample. As time of exposure cultures to high light increases, a decreased in number of active centers occurs, so fluorescence decreases. In these conditions reaction centers of photosystem II are gradually closed during exposure time lowering the photochemical efficiency of photosystem II. During treatment the reaction centers were inhibited at a rate of 52%.

Conclusions

1. Exposure to high light causes a decrease in maximum fluorescence after the first 15 minutes of exposure.
2. By adding diuron (DCMU) to the cell suspension it has been recorded an increase in amplitude compared to samples without DCMU. It can be seen more rapid growth of the fluorescence and higher amplitudes, suggesting the photosystem II efficiency in energy photons absorption.
3. Maximum fluorescence during treatment was significantly reduced to the samples with DCMU and without DCMU, reaching values lower than those of control sample. In these conditions the photochemical efficiency of photosystem II and electron transport chain efficiency have been reduced.

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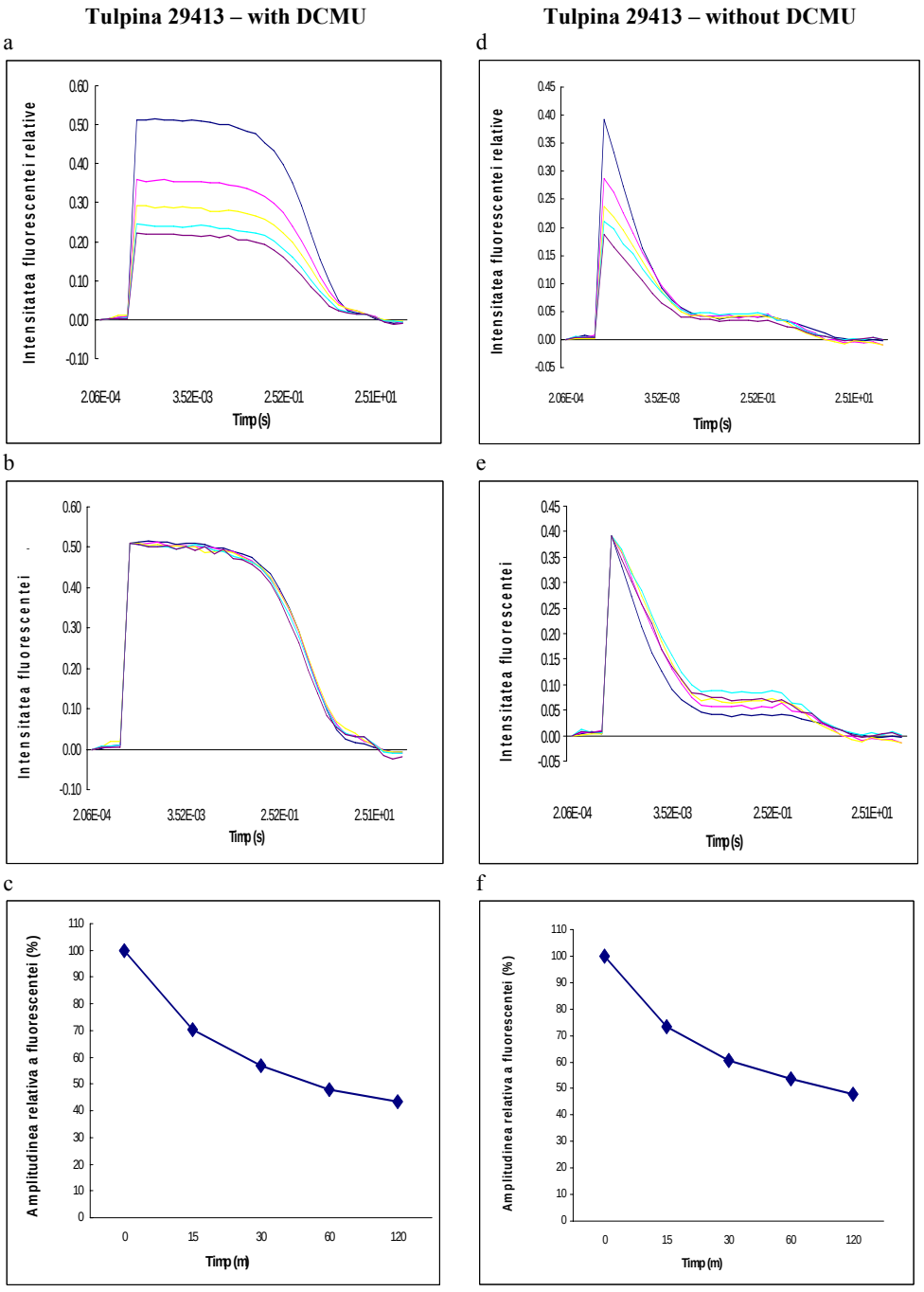

Fig. 1. Effect of high light intensity on cells of *Anabaena variabilis* sp.

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EFFICIENT MICROPROPAGATION AND EVALUATION OF GENETIC FIDELITY OF *IN VITRO* RAISED PLANTS OF *COMMIPHORA WIGHTII* ARN. (BHANDARI) – A MEDICINALLY IMPORTANT RED-LISTED SPECIES OF ARID REGIONS

PARMAR ASHOK KUMAR[1], KANT TARUN [1]

Abstract: A refined and an efficient protocol for *in vitro* clonal propagation of *Commiphora wightii*, a red-listed desert plant of medicinal importance, has been developed from nodal segment of mature plant. Nodal segments from new branches having 6-7 nodes were excised after discarding the initial 5-6 cm terminal portion and were surface sterilized with 2.5% NaOCl (sodium hypochlorite), (v/v). MS medium [MURASHIGE & SKOOG, 1962] with different concentrations of BAP (6-benzylaminopurine) was used alone and in combinations with IAA (indole-3-acetic acid), NAA (α-naphthalene acetic acid), Kn (kinetin) and other additives for shoot induction. Best bud break response (84.5%) was obtained on MS medium supplemented with 8.88 µM BAP, 0.57 µM IAA and additives (50 mg l^{-1} ascorbic acid, 25 mg l^{-1} citric acid and 25 mg l^{-1} adenine sulphate) within 2 weeks of inoculation. The micro-shoots were subcultured and maintained for further elongation on the same medium for 4 weeks. Best shoot multiplication was obtained on same medium as used for shoot initiation. Best rooting was obtained when the shoots were initially given a 24 h pulse treatment in liquid MS medium supplemented with 4.92 µM IBA (indole-3-butyric acid) and 5.71 µM IAA under dark condition, followed by transfer to semi-solid half-strength hormone-free MS medium supplemented with 2% (w/v) sucrose and 0.5% (w/v) activated charcoal. High (86.7%) percent rooting was achieved after 4-5 weeks with 3-4 multiple adventitious roots of 5-6 cm length. These *in vitro* raised well rooted plantlets were acclimatized in a two step manner. During *in vitro* hardening step, the survival was 61.5% and during *ex vitro* hardening step it was 100%. Hardened plants (10-12 cm in height) were transferred to polythene-bags filled with mixture of soil and FYM in the ratio of 2:1 (v/v) and were kept in 75% agro-net shade for one month, where they gained height up to 60 cm. Five month old hardened plants were planted in open field condition for evaluation of these tissue cultured raised plants. There is cent percent survival of these field grown plants over period of two years with no visible morphological abnormalities. Genetic fidelity test was carried out for these *in vitro* raised plants by using RAPD primers (OPA and OPN). Uniform banding pattern was observed in all plants without any polymorphism.

Key words: tissue culture, axillary shoot, rooting, hardening, oleo-gum-resin

Introduction

Commiphora wightii (Arn.) Bhandari (family Burseraceae) is well known by its vernacular names guggal (Hindi), guggul (English), guggulu (Sanskrit), etc. *C. wightii* is an endangered medicinal plant of arid and semi arid regions of Rajasthan, Gujarat, Maharashtra, Karnataka and Madhya Pradesh in India and also found Pakistan to gulf countries. It is now on the verge of extinction and predominant reasons for its fast diminishing population are over-exploitation, poor natural germination rate and slow growth. It has been listed in IUCN Red Data Book under Data Deficient Category [IUCN, 2012]. The species yield a valuable oleo-gum-resin from its shoot. The resin has

[1] Forest Biotechnology & Molecular Biology Laboratory, Forest Genetics and Tree Breeding Division, Arid Forest Research Institute, New Pali Road, Jodhpur 342005 – India; e-mail: tarunkant@icfre.org

tremendous value as cholesterol reducing agent and hence a favourite of Ayurvedic medicine industry [SATYAVATI, 1990]. In India, there is a gap between demand and supply of guggul gum and it is being imported from Pakistan and gulf countries moreover, the imported guggul gum is highly adulterated. The plant dies due to indiscriminate tapping for oleo-gum-resin [BHATT & al. 1989]. Natural regeneration is very poor and artificial propagation has limitations through seed as well as through stem cuttings [KANT & al. 2010a, 2010b]. Biotechnological tools have potential to overcome the problems of traditional methods of propagation for large scale production of high quality planting material, remove the gap between demand and supply in pharmaceutical market and also maintain the gene bank of this marvelous plant. *In vitro* propagation thorough axillary bud proliferation has tremendous scope of large scale production without losing the genetic identity of material.

Pioneer effort was made in this direction by BARVE & MEHTA (1993) through mature nodal segments of elite tree of *C. wightii*. Thereafter PRAJAPTI (2008) reported 40% bud break with only one shoot sprouted from each explant. These results depict that *C. wightii* tree is a recalcitrant and very slow growing species. An efficient protocol for *in vitro* clonal propagation of *C. wightii* is still a need for commercialization. Here we report a refined and efficient protocol for *in vitro* clonal propagation of *C. wightii* from nodal segments of mature plants, developed at Arid Forest Research Institute, Jodhpur with aim of selection of appropriate nodal segment explants, improvement in percent bud break response and multiplication rate, acclimatization and hardening of plantlets and growth performance in field condition. Morphological and Molecular characterization were done by collecting growth data from regular interval and by DNA finger printing of plants respectively.

Materials and methods

Plant material. Comiphora wightii nodal segments were collected from marked, visibly healthy trees growing in the natural population cluster in Kaylana region, Jodhpur district and Magliyavas, Ajmer district from where cuttings were brought, rooted and used as source of explants.

Explant selection and preparation. A young juvenile branch of mature *C. wightii* tree was categorized in three parts: immature, semi-mature and mature. Nodal segments from new branches having 6-7 nodes (semi-mature) were excised after discarding the initial 5-6 cm terminal portion (immature) of mature plant and used as an explant having 1-2 nodes.

Surface sterilization. Branches (15-20 cm) were washed under running tap water for 2 minutes to remove dirt and superfluous impurities. Explants were then shaken in 100 ml. RO water (produced from Millipore RiOS5) having 2 drops of tween-80 for 10 minutes, rinsed 3 times with sRO water (sterilized water from Reverse Osmosis). The cleaned explants were then treated for 10 minutes with a solution of 200 mg bavestein and 50 mg streptomycin in 100 ml sRO water with gentle shaking at 50 rpm and rinsed with sRO water once in a laminar flow clean air cabinet. Finally nodal segments were treated with 2.5% NaOCl (v/v) solution (5% available chlorine, Rankem) for 7-10 minutes and rinsed with sRO water thrice.

Autoclaving and growth room conditions. All media were adjusted at pH 5.8 and followed by autoclaving at 121 °C and 20 psi (137,900 pa) pressure for 15 minutes. All the cultures were aseptically inoculated and manipulated under a sterile laminar flow hood and incubated in tissue culture racks in an aseptic culture room having a temperature of 26 ± 2 °C, 16 h/d photoperiod and 1600 lux intensity light (via white cool florescent tubes).

Culture initiation. Sterilized nodal segments were vertically inoculated on autoclaved MS [MURASHIGE & SKOOG, 1962] medium supplemented with different concentrations of BAP (4.44, 8.88, 13.32, 17.75, 22.19 µM) and in combination with NAA (2.69 µM). Second treatment comprised of MS medium supplemented with different concentrations of BAP (4.44, 8.88, 13.32, 22.19 µM) and IAA (0.57 µM) with additives (50 mg l^{-1} ascorbic acid, 25 mg l^{-1} citric acid, 25 mg l^{-1} adenine sulphate) along with a positive control, earlier reported by BARVE & MEHTA (1993) on BAP (17.75 µM) and Kn (18.59 µM) with additives (glutamine 100 mg l^{-1}, thiamine HCL 10 mg l^{-1}, activated charcoal 0.3%). Further, most responsive treatments in terms of bud break response were tried on different media types MS, B5 [GAMBORG, 1968] and WPM [LLOYD & MCCOWN, 1968] media for suitability of the most responsive nutrient medium for improvement in terms of bud break response, length and numbers of shoots.

Multiplication & elongation of micro-shoots. After 3-4 weeks of inoculation, newly sprouted axillary buds were subcultured for multiplication on existing medium and on MS media supplemented with low concentrations of cytokinins (BAP and Kn) and combination with auxins (IAA and NAA) and with additives.

Rooting of micro-shoots. Micro-shoots were initially given a 24 h treatment in liquid MS and White's medium [WHITE, 1954] supplemented with 4.92 µM IBA and 5.71 µM IAA under dark condition, followed by transfer to semi-solid half-strength hormone-free MS and White's medium supplemented with 2% sucrose and 0.5% activated charcoal.

Acclimatization and hardening of plantlets. Well-rooted plantlets were transferred to glass jam jars filled-up to quarter level with vermiculite and soaked with half strength MS salt solution. After 4-5 weeks when plantlets showed new growth the plastic cap of the glass jar was unscrewed gradually over a period of 2-3 days to reduce relative humidity in the jar, then finally the caps were removed completely from the jars on the third day. The plantlets were then transferred to thermocol (Styrifoam) cups containing FYM : vermiculite :: 1:5 soaked with one fourth strength MS salt solution at one-week interval. These plantlets were placed in mist chamber. After two weeks these were then transferred to FYM : soil :: 1:2 mixture in plastic plantation bags (polythene-bags) of size 9x9x36 cm (2916 cm^3). In mist chamber, 90 second misting at ten minutes interval was given to maintain RH between 85 to 95%. The temperature of mist chamber was maintained between 28-30 °C. After one month of transfer to poly-bags plantlets were transferred under green-75% agro-net shade.

Field trial of in vitro raised plants. A field trial of *C. wightii* tissue culture raised plants was established in July, 2010 at AFRI Campus, Jodhpur in field area of 175.77 sq.meter (1891.9 sq.ft.), and an elevation at 725ft. above sea level and an altitude of N26˚13.865’ and latitude of E073˚01825’ in pit (size 1.5 x 1.5ft.) having distance of 2 x 2 meter. Watering was done on a monthly basis initially for a year. Later no watering was done and established plants have been growing on rain fed conditions. The trial comprised of 8 plants originated from axillary shoot proliferation based pathway (reported here) as

well as 42 plants somatic embryogenesis based pathway (results not reported here) and 10 from seedlings for comparative growth performance evaluation. Growth data (height, collar diameter, number of leaves, primary and secondary branches) are being collected at regular three month intervals.

Genetic fidelity test. Fresh semi mature leaves were collected from field trial of tissue culture raised *C. wightii* plants and from mother plant for DNA extraction and purification. DNA was isolated by following the protocol developed by SAMANTARAY & al. (2009) for *C. wightii* Arn. (Bhandari). RAPD analysis was performed based on the protocol of SAMANTARAY & al. (2009, 2010) by using six highly polymorphic randomly and arbitrarily 10-base primers (OPA 04, OPA 09, OPA 20, OPN 06, OPN 16 and OPN 20) synthesized from Xcelris genomics Labs Ltd., Ahmedabad (Gujrat) [WILLIAMS & al. 1990].

Statistical analysis. Experiments were set up in completely randomized design (CRD). Each treatment consisted of 15 replicates. Experiments were repeated thrice. The data were collected every 3-4 weeks and all requisite parameters were taken for data analysis. The data were analyzed statistically using SPSS ver 8.0 (SPSS, Chicago, IL). Significance of differences among means was compared by using analysis of variance and Duncan Multiple Range Test (DMRT) at $P \leq 0.05$.

Results and discussions

Plant materials. Abundance of undifferentiated cells in immature and semi-mature portion of young juvenile branch helps in establishing the culture quickly as compare to well differentiated mature portion of branch. This concept was kept in mind for selection of appropriate nodal segment explants which resulted in high percentage of bud breaks response. BARVE & MEHTA (1993) also suggested that mature nodal explants were collected after 8 days of excision of apical buds which resulted in high percent bud break response.

Explants sterilization. A study was carried out on responsiveness of different explants (nodal segment, leaf, internodes and semi mature fruits) to sterilization procedures for *C. wightii* by using NaOCl. It was concluded that semi-mature nodal segments treated with 2.5% NaOCl solution (5% available chlorine) for 7-10 minutes sterilize the explants up to 80-90%, which is comparable to the efficiency of $HgCl_2$ (mercuric chloride), Hence 2.5% NaOCl solution was used as an effective surfactant.

Culture initiation. MS medium supplemented with different concentration of BAP and combination with NAA were tried out for establishing *in vitro* culture, all concentrations resulted in axillary bud break, but the maximum of 46.7% bud break response was observed on MS medium supplemented with BAP (4.44 µM), NAA (2.69 µM), followed by 40.0% on MS medium supplemented with BAP (8.88 µM), NAA (2.69 µM). New shoots sprouted from each nodal area was not more than two which represent the effect of different concentrations of BAP and along with NAA were not significant. On the other hand, shoot length difference in two treatments was significant but not more than 0.5 cm in length which depict that the *C. wightii* is a recalcitrant and very slow growing species and these hormonal combination are not effective in further improvement of bud break response (Tab. 1).

Tab. 1. Effect of BAP and combination with NAA on bud break response

PGRs concentrations (μM)	Response (%)	Mean no. of shoots with ±SE	Mean shoot length (cm) with ±SE
©	6.7	1.00±00	0.20^{b}±0.0
4.44 BAP	20.0	1.33±0.3	0.20^{b}±0.0
8.88 BAP	13.3	1.00±0.0	0.50^{a}±0.0
13.32 BAP	20.0	1.67±0.3	0.40^{a}±0.1
17.75 BAP	6.7	1.33±0.3	0.40^{a}±0.1
22.19 BAP	6.7	1.00±00	0.50^{a}±0.0
4.44 BAP + 2.69 NAA	46.7	1.80±0.2	0.50^{a}±0.0
8.88 BAP + 2.69 NAA	40.0	1.50±0.3	0.48^{a}±0.0
13.32 BAP + 2.69 NAA	26.7	1.25±0.3	0.50^{a}±0.0
17.75 BAP + 2.69 NAA	26.7	1.33±0.3	0.17^{b}±0.2
22.19 BAP + 2.69 NAA	33.3	1.67±0.3	0.50^{a}±0.0

DMRT, Mean followed by different letters differ significantly at $p \leq 0.05$; © = control; SE, standard error.

Second treatment involved MS medium supplemented with different concentration of BAP and IAA with additives (50 mg l^{-1} ascorbic acid, 25 mg l^{-1} citric acid, 25 mg l^{-1} adenine sulphate) along with a positive control media which was reported by BARVE & MEHTA (1993) viz. MS medium supplemented with BAP (17.75 μM) and Kn (18.59 μM) with additives (100 mg l^{-1} glutamine, 10 mg l^{-1} thiamine HCL, 0.3% activated charcoal), (Tab. 2). After the end of 2nd week of inoculation, it was observed that a maximum 84.5% bud break response on BAP (8.88 μM) and IAA (0.57 μM) with additives (50 mg l^{-1} ascorbic acid, 25 mg l^{-1} citric acid, 25 mg l^{-1} adenine sulphate) was obtained. There was no further increase in the next two weeks (Tab. 2). After 3-4 weeks, these PGRs combination was found most responsive in terms of bud break with maximum shoot sprout (2.23±0.1 with 1.64±0.2 cm length of shoots). This response was better than the results earlier reported by BARVE & MEHTA (1993) and also used as positive control in the experiment. This positive treatment resulted in 75% bud break response, shoots sprouted up to 2.18±0.1with 1.59±0.2 cm length of shoot. These results clearly showed the effect of additives on bud break response and improve in the shoot number and shoot length (Fig. 1*a*, 1*b*). The positive effects of different additives on bud break response and shoot multiplication were reported earlier by BARVE & MEHTA (1993), SHEKHAWAT & al. (1993), NEELAM & CHANDEL (1992), KOMALAVALLI & RAO (1997). However our results are better than all earlier reports.

Tab. 2. Effect of BAP, IAA and additives on bud break response

PGRs concentrations (mg l^{-1})	Response (%)	Mean no. of shoots with ±SE	Mean shoot length (cm) with ±SE
©	75.0	2.18^{a}±0.1	1.59^{a}±0.2
BAP 1.0+IAA 0.1+ additives^{A}	54.8	1.67^{bc}±0.2	0.62^{b}±0.1
BAP 2.0+IAA 0.1+ additives^{A}	84.5	2.23^{a}±0.1	1.64^{a}±0.2
BAP 3.0+IAA 0.1+ additives^{A}	47.6	1.78^{bc}±0.2	0.94^{b}±0.2
BAP 5.0+IAA 0.1+ additives^{A}	28.6	1.33^{c}±0.3	0.77^{b}±0.1

DMRT, Means followed by different letters differ significantly at $p \leq 0.05$; ©- BAP 4.0+ Kn 4.0+ additives^{B} (BARVE & MEHTA, 1993); **A-** 50 mg l^{-1} ascorbic acid, 25 mg l^{-1} citric acid, 25 mg l^{-1} adenine sulphate; **B-** 100 mg l^{-1} glutamine, 10 mg l^{-1} thiamine HCL, 0.3% activated charcoal; SE, standard error.

Further experiment was carried out on different media (MS, B5 and WPM) supplemented with best responsive plant growth regulators (PGRs) and with additives in terms of bud break response among the aforesaid experiments for establishing the best nutrient medium (Tab. 3).

Tab. 3. Effect of different media on bud break response on best responsive PGRs combination

Media		Response %	Mean no. of shoots with ±SE	Mean shoot length (cm) with ±SE	Mean no. of leaves with ±SE
MS	A	84.6	1.64^{b}±0.2	0.65±0.2	4.50±0.7
	B	73.1	1.78^{ab}±0.3	0.72±0.2	4.00±1.0
	C	38.5	1.17^{b}±0.1	0.56±0.1	4.25±0.6
B5	A	53.8	1.50^{b}±0.2	0.35±0.1	5.00±1.1
	B	34.6	1.60^{b}±0.4	0.54±0.1	6.40±1.4
	C	19.2	1.11^{b}±0.1	0.31±0.0	4.33±0.5
WPM	A	65.4	1.40^{b}±0.2	0.28±0.0	4.40±1.0
	B	53.8	2.40^{a}±0.2	0.44±0.1	4.20±0.4
	C	23.1	1.40^{b}±0.2	0.34±0.1	5.60±0.9

DMRT, Means followed by different letters differ significantly at p≤ 0.05; **A**- BAP (8.88 µM)+IAA (0.57 µM)+additives: 50 mg l^{-1} ascorbic acid, 25 mg l^{-1} citric acid, 25 mg l^{-1} adenine sulphate; **B**- BAP (17.75 µM)+Kn (18.59 µM)+additives: 100 mg l^{-1} glutamine, 10 mg l^{-1} thiamine HCL, 0.3% activated charcoal; **C**- BAP (8.88 µM)+NAA (2.69 µM); SE, standard error.

Multiplication & elongation of micro-shoots. After 3-4 weeks of inoculation, newly sprouted axillary buds were sub-cultured for multiplication on existing medium and on various MS media supplemented with lower concentrations of cytokinin (BAP and KIN) and with combination of auxins (IAA and NAA) and additives (Tab. 4). After 3-4 weeks, no micro-shoots multiplied and leaves turned yellow. Defoliation was clearly seen in all cultures.

Tab. 4. Effect of different cytokinin, auxins and additives on multiplication of newly sprouted microshoot

S. no.	Treatment (µM)	Mean no. of shoots with ±SE	Mean shoot length (cm) with ±SE
1.	4.44 BAP	1.20^{a}±0.1	0.44^{c}±0.0
2.	4.44 BAP+0.54 NAA	1.53^{ab}±0.1	0.36^{abc}±0.0
3.	4.44 BAP+0.57 IAA	1.60^{b}±0.1	0.46^{c}±0.1
4.	4.44 BAP+4.65 Kn+0.54 NAA	2.20^{a}±0.1	0.24^{a}±0.0
5.	4.44 BAP+4.65 Kn+0.57 IAA	1.87^{bc}±0.1	0.38^{abc}±0.0
6.	4.44 BAP+4.65 Kn+activated charcoal	1.80^{b}±0.1	0.39^{bc}±0.1
7.	4.44 BAP+4.65 Kn+additives(Th. HCl+ glu +ads + AC)	1.93^{bc}±0.2	0.30^{ab}±0.0
8.	4.44 BAP+4.65 Kn+ additives(Th. HCl+ glu+ ads+ AC)	1.87^{bc}±0.1	0.28^{ab}±0.0
9.	4.44 BAP+4.65 Kn+ additives (Th. HCl+ aa +ca+ AC)	1.73^{b}±0.1	0.28^{ab}±0.0
10.	4.44 BAP+0.54 NAA+ additives (Th. HCl+ glu+ ads)	1.67^{b}±0.1	0.26^{ab}±0.0

DMRT, Means followed by different letters differ significantly at p≤ 0.05; Th. HCl- Thiamine HCL (10 mg l^{-1}), glu-glutamine (100 mg l^{-1}), ads- adenine sulphate(25 mg l^{-1}), AC- activated charcoal (0.3%), aa- ascorbic acid (50 mg l^{-1}), ca- citric acid (25 mg l^{-1}); SE, standard error.

The micro-shoots were subcultured and maintained for further elongation on the same medium for 4 weeks (Fig. 1c). Maximum three shoots were observed per explant. Only well elongated micro-shoots were found suitable for rooting. PREECE (1995) suggested that the accurate concentration of nutrients in the medium removes the stress in explants and help to improve the *in vitro* performance, which can not be achieved solely by the use of plant growth regulators. Further refinement is still needed for multiplication of micro-shoot.

Rooting of micro-shoots. Micro-shoots of 2-3 cm length were initially given a 24 h treatment in liquid MS and White's medium supplemented with 4.92 µM IBA and 5.71 µM IAA under dark condition, followed by transfer to semi-solid half-strength hormone-free MS and White's medium supplemented with 2% sucrose and 0.5% activated charcoal. High (86.7%) percent rooting was achieved after 4-5 weeks with 2.85 ± 0.5 multiple adventitious roots of 6.46 ± 0.4 cm length (Fig. 1d) on half-strength hormone-free MS medium with 0.5% activated charcoal, while 46.7% rooting was observed on White's medium with 0.5% AC (Tab. 5). Earlier, BARVE & MEHTA (1993) also reported these hormonal combinations were found best for rooting of micro-shoots of *C. wightii*. The marked promoting effect of auxins (IBA and IAA) and darkness on adventitious root formation in micropropagated shoots of different species were clearly reported by FOURET & al. (1986), GEROGE (1993), MONTEUUIS & BON (2000) and influence of agar and activated charcoal on *in vitro* morphogenesis in plants were reported by YASSEEN (2001).

Tab. 5. Effect of different media on *in vitro* hardening of microshoot.

S. No.	Media	Rooting %	Mean root length (cm) with ±SE	Mean root/ shoot with ±SE
1.	MS medium	86.7	6.46±0.4	2.85±0.5
2.	White's medium	46.7	6.29±0.4	2.29±0.2

SE, standard error

Acclimatization and hardening of plantlets. Chemoautotrophic well-rooted plantlets were gradually hardened in different phases; *in vitro* hardening, *ex vitro* hardening in plastic cups at mist-chamber and in polythene-bags in green-shade house to make them ready for field transfer as complete photoautotrophic plants. The well-rooted plants (4-5 cm) were gently removed from the vessels, washed initially to remove adhered traces of the depleted medium and then washed for 5-10 min in autoclaved distilled water [THIRUVENGADAM & al. 2006]. Within 4-5 weeks, these plants gained 3-5 cm height and showed 62% survival rate during *in vitro* hardening in culture room (Fig. 1e). During *ex vitro* hardening plants in mist-chamber and green-shade house remained showed 100% survival and attained 10-12 cm height (Fig. 1f). After five months, plants attained average height of 60 cm. Finally robust and healthy plants were transferred to field at the onset of rains (monsoon) season for field performance evaluation (Fig. 2a, 2b).

Field trial of in vitro raised plants. A comparative field trial of *C. wightii* tissue culture raised plants was laid out for growth performance evaluation and comprised of *in vitro* raised plants derived from mature axillary shoots (nodal segments), somatic embryogenesis pathway and seed derived plants. Plants are growing well in the field condition from last two years with 100% survival (Fig. 2c), (Tab. 6). Mean growth data in

terms of height (123.6±5.7 cm); collar diameter (2.1±0.1 cm); number of leaves (406.3±44.8); primary (24.4±2.4) and secondary (11.8±2.2) branches after two years and three month indicate robust plants with good growth rates compared to seedling derived plants.

Tab. 6. Growth performance of axillary shoot derived plant in experimental field over period of two years and three months.

Years	Months	Mean height (cm.) with ±SE	Mean CD (cm.) with ±SE	Mean no of primary branch with ±SE	Mean no of secondary branch with ±SE	Mean no of leave with ±SE
2010	June-August	86.9^{b}±7.5	0.8^{e}±0.1	1.6^{f}±0.4	0.0^{e}±0.0	167.3^{b}±40.1
	September-November	99.8^{ab}±9.5	1.1^{d}±0.1	6.4^{e}±0.9	1.0^{e}±0.6	172.5^{b}±11.9
	December-February	103.6^{ab}±9.6	1.2^{d}±0.1	5.5^{e}±1.4	2.0^{e}±0.8	24.4^{d}±8.4
2011	March-May	104.4^{ab}±10.3	1.2^{d}±0.1	8.8^{de}±0.7	$6.8\pm^{bc}2.1$	14.4^{d}±7.9
	June- August	105.5^{ab}±9.7	1.2^{d}±0.1	10.1^{d}±0.9	13.1^{ab}±4.9	53.1^{d}±10.2
	September-November	108.1^{ab}±9.4	1.3^{d}±0.1	11.4^{cd}±0.9	15.8^{a}±4.3	118.1^{bc}±11.6
	December February	109.0^{ab}±9.0	1.4^{cd}±0.1	13.9^{bc}±1.2	17.1^{a}±3.6	43.1^{d}±3.7
2012	March- May	110.3^{ab}±8.5	1.6^{bc}±0.1	16.9^{b}±0.9	17.1^{a}±2.2	77.5^{cd}±5.9
	June- August	112.0^{ab}±8.5	1.9^{ab}±0.1	21.3^{a}±1.3	18.3^{a}±2.5	125.0^{bc}±4.6
	September-November	123.6^{a}±5.7	2.1^{a}±0.1	24.4^{a}±2.4	11.8^{ab}±2.2	406.3^{a}±44.8

DMRT, Means followed by different letters differ significantly at $p \leq 0.05$; CD, collar diameter; SE, standard error.

Fig. 1. Axillary Shoot Proliferation (a-f): a, b - Axillary bud sprouted from mature nodal segment; c - Multiplication and elongation of micro-shoot; d - Rooting in micro-shoots; e - *In vitro* hardening of plantlets; f - *Ex vitro* hardening of plantlets in plastic cups.

Fig. 2. Axillary Shoot Proliferation (a - c): a, b - Plantlets in mist-chamber and in green-shade house; c - *C. wightii* tissue culture plants over a period of two years.

*Genetic fidelity test***.** DNA was extracted from semi-mature leaves of tissue culture *C. wightii* field growing plants after one year plantation and from mother plant growing at its natural sites by following the protocol developed by SAMANTARAY & al. (2010). RAPD analysis was performed based on earlier screened highly polymorphic RAPD primers for *C. wightii* by SAMANTARAY & al. (2009, 2010); WILLIAMS & al. (1990). These six highly polymorphic randomly and arbitrarily 10-base primers (OPA 04, OPA 09, OPA 20, OPN 06, OPN 16 and OPN 20) were used for genetic fidelity test. All banding profiles from micropropagated plants through axillary shoot proliferation were monomorphic and similar to those of the mother plant (Fig. 3). The genetic stability of *in vitro*-propagated plants has been confirmed in many numbers of species like *turmeric* [SALVI & al. 2001], *Pinus thunbergii* [GOTO & al. 1998], *Gerbera jamesonii* [BHATIA & al. 2011], *Chlorophytum arundinaceum* [LATTOO & al. 2006].

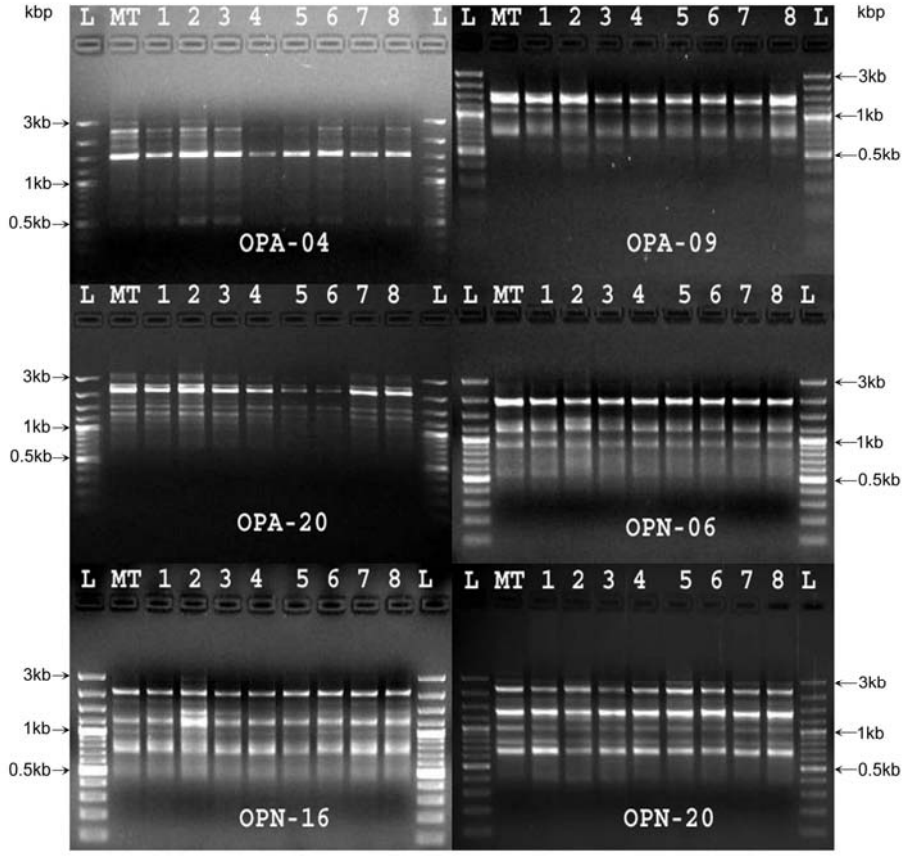

Fig. 3. Genetic fidelity test of axillary shoot proliferation derived tissue culture plants of *C. wightii* by using six highly polymorphic RAPD primers of Operon series (OPA-04, OPA-09, OPA-20, OPN-06, OPN-16 and OPN-20). L-100bp ladder, MT- Mother Tree (Mangliawas, Ajmer district, growing near AFRI Lab.)

Conclusions

Commiphora wightii is an important, endangered and sought after species due to its valuable oleo-gum-resin having tremendous medicinal importance. *In vitro* raised plants of *C. wightii* through axillary shoot proliferation was successfully regenerated at a good efficiency in this investigation by developing a complete protocol and their adaptability in field over a period of two years as proved. Evaluation of growth performance and genetic fidelity test of these *in vitro* raised plants has been successfully done. This is the first report on the field performance of tissue culture *C. wightii* plants and their genetic fidelity test. This protocol would provide an effective strategy for the conservation of this over-exploited medicinal plant. Multiplication rate of micro-shoots is still low. Bud break response has been improved compared to earlier reports. Hardening procedure which was not well established earlier has been fine tuned with the cent percent field performance of plants more than two years in open field.

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COMPARATIVE STUDY ON THE FIELD PERFORMANCE OF FHIA-01 (HYBRID DESSERT BANANA) PROPAGATED FROM TISSUE CULTURE AND CONVENTIONAL SUCKER IN GHANA

DZOMEKU BELOVED MENSAH[1], QUAIN MARIAN DORCAS[1],
BAM RALPH KWAME[1], DARKEY SOLOMON KODJO[1]

Abstract: Micro-propagated plants of FHIA-01 (exotic hybrid dessert banana) were grown and their shoot-tip cultures were produced following standard method. Suckers were taken from the same plants as with the shoot-tip culture samples. The design was the randomly complete block. The plant density was 1667 plants/ha. Plants were fertilized at the rate of 40 t/ha poultry manure per year split over 3 equal applications. Statistical analysis of data was performed with ANOVA. The field performance of *in vitro* propagated (tissue culture) tetraploid banana (FHIA-01) plants was compared with that of sucker-derived plants. In vitro-propagated plants established and grew faster, taller (240 cm) and bigger than the conventional sucker-derived plants. The former produced heavier bunches (39.1 t/ha) and could be harvested earlier. They however produced smaller number of fingers than the conventional sucker-derived plants. Significant differences were observed between the plant height and plant girth (48.6 cm) (at one meter above ground) at harvest. No significant difference was observed in bunch weight, number of hands, number of fingers and the number of leaves at harvest. The nutrient used in the Tissue culture medium may have played a significant role in the growth vigour of FHIA-01. It may also be having an influence on the performance of the hybrid. This influence may improve the yield of the crop thus improving the economy of farmers.

Key words: Banana (*Musa* spp.), micro-propagated, sucker-derived, *in vitro*

Introduction

Banana is a very important fruit in world commerce and is probably only surpassed by citrus in this regard [SAMSON, 1986]. In terms of gross value of production, banana is the fourth most important global food crop [TRIBE, 1994]. Export bananas are the fourth most important commodity and, as a fruit rank first [FRISON & al. 1997]. Bananas and plantains constitute a major staple food crop for millions of people in developing countries of the tropics [FAO, 1995]. They are grown over a harvested area of approximately 10 million hectares with annual production of about 86 million metric tons [FAO, 1995].

Bananas are asexually propagated by either separating the daughter suckers from the mother plant or by forcing the growth of the buds on the mother plant by stripping the older leaf sheaths (tissue culture) [BAKER, 1959]. Tissue culture method, however require sophisticated equipment and skills that are not easily accessible to farmers in developing countries. Tissue culture unlike the sucker-derived planting material has various advantages. These include producing uniform planting materials at the same time; clean, disease-free materials and many plants in a small space. *In vitro* micro-propagation of banana plants has been reported to be faster than the conventional propagation with sucker-

[1] Crops Research Institute, Council for Scientific and Industrial Research, P. O. Box 3785, Kumasi – Ghana, West Africa, e-mail: bmdzomeku@gmail.com, mdzomeku@yahoo.com

derived plants [DREW & SMITH, 1990]. However, there seems to be variety-dependency. For example, ZAMORA & al. (1989) reported that the yield from *in vitro*-propagated plants was significantly higher than that from sucker-derived plants in Lacatan banana (AAA), but the reverse was the case in Bungulan banana. DIRK & ORTIZ (1996) reported that *in-vitro* propagated *Musa* plants grew faster but the yield was not significantly different from that of the sucker-derived plants.

FHIA-01 hybrid banana is one of the breakthroughs in *Musa* international breeding programmes against the devastating fungal disease black sigatoka leaf spot (*Mycosphaeralla fijiensis* Morelet). In response to these production constraints efforts aimed at the genetic improvement of Musa have made significant strides [PERSLEY & DE LANGHE, 1987]. However, germplasm enhancement is burdened with obstacles typical of vegetative propagated crops, among which are low reproductive fertility and slow propagation are most conspicuous. One technique that has been identified for *Musa* germplasm handling and improvement is biotechnology [INIBAP, 1993]. Application of *in vitro* culture technique has significantly improved the germplasm handling. Large numbers of *in vitro* plants have been produced for rapid establishment of hybridization blocks and for international multi-site evaluation [VUYLSTEKE & al. 1993]. However, it has been observed that plants generated from *in vitro* culture exhibit various morphological and biochemical variations due to genetic change that LARKIN & SCOWCROFT (1981) termed somaclonal variation. Somaclonal variation rate of 0%-70% have been recorded in *Musa* produced from shoot - tip culture [SMITH, 1988; VUYLESTEKE & al. 1991].

FHIA-01 is hybrid banana from a cross between the wild banana and the domesticated banana. It was bred against black sigatoka and nematodes. It was introduced into Ghana in early 1990 from Hunduras to be evaluated and disseminated. Sufficient evaluation has been done and it has been released to farmers under the cultivar name *Kwedu bempa*.

This study was carried out to compare the field performance of the hybrid using conventional suckers and tissue cultured plantlets.

Material and methods

In-vitro derived planting materials (tissue culture) and conventional suckers taken from already growing FHIA-01 (*Musa* sp. AAA group) were chosen for the study. The conventional suckers were produced using the split corm techniques. The trial was planted in the rainy season of 2008 at Fumesua near Kumasi in the semi-deciduous forest zone of Ghana. The soil was yellowish-red, moderately drained gritty clay-loam containing quartz gravels. The annual rainfall averaged 2400 mm from (bimodal). Plants were spaced at 3 m x 2 m, providing a density of 1667 plants/ha. Plants were established in a randomized complete block design with three replications. Plants were fertilized at the rate of 40 t/ha poultry manure per year split over three equal applications. Field maintenance was the usual slashing with machete.

Twenty plants from each plot were tagged for data collection. Data was taken on growth parameters at two months after planting and thereafter. At harvest, the following parameters were considered plant height, yield, and number of leaves at harvest, number of daughter suckers, plant girth at one meter above ground, number of hands, number of fingers and the crop cycle. Statistical analysis of data on growth was done with ANOVA.

Results and discussions

The results of the study revealed that there was no significant difference in the number of leaves both at flowering and harvest between sucker-derived and tissue culture-derived plants (Tab. 1).

Tab. 1. Growth characteristics of in vitro-propagated and sucker-derived banana plants under field conditions

Planting Materials	Number of leaves at flowering	Number of leaves at harvest	No. of suckers
Sucker- derived	13.2	7.4	4.3
In vitro- Propagated	14.1	7.5	5.2
P< 0.01	ns	ns	ns

ns = not significantly different n = 20

The number of functional leaves on a banana plant at flowering plays a significant role in the yield at harvest. The higher number of leaves (about 12) is an indication of a heavy bunch. The high number of leaves at flowering compared favorably with the results of ALVAREZ (1997). In banana and plantain agronomy total number of functional leaves that a plant has at flowering time is a good indicator of its tolerance or susceptibility to diseases, with correlation existing between number of leaves and bunch weight.

There was a significant difference in the days to flowering, fruit filling and days to harvest between sucker-derived and *in-vitro*-derived plants (Tab. 2). The *in-vitro*-derived plants flowered earlier and also matured earlier than the sucker-derived plants.

Tab. 2. Yield characteristics of in vitro-propagated and sucker-derived banana plants under field conditions

Planting Material	Days to flowering	Days for fruit filling	Days to harvest	Bunch weight (t/ha)	No. of hands	No. of fingers
Sucker-derived	299.5±3	98.5±1	392.3±2	38.0±1	7.4±1	107.1±2
In vitro-Propagated	286.2±1	90.3±4	376.5±5	39.1±2	7.5±1	104.2±3
P< 0.01	**	**	**	ns	ns	ns

ns = not significantly different ** significantly different at P< 0.01 n=20

There was significant difference in plant height with the tissue-cultured plants producing taller plant (2.40 m) than the conventional sucker (2.14 m). Fig. 1 and 2 showed that the *in vitro*-propagated banana plants grew faster and their pseudostem circumference increased faster than that of the conventional sucker-derived plants. Plant height and pseudostem circumference increased more rapidly in the *in vitro*-propagated plants than the sucker-derived plants during the vegetative growth period. Optimum growth was observed at about the sixth month for the in vitro-propagated plants whereas the sucker-derived occurred at 5.7 months (Fig. 3). This may be a factor contributing to the earliness in the harvest of the in vitro-propagated plants. The number of hands per bunch and the number of fingers did not show any significant variation. The *in vitro*-propagated plants flowered about two weeks earlier than the sucker-derived plants. This was also reflected in the days

to harvesting as the *in vitro*-propagated were harvested about sixteen days earlier than the sucker-derived plants.

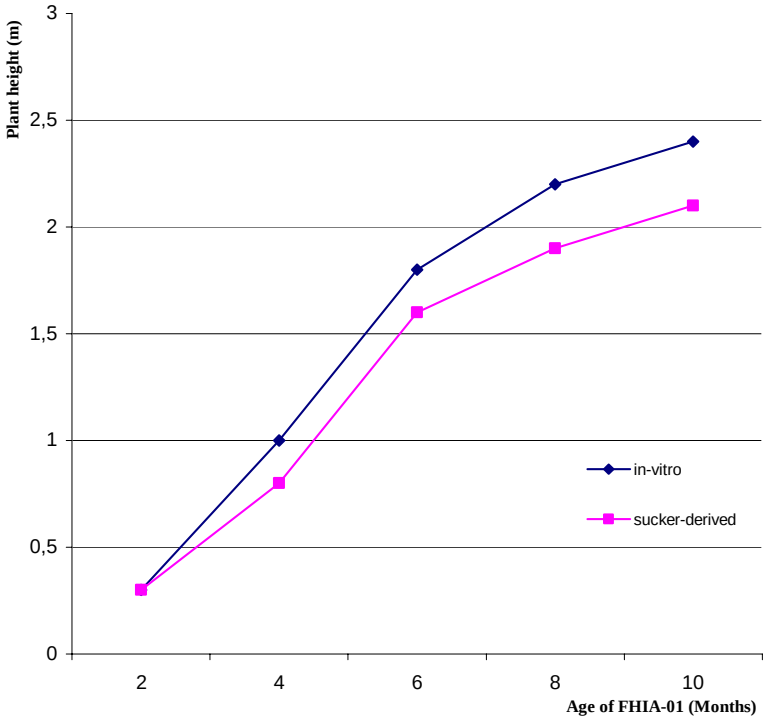

Fig. 1. Plant height of in vitro-propagated and sucker-derived banana plants grown in the field

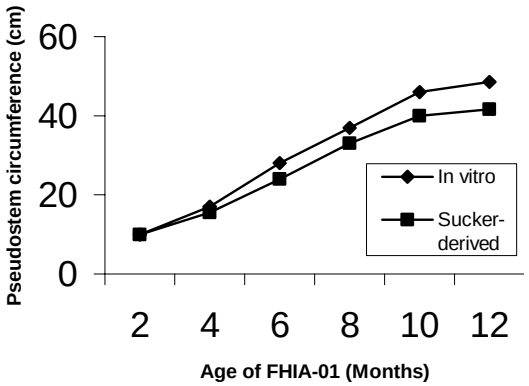

Fig. 2. Pseudostem circumference of in vitro-propagated and sucker-derived banana plants grown in the field

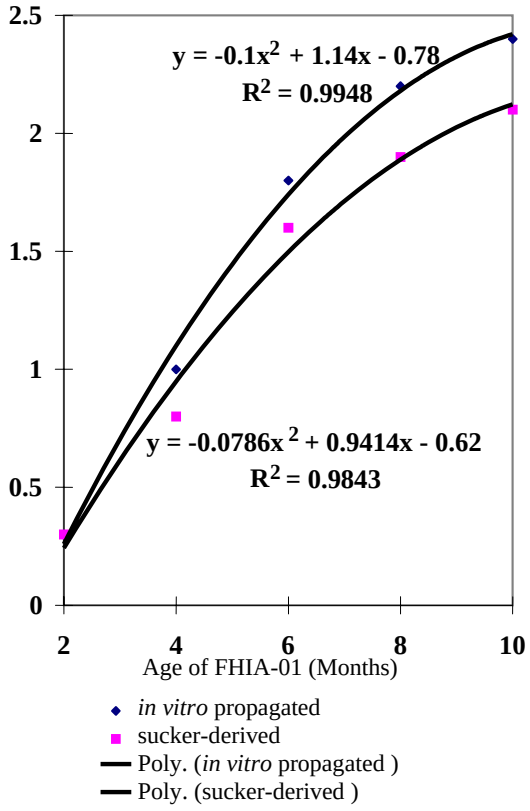

Fig. 3. Relationship between height and age of FHIA-01

The yield characteristics are presented in Tab. 2. The *in vitro*-propagated plants grew faster that the sucker-derived plants. This was reflected in the shorter crop cycle in the *in vitro*-propagated plants than the sucker-derived plants. The number of fingers in the *in vitro*-propagated plants was smaller compared to those of the sucker-derived plants yet they weighed heavier. This may be due to the bigger finger sizes observed in the *in vitro*-propagated plants.

Several authors have reported the superior growth and yield of *in vitro*-propagated banana plants compared to the conventional sucker-derived plants in various types of bananas and plantains [DREW & SMITH, 1990; ROBINSON & al. 1993]. These results are in agreement with their findings. DIRK & ORTIZ (1996) however could not find any significant different in yield between tissue culture and sucker-derived banana plants. These results seemed to follow the trend observed by DIRK & ORTIZ (1996). ROBINSON & ANDERSON (1991) also reported that faster growth and high yields were not always observed in the *in vitro*-propagated banana. However, pseudostem circumference and height of the *in vitro*-propagated plants were significantly higher than those of sucker-derived plants. The low yield of *in vitro*-propagated plants according to these authors was attributed to high incidence of diseases.

The faster growth of *in vitro*-propagated plants could be attributed to their intact active roots and shoot systems that can function almost immediately after planting. Unlike in the conventional method where paring is done on the sucker before planting the *in vitro*-propagated plants continue to photosynthesize immediately after planting. There is therefore a lag phase in the sucker-derived plants. This lag phase may take two weeks or more for the sucker to recover. Also the *in vitro*-propagated plants might have some carry-over nutrient stock upon which they could depend. The factors described above could explain the differences observed between the *in vitro*-propagated and the sucker-derived plants.

Conclusion

The study has revealed that tissue culture application in banana planting material production contributes significantly to crop performance. Tissue culture produced plantlets become robust in the field. It could be concluded that tissue culture produced planting materials perform agronomically better than conventionally produced materials.

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IMPACT OF METHYL JASMONATE ON PLB FORMATION OF HYBRID *CYMBIDIUM* (Orchidaceae)

JAIME A. TEIXEIRA DA SILVA[1]

Abstract: When methyl jasmonate (MeJA) was added at 1 mg/l, it could stimulate the development of protocorm-like bodies (PLBs) or PLB thin cell layers of hybrid *Cymbidium* Twilight Moon 'Day Light', when added to Teixeira *Cymbidium* (TC) medium without plant growth regulators. This is a simple means to mass produce PLBs for commercial purposes.

Key words: MeJA, PGR, protocorm-like body or PLB, Teixeira *Cymbidium* (TC) medium, thin cell layer or TCL

Introduction

Ethylene and methyl jasmonate (MeJA) pathways are often interlinked and related to plant development [SANIEWSKI & al. 2002] and defence [ALMAGRO & al. 2009]. MeJA stimulated the genes involved in lignin biosynthesis [YAQOOB & al. 2012]. MeJA at 1 μM stimulated protocorm-like body (PLB) formation (from shoots) and shoot formation in epiphytic *Cymbidium eburneum* Lindley and in terrestrial *Cymbidium kanran* Makino [SHIMASAKI & al. 2003] and this possibility served as the basis for assessing in this study whether the same would be true, and possible, for hybrid *Cymbidium*. Limited other studies on the effects of MeJA on plant growth *in vitro* exist although MeJA was shown to enhance flavonolignan production 300% in *Silybum marianum* liquid root cultures [ELWEKEEL & al. 2012], typical of jasmonates, which, in their capacity as a stress hormone, stimulate secondary metabolite production in a wide range of plant cell cultures [KOO & HOWE, 2009]. *Lilium* bulb formation was enhanced *in vitro* in the presence of MeJA [JASIK & DE KLERK, 2006] as was potato tuberization *in vitro* [SARKAR & al. 2006].

In vitro protocols for the induction and development of protocorm-like bodies (PLBs) of hybrid *Cymbidium* are well established. Despite this, no study exists yet on the use of and effect of MeJA *in vitro* on hybrid *Cymbidium*. Thus, using a newly developed ideal *Cymbidium* PLB regeneration medium, termed Teixeira *Cymbidium* (TC) medium [TEIXEIRA DA SILVA, 2012], the effect of MeJA on PLB formation was assessed with the expectation of increasing PLB formation.

Materials and methods

Chemicals and reagents

All chemicals and reagents were of the highest analytical grade available and were purchased from either Sigma-Aldrich (St. Louis, USA), Wako (Osaka, Japan) or Nacalai Tesque (Kyoto, Japan), unless specified otherwise.

[1] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Kagawa, 761-0795, Japan, e-mail: jaimetex@yahoo.com

Plant material and culture conditions

PLBs (either half-moon PLBs or PLB TCLs: see TEIXEIRA DA SILVA, 2013) of hybrid *Cymbidium* Twilight Moon 'Day Light' (Bio-U, Japan) originally developed spontaneously from shoot-tip culture on Vacin and Went (VW, 1949) agar medium without PGRs, were induced and subcultured (PLB induction and proliferation medium or VWPLB) every two months on TC medium [TEIXEIRA DA SILVA, 2012], which contains unique levels of macro- and micronutrients, and was supplemented with two plant growth regulators, or PGRs (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), according to procedures and advice outlined by TEIXEIRA DA SILVA & al. (2005) and TEIXEIRA DA SILVA & TANAKA (2006).

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^2s provided by plant growth fluorescent lamps (Homo Lux, Matsushita Electric Industrial Co., Japan). Longitudinally bisected PLB (3-4 mm in diameter) segments, 10 per flask, were used as explants for PLB induction and proliferation and for all experiments. Culture conditions and media followed the recommendations previously established for medium formulation [TEIXEIRA DA SILVA & al. 2005], biotic [TEIXEIRA DA SILVA & al. 2006b] and abiotic factors [TEIXEIRA DA SILVA & al. 2006a] for PLB induction, formation and proliferation.

Effect of ethylene inhibitors on PLB formation

Half-moon PLBs and PLB thin cell layers (TCLs) were cultured on PGR-free TC or PGR-containing TC medium in the presence of 1, 2, 4 or 8 mg/l MeJA. The control contained no PGRs and no MeJA. Solutions were made fresh and were filtered prior to the addition to TC medium.

Morphological parameters assessed

The number of PLBs formed per PLB segment or PLB TCL was measured after 45 days in culture since PLBs induce shoots spontaneously when left in culture on the same medium for more than 45 days. In this study, shoots are not desired, only PLBs since PLBs are the ideal propagative medium and somatic embryos for use in other studies. PLBs form on the surface of PLBs only [TEIXEIRA DA SILVA & TANAKA, 2006].

Statistical analyses

Experiments were organized according to a randomized complete block design (RCBD) with three blocks of 10 replicates per treatment (i.e., each medium). All experiments were repeated in triplicate (n = 90, total sample size per treatment). Data was subjected to analysis of variance (ANOVA) with mean separation by Duncan's new multiple range test (DNMRT) using SAS® vers. 6.12 (SAS Institute, Cary, NC, USA). Significant differences between means were assumed at $P \leq 0.05$.

Results and discussions

The addition of 1 mg/l of MeJA to PGR-free TC medium improved PLB formation significantly relative to PGR-free control TC, although PLB formation decreased as MeJA level increased (Tab. 1). MeJA is thus a simple and cost-effective way to improve PLB production for micropropagation purposes, serving as an alternative to PGRs (Fig. 1).

The response of MeJA, as a subset of jasmonates, is usually in response to a biotic or abiotic stress and is thus generally associated with growth inhibition (for example *Medicago sativa* somatic embryogenesis; RUDUŚ & al. 2006) rather than growth promotion, although this can be beneficial for secondary metabolite production *in vitro* where it serves as an elicitor [KOO & HOWE, 2009]. Consequently, MeJA is not frequently observed as a useful PGR and thus the number of studies remains limited. It is however, frequently used as a postharvest treatment to suppress fungal infections in fresh produce [e.g., GONZALEZ-AGUILAR & al. 2003]. Transgenic soybean plants with enhanced MeJA expression showed strongly different morphology in leaves and roots [XUE & ZHANG, 2007] while somatic embryogenesis-related genes (*Lea*) were activated in *Nicotiana plumbaginifolia* [REINBOTHE & al. 1994]. TCLs were also used to enhance adventitious root production in tobacoo after exposure to MeJA [FATTORINI & al. 2009] although, interestingly, it disrupted TCL-derived shoot formation, also in tobacco, by over-inducing mitotic activity and cell expansion [CAPITANI & al. 2005]. MeJA also reduced microrhizome biomass in turmeric cultures [COUSINS & ADELBERG, 2008]. PLBs and somatic embryos are synonymous in orchids [TEIXEIRA DA SILVA & TANAKA, 2006], and thus this protocol, using half-moon PLBs or PLB TCLs, represents a means of producing PLBs where a bioreactor is not available. Simple techniques that allow for increasing PLB production are central to orchid biotechnology [HOSSAIN & al. 2013].

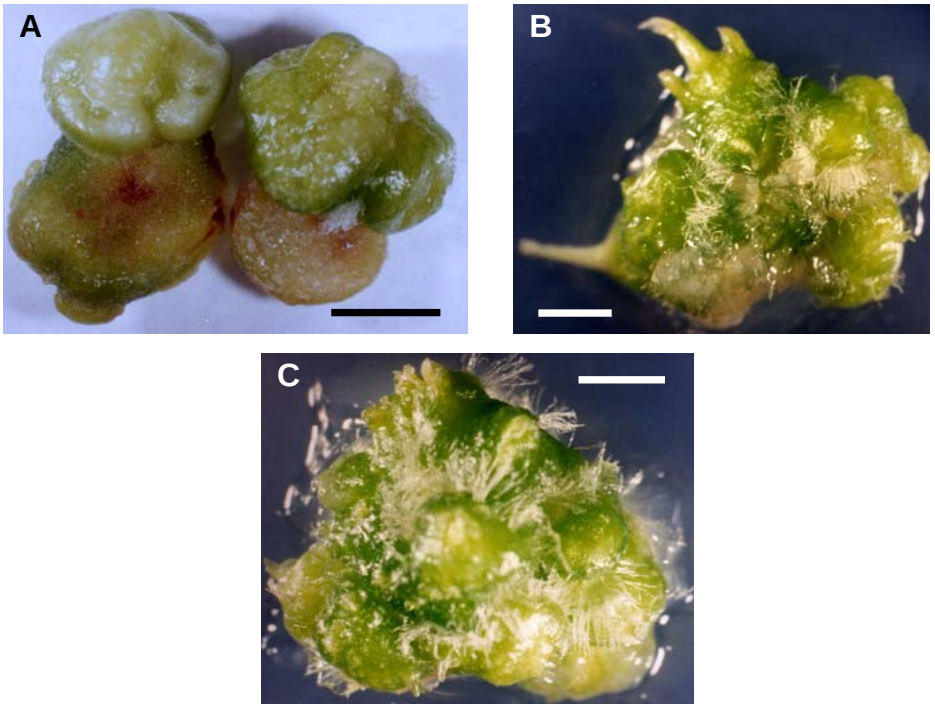

Fig. 1. (A) Thin cell layer forming PLBs 45 days after culture on Teixeira *Cymbidium* medium (TCPLB) with PGRs (0.1 mg/l α-naphthaleneacetic acid + 0.1 mg/l kinetin). (B) PLB induction from half-moon PLBs on TCPLB. (C) PLB formation on TCPLB without plant growth regulators but supplemented with 2.0 mg/l methyl jasmonate. Bars = 1 mm (A), 5 mm (B, C).

Tab. 1. Effect of methyl jasmonate on new/*neo*-PLB formation from half-PLB or PLB TCL culture of hybrid *Cymbidium* Twilight Moon 'Day Light'.

Medium composition	**MeJA concentration (mg/l)**	**Percentage of explants forming *neo*-PLBs (%)**	**Number of PLBs per explant**	**Fresh weight (mg) of PLB explant + *neo*-PLBs**
Half-moon PLBs on: TC (control) PGR-free TC		 100 a 81 ab	 8.3 b 6.1 bc	 526 a 431 ab
PLB TCLs on: TC (control) PGR-free TC		 100 a 76 ab	 1.2 e 0.4 f	 321 b 81 cd
Half-moon PLBs on: PGR-free TC + MeJA	1	100 a	9.6 a	518 a
	2	100 a	4.8 c	341 b
	4	67 b	2.6 d	186 c
	8	21 c	1.1 e	98 c
PLB TCLs on: PGR-free TC + MeJA	1	100 a	3.6 cd	324 b
	2	100 a	1.4 e	303 b
	4	78 b	0.8 ef	118 bc
	8	46 bc	0.3 f	68 d

Mean values followed by the same letter in the same column are not significantly different based on DMRT ($P = 0.05$). See text for media constituents. n = 90 (9 Petri dishes × 10 for each treatment).

MeJA, methyl jasmonate; PGR, plant growth regulator; PLB, protocorm-like body; TC, Teixeira *Cymbidium* medium [TEIXEIRA DA SILVA, 2012], includes 0.1 mg/l α-naphthaleneacetic acid and 0.1 mg/l kinetin, 2 g/l tryptone and 20 g/l sucrose (see reference for modified micro- and macro-nutrients); TCL, thin cell layer

Conclusions

Half-moon PLB explants or PLB TCLs can be used to derive new or *neo*-PLBs in hybrid *Cymbidium*. MeJA has shown to alter growth and developmental properties in plants, including other *Cymbidium* spp., and in this study, at least in hybrid *Cymbidium*, it could enhance PLB production relative to ideal PGR-containing medium. Although TCLs overall produce fewer *neo*-PLBs/PLB explant than half-moon PLBs, after conversion with the Plant Growth Correction Factor (TEIXEIRA DA SILVA & DOBRÁNSZKI, 2011), and based on surface area of explants, the potential number of PLBs in fact exceeds the recorded number. MeJA thus serves as an alternative means to derive *neo*-PLBs.

Acknowledgement

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Abbreviations:

MeJA – methyl jasmonate; NAA – α-naphthaleneacetic acid; PLB – protocorm-like body; PGR – plant growth regulator; TDZ – thidiazuron (N-phenyl-N-1,2,3-thidiazuron-5'-ylurea); VW – Vacin and Went

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MORPHO-CHEMICAL DESCRIPTION AND ANTIMICROBIAL ACTIVITY OF DIFFERENT *OCIMUM* SPECIES

KAKARAPARTHI PANDU SASTRY[1], RAMACHANDRAN RAMESH KUMAR[1], ARIGARI NIRANJAN KUMAR[1], GOGTE SNEHA[2], MARGARET ELIZABETH[2]

Abstract: Basil is a popular medicinal and culinary herb, and its essential oils have been used extensively for many years in food products, perfumery, dental and oral products. Basil essential oils and their principal constituents were found to exhibit antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria, yeast, and mould. The essential oils obtained from aerial parts of three different species of *Ocimum* comprising twenty one germplasm lines were investigated for their essential oil composition and antimicrobial activity during 2010. Essential oils from seventeen germplasm lines in *Ocimum basilicum* and two each in *Ocimum tenuiflorum* and *Ocimum gratissimum* were investigated for anti-microbial activity against four bacterial strains (*Staphylococcus aureus*, *Bacillus* sps., *Escherichia coli* and *Pseudomonas aeruginosa*). The morpho-chemotypes exhibited wide variability for morphological and chemical traits. Anti-bacterial activity was found to be high for *Staphylococcus aureus*, moderate for *Escherichia coli*, low for *Bacillus* and *Pseudomonas aeruginosa* was highly resistant. The essential oils of Pale Green-Broad Leaves (*O. basilicum*) and CIM Ayu (*O. gratissimum*) exhibited significant antibacterial activity against both *S. aureus* and *E. coli* signifying them promising for anti-bacterial activity. No relationship was observed between chemotype specificity and anti-bacterial activity, indicating that apart from major components of essential oil, minor components and other factors may be responsible for anti-microbial activities.

Key words: *Ocimum basilicum*, *Ocimum gratissimum*, *Ocimum tenuiflorum*, Methyl chavicol, Eugenol, Linalool, Antimicrobial activity

Introduction

There is a significant growth in the world trade for essential oils (growing approximately at 11% per year [BIZZO & al. 2009]). The exports and internal consumption of essential oils have shown a significant increase in India also in the recent past. In India essential oils are produced from many species and those belonging to the genus *Ocimum* also contribute modestly to the total exports.

The genus *Ocimum* L. (Lamiaceae) consisting of several species is very well distributed in tropical and subtropical Africa, Asia and South America [GUPTA, 1994]. They occur in various parts of tropical Asia, America and sub tropical regions of the world and found to grow from sea-level to an altitude of about 1800 m. The genus is represented by nine species in India. Among the various *Ocimum* species, Sweet basil (*O. basilicum* L.) is commercially and extensively cultivated for essential oil production in India and other countries. In India Sweet basil is cultivated in various States viz. West Bengal, Maharastra, Uttar Pradesh, Madhya Pradesh, Bihar, Jammu, Assam etc.

[1]Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Boduppal, Uppal (P.O), Hyderabad-500092, Andhra Pradesh – India. e-mail: sastry.kakaraparthi@gmail.com, crchyd@cimap.res.in
[2] St. Anns P. G. College, Mehdipatnam, Hyderabad, Andhra Pradesh – India

The oil of sweet basil is used extensively in condimentary products, cosmetics, and toiletry, perfumery and confectionery industries. The oil has moderate export value and the oil is reported to have bactericidal, insecticidal and also medicinal properties. The oil also contains protein, carbohydrates and relatively high concentrations of vitamins A and C.

O. gratissimum L. occurs throughout India and is also cultivated for it's essential oil. Several studies reported the chemical composition and antimicrobial activity of the essential oil of *O. gratissimum* L. [CHARLES & SIMON, 1990; CASTEELS & al. 1993; BASSOLE & al. 2005; ANANDA & al. 2010].

Ocimum essential oils are composed of aroma compounds such as eugenol, methyl eugenol, citral, linalool, geraniol and thymol which are required as raw materials for the pharmaceutical, cosmetics and food industries [GUPTA, 1994; BIZZO & al. 2009]. Many of these essential oils are used in folk medicine [GRAYER & al. 1996], exhibit other biological activities such as antimicrobial [PRASAD & al. 1986; NAKAMURA & al. 1999; BASSOLE & al. 2005], insecticide [DE PAULA & al. 2003; PAULA & al. 2004], antioxidant [GANIYU, 2008] and analgesic activities [FRANCA & al. 2008].

Even though numbers of reports on anti-microbial activities of *Ocimum* essential oil are available, studies across different *Ocimum* species are negligible. So the present investigation on the morphological features, chemical composition and antimicrobial activities of the volatile oils produced by twenty one chemotypes belonging to three different species, i.e. *Ocimum tenuiflorum*, *Ocimum basilicum* and *Ocimum gratissimum* was carried out.

Material and methods

Experimental site and design of the experiment

The present study was undertaken to evaluate the variability of twenty one chemotypes of *Ocimum* belonging to three different species for their suitability for commercial cultivation in the Deccan region of India during 2009-2010. The experiment was laid out at the research farm of Central Institute of Medicinal and Aromatic Plants (CIMAP), Research Centre, Boduppal, Hyderabad, Andhra Pradesh, India.

The experimental site is located at an altitude of 542 m above mean sea level with a geographical bearing of 78°8' longitude and 17°32' latitude. Semi-arid tropical climate zone of Hyderabad has an average rainfall of 800 mm per year. The soil of the experimental field is a red sandy loam (alficusto chrept) with pH 8.1 (1.25 soils to solution ratio), EC – 1.25 ds/m, organic C – 0.3%, total N – 0.03%, available P – 10 ug/g soil and exchangeable K – 128 ug/g soil.

Morphological variability

The seeds were sown during May-June, 2009. Three weeks old seedlings were transplanted in the main field with a row to row distance of 60 cm and plant to plant distance of 45 cm. There were twenty one plots replicated three times in a completely randomized design. Each treatment was accorded 3x4 m plots. In all there were 63 experimental plots. Morphological observations were recorded at the time of harvesting on ten randomly selected plots in each treatment.

Essential oil extraction

The aerial parts of *O. basilicum*, *O. tenuiflorum* and *O. gratissimum* were collected in flowering stage from experimental plots during 2010 – 2011 for the extraction of

essential oils. For the extraction of essential oils, freshly collected herbage of three *Ocimum* species were subjected to hydro-distillation using a Clevenger-type apparatus for 3.5 h.

The essential oils collected were dried over anhydrous sodium sulphate and stored at 4 °C until the analysis was carried out.

Antimicrobial cultures

Pure cultures of the strains obtained from National Collection of Industrial Microorganisms (NCIM), NCL, Pune, India were used in the experiment. Cultures are maintained on Nutrient Agar (Hi-Media, India) slopes at 4 °C and sub cultured before use.

The Gram-positive and Gram-negative bacterial strains used for the investigation are listed below:

a. *Staphylococcus aureus* – Gram-positive
b. *Bacillus* sps – Gram-positive
c. *Escherichia coli* – Gram-negative
d. *Pseudomonas aeruginosa* – Gram-negative

Determination of antibacterial activity

In vitro antibacterial activity of essential oils of twenty one *Ocimum* chemotypes was studied against four microbial cultures using Agar Diffusion Well Method.

Muller-Hinton medium was used for test assay. Essential oils were diluted to give 50 µg, 100 µg, 200 µg, 400 µg and 800 µg per 100 micro liters concentration. The overnight broth of inoculum was seeded on agar plates (1.5*108 CFU/ml). Eight mm diameter wells were prepared in seeded agar plates and test compound was introduced in each well. The solvent used for preparing essential oil solution was absolute alcohol. Solvent control well was run for every assay. All the plates were incubated at 37 °C for 24 hrs. The antimicrobial spectrum of the essential oils were determined in terms of zone sizes around each well i.e. diameter of inhibition zones. The experiment is replicated thrice following a completely randomized design.

GC analysis

GC analysis was carried out using Varian CP-3800 with Galaxie chromatography data system fitted with flame ionization detector (FID) and an electronic integrator. Separation of the compounds was achieved employing a Varian CP-Sil 5CB capillary column (ID: 50 m X 0.25 mm; film thickness 0.25 µm) with 5% dimethyl polysiloxane. Nitrogen was the carrier gas at 0.5 ml/min constant flow rate. The column temperature program was: 120 °C (2 min) to 240 °C (6 min) at 8 °C/min ramp rate. The injector and detector temperature were 250 °C and 300 °C respectively. Samples (0.2 µL) were injected with a 20:80:20 split ratio. Retention indices were generated with a standard solution of *n*-alkanes (C_6-C_{19}). Peak areas and retention times were measured by an electronic integrator. The relative amounts of individual compounds were computed from GC peak areas without FID response factor correction.

Results and discussion

Morphological description

Description about the morphological characters of the three *Ocimum* spp. are presented in Tab. 1. Among the three species, *O. gratissimum* was tall and *O. basilicum* showed wide variability for plant height ranging from short (44.00 cm) to medium plant height (125.00 cm). Canopy spread of tall *O. gratissimum* lines was relatively less than *O. basilicum* and *O. tenuiflorum* and both species recorded high number of leaves/plant. *O.*

gratissimum with less number of leaves/plant had large leaf area with lengthier and broader leaves. *O. basilicum* showed wide range for number of inflorescences/plant. *O. tenuiflorum* lines recorded higher number of inflorescences/plant. However, *O. tenuiflorum* with higher inflorescence number showed moderate number of whorls/ inflorescence (17) whereas *O. gratissimum* exhibited higher number of whorls/inflorescence (27-28). *O. basilicum* exhibiting wide variation for whorls/inflorescence.

O. gratissimum had lengthy peduncles, while the petiole lengths were high in *O. basilicum* and *O. tenuiflorum*. The flowers were relatively large in *O. basilicum* with large size bracts, sepals and petals. The stem colour was green or greenish purple in *O. basilicum*, whereas greenish purple in *O. tenuiflorum* and *O. gratissimum*. Flower colour of *O. basilicum* was white (some germplasm lines beared whitish purple flowers). *O. tenuiflorum* had purple/purplish white flowers, while the flower colour of *O. gratissimum* was purplish white. *O. basilicum* exhibited a range of colours for stamen colour *i.e.* white or creamy white or whitish purple. Stamen colours of *O. tenuiflorum* and *O. gratissimum* were similar to flower colour. Seeds were black in *O. basilicum* and brown in remaining two species.

Chemical description

The compositions of the essential oils of *O. basilicum* (seventeen chemotypes), *O. tenuiflorum* (two chemotypes) and *O. gratissimum* (two chemotypes) are presented in table (Tab. 2). In total twenty nine compounds were identified in the oils representing 78.0 - 99.6% of the oil. Methyl chavicol (5.2 - 87.0%), eugenol (0.0 - 50.0%), linalool (1.4 - 69.0%), limonene (0.1 - 10.3%), β-carophyllene (0.1 - 5.8%) and methyl cinnamate (only one chemotype - 61.0%) were the major constituents in *O. basilicum* oils.

Eugenol (0.9 - 84.0%), methyl eugenol (0.0 - 73.0%) and β-carophyllene (5.5 - 6.9%) were the major constituents in *O. tenuiflorum* oils. In *O. gratissimum* oils, eugenol and limonene + 1,8 cineole were found to be the major constituents. These eight major compounds were considered for a cluster analysis to identify possible chemotypes. The resulting dendrogram (Fig. 1) showed the existence of 3 main clusters and 3 single germplasm line clusters.

The first group was formed by *O. basilicum* germplasm lines 1, 3, 14, 10, 13 and 17, which could be identified as "methyl chavicol" chemotypes. Their oils, having very little variation in quantitative composition, showed high percentage of methyl chavicol (73.0 - 87.0%) with small quantities of linalool and eugenol. The second major cluster consisted of *O. basilicum* germplasm lines 2, 12, 4, 5, 7, 6 and 9, which comprised of three sub clusters (2 & 12; 4, 5 & 7 and 6 & 9) all having methyl chavicol as one of the major component along with linalool. The essential oil of lines 2 and 12 belonging to sub cluster-1 comprised of methyl chavicol (42.0 - 62.0%) as the major component along with linalool (18.0 - 24.0%) and line-2 had 25.0% eugenol in addition. Equal quantities of methyl chavicol (34.0 - 39.0%) and linalool (39.0 - 43.0%) were characteristic in the essential oils of germplasm lines 4, 5 and 7 belonging to second sub-cluster. On contrary, germplasm lines 6 and 9 in the remaining third sub-cluster possessed high amount of methyl chavicol (48.0 - 52.0%) in comparison to linalool (27.0 - 29.0%).

Third major cluster comprised of all the three species having eugenol or methyl eugenol as the major constituent. Germplasm line 11 belonging to *O. basilicum* consisted of equal quantities of eugenol (33.4%) and linalool (29.8%), while *O. gratissimum* lines were rich in eugenol (62.0 - 74.8%). Germplasm line 20 belonging to *O. tenuiflorum* had the highest eugenol (84.0 %) content and germplasm line 21 belonging to the same species was

rich in methyl eugenol (73.0%). *O. basilicum* germplasm lines 15, 8 and 16 in single genotype clusters were rich in specific component i.e. eugenol (15.0%), linalool (69.0%) and methyl cinnamate (61.0%) respectively.

Antimicrobial activity

Among the four bacterial strains tested, anti-bacterial activity was found to be high for *Staphylococcus aureus*, moderate for *Escherichia coli*, low for *Bacillus. Pseudomonas aeruginosa* was highly tolerant. Leading nosocomial agent *Staphylococcus aureus* a catalase positive, coagulase positive strain and gram positive cocci was found to be most sensitive to all the *Ocimum* oils tested. *O. basilicum* germplasm lines with wide differences for morphology and essential oil composition were highly variable for anti-bacterial activity also against *S. aureus* (Tab. 3).

Eugenol rich Exotic line and methyl chavicol rich Pale Green-Broad Leaves germplasm line showed profound anti- *S. aureus* activity (Fig. 2) with average inhibition zone of 45.6 mm and 41.2 mm, respectively. Moderate effects were exhibited by OB Tall, Globe P-15, Globe General, Citral-II and Dark Green-Broad Leaves. Anti- *S. aureus* effects were low for Linalool-II, MC-1, Linalool-III, Vikarsudha, MCN-9 and Kushmohak, while the inhibitory zones were very low for essential oils from MCN-10, MCN Narrow Leaves and Mulagu-II.

Eugenol rich essential oils obtained from *O. gratissimum* OG Original and *O. tenuiflorum* CIM Ayu exhibited very high inhibitory activity against *S. aureus* with average inhibitory zones of 46.4 mm and 34.6 mm respectively. Methyl eugenol rich *O. tenuiflorum* CIM Kanchan showed low antibacterial activity against *S. aureus*.

The essential oil of MCN-9 majoring in methyl chavicol (42.0%) along with eugenol (25.0%) and linalool (18.0%), which showed low anti- *S. aureus* activity exhibited relatively high anti- *E. coli* activity with average inhibition zone of 20.8 mm ranging 15.0 mm - 23.0 mm from 50-800 µg concentration (Tab. 4; Fig. 3). Relatively moderate resistance against *E. coli* were shown by essential oils from Citral-II (17.2 mm), Globe P-15 (16.8 mm), Pale Green-Broad Leaves (15.4 mm), Linalool-II (15.2 mm) and CIM Ayu (15.2 mm). The essential oils of Pale Green-Broad Leaves (*O. basilicum*) and CIM–Ayu (*O. gratissimum*) were more active against both *S. aureus* and *E. coli* signifying them promising for anti-bacterial activity.

Pseudomonas aeruginosa a highly adaptive aerobic Gram negative bacterium and a leading nosocomial pathogen, was highly resistant to the essential oils belonging to all the three species. In the present study, enhanced production of pigmentation i.e diffusible blue green pigment phytocyanin was noticed in the presence of *Ocimum* oils. The observation that production of diffusible pigment phytocyanin, which is highly enhanced in presence of *Ocimum* essential oils may be an indication that this pigment acts as a protective barrier for *Pseudomonas* against antimicrobial substances. The difference in the antimicrobial activity of Gram positive and Gram negative bacteria can be attributed to the difference in their cell wall composition. Gram negative bacteria are resistant due to presence of outer membrane acting as a barrier to many environmental or plant natural substances. The resistance exhibited by *Pseudomonas* can be attributed to the small pore size of its cell wall due to the outer membrane complexity [LOPEZ & al. 2005].

Conclusions

The different chemotypes of all the three *Ocimum* species exhibited variability for morphological characters, chemical composition and anti-bacterial activity. No relationship was observed between chemotype specificity and anti-bacterial activity, indicating that apart from major components of essential oil, minor components and other factors may be responsible for anti-microbial activities.

Tab. 1. Morphological characterization of different *Ocimum* species i.e. *O. basilicum* (17 genotypes), *O. tenuiflorum* (2 genotypes), *O. gratissimum* (2 genotypes)

S. No	Morphological trait	*Ocimum basilicum*	*Ocimum tenuiflorum*	*Ocimum gratissimum*
1	Plant height (cm)	44 - 125	95-120	96-150
2	Canopy spread (cm)	37 - 94	60-92.3	64-76
3	Leaves/ plant	350-4032	2210-2850	989-1200
4	Leaf length (cm)	3.2-11.6	5.4-5.6	10.4-15.7
5	Leaf width (cm)	1.4-5.1	2.9-3	6.4-6.7
6	Inflorescence/ plant	15-294	289-295	70-276
7	No. of Whorls/ Inflorescence	12 - 27	17	27-28
8	Petiole length (cm)	0.3-2.6	2.1-2.5	4-4.6
9	Peduncle length (cm)	13-33.5	14.2-30.2	15-17.8
10	Bract length (cm)	0.6-1.4	0.4	0.6-0.8
11	Bract width (cm)	0.3-0.6	0.3	0.2-0.3
12	Sepal length (cm)	0.4-0.9	0.4-0.5	0.4-0.5
13	Sepal width (cm)	0.1-0.3	0.1-0.2	0.1-0.3
14	Petal length (cm)	0.6-1.1	0.4-0.5	0.4-0.6
15	Petal width (cm)	0.2-0.4	0.2-0.3	0.2-0.3
16	Stem colour	Green / Greenish Purple	Greenish Purple	Greenish Purple
17	Flower color	White/Whitish Purple	Purple/Purplish white	Purplish white
18	Stamen color	White/Creamy white/Whitish Purple	Purple/Purplish white	Purplish white
19	Seed colour	Black	Brown	Brown

Tab. 2. Percentage composition of the essential oils of twenty one chemotypes belonging to three *Ocimum* species

Constituents	*Ocimum basilicum* Germplasm Lines										
	MC - 1	**MCN - 9**	**Broad dark green**	**Globe General**	**Linalool II**	**Mulugu II**	**Kushmohak**	**Citral II**	**Linalool III**	**Globe P-15**	**Exotic**
terpinen-4-ol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Methyl chavicol	73.0	42.0	87.0	34.0	39.0	48.0	38.4	5.2	52.0	75.5	0.2
(Z)methyl cinnamate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Eugenol	0.8	25.0	2.3	6.9	tr	0.1	3.3	nd	0.1	0.3	33.4
Methyl cinnamate	nd	nd	nd	nd	tr	nd	nd	nd	nd	nd	nd
geranyl acetate	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
methyl eugenol	0.6	1.6	0.6	1.5	0.1	0.4	2.7	nd	0.4	0.7	0.1
β-elemene	tr	nd	nd	nd	tr	tr	1.0	nd	tr	0.3	1.0
β-caryophyllene	0.6	0.5	0.2	1.6	0.8	1.1	1.4	1.7	0.8	0.6	5.7
α- humulene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
germacrene D	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
caryophyllene oxide	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
B-eudesmol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
α –thujene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
α-pinene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Campene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Sabinene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Myrcene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
limonene+1,8 cineole	5.3	1.3	3.0	2.7	1.7	6.3	1.4	4.7	2.7	2.7	10.3
(Z)-β-ocimene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
γ- terpinene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
terpinolene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Linalool	6.9	18.0	1.4	39.0	43.0	29.0	40.6	69.0	11.9	11.9	29.8
Camphor	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Borneol	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Citronellol	nd	nd	nd	nd	nd	nd	0.7	0.7	0.7	nd	nd
Citral-I	nd	nd	nd	nd	nd	nd	tr	2.5	1.5	nd	nd
Geraniol	nd	nd	nd	nd	nd	nd	1.5	0.2	0.7	nd	nd
Citral-II	nd	nd	nd	nd	nd	nd	tr	3.5	2.3	nd	nd
Total	87.2	88.4	94.5	85.7	84.6	84.9	91.0	87.5	73.1	92.0	80.6

Contd., **Tab. 2**

Constituents	*Ocimum basilicum* Germplasm Lines						*Ocimum gratissimum* Germplasm Lines		*Ocimum tenuiflorum* Germplasm Lines	
	CIM Saumya	**Vikarsudha**	**Broad Pale Green**	**MCN 10**	**MC Narrow**	**OB Tall**	**OG Variant**	**OG Original**	**CIM -AYU**	**CIM-kanchan**
terpinen-4-ol	nd	nd	nd	nd	tr	nd	tr	0.6	tr	tr
Methyl chavicol	62.0	87.0	87.0	6.6	6.0	40.0	0.4	0.1	tr	tr
(Z)methyl cinnamate	tr	tr	nd	nd	8.0	nd	tr	tr	tr	tr
Eugenol	1.3	tr	2.3	50.0	0.3	0.1	74.8	62.0	84.0	0.9
Methyl cinnamate	tr	tr	nd	nd	61.0	nd	tr	tr	tr	tr
geranyl acetate	tr	tr	nd	nd	tr	nd	tr	0.1	tr	tr
methyl eugenol	tr	1.1	0.6	1.3	0.3	0.5	tr	tr	tr	73.0
β-elemene	0.5	tr	tr	tr	0.9	tr	tr	0.5	7.5	0.5
β-caryophyllene	0.2	0.1	0.2	0.8	0.5	2.0	1.5	1.8	6.9	5.5
α- humulene	tr	0.1	nd	nd	0.3	nd	tr	0.2	tr	0.4
germacrene D	0.9	tr	nd	nd	0.6	nd	tr	tr	tr	tr
caryophyllene oxide	tr	0.2	nd	nd	tr	nd	tr	tr	tr	1.5
B-eudesmol	1.5	1.3	nd	nd	0.2	nd	tr	tr	tr	0.1
α –thujene	tr	tr	nd	nd	tr	nd	tr	0.1	tr	tr
α-pinene	0.1	0.4	nd	nd	0.1	nd	tr	tr	0.9	0.1
Campene	tr	tr	nd	nd	tr	nd	tr	0.1	0.1	tr
Sabinene	tr	tr	nd	nd	tr	nd	tr	0.2	tr	tr
Myrcene	0.4	0.1	nd	nd	0.3	nd	tr	0.2	tr	0.1
limonene+1,8 cineole	1.5	0.1	3.0	1.3	0.9	5.0	10.8	7.7	0.1	0.1
(Z)-β-ocimene	1.5	0.3	nd	nd	1.2	nd	tr	0.4	tr	0.1
γ- terpinene	tr	tr	nd	nd	tr	nd	tr	0.1	tr	0.1
terpinolene	tr	0.1	nd	nd	tr	nd	tr	tr	tr	tr
Linalool	24.0	1.8	1.4	18.0	16.0	32.0	tr	0.3	0.1	tr
Camphor	tr	tr	nd	nd	0.2	nd	tr	18.0	tr	tr
Borneol	0.6	0.1	nd	nd	tr	nd	tr	0.3	tr	tr
Citronellol	nd	nd	nd	nd	nd	nd	tr	tr	tr	tr
Citral-I	nd	nd	nd	nd	nd	nd	tr	tr	tr	tr
Geraniol	nd	nd	nd	nd	nd	nd	tr	tr	tr	tr
Citral-II	nd	nd	nd	nd	nd	nd	tr	tr	tr	tr
Total	94.5	92.7	94.5	78.0	96.8	79.6	87.5	92.7	99.6	82.4

nd: not detected tr: traces

Tab. 3. Antimicrobial activity of pure essential oils obtained from twenty one chemotypes belonging to three sp. of *Ocimum* tested at different concentrations on *Staphylococcus aureus*

S. No	Chemotype	Inhibition zone in mm (millimeters)						
		CONCENTRATION (µg)						
		50	100	200	400	800	Range	Average
1	MC - 1	8	8	17	20	22	8-22	15
2	MCN - 9	11	19	18	16	30	11-30	18.8
3	Broad Dark Green	11	20	28	34	48	11-48	28.2
4	Globe General	17	20	24	34	40	17-40	27
5	Linalool II	14	12	16	10	17	10-17	13.8
6	Mulugu II	2	4	10	10	20	2-20	9.2
7	Kushmohak	12	10	11	26	38	10-38	19.4
8	Citral II	20	16	30	27	48	16-48	28.2
9	Linalool III	8	8	20	12	40	8-40	17.6
10	Globe P-15	18	20	24	30	38	18-38	26
11	OB Tall	20	14	21	23	25	14-25	20.6
12	Exotic	38	42	50	50	48	38-50	45.6
13	CIM Saumya	18	18	20	34	40	18-40	26
14	Vikarsudha	4	20	20	20	30	4-30	18.8
15	Broad Pale Green	40	40	38	40	48	38-48	41.2
16	MCN 10	1	1	1	10	11	1-11	4.8
17	MCN Narrow	1	1	6	10	12	1-12	6
18	OG Variant	16	17	20	22	42	17-42	23.4
19	OG Original	30	50	50	50	52	30-52	46.4
20	CIM AYU	38	17	42	46	30	17-46	34.6
21	CIM Kanchan	10	12	8	14	14	8-14	11.6

Tab. 4. Antimicrobial activity of pure essential oils obtained from twenty one chemotypes belonging to three sp. of *Ocimum* tested at different concentrations on *Escherichia coli*

S. No	Chemotype	Inhibition zone in mm (millimeters)						
		Concentration (µg)						
		50	100	200	400	800	Range	Average
1	MC - 1	5	10	10	15	10	5 - 15	10
2	MCN - 9	15	18	28	20	23	15 - 28	20.8
3	Broad Dark Green	16	8	13	8	16	8 - 16	12.2
4	Globe General	10	12	14	16	17	10 - 17	13.8
5	Linalool II	11	16	13	17	19	11 - 19	15.2
6	Mulugu II	11	8	12	12	20	8 - 20	12.6
7	Kushmohak	2	12	11	10	14	2 - 14	9.8
8	Citral II	20	10	20	14	22	10 - 22	17.2
9	Linalool III	6	12	8	10	20	6 - 20	11.2
10	Globe P-15	6	16	10	24	28	6 - 28	16.8
11	OB Tall	4	10	8	12	28	4 - 28	12.4
12	Exotic	4	11	10	12	16	4 - 16	10.6
13	CIM Saumya	10	1	12	6	26	1 - 26	11
14	Vikarsudha	1	2	4	2	8	1 - 8	3.4
15	Broad Pale Green	8	15	12	12	30	8 - 30	15.4
16	MCN 10	6	7	8	12	12	6 - 12	9
17	MCN Narrow	1	1	1	10	11	1 - 11	4.8
18	OG Variant	2	6	6	10	12	2 - 12	7.2
19	OG Original	4	4	8	8	18	4 - 18	8.4
20	CIM AYU	8	12	12	16	28	8 - 28	15.2
21	CIM Kanchan	11	10	14	8	6	6 - 11	9.8

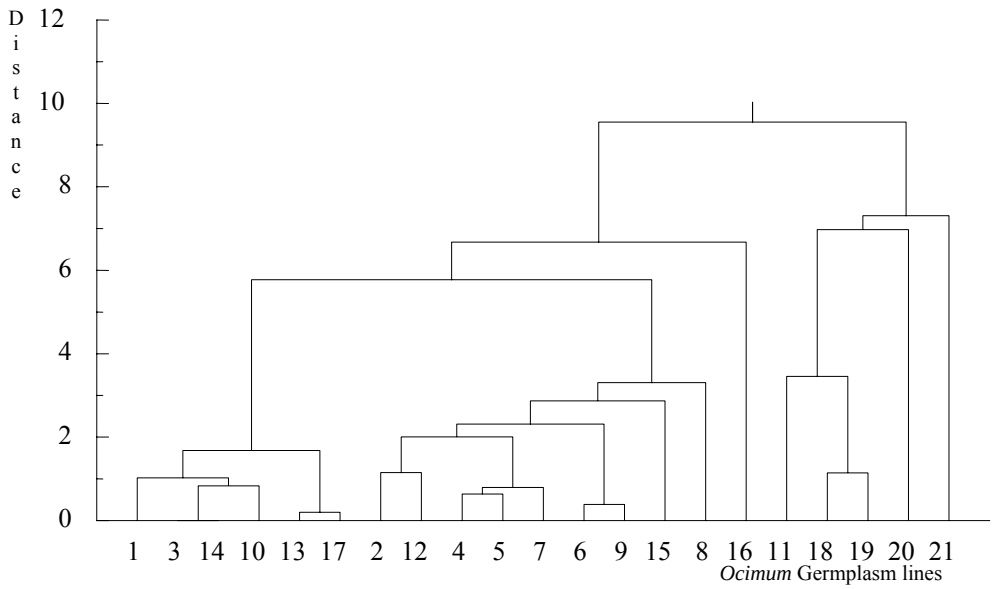

Fig. 1. Cluster analysis of twenty one chemotypes belonging to three species of *Ocimum* based on eight major essential oil components

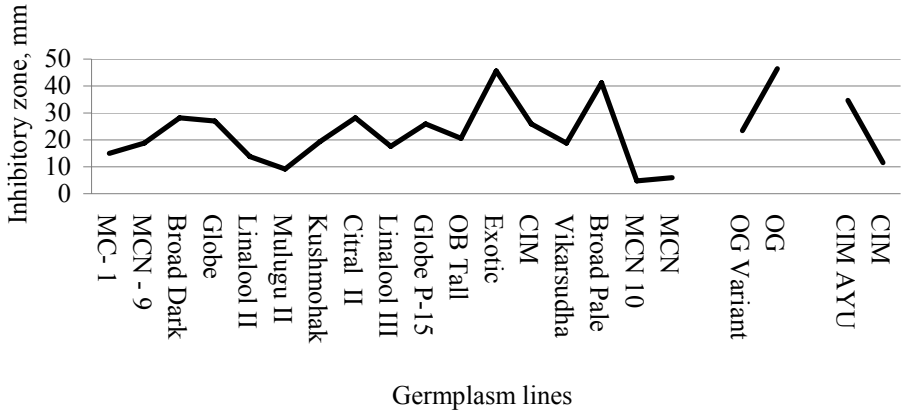

Fig. 2. Antimicrobial activity of pure essential oils obtained from twenty one chemotypes belonging to three species of *Ocimum* tested at different concentrations on *Staphylococcus aureus*

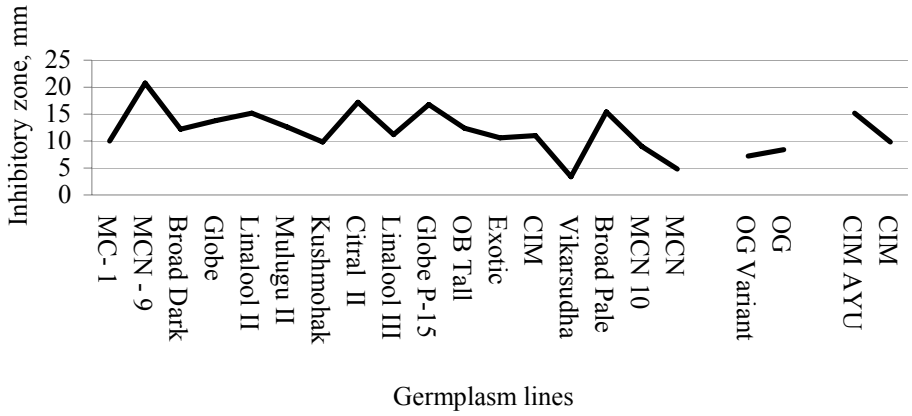

Fig. 3. Antimicrobial activity of pure essential oils obtained from twenty one chemotypes belonging to three species of *Ocimum* tested at different concentrations on *Escherichia coli*

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ASSESSMENTS OF GENETIC DIVERSITY IN COUNTRY BEAN (*Lablab purpureus* L.) USING RAPD MARKER AGAINST PHOTO-INSENSITIVITY

BISWAS MD. SANAULLAH[1], ZAKARIA MOHAMMAD[1],
RAHMAN MD. MIZANUR[1]

Abstract: RAPD marker was used to evaluate genetic relationships among 11 genotypes of country bean, including first three genotypes were photo-insensitive and the rests were sensitive. The genotypes were grouped into two major clusters where photo-insensitive genotypes remain in cluster I and sensitive genotypes remain in cluster II. A total of 26 bands were detected, of which 57.69% were polymorphic and the remaining were monomorphic across all genotypes. A highest level of genetic distance was observed between CB04 and CB06 while the lowest level of genetic distance showed between CB01 and CB03. The highest similarity index between the genotypes CB01 and CB03 indicated less divergence between them. Low similarity indices were observed between CB04 and CB06, which indicated more divergence. Crossing between the genotypes with low similarity coefficient will manifest high heterosis. The identified genetically distinct cultivars could be potentially important source of germplasm for further improvement of country bean.

Key words: country bean, RAPD marker, polymorphism, genetic diversity

Introduction

Country bean (CB) (*Lablab purpureus* (L.) Sweet, Syn. *Dolichos lablab* L.) belongs to the family Leguminosae (Fabaceae) and genus *Lablab* with varying chromosome number of 2n = 20, 22, 24. It originated from India [DEKA & SARKAR, 1990] and is a good source of minerals and vitamins [BASU & al. 2002] with antioxidants [BRADLEY, 1999]. Lablab bean (*Lablab purpureus* L.) is one of the major winter vegetables of Bangladesh. It is commonly known as country bean in Bangladesh and is widespread throughout the country.

Assessment of genetic diversity based on phenotype has limitations, since most of the morphological characters are greatly influenced by environmental factors and the development stage of the plant. In contrast, molecular markers based on DNA sequence polymorphism, are independent of environmental conditions and show a higher level of polymorphism. This necessitates the assessment of genetic diversity present in country bean using the modern molecular approaches. The immense genetic diversity of landraces of crops is the most directly useful and economically valuable part of biodiversity. Unlike high yielding varieties, the landraces maintained by farmers are endowed with tremendous genetic variability, as they are not subjected to subtle selection over a long period. Because of the limitations of morphological and biochemical markers, efforts are being directed to use molecular markers for characterizing germplasm diversity against high yield, year round, earliness and tolerant to high temperature and long day length. Molecular markers have demonstrated a potential to detect genetic diversity and to aid in the management of

[1] Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur-1706, Bangladesh, e-mail: sanaullah@bsmrau.edu.bd

plant genetic resources [VIRK & al. 2000, SONG & al. 2003]. In contrast to morphological traits, molecular markers can reveal differences among genotypes at the DNA level, providing a more direct, reliable and efficient tool for germplasm characterization, conservation and management. Several types of molecular markers are available today, including those based on restriction fragment length polymorphism (RFLP) [BOTSTEIN & al. 1980], random amplified polymorphic DNA (RAPD) [WELSH & al. 1990, WILLIAMS & al. 1990], amplified fragment length polymorphism (AFLP) [VOS & al. 1995] and simple sequence repeats (SSRs) [SINGH, 1999].

It is particularly useful for characterizing individual genotypes and selection of the parents for successful hybridization. Among the molecular markers, in the present study, we have used RAPD method of DNA fingerprinting, which is widely used in conservation biology because of quick results, cost-effectiveness and reproducibility.

The PCR-based RAPD approach using arbitrary primers requires only nanogram quantities of template DNA, no radioactive probes, and is relatively simple compared to other techniques [WILLIAMS & al. 1993]. However, morphological traits have certain limitations such as easily available of scorable markers, difficulty in scoring homozygous from heterozygous individuals, influence of environment in equating phenotypes with genotypes etc. On the other hand, molecular markers have many advantages such as abundance in polymorphism, no pleiotropic effect, less affected by environment and subjected to rapid detection [SINGH & al. 2005]. Therefore, RAPD has been using extensively for studying genetic diversity of crops in different parts of the world. However, no report on RAPD is available regarding the diversity of country bean in Bangladesh.

Materials and methods

Source of the genotypes

In the present investigation eleven genotypes (CB01 to CB11) of the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, were used for genetic diversity study against photo-sensitivity. Previously the genotypes were tested in the department using temporal planting and morphological markers and found that CB01, CB02 and CB03 genotypes were pho-insensitive and the rest were photosensitive but never tried with molecular markers.

DNA Extraction

Young leaves were used for extracting DNA by CTAB (Cetyl trimethyl ammonium bromide) with some modifications in protocol [DOYLE & DOYLE, 1990]. Fresh leaves from polybag raised plants were collected and immediately stored at 4 °C. One hundred and fifty mg of leaf tissue ground with 1 ml DNA extraction buffer using mortar and pestle. Extraction buffer (100 mM Tris HCl (pH 8.0), 20 mM EDTA, 1.4 mM NaCl and 2% CTAB per liter) was added in 2 ml tubes filled with ground leaf and mixed with vortex mixture. The tubes were incubated at 65 °C for 45 minutes with repeated shaking. Equal volume of chloroform : isoamylalcohol mix (24:1) was added and mixed thoroughly for 5 minutes, followed by centrifugation at 14000 rpm for 15 minutes. Two third volume of isopropanol was added to the supernatant. DNA was precipitated by centrifugation at 14000 rpm for 10 minutes and the pellet was washed in 70% ethanol and suspended in 100 µl of sterile distilled water with 10-15 µl (10 µg/µl concentrations) of RNAse and incubated at 65 °C for 30 minutes. The quality and quantity were checked through 1% agarose gel by electrophoresis. DNA concentration for PCR reaction was determined at 260 nm

wavelength with Spectrophotometer and the quality verified by electrophoresis on 1% agarose gel in TAE (Tris-acetate-EDTA) buffer.

RAPD analysis

Genomic DNA was used as template for PCR amplification as described by WILLIAMS & al. (1990). Several number of primers were used to get reproducible bands among them 4 arbitrary primers (OPERON Technologies, Inc. California, USA) were used to produce distinct marker profiles for 11 genotypes. The selected 4 primers generated in a much higher number of polymorphic bands. Amplification reactions were in the volumes of 25 µl volumes containing 10 µl of 5X Flexi Taq buffer, 4.0 µl $MgCl_2$ (25 mM), 2.5 µl dNTPs (4mM/µl), 2.5 µl random primer (10 pmol/µl), 0.25 µl Taq DNA polymerase (5 U/µl) and 4 µl of the extracted DNA (25 ng). Amplification was performed with Thermal cycler (Eppendorf) programmed for 45 cycles. After initial denaturation for two minutes at 94 °C, each cycle consisted of one minute at 94 °C, one minute at 42 °C and two minutes at 72 °C. The 40 cycles were followed by seven minutes of final extension at 72 °C.

The PCR product was mixed with 6 µl of loading dye (0.25% bromophenol blue, 0.25% Xylene Cyanol and 40% Sucrose, w/v) and spun briefly in a micro centrifuge before loading. The PCR products and 100 bp DNA ladder were electrophoresed using 1% agarose gel at 80 volts followed by staining with ethidium bromide then separated fragments and were visualized with an ultraviolet (UV) transilluminator (Biometra gel documentation system).

Data analysis

Data generated from the polymorphic fragments was scored as present (1) or absent (0) for each of the 11 genotypes. The diversity among the lines was worked out by subjecting the RAPD scores to cluster analysis. Percentage of polymorphism was calculated as the proportion of polymorphic bands over the total number of bands. The scores obtained using all the primers in the RAPD analysis were then pooled to create a single data matrix, to estimate polymorphic loci, gene diversity, genetic distance (D) and to construct a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among populations using a computer program, POPGENE (Version 1.31) [YEH & al. 1999]. Genetic similarity values defined as the fraction of shared bands between the RAPD profiles of any two individuals on the same gel were calculated manually from RAPD markers of the same weight on the data matrix. Similarity index (SI) was calculated from 2nxy/nx+ny, when nxy is the number of the common DNA bands in x and y plants, nx and ny are the total DNA bands of X and Y plant respectively [HILL & al. 1996].

Results and discussions

Four random primers were used in PCR reaction to amplify DNA fragments from 11 genotypes because these primers generated higher number of polymorphic bands. A total of 26 bands were detected, of which 42.31% (11 bands) was monomorphic across all genotypes (Tab. 1). The remaining 15 bands (57.69%) were polymorphic among the varieties tested. This accounted to an average of 3.75 polymorphic bands per primer indicating the medium level of polymorphism expressed by arbitrary primers. Among the Ten oligonucleotide primers, TIWARI & al. (2003) found all the primers produced polymorphic amplicons though the extent of polymorphism varied with each primer. KUMAR & al. (2008) found 95% percent polymorphism of the total 124 amplified products in twenty-six common bean using 15 primers and RAI & al. (2010) found 70.27%

polymorphism, indicating fair amount of variation at the DNA level among these accessions.

Tab. 1. Polymorphism detected by the use of 4 random RAPD primers on 11 country bean genotypes.

Name of primers	Sequence of primer (5'-3')	Total number of bands	Number of polymorphic bands	% of polymorphic bands
OPA 04	AATCGGGCTG	4	3	75
OPD 03	GTCGCCGTGA	10	6	60
OPD 20	ACCCGGTCAC	7	3	43
OPF 02	GAGGATCCCT	5	3	60
Total		**26**	**15**	
Average		**6.5**	**3.75**	

The primers OPD 03 produced maximum number of fragments (10) followed by OPD 20 and OPF 02 produced 7 and 5 fragments respectively. The primer OPA 04 recorded minimum number of fragment (4). The RAPD profiles for two primers viz., OPA 04 and OPD 03, which produced diagnostic markers are given in Fig. 1 (A&B). RAI & al. (2010) observed the number of amplification products produced by each primer varied from 4 to 9 with an average of 5.69 bands per primer. The simple matching similarity was calculated using RAPD score and dendrogram was constructed using unweighted pair group method with arithmetic averages (UPGMA).

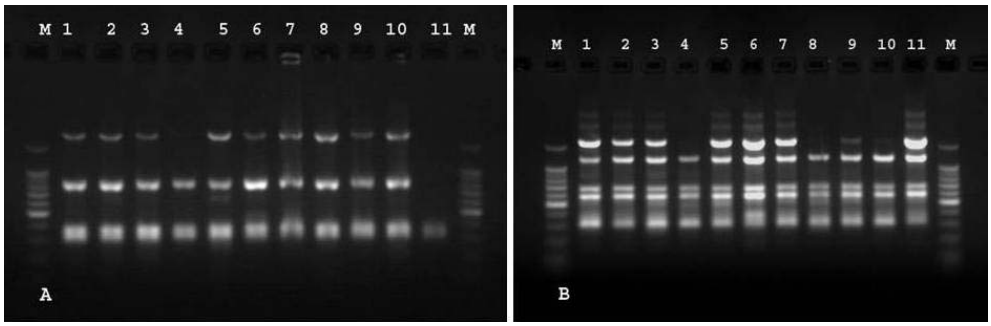

Fig. 1. RAPD profile of eleven genotypes of country bean produced using the random decamer primers OPA 04 (A) and OPD 03 (B). M, 100 bp DNA ladder and lane numbers correspond to the genotypes CB01 to CB11.

Cluster I consisted of two sub clusters, which included most of the testers. The sub cluster A of cluster I consisted of CB01, CB02, CB03, CB05, CB07 and CB11, while sub cluster B consists only one genotype, CB06. The sub cluster A of cluster II included CB04, CB08 and CB10 while sub cluster B of cluster II included only CB09 genotype (Fig. 2). The genotypes CB01, CB02 and CB03 were belong to the same cluster because they are photo-insensitive which is morphologically and temporally tested. BISWAS & al. (2010) found two major clusters based on Jaccard's similarity coefficient using UPGMA grouped among 14 genotypes of French bean.

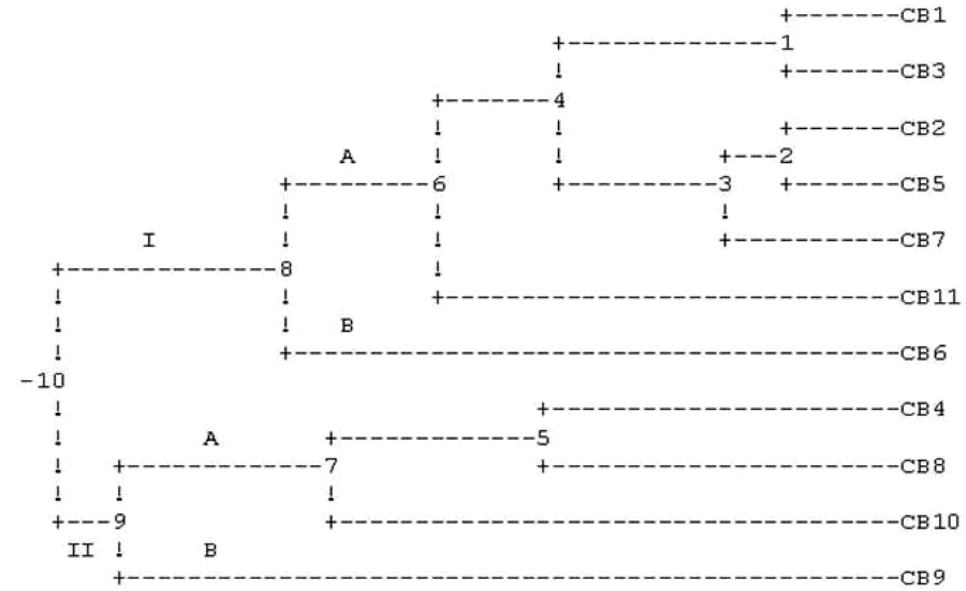

Fig. 2. UPGMA cluster analysis-based dendrogram showing genetic relationship among eleven genotypes of country bean.

Molecular markers analysis of the sixteen genotypes using 4 random RAPD primers produced polymorphism for most of the studied loci. As per the similarity index, the genotypes were grouped into two major clusters four sub clusters. A highest level of genetic distance (0.425) was observed between CB04 and CB06 while the lowest level of genetic distance (0.039) showed between CB01 and CB03 (Tab. 2). The highest similarity index between the genotypes CB01 and CB03 (0.97) indicated less divergence between them. Low similarity indices were observed between CB04 and CB06 (0.74) which indicated more divergence. RAI & al. (2010) showed RAPD based dendrogram showed similarity ranged from 0.38 to 0.96.

Tab. 2. Nei's genetic distance among eleven genotypes of country bean

genotypes ID	CB 01	CB 02	CB 03	CB 04	CB 05	CB 06	CB 07	CB 08	CB 09	CB 10	CB 11
CB 01	****										
CB 02	0.080	****									
CB 03	0.039	0.123	****								
CB 04	0.368	0.262	0.314	****							
CB 05	0.123	0.039	0.167	0.314	****						
CB 06	0.214	0.123	0.262	0.425	0.167	****					
CB 07	0.123	0.039	0.080	0.214	0.080	0.167	****				
CB 08	0.314	0.314	0.262	0.123	0.368	0.368	0.262	****			
CB 09	0.214	0.214	0.262	0.314	0.262	0.368	0.262	0.262	****		
CB 10	0.262	0.262	0.214	0.262	0.314	0.314	0.214	0.123	0.214	****	
CB 11	0.167	0.167	0.123	0.262	0.214	0.314	0.123	0.314	0.314	0.167	****

Crossing between the genotypes with low similarity coefficient will manifest high heterosis. The identified genetically distinct cultivars could be potentially important source of germplasm for further crop improvement programme in the country bean genotypes.

Conclusion

Country bean is an important vegetable crop in Bangladesh in terms of production and consumption. The cultivation of country bean in our country largely centralized in winter due to lack of high yielding and quality day neutral variety. The previous morphological characterization was not sufficient for the selection of parents to develop day neutral high yielding variety. In our study, RAPD marker was used to evaluate genetic relationships among 11 genotypes of country bean in our country. Therefore, the finding of the paper could help to develop high yielding variety designing appropriate breeding approach. The identified genetically distinct cultivars could be potentially important source for further improvement of country bean.

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MICROMORPHOLOGICAL AND CHEMICAL ASPECTS OF SOME LICHENIZED FUNGI SPECIES

PÎNDARU DIANA-MIHAELA[1], TĂNASE CĂTĂLIN[1], ARSENE CECILIA[2], OLARIU ROMEO-IULIAN[2]

Abstract: At present, lichenized fungi are used in biomonitoring studies of air quality, being good receptors in the climate change. This paper aims to investigate surface micromorphology of *Xanthoria parietina* and *Phaeophyscia orbicularis* species (Lecanoromycetes, Ascomycota). The study also includes the investigation of selected chemical parameters as *p*H and conductivity of the lichenized fungi samples collected from various locations in the Iaşi County (Romania). Measurements of the *p*H provide information on the degree of pollution in the location of interest. Bark trees *p*H was also investigated in order to see if our matrix substrate influences the *p*H of the interest lichenized fungi samples.

Key words: lichenized fungi, Ascomycota, micromorphology, *p*H, conductivity

Introduction

Lichenized fungi, as unique forms in the plant world, are the result of the symbiosis between a fungus (mycobiont) and a cyanobacteria or green algae (phycobiont). The result of this symbiosis is a stable vegetative body having a specific structure and is known as the thallus [BRODO & al. 2001].

Lichenized fungi are the most complex forms of life, commonly found on about 8% Earth surface [AHMADJIAN, 1995]. They can survive under extreme conditions such as Polar Regions and in high mountains. These locations are characterized by severe abiotic conditions such as dehydration, extreme temperatures and high light intensities. Some authors claim that what really makes them special and what separates them from most other eukaryotic organisms is their ability to tolerate extreme conditions. For this reason, lichenized fungi were called "extremophilic organisms", which can develop in such conditions that would kill other organisms, less specialized [YOUNG, 2005].

Many authors have repeatedly drawn attention to the problem of using lichenized fungi morphology as a visual indication of air pollution effects. Changes in thallus morphology were observed in lichenized fungi transplanted to polluted areas, exposed to simulated acid rain and fumigated by gaseous pollutants. Reported morphological changes include a reduction in size, detaching from the substratum with margins upturned, changes in coloration, and development of chlorotic and necrotic patches [BENNETT & al. 1996; OTNYUKOVA, 2007].

[1] "Alexandru Ioan Cuza" University of Iaşi, Faculty of Biology, Bd. Carol I, no. 11, 700506, Iaşi – Romania, e-mail: mihaela_dayana@yahoo.com, tanase@uaic.ro
[2] "Alexandru Ioan Cuza" University of Iaşi, Faculty of Chemistry, Bd. Carol I, no. 11, 700506, Iaşi – Romania

Given the importance of the above presented aspects, the purpose of this study is to observe in more details the structure of the interest lichenized fungi thallus (*Xanthoria parietina* and *Phaeophyscia orbicularis* species collected from Iaşi – Romania) to a better understand of those changes that occur in unfavorable environmental conditions for their development. Another aim is to investigate some chemical parameters (e.g., *p*H and conductivity), which might give us preliminary information about the chemical composition of the investigated matrix.

Material and methods

For an appropriate structural characterization of living organisms, microscopic investigations are requested. Thereby, specific information obtained from classical optical microscopy should be supplemented with information provided by Scanning Electron Microscopy (SEM) and Transmission Electronic Microscopy (TEM) techniques which are used depending upon the type and nature of the sample.

Microscopic investigations were performed on fresh lichenized fungi samples of *Xanthoria parietina* and *Phaeophyscia orbicularis* species (Lecanoromycetes, Ascomycota) collected from Iaşi (Romania). Surface micromorphology of the lichenized fungi species was investigated using a scanning electron microscope *FEI Quanta 250 FEG*.

Fresh thallus samples were cleaned, dried at room temperature and then segmented with a blade. Resulted segments were fixed on a copper stage using conductive carbon adhesive discs on both sides. Images were obtained using LFD (Large Field Detector) and GSED (Gaseous Secondary Electron Detector) detectors. Investigations on the chemical composition of the two lichenized fungi species was determined by EDX (Energy Dispersive X-ray Detector) detector.

Electrochemical parameters *p*H and conductivity of extracts can give preliminary indications on the chemical composition of the species of interest. Measurements of *p*H were made for both collected lichenized fungi and for the bark tree where sampling was conducted. *p*H was measured with a *p*H meter ION Meter Lab 450 and conductivity with a conductivity Lab Meter C 490. Each collected was analyzed in 10 replicates.

*Determination of lichenized fungi thallus p*H. Preparative steps were carried out in accordance with the suggestions from the literature [GAUSLAA, 1985; GILBERT, 1986; RIGA-KARANDINOS, 1998; CONTI & CECCHETTI, 2001; FRATI & al. 2006]. About 50 mg of lichenized fungi thallus was weighed with an *ADAM PW 254* balance. Weighed samples were homogenized, brought into contact with 10 ml of ultrapure water, ultrasonified 15 min and then centrifuged. After centrifugation at 1000 rpm for 10 min the supernatant was used for measurements the *p*H and conductivity.

*Determination of bark tree p*H. Bark tree samples were collected and used to determine the *p*H. 0.2 g of bark tree surface was placed in vials with 10 ml of ultrapure water and stirred for 1 h. Samples were then centrifuged for 10 min at 4000 rpm and clear liquid fraction was filtered (pore = 0.45 μm), and used for analysis.

Results and discussions

The thallus of *Xanthoria parietina* is usually 3–10 cm in diameter and closely adheres to the substrate. Apothecial discs (i.e. the hymenial layer) and the ostioles of pycnidia always revealed an intense yellow coloration due to the deposition of crystals of the anthraquinone parietin [SØCHTING, 1997]. It almost invariably produces apothecia (sexual reproduction organs) but no specialized organs for vegetative propagation. Fig. 1

clearly shows a lecanorin apothecia cup contoured on the lichenized fungi thallus surface of the *Xanthoria parietina* species.

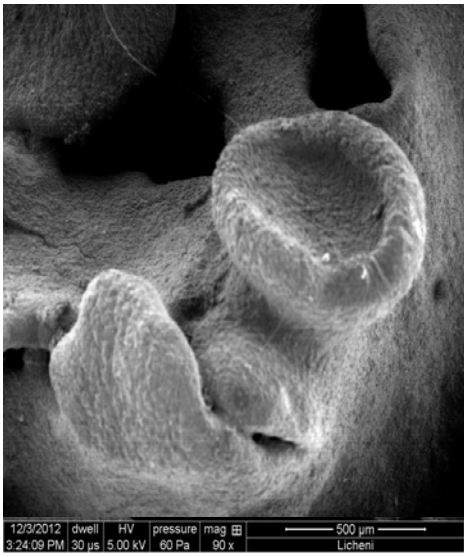

Fig. 1. Thallus apothecia on the surface of *Xanthoria parietina*

The images presented in Fig. 2a & 2b were obtained with LFD detector at a voltage of 3.00 kV. Fig. 2a shows cortex morphology, with details at peripheral part revealing the presence of filamentous hyphal growth at the periphery and conglutinates zones in the microaerobic central part. Fig. 2b presents hyphae dimensions between 1.750 µm and 1.922 µm.

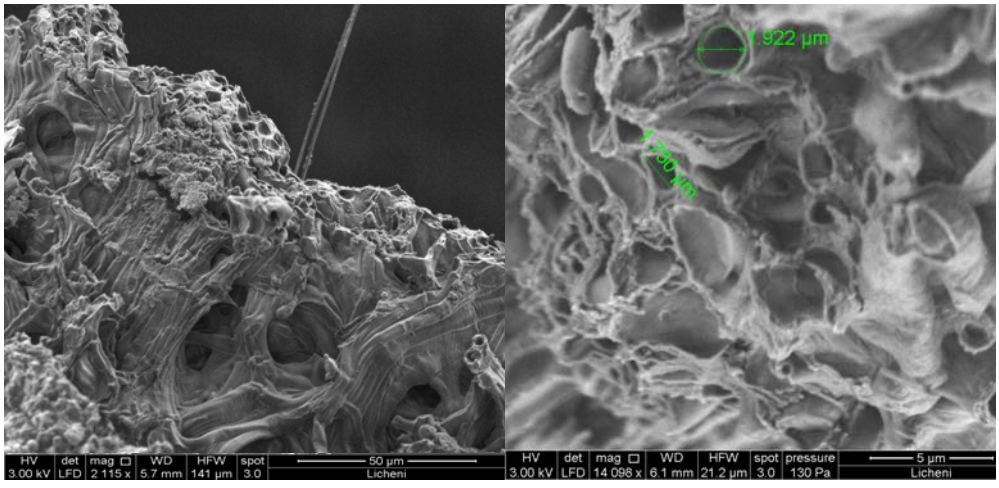

Fig. 2. (a, b) Surface micromorphology (upper cortex) of thallus of *Xanthoria parietina*

In Fig. 3, at a magnification of 470 x, the surface micromorphology highlighting the existence of a well-defined paraphyses, from *Xanthoria parietina* species with sizes of about 200 μm can be observed. It can be well observed that paraphyses present atmospheric particulate on the surface.

The thallus of the *Phaeophyscia orbicularis* is of foliose type, with a diameter up to 5 cm, irregular to more often orbicular lobes, in linear and discrete forms. Upper surface is gray to brown, sometimes with a whitish epinecral layer centrally formed. Apothecia are occasionally up to 2 mm in diameter, sessile, with circulars or lobules ascospores. Surface micromorphology of a *Phaeophyscia orbicularis* thallus is shown in Fig. 4. Existing lobes can be seen on the upper surface of the lichenized fungi.

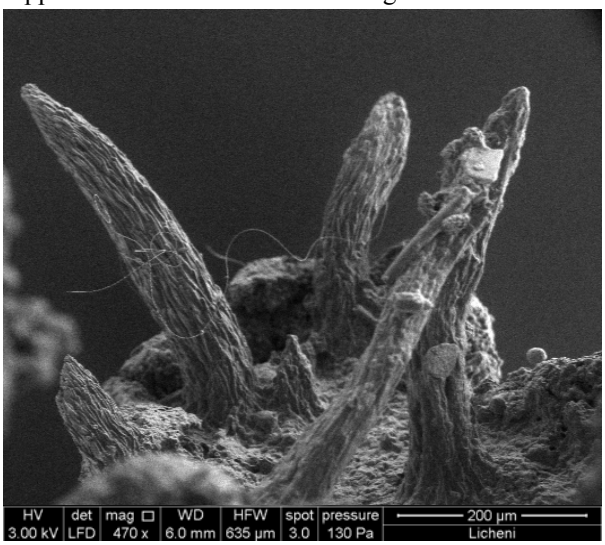

Fig. 3. Surface micromorphology of thallus: paraphyses

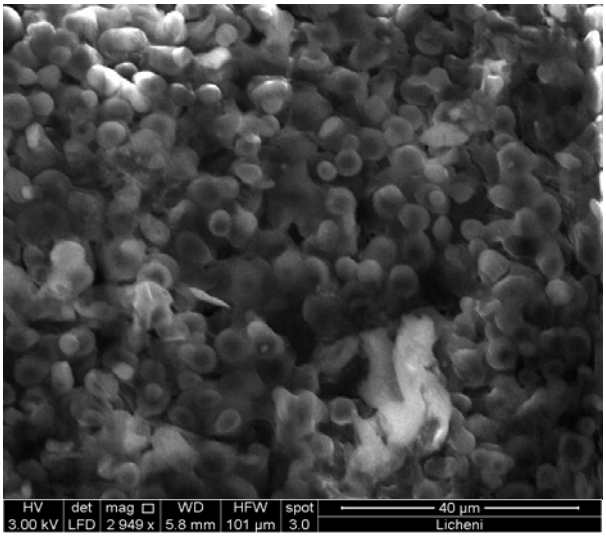

Fig. 4. Surface micromorphology of thallus of *Phaeophyscia orbicularis*

Investigations performed with an EDX detector for *Xanthoria parietina* (Fig. 5) and *Phaeophyscia orbicularis* (Fig. 6) species clearly reveal the presence of elements that probably can come directly from the thallus chemical composition (K, Mg, Ca, P, Fe) while the presence of elements such as Si and Al may be due to influences induced by the impact of atmospheric constituents (Si and Al are tracers of dust particles in the atmosphere).

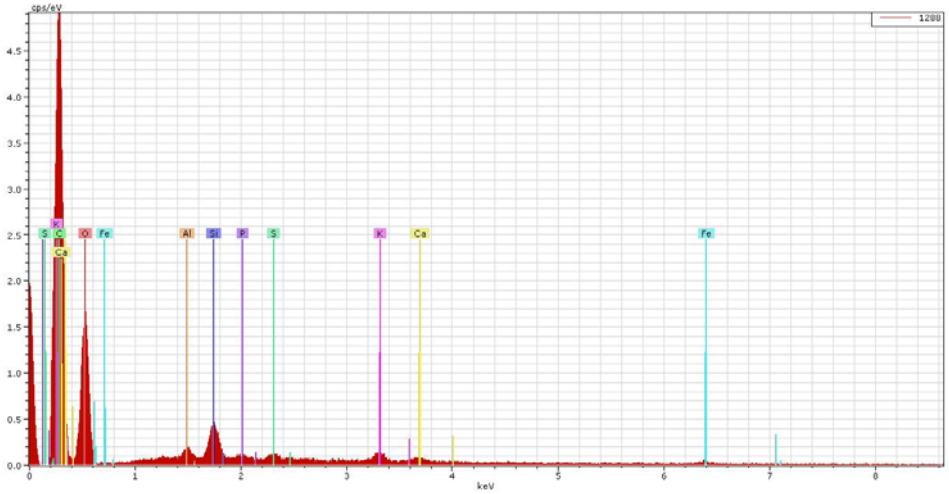

Fig. 5. The chemical composition of *Xanthoria parietina* thallus obtained using EDX detector

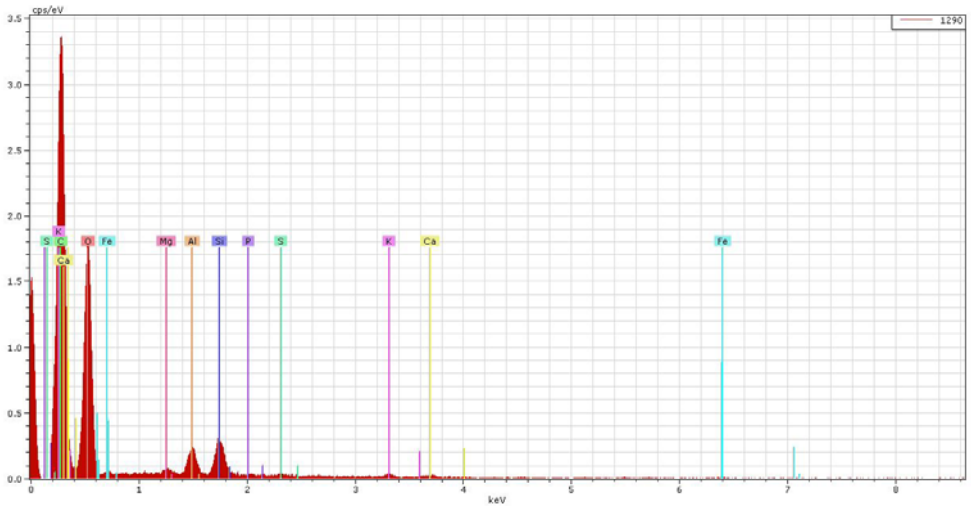

Fig. 6. The chemical composition of *Phaeophyscia orbicularis* thallus obtained using EDX detector

At Copou-Botanical Garden, Copou-Pârtie 1, Copou-Pârtie 2, Copou-Pârtie 3, Copou-Petru Poni, Copou-Penitenciar, Tudor Vladimirescu, Baza 3, Bucium, Dancu, Podul Roş, Galata sampling locations, sampling was performed for at least three month consecutively. At these sampling locations the results are presented as averages of the date obtained from the analysis of the total investigated replicates (i.e., 10 replicates). Especially the *p*H showed good stability within a three month period. For *Xanthoria parietina* the distribution of the *p*H (Fig. 7) and conductivity (Fig. 8) measurements of the samples collected are presented as averages of the total replicates (± SD-Standard Deviation) at the investigated sites.

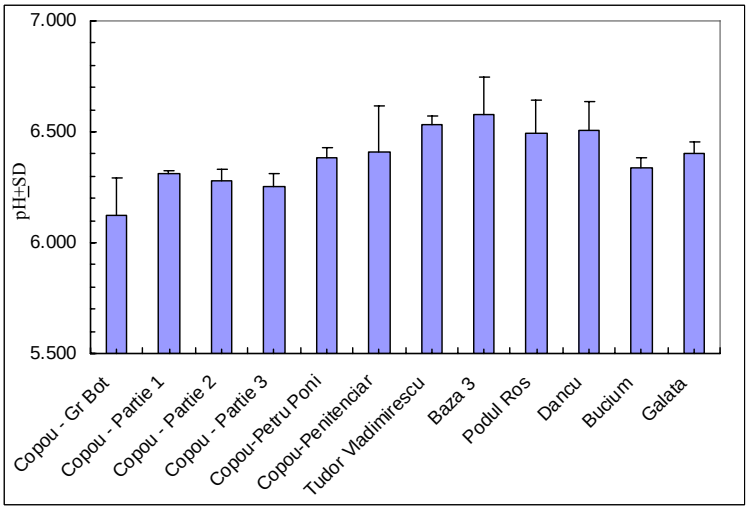

Fig. 7. Distribution of *p*H (mean ± SD) in *Xanthoria parietina* by sampling locations

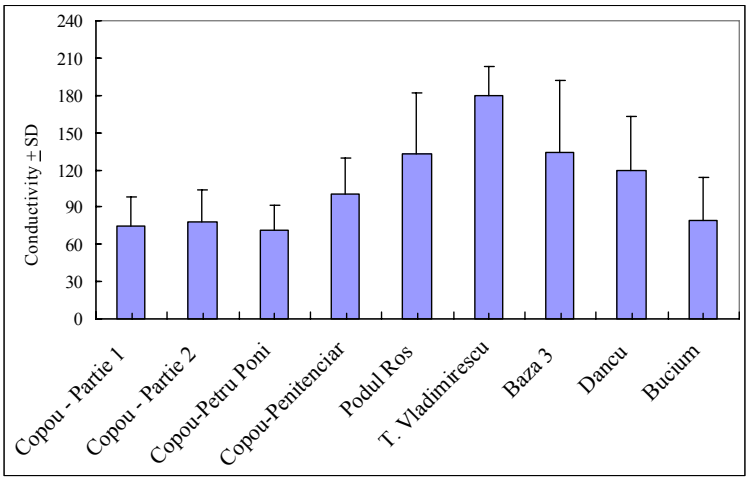

Fig. 8. Distribution of conductivity (mean ± SD) in *Xanthoria parietina* by sampling locations

Fig. 7 shows that statistically significant differences can be observed especially for samples collected from locations eventually heavily exposed to pollution (*e.g.* Copou-Penitenciar, Tudor Vladimirescu, Baza 3, Podul Roş which are locations susceptible to heavy car traffic induced pollution). The variability of the presented conductivity in Fig. 8 confirms the eventually intense exposure car traffic through a higher ionic components loading in the investigated species.

For *Phaeophyscia orbicularis* species simultan statistical calculations were performed, distribution of the interest species shown in Fig. 9 for *p*H and Fig. 10 for conductivity.

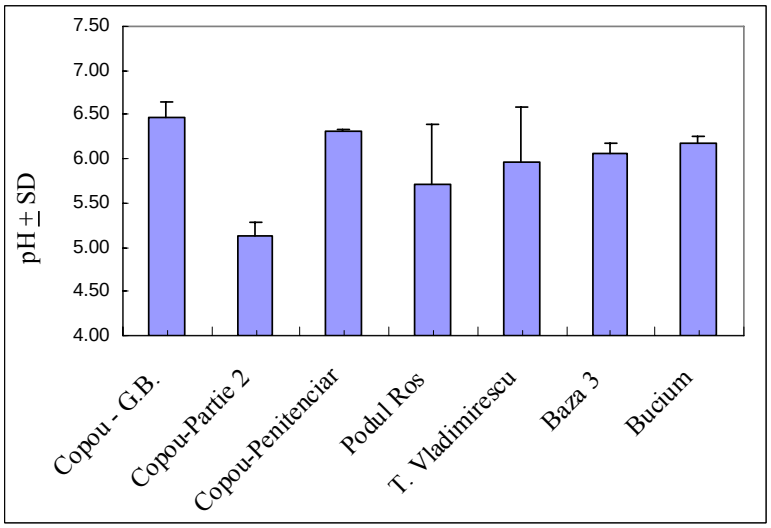

Fig. 9. Distribution of *p*H (mean ± SD) in *Phaeophyscia orbicularis* by sampling locations

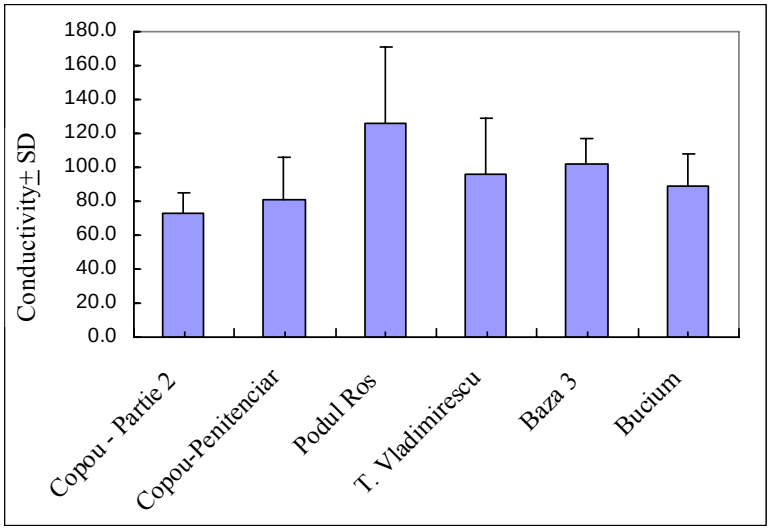

Fig. 10. Distribution of conductivity (mean ± SD) in *Phaeophyscia orbicularis* by sampling locations

For *Phaeophyscia orbicularis* species was observed that the *p*H variability ranged within the same limits with an exception of Copou-Pârtie 2 location. Distribution of the conductivity values shown in Fig. 10 suggests that, perhaps, also the pollution caused by an intensive road traffic Podul Roş, Tudor Vladimirescu, Baza 3 areas, could influence the values parameter in the collected samples from those areas.

Experimental investigations were also performed of the investigations in order to test the influence of the extraction matrix on the *p*H and conductivity. Fig. 11 presents the results obtained for *p*H variability in *Xanthoria parietina* species while Fig. 12 presents the results for the same investigations for the *Phaeophyscia orbicularis* species. Samples of interest species were extracted in ultrapure water and KCl 0.025 M solution.

The data presented in Fig. 11 and Fig. 12 allows us to suggest that the *p*H chemistry of the investigated biological samples can be experimentally performed by using as extraction agent either ultrapure water or potassium chloride solution.

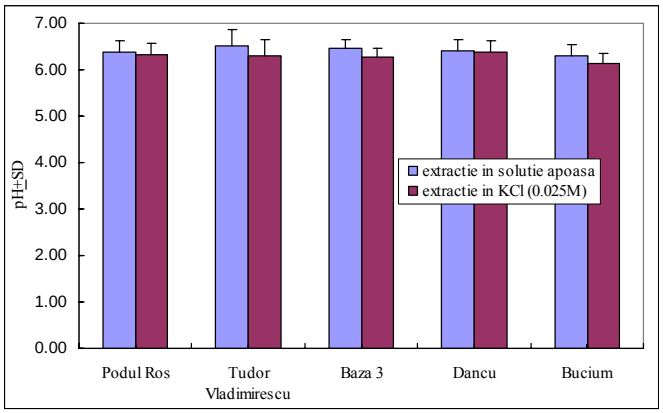

Fig. 11. *p*H variation (+ SD) in *Xanthoria parietina* species by sampling locations

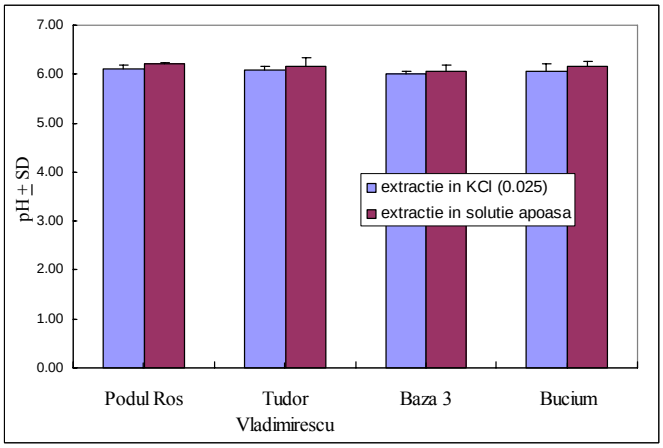

Fig. 12. *p*H variation (+ SD) in *Phaeophyscia orbicularis* species by sampling locations

Many studies [RIGA KARANDINO, 1998; VAN HERK, 2001; FRATI & al. 2006] report information on the possible influence of the substrate *p*H on the behaviour of the lichenized fungi thallus growing on that substrate. In the present work were undertaken also measurements of the bark tree *p*H from where lichenized fungi samples were collected. Data in Tab. 1 presents the *p*H values in the bark trees substrate and the corresponding collected lichenized fungi thallus. Tab. 1 shows the values of the interest statistical parameters, the average values for poplar bark substrate varying for example in the 5.6 to 6.3 range values. However, higher values were measured in bark substrates collected from locations with heavy traffic activities (*p*H = 6.035 in Podul Roş area and *p*H = 6.340 in Tudor Vladimirescu location). The variability shown by the average values specific for the investigated substrates and lichenized fungi samples highlights the existence of a statistically significant difference between the two types of matrices. This behaviour allow us to suggest that the chemical compositions of the investigated lichenized fungi samples were most probably affected mainly by the atmospheric deposition rather than the nature of the substrate on which it stands.

Tab. 1. Bark tree *p*H and thallus *p*H for investigated sampling locations.

Sampling location	Species	Type substrat	*p*H bark tree		*p*H thallus	
			mean	SD	mean	SD
Botanical G.	*Xanthoria parietina*	Oak	**5,591**	0,138	**6,125**	0,191
Botanical G.	*Phaeophyscia orbicularis*	Oak	**5,913**	0,204	**6,406**	0,146
Bucium	*Xanthoria parietina*	Poplar	**5,629**	0,048	**6,304**	0,231
T. Vladimirescu	*Xanthoria parietina*	Poplar	**6,340**	0,073	**6,513**	0,341
Baza 3	*Phaeophyscia orbicularis*	Poplar	**5,739**	0,167	**6,128**	0,190
Dancu	*Xanthoria parietina*	Lime	**6,035**	0,061	**6,385**	0,245
Podul Roş	*Phaeophyscia orbicularis*	Poplar	**5,817**	0,217	**6,412**	0,246

SD – Standard Deviation

Conclusions

Microscopic investigations performed in the present work highlighted interesting cortex morphology after sectioning the lichenized fungi thallus. The details observed at the peripheral part of a cross section of the thallus like fungal colony reveal filamentous hyphal growth at the periphery and conglutinate areas in the microaerobic central part. It was also possible to distinguish the existence of atmospheric particles on the thallus surface. The final assumption is that the morphology of lichenized fungi might have a complex structure that requires careful analysis.

Following the analysis of *p*H, it was observed that the bark tree nature does not influence the chemical behaviour of the interest lichenized fungi samples investigated in the present study.

Elevated values of the *p*H and conductivity chemical parameters were especially measured in *Xanthoria parietina* lichenized fungi samples collected from locations with particularly potential to be exposed to heavily pollution (Tudor Vladimirescu, Baza 3, Podul Roş locations susceptible to pollution induced by heavy car traffic).

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CULTURE DESCRIPTION OF SOME SPONTANEOUS LIGNICOLOUS MACROMYCETES SPECIES

BALAEŞ TIBERIUS[1], TĂNASE CĂTĂLIN[1]

Abstract. 24 species of lignicolous macromycetes from 4 taxonomic families and 2 orders, Class Agaricomycetes, Phyllum Basidiomycota, have been analyzed. The cultural characters of these isolates had been observed, some of them being little studied till now. The dikaryotic mycelium from the trama of the sporoms was used for the isolation purpose. The fungal isolates were cultivated onto malt extract-agar media (malt extract 20g l^{-1}) and incubated at 25 °C, in the dark, for 6 weeks. The cultures were observed directly and using a Nikon stereomicroscope in order to measure the growth rhythm and to observe the changes of the colonies: edge, surface, reverse, shape, colour, smell, presence or absence of the exudates. After 6 weeks from the inoculation, microscopic slides were made in order to investigate the types of hyphae, the colour and the structure of the mycelium and to note the presence of particular elements: cuticle, chlamydospors, arthrospores, conidia, and basidia. We noticed that the analyzed species present similar characters but also significant differences between them.

Keywords: lignicolous macromycetes, fungal growth, cultural characters

Introduction

Lignicolous macromycetes represent a diversified group of fungi in terms of ecological and morphological aspect but it also represents a heterogeneous taxonomic group. The traditional methods of identifying the lignicolous macromycetes based on collecting and analyzing the fruit bodies present the disadvantage of not being able to identify the macromycetes that do not form fruit bodies (due to local ecological and climatic conditions) or those with degraded fruit bodies. The isolation of lignicolous macromycetes in pure cultures and the analysis of the macroscopical and microscopical features of the mycelium grown *in vitro* offer the possibility to identify species without using fruit bodies (when the fungi are isolated from substratum) and offer, also, valuable resources to taxonomic studies.

The cultural features of some isolated lignicolous macromycetes have been studied by different authors [NOBLES, 1948; STALPERS, 1978, 1993]. Different authors [BAKSHI & al. 1969, 1970; NIEMELÄ, 1975, 1977] have realized ample studies on genera *Fomes*, *Phellinus* and *Trametes*. The lignicolous macromycetes present different characters *in vitro*, they can sometimes form fruit bodies, specialised structures for asexual reproduction, exudates, but their presence is not prerequisite for all the species/ isolates. The basidiomycetes may present hyphae with clamp connection, formed from the

[1]Department of Biology, "Alexandru Ioan Cuza" University of Iaşi, Bd. Carol I, No. 20 A, 700505 Iaşi – Romania; e-mail: tiberius_balaes@yahoo.com, tanase@uaic.ro

dikaryotic cells and avoid the septum for connecting with the proximal cell [TĂNASE & ŞESAN, 2006]. The presence of the clamp connection is a distinctive feature.

Many lignicolous macromycetes present economical importance, not only as agents of depreciation of the wood but also as agents that can be used in mycoremediation strategies, due to the presence of ligninolytic enzyme system involved in xenobiotics degradation. Studying the macromycetes grown *in vitro* is very important to the elaboration of mycoremediation strategies and the optimization of culture conditions. Some of the analyzed species from this paper have been little studied until now.

Materials and methods

Fungal strains and isolation procedures

All the tested strains were isolated using fruit bodies collected from deciduous woods in different stages of decay found in forest habitats in north-eastern Romania. The isolation process occurred under sterile conditions using the context mycelium of sporoms and the pure isolates thus obtained have been maintained by sub culturing them onto malt-extract media and stored in the dark at a temperature of 4 °C. The identification of the selected species was performed using classical macroscopic and microscopic methods according to the literature [BERNICCHIA, 2005; ERIKSSON & RYVARDEN, 1976; HANSEN & KNUDSEN, 1992, 1997; RYVARDEN & GILBERTSON, 1993, 1994; SĂLĂGEANU & SĂLĂGEANU, 1985; STALPERS, 1980], and the specimens were lyophilized (UniEquip lyophilizator, UNICRYO MIC 4 L model, Planegg, Germany) or dehydrated (using a dryer, Ezidri Ultra 1000 FD) and then deposited in the Faculty of Biology Herbarium [I], "Alexandru Ioan Cuza" University of Iaşi, Romania. All the tested strains and the corresponding herbarium number are listed in Tab. 1. The nomenclature used in this paper is according to the Species Fungorum database.

Culture conditions

In order to analyse the cultural characters it has been used the method established by STALPERS (1978). Consequently, the 9 cm diameter Petri dishes filled with 25 ml MEA (20 g malt extract, 15 g agar, distilled water – 1000 ml) have been employed for the purpose. All the media have been sterilized by autoclaving at 120 ºC in a 75 liters upright model autoclave (Raypas, Barcelona, Spain). Three replicates have been made for all the samples. The pH of media was adjusted with 0,1 M hydrochloric acid at the value 5.0. An electronic pH/ion-meter (model INOLAB, WTW, Weilheim, Germany) has been implied in the procedure. All the plates have been inoculated with small plugs of mycelium, placed at 1.5 cm from the edge of the plate and then incubated in the dark at 25 ºC, for 14 days, in an automated aeration incubator (Microbiotest, Gent, Belgium).

Cultural characters analysis

The cultures have been analyzed weekly in order to measure the growth rhythm and to observe the macroscopic changes of the colony, with the naked eye and with a stereomicroscope at 15-30 x magnification (stereomicroscope with phototube SZM2 Optika). After 6 weeks from the inoculation, microscopic observations have been made paying attention to the features / characters and type of the hyphal system from the advancing zone, the submerged and aerial mycelium. There have been studied: the type of

the hyphae, their colour and aspect; presence / absence of the crystals on the hyphae's surface; the diameters of hyphae; the presence, form and dimension of conidia, chlamydospores, cuticular cells; the formation *in vitro* of fruit bodies and their characters; the presence of other particular structures, of exudates etc.

The measuring of microscopic structures has been realized at magnification of 1000x with a trinocular microscope (Optika). For testing the amyloidal, dextrinoid or cyanophilic character of some microscopic structures there were used: Lugol solution, Melzer's reagent and Methylene blue. In order to verify whether some microscopic structures change the colour or swell, a solution of KOH 5% was used.

Results and discussion

The characters of some isolates of lignicolous basidiomycetes from four families and two orders (Tab. 1), included in Class Agaricomycetes, Subclass Agaricomycotina, Phyllum Basidiomycota have been analyzed in the present study. An accelerated growth rhythm was observed for most of the isolates and a moderate one for those from Hymenochaetaceae family. Some isolates presented a slow growth: *Merulius tremellosus*, *Postia caesia* and *P. stiptica.*

The fungal isolates studied presented in culture characters common to the genera or the families but also significant differences. Some cultures distinguished by the presence of asexual reproduction structures (arthrospores, chlamydospores) but also by forming some primordia of fruit bodies or even mature fruit bodies (*Skeletocutis alutacea*). The colour of the mycelium, the general aspect of the colony, the types of hyphae from the mycelium, the presence / absence of cuticle, the crystals in media or on the hyphae's surface and the presence / absence of the clamp connections have been factors of differentiation (Tab. 2).

The species from Hymenochaetaceae family dinstinguished by the presence of some strongly pigmented hyphae and the lack of the clamp connections whereas the species from Polyporaceae family distinguished by forming primordia and fruit bodies.

Macroscopic aspects and microscopic characters of mycelium grown on nutritive media

Bjerkandera adusta (Willd.) P. Karst. Mycelium is homogeneous, appressed, more lax in the centre, with white aerial hyphae, arranged radially. On the edge the mycelium forms a thick ring, felty, compact, cream, powdery, climbing on the wall plate. Some areas of the ring formed soft scabs, with shiny areas, sometimes confluent. Hyphal system is monomitic. Hyphae are thin, of 1.5-2.5 µm, sometimes with thickened segments of up to 5 µm, tangled and branched, with thick septa, hyaline. Presents clamp connections. The submerged mycelium and from the advancing zone have thin hyphae, sometimes thick, up to 7 µm diameter, with clamp connections. Other authors [BAKSHI & al. 1969] have observed the formation of chlamydospores in culture, but in our cultures chlamydospores were not found.

Bjerkandera fumosa (Pers.) P. Karst. Mycelium is irregular, loose in the centre, with rare aerial hyphae, appressed, translucent and slightly powdery. Mycelial mat becomes

dense and felty on the edge, forming a thick mycelial ring that continues on the plate wall, with compact. Aerial mycelium is white to cream. Primordia appear after four weeks, and they form mycelial cords to the edges. Mycelium presents: generative hyphae, hyaline, with septa and clamp connections, branched, both in the advancing zone and submerged or aerial mycelium; skeletal hyphae, of 5 mm diameter, with simple septa. The submerged mycelium has thin hyphae, with numerous lateral branches, finger-like.

Coriolopsis gallica (Fr.) Ryvarden. Colony shows different zones: an area with appressed mycelium, thin, translucent near the point of inoculation and an area with dense mycelium and interwoven aerial hyphae, powdery-white or cottony creamy, shaggy, matted later, yellow-brown with erect air cords, 20-40 mm high, on walls (Fig. 1A). The mycelium has generative hyphae, hyaline and skeletal hyphae, long, slightly pigmented (yellow-brown, beige) rarely branched or with septa, from 1.5 to 4 μm in diameter. Some hyphae are thicker than 6 μm in diameter (skeletal) but without septa, hyaline and branched, with narrow lumina. At the edge of the colony hyphae are long, sinuous, unbranched or rarely branched, without septa, cream, up to 4 μm in diameter, numerous, often grouped in bundles, sometimes with crystals, rarely dilated.

Daedalea quercina (L.) Pers. Mycelium is white, cottony, with slightly different zones, radially arranged in bundles, fuzzy-felty, forms uneven clumps, rhizomorphic or cottony, white to cream, which rises on the walls forming hirsute areas with densely intertwined hyphae. The mycelium presents generative hyphae, branched, with frequent clamp connections of 1.5 to 4.5 μm in diameter, the aerial hyphae are up to 6 μm thick. Skeletal hyphae are present in the aerial mycelium, hyaline, rarely branched, without septa, 2-3 μm in diameter. It was reported the formation of mature fruit bodies with basidia and basidiospores in the case of other isolated [NOBLES, 1948].

Daedaleopsis tricolor (Bull.) Bondartsev & Singer. Mycelium is felty, attenuated, white in the centre, and then forms an irregular crust, thick, brown, cottony areas alternating with smooth areas with different shades from light to dark brown with bumps, appressed scabs, thin felty zones with shades of brown, gray and even white felty crusts (Fig. 1B). On the plate wall the mycelium forms pigmented primordia. The hyphal system is trimitic. The advancing zone and the submerged mycelium have generative hyphae, branched, thin to moderately thick, with thick septa and clamp connections, hyaline. Aerial mycelium present, also, brown skeletal hyphae and thick cuticular cells.

Fomes fomentarius (L.) J. Kickx f. The advancing zone is straight with the aerial mycelium uniform and then appressed. Mycelium is apressed in the proximity of the inoculation point, felty, later forms white crusts with appearance of elongated spots, arranged radially, confluent, powdery-soft, compact, cream-beige-yellow to cream-brown. Mycelium shows generative hyphae, hyaline, with swollen septa of 1.5-3.0 (4.0) μm diameter and thick-walled skeletal hyphae, refringent, hyaline to brown, with narrow lumina, sometimes branched without septa of 1.5 to 3.0 μm in diameter, closely intertwined with cuticular cells forming pseudoparenchimatic layers. Hyphae do not have clamp connections. Isolates tested produced rare chlamydospores (Tab. 2), unlike the isolates evaluated in other studies [NOBLES, 1948].

Fomitopsis pinicola (Sw.) P. Karst. Mycelium is felty and smooth in the peripheral zone, white. In the central area forms a white and cottony mat, with dense

agglomerations spot like. Near the point of inoculation forms a very thick mat, compact, smooth, white, with protrusions (primordia of fruit bodies). The hyphal system is trimitic. In the advancing and the submerged mycelium there are generative hyphae, branched, with numerous septa and clamp connections, hyaline, diameter 1.5 to 3 µm. The aerial mycelium presents generative hyphae; skeletal hyphae, unbranched, without septa and by 1.5 to 4 µm diameters, long, sinuous and fibber hyphae, hyaline, branched, without septa, up to 4 µm in diameter. Some authors [NOBLES, 1948; STALPERS, 1987] reported the presence of chlamydospors, but our studied isolates did not produced.

Inonotus hispidus (Bull.) P. Karst. Mycelium presents concentric zones, waxy crusts, smooth, coloured in various shades of yellow to brown and concentric rings with aerial hyphae (Fig. 1C). The crusts are dark brown and present few aerial hyphae. Aerial mycelium areas are powdery and present yellow or yellow-brown hyphae, erect, short, sometimes with felty or powdery areas. Colony edge is straight. Submerged mycelium and from the advancing zone presents generative hyphae, long, simple-septate, rarely branched, from 2.5 to 5 µm diameter, hyaline. Aerial mycelium present generative hyphae and skeletal hyphae, 4-5 µm thick, straight, frequently branched, with simple septa, yellow, sometimes thin and sinuous. Connection hyphae and cuticular cells are also present.

Irpex lacteus (Fr.) Fr. Mycelium is lax, appressed with radially arrangement, mostly translucent with veins and white cords, especially in the periphery. It can be observed felty-cottony crusts, white, sometimes with small clusters of hyphae. Hyphal system is monomitic. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with rare septa, 4.5 to 6 µm diameter, with clamp connections. The aerial mycelium presents generative hyphae and fibber hyphae, 1.5 to 2 µm diameter, hyaline, rarely branched, with simple septa. The tested isolate produced arthrospors unlike isolates tested in other experiments [NAKASONE, 1990].

Lenzites betulina (L.) Fr. Colony forms a very thick and dense mycelial mat, white, with smooth edges, cottony in the middle, ± smooth, silky at the edge (Fig. 1D). A soft cottony mycelial ring is formed on the walls, 0.5 cm thick, 1 cm high, with denticulate edge, sometimes cream. Hyphal system is dimitic. Submerged mycelium and from the advancing zone presents generative hyphae, branched, septate, with clamp connections, hyaline, of 2.2 to 4.5 µm in diameter. Aerial mycelium present generative hyphae and skeletal hyphae very numerous, hyaline, thick refringenţ walls and with narrow lumina, without septa, occasionally branched, of 1.5-3 (4) µm diameter.

Merulius tremellosus Schrad. Mycelium is irregular, waxy, with dark green hyphae, radially arranged. Mycelium is appressed in the rest of the colony, waxy-levurian, translucent. Near the point of inoculation, mycelium is denser, forming a waxy broad crust, dark-green. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with simple septa, moderately branched, with thin wall which thickens with time, 2-5 (6) µm diameter. Aerial mycelium presents generative hyphae of 1.5 to 6 µm diameter, hyaline, moderately branched, with simple septa, frequently encrusted with numerous irregularly shaped crystals, some hyphae were opaque yellow.

Phellinus conchatus (Pers.) Quél. Mycelium presents concentric zones, with abundant aerial hyphae, cottony, orange-gray to cream-brown. Forms brown crusts around the edges, and a brown ring with numerous aerial hyphae, high, tangled, white, yellow or

orange-cream to brown. Mycelium mat is felty, thin near inoculum, but very heavy, felty-cottony, orange on the edges. The crusts are powdery, sometimes with small clusters of aerial hyphae. Submerged mycelium and from the advancing zone presents generative hyphae, thin, branched, with simple septa and rare, 2.5 to 4 μm thick, long. Aerial mycelium presents: generative hyphae and thick skeletal hyphae, with few septa, pigmented, sometimes inlaid.

Phellinus igniarius (L.) Quél. Mycelium mat presents concentric zones, with aerial brown-yellow-orange hyphae, cottony, lighter in the middle, forming brown crusts in the opposite side and denser and thicker networks on the edge. Aerial hyphae are easily felty, shorter, more frequent and longer in the sides. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with simple septa, from 1.5 to 6 μm in diameter. Aerial mycelium presents generative hyphae and also skeletal hyphae with walls thickened, yellow-gray, rarely with septa, 1-3 μm in diameter. Chlamydospores were observed, but other authors [NIEMELÄ, 1975; NOBLES, 1948] showed no chlamydospores production for this species.

Phellinus pomaceus (Pers.) Maire. Mycelium forms a dense mycelial network, very thick, felty-cottony, tangled, velvety-brown, yellow-brown to cream-gray-brown. Near the point of inoculation, mycelial network is thin and smooth, then forms higher areas, compact, irregular, cottony or felty, darker, sometimes with gray or cream hyphae. For edges, colony forms primordia (Fig. 1E). Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with simple septa, thin-walled, frequently branched, of 1,5-4,5 (6) μm diameter. Aerial mycelium presents generative hyphae and pigmented skeletal hyphae, thick walled, brown, rarely branched, without septa, of 1.5-3.5 μm diameter.

Polyporus arcularius (Batsch) Fr. Mycelium forms a waxy crust, brown, with a concentric wall-like mat, brown and wrinkled surface on external side and white-cottony on the inner side. The central wall-like cord is the highest (Fig. 1F). In the centre, mycelium is white and cottony and at the edges is appressed or with hyphal clusters, felty, white or cream. Aerial mycelium is trimitic and consists of highly branched generative hyphae with clamp connection, 2-4 μm thick; skeletal hyphae, thin, simple branched, septa without clamp connections, about 2 μm thickness and cuticular cells, up to 7 μm thick, amyloid, numerous.

Postia caesia (Schrad.) P. Karst. Mycelium is willing radially, translucent and colony edge is straight. Mycelium mat is appressed, thin and has several hyphal clusters and mycelial cords, small, white. Medium is deepened in the area covered by the colony. At the periphery, the mycelium is cream, arranged in the form of fine cords, radially branched. Mycelium is monomitic and consists of the generative hyphae, tree-like branched, with clamp connection, hyaline, up to 5 mm thick. Some lateral or terminal branches have crystals on their surface. Terminal branches are thin of 1-2.5 μm. Submerged hyphae are hyaline, with swellings.

Postia stiptica (Pers.) Jülich. Mycelium is appressed, only with submerged hyphae at the edge of the colony, translucent. Aerial mat is white, thin, with short hyphae. The medium is strongly deepened near the point of inoculation and faded. In this area develops a compact hyphal cluster, white, cottony. Mycelium is monomitic with generative hyphae,

with large clamp connection, of 1.5 to 3.5 μm thick. Aerial mycelium consists of generative hyphae, with clamp connection and many short side branches. Some hyphae have swollen septa or numerous blisters on their surface. The submerged mycelium has numerous and large irregular crystals.

Royoporus badius (Pers.) A.B. De. Mycelium mat forms dense networks, velvety, cottony, and thick near the point of inoculation and near wall plate, cream-brown or brown. From the center to the periphery forms thick crusts, soft, brown, which become thinner and less pigmented to edge, with wrinkles and projections. They are bounded to the centre by a white cord, thin and felty. In the centre, mycelial mat is thin and translucent. Submerged mycelium and from the advancing zone presents generative hyphae, strongly branched, 2-4 μm thick, with simple septa. Terminal branching of the submerged hyphae are tree-like. In the aerial mycelium are also found skeletal hyphae, 4-4.5 μm thick, curved or straight.

Skeletocutis alutacea (J. Lowe) Jean Keller. Mycelium mat presents concentric zones, is thin near inoculum, translucent, or with very fine mycelial cords, radially arranged. In the opposite side mycelium is whitish and easily felty. The two areas are separated by a mycelial ring that shows fruit bodies, shaped like thick crusts, compact, pored, or even bumps up to 1 cm high, with irregular angular pores, 2-4 (5) / mm, tubes long by 0.5 to 0.7 mm (Fig. 2A-D). Fruit bodies are cream-yellow, with light gray or greenish colour and are surrounded by sterile areas, white, silky-cottony. Basidia are of 8-10-13 x 1-3 mm. Basidiospors are cylindrical to allantoid, thin, 4-5 x 1 μm, numerous, nonamyloid. Corresponding to the pores, below, on the medium surface, are formed stalactite-like structures, with thick hyphae, up to 10 μm, with simple septa or short clamp connections, branching, sometimes with swellings, hyaline in KOH, sometimes at the end with cylindrical hyphae, thick-tipped, straight, dextrinoid. Hyphal system is dimitic, with long generative hyphae, up to 5 μm thick, with many clamp connection of 2-3 μm thick, and thick skeletal hyphae, tree-like branched.

Trametes gibbosa (Pers.) Fr. Mycelium forms a dense network, white, felty, with tall aerial hyphae, strongly branched and with compact areas, velvety, powdery to glabrous, thick and globular, more numerous and larger next to walls, white-cream or cream-ochre. In these areas fruit bodies are formed. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, thin-walled, with frequent clamp connections, of 1.5 to 4 μm in diameter. Aerial mycelium presents generative hyphae and skeletal hyphae, thick-walled and with narrow lumina, unbranched, without septa, from 2.5 to 5 μm in diameter.

Trametes hirsuta (Wulfen) Lloyd. Mycelium is appressed near the point of inoculation, felty in the opposite side and forms a mycelial ring very thick and cottony near the plate wall, compact white to cream-coloured. The mat of the ring touches the upper plate and forms many large fruit bodies, compact, soft and cottony, uneven, often globular, with protuberances up to 2 x 1.5 x 1.2 cm and many hyphal clusters (Fig. 2E-F). The fruit bodies have not matured and have not developed basidia or basidiospores, unlike isolates analyzed by other authors [BAKSHI & al. 1969; Noble, 1948]. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with clamp connections, of 1.8-4.3 μm diameter. Aerial mycelium consists of branched generative hyphae, hyaline with numerous septa and clamp connections, and skeletal hyphae, thin, long, hyaline,

unbranched, of 1.5-3 μm thick. Fruit bodies are made up of fibber hyphae, short, thick, heavily branched and generative hyphae.

Trametes pubescens (Schumach.) Pilát. Aerial mycelium is soft-felty in the peripheral zone, matted. In the median zone is slightly appressed and thin. Near the inoculum the mycelial mat has long aerial hyphae, highly branched, cream-gray. At the periphery, soft and powdery crusts or very cottony areas are formed and high and soft fruit body with powdery surface, creamy white or cream to gray. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with septa, 1.5 to 4.5 μm in diameter. Aerial mycelium consists of generative hyphae, thick, strongly branched and twisted, with clamp connection, from 3.5 to 6 μm in diameter and skeletal hyphae, thin, long, with few and simple septa, of 1.8 to 2, 2 μm thick.

Trametes suaveolens (L.) Fr. Mycelium is dense, felty-soft, smooth, white, with a thick mycelial ring at the periphery. Fruit bodies of 1-1.5 x 0.7 to 0.9 x 5 cm are formed near the plate wall. The fruit bodies are irregular, with large protrusions, creamy white, sometimes yellow shades. Mycelial mat is powdery. Submerged mycelium and from the advancing zone presents generative hyphae, thin, with septa, branched, with frequent clamp connections, hyaline, from 2.2 to 4.5 μm in diameter. Aerial mycelium presents generative hyphae of 1.5-3 μm diameter and numerous skeletal hyphae, rarely branched, thick-walled.

Trametes versicolour (L.) Lloyd. Mycelium is sparse and thin in the opposite side of inoculum, but in the median side of plate forms a soft and powdery crust, thin, creamy-white or beige. Near the inoculum point, the crust becomes very thick, soft or cottony, white, compact and develops primordia. The hyphal system is trimitic. Submerged mycelium and from the advancing zone presents generative hyphae, hyaline, with septa and clamp connections, 3.0 to 4.5 μm diameter. Aerial mycelium presents generative hyphae, long, hyaline; skeletal hyphae, thin, hyaline, long, unbranched, without septa, from 1.5 to 4 μm thickness and fibber hyphae, branched, without septa.

Conclusions

The macroscopic and microscopic characters of the 24 lignicolous basidiomycetes isolates were analyzed during *in vitro* cultivation of the mycelium. The malt-extract media were favourable for growth and the species from genus *Postia* used agar in their own metabolism.

There have been observed characters common to the families and genera but also significant differences between them, such as the growth rhythm, the presence of specialized reproduction structures and the mycelium colour. Some isolates formed fruit bodies or primordia, arthrospores or chlamydospores. Some isolates from the Hymenochaetaceae and Polyporaceae families formed cuticle on the surface of the nutritive media.

The isolates from Meruliaceae şi Polyporaceae families presented accelerated growth rhythm, and those from genus *Postia* had the slowest growth rhythm.

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Tab. 1. The tested fungal isolates, their taxonomic position and the number of the specimens in the Herbarium

ORDER	FAMILY	SPECIES	SPECIMEN
Hymenochaetales	Hymenochaetaceae	*Inonotus hispidus* (Bull.) P. Karst.	[I 137383]
		Phellinus conchatus (Pers.) Quél.	[I 137368]
		Phellinus igniarius (L.) Quél.	[I 137381]
		Phellinus pomaceus (Pers.) Maire	[I 137373]
Polyporales	Meruliaceae	*Bjerkandera adusta* (Willd.) P. Karst.	[I 137350]
		Bjerkandera fumosa (Pers.) P. Karst.	[I 137364]
		Irpex lacteus (Fr.) Fr.	[I 137351]
		Merulius tremellosus Schrad.	[I 137398]
	Polyporaceae	*Coriolopsis gallica* (Fr.) Ryvarden	[I 137358]
		Daedaleopsis tricolor (Bull.) Bond. & Sing.	[I 137365]
		Fomes fomentarius (L.) Fr.	[I 137376]
		Lenzites betulina (L.) Fr.	[I 137355]
		Polyporus arcularius (Batsch.) Fr.	[I 137362]
		Royoporus badius (Pers.) A.B. De	[I 137385]
		Skeletocutis alutacea (J. Lowe) J. Kell.	[I 137386]
		Trametes gibbosa (Pers.) Fr.	[I 137354]
		Trametes hirsuta (Wulfen) Lloyd	[I 137356]
		Trametes pubescens (Schumach.) Pilát	[I 137357]
		Trametes suaveolens (L.) Fr.	[I 137359]
		Trametes versicolour (L.) Lloyd	[I 137363]
	Fomitopsidaceae	*Daedalea quercina* (L.) Pers.	[I 137394]
		Fomitopsis pinicola (Sw.) P. Karst.	[I 137396]
		Postia caesia (Schrad.) P. Karst.	[I 137400]
		Postia stiptica (Pers.) Jülich	[I 137401]

Tab. 2. The principals macroscopic and microscopic characters of fungal isolates cultivated on synthetic nutritive media

SPECIES	GROWTH RHYTHM*	EXUDATES, PRIMORDIA AND FRUIT BODIES	SMELL	REVERSE OF COLONY**	REPRODUCTIVE STRUCTURES	PARTICULAR STRUCTURES AND CRYSTALS
Bjerkandera adusta	2	colourless exudates	decomposed vegetation	white	arthrospores, 3 x 3-10 µm, with thick walls	
Bjerkandera fumosa	2	primordia	anise like	white	arthrospores, 2 x 3-10 (13) µm; chlamydospors pear-shaped, of 4 x 6 µm	swollen septa; prismatic crystals
Coriolopsis gallica	2		hay like	yellow		swollen hyphae; small crystals, scarce
Daedalea quercina	3	primordia, scarce	mushroomy	unchanged	chlamydospors, spherical, numerous	numerous prismatic crystals
Daedaleopsis tricolor	2	brown exudates; primordia	indistinct	white	grouped chlamydospors, of 4-8 µm diameter	red-brown cuticular cells
Fomes fomentarius	3		indistinct	white	pink chlamydospors	swollen septa; numerous cuticular cells, 25 µm diameter, nonamyloid
Fomitopsis pinicola	2	primordia	rotten wood	unchanged		
Inonotus hispidus	6		indistinct	brown	yellow chlamydospors, 7-8 x 4-5 µm	cuticular cells
Irpex lacteus	2		indistinct	unchanged	arthrospores	swollen septa, scarce
Lenzites betulina	2	colourless exudates; primordia	mushroomy (*Agaricus*)	white		
Merulius tremellosus	6		scented	unchanged	arthrospores, 2-3 x 4-5 µm	crystals on hyphal surface

Phellinus conchatus	3		indistinct	white or brown		prismatic crystals
Phellinus igniarius	4		indistinct	brown	yellow chlamydospors	brown cuticular cells
Phellinus pomaceus	3	colourless exudates	indistinct	brown		brown cuticular cells
Polyporus arcularius	2		green apple	white or pigmented		hyphae with swellings; cuticular cells, amiloyd
Postia caesia	> 6		indistinct	white		hyphae with spherical swellings, 10 µm diameter; octahedral crystals, sometimes irregular
Postia stiptica	> 6		indistinct	white		hyphae with lateral swellings and vesicles; swollen septa; octahedral crystals, 10 x 10 µm, numerous
Royoporus badius	5		scented	white	chlamydospors, 3 x 7-15 µm, numerous	finger-like lateral branches; pigmented cuticular cells, 25 x 15 µm; octahedral or irregular crystals, of 11 x 11 µm
Skeletocutis alutacea	2	fruit bodies; basidia, 8-13 x 3 µm; basidiospors allantoid to cylindrical, 4-5 x 1 µm, numerous	indistinct	unchanged		hyphae with swellings, 5-6 x 10-15 µm; large octahedral crystals

Trametes gibbosa	2	fruit bodies	rotten wood	white		
Trametes hirsuta	2	fruit bodies	indistinct	white		
Trametes pubescens	2	fruit bodies	indistinct	white		
Trametes suaveolens	2	colourless exudates; fruit bodies	anise like, strongly	white	chlamydospors, 7-15 x 4,5-6 µm, numerous, with thin walls	
Trametes versicolour	2	primordia	indistinct	white		cuticular cells, hyaline

* The needed time for covering the entire plate (in weeks)

** Only the old part of the colony is considered. The recently covered medium remain, often, unchanged

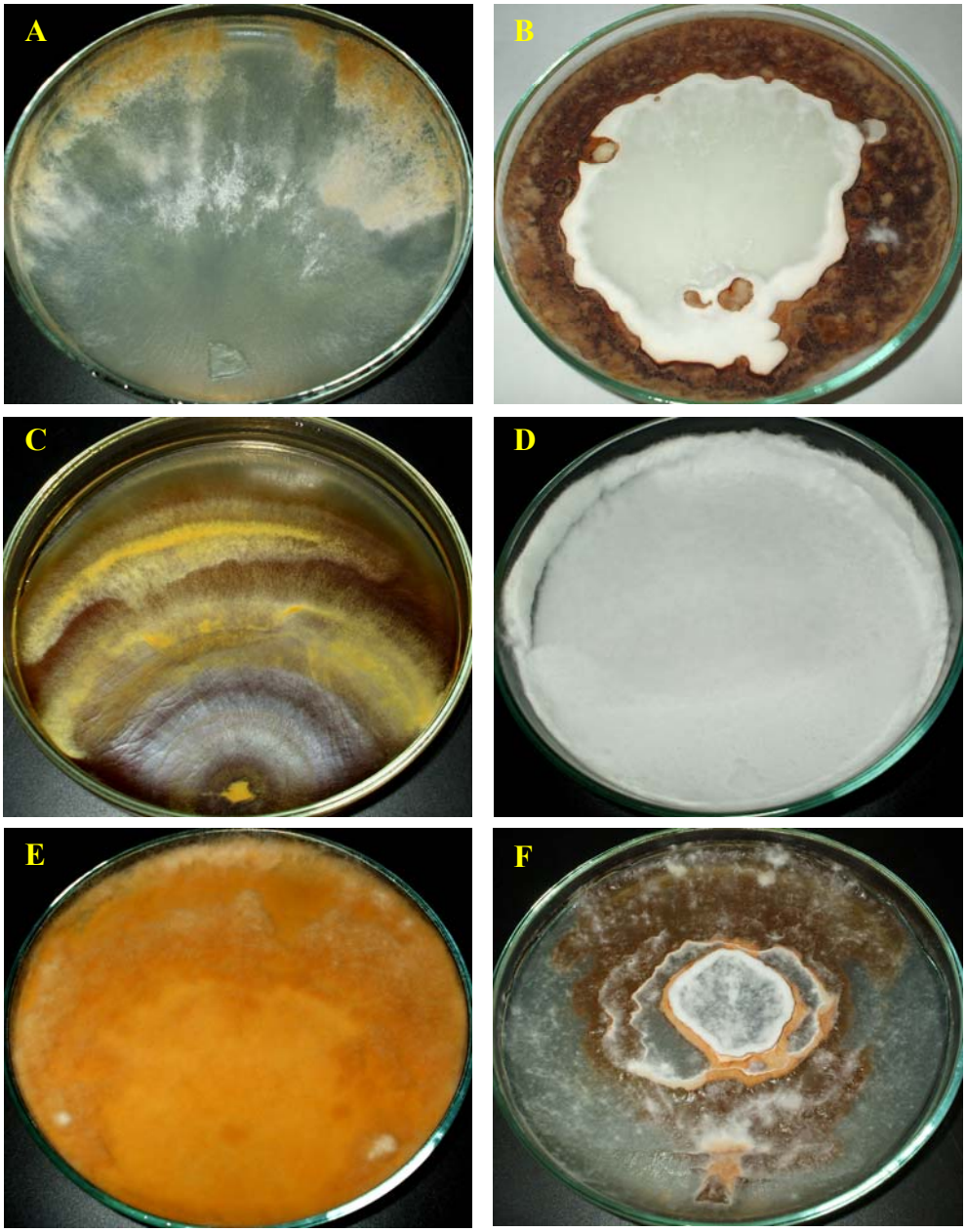

Fig. 1. General aspects of fungal colonies developed *in vitro*, in Petri dishes of 9 cm diameter: A - *Coriolopsis gallica*; B - *Daedaleopsis tricolour*, C - *Inonotus hispidus*; D - *Lenzites betulina*; E - *Phellinus pomaceus*; F - *Polyporus arcularius*.

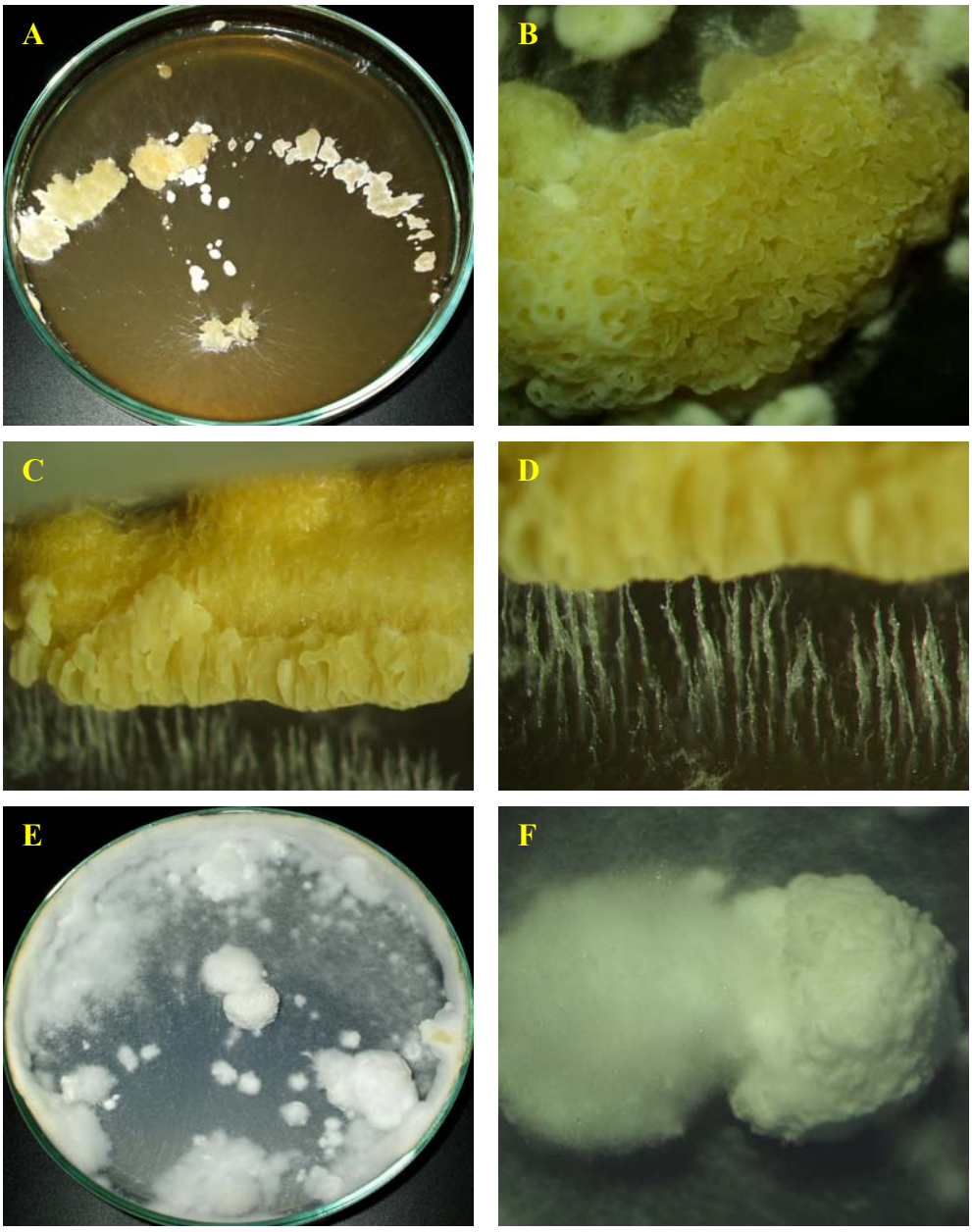

Fig. 2. *In vitro* development of fruit bodies, in Petri dishes of 9 cm diameter: A-D - *Skeletocutis alutacea*, general aspect (A) and details (B-D); E-F - *Trametes hirsuta*, general aspect (E) and detail (F).

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NEW ASPECTS OF THE ALPINE VEGETATION OF PARÂNG MOUNTAINS (SOUTH CARPATHIANS)

SIMON TIBOR[1], PÓCS TAMÁS[2]

Abstract*:* 4 plant communities unknown in the the European syntaxonomy are described from the alpine and subalpine belts of Parâng Mountains, based on vegetation studies of the authors during 1955–1960. These are: *Arabis alpina-Saxifraga aizoides*, *Arabis alpina-Delphinium elatum*, *Dianthus tenuifolius-Festuca dalmatica* and the *Primula minima-Dryas octopetala* communities. These communities could be described later as new associations in the possession of more relevés from different localities. The East and South Carpathian *Doronico carpatici-Festucetum pictae* association is distinguished under this new name from the *Festucetum pictae* Krajina described from the Tatra Mountains. 7 further associations are found as new to the Parâng Mountains.

Keywords*:* phytocoenology, alpine vegetation, Parâng Mountains, Southern Carpathians, Romania

Introduction

After the terrific II[nd] World War, during the years of 1955-'60 opened the opportunity the first time for a group of young Hungarian botanists (A. Borhidi, P. Juhász-Nagy, T. Simon, I. Skoflek, G. Vida, led by T. Pócs) to visit the Southern Carpathians, making acquaintance with its rich flora and vegetation. Their main goal was to study the vegetation of Parâng Mountains, scarcely known on those times, using the quadrat of Braun-Blanquet method. They offered their co-operation to the Romanian botanists working in the same area.

As a result of this activity, some papers were already published, containing records on the Parâng Mountains, as the account of VIDA (1963) on the beach forests (*Symphyto-Fagion*), the spruce forests (*Vaccinio-Piceetalia*) by BORHIDI (1971), the study on the subalpine bushes (*Rhododendro myrtifolii-Pinetum mughi*), pastures and swards by SIMON (2007, 2008-2009), but the rest of these vegetation studies (meadows and pastures by Juhász-Nagy and the subalpine and alpine vegetation studies of the present authors) remained unpublished. In the meantime a detailed monograph was published on the pastures of Parâng Mountains, including also the geography, plants, animals and fungi and their economic value, by BUIA & al. (1962). The monograph has a phytocoenology chapter, describing in details the majority of grassland communities. The alpine and subalpine vegetation of many other parts of the Romanian Carpathians were described in other modern monographs (e.g. by BOŞCAIU 1971, COLDEA 1990, 1991).

Some floristic data were published by PÓCS (1957) and by PÓCS & SIMON (1957) with the record of *Aubrietia croatica*, new to the whole Carpathians and Romania.

[1]Department of Plant Taxonomy and Ecology, Institute of Biology, Eötvös Loránd University, Budapest – Hungary, Pázmány P. sétány 1/C, H-1117
[2] Department of Botany, Institute of Biology, Eszterházy Károly College, Eger – Hungary, pf 43. H-3301, e-mail: colura@chello.hu

A complete flora of Parâng Mountains was also initiated, of which three volumes were published by PÓCS (1961, 1962, 1968). A very important new addition was the discovery of *Allium obliquum*, not far from the locality of *Aubrietia*, by PLOAIE (1990).

Our aim in this paper is to complete the picture on the rich alpine and subalpine vegetation of Parâng Mountains with our unpublished phytosociological relevés. Our investigations are new, especially demonstrating the rock and scree vegetation. The 5 synthetic tables contains information on 19 subalpine and alpine plant communities, among which 4 are new to European syntaxonomy. Several associations are described as new to Parâng or supplement previously known communities. We also tried to establish the level of naturalness of alpine vegetation more than 50 years ago to supply a reference base. This is comparable with the present situation, influenced by human activites, discussed by PLOAIE (1996), PLOAIE & TURNOCK (1999), PLOAIE & al. (2002).

Materials and methods

Alpine and subalpine plant communities were surveyed with the Central European phytocenological methods of Braun-Blanquet (1951). The phytosociological relevés were made by T. Pócs during 24-26 Sept., 1955, 12 July-5 Aug., 1956 and 3-19 Aug., 1960, and by T. Simon during 24 July-5 Aug., 1956. We used 5 × 5 m (25 m^2) sample squares if not otherwise mentioned. In the table the species are arranged alphabetically within each K groups. The nomenclature of vascular species is according to OPREA (2005), of the mosses follows OCHYRA & al. (2003), the liverworts ŞTEFĂNUŢ (2008), while that of the lichens BIELCZYK & al. (2004). The description of habitat of each relevé are included into this paper. After them, the proportion of geographical elements according to OPREA (2005), coenotaxonomic groups according to BELDIE (1967), BOŞCAIU (1971), BOROS (1968), COLDEA (1990, 1991) and POP & al. (1999-2000), IVAN & al. (1992), social behaviour according to BORHIDI (1995) and the conservational value according to the groups of SIMON (1988) and on the base of DIHORU & NEGREAN (2009) are summarized. The values of Simon demonstrate the ratio of groups referring to naturalness versus degradation. We also had to take into account that since the mid-fifties of the last century the concept of "plant associations" have changed considerably, giving more significance to the "local associations" distinguished by endemic and geographically differentiating species. In most cases the Carpathian or even West-, East- or Southern Carpathian associations became separated from those described from the Alps.

Results and discussion

The syntaxonomic position of the plant communities investigated (in **bold** letters):

RHIZOCARPETEA GEOGRAPHICI Wirth 1972
- *Umbilicarietalia cylindricae* Wirth 1972
  - Rhizocarpion alpicolae Frey 1933
    - **Rhizocarpetum alpicolae** Frey 1923 (Buellio sororiae-Rhizocarpetum geographicae Wirth 1972)
  - Umbilicarion cylindricae Frey 1933
    - **Umbilicarietum cylindricae** Frey 1933

ASPLENIETEA RUPESTRIS Br.-Bl. 1934
Androsacetalia vandelii Br.-Bl. 1926
Silenion lerchenfeldianae Simon 1957
Sileno lerchenfeldianae-Potentilletum haynaldianae Horvat, Pawł. & Walas 1937
THLASPIETEA ROTUNDIFOLII Br.-Bl. 1926
Thlaspietalia rotundifolii Br.-Bl. 1926
Papavero-Thymion pulcherrimi I. Pop 1968
Arabis alpina-Saxifraga aizoides community
Arabis alpina-Delphinium elatum community
Androsacetalia alpinae Br.-Bl. 1926
Festucion pictae Krajina 1933
Doronico carpatici-Festucetum pictae Pócs et Simon nomen nov. (non *Festucetum pictae* auct. roman., non Krajina 1933)
SALICETEA HERBACEAE Br.-Bl. 1947
Salicetalia herbaceae Br.-Bl. 1926
Salicion herbaceae Br.-Bl. 1926
Polytrichetum sexangularae Br.-Bl. 1926
Poo supinae-Cerastietum cerastoidis (Söry 1954) Oberd. 1957
Salicetum herbaceae Br.-Bl. 1913
Soldanello pusillae-Ranunculetum crenati Borza (1931) Boşcaiu 1971
JUNCETEA TRIFIDI Klika et Hadač 1944
Caricetalia curvulae Br.-Bl 1926
Caricion curvulae Br.-Bl. 1925
Primulo-Caricetum curvulae Br.-Bl. 1926 em. Oberd. 1957
Loiselurio-Vaccinion Br.-Bl. 1926
Cetrario-Loiseleurietum Br.-Bl. 1926
Primula minima-Dryas octopetala community
SESLERIETEA ALBICANTIS Br.-Bl. 1948 em. Oberd. 1978
Seslerietalia albicantis Br.-Bl. 1926
Festuco saxatilis-Seslerion bielzii (Pawł. et Walas 1949) Coldea 1984
Dianthus tenuifolius-Festuca dalmatica community
MONTIO-CARDAMINETEA Br.-Bl. et Tx. 1943
Montio-Cardaminetalia Pawł. 1928
Cardamino-Montion Br.-Bl. 1925
Cratoneuretum filicino-commutati (Kuhn 1937) Oberd. 1977
SCHEUCHZERIO-CARICETEA NIGRAE (Nordh. 1937) Tx. 1937
Caricetalia nigrae Koch 1926 em. Nordh. 1937
Caricion nigrae Koch 1926 em. Klika 1934
Carici dacicae-Plantaginetum gentianoidis Coldea 1981
BETULO-ADENOSTYLETEA Br.-Bl. et Tx. 1943
Adenostyletalia Br.-Bl. 1931
Adenostylion alliariae Br.-Bl. 1925
Heracleaetum palmati Puşcaru et al.
Salici-Alnetum viridis Colic et al. 1962

EPILOBIETEA ANGUSTIFOLII Tx. et Prsg. in Tx. 1950
Atropetalia Vlieg. 1937
Epilobion angustifolii (Rübel 1933) Soó 1933
Calamagrostetum arundinaceae subalpinum Csűrös 1962

Description of the studied plant communities

LICHEN DOMINATED COMMUNITIES OF SILICEOUS ROCKS
(RHIZOCARPETEA GEOGRAPHICI Wirth 1972)

Although they occur along the whole range of Carpathians in the montane, subalpine and alpine belts, on open siliceous rocks, hitherto we have relatively few coenological observations on these interesting communities. From Romania, MARDARI (2008) reports *Parmelietum conspersae* and *Umbilicarietum cylindricae* from the Bistrița Mountains in the Eastern Carpathians. We observed such communities in the Romanian Western Carpathians (Apuseni Mountains), Eastern Carpathians (Călimani Mountains) and in the Southern Carpathians, namely: Făgăraş, Retezat, and Parâng mountains. The following relevés represent two associations of this group. The lichens in these and in the other relevés were identified by the late dr. Ö. Szatala (Botany Department of the Hungarian Natural History Museum, BP).

Rhizocarpetum alpicolae Frey 1923 (*Buellio sororiae-Rhizocarpetum geographicae* Wirth 1972)

This community seems to prefer the cooler, longer snow covered rocks and especially the large siliceous boulders on scree slopes. The relevés in table 1 are from the SW exposed scree slopes, just below the Parâng Hut, at 1,750 m altitude, with inclination of 30° as average. The scree is composed of 0.2-2 m (in average 1 m) large gneiss stones and boulders. The size of relevés in each case is 50 × 50 cm (25 dm^2). This association is widespread in the glacial valleys of Parâng Mountains, up to 2,400 m altitude, where it fully covers the larger siliceous boulders and stones of the scree slopes and moraines, giving them a special greenish yellow color (Tab. 1, rel. 1-5).

Tab. 1. *Rhizocarpetum alpicolae* Frey 1923 (rel. 1-5); *Umbilicarietum cylindricae* Frey 1933 (rel. 6)

Aspect	SW	S	SW	S	W	K	S
Slope (°)	35	40	8	10	60		80
Coverage of lichens (%)	95	99	95	90	98		98
Surface (m^2)	0.25	0.25	0.25	0.25	0.25		0.25
Relevé no.	1	2	3	4	5		6
Rhizocarpon alpicola	5	5	3	4	5	V	1
Orthogrimmia donniana	+	1	1	+	1	V	+
Pertusaria lactea	1	3	1	1	3	V	-
Rhizocarpon badioatrum	1	2	2	+	2	V	-
Umbilicaria cylindrica	2	2	2	1	2	V	4
Lecidea auriculata	1	2	3	2	-	IV	-
Lecanora bicincta	-	-	+	+	1	III	1

Lepraria neglecta	-	+	-	+	+	III	-
Umbilicaria polyphylla	-	-	1	2	-	II	-
Cladonia squamosa	-	-	-	+	+	II	-
Cornicularia normoerica	-	-	1	+	-	II	+
Lecidea lithophila	-	+	-	-	+	II	-
Melanelia stygia	+	-	-	1	-	II	-
Deschampsia flexuosa	+	-	-	-	-	I	-
Lecidea conflueans	1	-	-	-	-	I	-
Parmelia omphalodes	-	-	-	+	-	I	-
Rhizocarpon geographicum	-	-	1	-	-	I	-
Umbilicaria crustulosa	-	-	-	2	-	I	+
Alectoria ochroleuca	-	-	-	-	-	-	+
Brodoa intestiniformis	-	-	-	-	-	-	+
Ceratodon purpureus	-	-	-	-	-	-	+
Evernia divaricata	-	-	-	-	-	-	+
Lecanora cenisia	-	-	-	-	-	-	1
Lecanora rupicola	-	-	-	-	-	-	+
Ophioparma ventosa	-	-	-	-	-	-	1
Platismatia glauca	-	-	-	-	-	-	1
Protoparmelia badia	-	-	-	-	-	-	2
Ramalina carpatica	-	-	-	-	-	-	2
Rinodina atrocinerea	-	-	-	-	-	-	+
Sphaerophorus fragilis	-	-	-	-	-	-	+

Place of relevés: 1-5. Parâng Mountains, the glacial valleys, up to 2,400 m. s. l.; 6. Badea rocks, at 1,820 m. s. l.

Umbilicarietum cylindricae Frey 1933

We observed this community on the near vertical, relatively dry, open, often S exposed siliceous cliffs in the Parâng Mountains, from the montane to the alpine belt, at 1,500-2,400 m altitudes. We made only one relevé, in the subalpine belt, at 1,820 m altitude, from the S facing micaschist cliffs of Badea rocks (inclination=80°). The size of sample square was 50 × 50 cm and the coverage of lichens 98%. It alternates with *Rhizocarpetum alpicolae* according to the aspect of the cliffs (Fig. 1).

If we compare this relevé with the table of MARDARI (2008), apart from the dominant species only one other species, *Umbilicaria crustulosa* is identical, but JAMES et al. (1977) distinguishes several "nodums" within this association, according to the environmental conditions (Tab. 1, rel. 6).

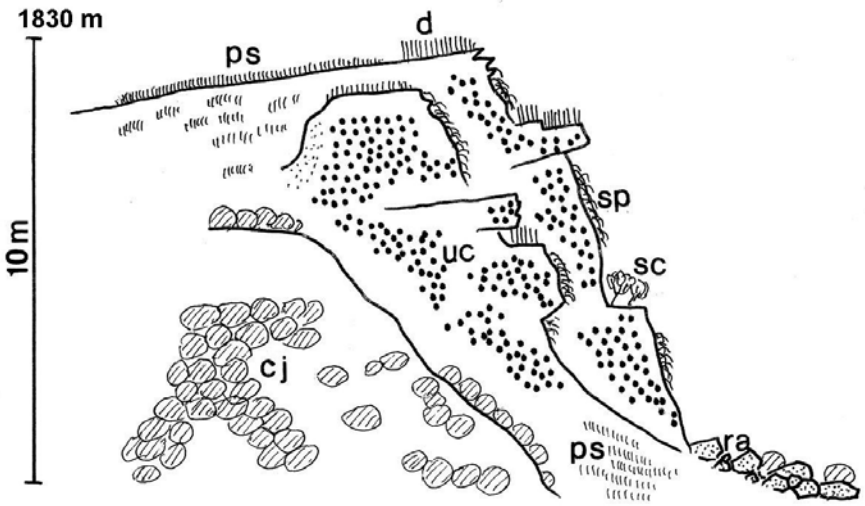

Fig. 1. The vegetation of the schistaceous Badea rocks in the subalpine belt, between the Parâng chalet and Parâng summit

cj: *Campanulo abietinae-Juniperetum*; **d:** *Dianthus tenuifolius-Festuca dalmatica* community; **ps:** *Potentillo-Festucetum airoidis*; **ra:** *Rhizocarpetum alpicolae*; **sc:** *Spiraeetum ulmifoliae*; **sp:** *Sileno lerchenfeldianae-Potentilletum haynaldianae*, type habitat of *Draba simonkaiana*; **uc:** *Umbilicarietum cylindricae* (drawn by T. Pócs)

ROCK CLIFFS (ASPLENIETEA RUPESTRIS Br.-Bl. 1934)

The open chasmophytic vegetation on the subalpine and alpine siliceous cliffs of Parâng Mts. have close relationships with those of the eastern part of Balkan Peninsula, encountered within *Silenion lerchenfeldianae* Simon 1958.

Sileno lerchenfeldianae-Potentilletum haynaldianae Horvat, Pawłowski et Walas 1937

This association was originally described from the Bulgarian Rila [HORVAT & al. 1937] and Pirin mountains [SIMON, 1958]. In the monograph of BUIA & al. (1962) it is not mentioned, but it was published by COLDEA (1991) from the siliceous rocks of Parâng Mountains in a synthetic table, represented by 6 relevés. In 1956 we made 5 relevés on the Badea rocks and in the Mândra circus, represented in Tab. 2 below. It is a "curtain community", dominated by chasmophytic species as *Silene lerchenfeldiana*, *Potentilla haynaldiana*, *Symphyandra wanneri* and *Thymus balcanus*, with III-V constancy value indexes, hanging on the mostly southern exposed siliceous (slate, gneiss, granite) cliffs. The proportion of Dacic-Balcanik species is important (12%) and this is the habitat of *Draba simonkaiana*, endemic to Parâng Mountains and critically endangered, according to DIHORU & NEGREAN (2009). The naturalness of community is relatively high, with only 7% of species referring to disturbance. Its occurrence also in other Southern Carpathian Mts. can be expected (Tab. 2, rel. 1-5).

SCREE SLOPE VEGETATION (THASPIETEA ROTUNDIFOLII Br.-Bl. 1926)

In the Parâng Mountains, this type of vegetation is very well developed on the "slope curtains" below rocky ridges and sometimes on the larger moraines, both on limestone and on siliceous bedrock. The scree slopes are special substrate, easily moving, poor in organic matter, with extreme water conditions. They are practically unsuitable for grazing, therefore their vegetation is well preserved. The extremities stimulate evolution, forcing the species to adjust themselves to these conditions. Many of them develop stolons or tussocks to fix the moving substrate (e. g. *Arabis* spp., *Cerastium* spp.), others store water and nutrients in their body (e.g. *Saxifraga* spp., *Sedum* spp.) or in underground organs (*Aconitum* spp., *Delphinium* spp.). In the monograph of BUIA et al. (1962) these communities were not published, except for "*Festucetum pictae*" from the siliceous screes.

CALCAREOUS SCREE SLOPES (THLASPIETALIA ROTUNDIFOLII Br.-Bl. 1926)

Arabis alpina-Saxifraga aizoides community

These communities belong to alliance *Papavero-Thymion pulcherrimi* I. Pop 1968, which is the Southern Carpathian equivalent of *Thlaspeion rotundifolii* Jenny & Lips 1930 em. Zollitsch 1968.

Arabis alpina-Saxifraga aizoides community is one of the representatives of the pioneer communities on the calcareous screes, especially in the limestone areas of central Parâng Mountains. It commonly occurs on the fine or medium large grained scree slopes. It has a relative high diversity, with only a few species of high constancy (K) value, as: *Saxifraga aizoides*, *Aconitum toxicum*, *Arabis alpina* and *Poa laxa* subsp. *pruinosa.* The Dacic-Balcanik geoelements have a considerable proportion (19%). Concerning the coenoelements, Seslerietea species are represented by 25%, Thlaspietea by 21%, as *Aconitum toxicum*, *Arabis alpina*, and *Delphinium elatum*, Asplenietea by 14% (*Saxifraga aiozoides* and *Pritzelago alpina* subsp *brevifolia*). Comparing this community with other Romanian Thlaspietalia associations, perhaps it is closest to *Acino-Galietum anisophylli* Beldie 1967. The occurrence of this new community can be expected also from other calcareous areas of the Southern Carpathians (Tab. 2, rel. 6-10).

Arabis alpina-Delphinium elatum community

It is a community of the coarse limestone scree, with some altiherbosa character. We discovered *Aubrietia columnae* subsp. *croatica* when made relevés from this community in 1956. In Parâng Mountains could not find *Linaria alpina*, which is so characteristic in similar habitats of Piatra Craiului and Bucegi Mountains. Spectacular species are in the Parâng Mountains the always present *Delphinium elatum* and *Aconitum tauricum.* Significant is the high proportion of Dacic-Balcanik geoelements (*Cerastium arvense* subsp. *molle*, *Campanula serrata*, *Alyssum repens*) and that of the Thlaspietea coenoelements (35%; for instance *Arabis alpina*, *Delphinium elatum*, *Aubrietia columnae* subsp. *croatica*). The naturalness of the community is demonstrated by the high proportion of protected and natural accessory species (Tab. 2, rel. 11-15).

Tab. 2. *Sileno lerchenfeldianae-Potentilletum haynaldianae* Horvat, Pawłowski et Walas 1937 (rel. 1-5); *Arabis alpina-Saxifraga aizoides* community (rel. 6-10); *Arabis alpina-Delphinium elatum* community (rel. 11-15)

Aspect	S-SE	S	S	SE	SE		SE	E-SE	NE	N	N		E	SE	SE	E	E	
Slope (°)	90	90	90	90	90		45	45	35	25	30		40	45	45	35	35	
Coverage of herb layer (%)	90	70	70	80	80	K	20	30	40	30	30	K	10 - 20	20	20	20	10	K
Coverage of moss layer (%)	20	10	5	20	15		-	-	5	5	15		0.5	0.5	0.5	0.5	0.5	
Height of herb layer (cm)	-	-	-	-	-		40	35	20	25	10		40	35	20	25	10	
Surface (m^2)	4	4	4	4	4		25	25	25	25	25		25	25	25	25	25	
Relevé no.	1	2	3	4	5		6	7	8	9	10		11	12	13	14	15	
Festuca airoides	1	1	2	2	2	V	-	-	+	-	-	I	-	-	-	-	-	-
Juncus trifidus	3	3	3	2	1	V	-	-	-	-	-	-	-	-	-	-	-	-
Luzula spicata	1	1	+	1	1	V	-	-	-	-	-	-	-	-	-	-	-	-
Potentilla haynaldiana	4	2	1	3	2	V	-	-	-	-	-	-	-	-	-	-	-	-
Thymus balcanus	+	1	1	+	+	V	-	+	1	-	-	II	-	-	-	-	-	-
Dianthus tenuifolius	2	1	2	-	+	IV	-	-	-	-	-	-	-	-	-	-	-	-
Silene lerchenfeldiana	-	2	3	+	+	IV	-	-	-	-	-	-	-	-	-	-	-	-
Saxifraga aizoides	-	-	-	-	-	-	+	+	1	2	2	V	+	+	-	1	+	IV
Aconitum toxicum	-	-	-	-	-	-	+	1	+	+	-	IV	-	-	-	-	-	-
Arabis alpina	-	-	-	-	-	-	+	1	-	+	+	IV	+	+	1	1	+	V
Aconitum tauricum	-	-	-	-	-	-	-	-	-	-	-	-	1	+	1	+	+	V
Cerastium arvense subsp. molle	-	-	-	-	-	-	-	-	-	-	-	-	+	1	1	1	1	V
Poa laxa subsp. pruinosa	-	-	-	-	-	-	+	+	-	1	+	IV	-	-	-	-	-	-
Acinos alpinus	-	-	-	-	-	-	+	+	-	+	-	III	+	+	+	-	-	III
Campanula serrata	-	-	-	-	-	-	+	+	+	-	-	III	+	+	+	-	-	III
Galium anisophyllum	-	-	-	-	-	-	+	1	+	-	-	III	1	+	1	+	1	V
Rhodiola rosea	-	-	-	-	-	-	-	-	-	-	-	.	+	-	-	+	+	III
Poa alpina	-	-	-	-	-	-	+	+	-	-	+	III	-	-	-	-	-	-
Poa alpina f. vivipara	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	II
Silene pusilla	-	-	-	-	-	-	+	+	-	1	-	III	-	-	-	-	-	-
Biscutella laevigata	-	-	-	-	-	-	+	+	-	-	-	II	-	+	+	-	-	II
Cerastium arvense subsp.	-	-	-	-	-	-	1	1	-	-	-	II	-	-	-	-	-	-

lerchenfeldianum																		
Delphinium elatum	-	-	-	-	-	-	2	2	-	-	-	II	2	2	2	2	2	V
Aubrietia columnae subsp. croatica	-	-	-	-	-	-	-	-	-	-	-	-	1	+	-	+	+	IV
Campanula rotundifolia subsp. polymorpha	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	II
Geranium sylvaticum subsp. caeruleatum	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1	-	II
Poa minor	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	II
Euphrasia salisburgensis	-	-	-	-	-	-	-	+	+	-	-	II						
Pritzelago alpina subsp. brevicaulis	-	-	-	-	-	-	-	-	-	1	+	II	-	-	-	-	-	-
Ranunculus montanus subsp. pseudomontanus	-	-	-	-	-	-	-	-	+	+	-	II	-	-	-	-	-	-
Saxifraga androsacea	-	-	-	-	-	-	-	-	-	+	+	II	-	-	-	-	-	-
Saxifraga moschata	-	-	-	-	-	-	-	-	-	+	+	II	-	-	-	-	-	-
Saxifraga rotundifolia	-	-	-	-	-	-	-	-	-	+	+	II	-	-	-	-	-	-
Scrophularia scopolii	-	-	-	-	-	-	+	+	-	-	-	II	+	+	+	+	-	IV
Seseli libanotis	-	-	-	-	-	-	+	+	-	-	-	II	-	+	+	+	1	IV
Urtica dioica	-	-	-	-	-	-	+	+	-	-	-	II	-	-	-	-	-	-
Agrostis rupestris	-	+	2	-	+	III	-	-	+	-	-	I	-	-	-	-	-	-
Alyssum repens	-	-	-	-	-	-	-	+	-	-	-	I	+	-	+	-	-	II
Anthoxanthum odoratum	-	-	-	-	-	-	-	-	1	-	-	I	-	-	-	-	-	-
Bellardiochloa violacea	3	1	2	-	-	III	-	-	2	-	-	I	-	-	-	-	-	-
Carex sempervirens	-	-	-	-	-	-	-	-	1	-	-	I	-	-	-	-	-	-
Cerastium alpinum	-	-	-	-	-	-	-	-	1	-	-	I	-	-	-	-	-	-
Doronicum carpaticum	-	-	-	-	-	-	-	-	-	1	-	I	-	-	-	-	-	-
Festuca picta	-	-	-	-	-	-	-	-	2	-	-	I	-	-	-	-	-	-
Galium mollugo	-	-	-	-	-	-	-	+	-	-	-	I	-	-	-	-	-	-
Geum montanum	-	-	-	-	-	-	-	-	1	-	-	I	-	-	-	-	-	-
Ligusticum mutellina	-	-	-	-	-	-	-	+	-	-	-	I	-	-	-	-	-	-
Minuartia verna subsp. collina	-	-	-	-	-	-	-	-	+	-	-	I	-	-	-	-	-	-
Myosotis alpestris	-	-	-	-	-	-	-	-	-	+	-	I	-	-	-	-	-	-
Oxyria digyna	-	-	-	-	-	-	-	-	-	2	+	II	-	-	-	-	-	-
Draba simonkaiana	-	1	-	1	+	III	-	-	-	-	-	-	-	-	-	-	-	-

Poa nemoralis var. agrostoides	+	2	2	-	-	III	-	-	+	-	-	I	-	-	-	-	-	-
Potentilla aurea subsp. chrysocraspeda	-	-	-	-	-	-	-	-	+	-	-	I	-	-	-	-	-	-
Rhododendron myrtifolium	-	-	-	-	-	-	-	-	+	-	-	I	-	-	-	-	-	-
Sesleria bielzii	-	-	-	-	-	-	-	-	1	-	-	I	-	-	-	-	-	-
Soldanella pusilla	-	-	-	-	-	-	-	-	-	+	-	I	-	-	-	-	-	-
Taraxacum alpinum	-	-	-	-	-	-	-	-	-	+	-	I	-	-	-	-	-	-
Veronica aphylla	-	-	-	-	-	-	-	-	-	-	+	I	-	-	-	-	-	-
Viola biflora	-	-	-	-	-	-	+	-	-	-	-	I	-	+	-	+	+	III
Viola declinata	-	-	-	-	-	-	-	-	+	-	-	I	-	-	-	-	-	-
Sedum alpestre	+	+	+	-	-	III	-	-	-	-	-	-	-	-	-	-	-	-
Symphyandra wanneri	3	3	1	-	-	III	-	-	-	-	-	-	-	-	-	-	-	-
Carex ornithopoda	+	+	-	-	-	II	-	-	-	-	-	-	-	-	-	-	-	-
Festuca dalmatica	2	1	-	-	-	II	-	-	-	-	-	-	-	-	-	-	-	-
Sedum atratum	-	+	+	-	-	II	-	-	-	-	-	-	-	+	+	-	-	II
Veronica baumgartenii	-	-	-	+	+	II	-	-	-	-	-	-	-	-	-	-	-	-
Allium ericetorum subsp. ericetorum	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Allium victorialis	-	-	-	1	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Cystopteris fragilis	-	+	-	-	-	I	+	+	-	+	-	III	-	+	+	-	-	II
Genista tinctoria var. oligosperma	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Juniperus commnis subsp. alpina	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Primula minima	-	-	-	+	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Saxifraga adscendens	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Saxifraga bryoides	-	-	-	1	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Saxifraga paniculata var. brevifolia	1	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Saxifraga pedemontana subsp. cymosa	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
BRYOPHYTA & MARCHANTIOPHYTA																		
Orthogrimmia donniana	+	+	2	2	1	V	-	-	-	-	-	-	-	-	-	-	-	-
Polytrichum piliferum	1	-	1	1	+	IV	-	-	-	-	-	-	-	-	-	-	-	-
Polytricum juniperinum	1	2	+	-	-	III	-	-	-	-	-	-	-	-	-	-	-	-

Radula lindenbergiana	-	+	+	-	-	II	-	-	-	-	-	-	-	-	-	-	-	-
Amphidium mougeotii	-	-	+	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Pohlia elongata	-	-	1	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Rhabdoweisia fugax	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Rhytidium rugosum	-	1	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Polytrichastrum alpinum	-	-	-	-	-	-	-	-	+	-	-	I	-	-	-	-	-	-
Preissia quadrata	-	-	-	-	-	-	+	-	-	-	-	I	+	+	-	+	-	III
LYCHENOPHYTA																		
Cetraria islandica	-	+	+	-	-	II	-	-	-	-	-	-	-	-	-	-	-	-
Parmelia sulcata	-	1	+	-	-	II	-	-	-	-	-	-	-	-	-	-	-	-
Cladonia pyxidata	-	-	-	2	+	II	-	-	-	-	+	I	-	-	-	-	-	-
Cladonia gracilis	1	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Cladonia squamosa	2	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-
Sphaerophorus fragilis	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-	-

Place of relevés: 1. Badea rocks, 1,830 m; 2. Badea rocks, 1,830 m, 1870 m; 3. Badea rocks, 1,860 m; 4-5. Groapa Mândrii, median ridge, 2,060 m; 6. Coasta lui Rusu, 2,130 m.s.l., on fine grained limestone scree; 7. Coasta lui Rusu, 2,140 m.s.l., on fine grained limestone scree; 8. Coasta lui Rusu, 2,180 m.s.l., on medium size serpentine scree; 9. Coasta lui Rusu, 2,080 m.s.l.; 10. Coasta lui Rusu, 2,130 m.s.l., on fine grained limestone scree; 11. Coasta lui Rusu, 2,165 m.s.l., coarse limestone scree; 12. ibid., 2,130 m.s.l.; 13. ibid., 2,130 m; 14. ibid., 2,080 m; 15. ibid., 2,080 m.s.l.

SILICEOUS SCREE SLOPES (ANDROSACETALIA ALPINAE Br.-Bl. 1926)

Widespread plant communities on the siliceous scree slopes of the subalpine and alpine belt of Carpathians, well summarized by COLDEA (1991) under the alliances of *Veronicion baumgartenii* Coldea and *Festucion pictae* Krajina. The first alliance units the associations of mobile screes in the Eastern and Southern Carpathians, with a number of endemic and Daco-Balcanik species, while the second units the association of the semi-fixed, fine grained siliceous screes of the Carpathians.

Doronico carpatici-Festucetum pictae Pócs et Simon nomen nov.
(=*Festucetum pictae* auct. roman., non Krajina 1933)

According to our opinion the Transylvanian association differs enough from the *Festucetum pictae* Krajina 1933, originally described from the Tatra Mountains, to be described as a separate association under the new name of *Doronico carpatici-Festucetum pictae*. The Eastern and Southern Carpathian association has several Daco-Balcanik geoelements missing from the Northern Carpathians, as: *Cerastium transsilvanicum*, *Doronicum carpaticum*, *Rhododendron myrtifolium*, and *Veronica baumgartenii.* It can be discussed whether the association should be classified on ecological basis into the alliance of *Festucion pictae* or on the basis of Daco-Balcanik geoelements better under *Veronicion baumgartenii.* The association is widespread in the Rodnei, Făgăraş, and Retezat Mountains [COLDEA, 1991] and is mentioned by BUIA (1962) from the Parâng Mountains without detailed description. DOMIN (1933) described an association also under the name of *Festucetum pictae* from the Bucegi Mountains, which, due to its calcareous substrate, differs from the above. Geographically it is transitional between the Tatras community and those described from the Bulgarian mountains by SIMON (1958) and by HORVAT & al. (1974). In the scree fixing succession it is transitional towards the *Caricion curvulae* mats. The association contains a good number of species worth to be protected (e.g. *Sedum atratum*, *Leucanthemopsis alpina*, *Poa laxa*, *Gentiana punctata*, *Cerastium transsylvanicum*) (Tab. 3, rel. 1-5).

Tab. 3. Ass. *Doronico carpatici-Festucetum pictae* Pócs et Simon nomen nov. (rel. 1-5); *Polytrichetum sexangularis* Br.-Bl. 1926 (rel. 6-7); *Poo supinae-Cerastietum cerastoidis* (Söry 1954) Oberd. 1957 (rel. 8-10); *Salicetum herbaceae* Br.-Bl. 1926 (rel. 11-13); *Soldanello pusillae-Ranunculetum crenati* Borza (1931) Boşcaiu 1971 (vel aff.) (rel. 14-16)

Aspect	**NW**	**E-NE**	**NW**	**S**	**SE**		**S**	**W**	**-**	**W**	**W**	**NE**	**NE**	**NE**	**N**	**N**	**N**
Slope (°)	**35**	**45**	**40**	**35**	**30**		**40**	**15**	**-**	**30**	**30**	**4**	**8**	**3**	**40**	**30**	**30**
Coverage of herb layer (%)	**10-20**	**60**	**60**	**10**	**15**	**K**	**5**	**10**	**8**	**30**	**30**	**70**	**60**	**60**	**70**	**60**	**60**
Coverage of moss layer (%)	**5-10**	**10-20**	**10**	**-**	**-**		**95**	**90**	**95**	**70**	**70**	**10**	**30**	**30**	**70**	**80**	**80**
Surface (m^2)	**25**	**25**	**25**	**25**	**25**		**1**	**4**	**0.2**	**1**	**1**	**1**	**1**	**1**	**?**	**?**	**?**
Relevé no.	**1**	**2**	**3**	**4**	**5**		**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Sedum atratum	+	+	+	+	+	V	-	-	-	-	-	-	-	-	-	-	-
Gnaphalium supinum	-	+	+	+	+	IV	-	-	-	-	-	-	-	-	+	-	-
Leucanthemopsis alpina	-	+	+	1	1	IV	-	-	-	-	-	-	-	-	+	+	1
Ligusticum mutellina	1	1	1	-	1	IV	-	-	-	-	-	-	-	-	1	1	1
Anthoxantum odoratum	+	2	1	-	+	IV	-	-	-	-	-	-	-	-	-	-	-
Poa supina	-	-	-	-	-	-	+	2	4	4	+	+	-	-	-	-	-
Salix herbacea	-	-	-	-	-	-	-	-	-	-	-	3	4	4	-	-	-
Poa laxa subsp. pruinosa	+	-	-	2	1	III	-	-	-	-	-	-	-	-	-	-	-
Campanula abietina	+	+	+	-	-	III	-	-	-	-	-	-	-	-	-	-	-
Doronicum carpaticum	1	1	1	-	-	III	-	-	-	-	-	-	-	-	+	-	-
Festuca picta	2	3	3	-	-	III	-	-	-	-	-	-	-	-	-	1	1
Geum montanum	+	1	+	-	-	III	-	-	-	-	-	-	-	-	-	-	+
Juncus trifidus	1	+	+	-	-	III	-	-	-	-	-	-	-	-	-	-	-
Luzula alpinopilosa subsp. obscura	-	+	+	-	+	III	-	-	-	-	-	-	-	-	4	4	3
Ranunculus montanus subsp. pseudomontanus	+	+	+	-	-	III	-	-	-	-	-	-	-	-	-	+	-
Rhododendron myrtifolium	+	+	+	-	-	III	-	-	-	-	-	-	-	-	+	-	-
Soldanella pusilla	+	1	+	-	-	III	-	1	-	-	-	-	-	-	2	2	2
Veratrum album	2	1	+	-	-	III	-	-	-	-	-	-	-	-	-	-	+

Aconitum toxicum	+	+	-	-	-	II	-	-	-	-	-	-	-	-	-	-	-
Arenaria biflora	-	-	-	+	1	II	-	-	-	-	-	-	-	-	-	-	-
Athyrium distentifolium	+	+	-	-	-	II	-	-	-	-	-	-	-	-	-	-	-
Campanula alpina	-	+	+	-	-	II	-	-	-	-	-	-	-	-	1	-	-
Carex pyrenaica	-	+	-	-	+	II	-	-	-	-	-	-	-	-	-	-	-
Festuca airoides	-	-	+	-	+	II	-	-	-	-	-	-	-	-	-	-	-
Gentiana punctata	+	+	-	-	-	II	-	-	-	-	-	-	-	-	-	-	-
Homogyne alpina	+	+	-	-	-	II	-	-	-	-	-	-	-	-	-	-	-
Oreochloa disticha	-	+	+	-	-	II	-	-	-	-	-	-	1	+	+	-	-
Pedicularis verticillata	-	+	+	-	-	II	-	-	-	-	-	-	-	-	+	-	-
Potentilla aurea subsp. chrysocraspeda	-	-	+	+	-	II	-	-	-	-	-	-	-	-	-	-	-
Primula minima	-	+	+	-	-	II	-	-	-	+	+	3	3	3	+	-	-
Ranunculus crenatus	-	+	+	-	-	II	-	-	-	-	-	-	-	-	-	+	2
Veronica alpina	-	+	+	-	-	II	-	-	-	-	-	-	-	-	-	-	-
Achillea distans subsp. stricta	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Agrostis rupestris	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Alchemilla glabra	-	1	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Campanula serrata	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Carex curvula	-	-	-	+	-	I	-	-	-	-	-	+	1	+	-	-	-
Cardamine resedifolia	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Cerastium transsylvanicum	-	-	1	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Cerastium cerastoides	-	-	-	+	-	I	+	+	2	2	2	+	-	-	-	1	+
Dryopteris filix-mas	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Hieracium alpinum	-	+	-	-	-	I	-	-	-	-	-	-	-	-	+	-	-
Luzula luzuloides var. cuprina	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Luzula spicata	-	-	-	+	-	I	-	-	-	-	-	-	-	-	-	-	-
Oxyria digyna	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	+	-
Polygonum viviparum	-	-	+	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Saxifraga carpathica	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Saxifraga moschata	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Saxifraga stellaris	-	-	-	+	-	I	-	+	-	-	-	-	-	-	-	+	-

Senecio subalpinus	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Silene vulgaris	-	+	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Taraxacum nigricans	-	-	+	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Vaccinium myrtillus	-	-	+	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Veronica baumgartenii	-	-	+	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Viola biflora	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Cerastium lanatum	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Deschampsia cespitosa	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
Leucanthemopsis alpinus	-	-	-	-	-	-	-	+	-	+	+	2	1	1	-	-	-
Persicaria vivipara	-	-	-	-	-	-	-	-	2	-	-	1	1	-	-	-	-
Plantago gentianoides	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
Saxifraga bryoides	-	-	-	-	-	-	-	-	-	+	1	1	1	-	-	-	-
Sedum alpestre	-	-	-	-	-	-	-	+	-	+	+	-	-	-	-	-	-
Silene acaulis	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
Taraxacum fontanum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	1
Anthemis carpatica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Phyteuma confusum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Poa media	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Sesleria bielzii	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
BRYOPHYTA																	
Polytrichum piliferum	+	-	+	+	+	IV	+	-	-	3	3	-	-	-	-	-	-
Polytrichastrum alpinum	-	-	-	+	1	II	-	-	-	-	-	1	2	3	3	-	-
Polytrichastrum sexangulare	-	-	-	+	+	II	5	5	1	1	1	-	-	-	-	-	-
Bartramia ithyphylla	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Bazzania trilobata	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Heterocladium dimorphum	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Kiaeria starkei	+	-	-	-	-	I	-	-	-	-	-	-	-	-	1	-	-
Polytrichum juniperinum	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-
Sanionia uncinata	-	-	+	-	-	I	-	-	-	-	-	-	-	-	+	-	-
Weissia wimmeriana	+	-	-	-	-	I	-	-	-	-	-	-	-	-	-	-	-

Bryum spp.	-	-	-	-	-	-	+	-	3	-	-	-	-	-	-	-	-
Paraleucobryum enerve	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
Warnstorfia exannulata	-	-	-	-	-	-	1	-	-	1	1	-	-	-	-	4	5
Anthelia juratzkana	-	-	-	-	-	-	-	+	4	-	-	-	-	-	-	-	-
Dicranum scoparium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Jungermannia sphaerocarpa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Hylocomium proliferum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Lophozia sudetica	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
Rhytidiadelphus triqueter	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Scapania undulata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
LICHENOPHYTA																	
Cetraria islandica	-	-	-	-	-	-	-	-	-	-	-	-	1	1	-	-	-
Cladonia rangiferina	-	-	-	-	-	-	+	-	-	-	-	+	-	+	-	-	-
Solorina crocea	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-
Stereocaulon alpinum	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
Thamnolia vermicularis	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-

Place of relevés: 1. Groapa Mândrii, 2,030 m.s.l., on coarse scree; 2. ibid., 2,000 m, medium sized scree; 3. ibid., 2,040 m.s.l., medium sized scree; 4. Piatra Tăiată, 2,160 m.s.l., fine grained scree; 5. Setea Mare - Piatra Tăiată, 2,340 m.s.l., fine graine scree; 6. Mândra-ridge, 2,500 m.s.l.; 7. ibid., 2,500 m.s.l.; 8. Cârja-circus, 2,100 m.s.l.; 9. Piatra Tăiata, 2,250 m.s.l.; 10. Mândra Ridge, 2,500 m.s.l.; 11. Vf. Mândra, 2,520 m.s.l.; 12. ibid., 2,525m.s.l.; 13. ibid., 2,525 m.s.l.; 14. N slope of Cârja-summit (2,400 m.s.l.), siliceous cliffs; 15. Groapa Mândrii, 2,030 m.s.l., cliffs of median ridge; 16. ibid.

SNOW VALLEYS (SALICETEA HERBACEAE Br.-Bl. 1926)

These pioneer communities (Fig. 2) develop in the alpine belt above the altitude of 2,000 m, in depressions of summits and in N exposed slopes or valleys where the snow melts only during summertime. They occur often along the eternal snow patches (firn). The habitat is usually wet from the melting water and has a lifespan of only 2-3 months per year. The communities living in this habitat are poor in species, have a considerable moss layer and their herb layer is often stunted. We studied in the Parâng Mountains three associations belonging to this group, namely *Polytrichetum sexangularis*, *Salicetum herbaceae*, and *Soldanello pusillae-Ranunculetum crenati*.

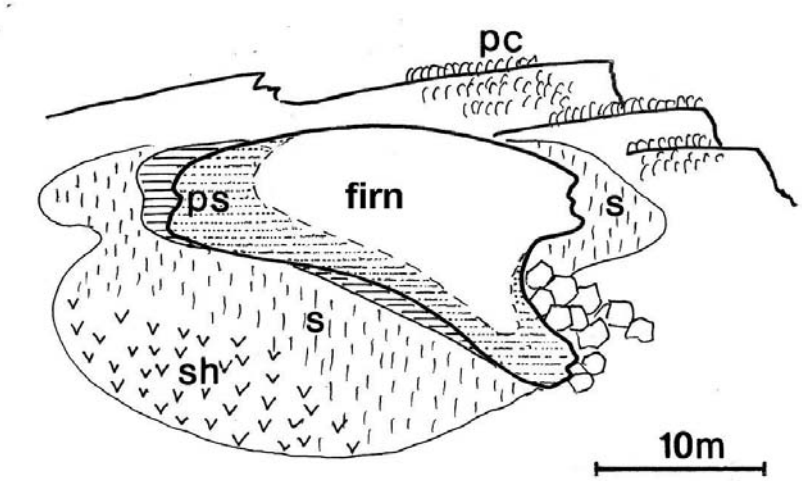

Fig. 2. Vegetation of a snow valley at 2,500 m altitude, on the W side of Mândra summit, on the 31st July, 1956
pc: Primulo-Caricetum curvulae; **ps:** Polytrichetum sexangularis; **s:** Poo supinae-Cerastietum cerastoidis; **sh:** Salicetum herbaceae (drawn by T. Pócs)

Polytrichetum sexangularis Br.-Bl. 1926

This association is located around the eternal snow patches of Cârja, Mândra and Tăiata summits, on fine, humus rich, muddy soil. Along the prevailing mosses, the occurrence of *Cerastium cerastoides* and at places the dominancy of *Poa supina* is striking. Among the geoelements, the European-alpine and Alpine-arctic types dominate (86%). Among the coenotaxa, the *Salicetea herbaceae* are prominent (64%). A number of species are worth to be protected, like *Cerastium cerastoides*, *Saxifraga bryoides* and *Soldanella pusilla.* The association is well known from the Southern Carpathians, but new to the Parâng Mountains (Tab. 3, rel. 6-7).

Poo supinae-Cerastietum cerastoidis (Söry 1954) Oberd. 1957

After *Polytrichetum sexangularis* this community has the longest snow cover, surrounding the snowfields. There is no sharp limit between these two communities. The succession reaches to *Salicetum herbaceae* only at places which are longer snowfree. It is a very poor community, consisting of phanerogams with the dominance of *Poa supina* and/or *Cerastium cerastioides*, which have relatively short active life cycles. According to COLDEA (1991) this chiono-hygrophilous community is known from the Rodnei, Retezat, Țarcu and Godeanu Mts., and new to Parâng Mts. (Tab. 3, rel. 8-10).

Salicetum herbaceae Br.-Bl. 1926

We observed the dwarf stands of this tundra like community on the Mândra summit at 2,520 m altitude. The snow cover in this habitat lasts a bit shorter time than the previous association. On the humus rich, fine, acidic soil, *Salix herbacea* is the dominant species, while the codominants are *Primula minima*, *Leucanthemopsis alpinus* and *Persicaria vivipara*; the moss layer is usually formed by *Polytrichastrum alpinum*. Among the geoelements, the circumboreal type is dominant (51%), while among the coenotaxons the Salicatea herbaceae and Caricetea curvulae are prevailing. *Plantago gentianoides*, *Primula minima*, *Saxifraga bryoides* and *Silene acaulis* are among the species worth to be protected. The association is known from the Rodnei, Bucegi, Făgăraş and Godeanu-Țarcu Mts. in Romania, being new to the Parâng Mts. (Tab. 3, rel. 11-13).

Soldanello pusillae-Ranunculetum crenati Borza (1931) Boşcaiu 1971 (vel aff.)

This plant community thrives on the northerly exposed rocks and slopes, with fine grained, wet and humus rich rubble. The coverage of herb and moss layer is considerable. Conspicuous species are: *Luzula alpinopilosa*, *Soldanella pusilla*, *Leucanthemopsis alpina*, *Ligusticum mutellina* and *Ranunculus crenatus*. In the moss layer *Polytrichastrum alpinum* and *Warnstorfia exannulata* are dominating. The circumboreal species are in majority, with their 38%, but the 23% Dacic-Balcanik species, like *Doronicum carpaticum*, *Poa media*, *Ranunculus crenatus*, *Rhododendron myrtifolium*, *Sesleria bielzii* underline the independent character of the Southern Carpathian association, hitherto known only from Godeanu-Țarcu and Făgăraş Mts. Among coenotaxa, the Caricetalia curvulae species with 26%, Vaccinio-Piceetalia with 23% and Thlaspietea species with 21% are in majority. The Parâng community slightly differs from the stands described from Țarcu and Făgăraş Mts. by the lack of *Plantago gentianoides* and *Gnaphalium supinum* and by the more prominent occurrence of *Luzula alpinopilosa* (Tab. 3, rel. 14-16).

MATS OF ARCTIC-ALPINE CHARACTER (JUNCETEA TRIFIDI Klika et Hadač 1944)

Communities of the wind exposed ridges and summits on siliceous ground above 2,000 m in the Carpathians and the Balkan Peninsula formed by pillow grasses and herbs and latticed dwarf shrubs.

Primulo minimae-Caricetum curvulae Br.-Bl. 1926

These climazonal alpine mats remained quite well in their original, natural state, except for the sides of regularly used tourist paths. Moderate grazing seems not causing serious degradation in these mats. It is widespread with some geographical variations all over the European Alpine-Carpathian-Balkanik mountain systems, on soils of siliceous bedrocks. In Parâng Mts. this is the most widespread alpine community. It was already studied by BUIA & al. (1962), represented by 15 relevés containing 34 species. We think that it will be a useful supplement to these our 10 further relevés with 50 species. The overall dominant species is *Carex curvula*, accompanied mostly by: *Agrostis rupestris*, *Festuca airoides*, *Geum montanum*, *Hieracium alpinum* and *Potentilla aurea* subsp. *chrysocraspeda.* Characteristic is the dominance of circumboreal geoelements, mostly from the coenotaxa of Caricetalia curvulae and of Vaccinio-Piceetalia, with the majority of specialists and competitors. *Campanula alpina*, *Cerastium cerastoides*, *Saxifraga bryoides*, *Sedum alpestre*, *Veronica baumgartenii* and *Pulsatilla alba* are partly unical species, worth to be protected (Tab. 4, rel. 1-10).

Primula minima-Dryas octopetala community

We observed this interesting association on the snow protected granitic layers of the highest summits. *Dryas octopetala* is a real edificator, community builder. It obviously belongs to Loiseleurio-Vaccinion alliance, without some of its species, but accompanied by several elements not occurring in Cetrario-Loiseleurietum, like *Carex curvula*, *Luzula spicata*, *Oreochloa disticha* and *Pedicularis verticillata.* It is different from *Dryadetum octopetalae* described by RÜBEL (1912) from the Bernina Alps, as the latter has a number of calciphilous species and the Parâng community has some Dacic elements, like *Cerastium transsylvanicum*. Observing this community from more places maybe justifies to describe it as a new association (Tab. 4, rel. 11).

Cetrario-Loiseleurietum Br.-Bl. 1926

This association is related to the previous one, although belongs to Loiselurio-Vaccinion alliance. It develops usually on the finely granulated gravely soils in northern exposure, usually under longer snow protection. It is dominated by circumboreal geoelements, like *Loiseleuria procumbens*, *Vaccinium gaultheroides* and *V. vitis-idaea.* Among the coenotaxa, those of Androsacetalia and some Salicetea herbaceae elements are dominant. A number of species are unicate and worth protection, like *Eritrichum nanum*, *Leucanthemopsis alpina*, *Huperzia selago* and *Campanula alpina.* BUIA & al. (1962) published 18 relevés from the altitude of 1,800-2,100 m. We think that our 5 relevés from 2,150-2,400 m complete the picture (Tab. 4, rel. 12-16).

Tab. 4. *Primulo minimae-Caricetum curvulae* Br.-Bl. 1926 (rel. 1-10); *Primula minima-Dryas octopetala* community (rel. 11); *Cetrario-Loiseleurietum* Br.-Bl. 1926 (rel. 12-16)

Aspect	**W-NW**	**W-NW**	**W-SW**	**W-NW**	**NW**		**NE-N**	**S**	**NE**	**NE**	**N-NE**	**N-NW**	
Slope (°)	**3**	**30-40**	**3-4**	**2-15**	**10**		**15-20**	**10**	**20**	**20**	**20**	**25**	
Coverage of herb layer (%)	**70**	**80**	**90**	**85**	**90**	**K**	**95**	**90**	**50**	**70**	**70**	**65**	**K**
Coverage of moss layer (%)	**20**	**15**	**15**	**5**	**20**		**3**	**10**	**20**	**20**	**20**	**20**	
Surface (m^2)	**4**	**1**	**1**	**1**	**1**		**1**	**1**	**1**	**1**	**1**	**1**	
Relevé no.	**1**	**2-3**	**4-5**	**6-8**	**9-10**		**11**	**12**	**13**	**14**	**15**	**16**	
Campanula alpina	1	+	+	1	1	V	+	1	-	-	-	-	II
Carex curvula	4	4	4	4	4	V	1	-	-	-	-	-	-
Hieracium alpinum	1	1	1	1	+	V	+	+	+	+	+	+	V
Phyteuma confusum	1	1	1	1	+	V	-	-	+	+	+	+	IV
Potentilla aurea subsp. chrysocraspeda	1	1	1	+	1	V	-	-	-	-	-	-	-
Primula minima	2	2	1	2	1	V	1	+	2	+	1	1	V
Festuca airoides	1	1	+	1	-	IV	+	+	1	1	1	1	V
Homogyne alpina	-	1	2	+	+	IV	-	-	-	-	-	-	-
Soldanella pusilla	+	-	+	1	+	IV	-	-	-	-	-	-	-
Vaccinium gaultherioides	+	-	+	1	+	IV	-	1	+	+	4	4	V
Vaccinium vitis-idaea	+	+	1	1	-	IV	-	-	1	1	1	1	IV
Dryas octopetala	-	-	-	-	-	-	5	-	-	-	-	-	-
Eritrichium nanum	-	-	-	-	-	-	-	-	1	+	+	+	IV
Leucantheopsis alpina	-	1	1	-	1	III	-	-	-	-	-	-	-
Omalotheca supina	+	-	+	+	-	III	-	-	-	-	-	-	-
Oreochloa disticha	-	1	-	1	-	II	+	-	-	-	-	-	-
Rhdodendron myrtifolium	+	-	+	1	-	III	-	-	+	+	-	+	III
Juncus trifidus	-	+	+	-	-	II	-	+	1	-	-	1	III
Ligusticum mutellina	-	+	+	-	-	II	-	-	-	-	-	-	-
Luzula spicata subsp. mutabilis	-	+	+	-	-	II	+	-	-	-	-	-	-
Persicaria vivipara	-	-	-	1	2	II	1	+	-	-	-	-	I
Salix herbacea	-	-	-	1	1	II	+	2	-	-	-	-	-

Agrostis rupestris	-	1	-	-	-	I	-	-	-	-	-	-	-
Avenastrum versicolor	-	-	-	+	-	I	-	-	-	-	-	-	-
Carex atrata	-	-	-	1	-	I	1	-	+	-	-	1	II
Cerastium cerastoides	-	-	-	1	-	I	-	-	-	-	-	-	-
Euphrasia minima	-	-	+	-	-	I	-	-	-	-	+	+	II
Geum montanum	-	-	1	-	-	I	-	-	-	-	-	-	-
Juniperus communis subsp. alpina	+	-	-	-	-	I	-	-	-	-	-	-	-
Loiseleuria procumbens	-	-	-	1	-	I	-	4	3	4	4	3	V
Luzula alpinopilosa	-	-	-	1	-	I	-	-	-	-	-	-	-
Poa annua subsp. supina	-	-	+	-	-	I	-	-	-	-	-	-	-
Pulsatilla alba	+	-	-	-	-	I	-	-	-	-	-	-	-
Saxifraga bryoides	-	-	-	+	-	I	-	-	-	-	-	-	-
Sedum alpestre	-	-	-	+	-	I	-	-	-	-	-	-	-
Silene acaulis	-	-	-	+	-	I	-	-	-	-	-	-	-
Vaccinium myrtillus	1	-	-	-	-	I	-	-	-	1	-	-	I
Veronica baumgartenii	-	+	-	-	-	I	-	-	-	-	-	-	-
Luzula sudetica	-	-	-	-	-	-	-	-	+	-	+	1	III
Cerastium transsylvanicum	-	-	-	-	-	-	+	-	-	-	-	-	I
Leucanthemopsis alpina	-	-	-	-	-	-	-	-	1	+	-	+	II
Agrostis rupestris	-	-	-	-	-	-	-	-	-	1	-	-	I
Pedicularis verticillata	-	-	-	-	-	-	+	-	-	-	-	-	I
Huperzia selago	-	-	-	-	-	-	-	-	-	-	-	+	I
BRYOPHYTA													
Paraleucobryum enerve	1	-	1	-	1	III	-	+	-	-	-	-	I
Polytrichastrum alpinum	-	+	+	-	-	II	-	-	-	-	-	-	-
Polytrichum piliferum	-	+	2	-	-	II	-	-	-	-	-	-	-
Diplophyllum obtusifolium	-	+	-	-	-	I	-	-	-	-	-	-	-
Hylocomium proliferum	-	-	-	-	+	I	-	-	-	-	-	-	-
Polytrichum juniperinum	-	-	-	-	+	I	+	-	-	-	-	-	-
Rhytidiadelphus triquetrus	-	-	-	-	+	I	-	-	-	-	-	-	-

Sanionia uncinatua	-	-	-	-	1	I	+	-	-	-	-	-	-
Anthelia juratzkana	-	-	-	-	-	-	-	-	1	-	-	-	I
Plagiochila asplenoides	-	-	-	-	-	-	+	-	-	-	-	-	-
Polytrichastrum sexing.	-	-	-	-	-	-	-	-	1	-	-	-	I
LICHENES													
Thamnolia vermicularis	1	+	1	-	-	III	-	1	1	1	+	1	V
Alectoria scalaris	-	1	1	-	-	II	-	-	-	-	-	-	-
Cladonia mitis	-	+	-	-	+	II	-	-	-	-	-	-	-
Alectoria nigrescens	+	-	-	-	-	I	-	-	-	-	-	-	-
Alectoria ochroleuca	+	-	-	-	-	I	-	-	-	-	-	-	-
Cetraria aculeata	-	-	-	-	-	-	-	-	+	-	-	+	II
Cetraria cucullata	-	-	-	-	-	-	-	+	-	-	-	-	I
Cetraria nivalis	1	-	-	-	-	I	-	1	+	-	-	+	III
Cetraria islandica	3	1	1	-	1	IV	1	3	3	2	1	1	V
Cladonia mitis	-	+	-	-	+	II	-	-	-	-	-	-	-

Place of relevés: 1. Parâng-Cârja Summit, 2,150 m.s.l., grazed; 2. Cârja Summit, 2,300 m.s.l.; 3. Cârja Summit, 2,400 m.s.l.; 4. Stoinița Summit, 2,350 m.s.l., grazed; 5. Gemănarea Summit, 2,380 m.s.l.; 6. Mândra Summit, 2,330 m.s.l.; 7. ibid.; 8. Mândra Summit, 2,519 m.sl.; 9. Mândra Summit, 2,440 m.s.l.; 10. ibid.; 11. Mândra Summit, 2,400 m.s.l.; 12.Groapa Mândrii, 2,200 m.s.l., median ridge; 13. Păpușa Summit, 2,150 m.s.l.; 14. ibid.; 15. ibid.; 16. ibid.

ALPINE ROCK SWARDS (SESLERIETEA ALBICANTIS)

Dianthus tenuifolius-Festuca dalmatica community

This community appears in closed mats on the South facing escarpment edges of Păpuşa and Parâng Summits, between 1,800 and 1,900 m. The soil, according to the vegetation, is somewhat calcareous. On the base of two relevés it can be classified in Festuco saxatilis-Seslerion bielzii alliance. With the dominance of *Festuca dalmatica* and with the presence of Dacic *Dianthus tenuifolius* and Dacic-Balcanik *Bupleurum diversifolium*, *Jovibarba heuffelii*, *Genista oligosperma* and *Lilium jankae*, it seems to be an independent association with Balcanik connections. Along the calciphilous species Caricetalia curvulae coenotaxa, also occur others, as *Euphrasia minima*, *Festuca airoides* and *Agrostis rupestris.* The junior author observed a similar community on the limestone rocks of Vânturariţa Mts., just 40 km E-SE from the central part of Parâng Mts. and named it provisionally as a xerothermic subalpine *Festuca dalmatica-Phleum montanum* association [PÓCS, 1963]. There, it occurs between 1,600 and 1,800 m, on the steep SE slopes of Mts. Albu and Buila (Tab. 5, rel. 1-2).

Tab. 5. *Dianthus tenuifolius-Festuca dalmatica* community (rel. 1-2); *Cratoneuretum filicino-commutati* (Kuhn 1937) Oberd. 1977 (rel. 3-4); *Carici dacicae-Plantaginetum gentianoidis* Boşcaiu et al. 1972 (rel. 5); *Heracleetum palmati* Puşcaru et al. 1956 (rel. 6); *Salici-Alnetum viridis* Colic et al. 1962 (rel. 7); *Calamagrostetum arundinaceae subalpinum* Csűrös 1962 (rel. 8)

Aspect	**S**	**S**	**E**	**E**	**S**	**E-NE**	**N**	**S**
Slope (°)	**30**	**30**	**2**	**2**	**15**	**30**	**15**	**50**
Coverage of upper canopy (%)	-	-	-	-	-	-	**3-4**	-
Coverage of lower canopy (%)	-	-	-	-	-	-	**85**	-
Coverage of herb (%)	**90**	**80**	**85**	**75**	**60**	**98**	**85**	**90**
Coverage of mosses (%)	**4**	**4**	**85**	**70**	**40**	**70**	**10**	-
Surface (m^2)	**20**	**2**	**5**	**5**	**5**	**25**	**25**	**25**
Relevé no.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Festuca dalmatica	5	4	-	-	-	-	-	-
Dianthus tenuifolius	2	2	-	-	-	-	-	+
Saxifraga stellaris	-	-	5	4	1	-	-	-
Heracleum palmatum	-	-	-	-	-	3	-	-
Sorbus aucuparia	-	-	-	-	-	-	2	-
Picea abies	-	-	-	-	-	-	+	-
Picea abies juv.	-	-	-	-	-	-	+	-
Pinus mugo	-	-	-	-	-	-	+	-
Alnus viridis	-	-	-	-	-	-	5	-
Juniperus communis subsp. alpina	-	-	-	-	-	-	+	-
Adenostyles alliariae	-	-	-	-	-	5	3	-
Dryopteris dilatata	-	-	-	-	-	-	2	-
Senecio nemorensis subsp. fuchsii	-	-	-	-	-	-	2	1
Oxalis acetosella	-	-	-	-	-	-	1	-
Ranunculus platanifolius	-	-	-	-	-	-	1	
Vaccinium myrtillus	-	-	-	-	-	-	1	
Veratrum album	-	-	-	-	-	2	1	1
Athyrium distentifolium	-	-	-	-	-	-	1	1
Geranium sylvaticum subsp. caeruleatum	-	-	-	-	-	-	-	1
Anthoxanthum odoratum	-	-	-	-	-	-	-	1
Hypericum maculatum	-	-	-	-	-	-	-	1
Viola declinata	-	-	-	-	-	-	-	+

Avenastrum versicolor	-	-	-	-	-	-	-	+
Homogyne alpina	-	-	-	-	-	-	1	-
Saxifraga rotundifolia	-	-	-	-	-	-	1	-
Aconitum paniculatum	-	-	-	-	-	-	+	-
Angelica archangelica	-	-	-	-	-	-	+	-
Avenella flexuosa	-	-	-	-	-	-	+	+
Rubus idaeus	-	-	-	-	-	-	+	
Aconitum tauricum	-	-	-	-	-	1	-	+
Rumex arifolius	-	-	-	-	-	1	+	-
Senecio subalpinus	-	-	-	-	-	-	+	-
Soldanella hungarica subsp. major	-	-	-	-	-	-	+	+
Streptopus amplexifolius	-	-	-	-	-	-	+	
Campanula rotundifolia subsp. polymorpha	-	-	-	-	-	-	-	1
Trisetum flavescens	-	-	-	-	-	1	-	-
Gentiana punctata	-	-	-	-	-	+	-	-
Saxifraga heucherifolia	-	-	-	-	-	3	-	-
Poa minor	-	-	-	-	-	2	-	-
Stellaria nemorum	-	-	-	-	-	2	-	-
Ligusticum mutellina	-	-	-	-	-	1	-	-
Primula elatior	-	-	-	-	-	1	-	-
Campanula abietina	-	-	-	-	-	+	+	
Geum montanum	-	-	-	-	-	+	-	-
Soldanella pusilla	-	-	-	-	-	+	-	-
Cardamine amara	-	-	2	2	-	-	+	
Carex dacica	-	-	+	1	-	-	-	-
Cerastium cerastoides	-	-	2	1	-	-	-	-
Deschampsia cespitosa	-	-	2	2	2	-	+	2
Epilobium nutans	-	-	+	+	-	-	-	-
Poa alpina f. vivipara	-	-	+	1	-	-	-	-
Plantago gentianoides	-	-	1	+	1	-	-	-
Aconitum napellus	-	-	+	-	-	-	-	-
Alchemilla glabra	-	-	1	-	-	1	-	-
Caltha palustris subsp. laeta	-	-	-	+		-	-	-
Ranunculus montanus subsp. pseudomontanus	-	-	+	-	-	+	-	-
Viola biflora	-	-	+	-	-	-	1	-
Juncus filiformis	-	-	-	-	4	-	-	-
Carex echinata	-	-	-	-	2	-	-	-
Alchemilla flabellata	-	-	-	-	+	-	-	-
Cardamine pratensis var. rivularis	-	-	-	-	+	-	-	-
Bellardiochloa violacea	-	1	-	-	-	-	-	-
Poa nemoralis var. agrostoides	2	2	-	-	-	-	-	2
Jovibarba heuffelii	1	-	-	-	-	-	-	-
Thymus alpestris	1	1	-	-	-	-	-	-
Allium ericetorum subsp. ericetorum	-	1	-	-	-	-	-	-
Allium victorialis	+	-	-	-	-	-	-	2
Bupleurum diversifolium	1	-	-	-	-	-	-	1
Calamagrostis arundinacea	1	1	-	-	-	-	-	5
Luzula luzuloides var. cuprina	1	-	-	-	-	-	-	+
Allium vineale	+	-	-	-	-	-	-	-
Agrostis rupestris	+	-	-	-	+	-	-	-
Bruckenthalia spiculifolia	+	-	-	-	-	-	-	-
Carex sempervirens	+	+	-	-	-	-	-	1
Crocus vernus subsp. vernus	+	-	-	-	-	-	-	+
Hieracium aurantiacum	-	-	-	-	-	-	-	+
Scrophularia scopolii	-	-	-	-	-	-	-	+

Euphrasia minima	+	-	-	-	-	-	-	-
Festuca airoides	+	+	-	-	+	-	-	-
Genista oligosperma	+	-	-	-	-	-	-	-
Lilium jankae	+	-	-	-	-	-	-	-
Seseli libanotis	-	-	-	-	-	-	-	+
Sesleria bielzii	-	-	-	-	-	-	-	+
Thesium alpinum	-	-	-	-	-	-	-	+
Seseli libanotis var. humilis	+	-	-	-	-	-	-	-
Saxifraga pedemontana subsp. cymosa	+	-	-	-	-	-	-	-
Sedum annuum	+	+	-	-	-	-	-	-
Thymus balcanus	-	+	-	-	-	-	-	-
Silene lerchenfeldiana	+	-	-	-	-	-	-	-
BRYOPHYTA								
Plagiobryum zierii	+	-	-	-	-	-	-	-
Polytrichum piliferum	1	-	-	-	-	-	-	-
Thallose liverworts (sterile)	+	-	-	-	-	-	-	-
Cratoneuron filicinum	-	-	5	4		-	-	-
Drepanocladus exannulatus	-	-	+	2		-	-	-
Philonotis seriata	-	-	2	2	4	-	-	-
Bryum schleicheri	-	-	1	-	2	-	-	-
Calliergonella cuspidata	-	-	-	+	-	-	-	-
Pseudoleskea incurvata	-	-	-	-	-	3	+	-
Plagiothecium succulentum	-	-	-	-	-	2	-	-
Pellia endiviaefolia	-	-	-	-	-	1	-	-
Polytrichum commune	-	-	-	-	-	+	1	-
Dicranum polysetum	-	-	-	-	-	-	1	-
Diplophyllum albicans	-	-	-	-	-	-	1	-
Dicranum scoparium	-	-	-	-	-	-	+	-
Isothecium myosuroides	-	-	-	-	-	-	+	-
Plagiothecium laetum	-	-	-	-	-	-	+	-
Pohlia cruda	-	-	-	-	-	-	+	-
Rhizomnium punctatum	-	-	-	-	-	-	+	-
Radula lindenbergiana	-	-	-	-	-	-	+	-
LYCHENOPHYTA								
Cladonia pyxidata	+	-	-	-	-	-	-	-
Cladonia rangiferina	+	-	-	-	-	-	-	-
Thamnolia vermicularis	+	-	-	-	-	-	-	-

Place of relevés: 1. Păpuşa ridge, 1,900 m.s.l.; 2. Parâng Summit, 1,830 m.s.l.; 3-4. Groapa Mândrii, 1,810 m.s.l.; 5. South from the western peak of Păpuşa, at 1,800 m.s.l.; 6. Mândra circus, 2,040 m.s.l.; 7. Jieţu Valley, 1,700 m.s.l.; 8. Mt. Păpuşa, 1,900 m.s.l.

SPRING BOGS (MONTIO-CARDAMINETEA Br.-Bl. & Tx. 1943)

Cratoneuretum filicino-commutati (Kuhn 1937) Oberd. 1977

We have found on the bottom of Mândra circus, among *Rhododendro-Pinetum mughi* stands, spring bogs similar to those shortly characterised by BUIA & al. (1962). These can be identified as *Cratoneuretum filicino-commutati* association. It is related also to *Chrysosplenio alpini-Saxifragetum stellaris* Pawł. et Walas 1949, but due to the complete lack of *Chrysosplenium alpinum* we could not classify them there. The other spring bog community, the montane-subalpine *Chrysosplenio-Cardaminetum amarae* Mass. 1959 seems to be bound to the forests belts at lower altitudes. The spring bog is surrounded by *Carici echinatae-Sphagnetum* transition bog, here and there with raised bog

characters. Along the brook flowing out from the bog, *Carex pauciflora* and *Scapania undulata* were found (Tab. 5, rel. 3-4).

MONTANE FENS (SCHEUZERIO-CARICETEA NIGRAE)

Carici dacicae-Plantaginetum gentianoidis Boşcaiu et al. 1972

Based on the composition of the only one sample, the community can be classified in the alliance of Caricion nigrae, with characteristics species as *Carex echinata* and *Plantago gentianoides*, which was described from the Parâng Mts. as *Caricetum dacicae* Buia et al. 1962. This fragment was observed South from the western peak of Păpuşa, at 1,800 m altitude, on the muddy gravel bank of a streamlet (Tab. 5, rel. 5).

ARCTIC-ALPINE ALTHERBOSA (BETULO-ADENOSTYLETEA)

Heracleetum palmati Puşcaru et al. 1956

The association was described first from the montane-subalpine belts of Bucegi Mountains, then from almost all other ranges of the Romanian Western Carpathians, South Carpathians, and even from parts of the East Carpathians [GERGELY & RAȚIU, 1986]. From the spruce belt of Parâng mountains BUIA & al. (1962) mentioned it briefly. We observed this endemic altherbosa community along streamlets in the alpine belt. It needs further studies whether the communities from the spruce belt and from the subalpine and alpine belts can be distinguished as separate associations. All these stands are rich in Adenostyletea elements. Our relevé is from the NE end of the median ridge dividing Mândra circus into two parts, altitude 2.040 m, exposure E-NE, inclination 30°, coverage of upper herb layer (80-90 cm high) is of 98%, and of lower herb layer is of (10-30 cm) 60%. Moss layer coverage is 70% (Tab. 5, rel. 6).

Salici-Alnetum viridis Colic et al. 1962

This bush community is widespread in the upper montane and subalpine belts of the Carpathians and takes over the place of *Pinus mugo* always on the wet or drenched rocks or gravels. It is known from Parâng Mts. [BUIA & al. 1962]. Our relevé is an addition from Jieţu Valley, 1,700 m altitude, on northern aspects, 15° slope. Upper canopy height is of 6-8 m, coverage 3-4%, lower canopy 2-2.5 m height, coverage 85%, herb layer 30-80 cm height, 85%, moss layer 10%. Litter is of 30%. Soils are situated on gravels, being very wet (Tab. 5, rel. 7).

FOREST CLEARING (EPILOBIETEA ANGUSTIFOLII Tx. et Prsg. in Tx. 1950)

Calamagrostetum arundinaceae subalpinum Csűrös 1962

An interesting community was observed on the South slope of Păpuşa Summit, above the timberline. Due to the lack of Epilobion angustifoliae, Sambucion, Atropion, or Fagetalia coenoelements and the presence of some subalpine and Asplenietea species (as *Carex sempervirens*, *Campanula polymorpha*, *Bupleurum diversifolium*, *Dianthus tenuifolius*, *Seseli libanotis*, *Allium victorialis*, *Luzula luzuloides* var. *cuprina*) it seems to be different from the Central European *Calamagrosti arundinaceae-Digitaletum grandiflorae* association. It probably can be identified with the *Calamagrostetum arundinaceae subalpinum*, described by CSŰRÖS & al. (1962) from the Bihor-Vlădeasa Mts., as both associations are more natural than the forest clearing communities and occur on the scree slopes of subalpine belt. Probably they develope at the place of *Campanulo abietinae-Juniperetum* after its burning. The clarification of its relation with the above communities needs further studies. Our relevé is from the south facing cliffs of Mt. Păpuşa, on wet, South aspects (slope of 50°!), scree slope among boulders, at 1,900 m altitude. Herb layer is of 40-60 cm high, with a coverage of 98%; there was no moss layer (Tab. 5, rel. 8).

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Fig. 3. The senior author makes a phytocoenological relevé on Parângul Mic summit, at 2,050 m.s.l., in an open *Potentillo-Festucetum airoides*. **Fig. 4.** *Primulo-Caricetum curvulae* on the Cârja summit, at 2,400 m.s.l. **Fig. 5.** The calcareous scree slope of the E slope of Coasta lui Rusu, at 2,200 m.s.l. **Fig. 6.** *Arabis alpina-Delphinium elatum* community on the coarse limestone scree of the previous locality. **Fig. 7.** *Doronico carpatici-Festucetum pictae* on the coarse siliceous scree in Groapa Mândrii, at 2,030 m.s.l. **Fig. 8.** *Aquilegia transsylvanica*, one of the most spectacular species in the alpine belt of Parâng Mts. **Fig. 9.** *Soldanello pusillae-Ranunculetum crenati* with the dominance of *Luzula alpinopilosa* on the N facing slopes of Groapa Mândrii, still partly snow covered by the end of July, 1956. **Fig. 10.** The N facing siliceous cliffs and scree slopes of Braiul summit (2,345 m.s.l.) (photos 3-7 and 9-10 by T. Pócs, photo 8 by T. Simon)

Fig. 11. Our generous hosts, the "ciobans" (shepherds) in Groapa Mândrii. **Fig. 12.** Morning in our camp near a sheepfold of Groapa Mândrii, at 1,915 m.s.l. (photos by T. Simon). **Fig. 13.** The Roşiile Tarn, at 1,978 m, with the scree slopes and summit of Ieşul Summit (2,375 m.s.l.) in background. **Fig. 14.** Gneiss rocks with *Rhizocarpetum alpicolae* and *Umbilicarietum cylindricae*, on the scree slopes below Parâng Hut, at 1,750 m.s.l. (photos by T. Pócs)

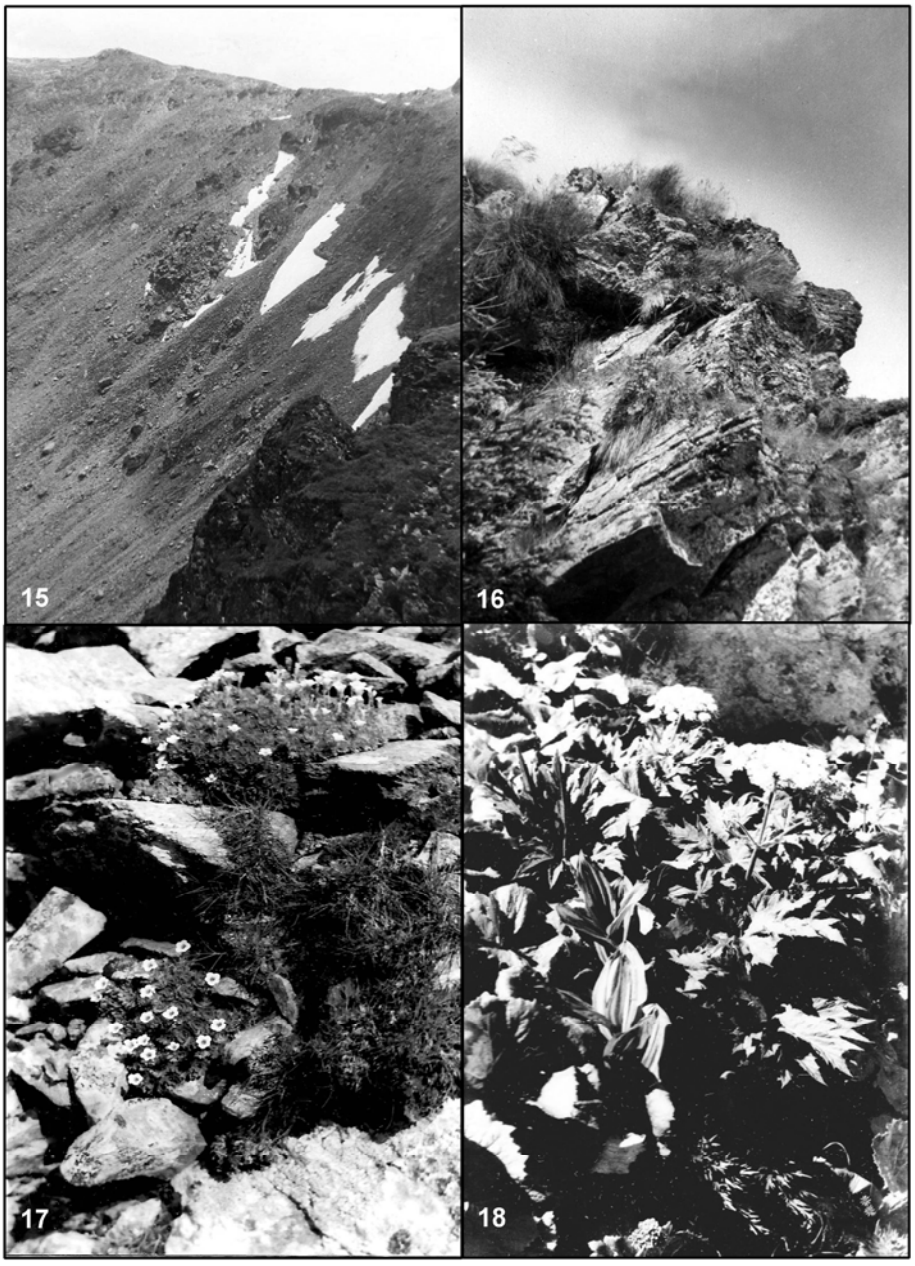

Fig. 15. Steep scree slopes on the NW aspects of Mândra, at 2,400-2,500 m.s.l. **Fig. 16.** *Sileno lerchenfeldianae-Potentilletum haynaldianae* on the Badea rocks, at 1,825 m.s.l. (photos by T. Simon). **Fig. 17.** *Saxifraga pedemontana* subsp. *cymosa* on the screes of Mândra, at 2,400 m.s.l. **Fig. 18.** *Heracleetum palmati* in Groapa Mândrii, at 2,040 m.s.l. (photos by T. Pócs) v

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INVASIVE PLANTS IN THE FLORA OF MUREŞ COUNTY

SĂMĂRGHIȚAN MIHAELA[1], OROIAN SILVIA[2]

Abstract: Invasive plants are those species that are capable of rapidly spreading at great distances from parent plants. Because of them, agriculture pays a huge annual tribute. These invasive plants might shortly become the most widely spread and destructive, as they seem to take best advantage from climate changes. They have a high phytocoenological competition capacity and rapidly adapt to new life conditions to the detriment of native plants.
This paper aims to inform of the phenomenon of invasion of these alien plants, of their distribution and abundance in certain areas in Mureş County. If the monitoring of invasive plants receives particular international attention, in the Mureş County there is no concern for monitoring their invasion, for limiting their negative effects on the environment and human economy.
The most rapacious and common invasive plants in the studied territory are presented in the paper.
Because these species already occupy extremely large surfaces, the measures for fighting them are difficult and costly, and long-term measures for their control and elimination are required.

Key words: invasive plants, flora, Mureş County

Introduction

Invasive species are an increasing major threat to indigenous biodiversity in Europe and worldwide.

Plant invasions are mainly caused by the intensification of economic branches such as transportation, trade, tourism, on the one hand, and by biological factors (absence of limiting factors) and climatological changes, on the other hand [ANASTASIU & NEGREAN, 2007; EASTWOOD, 2001].

Adventive plants are spontaneous or subspontaneous plants whose presence in a certain area is due to their accidental or intentional introduction as a result of human activity [RICHARDSON & al. 2000; PYŠEK & al. 2002].

Invasive plants are plants that are capable of rapidly spreading, at great distances from parental plants. Because of them, agriculture pays a huge annual tribute. Invasive plants might shortly become the most widely spread and destructive, as they seem to take best advantage from climate changes. They have a high phytocoenological competition capacity and rapidly adapt to new life conditions to the detriment of native plants.

These invasive plants manage to replace native species that have already adapted to local soil and climate conditions over the years, this invasion process being always correlated with the anthropic factor and inadequate ecosystem exploitation methods. Invasive species affect biodiversity by competing with other organisms and by changing the structure of the habitat [McNEELY & al. 2001], by the fact that they are toxic, carry parasites or are vectors for pathogenic agents, by hybridization with related species or

[1] Mureş County Museum, Natural Sciences Department, str. Horea 24, Târgu-Mureş – Romania, e-mail: msamarghitan@yahoo.com
[2] UMPh - Tg-Mureş, Faculty of Pharmacy, Department of Pharmaceutical Botany, Str. Gh. Marinescu 38, Târgu-Mureş – Romania, e-mail: oroianslv@yahoo.com

varieties, by altering the local trophic network; for example, invasive plants change the availability of nutrients, disturbing pollination and causing the disappearance of indigenous species; they alter the ecosystem by changing energy and nutrient flows, as well as physical factors within habitats and ecosystems [LEVINE & al. 2003].

According to LAMBDON & al. (2008), 5789 alien vascular plant species have been so far identified in Europe, of which 2843 are alien to Europe. Of these 1507 and 872, respectively, are casual in all regions where they occur, and 29 and 8, respectively, cryptogenic; for 504 and 183 species, respectively, the naturalization status is uncertain. There are in total 3749 naturalized aliens recorded in Europe and 1780 alien to Europe. Of adventive species, approximately 65% are naturalized.

PYŠEK et al. (2009) emphasize the presence of a continuous influx of allien species in Europe over the past decades.

If the monitoring of invasive plants receives particular international attention, in Mureş County there is no concern for monitoring their invasion, for limiting their negative effects on the environment and human economy.

Because these species already occupy extremely large surfaces, the measures for fighting them are difficult and costly, and long-term measures for their control and elimination are required.

In Romania, adventive species represent 11.5% of the national flora [CIOCÂRLAN, 2000], and their number increases, new alien species being continuously reported [CIOCÂRLAN & al. 2004; SÎRBU & OPREA, 2008a; ANASTASIU & NEGREAN, 2008].

According to ANASTASIU & NEGREAN (2005, 2007), 435 adventive plant species were identified in the Romanian flora, of which 384 neophytes and 51 archaeophytes, and the most important plant families represented in the Romanian adventive flora are: *Asteraceae, Brassicaceae* and *Poaceae*. Also, according to these authors, more than 50% of these adventive species are annual, and the proportion between deliberately and accidentally introduced adventive plants is approximately equal. If archaeophytes are predominantly of Mediterranean origin, most neophytes are of American origin.

In other evaluations [DIHORU, 2004], 61 adventive plant species are considered to be invasive.

These evaluations are mainly based on the number of locations in which a plant was reported and on the estimation of its relative impact on the affected habitats.

This is why systematic monitoring researches of the populations in their habitats are required.

Materials and methods

Mureş county is situated in central-northern Romania, having a surface area of 6696 km^2, which slowly descends in steps from the volcanic peaks of the Călimani and Gurghiu Mountains to the middle of the Transylvanian Plateaux. Over the past decade, extensive surface areas occupied by invasive species to the detriment of native species have been identified. They settled in massively due to the fragmentation of arable lands, left uncultivated, representing real infestation foci.

The inventory of invasive plant species in Mureş County was elaborated based on personal researches performed in the period 2000-2011, as well as on bibliographic

information [OROIAN, 1998, 2009; OROIAN & SĂMĂRGHIȚAN, 2006; PITEA, 1995, SĂMĂRGHIȚAN, 2005].

The presentation of each species is accompanied by ecological, chorological, coenological data, information regarding their belonging to different groups of bioforms and the number of chromosomes.

The aspects of chorology, ecology, physiognomy and floristic composition, was made on the *Vademecum ceno-structural privind covorul vegetal din România* [SANDA & al. 2001]. The establishment of the bioforms, floristical elements, ecological indices and number of chromosomes was made on the basis of *Flora cormofitelor spontane şi cultivate din România* [SANDA & al. 2004]. Seeds spreading types were established according to SOÓ (1964-1980).

The nomenclature of the species was given in accordance with *Flora Europaea* [TUTIN & al. 1964–1980, 1993] and OPREA (2005).

In order to establish the types of invasive plants, the terminology and the definitions recommended by RICHARDSON & al. (2000) and PYŠEK & al. (2004) were taken into consideration.

This study reports only the species with a significant degree of invasion.

Ecological indices used are:

U – humidity (1-1.5 xerophilous; 2-2.5 xero-mesophilous; 3-3.5 mesophilous; 4-4.5 meso-hygrophilous; 5-5.5 hygrophilous; 0 amphitolerant toward humidity)

T – temperature (1-1.5 cryophilous; 2-2.5 micro-termophilous; 3-3.5 micro-meso-termophilous; 4-4.5 moderate-termophilous; 5-5.5 termophilous; 0 amphitolerant toward temperature, eurythermic)

R – soil reaction (1-1.5 strong acidophilous; 2-2.5 acidophilous; 3-3.5 acid-neutrophilous; 4-4.5 low acid-neutrophilous; 5-5.5 basiphilous; 0 amphitolerant toward soil reaction, euryionic).

Results and discussions

In Mureş County, 21 invasive species (Tab. 1) were identified, more than 50% of these belonging to the Asteraceae family.

The most rapaceous and most common invasive plants in the studied territory are:

Amaranthus crispus (Lesp.et Thév.) Terracc. – *Sisymbrion*, *Onopordion*; Th, S-Am-Adv; 2n=34, D; $U_3T_4R_3$; anemochory, epizoochory.

Frequently found at the edge of farming fields, in anthropized, ruderalized fields.

Amaranthus retroflexus L. – *Sisymbrion*, *Arction*; Th, N-Am (changed to Cosm); 2n=34, D; $U_3T_3R_0$; anemochory, epizoochory.

It abundantly develops at the edge of farming fields in particular, but it is also present in semi-natural and natural habitats.

Ambrosia artemisiifolia L. – *Sisymbrietalia*, *Chenopodietalia albi*, *Eragrostetalia*, *Onopordetalia acanthi*; Th, Adv N-Am; 2n=36, P; $U_2T_0R_0$; antropochory, anemochory.

It is of North American origin. It forms local clusters in ruderal territories, along roadsides, on railway embankments, and has a high tendency to extend. It was identified in the studied territory in Târgu-Mureş, along the railway.

Conyza canadensis (L.) Cronq. (*Erigeron canadensis* L.) – *Sisymbrion*; Th-TH, N-Am Adv; 2n=18, D; $U_{2.5}T_0R_0$; anemochory, antropochory.

This plant of North American origin occupies extensive territories in Mureş County, rapidly invades ruderalized territories, abandoned farming lands, and has a great capacity to spread.

Echinocystis lobata (Michx) Torr. et Gray (*Echinocystis echinata* (Muhlb.) Britt., Sterns et Poggenb.) – Car. *Calystegion sepium*; Th, Adv; 2n=32, P; $U_4T_0R_4$.

Cultivated as an ornamental plant, the species prefers moist, shaded places, being frequently found on the bank of waters, as well as at the edge of forests, where it forms thick borders, in Târnavelor Plateaux and in the mountain area of the county.

Erigeron annuus (L.) Pers. (*Stenactis annua* (L.) Nees) subsp. *annuus* (Fig. 4) – *Arction*, *Sisymbrion*, *Calystegion sepium*, *Salicetea*, *Alno-Ulmion*; Th, N-Am Adv; 2n=27, 36, P; $U_4T_0R_4$; anemochory.

An annual-biannual-perennial plant, which produces rich, dense populations, occupying abandoned farming lands, present in both anthropic and semi-natural habitats, with a negative impact on the native flora.

Galinsoga parviflora Cav. – *Polygono-Chenopodietea*, *Panico-Setarion*; Th, S-Am Adv; 2n=16, D; $U_{3.5}T_0R_3$; anemochory, epizoochory, anthropochory.

It is of South American origin (Peru). It seems to have immigrated to the Romanian flora during World War I. It proliferates as a commensal plant in hill and mountain weed crops, particularly corn crops. It can be frequently found in Mureş County, from the plain to the mountain area.

Helianthus decapetalus L. – *Convolvulion*; H-G, N-Am-Adv; 2n=64, P.

It prefers moist soils in the river meadows. It occupies large surface areas along waters in the mountain area of the county. It was also reported in Târnavelor Plateaux.

Helianthus tuberosus L. – *Convolvulion*; H-G, N-Am-Adv; 2n=102, D.

A plant introduced in cultivation for food purposes, which has become wild almost throughout the country. In Mureş County, it is frequently found along water courses, where it forms thick borders that affect the structure of the invaded ecosystems. It can be present even in farming lands.

Impatiens glandulifera Royle (*Impatiens roylei* Walpers) (Fig. 3) – Car. *Salicion albae*, *Calystegion*; Th, Hymalaia (Adv); 2n=18,20, D; $U_4T_4R_4$; autochory, epizoochory.

Introduced in cultivation for its ornamental qualities, the species can be frequently found on the banks of rivers in the mountain and hill area. It forms significant populations, affecting the development of autochthonous species.

Juncus tenuis Willd. – Car. *Polygonion avicularis*; H, N-Am-Adv; 2n=84, P; $U_{3,5}T_3R_4$; anemochory, frequently epizoochory.

An accidentally introduced, North American adventive plant, *Juncus tenuis* is widely spread in the research territory. It can affect indigenous plants by competition and can disturb valuable habitats.

Lycium barbarum L. – *Arction lappae*, *Prunetalia*; mPh, Adv Asia; 2n=24-P; $U_3T_4R_0$.

Of Chinese origin, it was used for ornamental purposes, in hedges, particularly in rural areas, from where it was naturalized. It forms thick groups, in anthropized places, where compost is stored. It does not have a rapid propagation capacity.

Portulaca oleracea L. – *Polygono-Chenopodietalia*; Th, Asia-Temp-Adv; 2n=18,54, D-P; $U_3T_0R_0$; autochory, mirmecochory.

Portulaca oleracea is an aggressive weed in the majority of the crops. Having a rapid germination capacity, it competes with autochthonous plants. It is found in Mureş

County, on abandoned farming lands in the plain and hill area, as well as in semi-natural habitats, and even in the mountain area.

Reynoutria japonica Houtt. – G, Adv-Jap; 2n=44, P.

It is frequently found on the banks of waters in the hill and mountain area, where it forms dense populations that replace autochthonous species.

Robinia pseudacacia L. – *Bromo sterili-Robinietum*; MPh, Adv-Am-N; 2n=22, D; $U_{2,5}T_4R_0$; anemochory, endozoochory (subspontaneous).

The species invades semi-natural and even natural habitats, at various degradation stages, particularly in the hill and plain area, but it can also be found in the mountain area of Mureş County. It can have beneficial effects in soil fixation, but its presence needs to be monitored, particularly in the proximity of valuable habitats.

Rudbeckia laciniata L. (Fig. 1) – *Calystegion sepium*, Car. *Senecion fluviatilis*; H, N-Am Adv; 2n=76, P; $U_{4,5}T_4R_4$; anemochory.

A species of riparian habitats, which forms large groups that can suffocate other species. Having escaped from cultivation, it is extremely widespread in Mureş County, from the plain to the mountain area.

Solidago canadensis L. (Fig. 2) – *Calystegion sepium*, *Artemisietea*; H, Adv; 2n=18, D; $U_{3,5}T_3R_3$.

The species was introduced in cultivation for ornamental purposes, from where it spread to semi-natural and anthropized habitats. It occupies large surface areas in the Transylvanian Plateau and is even found at the edge of forests. The rich, monodominant populations replace the indigenous species.

Solidago gigantea Aiton subsp. *serotina* (Kuntze) McNeill – *Alno-Ulmion*, *Salicetea*, Car.*Calystegion sepium*; H, Adv; 2n=36, P; $U_{3,5}T_3R_3$.

Like *Solidago canadensis*, it was introduced for ornamental purposes and escaped from cultivation. It is frequently found in the Târnavelor Plateaux, forming compact groups on abandoned farming lands or degraded pastures.

Veronica persica Poiret (*Veronica buxbaumii* Ten.) – *Chenopodio-Scleranthea*, Car. *Polygono-Chenopodietalia*; Th, V-Asian (changed to Subcosmopolite); 2n=28, P; $U_3T_0R_4$, anemochory, endozoochory.

This species is remarkably enduring under difficult environmental conditions, particularly in superficial, compact, polluted, dry soils. As a result, it spreads rapidly along roadsides and railways, being present in degraded, anthropized soils, in competition with less enduring autochthonous species.

Xanthium italicum Moretti – *Bidentetea*, *Sisymbrion*, Car. *Chenopodion fluviatile*; Th, S-Eur; 2n=36, P; $U_{3,5}T_4R_0$; zoochory, antropochory.

It grows in ruderal places and highly grazed pastures, sometimes being found at the edge of farming fields.

Xanthium strumarium L. – *Sisymbrion*, *Onopordion*; Th, Cosm; 2n=36, P; $U_{3,5}T_{3,5}R_4$; zoochory, antropochory.

It is frequently found in the studied area on degraded or abandoned lands, on overgrazed pastures or at the edge of farming lands.

The majority of the identified species are integrated in anthropic habitats. Some of them also occur in semi-natural habitats: forest edge, river banks, meadows, etc., frequently having a strong negative impact on these.

Some of the invasive species are xenophytes, being accidentally introduced, while others were introduced by man for various purposes, particularly ornamental plants, and secondarily escaped from cultivation into the spontaneous flora (hemerophyte species).

Of the total number of invasive species described in Mureş County, 47.61% are xenophytes, and 52.38% are hemerophytes.

More than half of the identified invasive species are therophytes (57.14%), hemicryptophytes representing a third of these (28.57%).

The number of invasive plant species present in the studied locations is found to be correlated with the intensity of anthropic phenomena in the area. Thus, the greatest number of species was identified in urban areas such as Sighişoara (16 sp.), Saschiz (14 sp.), Reghin (11 sp.).

Tab. 1. The list of invasive species in Mureş county

Species	Geographic origin	Category	Way of introduction	Spreading area in Mureş County
Ambrosia artemisiifolia	N-Am	X	accidental	Târgu-Mureş
Amaranthus crispus	S-Am	X	accidental	Gurghiu, Reghin, Saschiz, Sighişoara;
Amaranthus retroflexus	N-Am	X	accidental	Band, Dubişte, Fărăgău, Moreşti, Reghin, Răstolița, Sighişoara;
Conyza canadensis	N-Am	X	accidental	Aluniş, Bereni, Daneş, Dubişte, Fărăgău, Herghelia, Ibăneşti, Lăpuşna, Mihai Viteazu, Moreşti, Răstolița, Saschiz, Sighişoara, Sovata, Şilea Nirajului;
Echinocystis lobata	N-Am	H	ornamental	Apold, Daia, Daneş, Dubişte, Răstolița, Reghin, Saschiz, Sighişoara, Solovăstru, Stejărenii, Vânători;
Erigeron annuus subsp. *annuus*	N-Am	H	ornamental	Aluniş, Apold, Archita, Băla, Beica de Sus, Caşva, Cloaşterf, Cozma, Criş, Daia, Daneş, Dubişte, Herghelia, Jabenița, Gurghiu, Lăpuşna, Lunca Mureşului, Miercurea Nirajului, Mihai Viteazu, Moreşti, Reghin, Răstolița, Saschiz, Săbed, Sighişoara, Stejărenii, Şaeş, Şilea Nirajului, Ulieş,Valea Secuieu;
Galinsoga parviflora	S-Am	X	accidental	Brădeţel, Daneş, Dubişte, Dulcea, Fărăgău, Gălăoaia, Gurghiu, Gura Fâncel, Herghelia, Lăpuşna, Păuloaia, Răstolița, Saschiz, Sighişoara, Sovata, Valea Secuieu;
Helianthus decapetalus	N-Am	H	ornamental	Apold, Borzia, Daia, Daneş, Ibăneşti, Răstolița, Reghin, Saschiz, Sighişoara;
Helianthus tuberosus	N-Am	H	food	Livezeni, Luduş, Moreşti, Reghin, Sântana de Mureş, Sâncraiu de Mureş, Solovăstru, Târgu-Mureş;
Impatiens glandulifera	Asia Himalaya	H	ornamental	Daneş, Dubişte, Gălăoaia, Lunca Bradului, Răstolița, Saschiz, Sighişoara;

Juncus tenuis	N-Am	X	accidental	Archita, Band, Daia, Deda, Fărăgău, Gurghiu, Ibăneşti Pădure, Jabenița, Lăpuşna, Lunca Bradului, Saschiz, Şarpatoc, Valea Sălardului, Vulcan;
Lycium barbarum	Asia (China)	H	ornamental	Apold, Băla, Cozma, Ercea, Luduş, Ogra, Sânpaul, Sighişoara, Ulieş;
Portulaca oleracea	Asia	X	accidental	Apold, Band, Cloaşterf, Daneş, Deda, Gurghiu, Jabenița, Reghin, Saschiz, Sânpaul, Sighişoara, Stejărenii;
Reynoutria japonica	Japan	H	ornamental	Apold, Daneş, Dulcea, Glăoaia, Ibăneşti, Răstolița, Sighişoara;
Robinia pseudacacia	N-Am	H	ornamental	Adămuş, Adrian, Aluniş, Androneasa, Apold, Band, Beica de Sus, Bereni, Bistra-Mureşului, Cloaşterf, Daneş, Deda, Dubişte, Fărăgău, Gălăoaia, Gurghiu, Herepea, Herghelia, Ibăneşti, Iod, Lunca Bradului-Sălard, Miercurea Nirajului, Moreşti, Răstolița, Săbed, Saschiz, Sighişoara, Sovata, Sângeorgiu de Mureş, Şilea Nirajului, Târgu-Mureş, Zau de Câmpie, Vânători, Ulieş;
Rudbeckia laciniata	N-Am	H	ornamental	Band, Brâncoveneşti, Caşva, Gurghiu, Lăpuşna, Reghin, Sighişoara, Solovăstru;
Solidago canadensis	N-Am	H	ornamental	Apold, Daneş, Saschiz, Sighişoara, Şaeş, Stejărenii;
Solidago gigantea subsp. *serotina*	N-Am	H	ornamental	Daia, Saschiz;
Veronica persica	V-Asia	X	accidental	Gurghiu, Lunca Bradului, Răstolița, Reghin;
Xanthium italicum	Am	X	accidental	Apold, Daia, Reghin;
Xanthium strumarium	N-Am	X	accidental	Aluniş, Bălăuşeri, Caşva, Daia, Fărăgău, Gălăoaia, Gurghiu, Herghelia, Jabenița, Răstolița, Reghin, Săbed, Saschiz, Sighişoara, Sighişoara-Platoul Breite, Şaeş, Târgu-Mureş, Ulieş

Am – America; N – North, V – West, S – South; X – xenophyte; H – hemerophyte

Conclusions

In Mureş County were identified 21 invasive plant species**.**

Given the number of localities in which they were identified, we may consider that the most widespread species are: *Conyza canadensis*, *Erigeron annuus* subsp. *annuus*, *Galinsoga parviflora*, *Juncus tenuis*, *Robinia pseudaccacia*, *Xanthium strumarium.*

The species with the best cover, with high density are: *Erigeron annuus* subsp. *annuus*, *Reynoutria japonica*, *Rudbeckia laciniata*, *Solidago canadensis*, *Solidago gigantea* subsp. *serotina*.

Fig. 1. *Rudbeckia laciniata* (Gurghiu) – photo Silvia Oroian

Fig 2. *Solidago canadensis* (Târnavelor Plateaux) – photo Silvia Oroian

Fig. 3. *Impatiens glandulifera* (Răstolița) – photo Silvia Oroian

Fig. 4. *Erigeron annuus* subsp. *annuus* (Târnavelor Plateaux) – photo Silvia Oroian

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NEW DATA ADDITION TO THE ROMANIAN ALIEN FLORA

OPREA ADRIAN[1], SÎRBU CULIȚĂ[2], ELIÁŠ PAVOL jun.[3], FERUS PETER[4]

Summary: A number of seven plant taxa are presented in this paper, some of them being now for the first time reported in Romania (e. g. *Cenchrus longispinus*, *Panicum miliaceum* subsp. *ruderale*; *Panicum miliaceum* subsp. *agricolum*; *Robinia neomexicana*), while other taxa are mentioned for the first time in the flora of some historical provinces (e. g. *Oenothera parviflora* L. in Oltenia, or. *Setaria faberi* R. A. W. Herrm. in Moldavia); other taxa are newly identified in the flora of various counties (e. g. *Reynoutria* × *bohemica* in Bihor, Sălaj, Cluj, Caraş-Severin, Vâlcea, Prahova, and Bacău counties; *Reynoutria sachalinensis* in Caraş-Severin County).

Keywords: alien plant species, flora, new records, Romania

Introduction

Biological invasions of alien species are large-scale phenomena of widespread importance and represent one of the major current threats to economic value, biological diversity and function of invaded ecosystems [HULME, 2007; LAMBDON & al. 2008].

Identification and reporting of alien species entering on a certain area, and assessing their invasive character, are the first important steps in developing national or regional strategies to prevent negative effects of biological invasions.

According to LAMBDON & al. (2008) and PYŠEK & al. (2009), a constant increase in the number of neophyte species was noticed in the whole Europe, especially in the last two centuries; on average, 6.2 new alien plant species are naturalized in Europe in each year.

Similar to the situation on the European level, research conducted in recent years also showed a continuous enrichment with neophytes of the Romania's flora. According to our estimations, through the contribution of many authors (see SÎRBU & OPREA, 2011 and NEGREAN, 2011, for extensive reference lists), a number of 47 new alien plant species were registered in the Romania′s flora, after the year of 2000. This suggests that further floristic investigations are required for a better knowledge of the alien flora.

Our paper is a new contribution in this regard, including: i) newly registered taxa in Romania′s flora, and, ii) new chorological contributions of some species previously reported in the literature.

[1] "Anastasie Fătu" Botanical Garden, "Alexandru Ioan Cuza" University, 7-9 Dumbrava Roşie St., 700487, Iaşi – Romania
[2] University of Agricultural Sciences and Veterinary Medicine Iaşi, Faculty of Agriculture, 3, Mihail Sadoveanu Alley, Iaşi – Romania, e-mail: culita69@yahoo.com
[3] Slovak Agricultural University, Faculty of Agrifood Resources, Tr. A. Hlinku 2, 949 76, Nitra – Slovakia
[4] Mlynany Arboretum, Slovak Academy of Sciences, Vieska nad Zitavou 178, 95152, Slepcany – Slovakia

Materials and methods

The floristic and chorological data in this paper are based on lately field investigations, conducted in different regions and localities of Romania. For each identified species, there are given informations concerning their general distribution all over the world, previously reported occurrence, as well as their current distribution in Romania′s flora.

The herbarium vouchers are deposited in the general herbarium of the University of Agricultural Sciences and Veterinary Medicine of Iaşi (IASI). The species nomenclature is given according to *Flora Europaea* [TUTIN & al. 1964-1980, 1993]. Terminology and definitions recommended by RICHARDSON & al. (2000) and PYŠEK & al. (2004) were used for establishing the status of alien plants.

Results and discussion

During our field surveys in the last six years, there have been identified other new vascular plants in the spontaneous and/or sub-spontaneous flora of Romania, as *Reynoutria × bohemica* Chrtek et Chrtková, *Reynoutria sachalinensis* (F. Schmidt) Nakai., *Panicum miliaceum* L. subsp. *agricolum* Scholz et Mikoláš, *Panicum miliaceum* L. subsp. *ruderale* (Kitag.) Tzvelev, *Robinia neomexicana* Gray, *Cenchrus longispinus* (Hack.) Fernald, *Oenothera parviflora* L., and *Setaria faberi* R. A. W. Herrm.

Among these alien taxa, two are xenophytes, being introduced accidentally (e. g. *Cenchrus longispinus* and *Setaria faberi*), while the others were intentionally introduced by humans for different uses (mainly as ornamental plants) and, subsequently, escaped from cultivation in the wild (= hemerophyte species).

Having in mind the number of localities in which these plants were identified in Romania, the following taxa show an invasive tendency, as they are: *Cenchrus longispinus*, *Reynoutria × bohemica*, *Reynoutria sachalinensis* etc.

All of these taxa are integrated into anthropogenic habitats. Some of these species, as: *Reynoutria × bohemica*, *Reynoutria sachalinensis* also penetrate into the natural habitats, as: riverbanks, river meadows, grasslands etc.

a) Newly registered taxa in Romania's flora

Cenchrus longispinus (Hack.) Fernald, *Rhodora* **45**: 388 (1943)

This species of Poaceae family is native in the United States of America and in southern parts of Canada, in Mexico, Central America and the West Indies [VERLOOVE & SÁNCHEZ-GULLÓN, 2012]. It is also naturalised in Australia, South Africa, the Mediterranean area of Europe, Asia in Middle East (Israel) and northern Africa (Morocco) [VERLOOVE & SÁNCHEZ-GULLÓN, 2012].

According to some authors [cited in VERLOOVE & SÁNCHEZ-GULLÓN, 2012] it seems that the naturalization of this species in Europe has begun on the Adriatic Sea coasts (in Italy), where it is known at least from the year of 1933, today being a fully naturalized species on a large scale. Still, the presence of this species in the European flora has been neglected until recently, being confused in literature, as well as in herbaria collections, with *Cenchrus spinifex* Cav. (*C. incertus* M. A. Curtis; *C. pauciflorus* Benth.) [VERLOOVE & SÁNCHEZ-GULLÓN, 2012].

Nowadays, in Europe, *C. longispinus* is spread in Italy, France, Greece, Croatia, Hungary and Ukraine [VERLOOVE & SÁNCHEZ-GULLÓN, 2012].

In Romania, *C. longispinus* has been identified by us on the Black Sea coasts, on sandy beaches along the seashore, at Mamaia resort (Constanța County) (leg. Oprea, 2007 & 2009); further, it has been identified in central railway station of Galați town, among the rails (leg. Sîrbu, Oprea, Eliáš, Ferus, 2011), being erroneously identified and published as *C. incertus* M. A. Curtis [SÎRBU & al. 2011].

Revision of our herbarium material collected in Romania led us to a correctly identification of this plant species, being a newly registered one in the Romanian vascular flora.

According to data in the taxonomical references on this species [CHASE, 1920; FERNALD, 1943; VERLOOVE & SÁNCHEZ-GULLÓN, 2012; WARD, 2010], *C. longispinus* differs by *C. spinifex* Cav., as it is stated in the next identification key:

Involucres with 30-50 spines, the outer ones numerous, shorter and slender (bristles-like), patents to reflexed; the inner spines longer and stout, terete, not or hardly flattened at base, at most 1 mm wide; spikelets of 5-6 mm long; plants always annuals .... *C. longispinus*

Involucres with c. 20-30 spines usually, shorter, very stout; the outer ones fewer to almost lacking, bristle-lile, reflexed; the inner ones stout, more or less conical, distinctly flattened, their bases up to 3 mm wide; spikelets of 5.8 mm long; plants annual or more often pauciennial ..... *C. spinifex*

In addition, in *Jepson Manual of Vascular Plants of California* [BALDWIN & al. 2012] there are showed other diagnemas, in order to help to discriminate between these two species (sheats strongly keeled at *C. longispinus* (Hack.) Fernald vs. sheats compressed, but not strongly keeled at *C. spinifex* Cav.).

Till now, in Romania′s flora, the species *Cenchrus incertus* M. A. Curtis (Syn. *C. carolinianus* Walter; *C. pauciflorus* Benth.) has been reported only, as being present along the Black Sea shorelines at Vama Veche [CIOCÂRLAN, 1991], Constanța harbour [CIOCÂRLAN & al. 2004], Jurilovca at Doloşman Cape (Tulcea County) [OȚEL, 1995 & 2006; DOROFTEI & al. 2011], Măcin (Tulcea County) [CIOCÂRLAN & al. 2004], Jijila (Tulcea County) [OPREA, 2005] (Fig. 1, a-d).

In *Flora Europaea* [CLAYTON, in TUTIN & al. 1980, p. 264] there are given three species of *Cenchrus*, namely: 1. *Cenchrus ciliaris* L. - originated in Africa and South-western Asia, distributed in Sicilia and Isole Lipari (Italy), only; 2. *C. incertus* M. A. Curtis – originated in tropical and warm-temperate regions of America's and naturalized in the centre of Mediterranean region (Italy, Corsica, Azores?, France?, Spain?); 3. *C. longispinus* (Hack.) Fernald – of North and Central American origins, naturalized in Southern Europe.

It is not excluded the possibility that other herbarium material collected in Romania to show the same confusion between the two species and therefore is need to be reviewed other herbarium collections.

Panicum miliaceum L. *Sp. Pl.* ed. I: **58** (1753)

(Proso) Millet is a species originating in China and Central Asia. It is an annual herbaceous, therophyte plant species, flowering in June-August.

It has been cultivated extensively in the past, before the advent of maize (*Zea mays* L.) in Romania (XVII century); at those times people prepared a kind of traditionally food made from the grains of (proso) millet, boiled in water. Nowadays, the (proso) millet is cultivated as a fodder plant, only. PRODAN (1935) cited this plant as a sub-spontaneous one in Danube Delta′s flora. It is sporadically met in many localities in Romania′s flora, being described also a plant association with this species as a dominant one, namely Ass. *Erigero canadensis-Panicetum miliacei* Ştefan 1993 [ŞTEFAN, 1993].

As a result of field surveys made in the last few years, there were identified two subspecies of *Panicum miliaceum*, one of these being a newly registered infrataxa in Romania′s flora, namely:

Panicum miliaceum L. subsp. ***agricolum*** Scholz et Mikoláš, *Thaiszia*, **1**: 33-36 (1991)

This wild subspecies of the (Proso) Millet has been described from Austria (Carinthia) [SCHOLZ & MIKOLÁŠ, 1991].

According to SCHOLZ & MIKOLÁŠ (1991), this subspecies differs of subsp. *ruderale* by relatively dense, nodding panicle, persistent glumes at maturity (glumes not disarticulating from the pedicel); from subsp. *miliaceum* (a taxa only in cultivation) it differs by fruits easily falling off at maturity, and lighter and thinner rippen cariopses.

It is distributed in the wild in Europe, as: Italy (identified early in 1842, in Herbarium Firenze, cf. SCHOLZ & MIKOLÁŠ, 1991), Austria [SCHOLZ & MIKOLÁŠ, 1991], Czech Republic [SCHOLZ & MIKOLÁŠ, 1991], Slovakia [SCHOLZ & MIKOLÁŠ, 1991], Slovenia [MELZER, cited by SCHOLZ & MIKOLÁŠ, 1991], Hungary [KOVÁCS & SZABÓ, cited by SCHOLZ & MIKOLÁŠ, 1991; TERPÓ-POMOGYI, 1976], France [SCHOLZ & MIKOLÁŠ, 1991].

In Asia it was found in Afghanistan [SCHOLZ & MIKOLÁŠ, 1991].

This infrataxa seems not to grow in South and North America (cf. SCHOLZ & MIKOLÁŠ, 1991).

This subspecies was identified in the next localities, in the Eastern part of Romania:

– Galaţi-West toward the village of Barboşi (Galaţi County), in ruderal places (leg. Sîrbu & Oprea, 01.08.2011);
– the village of Cudalbi (Galaţi County), in ruderal places (leg. Sîrbu & Oprea, 01.08.2011);
– the neighborhood of Borzeşti (Oneşti town, Bacău County), on fallow grounds (leg. Sîrbu, 09.07.2012) (Fig. 2);
– the village of Crişan (Tulcea County), in ruderal places (leg. Sîrbu & Oprea, 15.09.2011).

The genetic samples, analyzed on individuals from Slovakia, relieved the next results: 2n=36 [SCHOLZ & MIKOLÁŠ, 1991].

Flora Europaea counts only the species of (Proso) Millet (*Panicum miliaceum* L.) [CLAYTON, in TUTIN & al. 1980, p. 261].

Robinia neomexicana Gray, *Pl. Nov. Thurb*. 314 (1854) var. *neomexicana*
(Syn.: *Robinia luxurians* (Dieck) Schneid. ex Tarouca et Schneid., *R. neomexicana* var. *luxurians* Dieck, *R. neomexicana* var. *subvelutina* (Rydb.) Kearney et Peebles, *R. rusbyi* Wooton et Standl.)

New Mexican Locust, New Mexico, Southwest, Desert, Pink, or Rose Locust is a tree, up to 10 m high, often shrub; shoots glandular-hairy, with stipular-subulate spines;

leaves impari-pinnate up to 20 cm long, with (13-) 15-21 oblong-elliptical leaflets, 2-3.5 cm long, rounded or gradually acute at the apex, silky hairy on the underside, and pubescent, non-glandular rachis; flowers pink-whitish, ca 20 mm long, in multiflowers, pendent racemes, with peduncle and rachis glandulous-hairy; legumes of 6-10 cm long, glandular setaceous; V-VII.

Origins: Centre of the Northern America and Northern Mexico. Introduced in Europa.

Cultivated in Romania: the Arboretum of Gurahonț (Arad County) [ZANOSCHI & al. 2006], and the Botanic Garden "Anastasie Fătu" of Iaşi [DUMITRIU-TĂTĂRANU, 1960].

This taxa has been identified as a subspontaneous one, along the railways, in the western parts of Iaşi city, in the neighborhoods of "Canta" and "Alexandru cel Bun" [leg. Sîrbu & Oprea, 2012] (Fig. 3, a-c).

Flora Europaea does not mention this plant species in Europe [BALL, in TUTIN & al. 1968, p. 106].

b) Alien species identified in new localities of Romania

Panicum miliaceum L. subsp. ***ruderale*** (Kitag.) Tzvelev, *Zlaki S. S. S. R.* (1976)
(Syn. *P. miliaceum* L. var. *ruderale* Kitag., *P. spontaneum* Lyssov ex Zhuk., nom. illegit.)

This wild subspecies of the (Proso) Millet or Weed-Broomcorm Millet has been described from China (Manshuria), as var. *ruderale*, in 1937 [KITAGAWA, 1937]. It has structures developed for an effective natural fruit dispersal at maturity, as well as the brittle spikelets (cariopses are falling off together with the glumes), in contrast to the grains of the subsp. *miliaceum* (under cultivation in Romania, only), which are not readily falling off at their maturity [LYSSOV, 1975; BOUGH et al. 1986].

In Europe, this subspecies is distributed in the wild, in: Italy (the earliest record in Europe, in 1888) [LIPPERT, 1984], Austria [MELZER, 1982, 1983, 1984, 1987, 1988; SCHOLZ, 1983], Germany [LIPPERT, 1984], Hungary [TERPÓ-POMOGYI, 1976], Ukraine [MOSYAKIN, 1991], Czech and Slovakia [JEHLÍK, 1986]; also, probably exist in the wild flora of Poland [FREY & al. 1981], Latvia [TABAKA & al. 1988], and Lithuania [GUDZHINSKAS, in SCHOLZ & MIKOLÁŠ, 1991].

On other continents, *Panicum miliaceum* L. subsp. *ruderale* is distributed in: U. S. A. and Canada [CARPENTER & HOPEN, 1985; CAVERS & BOUGH, 1985; MOORE & CAVERS, 1985; BOUGH & al. 1986; COLOSI & al. 1988], and various Asian countries [TZVELEV, 1976].

This infrataxa has been identified also in the wild in Romania, since the early 1978-1980, when Sakamoto and Kobayashi made two field investigations in here [SAKAMOTO & KOBAYASHI, 1982a, 1982b]. These two Japanese authors cited this subspecies growing along the road from Iaşi toward the Experimental Farm of Agronomical Institute.

We also identified this infrataxa in other localities, in the Eastern part of Romania, as:

– the village of Leţcani (Iaşi County), in maize fields (leg. Sîrbu, 15.09.2007);
– between the villages of Ursoaia and Horleşti (Iaşi County), in maize fields (leg. Oprea, 24.10.2012);
– the village of Horleşti, in maize fields (leg. Eliáš, Ferus, Sîrbu, 16.08.2011);

– the village of Tăcuta (Vaslui County), in maize fields (leg. Sîrbu & Oprea, 02.08.2009) (Fig. 4).

The genetic samples, analyzed on individuals from Slovakia, relieved next results: 2n=36, and 2n=c. 36 [SCHOLZ & MIKOLÁŠ, 1991].

These three subspecies of *Panicum miliaceum* L. identified in Romania′s flora could be discriminated by the next traits (Tab. 1):

Tab. 1. Infrataxa determination of *Panicum miliaceum* L. (cf. Scholz & Mikoláš 1991):

Specifications	subsp. ***ruderale***	subsp. ***agricolum***	subsp. ***miliaceum***
panicle	erect, with branches erect-patent, lax	relative densely, nodding	densely, nodding
glumes	falling off	persistent	persistent
fruits	falling off	falling off, smaller	persistent, greater

Oenothera parviflora L. *Syst. Nat.* ed. 10, **2**: 998 (1759)

(Syn.: *Oenothera cruciata* Nutt. ex G. Don)

Small-flowered evening-primrose is a herbaceous biennial or short-lived perennial species, up to 2 m high, originated in North America [RAVEN, in TUTIN & al. 1968], introduced in Europe early in 1871 (in England) [MIHULKA & PYŠEK, 2001]. It is an adventive plant in ex-Czechoslovakia, France, Germany, Netherland, Hungary, Italy, Norway, Poland [RAVEN, in TUTIN & al. 1968], Great Britany (rare nowadays) [ROSTAŃSKI, 1982; SELL & MURRELL, 2009], R. of Moldova [BURAC & MITITELU, 1995]. On other continents, it is spread in Asia, Southern Africa and New Zealand [HOCH & WAGNER, 2007].

It grows in open habitats, disturbed grounds [WEAKLEY, 2007], along the roads, from the sea level to ca 1000 m alt. (in China) [HOCH & WAGNER, 2007].

In Romania′s wild flora, *O. parviflora* was previously reported in some localities from Danube Delta (Tulcea County), Moldavia, Maramureş, and Transylvania [different authors, cited by SÎRBU & OPREA, 2011].

However, as it was shown in some references of the romanian literature [e.g. SÎRBU & OPREA, 2011a], the name of *O. parviflora* is an misapplied synonyme for *O. muricata*. This is a reason for uncertainity of the presence and chorology of these two species in Romania.

It is a newly identified species for the historical province of Oltenie, in the villages of Stolniceni and Marcea (Vâlcea County) [leg. Sîrbu & Oprea, 2012] (Fig. 5).

A reliable identification key for all species of *Oenothera* in European flora is presented in a paper of ROSTAŃSKI (1982), while an identification key for all alien species of *Oenothera* in Romania′s flora is given in SÎRBU & OPREA (2011a).

O. parviflora is a diploid plant species (2n=14) [CIOCÂRLAN, 2009].

Setaria faberi R. A. W. Herrm. *Beitr. Biol. Pflanzen*, **10**: 51 (1910) (as "*faberii*")

The giant foxtail (Chinese foxtail, Chinese millet, giant bristlegrass, nodding foxtail) is a neophyte grass, originated in East Asia. It is distributed as an alien plant in Northern and Central America, Central Europe and Russia [HITCHCOCK, 1950; ZHENG-YI & RAVEN, 1994; DARBYSHIRE, 2003; NURSE & al. 2009].

S. faberi entered accidentally into European flora in the beginning of the last century, being mentioned till now from Czech Republic (1961) [PYŠEK & al. 2002], Belgium (1977) [VERLOOVE, 2006], Austria (1981) [ESSL & RABITSCH, 2002], Ukraine [MOSYAKIN & YAVORSKA, 2002], Greece and Creta [BERGMEIER, in GREUTER & RAUS, 2007], European part of Russia (1985), Lithuania (1988), Germany and Sweden [NOBANIS], France, Italy, Portugal, Slovenia, Spain (naturalized), Azore Islands, Great Britany, and Slovakia (as casual) [DAISIE], etc.

In Romania, it has been mentioned for the first time by COSTEA (1996), from the harbour of Constanța and along the railway between Medgidia and Constanța (leg. 1993-1995). Further on, it has been identified, also, in Brăila county [CIOCÂRLAN, 2000]. Giving its resemblance to the species of *S. viridis* [HITCHCOCK, 1950; ZHENG-YI & RAVEN, 1994; NURSE & al. 2009; CIOCÂRLAN, 2009], it is possible that its distribution in Romania′s flora is much larger actually, but remained unnoticed in the recent times (*S. faberi* has scabrid leaves on both parts, with long hairs on the above part; *S. viridis* is scabrid on the upper part of the leaves, only [CIOCÂRLAN, 2009].

S. faberi is an annual, polyploid species (2n=36) [NURSE & al. 2009].

It is a newly identified species for the historical province of Moldavia (being identified in the railway station of Bacău town [leg. Sîrbu, Oprea, Ferus, 2012]) (Fig. 6), as well as for Buzău County (it has been identified in the railway station of Buzău town [leg. Sîrbu, Oprea, Ferus, 2012]).

Flora Europaea does not mention this plant species [CLAYTON, in TUTIN & al. 1980, p. 263-264].

S. faberi is a tetraploid species (2n=36) [CIOCÂRLAN, 2009].

Reynoutria* × *bohemica Chrtek et Chrtková, *Čas. Nár. Muz. Praha*, *Ser. nat*., **152**: 120 (1983)

It is a perennial species, of hybrid origin, between *R. japonica* and *R. sachalinensis* [WEBB, in TUTIN & al. 1964], relatively newly described as a taxa, from Central Bohemia (Czech Republic) by CHRTEK & CHRTKOVÁ (1983). Nowadays, this taxa is one of the most invasive alien plant in Europe [PYŠEK & al. 2002; MANDÁK & al. 2004; SÎRBU & OPREA, 2008, 2011b], as well as in North America [ZIKA & JACOBSON, 2003; BARNEY & al. 2006; FNA].

R. × *bohemica* is known for some time in Romania, having already been identified in dozens of localities in the historical provinces of Tranylvania, Muntenia, Moldavia, and Oltenia [SÎRBU & OPREA, 2011a], being designed as a recogniton species (together with *R. japonica*) for the synanthropic perennial vegetation of humid habitats (*Galio-Urticetea*), of type "*Fallopia japonica* agg. DC." [KOVÁCS, 2004, 2006; FENESI, 2004].

This species is largely distributed as invasive in Romania′s flora, as it has been stated in many papers [In: SÎRBU & OPREA, 2011a].

It is a newly identified taxon in the following counties of Romania:

– Bihor County: Ștei, Petrileni, Sudrigiu, Beiuş, Răbăgani, Băile Felix, and Vaşcău (leg. Sîrbu & Oprea, 2012);
– Sălaj County: Jibou and Gârbou (leg. Sîrbu & Oprea, 2012);
– Cluj County: Frăsinet, Valea Ierei, Cerc, Caps, Buru (leg. Sîrbu & Oprea, 2012], Dej [leg. Sîrbu, 2012);
– Caraș-Severin County: Oțelu Roşu and Rusca Montană (leg. Sîrbu & Oprea, 2012);

– Vâlcea County: Râmnicu Vâlcea, Stolniceni (Fig. 7), and Munteni (leg. Sîrbu & Oprea, 2012);
– Prahova County: Comarnic, Valea Sarului, Sinaia, Azuga, and Buşteni (leg. Sîrbu, 2012);
– Vrancea County: West of Focşani (at the crossroad towards Odobeşti, Câmpineanca and Vidra) (leg. Sîrbu, Oprea & Ferus, 2012);
– Bacău County: Căiuți (leg. Sîrbu, 2012).

Reynoutria sachalinensis (F. Schmidt) Nakai, in Mori, *Enum. Pl. Cor.* **135** (1922) (Syn.: *Polygonum sachalinense* F. Schmidt; *Fallopia sachalinensis* (F. Schmidt) Ronse Decr.)

Giant Knotweed or Sakhalin Knotweed is a native species to East Asia (Sakhalin Islands, Kurile, Hokkaido, Honshu, Ullung-do, Korea) [MANDÁK & al. 2004; WITTENBERG, 2005; PYŠEK, 2006; ALBERTERNST & BÖHMER, 2006], similar to *R. japonica* var. *japonica* in many aspects, but is more vigorous (4-5 m height), with leaves much larger (up to 43 cm long and 27 cm wide), cordate at base, with multicells hairs on the lower side [BAILEY & al. 1996; ALBERTERNST & BÖHMER, 2006; BARNEY & al. 2006, etc.].

It is nowadays a naturalised or invasive alien species in most of Europe [WITTENBERG, 2005; MANDÁK & al. 2004; ALBERTERNST & BÖHMER, 2006], as well as in North America [ZIKA & JACOBSON, 2003; WESTON & al. 2005]

In Romania, this species is less known, being introduced early in 1901 (at Herăstrău, in Bucharest) [GRINȚESCU, in SĂVULESCU, 1952]. It was reported, till now, as an alien plant, in some localities from Transylvania [ȚOPA, 1947; OROIAN, 1998, cited by KOVÁCS, 2006; SĂMĂRGHIȚAN, 2000, 2005], South-West of Romania (in Mehedinți County) [MATACĂ, 2005], and North-East of Romania (Iași County) [SÎRBU & OPREA, 2011a].

R. sachalinensis is a newly identified species in the following county:
– Caraş-Severin: the villages of Rusca Montană, Ruşchița (and along the road between these two villages) (Fig. 8, a-c), and Vama Marga (along the railways) [leg. Sîrbu & Oprea 2012].

Flora Europaea mention this species as naturalized from gardens as *R. japonica*, but much less frequently [WEBB, in TUTIN & al. 1964, 1993].

A reliable identification key for the species of *Reynoutria* from the Romania's flora is given in SÎRBU & OPREA (2011a).

Conclusions

All the taxa presented in this paper are alien plants (neophytes, xenophytes and hemerophytes). Some of them are new for the Romania's flora (as *Cenchrus longispinus* (Hack.) Fernald; *Panicum miliaceum* L. subsp. *agricolum* Scholz et Mikoláš; *Robinia neomexicana* Gray), while the others (*Panicum miliaceum* L. subsp. *ruderale* (Kitag.) Tzvelev; *Oenothera parviflora* L.; *Setaria faberi* R. A. W. Herrm.; *Reynoutria* × *bohemica* Chrtek et Chrtková; *Reynoutria sachalinensis* (F. Schmidt) Nakai) have been identified in other regions and counties of Romania.

Some of them have an invasive character in Romania (e. g. *Reynoutria* × *bohemica* Chrtek et Chrtková; *Reynoutria sachalinensis* (F. Schmidt) Nakai; *Cenchrus longispinus* (Hack.) Fernald), while the others could become invasive in near future (*Panicum*

miliaceum L. subsp. *ruderale* (Kitag.) Tzvelev; *Panicum miliaceum* L. subsp. *agricolum* Scholz et Mikoláš; *Robinia neomexicana* Gray).

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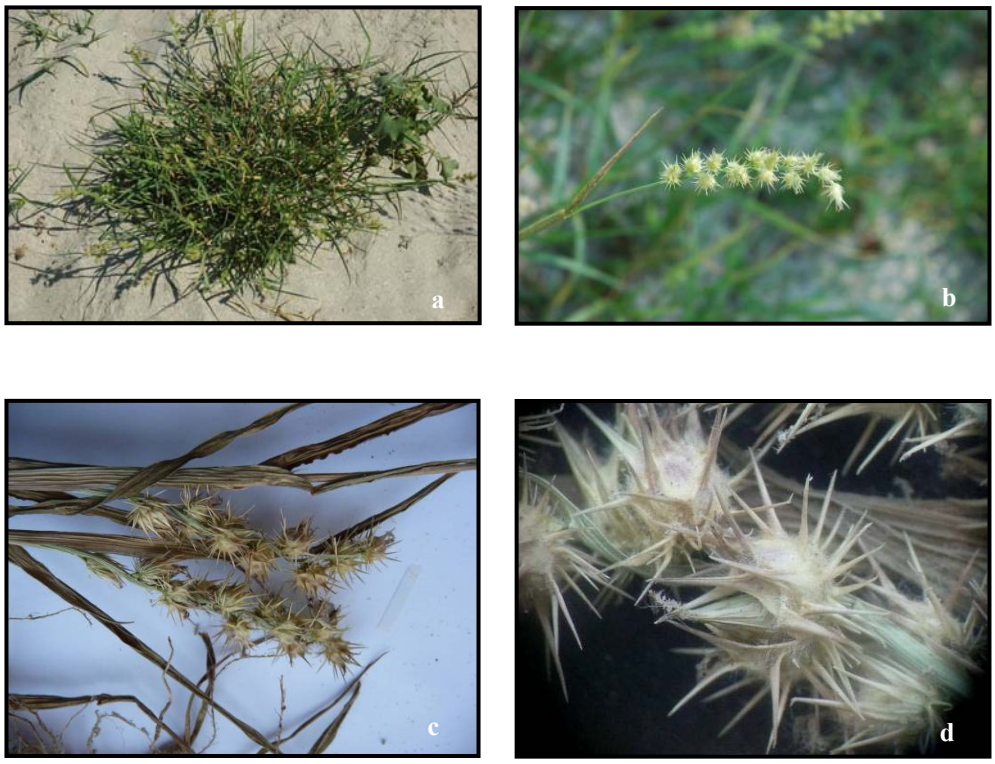

Fig. 1. *Cenchrus longispinus* (Hack.) Fernald at Mamaia (Constanța County), on sandy beaches
a. general habitus; b-c. panicle-like inflorescences; d. burs

Fig. 2. *Panicum miliaceum* L. subsp. *agricolum* Scholz et Mikoláš at Borzeşti (Bacău County), on fallow grounds

Fig. 3. *Robinia neomexicana* Gray var. *neomexicana* in Iaşi city
a. general habitus; b. shoots; c. racemes

Fig. 4. *Panicum miliaceum* L. subsp. *ruderale* (Kitag.) Tzvelev at Tăcuta village (Vaslui County), in maize crops

Fig. 5. *Oenothera parviflora* L. at Stolniceni village (Vâlcea County)

Fig. 6. *Setaria faberi* R. A. W. Herrm. in railway station of Bacău town

Fig. 7. *Reynoutria* × *bohemica* Chrtek et Chrtková at Stolniceni village (Vâlcea County)

Fig. 8. *Reynoutria sachalinensis* (F. Schmidt) Nakai between Rusca Montană and Ruşchița villages (Caraş Severin County)
a. general habitus; b-c. leaves

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THE COPĂCEL HILL FOREST, BETWEEN BĂLA AND ERCEA, A FUTURE RESERVE OF MUREŞ COUNTY

OROIAN SILVIA[1], COTOARĂ IONELA[1]

Abstract: The forest lies in the region known as the "Transylvanian Plain", on the Copăcel hill, between Băla and Ercea. The specific landscape of this region is characterized by medium altitude hills, with wide and soft slopes. In this forest, the presence of the *Delphinium simonkaianum* Pawł. var. *psilocarpum* (Simk.) Pawł species, a threatened endemic taxon, was reported in 1953. In 2011, this globally threatened taxon was identified, after 58 years, on the upper side of the Copăcel slope, in a mixed oak and hornbeam forest. These oak and hornbeam mixtures are the result of impacts exerted on oak forests. The identified association, *Melampyro bihariensis-Carpinetum* (Borza 1941) Soó 1964 em. Coldea 1975, has three distinct layers: the arborescent layer dominated by *Carpinus betulus* and *Quercus petraea,* along with *Quercus robur*, *Prunus avium*, *Acer campestre*, *Ulmus glabra*, etc., with good canopy cover (0.8-0.9); the shrub layer, represented by species such as: *Crataegus monogyna*, *Corylus avellana*, *Cornus mas*, *Ligustrum vulgare*, *Rosa canina*, *Sambucus nigra*, *Staphylea pinnata*, etc., is relatively poor in individuals, which are present particularly in forest clearings or at the edge of the forest. Grass synusia is well developed, sometimes forming an almost continuous cover (*Asarum europaeum*, *Convallaria majalis*, *Dactylis glomerata* ssp. *aschersoniana*, *Galium odoratum*, *Melampyrum bihariense*, *Stellaria holostea*, *Aconitum anthora*, *Aconitum moldavicum*, *Lilium martagon*, *Arum orientale*).

Key words: *Delphinium simonkaianum* Pawł. var. *psilocarpum* (Simk.) Pawł, rare plant, oak and hornbeam forest, Mureş County

Introduction

The Copăcel Forest is situated between the localities Băla and Ercea, in the Transylvanian Plain, a region characterized by medium altitude hills, with wide soft slopes, called "copârşaie" by the natives. It is from here that the species *Delphinium simonkaianum* Pawł. var. *psilocarpum* (Simk.) Pawł. was cited in Flora of Romania [SĂVULESCU (ed.), 1953].

Materials and methods

The list of the forest species was made based on trips in the field. For the nomenclature of the taxa, *Flora of Romania* [SĂVULESCU (ed.), 1952-1976] and *Flora Europaea* [TUTIN & al. 1964-1980, 1993] were used, and for the assignment of the oak and hornbeam forest to the type of habitat we used *Manual de interpretare a habitatelor Natura 2000 din România* [GAFTA & MOUNTFORD (coord.), 2008] as well as *Habitatele din România* [DONIŢĂ & al. 2005].

[1]UMPh - Tg-Mureş, Faculty of Pharmacy, Department of Pharmaceutical Botany, Târgu-Mureş, Gh. Marinescu Street 38, RO-540139, Târgu-Mureş – Romania, e-mail: oroianslv@yahoo.com

Results and discussions

The forest is a mixture of oak and hornbeam, being situated on the Copăcel hill between Băla and Ercea (Fig. 1). The oak mixed with hornbeam occupies a surface area of 24.1 ha, on a slope with north-western exposure and low grade inclination (15°). The substrate is formed by surface deposits derived from basic rocks, marl clays and limestone marl conglomerates. The dominant soils in the area are: brown eumesobasic, weakly acid, moderately humiferous soils, mesobasic soils with carbonates at the base of the profile, moderately to well supplied with organic nutrient substances [MAC, 1972]. The forest, private property, is of secondary origin, being anthropically conditioned. According to National Forest Administration **Romsilva** reports, the age of the trees is approximately 110 years. The forest flora is consisting of 124 cormophyte species.

The phytocoenoses were assigned to habitat **91Y0** ***Păduri dacice de stejar şi carpen*** [Dacian oak-hornbeam forests] CLAS. PAL.: 41.2C, [GAFTA & MOUNTFORD (coord.), 2008] **HdR 4124** [DONIȚĂ & al. 2005].

The identified association, *Melampyro bihariensis-Carpinetum* (Borza 1941) Soó 1964 em. Coldea 1975, has three distinct layers: the arborescent layer dominated by *Carpinus betulus*, and *Quercus petraea* along with *Quercus robur*, *Prunus avium*, *Acer campestre*, *Ulmus glabra*, *Populus tremula*, *Tilia cordata* etc., with good canopy cover (0.8-0.9); the shrub layer, represented by species such as: *Crataegus monogyna*, *Corylus avellana*, *Cornus mas*, *Ligustrum vulgare*, *Rosa canina*, *Sambucus nigra*, *Staphylea pinnata*, *Viburnum lantana* etc., is relatively poor in individuals, which are present particularly in forest clearings or at the edge of the forest. Grass synusia is well developed, sometimes forming an almost continuous cover (*Asarum europaeum*, *Convallaria majalis*, *Dactylis glomerata* ssp. *aschersoniana*, *Galium odoratum*, *Melampyrum bihariense*, *Stellaria holostea*, *Aconitum anthora*, *Aconitum moldavicum* (Fig. 7), *Lilium martagon*, *Arum orientale*) etc.

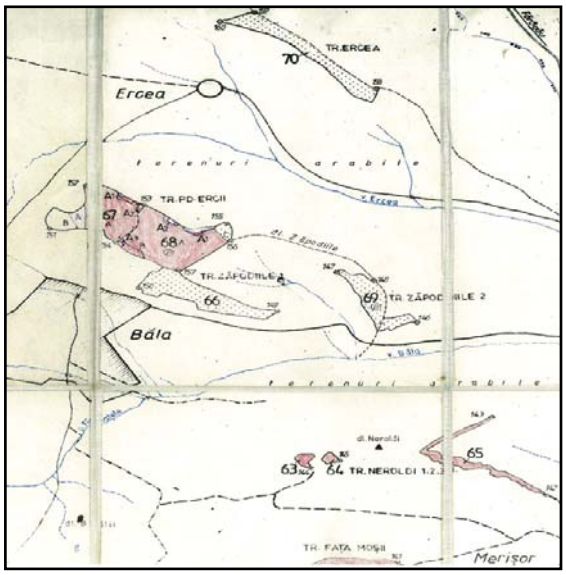

Fig. 1. Map of the location of Copăcel Forest

The majority of the plants (73%) are characteristic of the coenotaxa to which the phytocoenoses of the forest association are assigned [POP & COLDEA, 1987; SĂMĂRGHIȚAN, 2005]. Thus:

- **Lathyro hallersteinii – Carpinenion** (Boşcaiu 1979) Boşcaiu et al. 1982: *Carpinus betulus*, *Prunus avium*, *Pyrus pyraster*, *Tilia cordata*, *Carex pilosa*, *Dactylis glomerata* subsp. *aschersoniana*, *Festuca heterophylla*, *Galium schultesii*, *Helleborus purpurascens*, *Ranunculus auricomus*, *Stellaria holostea*, *Erythronium dens-canis*, *Vinca minor* etc.
- **Fagetalia** Pawł. in Pawł. et al. 1928: *Acer pseudoplatanus*, *Tilia platyphyllos*, *Rubus hirtus*, *Daphne mezereum*, *Aegopodium podagraria*, *Asarum europaeum*, *Ajuga reptans*, *Anemone nemorosa*, *Circaea lutetiana*, *Euphorbia amygdaloides*, *Galanthus nivalis*, *Galium odoratum*, *Galeobdolon luteum*, *Geranium robertianum*, *Isopyrum thalictroides*, *Lilium martagon*, *Luzula luzuloides*, *Maianthemum bifolium*, *Mercurialis perennis*, *Lathyrus vernus*, *Sanicula europaea*, *Salvia glutinosa* etc.
- **Querco – Fagetea** Br.-Bl. et Vlieger in Vlieger 1937: *Quercus petraea*, *Quercus robur*, *Acer campestre*, *Populus tremula*, *Pyrus pyraster*, *Cornus mas*, *Corylus avellana*, *Crataegus monogyna*, *Euonymus europaea*, *Ligustrum vulgare*, *Clematis vitalba*, *Ajuga reptans*, *Athyrium filix-femina*, *Brachypodium sylvaticum*, *Campanula trachelium*, *Cruciata glabra*, *Lathraea squamaria*, *Mycelis muralis*, *Poa nemoralis*, *Pulmonaria officinalis*, *Ranunculus ficaria*, *Scilla bifolia*, *Symphytum tuberosum*, *Tanacetum corymbosum*, *Viola reichenbachiana* etc.
- **Quercetalia pubescentis** Br.-Bl. (1931) 1932: *Cornus mas*, *Staphylea pinnata*, *Campanula persicifolia*, *Clinopodium vulgare*, *Convallaria majalis*, *Melittis melissophyllum*, *Polygonatum odoratum*, *Rosa canina*, *Sedum maximum*, *Stachys officinalis*, *Trifolium medium*, *Vincetoxicum hirundinaria* etc.

The analysis of the phytocoenoses according to the main ecological indices (Fig. 3) shows that the association has a mesophilic (63.70%), micro-mesothermal character (74.19%) and a predominantly acid-neutrophilic (37.09%) and weakly acid-neutrophilic (35.48) soil reaction. The predominant bioforms (Fig. 4) are hemicryptophytes (54.83%), phanerophytes (18.54%), followed by geophytes (16.93%). From a chorological point of view, the predominance of European species (17.74%) is found, along with a great number of Eurasian (16.93%), European-Caucasian (21.77%), Eurosiberian (12.99%) and Circumboreal (8.87%) elements. In addition to these, oak-hornbeam forests are contaminated by a significant number of Paleotemperate elements (7.25%) (Fig. 5). Regarding the distribution of polyploidy levels (Fig. 6), diploid species are predominant (64.51%), followed by polyploid species (32.25%).

In this forest, the presence of the species *Delphinium simonkaianum* Pawl. var. *psilocarpum* (Simk.) Pawł. (Fig. 2) [SĂVULESCU TR. (ed) 1953, Flora RPR, vol. II], an endemic and threatened taxon (globally threatened – 1997 IUCN Red List of Threatened Plants), was reported in 1953. The scientific name of the species was given in the honor of the great botanist *Lajos Simonkai* (1851-1910), the author of the work *Enumeratio Florae Transsilvanicae* (1886).

In 2011, this taxon was reported again, after 58 years, on the top of the Copăcel slope (on 28.06.2011, at 509 m altitude, lat. 46°43', long. 24°30', access to the area from DN 15 Târgu-Mureş-Reghin, DJ 153 B Târgu-Mureş-Dumbrăvioara-Glodeni-Fărăgău).

The species was assigned to:
Tracheophyta superdivision,
Spermatophyta division,
Angyospermophytina subdivision,
Dicotyledoneae class,
Ranunculales order,
Ranunculaceae family.

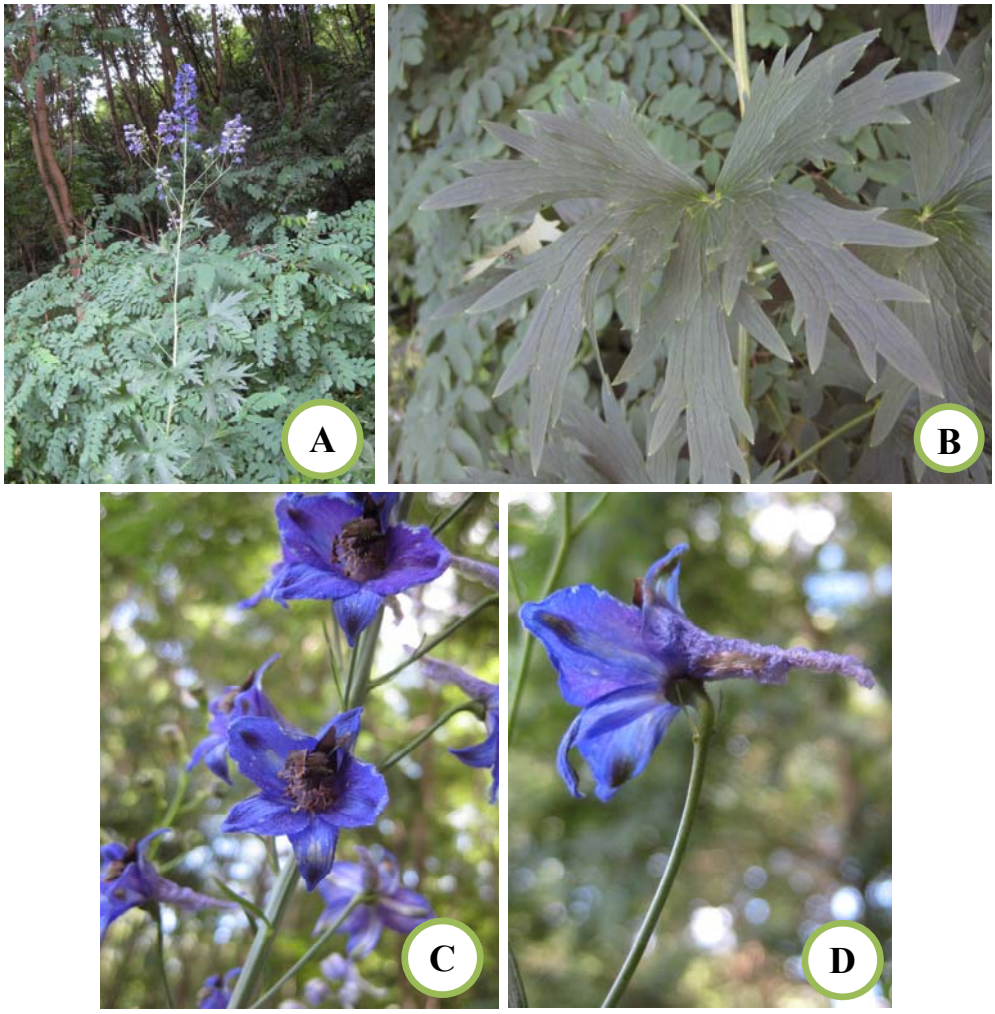

Fig. 2. A – *Delphinium simonkaianum*; B – leaf; C – inflorescence; D – flower.

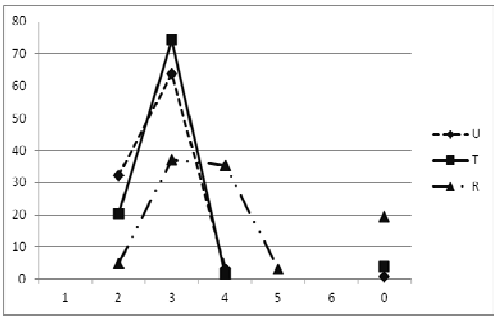

Fig. 3. Ecological indices of *Melampyro bihariensis – Carpinetum*

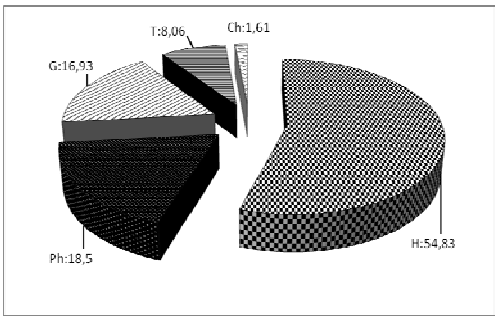

Fig. 4. Bioform spectrum of *Melampyro bihariensis – Carpinetum*

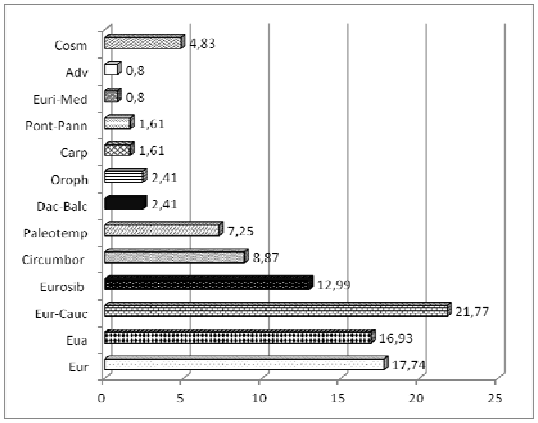

Fig. 5. Floristical elements *Melampyro bihariensis – Carpinetum*

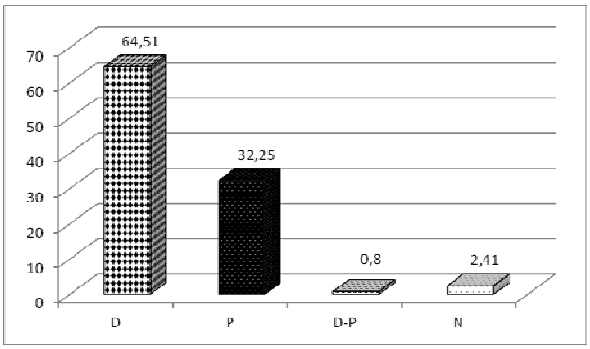

Fig. 6. Caryological spectrum of *Melampyro bihariensis – Carpinetum*

Regarding the **chorology of the species**, we mention some data provided by some specialized Romanian institutions:

IBB – mentions 24 herbarium sheets with the *Delphinium simonkaianum* Pawł. taxon, of which:

- 17 herbarium sheets from AB county (Piatra Cetii, Rachiş, Colţeşti-Piatra Urdaşului) collected by Ghişa E., Nyárády E.I. and Gergely I., in, 1938, 1960 (source IAŞI, BCHM, GLHM, PTHM, SMHM, TMMJ, PLHM, BV, ICAS, CJ);
- 1 sheet from BN county – Corongiş, collected by Baumgarten (1826, source SB);
- 1 sheet from BH county – Stâna de Vale, collected by Borza A. and Morariu I. (1936, source PLHM);
- 1 sheet from VL county – Albu Mountains – Piatra, collected by Ciurchea M. (1960, source CL);
- 1 sheet from IS county – Iaşi – Copou, collected by Marin E., Ursu E. (1968, source BCHM);
- 1 sheet from AG county – Dîmbovicioara Gorge, collected by Vlaicu N. (1978, source TMHM);
- 1 sheet from HD county – Hunedoara, collected by Bichigeanu (1961, source SIB);
- 1 sheet from DB county – Ialomiţa Valley, collected by Moşneaga M. (1970, PLHM).

The **HERBARIUM OF "BABEŞ-BOLYAI" UNIVERSITY CLUJ-NAPOCA**:

- 3 sheets with the site of collection in Alba county, Piatra Cetii and Colţeşti-Piatra Urdaşului, collected by Nyárády E.I. and Gergely I.
- 1 sheet with the site of collection in Cluj county, Turda district.

We mention that the plants of two herbarium sheets have their nomeclature changed from *D. elatum* and *D. intermedium* to *D. simonkaianum* (rev. Nyárády E.I.).

The **HERBARIUM OF THE FACULTY OF PHARMACY, TÂRGU-MUREŞ** also has a herbarium sheet with *Delphinium simonkaianum*, collected by E. Ghişa and E. I. Nyárády in 1938, from Piatra Cetii (AB).

The **"E. NYÁRÁDY" HERBARIUM OF THE NATIONAL BRUKENTHAL MUSEUM SIBIU** has several herbarium sheets collected by Nyárády E.I. with two *Delphinium* spp.: *Delphinium simonkaianum*, collected in AB county – Piatra Cetii, 1936 and *Delphinium elatum,* collected in 1917 on the Copăcel hill between Băla and Ercea. The nomenclature was changed by **W. Mucher** (Graz, 1992) to *Delphinium simonkaianum* var. *simonkaianum* (the species with the site of collection of interest for us).

The literature also reports other places where the species is present: **HR** – Tulgheş, Pietrele Roşii, **AB** – Feneş Valley, **CS** – Domogled Mt., **MH** – "Cazane", etc. [OPREA, 2005].

Other rare, endangered plants were also identified in the forest: *Fritillaria orientalis* (Fig. 8), *Galanthus nivalis*, *Adonis vernalis*.

Fig. 7. *Aconitum moldavicum*

Fig. 8. *Fritillaria orientalis*

The surroundings of the localities Băla and Ercea are also of particular scientific interest because on the sunny slopes in the proximity of the forest, a number of threatened European plant species: *Crambe tataria* (HD An IIb)*, Echium maculatum* (HD An IIb), as well as endemic [BELDIE, 1967] or threatened species, found in national Red Lists [OLTEAN & al. 1994; BOŞCAIU & al. 1994]: *Aconitum moldavicum*, *Salvia transsylvanica*, *Dictamnus albus*, *Orchis morio* etc., were identified.

This is not surprising because in the proximity of the two localities lies the Fărăgău Lake, the last natural lake in the Transylvanian Plain, which is declared a nature reserve.

Given that the Copăcel Forest is an area of botanical interest and has not yet been declared a botanical reserve, we propose to local authorities to take into consideration our suggestion that the identified area become a botanical reserve of scientific interest, where a number of rare plants in Romania or Europe can be protected in order for these beauties of the flora of Mureş county to be saved and preserved.

Abreviations:

– Counties:

AB	Alba
AG	Argeş
BH	Bihor
BN	Bistriţa Năsăud
BV	Braşov
CJ	Cluj
CS	Caraş-Severin
DB	Dâmboviţa
HD	Hunedoara
HR	Harghita

IS	Iaşi
MH	Mehedinţi
SB	Sibiu
VL	Vâlcea

– Herbariums:

BCHM	Herbarium of Bacău Museum
GLHM	Herbarium of Galaţi Museum
ICAS	Forest Research and Management Institute
PLHM	Herbarium of Ploieşti Museum
PTHM	Herbarium of Piatra Neamţ Museum
SMHM	Herbarium of Satu Mare Museum
TMMJ	Herbarium of Timişoara County Museum
IBB	Institute o Biology Bucharest

– HD An IIb - Habitats Directive, Annex IIb
– IUCN – *International Union for Conservation of Nature*

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ROCKERY AREA FROM BIOLOGICAL SECTION OF "ANASTASIE FĂTU" BOTANICAL GARDEN

ADUMITRESEI LIDIA[1]

Abstract: Rockery theme area from Biological Section groups a large variety of plants which are originating from different parts of the world and with different environmental requirements. There are presented both species with significant result during last years and those species which were not accustomed to physical and geographical conditions.

Keywords: casmophyte, rockery, Biological Section

Introduction

Rockery is one of the most attractive area both for public and teaching interests of Botanical Garden because it representing a space used for "ex situ" preservation.

Rockery from Biological Section has the main theme to illustrate some aspects of plants evolution which is an important subject because of scientific and aesthetic reasons.

Materials and methods

In according to the original theme (established during the '70s), we keep the collections that were well acclimatized to the local environment conditions of this place. During the last years, our concerns were focused on the improvement of plant funds both from quantitatively and especially qualitatively point of view.

The fossiliferous calcareous grit stones from Middle Sarmatian Age, from Repedea (Iaşi County) are populated with plants which illustrating aspects of the flower evolution from actinomorphic to zygomorphic symmetry plan or from dialipetalous to gamopetalous flowers.

In the group of rockeries, located near by the garden axis consists of sedimentary rocks from the quarry Mold (Câmpulung Moldovenesc – Suceava County), are presented few evidences of morphological evolution for some plants (roots, stems and metamorphosed leaves, progressive and regressive metamorphoses of flowers) and typical plant species for rockery areas (Plate I – a, b, c).

Results and discussions

To achieve the proposed goals, we pursued the following main directions:

- correlations between typical plants choice and the context (theme, rock type, degree of sunburn);

[1] Botanic Garden "Anastasie Fătu" Iaşi, Dumbrava Roşie str. no. 7-9, Iaşi – Romania, e-mail: lidia.adumitresei@yahoo.com

- ensuring of abiotic conditions as close to the specific requirements of chosen plants (it is already known the difficulties of ensuring that environmental conditions necessary for a large variety of plants are not neglected);
- creating a "whole" which has to accomplish aesthetic requirements reflected in harmony between flower and/or leaves colours, flowering period extension by using some new typical infrataxa created for alpine species which were been obtained through modern methods and acclimatized to cultivation conditions.

For a right answer to those mentioned problems, we undertaken the following:

- it maintain existing plant funds on the initial location, with a improved substrate, if necessary with calcareous soil (from Repedea, Drăgoiasa and Hagieni), fibrous peat (from Poiana Stampei), brown and blond peat (from Belarus), red soil with iron (from Albeşti - Constanța), sand with large grain (from plain Siret River), soil of leaves local product and soil of celery (from Rediu – Iaşi).

Species that have survived very well in these local climatic conditions and which maintaining the number of individuals are following: *Alyssum saxatile* L., *Arabis caucasica* Willd. ex Schlechtend. *'Rosea'*, *Arabis alpina* L. subsp. *alpina*, *Aubretia x hybriden*, *Anemone hupehensis* (Lemoine) Lemoine *'September Charme'*, *A. sylvatica* L., *Aster caucasicus* Willd., *Ajuga reptans* L., *Anchusa azurea* Mill., *Betonica officinalis* (L.) Trev., *Cerastium alpinum* L. subsp. *lanatum* (Lam.) Aschers. et Graebn., *Euphorbia myrsinites* L. subsp. *myrsinites* (Plate I - d), *Geranium macrorrhizum* L. (Plate I - e), *Helleborus odorus* Waldst. et Kit., *H. purpurascens* Waldst. et Kit., *Hosta sieboldiana* (Hook.) Engl., *Hypericum calicinum* L., *Iberis sempervirens* L., *Pennisetum alopecuroides* (L.) Spreng, *Polemonium caeruleum* L., *Potentilla recta* L., *P. reptans* L., *Salvia ringens* Sibth et Sm., *Sedum acre* L., *S. album* L., *S. hispanicum* L., *S. spurium* M. B., *S. stoloniferum* S. G. Smet., *S. telephium* L., *Stachys byzantina* K. Koch, *Veronica austriaca* L., *Vinca minor* L.

Taxa that have been maintained, but which registered a decreasing of individuals number with a positive responds are the following: *Ajuga reptans* L. *'Variegata', Hepatica transsilvanica* Fuss, *Lychnis chalcedonica* L., *L. coronaria* (L.) Desr., *L. viscaria* L., *Paeonia tenuifolia* L., *Phlox subulata* L., *Potentilla chrysantha* Trevir., *P. rupestris* L., *Pulsatilla vulgaris* Mill.

- the taxa from spontaneous flora, which had a good performance in culture conditions during the last 2-3 years, are the following: *Acinos alpinus* (L.) Moench., *Adonis aestivalis* L., *Asperula carpatica* Morariu, *Bassia prostrata* (L.) A. J. Scott, *Epimedium alpinum* L. (Plate I - f), *Euphorbia glareosa* Pallas ex Bieb. subsp. *dobrogensis* (Prodan) Ciocarlan, *Hepatica transsilvanica* Fuss, *Iris brandzae* Prodan, *Poa bulbosa* L., *Silene nutans* L. subsp. *dubia* (Herbich) Zapal, *Satureja caerulea* Janka, *Teucrium pollium* L., *Thymus balcanus* Borbas, *T. glabrescens* Willd., *T. pulegioides* L., etc.;
- taxa from spontaneous flora which not resist in our conditions are the following: *Campanula carpatica* Jack (Plate II - a), *Dryas octopetala* L., *Leontopodium alpinum* L. (Plate II - b), *Minuartia laricifolia* (L.) Schinz ex Thell.;
- plants from sowing (seeds from the international exchange of seeds and spontaneous flora) on some substrates with their specific ecological requirements and which had a good performance in vegetation process during the last 2-3 years, are the following: *Achillea ptarmica* L., *Alyssum murale* Waldst. et Kit, *Alyssum saxatile* L. *'Goldklügel'*, *Alyssum saxatile* L. *'Compactum'*, *Andryala integrifolia* L., *Anemone blanda* Schott et

Kotschy, *A. multifida* Poir. *'Rubriflora'*, *Aquilegia caerulea* James, *A. nigricans* Baumg., *Arabis soyery* Reut. et Huet subsp. *subcoriacea* (Gren.) Breistr., *Armeria maritima* (Mill.) Willd., *Asphodeline lutea* (L.) Rchb., *Aster alpinus* L. var. *dolomiticus*, *Azorina vidalii* (H.C.Wats.) Feer, *Saponaria ocymoides* L., *Thymus* × *citriodorus* (Pers.) Schreb., *Gentiana sinoornata* Balf., *Campanula glomerata* L. *'Alba'*, *C. glomerata L. 'Superba'*, *C. poscharskyana* Degen (Plate II - c), *C. rhomboidalis* L., *C. rotundifolia* L. subsp. *rotundifolia*, *C. collina* M. B., *C. cohleariifolia* Lam., *C. cohleariifolia* Lam. *'Weiss'*, *C. carpatica* Jack var. *turbinata*, *C. carpatica* Jack *'Deep Blue Clips'*, *C. carpatica* Jack *'White Clips'*, *C. latifolia* L., *C. ochroleuca* Mevr. V. Vollenhove, *C. punctata* Lam., *C. portenschlagiana* Schult., *C. punctata* Lam. *'Cherry Bells'* (Plate II - d), *C. thyrsoides L.*, *Cerinthe major* L. *'Atropurpureus'*, *Auebrieta* × *hybriden 'Royal Red'*, *Codonopsis clematidea* (Screng. ex Fisch. et Mey) C. B. Clarke, *Hypericum montanum* L., *Jasione montana* L., *Lagoecia cuminoides* L., *Papaver bursrei* Crantz, *Delosperma davyi* N. E. Br., *D. herbeum* N. E. Br., *Dianthus deltoides* L., *D. arenarius* L., *D. knapii* (Pant.) Aschers et Kan ex Borb., *D. carthusianorum* L., *D. spiculifolius* Schur, *D. superbus* L., *Gypsophylla cerastioides* D. Don, *G. muralis* L., *G. repens* L., *Legousia speculum-veneris* (L.) Chaix, *Leontopodium alpinum* Cass, *L. alpinum* Cass *'Materhorn'*, *L. linearifolium* Hand.-Mazz., *L. himalayanum* DC., *L. palibinianum* Beauverd, *Viola sororia* Willd., *V. labradorica* Schrank, *Aquilegia canadensis* L., *A. caerulea* James, *A. flabellata* Sieb. et Zucc. var. *pumila* (Huth) Kudo (Plate II - e), *Achillea ageratifolia* (Sibth et Sm.) Boiss., *Cymbalaria muralis* Ph. Gartn., *Gentiana septemfida* Pall. var. *lagodechiana* Kusn., *G. sinoornata* Balf. '*Alba*', *Sanvitalia procumbens* Lam., *Scutellaria alpina* L., *Silene zawadzkii* Herbich, *S. pendula* L. *'Compacta'*, *Hutchinsia alpina* (L.) R. Br., *Petrorhagia saxifraga* (L.) Link, *Platicodon grandiflorum* (Jacq.) A. DC., *Polemonium caeruleum* L. *'Alba'*, *Pulsatilla alpina* (L.) Delarbre, *P. halleri* (All.) Willd.

- taxa from trades with living plants or seeds material which have good performances and which expanding given space and number of individuals, are the following: *Anemone blanda* Scott et Kotsky *'Blue star'* (Plate II - f), *Armeria maritima* (Mill.) Willd. *'Leucantderosa'*, *Campanula cochleariifolia* Lam. *'Elisabeth Olivier'*, *C. portenschlagiana* Schult *'Alpines'*, *Carex buchananii* Berggr., *Festuca valesiaca* Schleich. ex Gaud. *'Glaucantha'*, *Fragaria vesca* L., *Hebe* × *andersonii* (Lindl. et Paxt) Cock., *Iris pumila* L., *Lamium maculatum* L. *'Silver Backon'*, *Lysimachia nummularia* L. *'Goldklügel'*, *Phlox subulata* L. *'Millstream Daphne'*, *Thymus vulgaris* L. *'Foxley'*, *Sedum lineare* Thunb. *'Lineamaculata'*, *Sempervivum arachnoideum* L., *S. tectorum* L., *Sempervivum* L. *'Raspbery Ice'*.

Conclusions

Specific activity for "ex situ" preservation representing a continuous process that we have to resume multiplying plants each year with other environmental requirements to achieve a sufficient required reserve stocking both for populate of rocks and botanical researches.

In the Biological Section are cultivated over 150 taxa belonging to 63 genera and 28 botanical families with different ecological requirements, which vegetate well in our conditions. Our efforts are rewarded by the beauty and tenderness of these special plants.

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Plate I

a, b, c, – overview; d – *Euphorbia myrsinites* subsp. *myrsinites;* e – *Geranium macrorrhizum*; f – *Epimedium alpinum*

Plate II

a – *Campanula carpatica*; b – *Leontopodium alpinum*; c – *Campanula poscarskyana*;
d – *Campanula punctata 'Cherry Bells'*; e – *Aquilegia flabellata* var. *pumila*;
f – *Anemone blanda*

100 YEARS SINCE THE BIRTH OF THE BOTANIST CONSTANTIN DOBRESCU (1912-1989)

Constantin Dobrescu was born on August 1, 1912 in Gura Sărăţii village (Merei Commune, Buzău County), in a peasants family (with six children) Vasile and Maria Dobrescu. He followed the primary school in his native village and then the gymnasium in Pătârlagele City. In 1933 he graduated from high school at "Bogdan Petriceicu Haşdeu" in Buzău City, and in 1937 he graduated the Faculty of Natural Sciences, at University of Iaşi. Until 1939 he conducted a military service and Reserve Officers School, after that being concentrated on the Eastern Front in the Second World War.

He started the botanist career as assistant at the Botanical Garden of Iaşi, as it appears in the Seed Catalogs of this institution in 1947 and 1948. Being a teacher at disciplines of Systematic Botany and Phytogeography he often used as teaching material for students, plants from the Botanical Garden. Since 1942 follow the university career, being appointed assistant professor at the Department of Botany, University of Iaşi (led at that time by Professor Dr. Constantin Papp), is advanced lecturer in 1950, associate professor in 1959 and in 1977 has retired. In 1974, Constantin Dobrescu held at the University of Bucharest, the PhD thesis entitled "Studies on flora and vegetation of Bârlad River upper basin", thesis prepared under the guidance of Professor Dr. Traian Ştefureac.

In 35 years of didactic activity in higher education from Iaşi, Constantin Dobrescu lectured on systematic botany and general botany, phytogeography, phytopathology, crop plants, modern research methods etc, both at the University of Iaşi and the Pedagogic Institute in the same city. He leaded the students practice in the field and guide over 50 license theses. Distinguished teacher, Constantin Dobrescu was appreciated by students for its clear, accurate and systematic courses, as well as thorough knowledge of the flora in the field.

Simultaneously with the teaching activity, Constantin Dobrescu has carried out an ample and efficient work in direction of nature protection in Moldova, as a member of the Nature Preservation Subcommittee of the Romanian Academy, Iaşi Branch and President of the Iaşi Branch of Biological Sciences Society in Romania. Thus, proposed and contributed to the designation of some nature reserves in Moldova, describing the flora and vegetation, such as: *Uricani Forest* (Iaşi County), *Georza-Dobrovăţ Lakes* (Iaşi County), *Hârboanca-Brăhăşoaia Forest* (Vaslui County), *Bălteni Forest* (Vaslui County), *Glodeni-Negreşti grasslands* (Vaslui County) and *Burcel Hill* (Vaslui County). He contributed to the

enrichment of the herbarium collections of national heritage of *Flora Romaniae Exsiccata* (20 species) and *Flora Moldaviae* et *Dobrogeae Exsiccata* (34 species), and contributed to seeds exchange collected from native plants by seeds catalog (*Delectus Seminum*) edited by Botanical Garden of Iaşi.

Constantin Dobrescu published about 100 studies, articles and scientific notes on the flora and vegetation of different regions of the country: the upper basin of Bârlad River, Nişcov-Buzău region and Red Lake - Harghita. As taxonomist Constantin Dobrescu was one of the 28 Romanian botanists who have edited the monumental botanical work *Flora României*, contributing to the drafting of 10 taxa of the *Poaceae* family (*Gramineae*).

Also, Constantin Dobrescu worked to describe some botanical reserves in collective works: *Monumente ale naturii din România* – Natural Monuments in Romania (1965) and *Călăuza monumentelor naturii din Moldov*a – Guide to Natural Monuments of Moldova (1969), and the elaboration of monographs *Pajiştile naturale din Moldova* - Natural Grasslands in Moldova (1956) and *Păşunile şi fânețele din R. P. Română* – Pastures and grasslands from RP Romania (1963). In collaboration, he published in German language the syntheses work *Conspectul asociațiilor vegetale din Moldova* – Conspectus of plant associations in Moldova, in 1972 (with Attila Kovács, currently Professor at the West University, Faculty of Natural Sciences and researcher at the Institute of Biology - Department of Botany, Zsombathely, Hungary).

Besides taxonomy, chorology and phytocoenology research, Constantin Dobrescu has studied the halophilic plant ecology, phytoteratology and mycology. In the mycology field he has discovered some fungal species and has described as new to the science, the species *Haplographium hispidulum* Dobrescu et Volcinschi (1961).

In the field of taxonomy and chorology of cormophytes in Moldavia, Constantin Dobrescu brought an important contribution by describing several species and hybrids new for the science: *Agrostis moldavica* Dobrescu et Beldie (1965), *Asperula moldavica* Dobrescu (1954), *Quercus* × *speciosa* Dobrescu et Beldie (1960), *Quercus* × *barnova* Georgescu et Dobrescu (1966), *Cirsium* × *moldavicum* Dobrescu et E. I. Nyárády (1964), all this taxa being maintained in *Romanian Flora* and *Flora Europaea* works. He identified some species as new for our country: *Schkuria abrotanoides* Roth, *Verbascum glanduligerum* Velen., *Agropyron orientale* Roem. et Schult., as well as 25 new taxa in the flora of Moldavia.

In phytocoenology field Constantin Dobrescu has described 18 coenotaxa new to the science, such as *Irido-Sietum latifoliae* Dobrescu et Vițalariu 1970 and *Galegetum officinalis* Dobrescu et Vițalariu 1981. He mentioned new choronyms and descriptions for more than a hundred plant associations, some of which are mentioned for the first time in Moldavia vegetation.

Should also be mentioned his concerns for the inventory and phenology of ornamental plants in parks and gardens, the mistletoe attacks, the reporting of some harmful plants for fisheries, mapping of the weeds in crops, forests and pastures mapping etc.

Being familiar with the botanical literature, Constantin Dobrescu has elaborated articles about "Society of Physicians and Naturalists of Jassy", about Flora of Moldavian principality – *Flora principatului Moldovei* (by J. Szabo), about Herbarium of the botanist Constantin Petrescu from Iaşi, about the scientific work of Professor Constantin Papp and about conferences of geobotany and some reviews of *Flora Europaea*, Hungary Flora (by R. Soó), etc.

He held the position of editorial secretary of the Scientific Annals of University of Iaşi - Biology series, since 1963.

A particular aspect of the life of Constantin Dobrescu is his involvement in the destiny of Iaşi Botanical Garden. Thus, based on the Order of the Ministry of Education no. 74.809/1960 on 1 August 1960 was convened a Commission consisting of Vice-rector Elena Jeanrenaud, Vice-dean Pierre Jeanrenaud, Associate Professor Constantin Papp, Associate Professor Constantin Burduja, Associate Professor Constantin Dobrescu and Engineer T. Vasilescu, which reached the following conclusions: because the botanical garden located near the "Alexandru Ioan Cuza" University could no longer meet the requirements of teaching and research, the Fora have provided a new location for future Botanical Garden. This terrain, located in the northwest of Iaşi city, behind the Exhibition Park had displays a diversified relief with slopes crossed by a transversal valley (Bădărău Valley). The surface was evaluated, first at about 60 ha. In the basement there is a groundwater that can be collected. It is also stipulated to be built greenhouses in the area of 5,000 square meters, all the actual outside sections, and a gradual work until 1970.

On the background of this activity, Constantin Dobrescu was appointed on 1 June 1962, Director of the the Botanical Garden of Iaşi. When he held this position, made available by Professor Constantin Burduja withdrawal, he already knew many of the problems to be solved and "for almost 1 year with diligent work he led the destiny of this institution and caring attention that plants material can reach in good condition the Garden from Copou hill". Even if he briefly led the activity of the Botanical Garden of Iaşi (until 1 March 1963), he has shown real leadership qualities and an openness to new challenges, such that the botanical garden to be moved in actual location.

Constantin Dobrescu died in Iaşi, December 22, 1989, and was buried in the Eternity Cemetery of Iaşi (parcel 31 / I, row 15, place 22).

Through the multitude of issues raised with scientific evidence, Constantin Dobrescu is an example of popular teacher, multilateral researcher, with a competent critical spirit, admired both by the students and his collaborators.

Adrian OPREA, Cătălin TĂNASE, Ana COJOCARIU

BOOK REVIEW

CULIȚĂ SÎRBU, ADRIAN OPREA, *Adventive plants species in Romania's flora*, "Ion Ionescu de la Brad" Publishing House, Iaşi, 2011, 733 p., 142 photos, 1584 ref.

At the end of year 2011 was published at the "Ion Ionescu de la Brad" Publishing House within the Agricultural and Veterinary Medicine University, the book *Adventive plants species in Romania's flora*, written by two botanists from Iaşi, Culiță Sîrbu and Adrian Oprea. Published in very good technical condition, the book contains a lot of data on non-native plant species entered over time in Romanian territory.

In the introduction chapter of this volume, the authors clarify terms which are being widely used in botanical foreign or Romanian literature, such as adventive plants species, synanthropic plants (anthropophytes), archaeophytes, neophytes, occasional species, naturalized, invasive etc.

In the volume's pages there are extensively treated, in systematic order, 671 species of adventive vascular plants mentioned from the country's territory, specifying, for each species, the following aspects: phylum, class, subclass, order, family, genus and species, scientific name (including the most common synonyms), popular name, geographical origin, general spreading area, short historical considerations on their introduction in Europe and Romania, historical, chorological, biological and phytocoenological aspects, the invasive character, their impact on natural ecosystems, agriculture or other economic sectors and human health, recommendations for management of invasive species, their spreading in Romania based on literature data and field surveys of the authors in the last 10 years. For each species, the invasive capacity, the potential and actual effects on natural and agricultural ecosystems, the impact on the economy and human health are emphasized.

Other issues are focused on the origin of the taxon, the global areal of the species, its first records in Europe and Romania. Then, the environmental requirements, preferred habitats which are in many cases invaded are presented. At the end of each sheet are presented the choronyms where the species was cited by various authors or even identified by the book's authors in the Romanian flora.

For certain species, some taxonomic considerations are made, the authors composing original identification keys of the related species, more difficult to identify, present in Romania's flora, for example *Azolla*, *Reynoutria*, *Oenothera*, *Amaranthus* etc. genera.

For many species which have become invasive in our country the possibilities of their control are discussed.

Although an extensive bibliography has been used, in some species cases it was not possible a detailed overview of all these aspects. It remains in the responsibility of the authors for any reprinting of this material in the future.

The authors indicate that, of the 671 adventive plant species inventoried in Romania at the time of this volume editing, a total of 112 species are considered invasive, due to their high capacity to spread in nature and, in some cases, to their proved negative impacts on natural biodiversity, economy and human health.

The volume includes 142 original color photographs of the most common species of adventive plants in our country and the localities where the photographs were taken.

The bibliography contains 1584 titles, older and actual, as well as various internet sites addresses consulted. Each paper/website is cited/quoted in the volume. Wherever was possible, the authors intended that the references cited for the species treated in this volume to be the primary ones, and only where it was not possible, data were taken by other authors.

The extensive work of both authors is the result of a long and hard activity of many coronyms registrations, of search and identification of the primary sources of citation for the species in the literature, of systematization and standardization of information etc., all with a critical look on each taxon included in this volume.

Taking into account the above, we believe that this volume will contribute to the information the scientific community and specialized institutions on the potential or on the actual impact of the invasion of adventive plants. It may guide also the development of certain policies at national level in order to prevent and combat the plant invasion phenomenon which will ensure that the principles set out in various international conventions to which Romania is a part are respected (*Convention on the Conservation of European Wildlife and Natural Habitats* – Bern, 1979; *European Strategy on Adventive Invasive Species* - Bern, 2002, etc.).

Aspects of migration history of the adventive species in Romania, the current geographic spreading, their impact on natural biodiversity, economy and human health etc., which are treated in this book, can concur to the satisfaction of the need for knowledge of those interested in plant biology, and to the enrichment of general knowledge of the public.

The paper is ending with the alphabetical index of scientific and popular names of species included in ample volume of the two authors.

Ion SÂRBU

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References
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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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