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PASSIFLORA CAERULEA L. TREATED WITH *TRICHODERMA* PLANT BIOSTIMULANTS CONSORTIUM. MORPHO-ANATOMICAL CONSIDERATIONS

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Abstract: Plant biostimulants are an emerging category of inputs into technologies for plant cultivation, which activate plant metabolism and nutrient use efficiency. A microbial plant biostimulants consortium was applied on *Passiflora caerulea* L., a medicinal and nutraceutical plant grown in greenhouse conditions. The treatments were applied during *P. caerulea* vegetation, as a foliar treatment with a *Trichoderma* consortium suspension of 10^8 cfu/ml, equiv. to 10^{13} spores/ha. The treatment determined significant quantitative changes on morpho-anatomical features, on the leaf lamina (lamina with 10-20% thicker, palisadic cells with 10-20% longer, larger stomata and stomatal index increased by 15%), on the leaf petiole (the diameter ~30% larger, conducting bundles, 20% more developed, the adaxial conducting bundles, ~30% increase) and on the stem (the diameter with 15-20% larger, central cylinder with 15-20% bigger, xylem vessels of more than 50 µm diameter, with 20% more present). These morpho-anatomical features demonstrate the plant biostimulants effects of *Trichoderma* consortium. The results presented here sustain with morpho-anatomical data the accumulation the bioactive compounds, mainly polyphenols and flavonoids with an increased antioxidant activity, which we already reported. Larger stems and leaves of *P. caerulea*, allow accumulation at a higher level of bioactives compounds.

Key words: *Passiflora caerulea*, nutraceutical crop, plant biostimulants *Trichoderma* consortium, structural effects, stems and leaves.

Introduction

The present scientific work is referring to *Passiflora caerulea* L. plants, which have been treated during the vegetation period, with a suspension of *Trichoderma*-plant biostimulants consortium, consisting of two strains applied together. This multifunctional consortium was demonstrated to stimulate the early stage of plant development and to promote the synthesis and accumulation of biologically active compounds [RĂUŢ & al. 2014, 2015; ŞESAN & al. 2018].

The morpho-anatomical researches carried out on the *P. caerulea* plants, treated with *Trichoderma*, aim to identify the biostimulants effects of the treatment on the leaves and stems development, reported in our previous paper [ŞESAN & al. 2018, *in press*], emphasizing on the development of the conducting tissues and the leaf structures, involved in photosynthesis. To the best of our knowledge such approaches haven't been yet performed

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till now and represent the scientific novelty of this work, both at national and international level of plant morpho-anatomical domain.

In this respect, two sets of data were used in this work: (i) the data of the *Passiflora caerulea* untreated plants, published in 2016 in the scientific approach "*Passiflora* spp. – new nutraceutical crop in Romania" [ȘESAN & al. 2016; SAVIN & al. 2016] and considered the control plant and ii) the data of *P. caerulea* plants treated with *Trichoderma* consortium bioproduct. It has to be mentioned here that both experimental treatments, untreated plants (control) and plants treated with suspension of *Trichoderma* consortium, have been carried during the 2015 vegetation period and the anatomical investigations have been performed in the laboratory during 2015-2017.

Material and methods

Biological material consisted in leaves and stems of *Passiflora caerulea* plants, treated on the early vegetation period, with a foliar suspension of *Trichoderma* (10^8µfc/ml), in the watering standard of 200 l/ha [RĂUȚ & al. 2014, 2015; ȘESAN & al. 2018].

The *Trichoderma* consortium contained in two plant biostimulants strains of *Trichoderma*, both deposited on the National Collection of Agricultural and Industrial Microorganisms (NCAIM), Budapest, Hungary, *T. asperellum* T36b, NCAIM F 001434 and *T. harzianum*, Td50b, NCAIM F001412 [RĂUȚ & al. 2014, 2015]. Preparation of *Trichoderma* consortium suspensions for the foliar treatments: The two strains of *Trichoderma* have been cultivated in Petri plates on the solid PDA medium, incubated for 5 days at 28 °C, spores having been collected in sterile distillate water. For each *Trichoderma* isolates spores having been counted at the value 10^8 cfu. The mixing rate of two isolates for obtaining *Trichoderma* consortium was 1:1.

The samples for anatomical analyses were collected on 23 of June 2015, from the Hofigal experimental field and preserved in 70% ethylic alcohol.

For histo-anatomical evaluation, the usual investigation and evaluation methods used in plant anatomy have been applied [ȘERBĂNESCU-JITARIU & al. 1983; SÂRBU & al. 2014; ȘESAN & al. 2016].

The *Passiflora* leaves and stems have been cross cut (anatomical knife), in the median zone of lamina segments, petiole and stems internodes from the median zone of the stems. Sections have been processed in accordance with the standard stages of the double staining technique [ȘERBĂNESCU-JITARIU & al. 1983]. Two differential and successive colorants have been used: Iodine green and Carmin Alum. To highlight the starch, IIK has been used. Paradermal sections were prepared, in order to observe the characteristics of the epidermis and the lacunose parenchyma cells, in apical view.

The microscopic analysis of the slides has been performed in normal and polarized lights (crystals study), with an optical microscope, DOCUVAL type. Microphotographs have been carried out, on the same microscope, using a Nikon D90 digital camera.

Results and discussions

Histo-anatomical evaluation

Leaves (PLATE I – II, Figures 1-9, Table 1)

Lamina. From the morphological point of view, the *Passiflora caerulea* lamina is simple, palmat-partite, with 5 unequally lobes (segments), ovat-lanceolate with acute apex, serrate border and penat nervation. The lamina segments are approximately 230-260 µm

width, hypostomatic and develop a dorsi-ventral structure. Tector or secretor trichoms were not observed on the level of the lamina epidermis (Figure 1, 2).

Median nervure of the leaf segment (500-550 µm width), has an adaxial part approximately flat and an abaxial one, with a proeminent semi-circular shape (Figure 1).

Epidermis presents proper epidermal cells, of a heterodiametric form (20-30 µm width x 60-80 µm length), with extern tangential wall thickened (~3-4 µm thickness) and covered with a cuticle of about 1-3 µm thickness (Figure 3, 5). At the level of the median nervure the epidermal cells are smaller and approximately isodiametric. Abaxial epidermis presents also stomata cells and stomatal annexes cells (Figure 4, 5).

Stomata (18 µm width x 24 µm length) are as anomocitic and anisocitic types and are present only at the level of abaxial epidermis (460- stomata / 1 mm^2).

Conducting tissues are organised in conducting bundles of collateral type: 1 – large bundle, disposed in the compact ground parenchyma of the median nervure of the leaf segment and 6-8 small bundles, located in the secondary nervures, at the mesophyll level (Figure 1). In all the conducting bundles, the xylem belt is adaxially oriented.

Mesophyll is differentiated in two zones: palisadic tissue, adaxially located and consisting in 1 – layer of vertically elongated assimilatory cells (90-120 µm length) and lacunose parenchyma, abaxially located and composed of spherical, slightly elongated cells and groups of more elongated cells (Figure 2, 4). Gaps of different sizes are present between the cells of the lacunose parenchyma.

Mechanical tissue consists in an angular collenchyma, observed / detected only in the median nervure of the leaf segment, where forms, 3-4 layers adaxially and 1-2 layers abaxially (Figure 1).

Calcium oxalate druses (15-25 µm diameter) are frequent in the structures of the lamina, where are arranged in rows, along the nervures (Figure 6).

Petiole (2.7 mm diameter) has a cylindrical form, is relatively circular in sectional view (Figure 7) and presents an adaxial shallow groove only in its lower zone. The petiole structure is of mono-symmetrical type, with separated conducting bundles.

Epidermis presents small cells, with an isodiametric shape in cross section, and extern tangential wall slightly thickened. Stomata are present, but tector or secretor trichoms missing (Figure 7, 8).

Mechanical tissue is represented by 3-4 subepidermal layers of angular collenchyma, with a discontinuous disposition (Figure 8).

Ground tissue consists of a lax parenchyma, composed of spherical and isodiametric cells, with thin walls (Figure 8).

Conducting tissues are organised in 10-12 conducting bundles of opened collateral type (vascular cambium is present) of different sizes: an adaxial large bundle (550 µm width x 700 µm length) and 10-12 smaller bundles (Figure 7, 9). Each of the conducting bundles has amilifera sheath (Figure 9).

Calcium oxalate druses are frequent in the petiole structure, being located both in the phloem parenchyma and ground parenchyma.

Stem (PLATE III, Figures 10-11, Table 2) has a cylindrical shape, irregular-ribbed, of about 2.8-3.3 mm diameter and developed a secondary structure (Figure 10).

Epidermis consists of approximately isodiametric cells in cross section, with slightly thickness walls, covered with a cuticle of 4-5 µm thickness.

Cortex is differentiated in an extern subepidermal zone, consisting in an angular collenchyma and an intern zone, represented by a lax parenchyma. The angular collenchyma it is more developed (3-4 layers) at the level of ribs. The last layer of the cortex is represented by the amilifera sheath (Figure 11).

Central cylinder is voluminous (2.5-3.0 mm diameter) and has in the center a large pity cavity (1.0-1.2 mm diameter), formed by the disorganization of the central zone of the ground parenchyma. From the activity of the vascular cambium are formed the secondary conducting tissues. These are organized in two rings: an outer ring of secondary phloem and an inner ring of secondary xylem (xylem vessels up to 150 µm diameter). The primary xylem is located in the inner part of the secondary xylem ring, near the pith cavity and the primary phloem at the periphery of the secondary phloem ring. Packs of sclerenchymatic periphloemic fibres of different sizes, accompany the phloem (Figure 11).

Calcium oxalate druses are abundant in the structure of the stem and are located in different types of cells as epidermal, cortical, phloemic parenchyma cells.

Storage cells (PLATE IV – Figures 12-14).

Possible storage cells for phytochemical products were identified in the lamina of *Passiflora caerulea*. These are some of the cells of the lacunose parenchyma and the adjacent cells of the stoma (Figure 12, 13, 14).

Comparative aspects

The comparative analyses of the morpho-anatomical data obtained from the two types of *Passiflora caerulea* plants, untreated plant (control plants – ŞESAN & al. 2016) and treated plants with *Trichoderma*, have allowed the demonstration of the biostimulatory effects of the treatment on the development of the leafs and the stems.

Some quantitative changes were observed in treated plants as compared to untreated plants (Tables 1-3).

Table 1. Analyzed *Passiflora caerulea* leaf parameters (control and treated with *Trichoderma* consortium bioproduct)

Leaf parameters	Measurements / size	
	Control (ŞESAN et al. 2016)	Treated with *Trichoderma* consortium bioproduct
Median nervure of leaf lobe	300-350 µm thickness	500-550 µm thickness
Leaf lamina	180-220 µm thickness	230-260 µm thickness
Cells in adaxial (superior) epidermis	20-30 µm width x 60-70 µm length	20-30 µm width x 60-80 µm length
Cells in abaxial (inferior) epidermis	20-40 µm width x 40-80 µm length	20-30 µm width x 40-80 µm length
Epidermis – extern wall	3.5 µm thickness	3.4 µm thickness
Cuticle	1.2 µm	1.3 µm
Stomata dimensions	10 µm width x 22 µm length	18 µm width x 24 µm length
Stomatal index	390 stomata/mm^2	460 stomata/mm^2
Palisadic cells dimensions	9-10 µm width 60-80 µm length	15-16 µm width 90-120 µm length
Median nervure bundle dimensions	150-160 µm width 170-180 µm length	200-230 µm width 220-240 µm length
Petiole diameter	1.5 mm (1.4-1.7 mm)	2.7 mm (2.5-3.0 mm)
Petiole bundle	8-9	10-12

Petiole adaxial bundle	250 µm width x 300 µm length	550 µm width x 700 µm length
Druses size	15-20 µm diameter	15-25 µm diameter

Table 2. Analyzed *Passiflora caerulea* stem parameters (control and treated with *Trichoderma* consortium bioproduct)

Stem parameters	Measurements / size	
	Control (ŞESAN et al. 2016)	Treated with *Trichoderma* consortium bioproduct
Stem diameter	2.4-3.0 mm	2.8-3.3 mm
Diameter of central cylinder	2.0-2.2 mm	2.5-3.0 mm
Diameter of pity cavity	0.8-0.9 mm	1.0-1.2 mm
Xylem vessels diameter	10-100 µm	10-150 µm
Number of xylem vessels larger as 50 µm	25	34
Epidermis cells cuticle	6-7 µm thickness	4-5 µm thickness

Table 3. Quantitative changes observed in the *Passiflora caerulea* plants treated with *Trichoderma* consortium bioproduct as compared to control plants

The organ	Quantitative changes of treated plants with *Trichoderma* consortium bioproduct
The leaf lamina	- Lamina with 10-20% thicker - Palisadic cells with 10-20% longer - Larger stomata - Stomatal index, 15% increase
The leaf petiole	- The diameter with ~30% larger - Conducting bundles with 20% more developed - The adaxial conducting bundles with ~30% bigger
The stem	- The diameter with 15-20% larger - Central cylinder 15-20% bigger - Xylem vessels of more than 50 µm diameter, 20% more developed

The data from tables 1-3, revealed that: (i) the mesophyll is more voluminous, palisadic cells and stomata are larger and the stomatic index is higher; (ii) the petiole has a larger diameter, has more conductive tissue and higher bundles and (iii) the stem is thick, and the conducting tissue from its secondary structure is better represented.

Morpho-anatomical data are important for the autentification of these features in different plants as *Passiflora caerulea* [VANDERPLANK, 2000; ULMAN & MacDOUGAL, 2004; ŞESAN & al. 2016], *Panax quinquefolius* [LI & al. 2014], *Buttia* [SANT'ANA-SANTOS & al. 2018] a.o.

Conclusions

Basic morpho-anatomical features of *Passiflora caerulea* plants, treated with *Trichoderma*, have not changed, compared with the untreated plants. In this respect, the following structural aspects are common both types of plants: (i) the leaf lamina has a dorsi-ventral structure, is hipostomatal and with lack of trichoms; (ii) the leaf petiole has a mono-symmetrical structure, with distinct conducting bundles of open collateral type; (iii) the stem has a circular outline, irregular ribbed and a secondary structure, with concentric rings of secondary xylem and phloem; (iv) the mechanical tissue consists of collenchyma in leaf and of collenchyma and sclerenchyma in stem; (v) calcium oxalate crystals of different size are present in both studied organs.

The treatment determined significant quantitative changes on morpho-anatomical features, on the leaf lamina (lamina with 10-20% thicker, palisadic cells with 10-20% longer, larger stomata and stomatal index increased by 15%), on the leaf petiole (the diameter ~30% larger, conducting bundles, 20% more developed, the adaxial conducting bundles, ~30% increase) and on the stem (the diameter with 15-20% larger, central cylinder with 15-20% bigger, xylem vessels of more than 50 µm diameter, with 20% more present). These morpho-anatomical features demonstrate the plant biostimulants effects of *Trichoderma* consortium.

Storage of phytochemical products is related to stomatal annexes cells and lacunose parenchyma cells from the leaf.

Morpho-anatomical data are important for the autentification of these features in different plants as *Passiflora caerulea* [VANDERPLANK, 2000; ULMAN & MacDOUGAL, 2004; ȘESAN & al. 2016], *Panax quinquefolius* [LI & al. 2014], *Buttia* [SANT'ANA-SANTOS & al. 2018] a.o.

To the best of our knowledge such approaches haven't been yet performed till now and represent the scientific novelty of this work, both at national and international level of plant morpho-anatomical domain.

Notes on contributors

Tatiana Eugenia ȘESAN – coordinated and monitorized the project 160/2014, performed designed experiments, collected samples from experimental field, gave general interpretation of the data, participate to the writing and finishing the manuscript. Anca SÂRBU and Monica Anca PARASCHIV - performed section of vegetal material and microscopic analysis of the slides, providing anatomical data, their interpretation in connection with the applied treatment in *Passiflora* crop, all the photos of optical microscopy, drafting and translation of the scientific work. Florin OANCEA - contributed and supervised the finalization of the manuscript.

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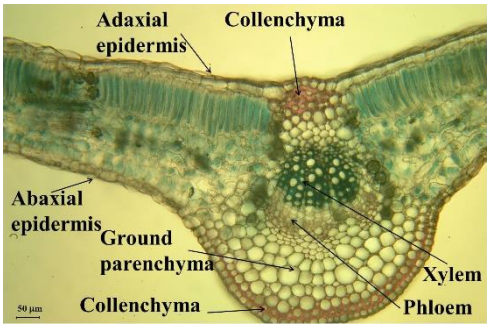
PLATE I

***Passiflora caerulea* – LAMINA**



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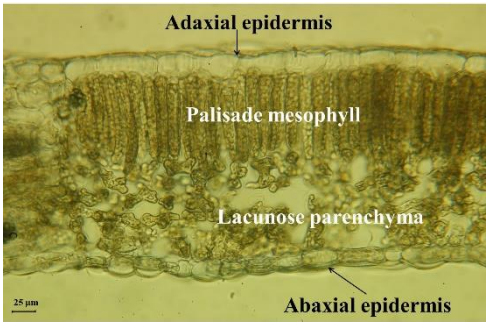

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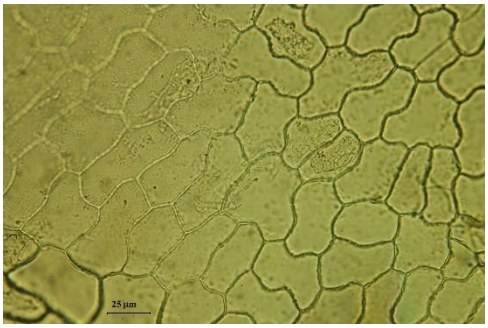

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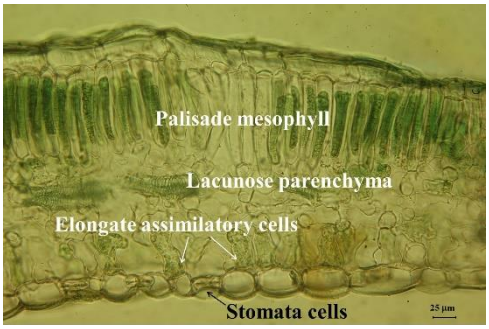

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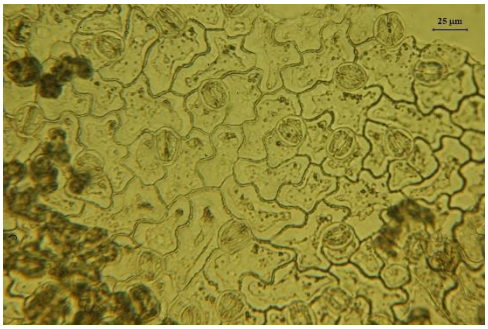

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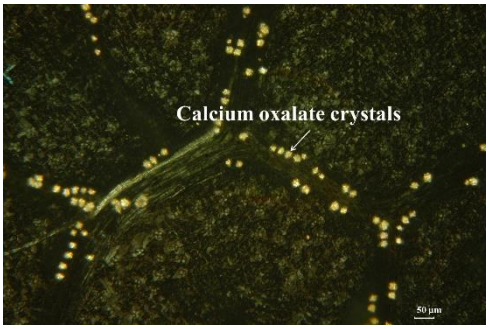
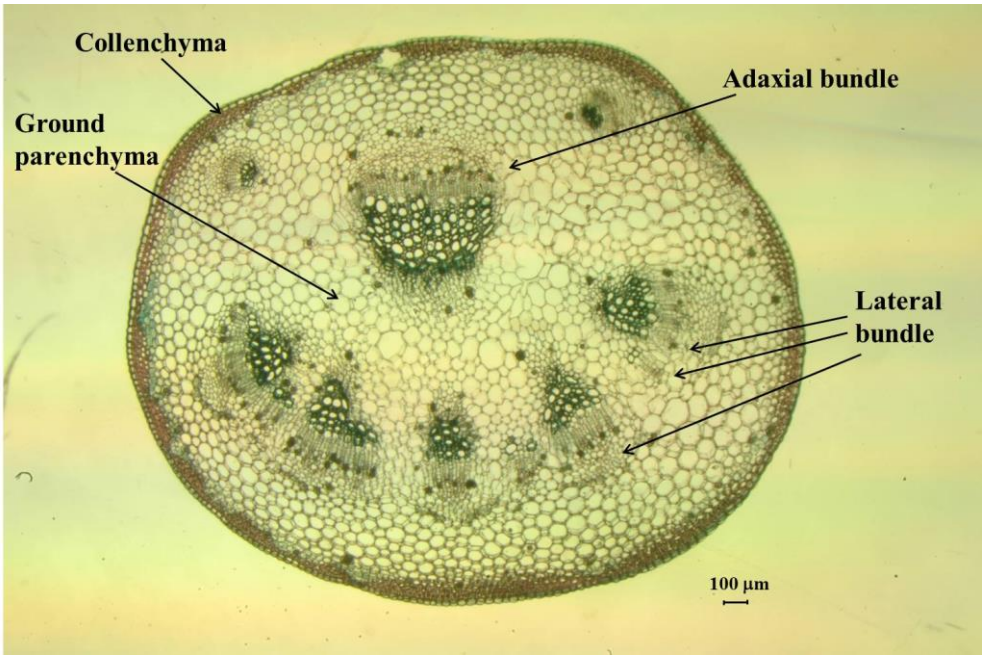


Figure 6

PLATE II

***Passiflora caerulea* – PETIOLE**



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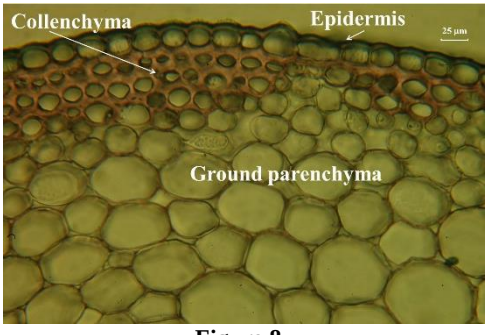

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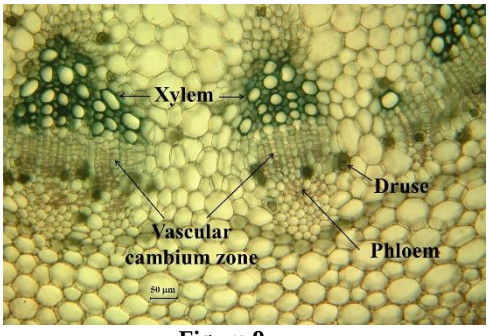
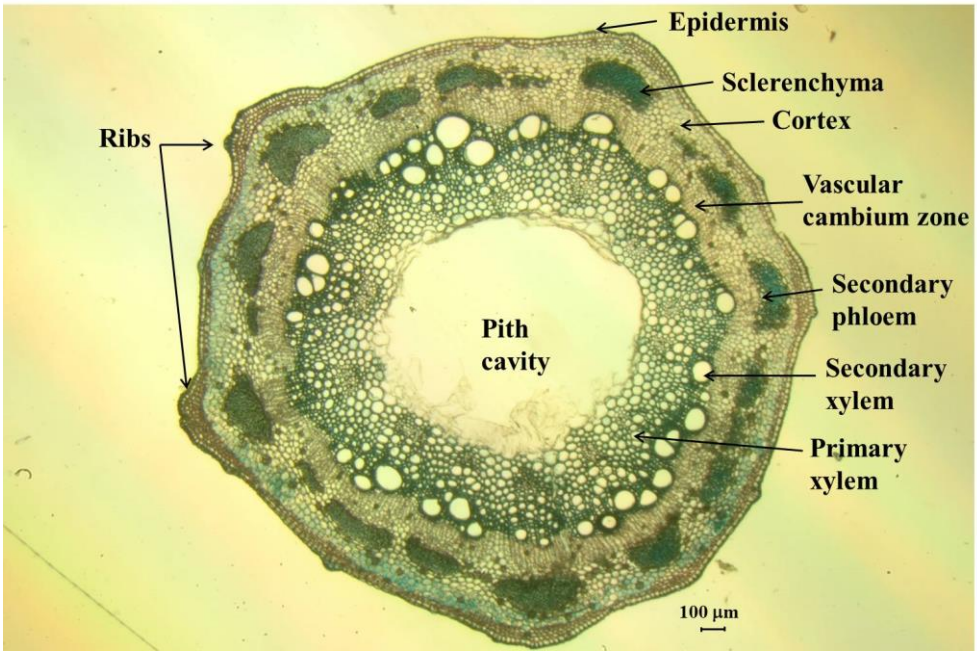


Figure 9

PLATE III

***Passiflora caerulea* – STEM**



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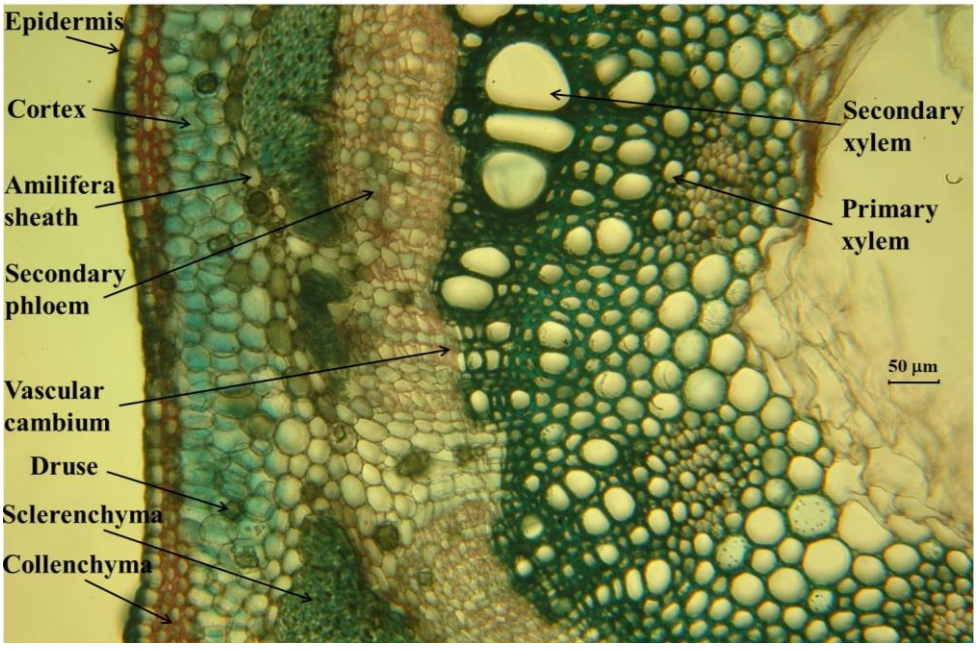

Figure 11

PLATE IV
***Passiflora caerulea* – STORAGE CELLS**

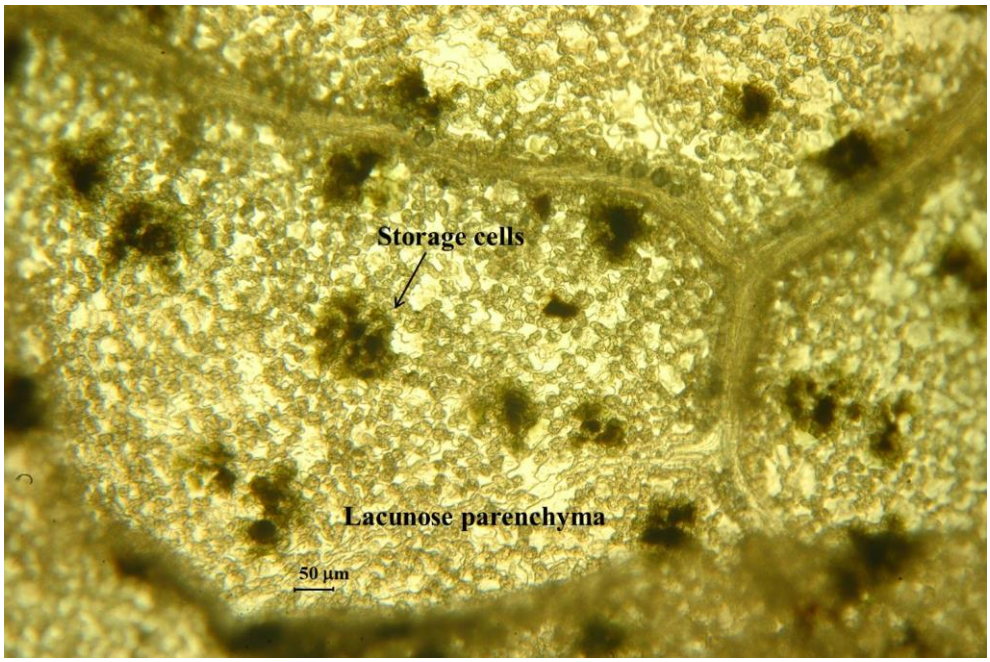

Figure 12

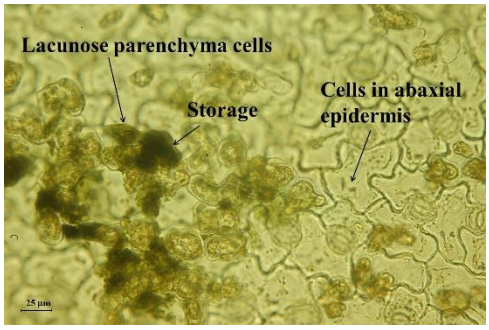

Figure 13

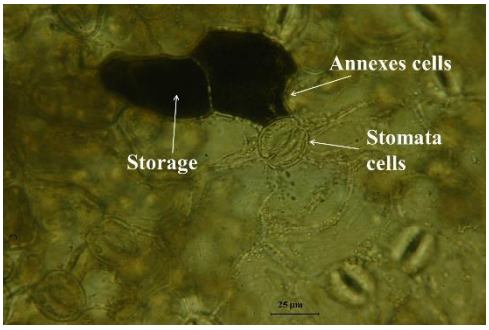

Figure 14

Explanation of plates and figures

PLATE I (photo: Anca Sârbu) – *Passiflora caerulea* – **LAMINA**
- Cross section through median zone of the leaf segment, with evidence of the median nervure, colorants Iodine green and Carmin Alum (Figure 1)
- Cross section through the median zone of leaf segment, with evidence of epidermis and mesophyll, colorants Iodine green and Carmin Alum (Figure 2)
- Adaxial epidermis (superior) in apical view (Figure 3)
- Cross section through the median zone of leaf segment with evidence of epidermis and assimilatory cells, colorants Iodine green and Carmin Alum (Figure 4)
- Abaxial epidermis (inferior) in apical view (Figure 5)
- Paradermal section through the leaf segment, with evidence of lacunose parenchyma, abaxial epidermis and calcium oxalate crystals in apical view, using polarized lights (Figure 6).

PLATE II (photo: Anca Sârbu) – *Passiflora caerulea* – **PETIOLE**
- Cross section through the petiole, colorants Iodine green and Carmin Alum (Figure 7)
- Cross section through the petiole, with evidence of epidermis, collenchyma and ground parenchyma, colorants Iodine green and Carmin Alum (Figure 8)
- Cross section through the petiole, with evidence of the lateral bundles, colorants Iodine green and Carmin Alum (Figure 9)

PLATE III (photo: Anca Sârbu) – *Passiflora caerulea* – **STEM**
- Cross section through the median zone of the stem, colorants Iodine green and Carmin Alum (Figure 10)
- Cross section through the median zone of the stem, with evidence of epidermis, cortex and of central cylinder elements, colorants Iodine green and Carmin Alum and IIK (Figure 11)

PLATE IV (photo: Anca Sârbu) – *Passiflora caerulea* – **STORAGE CELLS**
- Paradermal section through the leaf segment, with evidence of lacunose parenchyma and storage of phytochemicals products (Figure 12)
- Paradermal section through the leaf segment, with evidence of phytochemicals products accumulation in the cells (Figure 13)
- Paradermal section through the leaf segment, with evidence of the abaxial epidermis, the stomata cells and the accumulation of phytochemicals products in the adjacent cells (Figure 14)

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STUDIES ON EPIDERMAL APPENDAGES FROM VEGETATIVE ORGANS AT *EUPHORBIA* SPECIES CULTIVATED IN BOTANICAL GARDEN IASSY

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Abstract: The *Euphorbia* genus is unitary by the presence of the characteristic inflorescence named cyathium, but presents a remarkable morphological diversity of aerial vegetative organs. Species that originated in the tropical zones, especially South-African ones stand out by the presence of some epidermal appendages little known (trichomes, spines, leaf scars). Of those, the spines are the most conspicuous and intriguing from a structural point of view; the botanical terminology used for describing their features is critically presented. The representatives of the *Euphorbia* genus cultivated in Botanical Garden Iassy greenhouses were morphologically analysed and a detailed description of epidermal appendages resulted, which is useful in clearing taxonomical aspects.

Key words: epidermal appendages, *Euphorbia*, leaf scar, spine shields, vegetative organs.

Introduction

The *Euphorbia* genus presents an impressive morphological variability, correlated with the huge number of comprised species (approx. 2000) and the diversity of their habitats. Already vast, the genus was enriched by the immersion of taxa from *Monadenium*, *Pedilanthus* and *Synadenium* genera [BRUYNS & al. 2006; STEINMANN, 2003], determined by the last molecular genetic studies.

Characteristic for this genus is the presence of a particular inflorescence, named cyathium [BERRY & al. 2016; BROWN & al. 1925], which was intensively studied, morphologically and structurally. Quite frequently the inflorescence can't be seen at cultivated species, which originated from tropical and subtropical areas because of its lack of blooming. In this case the features of the aerial vegetative organs can be useful in identification, but in ecological, horticultural, pharmacological etc. studies as well. Anatomical and/or morphological studies on representatives of this genus are quite rare [ZAHRA & al. 2014] and focused mostly on species originated from the northern hemisphere [GALEŞ & TOMA, 2006; TALEBI, 2017]. The epidermal appendages are less studied at this genus, and sometimes these bear particular characteristics, rarely seen in the vegetal domain (especially at South African species).

The studied epidermal appendages from the vegetative organs are trichomes, spines and leaf scars.

The importance of the study of trichomes in resolving taxonomical [COURT, 2000] and ecological aspects is widely recognised, but in the case of the *Euphorbia* genus these studies are few and focused especially on herbaceous, non-succulent species [THAKUR & PATIL, 2012; ZAHRA & al. 2014].

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The spines present at succulent species which originate from arid areas (especially South Africa) are structures which frequently cause confusions with cactuses, these two groups of plants being the best known examples of convergent evolution. Otherwise spurges are considered homologous of cactuses in African arid areas. The presence of the spines together with the stem's appearance are the reasons for these expression used in the description of spiny succulent spurges: cactus-like [GILBERT & CARTER 1984; NEUWINGER 1996], cactiform [EVANS & al. 2014; ARCO AGUILAR & RODRÍGUEZ DELGADO, 2018]. The spines of the *Euphorbia* genus are different from those of cactuses (most of them are of stipelar origin and are developed on a specific structure named spine shields), and in comparison with them [DREZNER, 2017; GEBAUER & al. 2016] are less studied (or not at all), structurally and functionally. About the Euphorbia genus's spines general aspects are known, which are applicable for all spinescent species and just a few papers remember their ecological role [RABESANDRATANA, 1984], without a subject focused analysis.

Probably the reduced frequency of information about spines (comparatively with those refering to cactuses' spines) can be the source of the inexplicable mistake of some botanists who mentioned that in the case of *Euphorbia canariensis* the leaves turned into spines [ÁLVAREZ ESCOBAR & RODRÍGUEZ DELGADO, 2014]. This mistake is that much more regretable because it appeared in a paper that reached a great number of specialists from various domains (agriculture, cultural anthropology, biology, ecology, pharmacognosy, linguistics) comparatively with their valuable work [DIAZ HERNANDEZ & RODRIGUEZ DELGADO, 1995] used strictly in botanical domain.

Leaf scars can't be considered epidermal appendages in the classical sense, but at some of the analyzed species these represent obvious characters, easy to analyze and always presented. They are mentioned in the description of some species in specialty literature [CARTER & LEACH, 2001].

Material and methods

The studied material is represented by vegetative organs of 26 taxa from the *Euphorbia* genus belonging to the collection cultivated in greenhouses of "Anastasie Fătu" Botanical Garden Iassy.

The vegetal matter comes from species with ages between 30 and 40 years old (*E. aphylla* Brouss. ex Willd., *E. canariensis* L., *E. grandicornis* Goebel ex N. E. Br., *E. grandidens* Haw., *E. ingens* E. Mey ex Boiss., *E. ramipressa* Croizat, *E. tirucalli* L., *E. trigona* Mill.), between 15 and 20 years old (*E. guentheri* (Pax) Bruyns, *E. myrsinites* L.), between 10 and 12 years old (*E. bicompacta* Bruyns var. *rubra* (S. Carter) Bruyns, *E. bubalina* Boiss., *E. caerulescens* Haw., *E. cotinifolia* L., *E. flanaganii* N. E. Br., *E. ferox* Marloth, *E. globosa* (Haw.) Sims, *E. lactea* Haw., *E. milii* Des Moul., *E. pulcherrima* Willd. ex Klotzsch, *E. stenoclada* Baill., *E. umbellata* (Pax) Bruyns), and between 5 and 10 years old (*E. leuconeura* Boiss., *E. pteroneura* A. Berger, *E. tithymaloides* L.).

The number of items from each species varies: 1 (*E. ingens*, *E. grandidens* and *E. stenoclada*), 2 (*E. bubalina*, *E. caerulescens*, *E. ferox*, *E. lactea*, *E. ramipressa*), 3-15 (the rest of the species).

The study was focused on three types of epidermal appendages: spines, trichomes and foliar scars from vegetative aerial organs.

In order to observe the spines and the leaf scars 20 spiny structures were analyzed, along with scars from the mature sections (at least 2 years old). In order to observe trichoms 20 leaves collected from species whose leaves last for at least a few months were analyzed. In the case of *E. myrsinites*, the aerial leaf stems last only for one vegetative season, dying in winter.

The information from specialty literature [BERRY & al. 2016; CARTER, 2002; CARTER & LEACH, 2001; CARTER & RADCLIFFE-SMITH, 1988; CREMERS, 1978; JSTOR 2013; SWANEPOEL, 2013] was compiled with the observations realized with the help of an Optika stereo microscope (spines and leaf scars), and an Optika microscope (trichomes). The most relevant aspects have been photographed with a Canon A540 camera.

Results and discussion

The most obvious and interesting epidermal appendages at studied spurges are the spines. The succulent species of the *Euphorbia* genus from the arid areas of South Africa are considered homologous of the cacti in the American areas with similar weather conditions. In accordance with the theory of convergence evolution, spines are present as a result of adaptation to a harsh environment and often these two groups of plants are mistaken for each other. This confusion is facilitated as well by the fact that cultivated representatives of *Euphorbia* rarely bloom (ex. *E. trigona*, *E. canariensis*).

At the African species described in *Flora capensis* [BROWN & al. 1925], the spines are of three types conform to their provenance:

1. the apex of the branches turned into a sharp spine (ex. *E. stenoclada*);
2. the peduncle of the inflorescence turned into a sharp spine (ex. *E. ferox*);
3. spines in pairs named "stipular spines", differently disposed towards leaf scars (ex. *E. caerulescens*).

BROWN (1925) come into notice that this last type of spines can't be considered stipules in the real sense of the word, but the term is used in specialty literature [CARTER, 2002] referring to the *Euphorbia* genus. The second type of spines is designed by DOREEN COURT (2000) as peduncular spines, meanwhile MAPAYA (2003) names them "inflorescence thorns because they are homologous with inflorescences" or their withered and retained central axes.

The characteristic structure on which the spines develop is most frequently named spine shields [CARTER & LEACH, 2001]. In Flora of Tropical East Africa [BEENTJE & CHEE, 2014] it is shown that this term is rarely used and it is defined as a "horny pad from which the spines stick out". In a precedent work [SCHMIDT & al. 2002] this structure is identified by the term *warty cushions*, which appears in COATES PALGRAVE'S work (2013) as well, but apparently with a different signification. Spine shields are a defining character for African spiny succulent species and their features can be diagnosis characters.

DORSEY & al. (2013) point that spine shields "typically bears two or four spiny outgrowths". These outgrowths are interpreted by CARTER (1994) as "a pair of spines and a pair of stipules modified as prickles". These prickles [CARTER & LEACH, 2001] are named dorsal spines by MAPAYA or rudimentary spines by SWANEPOEL.

Secondary spines are named those which flank the floriferous eyes [CARTER & LEACH, 2001].

The features of epidermal appendages from species of the ***Euphorbia*** genus collection cultivated at Botanical Garden Iassy are presented below:

E. aphylla: leaf scars sparse, apparently punctiforme, approximately 0.2 mm diameter, whitish.

E. bicompacta var. ***rubra***: leaf scars elliptical, reniform or semilunar, 1 cm apart, obvious especially due to light brown colour on green surface of the photosynthetic stem; 3-18 mm length, on old stem they are bigger due to increase in thickness.

E. bubalina: leaf scars semicircular to triangular, approximately 1.5 mm length, 1.5-2 cm apart, disposed at apical end of tuberculate segments.

E. caerulescens: spine shields whitish-grey connected in a horny edge, on the most part of edges stem; spines in pair, 0.5-1.2 cm length, whitish with deep brown apex, straight or slightly curved, made an angle of 120 degrees.

E. canariensis (Plate I, D): spine shield almost circular or elliptical, decurent, approximately 3 mm diameter, approximately 1mm apart, reddish-brown becomes soon light brown. Spines in pairs shining brown disposed throughout angles of stem, ± 4 mm length, horizontal spreading, making an angle of approximately 180 degrees, straight or "cow-horn" shaped, very sharp.

E. cotinifolia (Plate II, B): non-glandular trichomes unicellular cylindrical reddish on the upper side of the petiole of the young leaves, soon caducous.

E. ferox (Plate I, H): spines (modified peduncles) solitary or rarely 3, disposed along the 8 angles, mostly 3.8-7.6 cm apart, 1.3-3.2 cm length, stout, very rigid, woody, straight or variably curved and more or less horizontally spreading except the few at the apex, brown becoming grey.

E. flanaganii: leaf scars semicircular, whitish, at the apical end of the tuberculate segment from the lower part of the branches or on the tubercles of the central stem.

E. globosa (Haw.) Sims: leaf scars irregular-ovate or semicircular, approximately 0.5 mm length, on the apex of the tubercle.

E. grandicornis (Plate I, A): spine shields fusiform, grey or brownish joined in a continuous horny edge. Spines in pairs very stout, 15-70 mm long, 2-3 mm thick at the base, but reduced to c. 3 mm length at the constrictions of the branches, 2-3 cm apart, widely diverging at an angle of 160-180 ^{0}, grayish or pale brown, the apex darker and sharp; prickles minute, 1-2 mm length, below the spines; secondary spines on one side and the other of the flowering eyes, 1–2 mm long.

Leaf scars between the spines, semicircular to triangular

E. grandidens: on main stem spine shield obovate, isolated, 1.5-2.5 cm apart, 1.5-8 mm length; spines pair, 2-9 mm length, black at the apex, rarely parallel, most frequent divergent (till 180 degree). On the terminal branches spine shields decurent transversely elliptic; spineless or with a pair of reduced spines, 1.5-2.5 mm length, 2.2 cm apart, divergent at an angle of 100 degrees, straight or cow-horn shaped; prickles minute, sometimes squamiform.

E. guentheri (Plate I, C): a cluster of 5 spines on each tubercle of the stem; 3 of them are disposed on a triangular shape, 1-2 mm length, solid, the central one reclined; the two other spines smaller (1mm length). Leaf scars approx. 1mm, almost circular, above and bordered by spines.

E. ingens (Plate I, J, K,): Spine shields oval or obovate, 12-18 mm apart, 4-5 mm in diameter, extending 5 mm above to include the flowering eye. Spineless or with a pair of spines 2-5 mm length, straight, divergent at an angle of 120 degrees. Spines and spine shields

are initially brown but soon becoming corky, rusty-brown and disintegrating or grey on old segment of the stem.

E. lactea: Spine shields light brown, decurrent, lanceolate, wider in medial zone, where are the spines, sometimes comprises flowering eyes on the upper part. Spines in pair, 2-6 mm length, divergent at an angle of 60 degrees, dark brown and very sharp at the apex, most of them down curved.

E. leuconeura (Plate II, C, D, I, J): cartilaginous stipules light brown or whitish-grey, divided in numerous bristles up to 5 mm length, joined and making a continuous edge.

Leaf scars linear to reniform, 1.5-2.5 cm apart; sometimes joined with branches' scars; obvious especially due to light brown colour on green surface of the photosynthetic stem.

E. milii (horticultural varieties) (Plate I, F, Plate II E, H): trichomes unicellular whitish on stem, petiole, base of leaf and lower half of spines, at the mature ones only in basal part. The most trichomes are simply, cylindrical, with acuminate or rounded apex; a few trichomes are clavate.

Spines in pairs (rarely 1 or 3) flanking the leaves or leaf scars, the pairs approximately parallel; spines 1-2 cm length, straight, grey with brown apex, very sharp, divergent at an angle of 45 degrees.

Leaf scars semicircular or reniform, approximately 2.5 cm length, gray.

E. myrsinites L.: leaf scars semilunar, approximately 3 mm length, light brown, alternate disposed and at an angle of 45 degree towards the stem.

E. pteroneura (Plate II, J): leaf scars semicircular or semilunar, 3-7 mm length, disposed on demarcation line of segments of the branches, 2-5 cm apart, alternate disposed.

E. pulcherrima (horticultural varieties): non-glandular trichomes multicellular uniseriate crisped, whitish, uniform disposed on lower side of leaf lamina, numerous; similar on upper side in lower third, on veins and edges of the lamina, otherwise rarely or absent.

E. ramipressa (Plate I, B, I): spine shields obovate (on old stem) or pentagonal, slightly decurent. Spines in pairs, darker at the apex, straight or curved (cow-horn shape), divergent at an angle of 120 degrees. Above the spines are two rudimentar prickles as a foliaceous squame, with approximately circular shape. Sometimes spine shields are reduced in dimension, the spines are very small or absent. On the old lignified stem, spine shields are more decurrent and sometimes joined in a subcontinuous series.

E. stenoclada (Plate I, G): on juvenile form of the plant terminal branches have the apex transformed in spine, brown, approximately 2 mm length.

E. tirucalli (Plate II, F): The extreme tips of young leafy branchlets sparsely tomentose with curled white hairs.

Foliar scar semicircular, light brown, approximately 1 mm length, perpendicular on the stem surface. Most often on one side and the other are stipular remnants, as two blackish squama.

E. tithymaloides: non-glandular trichomes multicellular uniseriate, whitish, frequent on petiol, midvein and edges of the foliar lamina, more rarely on foliar lamina.

Leaf scars elliptical or almost triangular, with angle rounded, 7 mm length, approximately 2 cm apart.

E. trigona (Plate I, E): Spines paired, 4 mm length, straight, divergent at an angle of 120 degrees, shining reddish-brown, become whitish-grey on old stems. The bases of the spines obovate, usually distinct, sometimes joined. On upper side rudimentary prickle.

Leaf scars bordered by bases of the spines, shape variable (circular, semicircular or polygonal), 2-3 mm length, whitish.

E. umbellata (Plate II, A, G): non-glandular trichomes bulbose on edges of the lamina.

Leaf scars semilunar or elliptic, up to 1 cm length, alternately disposed, approximately 2.5 cm apart, obvious especially due to light brown colour on green surface of the photosynthetic stem. At the apex of the branches scars are 7 mm long, but they can get to 12 cm due to increase in thickness, in this case the shape is almost elliptic.

Most of the species mentioned in specialty literature are glabrous or, on the contrary, only have non-glandular trichomes [LUZ & al. 2015; TALEBI & al. 2017; ZAHRA & al. 2014], as can be seen in the analysed taxa. In the case of *E. pulcherrima* the trichomes were only observed on the inner side, instead of both, as it appears in *Flore des Mascareignes* [RADCLIFE-SMITH, 1982].

The spiny structures are simple (*E. stenoclada*) or more complicated, a veritable armature (*E. grandicornis*, *E. guentheri*); in succulent species the "cow-horn" shape is frequent. All three types of spines mentioned by BROWN are present on our material, but for the type of spine represented by *E. stenoclada*, PEIRSON & al. (2013) uses the expression "spine tipped branches". In addition, only RADCLIFE-SMITH used the term *shields* ("écusson" in French) in the description of *E. milii* and *spinescent stipule* ("stipules épineuses" in French) for the spines.

Spine shields are characteristic for *Euphorbia* species and may be isolated or joined, making a continuous edge. The colour is mostly light brown, but can become rusty-brown (*E. ingens*) or gray. At *E. canariensis* CARTER (2002) mentioned a blackish colour, never seen in our material.

Leaf scars vary from almost indistinguishable (punctiform, about 1 mm length e.g. *E. tirucalli*) to conspicuous, visible from a considerable distance (e.g. 12 mm length on an old stem of *E. umbellata*). The disposition toward the stem is different: at *E. umbellata* the scars are parallel with axes of stem/branches, at *E. tirucalli* they are perpendicular, and at *E. myrsinites* they form a 45 degrees angle. When it comes to species with tuberculous stems, the scars are disposed on the apex of the tubercles, like in the case of *E. guentheri.*

The leaf scars of *E. canariensis* bear in the centre five green formations which can be rudiments of leaves, as VIERA Y CLAVIJO (1942) suggest. In fact at this species the leaves are never seen and are not mentioned in any botanical description.

Conclusions

Trichomes, spines and leaf scars were treated as epidermal appendages first of all from the point of view of their disposition towards the surface of the stem, and secondly from the point of view of their provenance.

The epidermal appendages of aerial vegetative organs from the *Euphorbia* species present distinctive features valuable from a morphological point of view. The trichomes are only non-glandular and present at few species, especially non-succulent. The spines are variable in shape, colour and position and some of them are developed on a special structure, named spine shields. The leaf scars can be a valuable character, by their different shape and disposition around the stem surface.

The results of the observations are mainly available for cultivated items.

Notes on contributor

Camelia IFRIM – is a botanist with a special interest in the study of the morpho-anatomy of exotic plants mainly from the Lamiaceae, Solanaceae and Euphorbiaceae families, as well as the study of the living collection in the greenhouses of the Botanical Garden Iassy.

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Explanation of the plates

Plate I (scale bars = 1 cm)

A. *Euphorbia grandicornis* – spines shields with spines on branche: 1 – spine shields, 2 – spines, 3 – prickles, 4 – leaf scar
B. *E. ramipressa* – spines shields with spines on tertiary branch
C. *E. guentheri* – clusters of spines and leaf scar with a drop of latex on stem
D. *E. canariensis* – spines shields with spines on secondary branch
E. *E. trigona* – spines and leaf scar on secondary branch
F. *E. millii* – tertiary branch with spines and leaf scar
G. *E. stenoclada* – spine on terminal branch
H. *E. ferox* – spine on stem
I. *E. ramipressa* – old stem with subcontinuous lines of spine shields and spines
J. K. *E. ingens* – stem with spines on rusty, respectively grey spine shields

Plate II (scale bars = 1 cm)

A. *E. umbellata* – trichome on margin of the leaf (700 x)
B. *E. cotinifolia* – trichomes on petiol of juvenile leaf (350 x)
C. *E. leuconeura* – cartilaginous stipules on edge of branche
D. *E. leuconeura* – cartilaginous stipules with bristles on edge of branche (100 x)
E. *E. milii* – clavate and cylindrical trichomes on surface of branche (700 x)
F. *E. tirucalli* – leaf scar with stipular remnants on terminal branch
G. *E. umbellata* – leaf scar on tertiary branch
H. *E. milii* – leaf scar on tertiary branch
I. *E. leuconeura* – leaf scar on secondary branch
J. *E. pteroneura* – leaf scar on secondary branch

Plate I

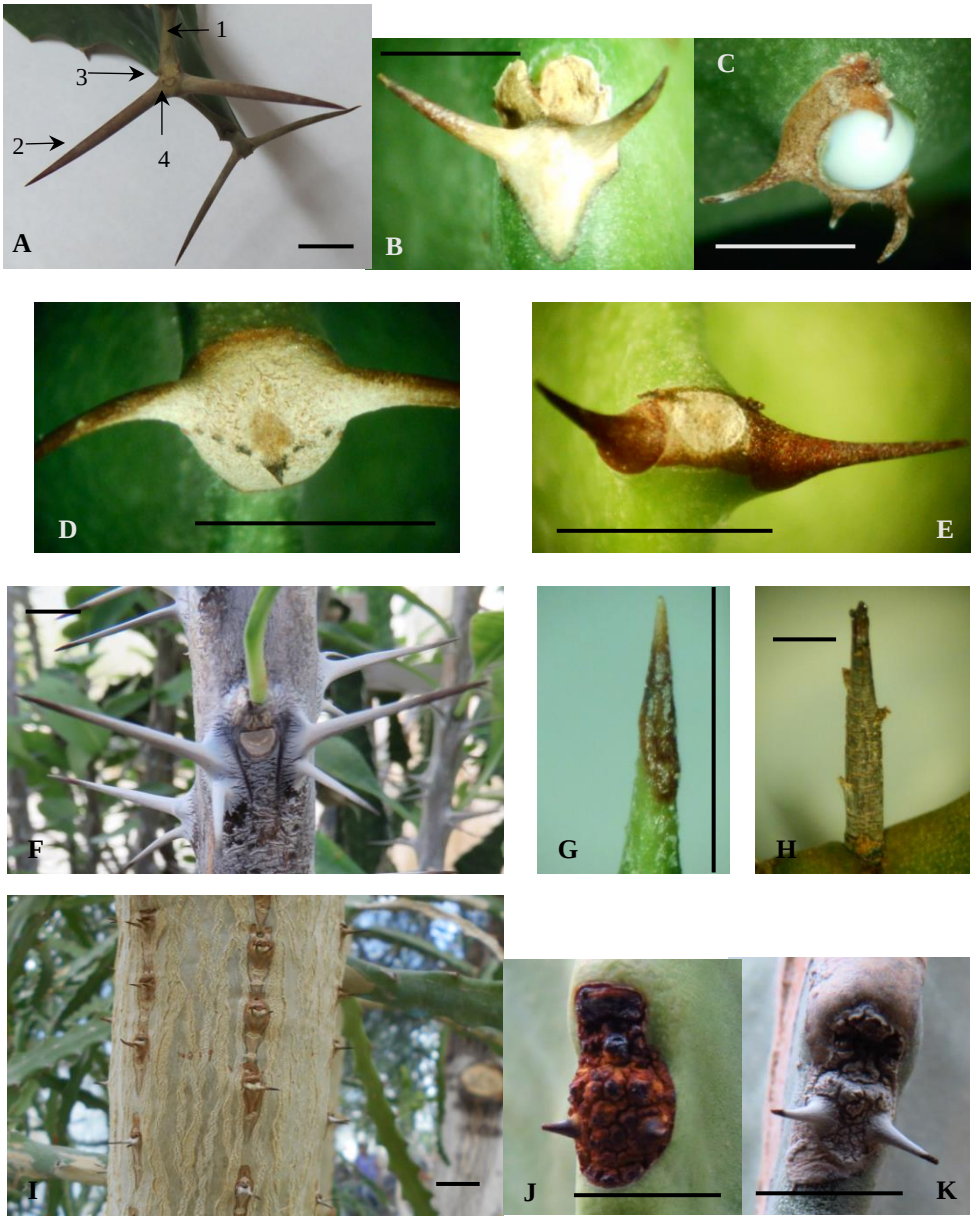
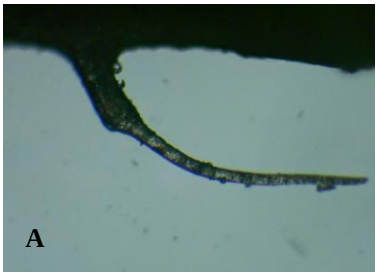
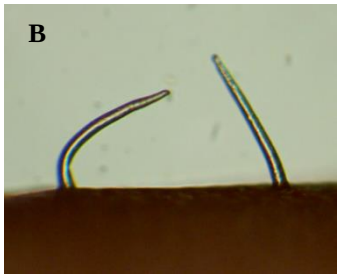
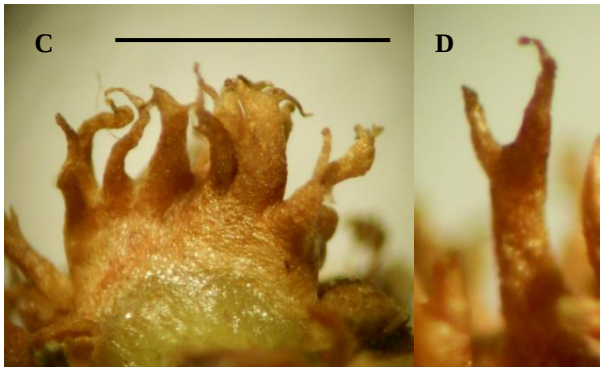
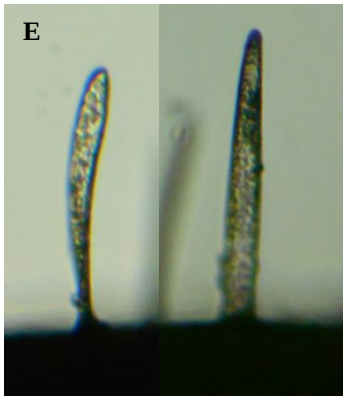


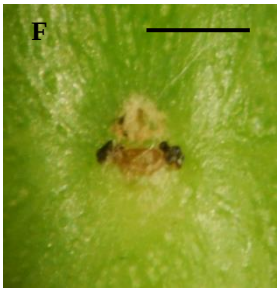
Plate II

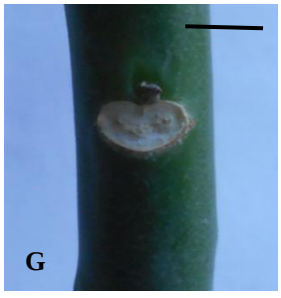


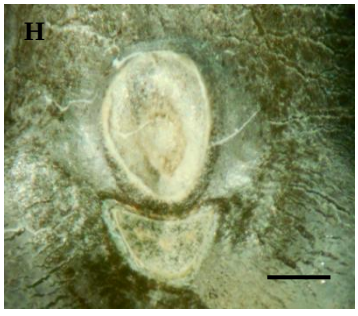












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THE NATURE OF THE INFERIOR OVARY IN SOME MONOCOTYLEDONOUS FAMILIES

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Abstract: The floral vasculature aspects of twenty-four species belonging to six monocotyledonous families are dealt with. These selected taxa include 23 horticulture species cultivated in Egypt and the remainders is among the wild flora of Egypt. A great attention has been focused by phylogenetists on the position of the ovary with respect to the other parts of the flower. In this connection, the nature of the inferior ovary is generally discussed on the basis of two theories; appendicular and axial. It is fortunate that the vascular skeleton can still be regarded as the most conservative character and that it may be rather conclusive in the determination of the nature of the inferior ovary. In the present study, the different patterns of floral vascularization are presented in cumulative tables and figures to facilitate the comparative study. Moreover, an interpretation to the observed variations is also tried to reach at the relations between the taxa studied and to determine the nature of the hypanthium.

Keywords: Epigynous flower, floral vasculature, hypanthium, inferior ovary, monocotyledons.

Introduction

More than other plant part the flower has always received a great attention on telling evolutionary pathways; and of all the floral phenomena that of the inferior ovary has doubtless been extensively discussed [DOUGLAS, 2003; BASSO-ALVES & al. 2017]. Since the nineteenth century two theories about its nature have received strong support and simply presented, these are the appendicular theory and the axial theory.

Under the appendicular theory, also called the Candollean theory and the concrescence theory; the ovary becomes inferior by the adnation of the bases of the outer floral whorls to the gynoecium; while the axis forms no part of the ovary wall. Supporters to this theory were VAN TIEGHEM (1871), DE CANDOLLE (1891), SWAMY (1948), BLASER (1954), DOUGLAS (1957), STEBBINS (1977), KHALIFA & al. (2009). Under the axial theory, also called the receptacular theory, the inferior ovary is considered to consist of tissues of the axis that form an invaginated floral receptacle surrounding the gynoecium and adnate to it. Among the holders of this theory were PAYER (1857), DOUGLAS (1957) and EAMES (1974).

Not easy as above either theory can be accepted since the whole situation is further complicated by the so-called hypanthium. This hypanthium is an expression of the tendency for the floral parts of different kinds to fuse together to form one morphological entity which often resembles the calyx in texture thus giving an impression of a calyx tube [GUSTALSSON & ALBERT, 1999]. The degree of fusion of petals (or inner perianth) and the stamens to this tube as well as their leveling is so diverse that many versions of epigyny do exist, and thus causing controversy about the nature of the inferior ovary whether totally axial, totally appendicular or partially this or that. More difficulty is confronted for the

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hypanthium, although appearing as a simple morphological structure, yet it is actually compound anatomically.

In spite of this, many cases in the dicots have been correctly comprehended and the nature of the inferior ovary has been satisfactorily assigned. In monocots, however the case is not as easy as in dicots. This is because as STEBBINS (1977), states, epigyny in monocots is derived by routes which differ from those of the dicots. However, he directed the attention towards the following points: 1- In most monocotyledonous hypogenous flowers the rule is the undifferentiating of the perianth into sepals and petals. If any differentiation occurs, it will be so weak. That the intercalary concrescence between the members produces a floral tube ending into a single series of free perianth lobes. 2- In some genera the stamen filaments are adnate to the perianth tube and the structure is somewhat, but not completely, analogous to the hypanthium of the Rosales. 3- The epigynous flowers in most of the Amaryllidaceae are probably derived from such perigynous flowers but the direct origin of epigyny from hypogyny, at least in monocots is certainly a possibility. 4- The determination of the nature of the inferior ovary represents a remarkable controversy if one has to decide whether it is axial, appendicular or both. The latter case originates through the adnation of the floral parts on one hand and the concavity of the receptacle makes at least a part of the ovary wall to be axial [PURI, 1951; KAPLAN, 1967; CRONQUIST, 1968].

Again and over a century the problem of the nature of the inferior ovary received its due attention. The comprehensive review by DOUGLAS (1944) represented a trigger for diverse interpretations and conclusions. In the same review it became clear that the internal region of the ovary wall is truly carpellary, but great controversy arose about the nature of its outer region. As regards this latter region one group of workers believed in its appendicular nature [DUCHARTRE, 1841; LESTIBOUDOIS, 1855; TREVIRANUS, 1861; KOEHNE, 1869; VAN TIEGHEM, 1870; BOUTINEAU, 1883; SAUNDERS, 1925 a,b,c; KOZO POLJANSKI, 1926; DOWDING, 1931; EAMES, 1931; EAMES & MAC DANIELS, 1947; OKIMOTO, 1948; SWAMY, 1948; BLASER & EINEST, 1950; ROSTOGI, 1951; KHALIFA & al. 2009]. The second group of workers considered it to be receptacular [DUCHARTRE, 1842; TREVIRANUS, 1859; HENSLOW, 1891; KIRKWOOD, 1905; JUDSON, 1929; SMITH & SMITH, 1942; SHARMA, 1949]. As a compromise, EAMES (1974) stated that the inferior ovary had developed in two morphologically different ways by adnation of the floral appendages and by hollowing the axis tip.

The interpretation of the nature of inferior ovary is not easy to explain when the force of connation has resulted in some events as syncarpy, and when the adnation produces unions of floral parts, delimitation of the receptacular tissue is mostly impossible and interpretation of the basic flower structure in syncarpous ovaries would differ widely. Thus, the validity of using the anatomical method in determining the nature of inferior ovary becomes of vital significance. It is fortunate that the vascular skeleton can still be regarded as the most conservative character and that it may be rather conclusive in the determination of the nature of the inferior ovary. Moreover, an interpretation to the observed variations is also tried to reach at the relations between the taxa studied and to determine the nature of the hypanthium.

Material and methods

Twenty-four species belonging to six monocotyledons families are dealt with. These taxa include 23 horticulture species cultivated in Egypt and the remainder is among the wild flora of Egypt. All taxa were identified after TÄCKHOLM (1974), BAILEY (1976), HUXLEY & al. (1992) and BOULOS (2000 & 2009). Herbarium specimens were kept at the Botany Department Herbarium, Faculty of Science, Ain Shams University. The families and the taxa studied are cited in Table 1 and arranged according to APG II (2003). The different localities of the taxa studied are also cited in Table 1. The flower buds of the studied taxa were fixed and preserved in F.A.A. embedded in paraffin wax, then serially sectioned at 10-15µ according to conventional method [JOHANSEN, 1940]. Sections stained in crystals violet-erythrosine (saturated in clove oil) combination. Some photos were presented to clarify critical cases.

Table 1. The different localities of the studied taxa [the taxa are arranged according to APG II (2003)]

Family	Genus	Species	Locality
Amaryllidaceae	*Agave*	*A. americana* L. *A. lophantha* Schiede ex Kunth. *A. sisalana* Perrine	OBG OBG OBG
	Clivia	*C. miniata* (Lindl.) Bosse	ZBG
	Crinum	*C. augustum* Roxb. *C. bulbispermum* (Burm.f.) Milne-Redh. & Schweick. C. x *powellii* Baker	ZBG BGA BGA
	Curculigo	*C. capitulata* Kuntze. = *Molineria capitulata* (Lour.) Herb.	OBG
	Furcraea	*F. foetida* (L.) Haw.	OBG
	Hippeastrum	*H. vittatum* (L'Hér.) Herb.	BGA
	Hymenocallis	*H. caribaea* (L.) Herb.	OBG
	Narcissus	*N. tazetta* L.	OBG
	Pancratium	*P. maritimum* L.	Mma.
Bromeliaceae	*Billbergia*	*B. nutans* H. Wendl. ex Regel *B.* x *windii* Baker	BGA BGA
Cannaceae	*Canna*	*C. indica* L.	BGA
Iridaceae	*Antholyza*	*A. aethiopica* L.	ABG
	Freesia	*F. refracta* (Jacq.) Klatt	BGA
	Gladiolus	*G.* x *gandavensis* Van Houtte	BGA
	Iris	*I.* x *germanica* L.	BGA
Musaceae	*Musa*	*M. nana* Lour.	BGA
	Strelitzia	*S. reginae* Banks	OBG
Zingiberiaceae	*Alpinia*	*A. speciosa* Schum. = *Etlingera elatior* (Jack) R. M. Sm.	OBG
	Hedychium	*H. coronarium* J. Koenig	OBG

Abbreviations: ABG = Alexandria Botanical Garden, Alexandria University, Alexandria; **BGA** = Botanical Garden of Ain Shams University, Cairo; **Mma** = The Western Mediterranean Costal Region (Sea shore, sand dunes), Alexandria-Matruh road; **OBG** = Orman Botanical Garden, Giza; **ZBG** = Zohria Botanical Garden, Giza.

Results and discussions

The results obtained in this study are concerned the different patterns of floral vascularization and their discussion is subsequently presented.

I. The pedicel vasculatures (Table 2)

Within the studied taxa the pedicel vasculature ranges between the numerous, scattered vascular bundles (14 taxa) (Figure 1 a) or in the form of number of vascular bundles definitely arranged. These are either 6 bundles in *Clivia miniata* and *Narcissus tazetta* (Figure 1 b); or the supply is formed of outer 6 bundles and one inner vascular mass in *Antholza aethiopica*, or outer 12 bundles and 6 inner vascular masses in *Crinum* x *powellii*; or outer 16 bundles and inner 7 vascular masses in *Hedychium coronarium* (Figure 1 c) or 9 vascular masses forming a ring in *Alpinia speciosa*; or a central hexagonal stele in *Freesia refracta* and *Gladiolus gandavensis* (Figure 1 d). In the remaining two taxa *Curculigo capitulate* and *Crinum bulbispermum* the pedicel vasculature is in the form of numerous vascular bundles arranged in a ring (Figure 1 e).

II. The perianth leaves vasculatures (Table 2)

A. The outer perianth leaves vasculatures

1. Outer median tepal bundle

The origin of these bundles is either independent in 17 taxa (Figure 1 a) or dependent in the remaining taxa. It could then be mentioned that the dependent state is either resulting from the incorporation of such bundles with outer staminal traces (*Agave americans*, *Anotholyza aethiopica* and *Gladiolous* x *gandavensis*) (Figure 2 e) or from the incorporation of such bundles with both the outer staminal traces and the carpellary dorsal bundles (*Hymenocallis caribaea*, *Narcissus tazetta*, *Billbergig nutans* and *Iris* x *germanica*) (Figure 2 d). The initial number and the final number of the outer median tepal bundles is one for each tepal in all the taxa under investigation.

2. The outer marginal tepal bundles

The origin of the outer marginal tepal bundles in the taxa studied is either independent in 12 taxa (Figure 1a) or dependent in the other 12 taxa. The dependent case is resulting from the incorporation of such bundles with the other bundles. Thus, the following forms are observed: with outer staminal strands in *Clivia miniata*, with outer staminal and carpellary dorsal strands in *Crinum* x *powellii* and *Hippeastrum vittatum*, with inner median and marginal tepal strands in the all studied taxa of the family Iridaceae (Figure 2 e & f) and with inner marginal and outer marginal tepal strands in *Crinum bulbispermum*, *Curculigo capitulata*, *Narcissus tazetta*, *Pancratium maritimum* and *Billbergia nutans* (Figure 2 d). The initial number of the outer marginal traces varies. It is either two traces in seven taxa (Figure 2 c), six traces in eight taxa, 2-5 traces in *Crinum* x *powellii*, nine traces in *Crinum bulbispermum* or numerous traces in seven taxa. Generally, in the taxa studied there are numerous outer marginal bundles (Figure 5 a) except in *Clivia miniata* where there are only six bundles.

3. The outer androperianth strands

Generally, absent in 15 of the 24 taxa studied. However, the results obtained indicated the presence of such strands in the remaining nine taxa (Table 2). The level of differentiation and separation of these strands to their components is different. Thus, the following types are presented: separation at the base of the ovary in *Agave americana*, *Hymenocallis caribaea* and *Billbergia mutans*, separation at the middle of the ovary in *Crinum* x *powellii*, separation at the top of the ovary in *Hippeastrum vittatum*, *Antholyza aethiopica*, *Gladiolus* x *gandavensis* and *Iris* x *germanica* (Figure 3 b), and separation at the top of the very long collar "Androperianth tube" in *Narcissus tazetta* (Figure 4 d, e).

B. The inner perianth leaves vasculatures

The characteristic features of the vasculature of the inner perianth leaves (median, marginal and androperianth strands) of the different taxa under investigation have been indicated and presented in cumulative and comparative patterns (Table 2).

III. Floral cup (Table 2)

A. Casing

Consequently, it could be said that the nature of casing in the present work is represented in the following three types:

1. The casing is totally appendicular in the three studied taxa of *Agave* species, *Crinum augustum*, *Curculigo capitulata* and *Furcraea foetida* (Figure 2 a, b, c).
2. The casing is nearly receptacular in *Narcissus tazetta* (Figure 2 d).
3. The casing is receptacular and appendicular in the remaining 17 taxa (Figure 2 e, f).

B. Collar

The collar is present in most of the studied taxa (21 taxa). In the remaining studied taxa, the collar is totally absent (*Crinum* x *powellii*, *Canna indica* and *Musa nana*). The nature of the collar if present is appendicular in all of the taxa studied except *Narcissus tazetta* (Figure 4 a-e). The extension of the collar above the ovary level is either short in 11 taxa, medium in *Clivia miniata* and *Strelitzia reginae*, long in 5 taxa, or very long in *Crinum bulbispermum*, *Narcissus tazetta* and *Pancratium maritimum*.

IV. Androecium vasculatures (Table 3)

In all the taxa studied of Amaryllidaceae and Bromeliaceae, the number of fertile stamen is six in two whorls. In Iridaceae it is only three fertile stamens whereas in *Canna indica* (Cannaceae) it is half stamen (Figure 5 e). Musaceae (*Musa nana* and *Strelitzia reginae*) possess five stamens; three outer and two inner and in Zingiberaceae (*Alpinia speciosa* and *Hedychium coronarium*) it is only one fertile stamen (Figure 5 f).

The plan of androecium vasculature in all the studied taxa is composed of one trace for each stamen. These traces remain unbranched in 18 taxa (Figure 5 a, b, c). The staminal bundles are branched in both filaments and anthers forming a few bundles which trespass and enter into the anther in 5 taxa (Figure 5 d, e, f). In *Gladiolus* x *gandavensis*, the staminal supply is originated as one bundle which is then splitted into three bundles in the staminal filaments.

The androgynoecium strands is recorded only in 14 out of the 24 taxa studied. The androgynoecium strands show considerable variation with regard to their differentiation and separation levels. Thus, the following levels can be observed:

Level 1: Separation at the middle of the ovary in *Crinum bulbispermum*, *Hymenocallis caribaea*, *Pancratium maritimum* and *Billbergia nutans*.

Level 2: Separation at the top of the ovary in 10 taxa refer to Table 3 (Figure 3 b, c).

V. Gynoecium (Table 3)

In all the taxa studied, the gynoecium consists of three fused (syncarpous) unlobed carpels. Each carpel has three locules with many ovules in each. It has long been established that the carpel is a 3-traced organ, one dorsal and two ventral bundles [EAMES, 1953] it is believed that this 3-traced condition is the most primitive and this has been modified by both reduction and fusion [EAMES, 1929; FAHN, 1982 & PANDEY, 1982]. In the studied taxa, the gynoecium and its vascular supply has been found to behave in the following manner:

A. Ventral bundles

Just below the detection of the locules the floral stele is differentiated showing the following types; forming numerous traces in 12 taxa (Figure 1 a), forming six traces in 3 taxa,

forming three vascular bundles or masses in 5 taxa, forming one vascular mass in 3 taxa and undetected vasculature in *Agave lophantha*. The behavior of ventral bundles has been found to behave in the following manner:

Type I. Ventral bundles arising separate and numerous for each carpel

Ia. The numerous bundles originate free. At a higher level, they undergo branching in the placental tissue for feeding the ovules and the septal regions. At the upper portion of the ovary or at the base of style, the ventral bundles fade out gradually afterwards (do not extend beyond the ovular supply).

Ib. Similar to the type Ia but the ventral bundles extend beyond the ovular supply, then fade out gradually till they completely become lost at the base of the stylar tissue.

Ic. The numerous ventral supplies fuse forming three pairs (two for each carpel) then branch for feeding the ovules and the septal region. Afterward the six ventral bundles are extended for the style and reaching the stigmatic tissue.

Id. The numerous ventral bundles originate free. At a higher level they undergo branching in the placental tissue for feeding the ovules only. At the upper portion of the ovary the ventral bundles extend beyond the ovular supply then fade out gradually till they become completely lost at the base of the stylar tissue.

Type II. Ventral bundles arising separate, two for each carpel

IIa. The ventral bundles originate free. At a higher level, they undergo branching in the placental tissue for feeding the ovules and the septal regions. Then at the base of the style the bundles fade out gradually till they are completely lost in the stylar tissue. i.e. do not extend beyond the ovular supply.

IIb. The ventral bundles originate free. At a higher level, they undergo branching in the placental tissue for feeding the ovules only. Then the ventral bundles are extended for the style and reaching the stigmatic tissue.

In type I and II, if they were the case, this character would be a primitive trend according to the principles of phylogenetic considerations [BESSEY, 1915; HUTCHINSON, 1948, 1973; TAKHTAJAN, 1969; STEBBENS, 1977; STACE, 1985]. Moreover, the feeding of one carpel in this case by three or more vascular bundles (2 or more separate ventral and one dorsal bundles) is regarded generally as the most primitive condition.

Type III. Ventral bundles arising fused, one for each carpel

IIIa. The two fused ventral bundles are first splitted forming 6 bundles (2 for each carpel). At the placental tissue, they undergo branching for feeding the ovules and the septal regions, then extend beyond the ovular supply and eventually fade out till they become completely lost at the base of the stylar tissue.

IIIb. Similar to the type IIIa, but the ventral bundles extend to feed the style up to the stigmatic tissue.

Type IV. Ventral bundles arising as a vascular mass for carpels

IVa. The vascular mass is first splitted forming six bundles (2 for each carpel). At the placental tissue, they undergo branching for feeding the ovules and septal regions. Then at the base of the style the bundles fade out gradually till they are completely lost in the stylar tissue, i.e. do not extend beyond the ovular supply.

IVb. The vascular mass is first splitted forming six bundles (2 for each carpel). At the placental tissue, they undergo branching for feeding the ovules and septal regions. Then extend beyond the ovular supply then fade out gradually till they completely lost at the base of the stylar tissue.

The ventral supply continues to the top of the ovary, after supplying the ovules. This case is recorded in most of taxa under study (15 taxa) (Figure 3 c). In the remaining taxa, it is

both extended beyond the ovular supply and feed the style (*Agave americana*, *A. sisalana*, *Furcraea foetida* and *Iris* x *germanica*) (Figure 5 b, c), or undetected (5 taxa) (Figure 3 b).

B. Septal bundles

These bundles, if detected, lie at the same radii of the ventral bundles. EAMES (1953) stated that occasional species having branching ventral bundles and branches of these bundles running towards the margin are rare. In this study, the septal are only undetected in *Agave americana*, *A. lophantha* and *Musa nana* (Figure 2 c).

C. Dorsal carpellary bundles

The ovarian dorsal supply consists of one dorsal carpellary bundle per carpel. In this study, all the taxa studied persist in the stylar tissue and extend for feeding the stigma (Figure 4 a-e) except in *Canna indica*. In general, the total number of carpels can be traced easily by counting the number of stylar bundles. The origin of the carpellary dorsal supply is either dependent (15 taxa) or independent (9 taxa). In the former case, the carpellary dorsal supply is conjoint with complex vascular cord (Table 2 and 3).

D. Carpellary wall bundles

The carpellary wall bundles are lying on either side of the dorsal carpellary bundles and adjacent to each other. The carpellary wall bundles, if present fade out at the base of the style. These bundles are recorded in 8 taxa out of 24 taxa studied (Table 3, Figure 2 b).

E. Stylar supply

EAMES (1953) mentioned that the traces of the carpel may pass as unbranched bundles to the style or the stigma, or may branch in varying degrees. The results obtained in this study, indicate various behaviour patterns of the stylar supplies. The main variations can be presented as follows: 1 - The stylar supply is formed of 3 bundles representing the extension of the dorsal carpellary bundles. This type has been recorded in most of the taxa studied (19 taxa) (Figure 4 a-d). 2 - The stylar supply is formed of 3 dorsal bundles and extension of the carpellary ventral bundles, this type is observed in four taxa (Figure 4 b, c). In *Canna indica* the stylar supply is not detectable (Figure 4 e).

The results obtained in this study, indicate the presence of a number of analytic characters of limited occurrence which help in the identification of different entities. They are also called diagnostic or key characters. Among these characteristics are presence of staminal cup (*Hymenocallis caribaea* and *Pancratium maritimum*), corona (*Narcissus tazetta*), half fertile stamen, petaloid stamens in *Canna indica* (Figure 4 e), leafy styles in *Iris* x *germanica* (Figure 4 c), posterior inner staminode in *Musa nana* and *Strelitzia reginae* and labellum in *Alpinia speciosa and Hedychium coronarium* (Figure 4 f).

The nature of floral cups in longitudinal sections (Figure 6 a-h) can be determined from the way in which their vascular traces are conjoined with the vascular stele of receptacle or the pedicel. Thus, in most perigynous and epigynous flowers, these vascular traces are separated from the receptacular stele and extend through the androperianth tube. This indicates that this organ is formed as a result of fusion of the bases of sepals, petals and stamens. In other flowers the vascular stele run off and gets fused upwards till the periphery of the receptacular tissues "Hypanthium". This means that such structure hypanthium is an extension of the receptacle i.e. does not result from the adnation of floral parts. Consequently, and because of the difficulty of differentiating between the true hypanthium and the floral cup, the floral vasculature behavior should be consulted. In this respect, the main floral patterns are recorded: 1- almost all traces run fused throughout the casing and very long collar, the separation of vascular cords or strands is taking place at the top of the collar tissue. Thus, the nature of epigyny is totally receptacular

(Figure 6 a). 2 - The vascular supply of outer whorls consisting of partial independent traces which separate from the common vascular cords (strands) at the middle or the top of the ovary. Thus, the nature of epigyny is semi-appendicular (Figure 6 b-g). 3 - The vascular supply consisting of totally independent traces which run off distinct below the level of the locules. Thus, the nature of epigyny is totally appendicular (Figure 6 h).

In view of the above discussion the subsequent suggested interpretation may be presented. It is of primary importance to draw attention towards what is meant by epigyny. The phenomenon of epigyny is the reversed topographical position of the ovary relative to the other floral organs. In hypogenous flowers the shoot apex is convex and its upper most part carries the gynoecium which is superior to the other floral organs. Evolution, as phylogenetis agree implies a cessation of the apical growth of the shoot apex and the activity of the sub-apical cells to grow and form a cup-shaped receptacle. The floral organs, other than the gynoecium occupy the rim of this concave receptacle while the ovary becomes immersed to various levels with the adnation of its wall to the receptacular tissue. The inferior ovary which results in a way other than this is believed to be a false case of epigyny. If this is to be convincing, then the hypanthium, whatever its aspect does not share in this way of transformation, viz. from hypogyny to epigyny. In this study, the controversial nature of the hypanthium decided according to the following view-points:

a. The term has been applied to the whole perianth-tube, which is a result of adnation of stamens to all the perianth leaves. In this study, this is not accepted and the whole structure is better termed the "androperianth".
b. The same tube is suggested, here to be formed of two parts; a lower one capping the ovary and termed a "casing" and an upper one termed a "collar".
c. Unless the ovary becomes inferior through the concavity of the receptacle, the phenomenon is considered as "False epigyny".
d. The nature of any organ is decided according to the above considerations.

The acceptances of the above inference, the different states of epigyny as a character are the following:

I. The true inferior flower, ovary wholly receptacular.

The outer floral whorls (androperianth tube) have no own vascular system. It consists of dependent traces which run off fused and later become separated from the common vascular cord "strands" at the top of the collar tissue. Thus, the nature of both casing and collar is receptacular (*Narcissus tazetta*).

II. The false inferior flower

A. The ovary semi-appendicular

The outer floral whorls "androperianth tube" have own vascular system consisting of partial independent traces which separate from the common vascular cord "strands" at the middle or at the top of ovary. Thus, the nature of casing is receptacular and appendicular whereas the collar, if present is appendicular (17 taxa).

B. The ovary wholly appendicular

The outer floral whorls (androperianth tube) have their own vascular system consisting of totally independent traces which run off distinct at a level lower than that of the locules. Thus, the casing and the collar, if present are appendicular (*Agave americana*, *A. lophantha*, *A. sisalana*, *Crinum augustum*, *Curculigo capitulata* and *Furcraea foetida*).

Table 2. The micromorphological aspects of the pedicel, perianth leaves and floral cup of the taxa studied

Attributes / Taxa	Pedicel	Perianth Leaves													Floral Cup			
		Outer							Inner							Collar "Androperianth tube"		
		Medium			Marginal			Androperianth Strand	Medium			Marginal			Androperianth Strand	Casing Nature	Occurrence	Nature	Extension
		Origin	I.	F.	Origin	I.	F.		Origin	I.	F.	Origin	I.	F.					
1. *Agave americana*	Num.	Dep.	1	1	Ind.	2	∞	B. Ov.	Ind.	1	1	Ind.	2	∞	-	Ape.	+	Ape.	Short
2. *Agave lophantha*	Num.	Ind.	1	1	Ind.	2	∞	-	Ind.	1	1	Ind.	2	∞	-	Ape.	+	Ape	Short
3. *Agave sisalana*	Num.	Ind.	1	1	Ind.	2	∞	-	Ind.	1	1	Ind.	2	∞	-	Ape.	+	Ape.	Short
4. *Clivia miniata*	6V.m.	Ind.	1	1	Dep.	2	6	-	Dep.	1	1	Dep.	2	∞	T. Ov.	Ape./Rec.	+	Ape	Medium
5. *Crinum augustum*	Num. /Scatt.	Ind.	1	1	Ind.	∞	∞	-	Ind.	1	1	Ind.	∞	∞	-	Ape.	+	Ape.	Very Long
6. *Crinum bulbispermum*	Num./Ring	Ind.	1	1	Dep.	9	∞	-	Ind.	1	1	Dep.	9	∞	T. Ov.	Ape./Rec.	+	Ape.	Long
7. *Crinum* x *powellii*	12+6V.m.	Ind.	1	1	Dep.	2-5	∞	M. Ov.	Dep.	1	1	Dep.	2.3	∞	T. Ov.	Ape./Rec.	-	-	-
8. *Curculigo capitulata*	Num./Ring	Ind.	1	1	Dep.	6	∞	-	Dep.	1	1	Dep.	6	∞	B.Ov.	Ape.	+	Ape.	Short
9. *Furcraea foetida*	Num. /Scatt.	Ind.	1	1	Ind.	2	∞	-	Ind.	1	1	Ind.	2	∞	-	Ape.	+	Ape.	Long
10. *Hippeastrum vittatum*	Num. /Scatt.	Ind.	1	1	Dep.	2	∞	T. Ov.	Ind.	1	1	Dep.	2	∞		Ape./Rec.	+	Ape.	Short
11. *Hymenocallis caribaea*	Num. /Scatt.	Dep.	1	1	Ind.	∞	∞	B. Ov.	Dep	1	1	Ind.	∞	∞		Ape./Rec.	+	Ape.	Long
12. *Narcissus tazetta*	6V.m.	Dep.	1	1	Dep.	6	∞-	Collar	Dep.	1	1	Dep.	6	∞	Collar	Rec.	+	Ape./Rec.	Very Long
13. *Pancratium maritimum*	Num. /Scatt.	Ind.	1	1	Dep.	6	∞	-	Dep.	1	1	Dep.	6	∞	T.Ov.	Ape./Rec.	+	Ape.	Very Long
14. *Billbergia nutans*	Num. /Scatt.	Dep.	1	1	Dep.	6	∞	B. Ov.	Ind.	1	1	Dep.	6	∞	B.Ov.	Ape./Rec.	+	Ape.	Short
15. *Billbergia* x *windii*	Num. /Scatt.	Ind.	1	1	Ind.	2	∞	-	Ind.	1	1	Ind.	2	∞	-	Rec.	+	Ape.	Long
16. *Canna indica*	Num. /Scatt.	Ind.	1	1	Ind.	∞	∞	-	Ind.	1	1	Ind.	∞	∞	-	Ape./Rec.	-	-	-
17. *Antholyza aethiopica*	6+1V.m.	Dep.	1	1	Dep.	6	∞	T. Ov.	Dep.	1	1	Dep.	6	∞	-	Ape./Rec.	+	Ape.	Short
18. *Freesia refracta*	Central Hex.	Ind.	1	1	Dep.	6	∞	-	Dep.	1	1	Dep.	6	∞	-	Ape./Rec.	+	Ape.	Long
19. *Gladiolus* x *gandavensis*	Central Hex.	Dep.	1	1	Dep.	6	∞	T. Ov.	Dep.	1	1	Dep.	6	∞	-	Ape./Rec.	+	Ape.	Short
20. *Iris* x *germanica*	Num. /Scatt.	Dep.	1	1	Dep.	6	∞	T. Ov.	Dep.	1	1	Dep.	6	∞	-	Ape./Rec.	+	Ape.	Short
21. *Musa nana*	Num. /Scatt.	Ind.	1	1	Ind.	∞	∞	-	Ind.	1	1	Ind.	∞	∞	-	Ape./Rec.	-	-	-
22. *Strelitzia reginae*	Num. /Scatt.	Ind.	1	1	Ind.	∞	∞	-	Ind.	1	1	Ind.	∞	∞	-	Ape./Rec.	+	Ape.	Medium
23. *Alpinia speciosa*	6vm+Ring.	Ind.	1	1	Ind.	∞	∞	-	Dep.	1	1	Dep.	∞	∞	T.Ov.	Ape./Rec.	+	Ape.	Short
24. *Hedychium coronarium*	16+7 V.m.	Ind.	1	1	Ind.	∞	∞	-	Dep.	1	1	Dep.	∞	∞	T.Ov.	Ape./Rec.	+	Ape.	Short

Abbreviations: Ape.: Appendicular, **B**.**Ov**.: Basic of Ovary, **Dep**.: Dependent, **F**.: Final number, **Hex**.: Hexagonal Shape, **I**.: Initial number, **Ind**.: Independent, **M**.**Ov**.: Medium of Ovary, **Num**.: Numerous, **Rec**.: Receptacular, **Scatt**.: Scattered, **T**.**Ov**.: Top of Ovary, **V**.**m**.: Vascular mass, -: Absent, +: Present.

Table 3. The micromorphological aspects of androecium and gynoecium of the taxa studied

Attributes	Androecium						Gynoecium								Characteristic Features
	Stamem Number		Staminal bundle behaviour		Androgynoecium Strand		Ventral bundles			Septal Bundles	Dorsal Supply		C.W.B.	Stylar Supply	
Taxa	Fertile	Staminode	Filament	Anther	Occurance	Separat.	Initial number	Behaviour***	Extension		Origin	Numbers			
1. *Agave americana*	3+3	-	Branch.	Branch.	-	-	6	IIb	Unde.	++	Ind.	3	-	C.D.B.+Ven.B.	-
2. *Agave lophantha*	3+3	-	Unbranch.	Unbranch.	-	-	Unde.	Unde.	Unde.	Unde.	Ind.	3	-	C.D.B.	-
3. *Agave sisalana*	3+3	-	Branch.	Branch.	-	-	Num.	Ic	+	++	Ind.	3	+	C.D.B.+Ven.B.	-
4. *Clivia miniata*	3+3	-	Unbranch.	Unbranch.	-	-	3	IIIa	+	+**	Ind.	3	-	C.D.B.	-
5. *Crinum augustum*	3+3	-	Unbranch.	Unbranch.	-	-	Num.	Ib	+	+**	Ind.	3	-	C.D.B.	-
6. *Crinum bulbispermum*	3+3	-	Unbranch.	Unbranch.	+	M.Ov.	1	IVa	+	Unde.	Dep.	3	-	C.D.B.	-
7. *Crinum* x *powellii*	3+3	-	Unbranch.	Unbranch.	+	T.Ov.	1	IVb	+	+**	Dep.	3	+	C.D.B.	-
8. *Curculigo capitulata*	3+3	-	Unbranch.	Unbranch.	-	-	6	IIa	+	Unde.	Ind.	3	+	C.D.B.	-
9. *Furcraea foetida*	3+3	-	Unbranch.	Unbranch.	-	-	Num.	Ic	+	++	Ind.	3	+	C.D.B.+Ven.B.	-
10. *Hippeastrum vittatum*	3+3	-	Unbranch.	Unbranch.	+	T.Ov.	6	IIa	+	Unde.	Dep.	3	-	C.D.B.	-
11. *Hymenocallis caribaea*	3+3	-	Unbranch	Unbranch	+	M.Ov.	Num.	Ib	+	+**	Dep.	3	-	C.D.B.	Staminal cup
12. *Narcissus tazetta*	3+3	-	Unbranch	Unbranch	+	T.Ov.	1	IVb	+	+**	Dep.	3	-	C.D.B.	Corona
13. *Pancratium maritimum*	3+3	-	Unbranch	Unbranch	+	M.Ov.	Num.	Ia	+	+**	Dep.	3	-	C.D.B.	Staminal cup
14. *Billbergia nutans*	3+3	-	Unbranch	Unbranch	+	M.Ov.	Num.	Ib	+	+**	Dep.	3	-	C.D.B.	-
15. *Billbergia* x *windii*	3+3	-	Unbranch.	Unbranch.	+	T.Ov.	Num.	Ib	+	+**	Dep.	3	-	C.D.B.	-
16. *Canna indica*	0.5	4+1.5	Branch.*	Branch.*	+	T.Ov.	Num.	Id	+	+**	Dep.	3	-	C.D.B.	Half-fertile stamen
17. *Antholyza aethiopica*	3	-	Unbranch.	Unbranch.	-	-	3	IIIa	+	+**	Ind.	3	+	C.D.B.	-
18. *Freesia refracta*	3	-	Unbranch.	Unbranch.	+	T.Ov.	3	IIIa	+	+**	Dep.	3	+	C.D.B.	-
19. *Gladiolus* x *gandavensis*	3	-	Branch.	Branch.	-	-	3	IIIa	+	+**	Ind.	3	+	C.D.B.	-
20. *Irisx* x *germanica*	3	-	Unbranch.	Unbranch.	-	-	3	IIIb	+	++	Dep.	3	+	C.D.B.+Ven.B.	Leafy styles
21. *Musa nana*	3+2	1	Unbranch.	Unbranch.	+	T.Ov.	3	Unde.	Unde.	Unde.	Dep.	3	-	C.D.B	Posterior inner staminode
22. *Strelitzia reginae*	3+2	1	Unbranch.	Unbranch.	+	T.Ov.	Num.	Ib	+	+**	Dep.	3	-	C.D.B.	Posterior inner staminode
23. *Alpinia speciosa*	1	3+2	Branch.*	Branch.*	+	T.Ov.	Num.	Ib	+	+**	Dep.	3	-	C.D.B.	Staminodiom"Labeellum"
24. *Hedychium coronarium*	1	3+2	Branch.*	Branch.*	+	T. Ov.	Num.	Ib	+	+**	Dep.	3	-	C.D.B.	Staminodiom"Labeellum"

Abbreviations: Branch.: Branched, **C**.**D**.**B**.: Carpellary Dorsal Bundle, **C**.**W**.**B**.: Carpellary Wall Bundle, **Dep**.: Dependent, **Ind**.: Independent, **M**.**Ov**.: Medium of Ovary, **Num**.: Numerous, **T**.**Ov**.: Top of Ovary, **Unbranch**.: Unbranched, **Unde**.: Undetected, **Ven.B**.: Ventral Bundle, (+)**:** Present, (++): The ventral bundles are extended for the style and reaching the stigmatic tissue, (-): Absent, (*): Either for one fertile stamen or for half stamen, (**): The ventral bundles extended beyond the ovular supply then fade out at the base of the style, (***): Refer to number of groups as result in the main text.

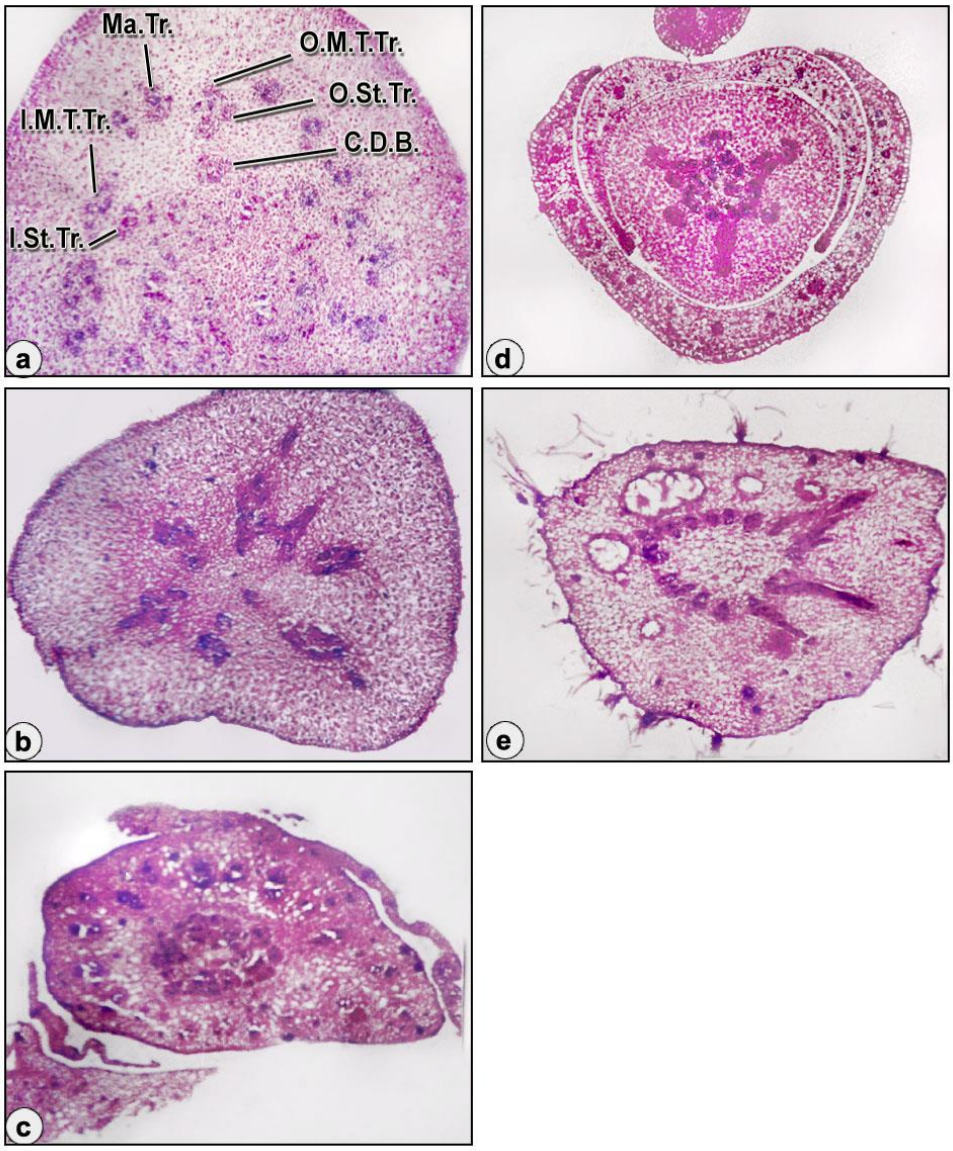

Figure 1. Variations in the pedicel vasculature in some of the taxa studied. a. *Agave americana*, b. *Clivia miniata*, c. *Hedychium coronarium*, d. *Freesia refracta*, e. *Curculigo capitulata*.
Abbreviations: C.D.B.: Carpellary Dorsal Bundle; I.M.T.Tr.: Inner Median Tepal Trace; I.St.Tr.: Inner Staminal Trace; Ma.Tr.: Marginal Trace; O.M.T.Tr.: Outer Median Tepal Trace; O.St.Tr.: Outer Staminal Trace.

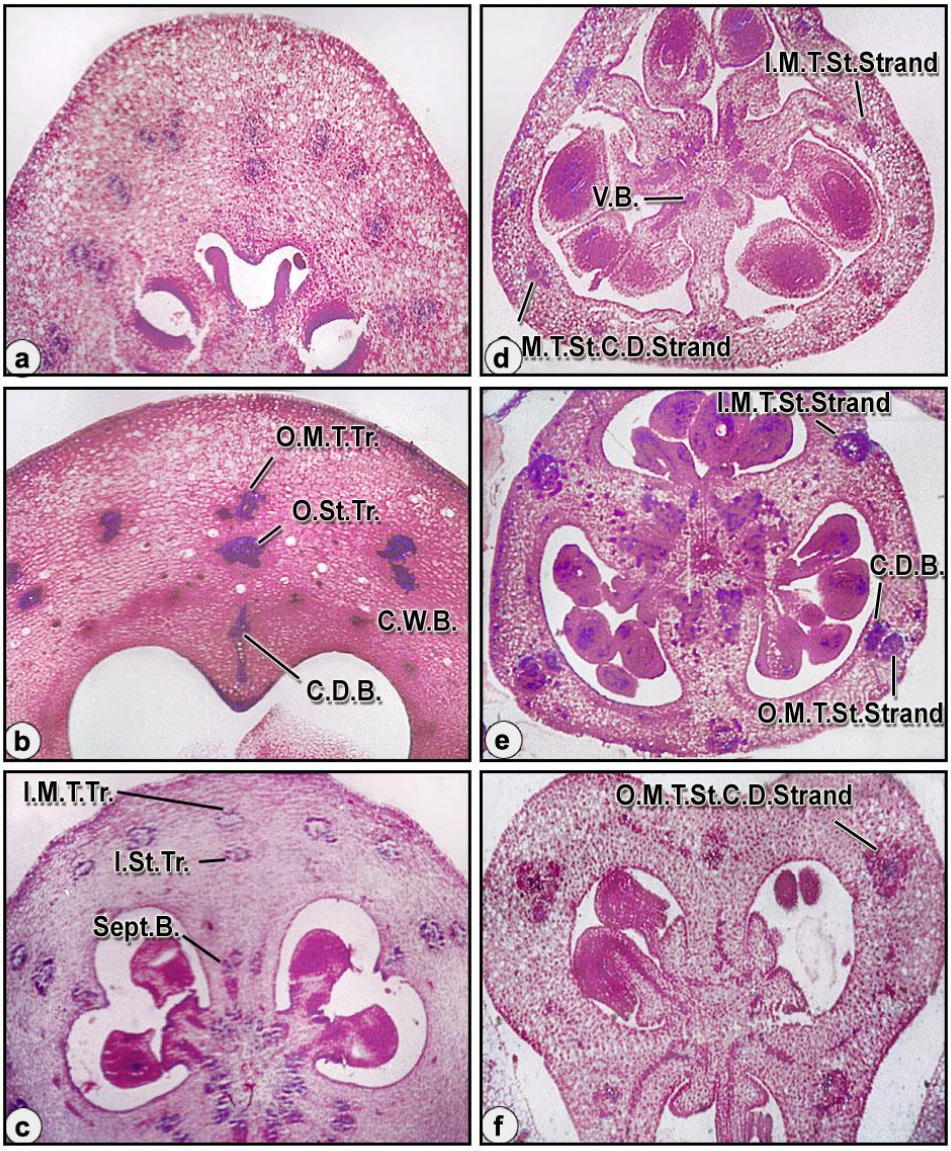

Figure 2. Variations in the casing vasculature in some of the taxa studied. a. *Agave americana*, b. *Agave sisalana*, c. *Furcraea foetida*, d. *Narcissus tazetta*, e. *Gladiolus* x *gandavensis*, f. *Iris* x *germanica*. ***Abbreviations*:** C.D.B.: Carpellary Dorsal Bundle; C.W.B.: Carpellary Wall Bundle; I.M.T.St.Strand: Inner Median Tepal Staminal Strand; I.M.T.Tr.: Inner Median Tepal Trace; I.St.Tr.: Inner Staminal Trace; O.M.T.St.C.D.Strand: Outer Median Tepal Staminal Carpellary Dorsal Strand; O.M.T.Tr.: Outer Median Tepal Trace; O.M.T.St.Strand: Outer Median Tepal Staminal Strand; O.St.Tr.: Outer Staminal Trace; Sept. B.: Septal Bundle.

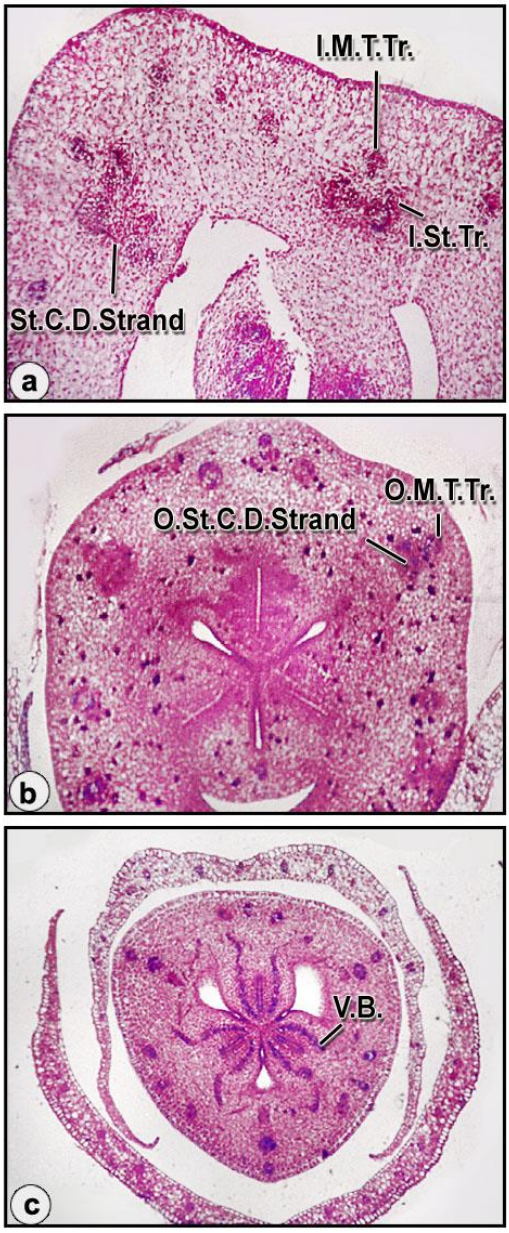

Figure 3. Variations in the vasculature at the top of the ovary in some of the taxa studied. a. *Hymenocallis caribaea*, b. *Antholyza aethiopica*, c. *Freesia refracta*.
***Abbreviations*:** I.M.T.Tr.: Inner Median Tepal Trace; I.St.Tr.: Inner Staminal Trace; O.M.T.Tr.: Outer Median Tepal Trace; O.St.C.D.Strand: Outer Staminal Carpellary Dorsal Strand; St.C.D.Strand: Staminal Carpellary Dorsal Strand; V.B.: Ventral Bundle.

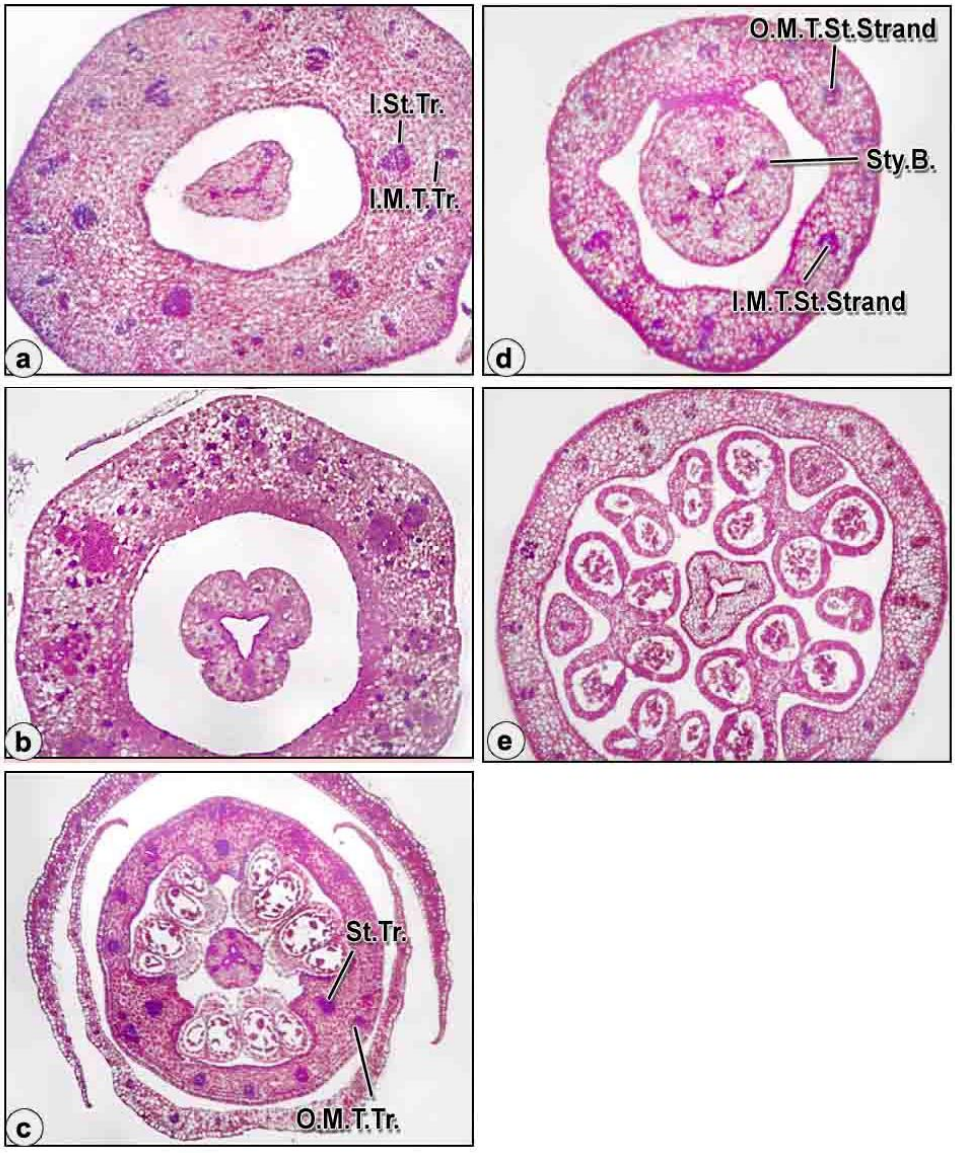

Figure 4. Variations in the collar vasculature in some of the taxa studied. a. *Crinum bulbispermum*, b. *Antholyza aethiopica*, c. *Freesia refracta*, d. & e. *Narcissus tazetta.*
***Abbreviations*:** I.M.T.Tr.: Inner Median Tepal Trace; I.M.T.St.Strand: Inner Median Tepal Staminal Strand; I.St.Tr.: Inner Staminal Trace; O.M.T.Tr.: Outer Median Tepal Trace; O.M.T.St.Strand: Outer Median Tepal Staminal Strand; St.Tr.: Staminal Trace; Sty.B.: Stylar Bundle.

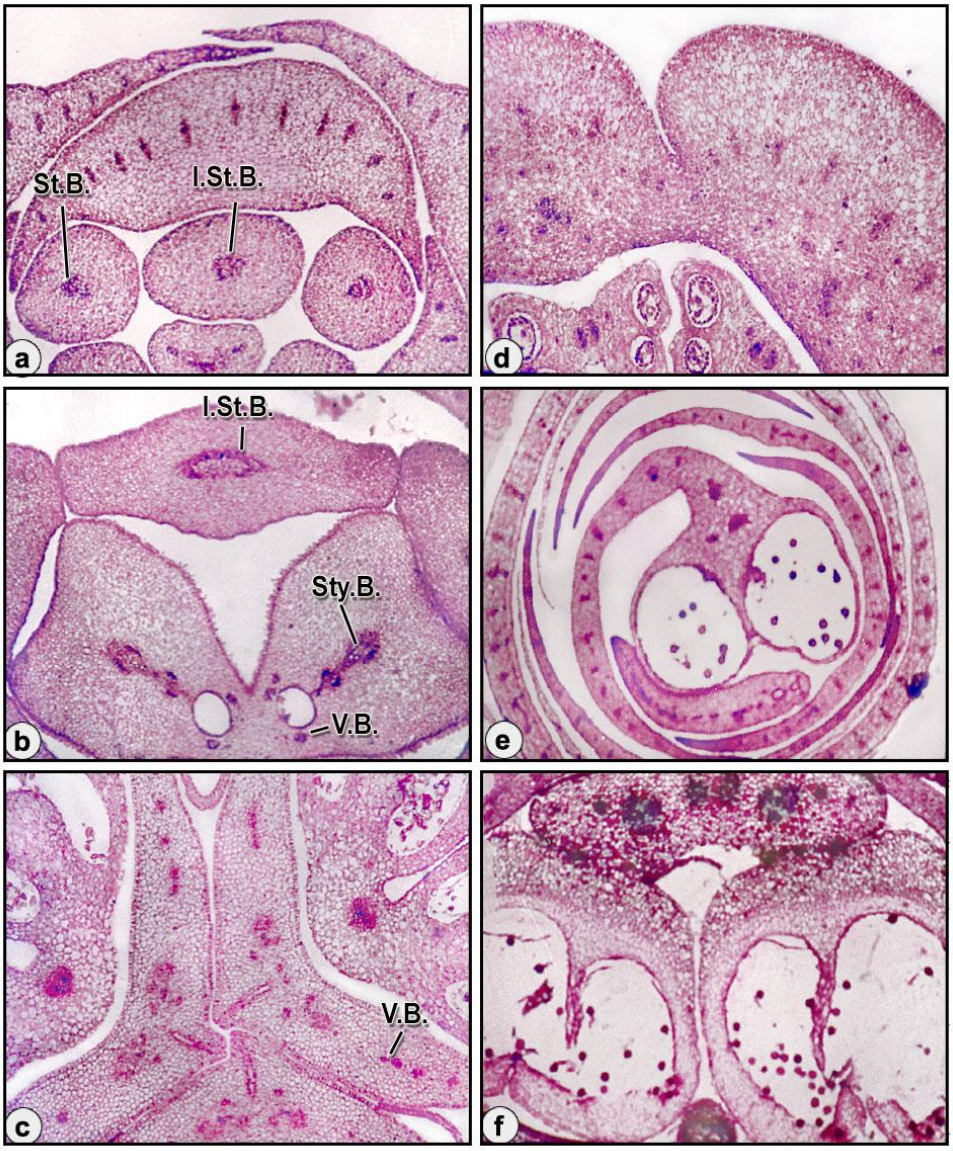

Figure 5. Variations in the stamens, style and stigma vasculature, in some of the taxa studied. a. *Crinum* x *powellii*, b. *Furcraea foetida*, c. *Iris x germanica*, d. *Agave americana*, e. *Canna indica*, f. *Alpinia speciosa*.
***Abbreviations*:** I.St.B.: Inner Staminal Bundle; O.St.B.: Outer Staminal Bundle; Sty.B.: Stylar Bundle; V.B.: Ventral Bundle.

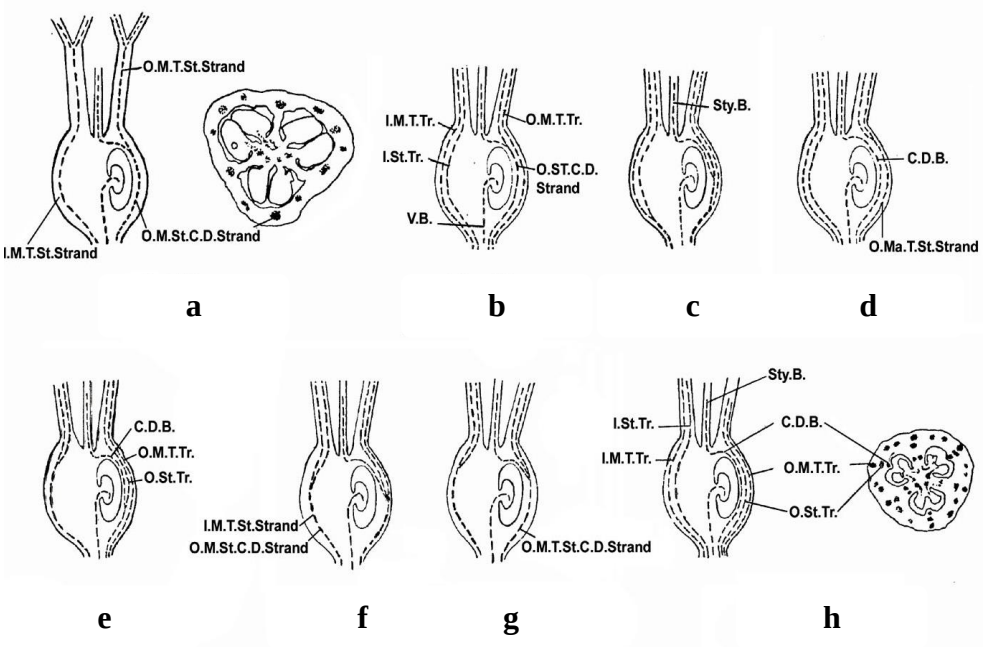

Figure 6. Longitudinal sections showing the significance of floral vascularization in the interpretation on the nature of epigynous flowers.

***Abbreviations*:** C.D.B.: Carpellary Dorsal Bundle; I.M.T.Tr.: Inner Median Tepal Trace; I.M.T.St.Strand: Inner Median Tepal Staminal Strand; I.St.Tr.: Inner Staminal Trace; O.Ma.T.St.Strand: Outer Marginal Tepal Staminal Strand; O.M.T.St.Strand: Outer Median Tepal Staminal Strand; O.M.T.St.C.D.Strand: Outer Median Tepal Staminal Carpellary Dorsal Strand; O.M.T.Tr.: Outer Median Tepal Trace; O.St.C.D.Strand: Outer Staminal Carpellary Dorsal Strand; O.St.Tr.: Outer Staminal Trace; Sty.B. Stylar Bundle; V.B.: Ventral Bundle.

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GENETIC AND AGRONOMIC EVALUATION OF RAINFED TOMATO (*SOLANUM LYCOPERSICUM* L.) ACCESSIONS IN IBADAN, NIGERIA

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Abstract: The extent of genetic variability among 19 accessions of tomato was studied using genetic variability parameters. Four-week old seedlings were transplanted in a well levelled field with 0.6 x 0.6 spacing and replicated three times in randomized complete block design. High significant differences among the accessions for all attributes studied. Cluster analysis based on 37 agro-morphological attributes separated accessions into two distinct groups according to the fruit types. Values for genotypic and phenotypic coefficients of variation showed variability among the accessions. Correlation analysis showed fruit/plant is positively and significantly correlated to plant height, number of branches/plant and leaf length. Very high genetic advance and heritability estimates for leaf length, leaf width, days to flower, days to 50% flowering, number of fruits/plant, fruit length, fruit diameter, fruit weight and 1000 seed weight suggest simple inheritance system and thus amenability for these attributes to selection in tomato improvement.

Keywords: attributes, genetic advance, heritability, inheritance, tomato, variance.

Introduction

Tomato (*Solanum lycopersicon* L. syn. – *Lycopersicon esculentum* Mill., *Lycopersicon lycopersicum* (L.) Karsten ex Farw.), is one of the most important vegetable crops grown over the world because of its wider adaptability, high yielding potential and suitability for variety of uses in fresh as well as processed food industries. It is one of the most important vegetable crops grown in Nigeria and utilized in almost every household for preparation of several dishes. Tomato plays an important role in human nutrition by providing essential amino acids, vitamins and minerals [SAINJU & al. 2003]. Its vitamin C content is particularly high [KANYOMEKA & SHIVUTE, 2005]. It also contains lycopene, a very potent antioxidant that may be an important contributor to prevention of cancers [AGARWAL & RAO, 2000]. With production of over 150 million tons of fresh fruit on 3.7 million hectares tomato exceeds the production of all other crops, with the exception of the potato and sweet potato [FAOSTAT, 2010]. Production in Nigeria has more than doubled in the last 10 years

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with the production in 2001, amounting to about 879,000 tonnes [AKANBI & OLUDEMI, 2003]. However, commercial tomato production in Nigeria relies mostly on exotic introductions. The production of which is essentially restricted to the Northern Guinea Savanna and the Sudan ecologies due to favourable climatic conditions, particularly high insolation and low relative humidity. In nearly three decades up to 2013, no tomato variety has been released in Nigeria [NACGRAB & NASC, 2013]. The need therefore, to explore the production capabilities and potentials of long forgotten indigenous land races and other ecotypes has never been more urgent.

The concept of heritability which specifies the proportion of the total variation among a species due to genetic components combined with genetic advance. These are good parameters for determining gene action involved in the inheritance of any trait and by extension help in deciding the best breeding method to apply for improving such trait. High heritability indicates less environmental influence in the observed variation [SONGSRI & al. 2008; EID, 2009], while high heritability accompanied by high genetic advance is an indication of additive gene action for such trait, making it most amenable to selection [TAZEEN & al. 2009]. Determining the variability of yield and yield related components will enable the plant researcher to deduce the extent of environmental influence on yield, considering that yield and its components are quantitative characters and are affected by the environment.

This study was carried out to determine the extent of genetic variation and agronomic evaluation among available tomato accessions with the specific objective to use suitable genetic parameters such as phenotypic and genotypic variances, phenotypic and genotypic coefficients of variation and genetic advance as a basis for future breeding work in tomato.

Material and methods

Nineteen (19) accessions of tomato held in National Genebank at the National Centre for Genetic Resources and Biotechnology (NACGRAB) were planted for 2013 rainy season. The accessions were first planted in nursery trays placed in a mesh house after relevant seed treatment with Mancozeb®. Top soil was used for planting. Seedlings were transplanted at four weeks after planting (WAP) to NACGRAB research field, Moor Plantation (224m, 7º23`, 3º50`), Ibadan, Nigeria. Seedlings were transplanted unto a well ploughed, harrowed and levelled field. Inter and intra-row spacing was 0.6 x 0.6m. Each treatment accession was in single 6 meter row plot. Total field size was 33m x 12m. The treatments were replicated thrice and laid out in a Completely Randomized Block Design. Recommended cultural practices were followed and irrigation was employed in the month of August when there was no rainfall. Data were recorded from five pre-tagged plants of each treatment. Attributes measured and recorded using descriptors for Tomato (*Solanum* spp.) [IBPGR, 1997] included: Leaf type, inflorescence type, stem pigmentation, stem pubescence, predominant fruit shape, colour of immature fruit, colour of ripe fruit, plant height (cm), number of branches per plant, leaf length (cm), leaf width (cm) number of leaflets, number of days to first flowering, number of days to first fifty per cent flowering, number of days to ripening of first fruit, number of days to maturity, number of fruits per inflorescence, number of fruits per pedicel number of fruits per plant, peduncle length (cm), fruit length (mm), fruit diameter (mm), weight per fruit (g), weight of 1000 seeds (g) and fruit yield per plant (g). Quantitative data obtained were subjected to Analysis of variance and significance means

were separated using Duncan multiple range test (DMRT) using PBTools [PBTOOLS, 2014]. Mean values were used to estimate Genotypic and Phenotypic coefficients of variation as by [SINGH & CHAUDHURY, 1985]. The broad sense heritability and genetic advance were calculated as proposed by [JOHNSON & al. 1955] and simple linear correlation coefficient was determined according to [SNEDECOR & COCHRAN 1967]. A correlation matrix was drawn up using the linear correlation coefficients. UPGMA cluster analysis was used to construct a dendogram to ascertain the genetic relationships among the tomato accessions.

Results and discussions

Growth parameters

There were statistically significant differences among the accessions on traits analyzed (Table 1). However, accession NG/Mr/MAY/09/005 had higher mean plant height while variety NG/AA/SEP/09/045 recorded the least values. The mean number of branches of accessions showed that NG/SA/01/10/002 recorded highest mean values while NHGB/09/113 recorded the least value. The mean number of leaflet showed significant difference among the accessions, NG/MR/MAY/09/005 recorded the highest values while NHGB/09/114 recorded the least. NG/MR/MAY/09/005 had highest mean leaf length value while NG/AA/SEP/09/042 had the least value. NG/0E/MAY/09/019 had highest mean leaf width value while NG/RM/JAN/10/001 had the least value. Accessions NG/0E/MAY/09/019; NG/AA/SEP/09/050 and NG/AA/SEP/09/013 had highest mean pedicel length while accession L00170 recorded the least values.

Yield and Yield Components of Different Tomato Accessions In Ibadan Nigeria.

Yield and Yield Components of the Different Tomato Accessions were statistically significant among the accessions (Table 2). However, accession NG/Mr/MAY/09/005 had least mean number of days to flowering while NG/AA/SEP/09/013 recorded the highest mean number of days to flowering. Least mean value of days to 50% flowering was recorded for NG/AA/SEP/09/037 while highest mean value was recorded for NG/AA/SEP/09/042. Accessions NG/AA/SEP/09/013 and NG/AA/SEP/09/044 recorded least mean value for number of fruit per pedicel per plant while NGHB/09/114 and NG/RM/JAN/10/001 recorded high mean values respectively. NG/0E/MAY/09/019 and NG/AA/SEP/09/013 recorded high mean values for fruit weight while NG/RM/JAN/10/001 and L00169 recorded least mean values. Highest mean value for fruit length was recorded by NG/AA/SEP/09/037 and the least mean value recorded by NG/RM/JAN/10/001. Highest mean value for fruit width was recorded by NG/AA/SEP/09/013 while NG/RM/JAN/10/001. Highest mean value for fruit per plant was recorded by NG/RM/JAN/10/001 and NG/01/MAY/09/019 recorded the least mean value for this trait. Mean highest number of days to maturity was recorded for NG/AA/SEP/09/045 while L00169 had the least mean values. NG/AA/SEP/09/050 had the highest mean value for days to first fruit ripening while L00169 had the least value. Highest mean value for fruit yield per plant was recorded for NG/SA/01/10/002 while L00169 recorded least mean value. NG/MR/MAY/09/005 recorded highest mean value for 1000 seed weight while NG/AA/SEP/09/050 recorded the least mean value.

Genetic diversity

Genetic diversity analysis for the accessions based on morphological characteristics measured, revealed that genetic distance ranging from 0.30 to 0.49 (Fig. 1). In this study, the cluster analysis based on 37 agro-morphological attributes that separated accessions into two distinct groups at 0.32 coefficient, which were according to fruit types – into cherry and classic fruit groups corresponding to varietal types (Figure 1). Cluster 1 included five accessions all of which are from south western Nigeria, while cluster 2 had 14 varieties from South-West Nigeria, South-South Nigeria and Republic of Benin (Table 3). Thirty-two of the 37 attributes recorded had morphological variation in 19 tomato accessions studied. The 20 qualitative attributes had two to nine numbers of observable types. Eight attributes (40%) had more than two types, of which fruit shape had the largest variation with six types (slightly flattened, flattened, cylindrical, rounded, ellipsoid and high rounded). There were no obvious differences for five attributes (leaf type, division of leaf blade, stem pigmentation, abscission layer and flower colour) among the accessions studied.

Genetic variability

Analysis of variance for the means of all the measured attributes showed significant differences ($P \leq 0.001$) among the accessions (Table 4). Values of genotypic and phenotypic variances were lowest in peduncle length and highest in fruit yield per plant. Higher values of genotypic and phenotypic variances were observed respectively for plant height (21.89, 40.75), number of branches (319.60, 499.91), leaf length (31.08, 31.95), number of leaflets (203.41, 441.06), days to flower (33.23, 41.75), days to 50% flower (86.43, 105.79), days to fruit ripening (21.27, 36.65) fruit per plant (4349.08, 4826.12), fruit length (144.05, 149.06), fruit diameter (144.09, 160.49), weight per fruit (672.56, 772.6), day to maturity (28.07, 31.94) and fruit yield per plant (775796.12, 1049841.90). The genotypic coefficient of variation (GCV) ranged from 6.06 in days to fruit ripening to 118.51 in fruit per plant. Similarly, PCV ranged from 7.96 (days to fruit ripening) to 124.84 (fruit per plant).

Estimates of broad sense heritability (H^2b) and genetic advance

Estimates of heritability in the broad sense were very high for leaf length (97%), leaf width (88%), days to flower (80%), days to 50% flowering (82%), fruit per plant (90%), fruit length (97%), fruit diameter (90%), fruit weight (100%) and 1000 seed weight (Table 3). Peduncle length (39%), number of leaflets per plant (46%) and number of days to fruit ripening (58%) had low to moderate heritability (Table 5). Very high genetic advance and heritability estimates were recorded for leaf length, leaf width, days to flower, days to 50% flowering, fruit per plant, fruit length, fruit diameter, fruit weight and 1000 seed weight.

Character association

Fruit yield per plant is positively and significantly ($P<0.05$) correlated to plant height ($r = 0.481$), number of branches per plant ($r = 0.471$) and leaf length ($r = 0.507$). Positive and significant association of number of fruits per plant with number of fruit per inflorescence ($r = 0.726$) is an indication of increased number of fruits with increased number of fruit bearing inflorescence. Weight per fruit which is a function of fruit size had predictably positive and significant association ($r = -0.582$) with fruit length and fruit diameter. In this work, number fruit per plant was negatively and significantly correlated with fruit diameter ($r = 0.582$). Number of branches had a significantly positive relationship with plant height ($r = 0.782$) and number of leaflets per plant ($r = 0.861$) while maintaining negative and

significant relationships with days to flower (r = -0.752), days to 50% flowering (r = -0.609), days to fruit ripening (r = -0.499), and days to maturity (r = -0.505) – Table 6.

Table 1. Means of agronomic attributes of nineteen tomato accessions in Ibadan, Nigeria

Accessions	Plant height (cm)	Number of branches	No of leaflet	Leaf length (cm)	Leaf width (cm)	Pedicel length (cm)
NG/SA/01/10/002	17.15	11.00	51.11	37.87	23.00	0.60
NGHB/09/120	16.2	7.28	51.0	29.07	21.80	0.50
NG/AA/SEP/09/045	5.90	6.00	25.0	27.60	20.47	0.53
NHGB/09/113	7.81	4.34	18.67	27.60	20.47	0.53
NG/AA/SEP/09/044	13.72	9.33	59.0	27.77	19.57	0.50
L00170	14.48	6.27	31.0	33.00	20.10	0.33
NG/OE/MAY/09/019	9.63	5.55	26.06	37.70	27.00	0.70
NG/AA/SEP/09/050	13.0	7.00	19.0	27.60	20.57	0.70
NG/SA/07/10/002	12.53	6.28	29.72	30.90	20.57	0.37
NG/AA/SEP/09/040	12.05	6.00	37.12	23.27	18.17	0.37
NG/MR/MAY/09/005	24.67	10.78	73.78	42.20	26.03	0.60
NG/AA/SEP/09/037	22.29	8.55	56.55	30.97	20.27	0.50
NG/RM/JAN/10/001	8.54	6.89	26.44	19.77	12.50	0.50
NG/MR/MAY/09/006	17.95	6.77	40.89	32.20	21.07	0.50
NGHB/09/114	6.14	4.55	18.55	26.00	17.10	0.42
NG/AA/SEP/09/013	13.09	7.66	41.18	29.90	20.30	0.70
NG/AA/SEP/09/042	6.91	5.06	20.89	19.30	13.7	0.40
L00169	9.60	7.17	45.0	28.20	16.7	0.37
NG/AA/SEP/09/053	18.01	8.67	63.56	34.37	25.10	0.50
F test	***	***	***	***	***	*
MSE	2.67	0.89	9.39	0.68	1.09	0.07
H^2	0.78	0.80	0.72	0.99	0.91	0.66

*, *** = significant at 5% and 0.1% probability levels respectively.
MSE = Standard error of mean
H^2 = Heritability

Table 2. Means of yield and yield components of nineteen tomato accessions in Ibadan, Nigeria

Accessions	Number of days to flower	Number of days to 50% flowering	Number of fruits per pedicel	Weight per fruit (g)	Fruit length (cm)	Fruit width (cm)	Number of fruits per plant	No of days to maturity	No of days to ripening of first fruit	Fruit yield per plant (kg)	1000 seed weight (g)
NG/SA/01/10/002	39.00	43.00	5.67	47.92	37.7	45.3	79.7	73.0	68	3.81	1.593
NGHB/09/120	40.00	46.67	3.33	64.14	47.0	54.0	17.7	78.0	75.33	1.21	2.673
NG/AA/SEP/09/045	51.00	53.33	6.0	27.28	51.7	27.3	31.0	87.7	83.67	0.85	2.443
NHGB/09/113	45.00	47.00	3.33	52.72	33.3	47.0	19.3	80.0	64.67	1.05	2.140
NG/AA/SEP/09/044	42.00	43.00	3.0	11.86	22.0	33.3	10.0	78.0	78.67	1.13	2.073
L00170	44.67	46.67	6.67	10.13	24.0	26.7	78.3	80.0	75.67	1.15	2.563
NG/OE/MAY/09/019	46.00	50.67	4.67	101.8	49.0	57.0	11.0	86.0	82.00	1.12	2.446
NG/AA/SEP/09/050	49.00	53.00	4.0	27.28	34.3	35.5	7.0	87.3	85	0.19	1.123
NG/SA/07/10/002	49.00	48.00	5.0	28.12	32.3	40.0	33.7	86.0	84	0.90	2.806
NG/AA/SEP/09/040	43.67	46.67	4.67	43.12	35.0	45.0	52.7	83.0	74	2.30	2.960
NG/MR/MAY/09/005	29.67	43.33	5.0	46.36	41.3	43.3	52.0	77.7	71.67	2.41	2.967
NG/AA/SEP/09/037	34.00	41.00	5.0	27.26	59.3	32.7	81.7	74.3	71.00	2.21	2.617
NG/RM/JAN/10/001	39.00	43.33	8.33	2.92	14.0	14,7	305.0	75.0	74.67	0.58	2.447
NG/MR/MAY/09/006	43.00	49.67	6.0	25.20	29.7	42.0	53.0	74.7	72.33	1.29	3.335
NGHB/09/114	51.33	68.33	7.0	48.51	40.7	42.0	39.0	81.0	79.67	1.30	2.123
NG/AA/SEP/09/013	55.67	43.00	3.0	86.33	42.3	58.5	17.7	75.0	73.00	1.52	2.207
NG/AA/SEP/09/042	46.00	80.00	5.0	19.18	27.0	28.5	14.0	78.0	75.33	0.27	2.120
L00169	41.67	46.00	6.33	2.72	16.7	17.0	80.0	71.7	63.67	0.21	1.153
NG/AA/SEP/09/053	32.67	42.67	5.67	25.13	46.3	32.3	41.3	79.7	71.00	1.01	1.623
F test	*	***	***	***	***	***	***	***	***	***	***
MSE	3.96	2.56	0.439	5.708	1.26	2.288	9.694	1.749	3.06	0.329	0.0076
H^2	0.65	0.93	0.92	0.95	0.99	0.96	0.98	0.88	0.75	0.88	0.98

*, **, *** = significant at 5%, 1% and 0.1% probability levels respectively. MSE = Standard error of mean. H^2 = Heritability

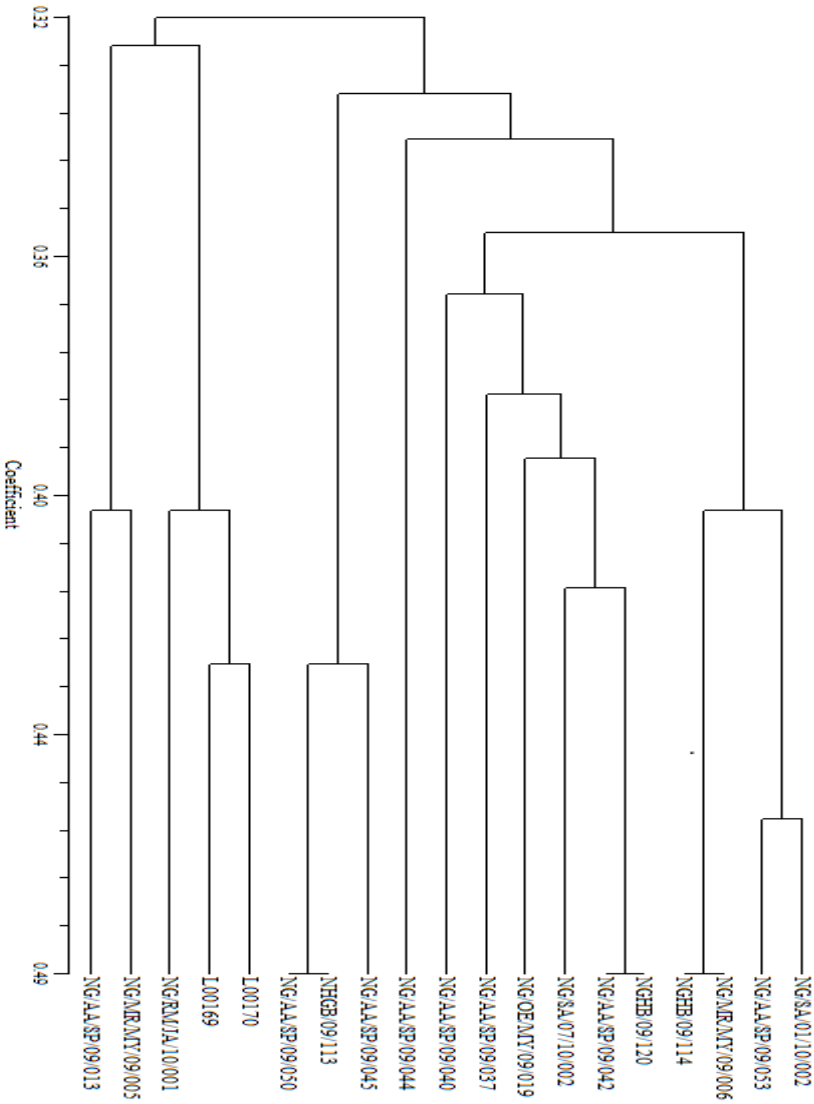

Figure 1. Dendogram of 19 tomato accessions based on 37 agro-morphological attributes and generated from average taxonomic distance matrix by UPGMA in NYSYSpc

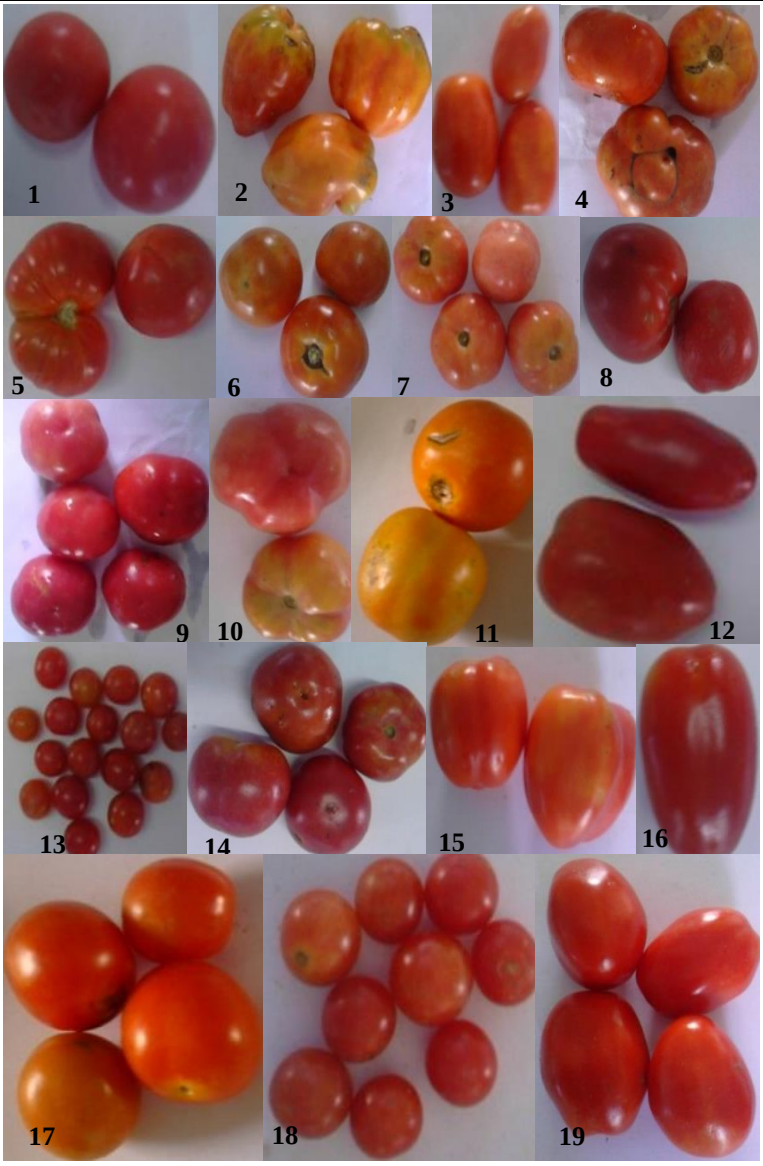

Figure 2. Genetic diversity as exhibited in fruits of 19 tomato accessions used for this study 1 – NG/SA/01/10/002, 2 – NGHB/09/120, 3 – NG/AA/SEP/09/045, 4 – NHGB/09/113, 5 – NG/AA/SEP/09/044. 6 – L00170, 7 – NG/OE/MAY/09/019, 8 – NG/AA/SEP/09/050, 9 – NG/SA/07/10/002, 10 – NG/AA/SEP/09/040, 11 – NG/MR/MAY/09/005, 12 – NG/AA/SEP/09/037, 13 – NG/RM/JAN/10/001, 14 – NG/MR/MAY/09/006, 15 – NHGB/09/114, 16 – NG/AA/SEP/09/013, 17 – NG/AA/SEP/09/042, 18 – L00169 and 19 – NG/AA/SEP/09/053.

Table 3. Accession names and sources of accessions used for the study

S. No.	Accession ID	Source	Region, Country
1	NG/SA/01/10/002	Quagbo market	Republic of Benin
2	NGHB/09/120	Agbo, Delta state	South-South, Nigeria
3	NG/AA/SEP/09/045	Igede, Ekiti state	South-West, Nigeria
4	NHGB/09/113	Agbo, Delta state	South-South, Nigeria
5	NG/AA/SEP/09/044	Igede, Ekiti state	South-West, Nigeria
6	L00170	Ido, Oyo state	South-West, Nigeria
7	NG/OE/MAY/09/019	Omi adio Oyo state	South-West, Nigeria
8	NG/AA/SEP/09/050	Ijero ekiti, Ekiti state	South-West, Nigeria
9	NG/SA/07/10/002	Quagbo market	Republic of Benin
10	NG/AA/SEP/09/040	Ika ejigbo, Osun state	South-West, Nigeria
11	NG/MR/MAY/09/005	Osiele, Ogun state	South-West, Nigeria
12	NG/AA/SEP/09/037	Osun state	South-West, Nigeria
13	NG/RM/JAN/10/001	Ido, Oyo state	South-West, Nigeria
14	NG/MR/MAY/09/006	Omida, Ogun state	South-West, Nigeria
15	NHGB/09/114	Sapele, Delta state	South-South, Nigeria
16	NG/AA/SEP/09/013	Osun state	South-West, Nigeria
17	NG/AA/SEP/09/042	Iloko ijesa, Osun state	South-West, Nigeria
18	L00169	Ido, Oyo state	South-West, Nigeria
19	NG/AA/SEP/09/053	Oja oba ado, Ekiti state	South-West, Nigeria

Table 4. Analysis of variance for different characters in tomato accessions

Attribute	Accession mean	F - value	P-value ≤	Coefficient of variation
Weight/fruit (g)	36.74	21.26	0.001	27.16
1000 Seed weight (g)	2.29	16753.0	0.001	0.35
No of days to flowering	42.40	12.69	0.001	12.98
No of days to 50% flowering	49.40	14.39	0.001	8.79
No of days to fruit ripening	76.04	5.15	0.001	5.16
No of days to fruit maturity	78.65	20.96	0.001	2.54
Plant height (cm)	13.14	4.48	0.001	33.05
Number of leaflets	38.66	3.57	0.001	39.87
Number of branches	7.11	4.38	0.001	14.06
Peduncle length (cm)	0.51	2.95	0.05	22.97
No of fruit/inflorescence	5.14	11.79	0.001	14.06
Fruit length (mm)	35.98	87.20	0.001	6.22
Fruit diameter (mm)	38.01	27.35	0.001	10.66
No of fruit/peduncle	55.69	28.35	0.001	39.25
Leaf length (cm)	29.63	108.36	0.001	3.15
Leaf width (cm)	20.07	22.02	0.001	6.49
Fruit yield per plant (g)	1269.64	15.75	0.001	2.54

Table 5. Estimates of phenotypic variance (σ^2p), genotypic variance (σ^2g), heritability (H^2b), genotypic and phenotypic coefficients of variability and genetic advance for various attributes of tomato accessions

Attributes	σ^2g	σ^2p	σ^2e	H^2b (%)	GCV	PCV	GA
Plant height	21.89	40.75	18.86	54	35.60	48.58	53.75
No of branches/plant	319.60	499.91	180.31	64	17.68	22.11	29.12
Leaf length	31.08	31.95	0.87	97	18.82	19.08	38.23
Leaf width	11.89	13.55	1.70	88	17.18	18.37	33.11
Number of leaflets/plant	203.41	441.06	237.64	46	38.90	57.29	54.43
No of days to flower	33.23	41.75	8.53	80	13.59	15.24	24.98
No of days to 50% flowering	86.43	105.79	19.37	82	18.82	20.82	35.04
No of days to fruit ripening	21.27	36.65	15.39	58	6.06	7.96	9.52
No of fruit/inflorescence	1.88	2.40	0.52	78	26.67	30.15	48.60
No of fruit/plant	4349.08	4826.12	477.04	90	118.51	124.84	231.74
Peduncle length	0.01	0.02	0.01	39	18.54	29.53	23.98
Fruit length	144.05	149.06	5.01	97	33.36	33.93	67.55
Fruit diameter	144.09	160.49	16.40	90	31.58	33.33	61.64
Weight/Fruit	672.56	772.6	99.6	100	75.58	75.63	135.69
Days to Maturity	28.07	31.94	3.88	88	6.72	7.17	12.98
1000 seed weight	0.35	0.35	0.0	100	25.85	28.85	53.24
Fruit yield/plant	775796.12	1049841.90	274045.48	74	63.37	80.70	122.85

σ^2g = genotypic variance, σ^2g = phenotypic variance, GCV = genotypic coefficient of variation, PCV = phenotypic coefficient of variation, GA= Genetic advance, H^2b = heritability in broad sense

Table 6. Genotypic (above) and phenotypic (below) correlation coefficients of vegetative and reproductive and yield attributes of 19 tomato accessions in Ibadan Nigeria

	NOL	NOB	PH	LL	LW	PDL	DTF	DTFF	DTFF Rp	DTM	FPI	WPF	FL	FD	FPP	FYPP	1000S W	WtPS
NOL		.960**	.875**	.732**	.579**	.149	-.998**	-.690**	-.400	-.541*	-.226	-.025	.259	.111	.055	.491	.149	.396
NOB	.861**		.804**	.727**	.462*	.423	-.877**	-.682**	-.261	-.523*	-.144	-.073	.135	.011	.106	.579**	-.131	.283
PH	.831**	.780**		.838**	.628**	.281	-.998**	-.639**	-.248	-.397	-.196	.031	.352	.223	.062	.577**	.288	.488*
LL	.630**	.653**	.733**		.892**	.419	-.532*	-.520*	-.218	-.109	-.122	.293	.388	.368	.255	.608**	.090	.620**
LW	.469*	.419	.558*	.854**		.675**	-.366	-.458*	.065	.289	-.404	.594**	.636**	.619**	.503*	.466*	.086	.831**
PEDL	.085	.313	.189	.336	.675**		-.106	-.297	.177	.155	-.528	.739**	.499*	.614	.221	.220	-.302	.510*
DTF	-.751**	-.637**	-.743**	-.433	-.366	-.106		.539*	.726**	.505*	-.209	.323	-.089	.268	.395	-.496*	-.153	-.257
DTFF	-.587**	-.589**	-.563*	-.498*	-.458*	-.297	.539		.342	.245	.165	-.029	-.080	-.096	.278	-.336	-.119	-.194
DTFFRp	-.406	-.276	-.276	-.187	.065	.177	.726**	.342		.908**	-.033	.093	.181	.055	.235	-.355	.075	.124
DTM	-.492*	-.455	-.359	-.110	.289	.155	.505**	.245	.908**		-.153	.230	.306	.177	.427.	-.317	-.007	.234
FPI	-.185	-.103	-.175	-.113	-.404	-.528*	-.209	.165	-.033	-.153		-.542*	-.316	-.678**	.759**	.038	.075	-.297
WPF	-.041	-.079	.020	.285	.594**	.739**	.323	-.029	.093	.230	-.542**		.601**	.925**	.455	.371	.135	.674**
FL	.214	.119	.314	.386	.636**	.499*	-.089	-.080	.181	.306	-.316	.601**		.535*	.435	.466*	.135	.996**
FW	.075	.013	.197	.360	.619**	.614**	.268	-.096	.055	.177	-.678**	.925**	.534*		.584**	.455*	.240	.614**
FPP	-.021	.114	-.033	-.250	-.503*	-.221	-.395	-.278	-.235	-.427	.759**	-.455	-.435	-.584		.018	.108	-.510*
FYPP	.408	.501*	.530*	.561*	.466*	.219	-.496*	-.336	-.355	-.317	.038	.371	.466*	.045	.018		.264	-.528*
SW1000	.121	-.114	.255	.089	.086	-.302	-.153	-.119	.075	-.007	.075	.135	.135	.240	.108	.264		.141
WtPS	.294	.234	.424	.554*	.831**	.510*	-.257	-.194	.124	.234	-.297	.674**	.998**	.614	-.510*	.528*	.141	

*, ** = significant at 5% and 1% probability levels respectively.

KEY:

NOL= number of leaflets per plant, NOB= number of branches per plant, PH= plant height, LL= leaf length, LW= leaf width

PDL= peduncle length, DTF= number of days to flower, DTFF = number of days to fifty percent flowering, DTFFRp= number of days to first fruit ripening, DTM= number of days to maturity, FPI= fruit per inflorescence, WPF= weight per fruit, FL= fruit length, FD= fruit diameter, FPP= number of fruit per plant, and FYPP= fruit yield per plant, 1000SW = 1000 seed weight and WtPS = weight per seed

Discussion

The low yield obtained for some of the accession used might be due to non-development of flowers into fruits on the plants pedicels. The poor fruit set may be as a result of high diurnal temperatures and relative humidity that are not conducive for good fruit set [OLANIYI & al. 2010] .The observed differences in growth and yield components of the various accessions suggest that this might be attributed to the differences in ecological distribution as revealed in the various collection locations. Also, the variation in the agronomic and yield components traits analysed is most likely to be due to genetic variability existing among the accessions since they were grown under the same environ-mental conditions.

Genetic diversity can be estimated using measurements of morphological attributes. This is a simple technique for quantifying genetic variation and assessing genotype performance under appropriate growing environments [FUFA & al. 2005; SHUAIB & al. 2007]. The precise, fast and reliable identification of important plant varieties is essential in agriculture and plant breeding purposes [WEISING & al. 2005]. Clustering of accessions used in this study into cherry and classic fruit groups corresponding to varietal types was similar to the results of KWON & al. 2009, who characterized 63 tomato varieties of Korea using SSR markers and morphological descriptors. Non-significant association between the clustering pattern and geographical origin of these materials is in agreement with the report by HU & al. 2012 in their work with 67 argentine tomato varieties. HU & al. 2012, also reported that fruit shape had the most variable types (seven). The 19 accessions used for this study may be identified as distinct varieties. However, molecular characterization using SSR markers is on-going to ascertain this result.

Highly significant differences among the accessions for all attributes measured is an indication of enough genetic variability and diversity of the accessions hence the scope for improvement of this crop. Similar observations have been reported on 14 characters [SINGH & RAJ, 2004; HIDAYATULLAH & al. 2008] in tomato. [MOHAMMED & al. 2012] also had similar findings of significant differences for all the traits they studied. Moreover, higher values of genotypic and phenotypic variances observed for plant height, number of branches, leaf length, leaf width, number of leaflets, days to flower, days to 50% flower, days to fruit ripening, fruit per plant, fruit diameter, fruit weight, day to maturity and fruit yield per plant indicate the existence of high magnitude of variability among the accessions with respect these attributes.

Smallest differences observed between PCV and GCV values of attributes such as leaf length, leaf width, days to flower, days to 50% flower, days to fruit ripening, fruit length, fruit per inflorescence, fruit diameter, fruit weight, days to maturity and 1000 seed weight suggest lesser influence of environmental factors on their expression. Selection for improvement of tomato for these attributes is likely to be most effective. Relatively higher differences between PCV and GCV values recorded for plant height, number of branches, number of leaflets, fruit per plant, peduncle length and fruit yield indicate more influences of environmental factors than other attributes studied.

Very high heritability estimates for leaf length, leaf width, days to flower, days to 50% flowering, fruit per plant, fruit length, fruit diameter, fruit weight and 1000 seed weight indicate possibility of improvement through selection. Similar results have been reported by TASISA & al. 2011 and ULLAH & al. 2012. However, PARNSE, 1957 stated that greater usefulness of considering estimate of genetic advance as an effective selection tool lies in

accompanied heritability estimates. Hence, very high genetic advance accompanied by high heritability estimates for leaf length, leaf width, days to flower, days to 50% flowering, fruit per plant, fruit length, fruit diameter, fruit weight and 1000 seed weight suggest simple inheritance system for these traits. FEHMIDA & AHMED, 2007 reported similar results for plant height, number of fruits per plant, fruit size and weight of 10 tomatoes.

Positive and significant association of fruit yield per plant ($P < 0.05$) with plant height, number of branches per plant and leaf length shows that taller plants, bearing more branches and longer leaves tend to yield higher as compared to shorter plants. This may be explained by the greater photosynthetic products available for partitioning to fruit production. Positive and significant association of number of fruits per plant with number of fruit per inflorescence is an indication of increased number of fruits with increased number of fruit bearing inflorescence. Weight per fruit which is a function of fruit size had predictably positive and significant association with fruit length and fruit diameter. MOHANTY, 2002 had reported positive and significant correlation of number of fruits per plant with fruit size and single fruit weight. More branching accessions of tomato tend to flower and mature late as shown in the negative and significant association of number of branches per plant with days to flower, days to fruit ripening and days to maturity. This may be due to the fact that much time is spent by the plant in growing more vegetative branches, hence extending its lifespan. Therefore, a breeder interested in improvement for early maturity in tomato may select plants with less number of branches.

Conclusion

The agronomic and genetic parameters discussed here are functions of the environment, so estimates may differ in other environments as well as agronomic performance. However, based on the high genetic advance accompanied by high heritability estimates for different attributes studied, especially, days to 50% flowering, fruit per plant, fruit length, fruit diameter, fruit weight and 1000 seed weight we could conclude that the determinant genetic effects of the phenotypic expression of these characters are fundamentally of the additive type. Hence, a high response should be achievable after several selection cycles.

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QUANTITATIVE INHERITANCE OF SPIKE CHARACTERS IN CASTOR (*RICINUS COMMUNIS* L.)

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Abstract: Castor oil plant (*Ricinus communis* L.) is an important oil crop with little research attention in Nigeria. In this research, studies on quantitative inheritance of spike characters in castor, through combining ability and generation mean analyses, were carried out. This was aimed at evaluating prominent seed yield related components for selection and hybridization in castor breeding in Nigeria. The result of combining ability analysis showed significant differences for both general combining ability (GCA) and specific combining ability (SCA) for number of spikes per plant (SPP), effective spike length (ESL), capsules per spike (CPS) and 100-seeds weight. Specific combining ability was important for seed yield (SY) and seed oil content. Broad sense heritability and narrow sense heritability of 28.02% to 99.64% and 10.28% to 72.19% respectively were recorded among the traits studied. The results of scaling and joint scaling tests revealed inadequacy of simple additive-dominance model to explain the mode of gene actions for all the studied traits. The six parameters model revealed that besides the additive and dominance gene actions, epistatic interaction mechanisms have also contributed significantly to the expressions of all the characters examined. These findings suggest heterosis breeding and recurrent selections as appropriate breeding techniques for the improvement of the traits. However, where additive gene actions are to be exploited in selection program for some of the traits, the problem pose by presence of epistasis gene interactions may be overcome through delay in selection to later generation when the major operating genes will be fixable.

Keywords: castor, combining ability, generation mean, inheritance, Nigeria, quantitative, spike characters.

Introduction

Castor oil plant (*Ricinus communis* L.) is one of neglected African crops with high economic values [GANA & al. 2013]. Castor oil, which is extracted from castor seed, is very critical to many industrial applications because of its ability to form many chemical derivatives [OGUNNIYI, 2006]. Castor has a wide-range of variability for qualitative and quantitative traits [WIESS, 2000]. However, the most prominent variation in castor is observed in the reproductive or spike characters such as seed size, number of capsules per spike, number of spikes, total spike length and effective spike length. These spike characters are important seed yield components in castor. RAMESH & VENKATE (2001) reported positive relationship and direct impact for spike length, capsules per spike and 100-seeds weight on the seed yield in castor. RAO & al. (2006) reported that the majority of the yield parameters in castor, aside from number of seeds per capsule, are normally inherited in a quantitative manner. Several authors have reported the importance of both general combining ability (GCA) and specific combining ability (SCA) in the expression of agronomic traits in castor [TANK & al. 2003; SOLANKI & al. 2004; PATEL & CHAUHAN, 2013]. RAMESH & al. (2013) reported the ratio of GCA:SCA variance in favour of non-additive gene action for all the traits evaluated in castor, except plant height to primary spike, number of nodes to

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primary spike, capsules per spike and total length of spike. However, more comprehensive genetic information for accurate decision on precise breeding methods and strategies may be obtained by combination of genetic analysis models. GRIFFING (1956a) and MATZINGER & KEMPTHORNE (1956) reported that estimates of GCA variance include, in addition to additive gene action, portions of higher order additive-type epistatic variance. Likewise, estimates of SCA variance include, in addition to dominance gene action, portions of all different types of epistatic variances. Therefore, precise decision on selection procedure may be accurately made when the decision is based on combining ability analysis in conjunction with generation mean analysis. MANIVEL (1994) reported, in a generation mean analysis experiment in castor, that simple additive – dominance model was not adequate for most of the traits in castor. MARINKOVIC & al. (2013) recorded highly significant values of epistatic gene effects (additive × additive and dominant × dominant) for number of capsules per spike, number of seeds per plant and 100-seeds weight in two crosses of castor. SAKHARE & al. (2017) reported duplicate type of gene action for seven out of nine characters in three crosses of castor. Among the epistatic interaction effects, both additive × dominance and additive × additive effects were reported governing number of spikes per plant and 100-seeds weight. Additive × additive epistatic interaction was predominance for effective length of spikes and additive × dominance interaction was reported for seed yield [MANIVEL, 1994].

Despite the huge economic benefits of castor, its genetic improvement in Nigeria has not been receiving much attention. In this research, studies on quantitative inheritance of spike characters through combining ability and generation mean analysis were carried out. This was aimed at evaluating prominent seed yield related components for selection and hybridization in castor breeding in Nigeria.

Material and methods

Plant materials

The plant materials used for this research were obtained from Castor Research Programme of National Cereals Research Institute (NCRI) Badeggi, Nigeria. Six (6) inbred lines developed by the Castor Research Programme of NCRI were used for the study. The six lines were developed for specific spike character such as high seed weight, high number of spikes, high number of capsules and high seed oil content. The lines include: (1) NCRICAS/Acc.005 – S5 – 2, a small seeded castor with high number of spikes per plant, (2) NCRICAS/Acc.010 – S4 – 5, a small seeded castor with high number of capsules per spike, long effective spike length and high seed oil content, (3) NCRICAS/Acc.036 – S5 – 8, a medium seeded castor with high number of spikes per plant (4) NCRICAS/Acc.001 – S6 – 10, a white large seeded castor, (5) NCRICAS/Acc.036M – S4 – 20, a medium seeded castor with high number of spikes per plant and high seed oil content, (6) NCRICAS/Acc.045 – S5 – 4, a black large seeded castor with high number of fruit bearing branches.

Mating design

Partial diallel cross among the six castor parental lines were carried out. Random seed samples from the six lines were used to establish diallel crossing block between January and May 2016, using irrigation facility at the Sugarcane Research Field of NCRI Badeggi, Nigeria. Two sets of blocks were established at different planting dates (15 days interval) to synchronize the flowering periods of the parents. The selected female flowers were covered with brown envelops prior to flower opening to control the pollination. Emasculation was effected prior to anthesis by the use of forceps. The pollens were collected in the evening (5 - 6 pm) and stored in white envelope for pollination in the next day. Pollination was effected 1 to 5 days after anthesis. Partial diallel crosses were made to generate 15 hybrids. After artificial pollination, the pollination envelops were replaced and appropriate labeling were ensued. For the purpose of this research, the six parental lines were coded as P1 for NCRICAS/Acc.005–S5–2, P2 - NCRICAS/Acc.010–S4–5, P3 - NCRICAS/Acc.036–S5–8, P4 - NCRICAS/Acc.001–S6–10, P5 - NCRICAS/Acc.036M–S4–20, and P6 - NCRICAS/Acc.045–S5–4.

Genetic Analyses

The six castor parental lines and their 15 F1 hybrids were evaluated for combining ability and heterosis between June and November, 2016. For each set of the crosses, 45 plant samples of F1 and parents were evaluated. The entries were laid out on Randomized Complete Block Design with three replications. The plot size was 3 m by 1.5 m with Inter-row and intra-row spaces of 75 cm. Two seeds per hole were planted and later thinned to one seedling per hole at four weeks after planting. Morphological data were taken according to Indian castor descriptor (INDIA, 2004). The parameters observed were: spike per plant, effective length of spike, number of capsules per spike, 100-seeds weight, seed yield and seed-oil content.

Three crosses (P1×P4, P2×P6 and P5×P6) with high overall specific combining ability and high heterosis for seed yield over the better parents were advanced to F_2 and backcrosses (BC) with the parents were made to generate F_1BC_1 and F_1BC_2 from parent 1 and parent 2 respectively. Seeds of successful crosses were harvested, processed and 20 individuals were self-pollinated to generate F_2 seeds. For the backcrosses (BC), F_1 plants were crossed with the two parents using the same procedure described above. The development of filial generation was done between June and November, 2016.

In 2017 growing season, the three crosses and their filial generations were evaluated for generation mean analysis on Compact Family Block Design with three replicated plots at NCRI Badeggi-Nigeria. Plant spacing, cultural practices and data taken described earlier were adopted.

Data analysis

General analysis of variance was carried out to test for significant of variability among the six parents and their hybrids. Combining ability analysis and variance component estimates were computed following the procedure of Plant Breeding Tools (PBTools, 1.3) for Griffing Diallel Method II. Ranking for overall general and overall specific combining ability was carried out according to SHIVANNA (2008).

Heterosis over mid parent (MP) and better parent (BP) were computed from mean of the treatments according to SHIVANNA (2008).

The standard error (SE) for the estimated heterosis was determined using mean squares due to error (MSe) as described by SHIVANNA (2008). The significance of the heterosis was determined using T-test.

SE (MP) = √(2 x MSe / 3r) for testing heterosis over MP

SE (BP) = √(2 x MSe / r) for testing heterosis over BP

Data from the two parents, F_1, F_2, F_1BC_1 and F_1BC_2 of all crosses was subjected to analysis of variance following the procedure of Statistical Tools for Agricultural Research (STAR 2.0.1, 2014) and the parameters that showed significant difference were used for scaling and joint scaling tests as described by DAVID (2006). Six parameters model of generation mean analysis was done according to TNAUSTAT General Breeding Procedure and DOSBox 0.74. Twelve (12) plant samples each of the two parents and F_1 populations; 120 individual plants of F_2 populations; 21 plant samples each of F_1BC_1 and F_1BC_2 populations were used for the analysis.

Results

The mean square values from analysis of variance (ANOVA) for the spike characters of the parents and their hybrids are shown in Table 1. The analysis of variance showed significant differences for all the studied traits among the entries. Analysis of variance for combining ability showed significant differences for both general combining ability (GCA) and specific combining ability (SCA) for number of spikes per plant (SPP), effective spike length (ESL), capsules per spike (CPS) and 100-seeds weight. Only specific combining ability was significant for seed yield (SY) and seed oil content (Table 2). Genetic variances due to general combining ability (GCA) and specific combining ability (SCA) ability are also presented in Table 2. Variance due to general combining ability was found to be larger than variance due to specific combining ability for spike per plant and 100 seeds weight. Higher SCA variances were recorded for effective spike length, capsules per spike, seed yield and seed oil content. Broad sense heritability and narrow sense heritability ranged from 28.02 % to 99.64 % and 10.28 % to 72.19 % respectively were recorded among the traits studied (Table 2). The highest broad sense heritability (99.64 %) and highest narrow sense heritability (72.19 %) were observed in 100-seeds weight. Broad sense heritability of 53.15 % and narrow sense heritability of 10.28 % were recorded for seed yield per hectare (Table 2).

Table 1. Mean squares from analysis of variance of six castor parents and their hybrids for seed yield and spike characters

Sources of Variation	DF	Spike per plant (SPP)	Effective Spike Length (ESL)	Capsules per Spike (CPS)	Seed Weight (SW) (g)	Seed Yield (SY) (kg/ha)	Seed Oil Content (SOC) (%)
All Genotypes	20	22.28**	135.95**	1366.94**	462.58**	647322.58**	247.98**
Parents	5	16.26ns	131.41**	2022.69**	996.96**	392915.21**	313.29**
Crosses	14	24.65**	142.34**	1224.12**	300.61**	674379.92**	106.27**
Parents vs Crosses	1	7.95ns	36.06*	114.72*	66.91ns	1540557.21**	1929.33**

Table 2. Mean square and variances for general and specific combining ability for seed yield and spike characters in castor

Characters	GCA	SCA	V*g*	V*s*	h^2	H^2
SPP	19.93**	3.65*	2.32	1.62	17.20	31.70
ESL	89.61*	29.45**	7.45	26.42	21.11	28.02
CPS	1041.20*	259.57**	97.71	176.15	27.23	86.22
100 SW	540.05**	25.57**	64.73	25.38	72.19	99.64
SY	284785.50ns	181456.80**	12916.21	181380.48	10.28	53.15
SOC	75.28ns	84.67**	0.02	81.21	56.82	96.91

General combining ability (GCA) effects of the parents on all the studied characters are presented in Table 3. All parents showed significant GCA effects for capsules per spike, 100-seeds weight, seed yield and seed oil content. All the parents, except P4, showed significant GCA effect for spikes per plant. The highest positive GCA effects for spikes per plant, effective spike length, 100 seed weight, and seed oil content were recorded in P1, P2, P6, and P5 respectively. Specific combining ability (SCA) effects for effective spike length was significant in eight out of 15 hybrids evaluated (Table 4). Six hybrids showed significant SCA effects for capsules per spike (Table 4). All hybrids, except P2xP3, showed significant effects for 100-seeds weight, however the maximum positive effect was observed in P4xP6 (Table 4). The highest positive SCA effects for seed yield and seed oil content was observed in hybrid P2xP6 and P1xP5 respectively (Table 4). Ranking status of each of the parents and hybrids, to determine good general and specific combiners across the studied character, is presented in Table 5. The result showed high overall general combining ability (> Norm) only in three parents, namely P1 (Score 24), P2 (Score 24) and P5 (Score 27). The best overall general combiner was parent P5 which has the highest total score. Other three parents (P3, P4, and P6) were found to be low (L) overall general combiners for the characters evaluated. Eight hybrids namely; P1XP2 (Score 54), P1XP3 (Score 64), P1XP4 (Score 57), P1XP5 (Score 67), P2XP6 (Score 64), P3XP5 (Score 55), P4XP5 (Score 55) and P5XP6 (Score 62) were found to be good specific combiners for the traits studied. The highest overall specific combiner was hybrid P1XP3 with the highest total score. The least average specific combiner (Score 26) among the hybrid was P1XP6.

Table 3. Estimates of general combining ability effects (*gi*) of each parent for seed yield and spike characters in castor

Parents	Spike per plant	Effective Spike Length	Capsules per Spike	Seed Weight (g)	Seed Yield (kg/ha)	Seed Oil Content (%)
P1	2.29**	-1.18**	10.21*	-7.52**	251.36**	-4.43**
P2	-2.03**	5.75**	15.46**	-6.79**	-86.14*	3.21**
P3	0.38**	-1.30**	-5.65ns	-4.17**	-147.63**	-1.37**
P4	-0.03ns	-1.16**	-5.47ns	8.32**	128.34**	1.14**
P5	0.83**	1.36**	1.45ns	-2.25**	97.69**	3.55**
P6	-1.56**	-4.18**	-15.76**	12.09**	-244.35**	-2.52**
SE (*gi*)	0.39	0.55	2.85	0.13	22.81	0.62

Table 4. Estimates of specific combining ability effects (*gi*) of 15 castor hybrids for seed yield and spike characters in castor

Hybrids	Spike per plant	Effective Spike Length	Capsules per Spike	Seed Weight (g)	Seed Yield (kg/ha)	Seed Oil Content (%)
P1XP2	0.24ns	-2.33ns	-3.71ns	2.22**	107.80**	5.34**
P1XP3	-0.25ns	-1.26ns	12.97ns	2.28**	231.09**	13.47**
P1XP4	2.89**	0.65ns	23.46**	-3.45**	439.25**	5.17**
P1XP5	2.16*	6.63**	-7.23ns	0.81*	175.20**	13.92**
P1XP6	-0.33ns	-4.99**	-16.96*	-7.96**	-346.27**	-4.34**
P2XP3	-0.92ns	-1.45ns	-19.75*	0.24ns	-74.97**	-2.14ns
P2XP4	-1.29ns	5.89**	4.21ns	-2.48**	94.97**	2.46ns
P2XP5	-2.42*	-7.60**	-15.96*	1.52**	-287.79**	4.47**
P2XP6	1.67ns	12.76**	38.51**	-6.79**	891.17**	-0.63ns
P3XP4	1.11ns	1.17ns	1.52ns	-6.99**	-153.91**	6.33**
P3XP5	-1.27ns	-0.71ns	-5.13ns	6.56**	-133.78**	7.25**
P3XP6	-0.43ns	1.29ns	7.86ns	-2.28**	13.50ns	3.99*
P4XP5	0.67ns	-2.74*	2.61ns	3.67**	564.54**	-4.27**
P4XP6	-1.72ns	-6.19**	-16.76*	6.62**	-519.95**	-0.76ns
P5XP6	2.78**	5.84**	6.66	-3.67**	524.27**	2.52ns
SE (S_{ii})	0.87	1.28	6.79	0.31	16.45	1.35

Table 5. Overall general combining and specific combining ability status of six parents and their hybrids for seed yield and spike characters in castor

Entries	SPP	SL	CPS	SW	SY	SOC	Total	Rank
P1	6	5	5	1	6	1	24	H
P2	2	6	6	2	3	5	24	H
P3	4	3	3	3	2	3	18	L
P4	3	2	2	5	5	4	21	L
P5	5	4	4	4	4	6	27	H
P6	1	1	1	6	1	2	12	L
Mean (Norm)							21	
P1XP2	9	10	4	12	8	11	54	H
P1XP3	8	6	13	11	12	14	64	H
P1XP4	15	5	14	5	11	7	57	H
P1XP5	13	13	8	9	9	15	67	H
P1XP6	7	3	2	1	7	2	22	L
P2XP3	5	4	5	8	4	3	29	L
P2XP4	3	8	12	6	10	10	49	L
P2XP5	1	1	1	10	2	8	23	L
P2XP6	12	15	15	3	15	4	64	H
P3XP4	11	11	7	2	3	12	46	L
P3XP5	4	12	6	14	6	13	55	H
P3XP6	6	9	9	7	5	9	45	L
P4XP5	10	7	11	13	13	1	55	H
P4XP6	2	2	3	15	1	5	28	L
P5XP6	14	14	10	4	14	6	62	H
Mean (Norm)							49	

The heterosis exhibited by the 15 hybrids over the mid parent (MP) and better parent (BP) values for the traits evaluated are shown in Table 6. None of the crosses, except P2XP4, recorded negative heterosis over mid-parents for number of spikes per plant (Table 6). Heterosis over better parent ranged between -48.62 % in P3XP6 and 34.58 % in P4XP5. Four crosses (P1XP4, P1XP5, P4XP5 and P5XP6) showed significant positive heterosis over better parent for number of spikes per plant. All the crosses recorded significant heterosis over both mid parents for effective spike length, however, only crosses P1XP5, P3XP5, P4XP5 and P5XP6 showed significant positive heterosis over better parent. Positive significant heterosis over the mid parent was found for seed weight in all the crosses except P1XP2 and P2XP3. Negative heterosis over better parent were observed for seed weight in all the crosses, except P2XP5 and P3XP5. All the crosses recorded positive mid parent heterotic values for seed yield, except P4XP6. Eight hybrids (P1XP3, P1XP4, P1XP5, P2XP4, P2XP6, P3XP5, P4XP5 and P5XP6) were found to have positive and significant heterosis over the better parent seed yield. Positive and significant heterosis over mid parents for seed oil content was recorded in all the crosses, except cross P4XP5 and P4XP6. Out of the fifteen hybrids, only three crosses (P1XP6, P2XP3 and P2XP6) recorded negative heterosis over better parent for seed oil content. The highest desirable heterosis (73.32 %) over better parent was observed in P1XP3.

The data collected from the generations of three crosses were subjected to individual analysis of variance. Significant differences were observed for all the traits studied in all the three crosses evaluated (Table 7). The mean performances of the generations from the three crosses are presented in Table 8. Average number of spikes per plant for F_1 ranged from 3·6 to 12·1 among the crosses. The parental mean for number of spikes per plant was from 3·3 to 15. In all the crosses, F_2 values for spikes per plant was only less than F_1 mean in Cross II. In BC_1, only Cross III recorded greater spikes per plant than its recurrent parent. In BC_2, Lesser mean to recurrent parents were observed in Cross I and Cross II while in Cross III greater mean than recurrent parent was recorded. For seed yield, the parental mean ranged from 125·26 g to 379·98 g per plant (Table 8). The F_1 recorded seed yield range between 62·60 g (Cross III) and 153·74 g (Cross II). The F_2 yield varied from 24.43 g (Cross III) to 84·89 g (Cross I). The BC_1 and BC_2 recorded yield ranging from 77·84 g to 189·57 g and 48·86 g to 140·13 g respectively. Among all the three crosses, none of the F_1 recorded seed yield greater than their better parents. The oil content of the parent ranged from 24 % to 50·33 % (Table 8). For F_1, the seed oil content ranged from 41·67 % to 54·00 %. In the F_2, the oil content ranged from 32·67 % to 43·33 % among the three crosses. Out of the three crosses, only F_1 of Cross III recorded oil content greater than its better parent. The F_2 oil content was lesser than F_1 mean in all the three crosses. The BC_1 mean was greater than recurrent parent values only in Cross I. The BC_2 recorded oil content greater than that of recurrent parent only in Cross III.

Table 6. Heterosis over Mid-Parents (MP) and Better Parents (BP) for Seed Yield and Spike Characters in Castor

	Spikes per Plant (RPP)		Effective Spike Length (RL)		Capsules per Spike (CPR)		100-Seeds Weight (SW)		Seed Yield (SY)		Seed Oil Content (SOC)	
Hybrids	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP	MP	BP
P1XP2	100.35**	-5.50**	16.53**	-4.22**	3.00ns	-11.82**	-0.08ns	-9.52**	101.37**	1.38ns	94.36**	5.77ns
P1XP3	0.00ns	-2.76**	4.33**	-2.96**	70.15**	-1.17ns	19.87**	-8.94**	95.22**	16.96*	103.34**	73.32**
P1XP4	104.86**	19.50**	5.38**	-10.95**	138.34**	12.14**	77.80**	-48.85**	73.60**	28.39*	78.30**	6.16ns
P1XP5	58.80**	27.83**	17.12**	7.72**	-1.39ns	-14.29**	24.37**	-4.36**	109.75**	12.46*	125.91**	35.96**
P1XP6	209.84**	-5.50**	44.63**	-23.53**	154.05**	-61.70**	72.45**	-62.27**	92.47**	-16.64*	39.06**	-14.04*
P2XP3	17.67**	-46.03**	14.26**	-12.66**	50.73**	-25.05**	-0.38*	-16.42**	15.17*	-3.22ns	51.61**	-3.21ns
P2XP4	-11.66**	-28.57**	26.50**	-12.15**	125.15**	-9.31**	74.53**	-44.55**	84.72**	25.75*	24.26**	13.57*
P2XP5	23.67**	-27.54**	9.48**	-17.24**	-19.37**	-40.00**	22.52**	4.04**	8.62ns	1.99ns	28.78**	16.45*
P2XP6	54.64**	0.00ns	121.50**	-3.74**	855.89**	23.37**	69.16**	-59.13**	166.70**	129.45**	10.84*	-2.42ns
P3XP4	62.00**	-8.10**	9.99**	-0.07ns	17.77**	-4.60*	33.67**	-49.38**	2.17ns	-17.24*	69.62**	18.49*
P3XP5	10.35**	-13.61**	11.16**	9.93**	6.57*	31.56**	52.57**	50.73**	31.00**	17.24*	80.61**	27.53*
P3XP6	73.22**	-48.62**	56.55**	-11.00**	233.30**	-13.50**	87.19**	-46.09**	9.22*	-21.04*	54.70**	12.19*
P4XP6	63.93**	-14.29**	25.41**	-21.52**	33.30**	-57.29**	22.02**	-7.21**	-24.07**	-55.53**	7.20ns	3.25ns
P4XP5	85.71**	34.58**	9.74**	0.82*	62.56**	-12.00**	116.82**	-18.88**	131.07**	27.53**	2.70ns	1.60ns
P5XP6	182.51**	7.04**	79.28**	3.07**	303.57**	-30.00**	94.80**	-44.58**	99.73**	61.34**	18.81**	15.68*
SE	0.28	0.16	0.40	0.23	2.17	1.26	0.11	0.06	3.01	4.99	2.88	4.61

* - Significant at $p < 0.05$, ** - Significant at $p < 0.01$, ns – not significant, t-value (0.05, df = 15) = 2.145, t-value (0.01, df = 14) = 2.977

Table 7. Mean Square Values from Individual Analysis of Variance for Seed Yield and Spike Characters of three Crosses of Castor

Parameters	P1×P4	P2×P6	P5×P6
Number of Spike Per Plant	8.719*	20.50**	44.30**
Effective Length of Spike (cm)	311.713**	994.135**	1080.63**
Number of Capsules Per Spike	8892.39*	16769.34**	10127.56**
100 Seed Weight (g)	3759.71**	8597.24**	4464.19**
Seed Yield Per Plant (g)	187356.94*	119682.72**	94502.23**
Seed Oil Content (%)	222.09**	71.12**	41.66**

Table 8. Mean Performance and Variance for Seed Yield and Spike Characters among Generations of three Crosses of Castor

		P1×P4		P2×P6		P5×P6	
	Generations	Mean ± S.E	Variance	Mean ± S.E	Variance	Mean ± S.E	Variance
Number of Spike per Plant	P_1	15.00±0.26^{ab}	0.77	12.30±0.21^{a}	0.46	9.70±0.33a	0.11
	P_2	9.60±0.16^{b}	0.33	6.40±0.16^{b}	0.27	3.30±0.30^{b}	0.09
	F1	12.10±0.90^{a}	0.81	6.30±0.15^{bc}	0.23	3.60±0.16^{b}	0.03
	F_2	12.64±0.17^{ab}	5.03	3.48±0.07^{d}	0.76	6.00±0.11^{b}	0.32
	BC_1	12.35±0.24^{b}	3.06	6.85±0.23^{b}	1.08	12.50±0.41^{a}	0.17
	BC_2	9.55±0.42^{ab}	2.76	3.75±0.17^{cd}	0.62	12.40±0.45^{a}	0.20
	Mean	2.83		1.88		2.52	
	SD	1.89		1.10		1.71	
Effective Spike Length (cm)	P_1	20.49±1.19^{a}	1.44	26.49±0.96^{b}	3.31	29.05±0.87^{a}	0.76
	P_2	10.11±0.31^{bc}	0.09	11.18±0.55^{d}	3.09	13.18±0.55^{d}	0.31
	F1	13.14±0.84^{b}	0.01	38.401.99^{a}	3.82	25.00±1.92^{b}	0.71
	F_2	8.78±0.39^{c}	0.16	18.44±0.67^{c}	47.13	11.43±0.34^{d}	6.92
	BC_1	10.11±0.57^{bc}	0.33	20.64±0.60^{c}	37.29	18.74±1.73^{c}	3.02
	BC_2	12.96±0.78^{b}	0.61	17.88±1.78^{c}	43.91	19.93±1.88^{c}	3.56
	Mean	10.21		19.68		14.66	
	SD	4.90		8.52		7.21	
Number of Capsules per Spike	P_1	77.00±1.14^{a}	12.89	49.30±3.91^{bc}	5.29	91.00±2.40^{a}	5.78
	P_2	20.00±0.36^{d}	1.33	19.60±3.08^{d}	9.49	21.60±3.08^{d}	9.49
	F1	35.90±1.91^{c}	16.32	121.60±8.58^{a}	3.58	52.90±4.33^{b}	8.81
	F_2	25.90±1.30^{d}	220.53	35.11±1.73^{c}	93.00	24.04±0.75^{d}	50.57
	BC_1	55.40±4.04^{b}	126.36	62.85±3.83^{b}	14.66	38.65±2.61^{c}	26.84
	BC_2	21.90±2.28^{c}	104.51	39.35±4.58^{c}	21.03	40.00±3.51^{c}	22.32
	Mean	29.71		42.57		31.77	
	SD	20.24		28.06		18.84	
100-Seeds Weight (g)	P_1	16.44±0.93^{b}	8.79	11.59±0.27^{d}	0.72	13.59±0.28^{f}	2.08
	P_2	58.28±0.72^{a}	5.26	51.24±0.65^{a}	4.29	53.34±0.65^{a}	1.43
	F1	14.34±0.68^{b}	4.58	18.24±0.43^{cd}	1.81	18.06±0.30^{d}	2.09
	F_2	13.62±0.41^{b}	22.28	49.04±1.23^{b}	97.74	15.79±0.16^{e}	18.92
	BC_1	13.03±0.39^{b}	3.19	13.31±0.16^{d}	0.49	25.99±0.73^{c}	13.54
	BC_2	15.78±1.03^{b}	21.24	25.77±1.57^{c}	49.09	39.96±1.18^{b}	13.40
	Mean	16.19		39.84		21.11	
	SD	10.59		18.69		10.87	
Seed Yield per Plant (g)	P_1	379.98±17.57^{a}	38.86	285.57±17.33^{a}	3.40	160.71±16.17^{a}	11.69
	P_2	133.21±5.80^{bc}	33.65	135.60±10.28^{b}	5.83	125.26±9.35^{bc}	13.50
	F1	110.04±3.99^{bc}	35.22	153.74±5.92^{b}	5.07	62.60±4.21^{d}	12.76
	F_2	84.89±9.40^{d}	288.39	56.49±5.66^{c}	162.04	24.43±2.24^{e}	55.05
	BC_1	189.57±41.57^{b}	98.18	77.84±10.35^{c}	37.25	102.88±18.11^{c}	37.82

	BC_2	129.20±37.90^{bc}	86.44	48.86±18.05^{c}	46.04	140.13±21.05^{ab}	43.30
	Mean	118.22		78.14		57.61	
	SD	138.67		82.06		66.63	
Seed Oil Content (%)	P_1	24.00±0.52^{e}	1.02	50.33±0.43^{a}	0.33	49.00±0.58^{b}	2.101
	P_2	47.33±0.33^{a}	0.33	42.33±0.35^{c}	0.33	40.00±0.58^{bc}	1.923
	F1	41.67±0.34^{bc}	0.33	48.33±0.35^{ab}	0.33	54.00±0.58^{a}	3.01
	F_2	32.67±1.54^{d}	12.33	36.67±2.84^{d}	24.33	43.33±2.19^{c}	18.33
	BC_1	39.00±1.42^{c}	7.00	45.33±1.43^{bc}	6.33	46.67±1.20^{bc}	17.33
	BC_2	44.67±2.03^{ab}	6.33	42.67±0.64^{c}	1.33	45.33±0.88^{bc}	15.33
	Mean	38.22		44.28		47.39	
	SD	8.28		4.98		3.88	

The results of scaling and joint scaling tests showed inadequacy of simple additive-dominance model to explain the model of gene actions for all traits evaluated in the three crosses (Table 9). At least two of the scales (A, B, C & D) and the chi-square (X^2) for joint scaling test were significant in all the three crosses studied. The residual genetic effect (m) was significant for all the traits studied in all the three crosses (Table 10). Significant additive effects (a) and additive × additive genes interaction (aa) were observed in the crosses for number of spikes per plant. Additive gene effects, dominance gene effects (d), additive × additive effects and dominance × dominance (dd) effects were significant for effective length of spike in the crosses studied (Table 10). Significant dominance gene effects and significant additive × additive interaction effects were positive and significant in all the crosses for number of capsules per spike. Duplicate gene effects coupled with significant additive × dominance genes interaction (ad) were observed for weight of one hundred seeds in all the crosses (Table 10). Significant additive gene effects, dominance gene effects (d), and duplicate interactions were observed for seed yield in two out of the three crosses. Two crosses out of the three crosses recorded duplicate gene interactions for seed oil content. Only one cross recorded complementary gene interaction for the oil content (Table 10).

Table 9. Scaling and Joint Scaling ($\chi 2$) Tests for Seed Yield and Spike Characters of Three Crosses of Castor

Traits	Crosses	A	B	C	D	*m*	*a*	*d*	$\chi 2$
Spike per Plant	P1×P4	-9.10**±1.06	1.40±1.24	-11.92**±1.95	-1.83*±0.59	5.79**±0.19	1.65**±0.15	2.04±3.37	33.49**
	P2×P6	-2.90*±0.53	-3.20**±0.41	-15.36**±0.50	-4.63**±0.32	3.27**±0.07	2.85**±0.13	10.11**±1.90	41.26**
	P5×P6	9.70*±4.08	15.90**±0.96	-0.60±0.71	-12.90**±0.65	21.90**±1.32	3.60**±0.22	84.30**±3.83	83.54**
Length of Spike	P1×P4	8.50*±3.29	45.40**±4.03	-5.72±5.35	-29.81**±2.57	35.06**±5.25	9.65**±1.08	167.68**±14.08	95.61**
	P2×P6	6.70±3.70	8.70±4.10	-46.08**±5.96	-30.74**±3.33	25.23**±6.76	8.55**±1.18	153.81**±17.29	284.77**
	P5×P6	33.90**±5.17	21.80**±2.75	-45.50**±5.66	-50.60**±2.68	70.75**±5.56	12.95**±1.48	263.25**±15.24	139.34**
Capsules Per Spike	P1×P4	17.90±8.37	17.90*±4.96	-15.20*±6.05	-25.50**±5.32	7.50**±1.66	29.49**±14.03	4.82**±0.46	123.69**
	P2×P6	-45.20*±12.14	-62.50**±12.93	-171.64**±19.16	-31.97**±6.90	33.50**±0.59	14.85**±2.48	34.70**±1.95	280.47**
	P5×P6	-66.60**±7.20	5.50±8.80	-122.21**±9.98	-30.55**±4.63	110.20**±29.85	107.33**±40.07	57.73*±27.92	196.15**
100 Seeds Weight	P1×P4	-4.71*±1.40	-41.06**±2.28	-48.92**±2.44	-1.57±1.38	34.20**±2.82	149.42**±5.86	-35.28**±2.87	80.83**
	P2×P6	-3.21**±0.59	-17.94**±3.22	96.85**±5.06	59.00**±2.92	-20.92**±0.59	-19.82**±0.35	-19.87**±0.35	133.14**
	P5×P6	20.34**±1.52	8.53*±2.47	-39.88**±1.12	-34.37**±1.42	-62.48**±7.65	-270.33**±13.71	150.96**±8.51	292.53**
Seed Yield per Plant	P1×P4	-110.87**±9.69	15.16±87.83	-393.71*±97.45	-149.00±59.31	-41.40**±8.98	183.15**±8.44	-245.28**±17.03	736.19**
	P2×P6	-283.62**±27.64	-191.61*±38.01	-502.65**±32.54	-13.71±23.69	123.38**±9.25	74.98**±10.07	17.72*±6.34	1625.48**
	P5×P6	-17.54±39.88	92.39±43.34	-313.42**±22.38	-194.13**±28.13	353.73±349.69	-477.21**±36.37	771.00**±69.96	717.18**
Seed Oil Content	P1×P4	12.33±9.53	0.34±2.83	-23.99**±7.25	-18.33*±6.00	10.00**±1.01	17.01**±2.16	36.82**±3.12	11.26**
	P2×P6	-8.00*±2.52	-5.32±3.37	-42.64**±4.37	-14.66*±4.44	11.67±9.56	4.00±2.98	1.50±1.21	18.33**
	P5×P6	-9.66*±3.75	-9.34±4.82	-29.68**±3.24	-5.34*±1.68	91.99**±5.84	47.32**±4.27	8.86**±1.56	14.54**

* - Significant at $p < 0.05$, ** - Significant at $p < 0.01$

Table 10. Six Parameters-Model Estimates for Seed Yield and Spike Characters of Three Crosses of Castor

Parameter	Crosses	*m*	*a*	*d*	*aa*	*ad*	*dd*	Type of Epistasis
Spike per Plant	P1×P4	12.64**±0.17	-3.60*±0.49	6.51±4.50	3.66*±1.19	-5.25**±0.51	4.44±2.75	ns
	P2×P6	3.48**±0.08	3.30**±0.29	6.61**±0.69	9.26**±0.66	0.45±0.32	-3.16±1.27	ns
	P5×P6	6.00**±0.11	0.40±0.61	24.90**±1.33	27.80**±1.29	-3.30±1.89	-57.40**±2.54	Duplicate
Length of Spike	P1×P4	20.39**±0.82	-8.80**±1.98	54.17**±5.56	59.62**±5.14	-18.45**±2.25	-113.52**±9.57	Duplicate
	P2×P6	32.45**±1.43	7.55**±5.36	76.93**±47.55	61.48**±6.39	-1.00±2.76	-76.88**±11.35	Duplicate
	P5×P6	21.65**±0.78	19.00**±2.17	106.35**±5.85	101.20**±5.36	6.05*±2.62	-156.90**±10.36	Duplicate
Capsules Per Spike	P1×P4	25.90**±1.30	33.50**±4.64	23.40*±10.83	51.00**±10.64	0.01±4.67	-86.80**±19.69	Duplicate
	P2×P6	35.11**±1.73	23.50**±5.97	151.09**±16.45	63.94**±13.81	8.65±6.47	43.75±30.63	ns
	P5×P6	24.04**±0.75	-1.35±4.37	57.71**±10.41	61.11**±9.26	-36.05**±4.79	-0.02±20.15	ns
100 Seeds Weight	P1×P4	13.61**±0.41	-2.74±1.10	-19.86**±2.90	3.15±2.76	18.17**±1.25	42.61**±5.05	Duplicate
	P2×P6	49.04**±1.23	-12.46**±1.57	-131.18**±5.89	-118.01**±5.85	7.36**±1.61	139.15**±8.07	Duplicate
	P5×P6	15.79**±0.15	-13.97**±1.39	53.34**±2.89	68.75**±2.85	5.90**±1.43	-97.62**±5.68	Duplicate
Seed Yield per Plant	P1×P4	84.89**±9.40	60.37**±6.25	151.44**±6.86	298.00*±118.62	-63.015±57.01	-202.29±245.21	ns
	P2×P6	56.49**±5.66	28.98*±10.82	-29.41*±8.81	27.42±47.39	-46.01±23.13	447.80**±89.39	Duplicate
	P5×P6	24.43**±2.24	-37.24**±4.76	307.88**±57.18	388.27**±56.26	-54.96±29.29	-463.12**±113.31	Duplicate
Seed Oil Content	P1×P4	32.67**±1.87	-5.67±4.01	42.67**±3.85	36.66**±4.25	5.99±6.02	-49.33**±2.15	Duplicate
	P2×P6	36.67**±2.22	2.66±1.88	31.32**±2.01	29.32**±3.14	-1.34±2.13	-16.00**±2.18	Duplicate
	P5×P6	43.33**±1.95	1.34±0.95	17.18**±1.67	10.68**±1.19	-0.16±0.92	8.32**±1.08	Complementary

Note: ns – not significant dominance effect, dominance × dominance effect or both, * - Significant at $p < 0.05$, ** - Significant at $p < 0.01$

Discussions

Analysis of variance (ANOVA) for the parents and their F_1 hybrids was significant in all the traits studied, indicating appropriateness of the data for genetic analysis. ANOVA for combining ability revealed significant differences of both general combining ability (GCA) and specific combining ability (SCA) for spikes/plant, effective length of spike, capsules per plant and 100-seeds weight. This is an indication of the importance of both additive and non-additive gene effects for the inheritance of the traits. RAMESH & al. (2013) reported similar presence of both additive and non-additive gene actions in castor for all the traits studied. The high magnitude of general combining ability variances observed for spikes per plant and 100 seed weight revealed the predominance of additive gene effects for the traits and thus intra-population improvement technique is suggested for the improvement of the two traits. The result revealed non-additive gene actions (SCA) as the major contributory gene effects for the expression of effective length of spike, capsules per plant, seed yield and seed oil contents. This suggest inter-population improvement technique for the improvement of these traits. Similar predominant variance due to SCA for the characters was reported by LAVANYA & CHANDRAMOHAN (2003), and SOLANKI & al. (2004) in their studies. Predominance of additive gene action in the expression of 100-seeds weight, and prevalence of non-additive gene action in the expression of seed yield, length of main spike and capsules per spike were also documented by PATHAK & al. (1989).

From the heritability results, high to low heritability due to both broad sense and narrow sense were obtained for the traits studied among the six parents and their hybrids. High broad sense heritability and high narrow sense heritability were obtained for 100-seeds weight, and high broad sense heritability and moderate narrow sense heritability were obtained for seed oil content. This gives insight on possible genetic progress and also indicating the level of environmental influence on the traits. The high heritability indicates least influence of environment and high possible selection progress while the low heritability revealed high environmental influence. The results obtained in this study are comparable to the reports of PATEL (1991), SERVUGAPERUMAL & al. (2000) and GOLAKIA & al. (2007).

General combining ability effects results revealed the appropriate parent for creating specific combination desired. The present research revealed P1 as best combiners to develop castor population with high number of spikes per plant. For increase in spike length and capsules number, P2 was the best combiner for development of useful segregating population. P4 and P6 were found to be the best combiners for increase in seed weight. For seed yield per plant, P1, P4 and P5 were found to be good combiner for creating promising segregating generations. Increase in seed oil content could be achieved from good combiners such as P2 and P5. Similar reports on GCA in castor have been made by DOBARIYA & al. (1989) and JOSHI & al. (2001) in their work on castor. Furthermore, an efficient hybridization or breeding programme requires parents that are good combiners for a considerable number of desired characters. Therefore, it is important to assess the overall status of the parents with respect to their GCA effects over all the studied traits. The results of overall GCA status of the parents revealed that parents P1, P2 and P5 were overall good combiners. This is evident from their high overall ranking status. The implication of this result is that these parents combined their genes in a desired direction for all the studied traits, suggesting their usefulness in castor improvement programme. DOBARIYA & al. (1992) reported two out of 12 castor parents to be best general combiners for yield and yield components. GOLAKIA & al. (2015) observed two parents to be best combiners for

earliness to maturity and seed yield per plant. RAMESH & al. (2013) observed one parent to be a good general combiner for most of the characters including seed yield in castor.

Based on SCA effects results, superior hybrids for different characters were identified among the crosses. The result revealed five hybrids to have significant SCA effects towards desired direction. Maximum desirable combination for spike length and capsules per spike was found in hybrid P2XP6. Desirable transgressive segregation for seed weight could be generated from P3XP5, P4XP5 and P4XP6. Desirable SCA effects for seed yield were found in eight out of the fifteen hybrids evaluated. The best combination to create population with increased seed yield was found to be P2XP6. For seed oil content, eight hybrids revealed significant desirable SCA effects with hybrids P1XP3 and P1XP5 being the best among others. All these results revealed the potentials of the hybrids for creating useful populations in later generations. Comparative findings have been documented by many researchers (NATARAJAN & al. 1993; MEHTA, 2000; JOSHI & al. 2001). RAMESH & al. (2013) reported that the best performing hybrids with high mean seed yield and positive significant SCA effects for seed yield were generated from either high × average, high × high, average × average, average × high combiners for seed yield. The involvement of at least one good general combiner (as a parent) to generate good hybrid was reported by MEHTA (2000), LAVANYA & CHANDRAMOHAN (2003), GOLAKIA & al. (2004) and BARAD & al. (2009). Eight hybrids namely P1XP2, P1XP3, P1XP4, P1XP5, P2XP6, P3XP5, P4XP5 and P5XP6 were good overall specific combiners for all the studied traits. These hybrids could be used for further studies and also to create promising segregating generations. Similar findings on overall specific ability have been stated by several authors [TANK & al. 2003; SOLANKI & al. 2004; PATEL & CHAUHAN, 2013].

The results revealed significant heterosis over mid and better parents for most of the traits studied. Eight out of 15 hybrids evaluated showed heterosis over both mid and better parents for seed yield. For seed oil content, also eight hybrids revealed heterosis over better parents. This is an indication of presence of transgressive individuals in the hybrids evaluated. The significant heterosis over mid-parents observed in most of the crosses is a reflection involvement of non-additive gene (specific combining ability effects) for the expression of the seed yield and oil content. This is in support of the results of combining ability for the traits. The result reported here on heterosis is similar to the reports of RAMANA & al. (2005) on three pistillate lines and nine testers of castor. Similar findings on heterosis in castor were also reported by TANK & al. (2003), and PATEL & PATHAK (2010).

The mean performance of the six generations, evaluated for generation mean analysis, revealed considerable inbreeding depression in all the three crosses for days to maturity, effective spike length, capsules per spike, seed yield and seed oil content. Evidence of transgressive segregation was found for spikes per plant in cross I and III, and for 100-seeds weight in cross II. This showed the possibility of obtained better individuals for these traits in the segregating populations. Similar patterns of inbreeding depressions and transgressive segregation had been earlier reported by MANIVEL (1994) in a study in castor. Considering the BC1 and BC2 mean together, none of the backcrosses registered increased mean over their respective recurrent parents for seed yield. This is an evidence for the presence of non-additive gene effects, pointing to dominance gene action and/or epistasis interaction for the inheritance of the trait. Such complexed inheritance similar to the present findings was also reported by ANNAPPAN (1981) in a study on castor.

The results of scaling and joint scaling tests revealed that simple additive-dominance model is inadequate to elucidate the mode of gene actions for the inheritance of all the characters studied, indicating the need for analysis of digenic interaction model involving the six genetic

parameters (m, a, d, aa, ad & dd). Had the simple additive-dominance model with three parameters (m, a & d) was adequate to explain the genetic system of any of the traits, none of the scales (A, B, C & D) as well as the chi-square values would have been significant (MATHER & JINKS, 1977). The findings reported here are related to those described by PATEL & PATHAK (2010), and MARINKOVIC & al. (2013). From the results, it was observed that besides the additive and dominance gene actions, epistatic interaction mechanisms have also contributed significantly to the inheritance of all the characters studied. However, the relative magnitudes of these effects varied for the characters and under different crosses evaluated. The genetic system operating for any character may be judged based on the magnitude of the six genetic parameters or judged by the magnitude and signs of dominance effect and dominance × dominance epistasis [EDWARDS & al. 1975]. The result revealed significant effects of additive coupled with significant effects of additive × additive interaction for number of spikes per plant in two (cross I and cross II) of the three crosses. However, the effects were coupled with predominant dominance effects in all the three crosses. The simultaneous presence of significant positive additive and dominance gene actions for effective spike length suggested recurrent selection technique to be adequate in harnessing the different kinds of gene effects. For the 100-seeds weight, the presence of significant additive × dominance and dominance × dominance interactions, which always reduce phenotypic dominance, would be a hindrance in simple selection technique in all the crosses. Additive gene influence was not significant for percentage seed oil content in all the crosses. The major gene effects for the trait include dominance, additive × additive and dominance × dominance gene effects. The absence of additive gene actions coupled with the presence of significant dominance × dominance effects in all the crosses would not be of advantage in simple selection procedure. However, the observed predominant dominance gene effects in the cross I and cross II presented opportunities for heterosis breeding exploitation. The significant dominance gene action, coupled with positive dominance × dominance interaction (indicating increasing dominant alleles), observed in cross III also presented similar opportunity. The results of the six parameters model reported here are similar to those reported by PATEL (1985), GONDALIYA & al. (2001), SOLANKI & al. (2003). PATEL & PATHAK (2010), MARINKOVIC & al. (2013) in their works on castor.

Conclusions

The results revealed adequate genetic variability and high potential for recombination in the parental lines evaluated. From the results, it showed that besides the additive and dominance gene actions, epistatic interaction mechanisms have also contributed significantly to the expressions of all the characters studied. High magnitude of general combining ability variance was observed for number of spikes per plant. Non-additive gene actions were found to be the major contributory gene effects for the expression of effective length of spike, seed yield and seed oil contents. However, varietal differential was observed on relative magnitudes of the gene effects for the characters under study.

The present research findings suggest that improvement of the traits evaluated may not be easily achievable by adopting simple selection technique, rather heterosis breeding and population improvement involving inter mating among promising divergent genotypes may be more appropriate.

Notes on contributors

Bolaji Zuluqurineen SALIHU (PhD) is a research scientist and a geneticist with a special interest in quantitative genetics. His work majorly focuses on castor population improvement.

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Benson Obukohwo APUYOR and Abolore Adijat AJADI are research scientists with research interest in castor processing and utilization. Their work including screening of castor germplasm for high seed oil content and good physicochemical properties

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STUDIES ON THE INTERRELATIONSHIP BETWEEN YIELD AND AGRONOMIC TRAITS IN SOME SELECTED SOYBEAN LINES AT YANDEV IN SOUTHERN GUINEA SAVANNAH OF NIGERIA

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Abstract: A field trial was conducted during the 2010 cropping season at the experimental farm of Akperan Orshi College of Agriculture, Yandev–Gboko, Benue State. The plots were laid in Randomized Complete Block Design with three replications. The analysis of variance showed significant in difference in nodule dry weight; days to 50% flowering; days to maturity; plant height at maturity; number of branches; number of pods per plant; number of seeds/ plant; and weight of 100 seeds among the genotypes studied. The result of this experiment conducted showed that there were highly significant correlation on number of pods per plant, number of seeds per plant, and significant correlation of one hundred seed with seed yield. The results of the path coefficient analysis also showed that number of pods per plant exerted the highest direct effect on seed yield. Weight (g) of one hundred seeds showed the least direct effect among the traits evaluated. The regression on seed yield and its component character also showed that number of pods per plant had the highest contribution to seed yield with the proportion due to regression being 48%. The relative contribution of number of branches per plant to seed yield was 2%.

Keywords: Correlation, genotypes, path analysis, regression, soybean.

Introduction

Soybean (*Glycine max* (L.) Merrill) is a legume that grows in tropical, subtropical, and temperate climates. Soybean is not an indigenous crop in Nigeria, although, it is gaining popularity in the country because of its numerous potentials that rank it even better than cowpea in the supply of high quality protein [AKANDE & al. 2007]. Soybean grains contain about 40% protein, 20% oil, an optimal supply of essential amino acids and nutrients, and a high calorie value [SINGH & al. 2008]. The main goal of growing crops is to maximize net profit through increasing grain yield [ALGHAMDI, 2004]. Hence, the primary goal of most soybean breeding programs is high grain yield [TOLEDO & al. 2000]. According to GRAFIUS (1959) increasing total yield would be made easier by selecting for the yield components because the yield components are more simply inherited than the total yield itself. Thus, studies on correlation enable the breeder to know the mutual relationship between various characters and determine the yield components to select, to improve the yield of the crop. Seed yield, as complex trait, is the result of the expression of the association of several plant growth components. So, selection for seed yield should take into account related characters. Hence, the knowledge of correlation between yield and yield components and among the components themselves is essential for yield improvement through selection programs. The objectives of this study was to determine

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the genetic variability that exist among the selected genotypes; to estimate the correlation coefficients between the seed yield of soybean and its components; and to identify traits that influenced yield of soybean and select them in order to improve its' yield.

Material and methods

The experimental material for the present study comprised of twenty(20) elite soybean genotypes. The sources and characteristics of these soybean genotypes used are presented in Table 1. A field trial was conducted during the 2010 cropping season at the experimental farm of the Akperan Orshi College of Agriculture, Yandev–Gboko, Benue State. Soil samples were taken from experimental site for analysis to determine the physical and chemical properties at the NICANSOL soil testing laboratory of the University of Agriculture, Makurdi. The textural class and the chemical properties of the soil are presented in Table 2a & Table 2b. The site is 117 m above sea level (a.s.l.m.) as altitude, 07°22` N and 08°37` E as longitude and latitude respectively.

Table 1. Soybean Genotypes, Sources and Characteristics.

S/N	Genotype Code	Sources	Variety's Characteristics
1	NCRI SOY 16	NCRI, Badeggi	Medium maturing, resistant to shattering
2	NCRI SOY 5	NCRI,Badeggi	Medium maturing, resistant to shattering
3	SAMSOY 2	UAM	Medium maturing, susceptible to shattering
4	TGX 1440 – 1E	IITA, IBADAN	Medium maturing, resistant to shattering
5	TGX 1448 – 2E	IITA, IBADAN	Medium maturing, resistant to shattering
6	TGX 1485 -1D	IITA, IBADAN	Early maturing, low shattering
7	TGX 1835 -10E	IITA, IBADAN	Early maturing, low shattering
8	TGX 1904 -6F	IITA, IBADAN	Medium maturing, resistant to shattering
9	TGX 1984 -17F	IITA, IBADAN	Late maturing
10	TGX 1984 19F	IITA, IBADAN	Late maturing
11	TGX 1984 -1F	IITA, IBADAN	Late maturing
12	TGX 1984 -22F	IITA, IBADAN	Late maturing
13	TGX 1984 -5F	IITA, IBADAN	Medium maturing
14	TGX 1985 -12F	IITA, IBADAN	Medium maturing
15	TGX 1986 -1F	IITA, IBADAN	Medium maturing.
16	TGX 1987 -10F	IITA, IBADAN	Extra early maturing, resistant to shattering
17	TGX 1987 -19F	IITA, IBADAN	Late maturing
18	TGX 1987 -57F	IITA, IBADAN	Medium maturing
19	TGX 1987- 62F	IITA, IBADAN	Extra early maturing, resistant to rust
20	TGX 923 -2E	IITA, IBADAN	Late maturing

Abbreviations: NCRI = National Cereals Research Institute, UAM = University of Agriculture, Makurdi, IITA = International Institute for Tropical Agriculture, Ibadan.

Tab. 2a. Physical properties of the **Yandev-Gboko, Benue State**.

Soil Parameters	Yandev-Gboko, Benue State	
Particle Size Distribution (%)	Sand	80.0
	Silt	15.9
	Clay	4.1
Textural Class	Loamy Sand	
pH	6.15	
H_2O	1:1	

Tab. 2b. Chemical properties of the soil at **Yandev-Gboko, Benue State**.

	Yandev-Gboko, Benue State	
Organic (%)	C	0.63
	M	1.09
	N	0.088
Available	P (ppm)	2.79
Exchangeable bases	(Cmol kg^{-1})	
	Ca	2.41
	Mg	1.92
	K	0.76
	Na	0.48
	CEC (Meq/100g)	5.87

Abbreviations: C = carbon, M = magnesium, N = nitrogen, CEC = cation exchange capacity

The experimental site was cleared, ploughed, harrowed, and ridged in mid-June to mid-July of the year 2010. Plots were laid in a Randomized Complete Block Design (RCBD) with three replications. Plot size was 5 m x 1.5 m, giving a plot area of 7.5 m^2. Inter-row spacing was 0.75 m apart. Planting was done by drilling 200 seeds in each plot. All entries were planted on 12th July, 2010. Pendametalin was applied as pre-emergence herbicide within 24hrs after sowing. Hoe weeding was done later as often as weeds were observed in the field. Side dressing was done using P_2O_5 (26%) at the rate of 30$Kgha^{-1}$. Cypermethrin (a broad spectrum insecticide) was applied to the crop as often as infestation was observed in the field. Data collected were on the following parameters: number of seeds per pod, and 100 seed weight. nodule dry weight; days to 50% flowering; plant height at flowering; days to maturity; plant height at maturity; number of branches; number of pods per plant; pod weight; number of seeds / pod; number of seeds / plant; weight of 100 seeds; and yield in tons per hectare. The data was subjected to statistical analysis as described by STEEL & TORRIE (1980).

Table 3. Analysis of variance of traits of elite Genotypes of Soybean evaluated at Yandev during the 2010 cropping season.

Traits	Meansquares		
	Block	Entries	Error
d.f	2	19	38
NDW (g)	1.12	0.15*	0.07
DF	2.15	10.14*	44.32
PHF (cm)	17.08	10.62	6.84
DM	77.27	95.45**	16.11
PHM (cm)	59.02	31.72	20.50
NB	3.99	0.81*	0.45
NPP	2589.4	341.7*	156.1
PW (g)	0.01	0.01	0.01
NSP^1	0.00	0.01**	0.00
NSP^2	10729.0	4895.0	4837.0
WHS (g)	1.62	2.85**	0.71
Yld(ton ha^{-1})	33.55	1.07*	0.75

Abbreviations: NDW(g) – Nodule dry weight; DF – Days to 50% flowering; PHF(cm) – Plant height at flowering; DM – Days to maturity; PHM(cm) – Plant height at maturity; NB – Number of branches; NPP – Number of pods per plant; PW(g) – Pod weight; NSP^1 – Number of seeds / pod; NSP^2 – Number of seeds / plant; WHS(g) – Weight of 100 seeds; Yld(ton ha^{-1}) – Yield in tons per Ha.

Results

The analysis of variance showed that, the genotypes were statistical different for all the traits studied exception of plant height at maturity, pod weight and number of seeds per plant - Table 3. Mean yield and agronomic performance of seed yield and its component characters in soybean evaluated at Yandev is presented in Table 4. From this Table, the genotypes that have highest yields are NCRI SOY 5 (3.24 t/ha), TGX 1904 – 6F (2.93 t/ha) and TGX 1984 – 22F (2.88 t/ha). The genotypes that served as check viz. TGX 1440 – 1E, TGX 1448 – 2E, TGX 1485 – 1D, TGX 1835 – 10E and TGX 923 – 2E yielded 1.36 t/ha, 1.66 t/ha, 1.95 t/ha, 1.62 t/ha and 1.4 t/ha respectively. Therefore, genotypes like NCRI SOY5 (3.24 t/ha), TGX 1904 - 6F (2.93 t/ha), TGX 1984 – 1F (2.48 t/ha) and TGX 1984 – 22F (2.88 t/ha) yielded more than the checkers.

Table 4. Mean performance of seed yield and its components in Soybean evaluated at Yandev during the 2010 Season.

Entries	NDW (g)	DM	PHM (cm)	NB	NPP	PW (g)	NSP^1	NSP^2	WHS (g)	Yld (ton h^{-1})
NCRI SOY 16	0.807	111.33	33.47	3.467	54.5	0.333	2.067	125.5	10.91	1.91
NCRI SOY 5	0.663	113.00	39.07	4.333	42.5	0.387	2.067	75.5	11.77	3.24
SAM SOY 2	0.700	114.33	37.90	4.467	52.5	0.330	2.000	73.7	11.05	1.21
TGX 1440-1E (check)	0.540	110.33	31.07	3.867	36.3	0.333	2.000	63.3	10.42	1.36
TGX 1448-2E (check)	0.503	112.00	28.73	3.933	43.2	0.277	2.000	93.3	10.50	1.66
TGX 1485-1D (check)	0.723	110.33	28.73	3.000	39.9	0.377	2.133	58.5	9.49	1.95
TGX 1835-10E (check)	0.843	96.67	31.80	3.800	32.2	0.387	2.067	63.9	10.87	1.62
TGX 1904-6F	0.313	106.67	32.73	3.400	62.3	0.277	2.000	133.7	10.46	2.93
TGX 1984-17F	0.367	106.33	34.40	4.267	47.6	0.417	2.133	186.5	8.81	1.68
TGX 1984-19F	0.360	103.00	37.73	3.067	36.9	0.347	2.000	46.6	9.56	1.65
TGX 1984-1F	0.323	112.67	27.87	3.333	55.1	0.443	2.000	117.5	10.18	2.48
TGX 1984-22F	0.873	108.00	32.33	4.333	71.3	0.347	2.000	139.9	9.56	2.88
TGX 1984-5F	0.483	103.00	31.67	3.733	48.5	0.390	2.000	60.5	9.56	1.77
TGX 1985-12F	0.747	107.00	31.13	4.533	43.0	0.390	2.000	60.7	9.17	1.23
TGX 1986-1F	0.983	108.33	35.60	4.000	53.0	0.387	2.000	80.6	10.64	1.76
TGX 1987-10F	0.440	100.33	35.73	2.600	27.6	0.443	2.000	49.8	11.17	1.11
TGX 1987-19F	0.727	114.00	33.00	3.80	52.2	0.387	2.000	101.5	9.27	1.91
TGX 1987-57F	0.993	105.33	34.47	4.133	46.9	0.360	2.133	56.9	9.63	1.60
TGX 1987-62F	1.007	103.67	38.40	3.467	61.3	0.387	2.000	53.6	8.81	1.40
TGX 923-2E (check)	0.753	121.33	34.07	3.867	43.7	0.207	2.000	157.3	7.80	1.40
Lsd (5%)	0.452	6.63	7.48	1.111	20.6	0.16	0.105	114.9	1.39	1.43
CV	41.6	3.7	13.5	17.8	26.3	26.9	3.1	77.3	8.5	47.2

Key: NDW(g) – Nodule dry weight; DM – Days to maturity; PHM(cm) – Plant height at maturity; NB – Number of branches; NPP – Number of pods per plant; PW(g) – Pod weight; NSP^1 Number of seeds / pod; NSP^2 – Number of seed / plant; WHS(g) – Weight of 100 seeds; Yld (ton ha^{-1}) – Yield in tons per Ha.

The genotypic correlation coefficient analysis for seed yield and components of soybean evaluated is presented in Table 5. This table shows that number of branches per plant, number of pods per plant, number of seeds per plant and weight of one hundred seeds were significantly correlated with seed yields.

Table 5. Correlation coefficients of seed yield and its components in soybean evaluated at Yandev during the 2010 season

Variables	PHM	NB	NPP	PW (G)	NDW	NSP[1]	NSP[2]	WHS	YLD
PHM		0.361*	0.216	0.209	0.288*	0.022	0.154	0.209	0.162
NB			0.573**	-0.007	0.316*	-0.125	0.407**	0.058	0.323*
NPP				0.024	0.359*	-0.129	0.579**	0.089	0.471**
PW (g)					-0.004	0.190	0.059	0.161	0.149
NDW (g)						-0.081	0.106	0.007	0.059
NSP[1]							-0.130	-0.186	0.058
NSP[2]								0.142	0.487**
WHS (g)									0.288*

* = significant at 5%, ** = significant 1%.

Key: PHM(cm) – Plant height at maturity; NB – Number of branches; NPP – Number of pods per plant; PW(g) – Pod weight; NDW(g) – Nodule dry weight; NSP[1] – Number of seeds / pod; NSP[2] – Number of seeds / plant; WHS(g) – Weight of 100 seeds; and Yld (ton ha^{-1}) – Yield in tons per hectare.

Path coefficient analysis for yield and yield component traits of soybean evaluated is presented in Table 6. Direct effects indicated in bold showed that number of seeds per plant had the highest direct effect (0.2872) with yield while number of pods per plant (0.0258) had the least direct effect with yield. Figure 1 revealed the path diagram for yield and yield components of soybean evaluated at Yandev during the 2010 season. The double-arrowed lines indicate the genotypic correlation between traits while the single- arrowed lines indicate the path coefficients (direct effect).

Table 6. Direct (bold) effect of seed yield and its component characters in soybean evaluated at Yandev during the 2010season.

Variables	NB	NPP	NSP2	WHS	YLD
NB	**0.0450**	0.1482	0.0116	0.0129	0.323
NPP	0.0258	**0.0258**	0.1664	0.0198	0.4708
NSP[2]	0.0184	0.1499	**0.2872**	0.0314	0.487
WHS	0.0026	0.0231	0.0407	**0.2210**	0.288

* = significant at 5%, **= significant 1%.

Key: NB – Number of branches; NPP – Number of pods per plant; NSP[2] – Number of seeds/ plant; WHS(g) – Weight of 100 seeds; YLD (ton ha^{-1}) – Yield in tons per hectare.

The stepwise regression analysis of the contribution of some agronomic characters to seed yield at Yandev is presented in Table 7. The regression of yield on number of pods per plant and weight (g) of one hundred seeds were significant while the regression of other yield components under study were not significant. The proportion of the variability that existed in the soybean when only number of pods per plant was involved in the yield equation was 48%. That of weight (g) of one hundred seeds was 6%.

Table 7. Regression of seed yield and its components in soybean evaluated at Yandev during the 2010 season.

Model	Variables	Coefficient of variation	R^2	R^2 change	Proportion due to regression	Cumulative regression	Significance
1	NPP	0.482[a]	0.232	0.219	48%	48%	0.05
2	WHS (g)	0.542[b]	0.293	0.269	6 %	54%	0.05

Where: Predictors: (Constant), NPP; Predictors: (Constant), NPP, WHS (g); Dependent variable: Yld (t/ha)

Discussions

The highly significant and significant variability exhibited in the twelve characters of soybean as revealed by analysis of variance showed that the genotypes are diverse. Such variations are useful in plant breeding as they provide heterogeneous population for a wide spectrum of genotypes for selection for the characters. These results agreed with the findings of GHATGE & KADU (1993) and RASAILY & al. (1986), SHAAHU & al. (2012, 2014) who obtained considerable genotypic variability for seed yield.

The results of the correlation coefficient analysis revealed that seed yield had highly significant positive correlation with number of pods per plant and number of seeds per plant. Similarly, seed yield had significant positive correlation with number of branches per plant and weight of one hundred seeds. Number of branches per plant had positive and highly significant correlation with the number of pods per plant and number of seeds per plant. Many researchers: NAKAWUKA & al. (1999), IQBAL & al. (2003) and MALIK & ADIPALA (2006) also found highly significant genotypic correlation coefficient of seed yield with number of pods per plant. MALIK & al. (2007), TAYYAR (2007), COPUR & al. (2009) and SHAAHU& al. (2012, 2014) showed that the seed yield was positively and significantly correlated with pod number, fruit branch and 1000-seed weight. These results are in agreement with other reports by AKTHER & SNELLER (1996) and BOARD & al. (1997) who found strong positive correlations between seed yield with pods per plant and seeds per plant.

The results of the path analysis indicated that the direct effect of number of branches per plant, number of pods per plant, number of seeds per plant and weight (g) of one hundred seeds. This confirms the results of KHAN & al. (2000); SINGH & YADAVA (2000) who reported that 100-seed weight, branches per plant, number of pods per plant and number of seeds per plant had direct effects on seed yield in soybean. This shows that selecting for these characters may lead to increase in seed yield.

The coefficient of determination (R^2) explains the amount of variability in yield due to number of pods per plant and weight of one hundred seeds. The F-test revealed a highly significant regression value for all levels of combination of the yield components as well as their combination have significant effects on yield. The F-test showed that only number of pods per plant and weight of one hundred seeds are statistically significant (regression coefficient of 0.48 and 0.06 respectively). This seems to indicate that only number of pods per plant and weight of one hundred seeds currently influence yield. The proportion due to regression for number of pods per plant on seed yield was 48% and that of weight of one hundred seeds was 6%.

The results of the experiment seem to suggest that, in breeding, the traits to consider for when breeding pods per plant, seeds per plant and weight of one hundred

seeds. Moreover, it may be recommended that more research should be conducted using the population in this experiment so as to get a more stable results over location and year.

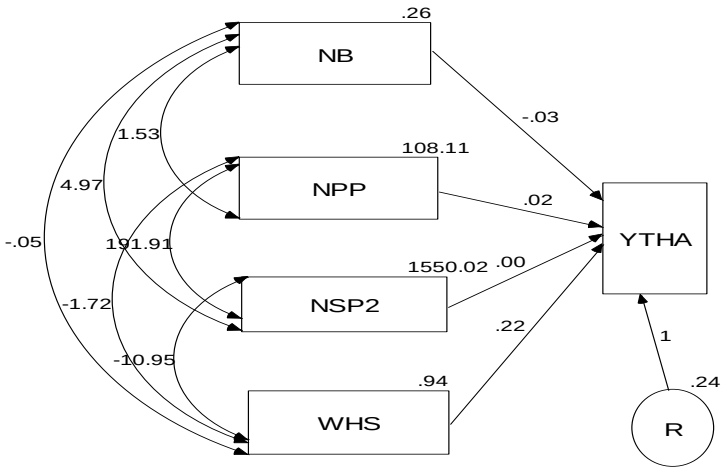

Figure 1. Path diagram for yield and yield components of soybean evaluated at Yandev during the 2010 season. [**Key**: Double-arrowed lines indicate the genotypic correlation between traits. Single-arrowed lines indicate the path coefficients (direct effect). NB – number of branches per plant; NPP – Number of pods per plant; NSP2 – Number of seeds per plant; WHS – Weight of one hundred seeds; YTHA – Yield in tons per hectare; and R – Error].

Conclusions

The analysis of variance showed significant in difference in nodule dry weight; days to 50% flowering; days to maturity; plant height at maturity; number of branches; number of pods per plant; number of seeds/ plant; and weight of 100 seeds among the genotypes studied. The result of this experiment conducted showed that there were highly significant correlation on number of pods per plant, number of seeds per plant, and significant correlation of one hundred seed with seed yield. The results of the path coefficient analysis also showed that number of pods per plant exerted the highest direct effect on seed yield. Weight (g) of one hundred seeds showed the least direct effect among the traits evaluated. The regression on seed yield and its component character also showed that number of pods per plant had the highest contribution to seed yield with the proportion due to regression being 48%. The relative contribution of number of branches per plant to seed yield was 2%.

Notes on contributors

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EFFECTS OF COPPER ON SEED GERMINATION AND SEEDLING GROWTH PERFORMANCE OF *LENS CULINARIS* MEDIK.

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Abstract: The discharge of heavy metals in the environment due to anthropogenic, industrial and automobile activities is a worldwide environmental pollution problem. Copper is widely used in different forms in fertilizer, fungicides. Industrial effluents and for the removal algal growth in ponds. In this study the toxic effects of copper (Cu) on seed germination and seedling growth of *Lens culinaris* were investigated. Germination rate of *L. culinaris* that showed that increased in concentration of copper treatment at 25 ppm significantly ($p<0.05$) reduced germination percentage as compared to control. Seedling growth variables i.e. root and shoot length, seedling size and root/shoot ratio also declined significantly ($p<0.05$) with the treatment of copper at 25 ppm as compared to control. Seedlings dry weight of *L. culinaris* gradually reduced with increased in all treatment of copper concentration as compared to control.

Tolerance indices and seedling vigor index of *L. culinaris* also decreased with increase in concentration of copper treatment. Low percentage of reduction in tolerance indices and seedling vigor index of *L. culinaris* was recorded at 25 ppm copper treatment as compared to control. A high percentage of reduction in seedling tolerance indices of *L. culinaris* was recorded at 100 ppm of copper treatment as compared to control.

Keywords: heavy metals, phytoxicity, seed germination, seedling growth, tolerance index.

Introduction

The group of elements have a density greater than $5 g/cm^3$ belongs to heavy metals group [AGORAMOORTHY & al. 2008]. The ever increase of heavy metal contamination in the environment has caused a serious environmental concern among researchers community. The effects of heavy metals on plant growth varied from species to species and the level of heavy metals available in the environment which may be beneficial or toxic to the plant growth environment. The essentials element likewise Fe, Zn, Cu or Mo are required in small quantities but at higher concentrations they may be poisonous for plant growth. Heavy metal contamination of soil and water causing toxicity/stress has become one important constraint to crop productivity and quality [SINGH & al. 2016]. Among the heavy metals copper can be considered important heavy metals for ecotoxicology concern. The effects of copper upon seedling growth of *Cucumis sativus*, carrot, maize and wild plant species on bioaccumulation, yield of tomato and mineral nutrients were reported [MOUSTAKAS & al. 1997; STOYANOVA & DONCHEVA, 2002; ROUT & DAS, 2003; AN & al. 2004; SONMEZ & al. 2006; MAHMOOD & al. 2005; AN, 2006; XU & al. 2006; AUDA & ALI, 2010; MUHAMMAD & al. 2011]. Copper is an essential micronutrient and is easily absorbed by plants. Its optimal content in plants tissues is reported to be 5-20 $\mu g\ g^{-1}$ [FERNANDEZ & HERNIQUES, 1991]. High concentrations of Cu become extremely toxic

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for root growth inhibition [FARGASAVA 2001]. Rice grain yields decreased exponentially and significantly with the increase of soil Cu levels. Rice grain yield was reduced about 10% by soil Cu level of 100 mg kg(-1), about 50% by soil Cu level of 300-500 mg kg(-1) and about 90% by soil Cu concentration of 1,000 mg kg(-1). Copper treatment at 0.5 mM inhibited the root growth of two lentil cultivars viz. cv. Krak and cv. Tina [JENAS & al. 2015].

Copper is toxic heavy metals and high concentration can be harmful for agriculture cops. The excess concentration of copper induced huge losses in agricultural crops viz wheat, iron, maize, sunflowers and cucumber production [ADREES & al. 2015]. Lentil is an edible pulse, oldest crop [SULTANA & GHAFOOR, 2006] and predominantly successfully cultivated in South East Asia and Turkey, Syria, Egypt, Iran and Pakistan. *L. culinaris* is annual leguminous popular crop due to its lens shaped seed and is a main source of vegetable protein. *L. culinaris* is a rabi legume plant and cultivated on large area in Pakistan [RAHMAN & al. 2013]. Little is known about the effects of copper on growth of an important crop lentil. Therefore, the aim of the present study was to evaluate the effects of copper on seed germination and seedling growth performance of *L. culinaris*.

Lentils (*Lens culinaris* Medik.) is an important legume pulse crop in world for millions of people as source of food. SZILAGY & al. (2011) examined the stability for seed yield in lentils (*Lens culinaris* Medik) in Romania. At present in Romania, lentils grown on the lower areas, the only lentil Romanian cultivar being 'Oana'. The temperature hydration kinetics of *Lens culinaris* was evaluated in water at different temperature [OROIAN, 2017].

Materials and methods

The healthy seeds of *Lens culinaris* Medik were collected from the local market. The seeds were surface sterilized with 0.2% dilute solution of Sodium-hypo chlorite for two minutes to prevent any fungal contamination. The seeds were washed with double distilled water. Ten seeds were placed in Petri dishes (90 mm diameter) uniformly on filter paper added at proper place and covered with lid to prevent loss of mixture through evaporation. Solutions of copper sulfate were prepared having five concentrations (0, 25, 50, 75 and 100 ppm) concentrations. At the start of the experiment, 3ml of solution of above treatment were applied to each set of respective treatment. After two days the old solution from every petri plate was sucked out and 2 ml fresh solution of respective treatment was added. The distilled water was added to each set of control treatment. The control received only 1ml of distilled water on alternate days. The experiments were designed on the basis of three replicates and the Petri dishes were kept at room temperature (32±2 °C) with 240 Lux light intensity and the experiment lasted for ten days. The experiment was completely randomized. Seed germination, root, shoot, seedling lengths, seedling dry weight and root / shoot ratio was recorded. Three seedlings having maximum from each petri plate was sampled to measure the seedling growth variable. The dry biomass was determined by placing the seedling in an oven at 80 °C for 24 hours. Seedling vigor index (S.V.I) was determined as per the formula given by BEWLY & BLACK (1982). Tolerance indices of seedlings were determined with the help of the following formula.

$$\text{Tolerance indices (T.I.)} = \frac{\text{Mean root length of treated seedlings}}{\text{Mean root length of control seedlings}} \text{ x } 100$$

The seed germination and seedling growth data were statistically analyzed by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to determine the level of significance at $p < 0.05$ on personnel computer using COSTAT version 3.

Results

The different concentrations of heavy metal treatment as copper sulfate on *Lens culinaris* indicated low percentage of seed germination, seedling growth, seedling dry weight and root/shoot ratio as compared to control. The results indicated that root is strongly affected as compared to shoot by copper treatment. An increased in concentration of Cu produced more toxic effects on seedling dry weight and root / shoot ratio of *L. culinaris* as compared to control treatments (Figure 1-2; Table 1).

Copper treatment at 25 ppm not significantly affected seed germination percentage of *L. culinaris* as compared to control (Table 1). The percentage of seed germination was significantly ($p<0.05$) reduced to 76.66, 66.66 and 56.66% at 50, 75 and 100 Cu treatment as compared to control (100%). Copper treatment showed that root growth of *L. culinaris* was decreased with increase in concentration up to 100 ppm. The treatment of Cu showed more adverse effects on shoot growth of *L. culinaris* as compared to control. Results showed that shoot growth parameters were also declined with increase in concentration from 25 to 100 ppm of Cu. The treatments of Cu at 25 ppm increased shoot length 12.72 cm of *L. culinaris* over control 8.87 cm and significantly decreased to 7.0, 5.87, and 5.07 with the treatment at 50, 75 and 100 ppm, respectively. Seedling size which consists on the length of root and shoot was recorded as 16.77 cm for control which decreased to 13.97 cm, 7.96 cm, 5.43 cm and 5.49 cm when treated with 25, 50, 75 and 100 ppm copper solution treatment. Copper treatment at all concentration influenced the seedling dry weight of *L. culinaris*. Seedling dry weights of *L. culinaris* when treated with different concentration of Cu was reduced to 0.03 g to 0.0133 g at 25 and 100 ppm concentration as compared to control. Results indicated that reduction was observed in root/shoot ratio with the increase in concentration copper particularly at 75 and 100 ppm.

Table 1. Effects of different concentration of copper on seed germination (%), seedling growth and seedling dry weight (g) of *Lens culinaris*

Treatments (Copper concentration ppm)	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling size (cm)	Seedling dry veight (g)	Root/shoot Ratio
00	100.00±3.33a	9.46±0.06a	8.87±0.06a	16.77±0.06a	0.034±0.98a	1.72±0.01a
25	100.00±5.77a	1.19±0.12b	12.72±0.9b	13.93±0.20b	0.030±2.64b	0.091±0.01b
50	76.66±3.33b	0.95±0.18b	7.00±0.2bc	7.96±0.4c	0.020±3.38b	0.133±0.02b
75	61.66±0.00c	0.28±0.14b	5.87±0.18c	5.43±0.33d	0.0133±2.96b	0.033±0.00b
100	51.66±3.33d	0.40±0.03b	5.07±0.25c	5.49±0.27d	0.0133±2.88b	0.091±0.006b
L.S.D.	8.13	2.25	1.09	2.28	0.01	0.12

Number followed by the same letters in the same column are not significantly different according to Duncan Multiple Range Test at <0.05 level. ± Standard error. L.S.D. Least significant difference.

The seedlings of *L. culinaris* showed lowest percentage of tolerance indices were recorded for 100 ppm of Cu treatment as compared to control (Figure 1). Similarly, Seedling Vigor Index (S.V.I.) for *L. culinaris* seedling was recorded highest in control and gradually declined with the increase in concentration of Cu treatments from 25 to 100 ppm (Figure 2).

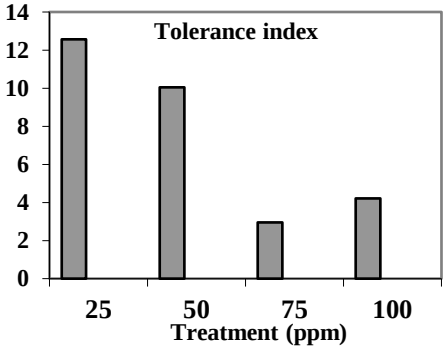

Figure 1. Percentage of tolerance in *L. culinaris* using different concentration (0, 25, 50, 75, 100 ppm) of copper (Cu)

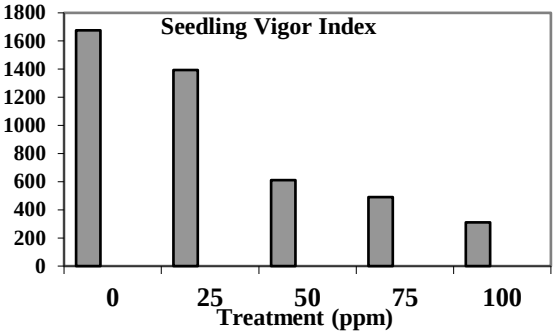

Figure 2. Seedling vigor index for *L. culinaris* using different concentration (0,25, 50, 75, 100 ppm) of copper

Discussion

Excessive concentration of copper generally produce common toxic effects on different growth variable of plants, such as low biomass accumulation, chlorosis, inhibition of growth and photosynthesis, altered water balance and nutrient assimilation, and senescence, which ultimately cause plant death. The plant under abiotic stress conditions are most likely to be adversely affected by heavy metals contamination. In present studies the toxicity and tolerance of copper on seed germination and seedling growth performances of *L. culinaris* were found significantly affected at higher concentration of copper treatment. Copper treatment at 25 ppm did not produce any significant effect on seed germination which

might be due to its resistance to copper. The effect of the Cu at 25 ppm concentration on root growth of *L. culinaris* was observed and agreed with the findings of ISMAIL & al. (2013). Exposure to 25ppm concentration of Cu reduced the root length of *L. culinaris* as compared with the root length of control. In another studies, the copper treatment in resin buffered solution culture at < 1 µ M produced toxic effects on the root morphology of a pasture species, Rhodes grass (*Chloris qayana* Knuth.) observed [SHELDON & MENZIES, 2005]. Similarly, the increasing concentrations of Cu significantly inhibited the growth of young sweet potato plants (*Ipomoea batatas*) reported earlier [KIM & al. 2010].

Results also showed that seedling dry weights of *L. culinaris* were also declined with increased concentration of copper sulphate and this reduction was more prominent with increasing concentration of copper in substrate. The increase in concentration of copper upto 100 ppm was found responsible for decreased the seedling growth of *L. culinaris* as compared to control treatment. Copper treatment at all concentration none significantly affected seedling dry weight as compared to control. The results indicated that increasing concentrations of Cu in seedlings tissues significantly ($p < 0.05$) reduced the seedlings growth. In addition to growth inhibition of *L. culinaris* copper treatment reduced root / shoot ratio.

The effects of Cu on the plant growth have been reported in some studies over the past few years. At the cellular level, oxidative stress has been reported as a common mechanism in both stress situations [SMEETS & al. 2009]. The treatment of copper produced toxic effects on germination and growth of *L. culinaris.* Lowest percentage of tolerance indices for *L. culinaris* seedlings were recorded for 100 ppm copper treatment.

Conclusion

It was concluded that the copper treatment produced toxic effects on seed germination and seedling growth of *L. culinaris* along with significant reduction in seedling dry weight as compared to control treatment. Similarly, the tolerance to copper treatment decreased the tolerance indices for *L. culinaris* seedlings with the increase in metal concentration in the substrate as compared to control. The difference in tolerance and seedling vigor index in response to copper toxicity should be considered while *L. culinaris* cultivated in copper contaminated areas. There is a need to be carried out further studies on other copper tolerant species for plantation in copper contaminated areas to overcome the shortage of agriculture crops.

Notes on contributors

Muhammad Zafar IQBAL - Ph.D., Professor, designed and supervised the experiment. Umm-e-HABIBA - M.Sc., Research scientist, performed the experiment. Sundus NAYAB - M.Sc., Research scientist, performed the experiment and assisted to collect the data. Muhammad SHAFIQ - Ph.D., Research scholar statistically analysed the experimental data, reviewed the literature and draft the manuscript.

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ADVENTITIOUS ROOTS DEVELOPMENT AND ROOT SYSTEM ARCHITECTURE OF *CHRYSANTHEMUM* CUTTINGS

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Abstract: *Chrysanthemum* have significant ornamental value and thus have a great economic importance and are also subjected to losses due to the insufficient rooting of the cuttings - the main method of vegetative propagation practiced for this crop. Insufficient understanding of the mechanisms that control adventitious root formation prevents the use of reliable technologies to improve the percentage of root adventitious development in cuttings of ornamental plants in general. Also, knowing the architecture of the root system helps correct application of chrysanthemum culture technologies. Two different cultivars of *Chrysanthemum* was investigated in order to describe the radicular architecture of cuttings, with emphasis on the root type and the influence of substrate on the developed model into four different variants of rooting substrate.

Keywords: adventitious roots, *Chrysanthemum*, cuttings, nutrients, root system, rooting substrate.

Introduction

The rooting of stem cuttings is one of the best methods of vegetative propagation in *Chrysanthemum*, to get high multiplication rates and also desired plants phenotypes. Propagation of *Chrysanthemum* and of other many ornamental plant species relies on adventitious root formation, which can be defined as roots developed under specific conditions from organs such as leaves and stems. Significant economic losses are still emerging as a result of insufficient rooting, despite of the intensive control over the environmental factors in the modern ornamental plant propagation industry.

Knowing the architecture of the root system helps correct application of chrysanthemum culture technologies in terms of properly positioning the crop on the appropriate fertility plots, ensuring soil mobilization according to plant requirements, application of fertilizers, and mechanical maintenance of crops in order keep intact the root system. The architectural model considers the root system as a set of connected axes, meaning a mathematical tree with nodes and branches. To describe the architecture of the radicular system, there are several models presented in the literature, but for studying the chrysanthemum cuttings in the proposed experiment was considered the hydraulic network method. This approach was developed by FITTER (1987), the general idea being to characterize the architecture of the root system and, in particular, the topology, using the mathematical tools developed in the field of hydrology to describe the drainage networks. For this purpose, the root system is defined as a set of links (linear segments between a

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terminal meristem area and a branch point or between two branch points), describing the topological parameters such as magnitude, altitude, topological index, etc. [FITTER & al. 1991]. Two main types of architecture can be distinguished: the "herringbone" model, where the branching occurs only on a main axis (high hierarchy) and the "dichotomic" model, where branching on each axis results in equivalent axes (low hierarchy). Among these models there are many other intermediate patterns found in plants in general, being useful in appreciating the functional potential of the root system. The model shows the efficiency of exploiting the resources present in the substrate, with the link between the herringbone model and poor nutrient soils.

Several parameters that can be used in interpretations related to the efficiency of soil nutrient use [PAGES & al. 2000] are indicated in the literature, of which, for the proposed experiment, those related to the total length of the roots formed on the cuttings, on the rooting period, the measurements taking place at the phase of transplantation of the cuttings into individual pots.

For determination of the total length of the roots, various measurement methods, whether manual or automated based on digital images, are available [DOWDY & al. 1995], among which more commonly used are direct measurement, intersected line method and visual estimation method [OLIVEIRA & al. 2000]. Direct measurements of the length of the roots can be practiced only on roots of large diameter, tree roots. Direct measurements of root lengths of herbaceous plants are rare and only for primary roots [SIVAKUMAR & SALAAM, 1994], since it is not possible to directly measure the length of the total root (including the root) of a plant or even a sample root.

Using the intersected line method, the root length of a sample can be estimated using intersection counting techniques of a given data line system [NEWMAN, 1966; TENNANT, 1975]. Newman's proposed method is based on the relationship between the length of the root and the number of intersections between the roots and a system of lines spread over a well-defined surface, and arranged randomly with the lines of known lengths on the given area. MARSH (1971) simplified the method of NEWMAN & TENNANT (1975), tested and popularized this modified method. The intersecting line method has become the standard manual technique for estimating the root length. The Intersected Line Method provides an estimate of the root length of the sample and is not a direct measurement. Sources of error arise only from random rooting on the grid, root visibility, definition of an intersection, and appreciation from the operator. With increased attention, it is possible to obtain coefficients of variation of the root length estimate of 5% or less [TENNANT, 1975], this being the minimum probable error. The variation coefficients for the Newman method are usually between 10 and 15% [BOHM, 1979; BLAND & MESARCH, 1990]. To minimize the error associated with the technique, is need to ensure proper lighting, the use of a magnifying glass to improve the visibility of the roots, improve the color contrast between the roots and the background, provide adequate breaks to avoid fatigue.

The purpose of the present study is to describe the radicular architecture of *Chrysanthemum* cuttings, with emphasis on the root type and the influence of substrate on the developed model into four different variants of rooting substrate.

Material and methods

Plant Materials and Growing Conditions. The investigations were conducted during the period 2016-2017 on experimental field of "Anastasie Fatu" Botanical Garden belonging to "Alexandru Ioan Cuza" University of Iasi, Romania, in the heated greenhouses, with two *Chrysanthemum* cultivars – *Chrysanthemum indicum* L. cv. Carmina, noted in our research as (I) and *Chrysanthemum* × *grandiflorum* Ramat. cv. Yellow Stardust (II) (The Plant List 2014, The Plant List 2014a) – Figure 1. The cuttings were set about February 10, collected from stock-plants maintained in a vegetative state, in cold greenhouses with a main temperature of 13.8°C and with moderate irrigation once for a week.

Experimental Model. The evaluation of the qualitative and quantitative characters of the cuttings in the two cultivars of chrysanthemums consisted in measurements and observations on the root system of the cuttings in order to estimate the root architecture of the chrysanthemum cuttings. The rooting substrates was represented by four variants (V), with organic (peat) and inorganic components (perlite and sand) combined into volumetric progressive proportions: V1 – peat 100%, V2 – peat+perlite (v/v), V3 – peat+perlite+sand (v/v/v) and V4 – perlite 100%. The experiment was set as bi-factorial, type 2x4, with first factor represented by species (two graduations – *Chrysanthemum indicum* L. and *Chrysanthemum* × *grandiflorum* Ramat.) and the second one by rooting substrate variant (four graduations - V1, V2, V3, V4).

In addition to the aspects regarding the development of the chrysanthemum cuttings, a number of parameters related to the development of the root system were taken into consideration, and the total length of the roots developed per cutting was measured after Newman method.

For roots density determination (D), the root length was reported at a given substrate volume, i.e. 60 ml (0.06 m^3), the results being expressed in m/m^3. Formula used in the calculation [ALI, 2010]: Root density (D) = total root length / substrate volume.

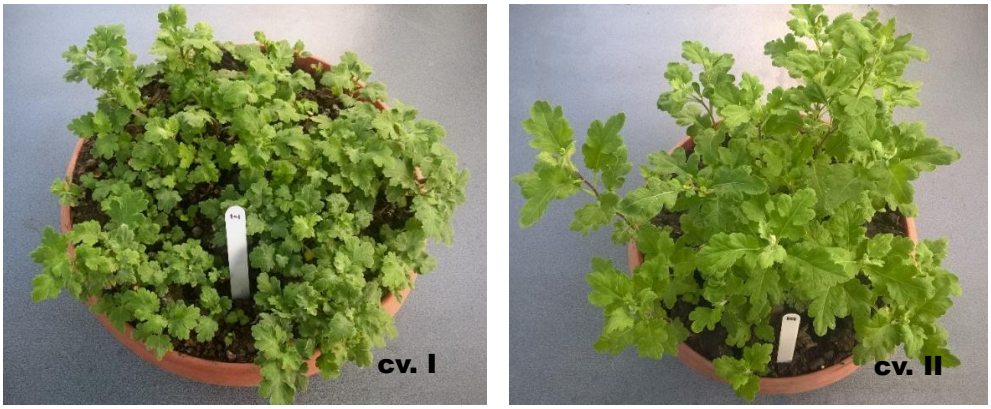

Figure 1. Stock-plants maintained in vegetative state: *Chrysanthemum indicum* L. cv. Carmina (I) and *Chrysanthemum* × *grandiflorum* Ramat. cv. Yellow Stardust (II)

Results and discussions

Our results for *Chysanthemum indicum* (I) and *Chrysanthemum* × *grandiflorum* (II) cultivars indicate a correlation between the species and the rooting capacity. From the total loss of 5%, it was 3.5% for cv. I (7 cuttings) and 1.5% for cv. II (3 cuttings). This difference, though insignificant, could be explained by the fact that *Chrysanthemum* × *grandiflorum* – a horticultural species cultivated for ornamental cut flowers, is a complex hybrid species less adapted to environmental variations, being cultivated only in protected areas, while *Chrysanthemum indicum*, is represented by rustic varieties with better tolerance to environmental variations, being cultivated successfully in open spaces.

Regarding the influence of rooting substrate type on total root length, one can notice differences both visually (Figure 2) and mathematics, based on data and calculation using the Newman formula.

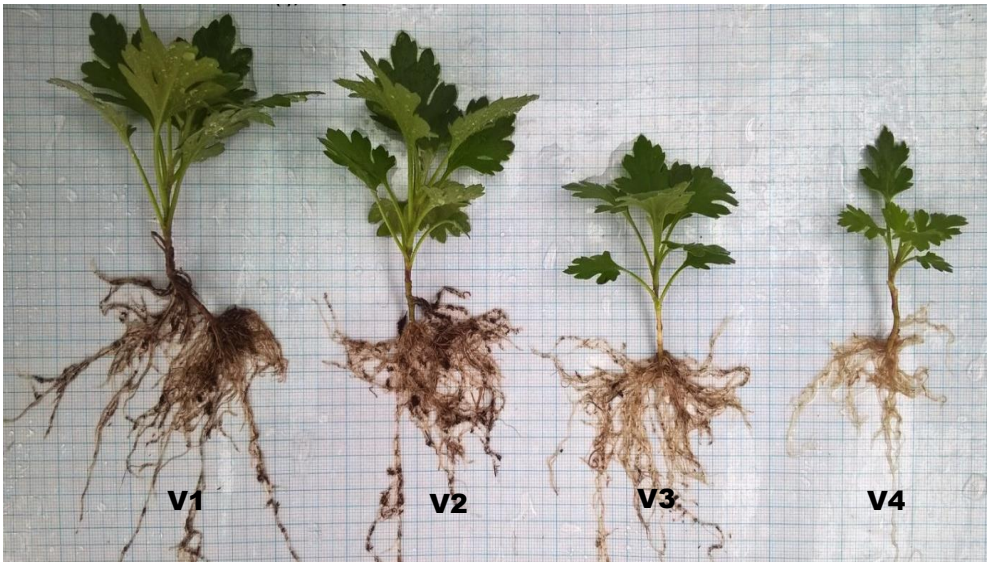

Figure 2. Influence of rooting substrate type on architecture and total root length of *Chrysanthemum indicum* cv. Carmina cuttings (V1–peat 100%, V2–peat+perlite (v/v), V3–peat+perlite+sand (v/v/v) and V4–perlite 100%; *Scale* - area of one minimal square is 1 mm^2)

Depending on the rooting substrate, for the *Chrysanthemum* cuttings it is noted that the most extensive root system was developed in the case of the V1 100% peat variant (62.55-65.88 cm), followed by the combined variants V2 and V3 (average length between 49.81-61.86 cm). Cuttings that rooted into an inorganic substrate (100% perlite – V4) showed a less developed root system (35.70-38.28 cm) – Table 1, 2 and Figure 3.

Radicular plant system is in contact with a variety of abiotic factors, including soil water and nutrient availability, which influences both primary root growth and lateral root formation [INGRAM & MALAMY, 2010]. These factors are constantly changing, and the ability of a plant to respond to these changes and to efficiently acquire the necessary water and nutrients has a direct impact on reproductive health and reproduction.

Table 1. Roots total length and roots density of *Chrysanthemum* cuttings (cm) – *Chrysanthemum indicum* 'Carmina'

cv. I	Rooting substrate variants			
	V1	**V2**	**V3**	**V4**
	Roots total length per cutting, in cm (Newman formula)			
A (cm)	62.55 ± SD	50.78 ± SD	49.81 ± SD	38.28 ± SD
D (m/m^3)	10.42	8.46	8.30	6.38
***SD**	*4.13	*3.55	*3.29	*4.43

Abbreviations: **I** – *Chrysanthemum indicum* L. cv. Carmina, **A** – average root length, **SD** – Standard Deviation, **D** – root density, **V** – variant of rooting substrate: **V1** – peat (100%), **V2** – peat+perlite (v/v), **V3** – peat+perlite+sand (v/v/v), **V4** – perlite (100%), **m** – meter, **cm** – centimeter.

Table 2. Roots total length and roots density of *Chrysanthemum* cuttings (cm) – *Chrysanthemum* × *grandiflorum* 'Yellow Stardust'

cv. II	Rooting substrate variants			
	V1	**V2**	**V3**	**V4**
	Roots total length per cutting, in cm (Newman formula)			
A (cm)	65.88 ± SD	61.86 ± SD	61.71 ± SD	35.70 ± SD
D (m/m^3)	10.98	10.31	10.28	5.95
***SD**	*3.13	*3.55	*3.09	*2.32

Abbreviations: **I** – *Chrysanthemum* × *grandiflorum* Ramat. cv. Yellow Stardust, **A** – average root length, **SD** – Standard Deviation, **D** – root density, **V** – variant of rooting substrate: **V1** – peat (100%), **V2** – peat+perlite (v/v), **V3** – peat+perlite+sand (v/v/v), **V4** – perlite (100%), **m** – meter, **cm** – centimeter.

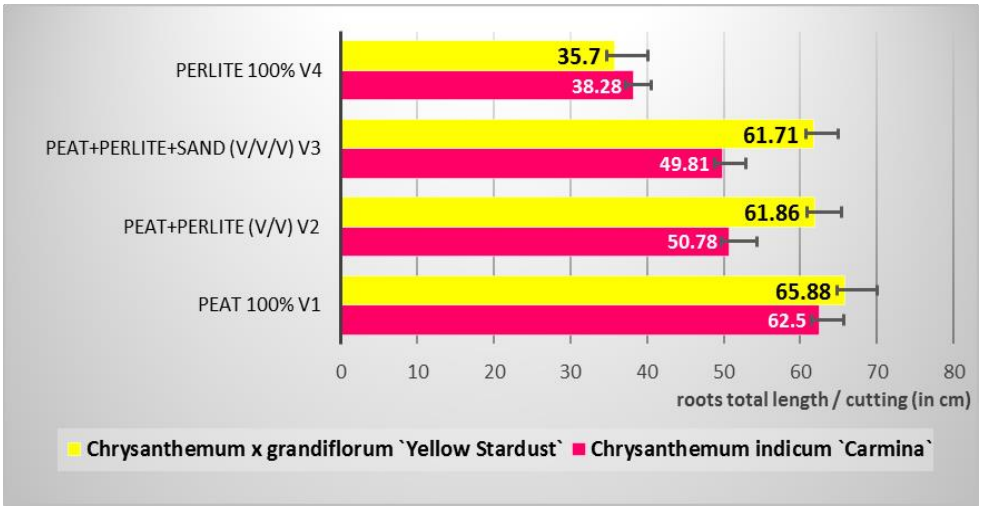

Figure 3. Roots total length of *Chrysanthemum* cuttings (average in cm) (*bars* – Standard Deviation)

Considering the fact that for rooting of cuttings, no substances have been used to stimulate the rhizogenesis, the total loss of the cuttings of the chrysanthemums was only 5%. This insignificant percentage indicates that the genus *Chrysanthemum* s.l. has a very good rooting capacity for cuttings, even in the absence of hormonal stimulators, which are indicated to be used for vegetative propagation, especially in heavily rooted plant species. Data from the literature indicates a correlation between the cultivar and the successful rooting of the cuttings, but with the use of IBA in different concentrations, the best results being obtained for *Chrysanthemum* cv. Crimson Robe, cv. Polario, cv. Escort, cv. Sterling and cv. Luysona at 50 ppm IBA, by spraying or immersing in the rooting stimulator, resulting in a much more compact radicular system [PETTER, 1992].

Knowing the architecture of the root system improve the correct application of chrysanthemum culture technologies in terms of properly positioning the crop on the appropriate fertility plots, ensuring soil loosening according to plant requirements, application of fertilizers, and mechanical maintenance of crops in order to does not destroy the root system. The investigation reveal the development of a "dichotomic" radicular system in the chrysanthemum cuttings – Figure 4, in which branching occurs on each axis, resulting in equivalent axes (low hierarchy). The radicular architecture model shows the efficiency of exploitation of the resources present in the substrate, the link between the developed radicular model and the substrate characteristics in terms of nutrient availability [FITTER & al. 1991]. While the herringbone model is associated with plants growing in poor nutrient soils, the dichotomic pattern observed at chrysanthemum cuttings indicate a nutrient rich substrate.

Figure 4. Dichotomic radicular architecture of *Chrysanthemum* cuttings (*Scale* – area of one minimal square is 1 mm^2)

There are a number of materials that can be used either separately or in various combinations to obtain rooting mixtures, choosing a particular material being determined by the availability, cost and local experience of its use. In northeastern Europe, peat is the most commonly used material as rooting substrate, and in the US the crumbly bark, vermiculite and perlite, which are also widely used materials. The most important factor in choosing a material for earth mixtures is that it must not contain toxic substances for plants. A large number of materials meet this requirement and can be successfully used, provided that the management of the substrate composition (i.e. the addition of nutrients or drainage material) is adapted to the requirements of the environment and culture [BUNT, 1988].

Our results support a relationship between the type of rooting substrate and the capacity to rooting, a lower rate being observed for peat and perlite+peat substrates (96%), while for the peat+perlite+sand and perlite, the rate was 99%. The explanation for this difference could be the fact that the peat is a substrate that becomes quite compact, which does not allow a good circulation of water and air at the root system level, while the perlite, through the porous structure allows the aeration and constant water drainage. KHER (1976) reported coarse sand for

chrysanthemums as the optimal rooting medium, also based on the high drainage and aeration capacity at the roots level. According to BARBAROSA & al. (2000) expanded clay can be considered an excellent medium for chrysanthemum culture into hydroponic system, with good aeration and drainage, and also the absence of pathogens.

Conclusions

The present study highlight several aspects related to *Chrysanthemum* vegetative multiplication and the influence of some factors (such as horticultural variety, type of rooting substrate) on the yield of planting material. Depending on the studied cultivars, the best results were obtained for *Chrysanthemum indicum*, indicating a better adaptation and a higher rooting capacity for this species that do not require special conditions for cultivation in enclosed spaces, as in the case of *Chrysanthemum* × *grandiflorum*.

Based on the researches made with the four variants of the rooting substrate, there was a better rooting in the peat+perlite (v/v) and perlite variant (V2, V4), characterized by a better drainage capacity and aeration. By correlating the results of the development of the root system and the growth rate after rooting, we can conclude that 100% organic or inorganic (peat or perlite) rooting substrate is not recommended. More variants and volumetric progression combinations are more appropriate to ensure drainage of water and aeration in the newly formed roots. In 100% peat, the percentage of initial losses is higher, while perlite, although rooted cuttings in a higher percentage than peat, fail to maintain the growth rate of an organic environment such as peat.

Knowing the particular type of radicular architecture can improve the correct application of chrysanthemum culture technologies as properly positioning of the crops into the appropriate fertility plots, adequate application of fertilizers, and mechanical maintenance of chrysanthemum crops in order to protect the root system of the plants.

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PROTOPLAST AS A TOOL TO ADDRESS QUESTIONS IN PLANT PHYSIOLOGY

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Abstract: A protoplast is a plant cell from which the cell wall has been removed by enzyme treatment. Tremendous utilization of protoplast in molecular analysis of plant growth and development has been observed in the past decades and the system has paved its way to significantly facilitate the comprehensive understanding of the complexity of underlying mechanisms. However, it should be kept in mind that a plant, like all systems, is composed of networks of interdependent components that integrate the system into a unified whole. In this mini review, we will re-explore protoplast approach in answering plant physiology questions through discussion of its application in the study of (1) photosynthesis and chloroplast-related process; (2) pollen tube growth; (3) sieve tube element protoplast for long distance translocation; (4) new regulatory metabolites from guard cell protoplast. This *in vitro* approach may open the way to further meaningful results at organismal level.

Key Words: guard cell, pollen tube, protoplast, photosynthesis, sieve tube element, stomata.

Introduction

Protoplasts are plant cells that are not enveloped by the protective cell wall. Physiological properties of plant protoplasts and application of plant protoplasts as a physiological tool were discussed a few decades ago reflecting the early purpose and effort of the use of protoplasts [GALUN, 1981; PILET, 1985]. With the advance in cellular and molecular technology, protoplast application in the analysis of plant signaling has been re-emphasized [SHEEN, 2001; XING & WANG, 2015; XING & al. 2017]. Educational effort was made through Annual Conference of American Society of Plant Biologists and published protocols are provided by Sheen Lab [http://molbio.mgh.harvard.edu /sheenweb/protocols_reg.html].

The concerns and drawbacks in using protoplasts for plant studies are mainly related to the single cell status isolated from *in planta* environment and the potential damage due to the isolation process [SHEEN, 2001]. Plant cell metabolism, growth, and development are modified by a large variety of internal and external signals. The ability of cells to respond to these signals is not confined to cells that are still growing and developing. Mature cells too can initiate metabolic responses and can even reinitiate growth and division in response to signal information. Many regulatory pathways are now characterized and the underlying genes are known. However, we should admit that contrast to animal cells, plant cells can easily change their identity when taken out of their environment or when cell lineages are disrupted [FARACO & al. 2011]. They may even change identity rapidly according to their new position when they are re-positioned [VAN DEN BERG & al. 1995]. Another indication comes with the totipotency and regeneration

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of an entire plant from cultured protoplasts as they must have first gone through dedifferentiation. It is not unreasonable to consider protoplasts as cells with loss of their identity and it makes protoplasts questionable in studies of cell type or tissue specific processes [FARACO & al. 2011].

On the other hand in this regard, two of the most significant supporting evidences are also provided in previous discussions: (1) Protoplasts retain properties of the cells and tissues they originate from for hours [BART & al. 2006; BIRNBAUM & al. 2003; FARACO & al. 2011; SHEEN, 2001; ZHAI & al. 2009]; (2) Protoplasts may have similar responses as intact whole plant tissues when responding to hormones, metabolites and environmental stimuli, and early experiments verified protoplast involvement in active photosynthesis and respiration, and protoplast retained their cell membrane potentials as endogenous cells [SHEEN, 2001]. Protocols have been well established for a large variety of plant species, so potentially some specific physiological functions could be tested in these species using protoplasts [XING & WANG, 2015]. Here, we will re-emphasize its usefulness in addressing physiological questions in plants through discussion of some representative cases.

Photosynthesis and chloroplast-related process

Biological process can often be a relay of events occurring from one specific tissue or cell type to another. While this could be a disadvantage for protoplast application, there are studies with cells which have specific functions but are surrounded by other cells that may interfere with these studies. The usefulness of protoplast techniques for physiological investigations is especially evident in physiological studies of photosynthetically active leaf cells.

The C_4 photosynthetic carbon cycle is evolved as an adaptation to high light intensities, high temperatures, and dryness, and it can be considered an elaborated addition to the C_3 pathway. The evolution also introduced biochemical and anatomical modifications that allow plants with this photosynthetic pathway to concentrate CO_2 at the site of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), thereby its oxygenase reaction and the following photorespiratory pathway are largely repressed in C_4 plants [GOWIK & WESTHOFF, 2011]. In most C_4 plants the CO_2 concentration mechanism is achieved by a division of labor between two distinct, specialized leaf cell types, the mesophyll and the bundle sheath cells [EDWARDS & al. 2004]. The bundle sheath cells enclose the vascular bundles and are themselves surrounded by the mesophyll cells. This wreath-like structure of C_4 leaf anatomy is termed Kranz anatomy, which is absent in C_3 plants. In the early attempt, protoplasts were isolated from mesophyll cells of C_3 plants and mesophyll protoplasts were separated from bundle sheath cells of C_4 plants [cf. GALUN, 1981]. It was shown that the carboxylation phase of the C_4 pathway is located in mesophyll protoplasts while the decarboxylative phase of this pathway as well as the carboxylative phase of the Calvin-Benson pathway is located in the bundle sheath cells. Ribulose-1,5-bisphosphate carboxylase was found only in bundle sheath cells and not in mesophyll protoplasts [cf. GALUN, 1981].

To further our understanding of sophisticated underlying regulatory mechanisms, a highly efficient rice green tissue protoplast system for studying light/chloroplast-related processes was established [ZHANG & al. 2011]. The feasibility of such studies was demonstrated by the observation that transient expression of the GFP tagged light-related transcription factor OsGLK1 markedly upregulated transcript levels of the endogenous photosynthetic genes *OsLhcb1*, *OsLhcp*, *GADPH* and *RbcS* [ZHANG & al. 2011].

Pollen tube growth

Pollen recognition or rejection is determined by the pollen surface protein and stigma surface protein. The process involves fluxes of multiple ions. The use of non-invasive methods has demonstrated a close link between intracellular ion fluctuations and ionic fluxes across the plasma membrane, and the cellular phenomena that occur during the formation and elongation of the pollen tube [FEIJÓ & al. 2001; HEPLER & al. 2001]. Transcriptomic studies have also demonstrated a number of anion /chloride membrane transporters and channels to be specifically and highly expressed in the pollen of *Arabidopsis* [PINA & al. 2005]. Protoplasts are favorable tools to study transport physiology as well as other physiological entities of the plasma membrane. The study in in the plasma membrane of *Lilium longiflorum* pollen protoplasts showed for the first time the presence of a large anionic conductance across the membrane of pollen protoplasts, resulting from the presence of Ca^{2+}-regulated channels, and a similar conductance was also found in germinated pollen [TAVARES & al. 2011].

Sieve tube element protoplast for long distance translocation

It is arguable whether protoplast approach is applicable to some highly coordinated physiological processes. One of the examples is phloem long distance translocation. In angiosperms, sieve elements (SE) lose many organelles, retaining only the plasma membrane and modified mitochondria, plastids, and smooth endoplasmic reticulum. Sieve elements are interconnected through pores in their end cell walls and form a longitudinal series called sieve tube. Companion cells aids the highly specialized sieve elements in three main ways (1) transport photosynthetic products from producing cells in mature leaves to the sieve elements in the minor veins of the leaf; (2) carry the protein synthesis, that is reduced/lost in sieve elements; and (3) supply ATP to sieve elements. The pressure-flow model explains phloem translocation as a bulk flow of solution driven by an osmotically generated pressure gradient between source and sink. These sieve element cells are transporting cells but with specialized subcellular structures. While protoplasts have yielded considerable insight into plasma membrane-bound ion channels and carbohydrate carriers in a variety of plant cells ranging from large parenchyma cells to guard cells, due to technical barriers, SEs were missing from other cell types that had been protoplasted successfully.

A technical fine tuning of cell wall digestion and the unequivocal identification of SE protoplasts led to isolation of functional SE protoplasts from *Vicia faba* as tested by osmotic treatment and the functionality examined by patch-clamp experiments [HAFKE & al. 2007]. At negative membrane voltages, the current-voltage relations of the SE protoplasts were found dominated by a weak inward-rectifying K^+ channel that was active at physiological membrane voltages of the SE plasma membrane [HAFKE & al. 2007]. This channel had electrical properties that are reminiscent of those of the AKT2/3 channel family, localized in phloem cells of *Arabidopsis* [DEEKEN & al. 2002; LACOMBE & al. 2000]. SE protoplasts could be an alternative in studying the membrane biology of SEs with inherent implications for the understanding of long distance transport and signaling. SE-mediated mass flow through phloem makes high demands on the physical properties of the SE plasma membrane, and isolation of SE protoplasts may thus facilitate the study of membrane biophysics in this long distance process [PATRICK, 2013; TURGEON & WOLF, 2009].

Phloem-associated K^+ channels had been located in the phloem by *in situ* analysis [LACOMBE & al. 2000], however, the exact cellular localization, ion channel densities, ion channel types, and particularly distribution along the phloem path were unknown. SE protoplasts isolated from the respective phloem sections would provide a unique tool for unequivocal information about these issues. Such successive phloem sectioning also enabled to identify, characterize, and quantify carbohydrate carriers in the SE plasma membrane at various sites along the phloem translocation pathway. Differential deployment of sugar carriers is likely essential for carbohydrate allocation in intact plants [HAFKE & al. 2007; KÜHN, 2003; PATRICK, 2013].

From metabolomics analysis of guard cell protoplast to leaf stomata bioassay

Among the environmental signals that guard cells transduce are light quality, light intensity, intercellular concentrations of leaf carbon dioxide, drought, and apoplastic concentrations of abscisic acid (ABA) [OUTLAW, 2003]. Drought can severely damage crops and at physiological level, vascular land plants conserve water via stomatal closure in response to drought. Guard cells have been highly developed as a model system to dissect the dynamics and mechanisms of plant cell signaling as well as for studies on guard cell ion transport [OUTLAW, 2003; TALLMAN, 2006; ZHU & al. 2016]. A recent comprehensive protocol on cellular, electrophysiological, and omics assays of guard cell function is a good reference [ZHU & al. 2016]. Small scale and large scale guard cell protoplast preparations are commonly used for electrophysiological and omics studies, respectively. One of the most significant approaches in gurad cell analysis utilized about 350 million guard cell protoplasts from about 30,000 plants of the *Arabidopsis* wild type and the heterotrimeric G-protein α subunit mutant, *gpa1*, which has ABA-hyposensitive stomata [JIN & al. 2013]. This metabolomics analysis has triggered further research questions and directions [JIN & al. 2013].

Recently, a non-targeted metabolomics utilizing gas chromatography mass spectrometry (GC-MS/MS) and liquid chromatography mass spectrometry (LC-MS/MS) were employed to identify metabolic signatures in response to ABA in *B. napus* guard cell protoplasts [ZHU & al. 2017]. The identified 390 distinct metabolites in *B. napus* guard cells fell into diverse classes. Of these, 77 metabolites, comprising both primary and secondary metabolites were found to be significantly ABA responsive, including carbohydrates, fatty acids, glucosinolates, and flavonoids. Secondary metabolites, sinigrin, quercetin, campesterol, and sitosterol were selected for stomatal bioassays in *Arabidopsis* and *B. napus*. Fully expanded leaves from *Arabidopsis* or *B. napus* leaf pieces were excised and incubated with stomata opening solution under white light to promote stomatal opening. All these tested secondary metalolites were confirmed to regulate stomatal closure in *Arabidopsis*, *B. napus* or both species [ZHU & al. 2017].

Conclusion

Considerable application of protoplast has been observed in the study of molecular mechanisms of plant growth and development due to the advantages of this *in vivo* cellular system [SHEEN, 2001; XING & WANG, 2015; XING & al. 2017]. Meanwhile, its potential in addressing physiological questions is also very promising, even though it could be challenging to interpret data obtained from protoplasts at physiological and organismal level.

A general response and specific developmental processes may require different approaches and strategies. For example, identification of specific cell types where phosphorylation is activated may require that the protoplasts are to be isolated from the specific cell types with cellular responses evoked. Mitogen-activated protein kinase (MAPK) activation during stress response and immunity can be measured biochemically using kinase activity assays or immunoblot detection of phospho-MAPKs because the activation of MAPKs is global. However, their activation during specific growth and developmental processes is likely to be limited to a specific tissue or even a few cells.

The spatiotemporal expression of receptor-like kinases (RLKs) and their ligands provides a mechanism to define the response only in a specific group of cells. A single MAPK cascade composed of YDA-MKK4/MKK5-MPK3/MPK6 functions downstream of ER/ERLs to regulate both stomatal development and localized cell proliferation [LEE & al. 2012; XU & ZHANG, 2015]. In this case, the signaling specificity is a result of limited tissue/cell-specific expression of the peptide ligands of ER-family receptors [HUNT & GRAY, 2009]. EPF1 and EPF2, which are expressed specifically within a subset of stomatal lineage cells, define the function of ER/ERL1/ERL2–YDA–MKK4/ MKK5–MPK3/MPK6 pathway in stomatal development [HARA & al. 2007; HUNT & GRAY 2009; LEE & al. 2012]. Protoplasts should then be isolated from sophisticatedly defined cell types.

Biological systems are composed of networks of interdependent components that integrate the system into a unified whole. We should keep in mind and in practice that after protoplast-based analysis, all results and hypotheses have to be verified at organism levels. ‘The whole is something over and above its parts and not just the sum of them all’ as stated by Greek physician Aristotle (384-322 B.C.).

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CHANGES IN MORPHOLOGY AND GROWTH RATE OF *FUSARIUM SOLANI* COLONIES EXPOSED TO VOLATILE COMPOUNDS SYNTHESIZED BY WOOD-ROTTING BASIDIOMYCETES

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Abstract: This study aims to determine the effects of the volatile metabolites synthesized by 53 species of wood-rotting basidiomycetes on the morphology and growth rate of *Fusarium solani* colonies. The fungi were cultivated in bi-compartmented Petri dishes. For every combination 4 different plates were prepared as well as a control Petri dish containing only *Fusarium solani*. The species were cultivated on PFMEA (potato flakes malt extract agar) and kept for 5 days at 25°C and further, the test plates were compared with the control, regarding the general aspect of *Fusarium solani* colony, pigmentation and differences in growth rate. The observations revealed that the volatiles synthesized by 42 species of wood-rotting basidiomycetes evidently influenced the development of the phytopathogenic species. The volatiles of *Neofavolus alveolaris* inhibited the most the growth of *Fusarium solani*. The GC-MS analysis of the volatile profile of *Neofavolus alveolaris* revealed the presence of compounds such as: 3-methyl-3-buten-1-ol, 2-methyl-1-butanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 1-octen-3-ol and benzaldehyde.

Keywords: *Fusarium solani*, growth inhibition, morphological changes, volatile metabolites, wood-rotting basidiomycetes.

Introduction

Fungi are a group of organisms that managed to developed specific adaptations which allow them to colonize different habitats and use trophic resources unavailable or hardly accessible to other species. Wood-rotting basidiomycetes form a particular ecological and functional group of higher fungi that due to an impressive enzymatic system can break down complex and resistant polymers such as cellulose or lignin [SCHMIDT, 2006]. In order to colonize a substrate, wood-rotting basidiomycetes must compete with other species. For that, they developed different repellent strategies, many of them involving the synthesis and secretion of secondary metabolites with antimicrobial properties that chemically signal the presence of a species on a certain substrate and inhibit the growth and development of other organisms.

The unique properties of the fungal enzymes and secondary metabolites are harnessed in various biotechnological processes [LORENZEN & ANKE, 1998], in the pharmacological industry (antibiotics, antibacterial and antifungal compounds, immunostimulators, antioxidants), cosmetics and perfumery (especially alcohols and terpenes) and agriculture (biopesticides). One particular class of secondary metabolites produced by wood-rotting basidiomycetes includes volatile organic compounds (VOC),

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lipophilic molecules that can easily penetrate the cellular membranes inhibiting, stimulating or disrupting the biological processes [WHEATLEY, 2002]. Due to their volatility, these metabolites have a wide range of action permitting the information to be precisely and exactly sent at various distances. The quantity and quality of these volatiles depends on several factors, including: media composition and pH [WHEATLEY, 2002], temperature and water content [TRONSMO & DENNIS, 1978], presence of other organisms [HYNES & al. 2006], age and stage of development [FÄLDT & al. 1999; LEE & al. 2015]. Fungal volatiles are not produced as sole molecules, but as complex mixtures of alcohols, ketones, aldehydes, terpenes, esters with various ecological functions [HUNG & al. 2015], such as intra- and inter-specific communication or insect, mites or herbivores attractants or repellents [JONSELL & NORDLANDER, 1995; RUESS & LUSSENHOP, 2005; BODDY & JONES, 2008; THAKEOW & el. 2008; DRILLING & DETTNER, 2009; ROHLFS & CHURCHILL, 2011].

This study aims to identify how the volatiles synthesized by 53 species of wood-rotting basidiomycetes influence the morphology and growth rate of *Fusarium solani* colonies. Moreover, after establishing which species of basidiomycetes inhibits the most the phytopathogen's growth, using a GC-MS analysis, the basidiomycete's volatile profile will be determined. By evaluating the inhibitory potential of the volatile metabolites, it is possible to understand the functioning mechanism of these compounds and finding the right circumstances in which they are produced and exhibit their maximum activity.

Material and methods

Fungal strains

The 53 species of wood-rotting basidiomycetes used in this study were collected from Romanian natural habitats and isolated within the Research Laboratory for Fungi with application in ecological reconstruction, Faculty of Biology, "Alexandru Ioan Cuza" University of Iaşi and are now part this laboratory's scientific collection. The phytopathogenic species *Fusarium solani* (Mart.) Sacc. was isolated from potato (*Solanum tuberosum*) tubercles. All fungi are maintained on malt extract agar 30% at 4 ºC.

Fungal screening

The effects of the volatile metabolites synthesized by the species of wood-rotting basidiomycetes on *F. solani* colonies were observed using the bi-compartmented Petri dishes in such way that the two mycelia didn't come into contact and the results was only due to the volatile compounds, as described in previous papers [PETRE & al. 2017]. The medium used in the screening activity was PFMEA (potato flakes malt extract agar): 20 $g \times l^{-1}$ potato flakes, 5 $g \times l^{-1}$ malt extract, 5 $g \times l^{-1}$ glucose, 15 $g \times l^{-1}$ agar [PETRE & al. 2017].

Every species of wood-rotting basidiomycete was inoculated in one compartment of the plate and the plant pathogen in the other. The plates were wrapped in two layers of Parafilm and incubated in the dark at 25 ºC, for 5 days. Four replicates were used for every combination. The control plate contained only the plant pathogenic species inoculated in one of the compartments. After 5 days, the test plates were compared with the control regarding the general morphology of *F. solani* colonies, pigmentation and inhibition of fungal growth. The inhibitory percentage was calculated for every plate: $IP=[C-T] \times 100/C$, where C represents the diameter of the control colony and T represents the diameter of the colony exposed to the VOC synthesized by the test fungi [NIDIRY & BABU, 2005]. The

medium inhibitory percentage (IP_{med}) was calculated as the average value of all four replicates inhibitory percentages. The first three species with the highest inhibition percentage were further tested on two different media: MEA (malt extract agar): 30 $g \times l^{-1}$ malt extract, 20 $g \times l^{-1}$ agar and KM media: 20 $g \times l^{-1}$ glucose, 2 $g \times l^{-1}$ peptone, 2 $g \times l^{-1}$ yeast extract, 0.25 $g \times l^{-1}$ KH_2PO_4; 0.25 $g \times l^{-1}$ $MgSO_4 \times 7\ H_2O$ [KAWABE & MORITA, 1993] in which 20 $g \times l^{-1}$ agar were added. This step was performed in order to determine if the media composition is influencing the production of antifungal volatiles. The species with the highest IP was selected for the analysis of volatile compounds produced *in vitro*.

Solid-Phase Extraction

Neofavolus alveolaris, species which showed the highest IP when tested *in vitro* was cultivated on a liquid medium containing 20 $g \times l^{-1}$ glucose, 2 $g \times l^{-1}$ peptone, 2 $g \times l^{-1}$ yeast extract, 0.25 $g \times l^{-1}$ KH_2PO_4; 0.25 $g \times l^{-1}$ $MgSO_4 \times 7\ H_2O$ [KAWABE & MORITA, 1993] and incubated in the dark at 25 ºC. After 25 days the surface culture of the wood-rotting basidiomycete was homogenized; 10 ml of homogenate was filtered and after mixed with 20 ml of pure water and 1 µl of 4-hydroxy-4-methyl-2-pentanone was added as an internal standard. The mixture was extracted on LiChrolut cartridges-EN (40-120 µm) 100 mg (bottom); RP-18 (40-63 µm) 200 mg (top) (Merck Millipore). These cartridges were previously conditioned with 2×6 ml *n*-hexane, 2×6 mL dichloromethane, 2×6 ml acetone, 2×6 ml methanol and 2×6 ml pure water allowing each solvent to pass completely before adding the next conditioning solvent. On the same filter used for the homogenate, 10 ml of pure water mixed with 1 g NaCl, 1 g Na_2SO_4 and 1 g of KH_2PO_4 were also passed in order to increase the ionic strength, thus facilitating the extraction of the compounds from the remaining biomass. The filtrate was later passed over the same cartridges. The SPE cartridges were completely dried using compressed air and later placed in a desiccator at 600 mbar for 24 h under a gentle nitrogen flow. Next, the cartridges were eluted with 1.5 ml of *n*-hexane, dichloromethane, acetone and acetonitrile respectively and eluate was further dried on anhydrous sodium sulfate [PETRE & al. 2017]. The extraction experiment was performed in duplicate. The eluents were collected in separate vials and analyzed by gas-chromatography with mass spectrometer detection (GC-MS).

GC-MS analysis

The GC-MS analysis of *N. alveolaris* extracts was done on a Shimadzu GC-MS 2010 equipped with a ZB WAXplus capillary column (10 m × 0.1 mm × 0.1 µm) operated in split mode injection (split ratio 1/10), as described in PETRE & al. (2017): the GC oven temperature was set from 35 ºC for 5 minutes, with an increase of 5 ºC/min to 220 ºC and hold for 5 minutes, with a total analysis time of 47 minutes. Helium was used as carried gas, with a total flow of 15.9 ml/min, column flow of 0.9 ml/min and a purge flow of 6 ml/min. The MS ionization source was operated in electron impact mode (EI) with the EI source temperature set at 200 ºC. The full scan mass-spectrums were acquired at every 0.1 seconds (equivalent with 5000 a.m.u.), between 30-500 Da (m/z).

Results and discussions

For this research 53 species of wood-rotting basidiomycetes were studied in order to determine the effects of their volatile metabolites on the morphology and growth rate of *F. solani* colonies. On the control plate, *F. solani* covered the compartment in 8 days. The colony was heterogeneous with areas where the aerial mycelium was well developed, white and downy, alternating with areas with submersed, pinkish mycelium (Figure 1).

Following the experiment, in 80% of the cases the morphology of the phytopathogen's colony was different from the one in the control plate (Figure 2-6), indicating that the volatile compounds synthesized by the wood-rotting basidiomycetes influenced the development of the mycelium (Table 1).

Moreover, in 9.5% of the cases, the aerial mycelium of *F. solani* was intensively pigmented unlike the control colony and in 30% of the cases, the mycelium was white, lacking the pigmentation.

All these observations underline the relation between the synthesis of volatile compounds and pigments during fungal interactions. The correlation between the productions of these categories of metabolites was recorded by literature [GRIFFITH & al. 1994; BRUCE & al. 2003; WALD & al. 2004; HYNES & al. 2006] and it was described as happening both prior and after the direct contact of the mycelia.

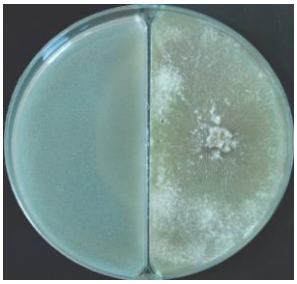

Figure 1. *F. solani* - control plate (PFMEA)

Figure 2. *L. arcularius* - *F. solani* (PFMEA)

Figure 3. *H. fasciculare* - *F. solani* (PFMEA)

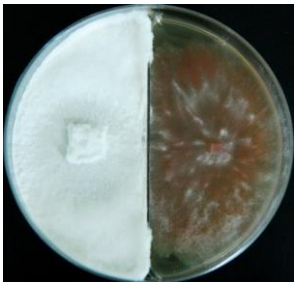

Figure 4. *L. betulinus* - *F. solani* (PFMEA)

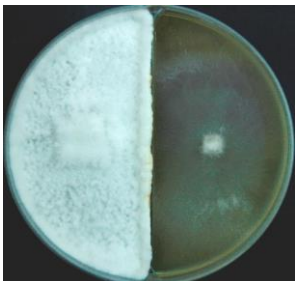

Figure 5. *N. alveolaris* – *F. solani* (PFMEA)

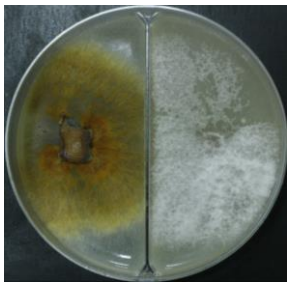

Figure 6. *P. igniarius* - *F. solani* (PFMEA)

Table 1. General aspect of *F. solani* colonies on the test plates

Species	IP_{med}	The morphology of *F. solani* colony on the test plates (PFMEA) compared with the control colony	Pigmentation of *F. solani* colony
Plicaturopsis crispa (Pers.) D.A. Reid	5%	poorly developed, heterogeneous aerial mycelium, submerged mycelium with hyaline hyphae	-
Crucibulum laeve (Huds.) Kambly	1.76%	unchanged	-
Cyathus striatus (Huds.) Willd.	5%	poorly developed, heterogeneous aerial mycelium, submerged mycelium with hyaline hyphae	+
Crepidotus applanatus (Pers.) P. Kumm.	3.23%	mycelium with concentric development: rings of dense, aerial hyphae around the inoculum point, submerged mycelium with marginal hyaline hyphae	+
Megacollybia platyphylla (Pers.) Kotl. & Pouzar	2.64%	homogeneous, lax aerial mycelium	+
Panellus stipticus (Bull.) P. Karst.	2.35%	homogeneous, lax aerial mycelium	+
Gymnopus dryophilus (Bull.) Murill.	2.05%	unchanged	+
Flammulina velutipes (Curtis) Singer	4.41%	unchanged	+
Hymenopellis radicata (Relhan) R. H. Petersen	4.41%	unchanged	+
Mucidula mucida (Schrad.) Pat.	7.64%	aerial mycelium relatively dense around the inoculum point and lax towards the margins	+
Psathyrella candolleana (Fr.) Maire	1.47%	aerial mycelium developed only around the inoculum point, submerged mycelium with hyaline hyphae towards the margins	-
Schizophyllum commune Fr.	4.7%	heterogeneous, lax aerial mycelium, submerged mycelium with hyaline hyphae towards the margins	+
Gymnopilus junonius (Fr.) P.D. Orton	5%	aerial mycelium absent, submerged mycelium with hyaline hyphae	+
Hypholoma fasciculare (Huds.) P. Kumm.	6.76%	well developed, dense aerial mycelium with a ring of hyaline hyphae around the inoculum point	+
Hypholoma lateritium (Schaeff.) P. Kumm.	2.94%	unchanged	+
Pholiota aurivella (Batsch) P. Kumm.	5%	heterogeneous aerial mycelium with concentric development	+
Auricularia mesenterica (Dicks.) Pers.	1.76%	relatively homogeneous, lax aerial mycelium	++
Inonotus hispidus	2.64%	relatively homogeneous, lax aerial mycelium	-

(Bull.) P. Karst.			
Phellinus igniarius (L.) Quél.	5.29%	well developed, heterogeneous aerial mycelium, with lax and dense areas	+
Phellinus pomaceus (Pers.) Maire	5.29%	aerial mycelium dense around the inoculum point and lax towards the margins	+
Daedalea quercina (L.) Pers.	5.58%	heterogeneous, adpressed aerial mycelium	-
Fomitopsis pinicola (Sw.) P. Karst.	5.29%	aerial mycelium with concentric development	+
Fomitopsis betulina (Bull.) B. K. Cui, M. L. Han & Y. C. Dai	3.82%	homogeneous, dense, well developed aerial mycelium	-
Ganoderma adspersum (Schulzer) Donk	2.94%	poorly developed aerial mycelium, submerged mycelium with hyaline hyphae	-
Ganoderma applanatum (Pers.) Pat.	5%	aerial mycelium dense around the inoculum point and lax towards the margins	++
Meripilus giganteus (Pers.) P. Karst.	2.64%	poorly developed aerial mycelium, submerged mycelium with hyaline hyphae	-
Bjerkandera adusta (Willd.) P. Karst.	1.47%	aerial mycelium developed only around the inoculum point, submerged mycelium with hyaline hyphae towards the margins	-
Bjerkandera fumosa (Pers.) P. Karst.	5.58%	poorly developed aerial mycelium around the inoculum point, submerged mycelia with hyaline hyphae	+
Phlebia radiata Fr.	3.82%	well developed, dense, downy aerial mycelium	+
Coriolopsis gallica (Fr.) Ryvarden	1.47%	unchanged	+
Daedaleopsis confragosa (Bolton) J. Schröt.	3.82%	aerial mycelium with concentric development	+
Daedaleopsis tricolor (Bull.) Bondartsev & Singer	4.11%	aerial mycelium with concentric development	+
Fomes fomentarius (L.) Fr.	2.94%	poorly developed aerial mycelium, submerged mycelium with hyaline hyphae	+
Lentinus tigrinus (Bull.) Fr.	5.58%	relatively homogeneous, dense, downy aerial mycelium	+
Lenzites betulinus (L.) Fr.	7.94%	relatively homogeneous, dense, downy aerial mycelium	-
Neofavolus alveolaris (DC.) Sotome & T. Hatt.	9.11%	poorly developed aerial mycelium around the inoculum point, submerged mycelium with hyaline hyphae	+
Panus neostrigosus Drechsler-Santos & Wartchow	5.29%	aerial mycelium with concentric development	+
Lentinus arcularius (Batsch.) Zmitr.	6.76%	well developed, homogeneous, dense aerial mycelium	-
Picipes melanopus (Pers.) Zmitr. & Kovalenko	5%	unchanged	+

Cerioporus squamosus (Huds.) Quél.	2.64%	unchanged	++
Cerioporus varius (Pers.) Zmitr. & Kovalenko	4.41%	unchanged	-
Picipes badius (Pers.) Zmitr. & Kovalenko	7.64%	well developed, homogeneous, downy aerial mycelium	+
Skeletocutis alutacea (J. Lowe) Jean Keller	1.47%	well developed, homogeneous, downy aerial mycelium	-
Trametes gibbosa (Pers.) Fr.	3.82%	aerial mycelium with concentric development	+
Trametes hirsuta (Wulfen) Lloyd	1.47%	well developed, dense, downy aerial mycelium around the inoculum point, submerged mycelium towards the margins	-
Trametes ochracea (Pers.) Gilb. & Ryvarden	4.11%	unchanged	+
Trametes pubescens (Schumach.) Pilát	2.94%	poorly developed aerial mycelium only around the inoculum point, submerged mycelium with hyaline hyphae	-
Trametes suaveolens (L.) Fr.	5.58%	downy aerial mycelium developed towards the margins, submerged mycelium with hyaline hyphae around the inoculum point	-
Trametes trogii Berk.	3.82%	well developed, homogeneous, downy aerial mycelium	+
Trametes versicolor (L.) Lloyd	3.82%	unchanged	++
Hericium coralloides (Scop.) Pers.	3.82%	heterogeneous aerial mycelium with concentric development	+
Stereum hirsutum (Willd.) Pers.	5%	poorly developed aerial mycelium around the inoculum point, submerged mycelium with hyaline hyphae	+
Stereum subtomentosum Pouzar	5%	heterogeneous aerial mycelium with concentric development	++

(-) unpigmented *F. solani* colony; (+) pigmented *F. solani* colony resembling the control; (++) intensively pigmented *F. solani* colony.

Following the exposure to the volatiles synthesized by the basidiomycetes, not only the morphology and pigmentation of *F. solani* colonies were influenced, but also the growth rate of the mycelia.

High IPs were observed for the volatiles synthesized by *P. badius*, *L. betulinus*, *L. arcularius*, *M. mucida* and *H. fasciculare*, while low IPs were calculated for species such as: *T. hirsuta*, *S. alutacea*, *B. adusta*, *C. gallica*, *C. leave*, *P. candolleana* and *A. mesenterica*.

The highest IPs were recorded for the volatiles synthesized by *M. mucida*, *L. betulinus* and *N. alveolaris*. When tested on MEA and KM media, the IPs for these three species were different compared with the ones recorded on PFMEA (Table 2).

For all three species the inhibition percentages were higher on the KM medium, fact that underlines the influence that the media composition has in the synthesis of the fungal volatiles [NORRMAN, 1971a, 1971b; BJURMAN, 1999].

Table 2. IP_{med} of the volatiles synthesized by *M. mucida*, *L. betulinus* and *N. alveolaris* on three culture media

Species	IP_{med} + SEM		
	PFMEA	MEA	KM
Mucidula mucida	7.64% ± 0.33%	10% ±0.33%	10.29% ± 0.29%
Lenzites betulinus	7.94% ± 0.56%	7.35% ± 0.56%	8% ± 0.29%
Neofavolus alveolaris	9.11% ± 0,29%	10.29% ± 0.29%	11.47% ± 0.29%

Compared to the other three media, KM had a greater complexity. The carbon source was represented by glucose (20 $g \times l^{-1}$), in the highest quantity, the nitrogen source by peptone (2 $g \times l^{-1}$), a mixture of amino acids rich in glutamic acid, proline, leucine aspartic acid and lysine, yeast extract (2 $g \times l^{-1}$) and several other essential elements such as potassium and phosphorus (0.25 $g \times l^{-1}$ KH_2PO_4), magnesium and sulfur (0.25 $g \times l^{-1}$ $MgSO_4 \cdot 7H_2O$), sodium and chloride (yeast extract with 0.5% NaCl).

The volatiles produced by *N. alveolaris* showed the highest IPs when cultivated on all three media, with a maximum on KM, thus being the species selected for the GC-MS analysis. By our knowledge this is the first study focused on the antifungal potential of the volatiles synthesized by this species.

Due to the large number of peaks present in the recorded mass-chromatograms of each analyzed final extract, we could not use only the comparison with NIST 2.0 mass-spectra database combined with retention times from previous literature in the field as acceptance criteria in order to declare a certain compound as present in our samples. In order to identify the classes of organic compounds synthesized by tested wood-rotting basidiomycete species, we applied some additional acceptance criteria described as described in PETRE et al. (2017). Since we tested each sample in duplicate against a control sample, we subtracted the results obtained for control samples from the chromatograms of each replicate. The results variability between replicates for the remaining identified compounds after blank-subtraction was further estimated (as relative standard deviation (RSD, %) between the results obtained for duplicate samples). Only results showing the lowest variability (RSD<30%) were later accepted as positively identified compounds in tested samples.

The highest number of compounds was identified within the dichloromethane fraction, while the lowest number was observed in the acetonitrile fraction. Table 3 presents data on the identified classes of organic compounds isolated from the culture fluids of *N. alveolaris* after elution with different solvents: *n*-hexane, dichloromethane (DCM), acetone and acetonitrile (AcN) together with their retention times (RT, minutes) recorded in specified GC elution conditions. Compounds marked with "*" were declared present in the analyzed samples.

One of the most abundant volatile compound synthesized by many fungi, 1-octen-3-ol was present in the culture fluid of *N. alveolaris* being previously recorded in other wood-rotting basidiomycetes [BERGER & al. 1986a; RAPIOR & al. 1996; ZIEGENBEIN & al. 2010], being responsible for the earthy / mushroom-like aroma [VENKATESHWARLU & al. 1999] and also acting as a signaling agent in the interspecific communication [THAKEOW & al. 2008].

Table 3. Volatile compounds isolated from *N. alveolaris*

Compound	RT (min)	Fraction (elution solvent)			
		***n*-hexane**	**DCM**	**Acetone**	**AcN**
Alcohols					
(R)-2-butanol	1,94		*		
(S)-2-octanol	5,59		*		
1-butanol	4,01		*		
1-octen-3-ol	13,4	*	*	*	
1-propanol	2,06		*		
2-hexanol	6,81		*		
2-methyl-1-butanol	6,19	*			
2-methyl-1-butanol	6,29			*	
2-methyl-1-propanol	2,84		*	*	
3-hexanol	5,97		*		
3-methyl-1-butanol	6,24		*	*	
3-methyl-3-buten-1-ol	7,31	*			
3-methyl-3-buten-1-ol	7,3		*		
4-methyl-2-pentanol	5,28		*	*	
Ketones					
2-hexanone	2,48		*		
3-hexanone	2,12		*		
3-penten-2-one	3,43			*	
4,6-dimethyl-2-heptanone	7,07	*			
4,6-dimethyl-2-heptanone	7,06		*		
4-hydroxy-4-methyl-2-pentanone	10,28		*	*	
4-methyl-3-penten-2-one	3,32		*	*	
Other					
Benzaldehyde	14,2	*	*		
Esther	10,75		*		
Phenol	30,95	*			

The specific almond like aroma observed for the *in vitro* cultures of *N. alveolaris* might be attributed to benzaldehyde which is synthesized by many other species of wood-rotting basidiomycetes [BERGER & al. 1986a, 1987; KAWABE & MORITA, 1993; FÄLDT & al. 1999], compound with applications in the cosmetic industry and perfumery [MORATH & al. 2012]. In the analyzed samples we noticed the presence of several alcohols including 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol which, as many compounds belonging to the same class, were reported to act at the cell membrane level by increasing its permeability for certain metabolites and ions [INGRAM & BUTTKE, 1984; HEIPIEPER & al. 1994]. Also the compounds 3-methyl-1-butanol and 2-methyl-1-propanol are considered responsible for the antifungal activity of several endophytic fungi such as *Muscodor albus* [STROBEL & al. 2001; EZRA & al. 2004], *Muscodor crispans* [MITCHELL & al. 2010] and *Phomopsis* sp. [SINGH & al. 2011], while 2-methyl-1-butanol was associated with the antifungal activity of *Trichoderma* sp. [WHEATLEY & al. 1997]. The same alcohols were also reported in the extracts of some wood-rotting basidiomycetes [BERGER & al. 1986a, 1986b, 1987; KAWABE & MORITA,

1993; SCHALCHLI & al. 2013]. WANG & al. (2004) isolated from *N. alveolaris* the polypeptide alveolarin with antifungal properties, but by our knowledge there is no record of testing this species volatiles for their antifungal activity.

Conclusions

The study shows that the volatiles synthesized by some species of wood-rotting basidiomycetes can induce noticeable changes in the morphology of *F. solani* colonies.

From the 53 species of basidiomycetes that were tested, only 18% of them synthesize volatiles that didn't influence the morphology of *F. solani*. Moreover, we noticed that the volatile metabolites of species such as: *A. mesenterica*, *G. applanatum*, *P. squamosus*, *S. subtomentosum* and *T. versicolor* increased the production of pigments, resulting in an intense pink coloration of the phytopahtogen's colony, while the volatiles of *C. leave*, *P. candolleana*, *I. hispidus*, *P. betulinus* and *L. arcularius* decreased the pigment synthesis, the phytopathogen's mycelium being white.

The volatiles produced by wood-rotting basidiomycetes also influence the growth rate of *F. solani*. Some of the highest inhibition percentages were recorded for species such as: *N. alveolaris*, *L. betulinus* and *M. mucida*, while the lowest inhibition percentages were calculated for *P. candolleana*, *B. adusta*, *C. gallica*, *S. alutacea* and *T. hirsuta*.

Also, when cultivated on different media, the inhibition percentages for *N. alveolaris*, *L. betulinus* and *M. mucida* varied, the antifungal effect of the volatiles being higher on the richer media, fact that underlines the importance of media composition of the synthesis of volatile metabolites. From all species, on all media, *N. alveolaris* had the highest inhibition percentages against *F. solani*.

Following the GC-MS analysis of *N. alveolaris* extracts we highlighted the major volatile metabolites synthesized by this species which belong mainly to the alcohols and ketones classes. By our knowledge this is the first study that focuses on the influence of volatile metabolites synthesized by *N. alveolaris* on *F. solani*.

The results encourage further research focused on determining the best media composition in order to increase the production of volatile metabolites, on identification of other bioactive molecules and testing the antifungal potential of these compounds against other phytopathogenic species.

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CLIMATIC PREDICTORS INFLUENCES VFWD FUNGAL DIVERSITY THROUGH DOMINANT TREE' ECOLOGY IN BEECH FORESTS IN THE NORTH-EASTERN ROMANIA

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Abstract: Lignicolous fungi plays are a vital part of forest ecosystems in Europe. They are involved in Carbon cycle, through decay processes of woody debris. Very fine woody debris (VFWD) forms an important component of this dead wood, being found in any forest in the World. Among European and Romanian forests, *Fagus* spp. dominating forests are the most important broadleaved ecosystems, of great biotic and abiotic complexity. The present distribution of lignicolous fungi is mainly linked to trees distribution. In the context of climate change, European beech forests will also shift in distribution, structure and composition, triggering changes in lignicolous fungal communities and diversity as well. Considering this background, VFWD lignicolous diversity might be a future beech forests climate change indicator. This will bring the necessity of assessing the main climatic factors that are influencing the lignicolous fungal diversity distribution across European beech forests in Romanian's North-East Region. In the present study, our findings confirms the fact that macroclimate have a great influence on lignicolous mycodiversity in beech forests. It seems that minimum temperature and Gams Continentality Index explains approximately 48% of the mycodiversity variation. While dropping minimum temperatures and increasing Gams CI values, the lignicolous fungal richness will rise. While minimum temperature of January might be linked to a complex ecological and phonological framework, Gams CI is a known ecological indicator for optimum habitat of beech forests, which in turn influence lignicolous diversity distribution. Those climatic variables might characterize the relation between plants-fungi-climate in the near future, as increasing atmospheric temperatures will manifest at different scales. Thereafter, VFWD mycodiversity might function as a valuable macroclimatic changing indicator.

Keywords: European beech, beech forests, climatic predictors, VFWD, lignicolous fungi, mycodiversity, BIO6, Gams Continentality Index.

Introduction

In the past millennia, humans have transformed the landscape, converting forests to agricultural land [FYFE & al. 2015] or changing the structure and composition of forests [ABREGO & SALCEDO, 2011]. The silvicultural practices of European forests have reduced the forest's biodiversity, including lignicolous mycodiversity through wood extraction [PREIKŠA & al. 2015] and lowering tree diversity [NGUYEN, 2015]. Forests have the ability of carbon-sequestration [WELLBROCK & al. 2017], and releasing it through deforestation leads to increasing atmospheric carbon [LISKI & al. 2000], with its known complex and negative effects, like global warming and precipitation changing [OLIVEIRA & al. 2016].

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Woody debris are an important component for forest ecosystems [HEILMANN-CLAUSEN & CHRISTENSEN, 2004], as they provide developing space and nutritional resources for a variety of lifeforms, including fungi [UNAR & al. 2017]. Many studies have pointed out the importance of large woody debris [BÎRSAN & al. 2014; ZHOU & DAI, 2012], with emphasis on the log category [HEILMANN-CLAUSEN & CHRISTENSEN, 2004; HEILMANN-CLAUSEN & CHRISTENSEN, 2005], which was shown to be an indicator to forest biodiversity [UNAR & al. 2017]. Less researches have pointed out the importance of fine woody debris to the saproxylic biodiversity of forests, and especially lignicolous fungal diversity [ABREGO & SALCEDO, 2011; JUUTILAINEN & al. 2014; KRUYS & JONSSON, 1999]. Still, FWD and VFWD may be of great diversity value for basidiomycetes, particularly in managed forests [KÜFFER & SENN-IRLET, 2004].

Lignicolous fungi are the only eukaryotic living being that are able to decay wood, through lignin degradation – the nature's most recalcitrant organic compound [KNEŽEVIĆ & al. 2013], and also the second most abundant carbon source on Earth [PALIWAL & al. 2015], and consequently introduce the nutrients back to the forest ecosystems [LONSDALE & al. 2008; BALDRIAN & al. 2016]. Besides, through the decay process carbon is released into the atmosphere [YANG & al. 2016]. Considering this, lignicolous fungi are the main trophic group among fungi in forest ecosystems [DVOŘÁK & al. 2017].

European beech is the most abundant tree species in Temperate Europe [KÜFFER & al. 2004], and especially in Central Europe [GEßER & al. 2007], and one of the most important tree species in Romania [MILESCU & al. 1967]. In Romania beech occupy large surfaces, forming pure stands or living besides coniferous or *Quercus* trees in their respective communities [MILESCU & al. 1967], in forests that occupy approximately 31% of Romania's forest territory [CÂMPU & DUMITRACHE, 2015]. In the North-East Romania, European beech is well represented, with a high presence probability in non-coniferous broadleaved forests [COPOȚ & al. 2016]. Pure beech or coniferous forests mixed with beech old-growth forests are of high value in representing European forest biodiversity [PETRITAN & al. 2012].

Even if Romania is considered a conservation hotspot for its large old-growth forests [MUNTEANU & al. 2016], which are known to host great lignicolous fungal diversity [DVOŘÁK & al. 2017], relative abundance of these forests have dropped in the past century [MUNTEANU & al. 2016]. While Romania is on 10 rank in Europe on forest ha / capita, the reforestation is gradually increasing forests surfaces [NIȚĂ, 2015].

Still, high logging rates in surrounding protected areas increase forest habitat loss and forest fragmentation [KNORN & al. 2012]. Also, harvest spikes have been observed after 2000 [MUNTEANU & al. 2016], which further increase the complex situation of Romania's forests, in terms of long-term mycodiversity conservation. As deforestation and forest disturbance are correlated with low lignicolous diversity in European broadleaved forests [BRAZEE & al. 2014], it is a sufficient trigger for assessing the status of fungal diversity.

Climate change might greatly affect European plant species distribution [THUILLER & al. 2005], including total replacement of some dominant tree species at regional scale, as in the case of European beech in Spain [PEÑUELAS & al. 2007]. In the Temperate Continental zone, forests are mainly influenced by precipitation patterns, which in the case of changing might trigger high level of water stress [LINDNER & al. 2010]. Recent decreased vigour has been observed in beech growth [AERTSEN & al. 2014], and it might be linked to precipitation pattern changing, as has been observed that both European

beech and oak are associated with negative growth depressions [SCHARNWEBER & al. 2011]. Also, recent findings [BOSELA & al. 2018] show that average beech growth declined across Europe, but this happened differently at regional scales. While in Romania, the summer drought did not influenced beech radial growth, in relatively closed regions – Balkans, it did [BOSELA & al. 2018].

In the near future, predicted intensive drought periods might continue influence beech negative growth [GEßER & al. 2007] or increase tree mortality, as it is a general trend [ANDEREGG & al. 2016], which can change beech forests structure and composition [RUIZ-BENITO & al. 2013], down to the fungal composition, as fungi are highly linked to forest composition [KUTSEGI & al. 2015], but also climatic characteristics, as annual temperature and precipitations [TEDERSOO & al. 2015]. Also, natural disturbances are linked to FWD increasing [FASTH & al. 2011] and CWD increasing [BÄSSLER & al. 2016], which contribute to woody debris profile changes, and lignicolous composition [BÄSSLER & al. 2016].

Therefore, our objective it is to find the climatic predictoris of lignicolous fungal diversity in European beech forests across North-East Romania.

Material and methods

The North-East Region is located in the north-eastern part of Romania, occupying the Romanian part of historical province of Moldavia. It is the most forests region of all, with approximately 600,000 ha [ANDRONACHE & al. 2017]. The climate is temperate continental, but inside the region, the climatic variability is high, as multiple general and local factors interacts, determining variations both of temperatures and precipitation distribution [ANM, 2008]. In general, precipitations are rising from February to July, followed by a decrease up to January [ANM, 2008].

At regional scale, precipitations are growing from southern part of the region to northern and from eastern to western part [ANM, 2008]. Carpathians presence is having a heavy effect on atmospheric mass movements, the biggest precipitations being found here [ANM, 2008]. Annual temperature varies temporal (determined by solar radiation variation) and spatially (determined by multiple factors, at different scales, e.g., elevation). The region is divided geo-morphologically in three major areas: the extra-Carpathians, the Carpathians and the transition one [MĂRGĂRINT, 2017]. For the hilly and mountain areas, annual temperature varies intra-regionally, according to altitude changing [ANM, 2008]. The mean annual temperature ranges from 0-4 °C to 10-11 °C, while mean annual precipitations in the range of approximately 1000-1200 to 500-600 mm [ANM, 2008].

A total of 25 circular plots of 1000 m^2 were randomly selected in North-Eastern Region, in broadleaf forests dominated by beech. Within each sample plot, all standing alive trees having a diameter at breast height (dbh) $\geq$ 10 cm were measured for dbh and genera was determined. A variable of beech dominance was computed the percentage of total basal area of all beech trees from the plot's total beech basal area. Afterwards, a filter was applied, and only the plots having at least 50% of beech basal area were keep, in total 23 plots (Figure 1).

The climatic variables used are representing the macroclimatic variability of beech forests in North-Eastern Romania (Table 1). All climatic variables were extracted from rasters downloaded from the cited databases, using QGIS software [Quantum GIS Development Team , version 2.18, 2017].

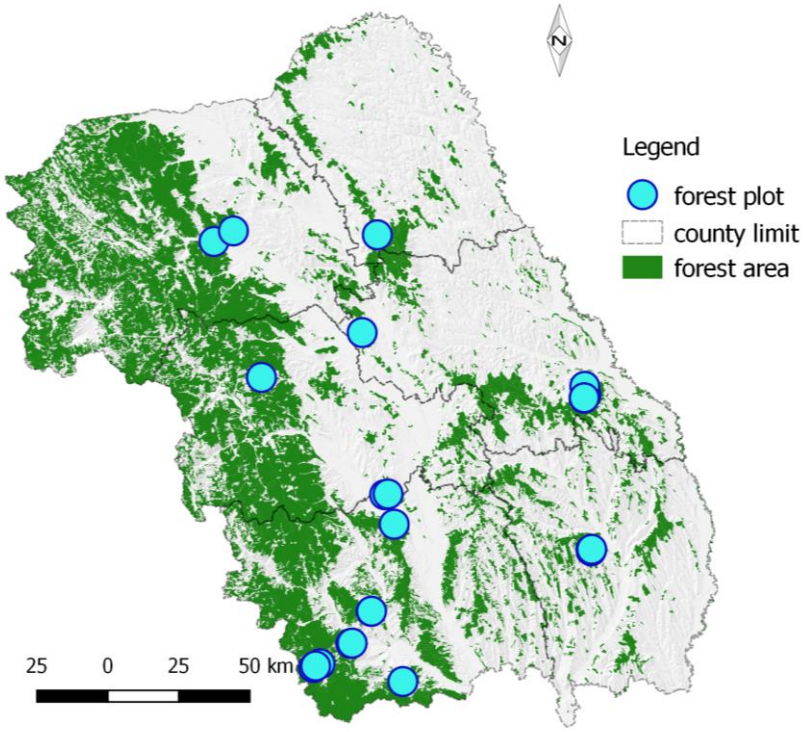

Figure 1. Plot distribution in beech forest ecosystems in North-East Romania (made in QGIS; the green forest area was obtained after ICAS Forest Type Map, 1997)

For climatic variables computing, WorldClim version 2.0 database was used at its finest resolution – 30 arcsec, equivalent to ~ 1 km [FICK & HIJMANS, 2017]. Bioclim variables and wind speed were used as *per se*, while other bioclimatic indices (e.g., water balance indexes) were derived from monthly average values for temperature and precipitations.

De Martonne aridity index (annual, seasonal, and monthly), Lang aridity index, and Koppen aridity index were selected among water balance indexes [SÁBITZ & al. 2014].

De Martonne aridity index was calculated via formula [QUAN & al. 2013]:

AI_{DM} = MAP / (MAT + 10), where MAP is the annual amount of precipitations (mm), and MAT the mean annual air temperature (°C). For seasonal periods, was used formula [HRNJAC & al. 2014]:

AIs_{DM} = 4*Ps / (Ts + 10), where Ps is the seasonal mount of precipitations (mm), and Ts the mean seasonal temperature (°C). As the values decrease, the aridity grows [CROITORU & al. 2012]. For monthly periods (e.g., January and July), was used formula [HRNJAC & al. 2014]:

AIm_{DM} = 12*Pm / (Tm + 10), where Ps is the monthly mount of precipitations (mm), and Ts the mean monthly temperature (°C).

Lang aridity index or *the rain factor of Lang* was calculated as the ratio between annual precipitations and mean annual temperature [QUAN & al. 2013]:

AI_{Lang} = MAP / MAT, where MAP is the mean annual precipitation (mm), and MAT is the mean annual temperature (°C).

Koppen aridity index was calculated following formula [QUAN & al. 2013]:

AI_{Koppen} = MAP / (MAT + 33), where MAP is the mean annual precipitation (mm), and MAT is the mean annual temperature (°C).

Potential Evapotranspiration (PET) was calculated as the sum of PET of each month, following formula [KARUNARATHNE & al. 2016]:

$PET = PET_1 + \ldots + PET_{12}$, where $PET_1 - PET_{12}$ is the monthly potential evapotranspiration, which were extracted from rasters downloaded from [ZOMER & al. 2007; ZOMER & al. 2008], at 30 arc sec. From the same source** values of Global Aridity Index (GAI) were extracted.

For climatic-tree growth, *Forestry aridity index* was calculated [FÜHRER & al. 2011]:

$FAI = 100 \times T_{7-8} / (P_{5-7} + P_{7-8})$, where T_{7-8} is the average temperature of July and August, P_{5-7} is the total precipitation (mm) in May, June, and July, and P_{7-8} is the total precipitation of July and August. For beech, the optimum values are < 4.75 [FÜHRER & al. 2011].

Another beech-related index, introduced by Ellenberg is the *Ellenberg Quotient* index is calculated according to formula [VLĂDUȚ & al. 2017]:

$EQ = 1000 \times (Tw / MAP)$, where Tw is the temperature of the warmest month of the year, and MAP is the mean annual precipitations. Values bellow 20 indicate pure beech forests, between 20 and 30, favourable to beech, but excellent to oak-hornbeam woodlands, and higher than 30, mesic oak forests to dry oak forests [VLĂDUȚ & al. 2017; SALAMON-ALBERT & al. 2016].

Finally, *Gams Continentality Index* (GCI) was calculated following formula [VLĂDUȚ & al. 2017]:

GCI = MAP / Alt, where MAP is the mean annual precipitation (mm), and Alt is the altitude (m). If values are between 1-2 then beech is favoured [SATMARI, 2010].

Snow-related variables (Table 1) values were extracted from rasters downloaded from Lifewatch-WB ecotope database [http://www.lifewatch.be/en/data], at 500 m resolution. As snow cover influences soil moisture [POTOPOVÁ & al. 2015], it might inference with VFWD decay and mycodiversity associated.

Table 1. The potential climatic variables influencing the fungal diversity.

Climatic variable	Description	Unit
BIO1	annual mean temperature	ºC
BIO2	annual mean diurnal range	ºC
BIO3	isothermality	-
BIO4	temperature seasonallity	%
BIO5	maximum temperature of warmest month	ºC
BIO6	minimum temperature of coldest month	ºC
BIO7	annual temperature range	ºC
BIO8	mean temperature of wettest quarter	ºC

BIO9	mean temperature of driest quarter	ºC
BIO10	mean temperature of warmest quarter	ºC
BIO11	mean temperature of coldest quarter	ºC
BIO12	annual precipitation	mm
BIO13	precipitation of wettest month	mm
BIO14	precipitation of driest month	mm
BIO15	precipitation seasonality	%
BIO16	precipitation of wettest quarter	mm
BIO17	precipitation of driest quarter	mm
BIO18	precipitation of warmest quarter	mm
BIO19	precipitation of coldest quarter	mm
wind	wind speed	m/s
DMAI	De Martonne aridity index (annual)	mm/ºC
DMAIspr	De Martonne aridity index (spring)	mm/ºC
DMAIsum	De Martonne aridity index (summer)	mm/ºC
DMAIaut	De Martonne aridity index (autumn)	mm/ºC
DMAIwin	De Martonne aridity index (winter)	mm/ºC
DMAIjan	De Martonne aridity index (January)	mm/ºC
DMAIjul	De Martonne aridity index (July)	mm/ºC
LAI	Lang aridity index (annual)	mm/ºC
KAI	Koppen aridity index (annual)	mm/ºC
FAI	Forestry aridity index (annual)	ºC/mm
EQ	Ellenberg Quotient	ºC/mm
GCI	Gams Continentality Index	mm/m
PET	Potential Evapotranspiration (annual)	mm
GAI	Global Aridity Index (annual)	-
SL	snow length / average snow duration	weeks
SS	snow start	weeks
SE	snow end	weeks

Fungal data was representing by collected fruit bodies on VFWD (Very Fine Woody Debris), defined as the woody debris with a diameter at large head between 1 and 5 cm. The lignicolous fungal species were detected at species or genera level, using literature [SĂLĂGEANU & SĂLĂGEANU, 1985; BREITENBACH & KRÄNZLIN, 1986; RYVARDEN, 1991; GERHARDT, 1999; BERNICCHIA, 2005; TĂNASE & al. 2009; COURTECUISSE & DUHEM, 2013]. The fungal nomenclature used was based on Index Fungorum [http://www.indexfungorum.org/Names/Names.asp].

The full variables set was tested for collinearity, using Spearman correlation |r| > 0.7. The 8 uncorrelated variables and the mycodiversity data were used to select the best models according to BURNHAM & ANDERSON (2002). This method uses the Akaike's information criterion (the delta AICc < 2), to find the best variables combination that explains the mycodiversity variation [BURNHAM & ANDERSON, 2002]. For the delineation of the best model we choose the adjusted R^2 with the largest value. For the final form of the model, we used the polynomial form of variable – diversity relation.

For statistical analyses, we used R version 3.4.0 software [R Development Core Team, 2005] with the packages *base*, *data.table*, *MuMIn*, *reshape*, *rsq*. Predictor's relations with mycodiversity were visualized using 2'nd degree polynomials plotting with PAST software [HAMMER & al. 2001].

Results and discussions

We recorded a total of 110 fungal taxons (in ca. 450 records). The species belongs to 84 genera, 46 families, 29 orders and 2 phyla. The taxa was distributed uneven, with Basidiomycota phylum dominating (66.4%), while Ascomycota taxons were lesser (33.6%). The most rich families (Figure 2) were Polyporaceae (10 taxons), Xylariaceae (7 taxons), Mycenaceae (7 taxons), Diatrypaceae (6 taxons), Hypoxylaceae (6 taxons), and Inocybaceae (6 taxons).

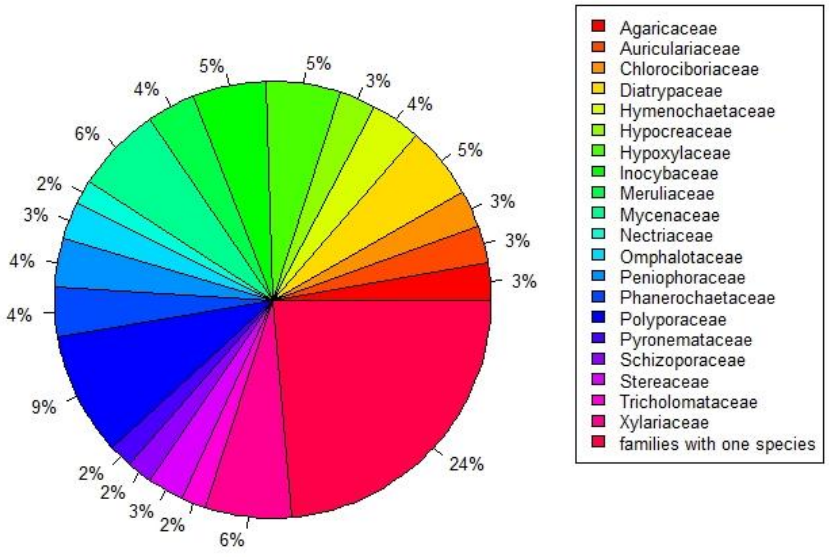

Figure 2. Families richness of lignicolous fungi found on VFWD in beech-dominated forests

The fungal lignicolous taxons varied from 7 to 25, with the mean of about 15 species. The most found species were: *Diatrype stigma* (82%), *Stereum hirsutum* (73%), *Biscogniauxia nummularia* (65%), *Schizophyllum commune* (60%), *Hypoxylon fragiforme* (60%), *Diatrype disciformis* (48%), and *Cerioporus varius* (48%). Taxons found only once

are representing approximately 52% of the diversity. Some of these are ubiquitous in broadleaved forests (*S. commune, D. stigma, S. hirsutum*) [TĂNASE & al. 2009].

Other (*B. nummularia, H. fragiforme, C. varius*) are specific to beech forests, as beech wood colonizers, in Hungary [KUTSEGI & al. 2015] or Romania [TĂNASE & al. 2009]. In Spain [ABREGO & SALCEDO, 2011], *D. disciformis, H. fragiforme, B. nummularia*, and *S. hirsutum* were also found frequently on beech wood. On beech VFWD, the fungal diversity represented 75% and had a 0.94 Spearman correlation with total diversity.

Through Burnham & Anderson analysis, we obtained 2 models that best explains the lignicolous fungal diversity (Table 3). The variables found were: BIO6 (minimum temperature of the coldest month) and GCI (Gams Continentality Index). According to the highest adjusted R^2, the best model was formed from BIO6, GCI and BIO12 (mean annual precipitations), together explaining 50.5% of lignicolous fungal diversity variability on VFWD in European beech dominated forests in North-Eastern Romania.

Table 2. Characteristics of final variables used in lignicolous mycodiversity.

Climatic variable	Importance	p-value <0.05	adj-R^2	Min	Max	Mean	Sd
BIO6	**0.769**	**0.001**	**0.39**	**-8.40**	**-6.10**	**-7.33**	**0.45**
GCI	**0.676**	**0.02**	**0.22**	**0.83**	**1.98**	**1.35**	**0.05**
BIO12	0.379	0.14	0.05	531	605	561.4	23.2
BIO11	0.319	0.07	0.14	-2.42	-0.70	-1.53	0.33
BIO2	0.307	0.35	0.04	8.09	9.08	8.46	0.31
DMAIwin	0.233	0.06	0.11	30.9	45.3	36.4	4.50
wind	0.199	0.40	0.03	2.2	3.2	2.7	0.28
SL	0.191	0.32	0.04	3	18	~11	4.69

GCI and BIO6 were statistic significant, while BIO12 was not found significant (**Table 2**). Thus, the final model was composed only from the two variables, which were also determined as the most important predictors for lignicolous fungal richness on VFWD in European beech forests, while explaining 48% of variation. Adding BIO12 (mean annual precipitations), the explaining power riches approximately 50%, thus having a little influence to interpret mycodiversity variation.

Table 3. Best explaining models of macroclimatic influence on lignicolous fungal diversity in beech-dominated forests.

Intercept	BIO11	BIO12	BIO2	BIO6	GCI	DMAIwin	SL	wind	R^2	AICc	delta	weight
-1.939	-	-	-	-0.55	0.365	-	-	-	0.70	136.07	0.0	0.68
-3.228	-	0.0029	-	-0.50	0.398	-	-	-	0.72	137.59	1.5	0.31

In Romania, recent spatial modeling [COPOȚ & TĂNASE, 2017] of another broadleaved forests-specific lignicolous fungal species – *Ganoderma lucidum*, found that elevation was the most important abiotic factor influencing its distribution in the same area.

Elevation is a proxy for temperature and precipitations [VAN GILS & al. 2012]. Particular bioclimatic variables – including mean average temperature (BIO1), mean precipitations (BIO11), and minimum temperature (BIO6) – were correlated with elevation, in North-Eastern Region [COPOȚ & TĂNASE, 2017]. It is clear that temperature influences the distribution of some lignicolous fungi in temperate broadleaved forests.

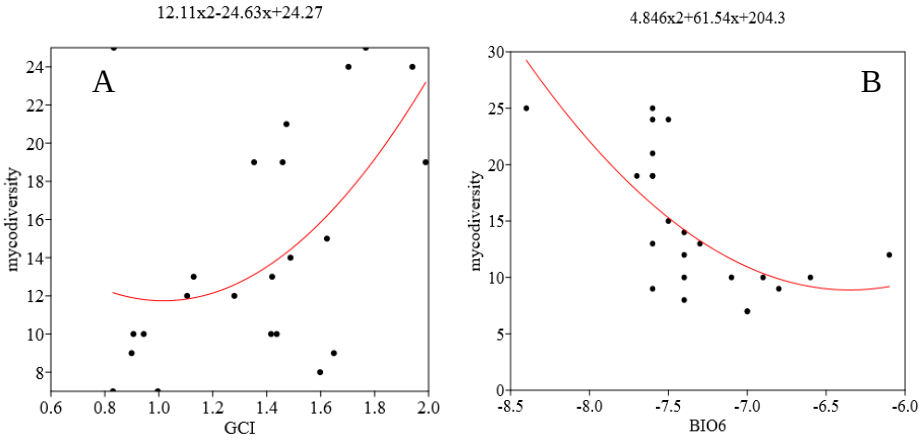

Figure 2. Graphic representation of the relations between fungal lignicolous diversity and: (A) Gams Continentality Index (GCI); (B) Minimum temperature of the coldest month (BIO6).

We found high lignicolous fungal diversity in plots where the Gams Continentality Index (GCI) was high. From Figure 2 (A), it can be seen that, as the GCI grows so is the mycodiversity. From the GCI interpretation, values bellow 1 shows stands where coniferous trees are favored, values between 1 and 2, are favorable to beech, and above 2, where thermophillous trees grows [SATMARI, 2010].

Approximately 74% of the plots were in a habitat favorable to beech. Consequently, starting from mixt beech-coniferous forests to beech optimum, the mycodiversity is growing. Optimal beech stands are associated with thick litter [MILESCU & al. 1967]. But high tree litter layer is associated with thermic amplitude decrease and reduced number of frost days [LOYDI & al. 2014]. Also, it keeps humidity on larger time periods [MILESCU & al. 1967]. Soil and litter high moisture have been linked to fungal mycelial spreading [KAŁUCKA, 1995; KUBARTOVÁ & al. 2009]. The thick-litter conditions creates a more stable microclimate, which will not permit to specialized pioneer fungi to engrossment the substrate for themselves. This assumption is confirmed by the well-known situation of above-ground VFWD, which have an instable microclimate, and therefore, particular fungal lignicolous species, with xeric adaptations to colonize the wood [HEILMANN-CLAUSEN & CHRISTENSEN, 2003].

Still, because many VFWD pieces are integrated in the litter, in the advanced stages of decay [OSTROGOVIĆ & al. 2015], a higher litter thickness will increase the chances of wood hindering. This process is influenced by multiple factors, as it is a multiannual process – beech FWD being found to full decay in approximately 18 years in Germany [MÜLLER-USING & BARTSCH, 2009]. Unlike above-VFWD, under- or partially buried VFWD will have a greater moisture, as the larger soil-contact is associated

with higher moisture [HEILMANN-CLAUSEN & CHRISTENSEN, 2003]. Another aspect shows that a higher surface-to-volume ratio – which characterize the VFWD [HEILMANN-CLAUSEN & CHRISTENSEN, 2004] – is increasing even furthermore the wooden surface-to-litter surface ratio. This means that litter-integrating VFWD will likely offer preferential conditions for fungal colonizing from a 3D-spatial context.

A question might be asked here: If the VFWD turnover is fast, than why the lignicolous diversity is still higher? The answer resides in the capacity of a humid and wood-rich litter to offer development space for grazing mycelium. Saprotrophic fungi are known for their capability to develop a net-like mycelium that will forage the substrate for water and nutrients [WEBSTER & WEBBER, 2007].

Even if fungi produces strong extracellular enzyme in order to decay organic material, these enzymes lack the ability to diffuse at large distances [PETRE & al. 2014]. Thereafter, the fungus must expand into the newly found wood substrate [PETRE & al. 2014], and thus, if the conditions permit, to develop reproductive mycelia. In turn, this will increase the chances of spore colonizing in the area, with increasing mycodiversity.

Observing the trend of the GCI-diversity relation (Figure 2), even if the GCI values are constantly growing, the mycodiversity will do the same. This triggers another question, whether the fungi are developing well in other beech forests, which have thermophilic tree species in them, like oaks and hornbeam.

In this case, some species will change the wood genera host, while others will be replaced by newly tree genera. High-GCI stands have genera (e.g., *Tilia*, *Acer*, *Quercus*) with numerous known associated lignicolous fungal species [GERHARDT, 1999; RYVARDEN, 1978; SĂLĂGEANU & SĂLĂGEANU, 1985; TĂNASE & al. 2009] that grows furthermore the beech forest's diversity.

While the first factor was characterizing the water balance of beech ecosystems, the second one is a measure of temperatures. European beech is sensible to low temperatures, both saplings and spring buds [BOLTE & al. 2007; MILESCU & al. 1967]. According to authors [BOLTE & al. 2007], the following values are necessary to fulfill the minimum winter temperatures requirements: January mean temperature above -3ºC, at most 148 days with daily mean temperature less than 7ºC, no severe winter frosts (<-35ºC) or severe late frosts.

Among this beech distribution factors, BIO6 (minimum temperature of the coldest month) which in this case is January, have been proved to significantly negative influence the lignicolous fungal diversity in *Fagus* dominated forests.

In the context of high trunk water content and winter temperature very suddenly dropping, frost cracks appears on beech trunk [CÂMPU & DUMITRACHE, 2015]. While the shallow cracks heal from year to year, the deep ones will not (every year, the cracks are re-opening), which will maintain a "proper" access to saproparasitic fungi [CÂMPU & DUMITRACHE, 2015]. Considering that beech have little resistance to strong winds [MILESCU & al. 1967], the frost cracks weakens even more the beech's standing. In time, the tree will dry and die, enriching constantly the downed woody debris pool. A higher dead wood on the soil will likely be correlated with higher lignicolous mycodiversity, as is proven that wood volume is well related with species richness [HEILMANN-CLAUSEN & CHRISTENSEN, 2004; KLOCKOW & al. 2014; LASSAUCE & al. 2011].

Another aspect consists in the beech saplings' sensitive to winter frosts, approximately 50% of beech saplings being killed by temperatures between -17 and -21 ºC [BOLTE & al. 2007]. Following this, in the spring, other tree species might take the

initiative and properly install in beech forests, where the canopy gap is large enough. In time, this will increase stand tree diversity, which have been linked to macrofungal richness [BUÉE & al. 2011].

In harsh-winters areas (delineated here by BIO6 low values), any fallen branch will enlarge the VFWD and FWD pool on the ground, without the possibility for lignicolous fungi to decay the wood, as low temperatures are known to hinder wood decay [KUBARTOVÁ & al. 2009]. On the contrary, mild-winters areas (BIO6 high values), the decay process is less discontinued during January, which continues wood decay. In the spring, higher temperatures and humidity might start a fast wood fungal colonization, as higher humidity and rising temperatures are associated with faster decay rates [FRAVOLINI & al. 2018].

Living wood has a higher concentration of nutrients in the winter – as a resource for spring sprouting –, and VFWD a higher nutrient concentrations than CWD [KLOCKOW & al. 2014]. Therefore, in harsh-winter stands, any fallen wood, will enlarge the resource availability for new and multiple colonizers, in the spring, more than areas with mild-winters. Starting the spring with an already installed community, the fungal richness might be lower than in the mild-winter stands, which in contrast, has an explosion of diversity. Consequently, because of good resources available, in the following spring and summer, newly installed fungal colonizers will be more diverse, increasing the overall VFWD mycodiversity.

Finally, beech is also sensitive to late frosts, which heavily affect seedlings and buds [MILESCU & al. 1967]. Afterwards, the trees are able to come back and form a new canopy, but at the expensive of fructification in that respective year [MILESCU & al. 1967]. This in turn, leaves the stand without a beech seedling year, which makes room for other's tree species' seedlings. The effects is the same as previous mentioned, with increasing tree diversity in time.

This hypothesis is sustained by the phonological temperament of beech, which starts sprouting earlier than other trees, especially on the lower part of slopes [MILESCU & al. 1967]. Usually, the lowest values of minimum January temperature are found in depressionary areas, valley corridors and high elevations, the North-Eastern part being associated with the a high number of days with frost [ANM, 2008].

Also, in mild-winter stands, beech starts sprouting and forming a canopy earlier, as the growing season has a larger number of days. The newly created shade will maintain humidity at soil level, but in the harsh-winter stands, the fact that canopy-derived shading is delaying might offer greater access to solar radiation, and warm up the litter and VFWD. Still, temperature droppings can occur in the early spring. This situation might trigger the cold-shock effect in lignicolous fungi, as some species initiated fruiting [PINNA & al. 2010]. Also, species from one group in particular – Ascomycota – are growing in the late-winter and spring [RUDOLPH & al. 2018]. On short-term, mycodiversity will increase, based on ascomycetes, which, according to a recent study can form up to 51% of total diversity [RUDOLPH & al. 2018].

Increasing temperatures will permit not only the colonization of new wood via spore germination, but also mycelial growth from one piece to another. The fact that lignin represent approximately 20% of plant litter in forests [PALIWAL & al. 2015], and VFWD density is higher than logs, that means that there is a greater chance for spreading mycelium to find a new substrate and colonize it, at least for the lignicolous fungi that prefer this type of colonizing strategy.

Conclusions

In conclusion, the lignicolous fungal diversity is influenced by macroclimatic characteristics. The mycodiversity is rising with minimum temperature dropping and with aridity index rising. The availability of good thermo-humid conditions is crucial for high lignicolous fungi diversity in European beech forests from North-Eastern Romania. This study shows that macroclimate is of great value in determining lignicolous fungal richness at regional scale. Also, the mycodiversity found in beech-dominated forests, are closely linked to the relation between the main host tree species and its ecological behavior. Therefore, macroclimatic characteristics must be included in diversity studies, even on very fine woody debris, which are often rejected from mycodiversity studies, even if this wood type represents the most ubiquitous downy wood component in forest ecosystems across the world.

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NEW RECORDS IN THE ALIEN FLORA OF ROMANIA: *EUPHORBIA SERPENS* AND *E. GLYPTOSPERMA*

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Abstract: Our recent field research and revision of some herbarium specimens led us to identify two species of *Euphorbia* (subgenus *Chamaesyce*), which we report now for the first time in the alien flora of Romania: *Euphorbia serpens* Kunth and *E. glyptosperma* Engelm. The first was collected in the city of Iași, north-eastern Romania, in September 2018. The second was collected, during 2005-2015, in several localities from the lower basin of the Siret river (Galați County), as well as from north-eastern Romania, near Ciurea (Iaşi County), but previously erroneously identified as "*Euphorbia chamaesyce* L.". Both species, originating in the New World, are xenophytes, more or less naturalized in Europe, perhaps in full process of expansion of their secondary area.

Keywords: alien plants, identification key, subgenus *Chamaesyce*, vascular flora.

Introduction

Euphorbia L. (Sp. Pl. 1: 450. 1753) is one of the most species-rich genus of flowering plants, with about 2,000 species distributed in all tropical or temperate regions of the world [PAHLEVANI & RIINA, 2011; BERRY & al. 2016].

The species of *Euphorbia* we further refer in the paper belong to the subgenus *Chamaesyce* Raf., section *Anisophyllum* Roeper. This clade of *Euphorbia* includes 300-350 species, most of them originating in the New World [YANG & BERRY, 2011; BERRY & al. 2016], that are remarkably distinct within the genus, by their prostrate herbaceous stems with sympodial branching, opposite leaves with asymmetric base and interpetiolar stipules, cyathia axillary, solitary or in small clusters or glomerules (not in pseudoumbels), without obvious bracts; cyathial glands 4, usually with petaloid appendages, seeds without caruncle, and the predominance of C_4 photosynthesis [SMITH & TUTIN, 1968; BENEDÍ & ORELL, 1992; JINSHUANG & GILBERT, 2008; PAHLEVANI & RIINA, 2011; YANG & BERRY, 2011; BERRY & al. 2016].

A number of 6 species of subgen. *Chamaesyce*, sect. *Anisophyllum* have been reported in the flora of Romania, so far [PRODAN, 1953; OPREA, 2005; CIOCÂRLAN, 2009; SÂRBU & al. 2013]. Two of them (*Euphorbia peplis* L. and *E. chamaesyce* L.) are native in the the Mediterranean region, probably occuring here at the limit of their native area; another one (*E. humifusa* Willd.) is a casual neophyte originating in the East and Central Asia; the other three species (*E. maculata* L., *E. nutans* Lag. and *E. prostrata* Aiton) are naturalized neophytes originating in North America, and among them at least *E. maculata* can be considered invasive [SÎRBU & OPREA, 2011].

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Our recent field research and revision of some herbarium specimens led us to identify two other neophytes species of *Euphorbia* (subgen. *Chamaesyce*, sect. *Anisophyllum*), which we report here for the first time in the flora of Romania.

Material and methods

Species were identified as a result of our field works (2005-2018), in north-eastern and eastern Romania and by checking of some herbarium specimens collected by the first author during 2005-2015. The geographic coordinates were recorded on the field using an eTrex Legend HCx GPS system. Voucher specimens were deposited in the Herbarium of the University of Agricultural Sciences and Veterinary Medicine Iaşi (IASI). For species identification we used various keys and descriptions published by SMITH & TUTIN (1968), HÜGIN (1998), JINSHUANG & GILBERT (2008), PAHLEVANI & RIINA (2011), BERRY & al. (2016), RADCLIFFE-SMITH (2018) and many other references as indicate below for each species. The nomenclature of the plant taxa follows SMITH & TUTIN (1968) and BERRY & al. (2016).

Results and discussion

a. Species of *Euphorbia* reported for the first time in the flora of Romania

1) *Euphorbia serpens* Kunth in A. von Humboldt & al., Nov. Gen. Sp. 2(fol.): 41; 2(qto.): 52. 1817. (Syn.: *Chamaesyce serpens* (Kunth) Small, Fl. S.E. U.S. [Small]. 709, 1333. 1903)

Distinctive features. It is easily recognizable among other related species of subgenus mainly by the following combination of characters [THELLUNG, 1907; BENEDÍ & ORELL, 1992; BENEDÍ, 1997; JINSHUANG & GILBERT, 2008; PAHLEVANI & RIINA, 2011; SILVA & al. 2014; WOLF & KIRÁLY, 2014; BERRY & al. 2016; RADCLIFFE-SMITH, 2018]: plant entirely glabrous; stems rooting at nodes; leaf-blades entire, suborbicular-ovate, rounded to emarginated at apex, obliquely shallowly cordate at base, without reddish maculae on faces; stipules whitish, united into a triangular scale (subsp. *serpens*), or almost free, linear, in subsp. *fissistipula* (Thell.) Hügin (var. *fissistipula* Thell.); cyathia single, axillary; appendages wider than cyathial glands; seeds gray to brown, smooth.

Identification keys: THELLUNG (1907), BENEDÍ (1997), HERNDON (1993), HÜGIN & HÜGIN (1997), HÜGIN (1998), PAROLLY & EREN (2007), RÖTHLISBERGER (2007), JINSHUANG & GILBERT (2008), HAND (2011), PAHLEVANI & RIINA (2011), PAHLEVANI & AKHANI (2011), SILVA & al. (2014), WOLF & KIRÁLY (2014), BERRY & al. (2016).

Iconography: i) ***drawings*** - the whole plants, with various details [BENEDÍ, 1997; JINSHUANG & GILBERT, 2008; PAHLEVANI & RIINA, 2011; RADCLIFFE-SMITH, 2018], habitus, seeds and stipules [HÜGIN & HÜGIN, 1997; HÜGIN, 1998], leaf, nectaries and cyathial appendages [BENEDÍ & ORELL, 1992]; ii) ***photos*** - plants in their habitat [WOLF & KIRÁLY, 2014; SILVA & al. 2014; PETROVA, 2018], plant habitus [SILVA & al. 2014], branches with cyathia [SILVA & al. 2014; PETROVA, 2018], seeds [HÜGIN, 1998; PAHLEVANI & AKHANI, 2011; WOLF & KIRÁLY, 2014]; ii) ***microscope photos*** - stipules, hypogynous disc, nectaries and petaloid appendages, capsules

and seeds [BENEDÍ & ORELL, 1992], leaf anatomy [BENEDÍ & ORELL, 1992; NOBARINEZHAD & al. 2018], mitotic chromosomes and pollen grains [NOBARINEZHAD & al. 2018].

Origin and general distribution. Native to tropical and subtropical Americas, *E. serpens* was introduced in the Old World (Europe, Asia, Africa, Australia) [JINSHUANG & GILBERT, 2008; BERRY & al. 2016], being nowadays a widely naturalized weed in temperate regions [BENEDÍ, 1997].

The first evidence on the presence of this species in Europe dates back from the 19th century. *E. serpens* subsp. *fissistipula* was found first, in 1842, in southern France, near Montpellier [THELLUNG, 1907; HÜGIN, 1999], while the typical *E. serpens* (subsp. *serpens*) was observed only after about 5 decades, in 1890, in Frankfurt, Germany [HÜGIN, 1999].

Although the subsp. *fissistipula* was found earlier in Europe, subsequently it was reported as a casual neophyte only in western regions, e.g. in France [THELLUNG, 1907; HÜGIN, 1999], Denmark (since 1974) [HÜGIN, 1999] and Belgium (since 1992) [VERLOOVE, 2006b], while the typical *E. serpens* seems to be more and more widespread on the continent, and naturalized in many regions (especially in the Mediterranean countries). If towards the end of the last century, *E. serpens* subsp. *serpens* has been known only in the Western and Central Europe (the United Kingdom, the Iberian Peninsula, Germany, France, Austria) [CLEMENT & FOSTER, 1994; HÜGIN & HÜGIN, 1997; HÜGIN, 1998; HÜGIN, 1999; FISCHER & al. 2008, DOMINGUES DE ALMEIDA & FREITAS, 2006; HOHLA, 2013; WOLF & KIRÁLY, 2014], in the last two decades it has been reported south-eastward from more and more countries, including Switzerland (2004) [RÖTHLISBERGER, 2007], Hungary (2013) [WOLF & KIRÁLY, 2014], Italy [CELESTI-GRAPOW & al. 2010; LAZZERI, 2015], Greece and Crete [GREUTER & RAUS, 2007; ARIANOUTSOU & al. 2010], Cyprus [HAND, 2011], Turkey [PAROLLY & EREN, 2007] and Bulgaria (2016) [PETROVA, 2018].

Distribution in Romania. We recently identified *E. serpens* in Romania (Figure 1), first in the central area of the Iași city (N 47º09'25.23", E 27º35'25.08", 42 m a.s.l., leg. C. Sîrbu, 08.09.2018; N 47º09'24.73", E 27º35'08.23", 53 m a.s.l., leg. C. Sîrbu, 28.09.2018) and subsequently in Bucharest, on the Calea Plevnei Street, near the intersection with the Știrbei Vodă Street (N 44º26'13.92", E 26º04'43.68", 77 m a.s.l., leg. C. Sîrbu, 08.11.2018).

All the specimens observed till now in Romania belong to *E. serpens* subsp. *serpens*.

Figure 1. *Euphorbia serpens* subsp. *serpens*, as a weed of flower platbands in the central area of the Iași city

Biology and ecology. *Euphorbia serpens* is a diploid species (2n=22) [BENEDI & ORELL, 1992; BENEDÍ, 1997; NOBARINEZHAD & al. 2018]. It flourishes and fructifies throughout the year, in warmer areas, or in summer only, in temperate regions [BERRY & al. 2016]. Most of the plants observed by us not only in September (Iași), but also in November (in Iași and Bucharest), had numerous mature cyathia and fruits, which shows a quite long period of time in which this species can produce mature seeds in Romania. But among those there were also many young plants, which suggests that *E. serpens* may have in Romania more than one biological cycle in each growing season as it was reported for the Iberian Peninsula [BENEDI & ORELL, 1992] and Bulgaria [PETROVA, 2018].

As other C4 photosynthesis plant species, *E. serpens* is adapted to warm, arid habitats, disturbed by natural or anthropogenic factors [YANG & BERRY, 2011; WOLF & KIRÁLY, 2014]. In North America it commonly grows on sandy or well-drained soils, desert scrub, coastal scrub, chaparral, woodlands, sand dunes, prairies, disturbed areas etc. [BERRY & al. 2016]. It was reported as a weed in ornamental gardens, lawns and roadsides in Iran [PAHLEVANI & RIINA, 2011], also on sandy places along roads and coastal areas, in China [JINSHUANG & GILBERT, 2008].

In Europe, *E. serpens* was reported from various anthropogenic habitats such as: trampled sites often associated with roads (the Iberian Peninsula) (BENEDÍ & ORELL, 1992; ČARNI & MUCINA, 1998), flagstone alleys (Hungary), irrigated vegetable gardens (Greece) [GREUTER & RAUS, 2007], flowerpots [DIRAN, 2016], cemeteries, sidewalks [HÜGIN, 1999; DIRAN, 2016], plant containers, plastic tunnels for plants (Bulgaria)

[PETROVA, 2018], flower beds [HAND, 2011] etc. It commonly participates in the structure of anthropogenic thermophilous plant communities of the order *Eragrostietalia* J. Tx. ex Poli 1966 [ČARNI & MUCINA, 1998; WOLF & KIRÁLY, 2014].

In Romania, we also identified *E. serpens* in anthropogenic habitats. In the central area of the Iași city it grows as a weed of parterres and flower platbands and along alignments of shrubs and trees, from the park of a shopping center, near the "Culturii" Palace. It had there rich populations forming alone or together with other exotic weeds (e.g. *Euphorbia maculata, E. prostrata, Veronica peregrina, Oxalis corniculata* etc.) dense patches, stretched to 2-5 m^2. In Bucharest the plant grows in some containers with ornamental exotic trees.

Introduction way. According to literature, the long-distance dispersal of *E. serpens* is mainly correlated to human activity (trade and transportation of infested crop seeds or/and soils) [HÜGIN, 1999; PAHLEVANI & RIINA, 2011; WOLF & KIRÁLY, 2014] and cannot be interpreted as a result of climatic change [HÜGIN, 1999; WOLF & KIRÁLY, 2014].

In the green areas of the shopping center from the Iași city, many exotic ornamental plants have been recently introduced (the park was inaugurated in 2012). Therefore we suppose that *E. serpens* was accidentally introduced there by seeds which might have been present in the contaminated soil imported together with the ornamental plants. The accidental introduction through the contaminated soil together with container exotic plants is more obvious for the smaller population of *E. serpens* found in Bucharest.

Unfortunately, we have not been able to find out from where the ornamental plants and their growth substrate were brought here. Importation of *Euphorbia serpens* with container plants from the Mediterranean region to Belgium [HOSTE & al. 2009] and Switzerland [RÖTHLISBERGER, 2007] was reported recently. This could be the case for plants arrived in Romania, too. PETROVA (2018) also reported recently *E. serpens* in containers with ornamental plants, in Bulgaria.

2) *Euphorbia glyptosperma* Engelm. in W. H. Emory, Rep. U.S. Mex. Bound. 2(1): 187. 1859 (Syn.: *Chamaesyce glyptosperma* (Engelm.) Small, *Fl. S.E. U.S.* [Small]. 712, 1333. 1903)

Distinctive features. *E. glyptosperma* differs from the other related species from the flora of Romania by the combination of the following characters [VERLOOVE, 2006; SOMLYAY, 2009; BERRY & al. 2016; GELTMAN & MEDVEDEVA, 2017]: plant entirely glabrous; stems prostrate, never rooting at nodes, branches somewhat ascending when young; leaves serrulate, at least to the apex; stipules distinct, linear-subulate; cyathia solitary or in small, cymose clusters at distal nodes; seeds brownish, sharply angular in cross section, apiculate at apex, with 3-4 (-6) prominent transverse ridges.

Iconography: i) ***drawings*** - the whole plants, with various details [BERRY & al. 2016], habitus and seeds [HÜGIN & HÜGIN, 1997]; ii) ***photos*** - plants in their habitat [AYMERICH, 2016], seeds [HÜGIN, 1998; SOMLYAY, 2009; GELTMAN & MEDVEDEVA, 2017]; capsules and seeds [KIRÁLY & al. 2009].

Identification keys: HÜGIN & HÜGIN (1997), HÜGIN (1998), RÖTHLISBERGER (2007), FISCHER & al. (2008), KIRÁLY & al. (2009), BÁTORI & al. (2012), BERRY & al. (2016), GELTMAN & MEDVEDEVA (2017).

Origin and general distribution. It is native in North America, where is one of the most widespread species of the genus [BERRY & al. 2016], and introduced in Europe [ROUX, 1992; HÜGIN & STARLINGER, 1997; HÜGIN 1999] and Asia [GELTMAN & MEDVEDEVA, 2017].

In Europe, *E. glyptosperma* was found first in Sweden (1911) [GELTMAN & MEDVEDEVA, 2017], and the Netherlands (1916; 1920) [HÜGIN, 1998] and later in Hungary (in 1957) [SOMLYAY, 2009]. However, being erroneously identified with other related species (*E. serpens* Kunth, *E. rhytisperma* (Klotzsch et Garcke) Boiss., *E. serpyllifolia* Pers., or *E. humifusa* Willd.), it remained long time unknown on the continent.

Towards the end of the last century, as a result of field works and revisions of older herbarium specimens, *E. glyptosperma* was first reported from France [ROUX, 1992], Austria (first time collected in 1964), Italy, Switzerland and the former Yugoslavia [HÜGIN & STARLINGER, 1997; HÜGIN 1999].

In the recent decades the species has been indicated in Hungary, by SOMLYAY (2009) and KIRÁLY & al. (2009) (naturalized populations), as well as in Belgium (first time collected in 2003) [VERLOOVE, 2006], Spain (2015) [AYMERICH, 2016] and the south-eastern European Russia (1987) [GELTMAN & MEDVEDEVA, 2017].

Although it was sometimes reported only as a casual neophyte, e.g. Switzerland [RÖTHLISBERGER, 2007], the species is currently naturalized at least in some European countries such as: Hungary [SOMLYAY, 2009; KIRÁLY & al. 2009], Italy [CELESTI-GRAPOW & al. 2010], Spain [AYMERICH, 2016], and Russia [GELTMAN & MEDVEDEVA, 2017].

Distribution in Romania. We first collected this species (Figure 2) near the Ciurea village (Iași County), along the railway (N 47º06'14.88", E 27º34'07.43", 59 m a.s.l., leg. C. Sîrbu, 11.07.2005), and later on in the Galați city (railway station, N 45º27'06.34", E 28º03'27.25", 7 m a.s.l., leg. C. Sîrbu, 01.08.2009), as well as on the lower basin of the Siret river, in the villages of Movileni-Șendreni (railway station, N 45º24'11.34", E 27º57'52.31", 9 m a.s.l., leg. C. Sîrbu, 01.08.2011) and Șendrenii Vechi (alluvial sands on the left bank of the Siret river, N 45º25'05.99", E 27º527'53.98", 14 m a.s.l., leg. C. Sîrbu, 09.09.2015). All specimens collected in the field were originally erroneously identified as *Chamaesyce canescens* (L.) Prokh.) (i.e. *E. chamaesyce* L.).

Biology and ecology. *Euphorbia glyptosperma* is a diploid species (2n=22) [BERRY & al. 2016]. It flourishes and fructifies from early summer to autumn [BERRY & al. 2016]. Specimens collected from Romania in August - September had both flowers and mature fruits and seeds, while the ones collected in early July were in bloom, only.

Like the previous species, *E. glyptosperma* is a weed of disturbed warm and arid habitats, often on sandy soils. In the native area (North America) it usually grows on river banks, sand prairies, loess hill prairies, meadows, ballast, open disturbed areas, roadsides (in North America) [BERRY & al. 2016]. In Europe it was found on various habitats such as: potato fields, ruderal places on stony or sandy soils (Hungary) [SOMLYAY, 2009], sand pioneer grasslands (Hungary) [KIRÁLY & al. 2009], river banks, ruderal places, lawns, roads (Austria) [HÜGIN & STARLINGER, 1997; ESSL & RABITSCH, 2002], unloading quays (Belgium) [VERLOOVE, 2006], trampled areas, sidewalks, pavements, river gravels (Italy) [BANFI & GALASSO, 2010], cemeteries (Italy) [HÜGIN, 1999], ruderal places near ports and roads, but also in more or less natural habitats (Russia) [GELTMAN & MEDVEDEVA, 2017].

In Romania, this species has similar preferences for open habitats with sandy or stony soils, disturbed by anthropogenic (train stations, railroads) or natural factors (alluvial sands on the Siret river bank).

Introduction way. *Euphorbia glyptosperma* has been introduced into Europe most likely with seeds of cereals or other plants imported from North America [VERLOOVE, 2006; GELTMAN & MEDVEDEVA, 2017]. In Romania it is possible that its introduction have occurred via the fluvial port or the railway station of the Galați city, from where it further spread by road or railway transportation. But, its introduction has not occurred very recently, given that it was first found here more than a decade ago and it grows not only in anthropogenic habitats. but also in those associated with river banks.

Figure 2. *Euphorbia glyptosperma*, on the gravels of railway foundation, at the Movileni-Șendreni train station

b. Identification key

The two species of *Euphorbia* subgenus *Chamaesyce* reported here for the first time in the Romania's flora, along with the other six related species previously known (as shown in *Introduction*) are easily identifiable using the following dichotomous key (see also identification keys indicated above):

1a. Mature seeds smooth (fine reticulated or granulated at high magnification) **2**

1b. Mature seeds irregularly wrinkled, tuberculate-rugulose, or with 3-4 transverse ridges on each face **4**

2a. Plants somewhat fleshy. Capsule of 3-4.5 × 4-5 mm. Seeds of ca. 3 mm long.....
..... ***Euphorbia peplis*** L. [*Chamaesyce peplis* (L.) Prokh.]

2b. Plants not fleshy. Capsules up to 2 mm in diameter. Seeds up to 1.5 mm long **3**

3a. Plants glabrous or sparingly pubescent, with prostrate stems, never rooting at nodes. Leaves serrulate at least toward the top. Stipules distinct, linear-subulate. Appendages as large as glands..... ***E. humifusa*** Willd. [*Ch. humifusa* (Willd.) Prokh.]

3b. Plants glabrous, usually with repent stems (rooting at nodes) or prostrate (non-rooting) but with conspicuous primordia of adventitious roots at nodes. Leaves entire. Stipules whitish, united into a triangular scale (var. *serpens*) or almost free, linear (var. *fissistipula* Thell.). Appendages twice larger than glands .... ***E. serpens*** Kunth [*Ch. serpens* (Kunth) Small]

4a. Stems ascending to erect, up to 40-60 cm high. Longest leaf blades 20-40 mm long. Cyathia at ends of branches forming in lax, corymbiforme cymes, sometimes solitary. Seeds blackish ..... ***E. nutans*** Lag. [*Ch. nutans* (Lag.) Small]

4b. Stems prostrate, not so high. Longest leaf blades less than 18 mm. Cyathia solitary or in small axillary pseudoracemose clusters or dense glomerules (not corymbiforme cymes). Seeds greyish or at most brown **5**

5a. Seeds irregularly tuberculate-rugulose, ± rounded at base. Capsule glabrous to uniform patent-pubescent..... ***E. chamaesyce*** L. (*Ch. canescens* (L.) Prokh.)
- subsp. ***chamaesyce***: glabrous or pubescent; leaf blade with margin entire or crenate, ± thickened; appendages about the same size as the glands
- subsp. ***massiliensis*** (DC.) Thell. [*Ch. massiliensis* DC.]: villous; leaf blade with margin serrulate, not thickened; appendages twice larger than the glands

5b. Seeds with 3-4 transverse ridges on each face, ± truncated at base. Capsule glabrous or hairy, but never uniform patent-pubescent..... **6**

6a. Capsule crisped-villous only along keels and toward base (glabrous between keels). Stipules united, triangular-subulate..... ***E. prostrata*** Aiton [*Ch. prostrata* (Aiton) Small]

6b. Capsule either entirely glabrous or uniformly appressed hairy. Stipules distinct, linear-subulate **7**

7a. Stems and leaves sericeous or villous. Capsules uniformly appressed hairy (sericeous). Seeds rounded at apex, with 3–4 low, transverse ridges. Glands green to yellow-green, turning pink with age, usually ± unequal ..... ***E. maculata*** L. [*Ch. maculata* (L.) Small]

7b. Stems, leaves and capsules glabrous. Seeds apiculate at apex, with 3-4 (-6) prominent transverse ridges. Glands red to purple, usually ± equal .... ***E. glyptosperma*** [*Ch. glyptosperma* (Engelm.) Small]

Conclusions

Two species of *Euphorbia* L. (sugenus *Chamaesyce* Raf., section *Anisophyllum* Roeper) are reported here for the first time in the spontaneous flora of Romania: *Euphorbia serpens* Kunth and *E. glyptosperma* Engelm.

Both species, originating in the New World, are xenophytes, more or less naturalized in Europe, perhaps in full process of expansion of their secondary area.

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SALICETUM ALBAE ISSLER 1924 *LEUCOJETOSUM AESTIVI* PÎNZARU SUBASS. NOV. IN THE REPUBLIC OF MOLDOVA

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Abstract: This article is focused on the description of the forests of *Salix alba* L. with *Leucojum aestivum* L., in the valley of Prut River, in the Republic of Moldova. Based on 19 relevés, the author has grouped these forests in a plant community newly described for science – *Salicetum albae leucojetosum aestivi* subass. nov. included in the alliance *Salicion albae* Soó 1951, the order *Salicetalia purpureae* Moor 1958, cl. *SALICETEA PURPUREAE* Moor 1958.

Key words: *Salicetum albae* Issler 1924 *leucojetosum aestivi* subass. nov. characteristic of phytocoenosis, ecology, range, Republic of Moldova.

Introduction

Salix alba L. riverside forests, in the Republic of Moldova, occur in patches in the valleys of the Dniester River and the Prut River, very rarely and along streams in the central part of the country. From 1960 to 1995, willow groves were studied according to the dominant method and grouped into the following associations: *Salicetum* (*albae*) *inundatum* and *Salicetum* (*albae*) *rubosum* (*caesii*) [GHEIDEMAN & al. 1964; PÎNZARU, 1991; POSTOLACHE, 1995].

Since the beginning of the 21st century, the methods of the Central European School [BRAUN-BLANQUET, 1964] have been used in the phytocoenological research on the floodplain forests in the Republic of Moldova. Thus, the *Salix alba* forests from the scientific reserves "Plaiul Fagului" [POSTOLACHE & CHIRTOACĂ, 2005], "Prutul de Jos" [POSTOLACHE & POSTOLACHE, 2012]; "Pădurea Domnească" [TOFAN-BURAC & CHIFU, 2002; POSTOLACHE, 2017] and from the "Nemţeni" forest reserve [COVALI, 2008] have been grouped in the association *Salicetum albae* Issler 1924 (= *Salicetum albae* Issler 1926) [GAFTA & al. 2008].

The association *Salicetum albae* Issler 1924 has been included in the priority habitats, EU code 91E0, cf. Annex 1 Habitats Directive 92/43/EEC.

This article contains the description of a new subassociation of *Salix alba* L. with *Leucojum aestivum* L. *leucojetosum aestivi* Pînzaru subass. nov. as part of the association *Salicetum albae* Issler 1924.

Materials and methods

The phytocoenological studies in the field were carried out in the spring and summer of 2018, in the floodplain of the Prut River, between Nemţeni commune, Hînceşti district, at the north and Sărata Răzeşi commune, Leova district, at the south. For the preparation of this paper, we examined the descriptions of the forest plots from "Amenajările silvice" ("Forest Planning"), 2011-2014, ICAS, Chisinau. Nineteen relevés were described according to the

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methods of the Central European School [BRAUN-BLANQUET, 1964]. The area of a relevé was 600 m^2, according to the school of Cluj-Napoca [CRISTEA & al. 2004]. The list of species is presented in accordance with recent publications [SÂRBU & al. 2013; PÎNZARU & SÎRBU, 2016]. Air temperature and atmospheric precipitation – according to the Atlas of Climate Resources of the Republic of Moldova [NEDEALCOV & al. 2013]. Information on phytocoenological relevés, published in the Republic of Moldova [TOFAN-BURAC & CHIFU, 2002; POSTOLACHE & CHIRTOACĂ, 2005; COVALI, 2008; POSTOLACHE & POSTOLACHE, 2012; POSTOLACHE, 2017], Romania [CHIFU & MITITELU, 1992; IVAN & al. 1993; GAFTA & al. 2008; CHIFU & IRIMIA, 2014; COLDEA, 2015], Germany [SEIBERT & CONARD, 1992]; Cehia [NEUHÄUSLOVÁ & DOUDA, 2013] and Slovenia [SÍLC, 2003], was examined for comparison.

Results and discussions

The association *Salicetum albae* Issler 1924 includes the phytocoenoses of *Salix alba* L. from the floodplains of rivers and streams in the Central and South-Eastern Europe. The following species are characteristic of the given association: *Salix alba* L., *Salix fragilis* L., *Populus alba* L., *Populus nigra* L., *Fraxinus angustifolia* Vahl, *Acer negundo* L., *Rubus caesius* L., *Urtica dioica* L., *Phalaris arundinacea* L., *Aristolochia clematitis* L., *Humulus lupulus* L., *Iris pseudacorus* L., *Symphytum officinale* L., *Calystegia sepium* (L.) Br. [CHIFU & MITITELU, 1992; SEIBERT & CONARD, 1992; IVAN & al. 1993; SÍLC, 2003; NEUHÄSLOVÁ & DOUDA, 2013; COLDEA, 2015].

Willow forests usually include few species, ephemeral plants are very few or absent. Analysing the list of species of the relevés of the association *Salicetum albae* Issler 1924 [CHIFU & MITITELU, 1992; SEIBERT & CONARD, 1992; IVAN & al. 1993; TOFAN-BURAC & CHIFU, 2002; SÍLC, 2003; POSTOLACHE & CHIRTOACĂ, 2005; COVALI, 2008; POSTOLACHE & POSTOLACHE, 2012; CHIFU & IRIMIA, 2014; COLDEA, 2015; POSTOLACHE, 2017], we didn't find any mention of the presence of summer snowflake (*Leucojum aestivum* L.), although it was cited as a characteristic species of the alliance *Salicion albae* Soó 1930 [SÂRBU & al. 2013]. This mesohygrophylic, geophyte is a central-european-mediterranean-atlantic geoelement, which occurs naturally in hilly areas of several countries and regions: from Spain to Ukraine, Turkey, the Caucasus and Iran. This species is considered characteristic of the coenotaxa *Calthion palustris* Tx. 1937 [ELLENBERG & al. 1992] and *Fraxinetalia* Scamoni et Passarge 1959 [AESCHIMANN & al. 2004].

In the Republic of Moldova, the presence of the species *Leucojum aestivum* L. has been identified only in the willow groves of the valley of Prut River, in the vicinity of Sărata Răzeşi commune, Leova district, and Cioara and Dancu communes, Hînceşti district. It is a critically endangered species (CR), included in the Red Book of the Republic of Moldova [GHENDOV & CIOCÂRLAN, 2015)]. As a result of the phytosociological studies carried out recently, we have found new places where this species occurs – near the communes Nemţeni, Cotul Morii, Leuşeni (near the custom house) and Călmăuţi, Hînceşti district. It grows sporadically or in abundant clusters (AD 2-4) in *Salix alba* L. riverside forests. Under the climatic conditions of the Republic of Moldova, *Leucojum aestivum* plants grow up to 75 cm tall and produce 3-6 leaves, which grow about 75 cm long and 10-16 mm wide. It blooms in April-May, produces by (1) 3-7 (8) pendant flowers, located unilaterally on pedicels of different lengths. The fruits are fleshy, ovate-triangular capsules, 10-25 mm long, 7-17 mm wide, with (1) 3-8 seeds in a fruit. It releases the seeds at the end of June – beginning of July. The seeds are spheroidal, about 6 mm in diameter, blackish. It propagates by seeds and vegetatively (Figure 1).

Figure 1. *Leucojum aestivum* L.: A. flowers – 21.IV.2018, B. fruits – 10.VI.2018

We group the plant communities of *Salix alba* L. with *Leucojum aestivum* L., accompanied by the constant species *Ficaria verna* Huds., *Iris pseudacorus* L. and *Carex riparia* Curtis in a new subassociation within the association *Salicetum albae* Issler 1924 *leucojetosum aestivi* subass. nov.

In Romania, the plant communities of the association *Salicetum albae* Issler 1924 are grouped in two subassociations – *typicum* and *amorphaetosum fruticosae* Morariu et Danciu 1970 [CHIFU & IRIMIA, 2014; COLDEA, 2015] (Table 1).

The manner in which *Salix alba* phytocoenoses, with high abundance of the species *Rubus caesius* L. or *Cornus sanguinea* L s.l.., are grouped is questionable. In Slovenia, these phytocoenoses are separated in subassociations: *cornetosum sanguinei* Wendeberger Zelinka 1952 and *rubetosum caesii* (Soó 1958) Sílc 2003 of the association *Salicetum albae* Issler 1924 [SÍLC, 2003]. While in Romania, some researchers treat them as associations: *Corno sanguinei-Salicetum albae* Dihoru et al. 1966 em. Chifu et Irimia 2014 and *Rubo caesii-Salicetum albae* Doniță et Dihoru 1961 em. Doniță et al. 1966 [CHIFU & IRIMIA, 2014]. On the other hand, other researchers do not recognize these coenotaxa [SEIBERT & CONARD, 1992; IVAN & al. 1993; NEUHÄSLOVÁ & DOUDA, 2013; COLDEA, 2015]. Comparing the lists of the species of these coenotaxa and of the relevés from the Republic of Moldova, we conclude that it is appropriate to group these *Salix alba* forests into a single association – *Salicetum albae* Issler 1924 (Table 1) with the subassociations *typicum*, *amorphaetosum fruticosae* Morariu et Danciu 1970 and *leucojetosum aestivi* Pînzaru subass. nov. (Table 2). In the *Salix alba* riverside forests of the Republic of Moldova, *Rubus caesius* L. and *Cornus sanguinea* L. s.l. often grow abundantly together, for which reason, it has been proposed to consider them as characteristic species of the given association

We include the *Salix alba* L. phytocoenoses from the "Nemțeni" forest reserve, which was included some time ago in the association *Salicetum albae-fragilis* Issler 1926 [nome ambiguum, GAFTA & al. 2008] by V. COVALI (2008), in the subassociation *leucojetosum aestivi*, because *Leucojum aestivum*, a species unregistered by the author, occurs in them (Table 2, rel. 17-18, h.l.). The description of the new coenotaxon is given below.

As. ***Salicetum albae*** Issler 1924 ***leucojetosum aestivi*** Pînzaru subass. nov. hoc loco

T y p e h.l.: Table 2, rel. 9. (Figure 2)

Table synthetic h. l.: Table 2, 19 relevés.

The total area of the subassociation is about 818 ha.

Syn.: *Salicetum albae-fragilis* auct. non Issler 1926, nome ambiguum: Covali, 2008

Locations: Altitude 20-25 m. Relief: the floodplain of Prut River. Soil: alluvial sandy, deep, rich in humus, with high trophicity. Climate – temperate continental, the average annual temperature is 10.5 °C, the average annual precipitation varies between 500 mm and 550 mm (Figure 6).

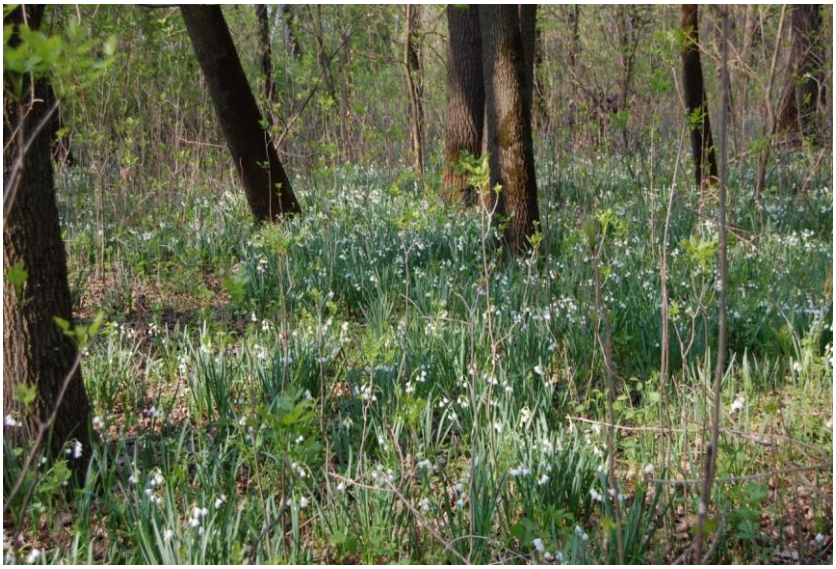

Figure 2. *Salicetum albae leucojetosum aestivi* subass. nov. (typus), Cioara commune, Hîncești district, 22.IV.2018

Characteristic species: *Leucojum aestivum*, *Ficaria verna*, *Iris pseudacorus*, *Carex riparia*, *Salix alba*.

Constant species: *Populus alba*, *Fraxinus excelsior*, *Acer negundo*, *Cornus sanguinea*, *Crataegus monogyna*, *Rubus caesius*, *Glechoma hederacea*, *Lysimachia nummularia*, *Symphytum officinale*, *Stellaria media*, *Urtica dioica*, *Arctium tomentosum*, *Elymus repens*, *Galium aparine*.

Structure: Vertically, three layers are distinguished in phytocoenoses (Figure 2, 3, 5):

1. The tree layer (A1), with a height of about (3-8)14-25 (-30) m, the coverage of the canopy is about 0.6-0.8. This layer consists of the dominant species *Salix alba*, with the cover-abundance (AD) of (1)2-4(5) points, the diameter of the stems varies between (4-14) 20-80 (-100) cm. Accompanying species: *Populus alba*, *Fraxinus excelsior*, *Salix fragilis*, in some places *Populus nigra*, *Quercus robur*, *Fraxinus americana*. The layer A2 is poorly defined, with a height of about 5-10 m: *Acer negundo*, *Pyrus pyraster*, *Malus sylvestris*, *Morus aba*, *Morus nigra*, *Ulmus minor*, *Ulmus glabra*, *Vitis sylvestris*.

Figure 3. *Salicetum albae-leucojetosum aestivi* subass. nov. summer, 18.VI.2018

2. The shrub layer (B) is 1.5-3 m high, unevenly developed, with coverage 10-60 (80) %. Constant species: *Cornus sanguinea*, *Crataegus monogyna*, in some places – *Prunus spinosa*, *Rosa canina*, *Acer tataricum*, *Amorpha fruticosa*; rarely – *Viburnum opulus*, *Coryllus avellana*, *Ligustrum vulgare*, *Euonymus europaeus*. At the level of the shrub layer, there are abundant *Acer negundo*, *Fraxinus excelsior*, *Fraxinus americana*.
3. In the herbaceous cover layer (C), two synusiae can be observed: ephemeroidal and ephemeral. The spring vegetation is very uneven; *Leucojum aestivum* occurs in clusters, accompanied by *Ficaria verna*, *Lamium purpureum*, *Anthriscus longirostris*, *Galium aparine*, *Stellaria media* and *Chaerophyllum temulum*. The summer synusia is richer, with the general coverage varying from (0) 30 to 100 %, such species as *Rubus caesius*, *Carex riparia*, *Glechoma hederacea*, *Elymus repens*, *Lysimachia nummularia* and *Poa palustris* are dominant and constant, but *Iris pseudacorus*, *Symphytum officinale*, *Arctium tomentosum* and *Valeriana officinalis* occur sporadically but constantly.

Floristic composition: in 19 relevés, 113 species of vascular plants have been detected: 11 species are characteristic of the above-mentioned association, another 11 – to the alliance *Salicion albae*, 4 – *Salicion triandrae*, 7 – *Salicetalia* and *Salicetea purpurae*, 7 – *Phragmitetea* s.l., 17 – *Molinio-Arrhenatheretea* s.l., 16 – *Querco-Fagetea* s.l., 8 – *Rhamno-Prunetea* s.l., 32 – Aliae.

Rare species protected by the state: *Leucojum aestivum* L. critically endangered (CR), included in the Red Book of R. Moldova [GHENDOV & CIOCÂRLAN, 2015], *Vitis sylvestris* C.C.Gmel. endangered (EN), included in the Red Book of R. Moldova. [CANTEMIR & ALEXANDROV, 2015] (Figure 4), *Cephalanthera damasonium* (Mill.) Druce, vulnerable (VU), included in the Red Book of R. Moldova [POSTOLACHE Gh & JARDAN, 2015], *Epipactis helleborine* (L.) Crantz, near threatened (NT) *Asparagus pseudoscaber* Grecescu, near threatened (NT), *Viburnum opulus* L. near threatened (NT) [Legea privind fondul ariilor…, Anexa 3, D(a), 1998].

Phytocoenological diversity. Within the given subassociation, in some areas, there is a great abundance of the species *Populus alba* L. (AD = 3-4), which is proposed to be grouped in facies *populosum albae* (Table 2. rel. 3, 6, 7, 14, 17, 19, Figure 5).

Range. The phytocoenoses of the subassociation *leucojetosum aestivi* occupy large areas in Leova (in the vicinity of Sărata-Răzeşi commune) and Hîncești districts (in the vicinity of Cioara, Dancu, Călmăuți, Leușeni, Cotul Morii and Nemțeni communes).

Limiting factors. Clearcutting and uncontrolled logging lead to changes in the composition of the stands, sometimes willow forests are replaced by pure plantations of *Gleditsia tricanthos* L., *Populus nigra* L., *Fraxinus excelsior* L., and the sectors where *Salix alba* is partially eliminated are invaded by *Acer negundo* L. and *Fraxinus americana* L.

Conservation value. These plant communities are of great importance, they represent the only habitat in the Republic of Moldova where *Leucojum aestivum* L. grows.

Conservation status. The phytocoenoses of this subassociation are protected in the Dancu Nature Reserve (131.57 ha) and in the Nemțeni Nature Reserve (20.9 ha) [Legea privind fondul ariilor…, Anexa 4, A, 1998].

Protection measures. It has been proposed include the floodplain forests with the above-mentioned subassociation, found near Sărata-Răzeși, Leușeni and Cotul Morii, in the network of protected areas. During the ecological reconstruction activities, the structure and composition of the tree species characteristic of the association should be improved.

Figure 4. *Vitis sylvestris* C. C. Gmel in the forest near Cotul Morii commune, Hîncești district, 19.VI.2018

Figure 5. Facies *populosum albae* in the "Nemțeni" nature reserve, 10.VI.2018

Figure 6. Locations of the subassociation *leucojetosum aestivi* subass. nov. in the Republic of Moldova

Conclusions

The subassociation *leucojetosum aestivi* Pînzaru represents rare mesohygrophylic phytocoenoses, and its entire range needs to be protected by the state. It is proposed to be included in the List of Rare Plant Associations of the Republic of Moldova.

The phytocoenoses with a great abundance of the species *Populus alba* L. and a rarer presence of the species *Salix alba* L. are proposed to be grouped in facies *populosum albae* (Table 2, rel. 3, 6, 7, 14, 17, 19).

The subassociation *leucojetosum aestivi* subass. nov. should be considered as part of the association *Salicetum albae* Issler 1924, the alliance *Salicion albae* Soó 1951, the order *Salicetalia purpureae* Moor 1958, cl. *SALICETEA PURPUREAE* Moor 1958.

Notes on contributor

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Table 1. Ass. *Salicetum albae* Issler 1924: a - *typicum*, b - *amorphaetosum fruticosae*

Subassociation:	**a**									**b**	
Number of the column	1	2	3	4	5	6	7	8	9	10	11
Number of the relevés	6	12	16	26	86	116	108	83	28	19	18
Number of species											
Constancy (K) or %	K	K	K	K	%	K	K	K	K	K	%
Characteristic species											
Salix alba	V	V	V	V	100	V	V	V	V	II	89
Salix fragilis	III	IV	IV	IV	47	III	V	I	V	I	33
Cornus sanguinea	V	IV	IV	V	27	III	I	I	-	-	28
Rubus caesius	IV	IV	IV	V	5	V	III	V	V	IV	-
Glechoma hederacea	-	I	II	V	38	II	II	I	IV	I	22
Urtica dioica	V	IV	I	V	50	-	II	I	IV	-	28
Vitis sylvestris	-	I	I	IV	6	II	-	I	II	II	44
Diff. subass.											
Amorpha fruticosa	-	-	-	-	9	-	-	I	I	V	72
Xanthium italicum	-	-	-	-	1	-	-	-	-	-	17
Asparagus officinalis	-	-	-	-	1	-	-	-	-	-	22
Asparagus tenuifolius	-	-	-	I	1	-	-	I	-	-	17
Asparagus verticillatus	-	-	-	III	-	-	-	I	II	-	17
Lycopus exaltatus	II	-	-	I	2	-	-	-	-	I	28
Leucojum aestivum	-	-	-	-	-	-	-	-	-	-	-
Iris pseudacorus	-	-	I	-	10	-	I	II	I	I	17
Carex riparia	-	-	-	-	-	-	-	-	-	-	-
Ficaria verna	-	-	-	I	-	-	-	-	I	-	-
Populus alba	-	II	I	III	51	III	II	I	III	-	33
Salicion albae											
Acer negundo	I	III	-	-	1	-	-	-	-	-	-
Valeriana officinalis	-	-	-	-	-	-	I	-	I	-	-
Viburnum opulus	II	I	II	III	10	II	I	I	III	-	17
Humulus lupulus	III	IV	III	V	35	III	II	I	V	-	28
Phalaris arundinacea	-	-	II	-	8	-	-	II	II	II	11
Frangula alnus	-	II	II	IV	8	I	-	I	I	-	28
Galium rubioides	-	-	-	-	-	-	-	I	I	I	-
Silene bacifera	II	II	I	III	8	I	I	I	I	-	11
Salix viminalis	-	I	I	-	14	I	II	I	I	-	-
Persicaria hydropiper	-	II	III	-	15	-	I	II	-	-	-
Salicion triandrae											
Galium aparine	III	II	II	II	28	I	-	II	-	I	-
Lysimachia vulgaris	-	I	I	-	13	II	II	II	-	II	28
Calystegia sepium	III	II	I	III	37	II	II	II	II	-	28
Rumex obtusifolium	-	-	-	IV	9	-	I	-	III	-	22
Salix triandra	-	I	I	II	15	I	I	I	-	-	6
Salix pentandra	-	I		-	-	-	I	-	II	-	-
Aegopodium podagraria	II	-	-	III	3	II	I	-	I	-	17
Epilobium hirsutum	-	-	-	-	-	-	I	-	-	-	-
Elymus caninus	-	-	-	I	5	-	-	-	I	-	-
Rumex crispus	-	-	-	-	I	-	-	I	-	-	-
Salicion elaeagno-daphnoides											
Alnus incana	-	-	-	-	12	-	I	I	-	-	-
Angelica sylvestris	II	-	-	I	8	I	-	I	-	-	-

Calamagrostis pseudophragmites	-	-	-	-	19	-	-	-	-	-	-
Chaerophyllum hirsutum	-	-	-	-	5	-	-	-	-	-	-
Hippophaë rhamnoides	-	-	-	-	-	-	-	-	I	-	-
Myricaria germanica	-	-	-	-	1	-	-	-	-	-	-
Salix daphnoides	-	-	-	I	-	-	-	-	I	-	-
Salix elaegnos	-	-	-	I	6	I	-	-	-	-	-
Saponaria officinalis	-	-	-	-	14	I	I	-	-	-	-
Salicetalia et Salicetea purpureae											
Lysimachia nummularia	IV	III	III	IV	33	II	II	II	III	I	39
Symphytum officinalis	III	II	II	IV	34	II	II	I	IV	II	56
Poa palustris	-	II		-	5	-	I	I	I	-	
Populus nigra	-	II	I	V	45	III	I	I	III	I	78
Solanum dulcamara	II	III	II	IV	30	I	I	-	IV	I	33
Salix purpurea	-	I	I	II	16	-	II	1	I	-	11
Rumex sanguineus	I	I	I	III	3	I	I	I	III	-	22
Anthriscus sylvestris	-	II	II	I	-	-	I	-	-	-	-
Tamarix ramosissima	-		I	-	5	-	-	-	-	-	-
Ranunculus repens	-	I	III	-	47	-	-	-	-	-	50
Stellaria aquatica	-	-	-	V	23	-	I	II	V	I	33
Periploca graeca	-	-	-	-	-	-	-	II	-	-	-
Phragmitetea s.l.											
Phragmites australis	II	I	I	I	13	-	I	I	I	III	44
Carex melanostachya	I	-	-	-	-	-	-	-	-	-	-
Inula helenium	II	-	-	-	-	-	I	-	-	-	-
Eupatorium cannabinum	I	I	-	II	12	I	I	I	I	I	22
Alisma plantago-aquatica	-	-	-	-	-	-	1	-	-	-	-
Alopecurus aequalis	-	-	-	-		-	-	I	-	-	-
Bidens cernua	-	-	I	-	-	-	-	I	-	-	-
Bidens tripartita	-	I	III	I	22	-	I	IV	I	I	22
Bolboschoenus maritimus	-	-	-	-	-	-	-	I	-	I	-
Caltha palustris	-	-	-	-	-	-	-	I	-	-	-
Carex acutiformis	-	-	-	-	-	-	I	I	I	IV	-
Carex vulpina	-	I	I	-	-	-	-	I	I	-	-
Cyperus glomeratus	-	-	-	-	-	-	-	I	-	-	-
Echinochloa crus-galli	-	I	II	I	-	-	-	I	I	-	11
Eleocharis palustris	-	-	-	-	-	-	I	I	-	-	-
Epilobium parviflorum	-	-	-	-	-	-	I	-	-	-	-
Filipendula ulmaria	-	-	-	-	-	-	I	-	-	-	-
Galium palustre	-	-	-	-	3	-	I	IV	I	II	-
Glyceria fluitans	-	-	-	-	-	-	-	I	-	-	-
Glyceria maxima	-	-	I	-	-	-	I	I	-	-	-
Glycyrrhiza echinata	-	-	-	-	6	I	-	I	-	-	17
Leersia oryzoides	-	-	-	-	-	-	-	I	I	-	-
Lycopus europaeus	-	I	I	I	-	-	I	III	-	II	-
Lythrum salicaria	I	I	I	-	35	-	I	III	-	II	17
Mentha aquatica	-	-	I	-	12	-	I	-	I	I	6
Myosotis scorpioides	-	I	-	-	-	I	II	I	I	I	-
Oenanthe aquatica	-	-	-	-	5	-	-	I	-	I	11
Oenanthe silaifolia	-	-	-	-	-	-	-	-	I	-	-
Persicaria lapatifolia	-	-	-	I	-	-	-	I	-	-	28
Persicaria maculosa	-	-	-	-	9	-	I	-	-	-	-

Persicaria mitis	-	-	-	-	-	-	-	I	-	-	-
Ranunculus scleratus	-	-	-	-	-	-	-	I	-	-	-
Rorippa amphybia	-	-	-	I	13	-	-	I	-	-	-
Rumex conglomeratus	-	I	I	I	-	-	I	I	I	-	-
Rumex hydrolapathum	-	-	-	-	-	-	-	-	-	I	-
Sagittaria sagittifolia	-	-	-	-	-	-	-	I	-	-	-
Scirpus sylvaticus	II	-	-	-	-	-	-	I	I	-	-
Scutellaria galericulata	-	I	I	I	21	-	-	I	I	-	-
Senecio paludosus	-	-		-	-	-	-	I	-	-	-
Sium sisarum	I	-	-	-	-	-	I	-	-	-	-
Sonchus oleraceus	-	-	-	-	-	-	-	I	-	-	-
Sparganium erectum	-	-	-	-	-	-	-	I	-	-	-
Stachys palustris	-	-	-	-	12	-	I	IV	I	-	11
Teucrium scordium	-	-	-	-	-	-	-	-	-	II	-
Typha angustifolia	-	-	-	-	-	-	-	I	-	I	-
Veronica anagalis-aquatica	-	-	-	I	-	-	-	-	I	-	-
Molinio-Arrhenatheretea s.l.											
Taraxacum camylodes	-	-	I	-	12	-	I	I	I	I	6
Euphorbia lucida	-	-	-	I	-	-	-	-	I	I	-
Potentilla reptans	-	I	I	-	-	-	II	I	I	I	-
Carex hirta	-	-	-	-	9	-	I	I	I	I	-
Daucus carota	-	-	I	-	13	-	I	I	I	-	-
Prunella vulgaris	-	-	I	II	7	-	I	-	-	-	17
Rorippa sylvestris	-	-	I	II	12	-	I	I	I	II	39
Scutellaria hastifolia	I	-	I	I	-	-	-	I	-	-	-
Thalictrum lucidum	-	-	-	-	-	-	-	I	-	-	-
Trifolium repens	-	-	I	-	15	15	II	-	I	-	-
Cerastium holosteoides	-	-	-	-	-	-	II	-	-	-	17
Equisetum arvense	II	II	-	-	20	-	II	I	I	II	11
Ranunculus polyanthemos	-	-	-	-	-	-	I	-	-	-	-
Trifolium campestre	-	-	-	-	-	-	I	-	I	-	-
Trifolium pratense	-	I	-	-	1	-	-	-	-	-	-
Achillea millefolium	-	-	-	-	1	-	I	I	-	-	-
Agrostis stolonifera	-	II	I	III	30	-	I	III	III	II	72
Alopecurus geniculatus	-	-	-	-	-	-	-	-	I	-	-
Alopecurus pratensis	-	-	-	-	-	-	I	-	-	-	-
Althaea officinalis	-	II	II	IV	10	I	I	I	III	-	28
Anthoxanthum odoratum	-	-	-	-	-	-	I	-	-	-	-
Barbarea vulgaris	-	-	-	-	-	-	I	-	-	-	-
Bellis perennis	-	-	-	-	-	-	I	-	I	-	-
Briza media	-	-	-	-	-	-	I	-	-	-	-
Carex brizoides	-	-	-	-	-	-	I	-	-	-	-
Cardamine pratensis	-	-	-	-	-	-	-	I	-	-	-
Centaurea phrygia	-	-		-	-	-	I	-	-	-	-
Centaurium erythraea	-	-	-	-	-	-	-	-	I	-	-
Centaurium pulchellum				I							
Dactylis glomerata	-	I	I	-	-	-	I	-	-	-	-
Equisetum palustre	-	-	-	I	-	I	-	II	-	-	28
Equisetum telmateia	III	-	-	-	-	-	I	-	-	-	-
Euphorbia palustris	-	-	I	I	-	I	-	-	-	I	-

Festuca pratensis	-	-	-	-	-	-	I	-	-	-	-
Galega officinalis	-	-	-	-	-	-	I	I	-	-	-
Geranium pratense	-	-	-	-	3	-	I	-	-	-	-
Heracleum sphondylium	-	-	-	-	7	-	II	-	-	-	-
Holcus lanatus	-	-	-	-	1	-	I	-	I	-	-
Inula britanica	-	-	-	-	10	-	II	I	-	-	-
Juncus articulatus	-	-	-	-	-	-	I	-	-	-	-
Juncus effusus	-	-	-	-	-	-	I	-	-	-	-
Mentha longifolia	-	I	I	I	16	-	I	I	I	-	11
Plantago major	-	I	-	I	-	-	-	-	-	-	-
Peucedanum carvifolium	-	I	III	-	-	-	-	-	I	-	-
Leucanthemum vulgare	-	-	-	-	3	-	I	I	-	-	-
Lolium perenne	-	-	-	-	1	-	I	-	-	-	-
Luzula campestris	-	-	-	-	-	-	I	-	-	-	-
Lysimachia punctata	-	-	-	-	-	-	-	-	I	-	-
Lythrum virgatum	-	-	-	-	-	-	I	-	-	-	-
Medicago lupulina	-	-	-	-	2	-	-	-	-	-	-
Mentha arvensis	-	-	-	-	-	-	I	-	I	-	-
Pastinaca sativa var. sylvestris	-	-	-	-	-	-	I	I	-	-	-
Petasites hybridus	IV	-	-	-	-	-	I	I	-	-	-
Picris hieracioides	-	-	-	-	-	-	I	-	-	-	-
Poa pratensis	-	-	-	-	-	-	I	-	I	-	-
Poa sylvicola	-	-	-	-	-	-	-	-	I	I	-
Poa trivialis	-	-	-	-	5	-	I	-	I	-	-
Potentilla anserina	-	-	-	I	-	-	I	-	-	-	-
Ranunculus acris	-	-	-	-	12	-	I	-	-	-	-
Ranunculus repens	-	-	-	III	-	-	III	II	II	II	-
Rhinanthus minor	-	-	-	-	-	-	I	-	-	-	-
Rumex acetosa	-	-	-	-	-	-	I	-	-	-	-
Rumex dentatus	-	-	-	-	2	-	-	-	-	-	-
Silene flos-cuculi	-	-	-	-	-	-	I	-	-	-	-
Silene vulgaris	-	-	-	I	-	-	-	I	-	-	-
Stellaria graminea	-	-	-	-	-	-	I	-	-	-	-
Thalictrum flavum	-	-	-	-	-	-	-	I	-	-	-
Thalictrum simplex	-	-	-	-	-	-	I	-	-	-	-
Trifolium hybridum	-	-	-	-	-	-	I	-	-	-	-
Trisetum flavescens	-	-	-	-	-	-	I	-	-	-	-
Vicia cracca	-	-	-	-	13	-	I	I	-	I	-
Querco-Fagetea s.l.											
Fraxinus excelsior	III	-	-	-	-	-	I	-	-	-	-
Quercus robur	-	-	-	I	-	-	-	-	-	-	-
Chaerophyllum temulum	-	-	-	-	-	-	-	-	I	-	-
Malus sylvestris	I	-	-	-	-	-	-	-	-	-	-
Epipactis helleborine	I	-	-	-	-	-	-	-	-	-	-
Convallaria majalis	I	-	-	-	-	-	-	-	-	-	-
Ulmus minor	-	-	-	III	12	I	-	-	I	I	11
Ulmus glabra	-	-	-	-	-	-	-	I	-	-	-
Euonymus europaeus	I	I	-	II	10	I	-	-	I	-	22
Geum urbanum	II	I	I	I	8	-	I	-	I	-	-
Lapsana communis	-	-	-	-	-	-	I	I	I	-	-
Salix cinerea	III	I	-	I	-	-	II	-	-	-	-

Populus tremula	III	-	-	-	-	-	I	-	-	-	-
Cerasus avium	I	-	-	I	-	-	-	-	-	-	-
Acer campestre	-	I	-	II	-	-	-	-	I	-	-
Acer pseudoplatanus	-	-	-	-	-	-	I	-	-	-	-
Ajuga reptans	-	-	I	-	-	-	-	-	-	-	-
Alnus glutinosa	-	-	-	-	-	II	-	I	I	-	-
Arctium nemorosum	-	-	-	-	-	-	-	-	I	-	-
Athyrium filix-femina	-	-	-	-	-	-	I	-	-	-	-
Brachypodium sylvaticum	II	-	I	I	8	-	I	-	-	-	-
Bromus benekenii	I	-	-	-	-	-	-	-	-	-	-
Campanula persicifolia	-	-	-	I	-	-	-	-	-	-	-
Campanula rapunculoides	-	-	-	I	-	-	-	-	-	-	-
Campanula trachelium	-	-	-	-	-	-	-	-	I	-	-
Carex pendula	I	-	-	-	-	-	-	-	-	-	-
Carex remota	-	II	-	-	-	-	-	-	I	-	-
Cardamine impatiens	I	-	-	-	-	I	-	I	-	-	-
Chrysosplenium alternifolium	-	-	-	-	-	-	I	-	-	-	-
Dryopteris filix-mas	-	-	-	-	-	-	I	-	-	-	-
Equisetum hyemale	-	-	-	I	-	-	-	-	I	-	-
Festuca gigantea	-	-	-	I	-	-	-	-	I	-	-
Fraxinus oxycarpa	-	-	-	I	10	-	-	-	I	-	-
Fraxinus pallisae	-	-	I	-	-	-	-	-	-	-	-
Geranium phaeum	II	-	I	-	-	-	I	-	-	-	-
Glechoma hirsuta	I	-	-	-	-	-	-	-	-	-	-
Impatiens noli-tangere	-	-	-	-	-	-	1	-	-	-	-
Lactuca muralis	-	-	-	-	I	-	I	-	-	-	-
Lamium galeobdolon	II	-	-	-	-	-	I	-	-	-	-
Lonicera xylosteum	-	-	-	I	-	-	I	-	-	-	-
Matteucia struthiopteris	-	-	-	-	-	-	I	-	-	-	-
Myosotis sparsiflora	-	-	-	-	-	-	-	-	I	-	-
Platanthera bifolia	-	-	-	-	-	-	-	-	I	-	-
Poa nemoralis	-	-	-	-	-	-	I	-	-	-	-
Pulmonaria officinalis	-	I	-	-	-	-	-	-	-	-	-
Ribes uva-crispi	-	-	-	-	-	-	I	-	-	-	-
Salvia glutinosa	I	-	-	-	2	-	-	-	-	-	-
Sambucus nigra	III	II	I	II	30	I	-	-	-	-	11
Scrophularia nodosa	-	-	-	-	6	-	I	I	I	-	-
Stachys sylvatica	II	-	-	-	-	-	I	-	-	-	-
Staphylea pinnata	-	-	-	-	-	-	-	I	-	-	-
Stellaria nemorum	-	-	-	I	-	-	I	-	I	-	-
Tanacetum corymbosum	-	-	-	I	-	-	-	-	-	-	-
Telekia speciosa	-	-	-	-	-	-	I	-	-	-	-
Tilia cordata	-	-	-	I	-	-	-	-	-	-	-
Tilia tomentosa	-	-	-	-	-	-	-	I	-	-	-
Ulmus laevis	I	-	-	-	-	-	-	I	-	-	-
Ulmus procera	-		-	-	-	-	-	-	I	-	-
Viola odorata	-	-	-	I	-	-	-	-	-	-	-
Viscum album	-	-	-	-	-	-	-	-	I	-	-
Rhamno-Prunetea s.l.											
Crataegus monogyna	III	I	-	IV	35	I	-	I	III	-	11

Prunus spinosa	-	-	-	II	-	-	I	I	I	-	-
Pyrus pyraster	III	-	-	I	-	I	I	I	-	I	-
Corylus avellana	IV	I	-	I	8	I	-	-	-	-	-
Acer tataricum	-	-	-	II	-	-	-	I	I	-	-
Rosa canina	-	II	I	II	20	I	I	I	I	-	-
Ligustrum vulgare	-	-	I	III	16	I	-	I	I	-	17
Clematis vitalba	-	I	I	IV	20	II	-	-	III	-	22
Physalis alkekengi	-	-	I	I	-	-	-	-	I	-	-
Rhamnus cathartica	-	-	I	I	-	-	-	-	-	-	-
Galeopsis pubescens	-	-	-	-	-	-	-	I	-	-	-
Euonymus verrucosus	-	-	-	-	-	-	-	I	-	-	-
Hypericum hirsutum	-	-	-	-	-	-	-	I	-	-	-
Cuscuta monogyna	-	-	-	-	-	-	-	I	-	-	-
Rosa caryophylacea	-	-	-	-	-	-	-	-	I	-	-
Aliae											
Stellaria media	-	I	I	I	-	-	I	I	-	-	-
Arctium tomentosum	-	I	I	-	-	-	I	-	-	-	-
Elymus repens	-	I	-	II	14	I	-	I	I	-	22
Anthriscus longirostris	-	I	-	-	-	-	-	-	-	-	-
Cirsium arvense	-	I	-	-	1	-	I	II	I	-	28
Aristolochia clematitis	-	-	I	I	17	-	I	-	II	-	-
Lathyrus tuberosus	-	-	I	-	-	-	I	-	-	-	-
Vicia hirsuta	-	-	-	-	-	-	-	-	I	-	-
Gleditsia triacanthos	-	-	-	-	-	-	I	-	-	-	-
Alliaria petiolata	-	I	I	I	-	-	I	-	I	-	-
Ballota nigra	-	I	I	-	-	-	I	-	-	-	-
Erigeron annuus	-	I	I	-	22	-	I	I	I	-	11
Robinia pseudacacia	-	-	-	-	-	-	I	I	-	-	-
Morus alba	-	I	I	-	7	-	-	I	-	I	-
Artemisia absinthium	-	I	I	-	3	3	3	I	I	-	-
Leonurus cardiaca	-	I	I	-	-	-	-	-	I	-	-
Tanacetum vulgare	-	-	-	-	1	1	1	I	-	-	-
Plantago major	-	-	-	I	-	-	-	I	I	-	-
Calamagrostis epigeios	-	II	I	IV	-	-	-	I	III	-	-
Vicia tetrasperma	-	-	-	-	-	-	I	-	I	-	-
Lamium maculatum	-	-	-	-	7	7	I	-	I	-	-
Achillea setacea	-	-	-	-	3	-	-	-	-	-	-
Aethusa cynapium	-	-	-	I	-	-	I	-	-	-	-
Agrimonia eupatoria	-	-	-	-	-	-	I	-	-	-	-
Allium vineale	-	-	-	-	-	-	I	-	-	-	-
Arctium lappa	-	-	-	I	15	-	-	I	-	-	-
Armoracia rusticana	-	-	-	-	-	-	I	-	-	-	-
Artemisia austriaca	-	-	-	-	-	-	-	I	-	-	-
Artemisia vulgaris	-	I	I	I	13	-	-	I	I	-	17
Astragalus glycyphyllos	-	-	-	-	-	-	-	I	-	-	-
Atriplex tatarica	-	-	-	-	-	-	-	I	-	-	-
Avena fatua	-	-	-	-	-	-	I	-	-	-	-
Bromus arvensis	-	-	-	-	-	-	-	-	I	-	-
Bromus inermis	-	-	-	-	-	-	I	-	-	-	-
Bromus sterilis	-	-	-	-	-	-	-	-	I	-	-
Calamagrostis arundinacea	-	-	-	-	-	-	I	-	-	-	-

Campanula abietina	-	-	-	-	-	-	I	-	-	-	-
Cannabis sativa	-	I	I	-	-	-	-	-	-	-	-
Cardamine amara	-	-	-	-	-	-	-	I	-	-	-
Carduus acanthoides	-	-	-	-	5	-	I	-	-	-	-
Carduus crispus	-	II	I	II	7	-	I	-	I	-	22
Centaurea micranthos	-	-	-	-	-	-	I	-	-	-	-
Chaerophyllum aromaticum	-	-	-	-	-	-	I	-	-	-	-
Chaerophyllum bulbosum	-	-	I	-	-	-	-	-	-	-	-
Chelidonium majus	-	-	-	I	-	-	I	-	-	-	-
Chenopodium album	-	-	-	-	-	-	-	I	-	-	-
Cichorium intybus	-	-	-	I	1	1	I	-	-	-	-
Cirsium vulgare	-	I	I	-	6	-	I	I	I	-	-
Clinopodium vulgare	-	-	-	I	-	-	I	-	-	-	-
Conium maculatum	-	-	-	I	-	-	I	-	I	-	-
Convolvulus arvensis	-	-	-	-	-	-	I	-	-	-	-
Cruciata laevipes	-	-	-	-	-	I	-	I	-	-	-
Cuscuta lupuliformis	-	-	-	I	-	-	-	-	I	-	-
Cynodon dactylon	-	-	-	-	1	-	-	I	-	I	-
Dipsacus fullonum	II	I	-	-	-	-	-	-	I	-	-
Dipsacus laciniatus	-	-	-	-	-	-	I	-	I	-	-
Dipsacus pilosus	-	I	-	-	-	-	-	-	-	-	-
Dysphania botrys	-	-	-	-	-	-	-	I	-	-	-
Echinocystis lobata	-	-	-	-	16	-	I	I	-	-	-
Echinops sphaerocephalus	-	-	-	-	-	-	-	I	-	-	-
Echium vulgare	-	-	-	-	-	-	I	-	-	-	-
Erigeron acer	-	-	-	-	9	-	-	-	-	-	11
Erigeron canadensis	-	-	-	-	1	-	I	I	I	-	-
Euphorbia cyparissias	-	-	-	-	13	-	I	I	-	-	-
Fallopia dumetorum	-	-	-	I	5	-	-	-	-	-	11
Festuca valesiaca	-	-	-	-	-	-	I	-	-	-	-
Filipendula vulgaris	-	-	-	-	-	-	-	-	I	-	-
Fragaria vesca	-	-	-	I	-	-	I	-	-	-	-
Fraxinus pennsylvanica	-	-	-	-	-	-	-	II	-	-	-
Galeopsis speciosa	-	-	-	-	-	-	-	I	I	-	-
Galeopsis tetrahit	II	-	-	-	-	-	I	-	-	-	-
Galinsoga parviflora	-	-	-	-	-	-	I	-	-	-	-
Galium verum	-	-	-	-	-	-	I	-	-	-	-
Gentiana asclepiadea	-	-	-	-	-	-	I	-	-	-	-
Geranium robertianum	-	-	-	-	-	-	I	-	-	-	-
Helianthus tuberosus	-	-	-	-	-	-	I	-	-	-	-
Hieracium aurantiacum	-	-	-	-	-	-	I	-	-	-	-
Hypericum perforatum	-	-	-	-	-	-	-	I	I	-	-
Lactuca saligna	-	-	-	-	-	-	-	I	-	-	-
Lactuca seriola	-	-	I	-	-	-	-	I	-	-	-
Lamium album	-	-	-	-	-	-	I	-	-	-	-
Leonurus marrubiastrum	-	-	-	-	-	-	-	I	-	-	-
Linaria vulgaris	-	-	-	-	-	-	I	-	-	-	-
Lipandra polysperma	-	-	-	-	-	-	-	I	-	-	-
Lithospermum officinale	-	-	-	-	-	-	-	-	I	-	-
Lotus tenuis	-	-	-	-	-	-	I	-	-	-	-

Medicago falcata	-	-	-	-	-	-	I	-	-	-	-
Mentha pulegium	-	-	-	-	-	-	I	I	-	-	-
Melilotus officinalis	-	-	-	-	-	-	-	I	-	-	-
Oenothera biennis	-	I	-	II	15	I	-	-	II	-	6
Oxalis stricta	-	-	-	-	-	-	-	I	-	-	-
Oxybasis ubrica	-	-	-	-	-	-	-	I	-	-	-
Parietaria officinalis	I	-	-	-	-	-	-	-	I	-	-
Plantago lanceolata	-	-	-	-	10	-	I	-	-	-	-
Plantago media	-	-	-	-	-	-	-	-	I	-	-
Poa annua	-	-	-	I	2	-	-	-	-	-	-
Polygonum aviculare	-	-	-	-	-	-	-	I	-	-	-
Potentilla argentea	-	-	-	-	-	-	I	-	-	-	-
Potentilla recta	-	-	-	-	-	-	-	-	I	-	-
Potentilla supina	-	-	-	I	-	-	-	-	-	-	-
Reseda lutea	-	-	-	-	-	-	I	-	-	-	-
Rumex pulcher	-	-	-	-	-	-	-	I	-	-	-
Sambucus ebulus	-	I	I	-	15	-	-	-	I	-	-
Senecio germanicus	-	-	-	-	-	-	-	I	-	-	-
Senecio sylvaticus	-	-	-	-	-	-	-	I	-	-	-
Setaria pumila	-	-	-	-	-	-	I	-	-	-	-
Setaria viridis	-	-	-	-	1	-	-	-	-	-	-
Silene alba	-	-	-	-	8	-	-	-	-	-	6
Solanum nigrum	-	-	-	-	9	-	I	-	-	-	-
Sonchus arvensis	-	I	-	-	6	-	-	-	-	-	6
Sonchus asper	-		-	-	-	-	I	I	-	-	-
Torilis japonica	-	-	-	I	-	-	-	I	I	-	-
Trifolium fragiferum	-	-	-	-	-	-	I	-	-	-	-
Tussilago farfara	-	I	I	-	3	-	I	-	I	-	-
Verbena officinalis	-	-	-	-	-	-	I	I	-	-	-
Veronica chamaedrys	-	-	-	-	-	-	I	-	-	-	-
Vicia pannonica	-	-	-	-	-	-	-	-	I	-	-
Xanthium spinosum	-	-	-	-	-	-	-	I	-	-	-
Xanthium strumarium	-	I	I	-	6	-	I	I	II	-	6

Ass. ***Saliceum albae*** Issler 1924 [= *Salicetum albae* Issler 1926]:

a. ***typicum*** [= Rubo caesii-Salicetum albae Doniță et Dihoru 1961 em. Doniță et al. 1966; Corno sanguinei-Salicetum albae Dihoru et al. 1966 em. Chifu et Irimia 2014]:

Col. 1. 6 rel. POSTOLACHE Gh & CHIRTOACĂ, 2005 (*Salicetum albae* Issler 1924)
Col. 2. 12 rel. POSTOLACHE Gh. 2017 (*Salicetum albae* Issler 1926)
Col. 3. 16 rel. POSTOLACHE Gh. & POSTOLACHE D., 2012 (*Salicetum albae* Issler 1926)
Col. 4. 26 rel.TOFAN-BURAC & CHIFU, 2002 (*Salicetum albae* Isseler 1924)
Col. 5. 86 rel. COLDEA, 2015 (*Salicetum albae* Issler 1926 *typicum*)
Col. 6. 116 rel. IVAN & al. 1993 (*Salicetum albae* Issler 1924)
Col. 7. 108 rel. CHIFU & IRIMIA, 2014 (*Salicetum albae* Issler 1926 *typicum*)
Col. 8. 83 rel. CHIFU & IRIMIA 2014 (Ass. *Rubo caesii-Salicetum albae* Doniță et Dihoru 1961 em. Doniță et al. 1966)
Col. 9. 28 rel. CHIFU & IRIMIA2014 (Ass. *Corno sanguinei-Salicetum albae* Dihoru et al. 1966 em. Chifu et Irimia 2014)

b. subass. ***amorphaetosum fruticosae*** Morariu et Danciu 1970

Col. 10. 19 rel. CHIFU & IRIMIA, 2014
Col. 11. 18 rel. COLDEA, 2015

Table 2. Ass. *Salicetum albae* Issler 1924 *leucojetosum aestivi* subass. nov.

Relevé no.	1	2	3	4	5	6	7	8	*9	10	11	12	13	14	15	16	17	18	19	K
Surface of relevé (m²)	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	600	
Altitudine	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	22	22	24	25	
Consistency	0,6	0,6	0,7	0,6	0,6	0,7	0,7	0,6	0,6	0,8	0,6	0,6	0,7	0,7	0,8	0,6	0,8	0,6	0,7	
Tree height (m)	12-16	16-20	18-21	15-17	19-22	18-25	17-24	14	14-16	3-5	21-24	6-24	19-24	19-25	17-19	24-27	8-9	17-21	26-30	
Tree diametr (cm)	24-36	24-34	22-30	20-28	28-36	26-42	24-38	18	16-20	4-7	32-40	10-40	26-38	30-42	24-30	52-80	10-14	30-36	30-60	
Shrub layer coverage (%)	10-30	25	35	45	30	55	15-75	15-40	10-55	25-65	20-45	35	15-45	30-60	30-75	30-80	25-75	10-15	10-30	
Herbaceous layer coverage		60-	45-		60-	70-	60-		35-		60-									
(%)	100	85	90	100	75	90	85	100	90	100	85	100	100	90	100	100	90	90	100	
Number of species	58	28	20	23	35	34	36	28	26	27	45	22	27	17	25	18	26	31	62	
Characteristic species ass. Salicetum albae																				
Salix alba	1-3	1	1	4	3	2	1-3	4	3-4	4	3	1	4	+	3	4-5	1	4	1	V
Cornus sanguinea	2	2	-	-	1-2	1	1-4	2	1-3	2-3	2	1	1	2-3	2-4	1	4	2	2	V
Rubus caesius	3-5	2-4	3-4	2-4	2-3	2-3	2-5	3-5	2-3	2-4	3	3-4	2-4	2-3	1	3-4	2-3	3-4	3-4	V
Glechoma hederacea	2	1	1	1-2	1	3	1-2	1	1-2	3-4	3	1-2	1-2	1-2	3	-	1	2	1-2	V
Urtica dioica	2	1	-	2	2	2-3	2-3	2	2	3	1	2	1	1-4	2-5	1	-	-	3-5	IV
Salix fragilis	1	1	-	-	1	1	1	-	-	-	1	-	-	1	-	-	-	1	1	III
Vitis sylvestris	-	-	-	-	+	+	-	-	-	-	-	-	+	-	+	-	1	1-2	+	III
Char.subass.																				
Leucojum aestivum	1-3	1-3	+	1-2	1-2	+	+	1-3	1-3	1	1-2	1-2	+	3-4	1	1	1-3	1-2	1	V
Iris pseudacorus	+	+	+	1	+	+	+	+	1-2	+	1	+	1	-	+	+	1	1	+	V
Carex riparia	-	-	-	1-2	-	2	2	2-3	2	-	2	2	2	-	2	-	3	3	2	IV
Ficaria verna	2-3	2	1-4	2	1	1-2	2-3	1	2	3-4	3	2	-	2	1	1	-	-	-	IV
Diff. facies																				
Populus alba	-	1	4	-	1	2-3	3-4	+	+	1	2	1	-	4	1-2	1-2	4	-	4	IV
Salicion albae																				
Acer negundo	1	1	1	1-3	1	3	1	1	-	1-2	2	+	+	1	1	-	1	-	3	V
Valeriana officinalis	+	+	+	+	+	-	+	1-2	-	-	+	-	-	-	-	-	-	-	-	III
Viburnum opulus	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	II
Humulus lupulus	1	-	1	-	+	-	-	-	-	1-2	1	-	-	-	+	-	1	-	-	II
Phalaris arundinacea	-	-	-	-	-	-	-	-	-	-	I	-	2	-	-	-	1	-	-	II

Amorpha fruticosa	+	-	-	-	-	-	-	-	1	2	-	-	1-2	-	-	-	1	-	-	II
Frangula alnus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	I
Viola elatior	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	+	I
Galium rubioides	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	2	I
Asparagus pseudoscaber	r	-	-	-	r	-	-	-	-	-	+	-	-	-	-	-	-	r	-	I
Silene bacifera	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	I
Salicion triandrae																				
Galium aparine	2	1	1	1	1	3-4	1-2	1	1-2	2	2	1-2	-	2	3	2-3	1	-	1	V
Lysimachia vulgaris	-	-	-	+	+	-	-	-	-	-	+	-	+	-	-	-	-	+	-	II
Calystegia sepium	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	I
Rumex obtusifolium	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	+	I
Salicetalia et Salicetea purpureae																				
Symphytum officinalis	+	1	+	+	+	+	+	+	+	+	+	-	+	-	+	+	1	+	+	V
Lysimachia nummularia	2	-	-	-	2	2	2	2-3	2	-	2	2-3	2	2	2-3	-	2	3	2	IV
Poa palustris	2	-	-	-	1-2	1-2	-	3	-	-	1	3	1	-	-	-	-	-	1	III
Populus nigra	1-3	+	-	-	1-2	1	-	-	2	-	1	-	1-2	-	-	-	-	-	-	II
Solanum dulcamara	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	I
Salix purpurea	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	I
Rumex sanguineus	-	1	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Phragmitetea s.l.																				
Phragmites australis	-	-	-	-	-	-	2	-	-	3	-	-	-	-	1	-	-	-	1	II
Carex melanostachya	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1-2	-	-	-	-	I
Epilobium ciliatum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	I
Inula helenium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	I
Eupatorium cannabinum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	I
Rorippa austriaca	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I
Alisma plantago-aquatica	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-		+	I
Molinio-Arrhenatheretea s.l.																				
Taraxacum camylodes	+	-	-	-	-	+	+	+	+	+	-	+	-	-	+	+	-	-	-	III
Euphorbia lucida	+	-	+	-	+	-	+	-	-	+	+	-	1	-	+	-	-	+	+	III
Phleum pratense	-	-	-	-	-	+	-	-	1	2	-	-	+	-	-	-	-	-	1	II
Potentilla reptans	-	-	-	-	-	-	-	-	1	+	-	-	1-2	-	-	-	-	-	2	II
Carex hirta	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	3	-	-	1-2	I
Daucus carota	+	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	I
Prunella vulgaris	+	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	+	I
Rorippa sylvestris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I

Scutellaria hastifolia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-		I
Thalictrum lucidum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1		I
Vicia sepium	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		I
Trifolium repens	-	1	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-		I
Cerastium holosteoides	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-		I
Equisetum arvense	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	2	-		I
Ranunculus polyanthemos	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		I
Trifolium campestre	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		I
Trifolium pratense	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		I
Querco-Fagetea s.l.																					
Fraxinus excelsior	-	3	1	1	2	-	-	-	-	-	1	3	-	1	1	1	-	1	+		IV
Quercus robur	1-2	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	1	1	1		II
Chaerophyllum temulum	+	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	1	-	1		II
Malus sylvestris	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-		I
Cephalanthera damasonium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r		I
Epipactis helleborine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	-	r		I
Convallaria majalis	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	2		I
Carex contigua	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1		I
Ulmus minor	-	-	1	-	-	1-2	1-2	-	-	-	-	-	-	-	-	-	-	-	1		I
Ulmus glabra	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1		I
Euonymus europaeus	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		I
Geum urbanum	1	+	+	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-		I
Lapsana communis	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+		I
Salix cinerea		-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		I
Populus tremula	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-			I
Polygonatum latifolium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-			I
Rhamno-Prunetea s.l.																					
Crataegus monogyna	2	2	2	+	1	1	1	1-2	-	+	1	+	-	1	+	1	+	+	1		V
Prunus spinosa	+	-	-	-	+	1	1-3	1	1	1-3	+	1	-	-	-	-	-	-	1		III
Pyrus pyraster	+	+	-	+	+	+	1	+	+	+	+	-	+	-	-	-	-	-	-		III
Corylus avellana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+		I
Acer tataricum	1	-	1-2	1	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-		I
Vicia tenuifolia	-	-	-	+	+	-	-	-	-	-	-	-	2	-	-	-	-	-	1-2		I
Rosa canina	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-		I
Ligustrum vulgare		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-		I
Aliae																					
Stellaria media	1	1-2	1	1	1	2-3	1-2	1-2	2-3	2-3	2	1-2	-	2	-	2	-	-	-		IV

Arctium tomentosum	+	+	-	-	+	+	-	+	+	-	+	+	-	+	+	-	+	-	+	IV
Elymus repens	2	2-3	2	3-4	3-4	-	3-4	3	4	-	-	-	2-3	-	-	-	-	3	3-4	IV
Morus nigra	+	-	-	-	-	-	1	-	-	+	+	+	+	-	+	-	+	+	+	III
Lamium purpureum	2	-	-	-	-	1	+	1	1-3	2-3	+	-	-	-	1-2	+	-	-	-	III
Anthriscus longirostris	1-3	-	-	-	3	2-3	2	1	-	1-2	+	-	-	-	2	-	-	-	2	III
Cirsium arvense	+	-	-	-	-	+	+	+	-	-	+	-	-	-	-	+	-	-	+	II
Aristolochia clematitis	2	-	-	-	1	-	2	2	-	-	-	-	-	-	-	-	2	2	2	II
Euphorbia virgata	+	+	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	II
Valerianella carinata	-	-	-	-	-	+	+	1	+	-	-	-	-	-	-	-	-	-	-	II
Lathyrus tuberosus	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	II
Vicia hirsuta	+	-	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	+	-	II
Gleditsia triacanthos	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	I
Alliaria petiolata	-	-	-	-	-	-	+	+	-	3	-	-	-	-	-	-	-	-	-	I
Ballota nigra	+	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	I
Erigeron annuus	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	I
Torilis arvensis	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I
Robinia pseudacacia	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	I
Morus alba	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I
Fraxinus americana	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1	I
Artemisia absinthium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I
Leonurus cardiaca	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Tanacetum vulgare	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Plantago major	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	I
Cardaria draba	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	I
Verbascum blattaria	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	r	I
Lathyrus sylvestris	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I
Calamagrostis epigeios	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	I
Odontites vulgaris	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	I
Vicia tetrasperma	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	I
Lamium maculatum	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	-	I
Equisetum ramosissimum	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	I
Plantago lanceolata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	I

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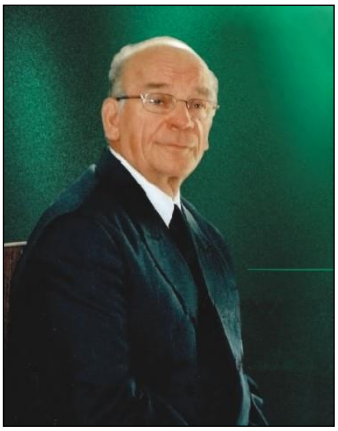
Professor univ. dr. eng. Mandache LEOCOV

(November 20, 1928 – October 16, 2018)



Just a few weeks ago, the man, professor and agronomist Mandache Leocov, left us discreetely and in dignity.

He was born on November 20, 1928, in the village of Prelipca, Salcea commune (Suceava County), in a family of peasants. After attending the Primary School in his native village (1936-1941), he graduated the Boys' High School (called now "Ştefan cel Mare" College) in Suceava (1949). After completing the high school he passed an entrance examination at the Faculty of Agronomy at the Polytechnic Institute of Iaşi, which he graduated in June, 1953, with the grade "very good". In the same year, on October, 15, he was appointed as assistant to the Department of Social Sciences and Political Economy of the Polytechnic Institute of Iaşi, where he worked until the autumn of 1954, after which he was transferred to the Agronomic Institute of Iaşi, at the Department of Social Sciences. In the academic year 1955-1956, as a result of the change of the educational plans, he was appointed as assistant at the Department of Pomiculture, discipline of Dendrology, within the Faculty of Horticulture, where he worked until 1958.

In the autumn of 1959, he was appointed as Deputy Director and Associate Professor of Agriculture at the Faculty of Natural and Agricultural Sciences, at the founding of the 3-year Pedagogical Institute in Iaşi. As Deputy Director, he skillfully contributed to the renovation and adaptation of the old building (former Normal School of Girls "Mihai Sturza") to the new requirements of education, improved the technical-material basis of the six established faculties (Faculty of Philology, Faculty of Mathematics, Faculty of Natural and Agricultural Sciences, Faculty of Physics-Chemistry, Faculty of Music-Design and Faculty of Physical Education), organizing the "the Laboratory of Agricultural Sciences", a school farming lot with herbal collections, other study facilities. He developed the *Agrofitotechnics Course*, which included Pedology, Agrotechnics and Fitotechnics, in 1964.

In 1962, when the agronomists, zootechnics and veterinary surgeons were redistributed, he was transferred as engineer at CAP / Agricultural Cooperative of Production / Micleşti (Vaslui County), taking in the cumulation the didactics, also.

In Micleşti, he set up new vineyards and orchards, expanded the vegetable culture and started the tobacco culture, made a lot of forest plantations on the degraded land, with an anti-erosive role. During the winters, he held classes in the agro-technological educational circles, for the professional training of the cooperative members.

In 1966 he returned to the chair, and in 1970, when the Pedagogical Institute was integrated into the University of Iaşi, he became a lecturer at the Faculty of Biology and the Faculty of Economic Sciences (for the subjects of Agrofitotechnics and the Basics of Agriculture). In 1977 he became professor.

He get the title of “Doctor of Agricultural Sciences” in 1972, with the thesis “Agro- and Geobotanic Study of Weeds in Vasluieț Basin”, under the direction of Professor Mihai Răvăruț, at the Agronomical Institute of Iași.

Between 1973-1990 he, also, was director of the “Anastasie Fătu” Botanical Garden in Iaşi, where he continued the activity of his ancestors, putting his personal footprint on many achievements within the institution, of which: opening the access of visitors directly from the "Exhibition Park", by taking over of the so-called land “Petrescu” within the Botanical Garden; installation of a main gate, made of sculptured oak timber (a masterpiece of Professor Dumitru Zaucă) and of a second gate, as a wooden sculptured in popular style; setting up environmental environments (peatland, rocks); consolidation works on slopes subjected to active landslides; take-over of Dutch-type greenhouses from the Agronomical Institute of Iași, of 900 m^2; the completion, in October 1974, of the construction and opening the “Palmarium” greenhouse (designed in early 1973); reorganizing the ring in front of the administrative pavilion; the organization of the Rosarium section on the ground of the former nursery, of 1.55 ha, where more than 700 varieties of roses were planted, and its opening in 1979, etc.

At the scientific level, he organized, together with the teaching staff of the Biology-Geography-Geology Faculty, in Iași, national scientific symposiums, the first being held on October, 16-17, 1976, occasioned by the celebration of the 120th anniversary of the establishment of the first university botanic garden in Romania; the second symposium took place on October, 10-11, 1981, when the bronze bust of Dr. Anastasie Fătu was unveiled in the front of the administrative pavilion, the creation and donation of Iaşi sculptor, Eftimie Bârleanu.

A special initiative of the director Mandache Leocov was the organization of floral exhibitions in the botanical garden, with its own plants of the botanic garden; thus, the first exhibition was the one of the azalea (in 1981), and the one of chrysanthemum was organized in 1982, after which these floral exhibitions being organized each year, until today.

As a specialist in agriculture, landscape dendrology, ecology of exotic and indigenous decorative plants, Professor Mandache Leocov has published, alone or in collaboration, more than 110 studies and scientific articles, in specialized journals in the country.

Many articles of Professor Mandache Leocov evoke the memory of the forefathers (Ion Ionescu de la Brad, Anastasie Fătu) and his contemporaries (Ion Tarnavschi, Valeriu Cotea, Constantin Ciopraga, Gheorghe Bălțatu, Emilian Țopa, Constantin Oescu, Petru Magazin, Mircea Hatman, Constantin Liviu Rusu, Vlad Bejan).

Professor Mandache Leocov has also written many articles on popularizing science, nature conservation, the Botanical Garden in Iaşi in particular. He presented at the local radio and television stations, at the Romanian Academy, Iasi branch, at the Romanian Literature Museum in Iași, the art galleries “Pod Pogor-fiul” and “Anticariat D. I. Grumăzescu”, at the “Costache Negruzzi” Museum in Hermeziu-Trifești, at the “Dimitrie Cantemir” High School, etc., interesting expositions in various fields of agro-biological sciences and nature protection.

As a result of his recognition as a landscaping specialist, the local administrative authorities asked him to solve some problems related to the city of Iași, as it appears from the list of his papers, from the correspondence received and from the conferences held on different occasions. We remind you the green space around the Agronomic Institute in Iași, the landscaping project of the Bahlui River, the organization of the green spaces from the

Polytechnic Institute in Iaşi between "Red Bridge" and "Tudor Vladimirescu", the embankments of the Bahlui River, the Eternity Iaşi Cemetery, and others. Also, he was concerned with the realization and installation of the statues of some cultural personalities of Iaşi or Moldavia, for the protection of the Eminescu's lime tree (*Tilia tomentosa*) in Copou Park in Iaşi, of the corner so-called "Plopii fără soţ" (*Populus alba*) at Bucium, the "Unirii" horse chestnut (*Aesculus hippocastanum*) at Vişan, the green spaces of the current "Pallas" complex in Iaşi, and, last but not least, the presentation of the importance of Iaşi as a city of flowers and parks.

For his work in over seven decades, he was rewarded with numerous diplomas of excellence, as: from the "Alexandru Ioan Cuza" University of Iaşi, from the Romanian Horticultural Society, from the "Ion Ionescu de la Brad" University of Agricultural Sciences and Veterinary Medicine in Iaşi, from the National Association of the Veterans of the War, the Iaşi branch, from the "Dimitrie Cantemir" High School of Iaşi, and from the Association of the Environmentalists Club in Iaşi, from the Egreta-Iaşi Association, from the parish "Descent of the Holy Spirit" of Bucium, and others.

Whoever remains impressed by the way in which Mandache Leocov contributed passionately and skillfully to the development of the Botanic Garden of Iaşi, but also to the establishment of the spiritual relations with all those who protect the nature and the perennial values of the Romanian people.

Professor Mandache Leocov was guided on his last journey by the family members, colleagues, friends and all who valued him, resting peacefully and reconciled himself and the others in the Eternity Cemetery of Iaşi.

Cătălin TĂNASE, PhD. Univ. Professor
"Alexandru Ioan Cuza" University of Iaşi, Faculty of Biology
Adrian OPREA, PhD.
"Alexandru Ioan Cuza" University of Iaşi, "Anastasie Fătu" Botanical Garden

JOURNAL OF PLANT DEVELOPMENT
GUIDE TO AUTHORS

AIMS AND SCOPE OF THE JOURNAL

Journal of Plant Development is the official scientific journal of the "Anastasie Fătu" Botanical Garden, which belongs to "Alexandru Ioan Cuza" University from IAŞI, ROMANIA. It was first published in 1979 (at that time as "Culegere de Studii şi Articole de Biologie"). The new series begins in 1993 under the name "Buletinul Grădinii Botanice Iaşi". From 2008 on, it has been published under its present name "**Journal of Plant Development**". It appears in one volume, with one or two issues per year.

Journal of Plant Development (JPD) is an international journal that acts as a medium for the exchange of ideas and provides publication (yearly) of articles in all areas of Plant Science and Botany (of all 'plant' groups in the traditional sense - including algae, cyanobacteria, fungi, myxomycetes). It covers topics in plant development field, as well as the plant ecology. The Journal also covers related fields such as: plant conservation, plant taxonomy, plant embryology, phytosociology, ecology, plant morpho-anatomy and histology, comparative and developmental morphology, physiology, ecophysiology, plant distribution, natural and artificial habitats, ornamental plants, pharmaceuticals uses of plants, plant molecular biology, plant cell, tissue and organ culture etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. All articles published in JPD are peer-reviewed.

TYPES OF MANUSCRIPTS AND LANGUAGE

The journal publishes original research articles, short communications and reviews in English. Journal of Plant Development also publishes book reviews and conference reports. Manuscripts may be of any length, but must be clearly and concisely written.

Three main *types* of manuscripts may be submitted:

Original research articles: should reports results of a substantial, completed and original work, and describe new and carefully confirmed findings. Experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short communications: are suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods or techniques. The style of main sections need not conform to that of original research articles. Short communications are 2 to 4 pages in length.

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All contributions are subject to a double-blind reviewing process. Acceptance of papers is supervised by an international Editorial Board. Manuscripts considered suitable for peer review by the Editorial Board are sent to at least two referees (members of the Board or external). The journal gives referees a target of four weeks for the return of their reports. The review process takes between three weeks and two months.

PROOFS AND REPRINTS

Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage. Because JPD will be published freely online to attract a wide audience, authors will have free electronic access to the full text (in both HTML and PDF) of the article. Authors can freely download the PDF file from which they can print unlimited copies of their articles.

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Manuscripts should be submitted electronically by sending a message to gbot.is@uaic.ro or ana.cojocariu@uaic.ro. The message should include:

(1) a cover letter, that should include the corresponding author's full name, address and telephone/fax numbers and should be in an e-mail message sent to the Editor. A Cover Letter is to be made upon submission, sending a revision or re-submission.

(2) a text file with the entire text, as an attachment, whose name should begin with the first author's surname.

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Details on types of contributions

1. Original research articles

The papers will be published only in a foreign language (English), structured as follows: title, authors, affiliation of the authors, abstract, keywords, introduction, material and method, results & discussions, conclusions, acknowledgements, references, tables, figure captions.

Title should be a brief phrase describing the contents of the paper.

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Abstract should be concise informative and completely self-explanatory, briefly present the topic, state the purpose of the research, indicate significant data, and point out major findings and conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. References should therefore be avoided, but if essential, they must be cited in full, without reference to the reference list. Non-standard or uncommon abbreviations should be avoided but, if essential, they should be defined at their first mention in the abstract itself.

Key Words: five to seven words, the most important ones, in alphabetical order, after someone could discover your paper on the internet engines. Key words should not repeat the title of the manuscript.

The **Introduction** should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines. The introduction should conclude with a brief statement of the overall aim of the experiments and a comment about whether that aim was achieved.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely

without referring to the literature. Discussion and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The **Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

Conclusions should spell out the major findings of the work and may include some explanation of the significance of these conclusions and what further research should be done. Authors should include a general interpretation of the results in the context of current evidence, and not restricted to that which supports the findings of the present study.

Notes on contributors in maximum 65 words, provide short biographical notes on all contributors.

The **Acknowledgments** of people, grants, funds, etc. should be brief. People who contributed to the work but do not fit criteria for authorship should be listed in the Acknowledgments, along with their contributions. It is the authors' responsibility to ensure that anyone named in the acknowledgments agrees to being so named.

References should be listed at the end of the paper in alphabetical order. In the text, a reference identified by means of an author's name should be followed by the date of the reference in parentheses. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text. Authors are fully responsible for the accuracy of the references.

2. Short communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. Short Communications follow the same format as for the original research papers, with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion sections should be combined into a single section.

3. Review articles

Review articles are critical evaluations of material that has already been published. By organizing, integrating, and evaluating previously published material, the author considers the progress of current research toward clarifying a problem. Reviews should be concise and no longer than 14-16 printed pages. Reviews are also peer-reviewed.

4. Book reviews and conference reports

These types of contributions would not exceed an A4 format page.

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Proposals for Special Issues of full research papers that focus on a specific topic or theme will be considered.

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☐	The **body of the paper** is well structured (preferred structure is: Introduction, Material and methods, Results, Discussion, Acknowledgments and References).
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JOURNAL OF PLANT DEVELOPMENT

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