Study on dynamic and effects of arbuscular mycorrhizae in some ornamentals from Cluj conditions

(SUMMARY OF PhD THESIS)

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INTRODUCTION

Arbuscular mycorrhiza is a symbiotic association between plant roots and fungi from phylum *Glomeromycota*. Over 70% of vascular plants can establish this type of symbiosis (BRUNDRETT *et* TEDERSOO, 2018).

Arbuscular mycorrhizal fungi enhance plant nutrition particularly in phosphorus and receive in exchange from the plant a part of the carbon fixed through photosynthesis (PARNISKE, 2008; BERRUTI *et al*., 2015). These fungi are obligate biotrophs and cannot complete their life cycle in the absence of a living host. Currently are known 234 species, classified in 11 families and 4 orders from the class *Glomeromycetes* (SOUZA, 2015).

The degree to which various plants can benefit from arbuscular mycorrhiza can vary from species to species. Average mycorrhizal dependency for field crops is 44%, for forage crops is 56% while for herbaceous wild plants is 70%. Perennial plants present a higher mycorrhizal dependency than annuals (TAWARAYA, 2003).

In order to be able to establish arbuscular mycorrhizae, plants must have a genetic "arsenal", represented by specific genes. Expression of these genes ensures the communication between symbiotic partners from pre-colonization and throughout all colonization stages (BONFANTE *et* GENRE, 2010; NAKAGAWA *et* IMAIZUMI-ANRAKU, 2015). Not all cultivated plants can establish mycorrhiza. Some botanic families such as *Brassicaceae*, *Caryophyllaceae* and *Cyperaceae* do not, while in other botanic families only some genera are able to establish arbuscular mycorrhiza (MOORE *et al*., 2011).

Arbuscular mycorrhiza is characterized by structures that occur inside roots (hyphae, spores, vesicles and arbuscules) as well as outside roots (hyphae, spores and auxiliary cells) (Fig. 1). Identification of these structures allows the observation and study of mycorrhiza (BRUNDRETT, 2008; BŁASZKOWSKI, 2012).

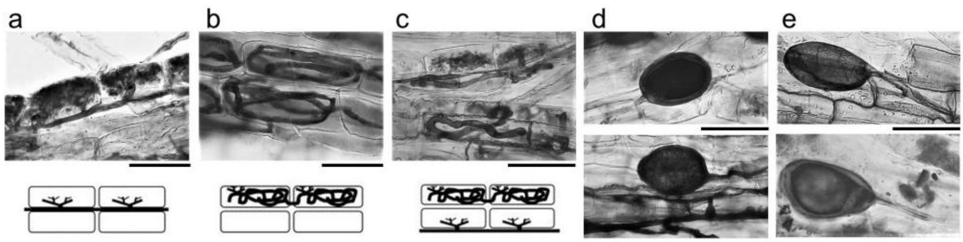

Fig. 1. Arbuscular mycorrhiza: *Arum* morphotype (a), *Paris* (b), *Arum+Paris* (c); intra-radicular spores (d) and vesicles (e); scale bar = 50 µm (Sources: IOANA CRIȘAN *et al*., 2018, 2019a, 2019b; DICKSON, 2004)

Following the root colonization, based on intra-radicular proliferation mode of the fungi, can be distinguished two main types: *Arum* and *Paris.* These were named after the plant species in which were first described: *Arum maculatum* and *Paris quadrifolia*. In *Arum* morphotype, hyphae are spreading between cells while inside cells are formed modified haustoria having the aspect of a "tree" (Fig. 1a). By contrast, in *Paris* morphotype, the hyphae are spreading from cell to cell forming intracellular coils (Fig. 1b). Through analysis of a large number of plants, was determined that there are several intermediate types, or cases when both main morphotypes are present in the same plant. These differences in colonization morphotypes between plant species are chiefly due to root histologic structure, in other words how restrictive the intercellular spaces are (DICKSON, 2004). Secondly, an influence is exercised by fungi species colonizing the plant. For example, endomycorrhiza in parsley (*Petroselium crispum*) corresponds to the intermediate morphotype when the plant is colonized by *Rhizophagus intraradices* and *Funneliformis mosseae*, and to *Paris* morphotype when is colonized by *Gigaspora margarita* and *Gigaspora rosea*. By contrast, the endomycorrhiza in leek (*Allium porrum*) corresponds to *Arum* morphotype regardless of colonizing fungi (DICKSON *et al*., 2007). In table 2 are presented the morphotypes found in main botanic families.

Table 2. Arbuscular mycorrhiza in main botanic families

Botanic Family	Morphotype[1]			
	A	I	A+P	P
Agavaceae, Begoniaceae, Vitaceae	x	-	-	-
Boraginaceae, Convolvulaceae, Cupressaceae, Geraniaceae, Iridaceae, Liliaceae, Rosaceae, Rutaceae, Sapindaceae, Ulmaceae, Violaceae	x	-	-	x
Oleaceae, Cucurbitaceae,	x	x	-	-
Zingiberaceae	x	x	-	x
Asteraceae, Myrtaceae, Polygonaceae, Primulaceae, Verbenaceae	x	-	x	x
Apiaceae, Fabaceae, Lamiaceae, Poaceae, Solanaceae	x	x	x	x
Caprifoliaceae	-	x	-	-
Araliaceae, Oxalidaceae	-	-	x	x
Adoxaceae, Equisetaceae, Gingkoaceae, Moraceae, Musaceae	-	-	-	x

[1](A) *Arum*, (I) Intermediate, (A+P) Both, (P) *Paris*
Sources: DICKSON, 2004; DICKSON *et al.*, 2007

Utilization of arbuscular mycorrhiza attracts a lot of attention in last years, reflected also by market expansion for mycorrhizal inoculants. Currently in Europe there are dozen companies producing inocula. Main sectors that employ mycorrhiza applications are gardening, landscaping, horticulture, agriculture and phytoremediation (CHEN *et al*., 2018).

Beneficial effects of arbuscular mycorrhiza are directly linked to root colonization. Thus, becomes evident the necessity to study colonization indicators in order to better understand: the factors of influence, the pattern in root system, the relationship existing between these parameters that evaluate the primary, secondary and tertiary colonization structures, as well as the significance of this relationship for biology of symbiosis and the plant. All these are essential to identify ways in which the utilization of arbuscular mycorrhiza may be optimized.

1. Structure of the PhD thesis

The PhD thesis comprises 148 pages and has two parts.

First part is dedicated to literature review and represents 32.43% from the thesis (48 pages), while the second part contains personal contribution representing 67.57% from thesis (100 pages).

Within PhD thesis are found 47 figures, 25 tables and 14 photo collages.

Part I of the thesis has two chapters:

Chapter 1. *Literature review of arbuscular mycorrhiza and other beneficial root fungal endophytes* is the most consistent part of the review section. This chapter summarizes most recent information on arbuscular mycorrhiza: definition, symbiotic plants, factors influencing spreading of fungi, beneficial effects for plants, taxonomy, biology of symbiosis and lastly are mentioned other important endophyte fungi groups that also colonize the roots of vascular plants.

Chapter 2. *Literature review of Iris assortment* is dedicated to the botanic characterization, importance and cultivation technology of rhizomatous iris assortment, because most important part of the researches were conducted on two species from this genus: *Iris germanica* and *Iris pseudacorus*.

Part II of the thesis contains five chapters:

Chapter 3. *Aim and objectives of research*, expresses the aim of the PhD research and names the specific objectives.

Chapter 4. *Material and method* contain detailed information regarding location and pedo-climatic conditions, biologic material, description of mycorrhizal inoculants used, organization of experiments, observations and determinations as well as laboratory techniques used. Last subchapter is dedicated to statistical methods.

Chapter 5. *Results and discussion* encompass the largest section of the PhD thesis, and presents the results of researches conducted on *Iris germanica* and *Iris pseudacorus*. Last subchapters of the thesis present the results of the complementary studies conducted on 25 plant species in local conditions.

Chapter 6. *Conclusions and recommendations* are organized in accordance with specific objectives of the research. Recommendations are related to the general use of arbuscular mycorrhiza or address producers of inoculants and researchers. Recommendations are in accordance with the results and main conclusions.

Chapter 7. *Originality and innovative contribution of thesis* mentions the novelty and contribution brought by the results of the PhD thesis to the knowledge within the domain of research.

References list contains 217 titles, from which 187 are books and articles most of them of recent date, while the rest of the titles (30) represent information bulletins, patents and web pages.

Annexes of the thesis contain 14 photo collages documenting different stages of the experiments.

2. Aim and objectives

Aim of the research was the identification of arbuscular mycorrhiza in the roots of an assortment of ornamental plants and the study of colonization dynamic and effects of inoculation in some species from genus *Iris* in conditions from Cluj-Napoca.

In order to accomplish the aim of the research, were conducted a series of researches each having specific objectives. The most important part of the research focused on two species from genus *Iris*, due the multiple importance these plants have: ornamental, medicinal and phytoremediation capacity. Not lastly, complementary researches on other plant species were meant to document the diversity of morphotypes and mycorrhizal potential in local conditions, as a reference point.

1). **Researches regarding arbuscular mycorrhiza of *Iris germanica***, had two specific objectives:

a) Evaluation of the effect of arbuscular mycorrhiza inoculation on *Iris germanica*, involving microscopic, biochemical and physiological determinations in regards with:
  - root colonization
  - biologic characteristics of the plants
  - composition of rhizomes from inoculated/non-inoculated plants
  - parameters that are in relation with assimilation rate: stomata.

b) Description of mycorrhiza dynamic in *Iris germanica*, respectively:
  - obtaining descriptive indicators for mycorrhiza continuity
  - identification of the influence of genotype and phenophase on colonization level.

2). **Researches regarding arbuscular mycorrhiza of *Iris pseudacorus*,** had two specific objectives:

a) Evaluation of the effect of arbuscular mycorrhiza inoculation on *Iris pseudacorus*, involving microscopic and morphometric determinations in regards with:
  - root colonization
  - biologic plant characteristics.

b) Description of mycorrhiza dynamic in *Iris pseudacorus*, respectively:
  - obtaining descriptive indicators for mycorrhiza continuity
  - assessment of the persistent effect of inoculation in second year after treatment.

3). **Researches regarding mycorrhizal status for an assortment of 25 plant species from local conditions**, had two specific objectives:

a) Identification of interspecific variation for root colonization by arbuscular mycorrhiza as well as AM morphotype diversity across a genotypic gradient

b) Comparison between AM incidence versus presence of other fungi groups inside roots (DSE and FRE).

3. Materials and methods

a) Location

Researches took place in Cluj-Napoca city, Romania, and experiments were located in Agro-Botanical Garden USAMV Cluj and in western part of the city.

Microscopic investigations were conducted at Microbiology Laboratory from Faculty of Agriculture, University of Agricultural Sciences and Veterinary Medicine from Cluj-Napoca.

Spectroscopic measurements were conducted at Laboratory of Vibrational and Raman Spectroscopy from “Life Sciences Institute King Michael I of Romania” from Cluj-Napoca.

b) Soil and substrates

Soil analysis was conducted at OSPA Cluj-Napoca.

Substrates used in pots were based on peat or bark humus.

c) Biologic material

Plant species used in the experiments were:

1) *Iris germanica* L. with six cultivars that present contrasting flower colors: 'Black Dragon' (black), 'Blue Rhythm' (blue), 'Sultan's Palace' (red), 'Lime Fizz' (yellow), 'Pinafore Pink' (pink), 'Pure As The' (white)

2) *Iris pseudacorus* L.

3) 25 species from Collections of Agro-Botanical Garden USAMV Cluj-Napoca: *Ageratum houstonianum, Aster dumosus, Coreopsis tinctoria, Cosmos bipinnatus, Crocus vernus, Dahlia variabilis, Galanthus nivalis, Gladiolus × grandiflorus, Helianthus annuus, H. tuberosus, Hemerocallis fulva, Hyacinthus orientalis, Iris reticulata, Leucojum aestivum, Liatris spicata, Lilium candidum, Muscari armeniacum, Narcissus poeticus, N. pseudonarcissus, Rudbeckia hirta, Scilla bifolia, Tagetes erecta, Tanacetum vulgare, Tulipa gesneriana, Zinnia elegans* (INDEX SEMINUM).

Mycorrhizal products used contained:

- 2 *Glomeromycota* fungi species (*Funneliformis mosseae, Rhizophagus intraradices*)
- 5 *Glomeromycota* fungi species (*Funneliformis mosseae, Rhizophagus intraradices, Claroideoglomus claroideum, Funneliformis geosporus, Rhizoglomus microaggregatum*).

d) Organization of experiments

Experiments took place between 2016-2019 according to table 2.

Table 2. Experiments regarding arbuscular mycorrhiza

Type	Plants	Study[1]	Interval
Experiments organized in randomized blocks	*Iris germanica* (6 cultivars)	Effect of inoculation (C)	2016-2018
		Colonization dynamic (C)	
	Iris pseudacorus	Effect of inoculation (G+C)	2018-2019
		Colonization dynamic (C)	
Collections of Agro-Botanical Garden USAMV Cluj-Napoca	25 plant species annuals/perennials	Mycorrhizal status	2018

[1]Experiments in field (C) or pots (G)

e) Laboratory analysis

Roots were stained for microscopy according to method by VIERHEILIG *et al*. (1998). Evaluation of root colonization was performed following methodology of TROUVELOT *et al.* (1986). In total, were assessed under microscope over 5000 root samples. Arbuscular mycorrhiza colonization indicators (AM) were obtained using Mycocalc software (https://www2.dijon.inrae.fr).

AM colonization indicators were:

- F% = frequency of colonization
- m% = intensity of colonization in root fragments
- M% = intensity of colonization in root system
- a% = abundance of exchange structures in root fragments
- A% = abundance of exchange structures in root system.

Stomata parameters were determined under microscope on leaf imprints.

Spectroscopic analysis (Fourier Transform Infrared Spectroscopy) for rhizome samples were conducted using KBr pellet technique.

f) Statistical methods

Data were analyzed using ANOVA, Duncan test, correlation and PCA.

4. Results and discussion

4.1. Results and discussion of researches regarding arbuscular mycorrhiza of *Iris germanica*

Iris germanica presented typical *Paris* colonization morphotype.

For *Iris germanica* plants was identified a positive significant correlation between inoculation treatment and development rate of the shoots (r=0.25*) but insignificant between inoculation and growth dynamic (r=0.12). Thus, development rate increased from 71% in non-inoculated plants to 88% for inoculated plants, while growth dynamic increased from 28% in non-inoculated plants to 31% in inoculated plants.

Potential stomatal conductance index for leaf stomata increased on average with 12.77% for inoculated plants compared to non-inoculated plants.

Interpretation of the FT-IR spectroscopic analysis of rhizome samples suggests that due to AM inoculation some cultivars showed an improved accumulation of starch in rhizomes ('Pinafore Pink') or of myristic acid ('Sultan's Palace', 'Lime Fizz', 'Pinafore Pink' and 'Pure As The').

In local conditions, phenophase exercised a highly significant influence over frequency of colonization and intensity of colonization in root fragments (F%, m%, p<0.001***) explaining over 50% from variance registered for these parameters (η^2= 0.69, 0.75). By comparison, the cultivar did not have a significant influence on AM colonization indicators (F% p=0.68, m% p=0.78, M% p=0.84).

Between frequency of colonization in spring and frequency of colonization in autumn was identified a significant correlation (r=0.34*), hinting to the importance of early root colonization as well as to the existence of mycorrhizal continuity during the entire growth season.

Towards autumn, the frequency of colonization decreased (-18.36%) but there was an increase of intensity of colonization in root system (56.30%) as well as of the frequency of intra-radicular spores and vesicles (22.73%) compared to spring, signaling the transition of the symbiose towards a latent endophytic phase in response to metabolic state of the plants.

4.2. Results and discussion of researches regarding arbuscular mycorrhiza of *Iris pseudacorus*

Iris pseudacorus presented typical *Paris* colonization morphotype.

In the first experimental year (2018), inoculation treatment explained 4.85% from the variance for frequency of colonization and 1.51% from variance for intensity of colonization in root fragments. In pot conditions, inoculation treatment was correlated significantly positive (r=0.47*) with intensity of colonization in root fragments, proving the effectiveness of mycorrhizal products. However, interaction between growth conditions (pots/field) and inoculation treatment exercised a significant influence on the frequency of colonization (p=0.03*).

Also, in pot conditions was identified a positive correlation between substrate type and all colonization indicators. This demonstrates that substrate type can be directly responsible for the success of symbiose.

Inoculation did not exercised a significant influence on colonization indicators during second year after treatment (F% p=0.45, m% p=0.65, M% p=0.95). But, the average value of intensity of colonization in root fragments was 12.05% higher in plants that were inoculated in previous year.

Phenophase exercised a highly significant influence over frequency and intensity of colonization in root fragments and root system (F%, m%, M% p<0.001***) and explained over 90% from variance registered for colonization parameters ($\eta^2 \geq 0.95$).

It was registered a decrease for all colonization indicators towards autumn: of 79.34% for intensity of colonization in root system, of 71.71% for intensity of colonization in root fragments and respectively of 45.73% for frequency of colonization, marking the decline of the symbiose towards the end of growth season.

It was identified that intensity of colonization in root fragments in a new vegetative year depends of intensity of colonization in root system in autumn (r=0.64*).

Also, it was identified a significant correlation between plant height and inoculation treatment from previous year (r=0.33*), demonstrating the long-term effect of mycorrhizal products.

4.3. Results and discussion of researches regarding mycorrhizal status for an assortment of 25 plant species from local conditions

In the conditions from Agro-Botanical Garden USAMV Cluj-Napoca, was identified that studied plants were colonized by fungi from phylum *Glomeromycota* (arbuscular mycorrhiza=AM) as well as by other micromycetes groups: from phylum *Ascomycota* (dark septate endophytes=DSE) and subphylum *Mucoromycotina* (fine root endophytes=FRE).

Based on principal component analysis (PCA), were distinguished 3 groups across the genotypic gradient (Fig. 2).

- First group (A) was characterized by high DSE colonization frequency (55-85%) and variable AM frequency (35-90%) while FRE occurred only in some species.
- Second group (B) was characterized by FRE presence simultaneous with moderate or high AM colonization (35-80%), but higher FRE frequency levels were associated with lower AM levels (such as for *Coreopsis tinctoria* and *Tagetes erecta*).
- Third group (C) was characterized by high AM colonization frequency (75-100%) concomitantly with moderate or low DSE levels (<20%) and variable FRE levels (<35%).

Orientation of the vectors (F% AM, DSE, FRE) suggests a potential negative interaction between these micromycetes groups (Fig. 2), that could be explained through competition for carbon from plant. Colonization morphotype corresponded to typical *Arum* for *Tulipa gesneriana* and *Dahlia variabilis*, typical *Paris* for *Helianthus tuberosus* and *Hemerocallis fulva*, while in some species were present intermediate morphotypes (*Cosmos bipinnatus*) or atypical ones (*Lilium candidum*).

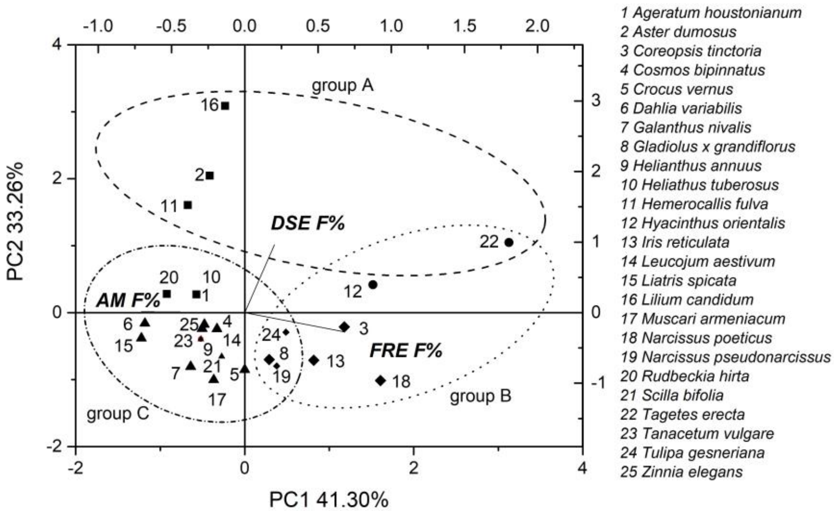

Fig. 2. Endophytic frequency (F%) for AM *(Glomeromycota)*, FRE *(Mucoromycotina)* and DSE *(Ascomycota)* in root of 25 plant species in conditions from Cluj-Napoca (2018)

5. Conclusion and recommendations

Iris pseudacorus and *Iris germanica* presented AM *Paris* colonization type.

The fact that phenophase exercised significant influence on colonization indicators demonstrates the control of plant metabolism over the symbiose.

It is recommended the AM inoculation of *Iris germanica* plants in order to obtain a good vegetative development before flowering and to improve the quality of rhizomes destined to processing.

It is recommended the AM inoculation of *Iris pseudacorus* plants in phytoremediation programs due to lasting positive effects on vegetative development.

In local conditions was notable the influence of genotypic gradient on root colonization by arbuscular mycorrhizal fungi, hinting to a potential specificity.

6. Originality and innovative contribution of the thesis

The thesis brings a contribution to the understating of biology, behavior, dynamic and effects of arbuscular mycorrhizae in local conditions.

Through the results obtained was demonstrated the possibility to use the AM colonization indicators to reconstruct the continuity of mycorrhiza between phenophases and vegetation seasons. This is very useful in field conditions.

Through the PhD research was obtained:

- first complete description of the morphotype and colonization dynamic of arbuscular mycorrhiza for *Iris germanica*, the most cultivated species from this genus
- first attempt to characterize *Iris germanica* rhizomes using FT-IR, and proposal of two spectral regions as quality markers for rhizomes
- first documentation of AM colonization morphotypes diversity in local conditions.

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