Research Journal of Agricultural Science, 49 (4), 2017

UTILIZATION OF ARBUSCULAR MYCORRHIZAE IN THE CULTIVATION OF ORNAMENTAL PLANTS

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Abstract. *Researches demonstrated that mycorrhizal inoculation determines beneficial effects on plants that can translate in economic advantages for flower growers. Suitable for inoculation with AMF are both greenhouse and field cultivated ornamental plants that are able to establish this kind of symbiose. The aim of the study was to review the available AM applications in cultivation of ornamental plants. Currently on the market can be found a variety of mycorrhizal products: gel, powder, granules and pellets with spores as well as fertilizer with low dose of spores. Confirmation of successful AM colonization could be conducted on farm by trained employees using cheap staining methods.*

Key words: *potted plants, nursery, commercial aspect, flower size, flower number, rooting*

INTRODUCTION

Since the roles of arbuscular mycorrhizae started to be distinguished in early researches, various studies explored the specific influence they have on plants. Thus, the beneficial effects identified early on, triggered the interest to further investigate the advantages they might bring for certain cultivated plants (LOVATO *et al.,* 1999). The results were promising, and the potential was used by the new emerging market segment of commercial inoculum that diversified its products just as the demands of producers came from different agricultural and horticultural sectors. Today are available a large variety of arbuscular mycorrhizal products (https://www.mycorrhizalproducts.com, http://www.agrocode.com, http://www.soilmoist.com, http://www.rootgrow.co.uk, https://www.planetnatural.com) while the information on their application might be more or less easy available. Firstly, the flower growers need to find out if the crops of interest for them are able to establish symbiotic relationships with arbuscular fungi (AMARANTHUS *et al.,* 2010; http://www.mycorrhizae.com). and to what degree each species might benefit from the investment in the inoculum. Once these settled, the next questions are related to the suitable type of product, its mode and frequency of application as well as quantities needed. Besides the assistance from the supplying company, most of this information is available on the label or application instructions.

Although to this day a wide range of high demand ornamental plants were studied in relation with inoculation, researches do not cover completely all the current cultivated assortment and this paper mentions the main flower species categories researched in relation to AMF inoculation.

MATERIAL AND METHODS

Various sources were reviewed for AMF applications in cultivation of ornamental plants. Firstly, the aim of the study was to highlight that beneficial effects of AMF inoculation for ornamental plants translate in advantages for flower growers and in this regard, results of a few researches conducted for the main ornamental categories: potted plants, cut flowers, and garden plants are discussed. Also, it is described on short the current AMF utilization for ornamental cultures together with the available products. The most accessible and low-cost methodology found in literature for AM root staining was tested on *Iris* sp. roots taken from plants grown in Botanical Garden UASVM Cluj-Napoca, and observations were conducted with a Biolux NV Bresser Microscope, in order to identify the potential for identification and quantification of successful AM colonization for ornamental plants with minimum equipment, that might be available for flower growers as well.

RESULTS AND DISCUSSIONS

Suitable ornamental plant cultures for AMF application are those species that can establish AMF symbioses. Main ornamental Angiosperm plant families that establish arbuscular mycorrhizal symbioses are presented in table 1 and can be divided in two groups. First one, includes the botanic families known to establish AMF for all their studied species so far (+). Some important ornamental plants from these families are balcony, garden and cut flower species from genera *Galanthus, Amaryllis, Hippeastrum, Hyacinthus, Convallaria, Allium, Bellis, Callistephus, Chrysanthemum, Cichorium, Dahlia, Tagetes, Antirrhinum, Clematis, Crocus, Cyclamen, Freesia, Gentiana, Iris, Lilium, Ranunculus, Nicotiana, Petunia, Pelargonium, Physalis, Primula, Tulipa* as well as indoor plants *Agave, Asparagus, Chlorophytum, Dracaena, Echinocactus, Opuntia* and common palm tree species grown indoors like *Chamaerops* and *Phoenix*. The second group includes ornamental plants from families that present both species able to establish symbiosis with arbuscular mycorrhizal fungi as well as plants unable to establish such relationships (+/-) and some ornamental genera found in these families are: *Sedum, Sempervivum, Abutilon, Hibiscus, Lupinus, Mimosa,* etc. At last, there are several families that are non-mycorrhizal (-) and reunite genera *Amaranthus, Alternanthera, Celosia, Gomphrena, Cistus, Helianthemum, Cerastium, Cleome, Dianthus, Alyssum, Portulaca* etc. (Table 1) (https://mycorrhizae.com, https://mycorrhizas.info).

Table 1

Arbuscular mycorrhizal status of main ornamental plant families

Angiosperm families	AM status
Amaryllidaceae, Apiaceae, Arecaceae, Asparagaceae, Asteraceae, Cactaceae, Euphorbiaceae, Gentianaceae, Geraniaceae, Hydrangeaceae, Iridaceae, Lamiaceae, Liliaceae, Paeoniaceae, Plantaginaceae, Primulaceae, Ranunculaceae, Rosaceae, Solanaceae, Verbenaceae, Violaceae	+
Malvaceae, Fabaceae, Crassulaceae, Cyperaceae, Araceae	+/-
Amaranthaceae, Brassicaceae, Bromeliaceae, Caryophyllaceae, Cistaceae, Cleomaceae, Portulacaceae	-

(+) AMF symbionts, (+/-) sometimes symbionts, (-) non-mycorrhizal
Sources: https://mycorrhizae.com, https://mycorrhizas.info

There can be identified three main types of cultures of ornamental plants suitable for mycorrhization: conventional cultures of nursery and potted plants as well as field cultivated annuals, biennials and perennials, but also non-conventional cultures such as hydroponic grown ornamental plants. The products used for AMF inoculation are found as: gel, powder, granules and pellets with spores or plant food with low dose of AMF spores for grown plants or geophytes. Also, the products can include several AMF species or just one. Regarding the method for inoculation, there can be identified six main ones: 1) on seeds before sowing; 2) on rooting or rooted cuttings, bare roots and seedlings; 3) at transplanting of plantlets or plants; 4) at crop starting of perennials in the field including geophytes; 5) by mixing pure mycorrhizal product with cultivation substrate; 6) through fertirrigation system (AMARANTHUS *et al.*, 2010;

MEIR *et al.*, 2010; https://www.mycorrhizalproducts.com, http://www.agrocode.com, http://www.soilmoist.com, http://www.rootgrow.co.uk, https://www.planetnatural.com).

Today, there is a wide range of methodologies for identification and measuring the degree of root colonization by arbuscular mycorrhiza. The simplest one is the non-destructive observation of the roots that provides only limited information (fig. 1) while the most revealing and used ones are the staining methods (VIERHEILIG *et al.*, 2005). There are several microscopy techniques that make use of either vital or non-vital staining methods, but there are also biochemical markers as well as molecular tools for investigation that because are costly are less routinely used (VIERHEILIG *et al.*, 2005; SUN *et* TANG, 2012). Among the current staining methods, the one developed by Vierheilig *et al*. (1998) and adapted by Stoian *et* Florian (2009) is the most available because it makes use of low-budget and less toxic agents like ink, vinegar and NaOH to obtain a good staining of the roots that allows the quantification of mycorrhizal colonization under a light microscope (fig. 2) (VIERHEILIG *et al.*, 1998; STOIAN *et* FLORIAN, 2009). When it comes to the quantification methods, although in the last decades many techniques were proposed, Sun *et* Tang (2012) indicate four methods as remaining the main ones used for assessing root AMF colonization: root segment ± method, the root-segment estimation method modified by Trouvelot *et al.* (1986), grid-line intersect method adopted by Giovannetti *et* Mossae (1980) and magnified intersections method designed by McGonigle *et al.* (1990) (SUN *et* TANG, 2012). Currently there are sources online that provide indications for assessing root colonization and calculation of key indicators (http://www2.dijon.inra.fr).

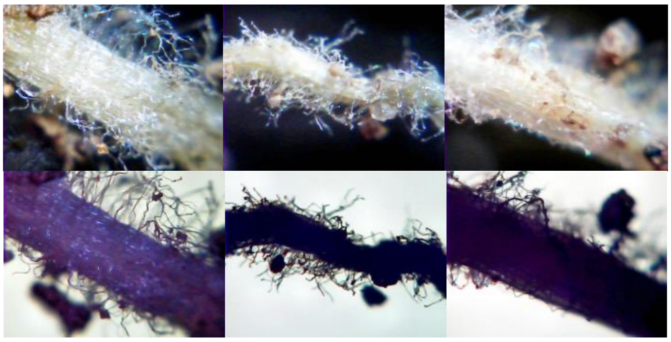
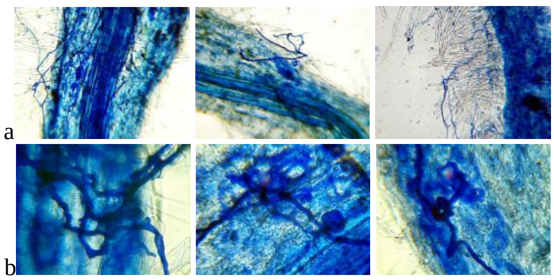


Figure. 1. Non-destructive observation of AM colonized *Iris* sp. roots (Original)



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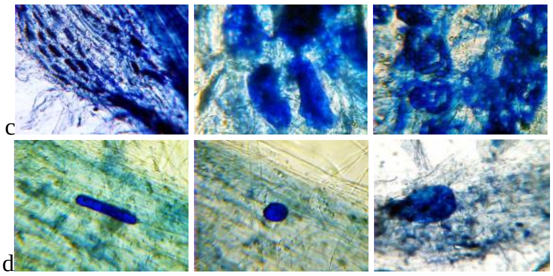

Figure 2. AMF structures in *Iris* root, evidenced by staining using NaOH, blue ink and vinegar: a) extra-radicular hyphae; b) intra-radicular hyphae; c) arbuscules and coils; d) vesicles (Original)

Arbuscular mycorrhizae application in floriculture can promote crop growth and yield, because the symbiosis enhances nutrient cycling, improves rooting, plant establishment, vegetative growth, accelerates budding and flowering, promotes the plant ability to resist drought, salt stress and pests (KOLTAI, 2010).

Some studies tried to identify how the potted cultivation in peat substrate might influence the response to inoculation of ornamental species. It was found that inoculation with commercial AMF significantly increased growth parameters important for ornamental plants. In two inoculated *Pelargonium* species grown on peat-based substrate was identified an increase in root and shoot dry weight, along other beneficial effects (PÜSCHEL *et al*., 2014) while another study identified that mycorrhizal colonization increased the number of buds and flowers in *Pelargonium peltatum* in substrate made of peat and compost (PERNER *et al*., 2007). The positive results of inoculation for peat grown potted plants were identified also for several other species. In *Sanvitalia procumbens* AMF increased number of flowers, shoot and root dry weight as well as the length of plant and branches; in *Impatiens hawkeri* inoculation lead to increased flower size while for *Verbena × hybrida* the inoculation lead to an increased flower number (PÜSCHEL *et al*., 2014).

Rosa hybrida is an important cut flower crop, and studies showed that although inoculation with *Glomus mosseae* and *Glomus intraradices* does not necessarily influence the plant biomass at harvest in this species, flower production was positively influenced by *Glomus mosseae* compared to uncolonized plants. Although this experiment was not able to link or identify a significant influence also for *Glomus intraradices* on flowering of rose plants (GARMENDIA *et* MANGAS, 2012), a previous experiment was able to identify the protective effect of *Glomus intraradices* in drought induced to roses plants up to a certain threshold (PINIOR *et al*., 2005). For *Eustoma grandiflorum* - another high income cut flower, growth and yield was positively influenced by AMF inoculation, especially when introduced to the growth medium during sowing and to the pit hole during planting. Inoculated plants presented significantly enhanced growth and yield parameters including flower stem length, number of flowering stems per square meter, as well as a slightly higher resilience to pathogenic fungi (MEIR *et al*., 2010).

For the plants used in green spaces and gardens, like *Tagetes patula*, growth enhancement was identified in controlled-environment experiments as response to inoculation with *Glomus intraradices* (LINDERMAN, 2003) as well as with species: *Glomus aggregatum*, *Glomus intraradices* and *Glomus mosseae* (SCHMIDT *et al*., 2015). Another research on this flower species failed to link inoculation to beneficial effects, on contrary heavy root colonization determined a carbon drain that might have caused fewer flowers (GAUR *et* ADHOLEYA, 2003).

In geophyte plants, like *Gladiolus grandiflorus*, vegetative and reproductive growth was linked to colonization in early growth stages (JAVAID *et* RIAZ, 2008) while the inoculated *Lilium* bulblets presented significantly higher growth variables (VARSHNEY *et al*., 2002). However, for several of the geophyte species studied (*Brodiaea laxa*, *Zephyranthes* sp., *Sparaxis tricolor*, *Freesia* × *hybrida*, *Zantedeschia* sp., *Canna* sp.), an earlier and more abundant flower yield in response to AMF inoculation was linked to the production of smaller daughter bulbs and more offsets than non-inoculated plants (SCAGEL, 2004). All these aspects might present great importance for producers of planting material.

Summarizing the consulted sources, firstly it can be noted that not all aspects regarding the influence the mycorrhization is having on plants is sufficiently clarified yet. However, it can be concluded that the importance of arbuscular mycorrhizae for ornamental crops stands in the fact that identified beneficial effects brought by AMF for ornamental plants can be translated in advantages for flower growers. From potted plants to cut flowers and garden flowers, five main such aspects linked to AMF inoculation can be defined: 1) improved plant nutrition leads to reduction in fertilization costs; 2) healthier plants attracts less pesticide application; 3) better rooting and survival rate at transplanting translates in reduced loses in seedlings and plantlets; 4) faster growth attracts a shortening of growing cycle for cultures; 5) better vegetative development and flower count means also improved commercial aspect of the plants or higher flower yield.

CONCLUSIONS

Utilization of arbuscular mycorrhizae has positive economic implications for flower growers because inoculation can attract a reduction in fertilization costs and improvement of the flower yield or commercial aspect of plants. Currently, on the market are available a variety of inoculation products for greenhouse and field cultivated ornamental crops. Confirmation and assessment of successful AMF colonization can be conducted on farm by trained employees utilizing staining methods that make use of cheap agents like sodium hydroxide, ink and vinegar followed by observation under dissecting or compound microscope at low magnification.

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