THE EFFECTS OF SUPPLEMENTARY MYCORRHIZATION REGARDING SOME VEGETATIVE CHARACTERISTICS AT *IRIS GERMANICA* L.

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Abstract

Arbuscular mycorrhizal fungi can establish associations with over 80% of land plants. Most important beneficial effects for plants are: phosphorus availability, increased resistance to abiotic and biotic stress. Studies regarding the effects of mycorrhization on ornamental plants in our local pedoclimatic conditions are almost inexistent. The aim of this study was the evaluation of mycorrhizal inoculum effect over some vegetative characteristics for six *Iris germanica* cultivars. The experimental field was settled in Botanical Garden of UASVM Cluj-Napoca. For each cultivar were established two treatments: non-inoculated and inoculated with mycorrhizae. Results indicate that regarding the percentage of buds that entered vegetation in spring was higher for inoculated plants compared to non-inoculated plants. Inoculated cultivars 'Sultan's Palace' and 'Pinafore Pink' had a higher number of shoots grown in spring than number of buds present in autumn. For most of the studied cultivars the mycorrhizae products exercised beneficial effects regarding the entering in vegetation of buds, or stimulating formation of new buds. This phenomenon is attributed both to mycorrhizal fungi and auxins added to inoculum.

Key words: arbuscular mycorrhizae, plant development, cultivars, bioproducts

Arbuscular mycorrhizal fungi are a group of obligate biotrophs that can establish mutual relationships with over 80% of plants (Berruti A. *et al*, 2015). They function as regulators of nutrient absorption and transfer in the rhizosphere (Stoian V. *et al*, 2014). The symbiosis improves root formation and plant establishment presenting importance for the cultivation of many ornamental plants (Koltai H., 2010). In exchange, the symbiotic fungus can receive up to 20% of the carbon fixed by the plant (Parniske M., 2008). Numerous studies were able to link specific beneficial effects involving vegetative and reproductive parameters to mycorrhizas for a variety of flowering species: *Rosa hybrida*, *Eustoma grandiflorum*, *Gladiolus grandiflorus*, *Brodiaea laxa*, *Pelargonium peltatum*, *Petunia hybrida*, *Tagetes erecta*, *Chrysanthemum morifolium* (Koltai H., 2010). Inoculation with mycorrhizae is appealing in particular to the flower growers because it can attract reduction in fertilization costs and improve the vigor and quality of plants that are important commercial characteristics for this category of plants. Because mycorrhizae increase physiological volume of the root and translates in faster growth and shortening of the growing cycle has potential impact on the production costs (Amaranthus M. *et al*, 2010).

In natural conditions of a subtemperate climate, Kumar A. *et al* (2012) identified 23 mycorrhizae species colonizing the roots of some ornamental plants. The dominant genera were *Glomus* and *Acaulospora* followed by *Sclerocystis* and *Gigaspora* while the rarest was the genus *Entrophospora*. The root colonization varied by flower species with the highest levels of colonization identified for species *Senecio cineraria*, *Rosa indica*, *Catharanthus roseus*, *Dahlia variabilis*, *Nerium indicum*, *Hydrangea paniculata* and the lowest levels of colonization for *Jacobinia carnea*, *Hibiscus rosa-sinensis*, *Lilium rubescens*. Intermediate levels of root colonization were identified for species *Chrysanthemum leucanthemum*, *Salvia splendens*, *Aster amellus*, *Gladiolus grandiflorus*, *Tagetes patula*.

Currently on the local market can be purchased inoculation products for ornamental species, yet studies of their effects in the local pedoclimatic conditions are lacking. In Romania, to this date the mycorrhizae in ornamental plants were researched in a controlled environment experiment with different nutritional conditions for *Tagetes* sp. (Schmidt B. *et al*, 2015). Studies conducted abroad could provide the cultivation technology bases for the inoculation of potted plants, but the inoculum application effects for plants outdoors in our conditions are unknown.

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In spring geophytes, most vegetative growth takes place prior to flowering and the plant is entering a semi-dormant phase during summer. For genus *Iris*, the buds that will develop in shoots next season start to form on geophytic organ from late summer. During winter, plants are in dormancy and shoots start to grow in spring. In bulbous species, temperature treatments cause the plants to sprout and flower earlier constituting the cultivation technology basis for cut flowers produced all year round (Rodrigues P.A.S., 1962; Okubo H., Sochacki D., 2013; Khodorova N.V., Boitel-Conti M., 2013). The geophyte plant depends on the photosynthetic mass for accumulating nutritional reserves in the geophyte organ that ensures the perennity (Khodorova N.V., Boitel-Conti M., 2013).

Regarding the mycorrhization effects on geophyte ornamental plants, previous studies were conducted on *Iris pseudacorus* (Wężowicz K. *et al*, 2015), *Lilium* sp. (Varshney A. *et al*, 2002), *Zantedeschia* sp. (Scagel C.F., Schreiner R.P., 2006), *Freesia hybrida* (Rezvanypour S. *et al*, 2015), all indicating improved vegetative parameters. For the genus *Iris,* arbuscular mycorrhizae applied as inoculum determined an accelerated metabolic rate and growth of geophytic organ. In particular it was demonstrated that mycorrhization of *Iris* plants increased the absorbing rate of both nitrogen and phosphorous (Chen Y. *et al*, 2014). From a perspective of phytoremediation, symbiosis between *Iris pseudacorus* and the following AMF species used for inoculation: *Diversispora epigaea*, *Glomus aureum*, *Rhizophagus irregularis*, *Rhizophagus clarus*, showed a good adapting potential to the presence of toxic metals in the environment (Wężowicz K. *et al*, 2015). Also, there were identified differences in the effect determined by different mycorrhizal fungi species. The promoting effect over photosynthesis rate of *G. mosseae* in *Iris* plants was significantly better than of *G. intraradices* (Chen Y. *et al*, 2014).

Although previous studies were able to highlight some beneficial effects of arbuscular mycorrhization on *Iris pseudacorus* plants, little or no studies were conducted so far in regards with other *Iris* species known for their economic importance, like *Iris germanica* (Crișan I., Cantor M., 2016). Also, studies on inoculation effect of ornamental plants in the local conditions are missing. Thus, the aim of this study was the evaluation of mycorrhizae inoculum effects regarding some vegetative characteristics for six *Iris germanica* cultivars in the conditions of Cluj County.

MATERIAL AND METHOD

The experimental field was settled in October 2016 in the Botanical Garden of UASVM Cluj-Napoca Romania, situated at 46°45´36´´ lat. N; 23°34´24´´ long. E; elevation (AMSL) 380-430 m. The average annual temperature is 8.1°C and average sum of annual precipitation 635 mm (Index Seminum). The physical—chemical analysis of the soil conducted at the O. S. P. A. Cluj indicated a clay loam soil type with 6.72 pH, low humus level (1.35%) and good NPK supply (N 0.461%, P 68 ppm, K 312 ppm).

A bifactorial experiment in randomized blocks with three replicates was established: factor A – the cultivar with six levels (a_1 = 'Black Dragon', a_2 = 'Blue Rhythm', a_3 = 'Sultan's Palace', a_4 = 'Lime Fizz', a_5 = 'Pinafore Pink', a_6 = 'Pure as The') and factor B – the treatment applied with two levels (b_1 = non-inoculated at planting and b_2 = inoculated at planting with mycorrhizal products). From the combination of the two factors resulted 12 experimental variants. Between the mycorrhized blocks and non-mycorrhized ones was ensured 7 m, that according to Powell C.L. (1979) considering the average spreading speed of mycorrhizal fungi through non-sterilized soil it would ensure the validity of the data collected for two-three years. On average was used 13 grams of mycorrhizal inoculum per plant.

The plant material was purchased from the company "Anthesis International" from Bucharest, and consisted of 108 rhizomes of six *Iris germanica* cultivars, imported from Holland. The inoculation products used were produced by PlantWorks from U. K. and ordered online. The two Empathy Root Grow products were: "After Plant" with general use for ornamental flower species, and "Bulb Starter" for ornamental geophytes. The products consisted of organic material, seaweed meal, vermiculite with added humates, organic acids and auxins among their ingredients, along with a mix of mycorrhizal spores.

Observed parameters were: weight of rhizome and number of buds at planting time, number of shoots in spring and height of the plants in two moments before flowering (IV.2017 and V.2017) as well as number of leaves (*figure 1*).

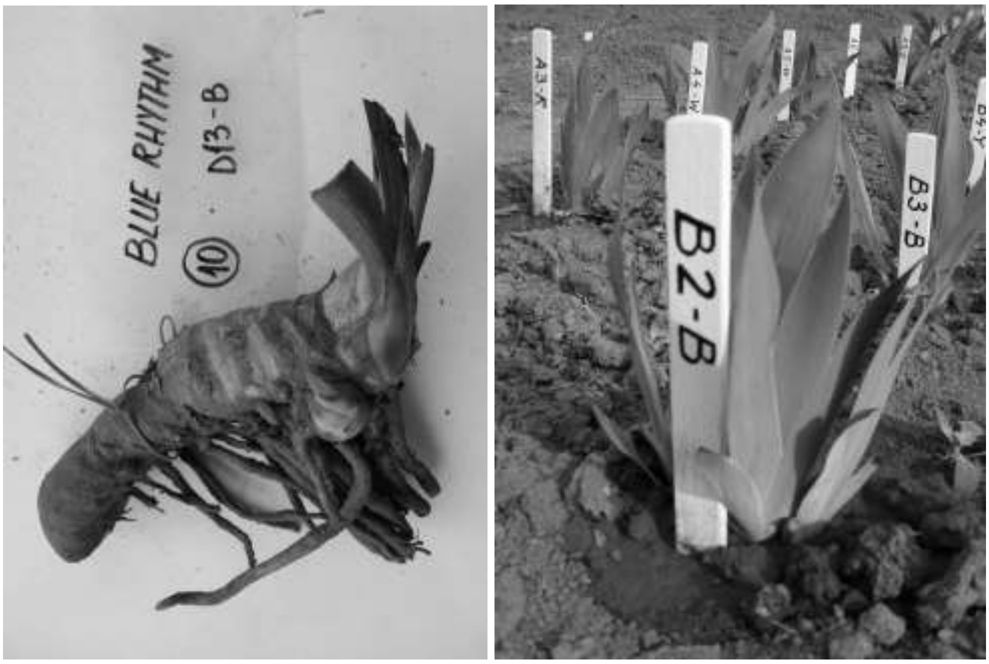

Figure 1 ***Iris germanica*** **rhizome with buds in autumn and plants with shoots and leaves in spring**

Two indicators were used to assess the studied plants in first phenophases: development rate and growth dynamic. Development rate was calculated using the formula: s/b x 100, where 's' represents the number of shoots per plant before flowering in spring and 'b' the number of vegetative buds present on rhizomes in autumn. The growth dynamic was calculated using the formula: $\Delta h/h_2$ x 100, where 'Δh' represents the difference between the height of the plant in May (h_2) and height in April (h_1). For statistical analysis were used Duncan test and Pearson correlation.

RESULTS AND DISCUSSIONS

Comparing the development rate and growth dynamic of the plants, on average was higher for the inoculated plants than non-inoculated plants (*figure 2*).

Analysis of development rate (*table 1*), revealed that variants V_9 and V_{11} were superior in value to all other variants, but statistically different from V_1 and V_8. For the inoculated plants of cultivars 'Sultan's Palace' and 'Pinafore Pink' the number of shoots grown in spring surpassed the number of buds counted in autumn, due to formation of new buds after planting, explaining a rate development exceeding 100%. The only cultivar that did not seem to exhibit a positive response in regards with development rate under supplementary mycorrhization was 'Blue Rhythm', which presented statistically different inferior values to variants V_9, V_{10}, V_{11}, V_{12} belonging to inoculated plants. Although V_8 is inferior different in value from variants V_1-V_7, the differences are not ensured statistically.

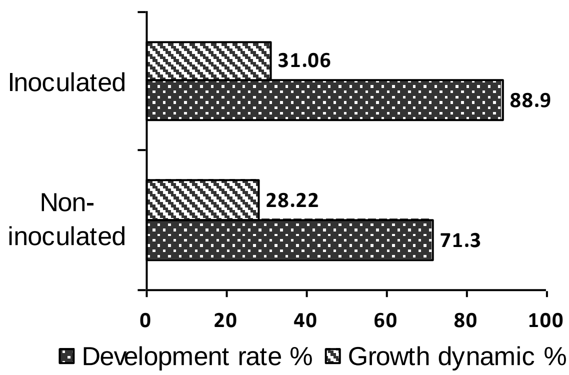

Figure 2 **Average development rate and growth dynamic of six *Iris germanica* cultivars**

It can be concluded based on Duncan test, that in regard with development rate the best response to inoculation was registered for cultivars 'Sultan's Palace' and 'Pinafore Pink' followed by 'Lime Fizz' and 'Pure As The'.

Analysis of growth dynamic (*table 1*) revealed variants V_2 and V_{10} were superior in value to all other variants but statistically different only to V_6 and V_9. The growth dynamic indicator shows that during the phenophase corresponding to leaf elongation, a faster growth was registered in four out of six inoculated cultivars: 'Black Dragon', 'Lime Fizz', 'Pinafore Pink' and 'Pure As The'.

Table 1

Vegetative development and growth indicators of six *Iris germanica* cultivars

Var.	Treatment	Cultivar	Development rate (%)	Growth dynamic (%)
V1	Non-inoculated	'Black Dragon'	63.52[ab]	26.13[ab]
V2		'Blue Rhythm'	69.72[abc]	36.71[b]
V3		'Sultan's Palace'	69.63[abc]	26.89[ab]
V4		'Lime Fizz'	73.61[abc]	27.19[ab]
V5		'Pinafore Pink'	72.22[abc]	29.03[ab]
V6		'Pure as The'	79.07[abc]	23.39[a]
V7	Inoculated with AM	'Black Dragon'	82.22[abc]	32.73[ab]
V8		'Blue Rhythm'	50.57[a]	34.66[ab]
V9		'Sultan's Palace'	107.41[c]	23.03[a]
V10		'Lime Fizz'	94.44[bc]	37.07[b]
V11		'Pinafore Pink'	105.56[c]	31.90[ab]
V12		'Pure as The'	93.70[bc]	26.96[ab]

Differences between values followed by at least one common letter are not significant at $p<0.05$

In *table 2* are presented the correlation coefficients for several variables with importance for entering in vegetation of *Iris* plants. It can be observed that regarding development rate there are several significant positive correlations with variables: cultivar, inoculation, number of shoots and leaves, and significant negative correlations with number of buds present on rhizomes at planting. Although the cultivar is exerting a statistically positive influence on the development rate, it must be noted that is slightly weaker than inoculation treatment. Also, a significant positive correlation between number of leaves and rate of entering in vegetation of buds could be explained through a good early start that ensures a greater number of shoots and thus also of leaves. When analyzing growth dynamic, it can be observed that inoculation has a positive influence but the correlation is not significant. There is a negative correlation between cultivar and growth dynamic indicating that cultivars have a different performance pattern in leaf elongation phenophase, since for the emergence of shoots corresponding to development rate indicator correlated with the cultivar was not obtained a negative coefficient.

Tabel 2

Correlation coefficients between two vegetative indicators and nine parameters of *Iris germanica* plants

Variables	Development rate	Growth dynamic
Cultivar	0.23*	-0.08
Inoculation treatment	**0.26***	**0.12**
Weight of rhizomes	0.04	-0.07
Number of vegetative buds	-0.34[o]	0.08
Number of shoots in April	0.28*	0.04
Height of plants in April	-0.19	-0.31[o]
Number of shoots in May	0.32*	0.07
Height of plants in May	-0.17	0.23*
Number of leaves	0.23*	0.08

The development rate presents a negative correlation with the height of plants in April and May explained by the fact that some plants with a greater number of shoots tend to be shorter, however, the correlation is not statistically significant. Also, a few plants did not present difference in height between the two determinations. The weight of the rhizome is correlating negatively with growth dynamic explained by the fact that during phenophase of leaf elongation the underground part of plant and above part are competing in regards with resource allocation. It is documented that allocation patterns vary greatly between plant organs at different growth stages especially in geophytes (Ruiters C. *et al*, 1993). At the start of growth, resources from rhizome are invested in growing of shoots but when leaves emerge and start to elongate also the plant invests part of energy stored in the geophytic organ to develop an adequate root system to balance increased demand of new leaves for "raw materials" used in photosynthesis. Also, *Iris* leaves are not good interceptors of available light especially at first stages of growth when their vertical position hinders proper light incidence on leaf (Elphinstone E.D., Rees A.R., 1987), fact that could also have a contribution to the observed phenomenon. Correlation coefficients obtained, show that effects of supplementary mycorrhization on plants during first vegetative stages cannot be studied separately from all other aspects that regard plant development and growth in order to be able to distinguish its influence from other factors and also to determine the phenophase in which is exercising the greatest influence on the plant characteristics. The correlation coefficient between inoculation treatment although statistically significant is not very strong and further studies involving more genotypes and other plant characteristics could bring further insight.

Results of this study indicate that development rate and growth dynamic were on average better for most of the inoculated *Iris germanica* cultivars. Inoculation correlates positively both with development rate and growth dynamic, but is statistically significant only for the development rate. This could be explained by supplementary mycorrhization exercising a greater influence in first phenophase or over certain characteristics. These findings indicating positive influence of AM on vegetative development are in accordance with results of previous studies conducted on ornamental geophyte plants. At stage of early development, highly positive correlation between mycorrhizal colonization and most vegetative and reproductive growth parameters of *Gladiolus grandiflorus* were put in evidence (Koltai H., 2010). Also, it was demonstrated that regarding growth parameters of *Freesia hybrida*, AMF application increased several leaf characteristics (size, length, width, number) as well as cormlet characteristics (diameter, weight number) compared with non-inoculated plants (Rezvanypour S. *et al*, 2015). Studies showed that inoculation of the *Zantedeschia* resulted in increased shoot production (Scagel C.F., Schreiner R.P., 2006). Also, the inoculated *Lilium* bulblets presented significantly better growth variables in regards with size, weight, shoot length, number of leaves and leaf area (Varshney A. *et al*, 2002).

Results obtained in the present study demonstrate positive influence of supplementary mycorrhization on early development of *Iris* plants and are of particular importance for rustic geophytes with spring flowering time, that depend for their subsequent development on early developed shoots and leaves. This could present importance for cultivation technology of both ornamental irises as well as for those destined to processing.

CONCLUSIONS

For most of the studied *Iris germanica* cultivars at the start of growing season, mycorrhizal inoculum exercised beneficial effects on entering in vegetation of buds or stimulated the formation of new buds and shoots. This fact could be partially attributed to mycorrhizae and part to auxins added to inoculation product. In next stages of vegetative development, inoculated plants presented on average a better growth dynamic, that could be attributed to improved plant nutrition causing a faster growth. The beneficial effects identified, recommend the utilization of supplementary mycorrhization on spring flowering geophytes like *Iris germanica* to ensure a good vegetative development prior to flowering.

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