Volume 15(1), 170- 174, 2011
JOURNAL of Horticulture, Forestry and Biotechnology

The influence of arbuscular mycorrhizal fungi on ornamental characters of *Tagetes patula* L.

Schmidt Brigitta[1,*], Şumălan R.[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine of Timişoara, Faculty of Horticulture and Forestry

*Corresponding author. Email: brigischmidt@yahoo.com

Abstract Arbuscular mycorrhizal fungi form with the host plants' root system one of the most wide-spread symbiotic relationships on Earth. The hyphal network surrounding the root system explores a great amount of soil and absorbs water, organic and inorganic compounds, and makes them accessible to the host plant. The fungus is an obligate symbiont and can't synthesize sugars so the host plant gives in exchange glucose. The micorrhizal fungi are more and more used as biofertilizers for their beneficial effects for the agriculture: enhanced plant nutrition and thus plant development, protection against pathogens or drought.
We conducted a pot experiment with controlled environmental conditions to observe the effects of a biofertilizer with arbuscular mycorrhizal fungi on the development of ornamental characters of French marigold (*Tagetes patula* L.). The plants were grown in hydroponic culture watered with normal or modified (poor in phosphates) Hoagland's solution and in soil system and for every variant, inoculated and non-inoculated variants were compared.
The investigated characters were: total leaf area, number of side shoots, number of flower buds and of fully developed flowers. We found significant differences between the nutritional treatments. Also between inoculated and non-inoculated plants there were highly significant differences at every investigated character, the mycorrhized marigold plants having enhanced ornamental features.
The study shows that arbuscular mycorrhizal fungi can be successfully used in plant production, especially for ornamental species, where is a need for a better nutrition with phosphates to develop the ornamental characters of plants (a higher number of flowers and total leaf area per plant).

Key words

arbuscular mycorrhiza, *Tagetes patula* L., ornamental characters

Arbuscular mycorrhizas is one of the most important mycorrhizal relationships on Earth, being present at over 80% of terrestrial plant species [5, 14]. This type of symbiotic relationship is described as an endomycorrhiza because the fungal hyphae are present in the root cortex, not only surrounding the roots like in case of ectomycorrhiza [13].
Mycorrhizal fungi contribute to the host plant's nutrition, absorbing and supplying with mineral elements, like phosphorus, nitrogen, potassium in various inorganic or even organic compounds, microelements (copper and zinc). Besides, the fungus offers a protection against drought by absorbing a greater amount of water then the roots. The smaller diameter and the faster growth of hyphae then of the roots permit the exploitation of soil particles with smaller pores and the surpassing of the nutritional depletion zone which appears around the rhizosphere [14]. By surrounding the root surface and covering the entrance sites for pathogens, the arbuscular mycorrhizal fungi are used in biological plant protection [3].
Marigold is an annual ornamental plant which is reported to develop arbuscular mycorrhiza, but the effects of arbuscular fungi on host plants' development are varied [1, 7, 8, 10].
The development of mycorrhiza is highly influenced by the concentration of nutrients in the growing substrate. If the soil is rich in nitrogen and especially phosphorous in accessible forms, the plant doesn't need the help of the symbiotic partner, thus in order to save the carbohydrates, the development of fungal hyphae in the root cortex will be suppressed by the plant [2, 9, 11, 15].
In this paper we present the results of the researches regarding phosphorous concentration's and colonization's effect with arbuscular mycorrhizal fungi on ornamental characters (total leaf area, number of

side shoots, flower buds and fully developed flowers) of the French marigold, an ornamental plant.

Materials and Methods

To study the effects of arbuscular mycorrhizal fungi on ornamental characters of *Tagetes patula* L. as the host plant, we established a pot experiment in controlled environmental conditions.

To obtain the plantlets, surface sterilized (with $HgCl_2$ 0,2 %, for 3 minutes, followed by 5 rinses with sterile distilled water) seeds of marigold (*Tagetes patula* "Palla D'oro") were germinated in Petri dishes.

We used as mycorrhizal inoculum the "MYCO GROW" commercial product (Fungi Perfecti® LLC, USA), which contains three fungal species, as following: *Glomus aggregatum, Glomus intraradices* and *Glomus mosseae*. The inoculation technique consisted in covering the plantlets' roots with spores by immersing them in the inoculum. After inoculation, plantlets were transferred in 0.5 l pots filled with the adequate type of substrate (perlite or soil, see below).

Two types of growing substrates were used in the experiment: steam sterilized perlite and soil with low phosphorous content. The soil is a chernozem collected from SDE Timişoara and the physical and chemical properties were analyzed. The soil's properties are presented in Table 1.

The pots with the plants were placed in green-house, where temperatures were maintained at 27/18^{o}C day/night, with a photoperiod of 12h daylength for the study period. Plants were watered with the adequate solution, using a quantity required by the relative size of the plant, and until the growing medium was saturated. To ensure the mycorrhization, supplemental inoculation was realized two weeks after planting for the mycorrhizal variants.

Table 1

Physical and chemical properties of the soil from SDE Timişoara – analysis realised at Agrochemistry Laboratory, BUASVMT

Soil characteristics	Gleic chernozem
Collected soil layer	0-25 cm
silt <0,02 mm	29,2
clay <0,01 mm	41,1
sand 0,2-0,02 mm	29,2
sand 0,2-2mm	0,5
pH in H_2O	6,45
humus (%)	4,09
Total N (%)	0,136
P (ppm)	28,8
K (ppm)	138

The following variants were tested: perlite plus Hoagland's solution with normal phosphorous concentration, perlite and no phosphorous addition, both variants with and without mycorrhizal inoculum (PM-P, PN-P, PM-0 and PN-0), sterilized soil with inoculum (SM) and non-sterilized soil without inoculum, with the native fungal community (SN). The soil was sieved before the use with a fine mash sieve and mixed with an equal quantity of steam sterilized perlite.

Complete Hoagland's solution was used for variants PM-P and PN-P, the same solution was prepared, but without phosphorous for PM-0 and PN-0, and for the variants SM and SN simple distilled water was used. For the variants PM-0 and PN-0, the first 7 weeks, we administrated the modified Hoagland solution, and after that, one with $Ca(H_2PO_4)_2$ as a phosphate source. $Ca(H_2PO_4)_2$ is more difficult to absorb by plants, because it has a low solubility in water.

Every experimental variant was used in nine repetitions, but data were collected from five representative plants per variant, because the variation among plants was high, excluding the plants which dried early because of non-experimental genetic or environmental factors.

The tests were realized 10 weeks after planting. We counted the number of flower buds, totally developed flowers and side shoots. Total leaf area per plant was determined using a portable area meter (ADC AM300, BioScientific Ltd.).

Statistical analysis of data was realized using the F and the t tests for statistical significance testing of a bifactorial experiment [6]. The "a" factor was represented by the presence or absence or mycorrhization and the "b" factor, the type of substrate/nutrient solution. For the "a" factor, the control was represented by the non-mycorrhized plants meanwhile for the "b" factor, the control is the soil culture.

Results

Analyzing the results, it can be observed that the nature of substrate and the phosphorous nutrition determined statistically significant differences between experimental variants. Regarding the number of side shoots of marigold plants, higher values were observed at plants grown in hydroponic culture. The easy absorption of nutrients from liquid solution determines a fast growth and development of plants.

Table 2

Number of side shoots of experimental plants

"b" factor substrate	"a" factor mycorrhization		Average of "a" factor	Relative "a" (%)	Difference to the control and significance
	Non-mycorrhized	Mycorrhized			
soil	1,11	1,89	1,5	100	Control
perlite without phosphorous	2,67	4,11	3,39	225,93	1,89***
perlite with phosphorous	7,44	8,78	8,11	540,74	6,61***
Average of "b" factor	3,74	4,93			
Relative "b" (%)	100	131,68			
Difference to the control and significance	Control	1,18***			

A factor: $DL_{5\%}$=0,54; $DL_{1\%}$=0,73; $DL_{0.1\%}$=0,95
B factor: $DL_{5\%}$=0,67; $DL_{1\%}$=0,89; $DL_{0.1\%}$=1,17

At p=0,1%, the number of side shoots were statistically highly significant at plants cultivated in perlite, with or without phosphorous addition, compared to those of plants grown in soil. The plants grown with complete Hoagland's solution had an over five times more side shoots then the control plants, meanwhile those watered with modified nutrient solution had over two times more side shoots then the same control plants cultivated in soil (Table 2).

Table 3

Number of floral buds per experimental plants.

"b" factor substrate	"a" factor mycorrhization		Average of "a" factor	Relative "a" (%)	Difference to the control and significance
	Non-mycorrhized	Mycorrhized			
soil	1,11	2,44	1,78	100	Control
perlite without phosphorous	0,78	1,67	1,22	68,75	-0,56
perlite with phosphorous	7,44	8,33	7,89	443,75	6,11***
Average of "b" factor	3,11	4,15			
Relative "b" (%)	100	133,33			
Difference to the control and significance	Control	1,04**			

A factor: $DL_{5\%}$=0,75; $DL_{1\%}$=1,00; $DL_{0.1\%}$=1,32
B factor: $DL_{5\%}$=0,92; $DL_{1\%}$=1,23; $DL_{0.1\%}$=1,62

The number of floral buds were significantly influenced by the level of nutrients from growing substrate. The values of plants grown in perlite with modified nutrient solution were not significantly different from those of control plants at p=5%, meanwhile the number of flowers from plants watered with complete Hoagland's solution were highly significantly greater at p=0.1% then those from the control plants. The use of complete Hoagland's solution compared to soil culture determined an over four times higher floral bud production (Table 3).

Table 4

Number of fully developed flowers per experimental plants

"b" factor substrate	"a" factor mycorrhization		Average of "a" factor	Relative "a" (%)	Difference to the control and significance
	Non-mycorrhized	Mycorrhized			
soil	0,2	2	1,1	100	Control
perlite without phosphorous	0	0,2	0,1	9,09	-1^{00}
perlite with phosphorous	1,4	2,4	1,9	172,72	$0,8^{*}$
Average of "b" factor	0,53	1,53			
Relative "b" (%)	100	287,5			
Difference to the control and significance	Control	1^{***}			

A factor: $DL_{5\%}$=0,51; $DL_{1\%}$=0,69; $DL_{0.1\%}$=0,94
B factor: $DL_{5\%}$=0,62; $DL_{1\%}$=0,85; $DL_{0.1\%}$=1,15

The number of fully developed flower is also depending on the phosphorous nutrition. Compared to control, the variants grown in perlite with modified nutrient solution had significantly lower number of flowers at p=1%. Even if the potential to develop flowers during the plant's life cycle is not significantly lower when modified nutrient solution is used, the number of floral buds being relatively high, the rate and speed of development is significantly lower compared to control. The good mineral nutrition of plants from hydroponic culture with the utilization of Hoagland's solution determines significantly higher number of fully development flowers at p=5% compared to control (Table 4).

Table 5

Total leaf area per experimental plants (cm^2)

"b" factor substrate	"a" factor mycorrhization		Average of "a" factor	Relative "a" (%)	Difference to the control and significance
	Non-mycorrhized	Mycorrhized			
soil	296,29	560,87	428,58	100	Control
perlite without phosphorous	817,08	1768,75	1292,91	301,67	$864,34^{***}$
perlite with phosphorous	4474,52	6575,08	5524,80	1289,09	$5096,22^{***}$
Average of "b" factor	1862,63	2968,23			
Relative "b" (%)	100	159,36			
Difference to the control and significance	Control	$1105,60^{***}$			

A factor: $DL_{5\%}$=336,81; $DL_{1\%}$=457,68; $DL_{0.1\%}$=620,44

B factor: $DL_{5\%}$=412,51; $DL_{1\%}$=560,54; $DL_{0,1\%}$=759,88

The leaf area of plants grown in hydroponic culture, no matter the type of nutrient solution, presented highly significant differences at p=0.1%, comparing to control (Table 5).

In all cases, ornamental characters were significantly or even highly significantly greater at mycorrhized plants. Thus, at inoculated plants, the number of side shoots was 131,68% of the control's value, for the number of floral buds, 133,33% of control, the number of fully developed flowers, 287,5% and the total leaf area per plants, 159,36% of the control.

The results show that mycorrhization is a useful tool for improving the productions in ornamental plant culture using ecological technologies. The mycorrhized plants have a higher number of side shoots, which bear the floral buds. The better mineral nutrition due to the arbuscular mycorrhizal fungi determines a faster development, so the flowers will open faster, a characteristic which is highly important in plant production. The faster the product is obtained, the higher the profits will be.

The $Ca(H_2PO_4)_2$ was faster metabolized by the mycorrhized plants, which is a very important characteristic of this symbiosis with applicability in plant production. Knowing that the high inputs of mineral fertilizers have negative effect on environment, the use of inaccessible phosphate compounds from soil (which represent around 99% of total P from soil [4]) can be a useful technology for the agriculture of the future.

Conclusions

The results clearly show the importance of phosphates not only in plants' generative development, but in forming the vegetative parts, too. The mycorrhiza had a beneficial effect on developing the ornamental characters of marigold these results confirming our earlier observations [12], where we clearly showed that inoculated plants had enhanced physiological processes.

A proper mineral nutrition of plants cultivated in hydroponic culture using Hoagland's solution determined significantly higher number of side shoots, flower buds, fully developed flowers and total leaf area compared to the plants cultivated in a poor soil.

Acknowledgements

We would like to thank to the colleagues from Agrochemistry laboratory for the soil analyses. We also thank for the chance to work in the laboratory of Plant physiology and Soil biology and microbiology, and for the help and support of the collective.

References

1.Aboul-Nasr A. 1996. Effects of vesicular-arbuscular mycorrhiza on *Tagetes erecta* and *Zinnia elegans*. Mycorrhiza 6:61-64.

2.Amijee F., Tinker P.B., Stribley D.P. 1989. The development of endomycorrhizal root systems. A detailed study of effects of soil phosphorous on colonization. New Phytol. 111:435-446.

3.Azcon-Aguilar C., Barea J.M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanism involved. Mycorrhiza 6:457-464.

4.Bolan N.S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorous by plants. Plant and Soil 134:189-207.

5.Brundrett, M. C. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. 154:275–304.

6.Ciulcă, S. 2002. Tehnică experimentală. Ed. Mirton, Timişoara, pp. 163-239.

7.Ezawa T., Yoshinda Y. 1994. Acid phosphatase specific to arbuscular mycorrhizal infection in marigold and possible role in symbiosis. Soil.Sci.Plant.Nutr. 40:655-665.

8.Koide R.T., Landherr L.L., Besmer Y.L., Detweiler D.M., Holcomb E.J. 1999. Strategies for mycorrhizal inoculation of six annual bedding plant species. Hort. Science 34:1217-1220.

9.Koide R.T. 1991. Transley review No. 29: nutrient supply, nutrient demand, and plant response to mycorrhizal infection. New Phytol. 117:365-386.

10.Linderman R.G., Davis E. Anne. 2004. Varied response of marigold (*Tagetes* ssp.) genotypes to inoculation with different arbuscular mycorrhizal fungi. Scientia Horticulturae 99:67-78.

11.Schmidt Brigitta, Domonkos Mónika, Şumălan R., Biró Borbála. 2010. Supression of arbuscular mycorrhiza's development by high concentrations of phosphorous at *Tagetes patula* L. Research Journal of Agricultural Science, 42(4):156-162.

12.Schmidt Brigitta, Şumălan R. 2010. Influence of arbuscular mycorrhiza on development of marigold using different phosphorous substrates. Conference proceedings of 2nd International Conference on Horticulure Post-graduate Study, Lednice, Czech Republic.

13.Siddiqui Z.A. Akhtar M.S., Futai K. 2008. Mycorrhizae – Sustainable Agriculture and Forestry. Springer, pp. 2-13.

14.Smith S.E., Read D.J. 2008. Mycorrhizal symbiosis. Third edition, Academic Press, Elsevier. London, pp. 13-15, 145-187.

15.Takács Tünde, Vörös Ibolya. 2003. Role of the arbuscular mycorrhizal fungi in the water and nutriend supply of their host plant. Növénytermelés 52:583-593 (In Hungarian).