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Effects of Arbuscular Mycorrhizal Fungi and Organic Fertilization on Growth, Flowering, Nutrient Uptake, Photosynthesis and Transpiration of Geranium (*Pelargonium hortorum* L.H. Bailey 'Tango Orange')

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Abstract

Greenhouse experiment was conducted to evaluate the effect of arbuscular mycorrhizal fungi (AMF) and organic fertilization on growth, flowering, nutrient uptake and some physiological traits of geranium. The roots of inoculated plants were colonised by mycorrhizal fungi regardless of preplant organic fertilization. Mycorrhizal inoculation increased the height of plants, irrespectively of organic fertilization. In plants unfertilised organically mycorrhizal inoculation increased leaf number, fresh and dry weight of leaves and roots. Mycorrhizal inoculation did not affect the numbers of flower buds and flowers but delayed flowering of plants unfertilised before planting. N, P, and K concentrations in the plants were higher in the mycorrhizal plants compared to the non-mycorrhizal ones when plants were not fertilised before planting. Transpiration and stomatal conductance were higher in mycorrhizal plants grown under lower level of organic fertilizer. Increased photosynthetic activity of mycorrhizal plants was connected with the increased ratio of variable to maximum chlorophyll fluorescence (F_v/F_m), which is directly proportional to the maximum quantum yield of primary photochemistry of PS II.

Keywords: *Pelargonium hortorum*, mycorrhization, organic fertilization, growth, flowering, photosynthesis, transpiration

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1. Introduction

Many bedding plants are mycorrhiza-dependent (Aboul-Nasr, 1995; Koide et al., 1999). Their propagation in greenhouses is usually started from seedlings or cuttings produced in sterile or partially sterilized substrates to minimize the risk of pathogenic diseases. Such conditions can eliminate or reduce the possibility of arbuscular mycorrhizal fungi (AMF) inoculation of the root system during greenhouse production. Due to the ability of AMF to increase plant growth through enhanced acquisition of mineral nutrients, water uptake, and resistance to stress conditions, early colonization of the root system may reduce transplant shock and improve plant quality in outdoor conditions.

The aim of this work was to investigate the effects of AMF inoculation and preplant organic fertilization on growth, flowering, and some physiological traits of geranium (*Pelargonium hortorum* L.H. Bailey 'Tango Orange') during cultivation under glass. It was shown earlier that mycorrhization of some bedding plants is significantly reduced by high P concentration (Koide et al., 1999). For this reason, granulated, slowly decomposed organic fertilizer was used before planting to avoid P accumulation in the growing medium.

2. Material and Methods

Rooted cuttings of geranium were used for the experiments. The cuttings were planted into sphagnum peat medium, pH 5.8. The substrate was inoculated by adding 1-liter mixture of osteospermum (*Osteospermum ecklonis* (DC.) Norl.) root pieces and substrate inoculated earlier with Endorize-TA AMF inoculum, containing a mixture of different *Glomus* species (Biorize Sarl, France) to 10 liters of inoculated peat substrate. The substrate was not sterilized. The peat used in this experiment was devoid of AM fungi as confirmed by the absence of colonization with the non-inoculated treatments. Mycorrhizal infection was estimated after one month of cultivation, after staining the roots with trypan blue (Phillips and Hayman, 1970). The roots analyzed for mycorrhization were sampled from parallel plants. Wholly organic granulated fertilizer Eco-Mix 1 (9-3-3) (DCM, Belgium) at 0, 1, and 4 g dm^{-3} was used as a preplant nutrient addition. Chemical characteristics of used peat substrates is presented in Table 1.

The plants were cultivated under glass from December 4th to March 27th without artificial lighting. During cultivation geranium was fertilized with Peters Professional (12-00-43 plus microelements) 1 g dm^{-3} once a week. All measurements were conducted at the end of experiment. The transpiration and stomatal conductance (g_s) was determined on 6 plants and 3 fully developed leaves per each plant using portable porometer (LICOR, 1600M, Nebraska,

USA). The measurements were taken when growing conditions were favorable for stomata opening and transpiration (on sunny day between 10–12 a.m.) after watering.

Evapotranspiration (EVPT) was determined by weighing plants with pots. There were 6 uniform plants randomly sampled from each treatment for these purposes. Plants were weighed in the morning, about 30 minutes after irrigation (when the excess of water drained out) and 7 hours later.

Net CO_2 assimilation rates were measured using a portable LCA-3 infrared CO_2 analyzer and the Parkinson Broad Leaf Chamber PLC-3B (ADC, England). Six fully developed leaves were used.

Chlorophyll fluorescence was measured on the ad axial surface of the attached leaves using a fluorescence measurement system (PEA, Hansatech Instruments, Ltd., England). The leaves were covered with clips, darkened for 20 min, and then illuminated with red light emitting diodes (peak at 650 nm, maximum irradiance at leaf surface 3000 μmol m^{-2} s^{-1}). Fast fluorescence kinetics parameters: F_o, F_m, F_v, F_v/F_m, were determined; where F_o - initial fluorescence, corresponding to all PS II reaction centers in the open configuration, F_m - maximal fluorescence, corresponding to all PSII reaction centers in the closed state, F_v - variable fluorescence ($F_m - F_o$), F_v/F_m - an indicator of the maximum quantum yield of primary photochemistry of PS II.

Sixteen weeks after treatments commenced, whole plants were harvested. Plant height, leaf number, fresh and dry weights of leaves and roots, numbers of flower buds and flowers, fresh and dry weight of flowers, and root colonization by AM fungi were determined.

Leaf nutrient contents were determined for 3 plants per replicate. There were 3 replications per treatment. All leaves were sampled. The leaf tissues were oven-dried at 78°C, milled to homogeneous samples, and then treated with the mixture of 65% HNO_3 and 60% $HClO_4$ (3.5:1, v:v). The concentrations of K^+, Ca^{2+} and Mg^{2+} were measured using atomic absorption spectrophotometry (PU 9100X; Philips, Holland), P was determined colorimetrically by using vanadium-molybdate complex, N was determined using Kjeldahl method with automatic distillation system with boric acid (Kieltec, Tecator, Sweden). The treatments were statistically analyzed by analysis of variance and means were compared with Duncan's multiple range test at 95% level of significance.

3. Results and Discussion

One month after inoculation the inoculated plants were infected by mycorrhizal fungi. The un-inoculated plants had no root infection. No significant differences in frequency and intensity of infection, were observed

Table 1. Chemical characteristics of peat substrates before planting of rooted cuttings of geranium 'Tango Orange'.

Chemical characteristics of peat substrates	Fertilization Eco-Mix 1 (g dm^{-3})					
	0		1		4	
	Mycorrhizal inoculation					
	–	+	–	+	–	+
pH	5.6	5.8	5.7	5.8	5.7	5.8
Total soluble salts (g NaCl dm^{-3})	0.091	0.091	0.105	0.112	0.245	0.224

between plants grown under different levels of organic fertilization. Percentage AMF-root colonization was about 30%.

Both, mycorrhizae and organic fertilization treatments affected growth and flowering of geranium (Table 2). Greatest growth and fastest flowering occurred at the highest fertility at 4 g dm^{-3}, and 0 g dm^{-3} had the poorest growth. At 0 g dm^{-3} mycorrhizal inoculation increased all growth parameters of geranium, decreased root/shoot ratio, and delayed flowering. Typical symptoms of P deficiency: reduced growth and a reddish coloration of leaves due to increase in anthocyanin production (Shuman, 1992) were observed on un-fertilized organically plants.

At 1 g dm^{-3} plants inoculated with AMF had higher DW of root system, similar root/shoot ratio, higher FW and DW of flowers than those un-inoculated with AMF. At 4 g dm^{-3} there were also no mycorrhizal effect on number of leaves, FW and DW of leaves, FW of roots, and root/shoot ratio. Mycorrhizal plants were higher and had greater DW of roots and flowers, although flowered later than those un-inoculated with AMF. The higher stem elongation found in inoculated plants probably indicates a change in hormonal balance induced by mycorrhizal symbiosis (Allen et al., 1985).

Enhanced host plant acquisition of mineral nutrients, especially P, as a result of mycorrhization is very well documented (Bolan, 1991; Marschner and Dell, 1994). In geranium, mycorrhizal plants un-fertilized with organic fertilizer took up more N, P, K, and less Ca (Table 3). Mycorrhizal plants grown at 1 g dm^{-3} had greater N, P, and K uptake, and lower Ca uptake than non-inoculated plants. At the highest fertility level of 4 g dm^{-3} inoculation with AMF slightly increased P content of leaves only, and decreased N content. The greatest effect of mycorrhizae on nutrient uptake at the intermediate fertility level has been earlier reported with other plant species (Maronek et al., 1982).

Table 2. The effect of fertilization and mycorrhizal inoculation on growth and flowering of geranium (*Pelargonium hortorum* 'Tango Orange').

Growth characteristics	Fertilization Eco-Mix 1 (g dm^{-3})					
	0		1		4	
	Mycorrhizal inoculation					
	–	+	–	+	–	+
Height of plants (cm)	5.8a	11.3c	9.2b	10.4bc	11.6c	16.8d
Leaf number	8.2a	16.0b	14.6b	18.0b	23.2c	26.2c
F.W. of leaves (g)	5.0a	18.3b	13.6b	17.0b	27.5c	33.1c
D.W. of leaves (g)	0.79a	2.65bc	1.69ab	2.11b	3.25cd	3.89d
F.W of roots (g)	4.67a	8.38b	6.53ab	7.14b	6.03ab	7.43b
D.W. of roots (g)	0.84a	1.83d	0.95ab	1.48c	0.78a	1.21bc
Root/shoot ratio	0.94c	0.46b	0.48b	0.42b	0.20a	0.22a
No of days from planting to coloured flower buds	106.8bc	114.0d	104.0ab	106.0bc	101.8a	109.0c
No of flower buds	0.4a	1.0a	0.6a	1.0a	0.8a	1.2a
No of flowers	0.4a	0a	0.2a	0.6a	0.2a	0.6a
F.W. of flowers (g)	1.26ab	–	0.50a	3.53c	0.96ab	2.99bc
D.W. of flowers (g)	0.17a	–	0.10a	0.50b	0.19a	0.57b

Means in rows with the same letter are not significantly different with probability of 95% according to the Duncan's multiple range test.

Table 3. The effect of fertilization and mycorrhizal inoculation on mineral element content of leaves of geranium (*Pelargonium hortorum* 'Tango Orange').

Mineral elements (% dry weight)	Fertilization Eco-Mix 1 (g dm^{-3})					
	0		1		4	
	Mycorrhizal inoculation					
	–	+	–	+	–	+
N	2.02a	2.45b	2.98c	3.44d	4.14f	3.66e
P	0.03a	0.04b	0.05c	0.07d	0.08e	0.09f
K	2.70a	3.21c	2.73ab	3.07c	3.02bc	3.21c
Ca	1.55c	1.01a	1.30b	1.05a	1.15ab	1.01a
Mg	0.16ab	0.15a	0.17ab	0.16a	0.18bc	0.17c

Means in rows with the same letter are not significantly different with probability of 95% according to the Duncan's multiple range test.

Table 4. The effect of fertilization and mycorrhizal inoculation on some physiological parameters of geranium (*Pelargonium hortorum* 'Tango Orange').

Physiological parameters	Fertilization Eco-Mix 1 (g dm^{-3})					
	0		1		4	
	Mycorrhizal inoculation					
	–	+	–	+	–	+
F_v/F_m	0.770a	0.810b	0.764a	0.813b	0.801b	0.807b
CO_2 assimilation rate (μmol CO_2 m^{-2} s^{-1})	0.84a	2.66c	1.16ab	2.86c	1.78b	2.64c
Evapotranspiration (g/plant)	13.32a	47.50d	16.74ab	28.40bc	46.60d	38.00cd
Transpiration (mmol H_2O m^{-2} s^{-1})	0.56a	0.74ab	0.95ab	1.41c	1.04c	1.13c
Stomatal conductance (mmol H_2O m^{-2} s^{-1})	27.88a	43.04abc	37.78ab	116.08d	68.50c	57.82bc

Means in rows with the same letter are not significantly different with probability of 95% according to the Duncan's multiple range test.

No mycorrhizal effect on nutrient uptake at high fertility level was observed in *Rosa multiflora* (Davies, 1987). Mycorrhization did not affect Mg uptake, irrespectively of fertilization level. Enhanced acquisition of Ca and Mg as a result of mycorrhization has been reported where plants have been cultivated under acidic conditions (Clark et al., 1999).

Better growth of mycorrhizal plants corresponded with increased photosynthetic rates (Table 4). Mycorrhization increased F_v/F_m ratio – an indicator of the maximum quantum yield of primary photochemistry of PS II, of plants grown at 0 and 1 g dm^{-3} but it did not affect F_v/F_m of plants grown at 4 g dm^{-3}. Earlier results on cucumber suggested that the effect of mycorrhization on photosynthesis activity can be indirect and connected with the increase in P content of leaves, rather than a consequence of mycorrhizal sink for assimilates (Black at el., 2000).

Mycorrhizal inoculation increased evapotranspiration, transpiration and stomatal conductance of plants unfertilized with Eco-Mix 1 and fertilized at 1 g dm^{-3}, but it did not affect these parameters of plants grown at 4 g dm^{-3}. These results suggest that the effect of mycorrhization on transpiration and evapotranspiration is due to the nutritional effect of AM fungi and better growth of inoculated plants. Higher transpiration and stomatal conductance was also measured in mycorrhizal *Helianthemum almeriense* (Morte et al., 2000).

4. Conclusions

Mycorrhization of geranium during cultivation under glass is possible using slowly decomposed organic fertilizer. In a low P fertilization regime mycorrhization enhances growth of geranium, increases mineral nutrient acquisition, photosynthetic activity, transpiration and stomatal conductance. The benefit of AMF inoculation to plants growing under higher P content is smaller. It seems to be possible that in P-supplemented fertilization regime un-inoculated plants would grow equally or even better than inoculated plants. It warrants further study to evaluate if inoculated geranium is better able to withstand the stress of transplanting and increase growth and flowering outside as a bedding plant in low maintenance conditions.

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