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Application of mycorrhizae to ornamental horticultural crops: lisianthus (*Eustoma grandiflorum*) as a test case

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Abstract

Ornamental crops are high-cash crops, grown under greenhouse conditions in semi-arid regions in Israel where a reduction in the native population of arbuscular mycorrhizal fungi (AMF) is expected due to routine soil disinfection. The application of AMF inoculum to the soil has been shown to be effective at improving plant growth and enhancing plant resilience to abiotic and biotic stresses. One of our aims is to introduce mycorrhizal application to ornamental crops, and a test case is presented here for two cultivars of lisianthus (*Eustoma grandiflorum*), one of the major ornamental crops grown in Israel. Several different methods of AMF application and their effects on growth, yield and vase life were examined in lisianthus grown in two different semi-arid locations in southern Israel. AMF enhanced lisianthus growth and yield, especially when introduced to the growth medium during seeding and to the pit hole during planting. Significantly enhanced growth and yield parameters included flowering stem length (58 ± 0.7 and 65.1 ± 0.7 cm for control and AMF treated, respectively) and number of flowering stems per square meter (73 ± 9 and 106 ± 6 for control and AMF treated, respectively); positive but non-significant effects were recorded on stem weight, number of flowers per stem and vase life of cut flowers. Yield enhancement was recorded under both low and regular phosphorus conditions. Although not significant, higher resilience against two pathogenic fungi was also recorded following AMF inoculation (23 ± 13 and 41 ± 10 surviving plants for control and AMF treated, respectively). Hence, AMF is suggested to be a useful growth amendment for promotion of lisianthus commercial production, and may potentially be applied to additional ornamental crops.

Additional key words: nursery, symbiosis, vase life.

Resumen

Aplicación de micorrizas a cultivos hortícolas ornamentales: lisianthus (*Eustoma grandiflorum*) como caso de prueba

Los cultivos ornamentales tienen una elevada rentabilidad comercial, y se producen en condiciones de invernadero en regiones semi-áridas en Israel, donde es de esperar una reducción en la población nativa de hongos micorrícicos arbusculares (HMA), debido a la desinfección rutinaria del suelo. Se ha demostrado que la aplicación de inóculo de HMA en el suelo es eficaz para mejorar el crecimiento vegetal e incrementar la resistencia de las plantas a estreses abióticos y bióticos. En lisianthus (*Eustoma grandiflorum*) cultivado en dos zonas semi-áridas del sur de Israel se examinaron varios métodos diferentes de aplicación de los HMA y sus efectos sobre el crecimiento, el rendimiento y vida como flor cortada. Los hongos formadores de micorrizas mejoraron el crecimiento y el rendimiento de lisianthus, especialmente cuando se incorporaron al medio de cultivo durante la siembra y/o en el hoyo de plantación en el trasplante. Los parámetros analizados que respondieron significativamente a la micorrización incluyen la longitud del tallo en flor (58 ± 0,7 y 65,1 ± 0,7 cm para el control C y tratadas con HMA, respectivamente) y el número de tallos por metro cuadrado (73 ± 9 y 106 ± 6 para C y HMA, respectivamente). Otros parámetros respondieron positivamente, aunque los datos no fueron significativos: el peso del tallo, número de flores por tallo y la vida como flor cortada.

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La mejora en el rendimiento se registró en condiciones de baja y moderada fertilización con P. También se observó una mayor capacidad de resistencia frente dos hongos patógenos después de la inoculación con HMA (23 ± 13 y 41 ± 10 plantas supervivientes para C y HMA, respectivamente). Por lo tanto, los HMA pueden considerarse como una mejora en la producción comercial de lisianthus, potencialmente aplicable a otros cultivos ornamentales.

Palabras clave adicionales: simbiosis, vida como flor cortada, vivero.

Introduction

Ornamental crops are grown in Israel and constitute one of its main fresh export products, mainly to Europe. As high-cash crops, enhancement of plant growth and development, in addition to yield promotion, may be highly valuable.

Lisianthus (*Eustoma grandiflorum*) is an ornamental plant originating in southern parts of North America (Halevy and Kofranek, 1984). The plant grows slowly during the winter, in the form of a rosette which elongates in the spring and flowers in the summer (Roh *et al*., 1989). Flowering has been shown to be affected by temperature and photoperiod (Harbaugh, 1995, 2000; Harbaugh and Scott, 1999), in a cultivar-dependent manner (Harbaugh, 2007), suggesting that lisianthus is a facultative long-day plant (Zaccai and Edri, 2002). Lisianthus has been the subject of intensive breeding efforts (Harbaugh, 2007), generating a number of new varieties annually with improved traits, such as flower color, size and form (Harbaugh, 2007). In Israel, lisianthus is one of the major ornamental crops exported to Europe. Hence, improvement in its growth and production may be of substantial benefit to Israeli growers. Lisianthus is especially propagated in arid and semi-arid regions, under greenhouse conditions. Greenhouses for lisianthus growth are usually plastic-covered, at least 4 m high, and covered with insect-proof netting.

Mycorrhizal symbiosis has been recognized to protect plants against environmental stresses, including drought and salinity (Porcel *et al*., 2003; Bolandnazar *et al*., 2007), and to serve as a biofertilizer and bioprotectant that can enhance crop productivity (Azcón-Aguilar and Barea, 1997).

The profound effect of arbuscular mycorrhizal fungi (AMF) on growth and development of a variety of crop plants has been studied and described in many research papers (Kapulnik *et al*., 1994; Azcón-Aguilar and Barea, 1997); however to date, in comparison to fruits and vegetables, ornamental crops have received only minor attention in this regard. Examples are studies examining the effect of AMF on roses, demonstrating alteration in the plant's drought response and osmotic adjustment following mycorrhizal colonization (Augé *et al*., 1986, 1987; Pinior *et al*., 2005). Other studies have examined other ornamental species, such as *Petunia hybrida*, *Tagetes erecta* and *Chrysanthemum morifolium* with respect to the effect of mycorrhizal application on growth and yield (Linderman, 2003; Sohn *et al*., 2003; Gaur and Adholeya, 2005).

AMF have been shown to benefit plants that may suffer from growth inhibition. This inhibition may be due to either chemical or physical soil manipulations at pre-planting stages of agronomical management, shortage in water supply, low quality of irrigation water, high day temperatures with high evapotranspiration rates, or soil salinity (Barea *et al*., 1993). Some of these conditions can typically be found in the northern Negev desert in Israel, which is one of the main cultivation areas for ornamental horticulture, including lisianthus.

Therefore, application of mycorrhiza to ornamental crops grown in arid areas in Israel may be an attractive approach for improving plant growth and yield. Of the factors that may determine the success of proper mycorrhizal application, dosage and time of inoculation are important. A sufficient amount of AMF infection units (i.u.) should be supplied to guarantee that the rate of colonization will be sufficient to have agronomic significance. In addition, the developmental stage of the plant to be inoculated may be critical: the earlier the inoculation, the greater the benefits to the plant (Barea *et al*., 1993).

Here, the effect of AMF on lisianthus growth and yield under greenhouse conditions in the Negev and Arava desert areas of Israel is presented. Several methods for AMF inoculum application were tested and the effects on growth, yield and vase life were recorded. These and additional applied studies are likely to lead to a precise and thorough utilization of AMF in arid and semi-arid regions.

Abbreviations used: AMF (arbuscular mycorrhizal fungi), EC (electrical conductivity), i.u. (infective units), RH (relative humidity).

Material and methods

To find an efficient and effective method for AMF application, several approaches were examined for their effect on lisianthus. Experiments were conducted at two experimental sites: MOP DAROM in the western Negev desert and Yair Station in the Arava desert, with the lisianthus cultivars and Echo White and Excalibur «pure white», respectively. Mycorrhizal inocula consisted of «whole inoculum», *i.e.,* a mixture of spores of *Glomus intraradices* species and inoculated root fragments, mixed with vermiculite (Wininger *et al*., 2003).

The different application methods tested were: ground application of bulk inoculum to the planting pit (10 mL into the planting hole, 1,000 i.u., designated G); application during seeding in the nursery, as 10% (v/v) of the seeding mixture (100 i.u. per plant, designated N); a combination of both N and G treatments (designated B); application by dipping seedling roots in inoculum, 70 days post-seeding, upon planting (designated D) and control, in which no inoculum was applied (designated C). All treatments were tested in MOP DAROM, whereas only treatment B was applied at Yair Station.

Lisianthus seedlings were grown at Hishtil Nursery for 70 days in Sussia, Israel (a cool climatic region) to prevent the need for seedling vernalization. They were then planted in local soils at the two experimental sites. MOP DAROM consisted of local sandy soil [electrical conductivity (EC) 2.73 at 0-20 cm depth, EC 1.72 at 20-40 cm depth, initial ground phosphate (P) level 42.5-52.5 mg kg^{-1} (soluble P determined by Olsen-sodium bicarbonate extract)]. Yair Station consisted of local sandy soil [EC 3, initial ground P level 40 mg kg^{-1} (soluble P determined by Olsen-sodium bicarbonate extract)]. Plants were grown under controlled greenhouse conditions. Four replicates were used for each AMF treatment, placed in randomized blocks, and in each plot, 100 plants were sampled for measurements. At MOP DAROM, fertilization during the experiment consisted of low P growth conditions (7:1:7 N:P:K), whereas at Yair Station the regular (7:3:7 N:P:K) P fertilization levels were used.

Several parameters were examined for evaluation of mycorrhizal effect on lisianthus growth and postharvest quality. These consisted of flowering stem number, length and weight, flower weight, vase life and number of surviving plants following exposure to two fungal diseases (pathogenic fungi identified by the Services for Plant Protection, Israeli Ministry of Agriculture). The effect of mycorrhiza on the vase life of cut flowers was evaluated as previously described (Meir *et al*., 2007) in a controlled standard observation room maintained at 20°C with 60 to 70% relative humidity (RH) and a 12-h photoperiod provided by cool-white fluorescent tubes and regular lamps at a light intensity of 14 µmol m^{-2} s^{-1}. Immediately after harvest, cut flowers were pulsed with a solution composed of 200 mg kg^{-1} 8-hydroxyquinoline citrate (8-HQC; TOG-4 Milchan Bros. Ltd., Israel), 0.22 mM 6-benzyl aminopurine (BA; TOG-L-101 Milchan Bros Ltd.), 0.225 mM silver thiosulfate (STS-75 Milchan Bros Ltd.) and 5% (w/v) sucrose. The pulsing was performed for 4 h in the ob-

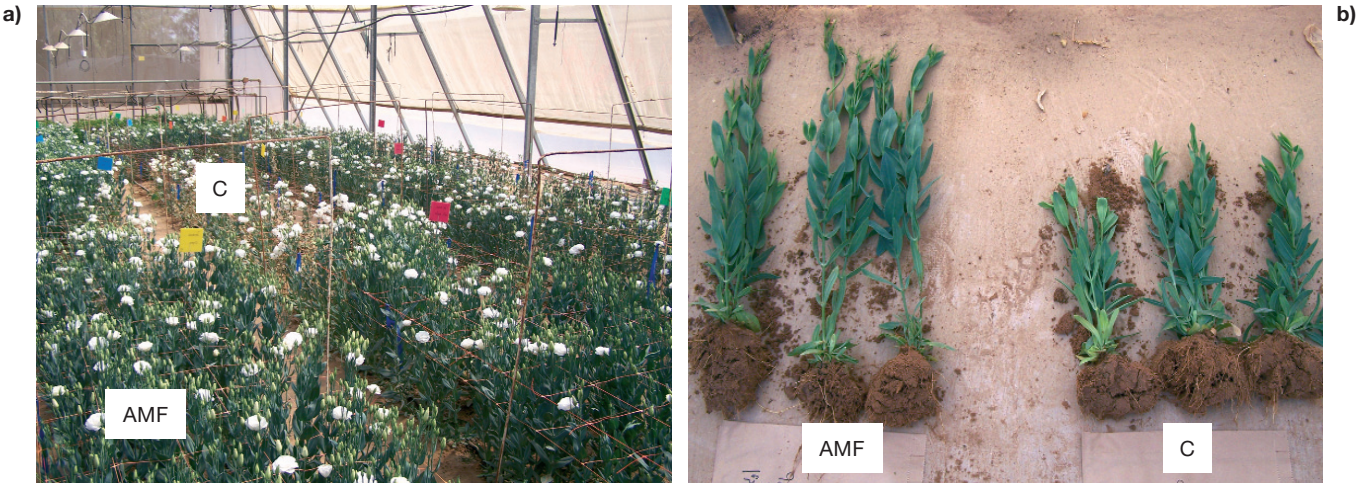

Figure 1. a) The experimental plot used in MOP DAROM, located in the western Negev desert.(b) Demonstration of AMF's effect on growth of lisianthus (*Eustoma grandiflorum,* cv. Excalibur) at Yair Station, during the second wave of growth, 24 weeks after planting. AMF: inoculated with mycorrhiza. C: non-inoculated control.

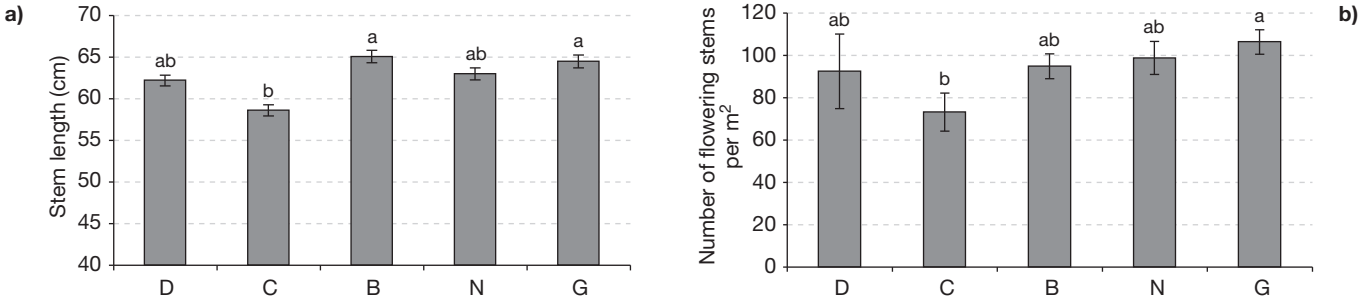

Figure 2. a) Length of lisianthus (*Eustoma grandiflorum,* cv. Echo White) flowering stems at harvest. b) Number of lisianthus flowering stems per m^2, following different mycorrhizal treatments under low P conditions at MOP DAROM. The different application methods consisted of ground application of bulk inoculum to the planting pit (G); application during seeding in the nursery, as 10% of the seeding mixture (N); a combination of both N and G treatments (B); application by dipping seedling roots in inoculum (D), and control, in which no AMF inoculum was applied (C). Different letters above columns designate significantly different means ($P \leq 0.05$).

servation room followed by an additional 20 h incubation at 6°C in the dark. After pulsing, the treated flowers were incubated for 24 h in boxes held in a cold storage room at 6°C and 80% RH to simulate air transport. Vase life was then evaluated by placing the flowers in a preservative solution composed of 50 mg kg^{-1} active chlorine complexed as sodium dichloroisocyanureate (TOG-6, Milchan Bros. Ltd.). Vase life of the flowers was considered over when three flowers had senesced. Means of replicates were subjected to statistical analysis by multiple-range test ($P \leq 0.05$), using the JMP statistical package (SAS, Cary, NC).

The presence of mycorrhizal colonization was examined in the roots for each of the treatments: all roots were collected, washed and stained with acid fuchsin, as described by Floss *et al*. (2008), and observed by confocal microscope (Olympus IX81,Tokyo, Japan) to detect the fluorescence signal.

Results and discussion

Application of AMF to lisianthus enhanced its growth and vigor (Fig. 1) under the examined conditions, especially during the second wave of growth and flowering. This positive effect was measured under both regular and low P growth conditions. Under low P, the ground (G) mycorrhizal application and the combined nursery and ground application (B) significantly increased yield parameters, especially in the second flowering wave. Significantly increased parameters included flowering stem length and number of commercial flowering stems per square meter (Fig. 2); other quality parameters, such as number of flowers within an inflorescence and flower weight improved with AMF treatment B, albeit not significantly (not shown).

Under regular P levels, at Yair Station, AMF was applied only as treatment B. In the second flowering wave, a marked enhancement, especially of stem length, was recorded for AMF-treated *versus* control plants (Fig. 3).

The MOP DAROM experimental plot was spontaneously infected with two pathogenic fungi: *Fusarium solani* and *Rhizoctonia solani*; this infection led to plant collapse, recorded at the end of the experimental period. A marked increase in plant survival was recorded for all AMF treatments relative to the control. A

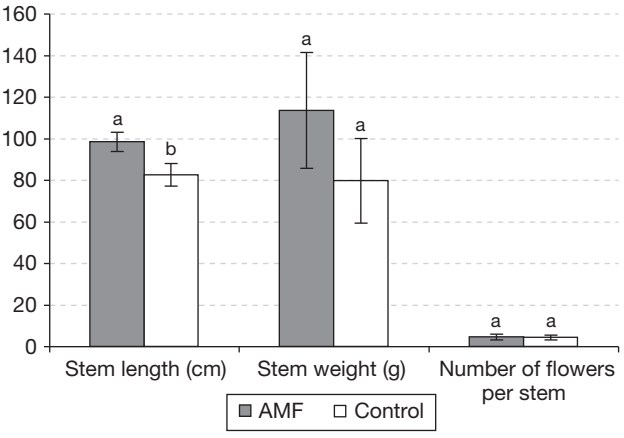

Figure 3. Stem length, stem weight and number of lisianthus (*Eustoma grandiflorum,* cv. Excalibur) flowers per stem following mycorrhizal treatment under regular P conditions at Yair Station. Application methods consisted of a combination of both nursery and ground treatments (AMF) and control, in which no AMF inoculum was applied. Different letters above columns designate significantly different means ($P \leq 0.05$) within each examined parameter.

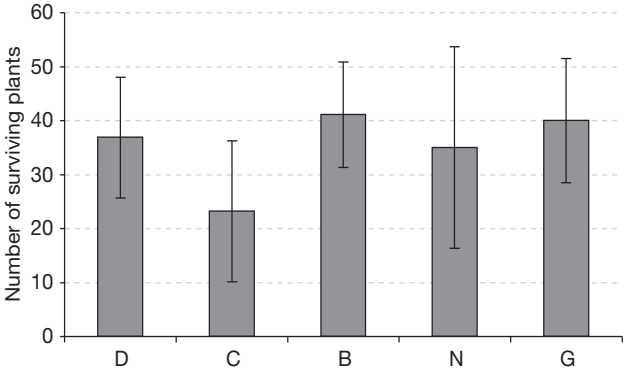

Figure 4. Number of surviving lisianthus (*Eustoma grandiflorum,* cv. Echo White) plants in MOP DAROM following spontaneous infestation with Fusarium solani and *Rhizoctonia solani*. The different AMF application methods consisted of ground application of bulk inoculum to the planting pit (G); application during seeding in the nursery, as 10% of the seeding mixture (N); a combination of both N and G treatments (B); application by dipping seedling roots in inoculum (D), and control, in which no AMF inoculum was applied (C). No significant differences were found between treatments ($P \leq 0.05$).

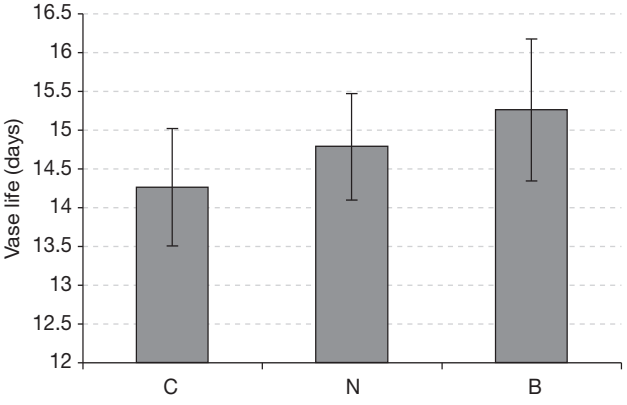

Figure 5. Vase life of lisianthus (*Eustoma grandiflorum,* cv. Echo White) cut flowers harvested from plants grown at MOP DAROM. The different AMF application methods consisted of ground application during seeding in the nursery, as 10% of the seeding mixture (N); a combination of treatments N and G (ground application of bulk inoculum to the planting pit) (B) and control, in which no AMF inoculum was applied (C). Data represent means of five replicates (five stems each) per treatment ± SE. No significant differences were found between treatments ($P \leq 0.05$).

similar protective effect of AMF has been recorded for other plant species (reviewed by Jeffries *et al*., 2003). However, probably due to the non-uniform spread of the fungal diseases, results were with high standard error and hence, were not statistically significant (Fig. 4).

Another parameter examined in our experiments was the effect of AMF treatments on the vase life of cut flowers. Following AMF treatments N and B, a trend of increasing vase life was observed compared to the control, but the differences were not statistically significant (Fig. 5). However, this may be regarded as a positive result as the AMF treatment improved yield without decreasing vase-life quality.

Lastly, the presence of mycorrhiza was examined in lisianthus roots. Following 2 months of lisianthus growth in the MOP DAROM experimental plot (AMF treatment N), hyphae and vesicles of AMF were clearly visible along the root's vascular system (Fig. 6).

To conclude, treatment with AMF enhanced several parameters of lisianthus yield and growth. The early mycorrhizal applications used in these experiments —at seeding in the nursery and/or during planting in the soil— are recommended. However, previous studies have suggested that the infectivity of mycorrhizal inoculants is influenced by the growing media and additi-

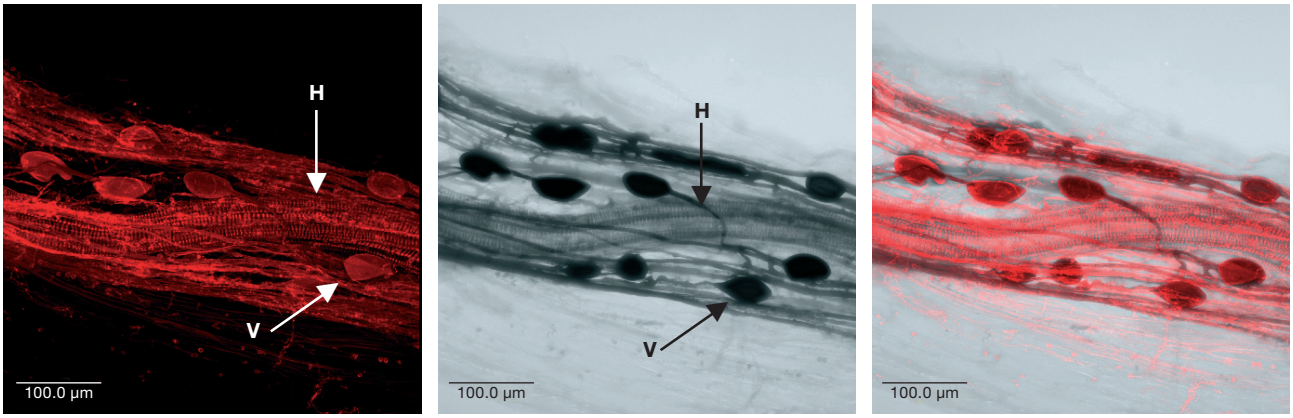

Figure 6. Microscopic images of lisianthus (*Eustoma grandiflorum,* cv. Echo White) roots following 2 months of growth in the MOP DAROM experimental plot (AMF application was performed in the nursery; treatment N); hyphae (H) and vesicles (V) are designated.

ves (Corkidi *et al*., 2004). Hence, optimization for specific conditions may be needed, especially for nursery application. Importantly, positive effects on lisianthus yield were also evident under regular P conditions, further suggesting that AMF may be useful for commercial growth of lisianthus. Studies are now being conducted on a semi-commercial scale, and examination of AMF's ability to promote yield of this high-cash crop in growers' farms is ongoing. Conceivably, AMF application may be extended to a variety of other ornamental crop species, and integrated as part of their growth protocols for enhancement of growth and yield.

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