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Original Article

Effects of Arbuscular Mycorrhizal Fungi on *Gazania rigens* Pot Plant Cultivation in a Mediterranean Environment

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Abstract

Herbaceous plants used in island beds and borders need to be rapid growing, high performing and maintaining good visual quality during the growing season. Arbuscular mycorrhizal (AM) fungi application is acquiring interest for its beneficial effects on ornamental bedding plants. *Gazania rigens* is a herbaceous ornamental plant grown for its large daisy-like flowers. The species thrives in the coastal areas of the Mediterranean region, particularly in the mild climate of southern Italy and Sicily, where performs well in summer bedding schemes in sea side gardens even in dry and windy conditions. The aim of this study was to evaluate the effect of inoculation with *Rhizophagus irregularis* on several ornamental parameters of *Gazania rigens*. Prior to transplanting, three-months-old plants received a mycorrhizal inoculum carrying 40 spores g^{-1} of *Rhizophagus irregularis*. Inoculum was applied at a rate of 10 g $plant^{-1}$. The AM application significantly increased number of flowers per clump by 100% and number of flowers per plant by 124.0%. *Rhizophagus irregularis* also positively influenced number of leaves per plant, plant height, and roots dry weight. Our findings indicated that mycorrhizal inoculation with *R. irregularis* may be beneficial to nursery growers wishing to produce high quality gazania for spring-summer bedding plant schemes.

Keywords: bedding plants; micorrhizal inoculation; ornamental quality; perennials; *Rhizophagus irregularis*

Introduction

Ornamental annuals and perennials are widely used in island beds and borders due to their functional and aesthetical qualities. As these planting schemes need to be attractive for as much of the year as possible, plants should be rapid growing, high performing and maintaining good visual quality along the growing season. *Gazania rigens* is a herbaceous ornamental plant grown for its large daisy-like flowers. Commonly grown as annual in cold regions, *G. rigens* is perennial in frost free areas such as those of the Mediterranean coastal regions. Garden design in Mediterranean climate regions nowadays relies mainly on native ornamental shrubs which are tolerant to the hot and dry local summer (Lopez *et al.*, 2006; Cassaniti *et al.*, 2009; Sabatino *et al.*, 2014). However, although not native from the Mediterranean Basin, *G. rigens* is drought tolerant, adapts to a wide range of soils, and performs well in summer bedding schemes in sea side gardens even in dry and windy conditions (Hamrick, 2003). There are reports proving that the application of commercial AM fungal-inoculants (AMF) can improve survival, plant growth and flowering performances of several ornamental annuals and perennials (Aboul-Nasr, 1996; Gaur *et al.*, 2000; Sohn *et al.*, 2003; Hayek *et al.*, 2012; Bhatti *et al.*, 2013; Püschel *et al.*, 2014). However, there is limited information concerning the response of *G. rigens* to mycorrhization. According to Puschel *et al.* (2014) AMF inoculation significantly increased root dry weight and number of leaves in *G. rigens*, but did not affect flowering-related parameters. However, in this study plants were observed only over a three-month period and since *G. rigens* are perennial in Mediterranean climates, it can be assumed that further researches performed in regions with mild winter and concerning a longer period of observation would provide useful information on plant response to AMF inoculation.

Therefore, the objective of the present study was to examine growth, development, flowering and plant quality response of *G. rigens* in relation to AM fungus *Rhizophagus irregularis* inoculation in the northern coast of Sicily.

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Materials and Methods

Experimental site, plant materials, growing conditions and treatment setting

The experiment was performed in the experimental farm of the Department of Agricultural, Food and Forest Sciences - University of Palermo - Italy (38° 9' 23" N, 13°20'2" E; altitude 48 m). On 11th May 2016, three-months-old *Gazania rigens* L. plants were transplanted in round plastic pots (15 cm in diameter and 13 cm in height) filled with 1.0 L of a commercial peat-perlite substrate mix (VIGORPLANT, SER FS V10-18, Italy) 80:20 (v/v). The substrate mix used had the following properties: pH 5.5, electric conductivity 0.20 dSm^{-1}, dry, bulk density 95 kg m^{-3}, total porosity 94%, NH_4 220 mg kg^{-1}, NO_3 833 mg kg^{-1}, P 40 mg kg^{-1}, K 631 mg kg^{-1}. Prior to transplanting, half of the pots received a mycorrhizal inoculum carrying 40 spores g^{-1} of *Rhizophagus irregularis* (formerly *Glomus intraradices*). Inoculum was applied before transplant at a rate of 10 g $plant^{-1}$. The plants were grown into a shadehouse [80% of shading (Retessrl, black shading net 80, MI, Italy)]. The pots were irrigated with tap water to ensure that the plants would not be exposed to water stress. Since fertilization application to soil mixes has been reported to suppress AM association (Biermann and Linderman, 1983a), no additional fertilizer was applied throughout the experiment. Air temperature and light level using a quantum light meter [LI-190 quantum sensor (Licor, Lincoln NE)] were recorded during the night and during the day. The average daily photosynthetic light integral (DLI) was calculated (Fig. 1).

Measurements and calculations

At 72, 87 and 102 days after transplanting (DAT) all plants were subjected to a not-destructive measurement, where the following parameters were estimated: number of clumps, number of leaves, number of flowers and plant height. After 102 DAT, flower diameter was also recorded. At the end of the experiment (102 DAT) each plant was divided in three fractions, flowers, clumps, and roots. Fresh and dry weights of each fraction were determined. Roots were washed to remove the substrate and then weighed. The dry weight was obtained, with a ponderable method, through the dehydration of the samples in a heater at 80 °C for 3 days until constant weight was reached. Root-to-shoot ratio (R/S) and dry matter partitioning in terms of percentage were also calculated.

To visualize the mycorrhizal development, the method described by Phillips and Haymann's (1970) and modified by Torta *et al.* (2003), was applied. In particular, three samples of lateral roots from each AM plant were collected and stained by acid fuchsine. The micorrhizal colonization [Mycorrhization Index (MI = % of stained tissue, with respect to hyaline portion, on the unit of length of the root)] was assessed on three fragments, obtaining the average value (Kormanik and McGraw, 1991). The weight of the root sub-sample used for the determination of micorrhizal colonization was added to root dry weight after recalculation of its fresh weight to dry weight.

Statistical analysis

The treatment was arranged in a randomized complete block design with four replicates per treatment of ten pot plants. All the data sets were analyzed by one-way analysis of variance. Percentage data were subjected to arcsin transformation before ANOVA analysis [$Ø= \arcsin (p/100)^{1/2}$]. The significance level $p < 0.05$ was used, and the significant differences between means were evaluated using Tukey's HSD test. All data were statistically analyzed using the SPSS software packageversion 14.0 (StatSoft, Inc., Chicago, USA).

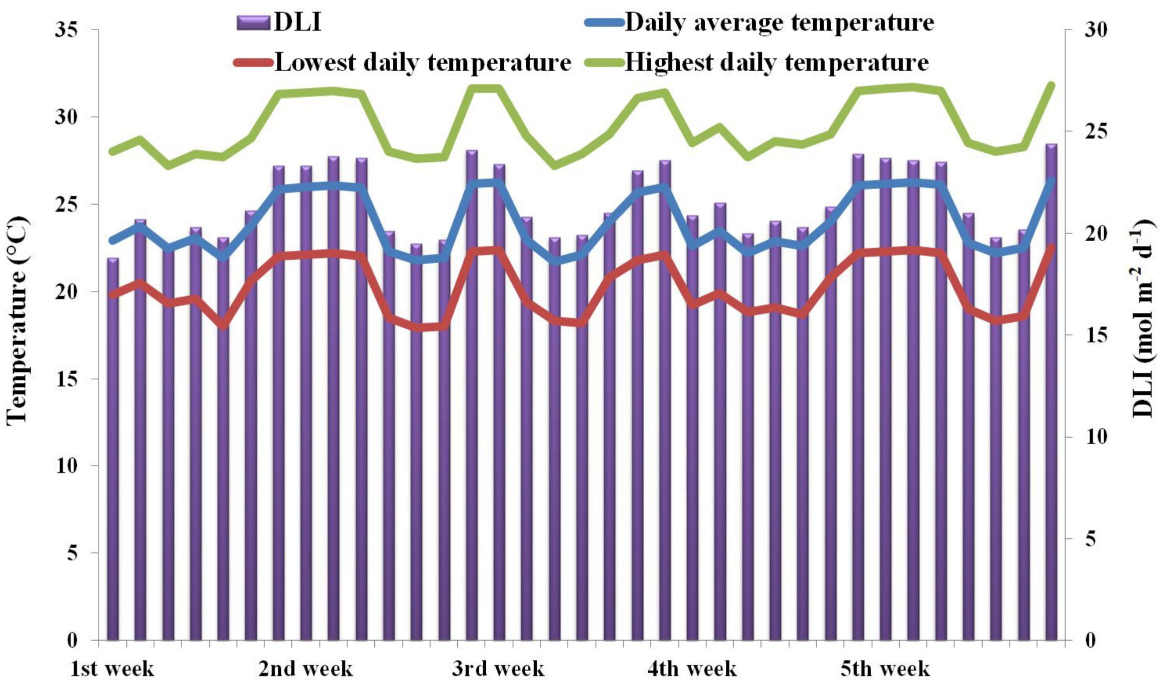

Fig. 1. Average, lowest and highest daily temperatures and DLI in Palermo, Sicily during the experimental period in a shadehouse covered with a 80% shade cloth

Results

Mycorrhizal inoculation reached 72.5% in AM+ plants and 16.2% in AM- plants (Fig. 2). Our observations revealed that 72 DAT the number of leaves in AM+ plants was significantly higher than in AM- plants (88.7 and 58.0 leaves plant^{-1}, respectively) (Table 1).

Data collected 87 and 102 DAT supported a similar trend. Inoculation significantly increased the number of flowers per clump both 87 and 102 DAT (Fig. 3). Data collected 72 DAT revealed that AM inoculation did not significantly affect number of flowers per plant. However, 87 and 102 DAT, ANOVA displayed significant differences between AM- and AM+ plants in terms of flowers per plant (2.3 and 4.3 flowers plant^{-1} after 87 DAT, respectively and 5.0 and 11.2 flowers plant^{-1} after 102 DAT, respectively). Data collected on plant height supported the trend established for the number of flowers per plant (Table 1) as 87 and 102 DAT, AM fungi inoculation positively influenced the above mentioned parameter (11.7 and 15.3 cm 87 DAT for AM- and AM+ plants, respectively and 13.8 and 18.8 cm 102 DAT for AM- and AM+ plants, respectively). Inoculation did not significantly affect flower diameter (Table 1).

AM inoculation significantly increased the percentage of DW of flowers, clumps and roots (Table 2). However, AM- plants revealed a significantly higher value in terms of R/S than AM+ plants (0.14 and 0.11, respectively) (Table 2). Inoculated plants had a higher flower dry mass ratio than not-inoculated ones (19.3 and 6.9%, respectively) (Fig. 2). AM- plants displayed a higher clump dry mass ratio than AM+ plants (80.7 and 70.8%, respectively) (Fig. 4). On the contrary, inoculate and not-inoculated plants had a similar root dry mass ratio (10.0 and 12.3%, respectively).

Table 1. Effects of arbuscular mycorrhizal fungi (AM) on number of flowers per clump, number of leaves, number of flowers, plant height and flower diameter in potted *Gazania rigens* L. plants

Treatment	Number of flowers clump $^{-1}$						Number of leaves plant $^{-1}$						Number of flowers plant $^{-1}$						Plant height (cm)						Flower diameter (mm)	
	72 DAT		87 DAT		102 DAT		72 DAT		87 DAT		102 DAT		72 DAT		87 DAT		102 DAT		72 DAT		87 DAT		102 DAT		102 DAT	
AM -	0.1	NS	0.2	b	0.4	b	58.0	b	67.4	b	76.6	b	0.7	NS	2.3	b	5.0	b	12.4	NS	11.7	b	13.8	b	47.9	NS
AM +	0.1		0.4	a	0.8	a	88.7	a	112	a	129	a	0.7		4.3	a	11.2	a	12.7		15.3	a	18.8	a	49.0	NS

In each column, values followed by same letters are not statistically different according to Tukey HSD Test (P≤0.05).
DAT, day after transplanting; AM-, not-inoculated; AM+, inoculated.

Table 2. Effects of of arbuscular mycorrhizal fungi (AM) on flowers, clumps and roots percentage of DW and root-to shoot ratio (R/S) in potted *Gazania rigens* L. plants

Treatment	Percentage of DW (%)						Root-to-shoot ratio (R/S)	
	Flowers		Clumps		Roots			
AM -	4.6	b	5.7	b	7.7	b	0.14	a
AM +	5.5	a	6.8	a	8.2	a	0.11	b

In each column, values followed by same letters are not statistically different according to Tukey HSD Test (P≤0.05).
DAT, day after transplanting; AM-, not-inoculated; AM+, inoculated.

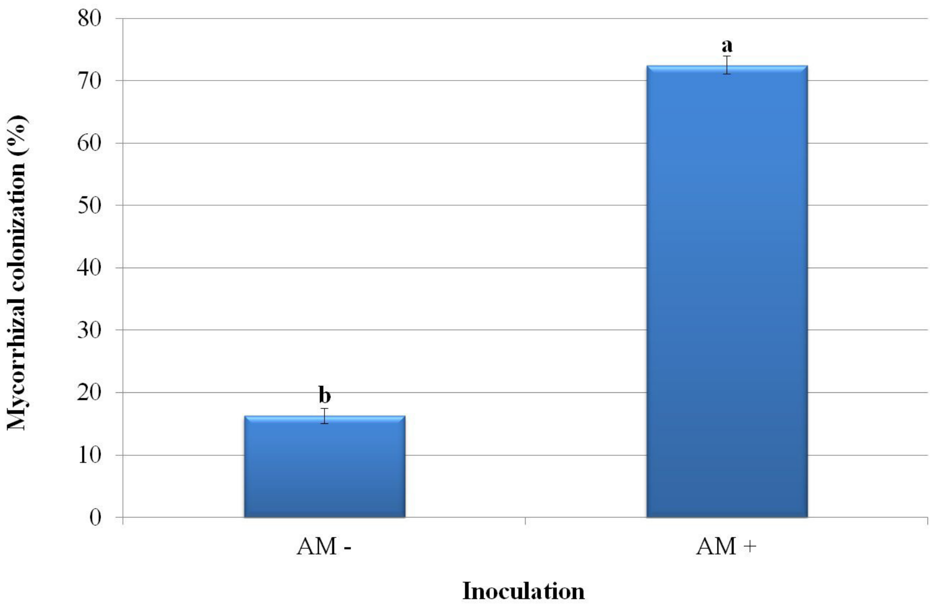

Fig. 2. Mycorrhizal colonization in potted *Gazania splendens* L. plants. Different letters indicate significantly different means at $p \leq 0.05$ (Tukey HSD test). Bars indicate the standard error of the mean (n = 4). AM-, not-inoculated; AM+, inoculated

Fig. 3. Not inoculated (a) and inoculated (b) 15 cm diameter pot *Gazania rigens* plants

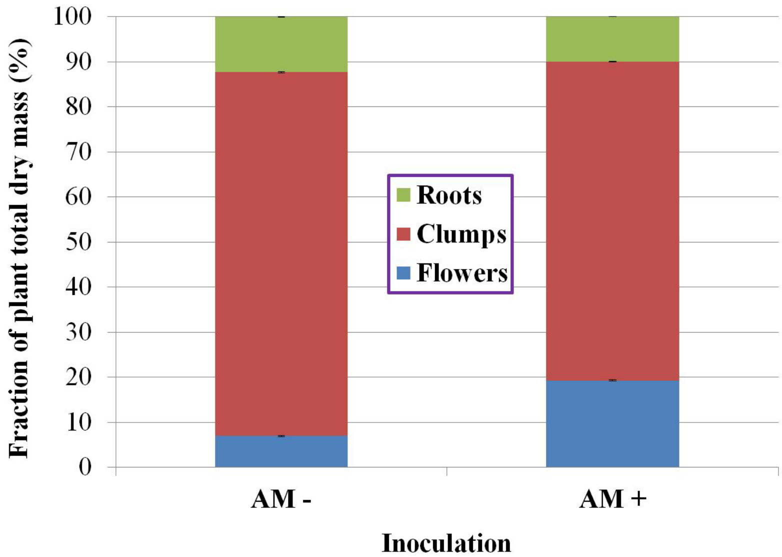

Fig. 4. Effects of arbuscular mycorrhizal fungi (AM) on dry mass partitioning among roots, heads and flowers in potted *Gazania rigens* L. plants. Different letters indicate significantly different means at $p \leq 0.05$ (Tukey HSD test). Bars indicate the standard error of the mean (n = 4). AM-, not-inoculated; AM+, inoculated

Discussion

In the present work, we analyzed the effects of arbuscular mycorrhizal fungi on vegetative and flowering response of *Gazania rigens* L. and their likely association with plant inoculation adaptation strategies. More than 70% gazania plants were successfully inoculated. Our results indicated that plants, AM fungi and substrate positively interacted and mycorrhizal symbiosis was established. However, our findings also revealed that mycorrhizal inoculation achieved 16.2% in AM- plants. Erratic presence of mycorrhizae in soilless media, although rare, has been previously attributed to airborn contamination in dust (Linderman and Davis, 2003). Our experiment showed that AM fungi may enhance growth parameters such as number of clumps per plant, number of leaves, plant height and percentage of DW. Our outcomes are partially consistent with those of Püschel *et al.* (2014), who reported an increase in terms of root DW and number of leaves in *Gazania rigens*, however, in line with our findings these authors report an increasing tendency in terms of root DW and number of leaves from AM+ plants. A positive effect of AM fungi on growth parameters has been revealed in various ornamental plants such as *Pelargonium peltatum*, *Pelargonium zonale*, *Sanvitalia procumbens* (Püschel *et al.*, 2014), *Tagetes erecta* (Asrar and Elhindi, 2011) and *Antirrhinum majus* (Asrar *et al.*, 2012). In contrast, Koide *et al.* (1999) found that AM inoculation determined growth depression in *Salvia rigens*, *Impatiens walleriana*, *Tagetes patula*, *Petunia* × *hybrida*, *Coleus* × *hybridus* and *Viola* x *wittrockiana*. These negative effects are probably due to a certain level of incompatibility among plants, AM fungi and substrate. Nevertheless, Püschel *et al*. (2014) revealed neutral effect of AM fungi inoculation on growth parameters in *Dimorphoteca sinuata*, *Impatiens hawkerii* and *Verbena* × *hybrida*. It should be mentioned that, although several authors often use the 'root-to-shoot dry mass ratio' to explain the treatment effects on the dry mass partitioning, we also used 'root dry mass ratio', 'clump dry mass ratio' and 'root dry mass ratio'. These data provide a more completed view on the effects of AM fungi on the partitioning of biomass among different plant parts, and should present the same direction of change as to using the 'root to shoot dry mass ratio' by early studies on dry matter accumulation and distribution in *Helianthus petiolaris* and *Helianthus annuus* (Sobrado and Turner, 1986). In this respect, our results revealed that inoculate and not-inoculated plants had a similar root dry mass ratio. Since our plants were grown on pots (15 cm diameter), this cultivation conditions might have mitigate a possible increase in root dry mass ratio. On the other hand, all gazania plants were irrigated to ensure that the plants would not be exposed to water stress. Thus, our results are also in line with the theory of the functional balance proposed by Brouwer (1963), which predicts that plants will react with a relative increase in the flow of assimilates to the root, leading, in the presence of limited water availability, to a consequent increase in root dry mass ratio. Our findings displayed a higher flower dry mass ratio of AM+ plants compared with AM- plants. The potentially most desirable effect of mycorrhizal inoculation of ornamental plants is the stimulation of flowering. We found a positive effect of AM fungi on the number of flowers after 87 and 102 DAT. Our results are consistent with those of Perner *et al*. (2007), Vosátka *et al*. (1999), Asrar *et al*. (2012) and Vaingankar and Rodrigues (2012), who reported an increase on the number of flowers in *Pelargonium peltatum*, *Verbena* sp., *Antirrhinum majus* and *Chrysanthemum morifolium* or *Tagetes erecta*. Furthermore, our outcomes are in accord with those of Püschel *et al*. (2014), who observed a tendency towards a higher number of flowers in *Gazania rigens*. Although our study showed that AM fungi did not significantly affect flower diameter, we observed a tendency towards a higher values in terms of flower diameter in inoculate plants (p = 0.059). Therefore, these results are partially consistent with those of Sohn *et al*. (2003) and Asrar and Elhindi (2011), who found that AM fungi inoculation increased the size of flowers in *Chrysanthemum morifolium* and *Tagetes erecta*.

Conclusions

In recent years there has been an increasing interest in the use of tender herbaceous perennials which are suitable for gardens in the spring-summer-fall months. Gazania and other herbaceous are now more widely grown than years ago. In cold regions gazania must be replaced each spring whereas in frost-free areas, once established, plants can survive winter and provide new growth in early spring. In the coastal areas of Sicily, gazania can be transplanted in the garden from 10 cm pots or from well-established plants grown in 13-15 cm pots.

The application of agronomical techniques which can improve bedding plant settling, survival and flowering performance in a variety of conditions can be crucial especially in regions with hot and dry summer. There are reports that AM may represent a promising and environment friendly tool to enhance biotic stresses tolerance (Cantrell and Linderman, 2001) such as thermal stresses (Zhu *et al*., 2011) and stressful edaphic conditions as soil salinity (Cantrell and Linderman, 2001), water stress (Püschel *et al*., 2014) and soil toxicity caused by heavy metals (Ouziad *et al*., 2005; Shahabivand *et al*., 2012; Kumar *et al*., 2015; Rouphael *et al*., 2015). Other authors demonstrated that mycorrhize can enhance plant post-transplant survival and growth (Biermann and Linderman, 1983b; Chang, 1992; Vosátka, 1995). In the present experiment, a high percentage of gazania plants were successfully inoculated. AM inoculation significantly enhanced both plant growth parameters (number of leaves per plant and plant height) and flowering-related parameters (number of flowers per clump and number of flowers per plant). Flower, clump, root DW and R/S were also positively influenced by AM inoculation. Therefore, our study suggests that in the Mediterranean region mycorrhizal inoculation with *Rhizophagus irregularis* may be beneficial to nursery growers wishing to produce high quality gazania plants to be used in spring-summer bedding plant schemes.

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