

REGULAR ARTICLE

# Arbuscular mycorrhizal fungi regulate flowering of *hyacinths orientalis* L. Anna marie

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## ABSTRACT

Arbuscular mycorrhizal fungi (AMF) represent positive effects on growth performance, nutrient absorption and stressed tolerance of host plants, whereas it is not clear whether AMF can affect flowering traits of ornamental plants. In this work, *Diversispora spurca*, *D. versiformis*, and *Funneliformis mosseae* were applied to rhizosphere of potted hyacinth (*Hyacinths orientalis* L. Anna Marie) plants. After four months of mycorrhizal inoculation, root could be colonized by exogenous AMF species, varied from 38% to 49%, whilst *F. mosseae* had the best mycorrhizal status. Out of these AMF species used, only *F. mosseae*-inoculated plants recorded greater raceme length and biomass production of single flowerlet, raceme, and flower stem. *F. mosseae* also induced the flowering earlier in 2 days and prolonged flowering time for 3 days. *D. versiformis* postponed 2 days for flowering. Mycorrhizal plants recorded considerably higher acetic acid (IAA) and zeatin riboside (ZR) levels in flowers, irrespective of AMF species. *F. mosseae*-inoculated plants had significantly higher methyl jasmonate (MeJA) concentrations in flowers than other AMF- or non-AMF-treated plants. These results thereby conclude that *F. mosseae* can be used to regulate flowering of *H. orientalis* L. Anna Marie, including flowering earlier and prolonging flowering time, which is closely associated with IAA, ZR and MeJA levels in flowers.

**Keywords:** Auxin; Flowering earlier; Mycorrhiza; Ornamental plants; Zeatin riboside

## INTRODUCTION

Arbuscular mycorrhizal fungi (AMF), a kind of soil inhabited microbes, can establish symbiotic association (namely, arbuscular mycorrhiza) with most of terrestrial plants (Bainard et al., 2011). The presence of arbuscular mycorrhiza shows lots of benefits in the host plants, including enhancement in quality and growth of crops, improvement in physical and chemical properties of soils, increment in survive rate of micropropagated plants, alleviation of magnesium deficiency and drought stress, and enhancement in disease and insect resistance of plants (Garmendia et al., 2004, 2005; Garmendia and Mangas, 2012; Zhang et al., 2015; Liu et al., 2016).

Hyacinth is native to the Mediterranean region and South Africa (Mabberley, 1997) and has 500 to 700 species, characterized by gorgeous flower color and a powerful bouquet. As a result, hyacinths extensively apply to parterre,

flower mirror, garden, and cut-flower (Xie and Wu, 2017). Under the artificial vegetation of florescence, there is being a new highlight in regulating flowering time by means of temperature, light, culture mediums, and plant growth regulators (Hayama and Coupland, 2003; Nazari et al., 2011; Abdulrahman et al., 2012; Miyamoto et al., 2015). In floriculture, soil AMF contributes positive effects on flowering regulation of ornamental plants. As reported by Scagel (2003, 2004), inoculation with *Funneliformis mosseae* notably enhanced the number of flowers and prolonged lifetime of flowers in cut flower roses. Usha et al. (2005) observed that *Glomus deserticola* markedly resulted in ahead of time in flowering of grapevines. Asara et al. (2012) also found the increase in the size of flowers in snapdragon (*Antirrhinum majus* cv. butterfly) plants after colonized by *G. deserticola* (Trappe and John). However, part studies also showed that AMF could make flowering time delayed (Saia et al., 2014) and had no effect on the number of flowers (Linderman and Davis, 2004).

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Even so, the information about AMF roles on flowering of decorative plants is poorly known. Therefore, the objective of this work intended to evaluate the AMF influences on flowering time and raceme of hyacinth, in combination with the changes in endogenous hormones of flowers.

## MATERIALS AND METHODS

### Experimental materials and plant set-up

Three AM fungal species were selected as the fungal materials, which included *Diversispora spurca*, *D. versiformis*, and *F. mosseae*, respectively. These AMF species were provided by the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry Sciences and further propagated by white clover under the condition of pots.

*Hyacinths orientalis* L. Anna Marie was selected as the plant material here. The seedballs (16–17 cm diameter) of the hyacinth with were surface-disinfected by 75% of alcohol solution for 10 min and sown into pots (16 × 11 × 13 cm, top diameter × bottom diameter × height) on October 23, 2015, where 2 kg of autoclaved mixture with soils and sands (3:1, v/v) were supplied. Approx. 1100 spores of AM fungus were mixed with the growth substrate of each pot. The same amount of autoclaved inoculants plus 2 mL inoculum filtrate (25 µm filter) was supplied into pots as the non-AMF control.

The AMF- and non-AMF-inoculated plants were grown in a controlled environment, where photosynthetic photon flux density was 768 µmol/m<sup>2</sup>/s, and day/night temperature was 18/10°C. To reduce the environmental effects, all of pots were weekly rotated the place.

### Experimental design

The experiment was designed with four AMF treatments, accompanied with a completely randomized arrangement: *Funnelformis mosseae*, *Diversispora spurca*, *Diversispora versiformis*, and the non-AMF control. Each treatment replicated nine times in a total of 36 pots.

### Determinations of flower traits

After 130 days of seedball planting, the hyacinth plants had more than 50% flowerlets bloomed, which is in full flowering stage. Raceme per plant was harvested, whose fresh weight and length were determined. In addition, fresh weight in single flowerlet and flower stem was also measured. The time in first-flowering (the first flowerlet bloomed), full-flowering (>50% flowerlets bloomed), and final-flowering (>95% flowerlet bloomed) in raceme was recorded.

### Estimation of root AMF colonization

Fresh root systems with 1-cm long were stained by 0.05% (w/v) of trypan blue, as per the protocol described by

Phillips and Hayman (1970). Root AMF colonization (%) = mycorrhiza-colonized root lengths/total observed root lengths × 100. Entry points were expressed as the number of entry points per cm root.

### Determinations of phytohormones levels in flowers

The fresh flowerlets with 0.20 g were extracted with 4 mL of 80% methanol solutions containing 1 mM 2,6-ditert-butyl-4-methylphenol at ice-bath, inoculated at 4°C for 4 h, centrifuged at 4000 r/min for 10 min, and isolated by an AccuBond C18 soild phase kit (Agilent Technologies Inc., USA) (Chen et al., 2009). Afterwards, the enzyme-linked immune kits were used to measure the concentrations of acetic acid (IAA), total gibberellic acids (GAs), brassin (BR), zeatin riboside (ZR), methyl jasmonate (MeJA).

### Statistical analysis

The collected data were analyzed by one-way variance (ANOVA) in the SAS software (8.1v). Duncan's multiple range test was used to compare significant difference between the treatments at 5% level.

## RESULTS

### Root mycorrhizal status

The roots of *H. orientalis* L. Anna Marie were colonized by *D. spurca*, *D. versiformis* and *F. mosseae*, and the significantly higher root colonization and entry points ranked as *F. mosseae* > *D. spurca* > *D. versiformis* in the decreasing order (Fig. 1).

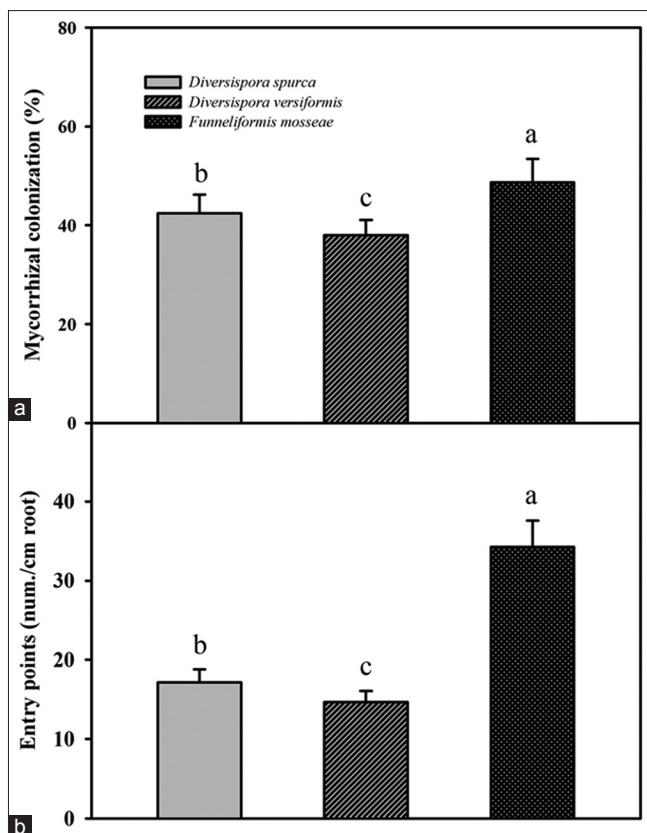
### Flower traits

Treatments with AMF represented diverse effects on the fresh weights of single flowerlet, raceme, and flower stem, dependent on AMF species (Fig. 2; Fig. 3). Meanwhile, plants inoculated only with *F. mosseae* possessed significantly higher single flowerlet, raceme, and flower stem biomass production than those with other inoculated and uninoculated treatments.

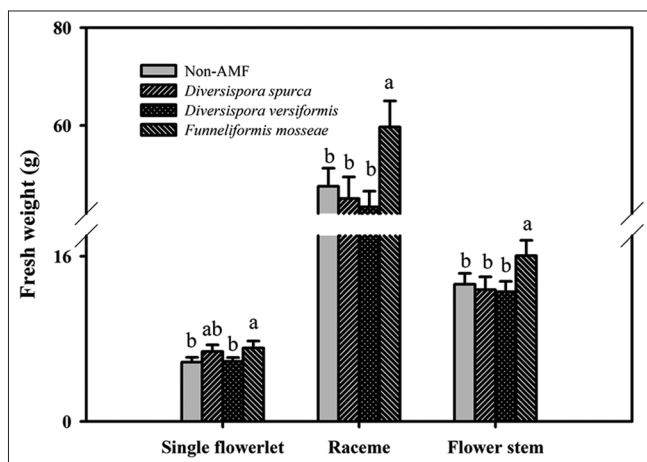
Amongst the used AMF species, the hyacinth plant inoculated only with *F. mosseae* recorded considerably greater raceme length (Fig. 3; Fig. 4). *D. versiformis* and *D. spurca* exhibited no significant effect on raceme length, relative to non-AMF-inoculated treatment.

### Flowering time

Compared with non-AMF plants, *D. spurca*-colonized plants showed longer days in the stage of final flowering, *D. versiformis*-colonized plants needed to have longer days to flower in first flowerlet, and *F. mosseae*-inoculated plants exhibited shorter days to flower in first flowerlet (Table 1). In addition, the daily number of blooming

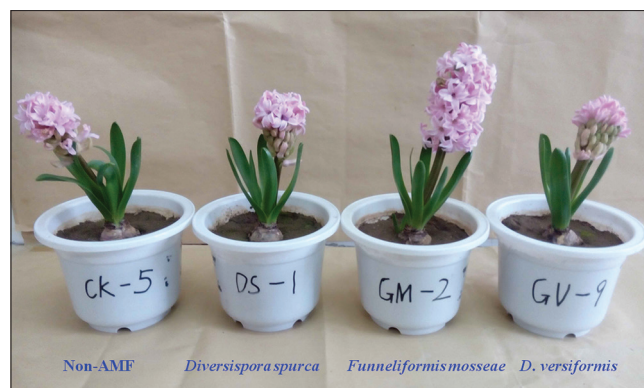


**Fig 1.** Root colonization (a) and entry points (b) in *Hyacinthus orientalis* L. Anna Marie inoculated with *Diversisporea spurca*, *D. versiformis* and *Funneliformis mosseae*. Data (means  $\pm$  SD,  $n = 3$ ) followed by different letters above the bars showed significant differences ( $P < 0.05$ ) between treatments.

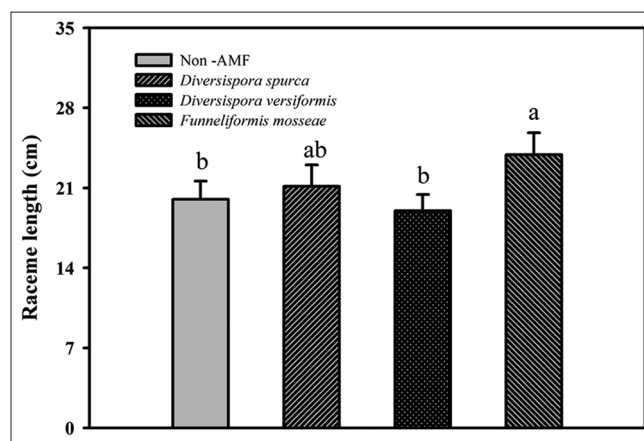


**Fig 2.** Effects of *Diversisporea spurca*, *D. versiformis*, and *Funneliformis mosseae* on biomass of single flowerlet, raceme, and flower stem in *Hyacinthus orientalis* L. Anna Marie. Data (means  $\pm$  SD,  $n = 3$ ) followed by different letters above bars showed significant differences ( $P < 0.05$ ) between treatments.

flowerlet was greater with *F. mosseae* inoculation while less with *D. spurca* and *D. versiformis* inoculation, compared with non-mycorrhizal inoculation in the whole flowering period.



**Fig 3.** Flowering status of *Hyacinthus orientalis* L. Anna Marie inoculated with *Diversisporea spurca*, *D. versiformis*, and *Funneliformis mosseae*.



**Fig 4.** Effects of *Diversisporea spurca*, *D. versiformis*, and *Funneliformis mosseae* on raceme length of flowers in *Hyacinthus orientalis* L. Anna Marie. Data (means  $\pm$  SD,  $n = 3$ ) followed by different letters above bars showed significant differences ( $P < 0.05$ ) between treatments.

### Endogenous phytohormone concentrations in flowers

Compared with non-AMF treatment, *F. mosseae*, *D. versiformis* and *D. spurca* significantly increased the IAA concentration of flowers by 19.6%, 19.4% and 17.7%, respectively (Fig. 5). Mycorrhizal plants colonized by *F. mosseae*, *D. versiformis* and *D. spurca* also represented 15.9%, 15.0%, and 12.7% higher ZR concentration of flowers in comparison with non-mycorrhizal plants. Inoculated plants had similar GAs and MeJA concentrations than un-inoculated controls, regardless of AMF species used, except a significant higher MeJA level in *F. mosseae*-inoculated plants.

### DISCUSSION

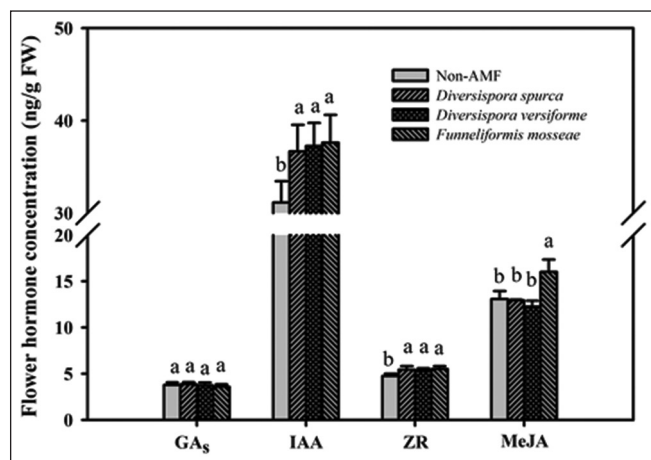
The present study indicated that inoculation with AMF exhibited different colonized status in roots and different effects on biomass production of flowers in *H. orientalis* L. Anna Marie. It is well known that AMF has a different capacity to colonize roots (Davoodian et al., 2012), dependent on the compatibility between AMF and host



**Table 1: The effects of *Diversispora spurca*, *D. versiformis*, and *Funnelformis mosseae* on flowering days of *Hyacinths orientalis* L. Anna Marie in full-bloom period**

Treatments	First-flowering days	Full-flowering days	Final-flowering days	Flowering time
Non-AMF	125.9±3.1bc	131.3±1.5ab	148.0±2.3b	22.1±2.0bc
<i>Diversispora spurca</i>	126.8±2.0ab	132.2±0.9a	150.3±0.5a	23.5±0.6b
<i>D. versiformis</i>	128.4±2.9a	132.5±0.3a	149.8±1.0ab	21.4±0.9c
<i>Funnelformis mosseae</i>	123.6±2.9c	130.2±1.1b	149.0±1.7ab	25.4±0.7a

Date (means±SD, n=6) followed by different letters indicated significant differences ( $P<0.05$ ) between treatments



**Fig 5.** Effects of *Diversispora spurca*, *D. versiformis*, and *Funnelformis mosseae* on total gibberellic acids (GAs), acetic acid (IAA), zeatin riboside (ZR), and methyl jasmonate (MeJA) concentration of flowers in *Hyacinths orientalis* L. Anna Marie. Data (means ± SD, n = 3) followed by different letters above bars showed significant differences ( $P < 0.05$ ) between treatments.

plants. In this work, only *F. mosseae* showed the highest root mycorrhizal colonization and entry points than the other AMF species, confirming that *F. mosseae* has the high-efficient compatibility with the hyacinth plant species. It is consistent with previous studies in harlequin flower (Scagel, 2004). Future works need to consider the compatibility between *F. mosseae* and other hyacinth plant species.

In this work, AMF inoculation showed diverse effects on raceme length and biomass of single flowerlet, raceme, and flower stem in *H. orientalis* L. Anna Marie. Hereinto, *F. mosseae* represented a positive effect, while two *Diversispora* species manifested no significant effect. Earlier study by Asrar and Elhindi (2011) reported that inoculation with AMF increased the size of the flowers of marigold. Mycorrhizal inoculation markedly increased flower height of *C. morifolium* (Vaingankar and Rodrigues, 2012) or had no significant effect (Linderman and Davis, 2004). AMF also enhanced the number of flowers of snapdragon and *Hypericum perforatum* (Asrar et al., 2012; Lazzara et al., 2017) or had no effect on it (Mora, 1990). It concludes that AMF can potentially improve flower morphology, heavily depended on the compatibility of both AMF and host plants, which is still needed to be considered in future.

Studies in the past indicated that AMF increased flowering earliness (Gaur et al., 2000; Garmendia and Mangas, 2012; Bona et al., 2015), postponed flowering (Dubsky and Sramek, 2002; Nowak, 2004), or had no effect on flowering (Adholeya, 2005). Meanwhile, it has also been found that AMF was able to prolong flowering duration (Jin et al., 2015) or shorten it (Banla et al., 2015). In our work, inoculation with AMF had diverse effects on flowering duration, strongly dependent on AMF species. *F. mosseae* had the effect on flowering earliness, and *D. versiformis* had the effect on postponed flowering. Moreover, *F. mosseae* also prolonged flowering duration than non-AMF controls. As a result, *F. mosseae* can be used to regulate flowering of hyacinth plants, including flowering earlier and prolonging flower duration. Hence, users can select a suitable AM fungus to regulate the flowering earlier and/or prolonging in field.

Endogenous phytohormones are one of the important factors to regulate flowering of ornamental plants (Shamshiri, 2012). Perner et al. (2007) proposed that AMF colonization may directly or indirectly modulate hormone balance for affecting flower development and blooming. Auxin plays an important role to plant morphogenesis (Alabadí et al., 2009). In our study, AMF colonization strongly stimulated the significant increment of IAA concentration in flowers than non-AMF colonization, regardless of AMF species, which is similar to Torelli et al. (2000) in onion, Meixner et al. (2005) in soybean, and Liu et al. (2016) in trifoliate orange subjected to well-watered and drought stress. This implies that AMF may regulate flower development and blooming by means of accelerating IAA synthesis. Total GAs are a kind of important hormones to promote the formation of flowers (Zhang et al., 2014). However, in the present study, mycorrhizal inoculation did not significantly affect GAs level in flowers of hyacinth plants, which is in agreement with previous study in trifoliate orange under mycorrhization with *F. mosseae* (Liu et al., 2016). It seems that GAs did not participate in flower regulation by mycorrhization. Earlier works showed the increase in ZR level by *F. mosseae* in *Citrus jambhiri* Lush (Dixon et al., 1988) and in *Poncirus trifoliata* (Liu et al., 2016), which is confirmed by our study in hyacinth plants, irrespective of AMF species. In our work, *F. mosseae*, but not *D. spurca* and *D. versiformis*, significantly increased MeJA level of flowers in hyacinth. Since ZR improved respiration of flowers to expand

petal and then induced blooming (Kobayasi and Atsuta, 2010), AMF-induced ZR increase might be the reason of AMF-stimulating flower development and blooming at an earlier stage, which depends on AMF species. In addition to phytohormones, plant nutrient status is an important factor in regulating flowering time and flower morphology in ornamental plants. As reported by Xie and Wu (2017), mycorrhizal inoculation induced greater concentrations of N, P, and K in flower, leaf, and roots of hyacinth plants. As a result, mycorrhiza-regulated flowering of ornamental plants is a complex issue by multiple physiological processes, which is still needed to be studied.

## CONCLUSION

AMF species exhibited positive or no significant effect on flower biomass, raceme length and flowering time in *H. orientalis* L. Anna Marie. Hereinto, *F. mosseae* can be used to regulate flowering of the hyacinth plant, including flowering earlier and prolonging flower duration. The mycorrhizal effect is possibly due to the changes in IAA, ZR and MeJA levels in flowers by mycorrhization. Future works need to highlight mycorrhizal effects on flowering and transcriptional levels of relevant flowering genes in different hyacinth plants and flower fragrance constitutions in hyacinth plants.

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## Author contributions

M-M. X and Q-S. W designed the study and took the data. M-M. X did the data analysis and wrote this paper. Q-S. W supervised the research project and also corrected the paper.

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