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Research Article
Mycorrhiza Modulates Morphology, Color and Duration of Flowers in Hyacinth

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Abstract
Background and Objective: Hyacinth, one of world-famous bulbous ornamental plants is widely planted in various habitats. The present work tried to evaluate whether and how inoculation with arbuscular mycorrhizal fungi (AMF) regulates flower morphology and flowering duration of hyacinth. **Materials and Methods:** Three AMF species, namely, *Diversispora spurca* (*D. spurca*), *Diversispora versiformis* (*D. versiformis*) and *Funneliformis mosseae* (*F. mosseae*) were inoculated into potted hyacinth (*Hyacinths orientalis* L. Anna Marie) plants for 130 days. Flower morphology, flowering duration, nutrient status, indole acetic acid (IAA), chlorophyll and anthocyanin concentration were measured. Data were analyzed by one-way analysis of variance (ANOVA) with SAS. Significances of treatments were compared by the Duncan's multiple range tests at $p<0.05$. **Results:** Root mycorrhizal colonization varied from 38.0-48.7%. Amongst three AMF species, only *F. mosseae* significantly ($p<0.05$) has increased flower biomass, opening flower number, flower stem height and floret diameter. *Diversispora spurca* and *F. mosseae*, respectively prolonged flower duration for 1.4 and 3.3 days. Mycorrhizal plants with *F. mosseae* had significantly ($p<0.05$) higher IAA concentration of flowers and roots, higher anthocyanin concentration of flowers, higher chlorophyll b and total chlorophyll levels of leaves and greater N, P and K levels of flowers and roots. *Diversispora spurca* colonized plants possessed significantly ($p<0.05$) higher flower IAA levels and leaf chlorophyll b and total chlorophyll concentrations. *Diversispora versiformis* generally did not affect these variables. **Conclusion:** Mycorrhizas could modulate morphology, color and duration of flowers in hyacinth, which is closely related with nutrient status, IAA levels and AMF species. Meanwhile, *F. mosseae* exhibited the best stimulated effects and can consider to be used in hyacinth cultivation.

Key words: Arbuscular mycorrhiza, auxin, chlorophyll, *Funneliformis mosseae*, hyacinth, nutrient

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soil arbuscular mycorrhizal fungi (AMF) can form mutualistic symbiosis with upto 80% of land's plants, arbuscular mycorrhizas. Arbuscular mycorrhizal symbiosis characterizes with mutual benefits: Host plants supply AMF with 3~20% of photosynthates and in return AMF provides water and nutrients to host plants by developed hyphal netmork[1,2]. Such mycorrhizal plants generally reproduce more successfully[3], effectively improve transplant survival and growth[4] and confer stronger resistance to abiotic and biotic stresses[5]. As a result, inoculation with AMF is regarded as an effecter to improve plant growth and development of ornamental and crop plants[6,7].

Hyacinth (*Hyacinthus orientalis* L.) is one of world-famous bulbous ornamental plants, which is widely applied in potted plants, cut-flower and nature plantation[8]. Particularly, hyacinth is well known for its gorgeous flower color and powerful fragrance. In floriculture, AMF shows positive effects on flower quality, flower growth and flowering time. Asrar *et al.*[9] reported that mycorrhizal inoculation with *Glomus deserticola* enhanced both flower biomass and flower size in snapdragon (*Antirhinum majus*). Scagel[10] reported that inoculation with AMF reduced flowering 7-8 days earlier in harlequin (*Sparaxis tricolori*). AMF increased the number of flowers in gerbera (*Gerbera jamesonii*)[11]. These results indicate that mycorrhizas have potential effects on flower growth and flowering time of ornamental plants. However, the information regarding AMF effects on hyacinth plants is poorly known.

The purpose of the present study was to analyze the effects of different AMF species on flower quality, flowering duration, nutrient status, indole acetic acid (IAA), chlorophyll and anthocyanin concentration of potted hyacinth plants.

MATERIALS AND METHODS

Plant set-up: *Hyacinths orientalis* L. Anna Marie was used in this study. The seed balls of hyacinths were chosen with 16-17 cm of the cross-section diameter and sterilized with 75% alcohol solution for 10 min and placed into a 3-L plastic pot on October 23, 2015. The pot was supplied with 2.0 kg autoclaved (121°C, 0.11 MPa, 2 h) substrates of soil and sand (3:1, v/v).

Three AMF species, namely, *Diversispora spurca* (C.M. Pfeiff., C. Walker and Bloss) C. Walker and A. Schüßler, *D. versiformis* (P. Karst.) Oehl, G.A. Silva and Sieverd and *Funneliformis mosseae* (T.H. Nicolson and Gerd.) C. Walker and A. Schüßler were used in this study. These AMF species were provided by the Bank of Glomeromycota in China (BGC) and propagated by pot culture in terms of identified spores and white clover under the host plant of *Trifolium repens* for 16 weeks. At the time of seedball planting, approximate 1100 spores of each AM fungus were inoculated into a pot as the AMF treatment. And the non-AMF control received same amount of autoclaved inoculums plus 2 mL inoculums filtrate (25 μm filter) to keep the similar microbial environment except the AM fungus. All treated plants were placed in a greenhouse with 768 μmol m^{-2} sec^{-1} photosynthetic photon flux density and 18/10°C day/night temperature. During acclimation of hyacinth plant, the pots were weekly rotated to avoid position effect. After 130 days, the test plants were in the full-bloom flowering period (>50% blooming of flowers) and then harvested.

Experimental design: The experiment consisted of 4 treatments with absolutely randomized blocked design. The 4 treatments included the inoculation with *D. spurca*, *D. versiformis*, *F. mosseae* and non-AMF control. Each treatment had 9 replicates, leading to a total of 36 pots.

Determinations of variables: The 1 cm long fresh root segments were cut and stained with 0.05% (w/v) trypan for 3 min by the protocol of Phillips and Hayman[12]. Root mycorrhizal colonization was estimated as the percentage of infected root lengths against total tested root lengths.

In the full-flowering period, flower morphological traits, including stem height, inflorescence length, floret diameter, opening floret number and total floret number were measured. The flowering duration was from the fist floret blooming to the last.

Leaf chlorophyll content was determined by the method described by Lichtenthaler and Wellburn[13]. Anthocyanin concentration in flowers was determined as per the protocol of Garcia-Viguera *et al.*[14]

The 0.2 g fresh samples of flowers, leaves and roots were used to determine IAA concentration in terms of the protocol of Chen *et al.*[15] under the Enzyme-Linked Immunosorbent Assay (ELISA) conditions.

N contention of flowers, leaves and roots was determined by the protocol of Zhao *et al.*[16]. P and K concentrations in tissues were determined by the protocol of Wu and Zou[17].

Statistical analysis: Data were analyzed by one-way variance of analysis (ANOVA) with SAS 8.1 software (SAS Institute Inc., Cary, NC, USA). Probabilities of significance were used to test

the significance among treatments. The significant differences between treatments were compared with the Duncan's multiple range (DMR) tests at $p<0.05$.

RESULTS AND DISCUSSION

Root mycorrhizal colonization and plant biomass production: No AMF structure (Fig. 1a) was observed in the non-inoculated hyacinth plants and the inoculated plants showed great root mycorrhizal colonization (Fig. 1b-d), which ranged from 38.0-48.7% (Table 1). Meanwhile, significantly (DMR, $p<0.05$) higher root mycorrhizal colonization was ranked as the trend of *F. mosseae*>*D. spurca*>*D. versiformis* (Table 1). This suggests different compatibility between AMF and hyacinth plants, which had been confirmed in previous studies[18].

Compare to non-AMF treatment, inoculation with *D. spurca*, *D. versiformis* and *F. mosseae* did not significantly ($p<0.05$) alter fresh and dry weight of roots (Table 1). In regard to flowers, amongst three AMF species used, only *F. mosseae*-inoculated treatment significantly ($p<0.05$) increased fresh and dry weight of flowers by 27.34 and 12.54%, respectively as compared with non-AMF treatment. In these AMF species, *D. spurca* did not affect leaf fresh and dry biomass and *D. spurca* and *D. versiformis* heavily decreased leaf fresh and dry biomass, in comparison with non-AMF inoculation. The result demonstrated that only *F. mosseae*, other than *D. spurca* and *D. versiformis*, had a positive effect on plant biomass production of hyacinth. It is in agreement with earlier studies in zinnia (*Zinnia elegans*)[19] and pot marigold (*Calendula officinalis*)[20]. It suggests that different AMF play different role on plant growth, due to functional diversity of AMF[21]. And, the compatablity of hyacinth plants with *F. mosseae* was greater than *D. spurca* and *D. versiformis.* As reported by the represent study, *F. mosseae* significantly promoted the growth of hyacinth, while *D. spurca* and *D. versiformis* were contrary. It is concluded that *F. mosseae* might elevate mineral nutrient absorption, increase IAA concentration and alter resource allocation. The results agreed with the research of Nadeem *et al.*[22]

Flower morphology and flowering duration: Among three AMF species used, only *F. mosseae* significantly (DMR, $p<0.05$) increased height of flower stem, floret diameter and the number of opening flowers and the other two AMF species did not alter flower morphology (Table 2). In addition, *F. mosseae* and *D. spurca* inoculated plants showed 3.3 and 1.4 days longer flowering duration than non-inoculated plants and *D. versiformis* shortened 0.7 days of flower duration. Previous studies had shown that AMF could increase, reduce or have no effect on flower traits and flowering duration of ornamental plants[23-26]. Inoculation with *Glomus intraradices* increased flower stem height of *Chrysanthemum morifolium*[23] while had no effect on marigold[24]. AMF also increased the number of flower in strawberry and *Hypericum perforatum*[25,26]. Similarly, AMF inoculation prolonged or also shortened flowering duration[27,28]. It is concluded that AMF-mediated flower morphology and flowering duration are strongly dependent on AMF species. As a whole, *F. mosseae* exhibited the best efficiency on flower than other two AMF species.

Chlorophyll concentration in leaf: Among three AMF species used, *D. versiformis* significantly (DMR, $p<0.05$) decreased

Table 1: Effects of AMF inoculation on root colonization and biomass production of hyacinth plants

	Fresh weight (g FW/plant)			Dry weight (g DW/plant)			AMF colonization
Treatments	Flowers	Leaves	Roots	Flowers	Leaves	Roots	(%)
Non-AMF	34.27±3.31^{b}	14.67±1.12^{a}	47.25±4.48^{a}	3.27±0.19^{b}	0.53±0.04^{a}	2.51±0.18^{ab}	0.0±0.0^{d}
D. spurca	32.27±3.17^{b}	12.94±1.14^{ab}	44.40±4.05^{a}	3.02±0.13^{b}	0.51±0.04^{ab}	2.27±0.11^{b}	42.4±3.8^{b}
D. versiformis	30.80±2.57^{b}	10.83±0.80^{c}	44.59±3.56^{a}	3.18±0.29^{b}	0.43±0.03^{c}	2.45±0.17^{ab}	38.0±3.1^{c}
F. mosseae	43.64±3.89^{a}	11.53±1.10^{bc}	46.01±4.16^{a}	3.68±0.14^{a}	0.44±0.03^{bc}	2.68±0.15^{a}	48.7±4.7^{a}

Data (Means±SD, n = 3) followed by different letters among treatments indicate significant differences (DMR, $p<0.05$) between treatments

Table 2: Effects of AMF inoculation on flower morphological traits and flowering duration of hyacinth plants in full-bloom period

Treatments	Flower stem height (cm)	Inflorescence length (cm)	Floret diameter (cm)	Opening floret number/plant	Total floret number/plant	Flowering duration (day)
Non-AMF	20.0±1.6^{b}	11.1±1.0^{ab}	2.9±0.2^{b}	24±2^{b}	55±5^{ab}	22.1±2.0^{bc}
D. spurca	21.2±1.9^{ab}	11.7±1.1^{ab}	3.1±0.2^{ab}	16±1^{c}	55±5^{ab}	23.5±0.6^{b}
D. versiformis	19.0±1.4^{b}	10.4±0.7^{b}	3.0±0.2^{b}	12±1^{c}	53±5^{b}	21.4±0.9^{c}
F. mosseae	23.2±1.4^{a}	12.3±1.0^{a}	3.3±0.1^{a}	45±4^{a}	59±5^{a}	25.4±0.7^{a}

Data (Means±SD, n = 9) followed by different letters among treatments indicate significant differences (DMR, $p<0.05$) between treatments

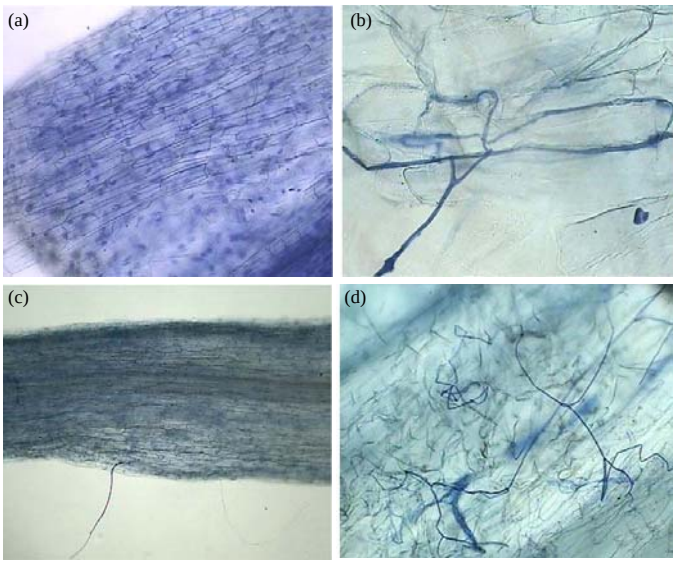

Fig. 1(a-d): Root mycorrhizal colonization of hyacinth plants by (a) Non-AMF, (b) *Diversispora spurca*, (c) *Diversispora versiformis* and (d) *Funneliformis mosseae*

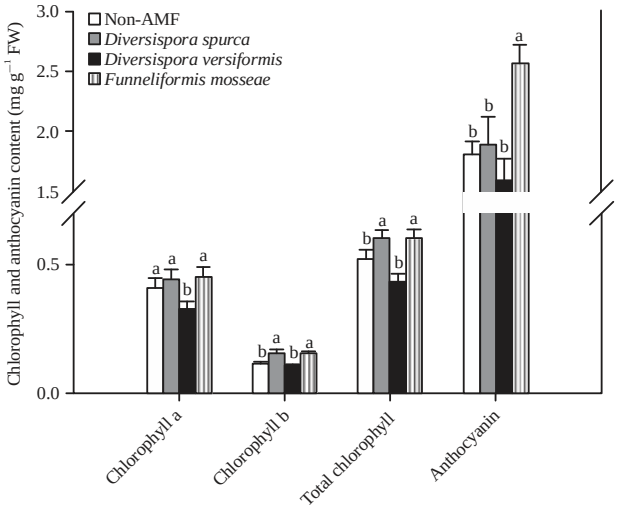

Fig. 2: Effects of AMF inoculation on leaf chlorophyll a, chlorophyll b, total chlorophyll and flower anthocyanin concentration in hyacinth plants. Data (Means±SD, n = 3) followed by different letters above bars showed significant differences (DMR, p<0.05) between treatments

leaf chlorophyll a concentration and *D. spurca* and *F. mosseae* considerably increased leaf chlorophyll b and total chlorophyll concentrations than non-AMF treatment (Fig. 2). *D. spurca* significantly (p<0.05) increased the content of chlorophyll b by 39.63% and *F. mosseae* significantly increased the content of total chlorophyll by 15.38%. The higher chlorophyll concentration in AM plants suggested higher photosynthetic capacity or greater N level[9]. The same result was reported by Lu *et al.*[29] in *Morus alba* plants.

Anthocyanin concentration in flowers: AMF inoculation with *D. spurca* and *D. versiformis* did not affect flower anthocyanin concentration as compared with non-AMF inoculation (Fig. 2). However, inoculation with *F. mosseae* possessed 81.1% significantly (DMR, p<0.05) higher flower anthocyanin concentration than non-AMF treatment. Such result is in accordance with the AMF-induced flower biomass production and morphology under *F. mosseae* conditions. *F. mosseae* increased the anthocyanin concentration that may be connected with the high N concentration in flowers,

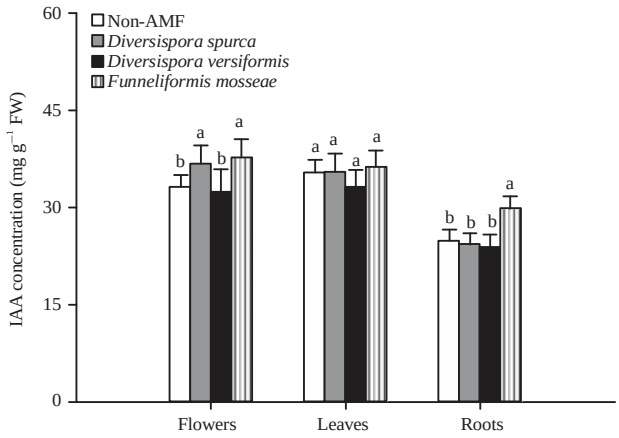

Fig. 3: Effects of AMF inoculation on IAA content of flowers, leaves and roots in hyacinth plants. Data (Means±SD, n = 3) followed by different letters above bars showed significant differences (DMR, p<0.05) between treatments

Table 3: Effects of AMF inoculation on N, P and K contents of flowers, leaves and roots in hyacinth plants

	Flowers (mg DW/plant)			Leaves (mg DW/plant)			Roots (mg DW/plant)		
Treatments	N	P	K	N	P	K	N	P	K
Non-AMF	68.09±7.92^{b}	7.90±0.11^{b}	54.91±1.71^{b}	25.78±2.28^{a}	2.88±0.26^{a}	26.35±0.88^{a}	34.94±1.51^{b}	4.65±0.55^{a}	31.00±0.70^{b}
D. spurca	65.22±7.76^{b}	7.88±0.24^{b}	56.80±0.30^{b}	22.19±1.55^{b}	2.07±0.21^{b}	22.21±2.64^{b}	35.87±3.85^{b}	4.49±0.25^{a}	30.28±3.02^{b}
D. versiformis	70.58±4.46^{b}	7.84±0.17^{b}	62.09±5.35^{b}	18.06±1.79^{c}	1.76±0.21^{b}	18.14±1.51^{c}	34.27±1.58^{b}	4.33±0.09^{a}	29.38±1.11^{b}
F. mosseae	96.84±6.67^{a}	11.12±0.13^{a}	74.10±5.95^{a}	18.73±1.86^{bc}	1.74±0.18^{b}	17.50±2.08^{c}	43.45±1.57^{a}	4.94±0.39^{a}	40.69±3.94^{a}

Data (Means±SD, n = 3) followed by different letters among treatments indicate significant differences (DMR, p<0.05) between treatments

because higher N concentration influenced anthocyanin production in *Hibiscus sabdariffa* plants[30].

IAA levels of tissues: In this study, compared with non-AMF treatment, three AMF species did not provide any effect on leaf IAA levels (Fig. 3). In roots, only *F. mosseae* exhibited 20.02% significantly (DMR, p<0.05) higher IAA concentration than non-AMF treatment, in flowers, *D. spurca* and *F. mosseae* markedly increased IAA levels. Changes in these IAA levels were coincident with changes in flower morphology, in this study. This implied that AMF-induced IAA changes are closely related with AMF-induced flower morphological changes in hyacinth. It is well documented that IAA plays an important role in plant morphogenesis, such as stem elongation, pollen tube growth, flower and floral transition[31,32]. Studies in the past also proved that inoculation with AMF significantly increased flower IAA concentration in onion[33] and soybean[34] and root IAA content in trifoliate orange[35].

Nutrient status in tissues: Compared to non-AMF, plants inoculated with *F. mosseae* had 42.22, 40.76 and 34.95% significantly (DMR, p<0.05) higher N, P and K concentrations in flowers and the other AMF species had no significant changes in flower N, P and K levels (Table 3). Conversely, in leaves, the AMF plants possessed lower N, P and K content than non-AMF plants. In roots, only *F. mosseae*-inoculated hyacinth plants exhibited relatively higher N and K content than other treatments. Therefore, in this study, *F. mosseae* had the best positive effect on N, P and K assimilating in flowers and roots. The similar results were shown in pepper[36], pelargonium[37] and *Miscanthus sacchariflorus*[38] plants. N nutrition may help to maintain chlorophyll synthesis and plant relative growth rate[39,40]. AMF played a vital role in enhanced P and K acquisition[41,42]. Meanwhile, K is involved in a wide range of function in plants as a carrier ion in hormonal stressed signals[37,43]. As a result, AMF-modulated N, P and K changes are associated with AMF-modulated biomass production changes in hyacinth plants.

CONCLUSION

In this study, mycorrhizal inoculation could heavily affect biomass production, chlorophyll, anthocyanin and IAA synthesis and nutrient acquisition of hyacinth plants. Meanwhile, only *F. mosseae* had strongly positive compatibility with hyacinth plants. AMF-modulated biomass production may be associated with AMF-modulated nutrient changes. AMF-induced flower morphological changes are due to AMF-induced IAA changes.

SIGNIFICANCE STATEMENTS

This study evaluated the effects of three AMF species, namely, *Diversispora spurca*, *Diversispora versiformis* and *Funneliformis mosseae* in flower morphology, color and duration of hyacinth. It found that only *F. mosseae* had the positive stimulated effects on morphology, color and lasting time of flowers, which is involved in IAA levels and N, P and K concentrations. This study provides new pathway to modulate flowering phase and flower quality of hyacinth plants through utilizing AMF as a stimulator in floriculture.

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