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EXOGENOUS POLYAMINES IMPROVE MYCORRHIZAL DEVELOPMENT AND GROWTH AND FLOWERING OF *FREESIA HYBRIDA*

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ABSTRACT

An experiment was conducted in order to investigate the effects of exogenous polyamines (PAs) on the development of mycorrhizae in roots, nutrient uptake and vegetative and reproductive growth of *Freesia hybrida* 'Golden Wave'. Corms of freesia were inoculated with *Rhizophagus intraradices* at sowing time and treated once a week by one of three PAs, putrescine (Put), spermidine or spermine, in concentrations of 0.05 and 0.1 mM each as foliar application or soil drench. Application of PAs, especially as soil drench, increased mycorrhizal colonization as well as the growth and development of inoculated plants. Among the three PAs, Put in 0.1 mM concentration was the most effective in increasing colonization, enhancing floral stem length and diameter, floral spike length, floret number on main and lateral spikes and increasing corm and cormlet weight, corm diameter and cormlet number. Sole application of arbuscular mycorrhizal fungi had no significant effect on the flowering time but soil drench with 0.1 mM Put accelerated flowering by about 17 days. Application of PAs elevated leaves N, P, K, Mg, Fe and Zn and corms' P, K, Ca, Fe and Zn concentration of inoculated plants. Our results suggest that soil drench application of PAs, especially Put, positively influenced mycorrhizal inoculation and nutrient uptake, which leads to improving growth, flower and corm production and quality of mycorrhizal plants of freesia.

Key words: flowering, putrescine, *Rhizophagus intraradices*, spermidine, spermine

INTRODUCTION

Freesia hybrida is a popular cool season plant belonging to *Iridaceae* family. Fragrant flowers of freesia are gathered in spike-type inflorescences on stem sprouting from corm and appear in wide range of colors. Freesia is mainly produced as a cut flower but is also suitable as a pot and bedding plant (Wang 2007). The main goal of most freesia growers is to produce more flowers or corms with maximum quality in a specific period of time. Recently, many floricultural growers have become interested in using arbuscular mycorrhizal fungi (AMF) because they are beneficial to vegetative growth, flower production and underground storage organs (Koltai 2010).

Roots of more than 80% of vascular plants are able to establish a symbiotic relationship (mycorrhiza) with arbuscular mycorrhizal fungi (AMF). AMF enhances water absorption and nutrient uptake from the soil via wide root external mycelia networks; in return, fungi obtain whole required carbon from the host plant (Smith & Read 2008). A number of mycorrhizal plants have a higher rate of photosynthesis (Birhane et al. 2012), accumulate more auxins and cytokinins than non-mycorrhizal plants (Fusconi 2014) and are more resistant to abiotic stresses due to improved osmotic adjustment

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(Al-Karaki 2006). The positive effects of AMF on vegetative and reproductive growth of some floricultural crops such as *Rosa hybrida* (Garmendia & Mangas 2012), *Chrysanthemum morifolium* (Sohn et al. 2003), *Eustoma grandiflorum* (Pivonia et al. 2010) and *Antirrhinum majus* (Asrar et al. 2012) were reported. Inoculation with AMF enhanced flower production of *Zantedeschia* and *Sparaxis tricolor* as well as increased nutrient concentration in *Zantedeschia* tuber and *Sparaxis tricolor* corms (Scagel 2004; Scagel & Schreiner 2006). Scagel (2003) showed that in pasteurized soil, mycorrhizal colonization of freesia plants was less than forty percent and inoculated plants did not differ significantly in terms of number of leaves, flowers and inflorescences and total corm and cormlet weight and numbers from non-inoculated plants. There is increasing evidence that the formation of mycorrhizal symbiosis is positively affected by the application of exogenous polyamines (PAs). Polyamines, mainly diamine putrescine (Put), triamine spermidine (Spd), and tetramine spermine (Spm), are ubiquitous nitrogenous compounds present in all living cells. PAs have important role in many physiological and developmental processes such as cell division and differentiation, growth, embryogenesis, rhizogenesis, flower development, fruit ripening, dormancy, senescence and also responses to biotic and abiotic stresses (Kaur-Sawhney et al. 2003).

El Ghachtouli et al. (1996) reported that PAs significantly stimulated frequency of mycorrhizal colonization of the root systems of different pea lines. Wu et al. (2010a) showed that increasing mycorrhizal colonization of *Citrus tangerine* by application of PAs, improved growth performance of inoculated plants. Among the three PAs, Put not only enhanced mycorrhizal colonization, but also increased plant growth and nutrient uptake of trifoliate orange seedlings (Wu & Zou 2009; Wu et al. 2012b). There are few studies of the effects of PAs on mycorrhizal development and none of these studies are related to ornamental geophytes or other floricultural crops. It is possible that dual application of AMF and PAs to ornamental crops may be an effective approach for improving plant growth, flower yield and quality and also underground storage organs. So, the objectives of the present study were to evaluate whether application of three PAs, Put, Spd and Spm can increase AMF colonization of freesia plants and whether combined application of AMF and PAs can improve nutrient uptake, flower and corm production and quality of freesia.

MATERIALS AND METHODS

Plant material, growth conditions and mycorrhizal inoculation

A pot experiment was done during 2013-2014 under greenhouse conditions at the University of Guilan. Corms of *Freesia hybrida* 'Golden Wave' were surface sterilized with 10% NaClO for 5 min, rinsed three times with distilled water and then planted into pots (16 cm diameter × 16 cm height). The substrate was a mixture of perlite, peat, sand and soil in the ratio of 1:1:1:1 by volume. The characteristics of soil were: sandy loam texture of pH 7.2, containing 0.38% organic carbon, 0.042% total nitrogen, 7.3% available phosphorus (Olsen et al. 1954), and 36 mg kg^{-1} available potassium. The mixture of sand, soil and peat was autoclaved two times at 121 °C for 20 min at an interval of 24 h. Pots were placed in a greenhouse under irradiation of 600-900 μmol m^{-2} s^{-1} and photoperiod 11h/13h lightness/darkness and 80±5% RH. The average day/night temperatures were 16 °C/12 °C for the first nine weeks after planting and 20 °C/15 °C until the end of the growth cycle. Plants were fertilized weekly per pot with 100 ml solution containing N:P:K (200:50:200 mg kg^{-1}) for the first ten weeks and N:P:K (100:50:250 mg kg^{-1}) until flowering. Mycorrhizal fungal inoculum *Rhizophagus intraradices* (recently renamed from *Glomus intraradices*) was obtained from a commercial laboratory (Turan Biotech Co., Shahroud Science & Technology Park, Iran), consisted of sterilized soil and sand, spores (an average of 5000 spores per 100 g dry soil), mycelium, and infected root fragments of *Trifolium repens*. 200 g of inoculum was placed 1 cm below the freesia corms at sowing time. For control pots, the same amount of inoculum was sterilized and added at the base of each corm.

Polyamine treatments and experimental design

Application of PAs (Sigma-Aldrich. Co.) started a week after corm sprouting. Inoculated plants were treated once a week by one of the three PAs, Put, Spd or Spm, in two concentrations 0.05 and 0.1 mM each as foliar application and also as

soil drench. For soil drench, 100 ml of PA solution per pot was used. A completely randomized design was used with three replicates and each replicate consisting of three pots.

Fourteen treatments included:

1. no AMF, no PAs
2. AMF + no PAs,
3. AMF + foliar application of 0.05 mM Put,
4. AMF + foliar application of 0.1 mM Put,
5. AMF + foliar application of 0.05 mM Spd,
6. AMF + foliar application of 0.1 mM Spd,
7. AMF + foliar application of 0.05 mM Spm,
8. AMF + foliar application of 0.1 mM Spm,
9. AMF + soil drench of 0.05 mM Put,
10. AMF + soil drench of 0.1 mM Put,
11. AMF + soil drench of 0.05 mM Spd,
12. AMF + soil drench of 0.1 mM Spd,
13. AMF + soil drench of 0.05 mM Spm,
14. AMF + soil drench of 0.1 mM Spm.

Growth parameters

Leaf length, leaf width (at the half length of leaf), leaf number per corm, time of flowering (days from planting to the emergence of the flower stalk), the length of floral stem, floral stem diameter (at the half of stalk length), floral spike length, number of florets per spike, number of lateral floral spikes and number of florets on lateral spikes were measured. At flower harvest time, three pots of each replicate were selected to take samples from the roots. Roots were rinsed with water and kept in lactoglycerol for mycorrhizal estimation. Also three other pots were considered per replicate for each treatment to score corm traits. After harvesting the flowers, irrigation gradually decreased until the leaves turned yellow and corms and cormlets were then removed from the substrate, and the number, weight and diameter of corms and cormlets were measured.

Estimation of AMF colonization

After washing, root samples of 1 g of fresh weight were taken from terminal parts (mostly fine roots). Root samples were cut into 1 cm segments and cleared in 10% KOH at 90 °C for 30 min, then acidified in 1% HCl for 10 min and stained with 0.05% trypan blue in lactoglycerol (Phillip & Hayman 1970). The total percentage of AMF colonization was measured by the method described by McGonigle et al. (1990).

Analysis of mineral nutrients

Two grams of each of the leaf and corm samples were dried at 70 °C for 48 h and used to analyze mineral compounds concentrations. Total nitrogen (N) was analyzed by the Kjeldahl method (Walinga et al. 1989). Phosphorus (P) was determined colorimetrically by the vanadate-molybdate-yellow method (Chapman & Pratt 1961), using a UV/visible spectrophotometer, potassium (K) by flam photometry (Walinga et al. 1989) and calcium (Ca), magnesium (Mg), iron (Fe) and zinc (Zn) by atomic absorption spectrophotometer (Perkin-Elmer 1982; Walinga et al. 1989).

Statistical analysis

Results were statistically analysed by using the analysis of variance (ANOVA) procedure in SAS software (9.0) to the Randomized Complete Design (RCD). Mean values were compared by least significant differences (LSDs) at the significance level $P \leq 0.05$.

RESULTS

AMF colonization

Except when the roots were treated with sterile inoculum, symbiosis was established between *Rhizophagus intraradices* and freesia plant roots. Mycorrhizal root colonization of plants not treated with PAs was 33%. Foliar application of 0.1 mM Put and Spd increased colonization by 72 and 40%, respectively, and soil drench application by 115 and 112%, respectively. Application of Spm as foliar spraying did not stimulate fungal colonization and applied as soil drench by 15% (Fig. 1).

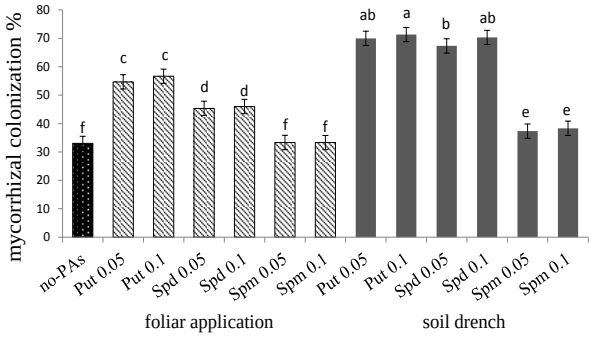

Fig. 1. Effect of foliar application and soil drench of polyamines on root colonization percentage of *Freesia hybrida* inoculated with *Rhizophagus intraradices*. Means followed by the same letter are not significantly different according to the least significant difference (P < 0.05)

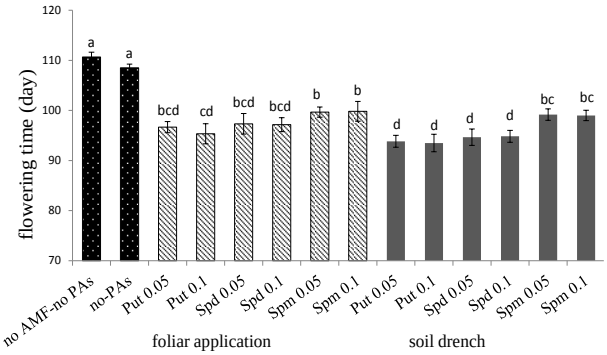

Fig. 2. Effect of foliar application and soil drench of polyamines applied on *Rhizophagus intraradices* inoculated *Freesia hybrida* plants on flowering time. Means followed by the same letter are not significantly different according to the least significant difference test (P < 0.05)

Growth Parameters

Leaf. AMF application without PAs increased leaf size, length, width and number compared with non-inoculated plants. Put and Spd application as soil drench on inoculated plants significantly increased leaf length and width compared with sole AMF inoculation. Foliar application of PAs did not affect leaf parameters nor the leaf number (Table 1).

Flowering time. Application of all tested PAs for inoculated plants shortened the time to flowering by 9 to 15 days as compared to inoculated control. There were no significant differences between foliar and soil drench application of PAs; nevertheless, Put and Spd were more effective than Spm, especially when applied as soil drench, flowers were produced about 15 days earlier than non-inoculated plants and 5 days earlier than Spm treated (Fig. 2).

Flower. AMF inoculation caused significant increase in floral stem length, floral spike length, floret number and floral stem diameter but no significant effect on lateral floral spike number and floret number on lateral spikes. The application of AMF and PAs positively influenced flower characteristics. The maximum floral stem length and diameter, floral spike length, floret number and floret number on lateral spikes were obtained after soil drench with Put although differences between 0.1 mM Put and Spd were not significant (Table 1).

Corm. The AMF inoculation jointly with or without PAs had no significant effect on daughter corm number. Inoculation with AMF increased corm weight and diameter, cormlet number and weight compared with non-inoculated plants. The parallel application of AMF and PAs improved corm and cormlet traits. Among tested PAs, Put as soil drench was the most effective in increasing corm and cormlet weight, corm diameter and cormlet number of inoculated plants (Table 1).

Nutrient concentrations in leaves and corms

Mineral nutrient analysis of leaves and corms of freesia showed that plants inoculated with AMF solely contained significantly more N, P, Mg, Fe and Zn in leaves and more P, K and Fe in corms (Table 2). Application of Pas, especially as soil drench, increased nutrient concentration in leaves and corms of inoculated plants. There were no significant differences between impacts of Put and Spd used as soil drench on nutrient concentrations. Calcium content in leaves and N and Mg contents in corms were not dependent on the AMF or PA treatments (Table 2).

Table 1. Leaf, flower and corm parameters of *Freesia hybrida* as influenced by *Rhizophagus intraradices* and foliar application and soil drench of polyamines

Treatments		Leaf length	Leaf width	Leaf number	Floral stem length	Floral spike length	Floret number per spike	Floral stem diameter	Lateral floral spike number	Floret number on lateral spikes	Corm weight	Corm number	Corm diameter	Cormlet weight	Cormlet number
		(cm)	(mm)		(cm)	(cm)		(mm)			(g)		(mm)	(g)	
	no-AMF + no-PA	29.8 f	13.0 d	7.7 d	36.0 g	5.2 e	5.5 e	2.4 f	1.3 a	4.2 e	2.7 g	1.7 a	17.2 g	0.47 g	1.7 e
	AMF + no-PA	38.5 de	16.8 c	10.0 bc	40. 7 f	6.9 d	7.0 d	2.7 e	1.8 a	5.5 de	4.6 f	1.7 a	22.1 f	0.82 f	3.3 cd
Foliar application	AMF + Put 0.05	41.3 bcd	18.5 c	10.8 a	46.3 de	7.9 abc	9.5 a	3.1 bcd	2.0 a	6.2 bc	6.3 ab	1.7 a	27.4 abc	0.84 cde	5.0 ab
	AMF + Put 0.1	42.5 abc	19.2 c	10.2 abc	47.3 cde	8.4 ab	9.7 a	3.0 cde	2.2 a	6.7 b	6.4 a	1.7 a	27.6 abc	0.87 c-f	5.0 ab
	AMF + Spd 0.05	40.2 cd	18.7 bc	10.7 ab	47.8 cde	8.1 ab	9.0 abc	3.1 cde	2.2 a	6.5 b	5.9 bc	1.7 a	26.5 bcd	0.84 def	3.3 cd
	AMF + Spd 0.1	41.7 a-d	19.3 abc	9.8 c	48.0 a-d	8.3 ab	9.2 ab	3.2 a-d	2.2 a	6.7 b	5.7 cd	1.7 a	26.6 bcd	0.88 bcd	3.7 cd
	AMF + Spm 0.05	38.8 de	17.3 c	10.7 ab	44.5 e	6.8 d	8.2 bcd	2.9 de	1.8 a	5.3 cd	5.3 de	1.3 a	25.1 de	0.83 def	3.0 cd
	AMF + Spm 0.1	38.5 de	17.0 c	10.2 abc	46.2 de	7.5 bcd	7.5 d	2.8 e	1.8 a	5.3 cd	5.0 ef	1.3 a	23.9 e	0.83 ef	2.7 de
Soil drench	AMF + Put 0.05	45.0 a	21.5 a	10.3 abc	50.8 a	8.7 a	9.8 a	3.5 a	2.0 a	7.7 a	6.5 a	2.0 a	27.7 abc	0.92 ab	5.3 a
	AMF + Put 0.1	44.5 ab	21.7 a	10.2 abc	50.3 ab	8.4 a	9.5 a	3.5 a	2.0 a	7.0 ab	6.6 a	2.0 a	28.3 a	0.94 a	5.7 a
	AMF + Spd 0.05	43.7 abc	20.2 ab	10.7 ab	49.8 abc	8.5 a	9.7 a	3.4 abc	2.0 a	6.8 ab	6.3 ab	1.7 a	28.0 ab	0.91 abc	4.0 a
	AMF + Spd 0.1	42.5 abc	20.3 ab	10.5 abc	50.7 ab	8.5 a	9.5 a	3.4 ab	2.0 a	6.7 b	6.5 a	2.0 a	27.9 ab	0.91 abc	5.0 ab
	AMF + Spm 0.05	38.3 de	16.8 c	10.5 abc	45.5 de	7.2 cd	7.8 cd	3.1 ade	1.8 a	5.3 cd	6.2 abc	1.3 a	25.7 d	0.87 def	4.0 bc
	AMF + Spm 0.1	36.5 e	16.8 c	9.8 c	44.7 e	7.2 cd	7.7 d	3.1 cde	1.8 a	5.7 c	6.0 abc	1.3 a	26.2 cd	0.85 def	3.3 cd

Note: Means followed by the same letter within a column are not significantly different according to the least significant difference test ($P < 0.05$).

Table 2. Leaf and corm mineral nutrient concentration of *Freesia hybrida* as influenced by *Rhizophagus intraradices* and foliar application and soil drench of polyamines

Treatments		N (%)	P (%)	K (%)	Mg (%)	Ca (%)	Fe (mg kg^{-1})	Zn (mg kg^{-1})
					Leaf			
	no-AMF + no-PA	2.10 f	0.35 e	2.63 c	0.26 d	0.78 a	54.3 g	46.1 f
	AMF + non-PA	2.34 e	0.47 d	2.85 bc	0.31 c	0.83 a	77.5 f	57.2 e
Foliar application	AMF + Put 0.05	2.96 a-d	0.59 c	3.32 a	0.37 ab	0.83 a	85.3 cde	68.7 abc
	AMF + Put 0.1	2.95 a-d	0.60 c	3.29 a	0.36 ab	0.84 a	87.7 b-e	70.0 abc
	AMF + Spd 0.05	2.83 cd	0.58 c	3.27 a	0.35 b	0.86 a	84.0 e	62.9 d
	AMF + Spd 0.1	2.90 bcd	0.59 c	3.26 ab	0.36 ab	0.88 a	85.3 cde	64.0 d
	AMF + Spm 0.05	2.77 d	0.57 c	3.24 ab	0.37 ab	0.86 a	84.7 de	62.7 d
	AMF + Spm 0.1	2.91 bcd	0.57 c	3.25 ab	0.35 ab	0.86 a	83.0 ef	62.5 d
Soil drench	AMF + Put 0.05	3.02 abc	0.66 ab	3.31 a	0.39 a	0.86 a	92.7 ab	71.7 a
	AMF + Put 0.1	3.08 ab	0.67 a	3.32 a	0.37 ab	0.84 a	95.7 a	73.1 a
	AMF + Spd 0.05	3.04 ab	0.61 abc	3.30 a	0.36 ab	0.84 a	93.3 ab	72.3 a
	AMF + Spd 0.1	3.10 a	0.62 abc	3.30 a	0.37 ab	0.84 a	91.0 a-d	70.7 ab
	AMF + Spm 0.05	2.92 a-d	0.60 bc	2.97 abc	0.35 b	0.83 a	91.7 abc	66.0 cd
	AMF + Spm 0.1	2.99 abc	0.60 c	3.30 a	0.35 b	0.86 a	90.7 a-d	67.0 bcd
					Corm			
	no-AMF + no-PA	4.2 a	0.55 e	1.10 g	0.06 a	0.43 f	34.2 e	60.7 f
	AMF + non-PA	4.3 a	0.61 cd	1.14 f	0.07 a	0.46 f	42.6 d	60.8 f
Foliar application	AMF + Put 0.05	4.5 a	0.69 b	1.20 bc	0.05 a	0.58 de	46.9 cd	64.1 cde
	AMF + Put 0.1	4.5 a	0.71 ab	1.19 bc	0.07 a	0.68 a	47.1 c	64.7 cd
	AMF + Spd 0.05	4.5 a	0.61 d	1.18 cde	0.06 a	0.59 cd	47.6 c	62.8 def
	AMF + Spd 0.1	4.5 a	0.63 cd	1.19 bcd	0.06 a	0.54 de	47.0 c	63.4 def
	AMF + Spm 0.05	4.4 a	0.62 cd	1.16 ef	0.07 a	0.47 f	43.5 cd	61.9 def
	AMF + Spm 0.1	4.3 a	0.61 d	1.17 de	0.06 a	0.49 ef	45.1 cd	61.5 ef
Soil drench	AMF + Put 0.05	4.5 a	0.72 a	1.21 ab	0.07 a	0.64 abc	55.7 ab	70.3 a
	AMF + Put 0.1	4.4 a	0.73 a	1.22 a	0.06 a	0.67 a	58.8 a	72.0 a
	AMF + Spd 0.05	4.2 a	0.72 a	1.20 abc	0.07 a	0.64 abc	54.0 b	69.3 ab
	AMF + Spd 0.1	4.3 a	0.71 a	1.20 abc	0.06 a	0.66 ab	56.1 ab	70.3 a
	AMF + Spm 0.05	4.5 a	0.63 cd	1.19 bcd	0.08 a	0.60 bcd	53.7 b	66.6 bc
	AMF + Spm 0.1	4.5 a	0.64 c	1.18 cde	0.06 a	0.62 abc	54.3 b	66.6 bc

Means followed by the same letter within a column are not significantly different according to the LSD test ($P < 0.05$).

DISCUSSION

In the present study mycorrhizal symbiosis between AMF *Rhizophagus intraradices* and roots of *Freesia hybrida* 'Golden Wave' was observed, but the percentage of colonization was low (about 33% of root length). A similar result obtained in pasteurized soil was reported by Scagel (2003). In the present study, application of PAs, especially Put, increased the percentage of mycorrhizal colonization. Similarly, Put increased mycorrhizal development and colonization in *Citrus tangerine* (Wu et al. 2010a), *Citrus limonia* (Yao et al. 2010) and *Poncirus trifoliate* (Wu et al. 2012b). The results from other investigations indicated that PAs increased mycorrhizal colonization by altering root morphology and mycorrhizal development. It was demonstrated that plants treated with PAs had a higher percentage of fine roots as well as longer and more lateral roots, so these changes will ultimately lead to an increase in the percentage of mycorrhizal colonization (Wu et al. 2010b; Yao et al. 2010; Wu et al. 2012b). On the other hand, exogenous PAs influenced AMF by stimulating spore

germination, hyphal elongation and branching (El Ghachtouli et al. 1996; Cheng et al. 2012). Our results have shown that inoculation with AMF increases flower and corm yield and quality of freesia plants. Similarly, Scagel and Schreiner (2006) showed that inoculation of *Zantedeschia* with *Glomus intraradices* increased shoot production and the number of flowers and tuber size. Nadeem et al (2014) reported that AMF increases plant growth by elevating mineral nutrient absorption, changing endogenous phytohormones and altering resource allocation. There is little information about the influence of interaction of AMF and PAs on plants. In our study, application of PAs, especially Put and Spd, significantly increased flower and corm yield and quality of freesia plants inoculated with AMF. Wu et al. (2010a) reported that growth and biomass production of both shoots and roots of *Poncirus trifoliate* plants were significantly enhanced in the result of Put application, whereas Spm and Spd were not effective. In contrast, our result showed no significant difference between Spd and Put in most evaluated traits and Spd was more effective than Spm in increasing mycorrhizal colonization and improving most vegetative and reproductive parameters. Previous research indicated that increased growth, flower production and quality, corm or tuber yield of *Freesia* and *Zantedeschia* were the result of increased nutrient levels in vegetative and storage organs of inoculated plants (Scagel 2003; Scagel & Schreiner 2006).

Our results showed that concentrations of P, K, Mg, Fe and Zn in leaves and P, K, Ca and Fe in corms of inoculated plants were higher than in non-inoculated plants. Similarly, previous studies reported that inoculation with AMF increased Zn and S concentrations in corms of freesia (Scagel 2003), N, K, Ca, Fe and Cu concentrations in *Zantedeschia* tubers (Scagel & Schreiner 2006) and N, Zn and S in corms of *Sparaxis tricolor* (Scagel 2004). Mycorrhization increased shoot P and K concentrations in pelargonium plants (Perner et al. 2007). Arbuscular mycorrhizal fungi help plants to acquire mineral nutrients and water by proliferating hyphae in the substrate, so mycorrhizal roots absorb more water and nutrients than non-mycorrhizal roots (Nadeem et al. 2014). In this experiment, mineral nutrient concentrations in leaves and corms significantly increased due to PA application, especially Put, applied as soil drench. Wu and Zou (2009) showed that PAs, especially Put, markedly increased leaf and root mineral concentrations by improving root system architecture and increasing mycorrhizal root colonization. This may be the main reason for the significant increase in mineral concentration of leaves and corms of inoculated plants of freesia after treatment with Put. As mentioned before, inoculation with AMF and also treatment with PAs had no significant effect on corm N concentration and leaf Mg and Ca concentrations. Probably, the amount of these nutrients in the medium was high and this did not limit the growth.

In our study, inoculation solely with the AMF had no influence on the time of flowering of freesia. However, parallel application of AMF and PAs as soil drench advanced the time of flowering. Mycorrhizal plants of freesia treated with Put as soil drench flowered 15 days earlier than sole AMF inoculated plants. Early flowering PA-treated mycorrhizal plants could be due to increased mineral nutrient concentrations in plants. Also, Scagel and Schreiner (2006) reported that increasing mineral nutrient concentrations in tubers of AMF inoculated *Zantedeschia* promoted early flowering.

The results of this study show that the effectiveness of PAs on mycorrhizal plants depends on their type and methods of administration. Soil drench with PAs was more effective than foliar application in increasing mycorrhizal colonization and also freesia vegetative and reproductive growth.

Furthermore, among all evaluated PAs, Put was the most effective. It can be explained that Put is the precursor of Spd and Spm biosynthesis and is more involved in the plant metabolism processes (Kaur-Sawhney et al. 2003). Put is known as one of the most important regulatory factors in plant-AMF interactions (El Ghachtouli et al. 1996). Wu et al. (2012a) showed that mycorrhization stimulated the synthesis of Put in roots and improved root system architecture. On the other hand, exogenous Put had the best effect on mycorrhizal development of citrus plants (Wu et al. 2010a).

When PAs are applied as soil drench, they affect directly both plant and mycorrhizal fungi. Previous studies have demonstrated that PAs stimulate germination of spores and mycelia of AMF, and PA biosynthesis inhibitors reduce mycelia growth and inhibit proliferation of fungal hyphae (El Ghachtouli et al. 1996; Cheng et al. 2012).

In conclusion, the results of this study suggest that PAs have important roles in mycorrhizal development and improvement of corm and flower yield and quality of freesia inoculated with AMF. Mycorrhizal colonization with *Rhizophagus intraradices* slightly improved some flower and corm characteristics of *Freesia hybrida* 'Golden Wave'. Application of exogenous PAs, especially Put, significantly increased root colonization. Dual application of AMF and Put at 0.1 mM concentration as soil drench, significantly accelerated flowering time and improved corm yield and quality, and at 0.05 mM concentration enhanced floral parameters. Positive effects of parallel application of AMF and PAs on freesia flower and corm yield and quality were evident, which suggests their usefulness in the commercial production of freesia. It is conceivable that dual application of AMF and PAs may be extended to a variety of other ornamental crop species, and implemented as part of their production management for the enhancement of growth and yield.

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