

Article

Growth and Nutritional Enhancement of *Lisianthus* (*Eustoma grandiflorum* (Raf.) Shinn.) via Dual AMF Inoculation Under Phosphorus Regimes

Morteza Sheikh-Assadi ¹ , Mohammadreza Taheri ¹, Azizollah Khandan-Mirkohi ^{1,*} , Mesbah Babalar ¹, Mahdi Khansefid ¹ , Mohammad Omid ¹ and Dionisios Gasparatos ^{2,*} 

¹ Department of Horticultural Science, Faculty of Agricultural Science and Engineering, University of Tehran, Karaj 31587-77871, Iran; m.asadi66@ut.ac.ir (M.S.-A.); mrtaheri@ut.ac.ir (M.T.); mbabalar@ut.ac.ir (M.B.); mkhansefid@ut.ac.ir (M.K.); m.omidighale@ut.ac.ir (M.O.)

² Laboratory of Soil Science and Agricultural Chemistry, Department of Natural Resources Management & Agricultural Engineering, School of Environment & Agricultural Engineering, Agricultural University of Athens, 11855 Athens, Greece

* Correspondence: khandan.mirkohi@ut.ac.ir (A.K.-M.); gasparatos@aua.gr (D.G.)

Abstract

The arbuscular mycorrhizal fungi (AMF) form symbiotic, mutually beneficial relationships in the rhizosphere, and modulate phosphorus (P) availability to regulate plant growth, nutrient uptake, and quality. However, their roles in cut-flower species remain poorly understood. The aim of this study was to evaluate the effects of single and dual inoculation with *Glomus intraradices* and *G. mosseae* on *Lisianthus* (*Eustoma grandiflorum*) grown under three P levels (10, 20, and 40 mg kg^{−1}) in greenhouse conditions. Under intermediate P, dual-inoculated plants exhibited the greatest above-ground vigor, with increases in stem length (+31%), dry shoot weight (+67%), and highest shoot P (+54%) and N (+23%) content, compared with non-inoculated controls. Under low P, dual inoculation maximized dry root weight (+63%) and mycorrhizal colonization, whereas AMF effects diminished at high P. Principal component analysis showed that there were distinct mycorrhizal interactions (PCA2, 20.3% variance) and a close integration between vegetative growth and nutrient accumulation (PCA1, 54.3% variance). For the first time, this study demonstrates that *Lisianthus* exhibits a strong response to dual AMF inoculation, offering a novel strategy to enhance growth, nutrition, and ornamental quality when P fertilization is optimized. By reducing chemical fertilizer use, this dual AMF–P management offers a sustainable framework for high-quality cut-flower production.

Keywords: fungal isolates; nutrient uptake; phosphorus fertilization; ornamental plants; sustainable floriculture



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1. Introduction

The area for cultivation of ornamental plants is increasing worldwide. More than 50 countries play a role in the production of cut flowers [1]. Issues arising from conventional agriculture have directed farmers to adopt organic farming [2,3]. Environmental issues and easier market access have persuaded some horticultural companies to follow organic agriculture [3,4]. In addition to offering significant environmental advantages—such as increased biodiversity, better soil health, and greater pollinator habitats—organic farming can be made financially sustainable by commanding higher premium prices [5]. Nevertheless, little is known about the organic production of cut flowers.

Floriculture, a multi-billion-dollar global industry [6] that depends on extensive supply chains, faces sustainability challenges as both an economic necessity and an ecological responsibility. Sustainable floriculture aims not only to reduce environmental harm but also to conserve finite resources, bolster growers' economic resilience, and improve social welfare—typically through integrated nutrient management, water reuse, biofertilizers, and energy-efficient systems. Given environmental pressures and market demand for sustainable products, biological strategies like biofertilizers are particularly important for reducing chemical inputs while maintaining flower quality and yield [7]. Consequently, in recent years, ensuring sustainability in floriculture has emerged as a major focus of research [8].

Soil microorganisms are important agents in plant industries, among which are arbuscular mycorrhizal fungi (AMF). The benefits of mycorrhizae for various aspects of plant growth and development have been described in numerous documents [9–15], although some mechanisms are still unknown in some cases. Arbuscular mycorrhizal fungi contribute to crop productivity by associating with plant roots to boost phosphorus and nitrogen uptake, and by improving hormonal status (cytokinins, auxins, gibberellins), which collectively enhance growth, nutrient content, and overall quality [15–18].

AMF significantly promote plant phosphorus acquisition through a coordinated interplay of structural, biochemical, microbial, and molecular processes [19]. The widespread hyphae assist plant roots in absorbing water and nutrients more efficiently [10,12]. This influence is particularly significant for P uptake [5,10,13]. Exploration of a wider soil zone by hyphae, faster transfer of P into the hyphae, and solubilization of soil P by organic acids and phosphatase enzymes are different mechanisms used by AMF to supply plants with more P [16,20]. The flow of P in colonized roots can be three to five times higher than in non-colonized roots, and even in hairy roots [14,16]. Compared to direct root absorption, this symbiotic P-acquisition route needs less carbon investment from the plant [21]. Meta-analyses indicate that AMF inoculation doubles plant P acquisition on average [22]. Mycorrhizae can play a role in N uptake, either as NH_4^+ or NO_3^- forms [17,18,23]. Inorganic nitrogen is absorbed by the mycelium and transferred to the plant primarily as arginine, which is subsequently converted to ammonium [24]. Poveda et al. (2025) proclaimed that mycorrhizal fungi release cytokinin-, auxin- and gibberellin-like substances that promote plant growth [15]. Cytokinins are integral to AM interactions; increased cytokinin levels in the host plant generally coincide with enhanced colonization. This can be achieved through the fungal production of cytokinins, stimulation of host Cytokinin biosynthetic pathways, or reduced cytokinin degradation, thereby establishing a feedback mechanism that supports colonization [25]. By enhancing IAA accumulation, modulating cytokinin levels (notably trans-zeatin riboside), and promoting GA_3 synthesis, AMF inoculation contributes to accelerated morphological growth in roots and shoots [26].

Thus, AMF are well documented to play a major role in crop yield and quality [12,27,28]. Some attempts have been made to incorporate AMF technologies in conventional methods [29], but ornamental plants have received little attention [30]. Mycorrhizal *Ajanina pacifica* plants developed more shoots, inflorescences, and longer-lasting flowers than non-inoculated controls, demonstrating that AMF inoculation frequently increases growth and flowering in ornamental species [31]. However, because plant mycorrhizal dependence varies and mycorrhizal isolates differ in their responses to a given host, results have been inconsistent [32,33]. *G. intraradices* and *G. mosseae* are commonly used AMF strains that seem to be broadly compatible in co-inoculation systems. Strains of both species have continuously improved flower quality and nutrient uptake across ornamental species [34,35], suggesting strong potential for combined inoculants. It has also been shown that, compared to single-species inoculation, co-inoculation with several AMF has syner-

gistic effects, increasing plant vigor through enhanced hormonal balance and increased nutrient absorption [34]. Therefore, studying plant behavior is important in introducing the best isolate [36].

The effectiveness of AMF on plant growth is estimated by measuring host growth responses. Different terms are used to describe these measurements: (1) “dependency of growth” [12]—the better term would be “inoculation effectiveness” [37]; (2) “mycorrhizal responsiveness” [33]; and (3) “mycorrhizal growth responses” [38]. Phosphorus is a key factor influencing mycorrhizal development and performance; the beneficial effect of AMF on P absorption and plant growth depends on P nutrition [37,39]. Several studies have shown that mycorrhizal inoculation improves P nutrition and the growth of host plants grown in low-P soils, whereas growth benefits reduce in high-P soils [40]. Watts-Williams and Cavagnaro (2012) and Dejana et al. (2022) studied the response of tomato plants to mycorrhizal inoculation under different P concentrations and found AMF to be more effective at lower P levels [38,41].

Despite the well-documented benefits of AMF in crops, limited studies have focused on economically important cut-flower species such as Lisianthus, leaving a critical knowledge gap. To our knowledge, the present investigation is the first to evaluate the response of Lisianthus to single and dual inoculation with two arbuscular mycorrhizal fungi (*Glomus intraradices* and *G. mosseae*) under different phosphorus (P) regimes. We hypothesized that (i) AMF inoculation would enhance nutrient uptake and growth performance, (ii) AMF effects would depend on P availability, and (iii) dual inoculation would differ from single-strain inoculation.

2. Materials and Methods

2.1. Treatments and Trial Design

The experiment was conducted in a greenhouse to evaluate the effects of AMF inoculation on ornamental Lisianthus (*Eustoma grandiflorum*). Three inocula were used: *Glomus mosseae* (M1), *G. intraradices* (M2), and their 1:1 (v/v) mixture (M3). A non-mycorrhizal treatment (M0) served as the control. These treatments were combined with three P concentrations—10 mg kg^{−1} soil (control, P1), 20 mg kg^{−1} soil (P2), and 40 mg kg^{−1} soil (P3)—resulting in 12 treatment combinations. Two AMF strains, *G. intraradices* and *G. mosseae*, were selected because they are well-studied, broadly compatible species commonly used in horticultural research, with documented synergistic effects when co-inoculated [34,35]. The trial was arranged in a factorial design (AMF × P levels) in a randomized complete block design. Each treatment was replicated three times, with five plants per replicate, totaling 180 plants (Figure 1). The experiment lasted approximately four months, from the transplant of seedlings to the harvest.

2.2. Soil Collection and Preparation

Soil was collected from the upper 0–20 cm layer of the experimental field (35°46′27.0″ N, 50°55′35.8″ E; 1249 m asl). The soil has a clay loam texture (hydrometer method—[42]); pH of 7.63; exchangeable K of 490 mg kg^{−1} (ammonium acetate extraction method—[43]); plant available P of 9.89 mg kg^{−1} (sodium bicarbonate extraction method—[44]) and total N, according to Kjeldahl’s method [45] 0.18%. The soil was mixed with fine-to-medium sand at a 2:1 (v/v) ratio. The prepared medium was autoclaved and considered as P1 (no added P). P2 and P3 were prepared by adding the required amount of triple superphosphate [Ca(H₂PO₄)₂] into the medium.

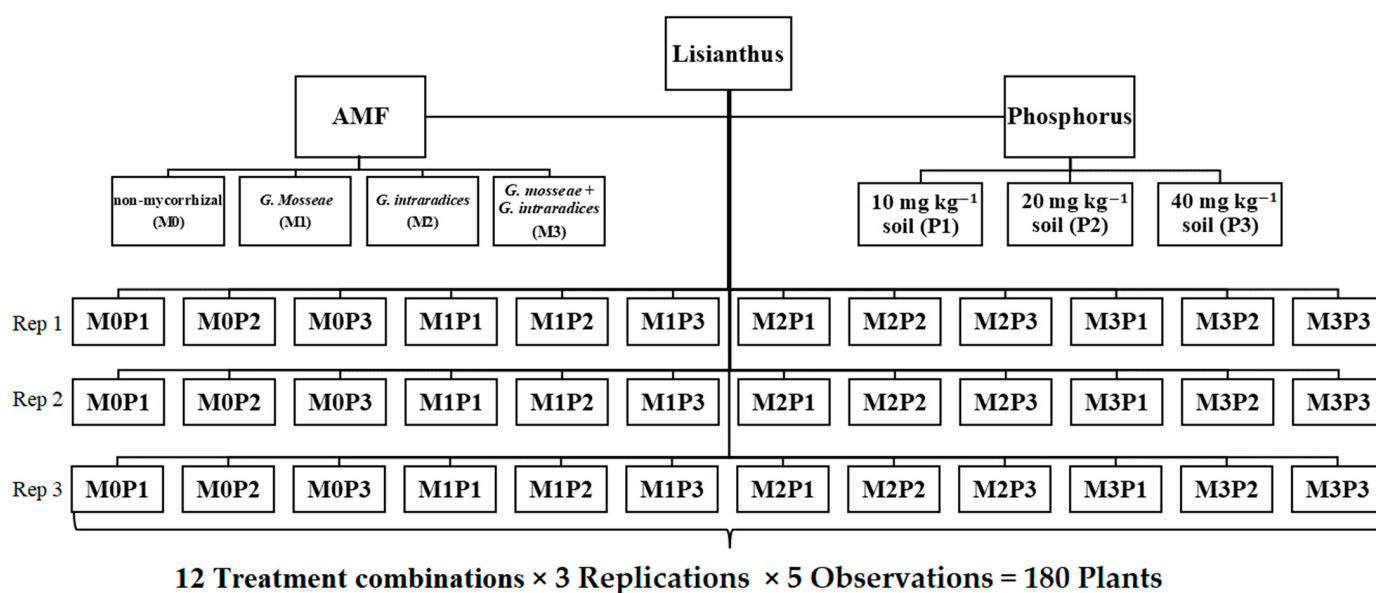


Figure 1. Flowchart of the greenhouse experiment.

2.3. AMF Inoculum Preparation

The pot-culture technique was used to increase the amount of inocula. Over a 4 month period, the isolates were propagated on sorghum roots in a sterile medium composed of top soil and fine-to-medium sand (1:1, *v/v*). To reduce contamination, the substrate and containers were sterilized before use. Sorghum seeds were cleaned, pre-soaked, and planted into pots containing inoculum combined with sterile substrate to establish cultures. The AMF strains (*G. mosseae* and *G. intraradices*) were obtained from the Department of Soil Science, University of Tehran. Following the first phase of root colonization, the materials were multiplied until an adequate amount of inoculum was acquired (approximately four months). The number of active fungal organs in the inoculum was quantified according to the most probable number (MPN). By thoroughly mixing the inoculum with sterile substrate, ten-fold serial dilutions (0 , 10^{-1} , 10^{-2} , and 10^{-3}) were created. Dilutions were placed into 70 cm³ tubes (five replicates per dilution) containing sterile medium. Sorghum seeds were planted in the tubes. Plants were watered and fed with 1/5 strength Hoagland solution as needed during the 30 day incubation, at about 23 °C with a 14 h photoperiod. To preserve fungal viability and avoid contamination, the remaining inoculum was stored in sealed containers at 4 °C in the dark throughout this period. The roots were collected, rinsed in 10% KOH for 10 min at 90 °C, and then stained with 0.05% trypan blue in a solution of glycerol, lactic acid, and water (2:2:1) for 15 min at 90 °C. For each replicate, presence or absence of colonization was scored under a microscope, and fungal active organs were determined using statistical tables [46].

2.4. Mycorrhizal Inoculation and Growing *Lisianthus*

Twelve-week-old uniform *Lisianthus* (*Eustoma grandiflorum* 'Mariachi Blue') seedlings were used. The seedlings had received standard cold treatment to prevent rosette formation and were transplanted into 2.5 L pots. For inoculation, 10 g of inoculum with 50 ± 10 active propagules g⁻¹ was added to the planting hole at transplanting, providing approximately 500 propagules per plant. Non-mycorrhizal treatments (M0) received 10 g of double-autoclaved inoculum at transplanting, serving as a sterile control. Pots were then transferred to the greenhouse. Over the growing period, the ambient temperature in the greenhouse naturally fluctuated between 10 and 28 °C. The pots were irrigated to 60–70% of the maximum water-holding capacity. Additionally, the plants were fertilized fortnightly

with a solution without P, composed of the following components in mM: 0.75 K as KNO_3 and K_2SO_4 ; 2.75 N as $\text{Ca}(\text{NO}_3)_2$, KNO_3 and $(\text{NH}_4)_2\text{SO}_4$; 2 Ca as $\text{Ca}(\text{NO}_3)_2$ and CaCl_2 ; 1.25 S as MgSO_4 , K_2SO_4 and $(\text{NH}_4)_2\text{SO}_4$; 1 Mg as MgSO_4 ; and in μM : 25 B as H_3BO_3 ; 40 Fe as Fe-EDTA; 1.5 Zn as ZnSO_4 ; 1.5 Mn as MnSO_4 ; 0.5 Cu as CuSO_4 ; and 0.1 Mo as NaMoO_4 . The plants were harvested from the first node above the soil when two open flowers with visible reproductive organs were present [47].

2.5. Measuring Growth and Ornamental Qualities

Stem length was measured from the base to the tip of the top flower or flower bud. Flower diameter was determined by measuring the distance between the apices of two opposite petals of a flattened flower [47]. Leaf area was measured by using Leaf Area Meter ($\Delta\text{T AREA METER MK2}$, Delta-T Devices, Cambridge, UK). The fresh weights of flowers, shoots, and roots were measured separately. They were then dried in an oven (70°C for 48 h) to determine dry weights. The total length of the entire root was calculated according to the line intersect method [48]. The number of days from transplanting to first open flowers emerging was recorded.

To determine vase life, stems with at least one flower beginning to open [47] were cut to 40 cm in length. They were placed in a transparent glass container with 400 mL of distilled water. Containers were transferred to a room at $23 \pm 2^\circ\text{C}$. The number of days to the wilting of the open flowers was recorded according to Harbaugh et al. (2000) [47]. Owing to differences in the time of emergence of the open flowers, the procedure was carried out at different times for each treatment.

2.6. Root Colonization Percentage

Roots were thoroughly rinsed. In order to estimate colonization percentage, the appropriate roots (<2 mm diameter) were cleared by KOH. They were then stained with trypan blue 0.05% in lactoglycerol [49]. After that, 50 fragments (1 cm) were separated from each sample. Finally, the root colonization percentage was quantified according to the gridline intersection method, using a stereomicroscope with magnification of $50\times$ [50].

2.7. Content of Nutrients

Shoots and roots were dried in an oven at 70°C for 48 h. For the mineral analysis, the samples were treated using standard procedures [51]. Nitrogen was determined according to the Kjeldahl method [45]. After extracting with HCl, P and K concentrations were determined by the spectrometry method [52] and flame emission spectroscopy, respectively. Moreover, Ca, Fe, and Zn concentrations were measured using atomic absorption spectrophotometry (Shimadzu AA-670, Kyoto, Japan).

2.8. Calculations and Statistical Analysis

Mycorrhizal growth responses (MGR) of plants were calculated using the total dry matter of each mycorrhizal plant (M) and average dry matter of whole non-mycorrhizal plants (NM) [38].

$$\% \text{MGR} = \frac{\text{dry weight (M)} - \text{mean dry weight (NM)}}{\text{mean dry weight (NM)}} \times 100.$$

The Shapiro–Wilk test was used to assess the normality of the data, and Levene's test was used to verify the homogeneity of variance. Two-way ANOVA ($\text{AMF} \times \text{P}$) was performed on the data, and Duncan's multiple range test was used to compare the means ($p < 0.05$) using SAS software (V9.1).

3. Results

3.1. Growth and Ornamental Traits

3.1.1. Shoot

The fresh and dry shoot weights and the stem length of *Lisianthus* were significantly affected by interaction between treatments (Figure 2A–C). Mycorrhizal inoculation had positive effects on the fresh and dry shoot weights, and also stem length. However, non-mycorrhizal and mycorrhizal plants cultivated at the highest P—40 mg kg^{−1} (P3)—and lowest P—10 mg kg^{−1} (P1)—concentrations did not differ significantly. Mycorrhizal plants obtained the highest fresh and dry stem weights and the highest stem length in the intermediate P concentration, 20 mg kg^{−1} (P2). Nevertheless, no significant differences were observed among the three mycorrhizal inocula with respect to the fresh or dry stem weight at any P concentrations (Figure 2A–C). Table 1 summarizes several traits, among which mycorrhizal inoculation significantly enhanced leaf area index, but *G. intraradices* alone did not have a significant influence on this index. Increasing the P concentration to 20 mg kg^{−1} soil positively affected the leaf area (Table 1).

Table 1. Effects of the phosphorus (P), AMF inoculation (M), and their interaction (P × M) on some growth parameters of *Lisianthus*.

Treatments		Leaf Area (mm ² Plant ^{−1})	Fresh Root Weight (g Plant ^{−1})	Root Length (cm Plant ^{−1})
P ^a	P1	36,100 ± 3815 ab	6.93 ± 0.36 a	2843 ± 215 a
	P2	40,000 ± 2928 a	5.38 ± 0.44 b	2184 ± 232 b
	P3	32,137 ± 1672 b	4.72 ± 0.10 b	1760 ± 68 b
M ^b	M0	31,119 ± 1496 b	4.86 ± 0.52 b	1783 ± 211 b
	M1	40,258 ± 4747 a	5.94 ± 0.71 a	2346 ± 349 a
	M2	33,331 ± 3705 ab	5.78 ± 0.83 ab	2413 ± 405 a
	M3	39,608 ± 1707 a	6.12 ± 0.66 a	2507 ± 341 a
Sig. ^c	P	*	***	***
	M	*	*	*
	P × M	ns	ns	ns

^a Phosphorus treatments—P1, P2, P3: 10, 20, 40 mg kg^{−1} soil, respectively. ^b AM fungal inoculum—M0: non-inoculated; M1: *Glomus mosseae*; M2: *G. intraradices*; M3: mixture of the two species. ^c *, **, and ns denote statistical significance from ANOVA at the 0.05 and 0.001 levels, and the absence of significance, respectively. Data correspond to the means ± standard error of independent replicates. Different letters in the columns indicate significant differences between treatments within the same factor (Duncan's multiple range test, *p* < 0.05).

3.1.2. Root

Both P concentration and mycorrhizal inoculation significantly affected fresh and dry root weights and root length. The results showed a significant decrease in the fresh root weight with increasing P concentration; the highest fresh root weight was observed in plants of P1 (Table 1). Mycorrhizal inoculation had a positive effect on fresh root weight. No significant differences in fresh root weight were found among the three fungal inocula (Table 1). Fresh root weight did not differ significantly between M1 and M0 plants. However, a significant P × M interaction was detected only for the dry root weight. Mycorrhizal plants (with no differences among three inocula) grown in the lowest P medium (P1, 10 mg kg^{−1}), had a greater dry root weight. There was no discernible difference between the dry root weight of mycorrhizal and non-mycorrhizal plants in P2 or P3 (Figure 2D).

Root length varied significantly among plants of P1, P2, and P3, and decreased with increasing P concentration. In other words, root length in P1 was approximately 1.6 times that in P3 (Table 1). Mycorrhization increased the root length by up to 1.4 times. The lowest root length was found in non-mycorrhizal plants. All three inocula had statistically similar effects on the root length (Table 1).

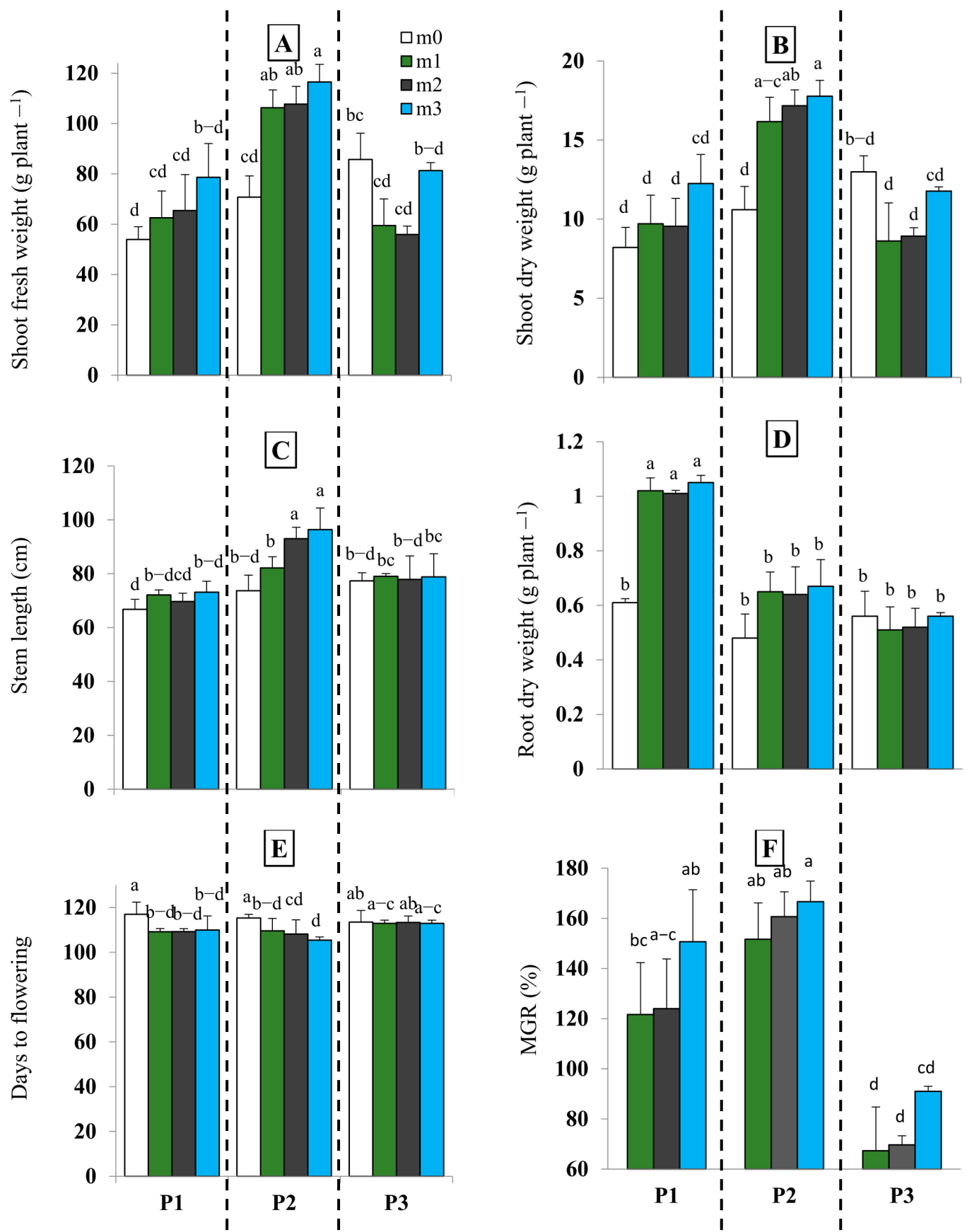


Figure 2. Effect of AMF inoculation (M0: non-inoculated; M1: *Glomus mosseae*; M2: *G. intraradices*; M3: mixture of *G. mosseae* and *G. intraradices*) on growth and ornamental characteristics, and mycorrhizal

growth responses (MGR) of *Lisianthus* at different levels of phosphorus (P1, P2, P3: 10, 20, and 40 mg p kg⁻¹ soil, respectively). (A) Shoot fresh weight; (B) Shoot dry weight; (C) Stem length; (D) Root dry weight; (E) Days to flowering; (F) MGR. Values are means and vertical bars are standard errors. Different letters indicate significant differences between treatments, Duncan's multiple range test ($p < 0.05$).

3.1.3. Flower

Non-mycorrhizal and mycorrhizal plants differed significantly in the number of flowering stems and number of flowers per stem (Table 2). The number of flowers per stem and the number of flowering stems increased with mycorrhizal inoculation, with the highest means recorded in M3 plants. However, the number of stems in the plants in M1 and M2 and the number of flowers per stem in M1 were not significantly influenced. Thus, the blended inoculum (M3) was more effective than either single inoculum. P concentration did not significantly affect the number of flowering stems; however, the number of flowers per stem increased with an increasing P concentration from 10 to 20 mg kg⁻¹ (Table 2). Further, mycorrhizal inoculation had a significant positive effect on the flower diameter and total fresh weight of flowers (Table 2). Flower diameter increased significantly with all three inocula, whereas the total fresh weight of flowers only increased significantly with M3. P concentrations did not influence flower diameter, but increasing the P concentration to 20 mg kg⁻¹ significantly enhanced the total fresh flower weight. None of the treatments were able to affect the vase life of *Lisianthus* cut flowers (Table 2). Overall, the blended inoculum (M3) produced the most favorable combination of flower number and biomass, highlighting the synergistic effect of dual AMF inoculation on *Lisianthus* ornamental performance (Table 2).

Table 2. Effects of the phosphorus (P), AMF inoculation (M), and their interaction (P × M) on ornamental characteristics of *Lisianthus*.

Treatments		Number of Stem/Plant	Number of Flowers/Plant	Flower Diameter (mm)	Total Fresh Flower Weight (g Plant ⁻¹)	Fresh Flower Weight (g)	Vase Life (Days)
P ^a	P1	1.83 ± 0.12 b	7.92 ± 0.99 b	90.92 ± 1.98 a	22.44 ± 3.12 b	2.84 ± 0.09 a	9.83 ± 0.50 a
	P2	2.19 ± 0.17 a	11.31 ± 1.34 a	90.59 ± 1.81 a	34.51 ± 4.15 a	3.02 ± 0.02 a	11.00 ± 0.14 a
	P3	2.00 ± 0.10 ab	9.37 ± 0.43 ab	91.30 ± 2.33 a	28.00 ± 2.32 ab	2.93 ± 0.18 a	11.83 ± 0.10 a
M ^b	M0	1.75 ± 0.14 b	7.36 ± 0.56 b	85.7 ± 0.86 b	21.61 ± 1.45 b	2.90 ± 0.12 a	10.44 ± 0.97 a
	M1	1.97 ± 0.14 ab	9.39 ± 0.91 ab	92.58 ± 0.90 a	28.85 ± 3.92 ab	3.04 ± 0.17 a	10.89 ± 0.80 a
	M2	2.03 ± 0.12 ab	10.07 ± 1.85 a	92.30 ± 2.38 a	28.09 ± 6.50 ab	2.73 ± 0.12 a	11.12 ± 0.29 a
	M3	2.28 ± 0.15 a	11.33 ± 1.03 a	93.11 ± 0.32 a	34.71 ± 3.25 a	3.05 ± 0.06 a	11.11 ± 0.29 a
	M3	2.28 ± 0.15 a	11.33 ± 1.03 a	93.11 ± 0.32 a	34.71 ± 3.25 a	3.05 ± 0.06 a	11.11 ± 0.29 a
Sig. ^c	P	ns	**	ns	**	ns	ns
	A	*	**	**	*	ns	ns
	P × M	ns	ns	ns	ns	ns	ns

^a Phosphorus treatments—P1, P2, P3: 10, 20, and 40 mg kg⁻¹ soil, respectively. ^b AM fungal inoculum—M0: non-inoculated; M1: *Glomus mosseae*; M2: *G. intraradices*; M3: mixture of the two species. ^c *, ** and ns denote statistical significance from ANOVA at the 0.05, 0.01 levels, and the absence of significance, respectively. Data correspond to the means ± standard error of independent replicates. Different letters in the columns indicate significant differences between treatments within the same factor (Duncan's multiple range test, $p < 0.05$).

Number of days to flowering of *Lisianthus* plants was significantly reduced by mycorrhizal inoculation (Figure 2E). No significant difference in days to flowering was observed between non-mycorrhizal and mycorrhizal plants in P3.

3.2. Root Colonization

Figure 3 shows a colonized *Lisianthus* root with the fungal structures clearly visible (Figure 3). No colonization was found in M0 plants (Figure 4A). The estimated colonization percentage in inoculated plants ranged from 19.5% to 37.8%, which significantly declined with increasing P concentration. The type of inoculum also affected the root colonization percentage: M3 performed better than M1 and M2 and the highest colonization was observed in plants grown in P1 (Figure 4A).

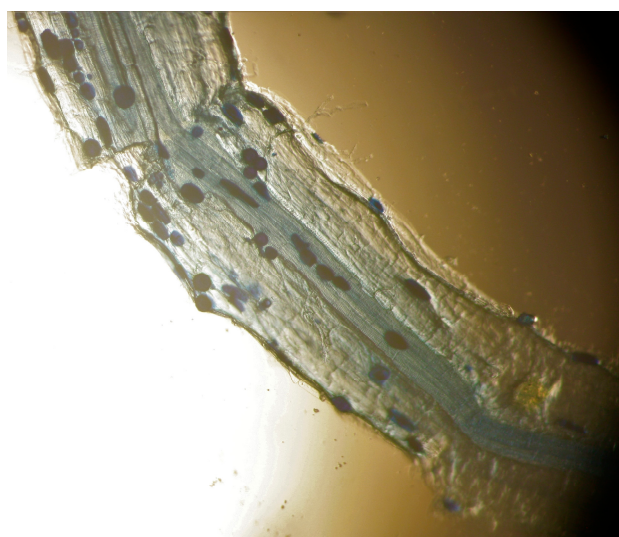


Figure 3. Mycorrhizal colonization of *Lisianthus* root.

3.3. Shoot Nutrient Concentrations

Concentration of various nutrients differed considerably between non-mycorrhizal and mycorrhizal *Lisianthus* plants (Figure 4B–E). The shoot concentrations of N, P, Fe, and Zn were influenced by the interaction between mycorrhizal inoculation and P (Figure 4B–E). Mycorrhization significantly increased the shoot concentrations of N, P, Fe, and Zn. M3 was more effective than M1 and M2 at an increasing P concentration, whereas the concentrations of N, Zn, and Fe did not differ among inoculum types. The highest shoot N and P concentrations occurred in P2 plants, while the highest concentrations of Zn and Fe were obtained in P1. In P3, non-mycorrhizal and mycorrhizal plants did not differ significantly in shoot N, P, and Zn concentrations. In P2, no significant differences were observed for Fe and Zn concentrations between non-mycorrhizal and mycorrhizal plants; however, mycorrhiza was beneficial to the P concentration. Regardless of the effect of mycorrhiza, the concentrations of Zn and Fe reduced with an increasing P concentration in the medium (Figure 4B–E).

The interaction of P and M factors for K and Ca elements was not significant, showing that phosphorus supply and mycorrhizal inoculation had separate impacts on K and Ca concentrations in *Lisianthus* shoots (Table 3). Mycorrhizal inoculation did not influence shoot K concentration. However, its concentration increased when the P concentration exceeded 10 mg kg^{-1} . Inoculation significantly enhanced the Ca concentration in *Lisianthus* aerial parts, whereas the medium P concentration (20 mg kg^{-1}) had no significant influence on the shoot Ca concentration (Table 3).

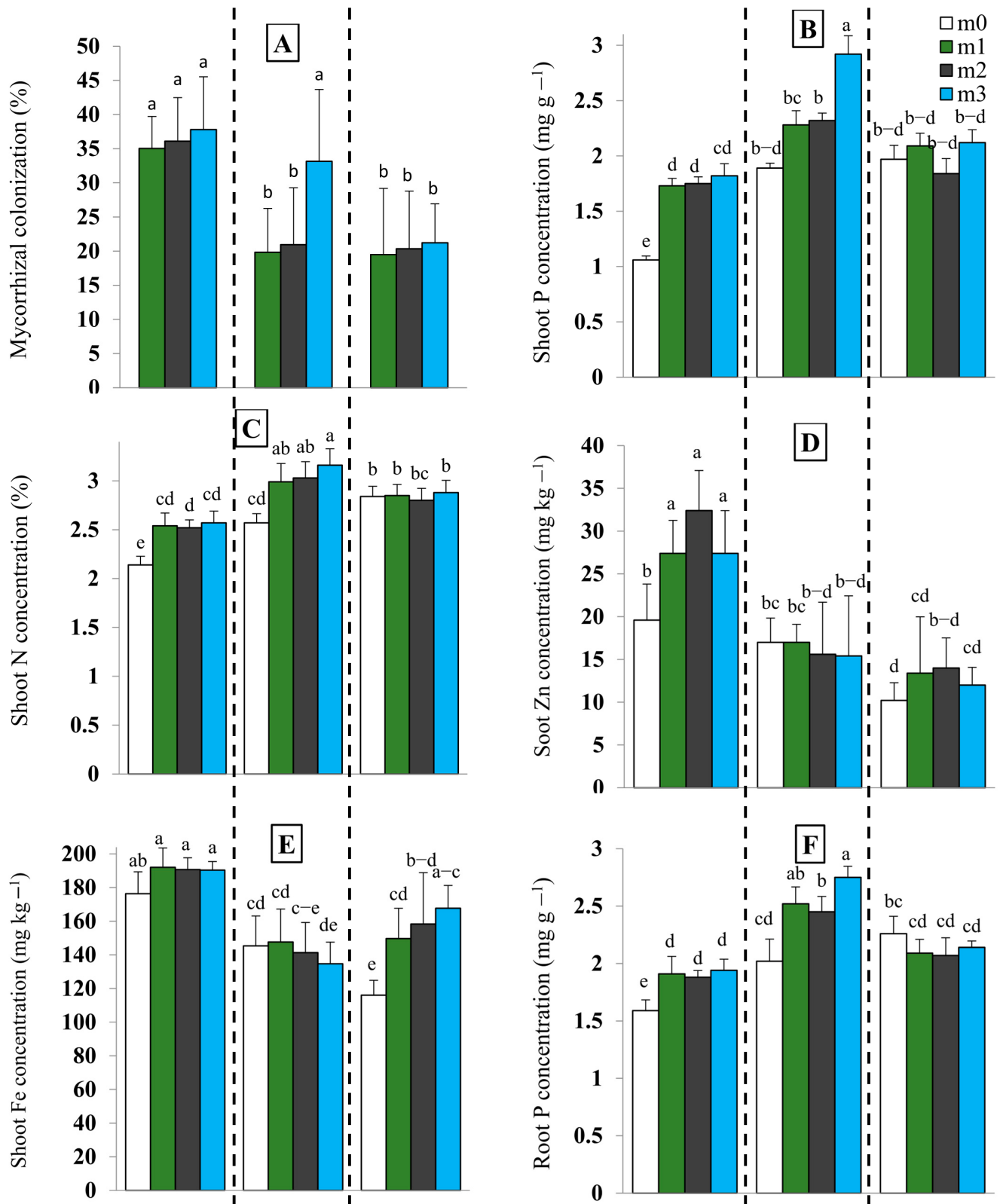


Figure 4. Effect of AMF inoculation (M0: non-inoculated; M1: *Glomus mosseae*; M2: *G. intraradices*; and M3: mixture of *G. mosseae* and *G. intraradices*) on mycorrhizal colonization and nutrient concentration of *Lisianthus* at different levels of phosphorus (P1, P2, P3: 10, 20, and 40 mg p kg⁻¹ soil, respectively). (A) Mycorrhizal colonization; (B) Shoot P concentration; (C) Shoot N concentration; (D) Shoot Zn concentration; (E) Shoot Fe concentration; (F) Root P concentration. Values are means and vertical bars are standard errors. Different letters indicate significant differences between treatments, Duncan's multiple range test ($p < 0.05$). No colonization was observed in non-inoculated plants.

Table 3. Effect of the phosphorus (P), AMF inoculation (M), and their interaction (P × M) on nutrients concentration of *Lisianthus*.

Treatments		Root					Shoot	
		N	K	Ca	Zn	Fe	K	Ca
		(%)	(%)	(%)	(mg kg ^{−1} D.W)	(mg kg ^{−1} D.W)	(%)	(%)
P ^a	P1	2.35	0.81	0.44	26.60	629.83	1.10	0.35
		± 0.09 b	± 0.05 a	± 0.01 a	± 0.70 a	± 19.69 a	± 0.02 b	± 0.02 a
	P2	2.71	0.89	0.41	18.40	409.83	1.23	0.37
		± 0.09 a	± 0.03 a	± 0.01 a	± 2.06 b	± 10.92 b	± 0.03 a	± 0.02 a
	P3	2.74	0.92	0.47	16.85	420.83	1.27	0.40
		± 0.01 a	± 0.02 a	± 0.00 a	± 1.06 b	± 31.12 b	± 0.03 a	± 0.01 a
M ^b	M0	2.45	0.81	0.45	23.53	458.89	1.17	0.32
		± 0.19 b	± 0.09 a	± 0.02 a	± 2.43 a	± 59.30 a	± 0.05 a	± 0.02 b
	M1	2.58	0.87	0.45	20.13	474.67	1.18	0.36
		± 0.12 ab	± 0.00 a	± 0.01 a	± 2.85 a	± 84.49 a	± 0.08 a	± 0.01 ab
	M2	2.65	0.90	0.43	18.53	518.67	1.25	0.38
		± 0.10 a	± 0.04 a	± 0.02 a	± 3.45 a	± 71.00 a	± 0.05 a	± 0.02 ab
	M3	2.72	0.92	0.44	20.27	494.00	1.21	0.41
		± 0.13 a	± 0.01 a	± 0.02 a	± 3.98 a	± 82.67 a	± 0.06 a	± 0.02 a
Significance ^c	P	***	ns	ns	***	***	**	ns
	M	**	ns	ns	ns	ns	ns	*
	P × M	ns	ns	ns	ns	ns	ns	ns

^a Phosphorus treatments—P1, P2, P3: 10, 20, and 40 mg kg^{−1} soil, respectively. ^b AM fungal inoculum—M0: non-inoculated; M1: *Glomus mosseae*; M2: *G. intraradices*; and M3: mixture of the two species. ^c *, **, ***, and ns denote statistical significance from ANOVA at the 0.05, 0.01, and 0.001 levels, and the absence of significance, respectively. Data correspond to the means ± standard error of independent replicates. Different letters in the columns indicate significant differences between treatments within the same factor (Duncan's multiple range test, $p < 0.05$).

3.4. Root Nutrient Concentrations

The P × AMF inoculation was significant only on root P concentration (Figure 4F). The highest root P concentration was observed in M1 and M3 plants grown in P2, while the lowest was recorded in non-mycorrhizal P1 plants. In addition, AMF effects were greatest at the lowest P concentrations 10 mg kg^{−1}; no significant differences between non-mycorrhizal and mycorrhizal plants were observed in P3 (Figure 4F). As shown in Table 3, both the phosphorus level and the mycorrhizal inoculation had a significant impact on the nitrogen (N) content in *Lisianthus* roots (Table 3). Root N content was greatly increased by raising the P concentration from 10 to 40 mg kg^{−1}, but the Fe and Zn contents dropped as P levels increased. When compared to plants that were not inoculated, the inoculation treatments M2 (*G. intraradices*) and M3 (dual inoculation) considerably enhanced root N. On the other hand, neither P nor mycorrhizal treatments had significant effects on the concentrations of K and Ca in the roots (Table 3).

3.5. Pearson Correlation Matrix

Pearson correlation analysis was used to investigate the connections between growth, flowering, and nutritional characteristics across treatments, aiming to find possible correlations between plant performance metrics, including nutrient levels and growth or blooming traits. The pairwise correlation matrix further elucidated the relationships among traits (Figure S2). Shoot fresh weight (SFW), Stem length (SL), and shoot dry weight (SDW) form a tightly integrated group (SL–SFW, $r = 0.84$; SL–SDW, $r = 0.85$; SFW–SDW, $r = 0.99$), which in turn correlates strongly with root- and shoot-level macronutrient pools (e.g., SL–root P

concentration, RPC, $r = 0.93$; SL–shoot P concentration, SPC, $r = 0.91$; SL–shoot N concentration, SNC, $r = 0.90$). Plants that grow taller and accumulate more shoot biomass also pack in higher concentrations of N, P, and other nutrients across both above- and underground tissues (RPC–SPC, $r = 0.95$; SPC–SNC, $r = 0.94$; SNC–RNC, $r = 0.95$).

Total fresh flower weight is strongly associated with measures of number of stems per plant (NSP–TFFW, $r = 0.79$), total flower number (NFP–TFFW, $r = 0.97$). Moreover, TFFW scales closely with overall plant size (stem length, SL–TFFW, $r = 0.90$; shoot dry weight, SDW–TFFW, $r = 0.78$), indicating that larger plants tend to produce more floral biomass. However, flower diameter itself is only weakly linked to total yield (FD–TFFW, $r = 0.28$), suggesting that variation in total fresh flower weight arises primarily through differences in flower number and individual flower mass rather than flower size (Figure S2).

Essential macro- and micronutrients co-accumulate within each plant compartment: strong positive correlations occur among shoot P, N, and K concentration (SPC–SNC, $r = 0.94$; SPC–SKC, SKC, $r = 0.64$), and among their root-tissue counterparts (RFeC–RZnC, $r = 0.80$). Root–shoot linkages are likewise robust (e.g., RNC–SNC, $r = 0.95$; RFeC–SFeC, $r = 0.93$), demonstrating coordinated nutrient uptake and partitioning across organs (Figure S2).

Several clear trade-offs emerged. Days to flowering (DTF) was inversely correlated with vegetative size (DTF–SL, $r = -0.64$; DTF–SDW, $r = -0.69$) and with floral yield (DTF–NSP, $r = -0.79$; DTF–TFFW, $r = -0.60$), indicating that earlier flowering tends to coincide with smaller plant stature and reduced reproductive biomass. Likewise, root Zn concentration (RZnC) trades off against nearly every growth dimension (RZnC–SL, $r = -0.78$; RZnC–SFW, $r = -0.43$; RZnC–SDW, $r = -0.43$), suggesting that high Zn accumulation may be associated with reduced overall size (Figure S2).

3.6. Principal Component Analysis

A principal component analysis (PCA) of the 27 assessed traits identified two primary axes explaining 54.3% (PCA1) and 20.3% (PCA2) of the total variance, respectively (Figure 5). PCA1 reflected above-ground vigor and reproductive potential, with strong positive loadings for fresh shoot weight, dry shoot weight, stem length, total fresh flower weight, shoot phosphorus concentration, and root phosphorus concentration. PCA2 primarily captured below-ground performance and mycorrhizal symbiosis, showing high positive loadings for dry root weight, fresh root weight, mycorrhizal colonization, and mycorrhizal growth responses. Under the intermediate phosphorus supply (P2), increasing AMF inoculation progressively enhanced PCA1 scores: P2M3 (*G. mosseae* + *G. intraradices*) and P2M2 (*G. intraradices*) occupied the furthest positive positions on PCA1, indicating that the 20 mg P kg^{−1} regime, combined with fungal inoculation, maximized shoot biomass, stem elongation, flower yield, and phosphorus uptake. In contrast, non-inoculated P2M0 clustered closer to the origin, confirming that the positive growth and nutritional characteristics were largely associated with AMF presence.

Along PCA2, the greatest underground response occurred at the lowest P, 10 mg kg^{−1} (P1) with inoculation: P1M3 and P1M2 were situated at the extreme positive end of PCA2, revealing that under phosphorus-limiting conditions, mycorrhizal fungi substantially promoted root growth and colonization. Treatments lacking AMF (M0 across all P levels) clustered near zero on PCA2, indicating minimal root benefit in the absence of inoculation. At the highest P, 40 mg kg^{−1} supply (P3), AMF effects on PCA2 diminished (e.g., P3M3 reverted toward neutral PCA2), suggesting that when soil P is abundant, additional fungal symbiosis contributes less to root biomass (Figure 5).

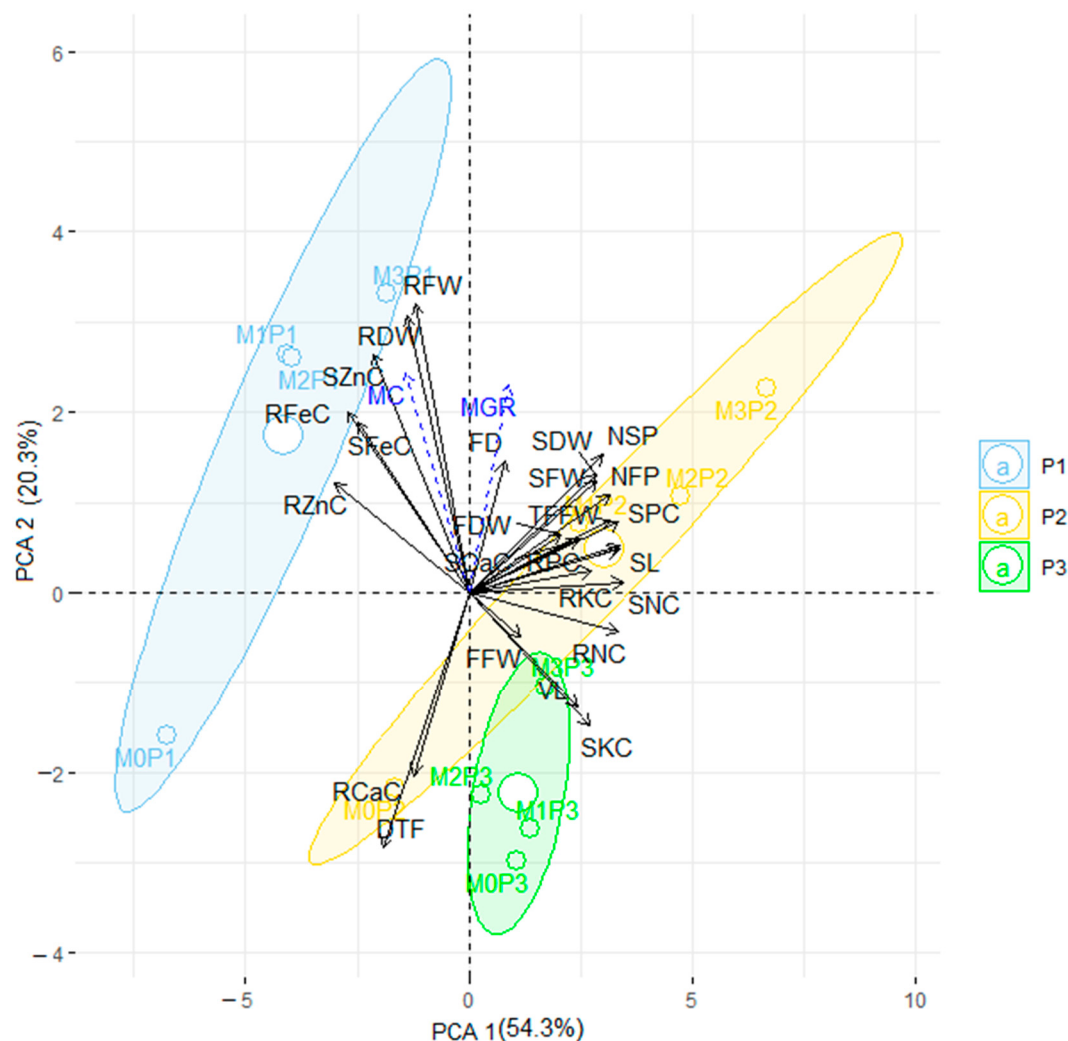


Figure 5. Principal component analysis of AMF treatments across varying phosphorus levels. Shoot fresh weight (SFW), shoot dry weight (SDW), stem length (SL), root dry weight (RDW), days to flowering (DTF), leaf area (LA), fresh root weight (RFW), root length (RL), number of stems per plant (NSP), flower diameter (FD), total fresh flower weight (TFFW), fresh flower weight (FFW), vase life (VL), shoot P concentration (SPC), shoot N concentration (SNC), shoot Zn concentration (SZnC), shoot Fe concentration (SFeC), shoot K concentration (SKC), shoot Ca concentration (SCaC), root P concentration (RPC), root K concentration (RKC), root Ca concentration (RCaC), root N concentration (RNC), root Zn concentration (RZnC), root Fe concentration (RFeC), mycorrhizal growth response (MGR), mycorrhizal colonization (MC).

Overall, P2M3 emerged as the top treatment for above-ground productivity and nutrient acquisition. Intermediate phosphorus treatments (P2M1, P2M2) achieved substantial, albeit slightly lower, gains in the same traits. These results demonstrate a complementary interaction between phosphorus availability and AMF inoculation: moderate P combined with a mixed-species inoculum maximized performance (Figure 5).

4. Discussion

4.1. Root Colonization

A reduction in root colonization with an increasing P concentration has been reported previously [14,38,53–56]. The lower colonization percentage found in the highest P concentration, 40 mg kg^{−1}, may result from reduced hyphal growth and spore production, which can suppress the symbiosis [55,57]. On the other hand, it is well known that in

high P soils, arbuscular mycorrhizal fungi may be effective in plants under several abiotic stresses (drought, salinity, extreme temperatures), but in our study that is not the case. The varied colonization percentages made by the three mycorrhizal inocula might be due to the fact that different species of mycorrhiza have different root colonization capacities [58]. Variation among different mycorrhizae is also reported by Linderman and Davis (2004) [33]. These colonization patterns likely underlie the P-dependent functional effects we detected on nutrient uptake, growth, and ornamental features.

4.2. Nutrient Contents in Root and Shoot

It was revealed that three different mycorrhizal inocula (*Glomus mosseae*, *G. intraradices*, and their mixture) affect absorption of N, P, Ca, Fe, and Zn under greenhouse conditions (Table 3 and Figure 4B–F). Widely increased uptakes of micro- and macronutrients by AMF have been stated [37,41,59–61], which exhibit the capability of AMF to enhance the nutrient status of the plants. Enhanced root nutrient uptake, increased transportation rate, and improved nutrient utilization are mechanisms through which plants benefit from mycorrhization. Positive effects of mycorrhization in enhancing P absorption have been reported [12,13,38,59,60,62]. Because root length strongly influences P uptake [63], the increased root length observed in mycorrhizal *Lisianthus* likely contributed to enhanced P absorption, consistent with studies by Schroeder and Janos (2005) and Sheikh-Assadi et al. (2023) [54,60]. AMF hyphae expand the soil volume explored by the plant, and mycorrhizal root exudates, such as organic acids and phosphatase, increase the release of P from rock phosphates and organic complexes [16,63,64]. Moreover, the fine diameter of extraradical hyphae increases the effective absorptive surface per unit volume compared to the root surface, improving P acquisition efficiency [63]. Furthermore, due to the greater tendency of mycorrhizal hyphae to absorb phosphate ions and the lower threshold for their absorption, mycorrhizal roots can acquire more P per unit of biomass than non-mycorrhizal roots [16]. Acid phosphatase and alkaline phosphatase play a key role in increasing P absorption [55]. Studies show that phosphatase activity is higher in mycorrhizal plants [55]; this can contribute to enhanced P concentrations in the tissues of mycorrhizal plants. Nonetheless, regarding plant P content, there was no difference between non-mycorrhizal and mycorrhizal plants in P3 (Figures 2B and 4F), which is compatible with the outcomes of Watts-Williams and Cavnaro (2012) [38]. Overall, mycorrhizal plants cultivated in P2 had the greatest P content (Figures 2B and 4F), which is in agreement with the outcomes of Prasad et al. (2012) [55]. One explanation for why plants grown at a high P concentration of 40 mg kg^{−1} did not show higher tissue P than those with an intermediate P concentration of 20 mg kg^{−1} could be the probability that high P may be deleterious to mycorrhizal colonization, thus restricting the absorption of P [65].

In addition, increased absorption of other nutrients by mycorrhization has been reported: Fe [62,66], Zn [37,58,62], and Ca [62]. In our study, mycorrhizal inoculation enhanced the concentrations of N, Zn, and Fe, but did not affect K concentrations (Table 3 and Figure 4B–F), which agrees with the result of Hart and Forsythe (2012) [66]. Exploration of the root zone by an extensive network of very thin extraradical hyphae of AMF—thinner than even the thinnest hairy roots—enables plant roots to access more water and nutrients [14,16,67]. Moreover, Bolan [16] reported that the uptake of phosphate by mycorrhizal roots was faster than by non-mycorrhizal roots. Therefore, mycorrhizal plants may have greater capacity for water and nutrient uptake, resulting in higher tissue concentrations [16]. The role of mycorrhizae in the absorption of Zn and Fe has been described [68]. AMF plays a significant role in the N nutrition of plants; they can absorb nitrate and ammonium forms, as well as amino acids [17,23,69], and can transfer them into the plant, thus improving the N status of the plant.

In this context, ornamental species are still relatively understudied, despite extensive documentation of AMF–P interactions in staple crops like maize, soybean, and wheat. The majority of current AMF–P studies focus on biomass or yield recovery under stress, but commercial floriculture requires a performance matrix—including stem strength, flowering earliness, floral biomass, and postharvest persistence—that is seldom assessed in mechanistic AMF research. By combining a factorial P-gradient design in a greenhouse, with accurate measurements of roots' and shoots' nutrients levels, flower growth, and vase life, the current work helps to fill this gap. This multi-trait assessment shows that AMF-mediated P acquisition is a useful horticultural technique that can improve fertilizer-use efficiency while enhancing marketable flower quality, providing both agronomic and physiological benefits.

4.3. Vegetative Growth and Biomass Responses

Our results indicate the significant influence of mycorrhizal colonization on *Lisianthus* production under greenhouse conditions. Mycorrhizal inoculation increased several growth parameters, although effects varied among inocula. Similar variability among inocula has been reported by Van Geel et al. (2016) [70]. Guo et al. (2022) studied four plant species and found a considerable increase in the growth of inoculated plants [32]. Gaur et al. (2000) reported that vegetative growth of *Callistephus chinensis*, *Petunia hybrid*, and *Impatiens balsamina* was increased by mycorrhizal inoculation [59].

The increase in stem length in mycorrhizal plants in our study is consistent with other investigations [55,59]. The highest stem length was obtained in mycorrhizal plants grown in P2, which is in agreement with the results of Prasad et al. (2012), who worked on *Chrysanthemum indicum* L. [55]. The increased stem length could be due to enhanced nutrient uptake [13,60] and promoted photosynthesis [61]. In our experiment, the highest concentrations of P and N were recorded in plants that had the highest stem length. Sohn et al. (2003) also suggested that mycorrhizal plants might have higher length, and they attributed this effect to the increased nutrient uptake by mycorrhizal roots [62].

Mycorrhizal inoculation increased *Lisianthus* biomass significantly (Figure 2A,B). Similar results have already been reported by Prasad et al. (2012) and Sohn et al. (2003) [55,62]. Further to this, the increased dry root and shoot weights are in agreement with earlier studies [33,58]. Mycorrhizal *Lisianthus* plants had a higher concentration of nutrients. The increased growth, which has already been reported by Watts-Williams and Cavagnaro (2012) [38], could be attributed to the increased nutrient uptake by mycorrhizal roots, which is in agreement with the results of Sohn et al. (2003) [62]. Mycorrhizal plants could absorb higher P from soil, which in turn promotes biomass production, thus enhancing plant growth [12,59]. AMF could also act as a metabolic sink, elevating the photosynthetic activity of their host [60]. Accordingly, biomass production would be promoted by AMF. Moreover, production of hormone-like substances by AMF and increased gibberellin and cytokinin levels in the host plant [15] can increase the photosynthetic rate [71], thus promoting plant growth. However, in some treatments, no statistical difference was detected between non-mycorrhizal and mycorrhizal plants, which is consistent with other findings [33,66]. The lowest growth was recorded in plants that had the lowest N concentration. Cytokinin promotes cell division and elongation, reduces N uptake, and can limit cytokinin synthesis in roots and subsequent transport to the shoots, thereby suppressing shoot cell division [72,73]. This may be a reason for observing the lowest growth in plants with the lowest N uptake. Additionally, these plants had higher Zn and lower P content, which has already been reported in [38]—lower biomass with higher Zn and lower P content. Enhanced leaf area, compatible with the results of Prasad et al. (2012) [55] and Sohn et al. (2003) [62], could also result from increased growth and development by the influence of

elevated P absorption [60]. In addition, N is one of the elements that can cause an increase in leaf area; therefore, the higher leaf area of inoculated plants may also be due to increased N in their tissues.

4.4. Root Length and Morphology

Besides the significant improvement of growth and P content, mycorrhization significantly affected the root length. Our findings regarding increased root length caused by mycorrhization are in good agreement with the results of several other researchers [12,54,55]. Sohn et al. (2003) reported an increase in the root length of *Chrysanthemum morifolium* in the transition stage by mycorrhizal inoculation [62]. Enhanced nutrient uptake by mycorrhizal roots likely contributed to this effect, as evidenced here by higher P and N concentrations. Mycorrhiza increased root branches, which could increase the total length of the root system [74]. Furthermore, mycorrhizal fungi may produce plant hormones or promote their production [15,61], thus influencing root growth. In addition, the lowest P, 10 mg kg⁻¹ concentration produced the longest roots (Table 1); this may reflect the induction of morphological changes in the root (increased production of hairy roots and cluster roots), mediated by auxin, ethylene, and cytokinin to enhance P acquisition [75,76].

4.5. Flowering and Ornamental Quality Parameters

Our results showed that mycorrhization lowered the number of days essential for the flowering of *Lisianthus* (Figure 2E). This beneficial effect of AMF has also been demonstrated by others [58,62]. Gaur et al. (2000) found that mycorrhizal *Callistephus chinensis*, *Impatiens balsamia*, and *Petunia hybrid* flowered 22, 16, and 12 days earlier, respectively, than non-mycorrhizal plants [59]; mycorrhizal inoculation can thus substantially affect time to flower initiation [59]. This fact may correspond to the role of P in flowering. Plants that flowered earlier had higher P concentrations in their tissues (Figure S1). What is more, mycorrhiza expedites development of the plant; accordingly, inoculated plants mature earlier [58]: a desirable outcome that may reduce production time and costs.

Several important floral characteristics of *Lisianthus*, including number of flowers per stem, number of flowering stems, flower diameter, and total fresh flower weight, were enhanced by AMF inoculation in the current study (Table 2). These results are in line with other studies on ornamental species like *Tagetes erecta* [77] and *Gerbera jamesonii* [78], where the application of AMF resulted in a significant increase in flower size, biomass, and flower production. These results align with prior reports that mycorrhizal inoculation remarkably improves both vegetative and generative growth [62]. Higher total fresh weight and flower diameter in mycorrhizal plants are also reported by Sohn et al. (2003) [62]. Gaur et al. (2000), mentioning the results of Lee and Bazzaz (1982) [79], reported that the number of flowers produced by the plant had been proportional to the plant growth and nutrient content [59]. Flores et al. (2007) reported that the inoculation of marigold (*Tagetes erecta*) plants with *Glomus fasciculatum* caused a significant increase in the production of inflorescence (22%), which the authors attributed to the improved P nutrition [80]. Some other authors ascribe an increased number of flowers to the altered photosynthetic rate [60,81]. In addition, some thought the stimulation of hormone synthesis to be involved in this influence [Ref. [82] cited in Flores et al. (2007)]. The results of Gaur et al. (2000), with reference to *Petunia hybrida*, confirm the increase in the number of flowers in mycorrhizal *Lisianthus* plants [59].

AMF inoculation had no significant effect on the vase life of *Lisianthus*, in contrast to flower size and number (Table 2). Cut roses (*Rosa hybrida*) treated with AMF under commercial circumstances have shown similar neutral effects on postharvest longevity [83], with no significant increase in vase life despite changes in growth characteristics. Although mycorrhization did not significantly affect the vase life of *Lisianthus* in our study, increased

yield without reduced postharvest quality is advantageous. Growers inevitably overuse nutrition to increase yield, which can reduce postharvest life; our results suggest that AMF may help to maintain yield while avoiding such quality penalties.

4.6. Mycorrhizal Growth Responses (MGR)

Earlier reports suggest that horticultural plant species are very responsive to mycorrhizal inoculation [37]. We quantified this effect as the percent of mycorrhizal growth response (MGR). It expresses that strongly mycorrhiza-responsive plants display better growth when grown in lowest P, 10 mg kg^{-1} . This is compatible with earlier studies showing that AMF plays a more important role when the host plant grows in lower concentrations of P [38]. Similarly to our findings (Figure 2F), Qin et al. (2024) showed that the lowest mycorrhizal growth response was observed in the highest P concentration [84]. Ortas (2012) states that the effect of mycorrhiza has been concealed in high concentrations of P [37]. Overall, the obtained values for *Lisianthus*' growth response in our study were higher than those of *Allium fistulosum* [12]. This discloses the better growth response of *Eustoma grandiflorum*.

4.7. Comparison of Inocula and Introducing the Best One

As is clear from the results, the blended inoculum (M3) was the most effective mycorrhizal treatment for increasing macronutrient uptake, growth, and biomass production. Mardukhi et al. (2011) also reported that mixed inocula (*Glomus etunicatum*, *G. mosseae*, and *G. intraradices*) were more efficient than single-species inocula, attributing the benefit to synergistic interactions among AMF species [85]. Furthermore, the mixed inoculum produced the best quantitative and qualitative outcomes (Tables 1 and 2 and Figure 2C,E). Accordingly, the blended inoculum of *G. mosseae* and *G. intraradices* can be ideal in order to be used in the cultivation of *Lisianthus*.

4.8. Agronomic and Environmental Significance of Findings

The current research provides precise recommendations for the commercial cultivation of *Lisianthus*. Our results show that mycorrhizal inoculation under moderate phosphorus supply (20 mg kg^{-1}) provides equivalent or even greater performance compared with high-P (40 mg kg^{-1}), non-inoculated plants, even though producers usually use high amounts of phosphorus fertilizers to enhance floral production. This suggests that application of AMF could partially substitute chemical P inputs while preserving vase life, flower quantity, and stem length—key attributes for cut-flower grading. Realistically, adding AMF inoculants to routine fertilization systems might save input costs and improve resource efficiency without sacrificing esthetic quality. Thus, in commercial floriculture, AMF technology is a viable instrument for sustainable intensification. However, these findings are derived from controlled greenhouse studies; field validation across diverse substrates, fertigation regimes, and seasons is required before commercial recommendations can be made.

5. Conclusions

Our study demonstrates that dual inoculation (M3) combined with intermediate phosphorus application (P2, 20 mg kg^{-1}) produced the strongest overall response in *Lisianthus*. Under this treatment, shoot biomass increased 2.16-fold, compared to the non-inoculated low-P control, and flowering occurred about 12 days earlier, indicating that balanced P management enhances AMF benefits. The advantages of AMF were significantly diminished at both extremes—low P (10 mg kg^{-1}) and high P (40 mg kg^{-1})—demonstrating that balanced soil fertility, not maximal fertilization, determines AMF efficacy. These results highlight that mixed-species AMF inoculation combined with a modest P supply can reduce fertilizer inputs while preserving or improving commercial flower quality, which is a useful

tactic for ornamental growers. This study supports a paradigm for sustainable horticulture by demonstrating that AMF-mediated nutrient acquisition can improve both nutrient-use efficiency and floral yield. However, field experiments across varied soils and climates are required to validate applicability under commercial conditions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy15112480/s1>. Figure S1: The relation between shoot phosphorus concentration and days to flowering. * Outlier, not included in the regression; Figure S2: Heat map of correlation among vegetative traits, ornamental features, and content of elements in *Lisianthus* plants.

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