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INFLUENCE OF AN ARBUSCULAR MYCORRHIZAL FUNGUS AND PHOSPHATE-SOLUBILIZING BACTERIUM INOCULATION AT STEM CUTTING STAGE ON P UPTAKE AND GROWTH OF *IMPATIENS WALLERIANA* PLANTS IN AN UNSTERILE FIELD SOIL

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ABSTRACT

Microorganisms play an important role in the propagation and growth of plants. Propagation of plants through stem cuttings is a popular method widely practiced in horticulture and forestry. As the information on the interactive role of arbuscular mycorrhizal (AM) fungi and phosphate-solubilizing bacteria (PSB) in the regeneration and growth of clonally propagated plants is limited, we inoculated stem cuttings of *Impatiens walleriana* with *Rhizophagus fasciculatus* and *Bacillus megaterium* var. *phosphaticum* individually or in combination in an unsterile Alfisol field soil. The inoculated cuttings were harvested after 45 days of cultivation and various growth parameters and phosphorus (P) uptake were measured. Inoculation with the AM fungus or PSB individually improved plant growth parameters, whereas the dual inoculation did not give synergistic results. Plants inoculated at cutting stage with *R. fasciculatus* accumulated the highest plant biomass and showed maximum microbial inoculation effect and efficiency of P uptake. Inoculation with the PSB stimulated symbiosis with native AM fungi but a synergistic effect has not been found when both AM fungi and PSB were co-inoculated. It could be concluded that screening for compatibility between microorganisms is essential before selecting the bioinoculants for dual inoculation.

Key words: *Bacillus megaterium*, *Rhizophagus fasciculatus*, clonal propagation, adventitious roots, specific root length, arbuscules

INTRODUCTION

In recent years rapid progress has been made in the large-scale vegetative propagation of several plant species. At present many plant species can be efficiently and cheaply propagated through rooted stem cuttings. The advantages of clonal propagation are well known. One of the major benefits of clonal propagation is that the progenies are true to type where the favorable characters are passed on to the subsequent generations (McKey et al. 2010). Clonal propagation through stem cuttings is one of the most popular and widespread methods of production of planting materials in horticulture and forestry. A large number of plant species that are propagated through seeds could also be easily propagated clonally. Generally, all the plant parts could act as propagules when suitable conditions are provided for their regeneration. For example, regeneration of plants from the stem, root, and leaf cuttings has been reported in a wide range of plant species (Read 2015; Gardner et al. 2019; Kapczyńska 2019). Phytohormones, especially auxins, are applied to the cuttings to increase rooting. One of the major problems in clonal propagation, however, is that the cuttings are normally rooted under the high substrate and atmospheric moisture. This highly humid condition favors the proliferation of pathogens resulting in plant loss (Copes & Blythe 2011; Maússe-Sitoe et al. 2016). Therefore cuttings are often rooted in soilless substrates or substrates that are sterilized (Koshila Ravi & Muthukumar 2019).

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Plants growing in natural soil are often associated with a wide range of microorganisms that influence their growth and health. Among them, the microorganisms that form an association with plants are most influential in affecting the growth and development of plants. Arbuscular mycorrhizal (AM) fungi belonging to *Glomeromycota* and *Mucoromycotina* are the most abundant soil fungi that associate with a wide range of plant species belonging to different groups. These fungi benefit plants in the uptake of nutrients, especially phosphorus (P) and other slow mobile nutrients in deficient soils. In addition to the nutritional benefit, AM fungi also protect host plants against various types of abiotic and biotic stresses thereby increasing plant growth and yield. Studies have shown that colonization of the adventitious roots of the stem cuttings by AM fungi could enhance their growth and fitness (Karagiannidis et al. 2011; Singh et al. 2013). One of the main drawbacks in many of the studies investigating the role of AM fungi in growth performance of cuttings is that these studies are often conducted in soilless substrates or disinfected soils.

Phosphorous is a major nutrient that is essential for growth and metabolism of plants. In soils, the availability of P is limited as this element is often complexed with other soil elements or exists in organic forms. This renders the soil deficient in available P, which is a common phenomenon in the tropics. Like AM fungi, phosphate-solubilizing bacteria (PSB) play an important role in increasing the availability of P to the plants by solubilizing the bound P in the soil. Previous investigations have shown that inoculation of PSB into the soil increases the availability of soil P thereby promoting plant growth (Kudoyarova et al. 2017). In addition to solubilizing P, PSB also produces phytohormones that are known to improve plant growth especially root proliferation (Kudoyarova et al. 2017). A few studies have examined the role of PSB in the growth of stem cuttings in different plant species (Erturk et al. 2010; Beneduzi et al. 2013).

Impatiens walleriana Hook. f., popularly known as balsam, is an ornamental plant belonging to the plant family Balsaminaceae and is native to East Africa. This species is widely cultivated for its ornamental value in many parts of the world and has naturalized in many of these areas (Lim et al. 2014). In addition to its ornamental value, the flowers of *Impatiens walleriana* are edible and the decoction prepared from the dried leaves and roots is used as an abortifacient (Kokwaro 2009). *I. walleriana* could be propagated through seeds as well as foliar stem cuttings (Lim et al. 2014). A previous study (Koide et al. 1999) has shown that the AM fungus *Rhizophagus intraradices* (*Glomus intraradices*) could improve the growth of *I. walleriana* plants raised from seeds. Though a large number of studies have examined the interactive influence of AM fungi and PSB on plants that were raised from seedlings, there is limited information on such effect on clonally propagated plants. Therefore in this study, we investigated the influence of AM fungi and PSB inoculation on rooting and growth of *I. walleriana* plants in unsterilized field soil. Further, we also examined if there were any relations between the uptake of P and growth of *I. walleriana* stem cuttings.

MATERIALS AND METHODS

Study site and plant material

The experiment was conducted at the shade house of the Botany Department (11°02′20.9″ N, 76°52′58.5″ E), Bharathiar University, Coimbatore, India. Stem cuttings of *I. walleriana* were prepared from the shoots collected in the Botanical Garden of the Bharathiar University. The healthy stem cuttings used in the study were 10 cm in length with almost uniform thickness and three leaves.

Determination of the soil characters

Surface Alfisol soil and sand were collected from Bharathiar University campus and were hand-mixed in the ratio of 1 : 1 (V : V). Soil pH and electrical conductivity (EC) were determined in 1 : 5 soil : water (v/v) suspension using respective digital meters The pH was 7.6 and EC 0.15 $dS \cdot m^{-1}$. The total nitrogen (N) and available P determined according to Jackson (1967) were 7.9 $mg \cdot kg^{-1}$ and 0.48 $mg \cdot kg^{-1}$, respectively. The exchangeable potassium (K) determined after extraction with ammonium acetate (Jackson 1967) was 17.8 $mg \cdot kg^{-1}$. The indigenous AM flora in the soil-sand mixture consisted of *Rhizoglomus aggregatum*,

Funneliformis geosporus, and *Sclerocystis sinuosa*, and the most probable evaluation technique indicated the presence of 53 AM propagules per gram (Muthukumar & Udaiyan 2006). Likewise, the indigenous PSB bacteria assessed using Pikovskaya's agar plates were 2×10^3 CFU (colony forming units) per gram of soil-sand mixture (Muthukumar & Udaiyan 2006).

Microbial inoculums

Commercial inoculums of PBS Stanes Symbion-[P]® and AM fungi Symbion VAM® recommended for field crops and ornamental plants were obtained from T. Stanes and Company Limited, Coimbatore, India. The liquid PSB inoculum contained 10^9 cells of *Bacillus megaterium* var. *phosphaticum* per milliliter of the inoculum. The AM fungal inoculum, prepared with exfoliated vermiculite as the carrier, contained 1000 propagules of *Rhizophagus fasciculatus* per gram of the inoculum in the form of spores, soil hyphae, and mycorrhizal roots.

Experimental design

The experiment was carried out in a completely randomized block design with four treatments – control, AM (2.5 g), PSB (1.25 ml), AM + PSB (2.5 g + 1.25 ml) – and ten replicates with a total of 40 containers (bags: $4 \times 10 = 40$). The microbial inoculum was placed in the planting hole prior to the insertion of the stem cuttings. Sterilized inoculum of AM and PSB at their specific application rate was added to the control and treatments not involving any of these microorganisms. The hormone-untreated stem cuttings were planted in polythene bags (15 cm height × 9 cm diameter), containing 105 g of the soil-sand mixture. The cuttings were planted at a depth of 2.5 cm and irrigated daily to the field capacity; no fertilizers were added.

Harvest

After 45 days of the experiment initiating, the plants were harvested destructively with their entire root system almost intact. The soil was washed from the roots and a weighed portion of each root sample from five randomly selected plants from each treatment (n = 5) was preserved in formalin–acetic acid–alcohol (FAA) solution for the assessment of AM colonization. Morphological parameters like plant height, number of branches, leaf numbers, and total root length (Newman 1966) were evaluated. Rooting efficiency was determined as the percentage of rooted cuttings to the total number of cuttings used in each treatment (Szabó et al. 2013). Shoots and roots were separated and dried at 40 °C for 72 hours to constant weight for the determination of dry mass. The root/shoot dry mass ratio was calculated from the respective dry mass. Specific root length [$cm \cdot mg^{-1}$] was calculated as the length to mass ratio: total root length [cm]/root dry weight [mg] (Ostonen et al. 2007).

Microbial inoculation effect (MIE) was calculated using the formula of Muthukumar and Udaiyan (2006):

$$\text{MIE [\%]} = \frac{\text{dry weight of microbial inoculated plant} - \text{dry weight of uninoculated plant}}{\text{dry weight of inoculated plant}} \times 100$$

Estimation of phosphorus

Phosphorus content in shoots (250 mg) and roots (20 mg) of *I. walleriana* was determined by Vandomolybdate method (Jackson 1967) using a spectrophotometer at 470 nm, after wet-ashing the plant sample in nitric-perchloric acid mixture. As the material of individual plants was small, especially for roots, we randomly pooled plants in each treatment into three groups (n = 3) of three plants each for the estimation of P.

The efficiency of P utilization (EPU) was calculated according to Gray and Schlesinger (1983) using the following formula:

$$\text{EPU} = \frac{\text{plant P content [mg]}}{\text{root biomass [mg]}}$$

Preparation of roots for AM assessment

Fixed roots were washed free of FAA and cut into 1-cm long bits, cleared in 2.5% potassium hydroxide (KOH) at 90 °C (Koske & Gemma 1989), acidified with 5N hydrochloric acid (HCl), and stained with trypan blue (0.05% in lactoglycerol). The roots were kept overnight in chlorozol black E lactoglycerol for staining. The stained roots were examined with a compound microscope (×400) for AM fungal structures and the percentage of root length colonization was determined according to magnified intersection method (McGonigle et al. 1990).

Statistical analysis

Analysis of variance (ANOVA) was performed on all the data to compare microbial inoculation effects on *I. walleriana* growth and P uptake after testing the data for homogeneity (Levene's test). Means were separated using Duncan's multiple range test (DMRT). Percentage data on AM colonization were arcsine square-root transformed prior to analysis. Regression analysis was used to assess the relationship between plant biomass and the efficiency of P uptake.

RESULTS

AM colonization

Adventitious roots of *I. walleriana* plants in all the treatments were invariably colonized by AM fungi. The fungal colonization was characterized by the presence of hyphae that transversed the cortex both intercellularly and intracellularly forming coils, arbuscules, arbusculate coils, vesicles and extramatrical hyphae that sometimes contained spores (Fig. 1). Abundant lipid droplets were observed in the intraradical fungal hyphae of the AM fungus and PSB co-inoculated plants.

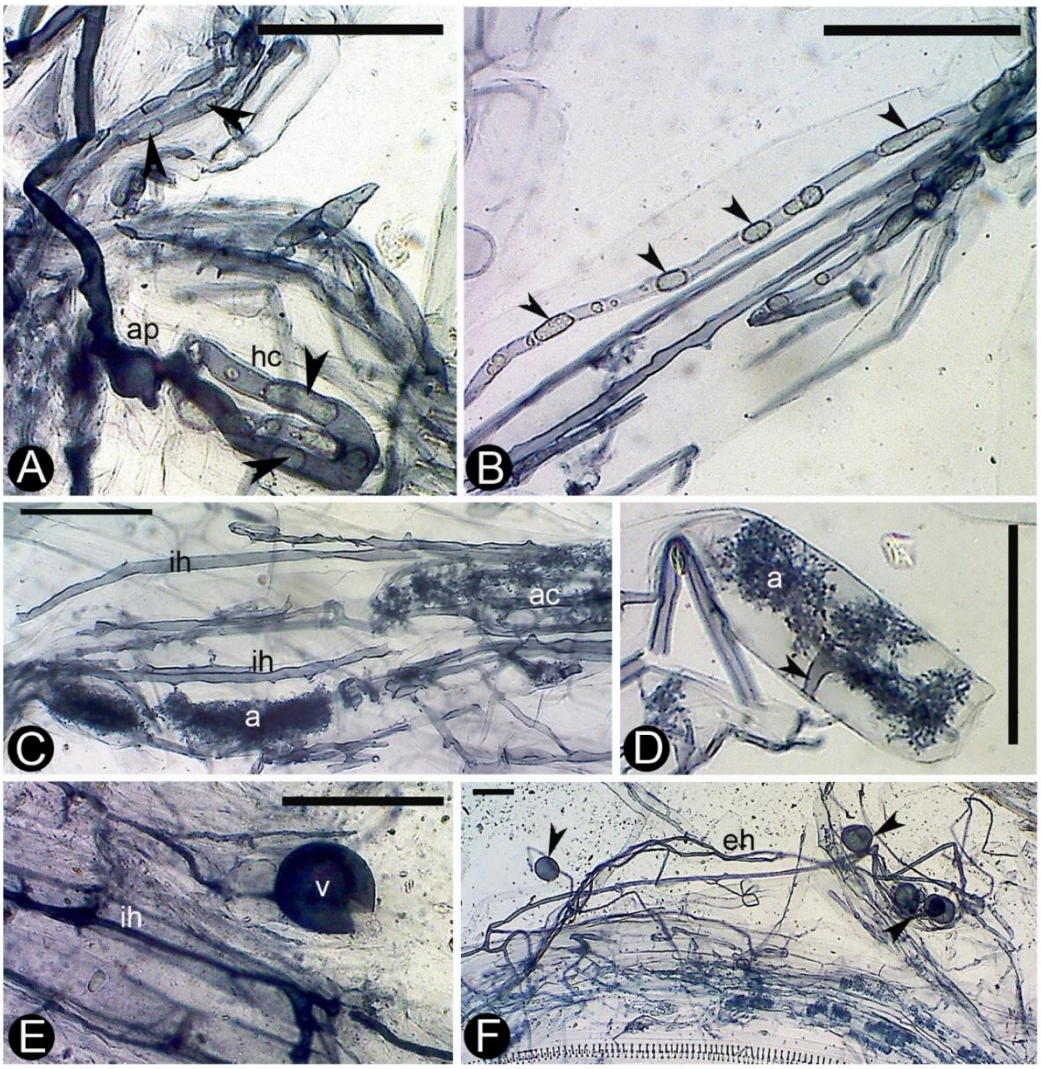

Fig. 1. Arbuscular mycorrhizal fungal colonization in adventitious roots of *Impatiens walleriana* plants: (A) appressorium (ap), hyphal coil (hc), and lipid droplets (black arrow heads) in *Rhizophagus fasciculatus* and *Bacillus megaterium* dual inoculated plants; (B) lipid droplets (black arrow heads) in intraradical hyphae of dual inoculated plants; (C) intraradical hyphae (ih), arbuscule (a), and arbusculate coil (ac) in *R. fasciculatus*-inoculated plants; (D) arbuscule (a) and the hyphal trunk (black arrow head) in cortical cell of dual inoculated plant; (E) fractured vesicle (v) and intraradical hyphae (ih) in *B. megaterium*-inoculated plant; (F) extraradical hyphae (eh) bearing spores (black arrow heads) in *R. fasciculatus*-inoculated plants. Scale bars = 50 μm.

There were significant differences in the extent of root length containing different AM fungal structures ($F_{2,48}$ = 182.724; $p < 0.001$) (Fig. 2). Inoculation of microorganisms also significantly affected the root length with different AM fungal structures ($F_{3,48}$ = 10.071; $p < 0.001$). The interaction among these factors (structures × inoculation) was also significant ($F_{6,48}$ = 3.579; $p < 0.01$) (Fig. 2).

The root length with AM fungal hyphae/hyphal coils and arbuscules/arbusculate coils in plants from cuttings inoculated with AM + PSB were respectively 20–52% and 6–75% higher compared to control and individual inoculation of these microorganisms (Fig. 2a, b). Vesicles were only observed in adventitious roots of *I. walleriana* inoculated with the AM fungus and PSB individually (Fig. 2c). In contrast to the root length colonized by different AM fungal structures, the total AM fungal colonization was highest in AM fungus-inoculated roots that were 3–64% higher than the control and other inoculations (Fig. 2d). The differences among treatments for root length containing different AM fungal structures and total colonization were significant (Table 1).

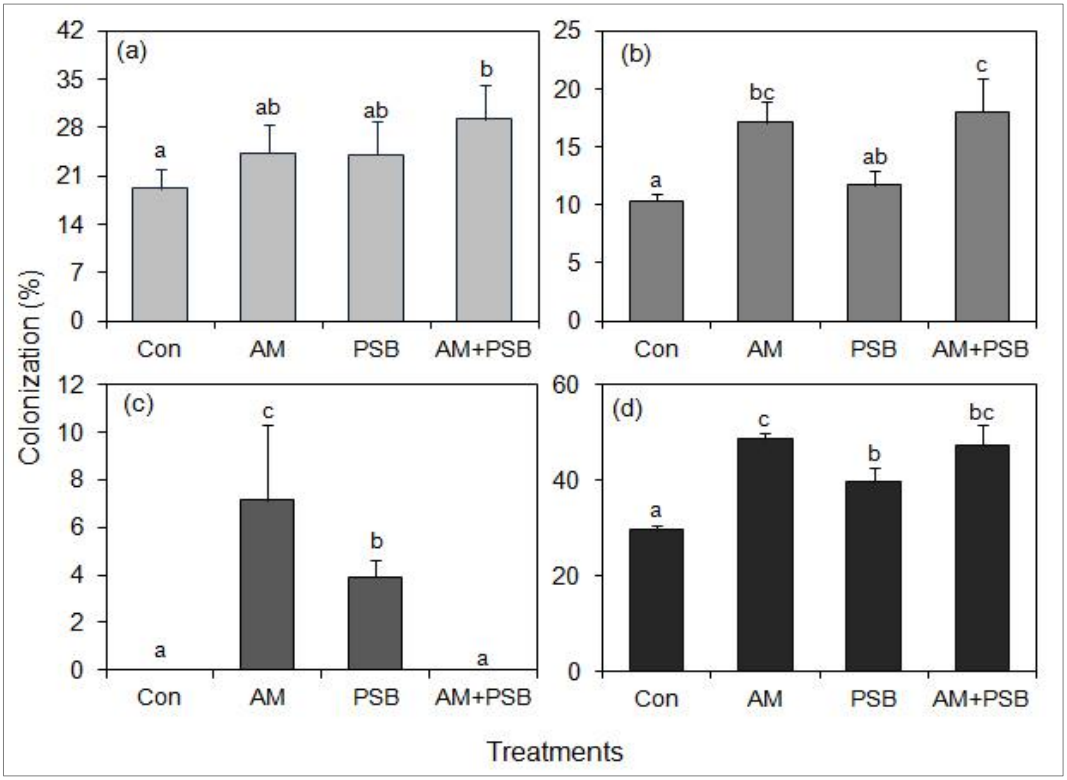

Fig. 2. Influence of arbuscular mycorrhizal (AM) fungus and phosphate-solubilizing bacterial (PSB) inoculation on the root length containing AM fungal hyphae/hyphal coils (a), arbuscules/arbusculate coils (b), vesicles (c), and total colonization (d) in *Impatiens walleriana* plants. Con, control. Bars ± standard error bearing same letter are not significantly ($p > 0.05$) different according to Duncan's multiple range test.

Table 1. Results of one-way analysis of variance for root length containing different arbuscular mycorrhizal (AM) fungal structures and total colonization. Differences (df) for the respective groups are presented in parenthesis

AM fungal variables	Sum of squares		Mean square		F-value	Significance
	between groups (df = 3)	within groups (df = 16)	between groups	within groups		
Hyphae/hyphal coils	251.502	292.261	83.834	18.266	4.590	0.017
Arbuscules/arbusculate coils	222.172	271.095	74.057	16.943	4.371	0.020
Vesicles	178.444	16.000	59.481	2.641	22.519	0.001
Total colonization	1143.711	616.915	381.237	38.557	9.888	0.001

Plant growth

The rooting efficiency was 100% as all the cuttings regardless of inoculation and including control had roots after 45 days. In general, microbial inoculation significantly affected all the plant growth parameters examined (Table 2), but only plants inoculated with PSB individually were significantly taller compared with control by 20% and compared with dual inoculated plants by 17%. The numbers of branches, leaves, and total root length were significantly higher in the treatment where both microorganisms were inoculated. The shoot biomass of cuttings significantly increased only when AM inoculation was applied. The shoot biomass of cuttings inoculated with AM fungus alone was 42–73% higher than that of cuttings in control or cuttings inoculated with PSB or AM + PSB. Contrary to this root dry weight was stimulated by PSB and dual inoculation (Table 2). The R/S ratio of PSB-inoculated cuttings was significantly higher than in other treatments. Inoculation of AM fungi and PSB individually or dually significantly affected the specific root length ($F_{3,36}$ = 12.614; $p < 0.001$). The specific root length was the highest in plants inoculated with both microorganisms (Fig. 3).

The differences in the MIE for microbial inoculated *I. walleriana* stem cuttings were significant ($F_{2,27}$ = 19.372; $p < 0.001$). The MIE value of plants inoculated as cuttings with the AM fungus was 146% and 176% higher than those inoculated with PSB and AM + PSB (Fig. 4).

Table 2. Influence of arbuscular mycorrhizal (AM) fungus and phosphate-solubilizing bacterial (PSB) inoculation on growth of *Impatiens walleriana* stem cuttings

Treatments	Plant height (per plant)	No. of branches (per plant)	Leaf numbers (per plant)	Total root length (cm)	Dry weight (mg per plant)		R/S ratio
					shoot (S)	root (R)	
Con	14.75 ± 0.33a[#]	1.00 ± 0.0001a	8.20 ± 0.47a	21.87 ± 0.91a	158.3 ± 15.02a	8.1 ± 0.60a	0.055 ± 0.007b
AM	16.52 ± 0.67ab	1.00 ± 0.0001a	8.20 ± 0.44a	24.51 ± 1.55a	273.4 ± 25.41b	9.1 ± 0.53ab	0.037 ± 0.006a
PSB	17.67 ± 0.62b	1.30 ± 0.1523ab	9.50 ± 0.40ab	25.10 ± 1.81a	192.3 ± 23.24a	10.9 ± 0.50c	0.064 ± 0.007b
AM + PSB	15.10 ± 0.90a	1.50 ± 0.1702b	10.80 ± 1.03b	31.72 ± 1.57b	182.7 ± 14.54a	10.6 ± 0.54bc	0.061 ± 0.005b
F-statistics							
df = 3,36	4.154**	4.696**	3.786**	7.818***	6.139**	5.790**	3.681*

*, **, *** Significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Means ± standard error in a column followed by the same alphabet are not significantly ($p > 0.05$) different according to Duncan's multiple range test.

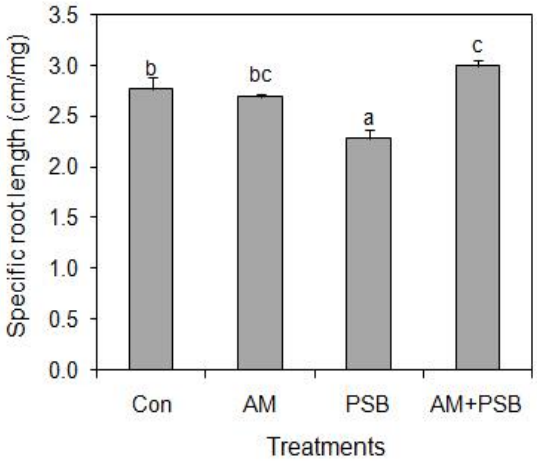

Fig. 3. Influence of arbuscular mycorrhizal (AM) fungus and phosphate-solubilizing bacterial (PSB) inoculation on specific root length of *Impatiens walleriana* plants. Con – control. Bars ± one standard error bearing same letter are not significantly ($p > 0.05$) different according to Duncan's multiple range test.

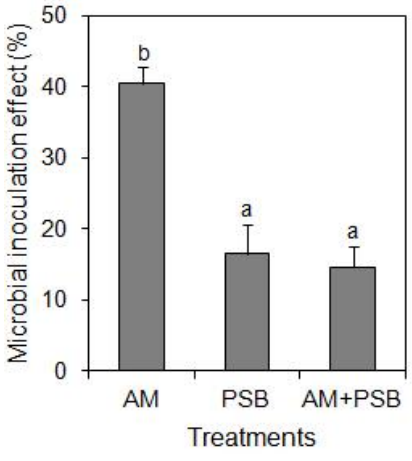

Fig. 4. Microbial inoculation effect of *Impatiens walleriana* plants inoculated with arbuscular mycorrhizal (AM) fungus and phosphate-solubilizing bacterium (PSB). Bars ± one standard error bearing same letter are not significantly ($p > 0.05$) different according to Duncan's multiple range test.

Phosphorus content and uptake

The P content significantly varied between the shoots and roots of *I. walleriana* ($F_{1,16}$ = 12.797; $p < 0.01$) (Fig. 5). The differences in P content in shoots were significant ($F_{3,8}$ = 5.467; $p < 0.05$). The highest content of P in shoots was recorded after PSB and AM + PSB inoculation, whereas the lowest in control. In contrast, the differences in P content in roots were not significantly different ($F_{3,8}$ = 1.167; $p > 0.05$).

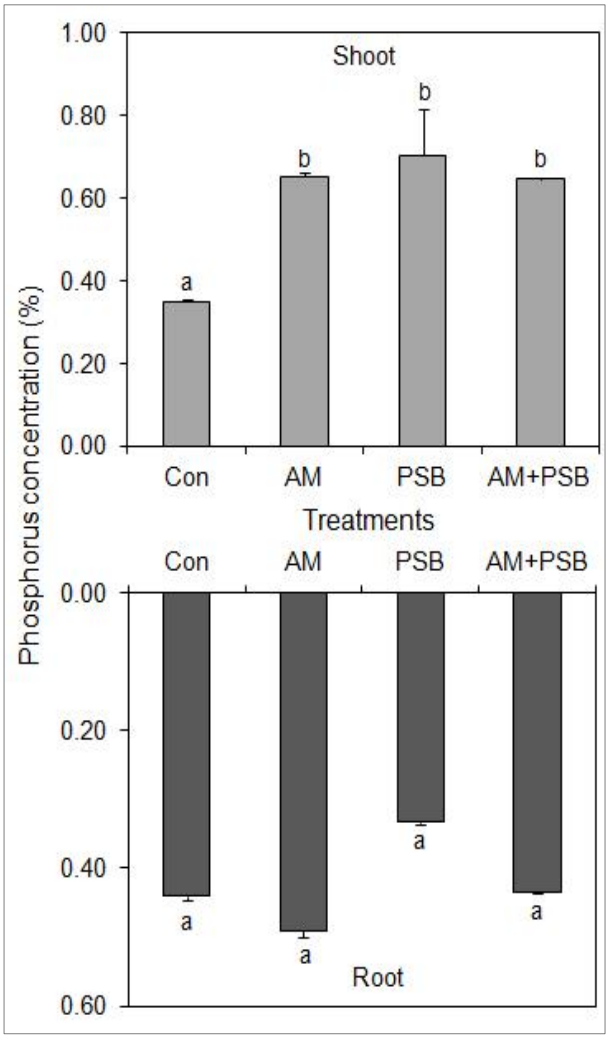

Fig. 5. Influence of arbuscular mycorrhizal (AM) fungus and phosphate-solubilizing bacterial (PSB) inoculation on P content in shoots and roots of *Impatiens walleriana* plants inoculated at stem cuttings stage. Con – control. Bars ± one standard error bearing same letter are not significantly ($p > 0.05$) different according to Duncan's multiple range test.

Single AM inoculation significantly increased the efficiency of P uptake, PSB increased this parameter in comparison with AM + PSB and control ($F_{3,36}$ = 11.052; $p < 0.001$) (Fig. 6). The P uptake per unit of the root in AM-inoculated cuttings was 63–179% higher than that of control, PSB- and AM + PSB-inoculated cuttings. A significant linear relationship existed between the efficiency of P uptake and the biomass of the cuttings (Fig. 7).

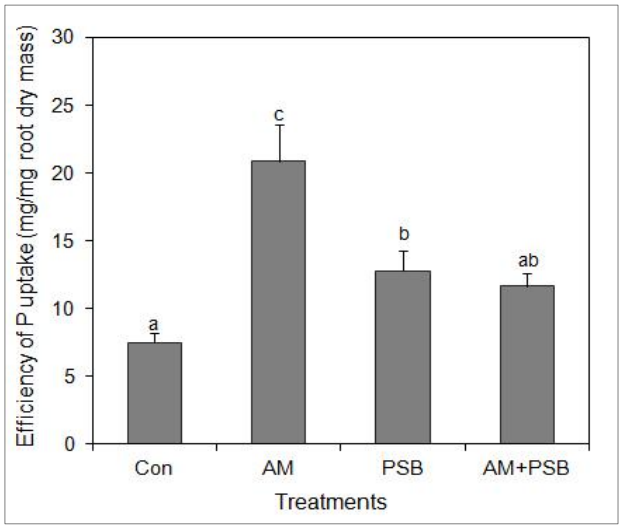

Fig. 6. Influence of arbuscular mycorrhizal (AM) fungus and phosphate-solubilizing bacterial (PSB) inoculation on the efficiency of P uptake in *Impatiens walleriana* plants. Con – control. Bars ± one standard error bearing same letter are not significantly ($p > 0.05$) different according to Duncan's multiple range test.

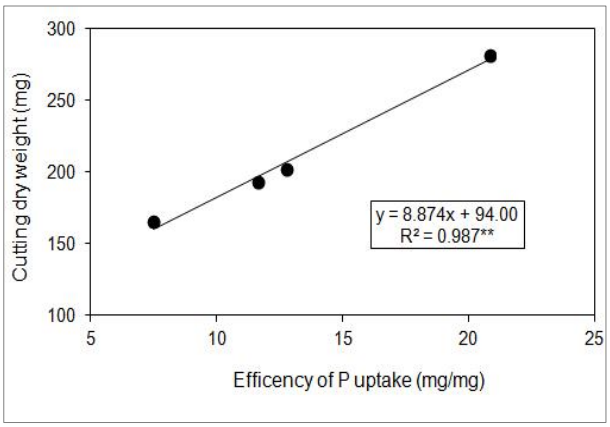

Fig. 7. Relationship between the efficiency of phosphorus (P) uptake and dry weight of *Impatiens walleriana* plants. **Significant at $p < 0.01$.

DISCUSSION

The results of this study clearly indicated that inoculation of *R. fasciculatus* or *B. megaterium* influenced some growth parameters and P uptake of *I. walleriana* stem cuttings. This is in accordance with studies where AM fungi or PSB are shown to improve the growth of different plant species (Vafadar et al. 2014; Koshila Ravi & Muthukumar 2019). Inoculation of PSB resulted in the tallest plants, which is in accordance with studies where inoculation of plant growth-promoting rhizobacteria (PGPR), including *B. megaterium*, has been shown to enhance plant height (López-Bucio et al. 2007; Vafadar et al. 2014). This increase of plant height could be attributed to the increased availability of nutrients and the production of phytohormones by the bacteria (Montero-Calasanz et al. 2013). In addition, it appears that the cytokinin signaling and production of volatiles like 2-pentylfuran play an important role in the stimulation of plant growth by *B. megaterium* (Ortíz-Castro et al. 2008; Zou et al. 2010).

The higher biomass accumulation in shoots of plants inoculated with AM fungus than those inoculated with PSB or co-inoculated by both microorganisms is in accordance with the observations of Marulanda-Aguirre et al. (2008) where lettuce (*Lactuca sativa* L.) plants accumulated more plant biomass when inoculated with *Glomus constrictum* alone than when co-inoculated with *B. megaterium*. This reduction in plant biomass upon AM fungus and PSB co-inoculation compared to the single inoculation with AM was attributed to the lack of compatibility between the inoculated pair of microorganisms (Marulanda-Aguirre et al. 2008).

There are reports that demonstrate synergistic interactions between co-inoculated AM fungi and PSB on plant growth (Abdel-Rahman & El-Naggar 2014; Vafadar et al. 2014). However, the benefits of dual inoculation of *R. fasciculatus* and *B. megaterium* to the growth of *I. walleriana* plants were not synergistic in this study. This is affirmed by the fact that, though dual inoculation of the AM fungus and PSB increased the number of leaves and branches, root length, and root dry weights of *I. walleriana* plants compared with uninoculated control, these increases were almost on par or only marginally higher compared to individual inoculation with these microorganisms. Moreover, the change in the R/S ratio, which is the measure to assess the overall health of the plants, also suggests this. Normally, the R/S ratio increases when plants are under nutrient stress, as plants allocate more resources for increasing the acquisition of the limiting resources and the R/S ratio tends to decrease under resource sufficiency. The increased R/S ratios of PSB- and AM fungus + PSB-inoculated cuttings in spite of increased plant P indicate that other nutrients than the one studied here may be the reason of limiting plant growth.

Total root length and specific root length are two important root architectural characteristics that determine the direct uptake of nutrients by plants. These characteristics not only vary with time and space but also are greatly influenced by the nutrient demand of the plant and the existing environmental conditions. Previous studies have shown that AM symbiosis or inoculation of PSB could significantly affect both total and specific root lengths of plants originating either from seeds or clonally propagated (Erturk et al. 2010; Abdel-Rahman & El-Naggar 2014). López-Bucio et al. (2007) showed that architectural changes in *Arabidopsis thaliana* roots induced by *B. megaterium* were produced by unidentified diffusates and were independent of auxin- and ethylene-signaling mechanisms. Similarly, a diffusible factor produced by AM fungi belonging to *Gigaspora* and *Rhizophagus* was shown to stimulate lateral root formation in *Medicago truncatula* (Oláh et al. 2005). Specific root length is a root parameter that is widely measured to ascertain the response of fine roots in the changing environment. This parameter is quite often used as an indicator of nutrient availability under specific conditions and usually increases under nutrient limitation conditions (Ryser 2006).

In this study, the specific root length of AM fungus-inoculated plants (individually or along with PSB) was higher suggesting that AM symbiosis results in finer roots. This is in accordance with Sinclair et al. (2014) who showed that strawberry plants inoculated with a cocktail of AM fungi like *Funneliformis caledonius*, *Funneliformis mosseae*, and *Rhizophagus irregularis* had increased specific root length compared to uninoculated plants. Increased AM colonization along with higher specific root length could help plants in the efficient uptake of nutrients.

In line with previous studies, microbial inoculated cuttings accumulated more P in their shoots and roots. This increased accumulation could be attributed to various microbial processes like the solubilization of P in the soil by the PSB, root fineness, and AM fungal hypha-mediated nutrient transport. It is well known that PSB produces a large number of organic acids, which solubilize the bound P and make it available to the plants. In addition, the AM fungal hyphae forage the soil for P more efficiently and acquire the P content that is made available by the PSB before it is taken up by other microorganisms or refixed in the soil. Moreover, the increased root length and specific root length in response to the microbial inoculation could also have facilitated the direct uptake of P from the soil. Any or all of these mechanisms could have contributed to the increased P content in microbial inoculated plants. This is clearly evidenced in this study where the uptake of P per given unit of the root (efficiency of P uptake) was higher for microbial inoculated plants than that for the uninoculated plants.

Inoculation with *B. megaterium* increased the colonization of *I. walleriana* roots by native AM fungi, which is in accordance with the fact that PSB could act as mycorrhizal helper bacteria (Lies et al. 2018). However, this stimulatory effect was not evident when *B. megaterium* was co-inoculated with *R. fasciculatus*. Several species of bacteria belonging to *Bacillus*, including *B. megaterium* can colonize the surface of the fungal hyphae and the hyphosphere and grow at the expense of AM fungi (Lecomte et al. 2011). Moreover, interesting observations were made in this study: the presence of large oil droplets in the intraradical AM fungal hyphae and restriction of vesicles to treatments involving individual inoculation of the microbes. Generally, vesicles are considered as storage structures of AM fungi and are large reservoirs of lipids. The available evidence indicates that AM fungi take up sugars (predominantly glucose) from their host and convert them into lipids in the intraradical hyphae, which are later exported to the extraradical mycelium and the spores (Rich et al. 2017). The development of the lipid droplets in the AM fungal hyphae coincides with the degeneration of the fine arbuscular branches (Kobae et al. 2014). Nevertheless, the abundant lipid droplets in the intraradical hyphae suggest that these could also act as storage structures or may be indicative of increased lipid synthesis from the host carbon (Bago et al. 2002). Further studies in this line would reveal if intraradical hyphae could be an alternative for fungal storage to vesicles.

CONCLUSIONS

The results of this study demonstrated that inoculation of AM fungus and PSB has the potential to increase the plant growth and P uptake of *I. walleriana* stem cuttings even in natural field soil. This enhanced plant growth was correlated with the increased P content in shoots and to some extent with uptake efficiency of microbial inoculated plants. Moreover, inoculation of the microorganisms affected the adventitious root structure with long and fine roots. The findings of this study also indicate that the interaction between the AM fungus and PSB may not be positive for plant growth. Therefore it is important to assess the compatibility of the co-inoculating microorganisms to achieve maximum benefit from their application. The results of the study could be important in organic plant production systems.

REFERENCES

Abdel-Rahman S.S.A., El-Naggar A.-R.I. 2014. Promotion of rooting and growth of some types of Bougainvilleas cutting by plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) in combination with indole-3-butyric acid (IBA). International Journal of Science and Research 3(11): 97–108.

Bago B., Pfeffer P.E., Zipfel W., Lammers P., Shachar-Hill Y. 2002. Tracking metabolism and imaging transport in arbuscular mycorrhizal fungi. Plant and Soil 244(1–2): 189–197. DOI: 10.1023/a:1020212328955.

Beneduzi A., Moreira F., da Costa P.B., Vargas L.K., Lisboa B.B., Favreto R., Baldani J.I., Passaglia L.M.P. 2013. Diversity and plant growth promoting evaluation abilities of bacteria isolated from sugarcane cultivated in the South of Brazil. Applied Soil Ecology 63: 94–104. DOI: 10.1016/j.apsoil.2012.08.010.

Copes W.E., Blythe E.K. 2011. Rooting response of azalea cultivars to hot water treatment used for pathogen control. HortScience 46(1): 52–56. DOI: 10.21273/hortsci.46.1.52.

Erturk Y., Ercisli S., Haznedar A., Cakmakci R. 2010. Effects of plant growth promoting rhizobacteria (PGPR) on rooting and root growth of kiwifruit (*Actinidia deliciosa*) stem cuttings. Biological Research 43(1): 91–98. DOI: 10.4067/s0716-97602010000100011.

Gardner J.A.G., Lubell J.D., Brand M.H. 2019. Propagation of *Comptonia peregrina* L. from stem cuttings. HortScience 54(3): 511–513. DOI: 10.21273/hortsci13770-18.

Gray J.T., Schlesinger W.H. 1983. Nutrient use by evergreen and deciduous shrubs in southern California: II. Experimental investigations of the relationship between growth, nitrogen uptake and nitrogen availability. Journal of Ecology 71(1): 43–56. DOI: 10.2307/2259962.

Jackson M.L. 1967. Soil Chemical Analysis. Prentice Hall India, New Delhi, 498 p.

Kapczyńska A. 2019. Propagation of *Lachenalia* cultivars from leaf cuttings. Acta Scientiarum Polonorum Hortorum Cultus 18(1): 189–195. DOI: 10.24326/asphc.2019.1.19.

Karagiannidis N., Thomidis T., Lazari D., Panou-Filotheou E., Karagiannidou C. 2011. Effect of three Greek arbuscular mycorrhizal fungi in improving the growth, nutrient concentration, and production of essential oils of oregano and mint plants. Scientia Horticulturae 129(2): 329–334. DOI: 10.1016/j.scienta.2011.03.043.

Kobae Y., Gutjahr C., Paszkowski U., Kojima T., Fujiwara T., Hata S. 2014. Lipid droplets of arbuscular mycorrhizal fungi emerge in concert with arbuscule collapse. Plant and Cell Physiology 55(11): 1945–1953. DOI: 10.1093/pcp/pcu123.

Koide R.T., Landherr L.L., Besmer Y.L., Detweiler J.M., Holcomb E.J. 1999. Strategies for mycorrhizal inoculation of six annual bedding plant species. HortScience 34(7): 1217–1220. DOI: 10.21273/hortsci.34.7.1217.

Kokwaro J.O. 2009. Medicinal plants of East Africa, 3rd ed. University of Nairobi Press, Nairobi, 478 p.

Koshila Ravi R., Muthukumar T. 2019. Perspectives on the role of arbuscular mycorrhizal fungi in the *in vivo* vegetative plant propagation. In: Giri B., Prasad R., Wu Q-S., Varma A. (Eds.), Biofertilizers for sustainable agriculture and environment. Soil Biology 55: 83–107. DOI: 10.1007/978-3-030-18933-4_5.

Koske R.E., Gemma J.N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research 92(4): 486–488. DOI: 10.1016/s0953-7562(89)80195-9.

Kudoyarova G., Vysotskaya L.B., Arkhipova T.N., Kuzmina L.Y., Galimsyanova N.F., Sidorova L.V., et al. 2017. Effect of auxin producing and phosphate solubilizing bacteria on mobility of soil phosphorus, growth rate, and P acquisition by wheat plants. Acta Physiologiae Plantarum 39(11): 253. DOI: 10.1007/s11738-017-2556-9.

Lecomte J., St-Arnaud M., Hijri M. 2011. Isolation and identification of soil bacteria growing at the expense of arbuscular mycorrhizal fungi. FEMS Microbiology Letters 317(1): 43–51. DOI: 10.1111/j.1574-6968.2011.02209.x.

Lies A., Delteil A., Prin Y., Duponnois R. 2018. Using mycorrhiza helper microorganisms (MHM) to improve the mycorrhizal efficiency on plant growth. In: Meena V. (Ed.), Role of rhizospheric microbes in soil. Springer, pp. 277–298. DOI: 1007/978-981-10-8402-7_11.

Lim T.K. 2014. *Impatiens balsamina*. Edible medicinal and non-medicinal plants 7: 537–547. DOI: 10.1007/978-94-007-7395-0_33.

López-Bucio J., Campos-Cuevas J.C., Hernández-Calderón E., Velásquez-Becerra C., Farías-Rodríguez R., Macías-Rodríguez L.I., Valencia-Cantero E. 2007. *Bacillus megaterium* rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in *Arabidopsis thaliana*. Molecular Plant-Microbe Interactions 20(2): 207–217. DOI: 10.1094/mpmi-20-2-0207.

Marulanda-Aguirre A., Azcón R, Ruiz-Lozano J.M., Aroca R. 2008. Differential effects of a *Bacillus megaterium* strain on *Lactuca sativa* plant growth depending on the origin of the arbuscular mycorrhizal fungus coinoculated: physiologic and biochemical traits. Journal of Plant Growth Regulation 27: 10–18. DOI: 10.1007/s00344-007-9024-5.

Maússe-Sitoe S.N.D., Chen S., Wingfield M.J., Roux J. 2016. Diseases of eucalypts in the central and northern provinces of Mozambique. Southern Forest 78: 169–183. DOI: 10.2989/20702620.2015.1126780.

McGonigle T.P., Miller M.H., Evans D.G., Fairchild G.L., Swan J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular–arbuscular mycorrhizal fungi. New Phytologist 115: 495–501. DOI: 10.1111/j.1469-8137.1990.tb00476.x.

McKey D., Elias M., Pujol B., Duputié A. 2010. The evolutionary ecology of clonally propagated domesticated plants. New Phytologist 186: 318–332. DOI: 10.1111/j.1469-8137.2010.03210.x.

Montero-Calasanz M.C., Santamaría C., Albareda M., Daza A., Duan J., Glick B.R., Camacho M. 2013. Alternative rooting induction of semi-hardwood olive cuttings by several auxin-producing bacteria for organic agriculture systems. Spanish Journal of Agricultural Research 11: 146–154. DOI: 10.5424/sjar/2013111-2686.

Muthukumar T., Udaiyan K. 2006. Growth of nursery-grown bamboo inoculated with arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in two tropical soil types with and without fertilizer application. New Forest 31: 469–485. DOI: 10.1007/s11056-005-1380-z.

Newman E.I. 1966. A method of estimating the total length of root in a sample. Journal of Applied Ecology 3: 139–145. DOI: 10.2307/2401670.

Oláh B., Brière Ch., Bécard G., Dénarié J., Gough C. 2005. Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. Plant Journal 44:195–207. DOI: 10.1111/j.1365-313x.2005.02522.x.

Ortíz-Castro R., Valencia-Cantero E., López-Bucio J. 2008. Plant growth promotion by *Bacillus megaterium* involves cytokinin signaling. Plant Signaling and Behavior 3: 263–265. DOI: 10.4161/psb.3.4.5204.

Ostonen I., Püttsepp Ü., Biel C., Alberton O., Bakker M.R., Lõhmus K. et al. 2007. Specific root length as an indicator of environmental change. Plant Biosystems 141: 426–442. DOI: 10.1080/11263500701626069.

Read P.E. 2015. Propagation by root cuttings. In: Beyl C.A., Trigiano R.N. (Eds.), Plant propagation concepts and laboratory exercises, 2nd ed. CRC Press, pp. 283–286. DOI: 10.1201/b17340.

Rich M.K., Nouri E., Courty P.E., Reinhardt D. 2017. Diet of arbuscular mycorrhizal fungi: bread and butter? Trends in Plant Science 22: 652–660. DOI: 10.1016/j.tplants.2017.05.008.

Ryser P. 2006. The mysterious root length. Plant and Soil 286: 1–6. DOI: 10.1007/s11104-006-9096-1.

Sinclair G., Charest Ch., Dalpé Y., Khanizadeh S. 2014. Influence of colonization by arbuscular mycorrhizal fungi on three strawberry cultivars under salty conditions. Agricultural and Food Science 23: 146–158. DOI: 10.23986/afsci.9552.

Singh R., Soni S.K., Kalra A. 2013. Synergy between *Glomus fasciculatum* and a beneficial *Pseudomonas* in reducing root diseases and improving yield and forskolin content in *Coleus forskohlii* Briq. under organic field conditions. Mycorrhiza 23: 35–44. DOI: 10.1007/s00572-012-0447-x.

Szabó V., Németh Z., Hrotkó K. 2013. Improved rooting by different plant growth regulator treatments on *Prunus mahaleb* L. cuttings. Acta Horticulturae 981: 431–436. DOI: 10.17660/actahortic.2013.981.68.

Vafadar F., Amooaghaie R., Otroshy M. 2014. Effects of plant-growth-promoting rhizobacteria and arbuscular mycorrhizal fungus on plant growth, stevioside, NPK, and chlorophyll content of *Stevia rebaudiana*. Journal of Plant Interactions 9: 128–136. DOI: 10.1080/17429145.2013.779035.

Zou Ch., Li Z., Yu D. 2010. *Bacillus megaterium* strain XTBG34 promotes plant growth by producing 2-pentylfuran. Journal of Microbiology 48: 460–466. DOI: 10.1007/s12275-010-0068-z.