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Dual Inoculation of Plant Growth-Promoting *Bacillus endophyticus* and *Funneliformis mosseae* Improves Plant Growth and Soil Properties in Ginger

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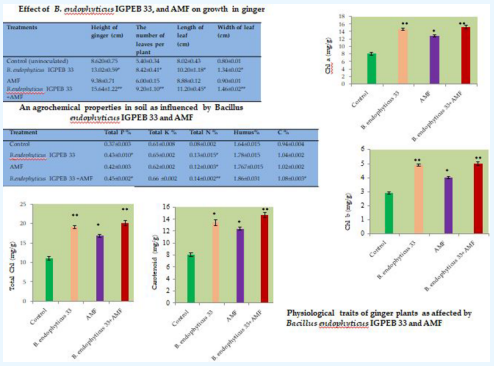
ABSTRACT: Co-inoculation with beneficial microbes has been suggested as a useful practice for the enhancement of plant growth, nutrient uptake, and soil nutrients. For the first time in Uzbekistan the role of plant-growth-promoting *Bacillus endophyticus* IGPEB 33 and arbuscular mycorrhizal fungi (AMF) on plant growth, the physiological properties of ginger (*Zingiber officinale*), and soil enzymatic activities was studied. Moreover, the coinoculation of *B. endophyticus* IGPEB 33 and AMF treatment significantly increased the plant height by 81%, leaf number by 70%, leaf length by 82%, and leaf width by 40% compared to the control. *B. endophyticus* IGPEB 33 individually increased plant height significantly by 51%, leaf number by 56%, leaf length by 67%, and leaf width by 27% as compared to the control treatment. Compared to the control, *B. endophyticus* IGPEB 33 and AMF individually significantly increased chlorophyll a by 81−58%, chlorophyll b by 68−37%, total chlorophyll by 74−53%, and carotenoid content by 67−55%. However, combination of *B. endophyticus* IGPEB 33 and AMF significantly increased chlorophyll a by 86%, chlorophyll b by 72%, total chlorophyll by 82%, and carotenoid content by 83% compared to the control. Additionally, plant-growth-promoting *B. endophyticus* IGPEB 33 and AMF inoculation improved soil nutrients and soil enzyme activities compared to the all treatments. Co-inoculation with plant-growth-promoting *B. endophyticus* and AMF could be an alternative for the production of ginger that is more beneficial to soil nutrient deficiencies. We suggest that a combination of plant-growth-promoting *B. endophyticus* and AMF inoculation could be a more sustainable and eco-friendly approach in a nutrient-deficient soil.



INTRODUCTION

Beneficial plant growth-promoting bacteria that root colonize plants and improve plant development are usually referred to as PGPB.[1−5] Indole-3-acetic acid (IAA), gibberellin, and cytokinin production by PGPB is one of the well-studied mechanisms of plant growth stimulation, leading to architectural and morphological changes in different plants.[6−8] PGPB directly improve plant growth through solubilizing phosphate,[9−13] enhancing nutrients,[14−16] biological control of plant pathogens,[17] and nitrogen fixation.[13] Bacteria from genera *Bacillus* synthesize phytohormones such as cytokinins, IAA, and gibberellins[18] and improve plant development.[19] *Bacillus* species, particularly *B. methylotrophicus*, *B. insolitus*, and *B. subtilis*, enhance shoot biomass, root biomass, and shoot and root lengths in wheat and fenugreek.[20,21] Alori et al.[22] reported that *Bacillus* species could also enhance the nitrogen (N) and phosphorus (P) in soil. *Bacillus subtilis* JW1 can improve plant height, nutrient uptake, and chlorophyll content in Chinese cabbage.[23] Mena-Violante and Olalde-Portugal[24] reported that *B. subtilis* BEB-13bs promoted fruit weight and length in tomato. Beneficial soil microorganisms such as arbuscular mycorrhizal fungi (AMF) symbiosis with plants is beneficial for plant growth, plant nutrition, and physiological properties of plants.[24−26] Several studies have indicated *Rhizophagus irregularis* increased colonization rate, shoot dry matter, root biomass, water contents, stomatal conductance, lipid peroxidation, chlorophyll content, osmotic potential, and potassium (K), magnesium (Mg), N, P, and iron (Fe) content in different plants such as *Leymus chinensis, Digitaria eriantha*, and *Cajanus cajan* L.[27−29] Chen et al.[30] reported that AMF promoted the root morphology that stimulated the N and P absorption and

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physiological properties in *Catalpa bungei* C.A.Mey. Liu et al.[31] and Liu et al.[32] reported that AMF enhanced photosynthetic activities and uptake of N and P promoted plant growth. Mathur et al.[33] observed that AMF stimulated plant growth (the leaf length, plant height, leaf number), and physiological properties (stomatal conductance, transpiration rate, photosynthetic rate, chlorophyll a) in *Zea mays* and improved the phosphorylation status, root hydraulic conductivity, aquaporin abundance, and photosynthetic capacity in *Solanum lycopersicum*.[34] Inoculation of AMF promoted the root biomass, proline biosynthesis, and plant nutrients such as Mg, Fe, P, and N in *Trigonella foenum-graecum* L.[35] Asrar et al.[36] reported that AMF improved plant growth parameters (leaf number per plant, shoot diameter, root diameter) and the chlorophyll content and water content in *Antirrhinum majus* L. AMF promoted soil nutrients, microbial activity, and soil enzyme activities.[37−39] Figueiredo et al.[40] indicated that AMF improved the colonization rate of AMF.

A combination of PGPB and AMF plays a major role in promoting plant development, plant nutrition, and yield in different plants.[41,42] El-Sawah et al.[43] reported that combination of PGPB and AMF significantly increased shoot length, root length, the number of branches per plant, and the number of pods and seeds of guar (*Cyamopsis tetragonoloba* (L.)) in field conditions.

Wahid et al.[44] reported that the combination of phosphate-solubilizing bacteria (PSB) and AMF can result in enhanced P uptake and development in maize (*Zea-mays* L.). Kavatagi and Lakshman[45] reported that coinoculating with *Rhizophagus fasciculatus*, *Pseudomonas fluorescens*, and *Azotobacter chrococcum* significantly increased plant growth parameters, percentage root colonization, spore number, and leaf number in tomato. PGPR and AMF coinoculation supported other interms of the improvement of plant development, nutrient solubility, and nutrient uptake informed by Zhang et al.[46] Yousefi et al.[47] reported that the combination of PGPB and AMF enhanced the shoot biomass and seed yield of wheat.

The Zingiberaceae family is the largest family; it is separated into about 52 genera and more than 1300 species of aromatic perennial medicinal plants.[48] Ginger and turmeric belong to the Zingiberaceae family; their rhizomes contain a rich amount of minerals like P and Ca, which are beneficial for health.[49−51] Rhizomes of ginger are found in compounds such as gingerol, gingerdiol, and gingerdione, vitamin C, flavonoids, and sesquiterpenes used traditionally for numerous health conditions including antioxidant, antipyretic, sedative, analgesic, cancer, insecticidal, vomiting, nausea, antidiabetic, and rheumatic diseases.[52−54] Ghayur et al.[55] reported that ginger rhizomes are used in cardiovascular diseases and traditional medicine. Chemical fertilizers have a negative impact on the environment and human health. At present, the production of organic products in the world is increasing year by year. This research will help to increase the organic production of ginger. There is very little information about the interaction of *Bacillus* and AMF on ginger. We aimed to test the following three hypotheses: (1) *B. endophyticus* and AMF can promote growth of ginger; (2) *B. endophyticus* and AMF can improve physiological properties of ginger; (3) *B. endophyticus* and AMF can interact to improve soil nutrients and soil enzymatic activities.

MATERIALS AND METHODS

Soil, AMF, and *B. endophyticus* IGPEB 33. The soil (typical irrigated gray soil) collected from Durmon Experimental Field Station of the Institute of Genetics and Plant Experimental Biology, Uzbekistan, was used for the experiment. The studied soil had the following agrochemical properties: soil organic carbon −0.960%, nitrogen −0.091%, phosphorus −0.170%, and potassium −0.69%.[50] The AMF (*Funneliformis mosseae*) fertilizer was purchased from the "Division of Microbiology", IARI, India. *B. endophyticus* IGPEB 33 strain was obtained from the "Laboratory of Medicinal Plant Genetics and Biotechnology". *B. endophyticus* IGPEB 33 was cultured nutrient broth and inoculated for the rhizome. *B. endophyticus* IGPEB 33 strain was isolated from ginger planted in Uzbekistan.

Phosphate Solubilization. Ability of bacteria phosphate to solubilize was determined by spot inoculating pure strain *B.* endophyticus IGPEB 33 on the Pikovskaya medium[56] and incubated at 28 °C. *B. endophyticus* IGPEB 33 inoculation was done in triplicate.

IAA Production. *B. endophyticus* IGPEB33 was grown for 48 h on nutrient broth at 28 °C. The grown *B. endophyticus* IGPEB 33 was centrifuged at 3000 rpm (revolutions per minute) for 30 min. Then the supernatant was mixed with orthophosphoric acid and Salkowski reagent. A pink color showed IAA production.[57]

Production of Enzymes. Protease production of *B. endophyticus* IGPEB33 was conducted on "sterile skim milk agar". The *B. endophyticus* IGPEB 33 strain was spot inoculated and grown at 28 °C for 2 days. Then incubation plates were observed for the appearance of the zone of clearance around the colony showing production of protease in *B. endophyticus* IGPEB 33.[58]

Lipase production of *B. endophyticus* IGPEB 33 strain was determined using the following medium (sodium chloride 5 g, calcium chloride 0.1 g, 10 mL of Tween 20, peptone 10 g, agar 15 g, water 1 L). *B. endophyticus* IGPEB33 was streaked in the medium described above and incubated at 27 °C for 2 days. Depositions around the *B. endophyticus* IGPEB 33 colonies indicted the production of lipase.[59]

ACC deaminase production of *B. endophyticus* IGPEB33 strain was grown for 48 h at 28 °C in a DF medium minimum saline medium with the addition of 2 g of $(NH_4)_2SO_4$. The appearance of *B. endophyticus* IGPEB33 colonies in minimal saline DF medium after the incubation period was taken as a positive result for the synthesis of ACC deaminase.[60]

To determine the amylase activity, the strain was cultured on the starch agar and incubated at 28 °C. After incubation, a 1% iodine solution was poured into Petri dishes and held for 1 min after which it was discarded. The iodine solution with the starch initially formed a blue color, and then the color quickly disappeared and colorless zones formed around the bacterial colonies. The formation of colorless zones around bacterial colonies indicates the presence of amylase activity of the isolates.[58]

The catalase was tested qualitatively.[61] H_2O_2 (6%) was added to the *B. endophyticus* IGPEB 33 colonies grown on NA (nutrient agar) in plates. After effervescences of O_2 released from the strain, colonies indicated the presence of catalase activity.

Experimental Design. The influence of *B. endophyticus* IGPEB 33 and AMF on the development of ginger was

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Table 1. Responses of Ginger Growth Indicators to *B. endophyticus* IGPEB 33 Alone, AMF Alone, and a Combination of *B. endophyticus* IGPEB33 and AMF[a]

Treatment	Height of ginger (cm)	Number of leaves per plant	Length of leaf (cm)	Width of leaf (cm)
Control (uninoculated)	8.620 ± 0.75	5.40 ± 0.34	8.02 ± 0.43	0.80 ± 0.01
B. endophyticus IGPEB 33	13.02 ± 0.59*	8.42 ± 0.41*	10.20 ± 1.18*	1.34 ± 0.02*
AMF	9.38 ± 0.71	6.00 ± 0.15	8.88 ± 0.12	0.90 ± 0.01
B. endophyticus IGPEB 33 + AMF	15.64 ± 1.22**	9.20 ± 1.10**	11.20 ± 0.45*	1.46 ± 0.02**

[a]The asterisk indicates significant differences compared to control treatment at $P < 0.05$, $P < 0.01$. The error values specify standard deviation.

conducted under net house at the Institute of Genetics and PEB. The study was conducted from April to August 2021. "Experimental treatments" included four treatments such as control, *B. endophyticus* IGPEB 33 alone, AFM alone, and combination with *B. endophyticus* IGPEB 33 and AMF. The AMF inoculum consists of 100 spores/g and 1200 inoculum potential (IP)/g. The AMF (*Funneliformis mosseae*) inoculum was placed at a depth of 5 cm from the surface of soil as a layer ensuring 10 spores for each rhizome. The *B. endophyticus* IGPEB 33 strain was used for inoculation of the sterilized rhizome of ginger. *B. endophyticus* IGPEB 33 inoculation was adjusted to approximately 10^7 cells/mL. Rhizome was planted in pots (diameter 26 cm, depth 22 cm) containing 8.0 kg of soil. Two ginger rhizome were planted per pot. Each pot was watered every 3 days. All treatment was replicated five times. After four months, plant height, leaf length, leaf width, and number of leaves were measured.

Physiological Parameters Measurement. Physiological parameters "total chlorophyll, chlorophyll a, chlorophyll b, and carotenoid contents in ginger were measured by the method of Hiscox and Israelstam.[62] A fresh leaf (50 mg) of the ginger sample was cut, and dimethyl sulfoxide (5 mL) was added to the test tubes. The test tubes were incubated at 37 °C for 4 h. Then absorbance of the extract was determined using a spectrophotometer. The relative water content of leaves in ginger was analyzed by the method of Barrs and Weatherly.[63] A fresh leaf (100 mg) of ginger sample was placed in petri plates and added water in plates for 4 h. After 4 h, the water content of leaf in ginger was measured. The samples were then taken out and blot dried, and the turgid weight (TW) was recorded. After that, the samples were kept in an oven at 70 °C overnight and the dry weight was recorded (DR). Relative water content was calculated as

$$\text{RWC}\ (\%) = [(\text{FW} - \text{DW})/(\text{TW} - \text{DW})] \times 100$$

Estimation of Agrochemical Properties of soil. The agrochemical parameters of the soil were analyzed after harvest. The carbon (C) content and humus content were analyzed using the modified method by Tyurin.[64] The phosphorus, potassium, and total nitrogen contents in soil were analyzed by the method.[65,66]

Determination of Soil Enzyme Activities. Enzyme (urease) activity in soil was determined using the method of Pancu and Gautheyrou.[67] Soil samples (2.5 g) were added with toluene (0.5) mL for 15 min. After mixing, urea (2.5 mL) and citrate buffer (5 mL) were added to the incubator at 38 °C for 24 h. Then, 4.0 mL of sodium phenate and 3.0 mL of sodium hypochlorite were added into 1.0 mL of filtrate and the mixture diluted with 50 mL and kept for 20 min at room temperature. The urease activity was determined at wavelengths of 578 nm using a spectrophotometer.

Soil enzyme (invertase and catalase) activities in soil were also assayed the method by Xaziev.[68] An invertase activity, dried soil (5.0 g) was used in the experiment. Then the dried soil, sucrose solution (8.0%) with 5.0 mL of double distilled water were added. After quantification of glucose content was performed using the colorimetric method at the wavelength of 508 nm on spectrophotometer. For soil catalase activity, a total of soil (2.0 g) was mixed with H_2O_2 (5.0 mL) and double-distilled water (40.0 mL). After this soil mixture with this solution was shaken for 20 min at 150 rpm. The remaining hydrogen peroxide (H_2O_2) was stabilized using 5.0 mL of sulfuric acid (1.5 M H_2SO_4) followed by centrifuging at 4000 rpm for 5 min. Then, the supernatant was used for titration with 0.05 M $KMnO_4$. The catalase activity of soil was determined at a wavelength of 480 nm in spectrophotometer.

The soil sample (2 g) was taken to which were added $CaCl_2$ (5 mL) and *p*-nitrophenyl phosphate (pNP) (1 mL) for phosphatase activity. The mixture was shaken, incubated at 30 °C for 1 h and centrifuged (5000 rpm for 5 min). All suspensions were filtered through Whatman No. 1 filter paper quickly, and the yellow color intensity was measured at a 440 nm wavelength in spectrophotometer.

STATISTICAL ANALYSES

The experimental data were analyzed with the StatView Software using ANOVA. The significance of the effect of treatment was determined by the magnitude of the *p*-value ($p < 0.05 < 0.01$).

RESULTS

B. endophyticus IGPEB 33 strain has indicated positive results for plant growth-promoting traits (P-solubilization, protease, amylase, catalase, IAA and ACC deaminase production). *B. endophyticus* IGPEB 33 strain showed no ability in producing lipase.

Inoculation with *B. endophyticus* IGPEB 33 alone increased plant growth parameters in Table 1. The *B. endophyticus* IGPEB 33 significantly developed plant height by 51%, respectively, compared with the control. When the *B. endophyticus* IGPEB 33 and the AMF inoculation were applied together, ginger growth indicators were significantly improved compared to *B. endophyticus* IGPEB 33 and AMF inoculation alone. The combined with *B. endophyticus* IGPEB33 and AMF significantly enhanced plant height by 81% than the control. Compared to the control, *B. endophyticus* IGPEB 33 ginger growth indicators (leaf width, leaf number and leaf length) were significantly promoted. The number of leaves (56%), the length of leaves (67%), and the width of leaves (27%) increased significantly with *B. endophyticus* IGPEB 33 treatment alone as compared to the control. Combination with *B. endophyticus* IGPEB 33 and AMF treatment increased the number of leaves, the length of leaves, and the width of leaves compared to other treatments. In comparison to the control, combination of *B. endophyticus* IGPEB 33 and AMF treatment significantly enhanced the number of leaves, the length of

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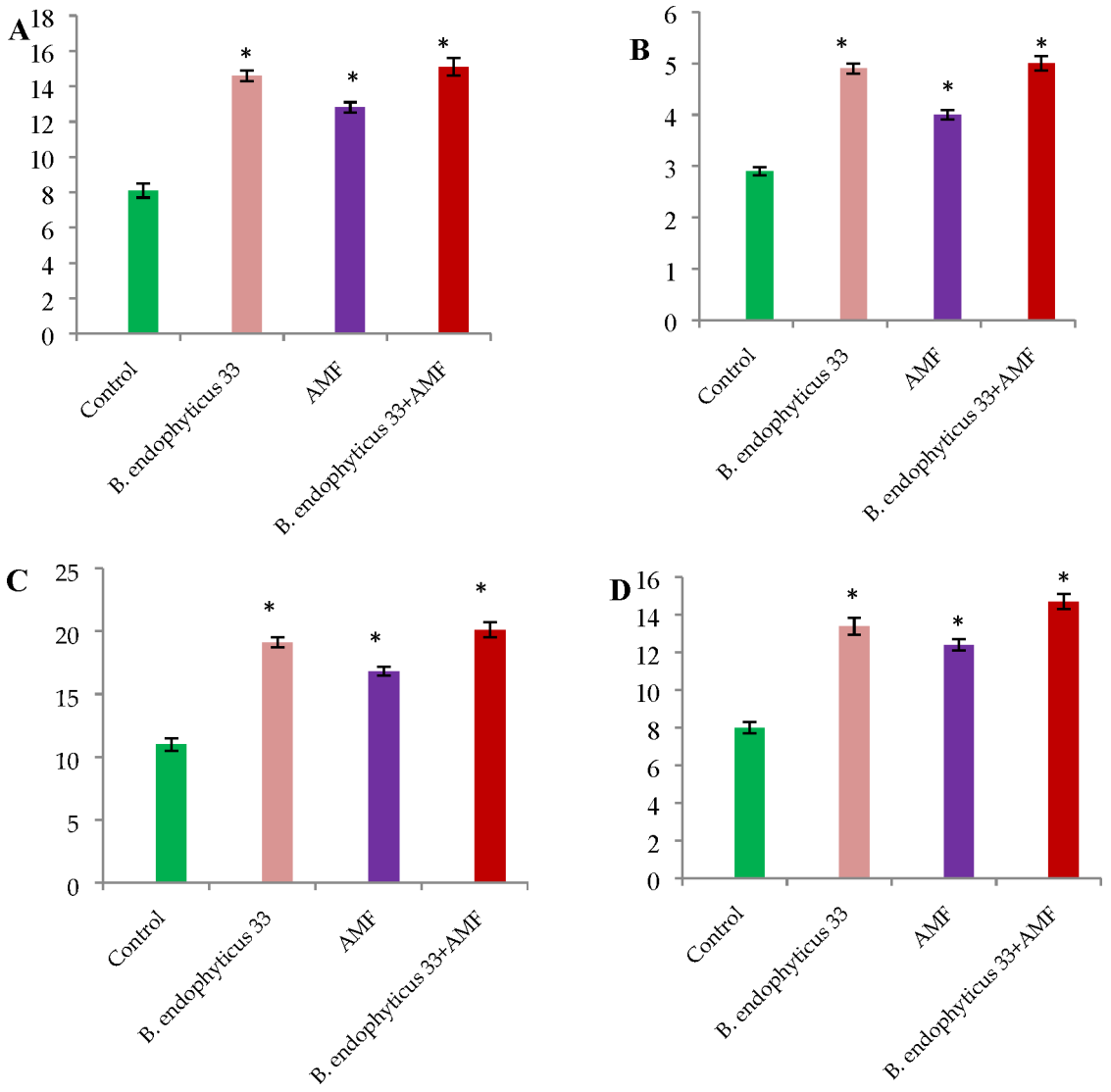

Figure 1. Physiological traits of ginger plants as affected by *B. endophyticus* IGPEB 33 and AMF: (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; (D) carotenoid content. Columns marked with an asterisk differed significantly compared to control treatment at $P < 0.05$, $P < 0.01$. The error bars shows standard deviation.

leaves, and the width of leaves by 70%, 82%, and 40% as shown in Table 1.

Data on the physiological properties as affected by *B. endophyticus* IGPEB 33 alone, AMF alone, and a combination of *B. endophyticus* IGPEB 33 and AMF are presented in Figure 1. As compared to the control, *B. endophyticus* IGPEB33 significantly promoted the chlorophyll a by 81%, chlorophyll b by 68%, total chlorophyll by 74%, and carotenoid contents by 67%. Inoculation by AMF alone promoted chlorophyll a (58%), chlorophyll b (37%), total chlorophyll (53%), and carotenoid content (55%). However, combination of *B. endophyticus* IGPEB 33 and AMF significantly promoted the chlorophyll a (86%), chlorophyll b (72%), total chlorophyll (82%), and carotenoid content (83%) compared to the control.

Data regarding relative water content of leaf as affected by *B. endophyticus* IGPEB 33 and AMF treatments alone and combination are presented in Figure 2. Compared to the control, *B. endophyticus* IGPEB 33 and AMF treatments alone stimulated the relative water content of leaf in ginger. However, the maximum relative water content of ginger was detected in combination of *B. endophyticus* IGPEB 33 and AMF, respectively.

The beneficial function of *B. endophyticus* IGPEB 33 and AMF treatments to improve soil agrochemical traits (total P, total K, total N, humus, and C content) in soil were studied (Table 2). The nutrients of total P and N contents in soil were stimulated by the application of *B. endophyticus* IGPEB 33 and AMF alone treatments. The P content increased by 16% and 13% *B. endophyticus* IGPEB 33 and AMF alone treatments compared to the control, respectively. The *B. endophyticus* IGPEB 33 and AMF treatments individually documented a significant increase in total N content by 62% and 50%, respectively, compared to the control. However, the highest total P and N content was observed with a combination of *B. endophyticus* IGPEB 33 and AMF treatment. Moreover, dual treatment of *B. endophyticus* IGPEB33 with AMF had a greater impact on increasing total P content (22%) and total N content (75%) as compared to the control. *B. endophyticus* IGPEB 33 and AMF together increased the humus content compared to the all treatments. In compared to the control, the

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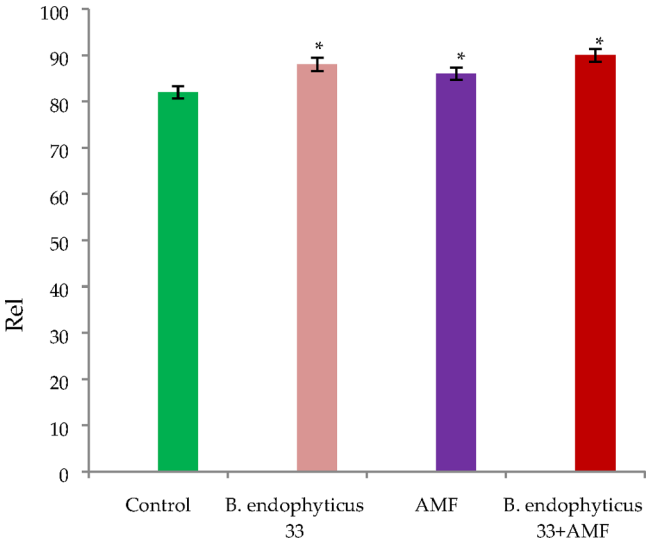

Figure 2. Relative water content of leaf as affected by *B. endophyticus* IGPEB 33 and AMF treatments. The asterisk differed significantly compared to control treatment at $P < 0.05$.

C content increased by 15% when coinoculated with AMF and *B. endophyticus* IGPEB 33 treatment, respectively.

Inoculation with *B. endophyticus* IGPEB 33 alone influenced soil enzyme (the catalase, invertase, urease, and phosphatase) activity to a significantly greater extent compared with the control (Table 3). Compared to the control, soil enzymes (the catalase, invertase, urease, and phosphatase) activities increased by 19%, 48%, 59%, and 48% compared to inoculation with *B. endophyticus* IGPEB 33 alone, respectively. In comparison to the control, the AMF treatment individually documented a significant enhance in the catalase activity by 19%, respectively. Application of AMF alone significantly promoted invertase activity by 35%, urease activity by 45%, and phosphatase activity by 40% as compared with the control. However, maximum values of the catalase and invertase activities of soil were observed in dual inoculation of *B. endophyticus* IGPEB 33, respectively. The combination of *B. endophyticus* IGPEB3 3 and AMF treatment further increased the catalase activity by 26% and the invertase activity by 74%. Both inoculation of *B. endophyticus* IGPEB 33 and AMF together increased the urease and phosphatase activities in soil compared with the all treatments. However, as compared to the control, dual application of *B. endophyticus* IGPEB 33 and AMF was more effective in enhancing the urease activity (84%) and phosphatase activity (55%).

DISCUSSION

Several PGPB benefits N fixation, phosphate solubilization, phytohormones, and enzymes production.[13,69] Phosphate-solubilizing bacteria releasing organic acids into the soil which solubilize the phosphate complexes converting them into orthophosphate which is available for plant uptake.[70] Similar scientists informed that PGPB exhibited plant beneficial properties.[71,72] The results agreed with the previous report on IAA phytohormone production by *Bacillus* sp.[73] Similarly, the *B. endophyticus*, *B. altitudinis*, and *B. megaterium* were detected to PGP properties (mineral solubilization, phytohormone production IAA).[74] Park et al.[75] found that *B. aryabhattai* was producing maximum phytohormones (IAA and gibberellins). Verma et al.[76] indicated production ability of IAA and GA by PGPB. Panda et al.[77] informed that isolated from maize rhizosphere in the eastern Himalayan, "*B. megaterium* M510" solubilize iron phosphate, aluminum phosphate, and tricalcium phosphate.

In our study, the response of the phosphate-solubilizing *B. endophyticus* IGPEB 33 strain inoculated on ginger plant growth was found to be significantly higher. Several scientists reported that *Bacillus* species promoted plant development and yield in lettuce[18] and tomato.[78] Similarly, Karnwal and Guleria[79] reported *Bacillus* ssp. enhanced the shoot length, leaf number, root length and root dry weight in turmeric. Dinesh et al.[80] investigated *Bacillus* increase in ginger plant growth. According to Chauhan et al.[81] *B. endophyticus* TSH42 significantly increased the shoot length (33.97%) and the biomass of fresh rhizome (56.77%) in turmeric (*Curcuma longa* L.).

Data regarding AMF alone treatment improved plant growth (the number of leaves, length of leaves, and width of leaves). Numerous studies have presented AMF-promoted plant development, plant nutrition, and yield.[82,83] Similarly, Kumar[84] reported that AMF improved plant growth parameters in *Jatropha curcas* L. These results are consistent with the findings obtained by Yamawaki et al.[85] who investigated that the application of AMF enhanced plant height, number of leaves, and number of stems in turmeric (*Curcuma longa* L) than that in control treatment, respectively. Similar findings were informed by Chen[30] single AMF significantly improved leaf area, plant height and the total biomass in *Catalpa bungei* C.A.Mey. AMF inoculation Cleopatra mandarin seedlings improved plant growth, biomass production and plant nutrition (K, P, Fe and Cu) in *Citrus reshni* Hort. Ex Tan. was documented Navarro.[86] Yang et al.[87] investigated AMF-promoted number of leaves, N and P concentrations in *Robinia pseudoacacia* L. under greenhouse conditions.

Several scientists reported that combination of PGPB and AMF promoted plant growth, plant nutrition, and yield in different plants such as *Solanum lycopersicum* L.,[45,88] *Eleusine coracana* L.,[89] *Sulla coronaria*[90] and *Triticum aestivum*.[42] Gao et al.[91] reported that combined application of the biofertilizer mixture (*A.chrocoocum*, AMF, and *B. circulans*) with organic fertilizers significantly increased the plant height, number of leaves per plant and leaf and root dry weight in maize (*Zea*

Table 2. Agrochemical Properties in Soil as Influenced by *B. endophyticus* IGPEB 33 and AMF[a]

Treatment	Total P (%)	Total K (%)	Total N (%)	Humus (%)	C (%)
Control	0.37 ± 0.003	0.61 ± 0.008	0.08 ± 0.002	1.64 ± 0.015	0.94 ± 0.004
B.endophyticus IGPEB 33	0.43 ± 0.010[a]	0.65 ± 0.002	0.13 ± 0.015[a]	1.78 ± 0.015	1.04 ± 0.002
AMF	0.42 ± 0.003	0.62 ± 0.002	0.12 ± 0.003[a]	1.767 ± 0.015	1.02 ± 0.002
B.endophyticus IGPEB 33 + AMF	0.45 ± 0.002[a]	0.66 ± 0.002	0.14 ± 0.002**	1.86 ± 0.031	1.08 ± 0.003[a]

[a]The asterisk indicates $P < 0.05$, $P < 0.01$. The error values specify standard deviation.

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Table 3. Soil Enzymes Activities as Influenced by *B. endophyticus* IGPEB 33 and AMF Treatments[a]

Treatments	Activity of catalase (mL $KMnO_4$ g^{-1} soil h^{-1})	Activity of Invertase (μg glucose·g^{-1} soil·h^{-1})	Activity of urease (NH_4/g of soil/h)	Activity of phosphatase (1 μg pNP/h/g of soil)
Control	3.14 ± 0.02	4.46 ± 0.02	7.86 ± 0.02	3.75 ± 0.03
B.endophyticus IGPEB 33	3.75 ± 0.03[a]	6.62 ± 0.02[a]	12.49 ± 0.05**	5.55 ± 0.06[a]
AMF	3.74 ± 0.02[a]	6.03 ± 0.02[a]	11.43 ± 0.11[a]	5.24 ± 0.05[a]
B.endophyticus IGPEB 33 + AMF	3.95 ± 0.03[a]	7.76 ± 0.05**	14.46 ± 1.01**	5.82 ± 0.08[a]

[a]The asterisk indicates $P < 0.05$, $P < 0.01$. The error values specify standard deviation.

mays L.). Sheteiwy et al.[92] reported significantly enhanced plant height (22.53%), leaf area (14.89%), fresh weight (49.76%), number of pods (35.15), and 100-seed weight (16.94%) of soybean (*Glycine max* L. Merril) under well-watered conditions. Similar results were informed by Nacoon[93] where combined PSB and AMF significantly increased development in *Helianthus tuberosus* L. Wahid et al.[44] showed that AMF and "phosphate solubilizing bacteria" improved P uptake and the growth in maize. AMF and PGPB together improve development and the nutrient uptake in sorghum was documented Dhawi et al.[94]

Positive effects of *B. endophyticus* IGPEB 33 alone on physiological traits in ginger were observed under net house conditions (Figures 1 and 2) as previously reported in turmeric.[95] Several researchers noticed that *Bacillus* species promoted the content of chlorophyll and relative water content in various plants.[96−98] Shi et al.[99] reported that *Bacillus pumilus* 2-1,*Chryseobacterium indologene* 2-2, and *Acinetobacter johnsonii* 3-1 promoted the chlorophyll content in sugar beet plants (*Beta vulgaris*). Similarly, Stefan et al.[100] reported that IAA-producing bacteria promoted chlorophyll content in bean plants (*Phaseolus coccineus*). Inoculation of *B. megaterium* stimulated the chlorophyll a, chlorophyll b, and carotenoids in *Lycopersicon esculentum.*[101]

Our current study also found that AMF can effectively increase physiological traits in ginger (Figures 1 and 2). Numerous studies have shown that photosynthetic rate and chlorophyll content in plants were enhanced by AMF.[102,103] Similarly, Hashem et al.[104] reported that inoculated AMF increased physiological traits such as chlorophyll a (27.21%), chlorophyll b (23.59%), and total chlorophyll (23.90) in chickpea (*Cicer arietinum*L.). Our results are in agreement with Chen et al.[30] where chlorophyll a and chlorophyll b improved in *Catalpa bungei* by AMF. Single inoculation of AMF improved chlorophyll content in maize.[39] Similarly, Sheteiwy et al.[105] informed that single inoculation of AMF significantly improved chlorophyll content of soybean plant. AMF improved plant development and photosynthesis in citrus.[106]

In this study, we found that dual inoculation with *B. endophyticus* IGPEB 33 and AMF significantly increased the physiological properties of ginger leaf (Figures 1 and 2). Numerous studies have presented that together PGPB and AMF improve plant physiological traits (the relative water content, contents of chlorophyll and carotenoids) in different plants.[107,108] Similarly, Ma et al.[109] reported that application of AMF or PGPB combination improved physiological traits in cowpea. Similarly, Vafadar et al.[110] reported that coinioculation with AMF and *P. putida* and *A. chroococcum* and *B. polymixa* significantly increased chlorophyll content in *Stevia rebaudiana.* Similar trends were noticed by Gao in maize.[91]

Inoculation of phosphate-solubilizing bacteria can improve the absorption of P, N, and K as reported by Lopez-Arredondo et al.[111] *B. megaterium* and *B. pumilus* solubilize the P in soil.[71,96] Akhtar et al.[112] found activities of urease and phosphatase in the soil increased significantly after covering the salty clay with straw. Ibarra-Galeana et al.[113] showed that *B. megaterium* and *B. flexus* improve phosphatase activities in soil. Similarly, Qaiser et al.[114] reported that *B. amyloliquefaciens* increased the total N and P in soil and significantly improved enzyme activity in soil.

In the present study, AMF alone improved soil nutrients and significantly enhanced soil enzyme activities (Table 2, 3). Numerous studies on soil enzymes such as catalase, invertase, FDA, phosphomonoestrase, and urease enzymes were enhanced by AMF applications.[84,85,115] Simlilarly, Li and Cai[39] and Ziheng et al.[116] reported that AMF inoculation improved soil the enzyme activities. Similar findings were reported by Zai et al.[117] AMF improved soil enzyme (urease and protease) activities. Our findings revealed that AMF application had the highest phosphatase and invertase activity. Similarly, El-Sawah et al.[43] found that AMF actively enhanced phosphatase and invertase enzymatic activities of soil. Many previous studies found that coinculation of PGPB and AMF improved soil nutrients and soil enzyme activities.[41,42] Similarly, Hidri et al.[90] reported that dual addition of *B. subtilis* and AMF significantly increased urease and phosphatase enzyme activities in soil.

CONCLUSIONS

From the present study, plant length, leaf length, number of leaves, soil nutrients (N, P), soil enzyme (catalase, invertase, urease, phosphatase) activities, and plant physiological properties of ginger were affected by the *B. endophyticus* IGPEB 33 and AMF alone and their combination of *B. endophyticus* IGPEB 33 and AMF. Inoculation of plant-growth-promoting *B. endophyticus* IGPEB 33 and AMF alone increased the plant growth, which positively affected the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content. Combination of plant-growth-promoting *B. endophyticus* IGPEB 33 and AMF inoculation improved plant development and physiological traits. The plant-growth-promoting *B. endophyticus* IGPEB 33 and AMF inoculation individually improved soil enzyme activities (catalase, invertase, urease, phosphatase) and soil nutrients such as total P and total N content. A positive impact of inoculation was indicated with combination of *B. endophyticus* IGPEB 33 and AMF. However, coinoculation of plant-growth-promoting *B. endophyticus* IGPEB 33 and AMF significantly increased the catalase, invertase, urease, and phosphatase activities and total P and N content in soil. We conclude that dual application of plant-growth-promoting *B. endophyticus* and AMF is most effective for environmental agricultural practices to promote ginger growth, soil enzyme activities, and soil nutrients in typical irrigated gray soil.

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Notes

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