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The effects of *Pseudomonas koreensis* IGPEB 17 and arbuscular mycorrhizal fungi on growth and physiological properties of ginger

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**Abstract:** The effect of *Pseudomonas koreensis* IGPEB 17 and arbuscular mycorrhizal fungi (AMF) on plant growth and physiological properties of ginger (*Zingiber officinale*) in net house conditions was investigated. The experiment included four treatments including *P. koreensis* IGPEB 17, arbuscular mycorrhizal fungi (AMF) biofertilizer and combination of *P. koreensis* IGPEB 17 and AMF treatment. The result indicated that *P. koreensis* IGPEB 17 had positive effects on plant growth-promoting traits such as P-solubilization, protease, amylase, IAA, and ACC deaminase production. Moreover, *P. koreensis* IGPEB 17 showed ability in producing lipase and cellulase. The *P. koreensis* IGPEB 17 significantly increased plant height (57.6%), the leaf number (28.1%), leaf length (23.3%) and leaf width (35.7%) compared to control treatment. However, the combination of *P. koreensis* IGPEB 17 and AMF treatment significantly increased the height of plant, the number of leaf, the length of leaf and the width of leaf by 68.4%, 42.3%, 30.2%, and 54.7%, respectively compared to control treatment. Furthermore, dual inoculation of *P. koreensis* IGPEB 17 and AMF significantly enhanced the chlorophyll a (76.5%), chlorophyll b (71.2%), total chlorophyll (73.3%), and carotenoid content (74.7%), respectively rather than control. In conclusion, results showed that dual inoculation of *P. koreensis* IGPEB 17 strain and AMF increased plant growth and physiological traits of ginger plants compared to inoculation with *P. koreensis* IGPEB 17 and AMF alone.

**Key words:** Ginger, *Pseudomonas koreensis*, arbuscular mycorrhizal fungi, total chlorophyll content, relative water content, soil nutrients

1. Introduction
Ginger belongs to the family *Zingiberaceae* and its rhizomes contain a high amount of minerals such as potassium (K), calcium (Ca), and phosphorus (P) for health benefits in human body (Gupta et al., 2014; Akhani et al., 2011; Jabborova et al., 2021a; Jabborova et al., 2022). Ginger rhizomes are rich compounds including flavonoids, vitamin C, gingerdiol, gingerol, and gingerdione used traditionally for numerous health conditions including sedative, antioxidant, cancer, analgesic, antipyretic, insecticidal, vomiting, nausea, anti-inflammatory, and rheumatic diseases (Park et al., 2008; Kundu and Surh, 2009; Dehghani et al., 2011; Niksokhan et al., 2014). Beneficial microorganisms such as plant growth-promoting rhizobacteria (PGPRs) play a main role in increasing the growth, and yield in plants (Santoyo et al., 2021). PGPRs directly promote plant growth such as solubilizing phosphate (Macleod et al., 2015; Jabborova et al., 2020a; Jabborova et al., 2020b; Jabborova et al., 2021b; Li et al., 2018), increasing nutrient uptake (Li et al., 2017; Liu et al., 2016;) and nitrogen fixation (Solanki et al., 2017; Li et al., 2018). *Pseudomonas* species could be able to produce Indole-3-acetic acid (IAA), cytokinins and gibberellins (Macleod et al., 2015). *P. protegens* Pf-5 and *P. nitroreducens*, have the capability to fixing the N (Setten et al., 2013; Solanki et al., 2017). *P. fluorescens*, *P. putida* and *P. koreensis* inoculation improve growth, development and yield in different crops such as cucumber (Liu et al., 2011), mustard (Ahemad et al., 2012), soybean (Jabborova et al., 2021c). Several studies have also indicated that PGPRs such as *Pseudomonas* spp. (Magnani et al., 2010), *P. aurantiaca* (Mehnaz et al., 2009a), *P. fluorescens* (Mehnaz et al., 2009b), *P. putida* (Costa-Gutierrez et al., 2021) and *P. stutzeri* (Lami et al., 2020) are isolated from plants. *Pseudomonas* sp. strain AK-1 enhances the shoot length and the root length in soybean (Kasotia et al., 2016). Inoculation of *P. koreensis* can promote plant biomass and the chlorophyll content in soybean (Adhikari et al., 2020).

Arbuscular mycorrhizal fungi (AMF) is beneficial for plant growth (Jabborova et al., 2021d, Jabborova et al., 2021e, Jabborova et al., 2021f), plant nutrition (Shao et al., 2017) and physiological properties (Hashem et al., 2019) in different plants. *G. intraradices*, *G. mosseae*, and *R. irregularis* enhanced colonization rate, root biomass,
chlorophyll content, osmotic potential and plant nutrients in several plants (Pedranzani et al., 2016; jixiang et al., 2017; Garg and Singh, 2018). Khan et al. (2021) reported that AMF symbiosis improved the metabolomics enzymes, the net photosynthetic rate and soluble protein of *Leymus chinensis*. Mathur et al. (2018) showed that AMF inoculation enhanced plant height, leaf width, root systems, and physiological traits in maize.

Combined inoculation of PGPRs and AMF exerted positive effects on plant nutrition, physiological traits and yield in different plants (Mdel et al., 2009; Dhawli et al., 2016; Yadav et al., 2020). Dual inoculation of PGPRs and AMF in promoting growth and yield in strawberry (Todeschin et al., 2018), sorghum (Dhawli et al., 2016), and lettuce (Kohler et al., 2010) have been informed. Laranjeira et al. (2021) reported that combined inoculation with PGPRs and AMF increased the growth and grain yield in chickpea. Coinoculation of both AMF and bacteria increase plant growth and colonization of plant roots (Selvakumar et al., 2016). Kavatagi and Lakshman, (2014) reported that coinoculating with *G. fasciculatum* and *P. fluorescens* significantly increased plant growth indices in tomato. There is little information about interaction of *Pseudomonas* species and AMF on ginger. The aim of this research was to investigate the effects of *P. koreensis* IGPEB 17 strain and AMF inoculants on several growth and physiological parameters in ginger under net house conditions.

2. Materials and methods

Soil collected from field (the Durmon Experimental field Station of the Institute of Genetics and Plant Experimental Biology, Kibray district, 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%, potassium –0.69% (Jabborova et al., 2021a). The AMF fertilizer was purchased from New Delhi, India. *P. koreensis* IGPEB 17 strain was obtained from the Laboratory of Medicinal Plants Genetics and Biotechnology. *P. koreensis* IGPEB 17 cultured on nutrient broth.

2.1. Effects of *P. koreensis* IGPEB 17 strain on Plant growth promoting traits

Phosphate solubilization ability was detected by spot inoculating of pure *P. koreensis* IGPEB 17 strain on the Pikovskaya medium (Pikovskaya, 1948), incubated at 28 °C for 5–7 days and the formation of clearing zones was evaluated.

*P. koreensis* IGPEB 17 was grown for 48 h on their respective media (nutrient broth) at 28 °C, and then centrifuged at 3000 rpm for 30 min. The supernatant was mixed with orthophosphoric acid and Salkowski reagent. The development of a pink color showed IAA production (Bric et al., 1991).

Protease production was measured on sterile skim milk agar by spot inoculation of *P. koreensis* IGPEB 17 strain and was incubated at 30 °C for 48 h. Then, plates were observed for the appearance of the clearing zone around the colony which indicated the enzymatic degradation of protease (Malleswari and Bagyanarayana, 2013).

Lipase production was measured on 10 mL medium containing peptone (10 g), agar (15 g), tween 20, sodium chloride (5 g), calcium chloride (0.1 g), in 1 L distilled water (Ghodsalavi et al., 2013). *P. koreensis* IGPEB 17 strain was streaked on this medium and incubated at 27 °C for 48 h.

Cellulose-degrading ability of *P. koreensis* IGPEB 17 was determined by streaking on the cellulose Congo-Red agar medium. Clearing halos around and beneath the colony indicating the enzymatic degradation of cellulose (Gupta et al., 2012).

The ACC deaminase production of bacterial strain was determined by culturing in minimal saline DF medium with the addition of 2 g of (NH₄)₂SO₄ at 28 °C for 48 h. The appearance of bacterial colonies in the minimal saline DF medium after the incubation period was considered a positive result for the synthesis of ACC deaminase (Mintoo et al., 2019).

To determine the amylase activity, the bacterial strain was cultured on starch agar medium and incubated for 28 h at 28 °C. Then, the iodine solution (1%) was added and kept for 1 min. The iodine solution reacts with the starch to initially form a blue color, then the color quickly disappears and colorless zones form around the bacterial colonies. The formation of colorless zones around bacterial colonies indicates the presence of amylase activity of the bacterial strain (Malleswari and Bagyanarayana, 2013).

2.2. Experimental design

The effect of *P. koreensis* IGPEB 17 and AMF on the growth of ginger was studied in pot experiments in a net house at Laboratory of MPG, IGPEB, Uzbekistan. Four treatments including control, *P. koreensis* IGPEB 17 strain, AFM biofertilizer and combination of *P. koreensis* IGPEB 17 strain with AMF were selected. The AMF (*Glomus mosseae*) biofertilizer consists of 100 spores/g and 1200 inoculum potential (IP) /g. The AMF biofertilizer was layered at a depth of 5 cm from the surface of soil, ensuring 10 spores for each rhizome. The *P. koreensis* IGPEB 17 strain was used for inoculation of the sterilized rhizome (10° cells/mL). Rhizome was cultivated into plastic pots (26 cm diameter, 22 cm depth) containing 8.0 kg in soil. The net house experiments were carried out in five replications. After four months, plant height, number of leaves, and leaf length and width were measured.

2.3. Physiological parameters measurement

Physiological parameters including carotenoids, total chlorophyll, chlorophyll a, and chlorophyll b contents in...
ginger were measured by using the method of Hiscox and Israelstam (1979). For this purpose, fresh leaf (50 mg) of ginger sample was cut in test tubes and dimethylsulfoxide (5 mL) was added. All tubes were incubated at 37 °C for 4 h. Then absorbance of the extract was taken at 470 nm, 645 nm, and 663 nm using a spectrophotometer. Relative water content (RWC) was analyzed by the method of Barrs and Weatherly (1962). Fresh leaf sample (100 mg) was placed in petri plates with water for 4 h. Then, RWC of leaf was measured.

2.4. Statistical analyses
The data were analyzed with the StatView Software using ANOVA. Impact of treatments was analyzed by the magnitude of the F value (p < 0.05 < 0.001).

3. Results and discussion
3.1. Effects of *P. koreensis* IGPEB 17 strain on Plant growth promoting traits
The results showed that *P. koreensis* IGPEB 17 strain had positive effects on plant growth promoting characteristics such as P-solubilization, protease, amylase, IAA, and ACC deaminase production (Table 1). Moreover, *P. koreensis* IGPEB 17 strain showed ability in producing lipase and cellulase.

The positive effects of bacteria on N fixation, phosphate solubilization, phytohormones production and enzymes production were reported (Kumar et al., 2016; Li et al., 2018; Awan et al., 2020). The phytohormone (IAA) production by *Pseudomonas* species was similar to our result (Macleod et al., 2015). Similarly, Zabihi et al. (2021) showed that *P. fluorescens* had positive effects on mineral solubilization and phytohormone (IAA) production. Liao et al. (1998) showed that *P. fluorescens* CY091 strain produced one major protease enzyme. Yoo et al. (2018) found that *P. koreensis* WA15 strain could be able to produce different enzymes such as cellulase, pectinase, and lipase.

3.2. Plant growth parameters
The *P. koreensis* IGPEB 17 had positive significant effects on plant growth parameters (Table 2). Results showed that *P. koreensis* IGPEB 17 treatment significantly enhanced plant height, leaf number, leaf length, and leaf width by 57.6%, 28.1%, 23.3%, and 35.7%, respectively compared with control treatment.

*P. koreensis* promoted plant growth, development, and yield in various plants including tomato (Shen et al., 2012), cucumber (Liu et al., 2011), and mustard (Ahemad et al., 2012). Similarly, Jaleel et al. (2017) reported that inoculation of *P. fluorescens* enhanced plant height by 12.2% in turmeric plant. Adhikari et al. (2020) also showed that *P. koreensis* MU2 strain increased plant biomass and root and shoot length in soybean. Kasotia et al. (2016) found that *Pseudomonas* sp. strain AK-1 enhanced the shoot length in soybean.

The AMF inoculation significantly increased the plant height, number of leaf and leaf width by 35.9%, 17.4%, and 26.1%, respectively compared with control treatment (Table 2). Several studies showed that AMF inoculation improved plant development and yield (Smith et al., 2011; Karasawa et al., 2012; Shao et al., 2017; Hashem et al., 2019). Mathur (2018) reported similar results that AMF

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Plant height (cm)</th>
<th>Leaf number</th>
<th>Leaf length (cm)</th>
<th>Leaf width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.06 ± 0.753</td>
<td>5.62 ± 0.40</td>
<td>8.32 ± 0.55</td>
<td>0.84 ± 0.02</td>
</tr>
<tr>
<td><em>P. koreensis</em> IGPEB 17</td>
<td>14.28 ± 1.02*</td>
<td>7.20 ± 0.84*</td>
<td>10.26 ± 0.63*</td>
<td>1.14 ± 0.10*</td>
</tr>
<tr>
<td>AMF</td>
<td>12.32 ± 0.52*</td>
<td>6.60 ± 0.30</td>
<td>9.02 ± 0.65*</td>
<td>1.06 ± 0.15*</td>
</tr>
<tr>
<td><em>P. koreensis</em> IGPEB 17 +AMF</td>
<td>15.26 ± 1.26**</td>
<td>8.00 ± 0.71**</td>
<td>10.84 ± 0.73*</td>
<td>1.30 ± 0.16**</td>
</tr>
</tbody>
</table>

* Asterisk (p < 0.05*, p < 0.01**)
inoculation could improve plant height and leaf width in maize. Moreover, inoculation with AMF increased plant development in turmeric (Yamawaki et al., 2013). Asrar et al. (2012) also found similar results that AMF significantly stimulated shoot length and number of leaf in *Antirrhinum majus* L. The plant growth and the number of leaves in *Antirrhinum majus* L. significantly increased by AMF inoculation (Yang et al., 2014).

Results showed that plant height, leaf number, leaf length, and leaf width significantly increased by 68.4%, 42.3%, 30.2%, and 54.7%, respectively with dual inoculation of *P. koreensis* IGPEB 17 and AMF compared with control treatment. Dual inoculation with PGPRs and AMF improved plant growth and development in various plants including strawberry (Todeschin et al., 2018), sorghum (Dhawi et al., 2016), lettuce (Kohler et al., 2010), and tomato (Kavatagi and Lakshman, 2014). Nacoon (2020) showed that inoculation by phosphate solubilizing bacteria and AMF together significantly increased plant height and leaf area of *Helianthus tuberosus* L. Moreover, coinoculation of AMF and phosphate solubilizing bacteria improved the growth parameters in maize (Wahid et al., 2018). Vafadar et al. (2014) investigated that dual inoculation with AMF (*Glomus intraradices*), and *Bacillus polymyxa* significantly increased plant growth in *Stevia rebaudiana*. Coinoculation with *Glomus mosseae* and *Bacillus subtilis* promoted plant growth in the medicinal plant *Artemisia annua* (Awasthi et al., 2011).

3.3. Leaf physiological parameters

The effects of inoculation by *P. koreensis* IGPEB 17, AMF, and combination of *P. koreensis* IGPEB 17 and AMF treatments on leaf physiological parameters are presented in Figures 1a–1d. Inoculation of *P. koreensis* IGPEB 17 significantly promoted chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids content by 64.1%, 52.0%, 55.7%, and 60.1%, respectively compared with control treatment.

The AMF inoculation also improved the chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents by 58.0%, 36.9%, 50.1%, and 53.4%, respectively, compared to the control. On the other hand, coinoculation by *P. koreensis* IGPEB 17 and AMF treatment significantly enhanced the chlorophyll a by 76.5%, chlorophyll b by 71.2%, total chlorophyll by 73.3%, and carotenoid content by 74.7%, respectively, compared to control.

The effects of *P. koreensis* IGPEB 17, AMF, and combination of *P. koreensis* IGPEB 17 and AMF treatments on leaf relative water content are presented in Figure 2. Among the treatments, only dual inoculation by *P. koreensis* IGPEB 17 and AMF had positive and significant effects on leaf relative water content.

![Figure 1](image-url). The effects of *P. koreensis* IGPEB 17, AMF, and combination of *P. koreensis* IGPEB 17 and AMF inoculation on chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), and carotenoids content (d). * Asterisk (p < 0.05, p < 0.01)**.
The positive effects of *P. koreensis* IGPEB 17 on photosynthetic pigments and leaf relative water content were previously reported in tomato (*Solanum lycopersicum*) (Mekureyaw et al., 2022). Similarly, Adhikari et al. (2020) showed that *P. koreensis* MU2 inoculation enhanced the chlorophyll content in soybean. Nosheen et al. (2018) found also that inoculation by *P. putida* increased the leaf chlorophyll and carotenoids contents of *Carthamus tinctorius* L.

Our current study also showed that AMF can increase the photosynthetic pigments (Figure 1). There are studies that show chlorophyll content in plants was enhanced by AMF inoculation (Shi-Chu et al., 2019; Jabborova et al., 2021d). Pedranzani et al. (2016) and Jixiang et al. (2017) showed that AMF (*G. intraradices*, *G. mosseae*, and *R. irregularis*) promoted chlorophyll content, osmotic potential and relative water contents in plants. Khan et al. (2021) also found that AMF inoculation increased photosynthetic pigments of *Leymus chinensis*. Similarly, Asrar et al. (2012) showed that inoculation of AMF improved leaf relative water content and chlorophyll content in *Antirrhinum majus* L.

Dual inoculation with *P. koreensis* IGPEB 17 and AMF significantly improved photosynthetic pigments and leaf relative water content (Figures 1 and 2). Several studies have shown that combination of PGPRs and AMF improve photosynthetic pigments and leaf relative water content in different plants (Diagne et al., 2020; Yadav et al., 2020). Sharma et al. (2021) reported that combination of *P. fluorescens* and AMF increased relative water content and photosynthetic efficiency in *Helianthus annuus* L. Diagne et al. (2020) also showed that application of AMF and PGPRs combination improved total chlorophyll content of *Casuarina obesa*. Angulo-Castro et al. (2021) reported that combined with AMF and *Pseudomonas tolaasii* P61 enhanced the chlorophyll content in bell pepper (*Capsicum annuum*).

**4. Conclusion**

Results showed that *P. koreensis* IGPEB 17 strain has positive results in some plant growth promoting traits including P-solubilization, protease, amylase, IAA, and ACC deaminase production. Inoculation with *P. koreensis* IGPEB 17 strain and AMF alone also increased photosynthetic pigments. However, combination of *P. koreensis* IGPEB 17 and AMF inoculation significantly promoted plant growth parameters compared to inoculation individually. Dual inoculation with *P. koreensis* IGPEB 17 and AMF significantly promoted photosynthetic pigments. Thus, using dual inoculation with *P. koreensis* IGPEB 17 and AMF can improve ginger growth and physiological traits.

**References**


