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Bacterial auxin signaling: comparative study of growth induction in *Arabidopsis thaliana* and *Triticum aestivum*

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Abstract: Auxin-producing rhizobacteria were evaluated for the role of their auxin signaling in enhancing the growth of *Arabidopsis thaliana* (L.) Heynh. and *Triticum aestivum* L. *Arabidopsis thaliana* wild type Columbia (Col-0) in comparison with phytohormone mutant lines *aux1-7* (insensitive to auxin and ethylene), *axr4-1* (insensitive to auxin), and *eir1-1* (insensitive to ethylene) was used as a model system. Bacterization of wild type Col-0 recorded significant increases in shoot length (38%) and number of siliqua (180%) with *Bacillus subtilis* TpP-1 over the controls. Inoculation with *aux1-7* and *axr4-1* showed statistically comparable results for growth parameters as compared to their respective controls. Treatment of *eir1-1* with *Pseudomonas* sp. AvH-4 and *Bacillus subtilis* TpP-1 recorded significant increases in shoot length (9%) and number of siliqua (20%), respectively. The pattern of growth response of *aux1-7*, *axr4-1*, and *eir1-1* indicated that bacterial auxin and ethylene signaling is involved in the growth promotion of *A. thaliana*. For *T. aestivum*, significant increases in number of tillers (74%) and seed weight (26%) were recorded with *Pseudomonas* sp. AvH-4. Results of this study suggested that growth induction by *B. subtilis* TpP-1, *Pseudomonas* sp. AvH-4, and *Pseudomonas aeruginosa* As-17 in *A. thaliana* is associated with bacterial auxin signaling.

Key words: *Arabidopsis*, bacterial auxin, bacteria, interaction, plant growth promotion

1. Introduction

Phytohormones are signal molecules that act as chemical messengers to control plant growth and development. A plant hormone is an organic substance synthesized in defined organs of the plant that can be translocated to other sites, where it triggers specific morphological and physiological responses. In addition to plants, the ability to synthesize phytohormones is also widespread among soil- and plant-associated bacteria (Baca and Elmerich, 2007). Indole-3-acetic acid (IAA) represents one of the most extensively studied and abundant types of auxin in plants. It regulates multidimensional developmental processes in plants, including cell division, cell elongation, cell differentiation, pattern formation, apical dominance, and tropic responses (Woodward and Bartel, 2005). In vitro experiments also demonstrated the role of auxin in combination with other phytohormones in plantlet development (Yıldız et al., 2011; Bektaş et al., 2013). Like plants, auxin is also quantitatively the most abundant phytohormone secreted by rhizobacteria as a secondary metabolite. Synthesis of IAA by plant-associated bacteria is probably one of the most important causes of improved growth and yield in various crops. For instance, in the case of *Azospirillum* auxin is quantitatively the most abundant

phytohormone, and it is generally agreed that auxin production, rather than nitrogen fixation, is the major factor responsible for enhanced plant growth (Bloemberg and Lugtenberg, 2001). The role of plant-growth-promoting rhizobacteria (PGPR) as biofertilizers to increase the growth and yield of agronomically important crops has been extensively studied (Vessey, 2003; Ali et al., 2009a, 2009b; Akhtar and Ali, 2011; Zafar et al., 2012).

Arabidopsis thaliana (L.) Heynh. is widely used as a model system in plant molecular biology. It has also been used as a model plant for understanding plant-microbe interactions because of its short life span and well-characterized genetic background (Pang and Meyerowitz, 1987; Persello-Cartieaux et al., 2001; Mantelin and Touraine, 2004). Mutants of *A. thaliana* have been used to evaluate the ability of PGPR to elicit growth promotion and induced systemic resistance against phytopathogens (Ryu et al., 2007; Domenech et al., 2007). Ryu et al. (2005) used different mutant lines defective in hormone response pathways to elucidate the role of bacterial phytohormone signaling in plant growth promotion. In the present study, the role of bacterial auxin signaling in mediating plant growth responses was investigated by using *A. thaliana* wild type Columbia (Col-0) and phytohormone mutant

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lines *aux1-7* (insensitive to auxin and ethylene), *axr4-1* (insensitive to auxin), and *eir1-1* (insensitive to ethylene). Bacterial strains of the genera *Bacillus*, *Pseudomonas*, *Micrococcus*, *Escherichia*, and *Staphylococcus* that showed in vitro auxin production were used to inoculate *Arabidopsis thaliana*. After PGPR–*Arabidopsis* interaction, the phytostimulatory effect of bacterial auxin on the growth and yield of wheat (*Triticum aestivum* L. var. Auqab-2000) was also demonstrated under different environmental conditions. Hence, the PGPR–*Arabidopsis* model was used to compare the effect of microbial auxin signaling on the growth of cereal crops.

2. Materials and methods

2.1. Bacterial strains

Bacterial strains used in this study include *Bacillus megaterium* MiR-4, *B. pumilus* DaR-2, *B. circulans* CaR-3, *B. licheniformis* BP-1, *B. subtilis* TpP-1, *Pseudomonas* sp. AvH-4, *P. aeruginosa* As-17, *Micrococcus* sp. AvR-5, *Escherichia hermannii* SnR-1, and *Staphylococcus saprophyticus* CdR-1. These strains were previously isolated from the rhizosphere, phyllosphere, or histoplane of natural plant settings (Ali et al., 2009a, 2009b). Strains exhibited plant-growth-promoting attributes and were evaluated further to elucidate the role of bacterial auxin signaling in plant growth promotion by using *Arabidopsis thaliana* as a model system.

2.2. In vitro auxin production by rhizobacteria

In vitro auxin production by rhizobacteria was determined in the presence of precursor L-tryptophan. Strains were grown in 100 mL of L-broth medium in 250-mL flasks supplemented with a filter-sterilized solution of L-tryptophan (1000 µg mL⁻¹). The flasks were inoculated with 100 µL of bacterial cell suspension adjusted to an optical density of 10⁷ CFU mL⁻¹. All inoculated flasks (in triplicate) were incubated at 37 °C for 72 h at 120 rev min⁻¹. After incubation, cells were removed from stationary phase cultures by centrifugation at 2300 × *g* for 15 min. One milliliter of supernatant was taken and mixed with 2 mL of Salkowski's reagent to determine auxin production in bacterial culture supernatant (Ali et al., 2009a). Auxin production was also quantified colorimetrically in a bacterial growth curve at different time intervals (Noreen et al., 2012).

2.3. GC-TOFMS analysis of bacterial IAA

Crude bacterial extracts were also screened for the presence of bacterial IAA by gas chromatography and time-of-flight mass spectrometry (GC-TOFMS). Flasks containing 100 mL of L-broth were inoculated with the bacterial suspension in triplicate, as mentioned above. Stationary phase cultures (10 mL) were extracted 3 times with an equal volume of ethyl acetate, then dried and redissolved in 2 mL of HPLC grade methanol. About 100

µL of methanolic extract and 1 mL of extraction medium (MeOH/H₂O; 90:10) were added to an Eppendorf tube. The extraction was performed using an MM 301 vibration mill at a frequency of 30 Hz S⁻¹ for 2 min. After extraction, 200 µL of the extract was transferred in GC vials and evaporated to dryness in a speed-vac concentrator. Then 30 µL of methoxyamine hydrochloride (15 µg µL⁻¹) in pyridine was added to each sample, followed by shaking for 10 min in a shaking machine. After 16 h of derivatization at room temperature, the samples were trimethylsilylated for 1 h at room temperature by adding 30 µL of MSTFA with 1% TMCS. After silylation, 30 µL of heptane was added. One microliter of the derivatized sample was injected splitless by CTC Combi Pal autosampler (Agilent, Atlanta, GA, USA) into an Agilent 6890 gas chromatograph, as described in Jonsson et al. (2004).

2.4. Seed sterilization

Seeds of wild type ecotype Columbia (Col-0) and phytohormone mutant lines of *A. thaliana* (obtained from Umeå Plant Science Center, Umeå, Sweden) were sterilized in 70% ethanol for 8–10 min and washed with 95% ethanol. Seeds were air-dried in a laminar flow cabinet under fully sterilized conditions. Certified seeds of *T. aestivum* L. var. Auqab-2000 were obtained from Punjab Seed Corporation, Lahore, Pakistan. Healthy seeds were surface-sterilized with 0.1% HgCl₂ for 5 min and washed 3–4 times in sterilized glass-distilled water.

2.5. Preparation of inoculum

For preparation of bacterial inoculum, bacterial strains were grown in 100 mL of L-broth for 24 h at 37 °C at 120 rev min⁻¹. Bacterial cells from cultures were harvested by centrifugation at 2300 × *g* and suspended in sterilized glass-distilled water. To ensure equal cell populations of each bacterial strain in the suspension, optical density was measured by spectrophotometer (CECIL CE 7200) to a final concentration of 10⁷ CFU mL⁻¹.

2.6. Bacteria–*Arabidopsis* interaction

Wild type ecotype Columbia (Col-0) and plant hormone mutant lines of *Arabidopsis thaliana* obtained from the Umeå Plant Science Center, Swedish University of Agricultural Sciences, Umeå, Sweden, were used to evaluate the potential of bacterial phytohormone signaling in plant growth promotion. In addition to *A. thaliana* wild type Columbia (Col-0), hormone mutant lines *aux1-7* (insensitive to auxin and ethylene), *axr4-1* (insensitive to auxin), and *eir1-1* (insensitive to ethylene) were used in the experiment. Sterilized seeds were inoculated on basal Murashige and Skoog (1962) medium supplemented with 1.5% sucrose and solidified with 1% agar. Seeds were vernalized by incubating plates at 4 °C in the dark for 48 h to enhance seed germination. After 48 h, plates were incubated (vertically) in a growth chamber for seed germination at 25 °C with a 12-h photoperiod and a light

intensity of $50\text{--}60\ \mu\text{mol m}^{-2}\text{s}^{-1}$. Initially, 5 seedlings (1 week old) were transplanted aseptically in pots ($6.5 \times 6.5\ \text{cm}$) containing autoclaved soil mixture and vermiculite in a 1:1 ratio. After transplanting, seedlings were inoculated with $500\ \mu\text{L}$ of bacterial cell suspension. For each strain 3 pots were inoculated, and the experiment was repeated twice. Experiments were conducted under fully sterile conditions. Control pots for wild type (Col-0) and mutant lines (*aux1-7*, *axr4-1*, *eir1-1*) were treated with autoclaved distilled water. Plants were irrigated with sterilized distilled water when required. Three days after transplanting, thinning was performed to leave 3 uniform seedlings in each pot, which were grown to maturity. Experiments were conducted in a greenhouse at $22\ ^\circ\text{C}$, 50% humidity, and a 12-h photoperiod with a light intensity of $150\text{--}200\ \mu\text{mol m}^{-2}\text{s}^{-1}$. After 6 weeks, growth parameters in terms of shoot length, shoot fresh weight, and number of siliqua were recorded.

2.7. Pot trials with *Triticum aestivum*

Pot experiments were also conducted to evaluate the phytostimulatory effect of *Bacillus pumilus* DaR-2 and *B. circulans* CaR-3, *B. megaterium* MiR-4, *Pseudomonas* sp. AvH-4, and *P. aeruginosa* As-17 on vegetative and yield parameters of *T. aestivum*. Sterilized seeds were treated with bacterial suspension for 15 min. In 1 set of experiments, seeds were inoculated under fully axenic conditions in pots ($6.5 \times 6.5\ \text{cm}$) containing autoclaved peat-based soil mixture and vermiculite, as mentioned above. Eight seeds were sown in each pot in triplicate, and the experiment was repeated twice. After germination,

thinning was performed to leave 5 uniform seedlings in each pot. After 2 weeks percent seed germination, shoot length, and fresh weight of plants were recorded. For a second set of experiments, seeds were inoculated in larger pots ($30 \times 30\ \text{cm}$) containing 10 kg of unfertilized garden soil. The soil had pH 7.2, EC $44\ \text{ds m}^{-1}$, and 0.60% organic content. Initially, 15 seeds were inoculated in each pot in 6 replicates, and after germination seedlings were thinned to 10 per pot. The pots were arranged in a completely randomized design in the wire house of the Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore, Pakistan, at ambient light and temperature. At full maturity shoot length, number of tillers, spike length, and 100 seed weights were recorded.

2.8. Data analysis

Data was subjected to analysis of variance (ANOVA) using SPSS 16, and means of different treatments were compared using Duncan's multiple range test ($P = 0.05$). The correlation coefficients between in vitro auxin production and cell densities in the growth curve experiment were also calculated ($P = 0.01$).

3. Results

3.1. Bacterial growth and auxin production

Growth experiments were conducted in L-broth medium supplemented with $1000\ \mu\text{g mL}^{-1}$ L-tryptophan to quantify bacterial auxin at different time intervals. In general, maximum auxin production in culture supernatant was observed during the stationary phase (Figure 1).

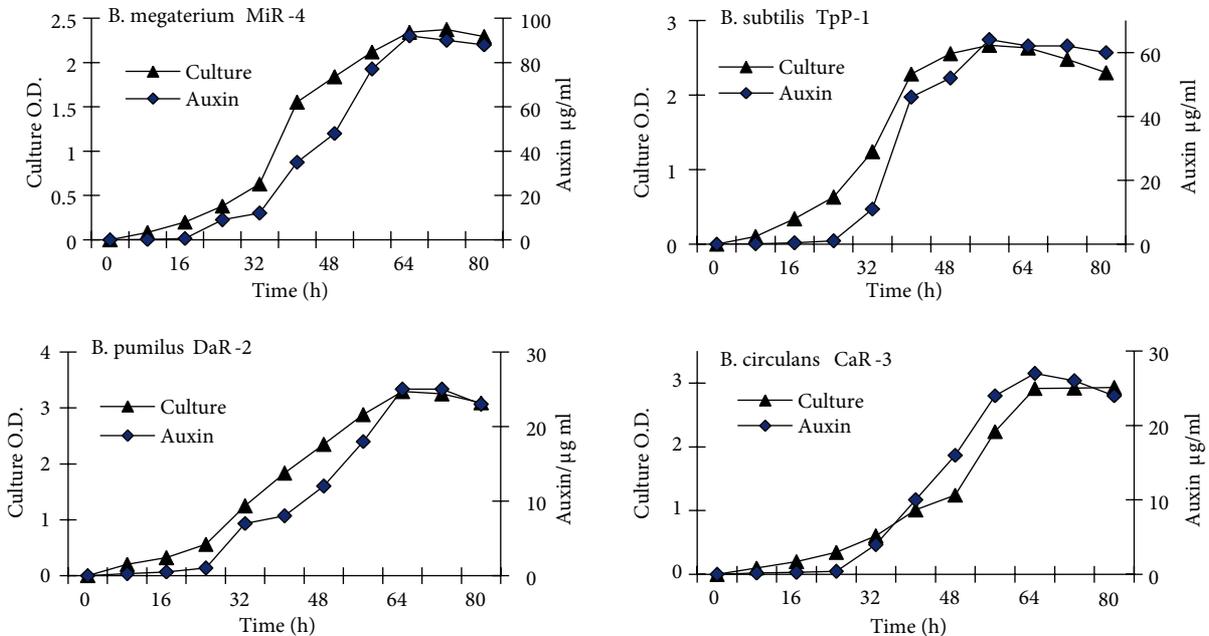


Figure 1. Auxin production by *Bacillus* strains in L-broth medium supplemented with $1000\ \mu\text{g mL}^{-1}$ L-tryptophan. The results shown are representative of 2 repetitions of the experiment. The value “*r*” in each figure indicates correlation between bacterial cell densities and auxin production ($P^{**} = 0.01$).

Experiments also indicated that auxin production for each strain is dependent on bacterial cell density; this is evident from the highly significant positive correlation between bacterial cell densities and auxin production (up to $r = 980$; $P = 0.01$). During the lag phase, very low levels of auxin were recorded; however, with entrance into the log phase auxin production also increased tremendously. From the growth curve it is also evident that the majority of the strains actively entered the log phase within 16 h of the growth period and showed continuous accumulation of IAA overtime. Among *Bacillus* strains, *B. megaterium* MiR-4 produced the maximum auxin concentration ($92 \mu\text{g mL}^{-1}$) after a 64 h growth period. Similarly, *B. subtilis* TpP-1, *B. circulans* CaR-3, and *B. pumilus* DaR-2 were 65, 27, and $25 \mu\text{g mL}^{-1}$, respectively, in the stationary phase (Figure 1). *Pseudomonas* sp. AvH-4 and *P. aeruginosa* As-17 showed maximum auxin production after 64 h ($72 \mu\text{g mL}^{-1}$) and 80 h ($106 \mu\text{g mL}^{-1}$), respectively. *Micrococcus* sp. AvR-5 and *Escherichia hermannii* SnR-1 showed maximum auxin production after 64 h and then showed a decreasing trend during the late stationary phase. *Staphylococcus saprophyticus* CdR-1 produced $35 \mu\text{g mL}^{-1}$ after a 72 h growth period.

3.2. GC-TOFMS analysis

The GC-TOFMS system enables spectra to be accumulated rapidly, thereby increasing the speed of analysis. The

chosen spectra accumulation speed was 30 spectra per second. Each analysis took only 15 min, and more than 200 compounds could be detected in each analysis. Analysis of bacterial crude extracts showed that strains released different types of metabolites in culture supernatant. The presence of IAA was confirmed qualitatively in the culture supernatant of all bacterial strains. Peaks corresponding to IAA were detected at 345.8 retention time(s). The mass spectrum of IAA was compared with the respective standard during a library hit for comparison (Figure 2).

3.3. Growth promotion of *Arabidopsis* by rhizobacteria

Treatment of *Arabidopsis thaliana* seedlings with bacterial cell suspension showed variable responses in different growth parameters. For *A. thaliana* ecotype Columbia (Col-0), significant increases in shoot length were observed with *Bacillus subtilis* TpP-1 (38%), *B. megaterium* MiR-4 (33%), and *B. pumilus* DaR-2 (32%). The auxin- and ethylene-insensitive mutant (*aux1-7*) recorded an absence of growth response for shoot elongation after bacterial treatment (Table 1). However, *aux1-7* recorded a significant improvement (10%) in shoot length with *B. subtilis* TpP-1 over the control. Inoculation of auxin-insensitive mutant *axr4-1* showed statistically comparable results to the control. On the other hand, ethylene-insensitive mutant *eir1-1* also showed statistically comparable growth responses to the control after inoculation with the majority

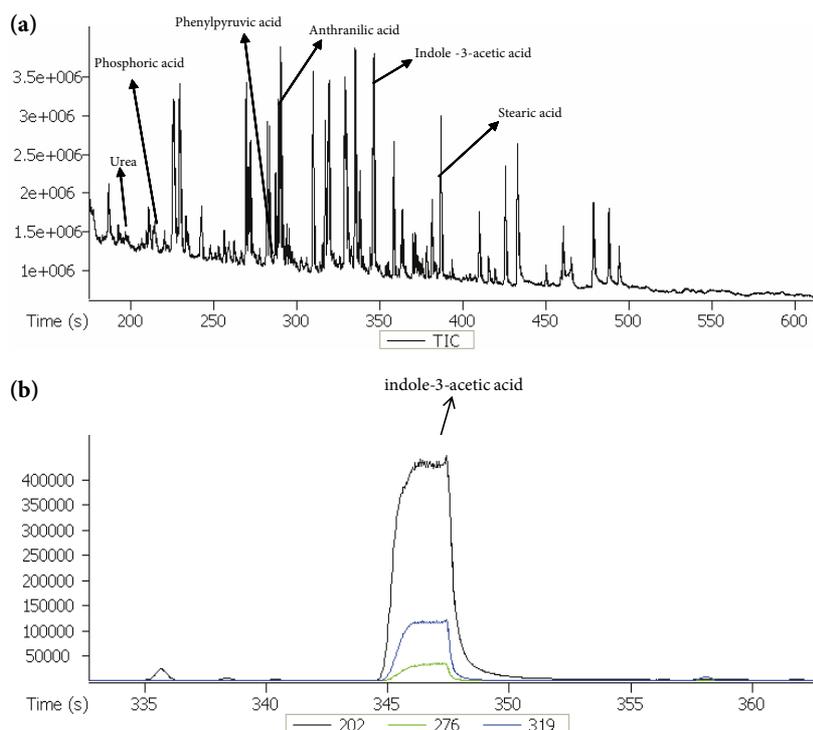


Figure 2. Ion chromatogram of As-17 generated by GC-MSTOF showing peaks of different metabolites at different retention time(s). (a) Total ion chromatogram. (b) Indole-3-acetic acid at 345.8 (s).

Table 1. Effect of rhizobacteria on shoot length (cm) of *Arabidopsis thaliana* L.

Strains	Wild type and mutant lines			
	Col-0	<i>aux1-7</i>	<i>axr4-1</i>	<i>eir1-1</i>
Control	22.61 ± 0.86 (a)	30.33 ± 0.72 (b)	26.38 ± 0.78 (ab)	28.66 ± 0.52 (bcd)
<i>B. megaterium</i> MiR-4	30.11 ± 0.84 (ef)	28.77 ± 0.81 (ab)	25.16 ± 0.68 (ab)	26.72 ± 0.76 (ab)
<i>B. pumilus</i> DaR-2	29.77 ± 0.93 (def)	27.33 ± 1.20 (a)	25.50 ± 0.79 (ab)	27.00 ± 0.63 (ab)
<i>B. circulans</i> CaR-3	27.94 ± 0.62 (bcd)	27.00 ± 1.00 (a)	26.27 ± 0.66 (ab)	27.83 ± 0.82 (abc)
<i>B. licheniformis</i> BP-1	28.77 ± 0.54 (cde)	28.66 ± 0.64 (ab)	25.44 ± 0.68 (ab)	26.22 ± 0.87 (a)
<i>B. subtilis</i> TpP-1	31.27 ± 0.62 (f)	33.27 ± 0.58 (c)	23.55 ± 0.66 (a)	30.22 ± 0.75 (de)
<i>Pseudomonas</i> sp. AvH-4	27.61 ± 0.85 (bcde)	29.22 ± 1.00 (ab)	27.83 ± 0.62 (b)	31.20 ± 0.54 (e)
<i>P. aeruginosa</i> As-17	25.72 ± 0.96 (b)	30.72 ± 0.83 (b)	25.55 ± 1.42 (ab)	30.88 ± 0.48 (e)
<i>Micrococcus</i> sp. AvR-5	29.16 ± 0.76 (cdef)	28.11 ± 0.47 (ab)	27.66 ± 1.00 (b)	28.11 ± 0.93 (abcd)
<i>E. hermannii</i> SnR-1	27.55 ± 0.88 (bcd)	29.61 ± 0.76 (ab)	26.00 ± 0.83 (ab)	29.77 ± 0.56 (cde)
<i>S. saprophyticus</i> CdR-1	26.83 ± 0.62 (bc)	28.77 ± 0.93 (ab)	26.44 ± 1.22 (ab)	28.72 ± 0.62 (bcd)

Mean ± SE of 2 repeated experiments (18 plants). Different letters within parentheses in same column indicate significant differences between treatments using Duncan's multiple range test (P = 0.05).

of bacterial strains. Nevertheless, significant increases for shoot elongation were shown by *Pseudomonas* sp. AvH-4 (9%) and *P. aeruginosa* As-17 (8%).

For wild type (Col-0), significant increases in shoot fresh biomass were observed with *Escherichia hermannii* SnR-1 (116%), *Pseudomonas aeruginosa* As-17 (107%), *Pseudomonas* sp. AvH-4 (95%), and *S. saprophyticus* CdR-1 (82%) (Table 2). Inoculation of *aux1-7* mutant plants (insensitive to auxin and ethylene) showed a stimulatory effect over the control only with *Pseudomonas aeruginosa*

As-17 (40%). Statistical analysis indicated that the majority of the treatments showed comparable results to those of the control. Auxin-insensitive mutant *axr4-1* did not show a growth response for shoot fresh biomass with most of the bacterial inoculations. For the majority of the bacterial strains, shoot fresh biomass of *eir1-1* (insensitive to ethylene) varied marginally from the control. However, significant increases in shoot fresh biomass were recorded with *B. subtilis* TpP-1 (36%) and *Pseudomonas* sp. AvH-4 (35%).

Table 2. Effect of rhizobacteria on shoot fresh weight (g) of *Arabidopsis thaliana* L.

Strains	Wild type and mutant lines			
	Col-0	<i>aux1-7</i>	<i>axr4-1</i>	<i>eir1-1</i>
Control	1.54 ± 0.12 (a)	2.86 ± 0.12 (a)	2.47 ± 0.17 (c)	2.43 ± 0.29 (abc)
<i>B. megaterium</i> MiR-4	2.38 ± 0.20 (bc)	2.65 ± 0.17 (a)	1.80 ± 0.09 (a)	2.00 ± 0.09 (a)
<i>B. pumilus</i> DaR-2	2.56 ± 0.08 (bcd)	2.97 ± 0.07 (a)	1.94 ± 0.11 (ab)	2.21 ± 0.11 (a)
<i>B. circulans</i> CaR-3	2.24 ± 0.12 (b)	3.18 ± 0.12 (a)	1.85 ± 0.16 (a)	2.16 ± 0.10 (a)
<i>B. licheniformis</i> BP-1	2.60 ± 0.30 (bcd)	3.00 ± 0.15 (a)	2.22 ± 0.12 (abc)	1.90 ± 0.07 (a)
<i>B. subtilis</i> TpP-1	2.71 ± 0.07 (bcde)	3.24 ± 0.08 (a)	1.81 ± 0.28 (a)	3.31 ± 0.19 (d)
<i>Pseudomonas</i> sp. AvH-4	3.00 ± 0.17 (def)	3.00 ± 0.25 (a)	2.50 ± 0.10 (c)	3.27 ± 0.14 (d)
<i>P. aeruginosa</i> As-17	3.19 ± 0.19 (ef)	4.00 ± 0.46 (b)	2.41 ± 0.15 (bc)	2.88 ± 0.10 (cd)
<i>Micrococcus</i> sp. AvR-5	2.42 ± 0.11 (bc)	2.95 ± 0.15 (a)	2.18 ± 0.11 (abc)	2.30 ± 0.23 (ab)
<i>E. hermannii</i> SnR-1	3.33 ± 0.13 (f)	3.24 ± 0.39 (a)	2.10 ± 0.06 (abc)	2.84 ± 0.21 (bcd)
<i>S. saprophyticus</i> CdR-1	2.80 ± 0.09 (cde)	3.18 ± 0.36 (a)	2.35 ± 0.10 (bc)	2.31 ± 0.17 (ab)

Mean ± SE of 2 repeated experiments. Mean values represents fresh weight (3 plants). Different letters within parentheses in same column indicate significant differences between treatments using Duncan's multiple range test (P = 0.05).

For number of siliqua, *Arabidopsis thaliana* (Col-0) showed highly significant increases with *B. subtilis* TpP-1 (180%), *Bacillus pumilus* DaR-2 (175%), *B. licheniformis* BP-1 (146%), *Micrococcus* sp. AvR-5 (141%), *Bacillus megaterium* MiR-4 (142%), and *B. circulans* CaR-3 (133%). Mutant plants of *aux1-7* recorded lack of growth in number of siliqua after inoculation with a majority of the bacterial strains, with the exception of *B. subtilis* TpP-1, which showed a 28% increase over the control (Table 3). The ethylene-insensitive mutant (*eir1-1*) showed variable growth responses; nevertheless, a significant increase (20%) was observed with *B. subtilis* TpP-1.

3.4. Growth experiments with *Triticum aestivum*

Growth response of *Triticum aestivum* was evaluated by inoculating plants under axenic and ambient conditions.

For seed germination, *Pseudomonas aeruginosa* As-17 recorded 26% and 13% increases under axenic and ambient conditions, respectively, over the control (Figure 3). Similarly, significant increases in shoot length (16%) and shoot fresh weight (40%) were also observed with *P. aeruginosa* As-17 (Figures 4 and 5). At final harvest, maximum increases in shoot length were shown by *Pseudomonas* sp. AvH-4 (29%), *P. aeruginosa* As-17 (22%), and *Bacillus pumilus* DaR-2 (19%) (Table 4). On the other hand, *Pseudomonas* sp. AvH-4 and *Bacillus megaterium* recorded 74% and 27% improvements, respectively, for number of tillers and spike length. Significant increases in seed weight were observed in *Pseudomonas* sp. AvH-4 (26%) and *Bacillus megaterium* MiR-4 (24%).

Table 3. Effect of rhizobacteria on number of siliqua of *Arabidopsis thaliana* L.

Strains	Wild type and mutant lines			
	Col-0	<i>aux1-7</i>	<i>axr4-1</i>	<i>eir1-1</i>
Control	34.66 ± 2.96 (a)	83.88 ± 3.49 (d)	93.22 ± 4.43 (b)	98.11 ± 7.00 (bc)
<i>B. megaterium</i> MiR-4	83.88 ± 6.47 (c)	72.33 ± 4.24 (bcd)	86.11 ± 2.51 (ab)	88.55 ± 5.79 (abc)
<i>B. pumilus</i> DaR-2	95.22 ± 7.00 (c)	72.88 ± 5.91 (bcd)	77.77 ± 4.91 (ab)	90.88 ± 3.53 (abc)
<i>B. circulans</i> CaR-3	80.77 ± 5.92 (c)	56.22 ± 6.50 (a)	81.00 ± 7.58 (ab)	80.77 ± 6.64 (a)
<i>B. licheniformis</i> BP-1	85.11 ± 4.38 (c)	64.33 ± 3.70 (abc)	84.11 ± 5.55 (ab)	84.66 ± 4.43 (ab)
<i>B. subtilis</i> TpP-1	97.00 ± 4.26 (c)	107.0 ± 4.13 (e)	69.11 ± 7.78 (a)	118.1 ± 6.23 (d)
<i>Pseudomonas</i> sp. AvH-4	42.44 ± 4.38 (ab)	76.66 ± 5.76 (bcd)	92.11 ± 6.00 (b)	100.4 ± 5.75 (bc)
<i>P. aeruginosa</i> As-17	41.66 ± 4.51 (ab)	70.55 ± 6.00 (abcd)	86.00 ± 6.88 (ab)	103.80 ± 2.54 (c)
<i>Micrococcus</i> sp. AvR-5	84.00 ± 6.73 (c)	77.33 ± 3.32 (cd)	70.88 ± 4.73 (a)	80.55 ± 3.98 (a)
<i>E. hermannii</i> SnR-1	50.44 ± 4.82 (b)	62.66 ± 2.28 (abc)	82.66 ± 7.71 (ab)	99.90 ± 3.81 (ab)
<i>S. saprophyticus</i> CdR-1	52.00 ± 2.96 (b)	62.00 ± 2.90 (ab)	94.44 ± 4.22 (b)	75.88 ± 5.67 (a)

Mean ± SE of 2 repeated experiments (18 plants). Different letters within parentheses in same column indicate significant differences between treatments using Duncan's multiple range test ($P = 0.05$).

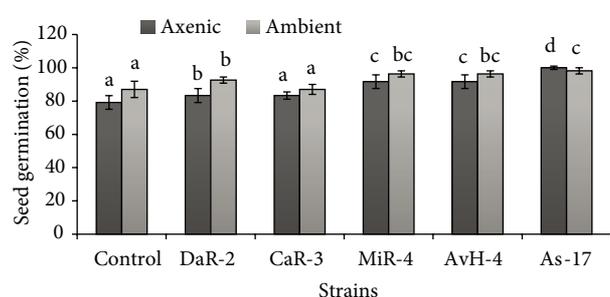


Figure 3. Effect of rhizobacteria on seed germination of *Triticum aestivum* under axenic and natural environmental conditions. Mean ± SE of 6 replicates showing the percent seed germination. Different letters on bars indicate significant differences between treatments using Duncan's multiple range test ($P = 0.05$).

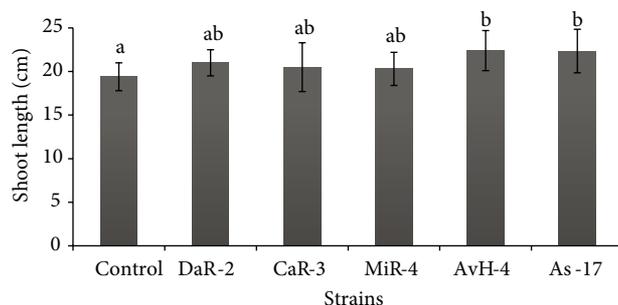


Figure 4. Effect of auxin-producing rhizobacteria on shoot length of *Triticum aestivum* under axenic conditions. Mean ± SE of 2 repeated experiments (30 plants). Different letters on bars indicate significant differences between treatments using Duncan's multiple range test ($P = 0.05$).

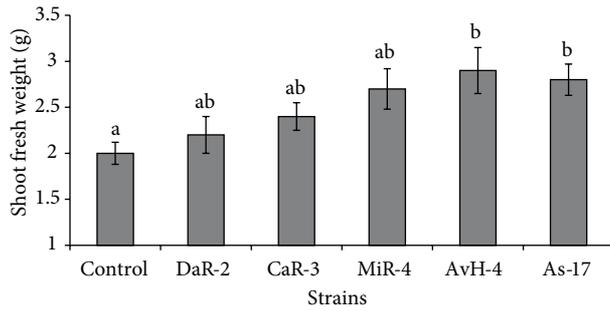


Figure 5. Effect of rhizobacteria on shoot fresh biomass of *Triticum aestivum* under axenic conditions. Mean \pm SE of 2 repeated experiments (30 plants). Different letters on bars indicate significant differences between treatments using Duncan's multiple range test ($P = 0.05$).

4. Discussion

The present study was conducted to evaluate the role of bacterial auxin signaling in enhancing the growth and yield of plants. *Arabidopsis thaliana* wild type (Col-0) and mutant lines were evaluated for their growth response to inoculation with auxin-producing rhizobacteria. The potential to elicit growth promotion of Col-0 and hormonal mutants of *Arabidopsis* varied among bacterial strains. All bacterial strains significantly enhanced shoot length of wild type (Col-0) over the control. However, inoculation of auxin- and ethylene-insensitive mutant (*aux1-7*) showed a lack of growth response that might indicate the involvement of auxin or ethylene signaling in growth enhancement. Similarly, growth promotion of auxin-insensitive mutant *axr4-1* was not elicited by the majority of bacterial strains, which also indicated the important role of bacterial auxin signaling in plant growth promotion. In ethylene-insensitive mutant *eir1-1*, analysis of data indicated that *Bacillus megaterium* MiR-4, *B. pumilus* DaR-2, *B. circulans* CaR-3, *B. licheniformis* BP-1,

and *Micrococcus* sp. AvR-5 were not significantly different than the control. However, growth promotion of *eir1-1* was elicited by *Pseudomonas* sp. AvH-4 and *P. aeruginosa* As-17. These results for shoot elongation in *eir1-1* have shown that growth elicitation by some strains may be mediated by ethylene and auxin (MiR-4, DaR-2, CaR-3, BP-1, AvR-5) or by auxin signaling alone (AvH-4, As-17). The majority of inoculations enhanced shoot fresh weight of Col-0, when compared with other plant hormone mutant lines. Increase in shoot fresh weight for *aux1-7* was recorded with *P. aeruginosa* As-17, whereas other treatments were not significantly different over the control. Auxin-insensitive mutant *axr4-1* recorded a statistically comparable response for fresh biomass in the majority of inoculations. In *eir1-1*, *Bacillus megaterium* MiR-4, *B. pumilus* DaR-2, *B. circulans* CaR-3, *B. licheniformis* BP-1, *Micrococcus* sp. AvR-5, and *Staphylococcus saprophyticus* CdR-1 did not stimulate shoot fresh biomass. However, *Bacillus subtilis* TpP-1, *Pseudomonas* sp. AvH-4, and *P. aeruginosa* As-17 showed enhancement in biomass. In Col-0 all bacterial strains enhanced the number of siliqua; however, *aux1-7* and *axr4-1* recorded lack of growth response in the majority of treatments. Ethylene-insensitive mutant *eir1-1* showed a growth response for number of siliqua with *Bacillus subtilis* TpP-1, which showed the role of auxin signaling in growth induction. It has been reported in a previous study that elicitation of growth promotion by rhizobacteria in *Arabidopsis* is associated with different signal transduction pathways. Moreover, in vitro growth involved auxin, gibberellins, brassinosteroids, and salicylic acid, whereas, in pot experiments (in vivo), ethylene signaling may be involved in growth promotion (Ryu et al., 2005). Our results also indicated that elicitation of growth by *Bacillus* strains (MiR-4, DaR-2, CaR-3, BP-1) and *Micrococcus* sp. AvR-5 may be mediated by ethylene

Table 4. Effect of rhizobacteria inoculation on the growth and yield of *Triticum aestivum* L.

Strains	Shoot length (cm)	No. of tillers	Spike length (cm)	Weight of 100 seeds (g)
Control	52.10 \pm 1.8 (a)	2.30 \pm 0.22 (a)	8.00 \pm 0.55 (a)	2.70 \pm 0.10 (a)
<i>B. pumilus</i> DaR-2	62.10 \pm 3.9 (b)	3.20 \pm 0.27 (ab)	10.15 \pm 0.70 (b)	3.20 \pm 0.17 (ab)
<i>B. circulans</i> CaR-3	51.30 \pm 3.7 (a)	2.40 \pm 0.30 (a)	9.50 \pm 0.45 (b)	3.00 \pm 0.11 (ab)
<i>B. megaterium</i> MiR-4	61.00 \pm 3.3 (b)	2.60 \pm 0.16 (a)	10.10 \pm 0.60 (b)	3.35 \pm 0.15 (b)
<i>Pseudomonas</i> sp. AvH-4	67.20 \pm 4.5 (c)	4.00 \pm 0.37 (b)	9.30 \pm 0.42 (b)	3.40 \pm 0.22 (b)
<i>P. aeruginosa</i> As-17	63.70 \pm 5.2 (bc)	2.60 \pm 0.18 (a)	9.65 \pm 0.30 (b)	3.20 \pm 0.12 (ab)

Mean \pm SE of 6 replicates (60 plants). Different letters within same column in parentheses indicate significant differences between treatments using Duncan's multiple range test ($P = 0.05$).

as well as auxin signaling. However, elicitation of growth in Col-0 and ethylene-insensitive mutant (*eir1-1*) by *B. subtilis* TpP-1, *Pseudomonas* sp. AvH-4, and *P. aeruginosa* As-17 suggested the involvement of auxin signaling alone in growth promotion. In other studies, bacterial volatile compounds have been shown to promote the growth of *Arabidopsis thaliana* (Ryu et al., 2003; Vespermann et al., 2007). On the other hand, bacterization of seeds of *Triticum aestivum* with auxin-producing rhizobacteria showed significant increases for different vegetative and yield parameters. At full maturity, *Pseudomonas* sp. AvH-4 was the most effective at enhancing shoot length (29%), number of tillers (74%), and seed weight (26%). Comparative analysis of results showed that auxin signaling of *Pseudomonas* strains (AvH-4, As-17) is involved in growth promotion of *Arabidopsis thaliana* and *Triticum aestivum*. Our results are in agreement with previous findings that auxins of microbial origin play a very vital role in plant growth promotion (Khalid et al., 2004; Ali et al., 2009a).

References

- Akhtar S, Ali B (2011). Evaluation of rhizobacteria as non-rhizobial inoculants for mung beans. *Aust J Crop Sci* 5: 1723–1729.
- Ali B, Sabri AN, Ljung K, Hasnain S (2009a). Auxin production by plant associated bacteria: impact on endogenous IAA content and growth of *Triticum aestivum* L. *Lett Appl Microbiol* 48: 542–547.
- Ali B, Sabri AN, Ljung K, Hasnain S (2009b). Quantification of indole-3-acetic acid from plant associated *Bacillus* spp. and their phytostimulatory effect on *Vigna radiata* (L.). *World J Microbiol Biotechnol* 25: 519–526.
- Baca BE, Elmerich C (2007). Microbial production of plant hormones. In: Elmerich C, Newton WE, editors. *Associative and Endophytic Nitrogen-Fixing Bacteria and Cyanobacterial Associations*. Dordrecht, Germany: Springer, pp. 113–144.
- Bektaş E, Cüce M, Sökmen A (2013). In vitro germination, protocorm formation, and plantlet development of *Orchis coriophora* (Orchidaceae), a naturally growing orchid species in Turkey. *Turk J Bot* 37: 336–342.
- Bloemberg GV, Lugtenberg BJJ (2001). Molecular basis of plant growth promotion and biocontrol by rhizobacteria. *Curr Opin Plant Biol* 4: 343–350.
- Domenech J, Ramos SB, Probanza A, Lucas GJA, Gutierrez MFJ (2007). Elicitation of systemic resistance and growth promotion of *Arabidopsis thaliana* by PGPRs from *Nicotiana glauca*: a study of the putative induction pathway. *Plant Soil* 290: 43–50.
- Jonsson P, Gullberg J, Nordström A, Kusano M, Kowalczyk M, Sjöström M, Moritz T (2004). A strategy for identifying differences in large series of metabolomics samples analyzed by GC/MS. *Anal Chem* 76: 1738–1745.
- In conclusion, the growth response of wild type (Col-0) and its comparison with mutant lines of *Arabidopsis thaliana* indicated that growth induction is mediated by more than one signal transduction pathway. In the present study, auxin and ethylene signaling are involved in growth promotion in the majority of the bacterial inoculations. Nevertheless, growth stimulation by *Bacillus subtilis* TpP-1, *Pseudomonas* sp. AvH-4, and *P. aeruginosa* As-17 is associated with bacterial auxin signaling. Results of this study also suggested that PGPR–*Arabidopsis* interaction is a good model for understanding the effect of rhizobacteria on the growth of agronomically important cereal plants.

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- Vespermann A, Kai M, Piechulla B (2007). Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. Appl Environ Microbiol 73: 5639–5641.
- Vessey JK (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255: 571–586.
- Woodward AW, Bartel B (2005). Auxin: regulation, action and interaction. Ann Bot 95: 707–735.
- Yıldız M, Sağlık Ç, Telci C, Erkiş EG (2011). The effect of in vitro competition on shoot regeneration from hypocotyl explants of *Linum usitatissimum*. Turk J Bot 35: 211–218.
- Zafar M, Abbasi MK, Khan MA, Khaliq A, Sultan T, Aslam M (2012). Effect of plant growth promoting rhizobacteria on growth, nodulation and nutrient accumulation of lentil under controlled conditions. Pedosphere 22: 848–859.