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Indole Acetic Acid (IAA) Mediated Plant Growth Promotion Activity of Fluorescent Pseudomonads and *Bacillus* spp.

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This investigation was made to study the involvement of IAA in plant growth promotion and vigor of wheat plants induced with plant growth promoting rhizobacteria. Nineteen isolates of fluorescent pseudomonads and 11 isolates of *Bacillus* spp. were subjected to germination percentage and seedling vigor index test. These parameters were determined according to recommended methods by ISTA (1985). The IAA production from selected bacterial isolates, which were showing the potential function of plant growth promotion were further subjected to quantitative analysis using

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Salkowski's reagent (2% of 0.5 M $FeCl_3$ in 34% $HClO_4$) for Colorimetric detection. The vigor index and plant growth were significantly increased in Pfa-65 (43.36%) followed by B-18 (42.58%), Pfa-77 (40.83%), B-24 (40.68), Pfa-37(38.82%) and B-38 (38.52). The maximum concentration of IAA was detected in the isolate B-24(36.66 µg/ml) followed by Pfa-65(36.16 µg/ml), B-18(35.98 µg/ml) and Pfa-77(35.72 µg/ml). A positive correlation was observed between concentration of IAA produced and germination percentage with PGPR treatments (correlation coefficient was 0.94). The correlation coefficient was 0.96 between IAA produced and percent increase in vigor index with the treatments. Our data suggests that IAA, a growth promoting hormone, might be the key factor to promote the plant growth directed by the rhizobacteria

Keywords: Bacillus spp.; fluorescent pseudomonads; germination percentage; Indole-3-Acetic Acid (IAA); Plant Growth Promoting Rhizobacteria (PGPR); vigor index.

1. INTRODUCTION

The rhizosphere is a highly favorable habitat for the proliferation of microorganisms and exerts a potential impact on plant health and soil fertility [1]. The volume of microbes in soil surrounding roots is influenced chemically, physically and biologically by the plant root and are attracted by the nutrients exuded from the plant roots. This "rhizosphere effect" was first described by Hiltner [2]. An important group of microbial communities existing in rhizosphere, that exert beneficial effects on plant growth upon root colonization were first defined by Joseph Kloepper and Milton Schroth and termed as plant growth-promoting rhizobacteria (PGPR) [3].

PGPR could directly or indirectly influence on plant growth and development. The direct growth promoting mechanisms involve nitrogen fixation, solubilization of minerals, production of phytohormones like auxins, gibberellins and cytokinins. The indirect approach occurs when PGPR prevent the deleterious effects of plant pathogens by production of certain metabolites such as siderophores, antibiotics and enzymes like glucanase or chitinase which may act as antagonistic products with the capability of inhibiting or terminating the growth of pathogenic microbes [4].

Plant growth benefits from the addition of PGPR include the increase in germination rates, root growth, yield including grain, leaf area, chlorophyll content, magnesium, nitrogen and protein content, hydraulic activity, tolerance to drought and salt stress, shoot and root weights and delayed leaf senescence. Auxin, one of the plant hormones, is known to be involved in cell division and cell enlargement, differentiation of phloem and xylem, root initiation on stem cuttings and also the development of branch roots. Auxin also mediates the tropism (bending) response to gravity and light [5]. Ahmed and Hasnain [6] reported that auxin-producing *Bacillus* spp. inflicts a positive effect on *Solanum tuberosum*'s growth. The most active and famous auxins in plants is indole-3-acetic acid (IAA) [7] which plays extensive roles in the physiological processes in plants. A low amount of IAA can stimulate primary root elongation, whereas high IAA levels decrease primary root length, increase root hair formation, and stimulate the formation of lateral roots [8,9]. Thus, plants have greater access to soil nutrients as IAA increases both the root surface area. This hormone is very commonly produced by PGPR. Colorimetric method is the simplest method and has long been employed for the detection of indole-3-acetic acid (IAA) produced by plants and microorganisms [10-12].

Depletion of nutrient supply in the agricultural soils, and consequently, the sizable gap between achievable and actual yields in various crops and crop protection are the major limitations in agriculture. The researches, therefore, are focused on restructuring the crop rhizospheres for improving and sustaining the nutrient supply in the soils to enhance the yield. Rhizosphere is a rich resource of microbes and should be explored for obtaining potential PGPR to enhance the plant growth and yield. Among different rhizobacteria, the fluorescent *Pseudomonas* spp. and *Bacillus* spp. have been studied for decades to understand mechanisms of plant growth promotion and suppression of soil borne plant pathogens. These PGPR have a tremendous potential to be involved in the integral part of sustainable agriculture.

2. MATERIALS AND METHODS

2.1 The Collection of Soil and Root Samples

Extensive collection of rhizospheric soil and root samples were carried out from wheat tomato,

onion, marigold and coriander plants from the plains of Uttarakhand. To collect rhizospheric soil plants were gently and carefully uprooted. The soil tightly adhering to the root was collected in polythene bags. Roots were cut from the tip into 2-3 cm bits and were used for isolation of rhizoplane inhabiting PGPR.

2.2 Serial Dilution of Soil and Root Samples

Soil samples were air dried for four hours at room temperature. Isolation of rhizobacteria was done by employing serial dilution technique [13]. 10 grams soil was suspended in 100 ml of sterile distilled water (1:10) and stirred well. The soil particles were allowed to settle down. The 10 ml of clear supernatant was then transferred to another flask containing 90 ml of sterile distilled water. Serial dilution in this way was carried out till a dilution of 1:1,000,000 was reached.

The root samples were washed with distilled water and dried with blotter paper. One gram of root sample was transferred to 100 ml sterile water in 250 ml conical flask and shaken for 20 minutes in a rotary shaker to dislodge the bacteria adhering to the root surface. Serial dilution of rhizoplane sample was carried out similar to rhizospheric soil to reach a dilution of 1:1,000,000.

2.3 Isolation of Fluorescent Pseudomonads

Kings B media [14] was used for selective isolation of fluorescent pseudomonads from the rhizosphere and rhizoplane. One ml of prepared soil suspension or root suspension was poured over Petri plates seeded with King's B media under sterile condition. The plates were incubated overnight at 28±1°C. Observation for appearance of fluorescent colonies under UV ray torch was recorded after 24 h. Individual colonies were picked up and maintained in pure culture for further study.

2.4 Isolation of *Bacillus* spp

Nutrient agar (NA) media was used for isolation of *Bacillus* spp. from the prepared soil and root suspension. The soil suspension and root suspension were subjected to heat treatment in water bath at 80°C for 10 min. Heat treatment was used to bias the selection towards spore forming bacteria [15]. The prepared media was poured in Petri plates and 1 ml of prepared soil and root suspension was poured over NA seeded plates. The plates were incubated for 24 h to 36 h at 30°C. Individual colonies were finally picked and further identification process was carried out using biology system.

2.5 Purification of Bacterial Isolates

Individual colonies of bacteria were picked up and streaked over media seeded Petri plates. When pure culture with single type of colony was obtained, it was transferred to slants and stored at 4°C. Pure cultures of isolates were maintained by sub culturing after every two weeks. Bacterial isolates grown in nutrient broth were transferred to 30% glycerol stock for long term storage in 2 ml screw capped vials and were stored at -20°C.

2.6 Seed Treatment

Seed treatment for growth promotion study was done with 50 ml suspension of bacterial cultures. A single 3 to 4 days old bacterial colony of each isolate was inoculated in100ml flask containing 50 ml nutrient broth and grown overnight at 28°C in an incubator shaker. Wheat seeds of variety UP 262 used for the study were obtained from Seed Production Centre, Pantnagar. The seeds were surface sterilized with 1% sodium hypochlorite solution for 2-3 min and then washed two to three times with sterile double distilled water. After complete drying, about 100 seeds were added in each flask containing bacterial cultures and were incubated for 24 h at room temperature. About 100 seeds were added in a flask containing sterile distilled water. It was also incubated for 24 h.

2.7 Efficacy of PGPR Isolates on wheat Seedling Growth *In vitro*

Plant growth promoting activity of PGPR strains were assessed based on the seedling vigor index using the standard blotter paper method. The experiment was carried in 90 mm diameter Petri plates over which three blotter paper moistened in distilled water were layered. Twenty five seeds treated with bacterial cultures were placed over each blotter paper laid Petri plates. Three replications were kept for each treatment. Wheat seeds treated with distilled water served as the control. Plates were incubated at 22 ± 2°C for seven days under alternate cycles of twelve hours of light followed by darkness. Germination percentage and seedling vigor index were determined according to recommended methods by International Seed Testing Association (1985).

Total number of germinated seeds were counted starting from third day after plating when radicals appeared till seventh day. Root length and shoot length of individual seedlings were measured and the germination percentage of seeds was recorded. Germination percentage and vigor index were calculated using the following formula:

Germination %= [total number of seeds germinated/ total number of seeds plated] x 100

Vigour index (VI) = Seedling length (Mean root length + mean shoot length) x Germination %

2.8 Study of Determinants Involved in Plant Growth Promotion: Indole Acetic Acid Detection

The thirty plant growth promoting bacterial isolates were subjected to quantitative screening for IAA production using Salkowki's reagent (2% of 0.5 M $FeCl_3$ in 34% $HClO_4$) for Colorimetric detection of IAA in liquid culture [16]. Three loops of freshly grown bacterial colonies (24- 48 h old) were suspended in 2 ml of sterile water. The OD of this bacterial suspension was adjusted to 0.6 at 660 nm (Khanna et al., 2010). 100 µl of this bacterial suspension was inoculated in 200 ml of Winogradsky's mineral solution and was maintained for 7 days at 28°C as stationary culture without shaking.

After seven days of inoculation under stationary conditions, the bacterial cultures were centrifuged at 10,000 rpm for 10 min at 4°C. One ml of the supernatant was mixed with 2 ml Salkowski's reagent in a test tube and was incubated for 30 min in dark at room temperature. One ml of sterile Winogradsky mineral solution was added in 2 ml Salkowski's reagent in a test tube. This was taken as control. IAA production in test medium was evidenced by a characteristic indication of reddish to pinkish color in the solution. Absence of IAA production was shown by pale yellow or colorless response. The amount of IAA produced was estimated by measuring the absorbance at 530 nm and using standard curve of IAA.

2.9 Statistical Analysis

Data presented are the averages of three replications of the treatments, obtained from two independent experiments. The experiments were performed in a completely randomized design. Statistical analysis was done using SPSS 16 software. Standard error of each mean was calculated to represent that on the tables. Difference of growth parameters between control and treated were determined by Paired t-test. Pearson's linear correlation coefficient between germination percentage and the concentration of IAA produced by PGPR treatments and also between percent increase in plant vigor and the concentration of IAA produced by the treatments were later analyzed.

3. RESULTS

3.1 Rhizobacterial Isolates

One hundred and twenty eight isolates of rhizobacteria were isolated from rhizosphere and rhizoplane soils of different crops (wheat, tomato, onion, marigold and coriander). Among them 80 isolates (Pfa-1 to Pfa-80) were of fluorescent pseudomonads and remaining 48 (B-1 to B-48) belonged to *Bacillus* spp. Among these 19 isolates of fluorescent pseudomonads (Pfa-2, Pfa-11, Pfa-22, Pfa-23, Pfa-24, Pfa-26, Pfa-27, Pfa-31, Pfa-35, Pfa-37, Pfa-40, Pfa-41, Pfa-46, Pfa-47, Pfa-50, Pfa-53, Pfa-65, Pfa-68 and Pfa-77) and 11 isolates of *Bacillus* spp. (B-1, B-2, B-3, B-18, B-19, B-20, B-24, B-36, B-38, B-46, B-48), which were earlier observed to be potential antagonists (unpublished research), were tested for their plant growth promotion activity.

3.2 Plant Growth Promotion Studies

According to the results of *in vitro* blotter paper tests (Table 1), there was an increase in shoot length, root length, germination percentage and vigor index in case of all the treatments. Germination percentage was highest in case of treatment with Pfa-37(85.33%) followed by Pfa-65(85%), B-18(85%), B-1(84.66%) and Pfa-77(84.33%). The best treatment in relation to plant growth promotion activity and increasing percent of vigor index was Pfa-65 (43.36%) followed by B-18 (42.58%), Pfa-77 (40.83%), and B-24 (40.68%). The rhizobacterial isolates Pfa-2, Pfa-11,Pfa-22, Pfa-23, Pfa-37, pfa-46, pfa-47, pfa50, pfa-53, pfa-77, B-2, B-18, B-19 were having germination percentage significantly higher than control ($p \leq 0.05$) as determined by paired t-test using SPSS 16 statistical software. A significant ($p \leq 0.05$) percent increase in vigor index compared to control was observed for the isolates Pfa-2, Pfa-11, Pfa-22, Pfa-27, Pfa-31, Pfa-37, Pfa-46, Pfa-50, Pfa-53, Pfa-65, Pfa-77, B-1, B-2, B-3, B-18, B-24, B-36, B-38, and B-46 as determined by paired t-test.

Table 1. *In vitro* growth promotion activity of fluorescent pseudomonads and *Bacillus* spp. on wheat as tested by blotter paper test

Bacterial isolate	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Germination %	Vigor index	%Increase in vigor index
Pfa-2	11.60 ±.680	12.50 ±.404**	24.10 ±.1.078*	80.33 ±.881*	1934.1 ±65.849*	20.60 ±2.708*
Pfa-11	11.86 ±.920	13.06 ±.753*	24.93 ±.1.673	81.33 ±.881**	2025.0 ±115.18*	26.19 ±5.705*
Pfa-22	12.06 ±.433*	13.30 ±.378*	25.36 ±.811*	83.33 ±.881**	2113.5 ±65.223*	31.92 ±4.768*
Pfa-23	10.83 ±.120	11.60 ±.251	22.43 ±.352	79.33, ±.881*	1780.3 ±47.939	11.17 ±4.376
Pfa-24	11.40 ±.550	12.53 ±.523*	23.93 ±.1.065*	79.66 ±.881	1908.6 ±105.405	18.96 ±5.371
Pfa-26	11.60 ±.624	12.46 ±.491	24.06 ±.1.109	83.00 ±1.15	1996.6 ±85.421	24.65 ±6.128
Pfa-27	12.23 ± .240*	13.26 ±.409**	25.50 ±.635*	83.66 ±.881	2134.6 ±75.204*	33.11 ±3.520*
Pfa-31	11.43 ±.523	12.56 ±.664	24.00 ±1.184	81.33 ±.881	1950.0 ±75.959*	21.69 ±4.995*
Pfa-35	11.33 ±.674	12.66 ±.638	24.00 ±.1.300	82.33 ±.881	1975.2 ±100.249	23.36 ±7.401
Pfa-37	12.36 ±.405**	13.73 ±.491**	26.10 ±.896**	85.33 ±.881**	2225.7 ±54.433**	38.82 ±2.073**
Pfa-40	11.26 ±.611	12.63 ±.523	23.90 ±.1.135	80.33 ±1.45	1916.8 ±57.736	19.70 ±5.108
Pfa-41	11.26 ±.633	12.26 ±.470	23.53 ±1.086	80.66 ±.881	1898.6 ±92.411	18.62 ±7.295
Pfa-46	11.93 ±.384	13.26 ±.392	25.20 ±.776	83.33 ±.881	2098.6 ±43.056*	31.01 ±4.073*
Pfa-47	11.53 ±.825	12.63 ±.845	24.16 ±1.669	82.00 ±1.15*	1984.1 ±158.169	23.68 ±9.101
Pfa-50	12.36 ±.545	13.56 ±.437*	25.93 ±.982	83.66 ±.881*	2171.5 ±104.170*	35.60 ±7.581*
Pfa-53	12.00 ±.404**	12.86 ±.317**	24.86 ±.705**	81.33 ±.881*	2021.9 ±50.212*	26.13 ±2.509**
Pfa-65	13.06 ±.352	13.96 ±.240**	27.03 ±.592**	85.00 ±1.154	2299.2 ±80.843**	43.36 ±3.381**
Pfa-68	11.10 ±.642	12.23 ±.698	23.33 ±1.337	81.00 ±.577	1890.4 ±112.342	17.82 ±5.776
Pfa-77	12.93 ±.260**	13.83 ±.348*	26.76 ±.523**	84.33 ±.881**	2256.7 ±33.893**	40.83 ±2.800**
B-1	12.76 ±.480	13.66 ±.233*	26.43 ±.683*	84.66 ±.881	2236.9 ±35.614**	39.64 ±3.933*
B-2	12.53 ±.638*	13.53 ±.497*	26.06 ±1.125*	84.00 ±.577*	2190.3 ±102.504*	36.68 ±6.535*
B-3	11.86 ±.260*	13.03 ±.185*	24.90 ±.435*	82.00 ±1.154	2042.8 ±64.481*	27.46 ±3.940*
B-18	12.86 ±.166*	14.00 ±.264*	26.86 ±.425*	85.00 ±1.15*	2283.9 ±51.81*	42.58 ±4.741*
B-19	11.76 ±.491	12.56 ±.592	24.33 ±1.074	81.33 ±.881**	1979. ±93.617	23.57 ±6.339
B-20	11.23 ±.480	12.20 ±.378	23.43 ±.817	80.66 ±1.201	1890.8 ±77.741	17.99 ±5.076
B-24	12.96 ±.371*	13.83 ±.284*	26.80 ±.655*	84.00 ±1.154	2252.4 ±82.480*	40.68 ±6.908*
B-36	11.63 ±.721	11.93 ±.466	23.56 ±.987*	80.66 ±1.452	1900.5 ±80.624*	18.49 ±3.866*
B-38	12.76 ±.440**	13.66 ±.328*	26.43 ±.735**	84.00 ±.577	2221.2 ±76.156*	38.52 ±3.631**
B-46	11.66 ±.448	12.43 ±.581	24.10 ±1.011	82.00 ±1.154	1978.5 ±110.659	23.45 ±6.808*
B-48	11.60 ±.556	12.56 ±.611	24.16 ±1.160	81.00 ±1.154	1956.8 ±90.821	22.06 ±5.251
CONT	10.10 ±.230	10.73 ±.328	20.83 ±.554	77.00 ±.1.154	1602.9 ±19.585	.00 ±.000

*Values are average of 3 replicates; ± = Standard error of mean; *Difference between control and treated significant at P = 0.05 as determined by Paired t-test using SPSS 16 statistical software; ** Difference between control and treated significant at P = 0.01 as determined by Paired t-test using SPSS 16 statistical software*

The germination percentage for the isolates Pfa-11, Pfa-22, Pfa-37, Pfa-77, B-19 was significantly higher than control at 1% level of probability as determined by paired t-test using SPSS 16 statistical software and for the isolates Pfa-2, Pfa-23, Pfa-46, Pfa-47, Pfa-50, Pfa-53, B-2 and B-18 it was significantly higher than control at $p \leq 0.05$. A significant percent increase in vigor index was noted at 1% level of probability for the isolates Pfa-37, Pfa-53, pfa-65, Pfa-77and B-38 compared to control. The isolates Pfa-2, Pfa-11, Pfa-22, Pfa-27, Pfa-31, Pfa-46, Pfa-50, B-1, B-2, B-3, B-18, B-24 and B-46 also showed significantly higher percent increase in vigor index than control at 5% level of probability.

3.3 Colorimetric Detection of Indole-3-Acetic Acid

The results of Salkowski's reagent test for Colorimetric indole-3-acetic acid detection (Table 2) showed that all the isolates produced some amount of Indole-3- acetic- acid. Maximum concentration of IAA was produced by the isolate B-24(36.66 µg/ml) followed by Pfa-65(36.16 µg/ml), B-18(35.98 µg/ml) and Pfa-77(35.72 µg/ml). Less amount of IAA was produced by Pfa-23(5.67 µg/ml) followed by Pfa-41 (5.87 µg/ml) and B-36 (7.4 µg/ml). This is indicating that tested isolates are bearing variable IAA production activity. The isolates which produced more amounts of IAA were also earlier (Table 1) reported to show significant increase in growth promotion parameters compared to control.

A positive correlation was found between germination percentage by different PGPR treatments and the amount of IAA (µg/ml) produced by those treatments (Table 3, Fig. 1). A correlation coefficient of 0.94 was observed between the two. Correlation was significant at the 0.01 level (2-tailed).

Table 2. Concentrations of IAA produced by different PGPR isolates as obtained from colorimetric Salkowski's reagent test

Bacterial isolate	IAA Concentration (µg/ml) *
Pfa-2	13.07 ±1.496
Pfa-11	14.23 ±1.758
Pfa-22	21.98 ±1.954
Pfa-23	5.67 ±.374
Pfa-24	11.50 ±.645
Pfa-26	19.01 ±2.317
Pfa-27	31.40 ±2.052
Pfa-31	12.53 ±.985
Pfa-35	14.15 ±1.084
Pfa-37	34.62 ±2.621
Pfa-40	8.50 ±.883
Pfa-41	5.87 ±1.315
Pfa-46	24.82 ±2.813
Pfa-47	12.39 ±.863
Pfa-50	29.69 ±2.928
Pfa-53	10.49 ±1.384
Pfa-65	36.16 ±3.374
Pfa-68	12.18 ±.701
Pfa-77	35.72 ±2.902
B-1	32.54 ±2.878
B-2	27.31 ±3.578
B-3	20.16 ±2.284
B-18	35.98 ±3.228
B-19	9.92 ±1.065
B-20	8.71 ±2.576
B-24	36.66 ±5.166
B-36	7.43 ±1.949
B-38	33.63 ±3.504
B-46	12.65 ±1.046
B-48	10.73 ±.852

** Values are average of three observations obtained from descriptive analysis by SPSS 16; ± = Standard error of mean*

Table 3. Pearson Correlation between Germination % and IAA concentration (µg/ml)

		Germination %	IAA concentration(µg/ml)
Germination %	Pearson Correlation	1	.940**
	Sig. (2-tailed)		.000
	N	30	30
IAA concentration	Pearson Correlation	.940**	1
	Sig. (2-tailed)	.000	
	N	30	30

**. Correlation is significant at the 0.01 level (2-tailed).

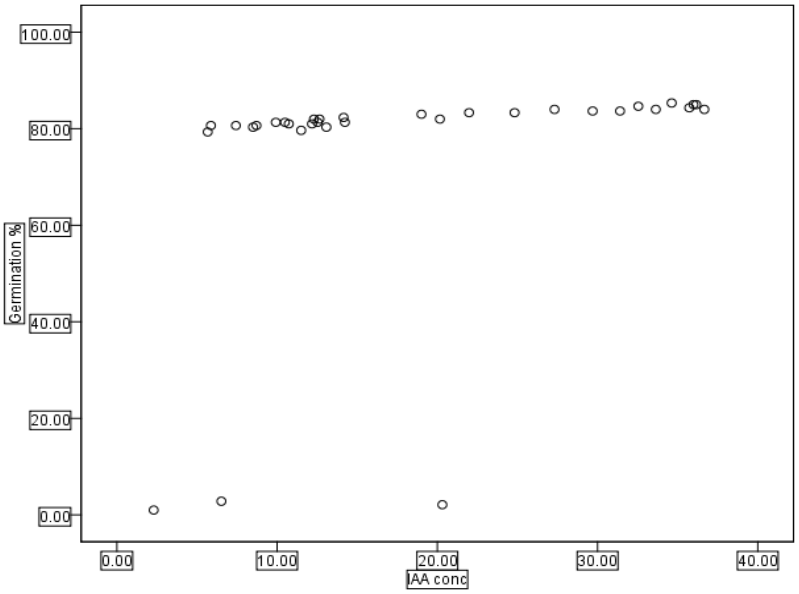

Fig. 1. A significant positive correlation between germination percentage on treatment with PGPR isolates and the concentration of IAA (µg/ml) produced by the isolates (Correlation Coefficient 0.94, p<0.01)

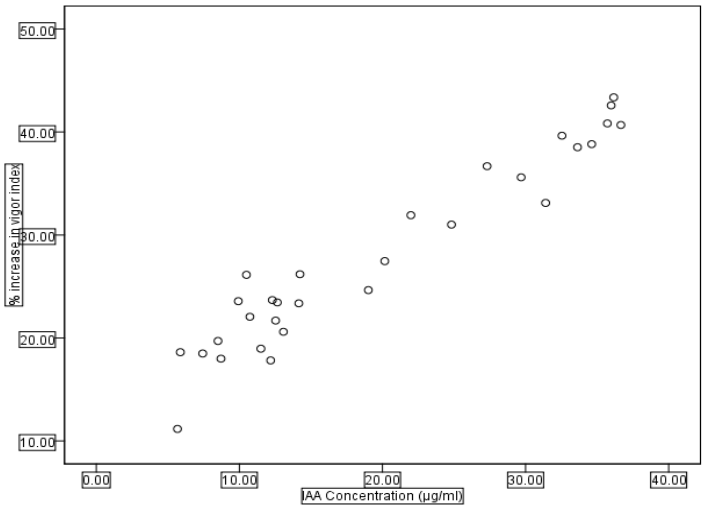

Fig. 2. A significant positive correlation between % increase in vigor index of wheat plants on treatment with PGPR isolates and the concentration of IAA (µg/ml) produced by the isolates (Correlation Coefficient 0.9636, p<0.01)

Table 4. Pearson Correlation between % increase in vigor index and IAA concentration (µg/ml)

		% increase in vigor index	IAA concentration
% increase in vigor index	Pearson Correlation	1	.964**
	Sig. (2-tailed)		.000
	N	30	30
IAA concentration	Pearson Correlation	.964**	1
	Sig. (2-tailed)	.000	
	N	30	30

A positive correlation was also found between percent increase in vigor index by different PGPR treatments and the amount of IAA (µg/ml) produced by those treatments (Fig. 2). A correlation coefficient of 0.96 was observed between the two. Correlation is significant at the 0.01 level (2-tailed).

4. DISCUSSION AND CONCLUSION

PGPR like fluorescent pseudomonads and *Bacillus* spp have high efficiency in host root colonization and plant growth metabolites production as well. Significant increase in plant growth parameters like shoot length, root length, germination percentage and vigor index in plants treated with PGPR have been reported from the production of plant growth regulators such as auxins, gibberellins, cytokinins and ethylene. It has been evidenced many times that auxins produced by Rhizobacteria are responsible for growth promotion. Shukla *et al.* [17] reported that fluorescent pseudomonad isolates induced crop specific growth promoting response because of their multiple PGPR traits. Wang *et al.* [18] reported treatment with the *Bacillus* spp. significantly enhanced the plant height and fresh weight of tobacco while clearly lowering the disease severity rating of the tobacco mosaic virus (TMV) at 28 days post-inoculation. Ramezanpour *et al.* [19] studied the plant growth promoting properties (indole acetic acid production, phosphate solubilization and siderophore production) and genetic diversity of isolated Pseudomonas strains. All the isolates were able to produce IAA but in variable amount. Khamna *et al.* [20] reported the production of IAA by a collection of *Streptomyces* spp. isolated from 14 Thai medicinal plants. Khin Mya Lwin *et al.* [21] reported isolation of 18 isolates of *Bacillus* spp. and *Serratia* spp. that produced IAA.

Chakraborty *et al.* [22] reported that the bacterium *Bacillus pumilus* showed positive PGPR traits *in vitro* such as phosphate solubilization, siderophore production, volatile production and IAA secretion. Rabha *et al*. [23] isolated numerous beneficial bacteria with multiple plant growth promoting attributes from the rhizosphere of some plant. Apart from other PGPR activities, four isolates produced the plant growth regulator IAA among which highest producer was the Bacillus isolate TSA-1A5. Patel *et al.* [24], Trivedi *et al.* [25] and Saranya and Sowndaram [26] reported that fluorescent Pseudomonas is a heterogenous group of growth promoting rhizobacteria that regulate plant growth by releasing secondary metabolic compounds viz., indole acetic acid (IAA), siderophores, ammonia and hydrogen cyanide. Indole-3-acetic acid (IAA), a key phytohormone, not only influences plant growth and development it also plays a vital role in plant-microbe interactions [27]. Eco-friendly plant disease management strategies provide great benefits farmers as well as consumers. IAA is one of the most common auxin which plays a crucial roles in plant growth promotion such as cell division, cell differentiation and development of fruits. Homeostasis of IAA is important to maintain optimum hormonal balance suitable for normal plant growth [28,29].

From the *in vitro* growth promotion test results (Table 1), it was observed that the isolates Pfa-65, B-18, Pfa-77, B-24, Pfa-37 and B-38 caused maximum increase in vigor index which is comparable to the results obtained from IAA test (Table 2) as the same isolates showed maximum production of IAA. A correlation coefficient of 0.94 (Table 3; Fig. 1) between germination percentage and the concentration of IAA produced by the PGPR isolates and a correlation coefficient of 0.96 (Table 4; Fig. 2) between percent increase in vigor index and the concentration of IAA produced by the PGPR isolates confirms the positive correlation between growth promotion activities and IAA producing

ability of the PGPR isolates. From the results it can be concluded that the plant growth promotion activity of the rhizobacteria might be due to production of IAA, which is a growth promoting hormone [30].

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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