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Effect of native strains of plant growth promoting rhizobacteria on growth and yield of Isabgol (*Plantago ovata*)

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ABSTRACT

An experiment was conducted to evaluate the growth promotion in isabgol *(Plantago ovata* Forsk.) with native rhizobacteria strains (ISB-2, ISB-5, ISB-8, ISB-9, ISB-10, ISB-125, ISB-15 and ISB-28) isolated from the rhizospheric soils collected from western semi-arid region of India. These isolates were tested for their indole acetic acid production, insoluble phosphate solubilization and ability to promote germination of isabgol seeds under controlled conditions. Three rhizobacterial isolates (IBS-5, IBS-9 and IBS-28) were found promising towards enhancing the growth and yield of isabgol plants. The isolate ISB-5 was identified as *Azotobacter vinelandii*, ISB-9 as *Pseudomonas stutzeri*, and ISB-28 as *Bacillus aryabhattai* based upon their biochemical and molecular characterization. The highest seedling vigour index was recorded for *B. aryabhattai* (316.52±3.02) followed by *A. vinelandii* (310.22±7.56) and the lowest seedling vigour was recorded with ISB-10 which was found to be at par with control. The maximum shoot dry weight at harvest was recorded with *B. aryabhattai* (2.33g) being at par with *P. stutzeri* and the minimum shoot dry weight was for control. The highest seed yield per plant (0.72 g) was observed with *B. aryabhattai* followed by *A. vinelandii* (0.61 g).

Key words: Indole acetic acid, Isabgol, Plant growth promoting rhizobacteria, *Plantago ovata*, Phosphate solubilization index, Seedling vigour index

Isabgol (*Plantago ovata* Forsk.), a native of Persia, is an annual herb grown as a cash crop in the western semi-arid region of India. Isabgol also known as Psyllium belongs to family Plantaginaceae with about 200 species out of which only two species, namely *P. ovata* and *P. psyllium* (2n= 8), have been commercially cultivated.

The biosynthesis of the secondary metabolites is controlled genetically and affected strongly by edaphic factors, of which plant microbes interaction is one of the important biotic factor besides the environmental conditions (Koocheki *et al*. 2007). Beneficial free-living rhizobacteria, which are the determinants of plant health and soil fertility, are usually referred to as plant growth-promoting rhizobacteria (PGPR). Nowadays, the use of PGPR is steadily increasing in agriculture, as it offers an attractive way to reduce the use of chemical fertilizers, pesticides, and related agrochemicals (Rana *et al*. 2012). PGPRs constitute a significant part of the protective flora that benefit plants by enhancing root function, suppressing disease and accelerating growth and development. The root system plays an important role in plant productivity because roots explore the soil for uptake of essential nutrients. It is clear that PGPR can change the endogenous levels of phytohormones such as auxins and other plant hormones including cytokinin and gibberellins and a particular PGPR strain may enhance plant growth and development using different mechanisms such as phytohormones synthesis, solubilization of phosphorus (P), fixation of atmospheric nitrogen (N), production of siderophore and inducing systemic resistance against plant pathogen (Glick 2004). Interaction of PGPR with host plants is an intricate and interdependent relationship involving not only the two partners but other biotic and abiotic factors of the rhizosphere region (Dutta and Podile 2010). Soil inoculations of beneficial microorganisms will not response positively unless the environment supports growth and survival of the introduced microorganisms in new area (Egamberdieva and Kucharova 2009). Therefore, to get significant benefits of microbial inoculation, the selection of native PGPR bacterial strains is a beneficial. However, reports regarding the bioinoculation effect of these PGPR strains in medicinal plants and particularly in *P. ovata* are scarce and little or no research work was reported on effect of native rhizobacteria for growth and yield of Isabgol crop from semi-arid western region of India. Hence, the present study was undertaken to investigate the growth promoting effects of native plant growth promoting

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rhizobacterial isolates on agro-morphological traits like plant height, root length, root weight and total seed yield in Isabgol.

MATERIALS AND METHODS

The soil samples were collected from Isabgol fields from western semi- arid region of India which is the prominent cultivation area of Isabgol. Seventy different rhizobacterial isolations were made from bacterial colonies grown on selective and specific growth media. Out of these rhizobacterial isolates eight isolates were found promising with respect to indole acetic acid (IAA) production and phosphate solubilization during preliminary laboratory studies. On the basis of plant growth promoting attributes these native rhizobacterial isolates were used for seed treatment of Isabgol under pot experiment for two consecutive years (2012-13 and 2013-14) during winter season under at ambient conditions at ICAR-National Research Centre on Seed Spices, Ajmer, India.

Production of IAA by the bacterial isolates was estimated using Salkowski reagent as descrided by Patten and Glick (2002). The rhizobacterial strains were grown in Luria Bertani medium at the L-tryptophan concentrations of 200 µg/ml and incubated at 25±1^{0}C. After 3 days of incubation, aliquots of bacterial cultures were centrifuged at 13 000 × g for 10 min. One ml of supernatant was mixed with 4 ml of Salkowski indicator (150 ml of concentrated sulfuric acid, 250 ml of distilled water, and 7.5 ml of 0.5 M $FeCl_3.6H_2O$). The solution was kept at room temperature for 20 min and light absorbance was immediately measured at 535 nm using spectrophotometer. The amount of auxin was calculated using the standard graph of indole-3acetic acid (IAA).

Phosphate solubilization assay was performed in Pikovskaya's agar medium containing tricalcium phosphate (Pikovskaya 1948). Bacterial culture was spot inoculated on the medium and incubated at 25±1^{0}C for 48 h. Development of a clear zone around the colonies indicates the P solubilization capacity of the isolates and the rhizobacterial strains showing clear zone around the colonies were measured for calculation of phosphate solubilization index [= the ratio of the total diameter (colony + halo zone) to the colony diameter].

The rhizobacterial isolates were grown on Tryptic Soy agar (TSA) medium for 48 h at 25±1^{0}C. Subsequently, the bacterial lawn was scraped into sterile distilled water and centrifuged at 6 000 rpm for 5 min and the pellet obtained was suspended in sterile distilled water. The optical density of the suspension was adjusted using a UV-visible spectrophotometer to obtain a final density of 1x10^7 colony forming units (cfu)/ml. Isabgol seeds (variety Bajrang-2), were surface sterilized with 5% (w/v) sodium hypochlorite for 5 min and rinsed thoroughly with sterile distilled water. Rhizobacterial inoculation of the seeds was achieved by soaking 1 g of seeds in 10 ml of bacterial suspension (with 1×10^7 cfu/ml), using 0.2% sterilized carboxy methyl cellulose (CMC) as sticker. They were incubated at 25±1^0 C in a rotary shaker for 30 min to facilitate attachment of bacterial cells to the seed coat. Later, the seeds were allowed to dry in an incubator at 30±1^0 C for 20 min. Seeds treated only with sterile distilled water followed by CMC represented control. Seedlings were watered as per need basis regularly. For germination test paper towel method was used and vigour index was calculated after 10 days of germination following the of AOSA (1991) method.

Vigor index (VI) = [Seedling length (cm) × Germination percentage]

The pot experiment was carried out to evaluate the growth promotion in Isabgol seeds treated with rhizobacterial isolates (strains designated as ISB-2, ISB-5, ISB-8, ISB-9, ISB-10, ISB-125, ISB-15 and ISB-28) along with a control without any bacterial inoculation. Seeds were sown in earthen pots filled with presterilized soil. The inoculated seeds were sown in each pot of 15 cm diameter filled with 3 000 g sandy loam soil obtained from the experimental farm of ICAR-National Research Centre on Seed Spices, Ajmer, India. The pot experiment was set up in a completely randomized design with four replications. At the end of the experiment, the plants were harvested and different growth parameters were recorded.

The experimental pot soil was sandy loam in texture. Physicochemical characteristics of the soil were EC 0.18 dS/m, pH 8.2 and organic carbon 0.23%. Soil available N, P_2O_5 and K_2O were 178.0, 12.0 and 85 kg/ha, respectively. The plants were harvested at maturity (~125 days after sowing) and then their seeds and straw yield were recorded. Different growth attributes namely plant height, root dry weight and shoot dry weight were recorded at 30 and 60 days after sowing (DAS). The dry weight (shoot and root) was determined after keeping the plant materials at 70°C for 72 hr. To determine yield and yield parameters five plants were sampled from each pot to measure yield components and per plant yield.

Total chlorophyll and carotenoids content in leaves were estimated using Dimethyl Sulfoxide (DMSO). For this 100 mg of fresh leaf portion was kept into a test tube containing 5 ml of DMSO. The test tube was then placed in an oven at 60^{o}C for about 2 hr or more (if required) to facilitate complete extraction of the pigments. After 2 hr and after attaining the room temperature, absorbance was recorded at 649, 665 and 480 nm with spectrophotometer (Spectrophotometer-119 Lab India UV 3000) running a multiple wavelengths programme. DMSO was used as blank. Calculations for different pigments were made according to Wellburn (1994).

Chl 'a' = $(12.19 \times A_{665})-(3.62 \times A_{646})$

Chl 'b' = $(25.06 \times A_{649})- (6.5 \times A_{665})$

Total chlorophyll = Chl 'a' + Chl 'b'

Total carotenoids Cx+c = $(1000 \times A_{480}–1.29$ Chl 'a' - 53.78 Chl 'b')/220

where Chl'a' = chlorophyll a, Chl'b'= chlorophyll b, Cx+c= concentration of xanthophylls and carotenes. Quantities of all these pigments were calculated in mg/g tissue fresh weight.

The activity of peroxidase (POX enzyme) was assayed

Table 1 Production of IAA and phosphate solubilization by rhizobacterial isolates from Isabgol field

Rhizobacterial isolates	IAA production (ppm)	Phosphate solubilization index	Molecular Identification	NCBI Genbank accession
ISB-2	40.60		NA*	
ISB-5	40.72		*Azotobacter vinelandii*	KF365886
ISB-8	27.76		NA	
ISB-9	11.92	2.04	*Pseudomonas stutzeri*	KF365887
ISB-10	11.04	1.25	NA	
ISB-12	32.56	1.36	NA	
ISB-15	34.82	1.64	NA	
ISB-28	26.85	2.35	*Bacillus aryabhattai*	KF365888
CD(P=0.05)	2.47	0.68		
SEm±	0.86	0.26		

*NA = not analyzed for molecular identification.

according to Chen *et al*. (2000) using plant extracts and measured by spectrophotometer at 470 nm, into which 7.5 µl guaiacol (50 mM in the mixture) and 792 µl Tris HCl buffer (0.05 M, pH 6.0) was added. The reaction was initiated by adding 100 µl of 0.6 M hydrogen peroxide. A blank consisting of guaiacol, Tris HCl buffer and hydrogen peroxide was used to set 100% absorbance. The enzyme activity was expressed as IU/min/mg fresh weight.

All pot experiments were performed in completely randomized block design with four replications in each treatment and the assay was repeated twice. The data were subjected to analysis of variance and mean values in each treatment by using SPSS package and the signiûcance of the treatments was calculated at 5% level of signiûcance.

RESULTS AND DISCUSSION

Plant growth promoting attributes of selected rhizobacterial strains

From initial 70 rhizobacterial isolates form Isabgol rhizosphere, eight were selected with respect to plant growth promotion. These promising rhizobacterial isolates were selected from the previous rhizobacterial isolations obtained from the isabgol cultivating field of western semi- arid region of India (Mishra *et al.* 2015). These were screened for auxin production and phosphate solubilization potential for selection of the promising isolates. The strain ISB-5 was identified as *Azotobacter*, ISB-9 as *Pseudomonas* and ISB-28 as *Bacillus*. Strain ISB-5 and ISB-9 were Gram negative, non-spore forming and non-capsulated, semi-translucent, rounded, smooth mucoid and rod shaped colonies with 2-4 mm in diameter while ISB-28 was gram positive and spore forming. All of them grew fast and showed positive oxidase activities. Strain ISB-5 and ISB-9 were able to grow in pH rage 5.0-9.0 and tolerated 4% NaCl on nutrient agar growth medium. Strain ISB-9 also grows up to 45^0 C temperature (data not presented). Strain ISB-28 had ability to form clear halo around spot inoculation on the Pikovskaya's agar plates. Such clearing zone around the bacterial colonies showed phosphate solubilization ability. All the isolates were found positive for production of IAA into culture filtrates obtained from the growth media containing tryptophan. Based on preliminary germination experiments on Isabgol seeds, IAA production assay and phosphate solubilization potentials three rhizobacterial isolates were found effective as PGPR for Isabgol and were also identified through molecular techniques. The rhizobacterial culture ISB-5 was found to be *Azotobacter vinelandii* (GenBank Accession Number: KF365886), ISB-9 was found to be *Pseudomonas stutzeri* (GenBank Accession Number: KF365887) and ISB-28 was found to be *Bacillus aryabhattai* (GenBank Accession Number: KF365888) based on nucleotide homology and phylogenetic analysis (Table 1)**.** The most effective rhizobacterial strain ISB-28 that was identified as *B. aryabhattai* has been submitted with Microbial type's culture collection at IMTECH, Chandigarh, India as MTCC-11834. Similarly, nitrogen-fixing Bacilli was isolated from the rhizospheres of wheat, maize, ryegrass, and willow in Beijing region of China to evaluate their potential use in plant growth improvement (Ding *et al*. 2005). One siderospore-producing strain, *Bacillus subtilis* CAS15, also reported as a growth-promoting agent that is effective on pepper growth (Yu *et al*. 2011). Rana *et al*. (2011) characterized a PGPR *Bacillus* sp. and evaluated its potential as inoculants for wheat. Strains of *Bacillus* that produce resistant spores cause long viability and can be easily applied as microbial inoculants which also show significant protective as well as growth-promoting activities (Ali *et al*. 2009). Many attempts have been made to isolate effective PGPR from various plants. However, bacteria are greatly diverse among different crops. The mechanisms underlying stimulation of plant growth may vary depending on plant and bacterial species along with other parameters including soil and environmental conditions and plant–microbe interactions may affect the ability of PGPR to express different attributes (Dey *et al*. 2004).

Effect of rhizobacterial inoculation on seed germination

The maximum germination percentage (66.80) was recorded with ISB-28 which was statistically at par with ISB-5 and ISB-15 while minimum was recorded with ISB-8 (50.34%) followed by control (Table 2). In case of radicle

Table 2 Effect of seed treatment with different rhizobacterial isolates on seed germination and seedling dry weight of Isabgol*

Rhizobacterial isolates	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling dry weight (g)
ISB-2	59.78	3.70	2.38	0.082
ISB-5	67.40	4.46	2.56	0.125
ISB-8	50.34	4.23	2.06	0.125
ISB-9	59.73	3.76	3.14	0.185
ISB-10	59.08	2.50	1.85	0.119
ISB-12	63.73	4.43	2.60	0.124
ISB-15	66.77	3.16	2.69	0.169
ISB-28	66.80	4.64	2.30	0.174
Control	53.41	3.00	1.51	0.086
Mean	60.78	3.76	2.34	0.132
CD(P=0.05)	1.97	0.57	0.49	0.008
SEm±	0.66	0.19	0.16	0.003

*10 days after germination under *in vitro* condition.

Table 3 Effect of different rhizobacterial isolates on the biometrical growth attributes of Isabgol plants

Treatment	30 DAS*			60 DAS*		
	Shoot length (cm)	Root length (cm)	Plant total dry weight (g)	Shoot length (cm)	Root length (cm)	Plant total dry weight (g)
ISB-2	5.76	3.34	0.264	11.17	5.28	0.58
ISB-5	5.23	2.98	0.210	10.84	5.65	0.61
ISB-8	5.21	3.24	0.288	11.87	6.45	0.46
ISB-9	6.89	3.99	0.355	13.6	7.98	0.68
ISB-10	5.12	3.56	0.250	10.35	7.21	0.74
ISB-12	4.76	2.89	0.218	9.50	5.12	0.57
ISB-15	5.01	2.75	0.225	12.63	4.85	0.71
ISB-28	8.23	3.82	0.412	15.67	6.95	0.92
Control	4.12	2.56	0.197	8.10	5.08	0.50
CD(P=0.05)	0.28	0.18	0.08	1.81	0.74	0.09
SEm±	0.10	0.09	0.03	0.76	0.23	0.03

*DAS-Days after sowing.

length, the longest was observed with ISB-28 (4.64 cm) which was at par with ISB-5, ISB-8 and ISB-12 and smallest radicle length was recorded with ISB-10 followed by control. Similarly, the maximum plumule length was recorded with ISB-9 (3.14 cm) and minimum plumule length was recorded with control (1.51 cm) which was at par with ISB-10 (Table 2). Seedling dry weight was recorded after 10 days of germination and maximum seedling dry weight was observed with ISB-9 (0.185g) followed by ISB-28 (0.174 g) and the minimum of same was recorded with ISB-2 which was at par with control as evident from Table 2. There was significant effect of seed treatment with native rhizobacterial isolates on isabgol seedling vigour index calculated after 10 days of germination under *in-vitro* conditions. The highest seedling vigour index was recorded for ISB-28 (316.52±3.02) followed by ISB-5 (310.22±7.56) and the lowest seedling vigour was recorded with ISB-10 to be at par with control. The results obtained for Isabgol seed germination experiment showed parity with Mahdavi (2013) reported similar range of isabgol seedling germination and vigour index during study of the chitosan and salinity effects on germination and vegetative growth.

Effect of rhizobacterial inoculation on growth and yield

Plant biometrical parameters such as shoot length, root length, total plant dry weight, chlorophyll content in leaves and peroxidase enzyme activity in root and shoot were analyzed at 30 and 60 DAS to assess the plant growth promotion potential of selected rhizobacterial isolates on Isabgol plants. At 30 DAS, the maximum shoot length (8.23 cm) was recorded with ISB-28 and that of minimum was observed with control. However, the maximum root length was observed with ISB-9 (3.99 cm) and the minimum shoot length was also recorded with control (Table 3) at 30 DAS. The total dry weight per plant was also recorded highest with ISB-28 and same was the minimum with control at 30 DAS. Similar trend of shoot length, root length and dry weight/plant was also observed at 60 DAS. Chlorophyll and carotenoids content are good biochemical parameters to assess the effect of rhizobacterial treatment on plant growth promotion. The maximum chlorophyll content 1.45 mg/g f. wt. and 1.47 mg/g f. wt. were recorded at 30 and 60 DAS, respectively, with rhizobacterial isolate ISB-28. Peroxidase enzyme activity was assayed to evaluate the plant defense potential as the peroxidase enzymes help plants to enhance their vitality and suppression of plant pathogen growth. The maximum peroxidase activity was observed in roots treated with ISB-5 followed with ISB-9 at 30 DAS. Similar pattern of peroxidase enzyme activity was recorded in the isabgol plant roots at 60 DAS. However, the minimum peroxidase activity was assayed with ISB-2 in case of root as well as shoot on both 30 and 60 DAS. Though, the control treatment showed higher peroxidase activity than ISB-2 which may be due to molecular signaling as a result of plant microbe interaction in the rhizosphere of treated isabgol plants. The isabgol plants matured at 120-125 DAS, showing the yellowing and senescence of leaves along with drying of spike bearing Isabgol seeds. The maximum shoot length (28.46 cm) was observed with ISB-28 which was at par with ISB-5 and ISB-9 and the minimum was recorded with control (22.25 cm). There was little variation with respect to root and shoot length at maturity stage except for control treatment (Table 4). The maximum shoot dry wt. at harvest was recorded with ISB-28 (2.33g) which was at par with ISB-9 and the minimum shoot dry wt. was for control (Table 4). Similar pattern was observed with root dry weight at harvest stage. The maximum no. of spikes per plant were recorded with ISB-9 (9.61) which was at par with ISB-5(8.45) and minimum spike per plant was recorded with control (5.50) (Table 4). However, maximum seeds per spikes were recorded with ISB-28 (53.56) which was followed with ISB-5 and the minimum seeds per spike were recorded with

Table 4 Effect of different rhizobacterial isolates on the growth and yield of Isabgol plants

Treatment	Length (cm)		Dry weight (g)		Spikes/plant	Seeds/spike	Seeds/plant	Yield/plant
	Shoot	Root	Shoot	Root	(no.)	(no.)	(no.)	(g)
ISB-2	26.46	7.18	1.71	0.07	7.32	40.26	286.3	0.52
ISB-5	27.33	8.2	1.81	0.06	8.45	43.47	350.0	0.61
ISB-8	25.8	7.67	1.76	0.05	7.88	40.94	344.0	0.58
ISB-9	27.5	8.2	2.29	0.04	9.61	35.75	205.5	0.43
ISB-10	26.5	7.5	1.69	0.04	7.12	37.25	210.2	0.42
ISB-12	26.8	7	1.65	0.05	5.74	32.70	158.0	0.38
ISB-15	25.5	7	1.73	0.09	5.35	28.65	207.0	0.43
ISB-28	28.46	7.8	2.30	0.08	7.28	53.56	402.0	0.72
Control	22.25	6	1.30	0.02	5.50	29.68	168.0	0.28
CD (P=0.05)	2.03	0.70	0.29	0.02	1.43	7.54	96.5	0.17
SEm±	0.88	0.18	0.11	0.01	0.67	2.84	38.2	0.09

control (29.68). The maximum number of seeds/plant was recorded with ISB-28 (402.0) followed by ISB-5 and the minimum number of seeds/plant was observed with control (Table 3). The highest seed yield/plant (0.72 g) was observed with ISB-28 and same was followed by ISB-5 (0.61 g). The minimum seed yield/plant (0.28 g) was recorded for control (Table 4). Similar results were reported by the earlier workers on PGPR in crops like maize, sorghum (Raju *et al*. 1999) and pearl millet (Niranjan *et al*. 2004) which revealed that under *in vitro* conditions, seed treatment with PGPR strains improved seed germination, seedling vigor, seedling emergence and seedling stand over the control. Similar improvement of seed germination parameters by rhizobacteria was reported in seed spices such as cumin and coriander that mainly grow in semi-arid western region of India (Kumar *et al*. 2013). Gholami *et al*. (2009) reported the effect of plant growth-promoting rhizobacteria (PGPR) on seed germination, seedling growth and yield of field grown maize with six bacterial strains (*P. putida* strain R-168, *P. fluorescens* strain R-93, *P. fluorescens* DSM 50090, *P. putida* DSM291, *A. lipoferum* DSM 1691, *A. brasilense* DSM 1690). Results indicated that seed inoculation significantly enhanced seed germination, seedling vigour, leaf and shoot dry weight, leaf surface area and maize seeds yield. These findings may be as a result of increased synthesis of hormones like auxins and gibberellins, which would have triggered the activity of specific enzymes that promoted early germination, besides, significant increase in seedling vigor causing better growth and yield of isabgol plants. Isabgol has been used in medicine since ancient times, but it has gained more importance as a medicinal plant in recent few decades due to its application as laxative and application of these native microbial inoculants may be useful in organic production of the isabgol crop.

Based on the findings of this research work , it may be concluded that the three native rhizobacterial isolates namely ISB-5 (*Azotobacter vinelandii)*, ISB-9 (*Pseudomonas stutzeri)*, and ISB-28 (*Bacillus aryabhattai)* indicated a congenial confirmation towards growth of Isabgol plants on the basis of effect of these on plant growth biometrical data (root length, shoot length, shoot weight and root weight) and yield attributing traits (number of spike/plant and number of seeds) were also enhanced in comparison to control. Isabgol seeds treated with these rhizobacterial strains enhanced shoot and root length as well as higher fresh biomass weight as compared to the control. The rhizobacterial isolate ISB-28 which culminated with maximum seed yield of isabgol plant, has been submitted at Microbial Type culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India as *B. arybhattai* MTCC-11834 for conservation and future application. These findings of the present study are unique of its type for Isabgol crop cultivation in India. The selected native rhizobacterial isolate *B. arybhattai* MTCC-11834 may also play a role to some extent in reducing the dependence on costly chemical fertilizers for production of Isabgol crop in western semi-arid region of India.

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