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Effect of UV-B Tolerant Plant Growth Promoting Rhizobacteria (PGPR) on Seed Germination and Growth of *Withania somnifera*

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria that colonize plant roots and enhance plant growth by a wide variety of mechanisms. Six bacterial isolates, efficient in producing PGP compounds like IAA, Ammonia, HCN, Catalase and Siderophore were successfully isolated, characterized and designated as PG1, PGB3, PG5, PG7, PG9 and PG10. Prior to seeds grown in plastic pots, seeds were treated with PGPR isolates and seedlings were harvested after 21 days of inoculation. Results of first study showed seed inoculation significantly enhanced seed germination and seedling vigour of Withania somnifera. Also application of PGPR isolates significantly improves the percentage of seed germination and seedling vigour index under UV-B exposure. Subsequently to investigate the effect of PGPR isolates on the growth characteristics, a pot culture experiment was conducted. Most of isolates resulted in a significant increase of plant height, fresh and dry weight, and leaf area and leaf number. Results confirmed that bacterial inoculation had significant effect on stimulation of root and shoot growth. Our findings suggest that the use of PGPR isolates PG10, PG9 and PG7 as inoculants biofertilizers might be beneficial for growth of Withania somnifera.

Keywords: IAA, Ammonia, HCN, PGPR and *Withania somnifera.*

INTRODUCTION

In recent years, a great deal of interest has been generated on studies related to increments in UV-B radiation. A decrease in the concentration of stratospheric ozone is enhancing the solar ultraviolet-B radiation on the earth's surface [1]. Bacteria are particularly vulnerable to UV-B damage because of their small size limits, effective cellular shading or protective pigmentation and their genetic material comprises a significant portion of their cellular volume [2]. Results from field studies on Rhizobacteria indicates that exposure to natural solar UV-B radiation results in a decrease in total cell abundance, a reduction in amino acids uptake, a depression of the activity of degrading enzymes and a significant inhibition of protein and DNA synthesis. Several studies have indicated that UV-B radiation can deleteriously affect the soil microbial diversity, physiological processes and overall growth in a number of plant species [3, 4]. Romanovskaia, [5] reported that total number of bacteria and the number of dominant species in soil samples exposed to UV-B radiation decreased. Thus, indicating the unfavourable effect of UV-B radiations on rhizobacteria and their plant growth promoting (PGPR) activities. The number of rhizobacteria bacteria in the rhizosphere soil and rhizoplane increases due to the progressive interaction between the roots and the microorganisms accompanied by continuous availability of nutrients for the growth of the microorganisms [6]. Plant growth promoting rhizobacteria are heterogenous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly or indirectly. The variability in the performance of PGPR may be due to various environmental factors that may affect their growth and exert their effects on plant [7].

In view of, varied therapeutic potential, Ashwagandha (*Withania somnifera* L. Dunal) is the subject of considerable modern scientific attention [8]. Also known as Indian Ginseng, this wonder herb belongs to family Solanaceae and is one of the most valuable herbs in the Ayurvedic and indigenous medical systems dating back more than 3,000 years. Ashwagandha, cultivated as an annual crop, is erect, 30-150 cm high and have fleshy and whitish-brown roots. Ashwagandha is known for its anti-carcinogenic properties. It has been used as an antibacterial, antioxidant, anti-carcinogenic, aphrodisiac, liver tonic, anti-inflammatory agent and is specific for wide range of conditions like arthritic inflammation, anxiety, insomnia, respiratory disorders, asthma bronchitis, extracts of roots increases RBC, WBC and platelets count in blood. This wonder herb has traditionally been used for rejuvenation, calming mind, relieving weakness and preventing impotency .As *Withania somnifera* is an important plant from medicinal point of view and UV-B has unfavorable effect on plant growth and its rhizobial associates, the present study was aimed to assess the effect of UV-B tolerant plant growth promoting rhizobacteria (PGPR) on seed germination and growth of *Withania somnifera.*

MATERIALS AND METHODS

Sampling and Isolation

The rhizospheric soil samples were collected from UV-B treated pots growing *Withania somnifera* (Ashwagandha) from west of Sam Higginbottom Institute of Agriculture, Technology and Sciences, Allahabad, India. Randomly selected plants were uprooted carefully and the excess of soil was removed by gentle shaking and the soil adhering to roots formed composite samples. The collected samples were placed in plastic bags and kept at 4^0C in the laboratory until processed. 10 gram of UV-B treated rhizospheric soil of *Withania somnifera* were taken into 250 ml of conical flask and 90 ml of sterile distilled water was added to it. After serial dilution up to 10^{-7} an aliquot of their suspension was spread on the plates of Luria Bartany (LB) agar medium. After 3 days of incubation at 28^0C bacterial colonies were spread to other LB agar plates and incubated at 28^0C for 3 days.

Morphological and Biochemical Characterization

Well isolated colonies were picked up and different characteristics of colonies such as shape, size, elevation, surface margin, colour, colony form, texture, pigmentation, odour and opacity etc. [9] were recorded. Selected isolates were biochemically characterized by Gram's reaction, carbohydrate fermentation, oxidase test, O-F test, H_2S production, IMVIC tests, NO_2 reduction and starch and gelatin hydrolysis as per the standard methods [10]. Motility of bacteria was observed by Hanging Drop method. A loopful of 2-day old culture was suspended in 1ml of nigrosin solution. A drop of suspension was taken on a cover slip. The cover slip was hanged on a hollow slide with Vaseline. The slide was then observed under microscope to test the motility of bacteria.

Characterization of Rhizobacteria for PGP Traits

Selected rhizobacterial isolates were characterized for plant growth promoting characteristics based on the standard procedures; IAA production was estimated by method of Bric [11] Ammonia, Catalase and HCN production by method of Bakker 1987 [12] and Siderophore production by method of Schwyn and Neilands [13]. Six potential isolates were selected on the basis of multiple PGP traits and were designated as PG1, PG3, PG5, PG7, PG9 and PG10. The culture of six isolates were streaked on LB agar plates and incubated at 10, 20, 28, 37 and 45^0C.

Seed Germination

Seeds of Ashwagandha (*Withania somnifera* L. Dunal), procured from Directorate of Extension, SHIATS, Allahabad were surface-sterilized with 0.02% sodium hypochlorite for 2 min, and rinsed thoroughly in sterile distilled water. For inoculation seeds were coated with 20% gum arabic as an adhesive and rolled into the suspension of bacteria (108 cfu ml-1) with perlit until uniformly coated. Germination tests were carried out by the paper towel method. 25 seeds for each treatment with three replications in completely randomized design and incubated in growth chamber at 28°C. After 7 days the number of germinated seeds was counted. Root and shoot length of individual seedling was measured to determine the vigor index with following formula: Vigor index= (mean root length +mean shoot length) × % germination [14]. For evaluation of ashwagandha's seedling growth promotion with PGPRs, above bacterial strains were tested in soil conditions. The plastic pots with 15cm diameter and capacity to hold 2Kg of soil were taken and PGPR inoculated seeds were sown at 4 to5cm depth of soil in each plastic pot. All treatments were arranged in 33 pots i.e., 3 replicates×11 pots per replication and a double seed per pot. Treatments were arranged in a factorial experiment based on completely randomized design. Seedlings were watered daily, and no artificial fertilization was used. After 30 days, fresh weight was determined and dry weight calculated by drying plants in an oven at 75°C until the weight remained constant. For leaf area determination, the area of each expanded leaf was calculated as K×length×width, where k =0.75 [15].

RESULTS AND DISCUSSION

Microbial diversity in soil is considered important for maintaining the sustainability of agriculture production systems [7].The quantity and activity of microorganisms are determining factor of the productivity of any kind of soil [16]. Near about 60 rhizobacterial isolates were isolated from the rhizosphere of *Withania somnifera*. After screening 6 potential isolates were selected for the present study and were designated as PG1, PG3, PG5, PG7, PG9 and PG10. As shown in Table 1, the morphological and biochemical characteristics of PGPR isolates varied widely. All the isolates were rod shaped with raised colonies having shiny surface and smooth margins. They differ in colour but all were odourless and no pigmentation was observed in the colonies of LB agar plates. Diameters of the colonies isolated varied from 0.2-2.0 mm. All the isolates were gram positive and most of them showed positive results for catalase, oxidase, O-F test, and starch hydrolysis and nitrate reductase. Results obtained in the present investigation were in agreement with the previous studies [17, 18, and 19]. Growth of isolates on LB agar plates varied with the temperature (Table 2). The growth of all isolates was good in the temperature range of 20^0C to 28^0C. In addition, PG3 showed maximum tolerance to temperature (45^0C). Plant rhizosphere is known to be preferred ecological niche for soil microorganisms due to nutrient rich availability. Plant growth promoting activities such as IAA production, ammonia, catalase, siderophore etc. are the characteristics of plant growth promoting rhizobacteria (PGPR). In the present study also IAA, Ammonia, Catalase and HCN production was shown by all (100%) the isolates. Among them PG7, PG9 and PG10 were strong IAA, Ammonia, Catalase and HCN producers, thus indicating their potential for plant growth promoting effects. However, production of Siderophore was detected less frequently than other PGP characteristics (Table 3). Among all the six isolates, PG9 and PG10 were strong siderophores producers. IAA secreted by a bacterium may promote root growth directly by stimulating plant cell elongation or division. Production of IAA by rhizobacterial isolates is also detected by other workers in *Bacillus*, *Pseudomonas* and other rhizobacterial isolates [18, 20, and 21]. Another important trait of PGPR, that may indirectly influence the plant growth, is the production of ammonia. Siderophore chelates iron and other metals contribute to disease suppression by conferring a competitive advantage to bio control agents for the limited supply of essential trace minerals in natural habitats [22, 23]. Production of catalase was exhibited by all the isolates of rhizobacteria. Catalase activity was detected in all the bacterial strains that may be potentially very advantageous Bacterial strains showing catalase activity must be highly resistant to environmental, mechanical, and chemical stress. However, Phosphate Solubilization activity was not detected in any of the rhizobacterial isolates under study (data not shown).

Table 1: Morphological and Cultural Characteristics of PGPR Isolates

PGP ISOLATES						
	PG1	PG3	PG5	PG7	PG9	PG10
Gram's Reaction	+ve	+ve	+ve	+ve	+ve	+ve
Cell Shape	Rod	Rod	Rod	Rod	Rod	Rod
Size (mm)	0.9-1.1	1.0-1.5	1.9-2.0	1.5-2.0	0.9-1.1	0.5-1.1
Elevation	Raised	Raised	Raised	Raised	Raised	Raised
Colour	Off-white	Brown	Brown	Yellow	Yellow	Off-white
Pigmentation	None	None	None	None	None	None
Oxidase	+	+	-	+	+	+
OF-test	+	+	-	+	+	+
H_2S Production	+	+	-	-	+	+
Indole	-	-	-	-	+	+
Methyl Red	-	-	-	+	+	+
Vogues Proskauer	+	-	+	+	+	+
Citrate Utilization	+	+	+	+	+	+
Nitrate Reduction	+	+	+	+	+	+
Starch Hydrolysis	-	-	+	+	+	+
Gelatin Hydrolysis	-	-	-	-	-	-

Table.2: Growth of PGPR Isolates at Different Temperatures

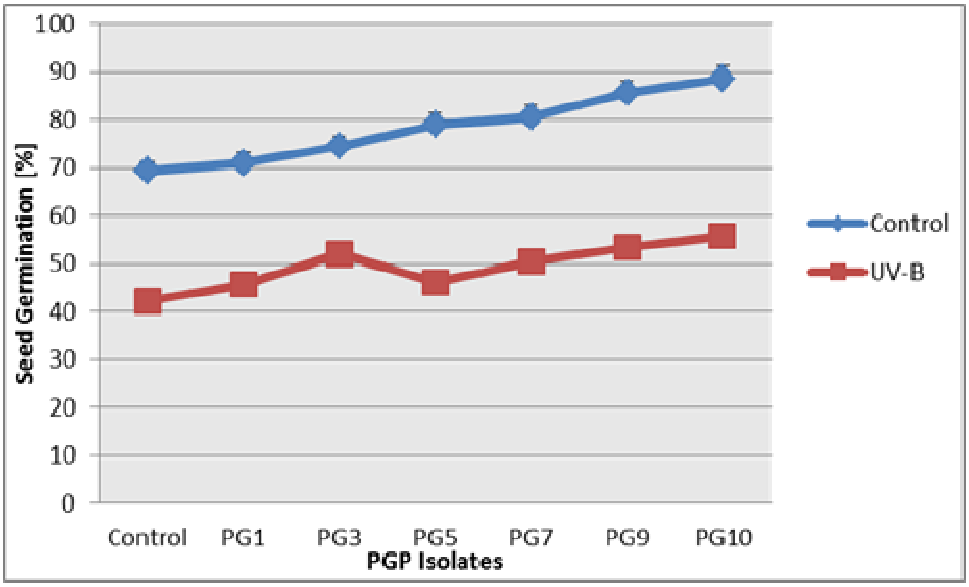
Temperature										
Isolates										
		10^0C		20^0C		28^0C		37^0C		45^0C
PG1		+		++		++		+		-
PG3		+		++		++		++		+
PG5		+		++		++		+		-
PG7		+		++		++		-		-
PG9		+		++		++		+		-
PG10		-		++		++		++		-

Table 3: Plant Growth Promoting Characteristics of Rhizobial Isolates

PGP Characteristics					
Isolates					
	IAA	AMMONIA	CATALASE	HCN	SIDEROPHORE
PG1	++	+	+	+	-
PG3	++	++	+	++	-
PG5	++	++	+	++	-
PG7	+++	++	+	++	+
PG9	++++	+++	+	+++	++
PG10	++++	+++	+	+++	+++

Some of the above-tested isolates could exhibit more than two or three PGP traits, which may promote plant growth directly or indirectly or synergistically. Similar to our findings of multiple PGP activities among PGPR have been reported by some other workers while such findings on indigenous isolates of India are less commonly explored [19, 24]. Plant growth promoting effects of PGPR strains in different crops were clearly demonstrated [25]. Bacterial inoculants are able to increase plant growth and germination rate, improve seedling emergence, responses to external stress factors and protect plants from disease [26]. In the present study also UV-B exposure significantly decreased seed germination and vigor index in *Withania somnifera* seeds. Effect of PGPR on germination percentage and vigor index also varied with bacterial isolates (Fig. 1 and Fig. 2). The effect of PGPR on germination rate of seedlings under UV-B exposure was statistically significant at $p<0.05$.This present investigation confirms the earlier works. Previous studies have documented adverse effects of UV-B on plant growth. Similar results were obtained by Sharon [27] while working on gamma irradiation on seedling growth of *Punica granatum*. Under in vitro conditions seed treatment with PGPR strains improved seed germination; seedling vigor, seedling emergence and seedling stand over the control both with UV-B and without UV-B. Similar results were found in in earlier reports [28, 29, 30, 31, and 32].

Fig. 1: Effect of PGPR inoculation on Germination Rate of *Withania somnifera* under UV-B exposure (60 minutes)



In pot experiment also, PGPR strains significantly enhanced plant growth as compare to control. Burd *et al*. [33] reported that plant growth promoting rhizobacteria might enhance plant height and productivity by synthesizing phytohormones, increasing the local availability of nutrients, facilitating the uptake of nutrients by the plants decreasing heavy metal toxicity in the plants antagonizing plant pathogens. Our results also revealed that plant height increased by 37%, 35% and 33% by isolates PG10, PG9 and PG7 respectively. Khalid *et al*., [34] showed that responses of wheat growth to inoculation with rhizobacteria depend on plant genotype and PGPR strains as well as environmental conditions. Observed data are presented in Table 4. Fresh weight and dry weight of *Withania somnifera* was significantly increased up to 17% and 14% by PG10 and PG9 respectively. PG10 produced highest plant biomass i.e. fresh and dry weight followed by PG9, PG7, PG5 and PG3. Most of the isolates significantly increased other growth characteristics such as leaf number, leaf area etc.

Fig.2: Effect of PGPR inoculation on Vigor Index of *Withania somnifera* under UV-B exposure (60 minutes)

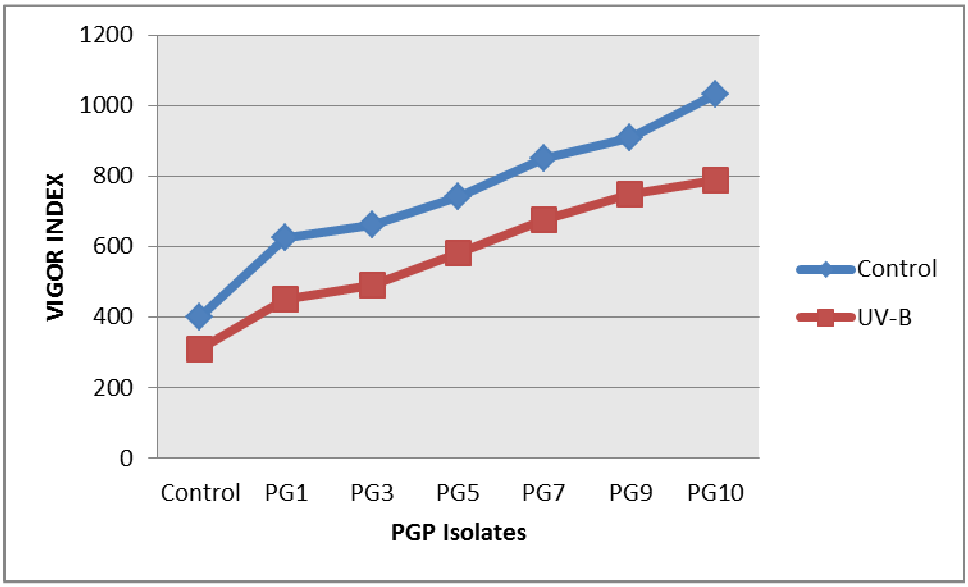


Table 4: Effect of PGPR Inoculation on Growth Characteristics of *Withania somnifera* Seedlings at 60 Days after Sowing

Treatments	Plant Height (cm)	No. of Leaves	Leaf Area (dm^2)	Fresh Weight (g/plant)	Dry Weight (g/plant)
Control	28.07^{f}	28.03^{f}	23.69^{e}	95.70^{g}	31.90^{g}
PG1	29.98^{e}	28.74^{ef}	24.63^{d}	99.28^{f}	33.08^{f}
PG3	31.09^{d}	29.21^{e}	25.11^{c}	101.43^{e}	33.81^{e}
PG5	31.66^{d}	29.88^{a}	25.46^{bc}	103.35^{d}	34.45^{d}
PG7	32.78^{c}	30.87^{d}	25.63^{bc}	107.19^{c}	35.73^{c}
PG9	34.46^{b}	32.56^{c}	25.84^{ab}	109.11^{b}	36.37^{b}
PG10	35.56^{a}	34.09^{b}	26.13^{a}	111.99^{a}	37.33^{a}
SE±	0.181	0.116	0.047	0.020	0.009
CD	1.008	0.805	0.498	0.375	0.156

Data are means ± standard error of three independent experiments. Different letters show significant difference at P<0.05

CONCLUSION

In conclusion, our result suggested that simultaneous screening of rhizobacteria for growth and yield promotion under pot and field experiment is a good tool to select effective PGPR for biofertilizer development biotechnology. PGPR are highly beneficial for plant growth and can serve as potential substitute for pesticides and chemical fertilizers. Even under unfavourable and stress conditions like UV-B exposure PGPR can enhance seed germination and can exert a beneficial effect on plant growth.

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