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Selection of an Efficient Plant Growth Promoting Rhizobacteria for Inoculating *Withania Somnifera*

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Withania somnifera is a medicinal plant well documented for its health benefits since ancient times. The present study was aimed at comparing the effectiveness of plant growth promoting rhizobacteria (PGPR) on the growth and yield of *W. somnifera.* Nine different PGPR were screened for their efficiency. Some of the PGPR used like *Bacillus subtilis, Pseudomonas fluorescens, Azotobacter chroococcum, Azospirillum brasilense,* are well known for their growth promotion in plants, some like *Methylobacterium radiotolerans, Exiguobacterium acetylicum, Paenibacillus polymyxa, Pantoea dispersa* and *Bacillus sonorensis* were used for the first time to see their influence on *W. somnifera*. Plants inoculated with PGPR showed significantly improved growth and yield compared to the uninoculated plants. The results of this study suggest that *Bacillus sonorensis* has a great potential to increase the growth and yield of *W. somnifera* and possesses all the PGPR traits and therefore can be used for inoculating *W. somnifera.*

Key words: PGPR, *W Somnifera, B Sonorensis,* Withanolide, Plant Growth

Introduction

Withania somnifera L. Dunal, also known as ashwagandha, is a medicinal plant used in the Indian medicine since ancient times for the treatment of many ailments such as inflammation of joints, nervous disorders, epilepsy, female disorders etc. Roots contain several pyrazole alkoloids like withasomnine, withaferin A, withanolide which are responsible for curing ailments[1].The overuse of chemical fertilizers and pesticides has led to environmental problems due to which importance is given to sustainable agriculture which uses organic inputs like microbial inoculants[2]. Microbial inoculants like plant growth promoting rhizobacteria (PGPR) are known to increase the growth and productivity of crop plants. These PGPR strains promote growth either by fixing atmospheric N or by solubilization of P^3 or by production of growth regulators such as IAA[4]. Therefore PGPR can be a best alternative to chemical fertilizer for sustainable and eco-friendly agriculture. In the present investigation pot culture experiments were conducted to screen and select the efficient PGPR on the basis of their ability to promote the growth, yield and withanolide concentration of *W. somnifera.*

Materials and methods

Bacterial strains and media used

The PGPR strains used in this study were *Bacillus subtilis* (National Bureau of Agricultural Insect Resources, Bangalore), *Pseudomonas fluorescens* (Central Institute of Medicinal and Aromatic Plants, Lucknow), *Azotobacter chrococcum* (University of Agricultural Sciences, Bangalore) *Azospirillum brasilense* (Microbial Type Culture Collection, Chandigarh), *Methylobacterium radiotolerans* (Tamil Nadu Agricultural University, Madurai), *Exiguobacterium acetylicum* (Microbial Type Culture Collection , Chandigarh), *Paenibacillus polymyxa* (National Agriculturally Important Microbial Culture Collection, Mau), *Pantoea dispersa* (National Agriculturally Important Microbial Culture Collection, Mau) and *Bacillus sonorensis* (University of Hyderabad, Hyderabad). All bacterial strains obtained from different resources mentioned above maintained at Centre for Natural Biological Resources and Community Development (CNBRCD), Bangalore were used in the

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present study.

M. radiotolerans was grown on ammonium mineral salt agar medium, *E. acetylicum* and *B. subtilis* were grown on nutrient agar medium, *P. fluorescens* on king's B agar medium, *A. chroococcum* on Ashby's agar medium, *A. brasilense* on N free bromothymol blue agar medium, *P. polymyxa* and *P. dispersa* on modified nutrient agar medium and *B. sonorensis* was grown on Luria Bertani agar medium.

Pot culture experiments

W. somnifera seeds used in the present study were obtained from Hyderabad. The variety used was Poshitha. The seeds were germinated in protrays using sterilized coirpith as the substrate. Nine different PGPR were used in the experiment for screening[5]. The organisms selected for the study are the ones which are well known for their beneficial effects and are used here to see their effect on the growth of *W. somnifera.* For inoculating plants the different media were prepared according to the organism's requirement. A single colony of each PGPR was transferred to 10ml of test tube with their respective medium and later transferred to 500ml flasks containing the specific medium and grown aerobically in flasks on rotating shaker (150rpm) at 32ºC for 2-5 days depending on the organism. The bacterial suspensions were then diluted with sterile water to a final concentration of 10^9 CFU/ml, and the resulting suspensions (10ml/plant) were used to treat *W. somnifera* plants. The poly bags of 5 kg capacity were filled with the mixture of soil, sand and vermicompost in the ratio of 1:1:0.25. Ten ml of different PGPR was added to the planting hole of each poly bag depending on the treatment. Forty day old healthy seedlings of uniform size were transplanted into poly bags. Each treatment was replicated seven times. The plants were maintained in a poly house and watered when necessary.

Plant parameters studied

The plants were harvested 145 days after transplanting (DAT)[6]. Plant height was measured from soil surface to the growing tip of the plant and stem girth was measured one centimeter above the soil surface using digital vernier calipers before harvest. Biovolume index (BI) was also calculated (height in cm x stem girth in mm). The shoot of the plants were cut and removed from soil surface and then roots of the plants were collected from soil, washed thoroughly and kept for drying at 60º C in an oven. .The dry weight of shoot and root was determined after drying the samples to a constant weight. The plant nutrient concentration analysis was done for N, P and K following the standard procedures outlined by Jackson[7]. Methanolic extract of roots were subjected to HPLC to determine withanolide concentration[8]. Statistical analysis was done by subjecting the data to analysis of variance (ANOVA) followed by Duncan's multiple- range test to differentiate the significant difference between different treatments at the probability level of $P<0.05$ using statistical software Assisstat 7.7 beta version.

Characterization of *Bacillus sonorensis* as PGPR

B. sonorensis was found to be best in increasing the growth, nutrition and yield of *W. somnifera*. Since not much information is available on *B. sonorensis* as a PGPR, it was characterized for its PGPR traits. Phosphate solubilization, Indole acetic acid (IAA), ammonia, hydrogen cyanide (HCN) and amylase production were determined by the methods outlined by Bhatt and Vyas[9]. Siderophore production was checked by Arnow assay[10]. Biofilm formation by *Bacillus sp.* during growth was examined in borosilicate glass tubes, as described by Yousef *et al.*[11]

Results and Discussion

The PGPR seem to have a great potential as bioinoculants to increase production in medicinal and aromatic plants[12]. In this experiment well known PGPR with few uncommon ones were used to see their effect on *W. somnifera*. *W. somnifera* plants varied in their response to inoculation with different PGPR. Plants inoculated with PGPR generally showed an increase in plant growth parameters and nutrient status compared to uninoculated plants. Earlier workers have observed similar response to inoculation with PGPR in other medicinal plants like *Catharanthus roseus*[13]. The plant height (Table 1) was highest in plants inoculated with *B. sonorensis* and on par with the treatments *M. radiotolerans, A. chroococcum* and *P. fluorescens* but differing significantly from the uninoculated control plant. The plant stem girth was maximum in *B. sonorensis* inoculated plants but not differing significantly from other inoculated treatments excepting *M. radiotolerans* and the control. The increased plant height and stem girth may be attributed to N fixation and production of plant growth hormones by these PGPR. The biovolume index (Table 1) was maximum in plants

inoculated with B. *sonorensis* and was statistically on par with *M. radiotolerans, A. chroococcum, P. polymyxa P. fluorescens* treated plants but differing significantly from plants treated with *E. acetylicum, B. subtilis, A. brasilense, P. dispersa* and the uninoculated plants. Plants inoculated with *B. sonorensis* had maximum shoot dry weight (Table 1) and did not differ significantly from other treatments except *E. acetylicum, P. polymyxa, P. dispersa* and control. Plants inoculated with *B. subtilis* showed maximum root dry weight closely followed by *B. sonorensis* (Table 1) and was statistically on par with *E. acetylicum* and *P. polymyxa* treatments. The least root dry weight was recorded in the control treatment. Total plant biomass (Table 1) was significantly superior in *B. sonorensis* compared to other treatments and the control but was statistically on par with plants inoculated with *M. radiotolerans, A. brasilense* and *B. subtilis.* Earlier workers have reported increase in shoot and root dry weight and total plant dry biomass in the medicinal plant *Coleus forskohlii* plants treated with the PGPR *Pantoea* sp. and *Pseudomonas* sp.[14]. Shoot N concentration (Table 2) was significantly high in plants inoculated with *E. acetylicum* and the root N concentration in *B.sonorensis* treated plants which differed significantly from all other inoculated and uninoculated treatments. The shoot P concentration (Table 2) was significantly high in all inoculated treatments excepting *M. radiotolerans, P. polymyxa* and uninoculated plants. The root P concentration was also significantly high in plants treated with *B. sonorensis* which was on par with the treatment *B. subtilis* and *E. acetylicum* but differing significantly from other inoculated treatments and control. The shoot K concentration (Table 2) was highest in *A. chroococcum* treated plants and least in control plants. The plants treated with *M. radiotolerans* showed the highest root K concentration (Table 2) but not differing from the treatments *E. acetylicum* and *B. sonorensis* and the least K concentration was recorded in control plants. *B. subtilis* and *P. fluorescens* enhancing growth of *Pelargonium graveolens* because of P solubilization

Table 1 — Effect of soil inoculation with PGPR on plant height, stem girth, biovolume index, dry weight of shoot and root and total dry biomass of Withania *somnifera*

. Treatment	Plant Height (cm/plant)	Stem Girth (mm/plant)	Biovolume Index	Dry weight of shoot (g/plant)	Dry weight of root (g/plant)	Total dry weight (g/plant)
Control	28.57^{e}	8.70^{c}	249.93^{c}	7.13^{d}	0.81^{c}	7.94^{c}
Methylobacterium radiotolerans	39.86^{ab}	10.19^{b}	409.18^{ab}	10.79^{ab}	1.19^{bc}	11.98^{ab}
Exiguobacterium acetylicum	36.86^{bc}	10.78^{ab}	397.89^{b}	8.68^{cd}	1.40^{ab}	10.08^{bc}
Bacillus subtilis	33.57^{d}	11.17^{ab}	376.44^{b}	9.37^{ab}	1.84^{a}	11.21^{ab}
Pseudomonas fluorescens	38.86^{ab}	10.85^{ab}	423.39^{ab}	9.14^{ab}	1.11^{bc}	10.25^{b}
Azotobacter chroococcum	38.14^{ab}	10.94^{ab}	419.65^{ab}	9.22^{ab}	1.29^{bc}	10.51^{b}
Azospirillum brasilense	34.57^{cd}	11.31^{ab}	391.41^{b}	9.53^{ab}	1.26b^{c}	10.79^{ab}
Paenibacillus polymyxa	37.29^{bc}	11.05^{ab}	412.06^{ab}	9.01^{bc}	1.41^{ab}	10.42^{b}
Bacillus sonorensis	42.07^{a}	11.44^{a}	480.33^{a}	11.09^{a}	1.80^{a}	12.90^{a}
Pantoea dispersa	34.64^{cd}	10.28^{ab}	355.27^{b}	8.93b^{c}	0.99^{bc}	9.92^{bc}

Values in each column followed by the same letter are not significantly different at P=0.05 level by Duncun's Multiple Range test. Values given are average of seven replications.

Table 2 — Effect of soil inoculation with PGPR on NPK concentration of *Withania somnifera*

Treatment	Shoot N (%)	Root N (%)	Shoot P (%)	Root P (%)	Shoot K (%)	Root K (%)
Control	1.40^{f}	1.41^{e}	0.21^{b}	0.08^{d}	1.22^{f}	1.01^{e}
Methylobacterium radiotolerans	2.10^{d}	1.78^{b}	0.16^{c}	0.10^{bc}	1.60^{e}	1.50^{a}
Exiguobacterium acetylicum	2.60^{a}	1.50^{cd}	0.24^{a}	0.12^{a}	2.28^{c}	1.44^{ab}
Bacillus subtilis	2.38^{b}	1.81^{b}	0.25^{a}	0.11^{ab}	2.30^{bc}	1.38^{bc}
Pseudomonas fluorescens	2.28^{bc}	1.58^{c}	0.24^{a}	0.10^{bc}	2.30^{bc}	1.36^{bc}
Azotobacter chroococcum	2.10^{d}	1.53^{cd}	0.24^{a}	0.09^{cd}	2.50^{ab}	1.30^{cd}
Azospirillum brasilense	2.24^{c}	1.49^{de}	0.25^{a}	0.04^{e}	2.40^{b}	1.30^{cd}
Paenibacillus polymyxa	2.24^{c}	1.80^{b}	0.21^{b}	0.01^{f}	2.15^{d}	1.28^{d}
Bacillus sonorensis	2.22^{cd}	1.94^{a}	0.25^{a}	0.12^{a}	2.40^{b}	1.45^{ab}
Pantoea dispersa	1.93^{e}	1.57^{cd}	0.25^{a}	0.10^{bc}	2.32^{bc}	1.34^{cd}

Table 3 — Effects of soil inoculation with PGPR on root withanolide concentration of *Withania somnifera*

Treatment	Withaferin A%	Withanolide A%	Total Withanolide%
Control	0.007[b]	0.025[de]	0.070[g]
Methylobacterium radiotolerans	0.009[ab]	0.026[cd]	0.090[cd]
Exiguobacterium	0.009[ab]	0.025[de]	0.077[fg]
Bacillus subtilis	0.008[ab]	0.024[e]	0.086[de]
Pseudomonas fluorescens	0.009[ab]	0.025[de]	0.097[bc]
Azotobacter chroococcum	0.009[ab]	0.025[de]	0.079[ef]
Azospirillum brasilense	0.013[a]	0.027[c]	0.087[de]
Paenibacillus polymyxa	0.011[ab]	0.030[b]	0.104[ab]
Bacillus sonorensis	0.013[a]	0.032[a]	0.109[a]
Pantoea dispersa	0.011[ab]	0.030[b]	0.094[cd]

and IAA production has been reported earlier[15]. Inoculation with all the PGPR studied significantly enhanced the withaferin A (Table 3) concentration in the roots of *W. somnifera* compared to uninoculated plants. The withaferin A concentration was highest in *B. sonorensis* treated plants and did differ significantly from other treatments. The withanolide A concentration was maximum in plants inoculated with *B. sonorensis* and differing significantly from all other inoculated treatments and control. The total withanolide concentration was also maximum in *B. sonorensis* treated plants which differed significantly from all the inoculated treatments except *P. polymyxa* and uninoculated control. The uninoculated plants had the least total withanolide concentration. Similar response to inoculation with PGPR has been reported earlier in other medicinal plants. Nidhi *et al.*[16] reported increase in plant growth and content of bacoside-A in *Bacopa monnieri* due to inoculation with PGPR *B. pumilus* and *Exiguobacterium oxidotolerans*. Increased plant height, root length, alkaloid content and N, P, K, Ca and Mg uptake in *Catharanthus roseus* because of inoculation with *P. fluorescens* and *B. megaterium* has been reported by Karthikeyan *et al.*[17]. Rajashekar and Elango[18] observed that a combination of PGPR strains *Azospirillum, Azotobacter, Pseudomonas* and *Bacillus* significantly increased plant height, root length and alkaloid content in *W. somnifera* compared to uninoculated control. In the present study PGPR (not studied earlier on *W. somnifera*) like *M. radiotolerence, E. acetylicum, P. polymyxa, P. dispersa* and *B. sonorensis* were found to increase the growth of *W. somnifera*. These bacteria have been reported to increase growth in other plants. Chinnadurai *et al.*[19] reported that foliar spray of *M. radiotolerence* an ACC deaminase producing bacterium enhanced the root and shoot length of rice and tomato seedlings and reduced ethylene levels in plants. Seed bacterization with *Exiguobacterium* sp. was found to enhance root growth and root dry weight of cow pea[20]. *P. polymyxa* promoting plant growth by producing IAA and also suppressing fungal phytopathogens has been reported in other plants[21]. Selvakumar *et al.*[22] reported that *P. dispersa* promoted shoot and root length, shoot and root dry biomass and N, P and K uptake in wheat. In the present study although plants inoculated with different PGPR increased growth when compared to uninoculated control, *Bacillus sonorensis* showed significant increase in most of the parameters like plant height, girth, root and shoot dry weight and withanolide concentration. Since not much information is available on the PGPR traits of this organism this aspect was also studied. The studies showed that it has PGPR traits like phosphate solubilization, IAA, siderophore, amylase, ammonia and HCN production and also biofilm formation which are contributing to the enhanced growth of *W. somnifera*.

Conclusion

It can be concluded that *B. sonorensis* possesses the PGPR traits and has a great potential to increase the growth and yield of *W. somnifera* and therefore appears to be a promising PGPR for inoculating *W. somnifera.*

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