© 2018 IJSRST | Volume 4 | Issue 5 | Print ISSN: 2395-6011 | Online ISSN: 2395-602X
Themed Section: Science and Technology

Effect on the Plant Growth Promoting Rhizobacteria (PGPR) Increasing Plant Total Alkaloid and Protein Content of Ashwagandha

S. Rajasekar[1], R.Elango[2], S. R. Paranthaman[3]

[1]Assistant Professor, Department of Microbiology, Sri Bharathi Arts &Science College for Women, Kaikuruchi, Pudukottai, Tamil Nadu, India

[2]Assistant Professor, Department of Microbiology, Annamalai University, Chidambaram, Tamil Nadu, India

[3]Head, Department of Microbiology, Umed Pharma Lab Private Limited, Hyderabad, Telangana, India

ABSTRACT

Withania somnifera (Ashwagandha), also known as Indian Ginseng, is a well-known Indian medicinal plant due to its antioxidative, antistress, antigenotoxic, and immunomodulatory properties. The present study was designed to assess and establish the cytoprotective potential of Ashwagandha Rhizobacteria from different medicinal plants viz., Withania somnifera, Coleus forskohlii and Vinca rosea grown in different parts of Tamil Nadu were isolated and characterized. Combined inoculation of all the four rhizobacteria viz., Azospirillum lipoferum-AAz-1, Azotobacter-AAt-13, Bacillus-APb-19 and Pseudomonas fluorescens-APf-5 enhanced the chlorophyll content, protein and total alkaloid content, especially Withaferin-A.

Keywords : Withaferin-A, PGPR

I. INTRODUCTION

In India, the use of several medicinal plants to cure specific ailments is in vogue from ancient times. The indigenous systems of medicine namely Siddha, Ayurveda and Unani have been in existence for several centuries. The most important pharmacological use of ashwagandha is as adaptogen with antistress antioxidant, antitumor, anti-inflammatory, mind boosting and has rejuvenating properties (Singh *et al.*, 1990). The rhizobiocoenosis is an important biological process that plays a major role in satisfying the nutritional requirement of these crops. Studies on the rhizobacterial population in the rhizosphere region and testing the suitability of the isolated rhizobacteria as seed and soil inoculant will be highly useful in improving the productivity and quality of this commercially important medicinal plant.

II. MATERIALS AND METHODS

2.1 Preparation of pots and seed inoculation

A pot culture experiment was conducted at the Department ofl Microbiology, Annamalai University ,Chidambaram to study the effect of combined inoculation of rhizobacteria on growth, yield and quality of ashwagandha (var. Jawahar 20). The rhizobacterial isolates *viz.*, *Azospirillum lipoferum*-AAz-1, *Azotobacter*-AAt-13, *Bacillus*-APb-19 and *Pseudomonas fluorescens*-APf-5 were prepared as carrier based inoculants as described earlier and used for this study.

The pots were filled with potting mixture (soil + sand + FYM) and the rhizobacteria treated seeds were sown at 25 seeds per pot and finally 5 seedlings were maintained. The experiment was conducted in completely randomized block design with three replications. The treatments are as follows:

2.2 Studies on biometric observation

Biometric observations like plant height, number of primary and lateral branches, leaf area, root length, root diameter, lateral roots, root fresh and dry weight, root yield and biochemical properties like total protein alkaloid content were recorded at 90, 120, 150 and 180 DAS.

1. RESULT AND DISCUSSION

Table 1. Effect of rhizobacterial inoculation on protein content of ashwagandha

Sl. No.	Treatments	Protein content (mg g^{-1})			
		90 DAS	120 DAS	150 DAS	180 DAS
1.	T_1 – Azospirillum (AAz-1)	12.06	12.56	12.37	11.81
2.	T_2 – Azotobacter (AAt-13)	11.87	12.31	12.18	11.62
3.	T_3 – Bacillus (APb-19)	11.94	12.37	12.25	11.69
4.	T_4 – Pseudomonas (APf-5)	12.00	12.43	12.31	11.75
5.	T_5 – Azospirillum (AAs-1) + Azotobacter (AAt-13)	12.18	13.00	12.62	12.12
6.	T_6 – Azospirillum (AAz-1) + Bacillus (APb-19)	12.37	13.18	12.81	12.31
7.	T_7 – Azospirillum (AAz-1) + Pseudomonas (APf-5)	12.43	13.25	12.87	12.37
8.	T_8 – Azotobacter (AAt-13) + Bacillus (APb-19)	12.12	12.93	12.56	12.06
9.	T_9 – Azotobacter (AAt-13) + Pseudomonas (APf-5)	12.25	13.06	12.68	12.18
10.	T_{10} – Bacillus (APb-19) + Pseudomonas (APf-5)	12.31	13.12	12.75	12.25
11.	Consortium T_{11} – Azospirillum (AAz-1) +Azotobacter (AAt-13) + Bacillus (APb-19) + Pseudomonas (APf-5)	13.12	14.37	13.00	12.56
12.	T_{12} – Uninoculated control	10.75	11.56	11.31	10.61
SED		0.0220	0.0298	0.0216	0.0249
CD (P=0.05)		0.0453	0.0614	0.0444	0.0513

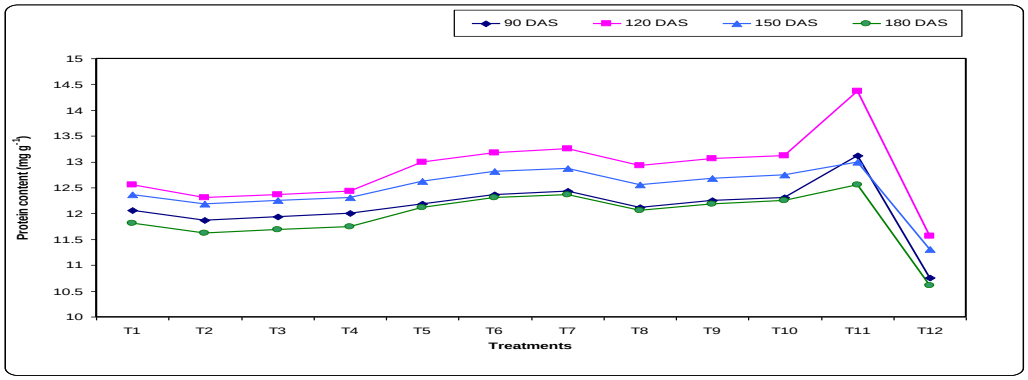

Figure 1. Effect of rhizobacterial inoculation on protein content of ashwagandha

Effect of PGPB Inoculation on the Protein Content of Ashwagandha

The protein content of Ashwagandha increased up to 120 days of PGPB inoculation and then gradually decreased with increasing age of the plants (Table 1) and (Fig.1). Among the different treatments, inoculation of PGPB Consortium (T_{11}) recorded the maximum protein content of 14.37on 120 DAS. The dual inoculant treatment of T7 (13.25 mg g^{-1}), T_6 (13.18 mg g^{-1}), T_{10} (13.12 mg g^{-1}) T_9(13.06 mg g^{-1}), T_5 (13.00 mg g^{-1}) and T_8 (12.93 mg g^{-1}). The single treatment of T_1 (12.56 mg g^{-1}), T_4 (12.43 mg g^{-1}), T_3 (12.37 mg g^{-1}), T_2 (12.31 mg g^{-1}).The uninoculated control recorded ($11.56x10^6$ mg g^{-1}) of Ashwagandha.

Table 2. Effect of rhizobacterial inoculation on total alkaloid content of ashwagandha roots

Sl. No.	Treatments	Total alkaloid (%)			
		90 DAS	120DAS	150 DAS	180 DAS
1.	T_1 – Azospirillum (AAz-1)	0.65	0.75	0.84	1.18
2.	T_2 – Azotobacter (AAt-13)	0.60	0.70	0.81	1.15
3.	T_3 – Bacillus (APb-19)	0.63	0.72	0.83	1.16
4.	T_4 – Pseudomonas (APf-5)	0.64	0.73	0.84	1.17
5.	T_5 – Azospirillum (AAz-1) + Azotobacter (AAt-13)	0.68	0.76	0.86	1.23
6.	T_6 – Azospirillum (AAz-1) + Bacillus (APb-19)	0.71	0.79	0.89	1.26
7.	T_7 – Azospirillum (AAz-1) + Pseudomonas (APf-5)	0.72	0.80	0.90	1.27
8.	T_8 – Azotobacter (AAt-13) + Bacillus (APb-19)	0.67	0.75	0.85	1.22
9.	T_9 – Azotobacter (AAt-13) + Pseudomonas (APf-5)	0.69	0.77	0.87	1.24
10.	T_{10} – Bacillus (APb-19) + Pseudomonas (APf-5)	0.70	0.78	0.88	1.25

11.	Consortium T_{11} – Azospirillum (AAz-1) + Azotobacter (AAt-13) + Bacillus (APb-19) + Pseudomonas (APf-5)	0.75	0.84	0.95	1.30
12.	T_{12} – Uninoculated control	0.59	0.68	0.80	1.12
SED		0.0030	0.0032	0.0021	0.0036
CD (P=0.05)		0.0063	0.0067	0.0043	0.0073

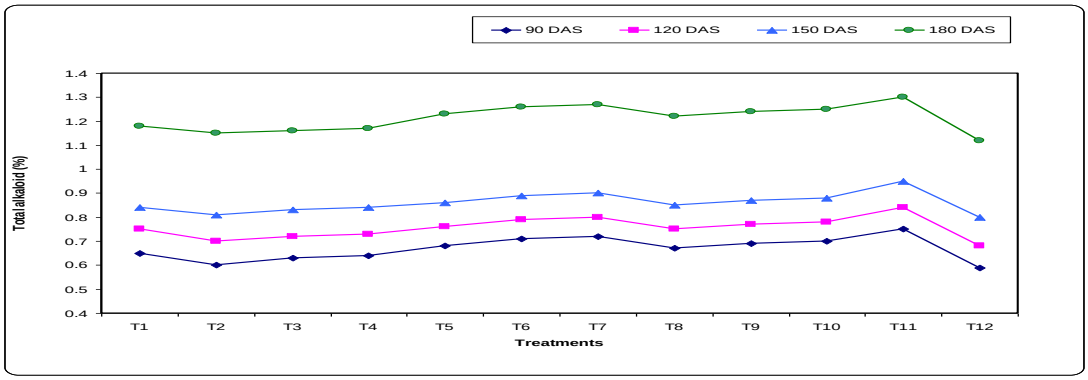

Figure 2. Effect of rhizobacterial inoculation on total alkaloid content of ashwagandha roots

Effect of PGPB Consortium Inoculation on the alkaloid content of Ashwagandha roots

The result on the root alkaloid content of Ashwagandha was presented in (Table 2) and (Fig.2). The inoculation with plant growth promoting bacteria significantly increased the alkaloid content of Ashwagandha. The alkaloid content of roots of Ashwagandha ranged from 0.55 to 1.50 mg g^{-1} of root.

The consortium(T_{11}) inoculated treatment, recorded the maximum alkaloid content of 1.30 mg g^{-1} in 180 DAS , followed by the dual inoculants treatment of T_7 (1.27 mg g^{-1}), T_6 (1.26 mg g^{-1}), T_{10} (1.25 mg g^{-1}), T_9 (1.24 mg g^{-1}), T_5 (1.23 mg g^{-1}) and T_8 (1.22 mg g^{-1}). Single inoculants treatments found comparitvely lower than consortium and dual inoculants treatments. The uninoculated control T_{12} recorded lowest of 1.12 mg g^{-1} in Ashwagandha.

III. DISCUSSION

In the present investigation, the inoculation of rhizobacteria enhanced the growth and yield parameters of ashwagandha. Inoculation with various rhizobacteria enhanced the root elongation and proliferation and this might be due to the production of IAA and GA by these organisms. The inoculated PGPR strains usually have been found to increase the root length and root biomass (Yan *et al.,* 2003; Chakraborty *et al*., 2003; Pal *et al.,* 2003 and Khalid *et al*., 2004) and this better developed root system may increase the mineral uptake in plants.

The present study indicated higher shoot and root length, dry matter, yield and alkaloid content of ashwagandha, when the mixed inoculant of *Azospirillum, Azotobacter, Bacillus* and *Pseudomonas* was applied. The free-living plant growth-promoting rhizobacteria (PGPR) can be used in a variety of ways

to increase the plant growth. The addition of PGPR increased the germination rate, root growth, leaf area, chlorophyll content, magnesium content, nitrogen content, protein content, hydraulic activity, tolerance to drought, shoot and root weights, and delayed leaf senescence which reflected in higher grain yield (Lucy *et al.*, 2004).

In general, production of growth promoting substances like gibberellins and auxins by the rhizobacteria enhanced proliferation of root system which in turn enhanced mineral uptake (N, P and K) and consequently increased dry matter accumulation (Okon, 1985 and Lakshmipriya, 1997). The positive effects observed in the present study may be attributed to considerable quantity of indole acetic acid production besides nitrogen fixation by the rhizobacteria. The rhizobacterial inoculation besides increasing yield also enhanced the alkaloid content of roots, especially Withaferin-A. This alkaloid is mainly responsible for the various pharmaceutical properties of ashwagandha.

IV. REFERENCES

[1]. Alagawadi, A.R. and A.C. Gaur. 1988. Interaction between Azospirillum brasilense and phosphate solubilizing bacteria and their influence on yield and nutrient uptake of sorghum (Sorghum bicolor L.). Zentralbl. Mikrobiol., 143: 637-643.

[2]. Anonymous. 2002. Ashwagandha. research achievements Annual report 2001-2002. National Research Centre for Medicinal and Aromatic Plants, Boriavi, Anand, 387 310, Gujarat. P: 11.

[3]. Anonymous. 2002. Ashwagandha. research achievements Annual report 2001-2002. National Research Centre for Medicinal and Aromatic Plants, Boriavi, Anand, 387 310, Gujarat. P: 11.

[4]. Atal, C.K., O.P. Gupta, K. Ragunathan and K.L.Dhar. 1975. Pharmacognosy and phytochemistry of Withania somnifera (Dunal). Central Council for Research in Indian Medicine and Homeopathy Publication, New Delhi, pp.6.

[5]. Barabara Lata. 2007. Cultivation, mineral nutrition and seed production of Catharanthus roseus (L.) G. Don in the temperature climate zone. Phytochem. Rev., 6: 403-411.

[6]. Bashan Y., de -Bashan L.E.(2010).How the plant growth -promoting bacterium Azospirillum pormotes plant growth - a critical assessment.Adv.Agron.10877-13610.

[7]. Bharti N., Yadav D., Barnawal D., Maji D., Kalra A.(2013).Exiguobacterium oxidotolerans , a halotolerant plant growth promoting rhizobacteria, improves ield and content of secandary metabolites in Bacopa monnieri (L.) pennell under primary and secondary salt stress. World J.Microbiol. Biotechnol. 29379-38710

[8]. Burr, T.J., M.N. Schroth and T. Suslow. 1978. Increased potato yield by treatment of seed pieces with specific strains of Pseudomonas fluorescens and P. putida. Phytopathol., 68: 1377-1383.

[9]. Chakraborty, U., B.N. Chakraborty, P. R. Chowdhury, C. Tongden and M. Basnet. 2003. Investigation on plant growth promoting rhizobacteria of tea rhizoshere.

[10]. 6th International workshop on PGPR, IISR, Calicut, Kerala, pp. 78-82.

[11]. Chezhiyan, N.S. Saraswathy and R. Vasumathi. 2003. Studies on organicmanures, biofertilizers and plant density on growth, yield and alkaloid content of bhumyamalaki (Phyllanthus amarus Schum and Thonn.). South Indian Hort., 51: 96-101.

[12]. Eastwood, F.W., I. Kirson, D. Lavie and A. Abraham. 1980. Analysis of hybrids of Withania somnifera. Part 2. New withanolides from a cross of South African chemotype by chemotype II (Israel) in Withania somnifera. Phytochem.,

[13]. 19: 1503-1507.

[14]. Gayathri, G. 2002. Studies on dynamics of soil microbes in rice rhizosphere with water saving irrigation and in-situ weed incorporation. Ph.D. Thesis,
[15]. Tamil Nadu Agricultural University, Coimbatore.
[16]. Kannaiyan, S. 2000. Biofertilizers key factor in organic farming. The Hindu Survey of Indian Agriculture, pp. 165-173.
[17]. Kloepper, J.W. and M.N. Schroth. 1978. Plant growth promoting rhizobacteria on radishes. Proc. 4th Int. Conf. Plant Path. Bact., 4: 879-882.
[18]. Kloepper, J.W., M.N. Schroth and T.D. Miller. 1980b. Effect of rhizobacteria on potato development and yield. Phytopathol., 70: 1078-1082.
[19]. Paul, D., V. Srinivasan, M. Anandaraj and Y.R Sharma. 2003. Pseudomonas fluorescens mediated nutrient flux in the black pepper rhizosphere microcosm and enhanced plant growth. In: 6th International PGPR workshop, Calicut, India, pp: 18-23.