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Influence of rhizobacterial inoculation on the growth parameters of ashwagandha

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Rhizobacteria from different medicinal plants *viz*., *Withania somnifera*, *Coleus forskohlii* and *Vinca rosea* grown in different parts of Tamil Nadu were isolated and characterized. A pot culture experiment was conducted at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. The results revealed that the rhizobacterial inoculation positively influenced the germination, vigour index, shoot and root length, biomass, dry matter production, root and alkaloid yield of ashwagandha. Inoculation of *Azospirillum lipoferum*-AAs-11, *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 as combined inoculant recorded the maximum growth and yield of ashwagandha.

Key words : Ashwagandha, Growth parameter, Rhizobacteria

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 WHO has estimated that over 80 per cent of the world population meets their primary health care needs through traditional medicine (Lambert, 1997). 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). Ashwagandha is presently cultivated in India under varied agro-climatic regions and soil types without much care on nutritional management. The major lacuna in the cultivation of ashwagandha is non availability of standard agrotechniques to increase the yield and quality. It is obvious that without clear understanding on the cultivation of ashwagandha, the use of various chemicals and inorganics may deteriorate the quality of the products. Novel solutions for plant growth enhancement are required to increase the yield and quality of ashwagandha and to reduce the burden on our resources and environment.The biofertilizers are ecofriendly and low cost technology and their application may play a major role in soil fertility, nutrient transformation, crop sanitation and sustainability. The rhizobiocoenosis is an important biological process that plays a major role in satisfying the nutritional requirement of these crops.

MATERIALS AND METHODS

A pot culture experiment was conducted at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore (T.N.) 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*-AAs-11, *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 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.

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 chlorophyll protein alkaloid content were recorded at 90, 120, 150 and 180 DAS.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following sub heads :

Shoot length :

Shoot length varied from 26.00 to 76.50 cm in various treatments at different growth stages. Among the single inoculant, *Azospirillum lipoferum*-AAs-11 recorded the maximum shoot length of 59.75 cm plant^{-1} at 180 DAI. Triple inoculation of *Azospirillum lipoferum*-AAs-11 with *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 showed better growth than the combination of *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1. There was significant difference in shoot length due to combined inoculation of *Azospirillum lipoferum*-AAs-11, *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 when compared to uninoculated control and individual inoculation at all the growth stages (Table 1).

Primary and lateral branches :

The results are presented in Table 1. All the inoculated treatments recorded higher primary and lateral branches than uninoculated control. Among the single inoculations, *Azospirillum lipoferum*-AAs-11 recorded maximum primary and lateral branches (2.65 and 16.10, respectively), whereas triple inoculation of *Bacillus*-APb-1, *Azotobacter*-AAz-3 and *Pseudomonas fluorescens*-APs-1 recorded 3.12 and 17.23 primary and lateral branches, respectively. Inoculation of *Azospirillum lipoferum*-AAs-11 with *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 showed maximum branches when compared to the combination of *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1. There was significant difference in primary and lateral branches due to combined inoculation of *Azospirillum lipoferum*-AAs-11, *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 when compared to uninoculated control and other treatments.

Leaf area :

Plants inoculated with rhizobacterial isolates either alone or in various combinations significantly increased the leaf area when compared to uninoculated control at all the days of sampling. Among the single inoculants, *Azospirillum lipoferum*-AAs-11 recorded maximum leaf area of 956.14 cm^2 at 180 days after sowing followed by *Pseudomonas fluorescens*-APs-1 (920.07 cm^2), *Bacillus* (869.78 cm^2) and *Azotobacter* (867.81 cm^2). Triple inoculation of *Azospirillum lipoferum*-AAs-11 with *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 increased leaf area (1194.72 cm^2), when compared to the combination of *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 (1126.47 cm^2). Combined inoculation of *Azospirillum lipoferum*-AAs-11, *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 resulted in maximum leaf area (1351.40 cm^2) over all the other treatments (Table 2).

Root growth :

The root length, root girth, lateral roots, fresh and dry root weight of ashwagandha plants significantly increased due to the inoculation of rhizobacterial isolates.

Table 1 : Effect of rhizobacterial inoculation on the shoot length, primary and lateral branches development of ashwagandha (var. Jawahar 20)

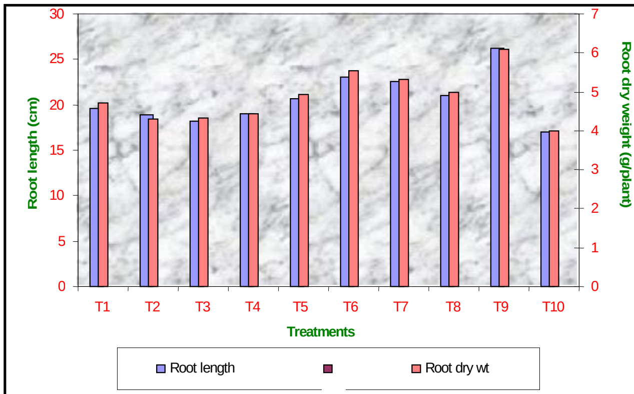
Treatments	Shoot length (cm plant^{-1})				No.of primary branches (plant^{-1})	No. of lateral branches (plant^{-1})			
	90 DAI	120 DAI	150 DAI	180 DAI		90 DAI	120 DAI	150 DAI	180 DAI
T_1 – *Azospirillum* (AAs-11)	30.25	43.55	52.11	59.75	2.65	9.00	12.86	13.00	16.10
T_2 – *Azotobacter* (AAz-3)	27.25	39.66	50.00	56.66	2.15	8.16	12.00	12.95	15.86
T_3 – *Bacillus* (APb-1)	27.00	38.44	48.00	55.00	2.05	8.00	11.86	12.85	15.33
T_4 – *Pseudomonas* (APs-1)	28.33	41.24	51.15	58.85	2.75	8.76	12.22	13.00	16.00
$T_5 – T_1 + T_2$	31.25	46.33	54.66	62.77	2.85	9.15	11.00	13.00	16.65
$T_6 – T_1 + T_3 + T_4$	35.25	62.55	64.65	71.33	3.25	11.00	12.10	14.25	18.00
$T_7 – T_2 + T_3 + T_4$	33.34	49.65	60.00	68.65	3.12	10.10	12.00	14.00	17.23
$T_8 – T_1 + T_2 + T_3$	32.15	48.25	57.15	66.45	3.00	9.85	11.86	13.25	17.00
$T_9 – T_1 + T_2 + T_3 + T_4$	38.47	56.22	69.47	76.50	3.52	11.44	13.24	16.32	20.25
T_{10} – Uninoculated control	26.00	37.33	45.66	53.00	2.00	7.96	10.00	11.85	15.33

	S.E. ±	C.D. (P=0.05)	S.E. ±	C.D. (P=0.05)
T	2.24	4.45	0.59	1.18
D	2.42	2.82	0.37	0.75
T x D	4.47	8.90	1.18	2.35

Table 2 : Effect of rhizobacterial inoculation on leaf area of ashwagandha (var. Jawahar 20)

Treatments	Leaf area (cm^2 $plant^{-1}$)			
	90 DAI	120 DAI	150 DAI	180 DAI
T_1 – *Azospirillum* (AAs-11)	110.99	306.13	772.77	956.14
T_2 – *Azotobacter* (AAz-3)	99.17	279.83	715.25	867.81
T_3 – *Bacillus* (APb-1)	97.15	279.32	691.96	869.78
T_4 – *Pseudomonas* (APs-1)	106.09	305.91	744.86	920.07
$T_5 – T_1 + T_2$	120.65	323.24	819.72	1009.93
$T_6 – T_1 +T_3+T_4$	167.85	437.65	1016.88	1194.72
$T_7 – T_2 +T_3+T_4$	143.21	389.48	953.32	1126.47
$T_8 – T_1+T_2+T_3$	138.87	361.29	877.43	1096.97
$T_9 – T_1+T_2+T_3+T_4$	201.10	549.80	1123.28	1351.40
T_{10} – Uninoculated control	83.77	241.38	690.32	747.83
	S.E. ±		C.D. (P=0.05)	
T	30.57		60.84	
D	19.35		38.48	
T x D	61.14		121.68	

Among the individual rhizobacterial inoculations, *Azospirillum lipoferum*-AAs-11 recorded higher root length (19.65 cm), root girth (1.78 cm), lateral roots (16.33 no.), root fresh weight (17.65 g $plant^{-1}$) and dry weight (4.72 g $plant^{-1}$) of ashwagandha on 180 DAS. Among the various treatments, maximum root length (26.17cm), root girth (2.32 cm), lateral roots (19.66 no.), root fresh weight (21.33 g $plant^{-1}$) and dry weight (6.10 g $plant^{-1}$) were observed with the combined inoculation of *Azospirillum lipoferum*-AAs-11, *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1. The uninoculated control plants recorded poor root growth and root weight (Table 3 and Fig. 1).



T_1 - *Azospirillum* (AAs-11)
T_2 - *Azotobacter* (AAz-3)
T_3 - *Bacillus* (APb-1)
T_4 - *Pseudomonas* (APs-1)
T_5 - $T_1 + T_2$
T_6 - $T_1 + T_3 + T_4$
T_7 - $T_2 + T_3 + T_4$
T_8 - $T_1 + T_2 + T_3$
T_9 - $T_1 + T_2 + T_3 + T_4$
T_{10} - Uninoculated control

Fig. 1 : Effect of rhizobacterial inoculation on the root growth of ashwagandha (var. Jawahar 20)

Dry matter production :

Inoculation of various bacterial strains significantly

Table 3 : Effect of rhizobacterial inoculation on the root growth of ashwagandha (var. Jawahar 20)

Treatments	Root parameters (180 DAS)				
	Root length (cm $plant^{-1}$)	root girth (cm $plant^{-1}$)	lateral roots (no. $plant^{-1}$)	root fresh weight (g $plant^{-1}$)	root dry weight (g $plant^{-1}$)
T_1 – *Azospirillum* (AAs-11)	19.65	1.78	16.33	17.65	4.72
T_2 – *Azotobacter* (AAz-3)	18.95	1.75	14.66	16.95	4.30
T_3 –*Bacillus* (APb-1)	18.20	1.75	14.00	17.00	4.33
T_4 – *Pseudomonas* (APs-1)	19.00	1.76	14.33	17.33	4.43
$T_5 – T_1 + T_2$	20.65	1.80	17.66	18.00	4.92
$T_6 – T_1 +T_3+T_4$	23.00	2.00	18.33	19.00	5.53
$T_7 – T_2 +T_3+T_4$	22.55	1.92	18.00	18.55	5.33
$T_8 – T_1+T_2+T_3$	21.00	1.85	17.00	18.20	5.00
$T_9 – T_1+T_2+T_3+T_4$	26.17	2.32	19.66	21.33	6.10
T_{10} – Uninoculated control	17.00	1.72	14.66	15.33	4.00
S.E. ±	1.76	0.16	1.31	1.53	0.41
C.D. (P=0.05)	3.69	0.33	2.76	3.22	0.87

Table 4 : Effect of rhizobacterial inoculation on dry matter production of ashwagandha (var. Jawahar 20)

Treatments	Dry matter production (g plant^{-1})			
	90 DAI	120 DAI	150 DAI	180 DAI
T_1 – *Azospirillum* (AAs-11)	0.580	4.72	9.65	15.18
T_2 – *Azotobacter* (AAz-3)	0.490	4.55	9.00	14.60
T_3 –*Bacillus* (APb-1)	0.513	4.60	9.15	14.64
T_4 – *Pseudomonas* (APs-1)	0.550	4.65	9.26	15.00
$T_5 – T_1 + T_2$	0.610	4.78	9.85	15.53
$T_6 – T_1 + T_3 + T_4$	0.693	5.11	11.12	16.17
$T_7 – T_2 + T_3 + T_4$	0.680	4.93	10.56	16.00
$T_8 – T_1 + T_2 + T_3$	0.650	4.82	10.00	15.92
$T_9 – T_1 + T_2 + T_3 + T_4$	0.703	5.47	12.56	17.27
T_{10} – Uninoculated control	0.410	4.16	8.50	13.26
	S.E. $\pm$		C.D. (P=0.05)	
T	0.42		0.84	
D	0.27		0.53	
T x D	0.85		1.68	

increased the plant dry weight over control (Table 4). Among the individual inoculants, *Azospirillum lipoferum*-AAs-11 recorded higher plant dry weight (15.18 g plant^{-1}) followed by *Pseudomonas fluorescens*-APb-1 (15.00 g plant^{-1}), *Bacillus*-APb-1 (14.64 g plant^{-1}), and *Azotobacter*-AAz-3 (14.60 g plant^{-1}). Inoculation of all the four rhizobacteria *viz.*, *Azospirillum lipoferum*- AAs-11, *Azotobacter*-AAz-3, *Bacillus*-APb-1 and *Pseudomonas fluorescens*-APs-1 together recorded maximum plant dry weight (17.27 g plant^{-1}).

Increase in the height of crop plants due to inoculation of rhizobacteria as observed in the present experiment was in conformity with the earlier reports in several other crops (Rajadurai *et al*., 2003; Chezhiyan *et al*., 2003). Inoculation of associative symbiotic diazotrophs *viz*., *Azospirillum* increased the dry matter production, grain yield and nitrogen content in most cereals and vegetable crops (Kapulnik *et al*., 1985). The effect of *Azospirillum* inoculation on the total yield increase in field grown plants ranged from 10-30 per cent (Kapulnik *et al*., 1981a; Okon and Labandera-Gonzalez, 1994).

Increased growth of plants might have been due to increased efficiency of roots in extracting the nutrients from the soil that are made available by the activity of inoculated bacteria. Many workers have reported the improvement of plant growth by the release of soluble 'P' from inorganic and organic phosphate due to phosphobacteria inoculation (Datta *et al.*, 1982; Banik and Datta, 1988). De Frietas and Germida (1990a) showed that inoculation of *P. aeruginosa, P. cepacia* and *P. fluorescens* (phosphate solubilizing microorganisms) strains on winter wheat increased the plant height, root and shoot biomass and number of tillers under growth chamber conditions. The phosphate solubilizing bacterial strains used in this study were able to solubilize inorganic phosphate and also mineralize organic phosphate as evidenced by high phosphatase activity.

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).

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