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INDUCTION OF DEFENSE ENZYMES IN FLOURESCENT PSEUDOMONADS AGAISNT WILT COPMLEX PATHOGENS OF MEDICINAL PLANTS

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ABSTRACT
The efficacy of seven potential fluorescent pseudomonads from different parts of Karnataka were tested for the induction of systemic resistance against wilt complex in two medicinal plants (coleus and ashwagandha) caused by *Fusarium* sp., *Ralstonia solanacearum* and *Meloidogyne incognita*. Among seven isolates of fluorescent pseudomonads, RB-50 and RB-31were enhanced the plant growth parameters with >85% germination and highest vigour index of 753.87 in ashwagandha. The highest number of branches and tubers per plant in coleus were observed in RB50 (13.33 and 10.33) followed by RB31 (11.00 and 8.00) respectively. Induced systemic resistance through as inferred biochemical analysis revealed the increased activities of the enzymes, *viz*. peroxidase (PO), polyphenol oxidase (PPO), phenylalamine ammonia lyase (PAL) and also phenolic compounds in the PGPR treated plants of coleus and ashwagandha challenged with individual and combinations of their pathogens. The increase in production, expression of defense enzymes and other compounds in PGPR treated plants upon challenge inoculation with different pathogens were comparatively higher when compared to uninoculated control. The observation revealed that, RB-50 and RB-31 fluourescent pseudomonads isolates were found effective, systemically induced resistance against wilt complex pathogens of coleus and ashwagandha by the accumulation of battery of enzymes in response to pathogens infection.

INTRODUCTION

Wilt complex of Coleus *(Coleus forskohlii* (Wild) Briq.) and Ashwagandha (*Withania somnifera* L. Dunal) are caused by a multitude pathogens either alone or in combination and distributed, wherever coleus and ashwagandha cultivation are persuade intensively. The major pathogens involved in wilt disease are species of *Fusarium* and a bacterium *Ralstonia solanacearum* (Smith) and root-knot caused by *Meloidogyne incognita* (Kofoid and White) Chitwood. All the pathogens are known to form complexes with nematodes, aggravating the disease. Nematodes alone are also potential pathogens of coleus and ashwagandha. The non availability of efficient appliances and pesticides (fungicide, bactericide and nematicide) and lack multiple disease resistant varieties also aggravated the problem. Therefore, a need for alternative methods of control of wilt complex and soil borne pathogens has become vital. Hence an attempt was made to manage these different groups of soil-borne pathogens using plant growth promoting rhizobacteria (PGPR), which induce resistance to wide range of pathogens (Liu *et al*., 1995). Fluorescent pseudomonads suppress the pathogens by various modes of actions namely competition for nutrients and space, antibiosis, siderophore, lytic enzymes, production of hydrogen cyanide and degradation of toxins, production of plant growth promoting substances (Sharma *et al*., 2014 a & b) and also induces systemic resistance (ISR) by enhancement of plant defense enzymes. Fluorescent pseudomonads activate ISR in plants against fungal, bacterial, viral diseases (Maurhofer *et al*., 1998), insects (Zehnder *et al*., 1997) and nematode pests (Sikora, 1988). Currently, attempts to protect plants from pathogens attack through PGPR have gained worldwide attention since; it is an ecofriendly and sustainable approach of plant disease management.

Plant enzymes are involved in defense reactions against plant pathogens. These include oxidative enzymes such as superoxide dismutase (SOD), peroxidase (PO) polyphenol oxidase (PPO) and phenylalanine ammonia-lyase (PAL) which catalyses the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure (Avdiushko *et al*., 1993). PGPR are involved in phytoalexin or phenolic compound biosynthesis (Bashan *et al*., 1985; Beaudoin-Eagan and Thorpe, 1985). Such enzymes have been correlated with defense against pathogens in several plants, including tobacco, tomato, cucumber and rice (Goy *et al*., 1992; Rajappan *et al*., 1995). These plant enzymes have long been thought to play an important role in the plant defense.

MATERIALS AND METHODS

Isolation of microorganisms

Samples of rhizosphere soils were collected from major coleus and ashawagandha medicinal plant growing parts of Karnataka. A total of fifty rhizobacteria were isolated by using

serial dilution technique on King's B Medium. Further, isolated rhizobacteria were characterized based on morphological and biochemical tests as fluorescent pseudomonads. Among fifty native fluorescent pseudomonads, seven strains were found highly inhibitory and commonly efficacious against the soil borne as well as vascular pathogens involved in wilt complex (*Fusarium*, *Ralstonia* and *Meloidogyne*) under *in vitro*. These pathogens were isolated from infected coleus and ashwagandha plants by using standard tissue isolation technique (Tuite, 1969).

Seed bacterization and plant growth promotion

Seeds of ashwagandha and fresh cuttings of coleus were surface sterilized with one per cent sodium hypochlorite and then seeds and cuttings are steeped/dipped in 10 ml of fluorescent pseudomonads suspension ($3X10^8$ cfu ml^{-1}) for 12 h. then seeds and cuttings are air dried and used further for sowing/planting in earthen pots containing 1 kg of sterilized soil: sand mixture under glasshouse. The germination percentage, shoot length and root length as well as fresh weight and dry weight of seedlings and vigour index were calculated (Abdul Baki and Anderson, 1973). Seeds/cuttings treated in sterile water and later were sown in a pot served as a control. After establishment of seedlings (30 DAP) in pot experiment a booster dose of talc based bioformulations of PGPR strains was also given at the rate of 30 ml of 10^8 cfu ml^{-1}.

Induction of defense mechanisms and experimental design

Seven potential fluorescent pseudomonads (RB01, RB10, RB13, RB22, RB31, RB43 and RB50) were used in the induction of defense reaction in coleus and ashwagandha. One day after bacterization, one set of bacterized plants was challenge inoculated with *Fusarium*, *Ralstoina* and *Meloidogyne* in individual or in combination interaction (as listed in Table 3 and 4) and bacterized plants without challenged with pathogens served as control. The experiment was conducted using randomized block design on greenhouse bench. The humidity in greenhouse was maintained at around RH 80% and temperature was adjusted to 28 $\pm$ 2°C. Observations were recorded after 5th days of challenged inoculation of fluorescent pseudomonads with different pathogens and their interactions.

Enzyme extract

The leaf, stem and root sample, collected from bacterized and pathogen inoculated coleus and ashwagandha plants were immediately homogenized with liquid nitrogen. One g of powdered sample was extracted with 2 ml of sodium phosphate buffer, 0.1M (pH 7.0) at 4ºC. The homogenate was centrifuged for 20 min at 10,000 rpm. This enzyme extract was stored at 2- 4ºC by adding a few drops of tolune for later use (Aneja, 2003). Protein extracts prepared from coleus and ashwaganda tissues were used for estimation of defense enzymes like peroxidase (PO) polyphenol oxidase (PPO), phenylalanine ammonia lyase (PAL) and total phenols.

Peroxidase activity was carried out as per the procedure described by Hammerschmidt *et al*. (1982). The enzyme activity was expressed as the increase in absorbance at 470 nm min^{-1} mg^{-1} of protein. Polyphenol oxidase activity was determined as per the procedure given by Mayer *et al*. (1965). A sample of one g was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) at 4ºC. The homogenate was centrifuged at 20,000 rpm for 15 min at 4ºC. The supernatant served as enzyme source and enzyme activity was expressed as changes in absorbance of reaction mixture at 495 nm min^{-1} change in absorbance min^{-1} mg^{-1} of protein. PAL activity (EC 4.3.1.5) was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid at 290 nm. Sample containing 0.5 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer pH 8.8 and 9.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30ºC. The amount of trans-cinnamic acid synthesized was calculated (Dickerson *et al*., 1984). Enzyme activity was expressed in fresh weight basis as nmol transcinnamic acid min^{-1} mg^{-1} of sample.

Phenol content was estimated as per the procedure given by Zieslin and Ben-Zaken (1993). A plant sample of one g was homogenized in 3ml of ice cold 0.1 M sodium borate buffer, pH 7.0, containing 1.4 mM of 2-mercaptoethanol and 50 mg of insoluble polyvinylpyrrolidene (PVP). The resulting extract was filtered through cheese cloth and the filtrate was centrifuged at 20,000 rpm for 15 min at 4ºC and the supernatant was used as the enzyme source. The content of the total soluble phenols was calculated according to a standard curve obtained from a Folin-Ciocalteau reagent with a phenol solution (C_6H_6OH) and expressed as catechol-equivalents mg^{-1} tissue weight.

RESULTS AND DISCUSSION

Plant growth promoting activity of PGPR strains

In ashwagandha plants under culture studies, the PGPR strains RB50, RB31 and RB1 showed maximum germination (>85%) and produced more shoot and root length with enhanced fresh and dry weight of seedlings compared to other strains

Table 1: Plant growth promoting activity of selected PGPR strains in ashwagandha seedlings in pot culture

PGPR strains	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	SVI
RB01	85.55 (67.63)*	4.55	3.83	0.53	0.04	691.24
RB10	79.33 (62.93)	3.36	3.53	0.45	0.03	525.95
RB13	84.33 (66.65)	4.17	3.92	0.63	0.05	682.22
RB22	70.33 (58.24)	2.77	3.37	0.25	0.03	4.31.82
RB31	86.00 (68.00)	3.91	3.93	0.54	0.06	679.41
RB43	82.66 (65.37)	3.39	3.05	0.41	0.04	532.33
RB50	87.66 (69.41)	4.67	3.93	0.58	0.07	753.87
Control	65.66 (54.10)	2.60	2.20	0.20	0.02	315.16
SEm $\pm$	0.40	0.16	0.14	0.02	0.00	
CD1%	1.67	0.64	0.60	0.10	0.01	

SVI- Seedling Vigour Index; *Figures in the parenthesis are arc sine transformed values

Table 2: Effect of PGPR strains on plant growth promoting activity in coleus under glasshouse conditions

PGPR strains	Shoot length (cm) DAP				No. of branches	No. of tubers	Tuber length (cm)	Shoot weight (g)		Root weight (g)		Total biomass (g)	
	60	90	150	180				Fresh	Dry.	Fresh	Dry	Fresh	Dry
RB 01	31.25	40.83	54.90	60.31	10.67	8.00	16.43	104.67	14.83	25.00	10.50	129.34	25.33
RB10	30.10	39.40	52.65	57.84	10.00	6.67	15.63	97.00	14.25	24.50	9.17	121.50	23.42
RB13	29.30	40.60	54.43	61.32	10.00	7.67	16.31	112.67	14.50	23.00	8.10	135.67	22.60
RB22	29.17	35.45	46.23	56.71	7.67	5.67	11.12	88.33	11.00	15.33	5.10	103.66	16.10
RB31	31.60	41.34	57.92	62.25	11.00	8.00	16.42	107.17	14.42	25.17	8.52	132.34	22.94
RB43	30.87	36.55	51.17	63.00	10.67	7.33	15.87	97.33	13.00	16.67	7.25	114.00	20.25
RB50	32.37	41.74	59.30	65.33	13.33	10.33	17.77	115.00	16.50	26.83	10.83	141.83	27.33
Control	24.80	27.30	29.67	34.95	6.67	5.33	9.33	60.33	7.33	10.83	2.08	71.16	9.41
SEm±	0.81	0.99	1.66	1.87	0.75	0.53	0.53	0.45	0.66	1.32	0.59	1.26	0.54
CD@ 1%	3.33	4.11	5.04	5.89	3.12	2.18	2.20	1.86	2.72	5.46	2.45	5.22	2.23

Table 3: Effect of PGPR strains on induced systemic resistance (ISR) in coleus plants, inoculated with different pathogens in pot culture

Treatment	PO *Δ in absorbance	% IOC**	PPO Δ in absorbance	% IOC	PAL Δ in absorbance	% IOC	Total phenols Δ in absorbance	% IOC
T1-RB01+F	0.098	30.66	0.017	54.54	0.061	35.55	0.66	50.00
T2-RB10+F	0.096	28.00	0.013	18.18	0.600	33.33	0.63	43.18
T3- RB13+F	0.098	30.66	0.017	54.54	0.065	44.44	0.69	56.81
T4 –RB22+F	0.095	26.66	0.012	9.09	0.051	11.76	0.60	36.36
T5 –RB31+F	0.100	33.33	0.018	63.63	0.075	66.66	0.79	79.50
T6 –RB43+F	0.097	29.33	0.016	45.55	0.052	15.55	0.65	47.70
T7 –RB50+F	0.102	36.00	0.019	72.72	0.080	77.77	0.87	97.72
T8 – F	0.075	-	0.010	-	0.045	-	0.44	-
T9-RB01+R	0.099	26.92	0.014	40.00	0.082	36.66	0.79	51.90
T10-RB10+R	0.094	20.51	0.012	20.00	0.076	26.66	0.70	34.61
T\11- RB13+R	0.097	24.35	0.014	30.00	0.088	46.66	0.80	53.80
T12–RB22+R	0.093	19.23	0.011	10.00	0.066	10.00	0.77	48.07
T13 –RB31+R	0.101	29.48	0.015	50.00	0.092	53.33	0.88	69.23
T14 –RB43+R	0.098	25.64	0.013	30.00	0.078	30.00	0.75	44.23
T15 –RB50+R	0.103	32.05	0.016	60.00	0.096	60.00	0.90	73.07
T16 – R	0.078	-	0.010	-	0.060	-	0.52	-
T17-RB01+F+R	0.100	47.05	0.018	38.46	0.098	63.66	0.80	33.33
T18-RB10+F+R	0.098	44.11	0.016	23.07	0.082	33.33	0.75	25.00
T19- RB13+F+R	0.099	45.58	0.017	30.76	0.092	53.30	0.81	35.00
T20-RB22+F+R	0.096	41.17	0.014	7.96	0.068	13.33	0.70	16.66
T21 -RB31++RF	0.102	50.00	0.019	46.15	0.108	80.00	0.82	36.66
T22-RB43+R+F	0.100	47.05	0.015	15.38	0.089	48.33	0.79	31.66
T23-RB5+R0+F	0.104	52.94	0.020	53.84	0.112	86.66	0.91	51.66
T24 - F+R	0.068	-	0.013	-	0.060	-	0.60	-
T25-RB01+M	0.099	41.42	0.020	33.33	0.088	51.72	0.81	62.00
T26-RB10+M	0.098	40.00	0.018	20.00	0.078	34.48	0.75	50.00
T27 RB13+M	0.100	42.85	0.020	33.33	0.085	46.55	0.82	64.00
T28–RB22+M	0.095	35.71	0.017	13.33	0.072	24.13	0.66	32.00
T29 –RB31+M	0.101	44.28	0.021	40.00	0.090	55.17	0.84	68.00
T30–RB43+M	0.097	38.57	0.019	26.66	0.082	41.37	0.79	58.00
T31-RB50+M	0.103	47.14	0.023	53.33	0.095	63.79	0.88	76.00
T32 – M	0.070	-	0.015	-	0.050	-	0.50	-
T33-RB01+F+M	0.098	28.94	0.025	47.05	0.091	44.44	0.72	30.00
T34-RB10+F+M	0.093	22.36	0.023	35.29	0.088	39.68	0.72	30.90
T35- RB13+F+M	0.099	30.26	0.026	52.94	0.093	47.61	0.73	32.72
T36-RB22+F+M	0.090	18.42	0.020	17.64	0.082	30.15	0.68	23.63
T37-RB31+F+M	0.100	31.57	0.028	64.70	0.095	50.79	0.75	36.36
T38-RB43+F+M	0.095	25.00	0.022	29.41	0.090	42.85	0.70	27.27
T39-RB50+F+M	0.101	32.89	0.030	76.47	0.098	55.55	0.80	45.45
T40-F+M	0.076	-	0.017	-	0.063	-	0.55	-
T41-RB01+R+M	0.099	52.30	0.023	43.15	0.101	48.52	0.78	39.28
T42-RB10+R+M	0.096	47.69	0.019	18.75	0.095	39.70	0.71	26.78
T43- RB13+R+M	0.093	50.76	0.022	37.50	0.102	50.00	0.80	49.85
T44-RB22+R+M	0.095	46.15	0.018	12.50	0.088	29.41	0.68	21.42
T45-RB31+R+M	0.100	53.84	0.025	56.25	0.105	54.41	0.83	48.21

Table 3: Cont.....

Treatment	PO *Δ in absorbance	% IOC**	PPO Δ in absorbance	% IOC	PAL Δ in absorbance	% IOC	Total phenols Δ in absorbance	% IOC
T46-RB43 + R + M	0.097	49.23	0.021	31.25	0.098	44.11	0.74	32.14
T47-RB50 + R + M	0.101	55.38	0.028	75.00	0.108	58.82	0.87	55.35
T48-R + M	0.065	-	0.016	-	0.068	-	0.56	-
T49-RB01 + F + R + M	0.109	12.37	0.029	61.11	0.105	40.00	0.86	43.33
T50-RB10 + F + R + M	0.106	9.27	0.024	33.33	0.100	33.33	0.80	33.33
T51-RB13 + F + R + M	0.103	11.34	0.028	55.55	0.108	44.00	0.87	45.00
T52-RB22 + F + R + M	0.105	8.24	0.020	11.11	0.092	22.66	0.72	20.00
T53-RB31 + F + R + M	0.113	16.49	0.030	66.66	0.109	45.33	0.90	50.00
T54 –RB43 + F + R + M	0.107	10.30	0.026	44.44	0.102	36.00	0.82	36.66
T55 –RB50 + F + R + M	0.124	27.83	0.032	77.77	0.110	46.66	0.92	53.33
T56- F + R + M	0.097	-	0.018	-	0.075	-	0.60	-

* Changes in absorbance /min/mg of protein;** Per cent increase over control; F- *Fusarium*, R- *Ralstonia*, M- *Meloidogyne*; Observations are recorded at 5 days after inoculation

Table 4: Effect of PGPR strains on induced systemic resistance (ISR) in ashwagandha plants, inoculated with different pathogens in pot culture

Treatment	PO Δ in absorb.*	% IOC**	PPO Δ in absorbance	% IOC	PAL Δ in absorbance	% IOC	Total phenols Δ in absorbance	% IOC
T1-RB01 + F	0.053	39.47	0.067	34.00	0.079	43.63	0.75	66.66
T2 + RB10 + F	0.048	26.31	0.065	30.00	0.073	32.72	0.70	55.55
T3-RB13 + F	0.055	44.73	0.070	40.00	0.081	47.27	0.78	73.33
T4-RB22 + F	0.045	13.15	0.061	22.00	0.069	25.45	0.60	33.33
T5-RB31 + F	0.058	52.63	0.072	44.00	0.085	54.54	0.79	75.55
T6-RB43 + F	0.050	31.57	0.064	28.00	0.075	36.36	0.73	62.22
T7-RB50 + F	0.060	57.89	0.080	60.00	0.089	61.81	0.80	77.77
T8-F	0.038	-	0.050	-	0.055	-	0.45	-
T9-RB01 + M	0.046	31.42	0.068	28.30	0.080	37.93	0.78	56.00
T10-RB10 + M	0.042	20.00	0.062	16.98	0.075	29.31	0.75	50.00
T11-RB13 + M	0.048	37.14	0.070	32.07	0.082	41.37	0.82	64.00
T12-RB22 + M	0.040	14.28	0.058	9.43	0.070	20.68	0.71	42.00
T13-RB31 + M	0.051	45.71	0.074	39.62	0.086	48.27	0.84	68.00
T14-RB43 + M	0.044	25.71	0.065	22.64	0.078	34.48	0.76	52.00
T15-RB50 + M	0.054	54.28	0.080	50.94	0.091	56.89	0.88	76.00
T16-M	0.035	-	0.053	-	0.058	-	0.50	-
T17-RB01 + F + M	0.058	45.00	0.085	41.66	0.085	37.09	0.84	52.72
T18-RB10 + F + M	0.051	27.50	0.076	26.66	0.079	27.41	0.76	38.18
T19-RB13 + F + M	0.066	65.00	0.088	46.66	0.088	41.-93	0.85	54.54
T20-RB22 + F + M	0.049	22.50	0.072	20.00	0.076	22.58	0.71	29.09
T21-RB31 + F + M	0.072	80.00	0.091	51.66	0.090	45.16	0.89	61.81
T22-RB43 + F + M	0.050	25.00	0.081	35.00	0.080	29.03	0.80	45.45
T23-RB50 + F + M	0.078	95.00	0.098	63.33	0.094	51.61	0.99	80.00
T24-F + M	0.40	-	0.060	-	0.062	-	0.555	-

*Changes in absorbance /min/mg of protein;** Per cent increase over control; F- *Fusarium*, M- *Meloidogyne*; Observations are recorded at 5 days after inoculation

and control. The highest vigour index 753.87, maximum shoot and root length (4.67 and 3.93 cm) was recorded in RB50 treated seedlings. Least vigour index (315.16), shoot and root length (2.60 and 2.2 cm) was registered in untreated control (Table 1 and Fig. 1).

In coleus highest shoot length of 32.37, 41.74, 59.30 and 65.33 cm at 60, 90, 150 and 180 days after planting and highest number of branches (13.33), highest number of tubers (10.33) and also highest biomass (141.83 g- fresh wt. and 27.33 g- dry wt. was recorded in RB50 treated plants followed by RB31 whereas lowest was observed in RB22 followed by untreated control (Table 2 Fig. 2). In this study, an increase in the plant growth by seed bacterization has been demonstrated. It is a well-established fact that overall plant growth and root development influenced by improved phosphorous nutrition. A large number of evidence suggests that PGPR enhance the growth, seed emergence and crop yield (Pradhan and Mishra, 2015). Significant increases in plant growth parameters in the present study may be attributed to the production of plant growth regulators such as auxins, gibberellins, cytokinins and ethylene (Frankenberger and Arshad, 1995). It has often been inferred that rhizobacterially produced auxins are responsible for growth promotion. Indole acetic acid promotes ethylene production by stimulating the enzyme in the ethylene biosynthetic pathway (Kende, 1993).

Induced Systemic Resistance (ISR)

Induced resistance is a state of enhanced defensive capacity against broad spectrum of pests and pathogens developed by a plant when appropriately stimulated (Van Loon *et al.,* 1998). The resulting elevated resistance due to biotic agents is referred

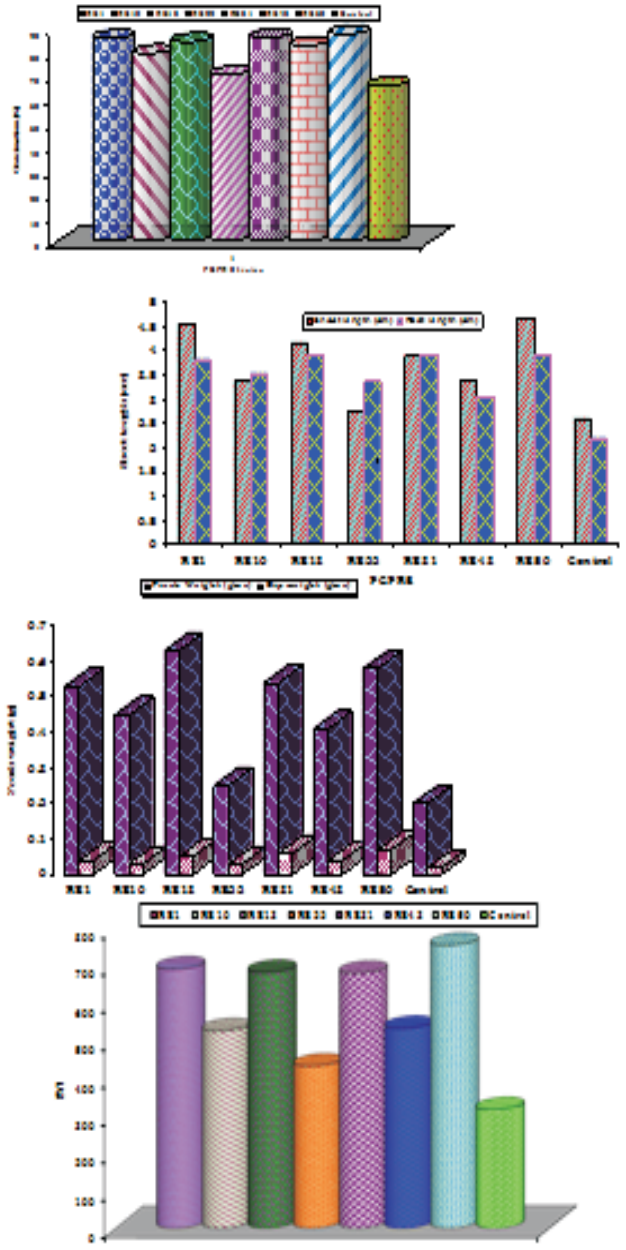

Figure 1: Plant groth promoting activity of selected PGPR strains in ashwagandha seedling in pot culture

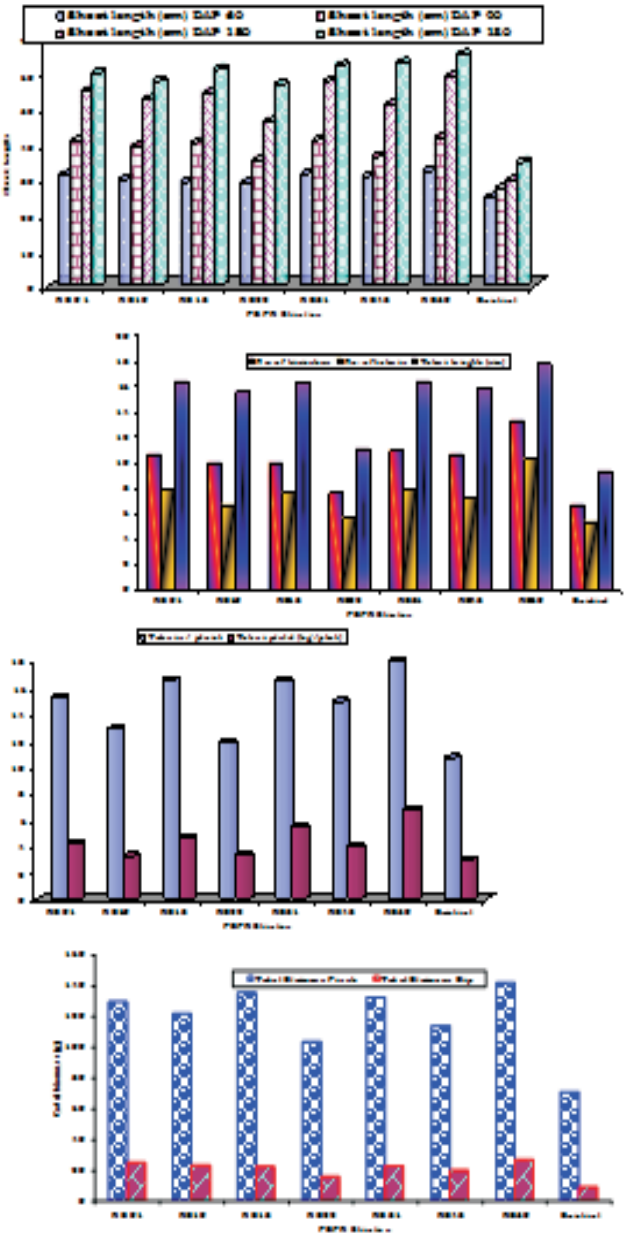

Figure 2: Effect of PGPR strains on plant growth promoting activity in coleus under glasshouse conditions

to as ISR whereas that by other than biological control agents is called systemic acquired resistance (SAR) (Zhu-Salzaman *et al.*, 2005).

In our study, we concentrated on biotic (Fluorescent pseudomonads) inducers for inducing the defense molecules challenged with *Fusarium, Ralstonia* and *Meloidogyne* in coleus and only *Fusarium* and *Meloidogyne* in ashwagandha. The ISR in this study was primarily focused for the defense related proteins, *viz*. PO, PPO, PAL and phenols (Table 3 and 4).

The results of the present study revealed that there was significant increase in the activity of PO, PPO, PAL and total phenolic contents in coleus and ashwagandha plants treated with PGPR strains RB50 and RB31 after 5days of inoculation. Similar studies, which showed an increase in PO, PPO and PAL activity were reported by Sandeep (2004) and Krishnaveni (2005) in *P. fluorescens* treated banana plants infested with *M. incognita* and *H. multicinctus*.

PO, PPO and PAL are linked to the ISR pathway regulated by jasmonates and ethylene that is activated by saprophytic microorganisms including rhizobacteria (Van Loon *et al.*, 1998). PAL is the first enzyme in phenylpropanoid metabolism involved in the production of phenolics and phytoalexins that prevent establishment of the pathogens (Daayf *et al.*, 1997). The present study also indicated enhanced activity of PO, PPO, PAL enzymes due to PGPR treatment with RB50 and

RB31, which might have prevented the establishment of nematodes, fungi and bacteria within the coleus and ashwagandha roots. Jonathan et al. (2006) also observed similar increase in plant growth and reduction in *M. incognita* population in banana plants treated with native isolates of *P. fluorescens* and also observed increased activity of PO, PPO and PAL enzymes.

In conclusion, the enzymes mentioned earlier played an important role in ISR. Enzyme accumulation could be involved not only in plant defense response, but may also be associated with induced resistance by PGPR RB50 and RB31 against wilt complex disease in medicinal plants caused by *Fusarium* sp., *Ralstonia solanacearum* and *Meloidogyne incognita*. It could be speculated that the enhanced expression patterns of these enzymes by fluorescent Pseudomonads might account for their ability to provide effective protection for coleus and ashwagandha from wilt complex of soil-borne pathogens. The induced ability of resistance to these pathogens was systemic. The use of PGPR as biofertilizers and biopesticide are a competent approach to replace chemical fertilizers and pesticides for sustainable crop cultivation in India. Thus, in the present study RB50 and RB31 found to be most efficient rhiobacterial strains to induce defense enzymes. The studies indicate that, PGPR strains as biopesticide are very much effective in controlling the wilt complex disease and also facilitating as plant growth promoter by triggering the defense mechanism.

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