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Growth Responses of *Vigna radiata* to Arid Land Bacteria Exhibiting Antimicrobial Activity

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Abstract: Twenty-four bacterial strains were isolated from rhizosphere and histoplane of four different arid plants, *Trianthema partulacastrum*, *Rumex dentatus*, *Chenopodium morale* and *Coronopus didymus*. These bacteria were inoculated to seeds of *Vigna radiata* var NM-92 which were germinated and grown for 15 days. Majority of strains had inhibitory effect on seed germination (2.2 to 13.0%). The increase in seedling length ranged from 17 to 38% with different inoculations. Bacterial strains TP1, TP3, TP4, RTP4 and RRD1 were the most effective ones for the different growth parameters such as seed length and seed weight whereas the most adverse effects were recorded with the inoculation of TP17 strain. Maximum increase in protein and auxin content was observed with strains TP5 and RCM2, respectively. Peroxidase and acid phosphatase showed maximum activity with the inoculation of strain TP1.

Key words: Antimicrobial activity, bacteria, *Trianthema partulacastrum*, *Coronopus didymus*, *Vigna radiata*

INTRODUCTION

Bacteria are ubiquitous in their distribution and are present in water (Klaus *et al*., 2007), soil (Sager *et al*., 2007) and play an important role in soil quality (Hill *et al*., 2000) by increasing organic carbon and moisture level (Eaton, 2000). They are also essential for various functions such as plant growth promotion (Humphry *et al*., 2007), inhibition of plant pathogen (antimicrobial activity) (Cho *et al*., 2007), improvement of soil structures and leaching metal (Van Veen *et al*., 1997). Tillage reduction in agriculture soil has been reported by microbial mass and microbial density (Lupwayi *et al*., 2000). The plant species, type and development influenced the variation of microbial communities in soil and association with roots (Wieland *et al*., 2001). These bacteria produce antimicrobial agents which inhibit/promote the growth of plants in the soil (Casida and Lukezic, 1992). Bacterial strains that enhance plant growth under heavy metal stress have been reported by Faisal *et al*. (2005) and Faisal and Hasnain (2006). Microbes exhibiting the antimicrobial behavior can effect their host in different ways. Their effect may be stimulatory (Alami *et al*., 2000) or inhibitory (Li *et al*., 2007). Vigna radiata is an important cash crop of Pakistan. Keeping the association of antimicrobial activity and plant promoting ability of bacterial strains, twenty-four bacterial strains were used for the present study, to evaluate the impact of antimicrobial activity exhibiting bacterial strains on the growth of *V. radiata*.

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Table 1: Bacterial isolates from histoplane and rhizoplane of four different plants *Trianthema partulacastrum*, *Rumex dentatus*, *Chenopodium morale* and *Coronopus didymus*

	Bacterial strains	
Source (plants)	Histoplane	Rhizoplane
Trianthema partulacastrum	TP1, TP2, TP3, TP4, TP5, TP7, TP8, TP9, TP11, TP13, TP14, TP15, TP16, TP17	RTP2, RTP4
Rumex dentatus	RD3, RD4, RD5	RRD1
Chenopodium morale		RCM2
Coronopus didymus		RCD1, RCD2, RCD3

MATERIALS AND METHODS

Bacterial Strains and Seeds Inoculation

The study was conducted in the University of the Punjab, Lahore, Pakistan in the year 2005 to 2006. Twenty four different strains were isolated from the roots of four weeds (*Trianthema partulacastrum*, *Rumex dentatus*, *Chenopodium morale* and *Coronopus didymus*) by Ahmed (2000), (Table 1). Bacterial strains were grown in L-agar medium (Gerhardt *et al*., 1994) at 37°C for 24 h. Healthy seeds of *V. radiata* were surface sterilized by soaking in 0.1% $HgCl_2$ and then washed thrice with distilled water. Seeds were then soaked in bacterial suspension for 15 to 20 min. For control, seeds were soaked in sterile glass distilled water for same duration.

Germination Experiments

Ten milliliter of autoclaved distilled water was poured in labeled petriplate (lined with double layer of Whattman filter paper No. 1 autoclaved and oven dried). With the help of sterilized forceps, seeds (control as well as inoculated) were spread in the respective labeled petri plate (15 seeds of *V. radiata* per petri plate) uniformly. Petri plates were kept in dark at 25±1°C for germination. Germination was recorded daily. After germination, plates were shifted to light (10D lux and 16 h day length) at 25±1°C after adding 10 mL of Hewitt nutrient solution in each plate (Hewitt, 1963). Seedlings were grown for 10 days after shifting to light. Experiment was repeated eight times. Seedlings were removed from the petri plates. Different growth parameters (seed germination, seedling length, fresh weight of seedlings, dry weight of seedling, dry weight per gram fresh weight of seedling) were recorded.

Biochemical Analysis

For biochemical analysis auxin (Mahadevan, 1984), soluble protein (Bhatti *et al*., 1993; Lowry *et al*., 1951), peroxidase (David and Murray, 1965) and acid phosphatases content (Iqbal and Rafique, 1986) were studied.

Statistical Analysis

Standard errors of the means and LSD were calculated following Steel and Torrie (1981).

RESULTS

Seed germination of *V. radiata* was affected with the inoculation of bacterial strains. The germination was increased over control with few bacterial strains such as TP17 (4.5%), RTP4, RCD1, RCD2, RD3 (4%). Majority of strains had inhibitory effects on this parameter and 1.04 to 2.7% reductions in germination of seeds were recorded (Table 2). Inoculation of TP2, TP9, RCD3 and RD4 and RCM2 did not cause any change in germination parameter. Seedling length was increased with inoculation of bacterial strains except TP17 where slight decrease (2.5%) over control was recorded

Table 2: Effects of bacterial inoculations on seed germination, seedling length, fresh weight, dry weight and dry weight g^{-1} fresh weight of *V. radiata* seedlings

Strains	Seed germination	Seedling length (cm)	Fresh weight (mg)	Dry weight (mg)	Dry weight g^{-1} fresh weight (mg g^{-1})
Control	91.66±5.53	15.80±0.64	391.10±2.55	13.71±0.13	35.04±0.57
TP1	84.99±2.89	19.60±0.26	439.70±12.79	18.39±0.065	41.90±0.32
TP2	91.66±7.26	9.74±0.48	410.35±9.65	17.57±0.43	42.02±0.02
TP3	89.99±3.33	20.86±0.08	407.14±7.14	18.21±0.36	44.71±0.09
TP4	84.99±5.52	19.67±0.21	418.00±2.25	19.60±0.40	46.81±0.081
TP5	86.66±6.42	18.48±0.44	355.55±1.17	14.72±0.74	41.59±1.64
TP7	79.99±6.42	18.94±0.27	371.66±9.02	12.96±0.38	34.87±0.05
TP8	83.33±1.37	19.21±0.64	400.30±8.97	16.21±0.21	40.53±0.35
TP9	91.66±5.26	18.56±0.39	403.83±8.33	16.51±0.09	40.89±0.67
TP11	89.99±5.45	20.47±0.58	393.93±9.12	16.74±0.59	42.50±0.51
TP13	88.33±5.52	19.08±0.28	394.13±6.63	16.91±0.24	42.92±0.07
TP14	89.99±3.33	20.96±0.45	382.13±4.28	15.19±0.19	39.74±0.05
TP15	89.99±3.33	20.31±0.56	378.38±2.38	15.19±0.19	40.14±0.25
TP16	89.99±5.45	19.28±0.28	373.92±6.78	10.14±0.07	27.10±0.43
TP17	95.83±2.76	15.40±0.38	400.33±0.33	11.74±0.07	29.32±0.12
RTP2	81.66±2.76	19.23±0.30	465.47±1.19	19.64±0.36	42.18±0.66
RTP4	95.83±6.66	20.21±0.24	374.48±7.82	15.58±0.25	41.60±0.20
RCD1	94.99±5.53	19.67±0.36	418.83±6.17	17.92±0.33	42.80±0.45
RCD2	94.99±5.53	19.84±0.25	408.66±1.33	19.66±0.33	48.11±0.66
RCD3	91.66±6.66	19.37±0.65	349.33±8.00	13.66±0.33	39.11±0.06
RD3	94.99±5.53	19.53±0.59	416.92±6.92	18.60±0.60	44.34±0.95
RD4	91.66±6.66	19.51±0.28	349.07±3.07	13.45±0.12	38.51±0.01
RD8	88.32±2.88	19.65±0.26	364.21±2.71	14.99±0.39	41.14±0.76
RRD1	89.99±5.45	21.86±0.12	404.40±7.26	18.50±0.65	45.74±0.80
RCM2	91.66±2.88	19.44±0.57	398.21±8.93	15.58±0.25	42.44±0.35
LSD at p = 0.05	4.075	0.17	9.28	0.24	0.495

Table 3: Effects of bacterial inoculation on the soluble protein, auxin, peroxidase and acid phosphatase activity of in *V. radiata* seedlings

Strains	Protein (µg g^{-1})	Auxin (µg g^{-1})	Peroxidases (Units g^{-1} fresh weight)	Acid phosphatases (Units g^{-1} fresh weight)
Control	667± 3.00	0.49±0.012	157±5.080	139±9.94
TP1	1076±4.00	1.28±0.080	481±2.360	352±6.85
TP2	1080±8.00	1.006±0.09	176±4.870	177±1.72
TP3	757±3.00	0.67±0.079	279±6.150	245±5.15
TP4	1047±9.00	1.21±0.050	299±0.260	223±10.40
TP5	1590±10.00	1.20±0.620	311±8.590	307±3.15
TP7	614±10.00	0.25±0.120	212±2.700	181±2.30
TP8	1070±6.00	1.245±0.22	230±4.700	199±4.63
TP9	740±8.00	1.042±0.12	87±1.920	115±2.30
TP11	780±11.00	0.76±0.100	350±7.510	313±1.85
TP13	1006±14.00	0.91±0.001	275±7.110	265±8.90
TP14	640±10.00	0.42±0.210	268±3.280	267±3.61
TP15	745±9.00	0.89±0.063	112±8.200	108±1.34
TP16	541±7.00	0.28±0.055	152±3.120	105±4.45
TP17	654±10.00	0.47±0.054	183±4.230	157±3.58
RTP2	1164±10.00	0.93±0.440	33±1.180	88±6.20
RTP4	743±7.00	0.85±0.029	38±0.090	61±1.18
RCD1	698±12.00	0.54±0.008	24±4.620	252±6.50
RCD2	649±7.00	0.38±0.008	199±5.007	148±5.81
RCD3	1028±12.00	1.046±0.12	98±0.056	118±1.28
RD3	1030±2.00	1.047±0.05	112±4.270	132±5.80
RD4	793±9.00	1.03±0.100	199±6.600	192±3.80
RD8	590±2.00	1.56±0.170	281±1.070	267±6.77
RRD1	436±2.00	0.40±0.100	256±1.480	219±46.77
RCM2	1194±12.00	1.77±0.153	126±9.570	121±4.66
LSD at = p 0.05	12.44	0.042	4.827	5.73

(Table 2). Maximum increase in the seedling was observed with inoculation of RRD1 (38.0%). Fresh weight of *V. radiata* was increased in the presence of bacterial strains TP1, TP2, TP3, TP4, TP8, TP9, TP17, RCD1, RCD2, RD3, RRD1, RCM2 and RTP2, when compared with non-inoculated control

(Table 2). The maximum increase in this parameter was observed with the inoculation of RTP2 (19.0%). Dry weight and dry weight per gram fresh weight increased with bacterial inoculation as compared to non-inoculated control.

Protein content was increased with the inoculation of majority of bacterial strains while TP7, TP14, TP16, TP17, RCD2, RD8 and RRD1 caused reduction in the soluble protein content over non-inoculated seedlings (Table 3). The maximum increase was observed with the inoculation of RCM2 (79.0%). The maximum increase in auxin content was observed with inoculation of RCM2 (26.7%). Peroxidase activity of *V. radiata* seedlings were increased with the inoculation of bacterial strains TP9 (44.0%). The activity of acid phosphatases increased with the inoculation of majority of bacterial strains. The maximum increase in the acid phosphatases activity was observed with the inoculation of TP1 (153.2%).

DISCUSSION

Microorganisms of soil are sensitive indicator of soil health because of the relationships between microbial diversity, soil quality and ecosystem sustainability (Doran *et al*., 1994). Hence the microorganism plays important roles in soil quality and plant productivity (Hill *et al*., 2000). The rhizospheric bacteria promote plant growth by inducing systemic resistance in host plant and protect plant from pathogens (Zheng *et al*., 2000). The strains used in present work were characterized and screened for antimicrobial activity against *Saccharomyces cervisae*, *E. coli* and *Bacillus subtilis* by Ahmed (2000). Majority of them showed antimicrobial activity against yeast but none of them showed any activity against *E. coli*. Strains TP13 and RD8 produce certain substances that inhibit *Bacillus subtilis* growth.

Germination of *V. radiata* was adversely affected by the inoculation of bacterial strains excluding TP13 and RTP4 (Table 2). The increase in fresh weight might be due to rise in water content (Hashim *et al*., 1996). Enhanced dry weight could be attributed to the accumulation of salts and nutrients, enhanced ion uptake and increased level of organic salts in cytoplasm (Sudhakar *et al*., 1993). Improved seedling length coupled with increased dry weight, which is clear indication of growth stimulation by bacteria. Decrease in fresh weight of seedlings may be due reduce water uptake under stress condition. Soluble protein content was also affected with the inoculation of bacterial strain. Maximum increase in this parameter was caused by the inoculation of RCM2 (79.01%). According to Staples and Dacher (1986) auxin and protein are formed from tryphtophan molecules with different molecular arrangement, so the amount of the auxin is directly related to the amount of soluble protein content. Auxin is the master hormone, exercising regulatory action over many different sorts of plant process. Campbell (1985) reported that bacterial strains stimulate plant growth by synthesizing and liberating growth hormone. Enhancement in the auxin contents with the inoculation of bacterial strains was reported in the present study. Enzymes are biochemical catalyst, which play important role. Two enzymes, acid phosphatases and peroxidase were studied for their activity vis-à-vis treatments. Activities of these enzymes were also improved with bacterial inoculation as compared to non-inoculated treatments (Table 3). Majority of bacterial strains caused improvements in the activity of peroxidase and acid phosphatase. The increase in antioxidant enzyme activity in plants under stress provides better protection against reactive oxygen activities (Meloni *et al*., 2003).

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