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Effect of Bacterial Mono Culture Inoculations on the Early Growth of *Triticum aestivum* Var. Inqlab-91 under NaCl Stress

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Abstract: Bacterial strains obtained from the rhizoplane, histoplane and phylloplane of *Achyranthus aspera* (RAa1, RAa2, HAa1, HAa2, HAa3, PAa1, PAa2, PAa3, PAa4, PAa5, PAa6), *Euphorbia heliscopia* (REh1, REh2, HEh1, HEh2, PEh1, PEh2), and *Malvestrum tricuspidatum* (RMt1, HMt1, HMt2, PMt1, PMt2) were used to inoculate the seeds of *Triticum aestivum* var. Inqlab-91. Inoculated as well as uninoculated seeds were germinated and grown under 0 and 100mM NaCl stress for 10 days. Different growth parameters and some biochemical characters were considered for these studies. Salinity reduced the percentage germination, root, shoot, seedling length as well as fresh weight per seedlings, whereas it increased the dry matter accumulation, Na^+, K^+ uptake, protein, auxin content and activity of peroxidases and acid phosphatases in uninoculated seedlings. While the association of many bacterial strains with wheat enhanced the seedling growth under 0 and 100mM NaCl stress. Bacterial inoculations improved different growth parameters by reducing the Na^+ uptake and improving the protein content, auxin production and alteration in enzyme activities.

Key words: Plant bacteria association, salt stress, *Triticum aestivum*, NaCl stress

Introduction

Suitable soil, good quality water and favourable climate are the three main components for plant growth. Any fluctuation in one of these factors badly affects the crop production. In Pakistan salt affected soils have caused a substantive decline in agricultural productivity. The salinity and sodicity can't be solved with complete success. Due to economic and/or environmental limitations such as inadequate drainage, it may not be possible to leach out salt from the soil. Therefore we have to live with this problem. Hence the management of such soils is more essential when the country is looking forward to big increase in food production (Ali *et al.*, 1997). Therefore at present greatest interest resides with the development and application of specific biocontrol agents to solve this problem. In this regard, bacteria have been proved valuable biocontrol agents. Many bacteria especially nitrogen fixing bacteria have shown to change the properties of salt affected soil (Gangewane and Salve, 1993; Velagaleti and Schweitzer, 1994).

Bacteria in the rhizosphere may enhance plant health and productivity by synthesizing phytohormones, increasing local availability of nutrients, facilitating uptake of nutrients by the plants, decreasing metal toxicity in plants, antagonizing plant pathogens and inducing systemic resistance in plants to pathogens (Benhamou *et al.*, 2000; Burd *et al.*, 2000; Ramamoorthy *et al.*, 2001). In previous studies we found that salt tolerant bacteria stimulated plant growth under salt stress (Afrasayab and Hasnain, 2000a,b; Afrasayab *et al.*, 2001). The purpose of this study was to determine the influence of salt tolerant bacteria isolated from different parts of plants on *Triticum aestivum* var. Inqlab-91 under salt stress.

Materials and Methods

Bacterial strains: Bacterial strains selected for this study were isolated from different parts of *Achyranthus aspera, Euphorbia heliscopia* and *Malvestrum tricuspidatum* growing in salt range of Pakistan (Table 1).

Bacterial inoculations and growth conditions: Bacterial strains were grown overnight at 37°C (150 rpm shaking) in L-Broth for preparation of bacterial inoculations. Bacterial suspensions, inoculation of seeds, experimental set up and growth conditions have been described earlier (Afrasayab and Hasnain, 2000b). Inoculated and non-inoculated seeds were germinated (in dark) and then supplied with nutrient solutions and grown in light (10 Klux for 16 hours) at 24± 2°C for 10 days. Experiment was arranged in completely randomized design with four replicates.

Table 1: Bacterial isolates from different sources used to study the inoculation effect on *Triticum aestivum* under NaCl stress.

Plant	Source	Bacterial isolates
Achyranthus aspera	Rhizosphere	RAa1, RAa2
	Histoplane	HAa1, HAa2, HAa3
	Phylloplane	PAa1, PAa2, PAa3, PAa4,
	Phylloplane	PAa5, PAa6
Euphorbia heliscopia	Rhizosphere	REh1, REh2
	Histoplane	HEh1, HEh2
	Phylloplane	PEh1, PEh2
Malvestrum tricuspidatum	Rhizosphere	RMt1
	Histoplane	HMt1, HMt2
	Phylloplane	PMt1, PMt2

Harvest and study of growth parameters: Seedlings were harvested and length (root, shoot seedling) as well as weight (fresh, dry, dry weight per gram fresh weight) parameters were studied

Sodium and potassium contents: Oven dried plant material (80°C) was acid digested (Humphries, 1956) and Na^+ /K^+ contents were determined with flame photometer (Jenway PEP7)(Furman, 1975).

Biochemical analysis: Biochemical studies of inoculated and non-inoculated seedlings including auxins (Mahadevan, 1984), soluble proteins (Lowry *et al.*, 1951), peroxidase (Racusen and Foote, 1965) and acid phosphatase (Iqbal and Rafique, 1986) analyses were carried out.

Statistical analysis: Data was analyzed statistically (Steel and Torrie, 1981). Mean, standard error of the mean, analysis of variance, and least significant difference (LSD) were calculated.

Results

Seeds subjected to salt stress showed significant reduction in germination. About 4.21% reduction in germination occurred at 100mM NaCl concentration (Table 2). In general bacterial inoculations provoked germination at 0 (1.01% increase) and 100 mM (1.05-4.21%) NaCl treatments. Enhancement in germination in most of the cases was significant at both treatments. A few bacterial inoculations dropped (RAa1, HAa1, PAa2) or had no effect (HAa2, PAa3, HEh1, HMt1) on germination at 0 mM, relative to non-inoculated respective treatment. The same trend was also reflected at 100 mM NaCl, where some of the bacterial inoculations (REh2, HEh2, PMt1, HMt1) had no stimulatory or inhibitory impact on germination. However enhancement in germination was more with bacterial strains isolated from *Achyranthus aspera.*

Although salinity affects plants at various growth stages, but

Table 2: Effect of inoculation of salt tolerant bacteria isolated from *Achyranthus aspera*, *Euphorbia heliscopia* and *Malvestrum tricuspidatum* on percentage germination and seedling lengths (cm) of *Triticum aestivum* var. Inqlab 91 at 0 and 100 mM NaCl concentrations (means of four replicates).

Bacterial inoculations	Percentage germination		Seedling lengths (cm)	
	0 mM	100 mM	0 mM	100 mM
Cont.	99± 0.89	95± 2.00	24.21± 1.96	13.80± 0.81
RAa1	98± 1.09	97± 1.09	26.88± 1.20	14.84± 0.83
RAa2	100± 0.00	97± 1.09	26.18± 1.95	13.37± 0.93
HAa1	96± 0.44	97± 1.09	26.34± 1.81	14.19± 0.70
HAa2	99± 0.89	97± 1.09	28.10± 1.34	14.25± 0.39
HAa3	100± 0.00	98± 1.79	25.95± 1.72	15.04± 0.81
PAa1	100± 0.00	97± 1.09	26.02± 1.50	12.91± 0.48
PAa2	98± 1.09	96± 0.44	25.51± 1.81	14.87± 0.74
PAa3	99± 0.89	99± 0.89	28.44± 1.12	14.29± 0.54
PAa4	100± 000	97± 0.44	25.45± 1.87	13.48± 0.43
PAa5	100± 0.00	96± 0.89	27.04± 1.62	14.19± 0.46
PAa6	100± 0.00	96± 0.89	27.43± 1.18	14.63± 0.70
REh1	100± 0.00	96± 3.74	22.72± 1.57	14.78± 0.96
REh2	99± 1.00	95± 1.58	23.02± 1.00	13.91± 0.90
HEh1	99± 1.00	96± 3.74	24.77± 0.94	15.22± 0.98
HEh2	100± 0.00	95± 1.58	23.88± 1.26	14.55± 0.65
PEh1	100± 0.00	94± 2.44	21.76± 1.01	13.55± 0.54
PEh2	99± 1.00	97± 1.22	24.12± 1.07	14.34± 0.82
RMt1	100± 0.00	98± 1.22	25.15± 1.24	12.95± 0.66
HMt1	99± 1.00	95± 1.58	23.39± 1.18	14.06± 0.67
HMt2	100± 0.00	94± 1.87	25.71± 1.60	13.98± 0.34
PMt1	100± 0.00	95± 1.58	26.29± 1.08	15.99± 1.08
PMt2	100± 0.00	93± 3.39	24.57± 0.98	14.58± 0.64
L.S.D. at	For Strain	0.82		0.76
p= 0.05	For Treatment	2.79		2.59

Table 3: Effect of inoculation of salt tolerant bacteria isolated from *Achyranthus aspera*, *Euphorbia heliscopia* and *Malvestrum tricuspidatum* on dry weight per seedling (mg), dry weight per gram fresh weight (mg/gm) of *Triticum aestivum* var. Inqlab 91 at 0 and 100 mM NaCl concentrations (means of four replicates).

Bacterial Inoculations	Dry weight per seedling		Dry weight per gram fresh weight	
	0 mM	100 mM	0 mM	100 mM
Cont.	31.93± 1.34	33.06± 1.33	139.80± 2.46	224.27± 2.42
RAa1	33.86± 0.98	35.12± 1.32	127.12± 1.26	222.58± 1.42
RAa2	20.88± 1.86	32.22± 1.45	90.63± 2.28	215.89± 1.89
HAa1	33.24± 2.30	38.36± 0.68	117.43± 2.27	230.36± 1.06
HAa2	32.80± 1.32	34.99± 2.11	131.99± 2.33	229.60± 1.73
HAa3	34.95± 0.84	36.20± 1.34	137.51± 1.70	214.82± 2.76
PAa1	32.40± 2.34	37.08± 1.69	127.90± 2.13	226.69± 1.99
PAa2	30.98± 1.09	38.18± 1.79	110.79± 2.68	225.13± 1.88
PAa3	35.25± 1.18	37.13± 2.80	130.99± 2.26	231.05± 2.44
PAa4	30.52± 2.22	33.17± 0.49	133.36± 2.04	221.28± 1.07
PAa5	32.00± 0.01	33.55± 1.33	127.44± 2.12	196.94± 2.68
PAa6	31.91± 1.19	34.69± 2.93	128.29± 1.76	214.09± 3.03
REh1	38.60± 1.39	34.36± 2.11	155.67± 1.18	207.55± 1.56
REh2	34.27± 0.92	41.23± 0.34	136.09± 0.76	295.02± 0.59
HEh1	28.97± 2.05	32.83± 1.13	142.17± 2.32	220.32± 0.63
HEh2	37.49± 0.03	37.96± 0.8	159.05± 0.71	241.86± 0.88
PEh1	34.07± 0.41	50.76± 0.08	135.37± 0.71	257.32± 0.77
PEh2	31.23± 0.63	39.60± 1.34	141.86± 1.68	221.55± 1.36
RMt1	35.08± 0.84	38.40± 1.98	149.20± 0.80	255.01± 1.97
HMt1	37.12± 1.82	33.50± 0.9	152.22± 1.37	197.91± 0.76
HMt2	32.78± 0.06	53.52± 1.9	132.98± 1.56	245.71± 1.38
PMt1	29.44± 0.12	42.86± 0.7	131.73± 1.47	251.03± 0.92
PMt2	33.13± 1.93	58.92± 0.9	146.74± 1.52	307.00± 0.86
L.S.D.	For Strain	3.18		12.21
	For Treatment	10.80		41.43

germination and seedling growth is the most critical ones. Salinity had inhibitory effects on root, shoot (data not shown) and seedling growth (62.13, 23.97 and 42.99% decreases, respectively) of *Triticum aestivum* var Inqlab 91. Root growth was more severely affected by salt stress. Generally, stimulation in root, shoot and seedling growth was recorded with the application of bacterial inoculations at 0 mM NaCl treatment. But negative impact on shoot growth was observed with strains isolated from *Euphorbia heliscopia* and *Malvestrum tricuspidatum* at this treatment. Bacterial inoculations increased shoot (3.21-12.85%) and seedling (0.80-15.87%)(excluding a few cases) growth at 100 mM NaCl treatments, but in case of root, half of the inoculations had growth promoting (3.72-13.12%) effect on this parameter. Significant improvement in seedling growth (most of the cases, maximum with RAa1, HAa3, PAa2, PAa6, HEh1, HEh2, PMt1, PMt2) with bacterial inoculations was detected under salt stress over non-inoculated respective treatment (Table 2).

Fresh weight per seedling had a decreasing trend under 100 mM NaCl treatment (data not shown). In general, with bacterial inoculations values of fresh weight of seedlings subjected to salt free (0.19-17.82) and salt stress (1.24-47.76% increases) conditions were higher as compared to non inoculated respective treatments. In most of the cases this value was highly significant over respective non-inoculated treatment. A few inoculations at 0 mM (PEh2, HEh1, PMt1, PMt2) and only one inoculation (REh2) at 100 mM NaCl treatment caused some reduction in this parameter, relative to non-inoculated respective treatments.

Seedlings subjected to salt stress a slight increase in dry weight, while a marked increase in dry weight per gram fresh weight (dry weight accumulation) was recorded (Table 3). In salt free conditions pattern of dry weight/dry weight per gram fresh weight parameters was not consistent with bacterial inoculations. At 100 mM NaCl, enhancement (0.33-78.22%) in dry weight (excluding RAa2, HEh1) whereas both increases (0.38-36.89 %) and decreases (0.75-12.19%) in dry weight per gram fresh weight were recorded.

At 0 mM, most of the bacterial inoculations enhanced (0.11-28.22%, except a few cases) Na^+ by seedlings. However at 100 mM NaCl situation was reverse. With majority of the bacterial inoculations decreases (0.08-27.95%) in Na^+ uptake by seedlings were recorded. More pronounced reductions were noted with bacterial isolates REh1, REh2, PMt1, PMt2 at 100mM NaCl, over non-inoculated respective treatment. With bacterial inoculations K^+ content of seedlings (except a few cases) decreased at 0 and 100 mM NaCl treatments, relative to non-inoculated treatments (Table 4).

Under salt stress seedlings showed significant increase (154.54%) in auxin content (Table 5). With most of the bacterial inoculations increased auxin level of seedlings was observed at 0 mM NaCl, while a few inoculations dropped auxins at this treatment. However at 100 mM most of the strains isolated from *Achyranthus aspera* plant dropped auxin level of seedlings, while rest of the inoculations elevated (0.71-149.29%) the auxins. In most of the cases this increase was significant. More pronounced stimulation in this parameter was recorded with phylloplane bacterial inoculations.

A significant increase (95.96%) in the soluble protein content of seedlings was observed (Table 5). Majority of the inoculations positively influenced the protein content of seedlings at both stress free and salt stress conditions. Increases (significant in most of the cases) of 0.86-84.43% at 0 mM (except HAa2, REh1, HEh2, PMt1, PMt2) and 0.59-48.96% at 100 mM (excluding PEh1, PEh2, PMt1, PMt2) with different bacterial inoculations were detected.

Peroxidase activity of *Triticum aestivum* var. Inqlab 91 seedlings was enhanced (8.45%) at 100 mM NaCl, relative to 0 mM NaCl treatment (Table 6). Bacterial inoculations variably influenced the peroxidase activity of seedlings under stress and stress free conditions. RAa2, PEh2, REh1, HEh1 showed reduction in peroxidase activity at 0 and 100 mM NaCl treatments over non-inoculated respective treatments. On the other hand bacterial inoculations HAa2, PAa1, PAa2, PAa5, PAa6, PEh1, REh2, HEh2, HMt1 enhanced the activity of this enzyme at both treatments. With rest of the bacterial inoculations pattern of peroxidase was not consistent.

Higher acid phosphatase activity was detected in seedlings subjected to salt stress. Majority of the bacterial inoculations inhibited activity of this enzyme at 0 mM, while at 100 mM increases (0.07-14.38%) as well as decreases (0.11-28.99%) were

Table 4: Effect of inoculation of salt tolerant bacteria isolated from *Achyranthus aspera, Euphorbia heliscopia* and *Malvestrum tricuspidatum* on Na^+ content (µg/gm. dry wt.) and K^+ content (µg/gm. dry wt.) of *Triticum aestivum* var. Inqlab 91 seedlings at 0 and 100 mM NaCl concentrations (means of four replicates).

Bacterial Inoculations	Na^+ contents		K^+ contents	
	0 mM	100 mM	0 mM	100 mM
Cont.	6698.62± 157.30	18637.39± 5.49	2810.46± 117.20	3117.08± 72.32
RAa1	8541.74± 195.50	19474.35± 253.87	2416.11± 41.59	3012.17± 3.17
RAa2	8588.69± 226.59	16908.26± 156.12	2531.70± 93.14	2448.48± 32.01
HAa1	7561.85± 92.26	17687.3± 166.71	2721.79± 38.98	2587.27± 38.91
HAa2	7510.94± 217.28	18779.75± 79.98	2320.66± 51.28	2631.67± 29.98
HAa3	8328.30± 36.37	19515.17± 144.85	2203.43± 40.86	2695.44± 62.62
PAa1	8151.08± 0.76	19419.95± 71.97	2405.70± 83.56	2681.52± 93.01
PAa2	8196.19± 28.74	16531.25± 22.10	2315.69± 91.25	2590.62± 2.21
PAa3	7785.71± 67.35	16455.40± 93.93	2536.9± 50.87	2695.01± 65.11
PAa4	8198.74± 68.39	16724.13± 24.38	4650.02± 92.24	2124.13± 73.16
PAa5	7143.49± 57.27	16747.12± 56.90	2224.59± 80.74	2148.84± 58.12
PAa6	7087.71± 45.58	19379.32± 17.70	2103.66± 17.39	3011.52± 5.53
REh1	7809.71± 49.78	13428.83± 724.84	1934.68± 42.48	4008.47± 102.01
REh2	6691.54± 350..20	13803.88± 712.68	2248.28± 58.38	2041.02± 21.18
HEh1	6997.76± 312.13	24758.76± 642.04	2780.30± 81.57	4126.22± 48.40
HEh2	7697.54± 205.48	18374.26± 931.42	2367.05± 68.19	3569.79± 39.76
PEh1	7541.64± 286.09	16511.92± 809.72	2197.45± 57.31	2485.54± 60.12
PEh2	7303.68± 388.99	21400.95± 529.66	2193.08± 38.64	3028.76± 20.06
RMt1	7717.27± 169.73	28775.08± 921.66	2989.01± 15.31	3070.88± 94.27
HMt1	8316.37± 311.06	18622.31± 765.56	2792.94± 102.95	2699.35± 117.84
HMt2	5764.65± 222.66	15124.18± 706.87	2113.58± 32.40	2478.41± 59.30
PMt1	6240.70± 136.12	14637.89± 762.37	2264.64± 47.63	2150.39± 41.81
PMt2	6637.37± 344.35	14643.43± 902.51	2860.71± 45.66	2915.21± 04.84
L.S.D. at p= 0.05	For Strain	490.10		256.43
	For Treatment	1662.04		1208.71

Table 5: Effect of inoculation of salt tolerant bacteria isolated from *Achyranthus aspera, Euphorbia heliscopia* and *Malvestrum tricuspidatum* on auxin content (µg/gm. fresh wt.) and soluble protein content (µg/gm. fresh wt.) of *Triticum aestivum* var. Inqlab 91 seedlings at 0 and 100 mM NaCl concentrations (means of four replicates).

Bacterial inoculations	Auxin content		Soluble protein content	
	0 mM	100 mM	0 mM	100 mM
Cont.	0.55± 0.03	1.40± 0.00	478.00± 1.38	936.70± 3.96
RAa1	1.05± 0.05	1.29± 0.07	743.49± 052	1202.60± 6.26
RAa2	0.59± 0.07	1.28± 0.004	759.64± 3.39	986.26± 4.29
HAa1	1.00± 0.09	1.59± 0.11	738.72± 1.80	1395.34± 7.39
HAa2	0.61± 0.03	1.03± 0.005	473.88± 2.45	931.15± 5.43
HAa3	1.32± 0.02	1.63± 0.03	739.72± 3.27	1009.34± 8.39
PAa1	1.00± 0.01	1.18± 0.02	595.12± 3.74	1067.41± 7.47
PAa2	0.62± 0.03	1.15± 0.05	496.32± 1.47	1317.07± 5.32
PAa3	0.71± 0.03	1.32± 0.003	755.44± 2.73	1258.06± 3.89
PAa4	0.58± 0.02	0.95± 0.04	66632± 5.27	1268.29± 9.93
PAa5	1.37± 0.09	3.49± 0.14	881.57± 3.78	1001.48± 8.45
PAa6	1.42± 0.10	2.59± 0.09	735.94± 3.43	1048.54± 7.37
REh1	0.60± 0.007	2.04± 0.19	449.47± 6.11	964.23± 7.03
REh2	0.38± 0.01	1.52± 0.02	581.06± 3.61	946.76± 5.24
HEh1	0.55± 0.03	1.85± 0.14	512.60± 2.41	1050.85± 9.34
HEh2	0.61± 0.08	1.90± 0.02	35397± 6.51	984.41± 4.11
PEh1	0.57± 0.02	2.05± 0.007	593.16± 6.93	896.89± 4.32
PEh2	0.36± 0.01	1.74± 0.01	505.13± 4.12	881.58± 1.98
RMt1	0.57± 0.01	1.50± 0.11	486.83± 2.12	1101.98± 4.39
HMt1	0.44± 0.006	1.41± 0.08	501.51± 4.01	988.11± 2.28
HMt2	0.54± 0.01	1.42± 0.004	573.12± 5.51	1005.04± 9.56
PMt1	0.76± 0.009	1.95± 0.06	404.47± 2.11	726.21± 2.68
PMt2	1.13± 0.04	3.26± 0.14	459.78± 4.13	705.33± 7.29
L.S.D. at p= 0.05	For Strain	0.22		70.17
	For Treatment	0.77		237.96

Table 6: Effect of inoculation of salt tolerant bacteria isolated from *Achyranthus aspera, Euphorbia heliscopia* and *Malvestrum tricuspidatum* on peroxidase (unit/gm. fresh wt.) and acid phosphatase (unit/gm fresh wt.) activities of *Triticum aestivum* var. Inqlab 91 seedlings at 0 and 100 mM NaCl concentrations (means of four replicates).

Bacterial inoculations		Peroxidase		Acid phosphatase	
		0 mM	100 mM	0 mM	100 mM
Cont.		230.29± 2.79	249.77± 2.57	761.00± 15.51	886.82± 2.46
RAa1		215.22± 9.81	313.85± 4.05	323.55± 3.82	884.20± 3.15
RAa2		224.00± 8.31	239.33± 302	475.54± 4.02	642.33± 5.67
HAa1		244.42± 3.51	223.68± 7.10	754.17± 21.7	1014.34± 14.65
HAa2		286.33± 4.86	293.69± 8.89	736.40± 11.88	926.86± 4.05
HAa3		224.76± 6.22	350.62± 2.57	851.35± 22.05	885.82± 5.39
PAa1		273.50± 3.75	342.34± 5.92	473.18± 17.77	988.27± 3.48
PAa2		242.12± 1.69	369.62± 2.58	435.12± 11.44	742.01± 4.99
PAa3		250.19± 2.11	225.06± 1.70	442.99± 21.38	775.67± 9.55
PAa4		221.10± 7.88	242.79± 3.27	424.24± 7.39	578.27± 5.49
PAa5		273.01± 4.04	257.29± 9.81	529.43± 9.52	736.65± 11.44
PAa6		319.17± 5.39	461.41± 5.41	749.94± 11.49	835.35± 3.01
REh1		219.22± 4.03	223.80± 1.39	640.59± 19.71	774.78± 5.45
REh2		232.70± 1.88	297.69± 5.06	391.53± 1948	629.74± 18.42
HEh1		214.77± 4.39	212.36± 3.98	853.24± 20.91	903.15± 19.44
HEh2		241.69± 3.62	291.72± 8.93	942.69± 16.74	1006.49± 29.31
PEh1		239.07± 1.96	292.53± 4.32	842.74± 20.74	854.30± 9.79
PEh2		186.22± 2.32	200.00± 1.02	223.33± 18.34	787.79± 11.41
RMt1		229.30± 1.98	303.26± 5.93	658.85± 15.49	1008.44± 35.16
HMt1		240.73± 4.68	229.76± 2.03	377.93± 10.32	947.47± 9.01
HMt2		244.47± 6.01	310.80± 3.68	519.15± 3.20	887.47± 5.68
PMt1		197.52± 1.46	364.02± 3.64	479.83± 15.93	807.79± 15.21
PMt2		235.81± 1.00	223.04± 8.81	579.83± 10.87	872.83± 16.03
L.S.D. at p= 0.05	For Strain		24.39		173.20
	For Treatment		82.71		587.36

recorded, as compared to non-inoculated respective treatment. Histoplane bacteria isolated from *Euphorbia heliscopia* (HEh1, HEh2) were found to stimulate acid phosphatase at 0 and 100 mM NaCl treatments, over non-inoculated respective treatments. HAa1, HAa2, PAa1, PMt2, RMt1, HMt1, HMt2 inoculations caused inhibition at 0 mM and stimulated acid phosphatase activity at 100 mM NaCl treatments, relative to non-inoculated respective treatments. In case of HAa3 and PEh1 inoculations, reverse situation was observed (Table 6).

Discussion

Bacterial strains isolated from different sources (rhizoplane, histoplane and phylloplane) of *Achyranthus aspera* (RAa1, RAa2, HAa1, HAa2, HAa3, PAa1, PAa2, PAa3, PAa4, PAa5, PAa6) *Euphorbia heliscopia* (REh1, REh2, HEh1, HEh2, PEh1, PEh2) and *Malvestrum tricuspidatum* (RMt1, HMt1, HMt2, PMt2, PMt2) were exploited for plant-bacteria interaction experiments (Table 1). The isolates were salt tolerant and were capable to deter 2.5-3.0M NaCl in solid as well as in liquid media. The bacterial isolates belonged to families Enterobacteriaceae (RAa2, HAa1, PAa6, REh1, REh2, HEh1, HEh2, PEh1, PEh2, HMt1, HMt2, PMt2), Pseudomonadaceae (PAa2, PAa3, PAa4, RMt1), Nesseriaceae (PMt1) and Bacillaceae (HAa2). A few strains RAa1, HAa3, PAa1 and PAa5 remained uncertain. Excluding HAa2, PEh1 (Gram positive) and PAa1, PAa5 (Gram variable) majority of the isolates were Gram negative motile rods (except RAa1, HAa3, PMt1, which were cocci). They gave

positive results for catalase and cytochrome oxidase enzymes and were facultative anaerobes (excluding PAa2, PAa3, PAa4, PAa5, RMt1 which were strictly aerobes). Seeds of *Triticum aestivum* var. Inqlab 91 (inoculated and non-inoculated) were germinated and grown under 0 and 100 mM NaCl treatments.

Germination and early seedling growth were more negatively influenced by salt stress (Table 2). A steep decrease in root, shoot and seedling growth was observed under salt stress. The reduction in percentage germination under salt stress may be due to increased osmotic pressure, retarding the water entry into the seed. High salinity decreases germination and growth by decreasing substrate water potential, cause ionic imbalance and toxicity and thus restricts water and nutrient uptake by the roots (Lambers *et al.*, 1998) and reduction in growth may also be related to the reduced physiological availability of water during vegetative period of the plant growth (Mehdi *et al.*, 2000). The decreased growth due to salinization has also been explained by a suppression of nutrient absorption due to uptake of NaCl in competition with nutrient ions, tissue dehydration or a combination of these effects (Carvajal *et al.*, 1999; Makela *et al.*, 1999) and metabolic disturbances (Soussi *et al.*, 1999). These results can be related to the findings of Ahmad *et al.* (2000) and Mehmood *et al.* (2000). Drastic effects of salt stress were more pronounced on roots. Salt stress induced reduction in root length and increase in root diameter. This morphological adaptation of roots is a typical change under stress environment (Degenhardt and Gimmler, 2000). With the application of bacterial inoculations significant stimulation in germination at both treatments (0, 100 mM NaCl) was recorded. More improvement in germination at 100 mM NaCl was with bacterial strains isolated from *Achyranthus aspera* plant, over non-inoculated respective treatment. Bacterial inoculations also induced enhancement in shoot (except bacterial isolates from *Euphorbia heliscopia*, and *Malvestrum tricuspidatum* at 0 mM NaCl), root (excluding some inoculations) and seedling growth. The isolates used in these experiments had the ability to take up Na^+ from the growth medium. Maximum Na^+ uptake by these strains was recorded at different optima (1.0-3.0M NaCl). One possibility for stimulation in germination and growth parameters might be that bacteria take up NaCl in their cells and may be capable of reducing the availability to seeds. Thus bacteria by lessening the harmful effects of NaCl provoked germination as well as seedling growth. The influence of salt tolerant bacteria on plant growth has been reported in earlier findings of Afrasayab and Hasnain (2000 a, b) and Afrasayab *et al.* (2001). In contrast to this, being the natural inhabitants, bacteria are found to colonize the rhizosphere (Lubeck *et al.*, 2000; Uregel *et al.*, 2000), have capability to increase the availability of nutrients to plants and regenerate the quality of soil (Alami *et al.*, 2000). In addition plant growth promoting bacteria are also involved in inducing cell wall structural modifications, biochemical and physiological changes leading to the synthesis of proteins and chemicals involved in plant defense mechanisms (Ramamoorthy *e t al.*, 2001).

NaCl stress caused significant reduction in fresh weights and enhancement in dry weight parameters (dry weight and dry weight per gram fresh weight) (Table 3). Alian *et al.* (2000) reported that water and salt stresses depressed the fresh weight accumulation in young leaves of different tomato cultivars. Enhancements in dry weight parameters have been attributed to osmotic adjustment mechanisms of plants by increasing level of organic and inorganic solutes in the tissues (Gulati and Jaiwal, 1996). Generally with bacterial inoculations stimulations in fresh weight as well as dry weight parameters were manifested at 0 and 100 mM NaCl, relative to non-inoculated respective treatments. Increased dry weights and plant heights of plants when inoculated with mixtures of mangrove rhizosphere bacteria and halotolerant *Azospirillum* sp. have been observed (Bashan *et al.*, 2000).

In the present case considerably higher values of Na^+ and K^+ in *Triticum aestivum* seedlings were determined under salt stress (Table 4). Plants respond to salt stress in two ways. Firstly, they exclude toxic ions such as Na^+ and Cl^- from leaves by different ways. Secondly, they absorb ions and accumulate in vacuoles (Shannon and Grieve, 1998). One possible explanation for increased Na^+ and K^+ uptake by seedlings subjected to salt stress maybe that increased ion concentration in the cells facilitates the water movement into the plant cells and saves energy required for the synthesis of organic solutes needed for osmotic adjustment (Lyenger and Reddy, 1994). Alian *et al.* (2000) has documented increased uptake of Na^+ ions in tomato cultivars under salt and water stress. However with bacterial inoculations significant reductions (except a few cases) in Na^+ (at 100 mM NaCl only) and K^+ (at both 0 and 100 mM NaCl), relative to non-inoculated respective treatments, were recorded. As described earlier bacterial strains used for these studies had the ability to take up NaCl from the growth medium. Hence the reduced Na^+ uptake by inoculated seedlings may be referred to the fact that bacteria check the Na^+ availability to plants.

Highly significant increase in auxin content of seedlings under NaCl stress was detected. IAA the most abundant in nature regulates a variety of developmental and cellular processes. The regulation of these processes by auxins is believed to involve auxin induced changes in gene expression (Broek *et al.*,1999). Besides plants, many soil and rhizosphere bacteria including phytopathogenic, epiphytic and plant growth stimulating bacteria also produce IAA. Bacterial inoculations also induced significant enhancement in auxin synthesis in seedlings at 0 and 100 mM NaCl, over non-inoculated respective treatments (Table 5). These results are in agreement with those of Afrasayab *et al.* (2001). Results show that stimulation in auxin synthesis may be an adaptation of plants to stress environment.

Salt stress induced significant enhancement in protein content of *Triticum aestivum* seedlings under salt stress (Table 5). The same results were reflected in seedlings inoculated with bacteria at both 0 and 100 mM NaCl (excluding few cases) treatments. Synthesis of certain amino acids and soluble nitrogenous compounds are also the adaptations of plants to salt stress (Soussi *et al.*, 1999; Holmstrom *et al.*, 2000). Plants growing under saline conditions must maintain high concentrations of osmotically active substances in order to compete successfully. In the present case high protein values of seedlings seem a mechanism of salinity tolerance .

Activation in peroxidase activity of *Triticum aestivum* seedlings was observed under salt stress (Table 6). The activities of superoxide dismutase (SOD) and catalase in *Bruguirea gymnorrhiza* were found to increase up to 1000mM NaCl (Takemura *et al.*, 2000) and activities of these enzymes were not affected by high NaCl concentration. Kumar *et al.* (2000) recorded varying trend of enzymes malate dehydrogenase and glutamate dehydrogenase in rice seedlings of differing salt tolerance. Inoculated seedlings exhibited a contrasting peroxidase response at 0 and 100 mM NaCl, relative to non-inoculated respective treatments.

Acid phosphatase activity was found to boost at 100mM NaCl (Table 6). Olmos and Hellin (1997) reported significant increase in acid phosphatase activity in salt adapted line of *Pisum sativum* and suggested a possible involvement of this activity in the maintenance of cell growth under salt stress. Inhibition in acid phosphatase activity at 0 mM (majority of cases) and increases as well as decreases in activity of this enzyme at 100 mM NaCl with bacterial inoculations was reflected.

These experiments led to conclude that generally bacterial isolates from different sources induced stimulation in plant growth. Some of the bacterial inoculations inhibited *Triticum aestivum* growth. This growth promotion involved enhancement in dry weight accumulation, auxins protein contents but decreased Na^+/K^+ contents. The results showed there was no relationship between growth and enzyme activities, as inconsistent pattern of enzyme activities was observed.

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