http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (6): 1016-1023, 2000

Synergistic Growth Stimulatory Effects of Mixed Culture Bacterial Inoculations on the Early Growth of *Triticum aestivum* Var Inqlab 91 under NaCl Stress

Shazia Afrasayab and Shahida Hasnain
Department of Botany, University of the Punjab, Q.A. Campus, Lahore-54590, Pakistan

Abstract: Salt tolerant bacteria were used to assess their potential for stimulating the growth of *Triticum aestivum* var. Inqlab 91. Certified seeds of *Triticum aestivum* var. Inqlab 91 were inoculated with monocultures (ST-1, ST-2, ST-3, ST-4, rhizosphere bacteria; HT-1, HT-2, HT-3, histoplane bacteria) and different combinations of mixed cultures of bacteria. Inoculated and non-inoculated seeds were germinated and grown under NaCl stress (0, 100 mM NaCl) for 10 days. After that growth measurements (length and weight parameters) were taken and Na^+/K^+ contents, soluble protein content, auxin content and enzyme activities (peroxidase and acid phosphatase) were determined. Generally, with the application of NaCl, reduction in germination and growth parameters, whereas increase in Na^+/K^+ contents, auxins content, soluble protein content as well as enzymes activities were observed. Bacterial inoculations stimulated germination and growth, over non-inoculated respective treatments, especially under saline conditions. Mixed culture inoculation with rhizosphere bacteria (some combinations) and histoplane bacteria was more effective in stimulating seedling growth than monoculture inoculations at 100 mM NaCl. Bacterial inoculations also enhanced fresh weight per seedling, auxin, soluble protein and K^+ contents of seedlings. Whereas decreases in dry weight accumulation, Na^+ content and activity of enzymes, at 100 mM NaCl with bacterial inoculations were recorded. From these results improved growth of seedlings could be related with increased auxin content but decreased dry weight increment, Na^+ content and enzyme activities.

Key words: Plant-microbe-interaction, NaCl stress, Na^+ contents

Introduction

Soil salinity is the most important devastating problem in the irrigated regions of the world. Salinity adversely affects the growth and metabolism of crops and serves as one of the major limiting factor for agricultural productivity. In Pakistan, like many other countries, vast areas suffer from salinity and associated problems. Production on salt affected lands require execution of biotechnological measures and the comprehension of osmoregulation, which can rather easily be studied in microorganisms. Interest in the biochemical and physiological processes of the microorganisms from hypersaline ecosystems has been increased (Ventosa *et al*., 1998). Halophilic and halotolerant bacteria have provided an authentic basis for studying the mechanism of osmoregulation. They are important for the comprehension of biochemistry , physiology and genetics of salt tolerance. Under osmotic stress, bacteria have evolved different strategies to cope with the changes in their environment (Ventosa *et al*., 1998). Bacteria can combat high osmolarity by enhancing transcription (Treuner-Lange *et al*., 1997) or by the presence of enzymes or organic and inorganic compounds (Ferguson *et al*., 1996; Ciulla *et al*., 1997; Galinski and Louis, 1998; Gouffi *et al*., 1998; Engelbrecht *et al*., 1999).

Many workers have reported plant growth promoting bacteria (Bashan and Holguin, 1994; Scupham *et al*., 1996). Bacteria stimulate seedling growth by decomposing mineral material and increasing the availability of nutrients to plants (Jimenez-Salgado *et al*., 1997), decreasing susceptibility to pathogens (Lutenberg and Dewegar, 1992) and synthesis and release of growth hormones (Campbell, 1985). Hasnain and Co-workers have isolated salt tolerant bacteria from different sources (Yasmin and Hasnain, 1993, 1997, 1998; Mirza *et al*., 1998). Their further studies revealed that inoculations of seeds with salt tolerant bacteria may stimulate seedling growth under NaCl stresses (Hasnain and Yasmin, 1993; Siddique *et al*., 1997; Hasnain and Afrasayab, 2000). Keeping in view the results of inoculation studies, the objectives of present studies are to determine the role of mono and mixed culture inoculations in stimulating the growth of *Triticum aestivum* var. Inqlab 91 and the mechanisms involved in this stimulation under salt stress.

Materials and Methods

Rhizosphere (ST-1, ST-2, ST-3, ST-4) and histoplane bacteria (HT-1, HT-2, HT-3), isolated from *Mazus* sp. inhabitant of salt range, were selected for mixed culture inoculation studies (Mirza *et al*., 1998). These strains could tolerate upto 2.5 M NaCl in the medium. Mono (7 mono cultures) and mixed (15 combinations) cultures of these salt tolerant bacteria were used to inoculate *Triticum aestivum* var. Inqlab 91. For preparation of cultures, bacterial strains were grown overnight in LB (Gerhardt *et al*., 1994) at 37°C. Bacterial cells were harvested, washed and resuspended in sterilized glass distilled water (10^7 cells/ml). Certified seeds of *Triticum aestivum* var. Inqlab 91, obtained from Punjab Seed Corporation, were surface sterilized with 0.1% $HgCl_2$ for about 5 minutes with continuous shaking. Seeds were washed (at least four times) with sterilized glass distilled water. Surface sterilized seeds were immersed in bacterial suspension (mono and mixed cultures) for 15 minutes. For control seeds were soaked in sterilized glass distilled water for the same duration. Inoculated and non-inoculated seeds were germinated and grown under salt stress (0, 100 mM) for 10 days. Experimental set-up and growth conditions has been described previously (Hasnain and Yasmin, 1993). After 10 days, seedlings were harvested and different growth measurements including length parameters and weight parameters were taken. Na^+ and K^+ contents of inoculated and non-inoculated seedlings were determined on flame photometer (Furman, 1975). Auxin content (Mahadevan, 1984), soluble protein content (Lowry *et al*., 1951), peroxidase (Racusen and Foote, 1965) and acid phosphatase (Iqbal and Rafique, 1986) activities of inoculated and non-inoculated seedlings were also analyzed. Experiments were repeated four times and data was subjected to statistical analysis (Steel and Torrie, 1981).

Results

Seven salt tolerant bacterial strains from rhizosphere and histoplane of *Mazus* sp., native plant of salt range Kallar Kahar, were selected for mixed culture inoculation studies. Seeds of

Triticum aestivum var. Inqlab 91 were inoculated with mono and mixed cultures of bacteria to assess the potential of salt tolerant bacteria for stimulating the growth of plants under salt stress.

Germination of *Triticum aestivum* var. Inqlab 91 was adversely affected under NaCl stress. About 8% decrease in germination, at 100 mM NaCl was observed (Table 1). Bacterial inoculations caused stimulation in germination under salt stress. Excluding a few cases, monocultures and mixed culture inoculations promoted germination at 0 mM, when compared with non-inoculated respective treatment. At 100 mM NaCl, stimulation in germination was manifested with almost all bacterial inoculations, relative to non-inoculated respective treatment. Maximum stimulation in germination, over non-inoculated respective treatment, with ST-4, ST-1, 2, 4; ST-1, 2, 3, 4 and HT-1 was observed at 100 mM NaCl. Synergistic effects of mixed culture inoculation on germination were recorded only in case of ST-1, 2, 4.

NaCl stress had detrimental effects on seedling growth (Table 2). Under salt stress reduction in seedling lengths (shoots as well as roots) was observed. Almost 44% decrease in seedling length was recorded. Roots were severely affected than shoots under NaCl stress. Mono and mixed culture inoculations of both rhizosphere and histoplane bacteria registered increase in seedling lengths at 0 and 100 mM NaCl, relative to non-inoculated respective treatment. Most of the mixed culture bacterial inoculations caused maximum stimulation and showed synergistic effects of mixed culture on seedling growth. Synergistic effects of mixed cultures were more pronounced in case of ST-1, 3, 4 and HT-1, 2, 3 under salt stress. Bacterial inoculations (mono and mixed cultures) promoted shoot lengths, at 0 and 100 mM NaCl relative to non-inoculated respective treatments. Some bacterial combinations, ST-3, 4; ST-1, 2, 4; ST-1, 3, 4; ST-2, 3, 4; HT-1, 2, 3, showed synergistic effects of mixed culture on shoot lengths. Maximum stimulation in shoot lengths was manifested with ST-1,3,4 at 100 mM NaCl. Stimulation in root lengths with bacterial inoculations at 0 and 100 mM NaCl treatments was observed when compared with non-inoculated respective treatments. At 100 mM NaCl maximum stimulation in root lengths, over non-inoculated respective treatment was recorded with ST-1, 3, 4 inoculation. Mixed culture inoculation of histoplane bacteria was more effective than monoculture inoculations. All histoplane bacterial combinations significantly enhanced root lengths. Maximum increase in root lengths was registered with HT-1, 2, 3 at 100 mM NaCl, when compared with non-inoculated respective treatments.

Weight parameters of *Triticum aestivum* var. Inqlab 91 were also affected by salt stress (Table 3). Fresh weight of seedlings decreased at 100 mM NaCl treatment. Bacterial inoculations significantly enhanced (in most cases) fresh weight per seedling at 0 and 100 mM NaCl treatments, relative to non-inoculated respective treatments. Under salt stress 28.59% increase in dry weight and 70.66% enhacement in dry weight accumulation was observed. With bacterial inoculations decreases, over non-inoculated respective treatments, in dry weight were manifested at 0 and 100 mM NaCl treatments. All bacterial inoculations caused decrease in dry weight accumulation at 0 and 100 mM NaCl treatments, relative to non-inoculated respective treatments. In general most mixed culture bacterial inoculations markedly reduced dry weight accumulation under saline conditions.

Na^+ content of seedlings increased about 3 folds at 100 mM NaCl (Table 4). With bacterial inoculations 31.93-78.38% increases in Na^+ uptake by the seedlings at 0 mM NaCl were observed. Mono and mixed culture inoculations caused 0.91-35.68% reduction in Na^+ uptake by the seedlings at 100 mM, when compared with non-inoculated respective NaCl treatment. All mono and mixed culture bacterial inoculations stimulated K^+ uptake by the seedlings at 0 and 100 mM NaCl treatment, when compared with non-inoculated respective treatments (Table 4).

A significant increase in the auxin content of *Triticum aestivum* seedlings, with the application of NaCl stress was observed (Table 5). Generally bacterial inoculations stimulated auxin synthesis of seedlings at both treatments. But some mixed culture inoculations caused reduction in auxin synthesis at 100 mM NaCl, when compared with non-inoculated respective treatment. At 100 mM NaCl treatment, stimulation in auxin synthesis was more pronounced with monoculture inoculations as compared to mixed culture inoculations.

Salt stress caused 30.67% increase in soluble protein content of *Triticum aestivum* var. Inqlab 91 seedlings (Table 5). Both decreases and increases in soluble protein content were observed at 0 and 100 mM NaCl with bacterial inoculations. At 0 mM NaCl, 11.08-40.05% increases, over non-inoculated respective treatment, in soluble protein synthesis with different bacterial inoculations, were manifested. Bacterial inoculations ST-2; ST-3, 4; ST-1, 2, 3; ST-2, 3, 4; ST-1, 2, 3, 4; HT-2, HT-3; HT-1, 2; HT-1, 3; HT-2, 3; HT-1, 2, 3 registered 2.53-16.86% decreases in soluble protein synthesis at 0 mM NaCl treatment, relative to non-inoculated respective treatment. At 100 mM NaCl, most of the bacterial inoculations enhanced soluble protein content of *Triticum aestivum* seedlings, when compared with non-inoculated respective treatment. While reduction in soluble protein content, over non-inoculated respective treatment, with some of the bacterial inoculations ST-1; ST-2; ST-1, 4; ST-2, 3; ST-3, 4; ST-1, 3, 4; ST-2, 3, 4; HT-1 was observed at 100 mM NaCl treatment. Maximum stimulation in protein content at 100 mM NaCl was recorded with ST-3; ST-4; ST-1, 2, 3; ST-1, 2, 4; HT-2; HT-3 and HT-2, 3, relative to non-inoculated respective treatment.

Enzyme study of *Triticum aestivum* seedlings revealed that the activity of peroxidase was affected with NaCl treatment as well as bacterial inoculations (Table 6). At 100 mM NaCl, 45% stimulation in peroxidase activity, over 0 mM treatment, was observed. At 0 mM, both increases and decreases, over non-inoculated respective treatment, with bacterial inoculations were recorded. 19.53-60.25% reduction in peroxidase activity, was manifested with bacterial inoculations at 100 mM NaCl, when compared with non-inoculated respective treatment. The activity of acid phosphatase increased significantly with salt treatment. At 0 mM, most of the rhizosphere bacterial inoculations and all histoplane bacterial inoculations enhanced acid phosphatase activity, relative to non-inoculated respective treatment. 2.24-35.02% decreases in acid phosphatase activity were manifested with mono and mixed culture inoculations at 100 mM NaCl, when compared with non-inoculated respective treatment. At 100 mM NaCl only ST-2, 4 and ST-3, 4 inoculations enhanced activity of this enzyme relative to non-inoculated respective treatment.

Discussion

Salinity drastically hampered germination and early growth of *Triticum aestivum* var. Inqlab 91. Percentage germination, seedling length (shoot and root lengths) and fresh weight of seedlings markedly decreased under salt stress. Whereas increase in dry weight parameters (dry wt. per seedling and dry wt. per gram fresh wt.), Na^+ and K^+ contents, auxin content, soluble protein content, and enzyme activities (peroxidase and acid phosphatase) was recorded. Inimical effects of salinity on germination and early growth have been reported previously (El-Saidi, 1997; Pareek *et al*., 1997; Siddique *et al*., 1997). Decreased germination has been considered due to toxicity of high concentration of salts to embryo and low water potential of the root zone (Acharya *et al*., 1990). Reduced seedling growth under salt stress has been implicated to reduced water uptake and an excessive ion accumulation in the plant tissues, possibly with the reduced uptake of essential mineral elements (Alam, 1994), altered permeability of plasma membrane

Table 1: Percentage germination of *Triticum aestivum* var. Inqlab 91 at 0 and 100 mM NaCl concentrations after inoculating with mono cultures and mixed cultures of salt tolerant bacterial strains (means of four replicates)

Bacterial strains	0 mM	100 mM
Control	96.25 ± 1.08	88.75 ± 3.69
ST-1	98.75 ± 1.08	88.75 ± 4.09
ST-2	98.75 ± 1.08	91.25 ± 2.07
ST-3	95.00 ± 1.76	93.75 ± 1.08
ST-4	97.50 ± 1.25	95.00 ± 1.76
ST-1, 2	100.00 ± 0.00	90.00 ± 2.50
ST-1, 3	95.00 ± 1.76	90.00 ± 1.76
ST-1, 4	95.00 ± 1.76	93.75 ± 2.07
ST-2, 3	100.00 ± 0.00	91.25 ± 2.07
ST-2, 4	92.50 ± 1.25	93.75 ± 2.72
ST-3, 4	98.75 ± 1.08	91.25 ± 3.24
ST-1, 2, 3	96.25 ± 1.08	92.50 ± 1.25
ST-1, 2, 4	93.75 ± 3.24	97.50 ± 1.25
ST-1, 3, 4	78.75 ± 1.08	91.25 ± 2.07
ST-2, 3, 4	97.50 ± 1.25	93.75 ± 2.07
ST-1, 2, 3, 4	95.00 ± 1.76	95.00 ± 3.06
HT-1	98.75 ± 1.08	97.50 ± 2.16
HT-2	93.75 ± 1.08	91.25 ± 2.72
HT-3	98.75 ± 1.08	88.75 ± 1.08
HT-1, 2	96.25 ± 2.07	92.50 ± 2.79
HT-1, 3	93.75 ± 2.07	92.50 ± 2.16
HT-2, 3	96.25 ± 2.07	93.75 ± 1.08
HT-1, 2, 3	97.50 ± 1.25	90.00 ± 3.95
LSD at p = 0.05	For Strain	1.45
	For Treatment	4.94

(Wu and Seliskar, 1998), impaired metabolism and accumulation of ammonia (Dubey, 1997). Increased dry weight parameters under salt stress have variously been related with osmotic adjustment mechanism of plants by augmented level of organic and inorganic solutes in the tissues (Gulati and Jaiwal, 1996; Ashraf, 1997). Increased K^+ (Yeo, 1998) and Na^+ uptake (Amtmann *et al*., 1997; Tyerman *et al*., 1997), proteins (Dubey, 1997), growth regulators (Stark, 1997) and altered enzyme activities (Wu and Seliskar, 1998) in plants growing under salt stress have been reported.

Bacterial inoculations enhanced the percentage germination of *Triticum aestivum* var. Inqlab 91 at 0 (excluding a few inoculations) and 100 mM NaCl treatments relative to non-inoculated respective treatments (Table 1). Enhanced germination with bacterial inoculations has been reported previously (Siddique *et al*., 1997; Hasnain and Afrasayab, 2000). Salt tolerant bacteria might be involved in extracting Na^+ ions from the solution, hence decreasing the stress level and increasing the availability of water in the medium, which may be a factor in causing stimulation in germination of *Triticum aestivum*. Mirza *et al.* (1998) reported the increased Na^+ uptake by salt tolerant bacteria. Bacterial inoculations registered stimulation in root, shoot and seedling lengths under saline conditions. Some bacterial inoculations have slightly deleterious effects on shoot, root and seedling lengths at 0 mM NaCl treatment. In many cases significant increase in growth parameters, over non-inoculated respective treatment, under stress condition was observed. Although all mono and mixed culture bacterial inoculations caused stimulation in seedling growth at 100 mM NaCl but some mixed culture inoculations had relatively more stimulatory effects on seedling growth relative to their respective monoculture inoculations (Table 2). It might be due to positive interaction between bacteria in which they enhance the activity of one another. On the other hand some bacterial inoculations caused relatively less stimulation in seedling length as compared to monocultures. It suggests that bacteria might be nullifying the effects of one another or their association is not beneficial for plant growth. According to reports growth stimulating bacterial strains colonize the roots, release some chemotaxis to root exudates, due to which competetion among microorganisms decreased and rate of root colonization with microbes increased (Bashan and Holguin, 1994; Croes *et al*., 1993). Growth promoting bacteria also improve plant growth by decomposing mineral material and making availability of nutrients to plants (Jimenez-Salgado *et al*., 1997). Previously we have reported improved seedling growth with bacterial inoculations under stress conditions (Siddique *et al*., 1997; Hasnain and Afrasayab, 2000). Bacterial strains also caused increase in number of roots and fresh weight of seedlings under salt stress, when compared with non-inoculated respective treatment. According to Bashan and Levanony (1990), *Azospirillum* strains could increase the water status of plants. The improved water status of plant with bacterial inoculations is evident by the increase in fresh weight and decrease in dry weight (dry weight accumulation as well) of seedlings both at 0 and 100 mM treatments (Table 3). ST-3,4 and ST-1,3,4 inoculations showed synergistic growth stimulating effects of mixed culture on fresh weight of seedlings. Mono and mixed culture bacterial inoculations decreased dry weight and dry weight per gram fresh weight of seedlings, relative to non-inoculated respective treatments, under salt stress. Bacterial strains may bind with inorganic ions and form ligands or organic complexes, thus restricting the bioavailability of these ions in the medium (Hughes and Poole, 1991) which is manifested by the decreased uptake of Na^+ in inoculated seedlings. Hence bacterial inoculation is alleviating the stress conditions in the medium which is manifested by the improved growth and low uptake of Na^+ by seedlings. Hasnain and Co-workers also noted decreased dry weight accumulation with bacterial inoculations (Siddique *et al*., 1997; Hasnain and Afrasayab, 2000) under salt stress. Bacterial combinations ST-3, 4; ST-1, 3, 4; HT-1, 2; HT-1, 3; HT-2, 3; HT-1, 2, 3 markedly reduced dry weight accumulation as compared to their mono culture inoculations.

Na^+ content of *Triticum aestivum* seedlings increased under salt stress. Bacterial inoculations caused increase in Na^+ uptake at 0 mM but decreased at 100 mM NaCl, when compared with non-inoculated respective treatments (Table 4). Decreased Na^+ uptake by the seedlings may be attributed to the unavailability of ions in the medium (Hughes and Poole, 1991). The decreased dry weight as well as Na^+ content reflect that bacteria bind with Na^+ ions and help in improving plant growth. K^+ uptake by the seedlings increased under NaCl stress. With bacterial inoculations, increase in K^+ content, over non-inoculated respective treatment, at 0 and 100 mM NaCl was manifested. It has been reported that salt tolerance in most micro-organisms depends on conserving a defined micro-environment in the cytoplasm. Salt tolerant bacteria achieve constant turgor pressure by increasing cytoplasmic K^+ concentration at high osmolarity (Ferguson *et al*., 1996). The rhizosphere bacteria mostly use plant exudates and inturn make availability of nutrients to plants (Jimenez-Salgado *et al*., 1997). The improved seedling growth and augmented K^+ content of seedlings suggested that bacteria were involved in deposition of K^+ around the roots, whereby it was taken up by the seedlings. Plant growth regulators are the substances, which co-ordinate and regulate growth, development and metabolic processes in plants. They also manipulate the reaction of plants against salt stress. Auxin is one of the major hormone, which regulates other plant hormones. Auxin content of *Triticum aestivum* seedlings increased under salt stress (Table 5). Bacterial inoculations stimulated auxin synthesis in seedlings at 0 and 100 mM (excluding some inoculations) NaCl treatments. Mutaftchiev *et al*. (1993) reported that auxins combine

Table 2: Impact of bacterial inoculations on shoot, root and seedling lengths (cm) of *Triticum aestivum* var. Inqlab 91 seedlings at 0 and 100 mM NaCl concentrations (means of four replicates)

Bacterial strains	Shoot lengths		Root lengths		Seedling lengths	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Control	12.97 ± 0.65	9.22 ± 0.51	12.15 ± 0.37	4.96 ± 0.36	25.12 ± 1.02	14.18 ± 0.87
ST-1	13.26 ± 0.72	10.29 ± 0.22	13.26 ± 1.03	6.22 ± 0.03	26.52 ± 1.75	16.51 ± 0.26
ST-2	14.00 ± 0.51	10.24 ± 0.52	12.60 ± 0.75	6.58 ± 0.24	26.60 ± 1.26	16.82 ± 0.77
ST-3	13.43 ± 0.54	10.59 ± 0.37	12.31 ± 0.34	6.07 ± 0.24	25.74 ± 0.88	16.66 ± 0.61
ST-4	13.30 ± 0.78	10.53 ± 0.68	12.30 ± 0.50	5.85 ± 0.16	25.60 ± 1.29	16.38 ± 0.85
ST-1, 2	13.84 ± 0.58	10.83 ± 0.66	12.88 ± 0.77	5.79 ± 0.54	26.72 ± 1.35	16.62 ± 1.20
ST-1, 3	12.37 ± 0.74	9.95 ± 0.30	11.91 ± 1.04	5.90 ± 0.24	24.28 ± 1.78	15.85 ± 0.54
ST-1, 4	13.63 ± 0.58	10.29 ± 0.71	13.04 ± 0.93	5.90 ± 0.38	26.67 ± 1.52	16.19 ± 1.09
ST-2, 3	13.16 ± 0.39	9.98 ± 0.67	12.48 ± 0.71	6.42 ± 0.40	25.64 ± 1.11	16.40 ± 1.07
ST-2, 4	13.26 ± 0.44	10.26 ± 0.36	13.50 ± 0.83	5.48 ± 0.27	26.76 ± 1.27	15.74 ± 0.63
ST-3, 4	13.59 ± 0.62	11.13 ± 0.96	13.10 ± 1.04	6.58 ± 0.38	26.69 ± 1.66	17.71 ± 1.34
ST-1, 2, 3	13.19 ± 0.45	10.35 ± 0.41	11.33 ± 0.32	5.59 ± 0.22	24.52 ± 0.77	15.94 ± 0.63
ST-1, 2, 4	14.40 ± 0.46	11.14 ± 0.18	14.49 ± 0.50	6.44 ± 0.14	28.29 ± 0.96	17.58 ± 0.32
ST-1, 3, 4	14.13 ± 0.81	11.37 ± 0.51	12.81 ± 0.71	7.11 ± 0.11	26.94 ± 1.52	18.48 ± 0.62
ST-2, 3, 4	14.31 ± 0.93	11.18 ± 0.38	13.07 ± 0.96	6.87 ± 0.59	27.38 ± 1.89	18.05 ± 0.97
ST-1, 2, 3, 4	13.66 ± 0.65	10.75 ± 0.49	13.58 ± 0.77	5.59 ± 0.31	27.24 ± 1.42	16.34 ± 0.80
HT-1	14.27 ± 0.85	10.74 ± 0.20	12.53 ± 1.11	6.06 ± 0.45	26.80 ± 1.96	16.80 ± 0.65
HT-2	13.61 ± 0.32	10.23 ± 0.25	8.34 ± 0.10	5.98 ± 0.38	21.95 ± 0.43	16.21 ± 0.63
HT-3	13.52 ± 0.39	10.36 ± 0.51	12.23 ± 0.42	5.96 ± 0.25	25.75 ± 0.81	16.32 ± 0.76
HT-1, 2	13.17 ± 0.25	10.98 ± 0.30	12.69 ± 1.08	6.24 ± 0.43	25.86 ± 1.33	17.22 ± 0.73
HT-1, 3	13.58 ± 0.59	10.67 ± 0.26	13.40 ± 0.81	6.69 ± 0.31	26.98 ± 1.40	17.36 ± 0.57
HT-2, 3	13.64 ± 0.46	10.55 ± 0.43	14.16 ± 0.81	6.50 ± 0.14	27.80 ± 1.27	17.05 ± 0.57
HT-1, 2, 3	14.02 ± 0.44	11.30 ± 0.11	13.31 ± 0.74	6.71 ± 0.27	27.33 ± 1.19	18.01 ± 0.38
LSD at	For Strain	0.16	For Strain	0.49	For Strain	0.50
p = 0.05	For Treatment	0.57	For Treatment	1.68	For Treatment	1.70

Table 3: Impact of bacterial inoculations on dry weight per seedling (mg), fresh 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 strains	Fresh weight per seedling		Dry weight per seedling		Dry weight per gram fresh weight	
	0 mM	100 mM	0 mM	100 mM	0 mM	100 mM
Control	250.59 ± 10.96	188.83 ± 5.43	36.33 ± 0.70	46.72 ± 0.90	144.97 ± 5.83	247.41 ± 3.16
ST-1	294.15 ± 5.0 1	222.98 ± 12.60	32.51 ± 0.45	36.64 ± 1.78	110.52 ± 2.73	164.32 ± 7.19
ST-2	277.0 ± 010.89	233.89 ± 11.79	37.35 ± 0.62	42.58 ± 1.38	134.83 ± 5.75	182.05 ± 6.58
ST-3	290.06 ± 9.04	224.87 ± 8.30	34.58 ± 0.49	41.63 ± 2.16	119.22 ± 4.76	185.12 ± 5.23
ST-4	321.77 ± 8.39	219.42 ± 11.65	34.94 ± 2.32	45.10 ± 0.51	108.58 ± 5.35	205.54 ± 6.08
ST-1, 2	339.31 ± 14.95	194.81 ± 6.88	34.02 ± 2.45	42.50 ± 1.76	100.26 ± 8.70	218.16 ± 4.32
ST-1, 3	319.93 ± 12.93	200.28 ± 6.69	33.33 ± 0.78	41.31 ± 0.48	104.17 ± 6.85	206.26 ± 3.58
ST-1, 4	358.29 ± 9.29	220.93 ± 5.99	33.55 ± 1.02	33.71 ± 1.61	93.64 ± 5.15	152.58 ± 3.80
ST-2, 3	303.39 ± 4.71	218.39 ± 8.07	30.90 ± 1.88	41.17 ± 0.83	101.84 ± 3.29	188.51 ± 4.45
ST-2, 4	332.47 ± 12.60	215.49 ± 8.11	36.12 ± 0.08	48.04 ± 0.20	108.64 ± 6.34	222.93 ± 4.15
ST-3, 4	334.26 ± 10.31	264.43 ± 9.94	32.08 ± 1.82	40.54 ± 2.27	95.97 ± 6.06	153.31 ± 6.10
ST-1, 2, 3	306.22 ± 6.03	212.99 ± 5.40	35.43 ± 1.01	38.68 ± 1.43	115.70 ± 3.52	181.60 ± 3.41
ST-1, 2,4	320.74 ± 8.62	221.18 ± 3.61	30.90 ± 2.66	40.17 ± 1.89	96.34 ± 5.64	181.61 ± 2.75
ST-1, 3, 4	344.81 ± 13.78	249.23 ± 10.51	35.27 ± 1.76	36.65 ± 0.78	102.28 ± 7.77	147.05 ± 5.64
ST-2, 3, 4	299.84 ± 12.81	200.10 ± 12.26	30.11 ± 2.44	38.75 ± 0.88	100.42 ± 7.62	193.65 ± 6.57
ST-1, 2, 3, 4	270.80 ± 9.75	202.56 ± 2.59	31.87 ± 1.32	35.57 ± 0.40	117.68 ± 5.53	175.60 ± 1.49
HT-1	333.21 ± 12.83	256.32 ± 10.90	35.94 ± 0.90	44.41 ± 2.18	107.86 ± 6.86	173.25 ± 6.54
HT-2	288.37 ± 7.33	217.02 ± 13.50	34.16 ± 2.94	38.66 ± 1.41	118.46 ± 5.13	178.14 ± 7.45
HT-3	302.27 ± 9.03	215.87 ± 12.50	33.74 ± 1.32	38.90 ± 1.94	111.62 ± 5.17	180.20 ± 7.22
HT-1, 2	295.99 ± 6.55	243.30 ± 5.54	32.64 ± 1.36	37.74 ± 0.76	110.27 ± 3.95	155.11 ± 3.15
HT-1, 3	331.29 ± 11.57	235.16 ± 8.91	33.74 ± 0.44	34.58 ± 1.83	101.84 ± 6.00	147.04 ± 5.37
HT-2, 3	329.47 ± 6.04	226.59 ± 5.19	32.10 ± 1.48	35.94 ± 2.03	97.43 ± 3.76	158.61 ± 3.61
HT-1, 2, 3	320.09 ± 6.89	214.99 ± 10.77	33.89 ± 0.98	36.17 ± 1.07	105.87 ± 3.93	168.24 ± 5.92
LSD at	For Strain	11.05	For Strain	1.46	For Strain	9.82
p = 0.05	For Treatment	37.48	For Treatment	4.95	For Treatment	33.32

Table 4: Impact of bacterial inoculations 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 strains	Na^+content		K^+ Content	
	0 mM	100 mM	0 mM	100 mM
Control	5444.69 ± 103.54	17146.63 ± 294.10	1618.25 ± 49.14	1846.17 ± 67.93
ST-1	7962.04 ± 231.56	16946.47 ± 641.06	1890.18 ± 116.75	2283.37 ± 94.06
ST-2	8248.63 ± 545.51	16697.65 ± 340.26	1803.34 ± 82.31	2008.23 ± 43.11
ST-3	8347.82 ± 399.72	15758.85 ± 318.52	1772.74 ± 56.12	1979.79 ± 74.35
ST-4	7183.42 ± 268.08	16896.00 ± 259.96	2126.96 ± 93.52	2185.06 ± 126.71
ST-1,2	9017.29 ± 239.13	16768.81 ± 354.98	1885.95 ± 76.19	2368.87 ± 158.25
ST-1,3	8922.47 ± 320.46	15188.85 ± 244.38	1640.93 ± 109.90	2410.33 ± 126.31
ST-1,4	8694.12 ± 232.37	16731.17 ± 467.84	1618.98 ± 116.26	2165.25 ± 23.73
ST-2,3	8606.94 ± 507.82	15242.73 ± 355.34	1824.46 ± 51.61	1896.05 ± 71.22
ST-2,4	9217.70 ± 219.81	16987.90 ± 224.62	1810.88 ± 13.20	2114.86 ± 25.45
ST-3,4	8321.55 ± 191.14	11028.69 ± 276.47	1621.16 ± 121.63	1858.94 ± 74.51
ST-1,2,3	8823.13 ± 318.13	16461.38 ± 431.72	1689.87 ± 75.94	2158.81 ± 96.55
ST-1,2,4	9380.33 ± 247.32	15577.34 ± 508.34	2054.17 ± 97.98	1950.84 ± 30.45
ST-1,3,4	8417.72 ± 251.71	15378.80 ± 329.54	1603.94 ± 86.32	2119.46 ± 63.01
ST-2,3,4	8553.60 ± 477.76	15153.67 ± 512.80	2090.01 ± 85.14	2277.19 ± 82.07
ST-1,2,3,4	9256.77 ± 245.63	16111.23 ± 365.41	1653.78 ± 110.37	2028.07 ± 58.46
HT-1	8924.63 ± 235.63	15132.35 ± 254.61	1651.09 ± 11.92	1876.45 ± 22.05
HT-2	9712.77 ± 269.21	14901.99 ± 515.96	1909.36 ± 38.38	1907.67 ± 11.89
HT-3	9125.00 ± 282.51	16989.67 ± 303.87	1666.56 ± 56.60	2198.26 ± 165.33
HT-1,2	7678.48 ± 213.39	15563.03 ± 301.45	1831.77 ± 117.46	2111.87 ± 104.80
HT-1,3	8158.82 ± 129.16	15975.00 ± 272.71	1692.38 ± 46.51	2092.96 ± 77.81
HT-2,3	8099.73 ± 325.19	15743.10 ± 283.94	1512.45 ± 53.21	2032.19 ± 71.50
HT-1,2,3	8326.08 ± 223.61	15487.55 ± 319.54	1647.87 ± 64.44	1896.85 ± 71.34
LSD at	For Strain	722.73	For Strain	88.51
p = 0.05	For treatment	2450.91	For Treatment	300.91

Table 5: Impact of bacterial inoculations 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 strains	Auxin content		Soluble protein content	
	0 mM	100 mM	0 mM	100 mM
Control	1.01 ± 0.09	2.81 ± 0.14	830.00 ± 24.01	1084.57 ± 17.98
ST-1	1.30 ± 0.07	3.62 ± 0.26	945.00 ± 16.97	1079.26 ± 30.81
ST-2	2.14 ± 0.12	3.70 ± 0.17	750.00 ± 24.25	1084.31 ± 25.45
ST-3	1.23 ± 0.08	3.72 ± 0.09	1116.00 ± 43.64	1330.50 ± 13.18
ST-4	1.50 ± 0.12	3.92 ± 0.18	1050.00 ± 24.86	1404.87 ± 10.35
ST-1,2	2.09 ± 0.16	2.69 ± 0.06	1132.50 ± 26.52	1176.00 ± 55.16
ST-1,3	1.48 ± 0.12	3.07 ± 0.05	1107.00 ± 14.85	1170.00 ± 34.86
ST-1,4	2.73 ± 0.12	2.79 ± 0.11	922.00 ± 15.47	975.50 ± 27.51
ST-2,3	1.47 ± 0.11	2.62 ± 0.12	1179.00 ± 48.79	1029.00 ± 28.50
ST-2,4	2.08 ± 0.10	2.80 ± 0.13	1093.50 ± 54.10	1131.00 ± 15.63
ST-3,4	1.31 ± 0.11	2.95 ± 0.11	792.00 ± 36.00	979.31 ± 16.07
ST-1,2,3	2.91 ± 0.13	2.88 ± 0.08	744.00 ± 26.32	1339.50 ± 13.18
ST-1,2,4	1.31 ± 0.09	2.81 ± 0.21	984.00 ± 12.00	1332.00 ± 18.70
ST-1,3,4	1.09 ± 0.09	2.88 ± 0.12	967.50 ± 35.00	937.50 ± 14.57
ST-2,3,4	1.43 ± 0.11	2.82 ± 0.11	741.00 ± 7.01	1056.00 ± 35.78
ST-1,2,3,	1.51 ± 0.10	2.94 ± 0.17	690.00 ± 38.18	1173.00 ± 44.55
HT-1	1.96 ± 0.15	3.04 ± 0.12	960.00 ± 38.89	966.00 ± 38.18
HT-2	1.41 ± 0.13	3.01 ± 0.03	750.00 ± 14.24	1242.00 ± 12.88
HT-3	1.85 ± 0.08	3.05 ± 0.11	809.00 ± 15.37	1253.50 ± 30.76
HT-1,2	1.59 ± 0.10	2.51 ± 0.09	775.35 ± 21.27	1095.00 ± 15.12
HT-1,3	2.28 ± 0.15	2.88 ± 0.07	777.00 ± 44.55	1192.50 ± 10.77
HT-2,3	2.54 ± 0.17	2.99 ± 0.08	693.00 ± 11.72	1203.00 ± 32.74
HT-1,2,3	1.37 ± 0.06	2.85 ± 0.08	772.00 ± 21.52	1152.00 ± 22.13
LSD at p = 0.05	For Strain	0.32	For Strain	87.13
	For Treatment	1.08	For Treatment	295.47

Table 6: Impact of bacterial inoculations 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 strains	Peroxidase		Acid phosphatase	
	0 mM	100 mM	0 mM	100 mM
Control	62.92 ± 3.69	91.30 ± 5.16	66.32 ± 2.68	101.44 ± 4.31
ST-1	46.14 ± 3.79	36.29 ± 2.24	81.05 ± 3.11	95.36 ± 4.23
ST-2	35.50 ± 2.12	67.04 ± 4.27	78.96 ± 2.78	83.64 ± 3.66
ST-3	30.13 ± 2.70	45.78 ± 3.36	72.45 ± 4.35	89.90 ± 3.72
ST-4	31.95 ± 2.27	46.50 ± 2.63	89.23 ± 3.62	99.16 ± 4.25
ST-1,2	32.07 ± 1.52	54.83 ± 3.16	54.16 ± 4.01	71.56 ± 4.51
ST-1,3	105.21 ± 2.54	73.46 ± 4.61	67.35 ± 3.51	90.29 ± 4.87
ST-1,4	48.91 ± 3.14	60.99 ± 3.72	65.48 ± 3.18	67.09 ± 2.78
ST-2,3	68.29 ± 3.87	61.69 ± 3.59	66.72 ± 3.25	84.95 ± 3.95
ST-2,4	52.53 ± 3.68	40.96 ± 1.69	91.35 ± 2.48	105.26 ± 6.12
ST-3,4	53.55 ± 3.49	53.48 ± 3.27	86.17 ± 3.67	112.07 ± 5.22
ST-1,2,3	75.00 ± 5.44	89.15 ± 2.89	67.56 ± 3.19	73.53 ± 3.12
ST-1,2,4	62.93 ± 1.12	39.61 ± 2.88	63.97 ± 2.66	91.86 ± 3.58
ST-1,3,4	44.01 ± 3.27	43.86 ± 3.83	55.89 ± 2.82	65.91 ± 3.64
ST-2,3,4	67.83 ± 3.45	57.77 ± 3.25	61.54 ± 2.17	87.64 ± 2.99
ST-1,2,3,4	70.21 ± 2.97	72.14 ± 4.49	52.14 ± 1.96	77.41 ± 2.89
HT-1	39.50 ± 2.32	43.16 ± 3.18	71.52 ± 3.71	93.62 ± 3.78
HT-2	63.20 ± 4.98	67.36 ± 1.97	80.36 ± 4.10	73.88 ± 2.67
HT-3	50.02 ± 2.30	47.81 ± 2.32	91.06 ± 4.33	88.53 ± 4.99
HT-1,2	81.57 ± 1.36	38.11 ± 1.58	75.44 ± 3.53	94.69 ± 3.81
HT-1,3	63.33 ± 2.46	48.48 ± 3.59	68.96 ± 2.22	84.51 ± 3.91
HT-2,3	59.56 ± 2.14	42.73 ± 2.73	67.91 ± 2.84	71.92 ± 2.97
HT-1,2,3	49.47 ± 2.28	45.27 ± 3.33	78.32 ± 3.63	67.99 ± 3.55
LSD at p = 0.05	For Strain	5.03	For strain	8.39
	For Treatment	17.39	For Treatment	28.48

with polysaccharides, proteins and biologically active compounds to minimize the harmful effects of salts. With the application of some mixed culture bacterial inoculations, auxin content of seedlings decreased, over non-inoculated respective treatment, at 0 mM NaCl. These results indicate that bacterial combinations might be involved in improving seedling growth by decreasing Na^+ uptake as well as auxin content of seedlings. Generally monoculture inoculations increased auxin content more efficiently under salt stress. The significant increase in auxin level of inoculated seedlings clearly demonstrates that auxin might be contributing towards plant growth stimulation under salt stress. With bacterial inoculations elevated auxin level in inoculated seedlings under stressed conditions has been reported previously (Siddique *et al*., 1997; Hasnain and Afrasayab, 2000).

Salinity also affected soluble protein content of *Triticum aestivum* seedlings (Table 5). Soluble protein content significantly increased swith the increase in salinity. According to Staples and Dacher (1986), the osmotic adjustment mechanisms of plants include, the ionic pumps of plasmalemma and tonoplast, internal transport mechanisms, the activities of the chloroplast and mitochondria and many metabolic activities of the cytoplasm, especially protein synthesis. Plants grown in saline environment show accumulation of soluble nitrogenous compounds such as amino acids, amides, betaines and polyamines to adjust their internal osmotic status (Takabe *et al*., 1998). Proline and glycine betaine play a wider role in protein stability in the presence of inhibitory ion concentration (Subbararo and Johansen, 1995). Under salt stress osmotic adjustment by plants are manifested by increased soluble protein content. Amount of protein content is directly related with auxin content as both are formed from tryptophane molecules with different arrangement. Hence auxins increase the soluble protein content by increasing the rate of metabolism. Majority of the bacterial inoculations caused increases in protein content (except some inoculations) of seedlings at 0 and 100 mM NaCl treatments. Siddique *et al*. (1997) has also reported enhanced soluble protein content with bacterial inoculations under salt stress.

The activity of enzymes peroxidase (Alexander, 1994) and acid phosphatase (Macaskie, 1995) depends upon metal accumulation by the cells. Enzyme study of *Triticum aestivum* seedlings revealed that activity of peroxidase and acid phosphatase was affected with NaCl treatment as well as bacterial inoculations (Table 6). Peroxidase and acid phosphatase activity increased under NaCl stress due to increased Na^+ uptake by the seedlings which inturn affected plant growth. Increased activity of plasma membrane ATPase enzyme enables the cells to tolerate higher cytoplasmic NaCl under salt stress (Wu and Seliskar, 1998). At 100 mM NaCl, increase, over non-inoculated respective treatment, in peroxidase activity was recorded. Both increases and decreases in peroxidase activity were manifested at 0 mM NaCl, when compared with non-inoculated respective treatment. Siddique *et al*. (1997) related improved seedling growth with reduced peroxidase activity and enhanced auxin level with the application of bacterial inoculations under salt stress. Bacterial inoculations also retarded acid phosphatase activity under salt stress. However at 0 mM most of the inoculations enhanced acid phosphatase activity, when compared with non-inoculated respective treatment. The activity of enzymes acid phosphatase and peroxidase is related with Na^+ content of seedlings. Under saline conditions, decreased Na^+ uptake and enzyme activities clearly indicate that bacteria might be involved in lowering the deposition of Na^+ around the roots, thus making less availability of salts to seedlings. These may be the stress relieving factors for seedlings under stress conditions. At 100 mM NaCl, ST-2, 4 and ST-3, 4 bacterial combinations enhanced acid phosphatase activity relative to non-inoculated respective treatment, which suggests that these bacteria interact to promote enzyme activity of one another. The

magnitude and extent of decrease or increase in enzyme activity varied with different strains. These results revealed that both rhizosphere and histoplane bacteria stimulate seedling growth especially under NaCl stress. Some mixed bacterial cultures (ST-3, 4; ST-1, 2, 4; ST-1, 3, 4; ST-2, 3, 4; HT-1, 2; HT-1, 3; HT-2, 3; HT-1, 2, 3) have synergistic growth stimulatory effects compared with the growth enhancement caused by monocultures. The growth promoting activity could be related with decreased dry weight accumulation, Na^+ content, enzyme activities and increased auxin content of seedlings.

Acknowledgements

Pakistan Science Foundation is acknowledged for financial support (Project No. P-PU/Bio-228).

References

Acharya, U.T., L. Prakash and G. Prathapasenan, 1990. Effect of gibberellic acid on seedling growth and carbohydrate metabolism during germination of rice (*Oryza sativa* L. var. GR 3) under saline condition. J. Agron. Crop Sci., 165: 6-13.

Alam, S.M., 1994. Nutrient Uptake by Plants under Stressful Conditions. In: Handbook of Plant and Crop Stress, Pessaraki, M. (Ed.). Marcel Dekker, New York, pp: 227-246.

Alexander, M., 1994. Bioremediation Technologies. In: Biodegradation and Bioremediation, Alexander, M. (Ed.). Academic Press, California, pp: 248-269.

Amtmann, A., S. Laurie, R. Leigh and D. Sanders, 1997. Multiple inward channels provide flexibility in Na^+/K^+ discrimination at the plasma membrane of barley suspension culture cells. J. Exp. Bot., 48: 481-497.

Ashraf, M., 1997. Improvement of Salt Tolerance in some Native Pulse Crops. In: Strategies for Improving Salt Tolerance in Higher Plants, Jaiwal, P.K., R.P. Singh and A. Gulati (Eds.). Oxford and IBH Publishing Company, New Delhi, Indai, pp: 413-433.

Bashan, Y. and G. Holguin, 1994. Root-to-root travel of the beneficial bacterium *Azospirillum brasilense*. Applied Environ. Microbiol., 60: 2120-2131.

Bashan, Y. and H. Levanony, 1990. Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. Can. J. Microbiol., 36: 591-608.

Campbell, R., 1985. Microbiology of Roots. In: Plant Microbiology, Campbell, R. (Ed.). Edward Arnold Inc., London, pp: 106-152.

Ciulla, R.A., M.R. Diaz, B.F. Taylor and M.F. Roberts, 1997. Organic osmolytes in aerobic bacteria from Mono Lake, an alkaline, moderately hypersaline environment. Applied Environ. Microbiol., 36: 220-226.

Croes, C.L., S. Moens, E. van Bastelaere, J. Vanderleyden and K.W. Michiels, 1993. The polar flagellum mediates *Azospirillum brasilense* adsorption to wheat roots. Microbiology, 139: 2261-2269.

Dubey, R.S., 1997. Nitrogen Metabolism in Plants under Salt Stress. In: Strategies for Improving Salt Tolerance in Higher Plants, Jaiwal, P.K., R.P. Singh and A. Gulati (Eds.). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp: 129-158.

El-Saidi, M.T., 1997. Salinity and its Effects on Growth, Yield and some Physiological Processes of Crop Plants. In: Strategies for Improving Salt Tolerance in Higher Plants, Jaiwal, P.K., R.P. Singh and A. Gulati (Eds.). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp: 111-127.

Engelbrecht, F., K. Marin and M. Hagemann, 1999. Expression of the *ggpS* gene, involved in osmolyte synthesis in the marine cyanobacterium *Synechococcus* sp. strain PCC 7002, revealed regulatory differences between this strain and the freshwater strain *Synechocystis* sp. strain PCC 6803. Applied Environ. Microbiol., 65: 4822-4829.

Ferguson, G.P., A.D. Chacko, C.H. Lee, I.R. Booth and C. Lee, 1996. The activity of the high-affinity K^+ uptake system Kdp sensitizes cells of *Escherichia coli* to methylglyoxal. J. Bacteriol., 178: 3957-3961.

Furman, N.H., 1975. Standard Methods of Chemical Analysis. Robert E Krieger Publishing Co., Malabar, Florida.

Galinski, E.A. and P. Louis, 1998. Compatible Solutes: Ectoine Production and Gene Expression. In: Microbiology and Biogeochemistry of Hypersaline Environments, Oren, A. (Ed.). CRC Press, Boca Raton, FL., pp: 187-202.

Gerhardt, P., R.G.E. Murray, W.A. Wood and N.R. Krieg, 1994. Methods for General and Molecular Bacteriology. 2nd Edn., American Society for Microbiology, Washington, DC., ISBN-10: 1555810489.

Gouffi, K., V. Pichereau, J.P. Rolland, D. Thomas, T. Bernard and C. Blanco, 1998. Sucrose is a nonaccumulated osmoprotectant in *Sinorhizobium meliloti*. J. Bacteriol., 180: 5044-5051.

Gulati, A. and P.K. Jaiwal, 1996. Effect of NaCl on nitrate reductase, glutamate dehydrogenase and glutamate synthase in *Vigna radiata* calli. Biol. Planta, 38: 177-183.

Hasnain, S. and A. Yasmin, 1993. Stimulated Growth of *Triticum aestivum* Seedlings with Salt Tolerant Rhizospheric Bacteria and their Na^+ Relations under NaCl Stresses. In: Plants for the Environment, Khan, M.S., M.A.A. Khan, S. Hadiuzzaman and A. Aziz (Eds.). Bangladesh Botanical Society, Dhaka, Bangladesh, pp: 27-36.

Hasnain, S. and S. Afrasayab, 2000. Evaluation of the role of salt tolerant bacteria in developing resistance of plants to salt stress conditions. Tech. Rep. PSF Project P-PU/Bio (228). Punjab University, Lahore, Pakistan.

Hughes, M.N. and R.K. Poole, 1991. Metal speciation and microbial growth-the hard (and soft) facts. Microbiology, 137: 725-734.

Iqbal, J. and N. Rafique, 1986. Toxic effects of $BaCl_2$ on germination, early seedling growth, soluble proteins and acid phosphatase in *Zea mays* L. Pak. J. Bot., 19: 1-8.

Jimenez-Salgado, T., L.E. Fuentes-Ramirez, A. Tapia-Hernandez, M.A. Mascarua-Esparza, E. Martinez-Romero and J. Caballero-Mellado, 1997. *Coffea Arabica* L., a new host plant for *Acetobacter diazotrophicus* and isolation of other nitrogen-fixing acetobacteria. Applied Environ. Microbiol., 63: 3676-3683.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Lutenberg, B.J.J. and L.A. Dewegar, 1992. *Pseudomonas*-Microbiology and Biotechnology. American Society for Microbiology, Washington, DC., pp: 13.

Macaskie, L.E., 1995. Use of Biological Processes for the Removal of Heavy Metals and Organophosphorus Compounds from Aqueous Solutions. In: Biotechnology for Environment and Agriculture, Ahmed, A., M. Ishaq, O.B. Khan and F. Sarwar (Eds.). B. C. C. and T. Press, University of Karachi, Karachi, Pakistan, pp: 127-142.

Mahadevan, A., 1984. Growth Regulators, Microorganisms and Diseased Plants. Oxford and IBH Publishing Co. Pvt. Ltd., India, pp: 31.

Mirza, T.Z., A.N. Sabri and S. Hasnain, 1998. Salt tolerant bacteria from rhizosphere, rhizoplane, histoplane and phylloplane of Mazus plant inhabitant of salt range. Sci. Int., 10: 151-156.

Mutaftchiev, S., A. Macaya, R. Prat, P. Devillers and R. Colberg, 1993. Early effect of plant cell wall fragment on plant cell growth. Plant Physiol. Biochem., 31: 459-467.

Pareek, A., S.L. Singla and A. Grover, 1997. Salt Responsive Protein/Gene in Crop Plants. In: Strategies for Improving Salt Tolerance in Higher Plants, Jaiwal, P.K., R.P. Singh and A. Gulati (Eds.). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp: 413-434.

Racusen, D. and M. Foote, 1965. Protein synthesis in dark-grown bean leaves. Can. J. Bot., 43: 817-824.

Scupham, A.J., A.H. Bosworth, W.R. Ellis, T.J. Wacek, K.A. Albrecht and E.W. Triplett, 1996. Inoculation with *Sinorhizobium meliloti* RMBPC-2 increases alfalfa yield compared with inoculation with a nonengineered wild-type strain. Applied Environ. Microbiol., 62: 4260-4262.

Siddique, S., A.N. Sabri and S. Hasnain, 1997. Growth Effect of *Triticum aestivum* Seedlings under NaCl Stress after Inoculating with Plasmid Bearing and Plasmid-Free Bacterial Strains. In: Endeavors in Biotechnology, Ilahi, I. (Ed.). University of Peshawar, Peshawar, Pakistan, pp: 97-112.

Staples, R.C. and R.F. Dacher, 1986. Salinity Induced Gene Expression in Crop Plants. In: Prospects for Biosaline Research, Ahmad, R. and A. San Pietro, (Eds.). Karachi University, Karachi, pp: 3-22.

Stark, C.J., 1997. A Plant Biochemical Regulator Improves Salt Tolerance. In: Strategies for Improving Salt Tolerance in Higher Plants, Jaiwal, P.K., R.P. Singh and A. Gulati (Eds.). Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp: 205-219.

Steel, R.G.D. and J.H. Torrie, 1981. Principles and Procedures of Statistics a Biometric Approach. 2nd Edn., McGraw Hill International Book Company, New york.

Subbararo, G.V. and C. Johansen, 1995. Physiological Mechanisams Relevant to Genetic Improvement of Salinity Tolerance in Crop Plants. In: Handbook of Plant and Crop Physiology, Pessarakli, M. (Ed.). Marcel Dekker, New York, ISBN: 9780824792503, pp: 807-831.

Takabe, T., T. Nakamura, M. Noumura, Y. Hayashi and M. Ishitani *et al*., 1998. Glycinebetaine and the Genetic Engineering of Salinity Tolerance in Plants. In: Stress Responses of Photosynthetic Organisms, Satoh, K. and N. Murata (Eds.). Elsevier, UK., pp: 115-131.

Treuner-Lange, A., A. Kuhn and P. Durre, 1997. The kdp system of *Clostridium acetobutylicum*: Cloning, sequencing and transcriptional regulation in response to potassium concentration. J. Bacteriol., 179: 4501-4512.

Tyerman, S.D., M. Skerrett, A. Garrill, G.P. Findlay and R.A. Leigh, 1997. Pathways for the permeation of Na^+ and Cl^- into protoplasts derived from the cortex of wheat roots. J. Exp. Bot., 48: 459-480.

Ventosa, A., J.J. Nieto and A. Oren, 1998. Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev., 62: 504-544.

Wu, J. and D.M. Seliskar, 1998. Salinity adaptation of plasma membrane H^+-ATPase in the salt marsh plant *Spartina patens*: ATP hydrolysis and enzyme kinetics. J. Exp. Bot., 49: 1005-1013.

Yasmin, A. and S. Hasnain, 1993. Salt tolerant pseudomonads exhibiting multiple resistances to heavy metals and antibiotics. Proc. Pak. Congr. Zool., 13: 293-305.

Yasmin, A. and S. Hasnain, 1997. Moderately halophilic bacteria associated with the roots of *Chenopodium album*, *Oxalis corniculata* and *Lycium edgeworthii*. Pak. J. Zool., 29: 249-257.

Yasmin, A. and S. Hasnain, 1998. Root associated osmo-/halotolerant bacteria from plants growing in saline soils. Proc. Pak. Congr. Zool., 18: 97-114.

Yeo, A., 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. J. Exp. Bot., 49: 915-929.