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SALT TOLERANT BACTERIA DIFFERENTLY AFFECTING THE GROWTH OF *TRITICUM AESTIVUM* VARIETIES UNDER NaCl STRESSES

SHAZIA AFRASAYAB AND SHAHIDA HASNAIN

Department of Botany, Uuniversity of the Punjab, Q. A. Campus, Lahore-54590.

Abstract

These studies were carried out to determine the role of bacteria in growth stimulation and improving salt tolerance status of plants. Salt tolerant bacteria isolated from rhizosphere (ST-9), rhizoplane (RT-9, RT-10), histoplane (HT-5) and phylloplane (PT-5, PT-6) of *Launea nudicaulis* plant, inhabitant of salt range were selected. Impact of bacterial inoculations was studied on two *Triticum aestivum* varieties (Inqlab 91 & Rawal 87). Inoculated and non-inoculated seeds of two varieties were germinated and grown under NaCl stresses (0, 50, 100 mM) for 10 days. After this period growth parameters (length & weight) were measured. Response of two varieties to salt stress was different. Under salt stresses reduction in germination, length (shoot, root, seedling) parameters and fresh weight was more in Inqlab 91 variety as compared to Rawal 87. Dry weight parameters, Na^+/K^+, auxin and soluble protein contents and enzyme activities were also affected under salt stresses. In general, bacterial inoculations stimulated germination, seedling growth and fresh weight, auxin and soluble protein contents of two varieties. Whereas reductions in dry weight parameters, Na^+ content and enzyme activities were manifested with bacterial inoculations. Although growth of Inqlab 91 was more affected by salt stress but enhancement in growth with the application of bacterial inoculations was more in Inqlab 91 relative to Rawal 87 variety.

Introduction

The plant kingdom is colonized by a diverse group of bacteria. Plant associated bacteria are not always detrimental; they also can have beneficial effects on plants by enhancing nutrient uptake and plant growth. Many bacteria in the rhizosphere (rhizobacteria) are stimulated by the presence of the plants. The main source of this stimulation is the availability of complex carbohydrates from plants. Plants on the other hand benefit from nitrogen compounds either fixed or released from organic material by bacterial metabolism. This is a beneficial association with no direct contact. Beneficial bacterial associations can stimulate plant growth (Andrews and Harris, 2000), induce systemic resistance in crop plants (Viswanathan and Samiyappan, 1999), enhance N_2 fixation and uptake by the plants (Viprey *et al.*, 2000; Alami *et al.*, 2000), exhibit biocontrol through the production of fungicides and plant hormones or relocation of metal ions and phosphates (Kunst *et al.*, 1997).

Many microorganisms especially nitrogen fixing bacteria introduced into salt affected soils have the ability to change the properties of such soils by adding additional nitrogen in the soil. *Rhizobia, Azotobacter* and *Sinorhizobium meliloti* have been found to increase the yield and nitrogen content of plants under saline conditions (Gangewane and Salve, 1993; Velagalati and Schweitzer, 1994). Rhizosphere bacteria exibiting high Na^+ uptake ability in growth medium have been reported (Afrasayab and Hasnain, 1999). It is a recognized fact that major damage due to salinity problem occurs in agricultural sector, where crop productivity is either completely eliminated or is greatly reduced. For sustainable production and

security of food, management of such lands is essential. Keeping the aforementioned situation in view, the present study was planned to utilize the plant associated salt tolerant bacteria for inoculation studies under saline conditions. As plant-bacterial combinations are host-specific and salt tolerance level of plant species is variable, therefore two different varieties of *Triticum aestivum* (Inqlab 91 and Rawal 87) were used.

Materials and methods

Certified seeds of *Triticum aestivum* varieties i.e., Inqlab 91 (obtained from Punjab Seed Corporation) and Rawal 87 (obtained from National Agriculture Research Center - Islamabad) were surface sterilized with 0.1% $HgCl_2$. Sterilized seeds were inoculated with salt tolerant bacteria (suspensions) isolated from rhizosphere (ST-8), rhizoplane (RT-9, RT-10), histoplane (HT-5) and phylloplane (PT-5, PT-6) of *Launia nudicaulis* inhabiting the area of salt range Kallar Kahar, Pakistan. The isolates could tolerate 2.5 M NaCl in the growth medium. The isolates were Gram negative motile rods (except RT-10 which was Gram variable cocci) having entire and circular colonies. The isolates were facultative anaerobes (except ST-8, RT-9). All of them showed strong catalase activity but gave negative results for urease enzyme. Preparation of bacterial suspensions, inoculations of seeds, experimental set-up and conditions for germination and growth have been described previously (Afrasayab and Hasnain, 2000b). Seeds were germinated and grown for 10 days. After this period, growth parameters (length and weight) were taken. Na^+ and K^+ contents of inoculated and uninoculated seedlings were determined photometrically (Furman, 1975). Seedlings were analyzed for auxins (Mahadevan, 1984), soluble proteins (Lowry *et al.*, 1951) and enzymes peroxidase (David and Murry, 1965) and acid phosphatase (Iqbal and Rafique, 1987). Data of these results was subjected to statistical analysis (Steel and Torrie, 1981).

Results

Germination and growth: Germination of both *T. aestivum* varieties was impeded with salt stress. Inqlab 91 variety showed more sensitivity to NaCl treatments than Rawal 87 (6.49-16.88% reduction in Inqlab 91, 6.25-11.25% reduction in Rawal 87)(Table-1). At 0 mM, bacterial inoculations ST-8, RT-10, PT-5, PT-6 provoked germination of *T. aestivum* Inqlab 91 (1.29-3.89%), but in Rawal 87, inoculations (except PT-5, where reduction was recorded) had no effect on germination. At 50 and 100 mM NaCl, all the inoculations stimulated germination of both varieties (5.55-8.33% at 50 mM and 14.06-18.75% at 100 mM in Inqlab 91, 1.33-4% at 50 mM and 1.41-5.63% at 100 mM in Rawal 87) over respective control treatment. Bacterial inoculations HT-5 and PT-5 provoked germination most significantly under all NaCl stresses in both varieties. ST-8 and PT-6 stimulated germination of Inqlab 91 at 100 mM NaCl treatment. With bacterial inoculations increases in germination were more in Inqlab 91 variety relative to Rawal 87.

Although shoot and root growth of *T. aestivum* varieties was affected by salinity (50-100 mM NaCl), but growth of Inqlab 91 (15.27-31.64% in shoot, 80.5-93.91% in root reduction) was more severely retarded as compared to Rawal 87 (7.94-17.68% in shoot, 14.35-20.14% reduction in root)(Table-1). Generally, the effect of bacterial inoculations on shoot growth was stimulatory in both varieties at

0 (except RT-10, HT-5, PT-6 in Rawal 87), 50 and 100 mM (except RT-10, PT-5 in Rawal 87). In Inqlab 91, ST-8, HT-5 inoculations at 50 mM and RT-9, HT-5, PT-5, PT-6 inoculations at 100 mM markedly promoted shoot growth. ST-8, RT-9, PT-6 had more stimulatory effect on shoot growth of Rawal 87 at both NaCl stresses (50 and 100 mM). Effect of bacterial inoculations on root growth was variable. Some bacterial inoculations caused reductions at 0 mM and most of the inoculations at 100 mM NaCl in Inqlab 91, whereas in Rawal 87 situation was reverse. However at 50 mM NaCl almost all bacterial inoculations (except PT-5) promoted root length in both the varieties. Growth stimulatory effect of bacterial inoculations in this parameter at 50 mM was more in Inqlab 91 (16.98-54.08%). Growth promoting effect of bacterial inoculations on root growth at two salt treatments in both varieties was also different. ST-8 inoculation at all NaCl treatments whereas HT-5, PT-6 at 50 mM markedly enhanced root growth in *T. aestivum* Inqlab 91. In Rawal 87 ST-8, RT-9, HT-5 inoculations had stimulatory effect on root growth at 50 and 100 mM NaCl treatments. Increasing salt concentration (50-100mM NaCl) adversely affected the seedling lengths of Inqlab 91 (29.12-39.59%) and Rawal 87 (10.81-18.78%) varieties of *T. aestivum* (Table-1). Almost all bacterial inoculations promoted seedling growth of Inqlab 91 variety at 0 (0.37-6.37% except RT-10), 50 (12.01-28.44%) and 100 mM (6.94-12.25% except RT-10) NaCl, relative to non-inoculated respective treatments. In Rawal 87, almost all inoculations at 0 mM (except ST-8), while some inoculations at 50 (PT-5) and 100 mM (RT-10, PT-5, PT-6) had deleterious effects on seedling growth. Increases (0.8-10.55%) at 50 mM and 100 mM NaCl (2.65-9.47%) in this parameter were recorded in Rawal 87 with bacterial inoculations over respective control. Enhancement in seedling growth in both the varieties with bacterial inoculations was pronounced at 50 mM than 100 mM NaCl. ST-8 (rhizosphere bacteria) inoculation promoted seedling growth significantly in both *T. aestivum* varieties as well as at all NaCl treatments. RT-9, HT-5, PT-5, PT-6 (at 50 and 100 mM NaCl) in Inqlab 91, while RT-9, HT-5 (at 50 and 100 mM) and PT-6 (at 50 mM only) promoted seedling growth more significantly.

Weight parameters of *T. aestivum* varieties were affected under salt stress conditions (Table-2). In Inqlab 91, 17.45-55.80% and in Rawal 87, 4.85- 29.70% reductions in fresh weight of seedlings at 50-100 mM NaCl treatments were observed. Generally, bacterial inoculations enhanced fresh weight per seedling (significant in most of the cases) at 0 (except RT-9, RT-10, HT-5, PT-5 in Rawal 87), 50 (except RT-9, RT-10 in Rawal 87) and 100 mM NaCl in both varieties. At 100 mM NaCl, increases in fresh weight were higher as compared to 0 and 50 mM NaCl. Salt stress also affected the dry weight parameters of seedlings. Dry weight per seedling increased in Inqlab 91 (19.96-37.76%) and Rawal 87 (4.15-12.71%) with 50-100 mM NaCl. Bacterial inoculations increased dry weight per seedling in Inqlab 91 at 0 mM (1.42-21.55%), while reductions were recorded at 50 (2.13-14.96% except RT-10, HT-5) and 100 mM (11.32-18.87%). In Rawal 87, generally reduction in this parameter at 0 (2.57-3.99%), 50 (1.06-18.99%, except ST-8, PT-5, PT-6) and 100 mM (1.54-9.00% except ST-8, RT-9) NaCl with the application of bacterial inoculations were observed. Dry weight accumulation (dry weight per gram fresh weight) also increased with the increase in salt stress. 45.33-127.27% increase in Inqlab 91 and 9.46-60.35% enhancement in Rawal 87 was recorded at 50-100 mM NaCl. Almost all bacterial inoculations decreased dry weight

accumulation of seedlings (significantly in most of the cases) at 0 (except RT-10, HT-5, PT-5, PT-6), 50 and 100 mM NaCl, over respective control, in both varieties. At 50 mM NaCl, PT-5, PT-6 and at 100 mM ST-8, RT-9 inoculations caused relatively more reduction in dry weight accumulation in Inqlab 91. However in Rawal 87 RT-10, PT-5 inoculations caused marked decrease in this parameter. With bacterial inoculations, reductions in dry weight accumulation were more in Inqlab 91 relative to Rawal 87. Similarly decreases were more at 100 mM than 50 mM NaCl treatment.

Na^+ and k^+ contents: Na^+ uptake by the seedlings increased under elevated NaCl concentration. Almost 3-5 folds increase in Inqlab 91 and 3-4 folds increase in Rawal 87 was observed (Table-2). Bacterial inoculations caused increase in Na^+ uptake by the seedlings of Inqlab 91 at 0 (47.54-62.07%) but reduced at 50 (3.06-16.56%) and 100 mM (8.49-28.12%) NaCl. In Rawal 87 variety 0.74-27.29% increase in Na^+ uptake by the seedlings at 0 mM whereas reduction (excluding ST-8, RT-9 where enhancement in this parameter was observed) at 50 (7.55-30.11%) and 100 mM (4.04-14.86%) NaCl, over respective non-inoculated treatment was recorded. ST-8, RT-9 in Inqlab 91 and PT-5, PT-6 inoculations in Rawal 87 showed marked reduction in Na^+ uptake of seedlings at 50 and 100 mM NaCl treatments. Inqlab 91 variety showed more reduction over Rawal 87 under 100 mM NaCl treatment. K^+ content of seedlings of both varieties increased progressively with the increase in NaCl stress in both *Triticum aestivum* varieties (Table-2). With the application of bacterial inoculations, K^+ uptake by the seedlings increased in both the varieties at 0 mM NaCl. While under NaCl stresses both the varieties behaved differently. Bacterial inoculations caused reduction in K^+ uptake by the seedlings in Inqlab 91 at 50 (6.33-25.10%) and 100 mM NaCl (7.26-18.32%), while caused enhancement in Rawal 87 (excluding ST-8, RT-9 which showed decrease) at 50 (2.24-16.93%) and 100 mM (13.06-21.54%) NaCl in this parameter.

Biochemical attributes: Salt stress caused enhancement in auxin content of *Triticum aestivum* varieties (25-137.03% in Inqlab 91; 47.12-152.87% in Rawal 87) under salt stress (Table-3). Elevated auxin content with bacterial inoculations in both varieties at 0 (except some cases), 50 (excluding PT-5 in Rawal 87) and 100 mM (except PT-5 in Rawal 87) NaCl was observed. With PT-5 (phylloplane bacteria) inoculation auxin content of *T. aestivum* Rawal 87 dropped at 50 and 100 mM NaCl. ST-8, RT-9, PT-5 (In Inqlab 91), RT-9, HT-5 (in Rawal 87) showed relatively higher enhancement in auxin content of seedlings at 100 mM NaCl treatment as compared to non-inoculated respective treatment. Enhancement in auxin synthesis was more in Inqlab 91 as compared to Rawal 87.

Enhanced soluble protein content in *T. aestivum* varieties i.e., Inqlab 91 (17.83-60.20%) and Rawal 87 (24.08-58.24%) at 50-100 mM NaCl was recorded (Table-3). Generally, bacterial inoculations enhanced soluble protein content of seedlings at all NaCl treatments in both varieties (except some cases in Inqlab 91 and Rawal 87), relative to non-inoculated respective treatmernts. At 50 and 100 mM NaCl increment with different bacterial inoculations was different. In both varieties increment in protein content with introduction of bacteria was higher at 50 mM, relative to 100 mM NaCl.

Activity of enzyme peroxidase enhanced in *Triticum aestivum* varieties (66.54-117.92% in Inqlab 91 and 48.54-114.43% in Rawal 87) in response to salt

stress (Table-3). At 0 mM 3.95-35.52% reduction in Inqlab 91 (except ST-8, PT-5) and 9.33-26.28% reduction in Rawal 87 (excluding ST-8, RT-10, HT-5) was manifested with bacterial inoculations, over non-inoculated respective 0 mM treatment. Bacterial inoculations (except some) dropped peroxidase activity of *T. aestivum* var Inqlab 91 at 50 (10.13-33.06%, excluding RT-9, RT-10) and 100 mM (1.72-24.72%, except RT-10) NaCl. Excluding RT-10 and PT-5 inoculations (which accelerated peroxidase activity) decreases in the activity of this enzyme with rest of the inoculations were recorded at 50 (11.05-20.42%) and 100 mM (10.32-28.62%) NaCl, relative to non-inoculated respective treatments.

Salt stress triggered acid phosphatase activity in *T. aestivum* varieties (84.55-155.41% in Inqlab 91; 139.39-169.60% in Rawal 87 at 50-100 mM NaCl)(Table-3). In general, altered activity of this enzyme with bacterial inoculations was analysed at all NaCl treatments (0, 50, 100 mM). Bacterial inoculations dropped activity of acid phosphatase at 0 (0.61-10.01%, except ST-8, RT-9, RT-10), 50 (1.60-23.84%, excluding HT-5) in Inqlab 91. In Rawal 87 increases (8.48-21.37%) in acid phosphatase activity with the application of bacterial inoculations were examined at 0 mM NaCl. Excluding RT-10, PT-5 (where 13.12, 3.41% respectively reduction in acid phosphatase activity was observed) rest of the inoculations enhanced activity of this enzyme at 50 mM. However at 100 mM, both increases (0.07-9.43%) and decreases (2.31-6.23%) in acid phosphatase activity were examined.

Discussion

NaCl treatments significantly affected the germination of *T. aestivum* varieties (Inqlab 91 and Rawal 87)(Table-1). High salt concentrations in the growth medium limited the availability of water, which affected germination. Adverse effects of salts on germination have been reported earlier (Nhiri *et al.*, 2000; Afrasayab and Hasnain, 2000ab). *T. aestivum* variety Inqlab 91 showed more sensitivity to NaCl as compared to Rawal 87. Reduction in germination was higher in Inqlab 91. The crop plants differ considerably with regard to salt tolerance at various growth stages but germination and seedling growth being the most critical ones. Inoculation with salt tolerant bacteria enhanced germination of two *T. aestivum* varieties Inqlab 91 and Rawal 87 under salt stress conditions. Bacterial inoculations HT-5, PT-5 (at all NaCl stresses in both varieties), ST-8, PT-6 (Inqlab 91 at 100 mM NaCl treatment) most significantly provoked germination, over respective control treatments. With bacterial inoculations increases in germination were more in Inqlab 91 as compared to variety Rawal 87. Stimulation in germination may be assigned to Na^+ uptake by bacteria. As mostly organisms regulate both the quantity and quality of inorganic ions to conserve a defined microenvironment in the cytoplasm (Serrano and Gaxiola, 1994). By taking up Na^+ ions from the medium, bacteria reduce the toxicity of ions hence make conditions favorable for seed germination.

Marked effects of salt stress on shoot, root and seedling growth of plants was also observed (Table-1). A linear decrease in these parameters with the increase in NaCl stress was recorded. Decrease in number of leaves, while increase in number of roots was also observed. Roots became brownish and hard in texture. Reduction in growth has been ascribed to adverse effects of higher amounts of salts on metabolic and enzymatic activities (Nhiri *et al.*, 2000; Takemura *et al.*, 2000;

Navarro *et al.*, 2000). Bacterial inoculations had stimulatory effect on seedling growth of *Triticum aestivum* varieties (except RT-10 in both varieties and PT-5, PT-6 inoculations in Rawal 87) under saline conditions. Growth promoting effect of bacterial inoculations varied with salt stress as well as with plant. In Inqlab 91 ST-8, HT-5 (at 50 mM) RT-9, HT-5, PT-5, PT-6 inoculations (at 100 mM) promoted shoot growth more efficiently relative to respective control treatments. ST-8, RT-9, PT-6 had stimulatory effect on shoot growth of Rawal 87 at both NaCl stresses (50 and 100 mM). Growth promoting effect of bacterial inoculations on root growth at two salt treatments was also different. With ST-8 inoculation at all NaCl treatments whereas with HT-5, PT-6 at 50 mM enhancement in root growth in *T. aestivum* Inqlab 91 was higher. In Rawal 87 ST-8, RT-9, HT-5 inoculations had more stimulatory effect on root growth at 50 and 100 mM NaCl treatments. ST-8 (rhizosphere bacteria) inoculation promoted seedling growth significantly in both *T. aestivum* varieties as well as at all NaCl treatments. RT-9, HT-5, PT-5, PT-6 (at 50 and 100 mM NaCl) in Inqlab 91, while RT-9, HT-5 (at 50 and 100 mM) and PT-6 (at 50 mM only) caused pronounced increase in seedling growth.

Plant growth promoting bacteria (PGPR) are considered to form part of a protective flora, which provide benefit to the plant of enhanced root function, disease suppression and accelerated plant development (Glick, 1995; Arshad and Frankenberg, 1998). Bacteria enhance plant growth by making availability of nutrients to the plants (Alami *et al.*, 2000), colonizing the rhizosphere (Leubeck *et al.*, 2000), control of plant pathogens (Nielson *et al.*, 1998), producing plant growth regulators (Vande Broek and Vanderleden, 1995) and regenerating the quality of the soil (van Veen *et al.*, 1997; Alami *et al.*, 2000). Bacteria when introduced into the rhizosphere of the plants grow successfully (Punja, 1997), as conditions for microbial survival and growth are favorable. Some bacterial inoculations had deleterious effects on shoot (in Rawal 87) and root (both varieties) growth. Reduction in plant growth may be due to poor bacterial survival in soil (van Veen *et al.*, 1997). The reason for decrease in microbial population includes insufficient nutrients available for maintenance and replication and environmental conditions such as pH, ionic strength and temperature (van Elas and van Overbeek, 1993). Enhancement in seedling growth was more at 50 mM NaCl in both varieties. Similarly bacterial inoculations promoted seedling growth of Inqlab 91 variety more as compared to variety Rawal 87, which indicates that plant bacterial combination in variety Inqlab 91 is most suitable.

Progressive decrease in fresh weight per seedling and increase in dry weight per seedling and dry weight accumulation was observed. Alami *et al.* (2000) reported that fresh weight of young leaves of tomato cultivars was adversely affected under water and salt stresses. Reduction in fresh weight of *T. aestivum* might be ascribed to scarcity of water in the growth medium. Under salt stress organisms adapt different strategies to survive in stress environment. For osmotic adjustment they accumulate organic and inorganic compounds in the cytoplasm. In the present case increase in dry weight seems to be due to such an adaptation. With the introduction of bacterial inoculations fresh weight per seedlings of *T. aestivum* varieties i.e., Inqlab 91 and Rawal 87 (except some inoculations at 0 and 100 mM NaCl) was stimulated under NaCl stresses. As under saline conditions bacteria take up inorganic ions to balance their internal osmotic potential equal to external environment, thus diluting the toxic effects of salts and making conditions favorable

for plant growth, which in turn results in fresh weight enhancement. Bacterial inoculations caused significant decrease in dry weight per seedling (except some cases in both varieties) and dry weight accumulation (excluding some cases in Rawal 87 at 0 mM). PT-5, PT-6 (at 50 mM NaCl) and ST-8, RT-9 (at 100 mM) inoculations caused relatively more reduction in dry weight accumulation in Inqlab 91. However in Rawal 87 RT-10, PT-5 inoculations caused marked decrease in this parameter relative to non-inoculated respective treatment. Bacterial strains have the ability to bind with inorganic ions and restrict the bioavailability of these ions by forming organic complexes (Hughes and Poole, 1991). In the present case decreased dry weight as well as decreased Na^+ uptake by the seedlings may be attributed to their behaviour of salt tolerant bacterial strains (Table-2).

Under osmotic stress increased ion concentration helps the plant cells by allowing water movement into the cells and saving energy that may be used in synthesis of organic solutes required for osmotic adjustment. Under saline conditions enhanced Na^+ (Alian *et al.*, 2000; Takemura *et al.*, 2000) and K^+ content (Yeo, 1998) in different plants has been reported. Bacterial inoculations caused reduction in Na^+ uptake by the seedlings in both varieties at 50 and 100 mM NaCl treatments. In Inqlab 91 variety reduction in Na^+ uptake by the seedlings was more as compared to Rawal 87 at 100 mM NaCl treatment, which indicates that in this variety plant-bacteria combination is most suitable.

As for as K^+ content of seedlings is concerned, both varieties of *T. aestivum* showed variation in K^+ uptake, when bacterial inoculations were applied (Table-2). With the application of bacterial inoculations reduced K^+ uptake by the seedlings in Inqlab 91 whereas enhancement in this parameter in Rawal 87 was recorded. Reduction in K^+ uptake in Inqlab 91 showed the sensitivity of the plants to inorganic ions or the replacement of K^+ by another monovalent cation such as Na^+ (Chow *et al.*, 1990). Similarly elevated K^+ in Rawal 87 suggests that under saline conditions plants usually retain cell volume by preferring K^+ over Na^+, their enzymes are sensitive to Na^+ (Leigh and Wyn Jones, 1984). Another factor in augmented K^+ uptake may be the deposition of K^+ by the bacteria around the roots. Plants use indifferently Na^+ and K^+ ions as osmotica for growth in saline environments (Amzallag and Lerner, 1994). However no correlation exists between salt tolerance and K^+: Na^+ ratio (He and Cramer, 1993).

Plant growth regulators regulate growth, development and metabolic processes as well as manipulate the reaction of plants against salt stress. Salt stress as well as bacterial inoculations enhanced the auxin content of seedlings of both varieties. In majority of the cases enhanced auxin content was associated with improved seedling growth. Plant growth promoting bacteria stimulate the growth of plants by synthesizing and liberating growth hormones. Auxins also minimize the toxic effects of salts by forming compounds with polysaccharides and proteins (Mutaftchiev *et al.*, 1993). Like auxins soluble protein content of seedlings also increased with salt stress as well as bacterial inoculations. Plants also accumulate organic compounds viz., soluble nitrogenous compounds such as amino acids, amides, betaines and polyamines in addition to inorganic compounds in extreme environments (Alian *et al.*, 2000; Holmstrom *et al.*, 2000). With the increase in amount of auxin, protein content in seedlings enhanced, as auxin regulates metabolic processes which inturn enhances soluble protein content. Enhancement in auxin and soluble protein content in *T. aestivum* Inqlab 91 was more at 100 mM

NaCl as compared to Rawal 87 and it was accompanied by improved growth in this variety. The results were in agreement with the findings of Afrasayab and Hasnain (2000a,b).

NaCl concentrations also affected the activity of enzymes peroxidase and acid phosphatase of both varieties (Table-3). Activity of these enzymes progressively enhanced with the increase NaCl concentration. Increased activity of different enzymes have been documented under metal (Cho and Park, 2000; Chien and Kao, 2000; Rao and Stresty, 2000) as well as salt (Barabas *et al.*, 2000; Takemura *et al.*, 2000) stresses due to acceleration in *de novo* synthesis of enzyme proteins. Decreased activity of different enzymes in rice seedlings (having different salt tolerance levels) grown under different salinity levels have been reported by Kumar *et al.* (2000). Increased enzyme activities protect the cells from stress damage caused by high endogenous level of ammonia (Gulati and Jaiwal, 1996) and enable the cells to resist salt stress. Generally, bacterial inoculations caused reduction in peroxidase and acid phosphatase activities in *Triticum aestivum* seedlings of var Inqlab 91 and Rawal 87 under salt stresses. Afrasayab and Hasnain (2000a,b) have reported decreased enzyme activity with decreased Na^+ uptake, enhanced auxin content and soluble protein contents. Under saline conditions, decreased Na^+ uptake and retarded enzyme activities show the clear relationship in these two parameters. These results suggest that bacteria might be involved in lowering the deposition of Na^+ in the root zone, thus making less availability of salts to seedlings. This may be an adaptation or a stress-relieving factor in plants in response to salt stress conditions. *T. aestivim* var Inqlab 91 showed more reduction in peroxidase and acid phosphatase activities relative to Rawal 87 under salt stress (100 mM). In this study it was found that although salt stress retarded the growth of *T. aestivum* varieties but bacterial inoculations caused stimulation in growth under NaCl stresses. Promotion in growth with the application of bacteria was more in *T. aestivum* var Inqlab 91.

Table-1. Percentage germination and growth parameters of *Triticum aestivum* varieties under different NaCl concentrations after inoculating with pure cultures of salt tolerant bacterial strains (means of six replicates).

	NaCl CONCENTRATIONS					
BACTERIAL	Var Inqlab 91			Var Rawal 87		
STRAINS	0 mM	50 mM	100 mM	0 mM	50 mM	100 mM
	PERCENTAGE GERMINATION					
Control	96.25 ± 1.08	90 ± 3.06	80 ± 3.95	100 ± 0	93.75 ± 1.36	88.75± 3.60
ST-8	100 ± 0	95.00 ± 3.06	95.00 ± 3.06	100 ± 0	95.00 ± 2.07	91.25 ± 2.07
RT-9	96.25 ± 1.08	96.25 ± 2.07	91.25 ± 2.07	100 ± 0	95.00 ± 1.25	90.00 ± 1.25
RT-10	97.50 ± 1.25	95.00 ± 1.76	91.25 ± 3.24	100 ± 0	95.00 ± 1.08	90.00 ± 2.79
HT-5	96.25 ± 1.08	97.50 ± 1.25	95.00 ± 2.50	100 ± 0	96.25 ± 1.25	93.75 ± 2.07
PT-5	100 ± 0	95.00 ± 1.76	93.75 ± 3.24	98.75 ± 1.76	96.25 ± 1.25	93.75 ± 2.07
PT-6	98.75 ± 1.08	95.00 ± 3.06	93.75 ± 2.07	100 ± 0	97.50 ±1.08	91.25 ± 1.08
L.S.D. At	For Strain		3.03	For Strain		1.42
p = 0.05	For Treatment		4.63	For Treatment		2.17
	SHOOT LENGTHS (cm)					
Control	12.83 ± 0.67	10.87 ± 0.15	8.77 ± 0.56	13.85 ± 0.35	12.75 ± 0.18	11.40 ± 0.09
ST-8	13.00 ± 0.25	12.18 ± 0.22	9.82 ± 0.14	14.23 ± 0.12	13.90 ± 0.53	12.28 ± 0.20
RT-9	13.58 ± 0.31	11.76 ± 0.27	10.75 ± 0.29	13.98 ± 0.14	13.83 ± 0.38	11.92 ± 0.49
RT-10	13.48 ± 0.12	11.83 ± 0.21	9.18 ± 0.15	13.30 ± 0.29	12.96 ± 0.59	11.19 ± 0.62
HT-5	13.68 ± 0.19	11.94 ± 0.37	10.25 ± 0.17	13.76 ± 0.52	12.81 ± 0.68	11.45 ± 0.53
PT-5	13.55 ± 0.27	11.43 ± 0.31	10.03 ± 0.14	13.94 ± 0.49	13.13 ± 0.34	11.36 ± 0.59
PT-6	13.48 ± 0.37	11.63 ± 0.25	9.97 ± 0.08	13.04 ± 0.31	12.90 ± 0.58	11.70 ± 0.51
L.S.D. At	For Strain		0.41	For Strain		0.29
P = 0.05	For Treatment		0.62	For Treatment		0.45
	ROOT LENGTHS (cm)					
Control	11.48 ±1.2	6.36 ± 0.57	5.92 ± 0.19	11.22 ± 0.46	9.61 ± 0.42	8.96 ± 0.10
ST-8	12.86 ± 0.47	9.80 ± 0.41	6.67 ± 0.43	12.83 ± 0.43	10.82 ± 0.60	10.01 ± 0.55
RT-9	12.20 ± 0.11	7.44 ± 0.42	5.49 ± 0.15	10.52 ± 0.74	9.67 ± 0.63	10.06 ± 0.52
RT-10	10.71 ± 0.37	7.56 ± 0.17	5.21 ± 0.04	8.64 ± 0.55	10.06 ± 0.75	8.66 ± 0.18
HT-5	11.67 ± 0.05	8.52 ± 0.24	5.71 ± 0.18	9.46 ± 0.50	9.73 ± 0.42	9.45 ± 0.42
PT-5	10.85 ± 0.24	7.87 ± 0.38	5.82 ± 0.14	10.21 ± 0.12	8.58 ± 0.44	8.52 ± 0.29
PT-6	11.88 ± 0.27	9.40 ± 0.20	5.74 ± 0.12	9.87 ± 0.33	11.33 ± 0.80	7.99 ± 0.20
L.S.D. At	For Strain		0.73	For Strai n		1.06
P = 0.05	For Treatmen t		1.12	For Treatment		1.62
	SEEDLING LENGTHS (cm)					
Control	24.31 ±1.87	17.23 ±0.73	14.69 ± 0.75	25.07 ±0.81	22.36 ±0.60	20.36 ±0.11
ST-8	25.86 ± 0.72	22.13 ± 0.63	16.49 ± 0.57	27.06 ± 0.55	24.72 ± 1.13	22.29 ± 0.75
RT-9	25.78 ± 0.44	19.57 ± 0.69	16.24 ± 0.45	24.50 ± 0.88	23.50 ± 1.01	21.98 ± 1.01
RT-10	24.19 ± 0.50	19.39 ± 0.38	14.39 ± 0.19	21.94 ± 0.84	23.03 ± 1.34	19.85 ± 0.80
HT-5	25.35 ± 0.27	20.46 ± 0.62	15.96 ± 0.35	23.22 ± 1.02	22.54 ± 1.10	20.90 ± 0.95
PT-5	24.40 ± 0.56	19.30 ± 0.69	15.85 ± 0.28	24.15 ± 0.61	21.71 ± 0.78	19.88 ± 0.88
PT-6	25.36 ± 0.64	21.03 ± 0.45	15.71 ± 0.20	22.91 ± 0.64	24.23 ± 1.38	19.69 ± 0.71
L.S.D. At	For Strain		0.79	For Strain		1.09
P = 0.05	For Treatment		1.21	For Treatment		1.66

Table-2. Impact of bacterial inoculations on dry weight per gram fresh weight (mg/gm), Na^+ and (μg/gm.d.wt.) K^+ content (μg/gm.d.wt.) of two varieties of *Triticum aestivum* seedlings under different NaCl concentrations (means of six replicates).

BACTERIAL STRAINS	NaCl CONCENTRATIONS					
	Var Inqlab 91			Var Rawal 87		
	0 mM	50 mM	100 mM	0 mM	50 mM	100 mM
	DRY WEIGHT PER GRAM FRESH WEIGHT (mg/gm.d.wt.)					
Control	150.80± 6.79	219.16± 8.43	338.21± 8.05	136.66± 6.62	149.59± 2.16	219.14± 0.58
ST-8	120.93 ± 3.36	158.38 ± 2.23	185.26 ± 4.00	128.40 ± 7.52	147.63 ± 2.88	181.39 ± 7.69
RT-9	128.22 ± 3.29	155.99 ± 2.1	195.67 ± 5.64	136.28 ± 9.16	148.72 ± 4.57	190.13 ± 5.37
RT-10	132.62 ± 5.54	175.88 ± 2.21	203.04 ± 5.02	143.07 ± 5.26	135.41 ± 5.58	160.77 ± 3.02
HT-5	127.89 ± 5.19	188.98 ± 2.44	223.05 ± 1.95	141.49 ± 6.58	128.98 ± 4.71	178.50 ± 5.74
PT-5	126.49 ± 4.6	146.35 ± 5.09	246.71 ±3.31	145.05 ± 5.38	148.68 ± 6.71	165.21 ± 7.24
PT-6	128.72 ± 7.42	145.16 ± 4.05	227.47 ± 4.47	143.43 ± 9.73	144.94 ± 2.26	173.87 ± 3.51
L.S.D. At	For Strain		29.13	For Strain		14.76
P = 0.05	For Treatment		44.51	For Treatment		22.56
	Na^+ CONTENT OF SEEDLINGS (μg/gm.d.wt.)					
Control	5306.43 ±107.59	19557.99 ±741.26	26654.76 ± 655.64	4622.85 ± 102.25	12410.70 ±489.92	13085.68 ± 302.08
ST-8	8600.25 ±350.06	16709.40 ± 423.11	19301.28 ± 376.27	5127.91± 143.45	13896.72 ±431.56	14209.93 ± 683.14
RT-9	7829.02 ± 182.17	16318.17 ± 953.66	19159.39 ± 149.95	5884.66 ± 192.76	13256.67 ±178.91	13372.47 ± 375.55
RT-10	8350.00 ± 636.49	16769.23 ± 544.01	21417.07 ± 75.48	4657.04 ± 234.11	11472.82 ±238.56	12555.97 ± 438.71
HT-5	7923.07 ± 235.74	19250.58 ± 926.03	21646.95 ± 372.67	5211.51 ± 154.98	8673.08 ± 224.79	12910.91 ± 456.87
PT-5	8191.73 ± 174.88	18958.64 ± 938.52	22608.46 ± 377.93	5796.99 ± 234.99	11172.64 ±345.66	11986.94 ± 728.92
PT-6	7877.27 ± 652 56	16764.28 ±186.90	24391.66 ± 571.66	4736.33 ±403.76	9018.49 ±243.45	11140.62 ± 578.44
L.S.D. At	For Strain		2265.81	For Strain		1260.59
P = 0.05	For Treatment		3461.08	For Treatment		1925.58
	K^+ CONTENT OF SEEDLINGS (μg/gm.d.wt.)					
Control	2022.50 ±26.09	3614.88 ±86.98	4203.57 ±53.57	1366.36 ±25.42	1682.78 ±65.81	1758.42 ±62.03
ST-8	2034.71 ± 40.87	2858.75 ±97.35	3433.33 ± 91.67	1419.64 ±84.68	1561.35 ± 38.87	1700.41± 81.37
RT-9	2363.15 ± 77.49	3385.98 ±66.31	3655.71 ± 87.91	1602.34 ±78.32	1637.93 ± 54.61	1737.18 ±62.13
RT-10	2268.12 ± 86.08	3285.71± 57.87	3548.60 ± 65.84	1369.41 ±53.41	1866.79 ± 45.98	2091.46 ±43.25
HT-5	2153.84 ± 39.89	3268.58± 86.44	3898.08 ± 67.41	1471.93 ±62.34	1720.62 ± 47.65	1988.23 ±76.02
PT-5	2310.80 ± 61.11	3267.29± 78.62	3708.33 ± 53.79	1578.90 ±91.32	1875.96 ± 65.73	2054.44 ±65.43
PT-6	2142.05 ± 99.38	2707.38± 41.33	3650.66 ± 48.27	1379.61 ±45.76	1967.78 ± 54.61	2137.17 ±56.09
L.S.D. At	For Strain		241.09	For Strain		143.98
P = 0.05	For Treatment		368.27	For Treatment		219.94

Table-3. Impact of bacterial inoculations on biochemical parameters of two varieties of *Triticum aestivum* seedlings under different NaCl concentrations (means of six replicates).

	NaCl CONCENTRATIONS					
BACTERIAL	Var Inqlab 91			Var Rawal 87		
STRAINS	0 mM	50 mM	100 mM	0 mM	50 mM	100 mM
AUXIN CONTENT OF SEEDLINGS (µg/gm.f.wt.)						
Control	1.08 ± 0.09	1.35 ± 0.07	2.56 ± 0.08	0.87 ± 0.08	1.28 ± 0.05	2.20 ± 0.14
ST-8	1.54 ± 0.06	2.00 ± 0.06	2.98 ± 0.04	0.61 ± 0.02	1.29 ± 0.07	2.40 ± 0.13
RT-9	1.37 ± 0.03	1.71 ± 0.06	2.87 ± 0.03	0.80 ± 0.01	1.47 ± 0.04	2.62 ± 0.11
RT-10	1.03 ± 0.05	2.72 ± 0.16	2.58 ± 0.07	1.00 ± 0.07	1.76 ± 0.12	2.21 ± 0.07
HT-5	1.48 ± 0.08	1.48 ± 0.08	2.63 ± 0.12	1.21 ± 0.01	1.89 ± 0.01	2.65 ± 0.15
PT-5	0.99 ± 0.02	1.68 ± 0.02	2.81 ± 0.08	0.82 ± 0.02	1.25 ± 0.05	1.98 ± 0.09
PT-6	1.01 ± 0.03	1.42 ± 0.11	2.60 ± 0.13	1.08 ± 0.06	1.42 ± 0.07	2.42 ± 0.05
L.S.D. At	For Strain	0.36		For Strain	0.18	
p = 0.05	For Treatment	0.55		For Treatment	0.28	
SOLUBLE PROTEIN CONTENT OF SEEDLINGS (µg/gm.f.wt.)						
Control	438.75 ± 24.47	517.00 ± 30.41	702.88 ± 19.32	629.00 ±2 8.79	780.50 ± 35.07	995.36 ± 21.37
ST-8	420.05 ± 21.03	587.50 ± 23.17	987.50 ± 46.32	574.03 ± 11.57	797.43 ± 23.44	1019.40 ± 29.45
RT-9	670.13 ± 16.29	1010.00 ± 7.07	635.71± 32.18	591.36 ± 23.41	898.67 ± 35.24	1002.35 ± 34.61
RT-10	770.00 ± 33.69	868.12 ± 57.99	769.33 ± 26.21	693.00 ± 24.15	821.24 ± 45.21	833.80 ± 22.71
HT-5	575.25 ± 27.62	682.50 ± 24.37	1375.00 ± 45.96	698.83 ± 13.85	882.89 ± 28.56	1023.77 ± 39.24
PT-5	767.00 ± 22.98	895.70 ± 18.95	1351.55 ± 41.33	564.54 ± 17.98	773.21 ± 34.65	924.26 ± 48.11
PT-6	562.50 ± 30.11	572.50 ± 15.35	838.36 ± 25.91	976.60 ± 25.67	996.63 ± 36.51	1066.05 ± 54.19
L.S.D. At	For Strain	235.42		For Strain	85.47	
P = 0.05	For Treatment	358.62		For Treatment	130.56	
PEROXIDASE ACTIVITY (unit/gm.f.wt.)						
Control	59.91 ± 2.91	99.78 ± 6.44	130.59 ± 8.22	89.36 ± 2.42	132.74 ± 3.46	191.62 ± 9.27
ST-8	68.89 ± 2.31	81.26 ± 6.11	117.28 ± 4.82	91.45 ± 3.23	113.61 ± 4.29	141.53 ± 6.34
RT-9	38.63 ± 2.16	102.11 ± 5.31	125.39 ± 4.92	81.02 ± 5.01	125.87 ± 4.91	136.77 ± 4.56
RT-10	41.78 ± 3.22	101.36 ± 5.79	147.88 ± 7.41	101.26 ± 3.96	146.83 ± 7.08	211.47 ± 6.07
HT-5	51.91 ± 3.07	79.18 ± 4.34	98.31 ± 5.22	95.15 ± 4.12	118.06 ± 5.11	164.92 ± 4.99
PT-5	72.01 ± 4.27	66.79 ± 4.05	110.46 ± 6.01	65.87 ± 4.51	150.91 ± 6.00	231.07 ± 11.34
PT-6	57.54 ± 3.18	89.67 ± 3.36	128.34 ± 8.12	70.44 ± 3.45	105.63 ± 4.25	171.83 ± 5.67
L.S.D. At	For Strain	17.10		For Strain	24.25	
p = 0.05	For Treatment	26.12		For Treatment	37.04	
ACID PHOSPHATASE ACTIVITY (Unit/gm.f.wt.)						
Control	43.96 ± 2.41	81.13 ± 4.79	112.28 ± 8.94	43.00 ± 0.82	102.94 ± 3.81	115.93± 8.59
ST-8	46.73 ± 3.71	71.39 ± 2.66	117.84 ± 4.61	39.72 ± 2.46	96.63 ± 5.11	116.02 ± 7.21

			4.61			
RT-9	58.21 ± 4.45	68.51 ± 3.58	109.25 ± 3.28	46.03 ± 1.97	87.09 ± 3.76	108.70 ± 6.55
RT-10	73.52 ± 3.36	79.83 ± 4.28	101.36 ± 5.91	52.19 ± 3.45	116.45 ± 4.88	126.87 ± 3.32
HT-5	39.56 ± 2.17	86.23 ± 3.12	127.31 ± 6.50	46.65 ± 2.34	90.28 ± 4.32	113.25 ± 7.11
PT-5	41.47 ± 3.59	61.79 ± 4.14	99.84 ± 4.71	38.08 ± 2.04	106.45 ± 7.04	123.23 ± 8.22
PT-6	43.69 ± 2.54	77.31 ± 5.75	103.35 ± 5.03	51.21 ± 2.98	89.95 ± 6.21	108.94 ± 5.01

$p = 0.05$

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