Role of Phytohormones in Root Nodulation and Yield of Soybean under Salt Stress

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Abstract

The present study was conducted to investigate the effects of Sodium Chloride (NaCl) 60 milli Molar (mM) and 80mM on inoculated and uninoculated plants of two cultivars (Williams-82 and its hypernodulating mutant NODI-3) of soybean [*Glycine max* (L.) Merr.]. The study comprised two sets of experiments. Experiment I was aimed at investigating the changes in Indole Acetic Acid (IAA) as auto regulatory factor for root nodulation and its response under NaCl stress. In this experiment, the changing level of IAA was monitored at 48 h after inoculation, in leaves of potted plants of soybean cultivars, grown under controlled conditions. A decrease in IAA content was found in both the cultivars with salt treatment in inoculated plants. The uninoculated plants showed an increase in IAA content with salt stress. The level of IAA and sugar content was also measured in phloem sap at 0 hour (h), 48 h --- the critical stage of nodule development, and at 192 h after inoculation, that is, at nodule initiation. Sugar content in phloem sap varied in both the varieties. Williams-82 showed more sugar contents in phloem sap while NODI-3 showed more accumulation of sugar in leaves. NaCI treatment reduced the sugar as well as IAA content in phloem sap in Williams-82, while, there was an increase in NODI-3. Experiment 2 was designed to study the effect of inoculation and NaCl (60mM and 80mM) treatment on the root nodulation, yield performance and nutritive quality of soybean. NODI-3 showed more accumulation of sugar in leaves, greater number of nodules plant^{-1}. Williams-82 exhibited higher protein content in leaves and seeds and greater number of flowers plant^{-1}, more seed weight and greater fresh and dry weight plant^{-1}. The inoculated plants showed vigorous growth and development than that of uninoculated ones.

Key Words: Phytohormones; Salt stress, Soybean, Nodulation

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Introduction

The soybean plant is an annual plant native to Southeast Asia. It has oblong pods that contain 2 to 4 seeds or beans. Soybeans are legumes, a member of the pea family, and have a high protein content of 40% by weight, 32% carbohydrate, 20% fat, 5% minerals and 3% fiber, and other trace substances. They are processed to make many foods and food additives. Soybean products are promoted for their protective properties against cancer. It an important crop that provides fatty acids and proteins for human beings and its production is severely affected by saline soils environmental conditions (Luo et al., 2005). Over the last two decades, Pakistan has been mostly importing edible oil due to low domestic production. Edible oil (1.467 million tons) having worth of Rs. 145 billion (US$ 1.654 billion) was imported during the year 2011-12. Local production during 2011-12 was 0.636 million tons (Anonymous, 2012).

Salinity is a major environmental constraint to crop production in the arid and semi-arid regions of the world (Jaleel et al., 2007a; 2007b). Han and Lee (2005) found that salinity in soil decreased plant growth, photosynthesis, stomatal conductance and mineral uptake in soybean plants compared to those from non-saline soil.

Plant hormones play an integral role in controlling the growth and development of plants. Hormones are not only frequently found as stress metabolites, but they are also part of signaling systems particularly under stress (Davies, 1995; Dobrev et al., 2005; Javid *et al*., 2011). Several workers (Elsheikh and Wood., 1995; Andrade et al., 1998;

Lucas-Gracia et al., 2004; Naz et al., 2009) have suggested involvement of plant growth promoting rhizobateria (PGPRs) and hormones in nodulation & N_2-fixation in legume plants. Phytohormones play a role in initiating and/or maintaining meristematic activity during nodule development.

Hirsch *et al.* (1989) & Davies (1995) presented a model describing the involvement of cytokinin and auxin in stimulating cell divisions in the inner cortex that leads to nodule formation. Abd-Allah *et al.* (1998) found that salt stress (0, 30 and 60 mM NaCI) significantly inhibited the nitrogenase activity, nodule number and dry matter accumulation plant $^{-1}$ of all the four cultivars (Williams 82, PI416937, DRI (an Egyptian cultivar) and NODI-3 (a hypernodulating mutant selected from Williams) when investigated in hydroponics culture and growth chamber environments. El-Fayoumy *et al.* (1996) showed that soil salinity >5.72 dS/m markedly affected biological N2 fixation, nodulation, growth, nitrogen content, water use efficiency and biomass of soybean.

The present investigation was undertaken to evaluate the role of phytohormones in root nodulation under salt stress and to study the effect of salt stress on nodulation, yield and biochemical contents of soybean.

Materials and Methods

Seeds of soybean cultivar Williams 82 and NODI-3 were used for the experiment, which was divided in two sets. In one set, the experiment was conducted at Department of Biological sciences, Quaid-I-Azam University Islamabad. The other set of experiment was conducted in green house at National Agriculture Research Centre, Islamabad.

Experiment in Growth Room

Seeds were surface sterilized with methanol for 1 min. followed by shaking in 10% Clorox (commercial grade) for 2-3 min. and finally successively washed with sterilized water before sowing. The seeds were raised in the controlled temperature room provided with 16 h light and 8 h dark period. Hoagland nutrient solution (Hoagland and Arnon, 1950) of quarter strength was added to the plants twice a week. Plants were inoculated with pure culture of *Rhizobium japonicum* strain USDA-110 at the trifoliate leaf stage. After inoculation, aqueous solution of NaCl (60 mM and 80 mM) was applied to the treated plants at the trifoliate leaf stage. Following parameters were studied:

1. Changes in the level of IAA in leaves of inoculated and uninoculated plants at 48 h after inoculation
2. Changes in the level of IAA in phloem sap of inoculated and unioculated plants at 0 h, 48 h and 192 h after inoculation.
3. Changes in the sugar content in phloem sap of inoculated and uninoculated plants at 0 h, 48 h and 192 h after inoculation.

To study the level of IAA and sugar content in phloem sap following procedure was adopted.

Phloem sap was collected as follows:

Shoots of plants were severed, approximately 3 cm below the cotyledon node, using a sharp razor blade against a paper towel bathed in a solution of 10 mM diSodium EDTA with 5 mM phosphate buffer (pH 6.0). Plant shoots were transferred to 1.5 ml micro centrifuge tubes containing 1 ml of 100 mM disodium EDTA (pH 7.0). The micro centrifuge tubes holding the shoots were placed in a water bath (25 °C) lined with H2O saturated paper towels and then covered to maintain high humidity and darkness for a 2 h EDTA pretreatment. After 2 h, plant shoots were removed from the pretreatment solution and thoroughly rinsed in distilled H2O. Plant shoots were transferred to 1.5 ml-micro centrifuge tubes containing 1 ml of distilled H2O and returned to the water bath for phloem collection. After 4 h, the collection period was terminated and the solution containing phloem exudates were pooled and stored at – 20 °C until further analysis.

IAA level was measured by the estimation of residual IAA by using Salkowski's reagent (Malik and Singh, 1980). Sugar estimation was done following the method of Dubo et al. (1956) as modified by Johnson et al. (1966).

To study the changes in the level of IAA and GA, fresh leaves were ground in 80% methanol with butylated hydroxy toluene (BHT) and extracted for 72 h with subsequent changes in solvent after every 24 h. The extracted sample was centrifuged and supernatant was taken and reduced to aqueous phase using rotary film evaporator (RFE). The pH of aqueous phase was adjusted to 8 with 0.1N NaOH to remove the basic and neutral compounds before it was partitioned three times with one-third volume of ethyl acetate. The pH of aqueous phase was readjusted to 2.5-3.0 and partitioned three times with one-third volume of ethyl acetate. The ethyl acetate phase was taken and dried down completely using Rotary Film Evaporator (RFE). The sample was redissolved in 1 ml of methanol (100%) and analyzed for the presence of phytohormones (IAA) using HPLC (LC-6A SCHIMADZU).

Experiment in Green House:

Seeds of soybean cultivars Williams-82 and NODI-3 were sown in plastic pots containing soil and farmyard manure. The parameters studied in this set of experiment were number of nodules plant^{-1}, sugar contents of leaves, protein contents of leaves and seeds, number of flowers plant^{-1}, number of pods plant^{-1}, number of seeds pod^{-1}, seed size (100 seed weight plant^{-1}), fresh and dry weight of shoot and root. Protein estimation of fresh leaves was done following the method of Lowry et al. (1951) using BSA as standard while protein contents of the seeds were measured by Kjeldhal Digestion following the method of AOAC (1982). Sugar estimation was done following the method of Dubo et al. (1956) as modified by Johnson et al. (1966).

Six different treatments were made to both sets of experiment

1. Inoculated Control denoted as T1
2. Uninoculated Control denoted as T2
3. Inoculated + NaC1 (60 mM) denoted as T3
4. Uninoculated + NaC1 (60 mM) denoted as T4
5. Inoculated + NaC1 (80 mM) denoted as T5
6. Uninoculated + NaC1 (80 mM) denoted as T6

Results and Discussion

IAA Content of soybean leaves at 48h after inoculation

Salt stress (80 mM) had a significant (P<0.05) effect on IAA level in soybean leaves at 48 h after inoculation. Maximum IAA level was observed in inoculated plants of cv. Williams-82 receiving no salt treatment whereas; minimum IAA level was observed in uninoculated plant of cv. NODI-3 receiving no salt treatment (Figure 1). The endogenous level of IAA was decreased in response to inoculation under salt stress. The observed differences in IAA content of soybean leaves at 48 h after inoculation may be attributed to the differences in the genetic make up of the two varieties. In a study it was observed that major changes in endogenous level of phytohormones occur between 48 to 96 h after inoculation (Asim *et al*., 2010). These phytohormones may be transported to the infected site in roots through phloem sap where they may promote a signal for nodulation as Dullart (1970) found large concentration of IAA in root nodules of *Lupinus luteus*.). IAA can strengthen the capacity of resistance of the soybean to saline environment (Wei and Chen, 2000). Root-produced organic compounds in the xylem sap, such as hormones and amino acids, are considered to be vital for plant development (Oda *et al*., 2003).

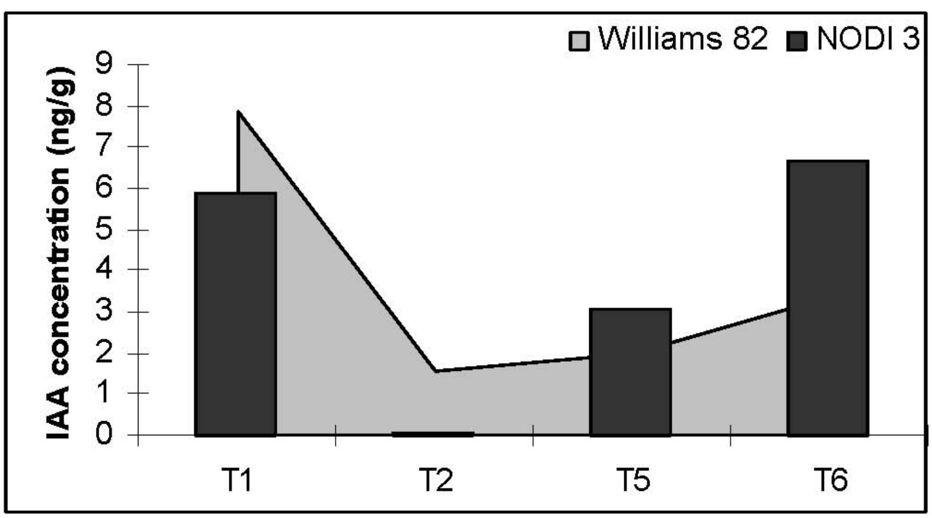

Figure 1. Effect of NaCl treatment on endogenous IAA concentration in leaves of Soybean at 48 h after inoculation

IAA and sugar content in phloem sap at 0h, 48h and 192h after inoculation

The major changes in the level of IAA occurred during 48-96 h after inoculation. Figures 2a & b showed that salt treatment affected the IAA content in phloem sap at 0h, 48h and 192h after inoculation. Both the varieties showed an increase in IAA content of phloem sap under induced salt stress. There was an increase in the IAA content in uninoculated plants as compared to inoculated plants. The observed increase in IAA content of phloem sap may be due to the transportation of IAA from leaves to roots where it may be involved in roots nodulation. This is in accordance with the previous findings where relatively large concentration of IAA has been found in root nodules of *Lupinus luteus* (Dullaart, 1970). Root initiation, adventitious root formation, and early development of root are also stimulated by auxin (Bellamine *et al*., 1998; Pan and Tian, 1999; Fisher et al., 2007).

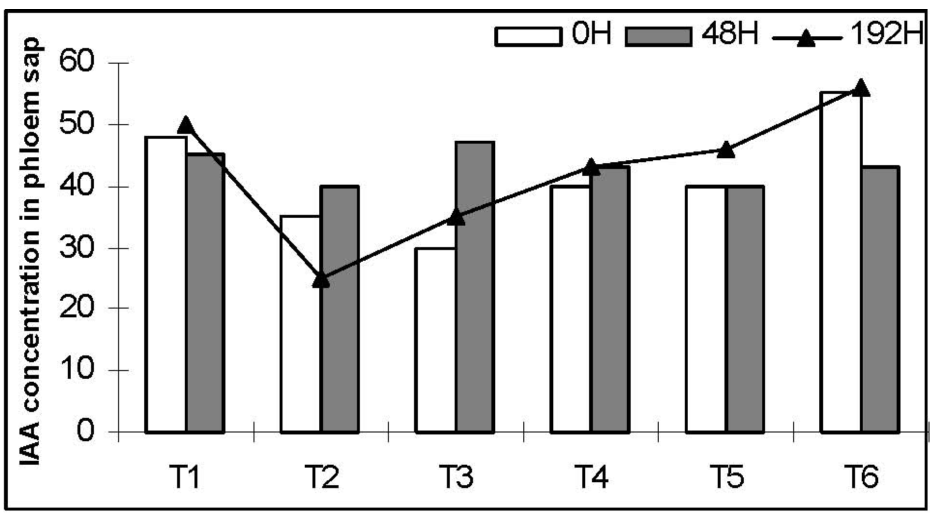

Figure 2a. Effect of NaCl treatment on IAA concentration (*ug/l)* in phloem sap of soybean cv. Williams 82 at 0, 48 and 192 h after inoculation

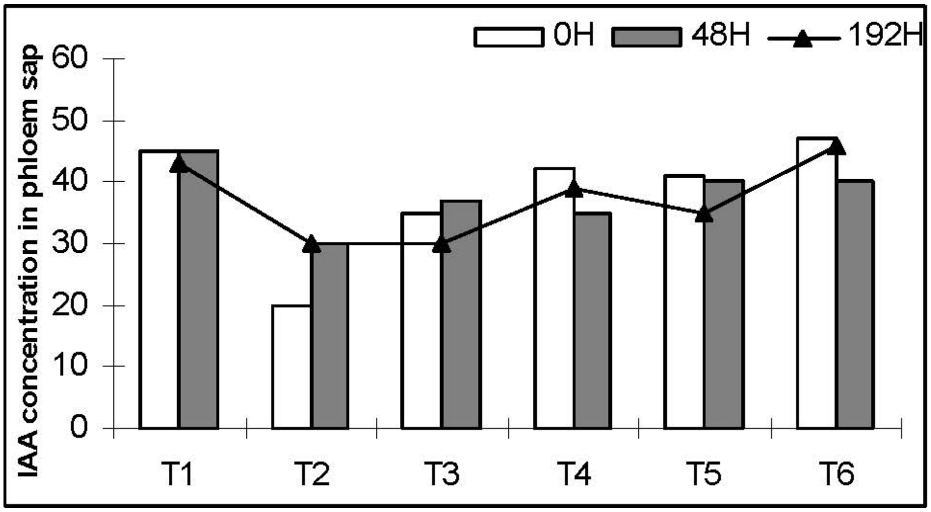

Figure 2b. Effect of NaCl treatment on IAA concentration (*ug/l)* in phloem sap of soybean cv. NODI-3 at 0, 48 and 192 h after inoculation

Salt treatment also affected the sugar content in phloem sap of both the varieties. Williams-82 showed more sugar contents in phloem sap at 0h, 48h and 192h after inoculation as compared to NODI-3. This might be attributed to more accumulation of sugar in leaves of NODI-3 as compared to Williams-82 due to more accumulation of sugar in leaves to other parts such as roots etc. through phloem sap (Figures 3a & b).

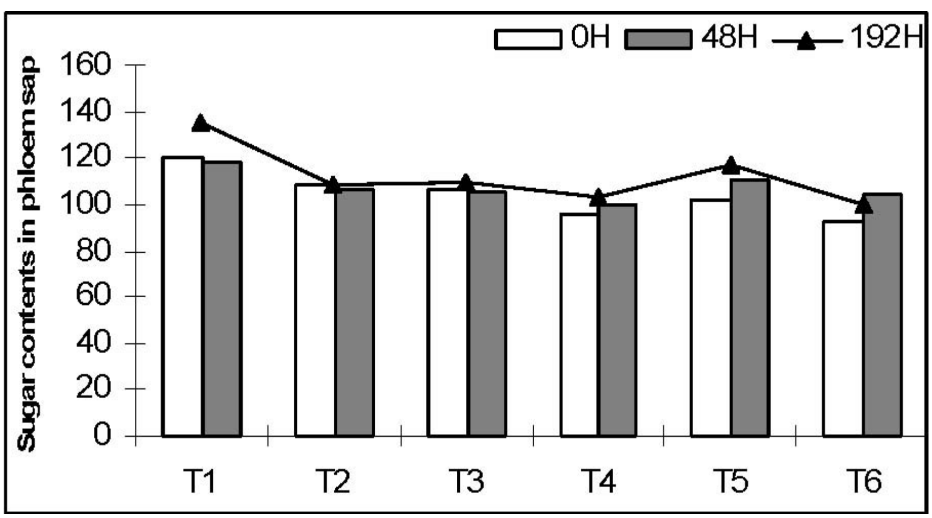

Figure 3a. Effect of NaCl treatment on sugar contents (*ug/l)* in phloem sap of soybean cv. Williams 82 at 0, 48 and 192 h after inoculation.

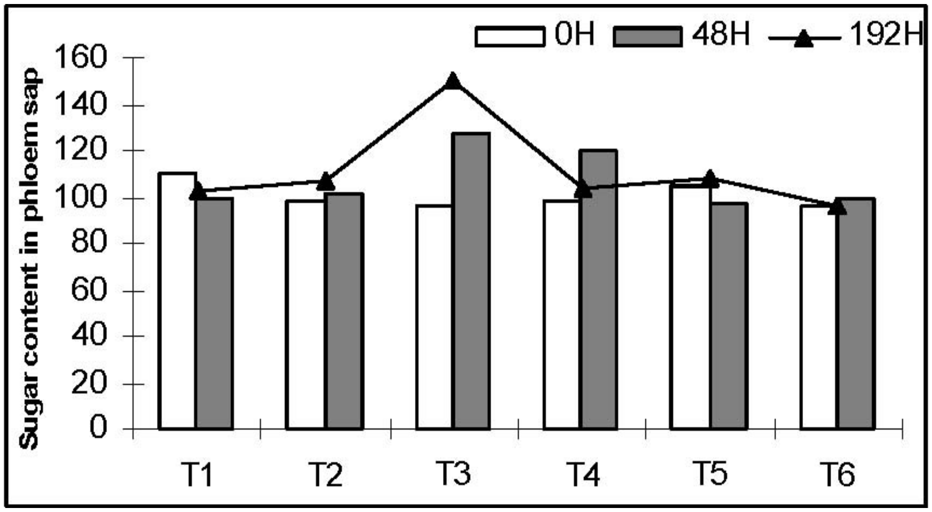

Figure 3b. Effect of NaCl treatment on sugar content (*ug/l)* in phloem sap of soybean Cv.NODI-3 at 0, 48 and 192 h after inoculation

Sugar Contents in leaves (Pod filling stage) and protein contents in Leaves (pod filling stage) and seeds (mature pod stage)

Salt stress significantly reduced the sugar and protein contents in soybean leaves at pod filling stage and protein contents of seeds at mature pod stage as compared to Williams-82 (Figures 4 & 5). The increased accumulation of sugar in leaves of the plants following NaCl treatment is perhaps an adaptation mechanism of plants to cope with the salinity by using sugar for osmoregulation. Alarcon *et al*. (1993); Perez-Alfocea *et al.* (1993) and Gao *et al.* (1998) reported that the organic solutes contributing to osmotic adjustment in the leaves of tomato plants grown under salt stress are organic acids and sugars. Cayuella *et al*. (1996) & Singh *et al*. (2000) reported that in leaves, salt treatment generally increased sugar concentration several folds over that of control plants.

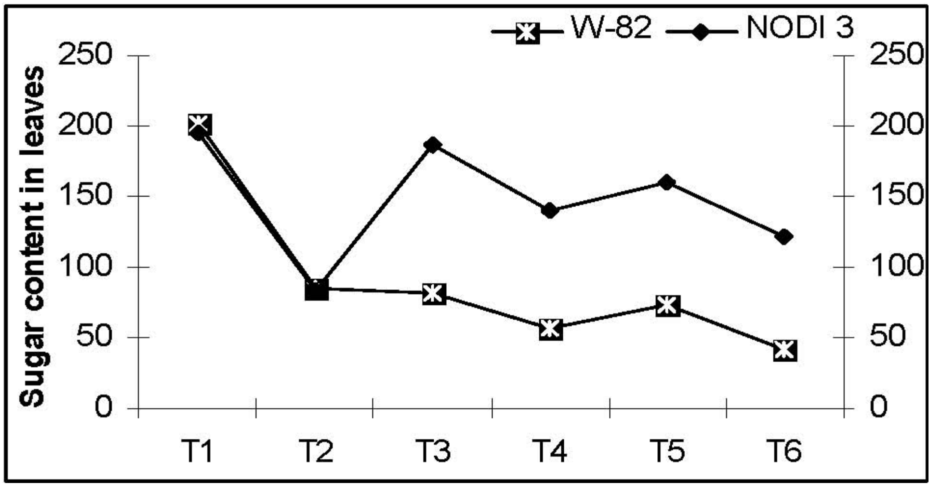

Figure 4. Sugar content (*ug/g FW)* in leaves of soybean cv. Williams-82 and NODI-3 as affected by NaCl treatment at pod filling stage

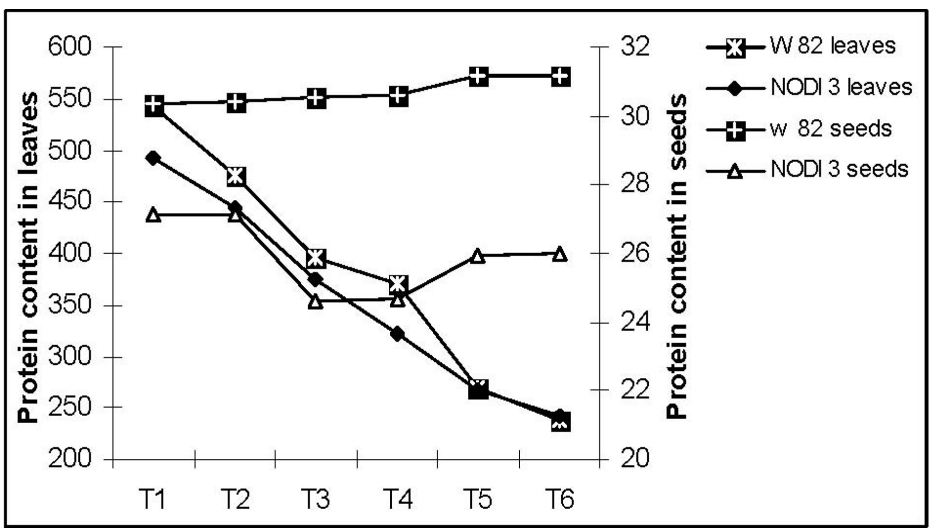

Figure 5. Protein contents (*ug/g FW)* in leaves and seeds (*ug/g)* of soybean cv. Williams-82 and NODI-3 as affected by NaCl treatment at pod filling stage

Juliano (1981) & Panneerselvam *et al.* (1998) reported decrease in the protein content of salt stressed plants. Salinity inhibits the incorporation of amino acids into proteins subsequently decreasing the protein content (El-Saidi *et al.*, 1986; Eder *et al.*, 1977; Stewart and Boggess, 1978; Chaudhuri and Chaudhuri, 1993). The salinity in rooting medium reduced incorporation of labeled amino acids into proteins of pea roots (Khane and Poljakoff-Mayber, 1968). Salt stress was also responsible for a decrease of the cytosolic protein content, specifically of leghemoglobin, in the nodules (Delgado *et al.*, 2006).

Number of nodules plant^{-1}

Salt stress significantly reduced the number of nodules plant^{-1} in booth the cultivars (Figure 6). Number of nodules in both the cultivars decreased with the increase in NaCl concentration. This may be due to the fact that nodulation was more sensitive than plant growth to salinity. The addition of NaCl had a negative effect on the initial growth and nodulation of soybean grown in pots (Tamura, 1992). Soil salinity decreased root nodulation and N^2-fixation (Qifu and Murray, 1993). NODI-3, however, showed two to three times number of nodules than Williams-82. This significant difference among the varieties may be attributed to the differences in genetic make up of the varieties.

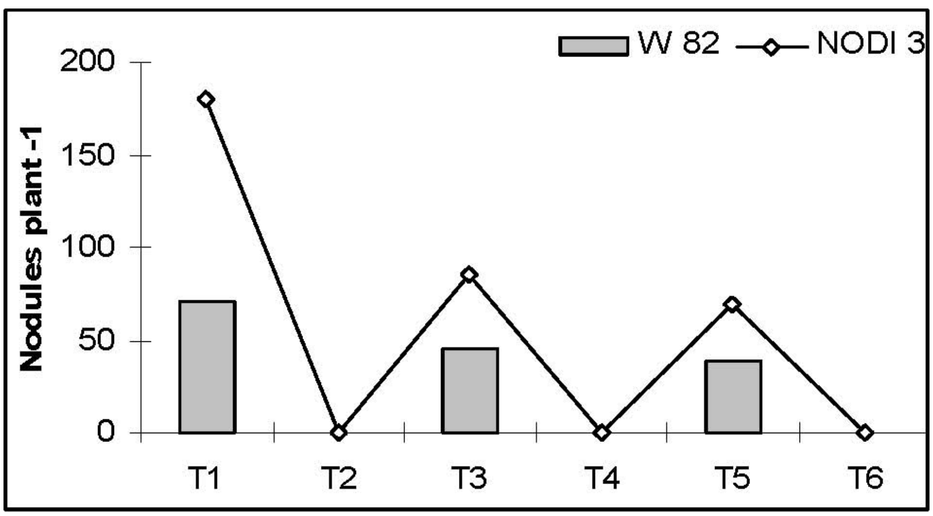

Figure 6. Number of nodules plant^{-1} of soybean cv. Williams-82 and NODI-3 as affected by NaCl treatment

Yield parameters

Maximum fresh and dry weight plant-1 was observed in inoculated plants of cv. NODI-3, receiving no salt treatment whereas minimum fresh/dry weight plant -1 was observed in uninoculated pants of William-82, receiving 80 mM NaCl treatment (Figure 7). A decrease in fresh and dry weight plant -1 was observed in both the cultivars (William-82 and NODI-3) with the increase in NaCl concentration. The reduction in fresh weight is probably due to decrease hydraulic conductivity of water form the growth medium as observed for other crops under saline conditions (Ahmed et al., 1985; Sharma, 1997; Ashraf and Bashir, 2003). The reduced dry weight also indicates decreased photosynthetic activity of seedlings. El-Tayeb, (2005) reported that decrease in photosynthetic pigments due to salt stress may result in decreased plant growth. Panneerselvam et al. (1998) reported that NaCl stress decreased root growth, shoot length and dry matter production of Glycine max seedlings.

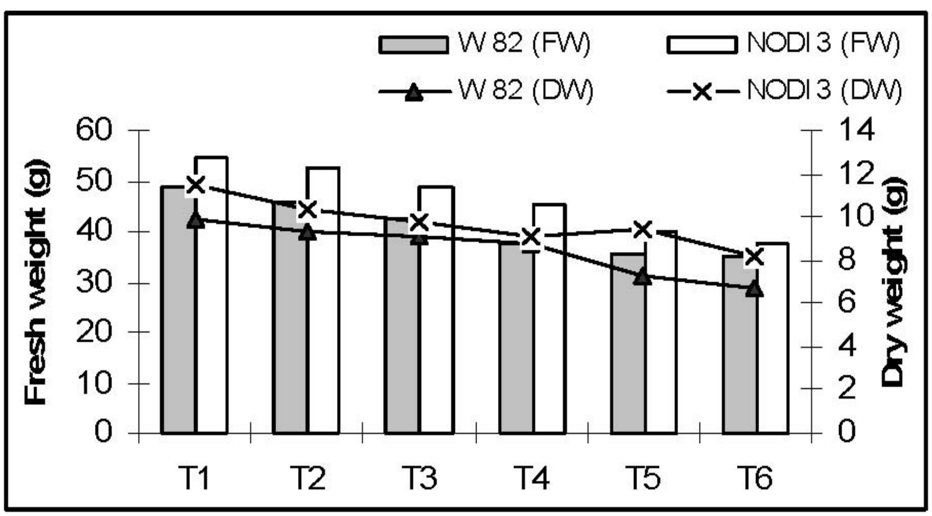

Figure 7. Effect of NaCl treatment on Fresh Weight (g) and Dry Weight (g) plant^{-1} of soybean cv. Williams-82 and NODI-3 as affected by NaCl treatment

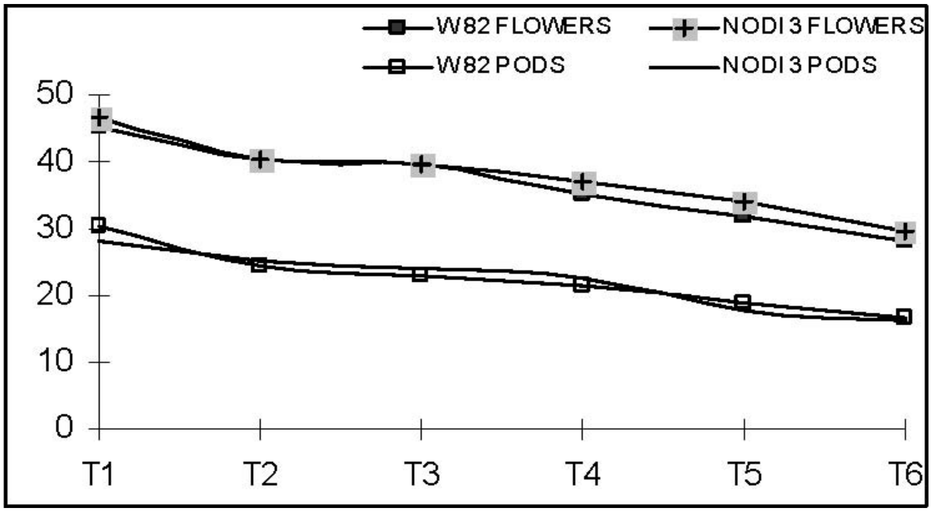

Figure 8. Number of Flowers and pods plant^{-1} of soybean cv. Williams-82 and NODI-3 as affected by NaCl treatment

Salt stress significantly reduced the number of flowers, number of pods, 100 seed weight, and number of seeds pod^{-1}, as evident from the Figures 8 & 9. The observed decrease in the seed size due to salt treatment may be due to the consequences of increased flowers drop resulting in the reduction in pods plant^{-1} and seeds plant^{-1}. Boguet *et al.*, (1987) and Hussain (1997) also observed differences in seed weight and number of pod plant^{-1} in soybean cultivars. This may be attributed to the fact that the presence of excessive salts in soil suppressed plant growth and development including inhibition in the weight of seeds and that water and salinity stress effect the seed yield due to plasmolytic effect of water and salinity stress in soybean (Salinas *et al.*, 1996). Singleton and Bohlool (1984) suggested that legumes grown in saline environment exhibited reduced yield potential.

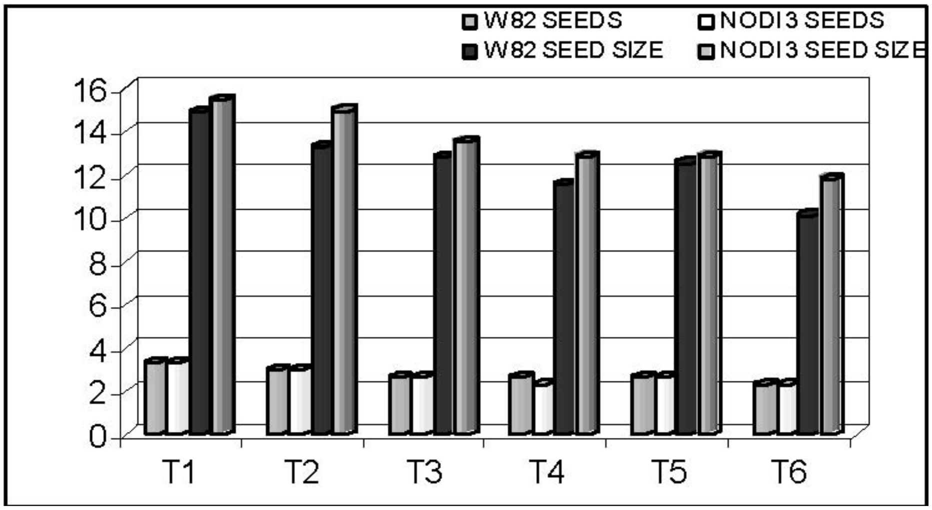

Figure 9. Number of seeds pod^{-1} and seeds size (100 seed weight (g)) plant^{-1} of soybean cv. Williams-82 and NODI-3 as affected by NaCl treatment

Conclusion

It is inferred from the present investigation that soybean is NaCl-sensitive crop but variation in sensitivity occurs in the cultivars. Both the NaCl concentrations (60mM and 80mM) showed reduction in all the physiological and biochemical parameters. Sugar content in leaves and phloem sap varies in both the varieties. Williams-82 showed more sugar content in phloem sap while NODI-3 showed more accumulation of sugar in leaves perhaps using sugar as osmoregulant.

References

Adb-Allah, M.H., T. D. Vuoug and J. E. Harper, 1998. Genotypic differences in dinitrogen fixation response to NaCl stress in intact and grafted soybean. *Crop Science*., 38: 72-77.

Ahmed, R., S. Ismail and D. Khan, 1985. Use of highly saline water for growing plant a coastal sandy belt of Pakistan. 4th Annual Report. PARC and University of Karachi. Coordinated Research Programme on Saline Agriculture. Pp. 115.

Alarcon, J.J.,M. J. Sanchez-Blanco., M.C. Bolarin and A.Torrecilla, 1993. Water relations and osmotic adjustment in *Lyconpersicon esculantum* L. Pennellii during Short-term salt exposure and recovery. *Physiol. Plant*., 89: 441-447.

Andrade, G., K.L. Mihara., R.G. Linderman and G.J. Bethlenfalvay, 1998. Soil aggregation status and rhizobacteria in the mycorrhizosphere. *Plant and Soil*., 202: 89–96.

Anonymous. 2011-12. *Economic Survey*. Finance Division, Economic Advisor's Wing, Govt. of Pakistan. Islamabad.

AOAC, 1982. Official methods for the determination of nitrogen and protein. 11th edition published by the Association of Official Analytical Chemists. Washington, DC: 788789.

Ashraf M., A. Bashir. 2003. Salt stress induced changes in some organic metabolites and ionic relations in nodules and other plant parts of two crop legumes differing in salt tolerance. Flora, 198: 486-498.

Asim. M., M. Asif, M. Munir, M. Aslam, 2010. Uptake and distribution pattern of applied Abscisic Acid (3H-ABA) in normal and hypernodulating soybean varieties. *Pakistan J. Agric. Res*. 23 (1-2): 31-36.

Bellamine, J., C. Penel., H. Greppin and Gaspar. T, 1998. Confirmation of the role of auxin and calcium in the late phases of adventitious root formation. *Plant Growth Regul*., 26: 191-194.

Boquet, D.J., B. Caco and D.E. Summers, 1987. Soybean variety tests. In Annual Progress Report, Northeast Res. Station and Macon Ridge Res. Station. Baton Rouge, Louisiana, USA. PP: 41-49.

Cayuella, E., F. Perez-Alfocea., M. Caro and M.C. Bolarin, 1996. Priming of seeds with NaCl induces physioklogical changes in tomato plants grown under salt stress. *Physiol. Plant*., 96: 231-236.

Chaudhuri,K and M.A. Chaudhuri, 1993, Differential tolerance of seedlings of two jute species to NACL stress seedlings.*Indian J.Expt. Biol*,, 31: 90-92.

Davies, P. J., 1995. Plant hormones Physiology, Biochemistry and Molecular Biology. Ed. P J Davies. Second Edition. Kluwer, Dorrecht, Netherlands.

Delgado, M.J., J.M. Garrido., F. Ligero and C. Lluch, 2006. Nitrogen fixation and carbon metabolism by nodules and bacteroids of pea plants under sodium chloride stress. *Physiologia Plantum*.,89(4) 824.829.

Dobrev, P. I., L. Havlicek., M. Vagner., J. Malbeck and M. Kaminek, 2005. Purification and determination of plant hormones auxin and abscisic acid using solid phase extraction and two-dimensional high performance liquid chromatography. *J. Chromatograghy*., 1075: 159-166.

Dubo, S.M., K.A. Giles., J. K. Hamiton., P.A. Rebers and F. Smith, 1956. Calorime

Calorimetric method for determination of sugars and related substance. *Anal. Chem.*, 28: 350.

Dullaart, J., 1970. The bioproduction of indole-3-acetic acid and related compounds in roots of Lupinus luteus L. and by its rhizobial symbiont. *Acta. Bot. Neerol.*, 19: 573-615.

Eder, A., W. Huber and N. Sankhla, 1977. Interaction between salinity and ethylene in nitrogen metabolism of pennisetum typhoides seedlings. *Biochem. Physiol. Pflanz.*, 171: 93-100.

El-Fayioumy, M.E., H. M. Ramadan and E.A. Koereish, 1996. Soybean biomass, N2 fixation and water use efficency as affected by frequency of irrigation, siol salinity and inoculatin with Bradyrhizobiyum. *Alexandria Jour of Agric Res.*, 41: 313-326.

EL-Tayeb, M. A, 2005. Response of barley to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.*, 45: 215-224.

Elsheikh EAE., M. Wood . 1995. Nodulation and N2 fixation by soybean inoculate inoculated with salt-tolerant Rhizobia or salt-sensitive bradyrhizobia in saline soil. *Soil Biol. Biochem.* 27(4/5): 657-661.

El-Saidi, M.T., N.A. Nour-Ed-Den and A.K. Abdul Haleem, 1986. Effect of different Levels of salinity in irrigation water on some physiological processes of cotton plants. Prospects for Biosaline Research., 157-165.

Fischer U., Y. Ikeda and M. Grebe, 2007. Planar polarity of root hair positioning in Arabidopsis. *Biochem Soc Trans.*, 35**:** 149–151

Gao, Z. F., M. Sagi and S. H. Lips, 1998. Carbohydrate metabolism in leaves and assimilate partitioning in fruits of tomato (*Lycopersicum esculentum* L.) as affected by salinity. *Plant Sci.*, 135: 149-159.

Han, H. S. and K. D.Lee, 2005. Physiological responses of soybean inoculation of *Bradyrhizobium japonicum* PGPR in saline soil conditions. *Res. J. Agri.Biol. Sci.*, 1(3): 216-221.

Hirsch, A.M., T.V. Bhuvaneswari., J. G. Torrey and T. Biessling, 1989. Early nodulin genes are induced in alfalfa root out growths elicited by auxin transport inhibitors. PNAS. USA., 86: 1244-1248.

Hoagland, D.R. and D. I. Arnon, 1950. The Water culture method for growing plants without soil. *California Agric. Expt. Stn.* Circle. No. 347.

Hussain, F., 1997. Comparative studies on yield and yield components of five soybean (Glycine max L.) cultivars sown under Faisalabad conditions. M.Sc. Thesis. Univ. Agric. Faisalabad.

Jaleel, C. A., R. Gopi., B. Sankar., P. Manivannan., A. Kishorekumar., R. Sridharan and Panneerselvam, R. 2007a. Studies on germination, seedling vigour, lipid peroxidation and praline metabolism in *Catharanthus roseus* seedlings under salt stress. *South Afri. J. of Bot*., 73: 190–195.

Jaleel, C. A., P. Manivannan., A. Kishorekumar., B. Sankar and R. Panneerselvam, 2007b. Calcium chloride effects on salinity induced oxidative stress, proline metabolism and indole alkaloid accumulation in *Catharanthus roseus,*Comp. Ren. Biol., 330: 674–683.

Javid, M.G., A. Sorooshzadeh.,F. Moradi.,S.A. Sanavy.,I. Sanavy. 2011. The role of phytohormones in alleviating salt stress in crop plants. *Aust. Jour of Crop Sci*., 5(6):726-734.

Johnson, R.P., T.L. Balwani., L .J. Johnson., K.E. McClur and B.A. Dehority, B.A. 1966. Com plant maturity. II. Effect in vitro cellulose digestibility and soluble carbohydrate content. *J. Anim. Sci*., 25: 617.

Juliano, B.O., 1981. National Congress of Biochemistry, Cairo. 104-105.

Khane, I and A. Poljakoff-Mayber., 1968. Effect of substrate salinity on the ability of protein synthesis in pea roots. *Plant phsiol*., 43: 1115-1119.

Lowry, O.H., N. R. Rosenbrough., A. L. Farr and J. R. Randalt, 1951. Protein measurement with folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Lucas-Garcia, J.A., A. Probanza., B. Ramos., J. J. Colón-Flores and F. J. Gutierrez -Mañero, 2004. Effects of plant growth promoting rhizobateria (PGPRs) on the biological nitrogen fixation, nodulation and growth of *Lupinus albus* I. cv. Multolupa. *Engineering Life Sciences*,. 4**:** 71–77

Luo, Q.Y.,B.J. Yu and Y.L. Liu. 2005. Differential sensitivity to chloride and sodium ions in seedlings of *Glycine max* and *G. soja* under NaCl stress. *J Plant Physiol.* 162(9):1003–1012. doi: 10.1016/j.jplph.2004.11.008.

Mailk, D. and M. B. Singh, 1980 Plant enzymology. Plant Enzymology and Histo -enzymology. Karachi Kayani Publisher, New Delhi, Ludhiana., 51-53.

Naz, I.,A.Bano and T. Hussan. 2009. Isolation of phytohormones producing plant Growth promoting rhizobacteria from weeds growing in Khewra salt range, Pakistan and their implication in providing salt tolerance to *Glycine max L. Africn Jour of Biotech* 8 (21), pp. 5762-5766

Oda, A., C. Sakuta., S. Masuda., T. Mizoguchi., H. Kamoda and S. Satoh, 2003. Possible involvement of leaf gibberellins in the clock-controlled expression of *XSP30*, a gene encoding a xylem sap lectin, in cucumber root. *Plant Physiol*., 133:1779–1790.

Pan, R. and X. Tian, 1999. Comparative effect of IBA, BSAA, and 5,6-Cl-IAA-Me on the rooting of hypocotyls in mungbean. *Plant Growth Regul*., 27: 91-98.

Panneerselvam, R., M. Muthukumarasamy and S. N. Rajan, 1998. Amelioration of NaCl Stress by Triadimefon in Soybean Seedlings. *Biologia Plantarum*., 41(1) 133-137.

Perez-Alfocea, F., M.T. Estan., M. Caro G. Gueerier, 1993. Osmotic adjustment in Lycopersicon esculantum and L. Pennelli under NaCi and polyethylene glycol 6000 iso-omotic stress. *Plant Physiol*., 87: 493-498.

Qifu, M and F.Murray, 1993 Effects of SO and salinity o nitrogenase activity, nitrogen concentration and growth of young soybean plants. *Env. Exp. Bot*., 33: 529-537

Salinas, A.P. N. Zelener., R. M. Craviotto and V. Bisaro, 1996. Physiological responses of different soybean cultivars to soil water stress. *Pesquisa Agropecuaria Brasilierira*., 31: 531-538

Sharma, S. K., 1997. Plant growth, photosynthesis and ion uptake in chickpea as influenced by salinity. *Indian J. Plant Physiol*., 2: 171-173.

Singh, S. K., H. C. Sharma., A. M. Goswami., S. P. Datta and S. P.Singh, 2000. In vitro growth and leaf composition of grapevine cultivars as affected by sodium chloride. *Biol. Plant*., 43: 283-286.

Singleton, P.W. and B. B. Bohlool, 1984. Effect of salinity on nodule formation by soybean. *Plant Physiol*., 74: 72-76

Stewart, C.R. and S. P. Boggess, 1978. Metabloism of 5-H3 proline by barley leaves and its use in measuring the effect of water stress on proline oxidation. *Plant Phsiol*., 61: 654-657.

Tamura, Y., 1992. Effect of chlorine in sodium on initial growth and nodulation of soybean. *Jour of Soil Sci and Plant Nutr*., 63: 411-414.

Wei, A.L and Y.Z. Chen. 2000. Effect of IAA on soybean seedling's membrance injury and salt resistance. *Acta Bot Boreal Occident Sin.*;20(3):410–414. (in Chinese)