Journal of Bio-Molecular Sciences (JBMS) (2014) 2(4): 100-107.

Journal of Bio-Molecular Sciences
(JBMS)
ISSN: 2311-4630
www.directsciences.com/jbms

The Effect of Sodium Chloride (NaCl) stress on Seed germination and Seedling Growth of Rice (*Oryza Sativa* L.)

Muhammad Hafeez Ullah Khan, Ijaz Malook, Amir Atlas, Mehmood Jan, Sami Ullah Jan and Gulmeena Shah

Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat 26000, Pakistan

Received 15 Oct. 2014; Accepted 22 Dec. 2014; available online 25 Jan. 2015

Abstract: Abiotic stress is a major problem around the world causing huge losses to cultivated crops. To overcome this problem, a study was carried out to examine the effect of Sodium chloride (NaCl) stress on germination of seeds, plantlet development (root, shoot length and fresh, dry weight), cell damage (cell membrane stability) and SDS-PAGE analysis of two rice cultivars i.e. Basmati-385 and Shaheen Basmati. It was observed that salt stress had negative effects on seed germination and germination rate. In both rice varieties, gradual reduction in root and shoot length was observed from control to 100 mM Sodium chloride stress while a considerable decrease in shoot and root length was examined at 150 mM. Root and shoot dry weight showed progressive decrease with increasing salt stress. Cell injury also showed progressive increase from control to 150 mM salt concentration. In SDS-PAGE analysis, no variation was found in proteins banding pattern of rice leaves proteins under salt stress and control.

Key words: Salinity, Cell membrane stability, Rice, SDS-PAGE

Introduction

Salinity is the most severe concern to crop production worldwide (Sahi, 2006). Natural and salty resources of water are the chief damaging factors in waterless and semi-arid area of the global (Binzel, 1994). Salinity is one of the important ecological issues that damage yield development and cultivated efficiency**.** Salinity covered 953 million ha of total area that is about 8 percent of cultivated region (Szabolcs, 1979; Singh, 2009). Among all the other crop species, rice is temperately sensitive to salt in the field. Rice is one of the most commonly cultivated crops in seaside regions normally under water with salty seawater (Akbar et al., 1972; Mori and Kinoshita, 1987).

The salt concentration increased the germination rate and reduced germination percentage (Ashraf et al., 2002). The fresh and dry weight of roots and shoots decrease with increase of salt stress (Dkhil and

*Correspondence to: Muhammad Hafeez, Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat 26000, Pakistan, E-mail; hafeezkhan_88@yahoo.com

Denden, 2010). In high salt concentration germination, root and shoot length and fresh and dry weight of rice reduced upto 50 percent (Anuradha and Rao, 2002).

In Pakistan sustainable crop production is endangered by several factors including soil degradation, moderate salinities and use of salty ground water. In Pakistan 6.8 million ha area are effect from salinity and about 40,000 ha area are affected from salinity each year (Khan 1998). Rice (*Oryza sativa* L.) is the most important staple food globally, and Asia contributes more than 90% of global rice production and consumption. In Asian countries, Pakistan produced different kinds of fragrant rice varieties (Rabbani et al., 2008). Rice grows as a major crop for 11,500 years and it presently sustains almost one half of the world population (Wu et al., 2004). It has also been employed as a foreign exchange earning product (Anonymous, 2009). Production of rice is badly affected due to deterioration of soil and water reservoirs around the world; there is an urgent demand to combat these environmental factors. Therefore, the current study was designed to investigate the effect of salt stress on seed germination and seedling growth of Basmati-385 and Shaheen Basmati.

Materials and Methods

Plant Materials

Two rice varieties seeds; Basmati-385 and Shaheen Basmati (SB), were collect from National Agricultural Research Center (NARC) Islamabad.

Sterilization

Vigorous and healthy seeds with the same size were taken; seeds were surface sterilized in 3.5 percent Sodium hypochlorite for 5 min.

Seed Germination and Seedling Growth

To study the effect of salt stress on germination and seedling growth, healthy and mature seeds were germinated in Petri plates contained double filter paper soaked with 20 mL distilled water or 50, 100 and 150mM NaCl solution. During that period seeds were kept in dark condition under room temperature (30 °C) for 4 days so that all seeds can germinate. Seeds germination reading was taken after every 12 hours up to 4 days (96 hours). After 4 days seeds were transferred to white fluorescent light (16 hours light period/ day) at room temperature (32 °C) and allowed to grow for further 3 days. Root, shoot length and root, shoot fresh and dry weight of 10 days old seedling were then measured and recorded.

Hydroponics culture Experiment

The seeds were primarily germinated in distilled water for conducting hydroponics culture**.** The seeds were kept in distilled water until they were fully germinated. After ten days the young seedling with two leaves were then transferred to plastic trays filled with Hoagland's solution (Hoagland and Arnon, 1950). The growing seeds with shoots and roots were allowed to be fully grown in Hoagland's solution for three weeks**.** During these weeks the Hoagland solution was renewed after every week and with continuous maintenance of pH at 5.6 to 5.9.

After three weeks, stress of sodium chloride of different concentration i.e. control, 50, 100, and 150 mM were given to the sterilized fully growing plants for one week. Whole experiment was conducted under green house condition. After one week of stress treatment, the plant were harvested and chopped in liquid nitrogen to get fine powder for further analysis.

Cell Membrane Stability

From each treatment fresh leaves were taken and sliced in twenty pieces of 1cm. These fine strips were placed in test tube with twenty ml distilled water. The test tubes were incubated at 10 ºC for 24h, followed by warming at 25 ºC. The electro-conductivity (C_1) was measured and then autoclaved for 40 min to determined the electro-conductivity (C_2) again. Cell membrane stability was calculated by using formula, where C refers to electro-conductivity one and two (Jamil et al., 2011).

$EC = C_1/C_2 \times 100$

Analysis of Proteins by SDS-PAGE

Rice plant proteins were analyzed by SDS-PAGE (Sodium dodecyle sulfate Polyacrylamide Gel Electrophoresis) using the standard protocol of Laemmli (1970) with slight modfication. Electrophoresis was carried out in discontinuous system using 10% separating gel and 4% stacking gel.

Results

Effect of Salt stress on seed germination

Result showed that there were slight differences in the germination pattern of untreated and salt treated seeds. In Basmati-385 maximum germination percentage for untreated seeds was 100 percent while it decreased to 90 percent at 150 mM salinity stress (Fig. 1A). In control, seeds started to germinate within thirty six hours, while the seeds in rest of salt treatment started after fourty eight hours.

Result showed that there were slight differences in the germination pattern of untreated and salt treated seeds. In Basmati-385 maximum germination percentage for untreated seeds were 100 percent while it decreased to 90 percent at 150mM salinity stress (Fig. 1A). In control, seeds started to germinate within thirty six hours, while the seeds in rest of salt treatment started after fourty eight hours (Fig. 1B). Salt stress also reduces seed germination in Shaheen Basmati as the salt concentration increased. Maximum germination percentage in control was 100% while it decreased to 90% at 150mM salinity (Fig. 1A). In control and 50mM salt stress, seeds started to germinate after thirty six and fourty eight hours while seed germination in 100 and 150mM salt stress was observed after 72 hours (Fig. 1B).

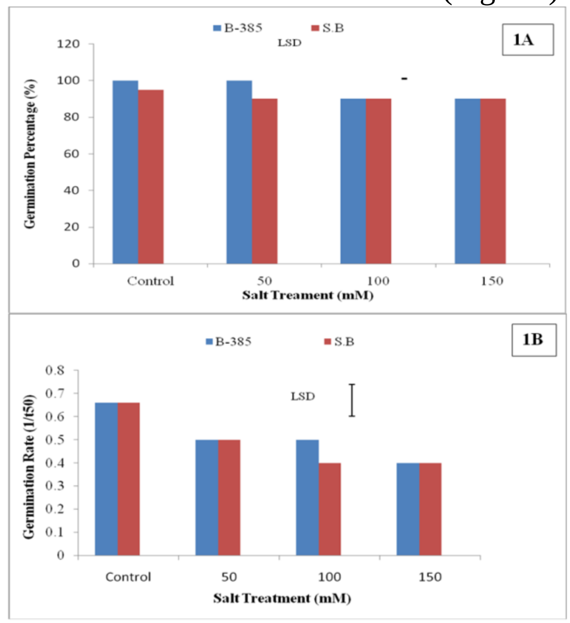

Figure 1. Effect of salt stress on seed germination (A) and germination rate (B) of B-385 and S.B.

Effect of Salt Stress on Root and Shoot Length

Ten days old seedlings were grown under different salt concentrations for three weeks. Root and shoot lengths were measured after harvesting. Results showed that salt concentration decreased the lengths of plantlet roots and shoots. In Shaheen Basmati, continues declined in root length was observed from control to 100mM NaCl stress while a regular decrease in root length was observed from 100 to 150mM NaCl (Fig. 2B). Effect of NaCl on the shoot length of SB was similar to the effects NaCl on its roots. There was gradual decrease in the length of shoots from control to 100mM while there was significant reduction in length of shoots at 150mM (Fig. 2A).

In Basmati-385, gradual reduction in root length was observed from control to 100mM NaCl stress while a major reduction in length was observed at high salt concentration (Fig. 2B). Effect of NaCl on the shoot length of Basmati-385 was similar to the effects NaCl on its roots. There was gradual decreased in the length of shoots from control to 100mM while there was significant reduction in length of shoots at 150mM (Fig. 2A).

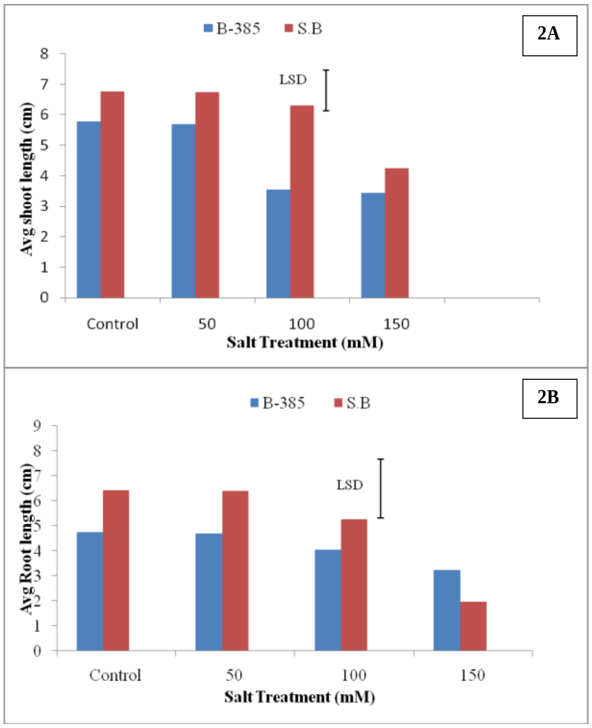

Figure 2. Effect of salt stress on shoot (A) and root length (B) of B-385 and S.B.

Effect of Salt Stress on Root/Shoot Fresh and Dry Weight

After measuring lengths, the roots and shoots were separated and their average weights were measured on digital balance. Results showed that salinity caused considerable reduction in fresh weight of root and shoots of both varieties as compared with control (Figs. 3A and 3B).

As increase in the salt concentration, dry weights of root and shoot also showed gradual reduction in Shaheen Basmati and Basmati-385. Results showed that salinity effect was high on dry weights of Basmati-385 as compared to Shaheen Basmati (Figs. 3A and 3B).

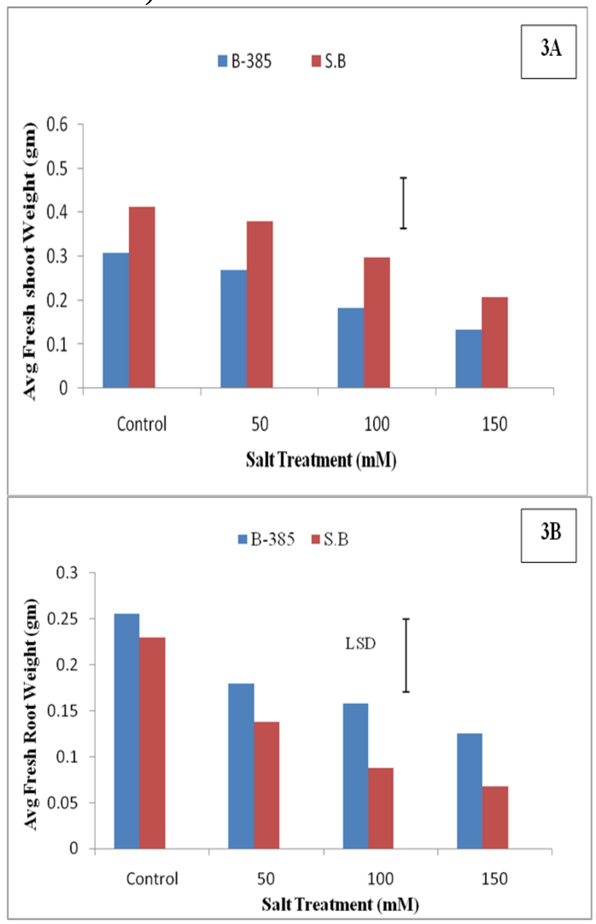

Figure 3. Effect of salt stress on fresh shoot weight (A) and fresh root weight (B) of B-385 and S.B.

Effect of Salt Stress on Cell membrane stability

Salt stress showed considerable effect on cell membrane stability of rice leaves of both varieties. The cell membrane was ruptured due to high salt concentration. High electric conductivity was noticed with the increasing concentrations showing increase in cell injury. The cell injury was high at 150 mM NaCl solution as compared to other salt solution in both varieties (Fig. 5).

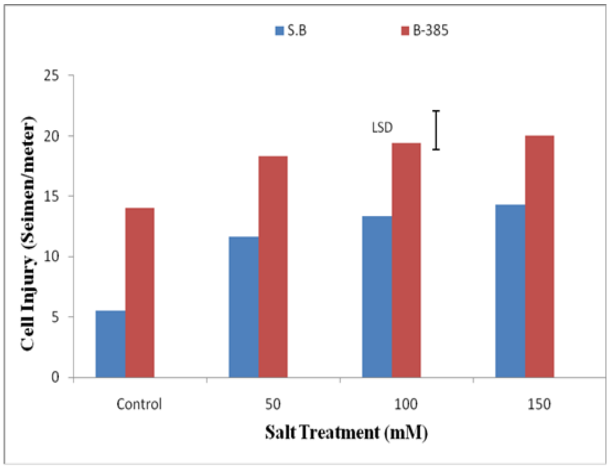

Figure 5. Effect of salt stress on cell membrane stability of B-385 and S.B.

Effect of Salt Stress on Protein analysis by SDS-PAGE

Protein profile analysis of both varieties (Basmti-385 and Shaheen Basmati) were between 20 kDa and 60 kDa. There were total four protein bands observed in all treatments (Control, 50, 100 and 150mM) in the electrophoretic gel. In four protein bands, three major bands and one minor band were observed. The protein banding was compared with a standard molecular weight marker. First two major bands lied in 60 kDa and 58 kDa. The third minor band lied in 40 kDa and fourth major band lied in 20 kDa. Salt stress showed no significant variation on protein banding pattern of both varieties (Figs. 6A and 6B).

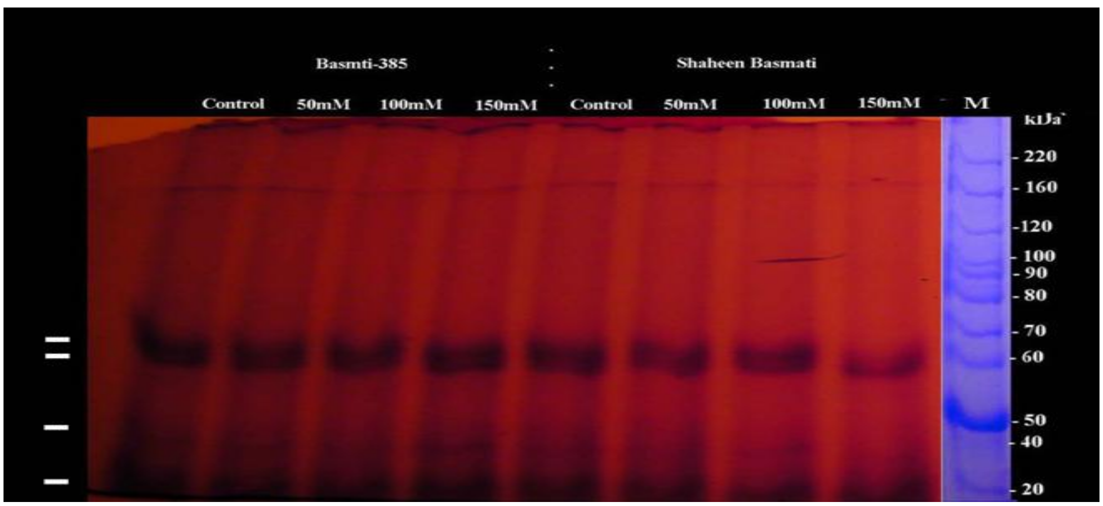

Figure 6A: SDS-PAGE page profile of Basmati-385 and Shaheen Basmati

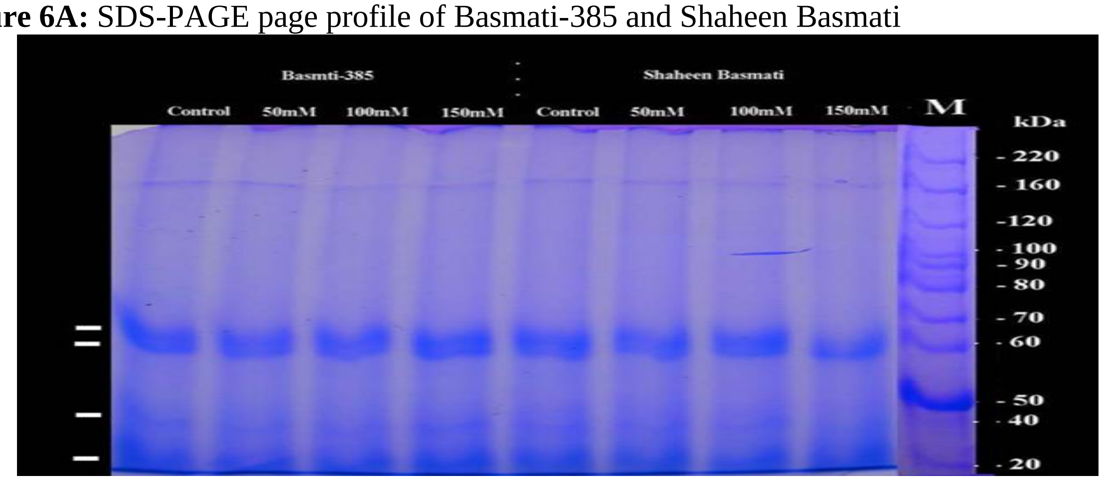

Figure 6B: SDS-PAGE page profile of Basmati-385 and Shaheen Basmati.

Discussion

Seed germination is a complex process and it plays a major role in the life cycle of plant (Saritha et al, 2007). Seed germination decreased as the level of salt increases (Dkdil et al, 2010; Saddiqi et al, 2007). The germination of the seeds were negatively affected by salt concentration, whereas the seeds germinated in salt solution took more time to germinate as compared to control (Figure 1). Inhibition of seed germination occurred with salt concentration which could be due to ion toxicity (Huang and Redmann, 1995). Seed germination delayed as the level of salt increases whereas higher level of salt concentration reduced the final germination percentage (Gloulam and Fares, 2001). Similar kind of result had also been shown by Lima et al. (2003), according to them the viability of seeds under salt stress was decreased as the concentration of salt increased. The germination and final seed germination percentage decreased with the decrease of the water movement into the seeds during imbibitions under salt stress (Hadas, 1977). As the salt concentration increased to 150mM, the germination rate and percentage of seed germination is reduced (Alam et al., 2004). It has been investigated that the germination of seeds, survival of rice seedling and overall plant growth is reduced due to high salt concentration (Zing et al., 2000, Narale et al., 1969). Salt also increases the time for germination (Jamil et al., 2007). A decrease in both percentage and rate of seed germination at high level of salinity could be attributed to process of osmosis, ion toxicity and lack of nutrients in the soil (Redmann, 1994).

For salt stress the root and shoots are most important parameters because roots are in direct contact with soil and absorb water from soil while shoot supply it to the rest of the plant (Jamil and Rha, 2007). As the roots are in the direct contact with surrounding, they are first to encounter the saline medium and are potentially the first site of damage or first line of defense under salt stress. Our result showed that there was decreased in root length of both the varieties with the increasing concentration of salt stress (Figure 2B). Mer et al. (2005) reported that by increasing the salinity, plomule length in wheat, barley pea and cabbage seeds decreased. Our results showed that there was decrease in shoot length of both varieties with the increasing concentration of salt stress (Figure 2A). However these results are not similar with those of Cramer et al. (1985) who reported that roots were less sensitive to salt than shoots.

The observed reduction in shoot length in salinized conditions was possible due to many reasons. One possibility is that photosynthesis reduced by salinity which in turn limited the supply of carbohydrate needed for growth (Alam et al., 2004). A second possibility is that salinity decreased the root and shoots growth by decreasing turger in expending tissue resulting from lower water potential in root medium (Alam et al,. 2004). A third is that the root show response to salinity was to down regulate shoot growth via long distance signal (Alam et al,. 2004). The fourth possibility is that a disturbance in mineral supply, either an excess or deficiency, induced by changes in concentration of specific ions in the growth medium, might have directly affected growth (Alam et al,. 2004).

Cell injury analysis is another parameter which is affected by salt concentration. According to Jamil et al. (2010) the cell injury increases with increasing salt concentration. Our result showed that salt concentration increases the cell injury (Figure 5). Our result of cell injury analysis correlated with Jamil et al. (2010).

SDS-PAGE is considered to be a particularly reliable method because storage proteins are largely independent of environmental fluctuations (Hanada et al., 2002). In the present study no significant variation was observed in both varieties (Basmati-385 and Shaheen Basmati) in all treatments (Figure 6A and 6B). A total of four bands were obtained and all the bands were common in both varieties. The bands were appeared on the gel divided into two categories; category 1st consists of major bands and second encircles minor bands on the glance (sharpness) of the bands. In four protein bands, three major bands and one minor band were observed. Kong-ngern *et al*. (2005) compared salt treated rice plants to control plants. In opposite, the level of sensitive rice remained unaffected with salt treatment. One of the major band in their banding patterns which exactly lies on 21 kDa in control and salt treatment. In our results one major band lies on 20 kDa in control and salt treatments which indicate that our results show resemblance to some extent with the Kong-ngern et al.

References

Akbar, M., Yabuno, Y. D. and Nakao, S. 1972. Breeding for saline resistant varieties of rice. Variability for salt-tolerance among some rice varities. Jpn. J. Breed. 22: 277-284.

Alam, M. Z., Stuchbury, T., Naylor, R.E.L. and Rashid, M. A. 2004. Effect of salinity on growth of some modern rice cultivars. J. Agron. 3(1): 1-10.

Alam, S.M., Ansari, R. and Athar Khan, M. 2000. Saline Agriculture and Pakistan. Industry and Econ. 56: 45-53

Anonymous. 2009. Economic Survey, 2008-2009. Ministry of Food, Agriculture and Livestock. Federal Bureau of Statistics, Islamabad, Pakistan.

Anuradha, S. and Rao, S. 2002. alleviating influence of brassinolide on Salinity stress induced inhibition of germination and seedling growth of rice. Ind. J. Plant Physiol. 7(4): 384-387.

Ashraf, M. 2010. Inducing drought tolerance in plants: some recent advances. Biotechnol. Adv. 28: 169-183.

Bernstein, N. and Kafkafi, U. 2002. Root growth under salinity stress. p. 787-819.

Binzel, M.L. and Reuveni, M. 1994. Cellular mechanisms of salt tolerance in plant cells. Hortic. Rev. 16: 33-70.

Coata, P. and Plomion, H. 2005. Genetic analysis of needle proteins and proteins variations in maritime pine. Silvae-Genet. 49(4): 146-150.

Cramer, G. R., Lauchli, A. and Polito, V. . 1985. Displacement of Ca2+ by Na+ from the plasmalemma of root cell. A primary response to salt stress. Plant physiol. 79: 207-211.

Cuartero, J. and Munoz, R. 1999. Tomato and salinity. Sci. Hort. 78: 83–125.

Dkhil, B. B. and Denden, M. 2010. Salt stress induced changes in germination, sugars, starch and enzyme of carbohydrate metabolism in Abelmoschus esculentus (L.) Moench seeds. Afr J. Agric. Res. 5(6): 408-415.

Ghoulam, C and Fares, K. 2001. Effect of salinity on seed germination and early seedling growth of sugar beet. Seed Sci. Technol. 29: 357–364.

Hadas, A. 1977. Water uptake and germination of leguminous seeds in soils of changing matrix and osmotic water potential, J. Exp. Bot. 28: 977-985.

Hanada, H., Kayano, T., Tanaka, H. and Tabei, Y. 2002. Digoxigenin labeled RAPD (DIG-RADP) analysis on genetic relation of sweet pea (Lathyrus odoratus L.) cultivars. J. Jap. Soc. Hort. Sci. 71(5): 719-721

Hoagland, D. R. and Arnon, D. I. 1950. The water culture method for growing plants without soil. Calif. Agr. Expt. Sta. Circ. 347.

Huang, J. and Reddman, R. E. 1995. Salt tolerance of *Hordeum* and *Brassica* species during germination and early seedling growth. Can. J. Plant. Sci. 75: 815-819

Irfan Ullah, Pervez Khaliq, Abdullah, and Ijaz Malook., 2009. Genetic Diversity in Pea (Pisum Sativum) based on SDS-PAGE. Pakistan J. Agric. Res. 22: 3-4.

Jamil, M. and Rha, E. S. 2007. Gibberellic acid enhances seed water uptake, germination and early seedling growth in suger beet under salt stress. Pak. J. Bio. Sci. 10 (4):654-658.

Khan, G.S. 1998. Soil Salinity/Sodicity Status in Pakistan. Soil Survey of Pakistan, Lahore. P. 59.

Khan, I., Shuib, M., Ali, Z., Imran, M. and Zeb, A. 2006. Molecular and Physiochemical Evalution of Wheat. JCSP (Accepted).

Kong-ngern, K., Daduang, S., Wongkham, C., Bunnag S., Kosittrakun, M. and Theerakulpisut P. 2005. Protein profiles in response to salt stress in leaf sheaths of rice seedlings. Sci. Asia, 31: 403-408

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **227** (5259): 680–685.

Lima, M., Lopes, G. S., Moraes, D. M. and Abreu, C. M. 2003. Physiological quality of rice seeds submitted to salt stress, Associacao Brasileira de Technologia de Sementes. 93:324-336.

Mer, R. K., Prajith, P. K., Pandya, D. H. and Dandey, A. N. 2000. Growth of young plants of Hourdeum vulgare, Triticum aestivum, Cicer arietiumand Brassica juncea. J. Agron. Crop. Sci., 185: 209-217.

Mori, I. K. and Kinoshita, T. 1987. Salt tolerance of rice callus clones. Rice Genet. Newslett. 4: 112-113.

Jamil, M. Iqbal, W., Bangash, A., Rehman, S., Imran, Q. M. and Rha, E. S. 2010. Constitutive Expression of *OSC3H33*, *OSC3H50* AND *OSC3H37* Genes in Rice under Salt Stress. Pak. J. Bot. 42(6): 4003-4009.

Narale, R. P., Subramanyum, T. K. and Mukherjee, R. K. 1969. Influence of salinity on germination, vegetative growth, and grain yield of rice (Oryza sativa var. Dular). Agron J. 61: 341-344.

Rabbani, M. A., Pervaiz, Z. H. and Masood, M. S. 2008. Genetic diversity analysis of traditional and improved cultivars of Pakistani rice (Oryza sativa L.) using RAPD markers. Elect. J. Biotech. 11: 1-10.

Redmann, R.E. 1974. Osmotic and specific ion effects on the germination of alfalfa. Canad. J. Bot. 52: 803-8.

Sahi, C., Singh, A., Kumar, K., Blumwald, E. and Grover, A. 2006. Salt stress response in rice: genetics, molecular biology, and comparative genomics. Funct. Integr. Genomic. 6(4): 263-284

Saritha, V., Kuriakose and Prasad, M. N. V. 2007. Cadmium stress affects seed germination and seedling growth in *Sorghum bicolor* (L.) Moench by changing the activities of hydrolyzing enzymes. Plant growth regul. 54: 143-156.

Siddiqi, E.H., Ashraf, M. and Akram, N. A. 2007. Variation in seed germination and seedling growth in some diverse lines of safflower (*Carthamus tinctorius* L.) under salt stress. Pak. J. Bot. 39 (6): 1937-1944.

Singh, G. 2009. Salinity-related desertification and management strategies: Indian experience. Land Degrad. Develop. 20: 367-385.

Szabolcs, 1979. Review of research on salt affected soils. Natural Res. Res. 15. Paris: UNESCO.

Wu, C. J., Cheng, Z. Q., Huang, X. Q., Yin, S. H., Cao, K. M. and Sun, C. R. 2004. Genetic diversity among and within populations of *Oryza* granulate from Yunnan of China revealed by RAPD and ISSR markers: implications for conservation of the endangered species. Plant Sci., 167: 35-42.

Zeng, L. and Shannon, M. C. 2000. Salinity effects on seedling growth and yield components of Rice. Crop Sci. 40: 996-1003.