

Impact of salinity (NaCl) stress on Rice CO-43's photosynthetic pigment properties (*Oryza sativa* L.)

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Abstract

Environmental factors like salinity frequently have a negative impact on crop growth, productivity, and yield. This study examined the impact of salt stress on physiological traits of rice (*Oryza sativa* L.) at the early seedling stage of the seventh day. The seeds for Rice (CO43) were grown in plastic containers that contained culture solution and a nylon netted frame (5 x 7") that was fitted with a 2.5 L solution capacity. The culture solution's pH was kept at 5.0. For seven days, these boxes were stored individually at 30°C in an incubator. Salinity cause decrease leaf area, chlorophyll content, and corotenoid content of rice. However, membrane injury, total sugars of all rice increased under salinity stresses. Treatments are T1-50Mm, T2-100mM, T3-150mM, T4-200mM, T5 -250mM high salinity Treatments decrease, chlorophyll-a, b, and corotenoid under high salt stress on Rice (CO-43).It was concluded that salinity increased plant was growth and photosynthetic pigments is affected compare to the control.

Key words: salinity, seedling growth, rice genotypes chlorophyll, membrane permeability.

Introduction

Rice is one of the most important cereal crops in the world, with exceptional agricultural and economic importance as a staple food for more than half of the world's population, and Asian farmers produce more than 90% of total rice production (IRRI, 2011). Salinity is a common environmental stress that has a significant impact on crop growth, food production, and crop yield in many regions, particularly arid and semi-arid areas (Jamil et al., 2010; Osakabe et al., 2011; Hussain et al., 2013). It is estimated that over 800 million hectares of land worldwide are affected by salinity and sodicity (Munns, 2005; Kumar et al., 2010 and Tavakkoli et al., 2011). Salt contaminated soils ($E_{c} > 4 \text{ dS m}^{-1}$ or 40 mM NaCl or osmotic potential 0.117 MPa) are classified as saline land, which has a direct impact on plant growth and development prior to reproductive stage, particularly crop species (Chinnusamy et al., 2005; Ashraf et al., 2008; Ashraf, 2009). Some crop species, such as rice, corn, bean, eggplant, onion, potato, pepper, sugarcane, and cabbage, are susceptible to salt stress ($E_{c} 1.0-1.8 \text{ dS m}^{-1}$), which reduces crop growth and productivity by 6-19%. In general, biochemical, physiological, anatomical and morphological characteristics of plants directly affected by soil salinity is ascribed as (Chinnusamy et al., 2005; Parida and Das, 2005). Abiotic stresses such as high or low temperature, water scarcity, high salinity, and heavy metals have a significant antagonistic effect on crop metabolism and thus plant growth, development, and ultimately crop productivity via an osmotic effect on plant water uptake and specific ion toxicities (Munns et al., 2006 and Solangi et al., 2015). Salt stress affected Proline and sugar synthesis and accelerated the rate of biosynthesis, and higher concentrations of chlorophyll b than chlorophyll a were observed in many crop plants during vegetative growth (Khan et al., 2000; Asch et al., 2000; Santo, 2004 and Akram et al., 2007). The current study sought to investigate the effect of salinity (NaCl) stress on seed germination, seedling

growth, and various physiological parameters such as total sugars, ions concentration, chlorophyll content, shoot membrane permeability, and Proline accumulation in four rice genotypes: CO 43, TRY 1, TPS 2, TPS 3, and ADT (R) 44.

Materials and methods

Seed source:

Plant Materials

Rice CO-43 variety seeds were obtained from the Coimbatore campus of the Tamil Nadu Agricultural University (TNAU).

Sterilization

After being taken from strong, healthy plants, the seeds were surface sterilized in 3.5 percent sodium hypochlorite for 5 minutes.

Seed Germination and Seedling Growth

250mM NaCl solution to study the effect of salt stress on germination and seedling growth. During that time, seeds were kept in the dark at room temperature (30 °C) for four days to ensure that all seeds germinated. Readings on seed germination were taken every 12 hours for up to 4 days (96 hours). During the year 2018, this research was carried out in the Plant Physiology Laboratory of L.N.Government Arts College, Ponneri, Tamil Nadu. The experiment used a Complete Randomize Design (CRD) with three replicates. There are four salinity levels (50, 100, 150, 200, and 250mM NaCl) on the rice.

Chlorophyll and Carotenoid content

A test tube containing 0.1 grammes of homogenized fresh leaf tissue was filled with 10 ml of 80% acetone, aluminum foil was placed over the test tube, and the tube was incubated at room temperature in the dark for an entire night. The following day, samples were taken while waiting for the particulates to settle at the bottom of a vortex. With a spectrophotometer, the extract absorbance was measured at wavelengths of 470.0 nm, 646.8 nm, and 663.2 nm using 80% acetone as a blank. The content of carotenoid and chlorophyll (Chl. a, Chl. b, and Total Chl.) was measured in (mol. g-1 fresh weight).using method elaborated by Lichtenthaler (1987).

Proline content (μ mol. g-1 fresh weight)

Free Proline content was measured in (μ M g-1 fresh weight) using method elaborated by Bates et al. (1987).

Reagents:

In order to make acid-ninhydrin, 1.25 g of ninhydrin was heated with agitation in 60 ml glacial acetic acid and 40 ml 6 M phosphoric acid until dissolved. The reagent is stable for 24 hours when kept cool (stored at 4°C). The homogenate of 0.25g of plant material was filtered using Wattman filter paper #2 after being ground and homogenized in 10 ml of 3% aqueous sulfosalicylic acid. In a test tube, 2 ml of filtrate was combined with 2 ml of acid-ninhydrin and 2 ml of glacial acetic acid. The reaction was heated to 100 °C for 1 hour before being stopped in an ice bath. The reaction mixture was extracted with 4 ml of toluene and aggressively stirred for 20–25 seconds in a test tube stirrer. Toluene was used as a blank to measure the absorbance of the chromospheres that contained it at 520 nm after it was aspirated from the aqueous phase and warmed to room temperature.

Statistical investigation

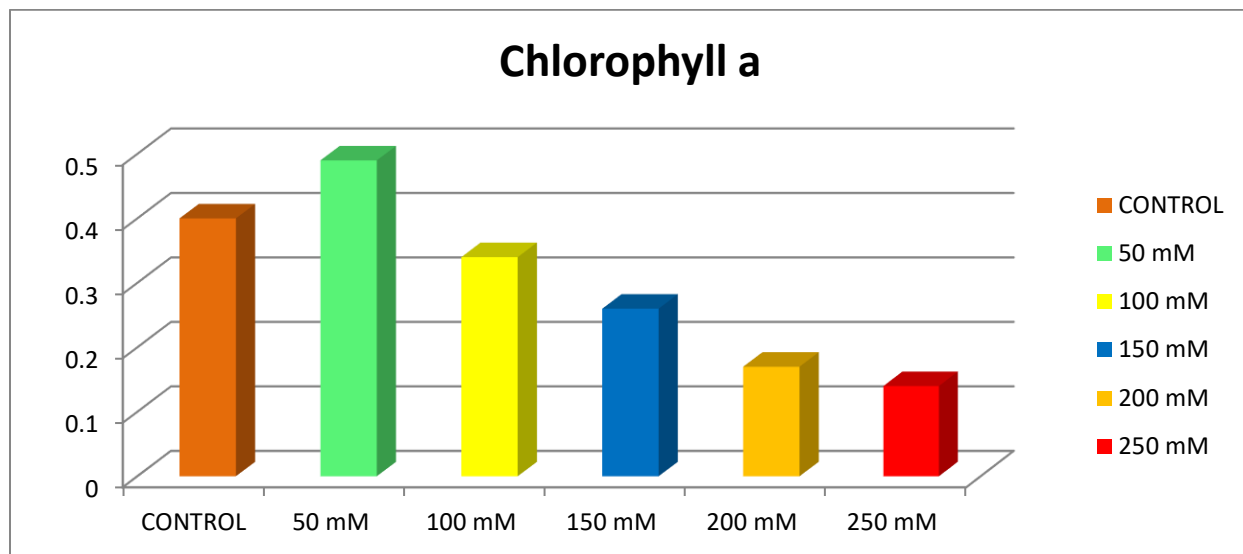
All parameter data were subjected to analysis of variance (ANOVA) to determine the superiority of treatment means, and the least significant difference test was performed at alpha 0.05. A Microsoft computer package called "Statistics 8.1" was used for this purpose.

Result and discussions

At the Plant Physiology Laboratory of L.N.Government Arts College, Ponneri, Tamil Nadu, a laboratory experiment was conducted in 2018 to investigate the effect of salinity on the photosynthetic pigment characteristics of rice (*Oryza sativa* L.) at the germination and seedling stages. The effect of different salinity concentrations (50, 100, 150, 200, and 250mM NaCl) on chlorophyll content, Proline, shoot membrane permeability, and Proline was determined in one rice genotype after 07 days of incubation (CO-43).

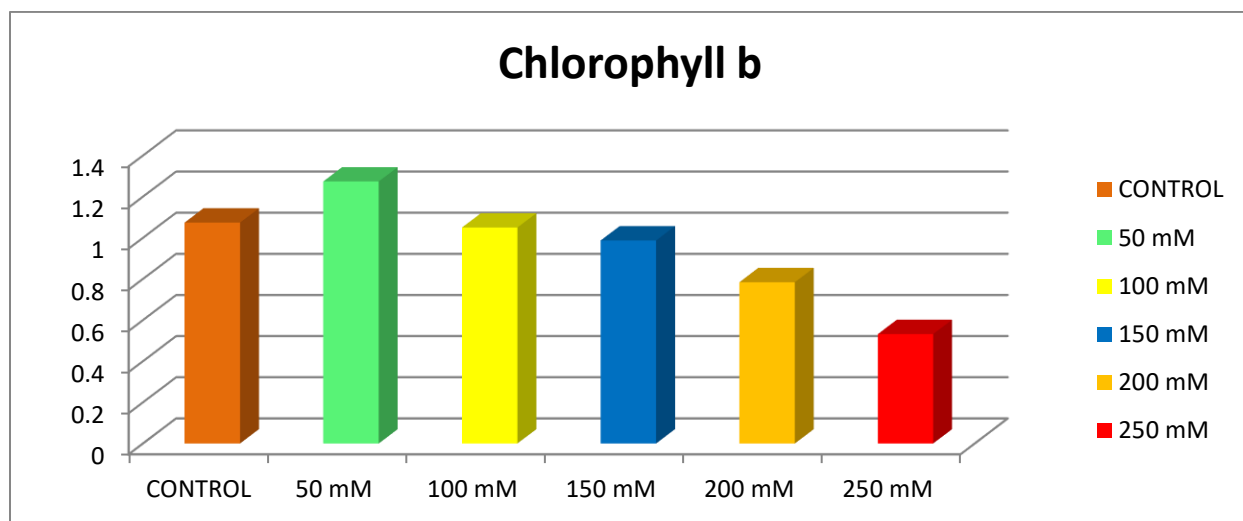
Chlorophyll a concentration (mg g⁻¹ fresh weight)

In terms of chlorophyll a concentration under salinity stress, as shown in Figure 1, all treatments responded significantly differently. In 50mM NaCl stress, rice had the highest chlorophyll a concentration (0.49mg g⁻¹ fresh weight). The lowest chlorophyll a concentration (0.14 mg g⁻¹ fresh weight) was measured in 250mM NaCl stress and compared to the control. It is consistent with previous findings that photosynthetic pigments in Pokkali (salt-tolerant genotype) salt stressed seedlings (200 mM NaCl) can be stabilized better than those in IR29 (salt-sensitive genotype) (Theerawitaya et al., 2012; Boriboonkaset et al., 2012; Zhenhua et al.,2012 and Saeedipour, 2014).



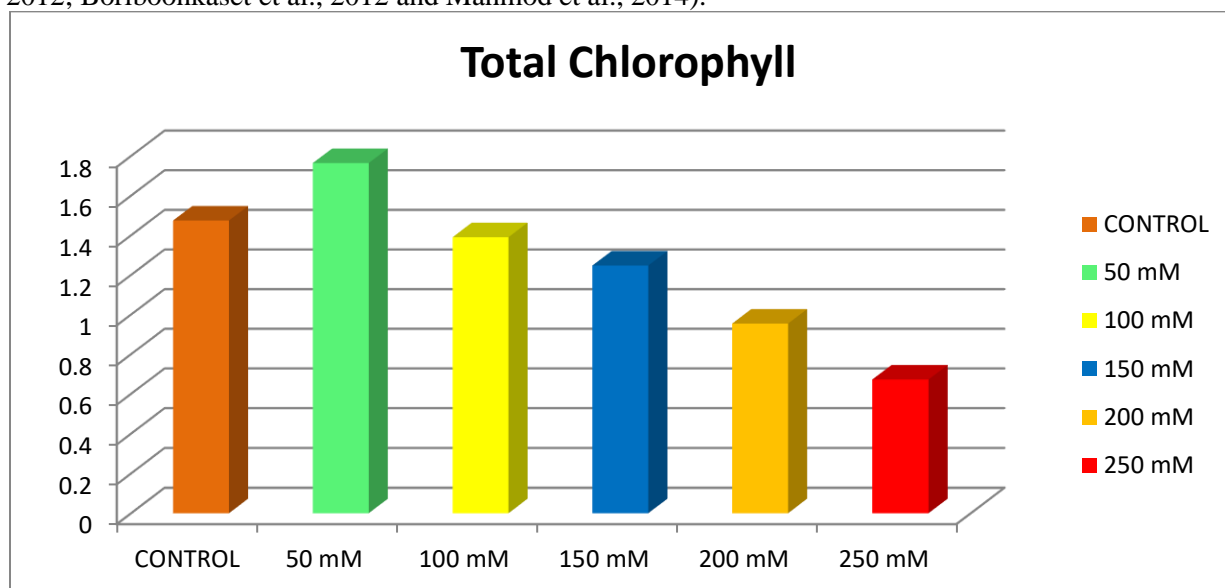
Chlorophyll b concentration (mg g⁻¹ fresh weight)

As seen in Figure 2 for chlorophyll b concentration under salinity stress, each treatment had a significantly distinct response from the others. Less than 50mM NaCl stress, rice's highest chlorophyll B content (1.274 mg g⁻¹ fresh weight) was noted. Under 250mM NaCl stress, the lowest chlorophyll b content was observed (0.532 mg g⁻¹ fresh weight), and this was compared to the control, respectively. The scientists have also revealed that colors can change under conditions of high salt (Santo, 2004; Akram et al., 2007; Theerawitaya et al., 2012; Boriboonkaset et al., 2012 and Saeedipour 2014).



Total Chlorophyll concentration (mg g⁻¹ fresh weight)

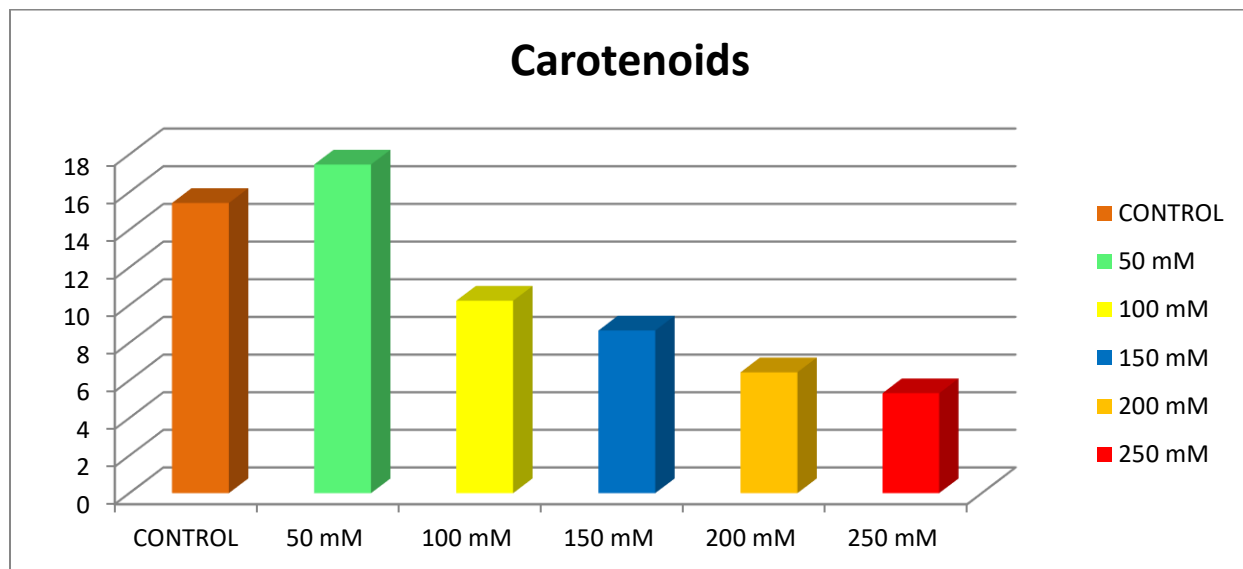
Figure 3 shows the total chlorophyll content under salinity stress, and all of the treatments had significantly distinct responses to one another. Less than 50mM NaCl stress, rice's highest Total Chlorophyll concentration (1.764 mg g⁻¹ fresh weight) was noted. Less than 250mM NaCl stress, the lowest total chlorophyll concentration (0.672 mg g⁻¹ fresh weight) was discovered and compared to the control, respectively. Findings that support (Iqbal et al., 2006; Ashraf et al., 2005; Theerawitaya et al., 2012; Boriboonkaset et al., 2012 and Mahmud et al., 2014).



Carotenoids concentration (mg g⁻¹ fresh weight)

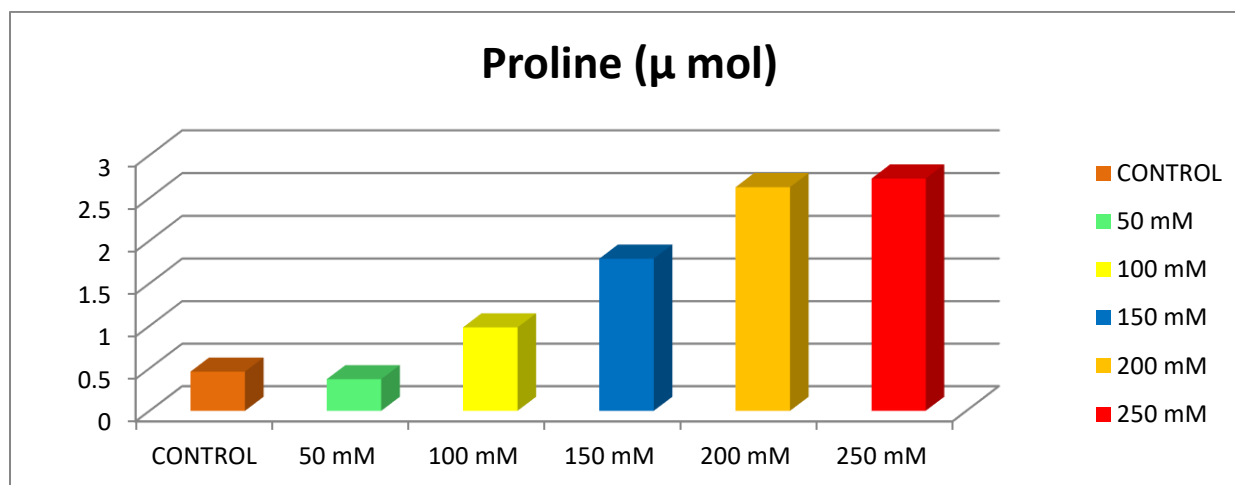
Figure 4 shows the concentration of carotenoids during salt stress. Each treatment had a significantly different response. Less than 50mM NaCl stress, the highest quantity of carotenoids in rice (17.46 mg g⁻¹

fresh weight) was noted. The lowest concentration of carotenoids was measured (05.32 mg g⁻¹ fresh weight) under stress with 250 mM sodium chloride, and it was compared to the control, respectively. Iqbal et al. (2006) and Ashraf et al. (2005) support the findings. Boriboonkaset et al., 2012; Theerawitaya et al., 2015).



Proline content (μ mol. g⁻¹ fresh weight)

Figure 4 shows the Proline concentration under salinity stress, and all the treatments had significantly varied responses to it. Less than 250mM NaCl stress, the highest concentration of Proline in rice (2.726 mg g⁻¹ fresh weight) was noted. Proline's lowest concentration was measured under conditions of stress with 50mM NaCl and compared to the control, respectively (0.372 mg g⁻¹ fresh weight). A well-known osmoprotectant, proline is crucial for maintaining osmotic balance, safeguarding subcellular structures and enzymes, and raising cellular osmolarity (turgor pressure), which provides the turgor required for cell growth under stressful circumstances (Matysik et al. 2002; Sairam and Tyagi 2004). The essential osmolyte for maintaining cell turgor and preventing salinity in plants is proline (Farkhondeh et al., 2012). The outcomes are consistent with those of (Zayed et al., 2004; Chutipaijit et al., 2009 Kumar et al., 2009 Danai- Tambhale et al., 2011 and Hakim et al., 2014).



Conclusion

In general, CO-43 demonstrated superior tolerance to salt stress than TRY 1, TPS 2, TPS 3, and ADT (R) 44, under the influence of salinity levels on physiological response at seedling stage, according to the findings of the current study. Showed greater proline and chlorophyll content with varying salinity levels, and all of these physiological traits are significant in the environment's salt tolerance. Chlorophyll "b" was shown to be more potent than chlorophyll "a" in this investigation. There was an increase in chlorophyll b concentration with rising salinity levels, which resulted in a high capacity for photosynthetic activity (chlorophyll a, b total, and carotenoids). Furthermore, at high salt concentrations, biomass, vegetative growth, and germination were drastically decreased (250mM NaCl). However, at high salinity levels, a considerable reduction was seen. The results of the experiment show that salinity stress has negative impacts, including osmotic pressure-induced root and shoot development, leaf rolling, membrane injury, and tip burning.

References

- Abdelgawad, Z. A., T. A. Hathout, S. M. El-Khallal, E. M. Said and A. Z. Al-Mokadem. 2014. Accumulation of Trehalose Mediates Salt Adaptation in Rice Seedlings American-Eurasian J. Agric. and Environ. Sci., 14(12):1450- 1463.
- Ali, Y. Z. Aslam, M. Y. Ashraf and G. R. Tahir. 2004. Effect of salinity on chlorophyll concentration, leaf area, yield and yield components of rice genotypes grown under saline environment, 1(3):221-225.
- Akram, N. A., M. Shahbaz and M. Ashraf. 2007. Relationship of photosynthetic capacity and proline accumulation with the growth of differently adopted population of two potential grasses (*Cynodon Dactylon* (L.) Pers. and *Cenchrus ciliaris* (L.) to drought stress. Pak. J. Bot., 39: 777-786.
- Asch, F., M. Dingkuhn, and K. Dorffling. 2000. Salinity increases CO₂ assimilation but reduces growth in field-grown, irrigated rice. Plant and Soil 218, 1-10.
- Ashraf, M. and M. R. Foolad. 2005. Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth, and crop yield under saline and non- saline conditions. Adv. Agron., 88: 223-271.
- Ashraf, M., H. R. Athar, P. J. C. Harris and T. R. Kwon. 2008. Some prospective strategies for improving crop salt tolerance. Adv. Agron., 97: 45-110.

- Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotech. Adv.*, 27: 84-93.
- Bagheri A. A., S. Saadatmand., V. Niknam, T. Nejadshari, V. Babaeizad. 2014. Effects of *Piriformospora indica* on biochemical parameters of *Oryza sativa* under salt stress *IJB*, 4(4): 24-32.
- Bates, L. S., R. P. Waldern and L. D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant Soil*, 39: 205-207.
- Boriboonkaset, T., C. Theerawitaya, A. Pichakum, S. Cha-um, T. Takabe and C. Kirdmanee. 2012. Expression levels of some starch metabolism related genes in flag leaf of two contrasting rice genotypes exposed to salt stress. *AJCS*, 6(11):1579-1586.
- Danai-Tambhale, S., V. Kumar and V. Shriram. 2011. Differential response of two scented indica rice (*Oryza sativa*) cultivars under salt stress. *J. Stress Physio. & Biochem.*, 7(4):387-397.
- Djanaguiraman, M., J. A. Sheeba, A. K. Shanker, D. D. Devi and U. Bangarusamy. 2006. Rice can acclimate 229 to lethal level of salinity by pretreatment with sublethal level of salinity through osmotic adjustment. *Plant Soil*, 284: 363- 373.
- Chinnusamy, V., A. Jagendorf and J. K. Zhu. 2005. Understanding and improving salt tolerance in plants. *Crop Sci.*, 45: 437-448.
- Chutipaijit, S., S. Cha-um, and K. Sompornpailin. 2009. Differential accumulations of proline and flavonoids in indica rice varieties against salinity. *Pak. J. Bot.*, 41(5):2497-2506.
- Flowers, T. J., Yeo, A. R., 1986. Ion relations of plants under drought and salinity. *Aust. J. Plant Physiol.* 13, 75-91.
- Hakim, M. A., A. S. Juraimi, M. M. Hanafi, M. R. Ismail, A. Selamat, M. Y. Rafii, and M. Ikram-ul-Haq, A. M. Dahri, M. U. Dahot, N. Parveen, A. Ghaffar and A. L. Laghari. 2010. Growth responses of NaCl stressed rice (*Oryza sativa* L.) plants germinated from seed in aseptic nutrient cultures supplemented with proline. *Afr. J. Biotechnol.* 9: 6534-6538.
- Iqbal, N., M. Y. Ashraf, Farrukh Javed, Vicente Martinez and Kafeel Ahmad. 2006. Nitrate reduction and nutrient accumulation in wheat (*Triticum aestivum* L.) grown in soil salinization with four different salts. *Plant Nutrition J.*, 29:409-421.
- Jamil, M., W. A. Iqbal, Bangash, S. Q. M. RehmanImran, E. S. Rha. 2010. Constitutive expression of OSC3H33, OSC3H50 and OSC3H37 genes in rice under salt stress. *Pak. J. Bot.* 42: 4003-4009.
- Khalil, S. K., K. Zeb and A. Z. Khan. 2002. Changes in leaf area, assimilate accumulation and partitioning of wheat varieties planted on different dates. *Pak. J. Soil Sci.*, 21: 15-19.
- Khan, M. A., I. A. Ungar and A. M. Showalter. 2000. Salt tolerance in the subtropical perennial halophyte *Atriplex griffithii* Moq. var. *stocksii* Boiss. *Ann. Bot.*, 85: 225-232.
- Kumar, V., V. Shriram, T. D. Nikam, N. Jawali and M. G. Shitole. 2009. Antioxidant enzyme activities and protein profiling under salt stress in indica rice genotypes differing in salt tolerance. *Arch. Agron. Soil Sci.*, 55(4):379- 394.
- Kumar, V., V. Shriram, Kavi, P. B. Kishor, N. Jawali, and M. G. Shitole. 2010. Enhanced proline accumulation and salt stress tolerance of transgenic indica rice by over expressing P5CSF129A gene. *Plant Biotech Rep.*, 4(1):37-48.
- Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods Enzymol.*, 148: 350-382.
- Mahmood A., T. Latif and M. A. Khan. 2009. Effect of salinity on growth, yield and yield components in basmati rice germplasm. *Pakistan J. Bot.* 41: 3035-3045.
- Mahmod, I. F., S. S. Barakbah, N. Osman and O. Omar. 2014. Physiological Response of Local Rice Varieties to Aerobic Condition *Int. J. Agric. Biol.*, 16(4): 738- 744.
- Mansour M. M. F. 1997. Cell permeability under salt stress. In: Jaiwal PK, Singh RP, Gulati A (Eds.). *Strategies for Improving Salt Tolerance in Higher Plants*. Oxford and IBH, New Delhi. pp. 87-110.

- Matysik, J., A. B. Bhalu, and P. Mohanty. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.*, 82: 525-532.
- Meloni, D. A., C. A. Oliva and J. Cambraia, 2003. Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Braz. J. Plant Physiol.*, 15: 12-21.
- Munns, R. 2005. Genes and salt tolerance: bringing them together. *New Phytology*, 167: 645-663.
- Munns, R., R. A. James and A. Lauchli. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *Journal of Experimental Botany*, 57: 1025- 1043.
- Nemati, F. Moradi, S. Gholizadeh, M. A. Esmaili, M. R. Bihamta. 2011. The effect of salinity stress on ions and soluble sugars distribution in leaves, leaf sheaths and roots of rice (*Oryza sativa* L.) seedlings. *Plant soil environ.*, 57(1):26- 33.
- Osakabe, Y., S. Kajita, and K. Osakabe. 2011. Genetic engineering of woody plants: current and future targets in a stressful environment. *Physiol. Plant.* 142: 105-117.
- Parida, A. K. and A. B. Das. 2005. Salt tolerance and salinity effects on plants: a review. *Ecotoxicol. Environ. Safe*, 60: 324-349.
- Riazi, A., K. Matruda and A. Arslam. 1985. Water stress induces Changes in concentration of proline and other solutes in growing regions. *J. Exp. Bot.*, 36(172):1716-1725.
- Saeedipour, S. 2014. Effects of salinity stress on growth, chlorophyll content and ion accumulation in two indica rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. *Int. J. Biosci.* 4(4):33-40.
- Sairam, R. K. and A. Tyagi. 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Curr. Sci.*, 86(3):407-421.
- Santo, C. V. 2004. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Science of Horticulture* 103, 93-99.
- Solangi. 2015. Genotypic responses of rice under salinity and high temperature stresses on seed germination and seedling growth. *IJAT*, 11(5):1129-1143.
- Theerawitaya, C., T. Boriboonkaset, S. Cha-um, K. Supaibulwatana, C. Kirdmanee. 2012. Transcriptional regulations of the genes of starch metabolism and physiological changes in response to salt stress rice (*Oryza sativa* L.) seedlings. *Physiol. Mol. Boil. Plant*, 8:197-208.
- Theerawitaya, C., N. Yamada, T. Samphumphuang, S. Cha-um, C. Kirdmanee, and T. Takabe. 2015. Evaluation of Na⁺ enrichment and expression of some carbohydrate related genes in indica rice seedlings under salt stress *POJ* 8(2):130-140.
- Yan, B., Q. Dai, X. Liu, S. Huang and Z. Wang. 1996. Flooding induced membrane damage, lipid oxidation and activated oxygen generation in corn leaves. *Plant Soil.* 1996; 197:261-268.
- Zayed, B. A., A. El-azeem K. Salem, and O. A. M. Ali. 2014. Physiological characterization of Egyptian salt tolerant rice varieties under different salinity levels *Life Science Journal*; 11(10):1264-1272.
- Yoshida, S., D. A. Forno, J. H. Cock and K. A. Gomez. 1976. *Laboratory Manual for physiological studies of rice*. IRRI, Las Bano. Laguna, pp. 83.
- Zhen-hua, Z., L. Qiang, S. Hai-xing, R. Xiang-min and A. M. Ismail. 2012. Responses of different rice (*Oryza sativa* L.) genotypes to salt stress and relation to carbohydrate metabolism and chlorophyll content. *African J. Agril. Res.*, 7(1):19-27.