Original Research Article

Morpho-Physiological Study of Four Varieties of Rice (*Oryza sativa* L.) to Salinity Stress

ABSTRACT

Abiotic stress, especially salinity is one of the major restraints for attaining self-sufficiency in the food grain production. The saline soils affect the crop growth both directly and indirectly by interfering with plant metabolism resulting in reduction in yield potential of various crops. Rice (*Oryza sativa*), the staple food of much of the world's population, is categorized as a salt-susceptible crop. Improving the salt tolerance of rice would increase the potential of saline-alkali land and ensure food security. To investigate the effect of different levels of salinity on morpho-physiological characters and yield of four rice variety *viz*. Pokkali, Narendra Ushar Dhan 3, IR 28 and IR 29. A Pot experiment was conducted during *Kharif* 2011 and 2012 at the Department of Crop Physiology, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.) under normal soil and saline condition with EC 6.0 dSm^{-1} and EC 10.0 dSm^{-1}. The three levels of saline soil, *i.e.* Normal soil; EC 6.0 and EC 10.0 dSm^{-1} were imposed at 21 days after sowing. Gradual increase on plant height as well as biomass with the advancement in the age of crop up to dough grain in all the varities under both normal and salinity treatment. Maximum grain yield recorded in tolerant varieties Narendra Usar Dhan 3 followed by Pokkali, whereas, minimum in IR 28. Under saline condition regarding sink potential the tolerant varieties recorded less reduction in yield attributes like length of panicle (cm), No. of panicle $plant^{-1}$, No. grain $panicle^{-1}$ and grain yield $plant^{-1}$ as compared to normal condition. The salinity tolerance capacity of tolerance varieties was associated with increased the activity of catalase, superoxide dismutase enzyme batter accumulation of proline and total carbohydrate as compared to normal condition.

Keywords: Antioxidant Enzyme; SOD; MSI; Salinity; Grain Yield; Rice

1. INTRODUCTION

Rice is one of the important staple food crops and is consumed by approximately 50% of the global population as their main source of energy [39, 40]. The world population is increasing and will reach 9.5 billion by year 2050 and world food production will need to increase by 70% [(1, 37, 38)]. Salinity is one of the most important abiotic stresses, limiting crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (2). According to the FAO Land and Nutrition Management Service (2008), over 6% of the world's land is affected by either salinity or sodicity which accounts for more than 800 million ha of land. Saline soils are defined as those contain sufficient salt in the root zone to impose the growth of crop plants (4). However, since salt injury depends on species, variety, growth stage, environmental factors, and nature of the salts, it is difficult to define saline soils precisely. The USDA Salinity Laboratory defines a saline soil as having an electrical conductivity of the saturation extract (EC) of 4 dS m^{-1} or more. EC is the electrical conductivity of the 'saturated paste extract', that is, of the solution extracted from a soil sample after being mixed with sufficient water to produce a saturated paste. The most widely accepted definition of a saline soil has been adopted

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from as one that has an EC of 4 dS m^{-1} or more and soils with EC exceeding 15 dS m^{-1} are considered strongly saline.

Soil salinity, particularly due to NaCl, can be considered the single most widespread soil toxicity problem that global rice production faces at present. Salinity influences a number of physiological processes. These processes include photosynthesis, nutrient uptake, water absorption, root growth, and cellular metabolism (*5,* 6 and 7).

Rice is related as salinity sensitive crop (8). Although, salinity affects at all ~~stages of the~~ growth and development stages of rice ~~plant~~ crop and ~~crop~~ it ~~respons~~responses ~~es~~ to salinity varies with ~~growth~~ these stages and, concentration and duration of exposure to salinity. In the most commonly cultivated rice young seedlings were very sensitive to salinity (9). Salinity stress ~~is one of the crucial factors limiting growth and development of the plant and even~~ causes premature termination of life cycles by altering their morphological, physiological and biochemical features. The detrimental effect of salinity can be attributed to the decrease in osmotic potential of the medium and disturbance of the ion balance on plant growth and metabolism (10). Salinity in the growth medium causes significant ~~reduction~~ impacts ~~in~~ on plant growth parameters like leaf area, leaf length, root and shoot dry weight (11), decrease carotenoid and induce reduction in chlorophyll and photosynthetic activity. The harmful effect of salinity on growth, yield and metabolism depends on the type and levels of salinity (12), the stage of growth (13) and the plant species (14).

~~The objective of~~Therefore, this study ~~was~~ aims to compare growth and physiological responses, among four rice cultivars differing in salinity tolerance ability in order to gain more understanding of the mechanisms of salinity tolerance at the cellular levels which in turn determined growth and yield. The parameters which showed highly different pattern of responses to salinity between the salinity tolerant and salinity sensitive varieties are expected to be useful as potential targets for improvement of salinity tolerant rice.

2. MATERIALS AND METHOD

A Pot experiment was conducted during Kharif 2011 and 2012 at the Department of Crop Physiology, Narendra Deva University of Agriculture and Technology, Kumarganj, Faizabad (U.P.) under normal soil and saline conditions with EC 6.0 dSm^{-1} and ~~EC~~ 10.0 dSm^{-1}. The soil was silty loam in nature with having pH 8.7, organic carbon 0.22%, EC 3.6 dSm^{-1} and ESP 71 % concentration in soil solution and normal was sandy loam in nature soil having pH 7.8, organic carbon 0.48%, EC 1.9 dSm^{-1} and ESP 13 %. Three soil salinity level (*i.e.* Normal, EC 6.0 dSm^{-1} and EC 10.0 dSm^{-1}) and ~~for~~ four variety, namely, Pokkali, Narendra Ushar Dhan 3, IR 28 and IR 29 were taken ~~in~~ for thise experimentation. The experiment was laid down in CRD factorial with three replications.

EC 6.0 and 10 dSm^{-1} maintained the amount of NaCl for 30 liters of water. Soil saturation being 30%, NaCl 44.86 and 119.61 g, dissolved in demineralized water, respectively. Spread 100 Kg soil on a thick polyethylene sheet and spray NaCl solution so as to saturate it similar to that mentioned in the preparation of alkali soil. After completion of spraying, leave this soil for a few days for equilibration. Finally mix the soil and use or filling in pots or trays for experiment. Before filling in the pots check for the salinity level in the soil as per standard procedure.

The total chlorophyll content was estimated by using the method of Arnon~~,~~ ~~1949~~ (15) and expressed as mg g^{-1} fresh weight. The membrane stability index (MSI) and total carbohydrate (mg g^{-1} dry weight) were determined according to the specified methods described by Saadalla *et al*.~~,~~ ~~1990~~ (16) and Yemm and Willis~~, 1954~~ (17) ~~respectively~~. Catalase activity (unit g^{-1} Fr. Wt.) was assayed by calorimetrically method given in analytical biochemistry (18). Estimation of SOD spectrophotometrically according to the methods of Asada *et al*.~~;~~ ~~1974~~ (19) and expressed as

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enzyme unit g^{-1} fresh weight. Free proline content (mg g^{-1} fresh weight) in embryo axis was estimated spectrophotometrically according to the method~~s~~ of Bates *et al.*~~, 1973~~ (20).

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3. RESULT AND DISCUSSION

The results indicate that the plant height decreased both tolerant and susceptible varieties of rice in saline to normal soil. Minimum reduction in plant height was found in tolerant varieties i.e. Pokkali and Narendra Usar Dhan 3 at EC 6.0 and 10.0 dSm^{-1}. However, the magnitude of reduction was higher in plant height in susceptible varieties IR 28 and IR 29. Bhowmik *et al.*~~, 2009~~ (21) also observed differences in plant height probably results due to slow growth caused by osmotic stress imposed by a high concentration of salinity in rooting zone. The reduction of rice height in proportion to the increases salinity is due to the effect of osmosis stress in reduction of water and nutrient absorption including nitrogen which is necessary for plant height.

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Table 1-: Effect of different salinity levels of different growth and biochemical parameters of rice varieties

S. No.	Variety	Plant Height (cm) at maturity	Dry biomass plant^{-1}	Superoxide dismutase (unit g per g fresh weight per min)	Proline content	Membrane stability index (%)
1	Pokkali	131.13	30.36	493.55	475.69	47.99
2	Narendra Ushar Dhan 3	68.93	18.95	488.8	456.58	46.15
3	IR 28	63.50	16.61	402.37	404.60	40.51
4	IR 29	68.35	18.81	414.15	402.71	40.98
1	Normal	100.54	24.82	385.4	365.22	50.85
2	EC 6.0	84.02	21.81	457.1	445.81	43.89
3	EC 10.0	64.37	16.92	506.7	493.66	36.98
Sem±		V= 1.83 T=1.58 VXT= 3.16	V= 7.38 T=6.39 VXT=12.8	V= 7.45 T=6.46 VXT=12.9	V= 12.36 T=10.7 VXT=21.41	V= 36.1 T=31.3 VXT=62.6
CD at 5%		V= 5.36 T=4.64 VXT=NS	V= 7.38 T=6.39 VXT=12.79	V= 21.88 T=18.95 VXT=NS	V=36.29 T=3143 VXT=NS	V=106.2 T=31.3 VXT=NS

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Under the saline condition, reduction in biomass production is a common feature in crop plants. In this study; salinity caused a significant reduction in shoot dry weight of all the varieties compared with normal condition. Among varieties, the lowest reduction in biomass was found in Pokkali followed by Narendra Usar Dhan 3 indicating their tolerance to salinity, whereas, the most sensitive varieties are IR 28 and IR 29, which showed a higher reduction in plant biomass. Under both the treatment, *i.e.* E.C. 6.0 and 10.0 dSm^{-1}, the minimum reduction was observed by Pokkali followed by Narendra Usar Dhan 3, respectively in comparison to control. While, the maximum reduction was observed by IR 28 ~~respectively~~ in comparison to control. Under salinity stress, plant cell turgor pressure decreased and stomatal closure took place, resulting in a decrease in photosynthesis (22). Another cause of growth inhibition under NaCl stress could be an imbalance in uptake of mineral nutrients due to competition with Na^+ (21).

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Total chlorophyll content decreased with ~~increasing~~ increase in ~~of~~ salinity stress in all rice varieties (Fig.-1). At 6.0 dS–m^{-1}, Pokkali and Narendra Usar Dhan 3 consisted of comparatively higher amounts of total chlorophyll. Pokkali and Narendra Usar Dhan 3 showed lesser reductions, while severe reductions were observed in IR 28 and IR 29 due to salinity stresses at 6.0 and 10.0 dS~~-~~m^{-1}. The results however, clearly indicated that chlorophyll content was significantly influenced by increasing salinity. The reduction in chlorophyll content under salinity might be due to the loosened binding between chlorophyll and chloroplast protein (23). The reduction in chlorophyll depends on the varietal tolerance to salinity.

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Fig.1: Effect of different EC level of Height, Chlorophyll, Carbohydrate and Catalase activity of different rice varieties

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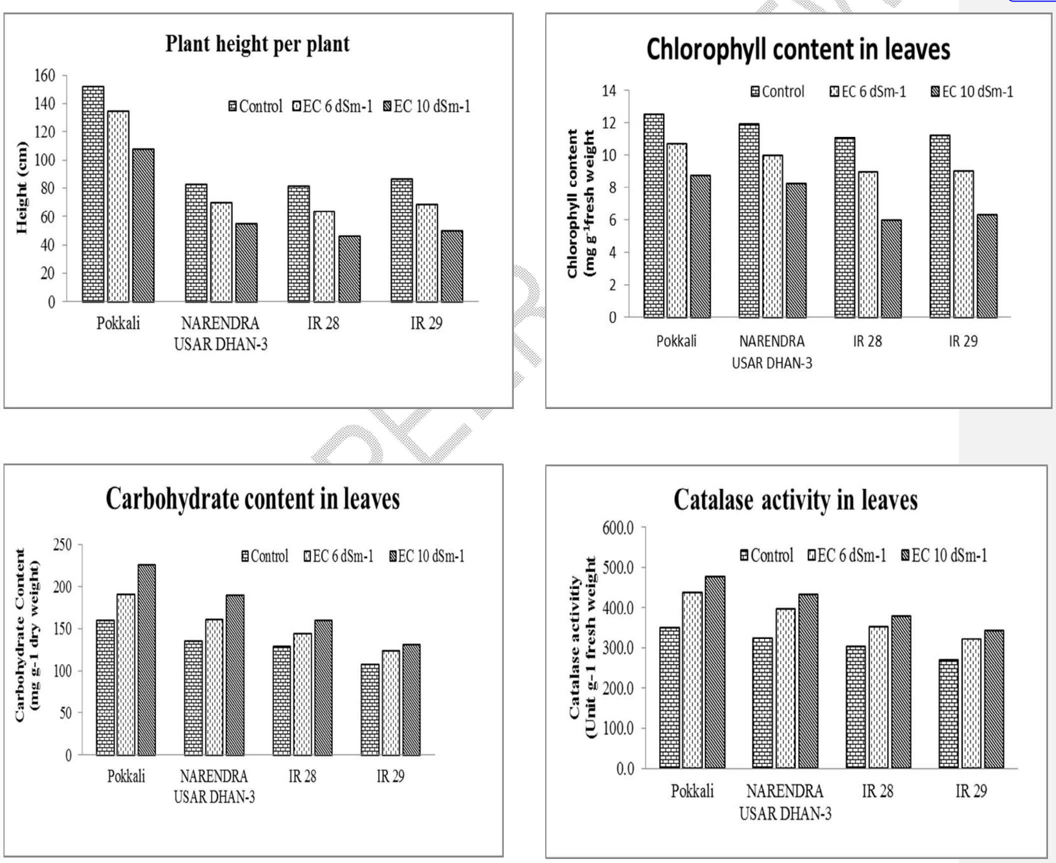

Under salinity levels, (E.C. 6.0 and 10.0 dSm^{-1}), highest membrane stability index was observed ~~by~~ in tolerant varieties *i.e.* Pokkali followed by Narendra Usar Dhan 3, while lowest membrane stability index was observed ~~by~~ in susceptible varieties IR 28. The amount of electrolyte leakage from leaf cells was greatly affected by salinity indicating that salinity leads to an increase in cell membrane permeability. The electrolyte leakage of all the varieties increased with increasing NaCl concentrations. Although under non-stressed conditions, electrolyte leakage of leaves did not vary among the varieties (24).

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The total carbohydrate in shoot decrease significantly under saline condition in all the rice varieties, however, highest total soluble carbohydrate was observed in Pokkali and Narendra Usar Dhan 3. IR 28 and IR 29 showed comparatively less carbohydrate content. Reduction in soluble carbohydrate under salinity may due to reduced hydrolysis of reserve polysaccharides or rapid utilization of total soluble sugars. Decrease in sugar content under salt stress has been also reported by a number of workers Prasad~~, 1990~~ (25) and Amer~~, 1999~~ (26).

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Table 2-: Effect of different salinity level of different ions ~~and~~ on yield attribute~~s~~ ~~parameters~~ of rice varieties

S. No.	Variety	length of panicle (cm)	No. of panicle ~~/~~plant^{-1}	No. grain / panicle^{-1}
1	Pokkali	18.72	3.76	156.49
2	Narendra Ushar Dhan 3	17.3	3.75	157.2
3	IR 28	14.73	3.41	141.73
4	IR 29	15.95	3.4	145.21
1	Normal	19.19	4.07	180.4
2	EC 6.0	16.79	3.66	145.63
3	EC 10.0	14.04	3.02	124.45
Sem±		V= 0.37 T=0.32 VXT= NS	V= 0.09 T=0.08 VXT= 0.16	V= 3.33 T= 2.88 VXT=5.76
CD at 5%		V= 1.08 T=0.94 VXT= NS	V= 0.27 T=0.23 VXT=NS	V=9.77 T=8.46 VXT=NS

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The minimum accumulation of proline content was observed by IR 29, where, as maximum accumulation in Pokkali at both the EC 6.0 and 10.0 dSm^{-1} with comparison to control. Proline has been assigned the role of cyst solute, a storage compound or a protective agent for cytoplasmic enzymes and cellular structure (27). Proline accumulation is a consequence of stress induced damage to cells (28).

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The activity of catalase and super oxide dismutase in rice leaves increased under saline condition. However, the magnitude of increase in above enzymes was higher in tolerant varieties than susceptible one. Reactive oxygen species (ROS) are regarded as the main source of damage to the cell of abiotic stress, such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl anion (OH^-) particularly synthesized in the chloroplast and mitochondria (29). Plant processes, antioxidant enzyme like SOD and CAT to protect against the damaging effect of ROS (19). Several factors associated with salinity stress can lead to an increase in reactive oxygen species (30).

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In plants, the role of proline may not be restricted to that of compatible osmolytes, but proline synthesized during water deficit and salinity stress may serve as an organic nitrogen reserve that can be utilized during recovery (31). The increase in accumulation of free proline plays an important in imparting a certain degree of salinity tolerance through osmoregulation.

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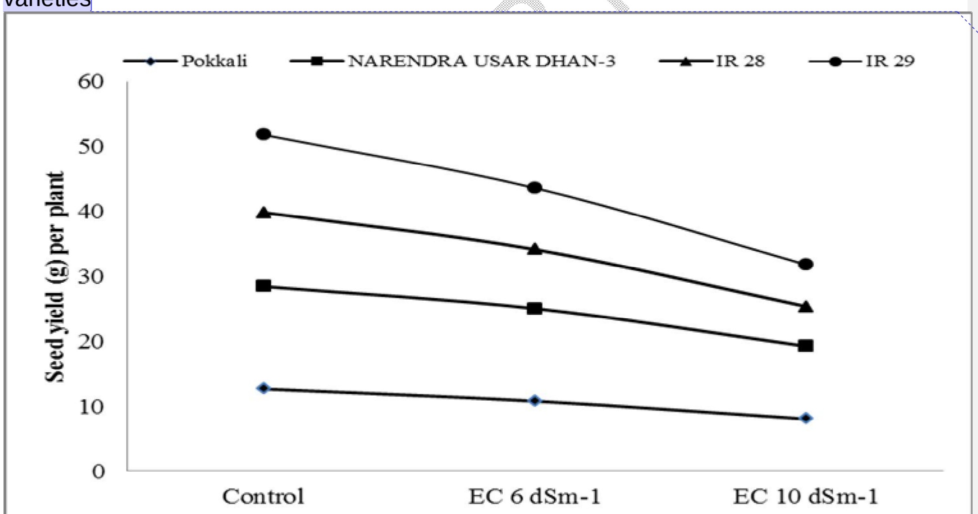
Free radical scavenging systems such as SOD can be a critical component of salinity tolerance because of their protection of chloroplast function under high salinity. Antioxidant enzyme plays a significant role in plants to protect them against the damaging effect to ROS generated during salinity stress (19). SOD catalyses, the dismutation of superoxide to H_2O_2, which is detoxified by catalase to water and oxygen. The minimum reduction catalase as well as SOD was observed by IR 29, whereas, maximum reduction was observed by Pokkali followed by Narendra Usar Dhan 3.

Salinity significantly reduced in the number of panicles plant^{-1}, number of grains panicle^{-1}, panicle length, and grain yield plant^{-1} of all varieties as compared to normal soil. The magnitude of reduction in all the traits was found very high in susceptible varieties (IR 28 and IR 29) as compared tolerant varieties (Pokkali and Narendra Usar Dhan 3) at both the levels of salinity (EC 6.0 and 10.0 dSm^{-1}). In this study, grain yield loss occurred due to effect of salinity in all varieties but yield performances of Narendra Usar Dhan 3 was better in all salinity levels than the other varieties in comparison with salt tolerant check Pokkali. Probable cause for lower grain yield in susceptible varieties was reduction in cell metabolic activities which limit the cell wall elasticity, and thus cell walls become rigid and consequently the turgor pressure of cell decreases. The other possible causes could be the shrinkage of cell contents, reduced development and differentiation of tissues, imbalanced nutrition, damage of membranes and disturbed avoidance mechanisms (32, ~~and~~ 33). The grain yield plant^{-1} of rice genotypes was significantly reduced under salinity stress (33). Similar result was also found by Mahmood et al.~~, 2009~~ (34) where rice grain yield of rice was significantly decreased with increasing salinity levels.

Fig-2. Effect of different levels of salinity on seed yield plant^{-1} of rice varieties



The severe inhibitory effects of salinity on fertility may be due to the differential competition in carbohydrates supply between vegetative growth and constrained its distribution to the developing panicles, whereas, the other is probably linked to reduced viability of pollen under stress condition, thus resulting failure of seed set (35). The decreased harvest index with the increase of salinity was consistent with this hypothesis. The reduction in yield and yield components under saline condition might be due to reduced cell division and differentiations leading to poor sink development. In addition, adverse effect of salinity stress on photosynthesis and translocation of metabolites to the reproductive sink could be some of the possible reasons for lower yield (36).

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4.CONCLUSION

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