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RESEARCH ARTICLE

VARIATION IN SODIUM, POTASSIUM AND CALCIUM CONTENT AND SODIUM/POTASSIUM RATIO IN SOME NATIVE RICE CULTIVARS OF NORTH KERALA, INDIA UNDER SALINITY STRESS

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ABSTRACT

NaCl induced salinity stress showed significant effects on sodium, potassium and calcium content and sodium/ potassium ratio in rice. There was a positive rise of sodium and calcium content whereas potassium content got significantly reduced due to salt treatment. The general trend was same in all the cases, but the quantum of variation differed from cultivar to cultivar. The concentration of sodium and calcium got increased in relation to increase in salt stress and the concentration of potassium and sodium/potassium ratio got reduced in the same pattern. The cultivars show different levels of variations in the case of such responses and these mechanisms make them differentially adapted to salt stress.

Key words: Salt stress, Sodium content, Potassium content, Calcium content, Sodium/potassium ratio.

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INTRODUCTION

Salinity is one of the causes of abiotic stress, ultimately leading to severe inhibition of plant growth, development and productivity[1]. High concentration of salts in soil accounts for large reduction in the yield of a wide variety of crops all over the world. About 1,000 million ha of land is affected by soil salinity, 7% of all land area[2]. Of the 1.5 billion ha that is cultivated, about 5% (77 million ha) is affected by salt[3]. Irrigated farming system provides about a third of the world's food requirement and it is estimated that about 20% of the irrigated area is salt affected especially in coastal areas as salt water enters them during high tide[4,5,6]. Plant response to external cues can involve molecular, biochemical, physiological or morphological changes, which must be balanced to attain optimal plant growth and productivity[7,8]. It includes ion regulation and compartmentalisation, antioxidant systems, plant hormones and osmoregulation[9,10]. Salt stress leads to severe inhibition of plant growth and development, membrane damages, ion imbalances due to Na^+ and Cl^- accumulation, enhanced lipid peroxidation and increased production of reactive oxygen species like superoxide radicals, hydrogen peroxide and hydroxyl radicals, which are scavenged by both enzymatic and non-enzymatic mechanisms and compartmentalisation of ions[7,11-16]. Salt stress damage in rice is mostly caused by the accretion of more Na^+ than that of Cl^-, unless the latter accumulates to very high concentrations in plant tissues[9,10]. Excessive amounts of salts, particularly sodium chloride (NaCl), in the soil induce osmotic and ionic effects, leading to modification in plant metabolism[8,17]. Almost in all the cases, salinity induced oxidative stress has been marked as a potent limiting factor for sustainability and proper growth and development in plants in irrigated and rain fed agriculture. Salt stress is also a problem in rain fed agriculture in coastal areas as salt water enters them during high tide[6]. Salinity is a major environmental threat for agricultural production and that affects ionic and osmotic as well as nutritional relation of plants. Ion channels are key players in maintaining ion homeostasis under salinity. Rice is considered relatively as a salinity tolerant species at germination stage, whereas the vegetative and early reproductive stages are most sensitive to salinity[6,18-23]. Under salty conditions, the mineral nutrition of most plants can be expected to be damagingly affected. The high concentration of sodium in soil solution alters the uptake of other nutrients like Na^+, K^+, Ca^{2+}, and Mg^{2+}, particularly potassium, and its accumulation in the protoplasm of plant tissues causes toxic effects[24]. Calcium is an essential plant nutrient and it plays a crucial structural role in cell walls and maintains membrane integrity[25]. The effects of salt stress on crop plants are influenced by the availability of

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plant nutrients, however, these effects vary with the type of each nutrient element[26]. The uptake and distribution of Na^+ and K^+ are affected by the availability and uptake of other nutrients such as Ca^{2+} and Mg^{2+} and their ultimate concentrations in plant tissues[27]. The ability to maintain favourable ratios of these essential nutrients in plant tissues affects the extent of their tolerance of salt stress[10]. This is substantiated by the considerable variation in concentrations of these elements (Na^+, K^+, Ca^{2+}, and Mg^{2+}) in tissues of sensitive and tolerant genotypes[28]. Many studies focused mostly on the effect of salt stress on the uptake and distribution of major plant nutrients (e.g. Na^+, K^+, Ca^{2+}, and Mg^{2+}) in plant tissues[29,30]. Low Ca^{2+}: Na^+ ratio in a saline environment may impair the selectivity of roots for ion uptake and it results in the passive accumulation of toxic ions such as Na^+ in the root and shoot[31]. Adequate calcium is, therefore, required in the external medium to maintain selectivity and integrity of plasma membrane[29,32]. In saline environments where plants take up excessive amounts of sodium at the cost of potassium, calcium ions play an important role in reducing sodium ion accumulation in plants and thus alleviate the deleterious effects by mitigating the toxic effects of sodium ion rather than the osmotic effects associated with salt stress[33-35].

Increased sodium content generally disturbs the nutrient balance and osmotic regulation and causes specific ion toxicity. Na^+ ion accumulation was significantly higher in salinity treatments compared with control for barley cultivars, and they were of the order of about 10 fold at 20 dSm^{-1} salinity level[36]. Reduction of sodium ions in the cytoplasm and the accumulation of osmolytes have been suggested as the two major mechanisms that underlie the tolerance mechanism[11]. Increase of Na and Cl concentration and decrease of potassium, phosphorus, nitrogen, calcium, and magnesium in different tissues of olive plant by increasing NaCl concentration in growing medium has been reported[37,38]. K content decreased significantly with increasing NaCl concentration in nutrient solution[39]. Potassium is a macronutrient for plants that is required for physiological processes such as the maintenance of membrane potential and turgor, activation of enzymes, regulation of osmotic pressure, stomatal movement and tropisms. Potassium uptake is usually inhibited under salt stress, because of its molecular resemblance to sodium ions, causing competition during active uptake. This could affect the rate of conversion of soluble sugars into starch when the uptake of K^+ and its concentration in plant tissues is reduced, as K^+ is needed for the catalytic activities of starch biosynthesis enzymes[40,41].

Studies have shown that Ca^{2+} is an important second messenger in eliciting responses to various signals, including many biotic and abiotic signals[42-46]. It appears that plants use Ca^{2+} as a second messenger more than any other known messengers in plants and animals. This is evident from the fact that nearly all signals (developmental, hormonal, and stresses) cause changes in cellular Ca^{2+}, primarily in the cytosol, and, in some cases, in the nucleus and other organelles[47]. Elevated levels of external Ca^{2+} can increase both growth and Na^+ exclusion of plant roots exposed to NaCl stress[48]. Thus adequate Ca^{2+} is required in the external medium to maintain the selectivity and integrity of cell membrane[49]. Supplemental Ca^{2+} may also have effects on intracellular membranes of root cells exposed to NaCl stress and may decrease NaCl induced vacuolar alkalization in root tissues by a Ca^{2+} effect on Na^+ efflux at the plasma membrane[50,51]. Proportion of Ca^{2+} in the external solution that is adequate under non-saline conditions becomes inadequate under saline-sodic conditions and may result in reduced yields due mainly to ion imbalance[52]. The osmotic pressure in the soil surpass the osmotic pressure inside the plant cells due to the presence of high salt content and thus, reduces the capability of plants to absorb water and minerals[53,54]. Excessive accumulation of salt ions, mainly Na^+ and Cl^-, in the leaves is a major contributory factor causing salt stress[55]. Plant cells have the ability to compartmentalise these ions in the vacuole. When Na^+ and Cl^- are sequestered in the vacuole of a cell, K^+ and organic solutes should accumulate in the cytoplasm and organelles to balance the osmotic pressure of the ion in the vacuole. The ionic balance of a growth medium rather than absolute sodium content determines the salt tolerance of a plant[40]. Almost in all the cases, salinity induced oxidative stress has been evident as a potent limiting factor for sustainability and proper growth and development in plants. Plants have developed several physiological and biochemical strategies to adapt to or tolerate osmotic stress conditions and deal with salt injury. The most sensitive plant component to salt stress has been identified as the photosynthetic apparatus[56]. Photosynthetic capacity which can be determined through the gaseous exchange and chlorophyll fluorescence measurements provides a good way to assess the effects of salt stress on plants and to gain insight on the behaviour of the photosynthetic machinery under stress[57-59]. It is reported that salinity inhibits photosynthesis and gas exchange capacity[60-63]. Under the above circumstances, an experiment has been carried out presently to study the variation in sodium, potassium and calcium ions and Na+/K+ ratio in some native rice cultivars of North Kerala, India.

MATERIALS AND METHODS

Germination of seeds and growing of plant materials

The experiment was conducted in the experimental rainout poly house of Department of Botany, University of Calicut, Kerala, India during the first crop season of 2013. Seven native cultivars of rice including five cultivars namely *Orthadian, Orkazhama, Kuthiru, Kuttusan* and *Chovvarian* collected from one of the saline rice habitats of North Kerala and two native rice cultivars namely *Kunhutty* and *Veliyan* collected from one of the non-saline rice habitats of North Kerala were used for the study. Plants were grown in Randomized Block Design with three replications.

Experimental treatments

The experimental treatment was started from the 45th day onwards using aqueous solutions of sodium chloride as described earlier Joseph and Mohanan[64].

Digestion of the plant materials

Leaves from all the treated rice plants and control were collected on the 90th day. Digestion and preparation of the plant material for the analysis was adopted from known protocols[65-67]. The leaves were washed to remove the dust particles and dried in hot air oven at 80^{o}C for 48 hrs. It was then crushed into fine powder using a grinding machine. 0.5 g of powdered plant leaf material was taken for digestion in a digestion tube and 10 ml conc. H_2SO_4 and a pinch of salicylic acid were added. The mixture was kept overnight and digested using a block digester in a digestion chamber by adding 5ml

H_2O_2 in every two hours, at a temperature of 340°C till the samples became clear. After the completion of digestion, the digestion tubes were taken from the blocks and allowed to cool. Each digested sample was transferred to 100 ml standard flask and the volume adjusted to 100 ml with deionised water. The salicylic acid forms a compound with the nitrates present to prevent loss of nitrogen. Actual digestion is started with H_2O_2 and in this step the larger part of organic matter is oxidized. After the decomposition of excess of H_2O_2, the digestion is completed by conc. H_2SO_4 at elevated temperature. In this process nitrogen is converted to NH_3 and phosphorus is converted to phosphate. It was kept to stand for settling the debris at the bottom and transferred to a small bottle and used for the estimation of the minerals using atomic absorption spectrophotometer.

Estimation of sodium, potassium and calcium

NaCl, KCl and $CaCO_3$ were used for the preparation of sodium, potassium and calcium standards respectively. They were dissolved in deionized water and made up to 1000 ppm stock solution. From the above stock solution standard solutions were prepared with double distilled water. Sodium and potassium were estimated with the help of flame photometer (Elico CL378) with LPG and oil free dry air. The equipment was first standardized with the above standards and after that, samples were fed to the instrument. The amount of minerals present in the leaf samples were expressed in mg/g dry weight of the tissue. The content of calcium was determined by atomic absorption spectroscopy using Atomic Absorption Spectrophotometer (Varian AA 240) with wavelength 422.70 nm, slit width 0.1 nm, acetylene - nitrous oxide gas. Standard solutions of 1, 2.5, 5, 10, 20 and 30 ppm were prepared from the standard solution of $CaCO_3$. The equipment was first standardized with the above standards and after that, samples were fed to the instrument. Based on the reading obtained for each sample and the concentration of each sample, the concentration of calcium was calculated. The amount of calcium present in the samples is expressed in mg/g dry weight of the tissue.

Statistical analysis

The analyses were repeated three times and the results were analysed statistically for analysis of variance. All data were represented by an average of the three replicates and the standard error (S.E.).The significance level was $P < 0.05$.

RESULTS

Salinity induced by sodium chloride showed significant effects on different ion concentrations in the rice plants studied (Table 2 and Fig. 1,3,5). Mean concentrations of sodium, potassium, and calcium and sodium/potassium ratio got significantly changed due to the effect of salinity treatments. There was a significant positive raise of sodium and calcium content in all the rice plants in relation to the variation of NaCl salt concentration in the growth medium. Both the salt tolerant and susceptible rice cultivars showed the above result. The highest accumulation of sodium was observed in *Orkazhama* followed by *Chovvarian,* two cultivars from the saline rice tracts of north Kerala. In contrast, potassium content was significantly reduced due to salt treatment in the case of all the cultivars. The highest reduction in potassium content was noted in *Kunhutty* followed by *Veliyan*, the cultivars from the non-

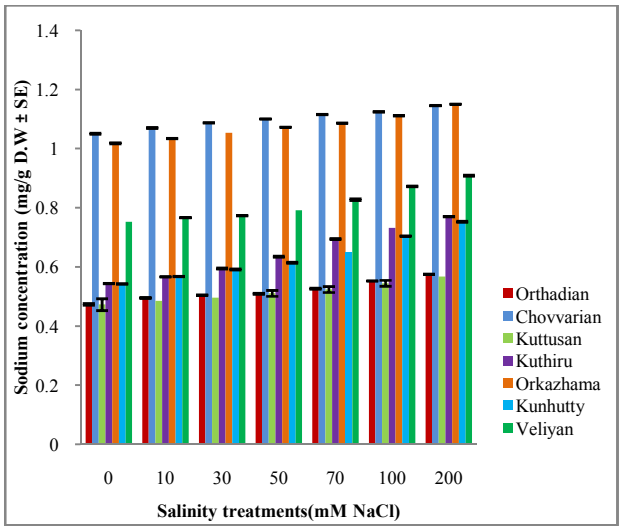

Fig. 1. Variation in Na^+ content in different rice cultivars under salt stress

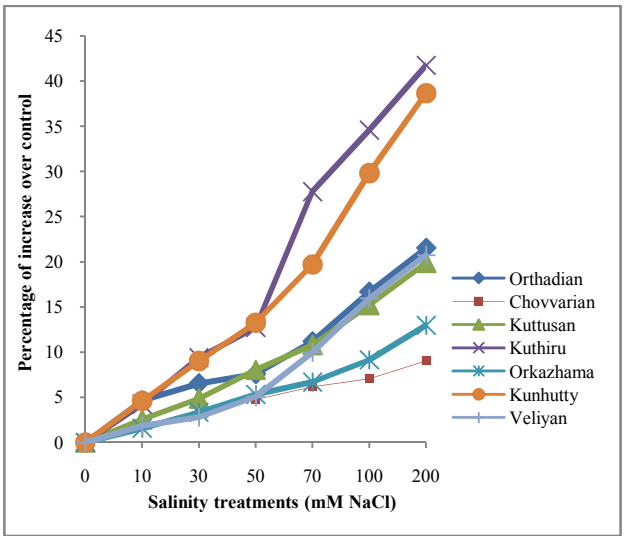

Fig. 2. Percentage of increase in Na^+ content in different rice cultivars under salt stress

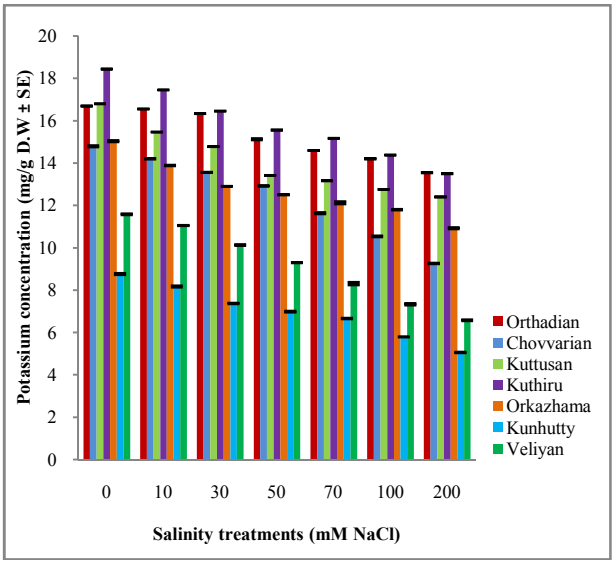

Fig. 3. Variation in K^+ content in different rice cultivars under salt stress

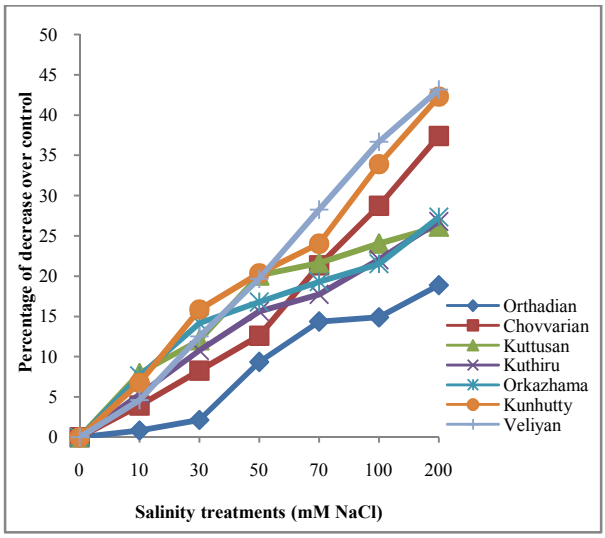

Fig. 4. Percentage of reduction in K^+ content in different rice cultivars under salt stress

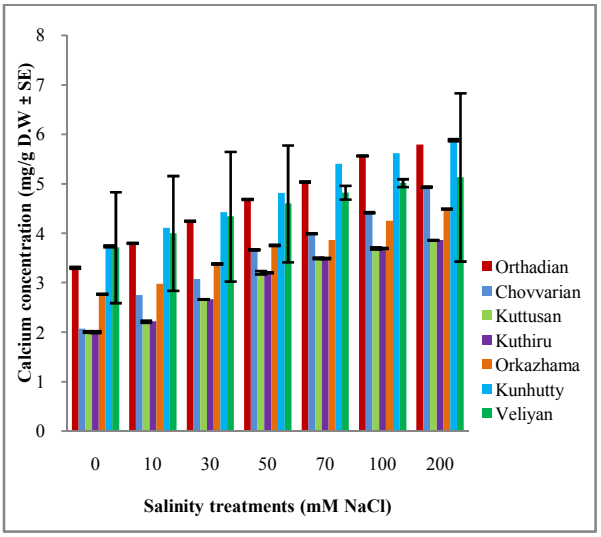

Fig. 5. Variation in Ca^{2+} content in different rice cultivars under salt stress

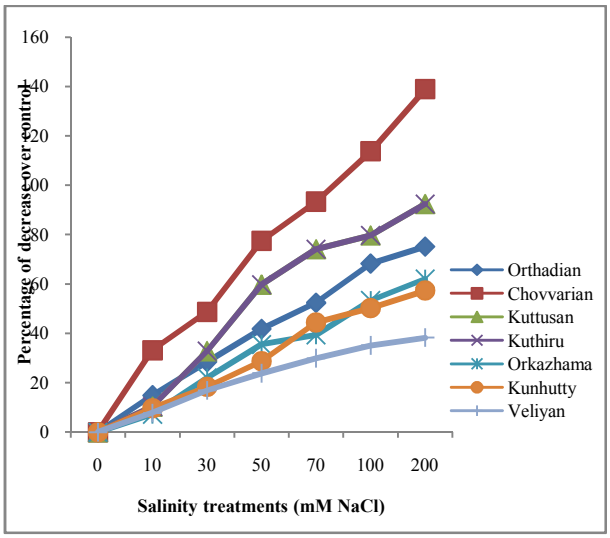

Fig. 6. Percentage of increase in Ca^{2+} content in different rice cultivars under salt stress.

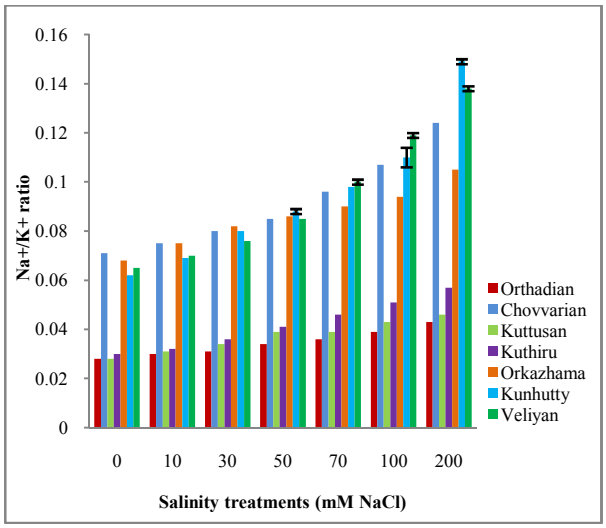

Fig. 7. Variation in Na^+/K^+ ratio in different rice cultivars under salt stress

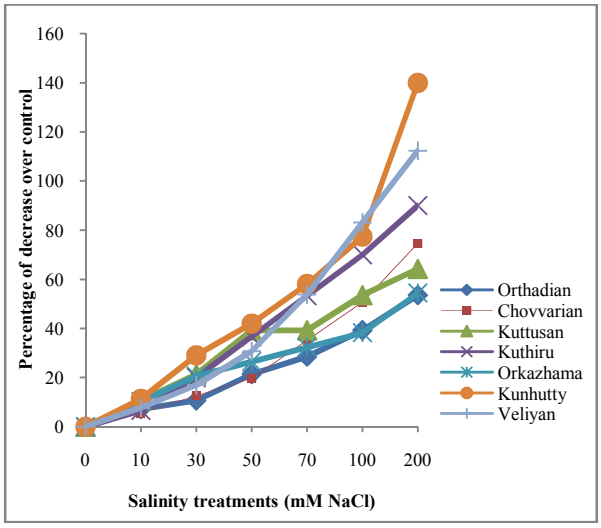

Fig. 8. Percentage of increase in Na^+/K^+ ratio in different rice cultivars under salt stress

saline area. There is a competition between Na and K absorption in rice plants under stress, resulting in a rise in Na^+/K^+ratio. With the increase in the mean concentration of sodium in the leaves due to salinity, the concentration of potassium is decreased significantly in all the cultivars studied in relation to the control plants. The highest mean concentration of calcium was observed in *Kunhutty* followed by *Orthadian* and the lowest concentration was observed in *Kuttusan* followed by *Orkazhama.*

DISCUSSION

Rice plants are relatively susceptible to salinity stress [68,69]. However, reports have indicated that both growth and yield characters of the plant are affected by salt stress[70]. The raise in Na^+ ion content and decline in K^+ion uptake disturbs ionic imbalance as observed in most species exposed to salt stress. The lowering of K^+ concentration in tissue may be due to direct competition between K^+ and Na^+ at plasma membrane, inhibition of Na^+ on K^+ transport process in xylem tissues and/or Na^+ induced K^+ efflux from the roots. K^+ and Ca^{2+} have been reported to be the major cations in cell organization as well as the major contributors to osmotic adjustment under stress conditions in several plant species[71,72].

Table 2. Concentration of Na^+, K^+, Ca^{2+} (mg g^{-1} Dry Weight) and Na^+/K^+ ratio in different cultivars under varying NaCl concentrations

Variety name/ Treatments	Na^+		K^+		Ca^{2+}		Na^+/K^+ ratio	
ORTHADIYAN	Mean±SE	CD @ 5%	Mean±SE	CD @ 5%	Mean±SE	CD @ 5%	Mean±SE	CD @ 5%
0 mM/0dSm^{-1}	0.474±0.003	0.014	16.695±0.004	0.142	3.308±0.02	0.086	0.028±0.000	0.001
10 mM/0.91 dSm^{-1}	0.496±0.002*		16.557±0.012		3.802±0.01*		0.030±0.000*	
30 mM/2.74 dSm^{-1}	0.505±0.001*		16.338±0.008*		4.249±0.01*		0.031±0.000*	
50 mM/4.57 dSm^{-1}	0.510±0.002*		15.133±0.038*		4.691±0.01*		0.034±0.000*	
70 mM/6.39 dSm^{-1}	0.527±0.002*		14.599±0.008*		5.042±0.01*		0.036±0.000*	
100 mM/9.13 dSm^{-1}	0.553±0.001*		14.210±0.019*		5.568±0.01*		0.039±0.000*	
200 mM/18.26 dSm^{-1}	0.576±0.001*		13.544±0.013*		5.794±0.00*		0.043±0.000*	
CHOVVARIAN								
0 mM/0dSm^{-1}	1.051±0.002	0.014	14.791±0.035	0.186	2.068±0.00	0.047	0.071±0.000	0.002
10 mM/0.91 dSm^{-1}	1.071±0.002*		14.209±0.016*		2.754±0.00*		0.075±0.000*	
30 mM/2.74 dSm^{-1}	1.088±0.001*		13.568±0.005*		3.076±0.00*		0.080±0.000*	
50 mM/4.57 dSm^{-1}	1.101±0.001*		12.927±0.022*		3.671±0.01*		0.085±0.000*	
70 mM/6.39 dSm^{-1}	1.116±0.001*		11.635±0.030*		3.998±0.01*		0.096±0.000*	
100 mM/9.13 dSm^{-1}	1.125±0.001*		10.542±0.026*		4.420±0.01*		0.107±0.000*	
200 mM/18.26 dSm^{-1}	1.146±0.001*		9.260±0.016*		4.940±0.01*		0.124±0.000*	
KUTTUSAN								
0 mM/0dSm^{-1}	0.473±0.001	0.010	16.803±0.014	0.163	2.009±0.02	0.075	0.028±0.000	0.001
10 mM/0.91 dSm^{-1}	0.485±0.001*		15.468±0.023*		2.217±0.00*		0.031±0.000*	
30 mM/2.74 dSm^{-1}	0.496±0.001*		14.779±0.002*		2.668±0.00*		0.034±0.000*	
50 mM/4.57 dSm^{-1}	0.511±0.001*		13.425±0.034*		3.209±0.01*		0.039±0.000*	
70 mM/6.39 dSm^{-1}	0.524±0.002*		13.167±0.015*		3.497±0.01*		0.039±0.000*	
100 mM/9.13 dSm^{-1}	0.545±0.001*		12.759±0.024*		3.699±0.01*		0.043±0.000*	
200 mM/18.26 dSm^{-1}	0.567±0.002*		12.407±0.008*		3.865±0.00*		0.046±0.000*	
KUTHIRU								
0 mM/0dSm^{-1}	0.544±0.001	0.012	18.443±0.002	0.064	2.009±0.02	0.591	0.030±0.000	0.001
10 mM/0.91 dSm^{-1}	0.567±0.001*		17.458±0.010*		2.217±0.00*		0.032±0.000*	
30 mM/2.74 dSm^{-1}	0.595±0.002*		16.456±0.008*		2.668±0.00*		0.036±0.000*	
50 mM/4.57 dSm^{-1}	0.635±0.002*		15.560±0.005*		3.209±0.01*		0.041±0.000*	
70 mM/6.39 dSm^{-1}	0.695±0.002*		15.172±0.004*		3.497±0.01*		0.046±0.000*	
100 mM/9.13 dSm^{-1}	0.732±0.000*		14.379±0.012*		3.699±0.01*		0.051±0.000*	
200 mM/18.26 dSm^{-1}	0.771±0.001*		13.504±0.009*		3.865±0.00*		0.057±0.000*	
ORKAZHAMA								
0 mM/0dSm^{-1}	1.019±0.002	0.008	15.035±0.035	0.252	2.775±0.01	0.056	0.068±0.000	0.002
10 mM/0.91 dSm^{-1}	1.035±0.001*		13.888±0.026*		2.978±0.00*		0.075±0.000*	
30 mM/2.74 dSm^{-1}	1.053±0.000*		12.907±0.007*		3.385±0.01*		0.082±0.000*	
50 mM/4.57 dSm^{-1}	1.073±0.001*		12.512±0.014*		3.760±0.01*		0.086±0.000*	
70 mM/6.39 dSm^{-1}	1.087±0.001*		12.135±0.058*		3.867±0.00*		0.090±0.000*	
100 mM/9.13 dSm^{-1}	1.112±0.001*		11.799±0.019*		4.255±0.00*		0.094±0.000*	
200 mM/18.26 dSm^{-1}	1.151±0.001*		10.928±0.030*		4.496±0.01*		0.105±0.000*	
KUNHUTTY								
0 mM/0dSm^{-1}	0.543±0.001	0.073	8.767±0.036	0.197	3.741±0.02	0.092	0.062±0.000	0.013
10 mM/0.91 dSm^{-1}	0.568±0.001		8.176±0.036*		4.112±0.00*		0.069±0.000	
30 mM/2.74 dSm^{-1}	0.592±0.002		7.377±0.017*		4.428±0.00*		0.080±0.000*	
50 mM/4.57 dSm^{-1}	0.615±0.002		6.983±0.029*		4.815±0.00*		0.088±0.001*	
70 mM/6.39 dSm^{-1}	0.650±0.000*		6.660±0.016*		5.402±0.00*		0.098±0.000*	
100 mM/9.13 dSm^{-1}	0.705±0.001*		5.793±0.004*		5.620±0.00*		0.110±0.004*	
200 mM/18.26 dSm^{-1}	0.753±0.002*		5.059±0.016*		5.885±0.02*		0.149±0.001*	
VELIYAN								
0 mM/0dSm^{-1}	0.753±0.000	0.012	11.586±0.026	0.276	3.714±1.12	0.089	0.065±0.000	0.004
10 mM/0.91 dSm^{-1}	0.767±0.001*		11.053±0.018*		4.002±1.16*		0.070±0.000*	
30 mM/2.74 dSm^{-1}	0.774±0.001*		10.137±0.025*		4.342±1.31*		0.076±0.000*	
50 mM/4.57 dSm^{-1}	0.791±0.000*		9.305±0.013*		4.600±1.18*		0.085±0.000*	
70 mM/6.39 dSm^{-1}	0.828±0.003*		8.313±0.063*		4.825±0.14*		0.100±0.001*	
100 mM/9.13 dSm^{-1}	0.873±0.001*		7.336±0.040*		5.018±0.08*		0.119±0.001*	
200 mM/18.26 dSm^{-1}	0.909±0.002*		6.585±0.029*		5.134±1.70*		0.138±0.001*	

* shows significant variation from the control value

In the present study, concentration of sodium and calcium got gradually increased in relation to increase in salt stress in all the cultivars studied. High levels of Na^+ inside the cells inhibit K^+ uptake and as a result it causes an increase in the Na^+/K^+ ratio[73]. Many of the deleterious effects of Na^+ seem to be related to the structural and functional integrity of membranes[74]. Plants under salt stress usually absorb Na^+ and simultaneously inhibit K^+ absorption. We observed that salt stress increased Na^+/K^+ ratio and the increase in Na^+ content reduced K^+ content in rice cultivars studied.

Salt stress reduces crop growth and yield in many ways. NaCl is the dominant salt, causes osmotic stress and ionic toxicity in soil. Under ordinary conditions the osmotic pressure inside the plant cells is higher than that in soil. Plant cells use this higher osmotic pressure to absorb water and other necessary minerals through the root cells from the surrounding soil[10,53]. On the other hand, Na^+ and Cl^- ions can enter into the cells and have direct toxic effects on cell membranes, as well as on metabolic activities in the cytosol[8,75]. These primary effects of salinity stress causes various secondary effects[76].

Potassium ion (K^+), one of the essential and most abundant cations in cells, and needs to be maintained within 100-200 mM range in the cytosol for efficient metabolic functioning[77-79]. K^+ activates more than 50 enzymes, which are not resistant to high cytosolic Na^+ and high Na^+/K^+ ratios[60]. So, maintenance of a low cytosolic Na^+/K^+ ratio is also significant for normal functioning of cells[80,81]. Apart from the external abiotic signals, a range of internal signals also modify plant cell growth and development. A cascade of complex events occurred for initial detection of signals and subsequent transduction of these signals to various physiological response is activated. The signal transduction normally triggered through second messengers leading to physiological response through modification of gene expression[82]. For the development of salinity tolerant crop species it is necessary to have very clear knoledge of the tolerance mechanisms available in plants. Changes in cellular Ca^{2+} and pH in specific organelles play very crucial role for activating various defence mechanisms for salinity tolerance. Recent reports suggest that such changes occur not only in cytosol, but also in other organelles including mitochondria, nucleus, vacuole, etc. It is likely that salinity stress elicits different parts of the cell to activate specific tolerance mechanisms and may vary between salinity tolerant and sensitive plant species[76].

Conclusion

Salinity tolerance of rice is cultivar specific and such genotypes have developed several adaptations varying from morphological to molecular so as to overcome the adverse effects of salinity stress. This variability provides valuable raw material both for crop improvement and physiological augmentation of certain desirable traits in rice.

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