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Cation dynamics and growth morphology of aromatic rice under salt stress

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Abstract

Salinity is an abiotic stress which affects plant's growth, morphology and cellular functions, and alters the ionic balance in the cytosol and vacuole in various crops. In the present investigation, salt stress was tested with three rice (*Oryza sativa* L.) varieties/genotypes viz. Pokkali, Sunduri Samba, and Khasa with three levels of salinity (0, 6, and 9 dSm^{-1}). In the investigation,growth, morphology, and ionic status in roots, stem, and leaf were observed under pot culture with three replicates in each treatment. The results of the experiment revealed that salt stress decreased in growth attributes namely, root dry matter, shoot dry matter, total dry matter and root: shoot ratio. Sunduri Samba showed higher values, but Khasa the lowest. Mineral ion content especially the cation in the plant tissue namely Na^+, K^+, Ca^{2+}, Mg^{2+} differed significantly due to salt stress as well as the K^+/ Na^+ ratio decreased in root and shoot due to salinity. Sunduri Samba maintained quite higher K^+/Na^+ than the susceptible genotype. Due to salinity, different growth characteristics and K^+ concentrations decreased but Na^+ concentration increased. Here the tolerant genotype Sunduri Samba accumulated higher amounts of K^+ than the susceptible genotype Khasa and altered the ionic ratios through distribution of Na^+ ion in shoot.

Keywords: Salinity; Aromatic rice; Growth; Morphology; Cation dynamics

Introduction

Among rice (*Oryza sativa* L.) aromatic varieties rank most sensitive to salinity. Not only is rice considerably less tolerant to salinity than other species, but salinity affects its growth, morphology and reproductive development quite differently. Photosynthesis inhibition (Sharma *et al*.,2005), water deficit, ion toxicity associated with excessive Cl^- and Na^+ (Afzal *et al.,* 2008; Patel and Pandey, 2008), interference with nutrition leading to nutrient imbalance (Misra *et al*., 1997). The Na^+ ion resulting from salinity is one of the major toxic components for most plants, including the major cereals (Tester and Davenport, 2003). Because Na^+ is chemically similar to K^+, a certain amount of its harmfulness is due to interference at some level with the transport and cytoplasmic functions of K^+. On the other hand, uptake of Na^+ badly affects the cellular K^+/Na^+ ratio and may lead to cell death.

Physiologically, NaCl-based salinity has several implications on plant growth and development which enforces an initial water deficit that results from the relatively high solute concentrations in the soil. This phenomenon causes ion-specific stresses resulting from altered K^+/Na^+ratio and builds up detrimental levels of cellular Na^+ and Cl^- concentrations finally the ion toxicity (Gupta and Huang, 2014). Growth and survival of vascular plants at high salinity depends on adaptation to both low water potentials and high Na^+ concentrations, as high salinity in the external solution of plant cells produces a variety of negative consequences. Considering NaCl as the primary inflictor of salinity, the penalty of a high extracellular NaCl concentration on plant is obvious (Parida and Das, 2005). Cellular ionic imbalance generated from excess Na^+ and Cl^- ions affect life processes negatively.

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Na^+ enters cell efficiently through K^+ transport channels, effectively replaces monovalent of cations like K^+ as cofactors of several enzymatic processes, and in doing so inhibits such enzyme activities. Therefore, plant survival and growth depend on adaptations to re-establish ionic homeostasis (Sengupta and Majumder, 2010).

High salinity results in increased cytosolic Ca^{2+} that is transported from the apoplast and intracellular compartments. The resultant transient Ca^{2+}increase potentiates stress signal transduction and leads to salt adaptation. A prolonged elevated Ca^{2+}level may, however, also pose a stress; if so, reestablishment of Ca^{2+} homeostasis is a requisite (Hasegawa *et al.*, 2000). Ca^{2+} can facilitate higher K^+/Na^+ selectivity, and interestingly, external Ca^{2+}can suppress the K^+ acquisition deficiency of salt overly sensitive (SOS). The transport systems involved in K^+ acquisition by roots and loading to the xylem have been summarized recently (Maathuis *et al.*, 1997). Na^+ efflux across the plasma membrane and compartmentalization into vacuoles or pre-vacuoles is mediated presumably by Na^+/H^+ antiporters, regardless of whether or not the membrane potential is inside positive or negative.

The role of Mg^{2+} is unclear under salt stress, since responses have been variable *in vitro*. For instance, it increases in rice callus (Ahmad *et al.,* 2009), decreases in soybean callus (Liu and Staden, 2001), and is unaffected in callus of *Sonneratia alba* Sm. (Yasumoto *et al.*, 1999).

Net Na^+ fluxes in anatomically distinct root zones of rice seedlings and analyzed parts of individual roots showing different Na^+ uptake where the anatomically distinct root zones contributed differently to the overall uptake of Na^+(Zhou *et al.*, 2011). The average Na^+ uptake in root zones in which Casparian strip of the endo-and exo-dermis were interrupted by initiating lateral root primordia was significantly greater than that at the root apex, where Casparian strip were not yet formed, or in the region where endo and exodermis with Casparian strip were not well developed. The major role of this specialized structure is to block the nonselective apoplastic bypass flow of ions and water through the cortex into the stele of the root (Yadav*et al.*, 1996). However, Casparian strip of the endo-and exo-dermis are not a perfect barrier to apoplastic fluxes of water, dissolved solutes and ions, as well as apoplastic tracer dyes.

Due to several complexities, the mechanisms of genetic control of salt tolerance in plants have not yet fully understood. In fact, there are several genes controlling salinity tolerance in the different species whose effect interacts strongly with environmental conditions. Thus, genetic variation can only be demonstrated indirectly, by measuring the responses of different genotypes. Probably the most suitable response to measure is growth or yield, especially at moderate salinities (Carillo *et al.*, 2011). Salt tolerance, in fact, can be usually assessed as the percent biomass production in saline versus control conditions over a prolonged period of time (this usually correlates with yield) or in terms of survival, which is quite appropriate for perennial species (Munns, 2002).Therefore, the present experiment was conducted to investigate the effect of salinity on growth, morphology and cation dynamic of aromatic fine rice varieties/genotypes under salt stress condition.

Materials and methods

This experiment was conducted at the shade-house of the department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh. Three rice genotypes viz. Sunduri Samba, tolerant check Pokkali and susceptible Khasa were used with three levels of salinity (0, 6, 9 dSm^{-1}) where salt was applied in three equal splits at two days interval.Plastic pots containing about 8 kg soils were used in this experiment. Fertilizers namely urea, triple super phosphate (TSP) and muriate of potash (MoP) were used for N, P and K at the rate of 0.19, 0.06 and 0.07 g kg^{-1}soil,respectively. The MoP and TSP were applied as basal doses before transplanting where urea was applied at topdressing in three equal splits. The experiment was laid out in two factorials completely randomized design(CRD) with three replications, where factor: 1 genotype-3, factor2: salinity level-3 (0, 6, 9 dSm^{-1}). The three genotypes in combination with two salinity levels were randomly assigned in 27 pots. After 14 days of salt deposition different physiological parameters were taken from the fresh leaf samples. At final harvest, roots were carefully cleaned with running tap water and finally washed with distilled water. Rice plants were separated into roots, shoots and seeds and rinsed repeatedly with tap water and finally with distilled water and thereafter dried in an electrical oven (Froilabo, France) at 71ºC to obtain a constant weight. The mean root dry weight pot^{-1} was calculated for each treatment. Shoot dry weight was calculated from the summation of leaf, stem and panicle dry weight. The total dry matter (TDM) was calculated from the summation of root and shoot dry matter.

For estimation of Na^+, K^+, Ca^{2+} and Mg^{2+}, oven-dried plant samples were ground in a Wiley Hammaer Mill (Willy PULVERIZB, Yoshida Seisakusho Co.Ltd), passed through 40 mesh screens, mixed well and stored in plastic vials.For acid digestion, oven-dried ground plant tissues (0.5g) and 5ml of di-acid (HNO_3 and $HClO_4$) mixture were taken in a digestion tube and left for 20 min then transferred to a digestion block and continued heating at 100ºC and left to digest until yellowish color of the solution turned to whitish color. Then the tubes were removed from heating chamber and left it to cool to room temperature. About 40ml of de-ionized water was carefully added to the digestion tubes and the contents filtered through Whatman no. 40 filter paper into a 100 ml volumetric flask and the volume was made up to the mark with de-ionized water. Content of Na^+, K^+, Ca^{2+}, Mg^{2+} were determined by Atomic Absorption Spectrophotometer (Model-170-30, Hitachi, Japan).

Results and discussion

The shoot dry weight (SDW) showed serious reduction with the increase of salinity however Pokkali recorded the tolerance reflecting the highest values in all the salinity levels (Table I). However, other two genotypes did not show any significant difference both at 6 and 9 dSm^{-1}.

Similarly,root dry weight (RDW) reduced the increment of salt stress both the genotypes Sunduri Samba and Khasa did not show significant difference both at 6 and 9 dSm^{-1} (Table I).

There was a similarity among SWD, RWD and total dry matter (TDM) where rise of salt stress declines the TDM (Table II). However, the genotypes Sunduri Samba and Khasa did not show significant difference both at 6 and 9 dSm^{-1}. But the genotype Pokkali maintained the highest scores among all the salinity levels.

It is apparent that salt stress resulted in marked change in root: shoot ratio (Table II). Saline conditions significantly altered the tissue concentration of different ion content both in shoot and root (Fig. 1). There was a sharp decrease in K^+ content as the salt stress increased. Here, the tolerant genotype Sunduri Samba had quite a large %K^+value at 6 dSm^{-1}than the susceptible genotype Khasa. However, at 9 dSm^{-1} both the tolerant (0.51) and susceptible (0.42) genotype showed little difference. Other than shoot, Sunduri Samba had higher % K^+ value than the susceptible genotype Khasa both at 6 dSm^{-1} and 9dSm^{-1}. One thing is clear that shoot maintained a higher concentration of K^+ than root.

Salinity caused dramatic increases in both root and shoot Na^+ content. Unlike the K concentration Pokkali had the lowest value of Na concentration both at 6 and 9dSm^{-1} (Fig. 2). However, the tolerant genotype Sunduri Samba and susceptible Khasa had statistically similar %Na^+ value both at 6 and 9dSm^{-1}.

In root, a different type of trend was observed, where Pokkali and Khasa had the highest %Na^+ value both at 6 and 9dSm^{-1} which were statistically non-significant. Moreover, Sunduri Samba maintained the lowest %Na^+ value both at 6 and 9dSm^{-1}.

Hussain *et al.* (2003) observed that Na^+ concentration increased in root and shoot of rice due to increase of its concentration in root medium and it significantly increased the Ca^{2+} concentration in root and tissues at higher salinity.

Table I. Effects of different level of salt stress on shoot and root dry weight of three selected rice genotypes

Treatment/ genotype	Salinity levels (dSm^{-1})			Salinity levels (dSm^{-1})		
	0	6	9	0	6	9
	Shoot dry weight (g pot^{-1})			Root dry weight (g pot^{-1})		
Sunduri Samba	12.07 a	2.33 b	0.93 b	3.13 b	0.42 b	0.15 b
Khasa	11.80 a	1.87 b	0.63 b	4.64 a	0.39 b	0.13 b
Pokkali	13.03 a	5.63 a	4.03 a	4.91 a	1.41 a	0.39 a
LSD	2.04	0.80	0.58	1.08	0.29	0.06
CV (%)	8.30	12.20	15.46	12.77	19.47	11.41

Values having same letter(s) in a column do not differ significantly at 5% level of significance by DMRT

Table II. Effects of different level of salt stress on total dry matter and root: shoot ratio of three selected rice genotypes

Treatment/ genotype	Salinity levels (dSm^{-1})			Salinity levels (dSm^{-1})		
	0	6	9	0	6	9
	Total dry matter (TDM) (g pot^{-1})			Root: shoot ratio		
Sunduri Samba	14.86 b	2.75 b	1.09 b	0.26 b	0.18 b	0.16 a
Khasa	16.77 a	2.25 b	0.76 b	0.38 a	0.17 b	0.13 a
Pokkali	17.61 a	7.05 a	4.42 a	0.38 a	0.26 a	0.18 a
LSD	1.764	1.03	0.46	0.06	0.02	0.06
CV (%)	5.38	12.78	10.91	7.33	13.26	16.82

Values having same letter(s) in a column do not differ significantly at 5% level of significance by DMRT

Table III. Effects of different levels of salt stress on Ca and Mg ion content in the shoot of three selected rice genotypes

Treatment/ genotype	Salinity levels (dSm^{-1})			Salinity levels (dSm^{-1})		
	0	6	9	0	6	9
	% Ca			% Mg		
Sunduri Samba	0.38 a	0.66 a	0.74 b	0.28 a	0.37 a	0.48 a
Khasa	0.39 a	0.59 ab	0.68 b	0.28 a	0.35 a	0.47 a
Pokkali	0.36 a	0.52 b	0.82 a	0.29 a	0.38 a	0.49 a
LSD	0.06	0.09	0.06	0.06	0.06	0.06
CV (%)	7.75	6.59	4.02	10.97	9.75	7.96

Values having same letter(s) in a column do not differ significantly at 5% level of significance by DMRT

It is apparent that K/Na ratio decreased with the increase of salinity and in that case, the shoot and root showed quiet similar type of results (Figure 3). Both in shoot and root, Pokkali had the highest values than the other genotypes both at 6 and 9 dSm^{-1}. On the other hand, in shoot, the tolerant variety Sunduri Samba had the higher value than Khasa at 6 dSm^{-1} level of salinity. However, at 9 dSm^{-1} both the genotypes showed similar type of results. In root, Sunduri Samba had the higher value than Khasa both at 6 dSm^{-1} and 9 dSm^{-1}.

It is apparent from Table (III,IV) that there was a significant variation in the relative value on Ca^{2+} content in shoots and roots of different rice genotypes due to salinity. However, shoot maintained the higher value than root. Both in shoot and root % Ca^{2+} content increased with the increment of salinity level (Table III, IV).

In this experiment, as the NaCl stress increased the % Mg^{2+} concentration increased in shoot, whereas in root there was a decreasing trend (Table III, IV). Here both in root and shoot, there was not any genotypic variations among all three genotypes both at 6 and 9 dSm^{-1}.

The key feature of the research indicates that the genotype Sunduri Samba maintained higher K^+/Na^+ ratio than the genotype Khasa. Again, in other measuring characteristics it got a convincing figure to tag it as a tolerant genotype. As the salt stress increases the morphological characteristics showed reduced values. In that case, the tolerant genotype Pokkali had higher values than the other genotypes. However, Sunduri Samba showed quiet higher values than Khasa. It is reported that shoot growth is more sensitive than root growth to salt- induced osmotic stress (Ehlting *et al.*, 2007) probably because a reduction in the leaf area development relative to root growth would decrease the

Table IV. Effects of different levels of salt stress on Ca and Mg ion content in the root of three selected rice genotypes

Treatment/ genotype	Salinity level (dSm^{-1})			Salinity level (dSm^{-1})		
	0	6	9	0	6	9
	% Ca			% Mg		
Sunduri Samba	0.26 a	0.39 a	0.48 a	0.32 a	0.23 a	0.13 a
Khasa	0.24 a	0.34 a	0.45 ab	0.33 a	0.24 a	0.16 a
Pokkali	0.25 a	0.33 a	0.41 b	0.37 a	0.22 a	0.18 a
LSD	0.06	0.06	0.06	0.06	0.06	0.06
CV (%)	10.98	7.71	8.38	8.21	9.80	14.77

Values having same letter(s) in a column do not differ significantly at 5% level of significance by DMRT

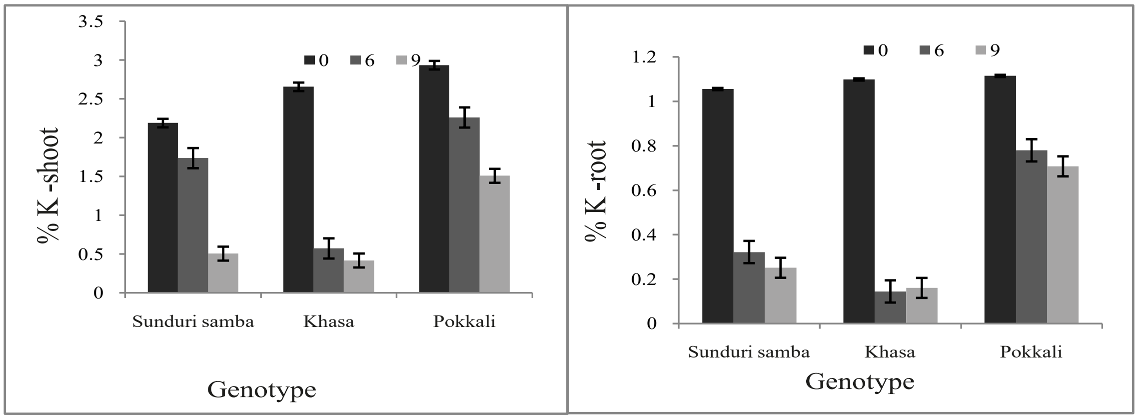

Fig. 1. Effects of salt stress on K^+ content of shoots and roots of three different rice plants. Level of salt stress 0, 6, 9 dSm^{-1}

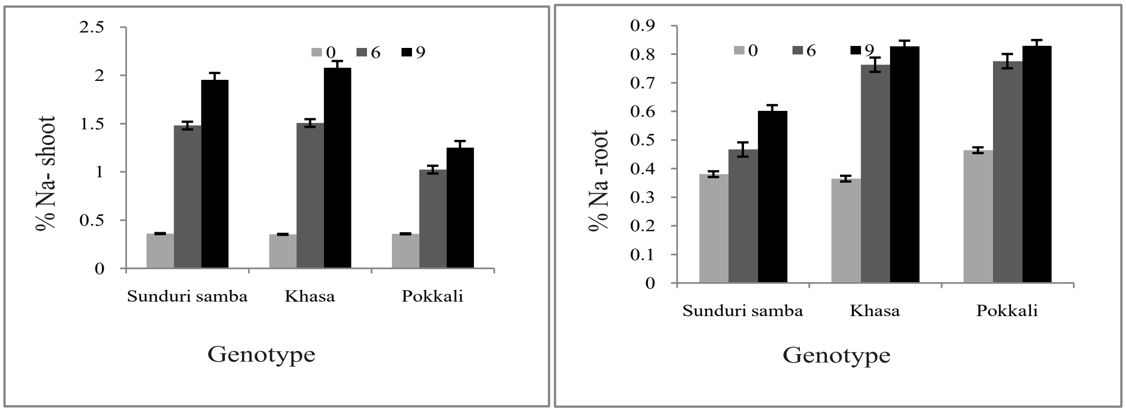

Fig. 2. Effects of salt stress on Na^+ content of shoots and roots of three different rice plants. Level of salt stress 0, 6, 9 dSm^{-1}

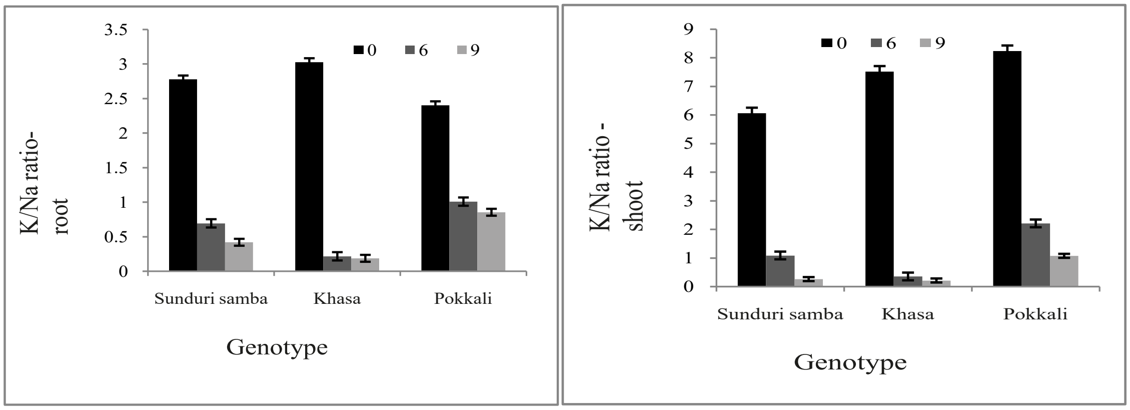

Fig. 3. Effect of salt stress on K^+/Na^+ ratio of shoots and roots of three different rice plants. Level of salt stress 0, 6, 9 dSm^{-1}

water use by the plant, thus allowing it to conserve soil moisture and prevent salt concentration in the soil (Munns and Tester, 2008). The reduction of root growth by NaCl was closely correlated with the increase in ionically bound peroxidase activity in roots of NaCl-stressed rice seedlings (Lin and Kao, 2001). Asch *et al.* (2000) reported that the growth reductions were attributed to unknown mechanisms and the apparent losses might be due to several factors, such as root decomposition and exudation, and energy consuming processes such as osmoregulation and interaction of Na and K from the transpiration stream and subsequent storage in leaf sheaths. The striking feature of this experiment was to measure different ion concentration against different salt stress. Here we demonstrated that all the genotypes got reduced amount of K^+ ion contentas the salinity level increased. In normal conditions, with cytosolic K^+ being around 150 mM and cytosolic K^+/Na^+ in a much lower range, this ratio is rather high (~100), enabling normal cell metabolism which includes cell osmoregulation, turgor maintenance, stomatal function, activation of enzymes, protein synthesis, oxidative metabolism and, in particular, photosynthesis (Marschner, 1995; Shabala, 2003). In terms of genotypic difference, the K^+ results appear to be much more sensitive indicators of salt tolerance than Na^+. It is not surprising therefore that the K^+/Na^+ ratio in plant tissues has often been suggested as a potential screening tool for plant breeders (Chen *et al.,* 2005). On the other hand, the K^+ accumulation in roots and shoots was slightly inhibited in response to salinity (Trinchant *et al.,* 2004).

The second pertinent point of this experiment was to measure Na^+ ion contentincrement with the increase of salinity.In terms of Na^+ content the scenario was indifferent than the K^+ content. Here Na^+ content increased with the increase of salinity and the genotype Khasa had the highest amount other than two genotypes which is not acceptable to select as a tolerant genotype. On the other hand, Sunduri Samba showed a bit agreeable value here. Salinity treatment caused immediate changes in net Na^+, K^+, H^+ and Ca^{2+} fluxes from barley root surface (Chen *et al*., 2005). They also stated that, salt stress significantly lowered K^+ content in both roots and shoots of all the cultivars studied, but the inhibitory effects of NaCl were much less in the tolerant cultivars than in the susceptible ones. Orcutt and Nilsen (2000) stated that higher amount of Na^+ in root than shoot was the result of the process of re-absorption. Velu and Srivastava (2000) found that Na^+ content increased K^+ content decreased in plant tissue irrespective of varieties at all stages of growth. They also found that the sensitive variety had high Na^+ content coupled with low K^+ content compared to moderately tolerant and tolerant variety. Increasing concentrations of NaCl from 50-150 mM progressively decreased root growth and increased Na^+ (Lin and Kao, 2001). Bal and Chattopadhyay (1989) observed that salt tolerant cultivar had greater Na^+ retention in roots and shoots. Salt tolerant cultivars had lower Na^+ and higher K^+ contents due to salinity (Won *et al*., 1992).

One of the key features of plant to salt tolerance is the ability of plant cells to maintain optimal K^+/Na^+ ratio in the cytosol.Under salinity, however, the K^+/Na^+ ratio falls dramatically. This occurs as a result of both excessive Na^+ accumulation in the cytosol and enhanced K^+ leakage from the cell, the latter resulting from NaCl-induced membrane depolarization under saline conditions. In that case, the genotype Pokkali and Sunduri Samba showed higher value than the susceptible genotype Khasa. Na^+/K^+ ratio may serve as an indicator of crop tolerance to stress as the increase of Na^+ in salt tolerant species is generally associated with a decrease in K^+ (Greenway and Munns, 1980). They also said that with the increase of NaCl in the medium the Na^+/K^+ ratio increased in stem and leaf and root up to 120mM NaCl and decreased back again at 160mM NaCl.

Ottow *et al.* (2005) found that the vacuolar Na^+ concentrations increased only after prolonged exposure and after cytosolic increases had occurred. However, in both apoplast and vacuole, accumulation of Na^+ resulted in decreases in Ca^{2+}. This might imply that Ca^{2+} is important in mediating salt adaptation and that this process occurs independently of osmotic stress or the necessity of acute defense against Na^+ toxicity. However, in our study, we administered the Ca^{2+} content increase with the increase of salinity. But the genotypic variation for Ca^{2+} content was no significant. In our study the increase of Mg^{2+} content was observed with the increase of salt stress. It is reported that the accumulation of Mg^{2+} in the leaf of the plant was helpful to maintain osmoregulation to protect the plant cells from osmotic shock caused by salinity (Greenway and Munns, 1980).

Conclusion

Due to salinity Na^+ concentration increased with the decrease in K^+ concentrations. Again K^+/ Na^+ ratio decreased due to salinity. In that case, tolerant genotype showed lower Na^+ concentration, higher K^+ concentrations and higher K^+/ Na^+. Concentration of Ca^{2+}, Mg^{2+} in shoot increased with the increase of salinity. In shoot, Sunduri Samba had the highest Ca^{2+} value and Pokkali had the lowest value at 6 dSm^{-1}. On the other hand, at 9 dSm^{-1} Pokkali had the highest value and Khasa had the lowest value. In root, at 6dSm^{-1} all three genotypes showed statistically similar values. But at 9 dSm^{-1} Sunduri Samba had the highest value and Pokkali had the lowest value. In case of Mg^{2+} both in root and shoot, there was not any genotypic variations among all three genotypes both at 6 and 9dSm^{-1}. But there was significant increase in Ca^{2+} Mg^{2+} content both in root and shoot as the salinity increases.

The tolerant genotype (Sunduri Samba, Pokkali) maintained higher K^+ ion concentration, K^+/Na^+ ratio and lower Na^+ ion concentration. Therefore, it seems that tolerant genotypes could adapt better in salt stress than the susceptible one.Considering the changes in different ion concentration and K^+/ Na^+ ratio Sunduri Samba can be used for further experimentation in salt tolerance mechanism.

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