

## MORPHO-PHYSIOLOGICAL RESPONSE OF RICE (*ORYZA SATIVA* L.) VARIETIES TO SALINITY STRESS

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### Abstract

Morpho-physiological response of 7 rice (*Oryza sativa* L.) varieties viz., Moroberekan, Co39, Azucena, Bala, IR64, Kalinga-III and Nipponbare was assessed under control and 100 mol m<sup>-3</sup> NaCl + 5 mol m<sup>-3</sup> CaCl<sub>2</sub> stress in a flood bench system in greenhouse. Physiological and growth parameters were recorded after 21 or 42 days of salt stress. The statistical analysis revealed significant differences among varieties for various traits associated with salt tolerance under salinity stress. Regarding physiological parameters significant higher accumulation of Na<sup>+</sup> was in Moroberekan (52.9 mol m<sup>-3</sup>), whereas lowest (14.1 mol m<sup>-3</sup>) in IR64, followed by Co39 (14.6 mol m<sup>-3</sup>) and Azucena (14.7 mol m<sup>-3</sup>). There was a significant negative correlation between leaf Na<sup>+</sup> and K/Na ratio in varieties under salt stress; however salinity and salinity×variety interaction had non-significant effect on leaf K<sup>+</sup> concentration in this study. Growth parameters were also negatively correlated with salinity stress. Among varieties the lowest reduction in shoot fresh (58%) and dry weight (36.5%) was in Co39, indicating its tolerance to salt stress, whereas, the most sensitive genotype was Azucena with highest reductions in shoot fresh (82%) and dry weights (77%). The use of salinity damage scores as assessment criteria in the appraisal of salt tolerance is discussed. A contrasting response of Co39 and Moroberekan to salt stress suggests the use of available recombinant inbred lines (RILs) population from Co39×Moroberekan, for mapping of QTLs associated with salinity tolerance.

### Introduction

Growth and yield reduction of crops is a serious issue in salinity prone areas of the world (Ashraf *et al.*, 1994; Ashraf, 2009). In addition a severe shortage of good quality water is also forcing growers to use poor quality ground water for irrigation, which further aggravated the salinity problem. Rice is a major cereal crop in Asia, providing food to more than half of the world population (Ma *et al.*, 2007). Salinity happens to be a major constraint to the sustainability and expansion of rice cultivation in areas where rice production has not kept up with increasing demand from a growing population. In Pakistan one million hectares of the rice growing area is salt-affected (Qureshi *et al.*, 1991) and salinity accounts for reductions of 64% in crop yield on these soils (Afzal *et al.*, 2005). Although rice is a salt sensitive crop (Maas, 1990) yet considerable genetic variability has been observed among and even within rice cultivars (Akbar *et al.*, 1972; Flowers & Yeo, 1981) which is helpful in selection and development of tolerant rice to salinity stress through genetic means (Nejad *et al.*, 2008). The development of salt tolerant cultivars of rice through conventional and modern molecular techniques would help solve the problem of world food security. The cultivation of tolerant varieties on saline fields may also reduce salinity by the process of biological reclamation.

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The selection of salt tolerant plants based on morphological markers has met with limited success (Ashraf *et al.*, 2008) and only few salt tolerant rice varieties have been released for use in the world (Flowers & Yeo, 1995). With advents in plant biotechnology as an aiding tool to conventional breeding, molecular markers technique has become a powerful tool to improve salt tolerance of rice by mapping and tagging of genes involved in the control of growth and yield under stressful environments.

It is prerequisite for any mapping experiment that parents of mapping population must have sufficient variation in the traits of interest. The development of new mapping populations is a laborious and time gaining process such as in the production of recombinant inbred lines (RILs) six to eight generations are required for the production of stable lines. It is possible that existing mapping populations can be used for mapping of different traits for which there is considerable genetic variation among the parents of a particular mapping population e.g., a mapping population developed for drought tolerance may be used for salt tolerance studies.

This study was designed with the objective to investigate the variation in salt tolerance of parental varieties of the available mapping populations at seedling stage under salt stress in greenhouse, because salt tolerance observed at early seedling and vegetative growth stages is of immense value in determining the ultimate salt tolerance and performance of plant species (Shannon, 1985).

## Materials and Methods

**Plant material:** Seven varieties of rice (*Oryza sativa* L.) included in this study were Azucena, Bala, Co39, IR-64, Kalinga-III, Moroberekan and Nipponbare. Azucena is a Japonica upland race from the Philippines and is drought resistant. Bala is an Indica, improved upland variety from East India and is also drought resistant. Co39 is a lowland Indica variety with medium height having originated from India. IR-64 is also Indica and improved paddy rice from IRRI, Kalinga-III is also japonica tall variety has its origin in CRRI, India. The Moroberekan is tropical upland japonica variety of long stature and also drought resistant. It has its origin in West Africa, while Nipponbare is also a japonica originated from Japan.

**Sowing of nursery:** The rice seeds of 7 varieties were sown in seed plug (P-84) trays (Plantpak, Malvern, England) having John Innes compost No. 1 (Corwen, Clwyd, U.K.) as growth medium. These trays were kept in the greenhouse on a bench and watered every day until transplantation. Twenty-three days after seed sowing, healthy and uniform seedlings of all varieties were transplanted in 2 litre capacity plastic pots (one seedling/pot) lined with porcelaine cloth and filled with John Innes No. 1 compost. After transplantation plants were left on the benches in green-house for one week to establish their root system.

**Growth conditions in greenhouse:** Supplementary 400 Watt high-pressure sodium vapor lamps were used to maintain a minimum photon flux density of photosynthetically active radiation (400 – 700 nm) of  $350 \mu\text{mol m}^{-2} \text{s}^{-1}$  during the 16 hour photoperiod. The minimum temperature was maintained at 25°C during the photoperiod and 20°C during the dark period.

**Experimental protocol:** Flood bench system is comprised of plastic tanks (1.0×1.0×0.25 m<sup>3</sup>) each containing 24 pots of two liter capacity. Tanks with plant pots were placed on an iron frame, approximately one meter high from the greenhouse floor. Water reservoirs

were placed on the floor underneath the tanks. Submersible electric pumps were placed in water reservoirs to pump water into tanks to a height of one meter. There were two connections of the water to each tank, one for water to enter into tank from the reservoir and the other for water to drain from the tank to reservoir. Pots were placed into each flood bench tank, on upturned empty p-576 plug trays at the bottom of each tank, to improve drainage in a randomized layout with three replications. The plants were flooded once a day for at least 15 minutes, twice on hot days and also twice when plants were fully grown in size.

Nutrient solution having plant food Phostrogen (Phostrogen, Corwen, Wales, U.K.) @  $1\text{ g L}^{-1}$  plus  $0.5\text{ ml L}^{-1}$  micro-nutrients (Hoagland & Arnon, 1950). Consideration was also given to the addition of silicon to solutions (Epstein, 1994; Gong *et al.*, 2006) and  $0.1\text{ ml L}^{-1}$  Sodium silicate were supplied to the plants with the irrigation solution. Silicon has role in protection from fungal diseases in rice.

Phostrogen is a fertilizer containing, N=14%, P=10%, K=27%, MgO=2.5% and  $\text{SO}_3=11\%$ . After establishment of seedlings salt stress was started in daily increments of  $25\text{ mol m}^{-3}$  and  $1.25\text{ mol m}^{-3}$  for NaCl and  $\text{CaCl}_2$  respectively to reach the final level of  $100\text{ mol m}^{-3}$  NaCl +  $5.0\text{ mol m}^{-3}$   $\text{CaCl}_2$ . Except for the controls, salts were added to the water reservoirs and quantity of salts was dependent on the required concentrations of salinity that has to be maintained. Salinity level was maintained in the solution by recording the electrical conductivity (EC) with portable waterproof conductivity meter (Hanna Instruments, U.K) in each reservoir. Seven rice varieties including Co39 and Moroberekan were evaluated for salt tolerance in a flood bench system at Pen y Ffridd Research Station, University of Wales, Bangor, UK.

**Sample collection of leaf:** After 21 days of salt stress ( $100\text{ mol m}^{-3}$  NaCl +  $5.0\text{ mol m}^{-3}$   $\text{CaCl}_2$ ), youngest fully expanded leaf was sampled from each plant for ion analysis. The samples were washed with distilled water, dried with tissue paper and stored in labeled,  $1.5\text{ cm}^3$  microcentrifuge tubes (Sigma Chemicals, UK) at  $-20^\circ\text{C}$  in the commercial freezer at least for one week.

**Sap extraction:** The microcentrifuge tubes having frozen samples were taken out of the freezer and after thawing for some time, the sample was crushed with a stainless steel rod with a tapered end. Two holes were made with the help of a pin, one at the base and another at the top in the cap of the tube. Each tube was then placed in another labeled empty microcentrifuge tube of the same dimensions ( $1.5\text{ cm}^3$ ) and samples were centrifuged at 8000 rpm (Model 5804R, Germany) for 10 minutes. The tissue sap was collected in the lower microcentrifuge tube and upper tube was discarded (Gorham *et al.*, 1997). The leaf sap was analyzed or stored frozen for subsequent analysis.

**Analysis of ionic concentrations:** The sap samples were diluted in dilution tubes with distilled water and  $\text{Na}^+$  and  $\text{K}^+$  content in sap of tissues were analyzed with a PFP-7 flame photometer (Jenway, UK).

**Measurement of biomass production:** After 42 days of salt stress, ( $100\text{ mol m}^{-3}$  NaCl +  $5.0\text{ mol m}^{-3}$   $\text{CaCl}_2$ ), the shoot portion of plants was harvested and shoot fresh weight (g/plant) was taken on digital balance. The fresh shoot samples were stored in labeled paper bags and dried in oven at  $70^\circ\text{C}$ , until constant weight and oven dried weight (g/plant) was recorded. The data about number of tillers per plant were also recorded at the time of harvesting.

**Measurement of % shoots water content:** Water contents of the shoot were calculated on fresh weight basis by subtracting the oven dry weight from shoot fresh weight and dividing with shoot fresh weight.

**Damage scoring of leaves:** After 21 days of salt stress damage scoring was done using the scale (Modified Lee, 1995) from 1 to 5. The plant with no visible damage was scored as 1 and with maximum damage due to salts as 5.

**Statistical analysis:** The factorial experimental design with 7 genotypes and two salinity levels was arranged in a completely randomized design (CRD) with three replications. The data were analyzed using the software package MSTAT-C or SPSS for Windows release 11.5.1 (SPSS, 2002). Relationships between the physiological and growth parameters were analyzed.

## Results

**Salinity effects on growth parameters:** Salinity caused a significant reduction in shoot fresh weight of the varieties, compared with control (Fig. 1a). Under salt stress the maximum shoot fresh weight was produced by Kalingha-III (31 g/plant), whereas, minimum in Bala (18.5 g/plant) after 42 days of stress. The highest reduction in shoot fresh weight under salinity compared to control was recorded in Azucena (82%), whereas, lowest in Co39 (58%).

With increase in salinity (100 mol m<sup>-3</sup> NaCl) a significant reduction in shoot dry weight has been observed in all varieties (Fig. 1b). Among varieties the highest shoot dry weight was obtained in Kalinga-III (8.45 g/plant), whereas, minimum in Moroberekan (3.85 g/plant) under salt stress, however on relative basis the minimum reduction in shoot dry weight in response to applied salt stress was recorded in Co39 (36.5%) which was at par with IR64.

The data about shoot fresh/dry weight ratio (Fig. 1c) was highest in Moroberekan (4.42) and minimum in Nipponbare (3.06) under salt stress. On relative basis, the minimum reduction in shoot fresh/dry weight ratio was observed in Azucena (17%) and maximum in IR64 (37.2%).

Salinity caused a significant reduction in number of tillers plant<sup>-1</sup> compared with control (Fig. 2a). The maximum number of tillers was recorded in IR64 (15.3) at 100 mol m<sup>-3</sup> NaCl, whereas, minimum in Azucena (5.7). On relative basis, the maximum reduction in tillers was observed in KIII (49.4%), whereas, minimum in Co39 (3.6%). A decrease of 37.5% in tillers was observed in Moroberekan under salt stress, compared with control.

**Ranking of cultivars for salinity damage scores:** Salinity damage scoring (SDS) was done after 21 days growth after salt stress. The data regarding damage scoring are presented in Fig. 4. There is no unit for leaf damage scores because these are the comparative values which were given based on plant vigor, chlorosis, necrosis and their general physical health conditions. The varieties were classified into five different categories from no visible salinity damage (Score-1) to severe salinity damage (Score-5). All plants were scored following a modified standard evaluation system (MSES) (Lee, 1995) for salt tolerance (Table 1). The maximum value of salinity damage score (SDS) was observed in Moroberekan (4.33) whereas minimum in Co39 (1.33) after 21 days exposure to full salt stress.

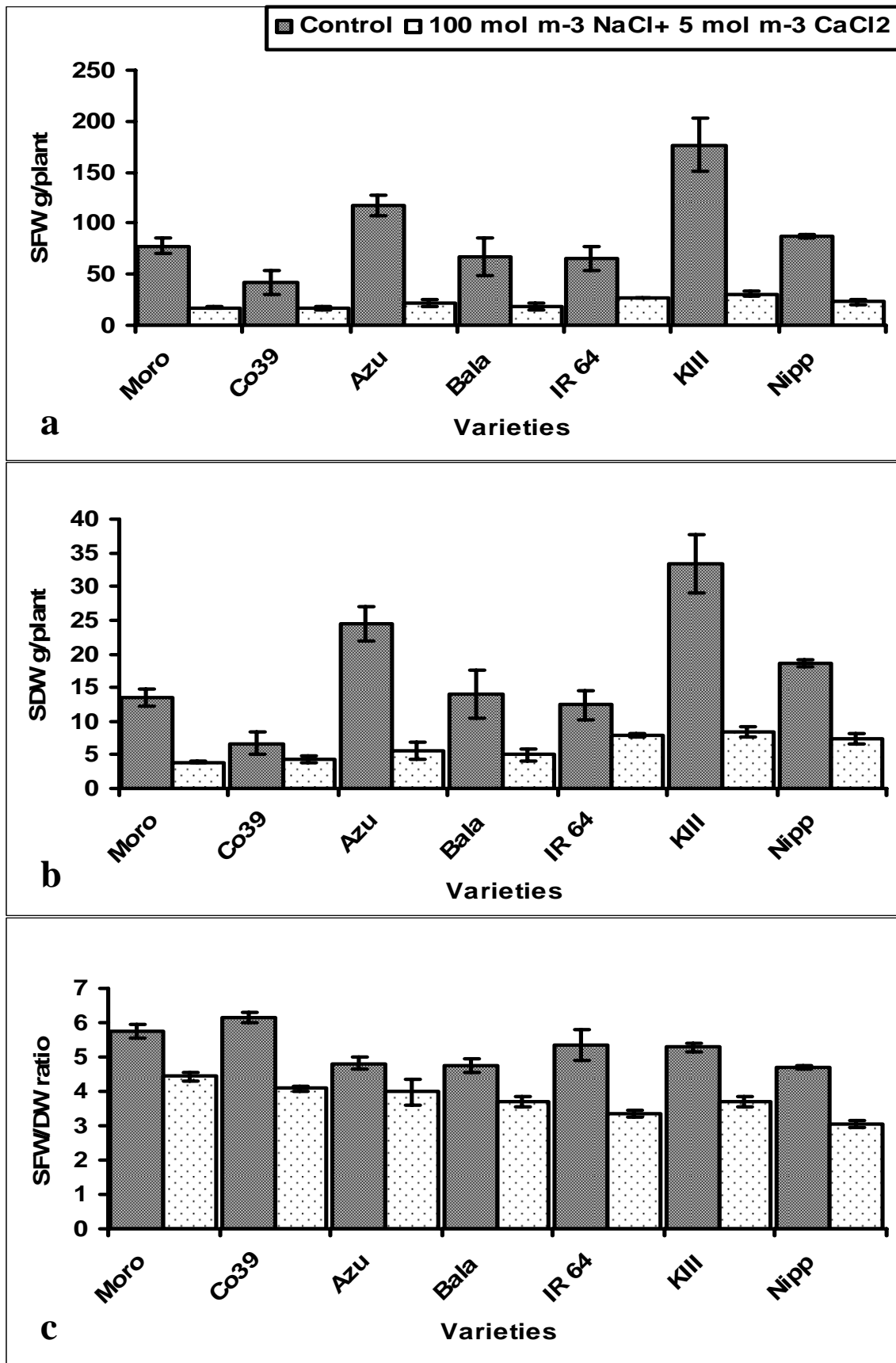


Fig. 1. Effect of salinity (100 mol m<sup>-3</sup> NaCl + 5 mol m<sup>-3</sup> CaCl<sub>2</sub>) on shoot fresh, shoot dry weight and shoot fresh - dry weight ratio of seven rice varieties. Error bars represent standard error of mean (n = 3).

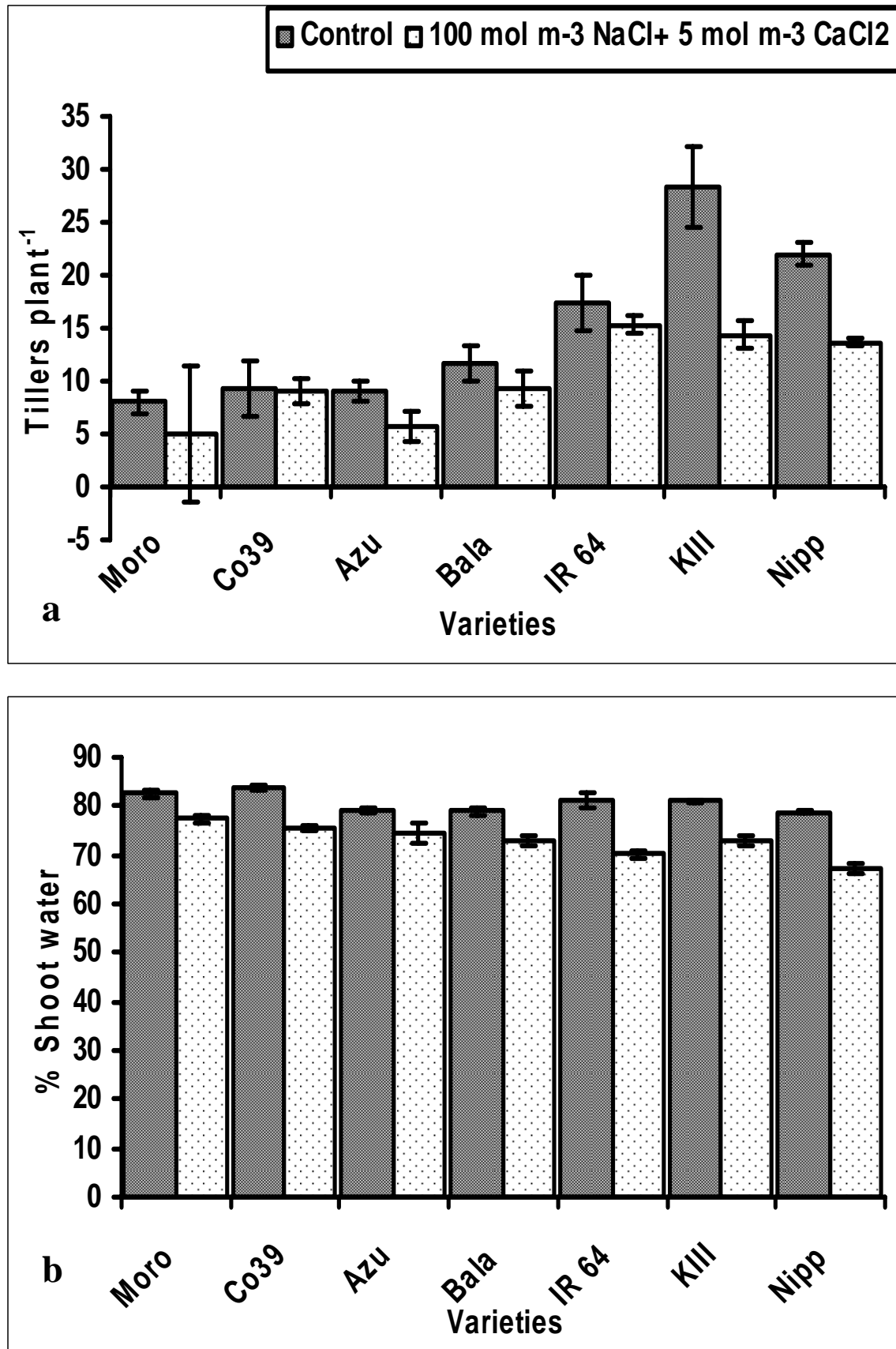


Fig. 2. Effect of salinity (100 mol m<sup>-3</sup> NaCl + 5 mol m<sup>-3</sup> CaCl<sub>2</sub>) on number of tillers plant<sup>-1</sup> and shoot water contents (%) of seven rice varieties. Error bars represent standard error of mean (n = 3).

**Table 1. Scale used for the salinity damage scoring (SDS) of plants under salt stress (Modified from Lee, 1995).**

Sr. No	Details of salinity damage	Given score
a	No visible salinity damage to leaves	1
b	Slight salinity damage to leaves	2
c	Moderate salinity damage to leaves	3
d	High salinity damage to leaves	4
e	Severe salinity damage to leaves, almost all leaves were dying	5

**Table 2. Mean squares of different traits studied in rice under control and 100 mol m<sup>-3</sup> NaCl + 5.0 mol m<sup>-3</sup> CaCl<sub>2</sub> salt stress (n = 3).**

Source of variation	Na <sup>+</sup>	K <sup>+</sup>	K <sup>+</sup> /Na <sup>+</sup>	SFW	SDW	SFW/DW	Tillers plant <sup>-1</sup>	Shoot water %	SD score
Salinity	6377.6**	2374.4 <sup>NS</sup>	397460.8**	49087.3**	1391.6**	23.4**	238.1**	634.7**	36.21**
Variety	315.0**	13166.4**	3150.6**	3502.8**	152.1**	1.24**	198.7**	35.1**	1.71**
Salinity × Variety	328.4**	824.1 <sup>NS</sup>	2451.0**	2487.8**	93.5**	0.32*	34.1**	10.7*	1.71**

\*\* = Highly significant at p≤ 0.01 level

\* = Significant at p≤0.05 level

NS = Non significant

**Salinity effects on physiological parameters:** Data regarding Na<sup>+</sup> concentration (mol m<sup>-3</sup>) in expressed leaf sap of seven rice varieties (Moroberekan, Co39, Azucena, Bala, IR64, Kalinga-III and Nipponbare) after 21 days exposure to salt stress of 100 mol m<sup>-3</sup> NaCl + 5.0 mol m<sup>-3</sup> CaCl<sub>2</sub> and non saline conditions are shown in (Fig. 3a). Analysis of variance revealed that salinity, varieties and salinity×variety interaction had highly significant (p<0.01) effect on sap leaf Na<sup>+</sup> (Table 2). The variation in leaf Na<sup>+</sup> was highly significant among varieties under salt stress; however less variation was recorded in control [Fig. 3 (a)]. Among rice varieties, the highest conc. of Na<sup>+</sup> was found in expressed leaf sap of Moroberekan (52.9 mol m<sup>-3</sup>) which is significantly higher than other rice varieties, however, the lowest Na<sup>+</sup> (14.1 mol m<sup>-3</sup>) was observed in the leaf of IR64, followed by Co39 (14.6 mol m<sup>-3</sup>) and Azucena (14.7 mol m<sup>-3</sup>). The varieties KIII, Bala and Nipponbare were intermediate (range 20 –37 mol m<sup>-3</sup>) in the accumulation of leaf Na<sup>+</sup> under salt stress. Moroberekan accumulated approximately 5 times higher Na<sup>+</sup> in expanded leaf compared with Co39 under salt stress.

Fig. 3 (b) shows the varietal differences in the maintenance of K<sup>+</sup> concentration in the leaf sap under saline and non-saline (control) conditions. For K<sup>+</sup> concentration in expressed leaf sap, the analysis of variance showed that the effect of variety was highly significant (p<0.01), while salinity and salinity×variety interaction was non significant, respectively (Table 2). The leaf K<sup>+</sup> was decreased under salinity compared to control in Moroberekan, Co39, Azucena, Bala and Nipponbare, whereas, it was increased in IR64 and Kalinga-III (Fig. 3. b). Under salt stress the highest K<sup>+</sup> was recorded in the leaf sap of Co39 (335.9 mol m<sup>-3</sup>), whereas, minimum in Azucena (192.9 mol m<sup>-3</sup>).

The K<sup>+</sup>/Na<sup>+</sup> ratio in the leaf sap of different rice varieties, under control and salinity is exhibited in Fig. 3(c). There is large reduction in K<sup>+</sup>/Na<sup>+</sup> ratio under salt stress compared to control, however, varieties showed variation in the reduction of K<sup>+</sup>/Na<sup>+</sup> ratio in response to applied salt stress. Under salt stress the highest value of K<sup>+</sup>/Na<sup>+</sup> ratio was recorded in Co39 (33.0), whereas, lowest in Moroberekan (4.22).

In response to applied salinity there was decrease in the shoot water contents (%), compared with control in all the varieties (Fig. 2b). The maximum shoot water (%) was recorded in Moroberekan (77%) followed by Co39 (76%), whereas, minimum in Nipponbare (67%) under salt stress.

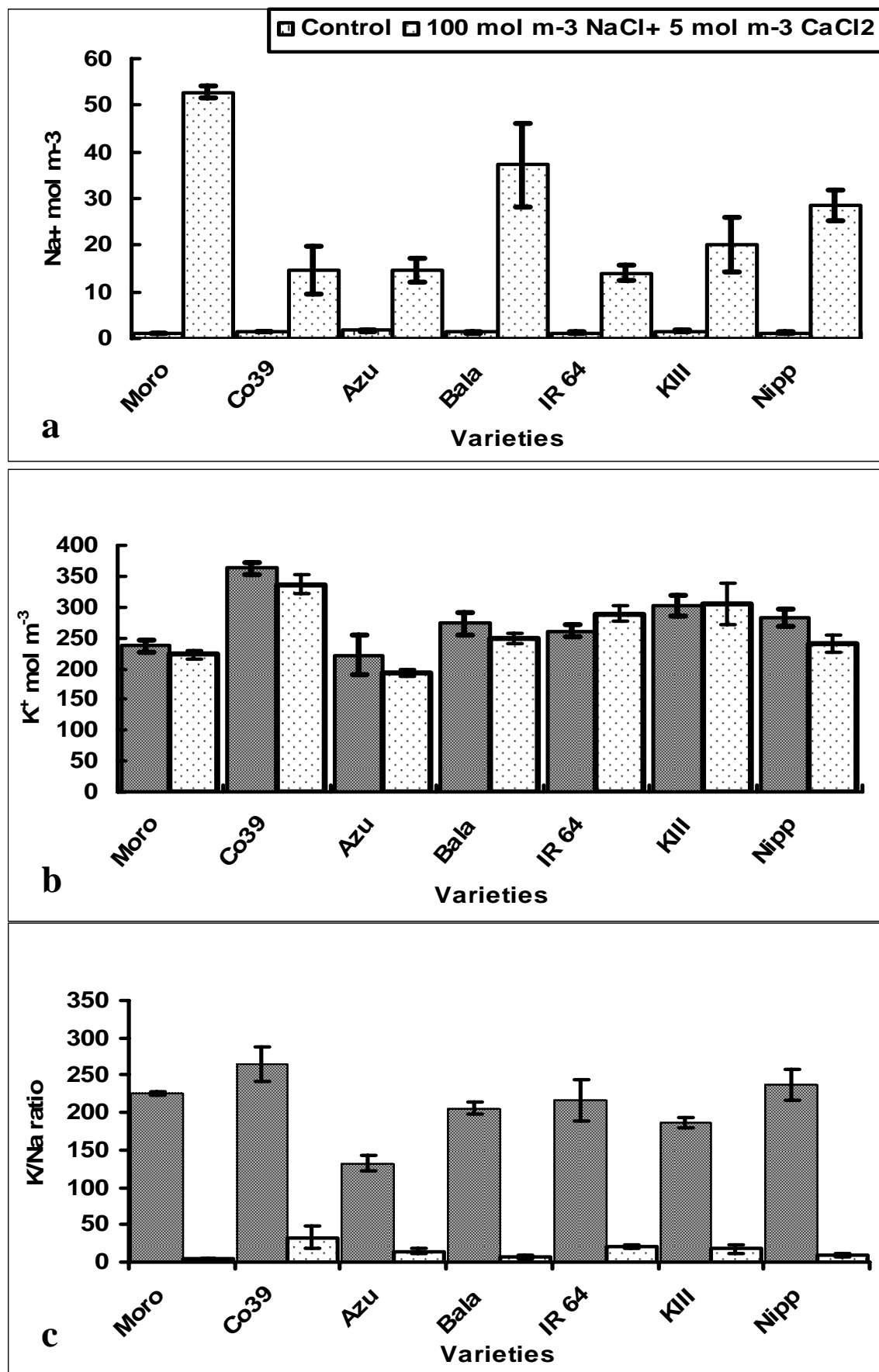


Fig. 3. Effect of salinity (100 mol m<sup>-3</sup> NaCl + 5 mol m<sup>-3</sup> CaCl<sub>2</sub>) on sap Na<sup>+</sup>, K<sup>+</sup> and K<sup>+</sup>/Na<sup>+</sup> ratio of seven rice varieties. Error bars represent standard error of mean (n = 3).



**Table 3. Pearson's correlation coefficients among various parameters in flood bench experiment.**

Traits	Na <sup>+</sup>	K <sup>+</sup>	K <sup>+</sup> /Na <sup>+</sup>	SFW	SDW	SFW/DW	Tiller plant <sup>-1</sup>	Water %
K <sup>+</sup>	-0.261 <sup>NS</sup>							
K <sup>+</sup> /Na <sup>+</sup>	-0.755**	0.288 <sup>NS</sup>						
SFW	-0.556**	0.031 <sup>NS</sup>	0.586**					
SDW	-0.524**	0.007 <sup>NS</sup>	0.505**	0.985**				
SFW/DW	-0.551**	0.178 <sup>NS</sup>	0.841**	0.504**	0.368*			
Tiller plant <sup>-1</sup>	-0.199 <sup>NS</sup>	0.254 <sup>NS</sup>	0.616**	0.616**	0.632**	0.111 <sup>NS</sup>		
Water %	-0.533**	0.143 <sup>NS</sup>	0.810**	0.525**	0.396**	0.977**	0.105 <sup>NS</sup>	
LD Score	0.754**	-0.525*	-0.672**	-0.162 <sup>NS</sup>	-0.122 <sup>NS</sup>	-0.006 <sup>NS</sup>	-0.048 <sup>NS</sup>	-0.030 <sup>NS</sup>

\*, \*\* = Correlation was significant at  $\leq 0.05$  and  $0.01$  levels

NS = non significant

LD = Leaf damage

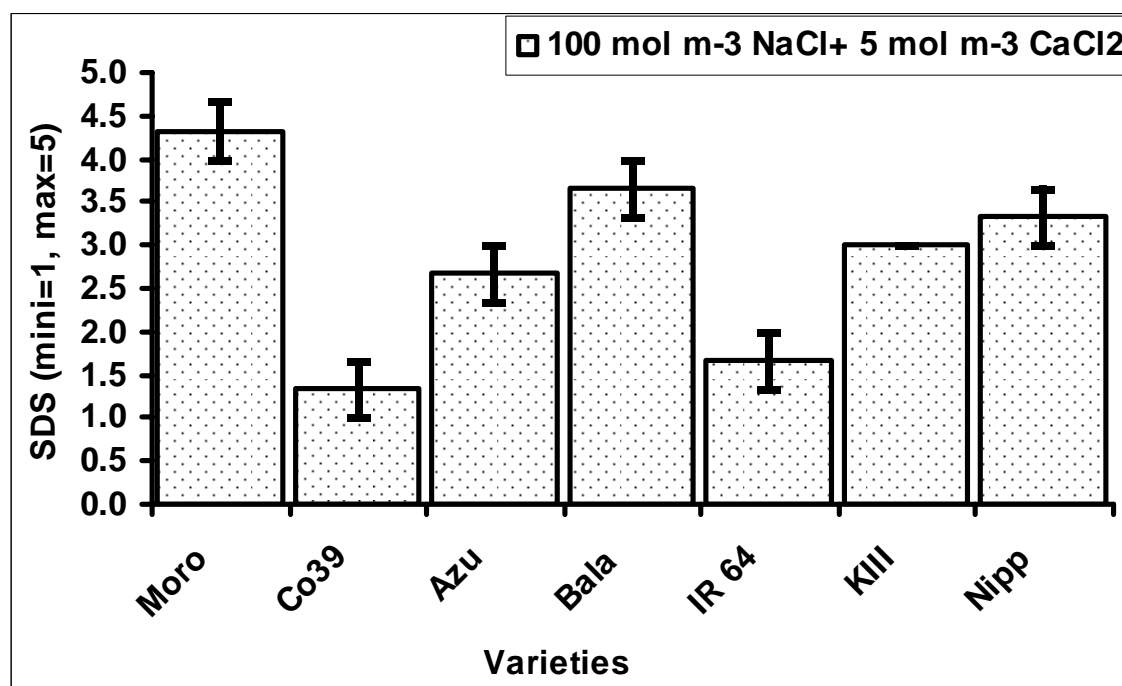


Fig. 4. Effect of salinity ( $100 \text{ mol m}^{-3} \text{ NaCl} + 5 \text{ mol m}^{-3} \text{ CaCl}_2$ ) on leaf damage scores of seven rice varieties. Error bars represent standard error of mean ( $n = 3$ ).

## Discussion

Salt tolerance is a complex trait and understanding of its molecular basis is essential in the efforts to develop salt tolerant varieties (Chinnusamy *et al.*, 2005). Genetic variation for salt tolerance has been recognized in many crop species (Flowers *et al.*, 2000; Ashraf *et al.*, 2002). Morphological parameters such as shoot fresh weight and shoot dry weight are well correlated with crop salt tolerance at early growth stages and can be used as an indicator for salt tolerance (Ashraf *et al.*, 1999; Noreen & Ashraf, 2008). Under salt stress reduction in biomass production is a common feature in crop plants. In this study salinity caused a significant reduction in shoot fresh and dry weights of the varieties compared with control (Fig. 1). Among varieties the lowest reduction in shoot fresh (58 %) and dry weight (36.5 %) was in Co39, indicating its tolerance to salt stress, whereas, the most sensitive genotype was Azucena with highest reductions in shoot fresh (82 %) and dry weights (77 %). Shereen *et al.*, (2005) also observed significant reduction in seedling growth under salinity.

Reduction in shoot fresh weight of the genotypes was also attributed to decreased water potential of rooting medium and growth inhibition related to osmotic effects under salt stress (Munns *et al.*, 1995). Under salt stress plant cell turgor pressure decreased and stomatal closure took place resulting in decreased photosynthesis (Gale & Zeroni, 1984). Another cause of growth inhibition under NaCl stress could be imbalances in uptake of mineral nutrients due to competition with Na<sup>+</sup> (Ashraf & Sarwar, 2002). High concentration of Na<sup>+</sup> is detrimental for plant growth and could be responsible for the reduction in shoot fresh and dry weight. A highly significant negative correlation between Na<sup>+</sup> and growth parameters (Table 3) also indicated that its high concentration in tissues cause serious growth and yield reduction by impairing different metabolic processes in the cytoplasm. An inverse relationship between growth and Na<sup>+</sup> concentration was also observed by Flowers *et al.*, (1985). The reduction in seedling growth under salinity stress may be due to accumulation of salt in older leaves which cause premature senescence, and reduced the photosynthetic leaf area of a plant to a level that cannot maintain growth (Munns, 2002). The synthesis of osmotica to withstand salinity stress utilizes much of carbon and reduces metabolite formation and thus ultimately decreases biomass production (Cheeseman, 1998).

The number of tillers plant<sup>-1</sup> is also an important yield parameter under salinity because it determines the grain bearing panicles. Salinity caused a significant reduction in number of tillers plant<sup>-1</sup> compared to control treatment (non saline) in this study (Fig. 2a). On relative basis, the maximum reduction in tillers was observed in Azucena (51.4%), whereas, minimum in Kalinga-III (34.9%) under salt stress. A highly significant reduction in number of tillers plant<sup>-1</sup> under salinity was also reported in rice (Zeng *et al.*, 2000). The decrease in tillering capacity might be due to the toxic effect of salt on plant growth. The development of more tillers may be a mechanism of salt tolerance by dilution of salts in plants (Aslam *et al.*, 1989).

It is clear from the results that all the varieties showed differing responses to salt stress for the accumulation ions. Sodium (Na<sup>+</sup>) increased under salinity stress in all the rice varieties (Fig. 3a). The varieties IR64 and Co39 were salt tolerant due to less accumulation of Na<sup>+</sup> in leaves under salt stress. Under salt stress the variety Moroberekan accumulated 5 times higher Na<sup>+</sup> in expanded leaf compare with Co39. The sensitivity of some crops (Flowers & Hjiabagheri, 2001) to salinity is the result of the inability to keep Na<sup>+</sup> and Cl<sup>-</sup> out of the transpiration stream (Khan *et al.*, 2006). Generally, the salt tolerant varieties of rice maintain low concentration of Na<sup>+</sup> in their leaves than those of salt sensitive lines, when exposed to salt stress (Lutts & Guerrir, 1995; Lutts *et al.*, 1995), therefore Na<sup>+</sup> in the leaves of crop plants can be used as an important indicator of salinity tolerance; and breeding for low ion accumulation could be a simple way to improve salt tolerance. Selection within varieties or lines with low Na<sup>+</sup> transport has been made in rice (Yeo *et al.*, 1988). Many enzymatic activities of plants are adversely affected by high Na<sup>+</sup> concentration (Maathuis & Amtmann, 1999). Salt tolerance is related to exclusion of Na<sup>+</sup> ion and distribution of almost uniform concentration of this ion in all leaves (Ashraf & O'Leary, 1995). The findings of Epstein *et al.*, (1980) and Ashraf & McNeilly (1990) also support our results.

It can be seen from the results (Fig. 3b) that under salt stress the concentration of K<sup>+</sup> in the leaf sap was decreased in all varieties, except IR64 and Kalinga-III. A positive but non-significant relationship between leaf K<sup>+</sup> and growth traits have been found in this study (Table 3). A good supply of K<sup>+</sup> to plants can minimize injurious effects of high Na<sup>+</sup> under salinity. Influx of Na<sup>+</sup> into shoot tissues is often accompanied by a decrease in leaf K<sup>+</sup> concentrations, leading to a decrease in K<sup>+</sup>/Na<sup>+</sup> ratios. The decrease in K<sup>+</sup> contents was due to the presence of excessive Na<sup>+</sup> in growth medium because high external Na<sup>+</sup> content is known to have an antagonistic effect on K<sup>+</sup> uptake in plants (Sarwar & Ashraf, 2003).

In present study there were no significant correlations observed between leaf  $K^+$  and any of the growth traits (Table 3). There are some reports that show non-significant or negative relationship between these attributes in barley, sorghum and mungbean (Khan & Ashraf, 1988; Ashraf & Naqvi, 1996). Lee *et al.*, (2003) reported that there were no significant differences between Japonica and Indica rice types for shoot  $K^+$  concentration. Lee & Senadhira (1999) also reported that shoot  $K^+$  conc. in Japonica rice showed no relation to salt tolerance supporting our result.

One of the key features of plant salt tolerance is the ability of plant cells to maintain optimal  $K^+/Na^+$  ratio in the cytosol, when exposed to salt stress (Maathuis & Amtmann, 1999; Carden *et al.*, 2003; Gollmack *et al.*, 2003; Tester & Davenport, 2003; Ashraf, 2004; Peng *et al.*, 2004). The K/Na ratio also decreased significantly because of increase in leaf  $Na^+$  (Table 3). This decrease in  $K^+/Na^+$  ratios may relate directly to a decrease in yield in some conditions (Asch *et al.*, 2000). There was a positive relationship between high K/Na ratio and salinity tolerance (Gregorio & Senadhira, 1993) making it best indicator of growth and yield under salt stress (Gill & Singh, 1995).

Highly significant positive correlation between  $Na^+$  and leaf damage score (SDS) may be attributed to more accumulation of toxic ions such as  $Na^+$  in the leaves of sensitive rice varieties; Moroberekan and Bala. Visual scores of salinity damage were negatively correlated with K/Na ratio in the leaf (Table 3). Similar relationship was also reported by Lee & Senadhira (1999). The score based on visual injury symptoms were well related to salinity-induced reduction in grain yield (Gregorio *et al.*, 1997). This shows the reliability of using visual scoring for salinity screening. It can be concluded that variety Co39 was salt tolerant on SFW basis and low salinity damage score basis and is also low  $Na^+$  accumulator whereas Moroberekan was salt susceptible on SFW and high salinity damage score basis and is a high  $Na^+$  accumulator. Based on above results it can be hypothesized that the QTLs for the traits measure would have been existed in the mapping population derived from the cross between Co39 and Moroberekan. Studies may be performed to identify these QTLs associated with salt tolerance and to unravel functions underlying those QTLs.

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