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Effects of salinity stress on growth, chlorophyll content and ion accumulation in two indica rice (*Oriza sativa* L.) cultivars differing in salinity tolerance

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Abstract

Rice (*Oryza sativa*) is particularly sensitive to salt stress during the reproductive stage. Physiological responses to salinity were evaluated for contrasting genotypes, during the reproductive stage. Two rice genotypes differing in their tolerance of salinity were evaluated in a set of greenhouse experiments under o and 6 dSm^{-1} of salinity during reproductive stage. Salt stress increased chlorophyll b concentration in leaves of a tolerant (FL485) rice genotype, but significantly decreased chlorophyll a in both cultivars and reduced chlorophyll *a*/*b* ratio just in susceptible cultivar and this is probably one of the reasons for the higher tolerance of FL485 compared with IR29. Salinity caused higher accumulation of K^+ in sensitive cultivar than tolerant one but the Na^+ level in leaf of IR29 was more than FL485. Grain yield and 1000 grain weight of both genotypes decreased with the application of NaCl. Our results indicated that the tolerant genotype had mechanisms to prevent high Na^+ accumulation in leaf. These mechanisms help plant to prevent tissue death and enable to continue its growth under saline conditions.

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Introduction

Salinity, affecting land, is one of the most serious abiotic stresses limiting plant growth and development, as well as causing low productivity, especially in salt-sensitive crop species (Pitman and La¨uchli 2002). Certain rice varieties have been reported as being salt sensitive in their seedling and reproductive stages (Zeng *et al.* 2001; Moradi and Ismail 2007), leading to a reduction in crop productivity of more than 50% when exposed to 6.65 dS m^{-1} electrical conductivity (EC) and soil salinity (Zeng and Shannon 2000). Generally, salinity affects the growth of rice plant at all stages of its life cycle. But it is more pronounced on reproductive stage than on vegetative stage consequently decreased the grain yield (Afridi *et al.,* 1988). Total number of tillers, grain weight per panicle, 1000-seed weight and quality and quantity of grains decreased progressively with increase in salinity levels (Abdullah *et al.,* 2001). Salinity affects plant growth and development generally through osmotic stress limiting water uptake and the excessive uptake of ions, particularly Na^+ and Cl^- that ultimately interfere with various metabolic processes (Munns and Tester, 2008). Salinized plants may suffer from metabolic toxicity, nutrient deficiencies and imbalances, membrane dysfunction, and antioxidative stress, which damage tissue and induce early senescence (Essah *et al.*, 2003). Exclusion of Na^+ and tolerance of high cellular Na^+ accumulation play an important role in minimizing Na^+ toxicity above and beyond osmotic tolerance (Munns and Tester, 2008). In addition, avoiding Na^+ accumulation in saline environments is an important mechanism contributing to ionic tolerance. The cytosolic K^+/Na^+ may also be critical for salinity tolerance of plants (Thalji and Shalaldeh, 2007; Azadi *et al.*, 2011). It can be generalized that plants may restrict uptake of ions like Na^+, Cl^- or take up only selective ions to maintain a higher K^+/Na^+ ratio when exposed to salinity stress. Tolerant wheat genotypes exhibited low Na^+, high K^+ and high K^+/Na^+ in the leaf blade (Munns *et al.*, 2000). Under salinity, however, the K^+/Na^+ ratio falls dramatically (Maathuis and Amtmann 1999). This occurs as a result of both excessive Na accumulation in the cytosol (Leigh 2001; Zhu 2000) and enhanced K^+ leakage from the cell (Shabala 2000; Shabala 2003; Shabala and van Volkenburgh 2003), the latter resulting from NaCl-induced membrane depolarization under saline conditions (Cakirlar and Bowling 1981; Shabala *et al.* 2003). (7)The effects of salinity on chlorophyll synthesis and integrity seems to vary with the level of salt stress, as few reports suggested an accelerated rate of biosynthesis and higher concentrations during vegetative growth (Asch *et al.*, 2000; Santo, 2004), however, significant differences between genotypes were sometimes observed regarding the effects of salt stress on chlorophyll concentration in leaves (Rout *et al.*, 1997; Datta *et al.*, 2009). The chlorophyll b content showed a reduction in salt stress but with a greater magnitude than a chlorophyll a content. Salt stress was found to be more deleterious to chlorophyll b indicating the susceptible nature of this compound towards the stress situation. The instability of chlorophyll b content for salt stress can be regarded as an index of tolerance, which might produce higher photosynthetic rate and eventually show higher yield. Chlorophyll a/b has often considered as a measure of the activity of chlorophyll synthesizing mechanism in plant under stress condition (Kupke and Huntington, 1963). The present experiment was conducted to identify the characters responsible for salinity of two rice genotypes differing in tolerance to salt stress and to study the association of the physiological traits such as ions and chlorophyll contents with the salt tolerance.

Material and methods

Plant materials, growth conditions and stress treatments

Two rice cultivars contrasting in tolerance of salt stress during reproductive stages (Moradi *et al.*, 2003) were selected for this investigation. FL485 is breeding line tolerant of salt stress at both the seedling and reproductive stages, and IR29 is a cultivar sensitive to salt stress during both stages and is commonly used as a sensitive check in breeding

nurseries. Salt stress starting at about 10–7 d before panicle initiation and continuing through harvest. The experiment was carried out in a greenhouse with air temperature in the range of about 25 to 35 ^{o}C and light intensity in the range of about 600–1000 mmol m^{-2} $s^{-1}$and with 20 pots per cultivar in each replication. Pre-germinated seeds were sown in 1 L perforated plastic pots filled with fertilized (50 N, 25 P and 25 K mg kg^{-1}) Maahas clay soil (43 % clay, 44 % silt and 13 % sand; pH 5.9; Tirol-Padre and Ladha, 2004) and were kept in concrete tanks filled with tap water. The level of water was maintained at 3 cm below the soil surface for 2 d. Five seeds of each of the two cultivars were sown in each pot, thinned to one seedling 2 weeks later, and the water level was raised to about 1–2 cm above the soil surface. When the seedlings were 28 d old, water was siphoned out and the pots were drained for 12 h, then flooded with tap water (control) or with a saline solution with EC of 3 dS m^{-1} using NaCl for 3 d, then increased progressively to 4 and 5 dSm^{-1} at 3 d intervals, and finally stabilized at 6+0.3 dS m^{-1} through harvesting. The pots were kept flooded thereafter for the duration of the experiment, and the EC of the water was monitored daily and adjusted when necessary using NaCl and tap water.

Sampling

All parameters were measured on flag leaves and panicle of the first two tillers that were tagged 25 d after sowing. sampling of the flag leaves and panicles were removed from anthesis up to full grain maturity for the various biochemical analyses. For the biochemical assays, samples were cut into small pieces after measuring their fresh weight, frozen in liquid nitrogen, and stored at -80 ^{o}C. Three replicates were maintained for all measurements. The various plant parts were dried in oven at 80 ^{o}C for dry matter analyses and various estimations.

Chemical content

Photosynthetic pigments

One gram of fresh tissue was extracted by grinding in a mortar using 20 ml 80% acetone, a small amount of pure (Silica Quartz), and 0.5 g calcium carbonate to equalize the cellular sap acidity. The extract was filtered using a glass funnel (Sentered glass funnel G4) and collected in a conical flask. The residue was re-extracted using the same method, until it became devoid of color. All the filtrate was collected in a standard flask and the volume completed to a specific amount by adding 80% acetone. The optical density (O.D.) of the extract was measured at wave lengths 663, 645, and 440.5 nm (Smith and Benitez, 1955) to estimate chlorophyll 'a' and 'b', and carotenes respectively, using a Spectrophotometer (Spectronic 21D) and a vitreous cell (thickness of photo route 1 cm). Three replicates were used for each treatment, and the amount of pigment present in each sample was calculated according to the following equations:

mg chlorophyll a/g-tissue

$$= 12.7\ (O.D.)\ 663 - 2.69(O.D.)\ 645 \times \frac{v}{w \times 1000}$$

mg chlorophyll b/g-tissue

$$= 22.9\ (O.D.)\ 645 - 4.68(O.D.)\ 663 \times \frac{v}{w \times 1000}$$

mg carotenoids/g-tissue

= 46.95 (O.D. 440.5 - 0.268 - chlorophyll 'a' + 'b')

whereas W, the fresh weight by grams for extracted tissue; V, the final size of the extract in 80% acetone; O.D., optical density at specific wave length.

Determination of $Na^{+}$and K^{+}

Known weight of dried samples were ground to a fine powder and about 0.1 g was transferred to a test tube containing 10 mL of 0.1 N acetic acid, and heated in a water bath at 80 ^{o}C for 2 h. The extracted tissue was cooled at room temperature and left overnight, and then filtered using Whitman filter paper number 40. Sodium and potassium concentrations were then determined using an atomic absorption spectrometer (Perkins Elmer, Norwalk, CT,
USA) (Gadallah, 1999).

Statistical analysis

The experiment was a completely random design with three replications. The main effect of factors (salinity and cultivars), and their interaction (salinity × cultivars) were evaluated by analysis of variance (ANOVA) using IRRISTAT version 92 (IRRI, 1992). The comparison of treatment means was made by least significant difference (LSD) at p = 0.05.

Results and discussion

Under salt stress one of the mechanisms of salt tolerance is accomplished by uptake and accumulation of inorganic ions, mainly Na^+, K^+ and Cl^- (Alian *et al.*, 2000). In our study, regardless to salinity treatment absolute leaf Na^+ content was greater in susceptible cultivar than tolerant one (Table 1). Salinity cause to more leaf Na accumulation in both cultivars, however this increment was not significant in each cultivar in respect to control treatment (Table3). Potassium content on the other hand has been raised in IR29 cultivar but fall down in FL485 cultivar. Consequently a similar trend like K^+ concentration was found for K^+/Na^+ ratio (Table 3), as the reduction values were 17.61 and 13.94% for IR29 and FL485 respectively. The uptake of Na^+ and K^+ or the ratio of K^+/Na^+ have been associated with salinity tolerance in some plant species (Tajbakhsh *et al.*, 2006; Thalji and Shalaldeh, 2007; Dasgan and Koc, 2009; Azadi *et al.*, 2011). K^+/Na^+ 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). The tolerant wheat genotypes maintained low Na^+ and high K^+ and high K^+/Na^+ in the leaf blade (Munns *et al.*, 2000). However, concentration of Na^+ and K^+ were not associated with the degree of salinity tolerance in other species (Marcar, 1987; Munns and James, 2003). Our results indicated that K^+/Na^+ was not consistent under salinity stress and may not well represent salinity tolerance. However, the tolerant cultivar (FL485) had less accumulation of Na^+, compared to the sensitive IR29, suggesting that avoiding excessive accumulation of Na^+ or tolerance to accumulated Na^+ facilitated salinity tolerance in rice. The inconsistent results of salinity tolerance in relation to K^+ or Na^+ accumulation found in different studies may be due to variations of salinity level, duration, species or cultivars.

Table 1. Varietal performance on yield and some physiological parameters under salinity (6 dSm^{-1}) in reproductive stage of two rice genotypes differing in salinity tolerance.

Variety	No. of tillers	Panicle DW (g)	Shoot DW (g)	1000 grain weight (g)	Grain yield ($gplant^{-1}$)	Chla (mg/gfw)	Chlb (mg/gfw)	Chla/Chlb	Total chl (mg/gfw)	Car. (mg/gfw)	Na^+ Flag leaf ($mgg^{-1}dw$)	K^+ Flag leaf ($mgg^{-1}dw$)	K^+/Na^+
IR29 (A_1)	5.8^a	0.668a	10.63a	17.71^b	9.54^b	0.739^b	0.099^b	7.46^a	0.837^b	0.076^a	1.99^a	31.23^a	15.69^b
FL485(A_2)	7.3^a	0.805a	12.74a	22.13a	11.87^a	0.878^a	0.356^a	2.47^b	1.235^a	0.083^a	1.29^b	24.15^b	18.72^a
LSD $_{(0.05)}$	2.11	0.462	3.195	0.502	2.097	0.045	0.045	1.04	0.045	0.045	0.253	3.559	2.35

Table 2. Effect of salinity (6 dSm^{-1}) on yield and some physiological parameters in reproductive stage of two rice genotypes differing in salinity tolerance.

Salinity levels (dSm^{-1})	No. of tillers	Panicle DW (g)	Shoot DW (g)	1000 grain weight (g)	Grain yield ($gplant^{-1}$)	Chla (mg/gfw)	Chlb (mg/gfw)	Chla/Chlb	Total chl (mg/gfw)	Car. (mg/gfw)	Na^+ Flag leaf ($mgg^{-1}dw$)	K^+ Flag leaf ($mgg^{-1}dw$)	K^+/Na^+
Salinity (B_1)	6.7^a	0.32b	10.39a	1921^b	6.96^b	0.734^b	0.254^a	2.89^b	0.989^b	0.072^a	1.75^a	28.27^a	16.15^b
Control (B_2)	6.5^a	1.16a	12.98a	20.63^a	11.91^a	0.883^a	0.201^b	4.39^a	1.083^a	0.087^a	1.53^a	27.11^a	17.72^a
LSD $_{(0.05)}$	2.11	0.462	3.195	0.502	2.097	0.045	0.045	1.04	0.045	0.045	0.253	3.559	0.982

Pigment degradation in salt stressed rice is one of the most effective parameters to be a criterion in screening for salt tolerance (Wanichananan *et al.*, 2003). There are several reports, which have stated that pigment stabilization in salt-tolerant rice varieties, HJ salt tolerant (Cha-um *et al.*, 2007), FL478 (Demiral and Tu¨rkan 2006) and Pokkali (Walia *et al.*, 2005), is more reliable than in salt-sensitive varieties, HJ salt sensitive, IR29 and IR28. In this study, the photosynthetic pigments, Chla and

chlorophyll a+b contents in both salt-tolerant and salt-sensitive varieties decreased when exposed to salt stress. The degradation of Chla and TC in salt-stressed IR29 was 19.43 and 13.06%, while that in FL485 was 14.66 and 5.51%, respectively (Table 3). The data showed that the chlorophyll b concentration in leaves increased under salt stress in both cultivars, with more extent in sensitive genotype (45.68%) than tolerant cultivar (30.07) (Table 3). Consequently, chlorophyll *a*/*b* ratio decreased substantially in IR29 (44.75%) under salt stress, while in FL485 this value was 30.07%, suggesting greater effects of salt stress in reducing chlorophyll *a* than chlorophyll *b*. Considering that chlorophyll *a* is the main photosynthetic pigment (Daiz *et al.*, 2002; Santo, 2004), this reduction in ratio could probably be one of the main reasons for reduced photosynthesis under salt stress as reported in rice before (Moradi and Ismail, 2007). Significant differences in chlorophyll concentrations under salt stress were also observed between genotypes, with the tolerant genotype having higher chlorophyll *a*, ability of the tolerant genotype to maintain higher concentration of chlorophyll *a* is probably one of the important mechanisms contributing to salinity tolerance in this genotype, which could consequently result in higher photosynthetic capacity and shoot dry weight (Moradi and Ismail, 2007; Rout *et al.*, 1997; Datta *et al.*, 2009.). No significant differences were observed between cultivars in carotenoids concentration due to effects of genotype, salinity and the interaction effect of genotypes and salinity stresses (Table1 3).

Table 3. Interaction between genotypes and salinity (6 dSm^{-1}) on yield and some physiological parameters in reproductive stage of two rice genotypes differing in salinity tolerance.

Interaction (genotypes × salinity levels)	No. of tillers	Panicle DW (g)	Shoot DW (g)	1000 grain weight (g)	Grain yield ($gplant^{-1}$)	Chla (mg/gfw)	Chlb (mg/gfw)	Chla/Chlb	Total chl (mg/gfw)	Car. (mg/gfw)	Na^+ Flag leaf (mgg^{-1}dw)	K^+ Flag leaf (mgg^{-1}dw)	K^+/Na^+
A_1B_1	6[a]	0.217[b]	9.23[b]	16.74[d]	6.02[c]	0.659[c]	0.118[c]	5.58[b]	0.779[d]	0.07[a]	2.09[a]	34.19[a]	16.36[b]
A_1B_2	5.7[a]	0.413[b]	11.56[b]	18.68[c]	10.77[ab]	0.818[b]	0.081[c]	10.1[a]	0.896[c]	0.08[a]	1.89[a]	28.17[b]	14.9[b]
A_2B_1	7.3[a]	1.12[a]	12.04[ab]	21.68[b]	7.9[bc]	0.809[b]	0.39[a]	2.07[c]	1.2[b]	0.08[a]	1.42[b]	22.34[c]	15.73[b]
A_2B_2	7.3[a]	1.2[a]	13.92[a]	22.57[a]	13.06[a]	0.948[a]	0.32[b]	2.96[c]	1.27[a]	0.09[a]	1.18[b]	25.96[bc]	22[a]
LSD $_{(0.05)}$	2.98	0.653	4.519	0.709	2.965	0.063	0.063	1.02	0.063	0.063	0.357	5.033	2.1

No significant differences were observed between the two genotypes in number of tillers due to effects of genotype, salinity and the interaction effect of genotypes and salinity stresses. In finding of several researchers the number of tillers per plant decreased with increasing salinity levels as stated by (WeonYoung *et al.*, 2003) and (LingHE *et al.*, 2000) in rice. The lack of variability in tiller number in our experiment illustrates the importance of timing treatments since imposed salinity treatment 10–7 d before panicle initiation on tiller is unaffected. Salinity reduced the 1000-grain weight by 11.19% (Table 2). Zaman *et al.* (1997) and Aoki and Ishikawa (1971) reported that 1000-grain weight decreased with increasing the levels of salinity. The 1000-grain weight of IR485 (22.13) was more than IR29 (17.71) (Table1). The interaction effects between salinity levels and varieties showed that IR485was dominant in producing grain weight under salinity stresses, as salinity cause to more reduction in 1000-grain weight in sensitive cultivar (10.38%) than tolerant cultivar (4%). Grain yield of IR29 and FL485 grown under salt stress were significantly reduced when compared with those grown under the control condition (Table 3), however the reduction was more in IR29 (44%) in compare to FL485 (39.5%). The different varieties, salt stress treatment and different combinations of these factors were shown to have significant effects on panicle dry weight and shoot dry weight and as the growth performances in respect to yield and yield contributing characters of FL485 salt-tolerant rice were better than that of IR29 salt sensitive rice when exposed to salt stress.

Conclusion

This study showed that tolerant rice cultivar maintained a relatively higher photosynthetic function after exposure to salt stress. A careful consideration of cations in this study demonstrated that, the tolerance of FL485 might come from its cation absorption selectivity or ability keep far away Na^+ from young leaves. Na^+ was lower in leaves of tolerant cultivar (FL485) than susceptible one.

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