

Physiological responses of two Turkish rice (*Oryza sativa* L.) varieties to salinity

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Abstract: Effects of salinity (60 mM NaCl) on 2 Turkish rice varieties (Kıral and Yavuz) were studied in comparison to 2 international check varieties (IR4630-22-2 tolerant and IR31785-58-1-2-3-3 susceptible) in 2 hydroponic and 1 outdoor soil-based pot experiments. Partitioning of dry matter, leaf chlorophyll concentration, K⁺ and Na⁺ uptake, and proline accumulation in the leaves were investigated under both control and salt stress conditions. Dry matter accumulation decreased with salt stress in all varieties, with the decreases being more pronounced in IR31785 and Kıral. The results showed that the varieties tested expressed different adaptation mechanisms under salt stress; however, the increase of leaf proline accumulation was a general indicator for responses to salt stress in all cultivars.

Key words: Chlorophyll, K/Na ratio, proline, rice, salinity

İki Türk çeltik çeşidinin (*Oryza sativa* L.) tuzluluğa karşı fizyolojik reaksiyonları

Özet: Tuzluluğun (60 mM NaCl) 2 Türk çeltik çeşidi (Kıral ve Yavuz) üzerine etkileri, 2 adet uluslar arası olarak onaylanmış dayanıklı (IR4630-22-2) ve hassas (IR31785-58-1-2-3-3) çeşit ile karşılaştırmalı olarak 2 besin ortamı ve 1 açık alan saksı denemesi ile çalışılmıştır. Kuru madde dağılımı, yaprak klorofil içeriği, K⁺ ve Na⁺ alınımı ve yapraktaki prolin birikimi hem kontrol hem de tuzluluk koşulları altında incelenmiştir. Tuzluluğun etkisi ile kuru madde üretimi tüm çeşitlerde düşmüştür ve tuzluluğun düşüşü IR31785 ve Kıral çeşitlerinde daha belirgin olmuştur. Denenen çeşitlerin tuzluluk stresi altında farklı adaptasyon mekanizmaları geliştirmekte olduğunu sonuçlar göstermektedir ancak yapraktaki prolin birikimindeki artış tüm çeşitlerde tuzluluk stresine reaksiyonlar için genel bir gösterge durumundadır.

Anahtar sözcükler: Klorofil, K/Na oranı, prolin, çeltik, tuzluluk

Introduction

Salinity is one of the most important environmental stress factors limiting plant growth and productivity. Almost 10% of arable land and 24% of irrigated areas are affected by sodium chloride based salinity (Pessarakli and Szabolcs 1999). Higher plants

such as rice (*Oryza sativa* L.) are glycophytes and growth and yield are substantially reduced by salinity.

The detrimental effects of salinity on plant growth are associated with low osmotic potential of the soil solution (secondary drought), nutritional imbalances (nutrient disorders), specific ion effects (sodium or

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chlorine toxicity) or a combination of those factors (Ashraf 1994; Marschner 1995; Ashraf and Harris 2004).

Early responses during the first phase of salt stress are often a rapid decrease in cell water potential, a reduction in cell division, and an expansion as well as an increase in compatible compounds such as proline, polyols, and sugars (Pollard and Wyn Jones 1979; Garcia et al. 1997; Munns 2002). Proline is considered one of the most important compatible solutes in higher plants, although the specific functions of proline are still not clear (Ashraf 1994; Ali et al. 1999; Abraham et al. 2003). Some studies suggested that proline acted as a protective agent for enzymes (Bandurska 1993; Solomon et al. 1994), contributing to membrane stability (Ashraf and Harris 2004), whereas Lin and Kao (2001) reported that root-zone application of proline resulted in the inhibition of root growth and an increase in cell-wall peroxidase activity associated with an increase in cytosolic H_2O_2 concentration in rice seedlings.

Salinity-induced accumulation of proline correlated to salt tolerance was found in alfalfa (Petrusa and Winicov 1997), green gram (Misra and Gupta 2005), and rice (Hien et al. 2003). In contrast, several other studies reported lower or decreased levels of proline for salt-tolerant rice genotypes (Garcia et al. 1997; Lutts et al. 1999; Maiale et al. 2004; Demiral and Türkan 2005). Thus, proline accumulation clearly indicates a stress response; however, at least in rice, it is still not clear whether it is involved in salinity resistance or not.

Visual symptoms of salt stress in rice are signs of a reduction in leaf size, leaf rolling, and precipitation of salts on the stem base, as well as early senescence and necroses starting on old leaves. Salt-induced leaf senescence has been shown to be an effect of the accumulation of toxic ions (Na^+ and Cl^-) or the depletion of K^+ and Ca^{2+} (Yeo and Flowers, 1983; Leidi et al. 1991; Yeo et al. 1991; Lutts et al. 1996a). Consequently, Asch et al. (2000b) developed a tool to assess potential yield losses due to salt stress in rice on the basis of the K/Na ratio of the youngest fully developed leaves in 8-week old rice seedlings.

Decreases of chlorophyll content of rice leaves under salinity were reported by Lutts et al. (1996a), Singh and Dubey (1995), and Mitsuya et al. (2003).

Based on the relationship between leaf Na and chlorophyll content, Yeo et al. (1990) suggested evaluating genotypic salt-resistance on the basis of both higher chlorophyll and lower Na^+ content at the leaf tissue level. In contrast, Singh and Dubey (1995) and Asch et al. (1997a, 2000a) reported that the chlorophyll concentration of young and photosynthetically active rice leaves did not decrease but rather increased with increasing Na^+ or Cl^- concentrations. In addition to these quite contrasting effects of salt stress on chlorophyll concentration, there is some evidence of changes in the ratio between Chl a and Chl b. Lutts et al. (1996a) reported that the Chl a concentration of 32-day-old rice leaves decreased markedly after 7 days of 30 mM NaCl treatment, whereas the concentration of Chl b was not reduced. Singh and Dubey (1995) also reported that the decrease in total chlorophyll concentration of susceptible rice cultivars was mainly attributed to the destruction of Chl a.

Compared to the intensive work on salt tolerance in Asian rice genotypes over the last few decades, characterization of salt tolerance of newly developed rice varieties from Turkey is less comprehensive. Focusing on rice cultivars from Turkey and taking into account the contradicting reports on the effects of salt stress on proline accumulation and leaf chlorophyll content, we compared some varieties with international, Asian check varieties subjected to salt stress under controlled conditions. We investigated the relationship between salt tolerance, leaf proline, sodium, potassium, chlorophyll concentration, Chl a/b ratio, biomass production, and dry matter partitioning.

Materials and methods

Rice cultivars Kiral (KRL) and Yavuz (YVZ) from Turkey and IR 31785-58-1-2-3-3 (IR31785) and IR 4630-22-2 (IR4630) from IRRI (International Rice Research Institute) were used in experiments. Tatar (2006) determined Turkish rice varieties including KRL and YVZ and Asch et al. (2000a); Asch and Wopereis (2001) also reported the international rice varieties, IR31785 and IR4630, as susceptible and as tolerant, respectively. The 2 nutrient solution experiments and chemical analyses were conducted at the greenhouse and laboratories in Germany from

October 2005 to March 2006.

Seeds were germinated on filter paper moistened with de-ionized water in petri dishes at 28 °C in the dark. Three days after sowing (DAS), uniformly germinated seeds were selected and transferred to trays containing 5 L of de-ionized water. The mean air temperature in the greenhouse was 28 °C and 22 °C during the day and the night, respectively. Plants were, in addition to the natural light, illuminated for 12 h with SON-T-Agro 400 W bulbs, keeping the light intensity at approximately 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at leaf level. Relative air humidity varied between 40% and 50%. At 8 DAS, seedlings were transferred to PVC pots containing 40 L of half-strength nutrient solution. Six seedlings for each variety were sown in all pots. Plants were fixed by wrapping seedlings with foam strips. At 15 DAS, plants were provided with full-strength modified Yoshida solution (Yoshida et al., 1976) containing millimolar concentrations of: 2.86 N (NH_4NO_3), 0.32 P (KH_2PO_4), 1.02 K (K_2SO_4), 1.67 Mg (MgSO_4), 1 Ca (CaCl_2), 0.1 Fe (FeEDTA), 2 Si (Na_2SiO_3), (μM): 9.1 Mn (MnCl_2), 18.0 B (H_3BO_3), 0.52 Mo ($(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$), 0.15 Zn (ZnSO_4) and 0.156 Cu (CuSO_4). Nutrient solutions were renewed every 4 days. On the 30th day, DAS 0 and 60 mM NaCl were added to the nutrient solution. Plants were harvested at 40 DAS and separated into roots, stems, and leaves. The leaf blade and roots samples of the seedlings were rinsed in distilled water, blotted on filter paper, and the fresh weight was recorded. Samples were transferred to liquid nitrogen (within 2 min of harvesting) and kept at -25 °C for further analyses. Samples were freeze-dried and ground using a ball-mill.

Proline, chlorophyll, sodium, and potassium concentrations were measured. The same experiment was replicated to confirm the data in the second experiment.

Additionally, the salt tolerance of the 4 varieties was validated in an outdoor pot experiment with soil as substrate at Ege University, Turkey, from May to July in 2006. Seeds were germinated in petri dishes and 3 seedlings were transferred into pots with 14 cm diameter and 3 L volume filled with sandy loam. Plants were supplied with nutrient solutions containing 5 times higher concentration of N, P, K, and salt (17.5 g L⁻¹ NaCl) as indicated above by using

the same experimental schedule. Plants were regularly watered. For aerobic conditions, the soils were continually kept at field capacity by weighing. All experiments were performed as a randomized block design with 4 replicates. Statistical analyses were performed with SAS, Proc GLM with varieties and salt treatments as fixed effects. The test of normality of residuals was performed with Proc Univariate.

Analyses

The proline content of leaf blades of the bulk sample of the 6 seedlings from each pot was determined according to Bates et al. (1973). Approximately 0.5 g of plant material was ground in liquid nitrogen, homogenized in 10 mL of sulfosalicylic acid, and filtered through Whatman #2 filter paper. Then 2 mL of the filtrate was incubated with 2 mL of acid ninhydrine and 2 mL of glacial acetic acid for 1 h at 100 °C. The reaction was stopped by transferring the samples into an ice bath. The reaction mixture was extracted with 4 mL of toluene and the absorbance of the aqueous phase was measured at 520 nm. Proline concentration was calculated on a fresh weight basis. Samples were extracted in 80% acetone and the absorbance of the centrifuged extract was recorded at 480, 645, and 663 nm using a spectrophotometer. Chlorophyll contents were calculated according to Arnon (1949) and Jayaraman (1988). Samples were extracted for 2 h in 100 mM acetic acid at 90 °C (20 mL of extract solution g⁻¹ dried material) according to Yeo and Flowers (1983) and the concentration of Na⁺ and K⁺ was measured with an atomic absorption spectrometer.

Results

In all experiments, salt stress reduced the total dry matter (DM) significantly when compared to control conditions (Table 1). The DM response of all varieties was similar in 2 independent greenhouse nutrient solution experiments conducted in Germany and the pot experiment conducted in Turkey. In line with their earlier classification (Asch and Wopereis 2001; Tatar 2006), YVZ and IR4630 (tolerant) were the least affected by salt stress; however, KRL and IR31785 (susceptible) were the most affected by salt stress. Salt-induced growth reductions were between 27% and 45% for the 2 susceptible cultivars and between 8%

Table 1. Total dry weight (DW) and percentage decrease under salt stress relative to control conditions of 4 rice varieties in 2 hydroponic and 1 soil culture experiments. KRL and YVZ are 2 Turkish varieties and IR4630 (tolerant) and IR31785 (susceptible) are international salt-stress check-varieties. Duncan’s multiple range test: Mean values sharing the same letter are not significantly different ($P < 0.05$). Error given as standard error of means, $n = 4$. ns = not significant. * $P < 0.05$, ** $P < 0.01$

| Cultivars | Salt Treatments (mM) | Exp I (Hydroponic) | | Exp II (Hydroponic) | | Exp III (Soil) | |
|-----------------|----------------------|--------------------|--------------|---------------------|--------------|----------------|--------------|
| | | DW (g) | Decrease (%) | DW (g) | Decrease (%) | DW (g) | Decrease (%) |
| KRL | 0 | 0.77 ± 0.17 | | 0.85 ± 0.05 | | 1.02 ± 0.08 | |
| | 60 | 0.51 ± 0.12 | 32.3 ab | 0.54 ± 0.05 | 37.0 a | 0.74 ± 0.05 | 27.9 a |
| YVZ | 0 | 0.68 ± 0.22 | | 0.66 ± 0.05 | | 1.00 ± 0.07 | |
| | 60 | 0.59 ± 0.10 | 7.9 b | 0.56 ± 0.03 | 15.6 b | 0.91 ± 0.07 | 9.3 b |
| IR4630 | 0 | 0.32 ± 0.07 | | 0.31 ± 0.03 | | 0.36 ± 0.03 | |
| | 60 | 0.28 ± 0.03 | 11.3 ab | 0.27 ± 0.03 | 11.8 b | 0.31 ± 0.02 | 12.5 ab |
| IR31785 | 0 | 0.49 ± 0.10 | | 0.32 ± 0.05 | | 0.46 ± 0.05 | |
| | 60 | 0.28 ± 0.08 | 44.6 a | 0.21 ± 0.02 | 33.6 a | 0.32 ± 0.05 | 27.3 a |
| Mean | | 0.49 | 24.0 | 0.47 | 24.5 | 0.64 | 18.7 |
| Cultivar | | ** | * | ** | ** | ** | ** |
| Salt | | ** | - | ** | - | ** | - |
| cultivar × salt | | ns | - | ** | - | ** | - |

and 16% for the tolerant ones. Although the relative effects of salt stress on biomass accumulation were similar in susceptible and tolerant cultivars separately, the 2 Turkish varieties accumulated twice the biomass relative to the international check-varieties under both control and stress conditions (Table 1).

Salt effects on biomass accumulation resulted in differences in the partitioning patterns among the varieties (Figure 1). Salt stress decreased the leaf weight ratio (LWR) significantly and increased the stem weight ratio (SWR) in YVZ, whereas no effects were observed in the other cultivars. The share of roots in the overall biomass (RWR) significantly increased under salt stress in KRL, YVZ, and IR4630, whereas in IR31785 growth was equally reduced in all organs.

The highest K/Na ratio was found in leaves and roots of salt-tolerant IR4630, while it was the lowest in salt-susceptible IR31785 under salt stress (Figure 2).

The K/Na ratio of the 2 salt affected Turkish varieties was not significantly different. Salt stress induced a significant decrease in the ratio between chlorophyll a and chlorophyll b of all varieties (Figure 3a). The ratio was reduced by a factor of 2.64 in KRL, 3.57 in YVZ, 4.29 in IR 4630, and 5.2 in IR 31785, indicating a higher capacity of the Turkish rice varieties to maintain a high Chl a/b ratio under salt stress conditions than the international check varieties. Proline accumulation in leaves increased under salt stress (Figure 3b). The susceptible variety IR31785 exhibited the highest concentrations of proline content under both control and stress conditions. As indicated by the significant variety x salt interaction, the response of proline concentration to salt stress was different from varieties with a lower relative increase in 2 tolerant varieties. The relative decrease K/Na ratio was significantly correlated with the DM response to salt stress (Figure 4). However, the ratio was negatively correlated with the relative increase in proline

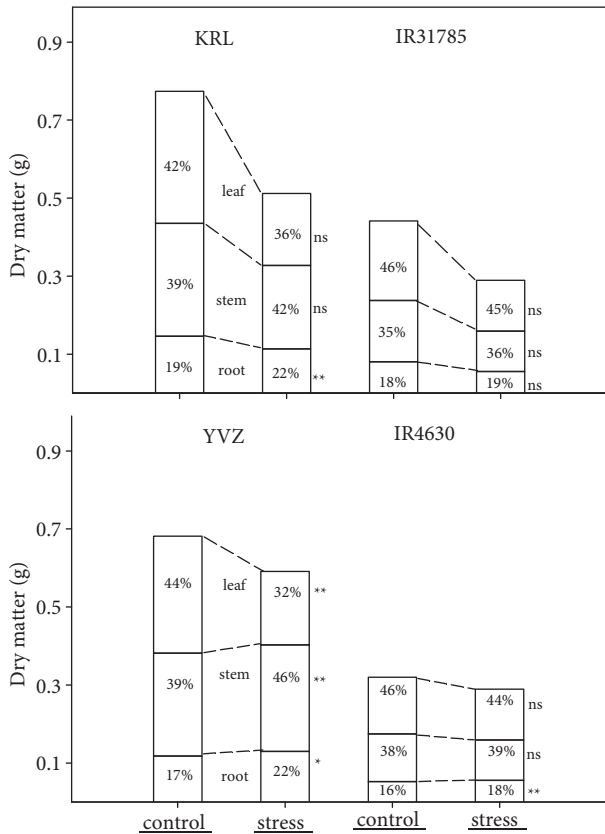


Figure 1. Dry matter partitioning values of 4 rice varieties (KRL, YVZ, IR4630, and IR31785) under control (0 mM NaCl) and salt stress (60 mM NaCl) conditions. ** P < 0.01, * P < 0.05, ns = not significant. Significant differences tested with one way ANOVA for each genotype.

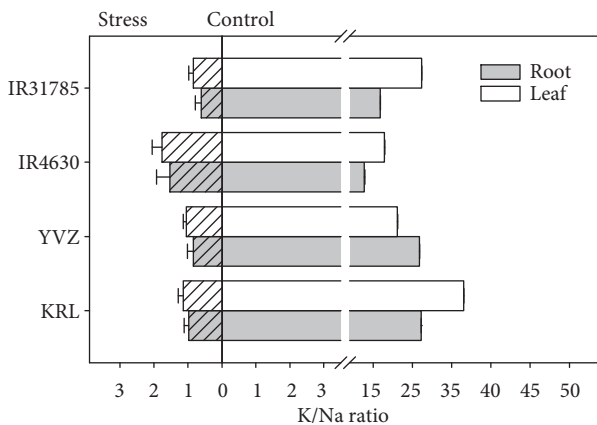


Figure 2. K/Na ratios of leaves and roots for 4 rice varieties (KRL, YVZ, IR4630, and IR31785) under control (0 mM NaCl) and salt stress (60 mM NaCl) conditions. Error bars = standard error of mean.

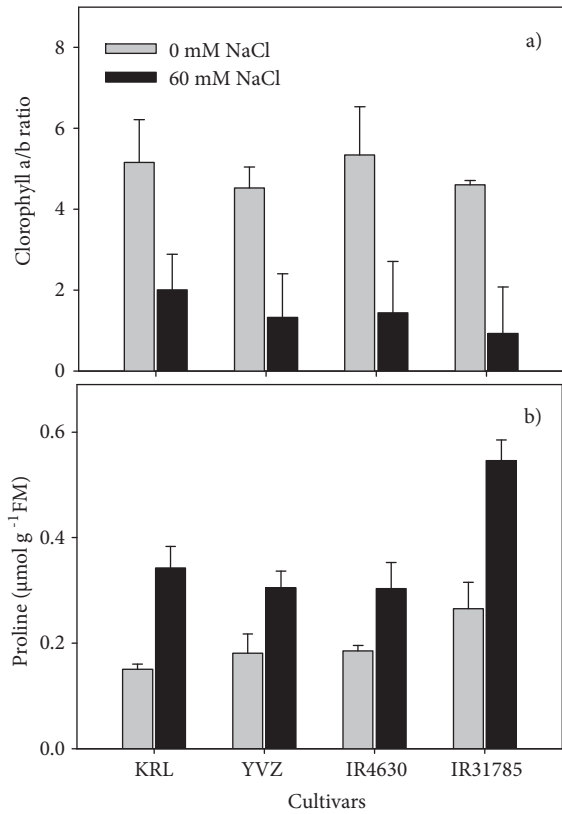


Figure 3. Effect of salt stress on a) chlorophyll a/b ratio and b) proline concentration for 4 rice cultivars (KRL, YVZ, IR4630, and IR31785). Error bars = standard error of mean.

concentration, with the tolerant varieties exhibiting a higher decrease in K/Na ratio.

Discussion

Salinity is one of the most important yield limiting factors in rice production areas (Zeng and Shannon 2000). Due to water lost through evaporation, the salinity level increases with time. In Turkey, salinity also became an important environmental problem as sound irrigation management in agricultural areas is not yet fully established and removal of the salt load by drainage is not a common practice (Alpaslan et al. 1998). Although Turkey has one of the highest rice yield potentials in the world, the rice production areas are rather limited. In order to expand rice production areas, salt-affected lands are increasingly being used, requiring the development of salt tolerant varieties.

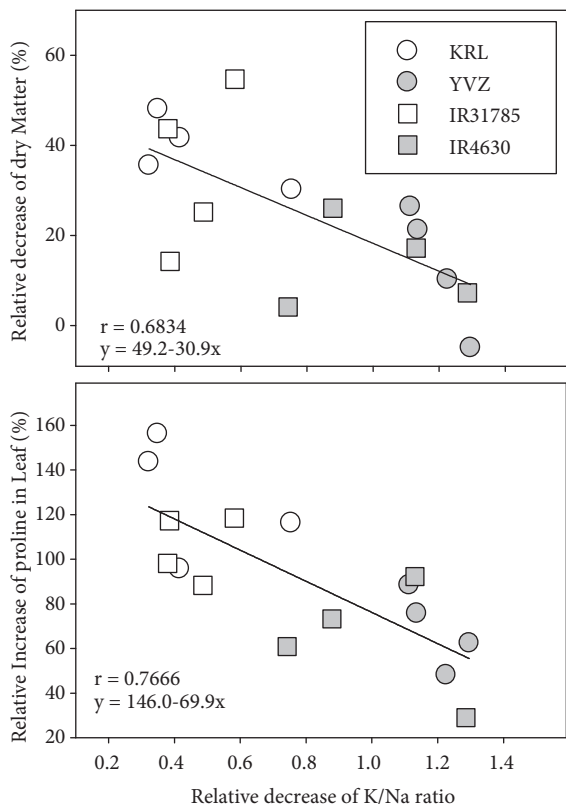


Figure 4. Salinity induced changes in dry matter and leaf proline concentration in relation to salinity induced changes in leaf K/Na ratio. All values expressed relative to the unstressed control. Values for tolerant cultivars are given in gray-shade.

Salinity effects on plants are complex. Munns and Tester (2008) reported 3 adaptation mechanisms of plants under salt stress: osmotic stress tolerance, Na⁺ or Cl⁻ exclusion, and tissue tolerance to accumulated Na⁺ or Cl⁻. The variation in salinity tolerance may change depending on the species, the salinity level, or the phenological stage of the plant. Rice is highly susceptible to salt stress at the seedling stage and screening for salt tolerance in rice at this stage was successfully implemented with regard to agronomic performance under field conditions (Zeng 2005; Asch et al. 2000b). Screening tools for salt tolerance in rice have been successfully used in breeding programs of the International Rice Research Institute (Glenn et al. 1997). However, information on tolerance levels and salt stress responses of Turkish rice cultivars is limited (Alpaslan et al. 1998; Alpaslan et al. 1999).

The dry matter accumulation of the 2 Turkish cultivars was about twice as high as that of the international check-varieties used in this study. However, the relative DM decrease under salt stress was similar in the susceptible and tolerant cultivars from Turkey and IRRI, indicating that salt tolerance did not depend on the growth potential per se. Our results clearly show that there are varietal differences in salt tolerance among Turkish rice germplasm.

Ali et al. (2002) reported effects of salt stress on dry matter partitioning in rice and suggested that a preference for dry matter partitioning to the root system rather than to the shoots should serve as a selection parameter for salt tolerant genotypes. In the present study, partitioning of dry matter to the different organs varied strongly among the varieties and was not consistently related to salt tolerance. No significant differences in partitioning parameters were found between the 2 tolerant genotypes and the susceptible IRRI variety, whereas the tolerant Turkish variety YVZ under salt stress significantly reduced the share of dry matter invested into transpiring organs. This adaptation may indicate a response to the primary stress event of salt-induced water deficit. To what extent this adaptation could represent a useful mechanism to salinity resistance requires further evaluation of a broader range of varieties.

Salinity effects on ion uptake have been investigated in many species (Sharma 1996; Zhao et al. 2007) including rice (Zafar et al. 2004; Wimmer and Asch 2005). Lutts et al. (1996b) reported that a salt susceptible check-variety accumulated more Na⁺ and less K⁺ than the tolerant varieties under salt stress. Similarly in this study, the K/Na ratio was lower in the susceptible but higher in the tolerant international high yielding check-variety; however, YVZ and KRL had similar K/Na ratios under salinity. Considering the 2 main mechanisms to avoid detrimental effects of salt stress, namely salt exclusion and tolerance on the cell-level (Parida and Das 2005), the results confirm earlier reports by Asch et al. (1997b) who showed a salt exclusion mechanisms superior to IR31785 in IR4630 due to high water use efficiency and a low apoplectic by-pass for sodium, leading to a reduced salt load in the leaves. Thus, they supported the findings of Sharma (1986), who found a correlation between Na accumulation in leaf tissue

and salt tolerance. Contrarily, YVZ had a similar K/Na ratio in leaves with susceptible Turkish variety KRL indicating that YVZ must possess an intercellular adaptation mechanism rendering the accumulation of sodium in the tissue non-toxic. These findings are similar to those of Lutts et al. (1996b), who found no correlation between tolerance and Na concentration level in leaf tissue.

A stress-induced reduction in chlorophyll concentration was previously reported in rice (Ali et al. 2004). Lutts et al. (1996a) suggested that Chl a is affected more than Chl b under salinity and Kura-Hotta et al. (1987) proposed Chl a/b as an indicator of leaf senescence. Although our results generally confirm the finding of Lutts et al. (1996a), varietal differences were not found in Chl a/b ratio. These results indicate that the Chl a/b ratio may not be a suitable selection criterion.

In all cultivars, a more than 1.5-fold increase in the leaf proline concentration was found as a result of salt stress. The increase was more pronounced in the susceptible varieties IR31785 and KRL. A higher proline content of salt-stressed plants was previously reported in different species (Madan et al. 1995; Garcia et al. 1997; Özcan et al. 2000). Lutts et al.

(1999) suggested that proline accumulation in rice under salinity was most likely a symptom of injury rather than an indicator of increased tolerance. However, Singh et al. (1996) emphasized the protective role of proline under salinity. Mansour et al. (1998) also suggested additive effects of proline upon salt tolerance via increased cell membrane stability. Based on our results, we cannot distinguish between higher proline concentrations in leaf cells being an indicator of injury or a protection mechanism. The decrease in K/Na ratio and proline content in leaves (Figure 4) indicated that the proline content of leaves may act as an indicator of Na-uptake and allow the identification of tolerant varieties, which effectively exclude Na from leaf tissue either by exclusion or compartmentalization in root and stem tissues.

In conclusion, the results presented here show that salt tolerance of 1 Turkish cultivar is related to the rapid reduction in transpiring leaf surface and intercellular adaptation of leaf Na. Different cultivars showed different adaptation mechanisms under salt stress, whereas the increase in proline accumulation was an indicator of a response to salt stress in all cultivars.

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