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The effect of salinity stress on ions distribution in panicle, flag leaf and leaf sheaths of two rice (*Oryza sativa* L.) genotypes differing in salt tolerance

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Abstract

The influence of NaCl on ions accumulation was investigated in panicle, leaf sheath and flag leaf of two rice genotypes differing in salt tolerance. In comparison, Na⁺ content in all part of sensitive cultivar (IR29) was more than tolerant one (FL485) under salt condition. There was a significant ($P \leq 0.01$) reduction in flag leaf, leaf sheath and panicle K⁺ concentration in both cultivars result in salt treatment. Salinization of the medium caused a significant accumulation of Cl⁻ ions in all parts of stressed IR29 especially in flag leaf and leaf sheath in compare to their control treatment. Opposite to that observed in stressed IR29 Cl⁻ accumulation, a marked decline was found during experiment in all part of salt-tolerant cultivar FL485 except in leaf sheath. Ca²⁺ accumulation in tolerant FL485 cv. was significantly more than sensitive IR29 cv. at all part of plant. Upon salinization, the Mg²⁺ content of leaf sheath and flag leaf of both cultivars significantly reduced in contrast to those control treatment, while no significant changes was observed in Mg²⁺ panicle concentration of both cultivars.

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Introduction

Salinity and water deficits are among the most important agricultural problems in semi-arid and arid regions (Flowers *et al.*, 1977; Glenn and Brown, 1998). These conditions cause significant yield reductions on cultivated lands, inducing a wide range of perturbations at the cellular and whole-plant levels that can lead to plant death and decreases in productivity. However, it has been well established that plants growing naturally in arid and semiarid areas have evolved many adaptations to counteract salt stress (Khan *et al.*, 2000). The ability to maintain turgor despite the lack of water induced by salt stress may preserve the metabolic processes, and therefore the growth, of a plant (Martinez *et al.*, 2004). Adaptation of plants to high levels of sodium salts can be achieved in two very different ways. Plants can either exclude salts from the interior of their cells, or include salts within the leaf cells but at the same time sequester them in the vacuoles. The result of this adaptation process is maintenance of cytoplasmic salt concentration at a relatively low level (O'Leary, 2002). Ion selectivity enables the plant to control uptake of toxic ions like Na^+ and Cl^- and their accumulation in the cytoplasm (Shannon and Grieve, 1999). Plants do this either through "strict ion regulation" to keep the Na^+ and Cl^- out of the transpiration stream and subsequently the cytoplasm of the aerial parts of the plants (Harvey, 1985), or through ion discrimination, enabling the plant to discriminate between chemically similar ions such as Na^+ and K^+ (Gorham *et al.*, 1997). Chloride ions can affect plant growth directly by influencing photosynthesis, as it is required for the reactions of photosystem II, and indirectly, *via* stomatal regulation (Marschner, 1995). On a worldwide basis, the problem of chlorine toxicity is much greater than that of chlorine deficiency. On average, concentrations of chloride in the external solution of more than 20 mM can lead to toxicity in sensitive plant species, whereas in tolerant species the concentration can be four to five times higher without reducing growth (Marschner, 1995). The concentration at which Cl^- becomes toxic is probably in the same range as that for Na^+ (Greenway, 1972).

High levels of Ca^{2+} in rice root environment are essential for the maintenance of high root uptake and shoot accumulation of Ca^{2+} and K^+ in saline soils and thus for avoiding salinity damage in plants as shown in rice plants (Song *et al.* 2006). Magnesium, by comparison, has received little attention, although it could play a central role in senescence-related processes. Mg^{2+} is implicated in the regulation of protein synthesis (Flowers and Dalmond, 1992). A decrease in Mg^{2+} absorption could also be responsible for decreased chlorophyll content (Leidi *et al.*, 1991) and quenching of variable fluorescence due to increased 'spillover' of excitation energy from PS II to PS I (Krause and Weis, 1991). The objectives of this experiment were to compare responses of rice genotypes differing in tolerance to salt stress in relation to ions accumulation in leaves, leaf sheaths and panicle during reproductive phase.

Materials 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 °C and light intensity in the range of about 600–1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 dS m⁻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

Ions concentration were measured on flag leaves, leaf-sheath and panicle of the first two tillers that were tagged 25 d after sowing. Sampling of the flag leaves, leaf-sheaths and panicles were removed from anthesis up to full grain maturity at seven day interval [7, 14, 21 and 28]. The various plant parts were dried in oven at 80 °C for dry matter analyses and various estimations.

Determination of Na⁺, K⁺, Cl⁻, Mg⁺² and Ca⁺² ions

Samples digestion was done for Na⁺, K⁺, Ca²⁺ and Mg²⁺, determination via wet digestion using sulfuric acid (96%), H₂O₂ (30%) and salicylic acid. The concentration of Na⁺ and K⁺ were determined after digestion with a Flame photometer (Corning-410), Ca²⁺ and Mg²⁺ were determined with an Atomic Absorption Spectrometer (Perkin Elmer, Analyst 300, California, USA). Chloride was measured with Ion-Analyzer using a chloride electrode (ISM146-Cl, Los Angeles, USA) after digestion with distilled deionized water. Data were analyzed in a factorial based on completely randomized design with four replications. Means were statistically compared among treatments by the Least Significant Difference (LSD) at the $P \leq 0.01$ level using the SAS (Ver. 6.12) software.

Results and discussion

Irrespective to salt stress, absolute Na⁺ concentration in different parts of IR29 cultivar was more than FL485 (Fig 1). Na⁺ concentration significantly increased in rice tissues in the presence of NaCl (Fig. 1). Nevertheless, the degree of accumulation was not the same in all tissues. Leaf sheath Na⁺ concentration

was higher than in other parts in both genotypes (Fig. 1). In comparison, Na⁺ content in all part of sensitive cultivar (IR29) was more than tolerant one (FL485) under salt condition and the differences between the amount of Na⁺ accumulation in the panicle was higher than in other part at the end of experiment (Fig. 1A). Results showed that plant growth suppression in studied genotypes was related to the rate of Na⁺ accumulation in leaves, leaf sheaths and panicle. Salt tolerant genotype was able to prevent high Na⁺ accumulation in all part of plant and thereby decrease Na⁺ damages to active tissues. Na⁺ toxicity is strongly linked to the plants' ability to sustain the acquisition and in plant a distribution of K⁺ (Kader and Lindberg 2005).

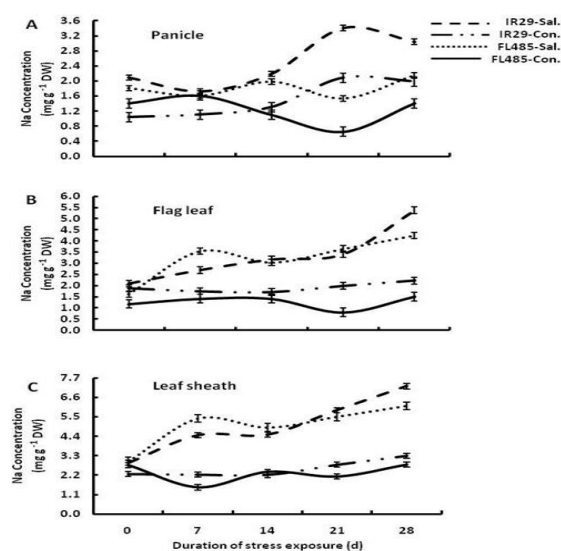


Fig. 1 (A-C). Effects of salinity on Na⁺ concentration in panicles (A), flag leaves (B) and leaf sheaths (C) in two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent \pm SE of the mean (n=4).

Similar to Na⁺, absolute K⁺ concentration in all part of unstressed IR29 cv. was higher than FL485 cv. at all sampling stages in the flag leaf and leaf sheath and at the end of experiment in the panicle (Fig. 2). There was a significant ($P \leq 0.01$) reduction in flag leaf, leaf sheath and panicle K⁺ concentration in both cultivars under saline condition in compare to those respective control treatment (Fig. 2). Results also showed that there were more reduction in all sampling stages of ($P \leq 0.01$) flag leaf, leaf sheath and panicle of susceptible IR29 cv. K⁺ concentration except the last sampling

under stress conditions in compare to tolerant FL485 cv. (Fig. 2). As shown in figure 2B, K^+ concentration in flag leaf of both genotypes was higher than in leaf sheath and panicle. Interactions between cations with respect to uptake and accumulation rates in different plant parts are very complex (Gorham *et al.*, 2007). In addition, more than 50 plant enzymes require K^+ as a cofactor, and these are particularly susceptible to high Na^+ and high Na^+/K^+ ratios (Munns and Tester., 2008). Excessive Na^+ inhibits the uptake of K^+ and Ca^{2+} in many plant species, such as wheat, cotton and tomato (Box and Schachtman, 2000 : Al Karaki, 2000). The reduction in K^+ concentration observed in both cultivars with NaCl regime can be explained by the antagonism between K^+ and Na^+ during the uptake process (Box and Schachtman, 2000). Although Na^+ and K^+ are chemically similar elements, they have very different roles in plant metabolism. One of the main roles of K^+ is in generating turgor, which can be partially fulfilled by Na^+ , but the estimated extent to which Na^+ can replace K^+ is very variable in different species (Subbarao *et al.*, 1999; Subbarao *et al.*, 2000). There is evidence that shoot Na^+ concentrations (altered by spraying Na^+ onto leaves) can affect the transport of K^+ to the shoots, or at least leaf K^+ concentrations (Song and Fujiyama, 1998). It was shown that cells of higher plants are able to discriminate against Na^+ (Higinbotham *et al.*, 1967).

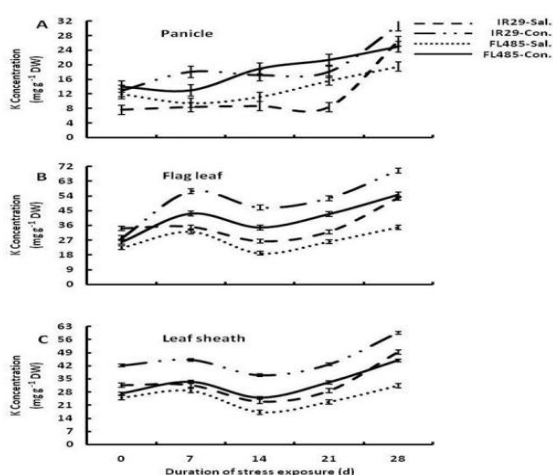


Fig. 2 (A-C). Effects of salinity on K^+ concentration in panicles (A), flag leaves (B) and leaf sheaths (C) in two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent \pm SE of the mean (n=4).

Salinization of the medium resulted in a significant accumulation of Cl^- ions in all parts of stressed IR29 especially in flag leaf and leaf sheath at all sampling stages in compare to their control treatment (Fig. 3b&c). Opposite to that observed in stressed IR29, Cl^- accumulation, a marked decline was found during experiment in all part of salt-tolerant cultivar FL485 except in leaf sheath (Fig. 3). FL485 was able to preserve Cl^- in leaf sheath while a high rate of this ion was observed in the flag leaf of IR29. Thus, resistant cultivar had the ability to avoid Cl^- accumulation, which probably could be a good tolerance determinant for NaCl stress. Our result is in line with Lacerda *et al.* (2003), who showed that Cl^- accumulation in shoot of salt tolerant plant was lower compared to salt-sensitive ones under saline condition.

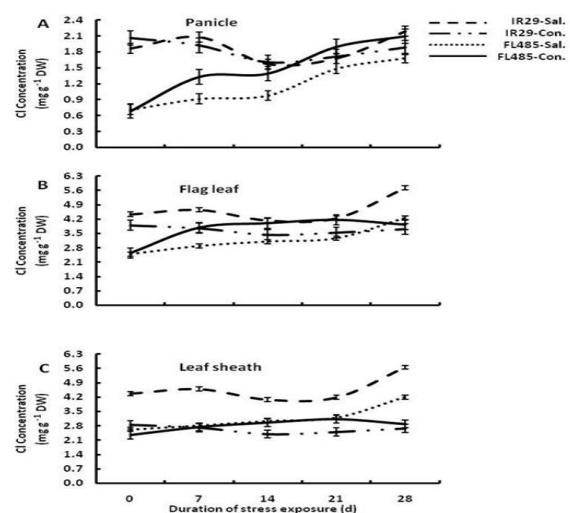


Fig. 3 (A-C). Effects of salinity on Cl^- concentration in panicles (A), flag leaves (B) and leaf sheaths (C) in two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent \pm SE of the mean (n=4).

Regardless to salinity, Ca^{2+} accumulation in tolerant FL485 cv. was significantly more than sensitive IR29 cv. at all part of plant. Over time, Ca^{2+} concentration in all part of both cultivars increased (Fig. 4). Changes in Ca^{2+} concentration in the leaf sheath and flag leaf of susceptible cultivar under salinity during the experimental period was not significant whereas salinity cause to a markedly reduction in panicle Ca^{2+} concentration of susceptible cultivar during all

sampling stages but this reduction was not evident in tolerant variety (Fig. 4a). Ca^{2+} functions in plants include alleviation of ionic stress, activation of enzymes, sensing and responding. It was shown in previous studies that increasing Na^+ content in plant environment caused reduction of Ca^{2+} content in salt-sensitive plants (Lacerda *et al.* 2003). In previous studies on soybean and cucumber, an additional supply of Ca^{2+} to salt-stressed plants improved the salt tolerance of plants by reducing Na^+ uptake and transport (Dabuxilatu and Ikeda 2005). According to Husain *et al.* (2004) the major role of Ca^{2+} for increasing salt tolerance of plants was related to its inhibitory effect on the xylem loading of Na^+ . Song *et al.* (2006) reported that high levels of external Ca^{2+} are essential for the maintenance of high root uptake and shoot accumulation of Ca^{2+} and K^+ on saline soils. It also plays a key role to avoid salinity damage to plants. Despite the accumulation of calcium, Mg^{2+} accumulation trend in different part of both cultivars and regardless of treatments decreased during the experimental period. It is interesting to note that the level of Mg^{+2} was higher in the tolerant FL485 cv. as compared to the susceptible IR29 cv. (Fig. 5). Upon salinization, the Mg^{2+} content of leaf sheath and flag leaf of both cultivars significantly reduced in contrast to those control treatments by time, while no significant changes was observed in Mg^{2+} panicle concentration in respect to control conditions in both cultivars (Fig. 5a). Salinity has various effects on Mg^{2+} content in plants. Mitochondrial SOD (superoxide dismutase) is scavenged with Mg^{2+} that can play an important role to remove toxic effects of oxidative stress. Mg^{2+} concentration decreasing under salinity lead to a decrease of micro elements solubility in saline and sodic soils (Lacerda *et al.* 2003).

Over all, this study has highlighted a relationship between plant ionic status and salinity tolerance in studied rice cultivars. Tolerant genotype was able to avoid Na^+ excess accumulation and reduced Cl^- content in panicle and flag leaf and yet, under stressed or unstressed conditions Mg^{2+} and Ca^{2+} levels of all part of tolerant plant was more than sensitive one.

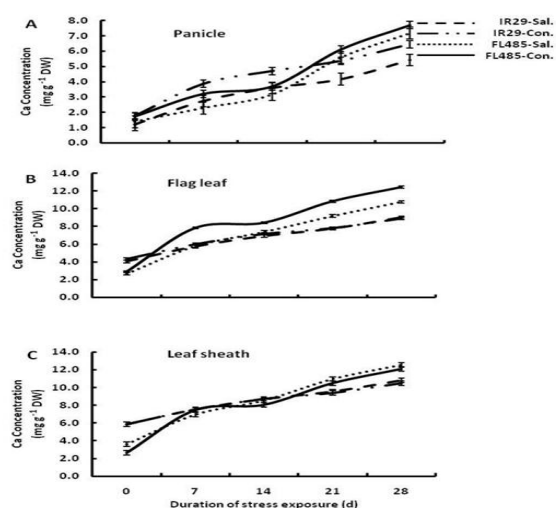


Fig. 4 (A-C). Effects of salinity on Ca^{2+} concentration in panicles (A), flag leaves (B) and leaf sheaths (C) in two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent \pm SE of the mean (n=4).

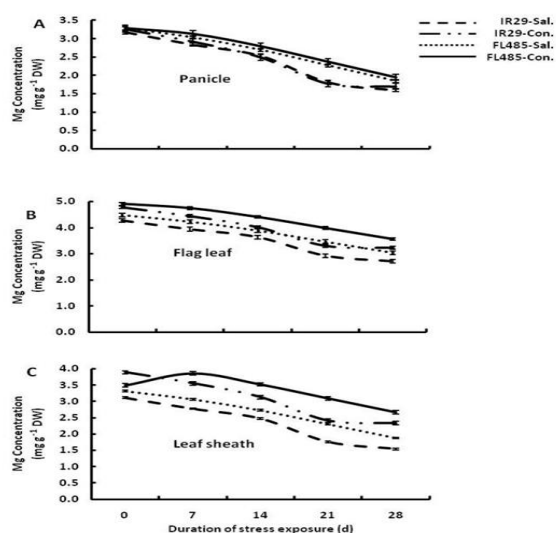


Fig. 5 (A-C). Effects of salinity on Mg^{2+} concentration in panicles (A), flag leaves (B) and leaf sheaths (C) in two rice cultivars (Salt-Sensitive cv. IR29 and Salt-Tolerant cv. FL485). Vertical bars represent \pm SE of the mean (n=4).

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