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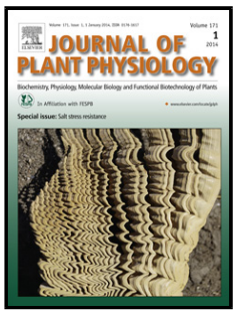

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SECTION: PHYSIOLOGY

A differential tolerance to mild salt stress conditions among six Italian rice genotypes does not rely on Na^+ exclusion from shoots

Running title: Cation homeostasis under salt stress in six Italian rice genotypes

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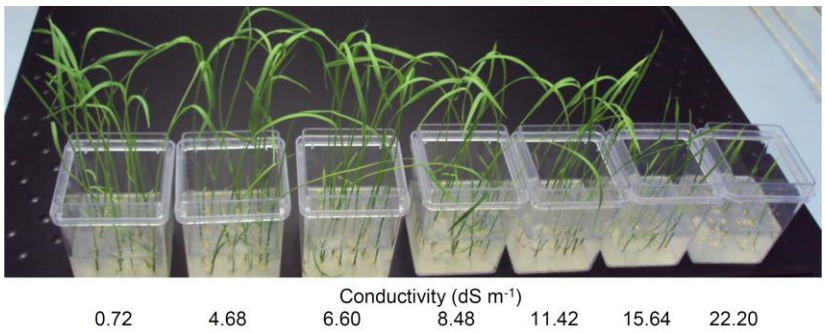
Graphical abstract



ABSTRACT

Rice is very sensitive to salt stress at the seedling level, with consequent poor crop establishment. A natural variability in susceptibility to moderate saline environments was found in a group of six Italian temperate japonica rice cultivars, and the physiological determinants for salt tolerance were investigated. Cation (Na^+, K^+ and Mg^{++}) levels were determined in shoots from individual rice plantlets grown in the absence or in the presence of inhibitory, yet sublethal salt levels, and at increasing time after salt treatments. Significant variations were found among genotypes, but these were unrelated to the relative tolerance, which seems to result from neither mechanism(s) for reduced Na^+ translocation to the aerial part, nor its increased retrieval from the xylem mediating Na^+ exclusion from leaves. Accordingly, thiobarbituric acid reactive substance levels raised in leaf tissues of salt-treated seedlings, and osmo-induced proline accumulation was found in all genotypes. Data suggest that the difference in salt tolerance most likely depends on mechanisms for osmotic adjustment and/or antioxidative defence.

Abbreviations: ROS, reactive oxygen species; TBARS, thiobarbituric acid reactive substances.

Keywords:
rice (*Oryza sativa* L.)
salinity tolerance
damage threshold
cation homeostasis and translocation
osmo-induce proline accumulation
thiobarbituric acid reactive substances

1. Introduction

Salt accumulation in the soil, deriving from improper farming practices or irrigation with poor quality water (Rengasamy, 2010), represents an increasing threat to agricultural productivity (Pitman and Läuchli, 2004). Because of the climate change, this problem is expected to grow steadily (Munns and Gilliham, 2015). Moreover, in proximity to deltas and estuaries the ingression of saline cones may occur, leading to remarkable seasonal raising of salt content in the soil solution. This is the case of Northern Italy (Antonellini et al., 2008), where rice is grown as an irrigated crop in Po delta.

By definition, a soil is considered saline when ionic concentration exceeds an electrical conductivity of 4 dS m^{-1}, a value that corresponds to about 30-40 mM ions, depending on composition (Munns and Tester, 2008). Plants can cope with moderate salinity conditions either by limiting ion uptake at the root level, by compartmentalizing/extruding ions in the vacuole/apoplast, or by counteracting the consequent water withdrawal through the intracellular accumulation of compatible osmolytes (Zhu, 2001; Zhang et al., 2012). At increasing soil conductivity, however, salt stress causes a progressive reduction of the photosynthetic rate and stimulates the production of reactive oxygen species (ROS), which in turn can lead to oxidative damages at the cellular level (Chaves et al., 2011).

Rice (*Oryza sativa* L.), the second most important cereal crop worldwide feeding almost one half of the population, is very sensitive to excess salt (Zeng and Shannon, 2000).

At the seedling level, salt stress can lead to poor crop establishment, whereas at the onset of flowering, salinity can severely disrupt grain formation and yield. It has been showed that a conductivity value as low as 3 dS m^{-1} was already inhibitory, and that a 12% yield reduction occurred for each further 1 dS m^{-1} increase (Maas, 1990). More recently, experimental evidence accounted for an even higher sensitivity, with a damage threshold of 1.9 dS m^{-1} (Grattan et al., 2002). Because of the agronomical relevance, many researchers investigated the occurrence of a differential sensitivity to salt stress among rice cultivars (Negrão et al., 2011), as well as the mechanisms involved (for a recent review see Reddy at al., 2017). However, virtually all studies dealt with Asian rice genotypes, whereas scarce information has been made available to date with respect to the Italian rice germplasm. Italy is the only country in Europe with a significant land area used for rice production (about 250,000 ha; www.enterisi.it), sustaining a market worth more than 1 billion €. Italian germplasm comprises not less than two hundred varieties belonging to the *japonica* ssp. (Faivre-Rampant et al., 2011), and a valued rice is produced in paddy fields in the Northern Adriatic coastal region that is vulnerable to salt inflow from the sea. This notwithstanding, the occurrence of a natural variability among these cultivars with respect to salt tolerance has never been investigated.

In the frame of a research project for integrated genetic and genomic approaches for new Italian rice breeding strategies, we aim at a better understanding of the biochemical mechanisms underlying salt tolerance in rice. To achieve this goal, cultivars with a contrasting ability to cope with salt stress need to be identified. Here we report the occurrence of a mild, yet significant natural variability during early growth under moderate stress conditions among a set of six Italian rice varieties. The possibility that the differential tolerance may arise from different ion homeostasis in leaves was investigated.

2. Materials and Methods

2.1. Plant material and growth conditions

Rice (*Oryza sativa* L.) genotypes were obtained from the *Unità di ricerca per la risicoltura* of the Consiglio per la Ricerca in Agricoltura e l'Analisi della Economia Agraria (CREA; Vercelli, Italy). Seeds were surface-sterilized by sequential treatment for 5 min with absolute ethanol and for 10 min under vacuum with a 3% NaClO solution containing 0.04% (v/v) Triton X100. After extensive washing with sterile distilled water, seeds were allowed to imbibe for 5 h in the dark, then sown in Magenta vessels (6⇨ x 6⇗ x 20⇧ cm) filled with 75 mL of agarized (6‰) water, 16 seeds for vessel, and covered with the same volume of 2X nutrient solution (Table 2), pH 5.8, containing 2 mL L^{-1} Plant Preservative Mixture (Plant Cell Technology). Vessels were incubated under a photoperiod of 16 h light (250 µmol m^{-2} sec^{-1}) and 8 h dark at 26 ± 1°C (day) and 22 ± 1°C (night) in an incubator equipped with E27 ES 1700 lumen daylight lamps (GE Lighting).

2.1.1. Salt treatments

The effect of increasing conductivity upon seed germination and seedling growth was assessed by complementing the nutrient solution with a salt mixture (NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 in a 10:1:2:1 molar ratio) (Table 2). The actual value of conductivity in each sample was measured 24 h after sowing and at the end of the incubation period with a conductivity-meter (Hanna HI 8733). A randomized complete block design with four replications was used, each block consisting of 7 vessels of 6 salt rates (from 4.7 to 22.2 dS m^{-1}) and untreated controls. Destructive harvest was carried out 14 to 16 days after sowing, when controls had reached the three-leaf stage. After discarding seed residues, roots and shoots were weighted separately for each seedling ($n = 30 \leq x \leq 60$ depending on germination rate). Then the material was treated in an oven at 90°C for 48 h for the determination of dry weight.

2.2. Determination of tissue cation content

For the evaluation of cation concentration in the rice seedling tissues, the experimental design consisted of a randomized complete block with four replicates. Each block comprised 24 Magenta vessels of 6 genotypes and 3 salt rates (4.7, 8.5 and 15.6 dS m^{-1}), plus untreated controls. In a first experiment, seeds were germinated and grown for 13 days in nutrient solution. Thereafter, 50 mL of salt-containing nutrient solution were added, so as to obtain the desired final concentrations. Plant materials were harvested 24 and 48 h after the addition. In a second experiment, seedlings were sown directly in the presence of salts at a given conductivity value, and harvested 14 days after sowing. At the harvest, ten uniformly-grown seedlings were collected for each treatment, and roots were extensively washed with deionized water. Seed residues were discarded, and roots and shoots from each seedling were weighted and dried in an oven at 90°C for 2 days.

Dried material was then digested by a microwave digester system (Anton Paar Multiwave 3000) in Teflon tubes filled with 10 mL of 65% HNO_3 by applying a two steps power ramp (Step 1: at 500W in 10 min, maintained for 5 min - Step 2: at 1200 W in 10 min, maintained for 15 min). After 20 min cooling, the mineralized samples were transferred into polypropilene test tubes. Samples were diluted 1:20 with Milli-Q water and the concentration of elements was measured by ICP-MS (Varian 820 ICP-MS). An aliquot of an internal standard solution (2 mg L^{-1} ^{45}Sc, ^{89}Y, ^{159}Tb) was added both to samples and standards for calibration curve to give a final concentration of 20 mg L^{-1}. Operating conditions are reported in Table 2.

2.3. TBARS and proline quantitation

To measure thiobarbituric acid reactive substances (TBARS), fresh plant material from seedlings grown under the same experimental conditions was treated with 2 mL g^{-1} of 0.1% (w/v) TCA solution, and extracted with 1 g g^{-1} quartz sand in a mortar equilibrated on ice. Following centrifugation for 3 min at 14,000 *g*, TBARS were determined by mixing proper dilutions of the supernatant in a final volume of 40 µL with 80 µL of 20% TCA and 80 µL of 0,67% (w/v) thiobarbituric acid. Samples were incubated at 70°C for 30 min, and then read

at 532 nm. TBARS were estimated by comparison with a calibration curve obtained with an authentic malondialdehyde standard.

For the determination of free proline levels, plant material was extracted as above with 2 mL g^{-1} of a 3% (w/v) solution of 5-sulphosalicylic acid. Proline content was quantified with the acid ninhydrin method, as previously described (Forlani et al., 2013).

2.4. Statistical analysis

The conductivity values causing 50% inhibition (IC_{50}) of either growth or germination, as well as the thresholds of damage (herein defined as the conductivity values at which 10% inhibition was caused, IC_{10}) and their confidence intervals were estimated by non-linear regression analysis (variable slope) of dry weight values, expressed as percentage of untreated controls, plotted against the logarithm of conductivity, using Prism 6 (version 6.03, GraphPad Software, Inc., USA). The same software was used for analysis of variance. Where differences are reported, they are at the 5% level ($P < 0.05$), unless stated otherwise. In order to evaluate the possible existence of correlations among the concentrations of Na^+, K^+ and Mg^{++} in the shoot of each cultivar grown at increasing conductivity values in the medium, the Pearson coefficients were calculated in R environment (www.r-project.org) using the standard “cor.test” function. The resulting correlation matrixes were plotted using the "corrplot" function and the "circle" method; the significance of the correlations was assessed with the *t*-test implemented in the “cor.test” function.

3. Results

3.1. Seedling growth of six Italian rice cultivars was differentially affected by mild salt stress conditions

Based on preliminary results obtained on a wider set of cultivars, a group of six Italian rice varieties were considered for the ability to grow in the presence of increasing salt levels.

Because the composition of the soil solution in saline areas comprises significant amounts of other salts besides NaCl, a salt mixture mimicking field conditions (Grattan et al., 2002) was used (Table 1). When seedling weight was measured 2 weeks after sowing, in some instances a significant reduction of dry biomass was found at the lowest conductivity value tested (Table 3; Fig. 1A). Result analysis showed the occurrence of a significant variability among genotypes. A non-significant interaction between genotype and treatment was on the contrary found, suggesting that these rice varieties respond comparably at varying salt concentration. Regression analysis of data allowed the estimation for each genotype of the IC_{50} and IC_{10} values (Fig. 1B). Genotypes were divided accordingly into three groups: highly salt sensitive (IC_{10} < 5 dS m^{-1}: Vialone nano and Thaibonnet), sensitive (IC_{10} in the range 5-8 dS m^{-1}: Carnaroli, Volano and Baldo) and mildly tolerant (IC_{10} > 8 dS m^{-1}: Loto). The effects on plant growth were already evident at a substrate conductivity that may realistically occur in salt-affected agricultural areas (2 to 6 dS m^{-1}; Maas, 1990) as showed in Figure 1A, considering both the most sensitive (Vialone nano) and the most tolerant (Loto) cultivars.

3.2. Na^+ concentrations in leaves of salt-treated seedlings increase rapidly in all genotypes following the exposure to stress conditions

In order to obtain some insight into the mechanisms underlying the differential susceptibility to excess salts, cation content was measured in seedlings grown under normo-osmotic conditions up to the three-leaf stage, and then exposed to salt stress. Although roots had been extensively washed at harvest, the level of cations in the root system merely reflected the ion composition of the medium (data not presented), most likely because of apoplastic diffusion in root cortex.

Data obtained for shoots 24 and 48 h following salt exposure are summarized in Table 4. In all cases the levels of Na^+ increased steadily with time, coherently with the increasing of the substrate conductivity. Interestingly, among the most sensitive cultivars Thaibonnet showed the larger and faster accumulation of Na^+ in the shoot. However, it was not observed in the case of Vialone nano, which behavior was not markedly different to those of the most

tolerant cultivar Loto. Therefore, although significant differences resulted among genotypes (Table 4), the dynamic of Na^+ accumulation in the shoots seems unrelated to the relative tolerance to salt stress conditions. Concerning K^+ concentration, which usually shows a significant decrease in salt-treated plants due to a competition between the two cations for the transporters mediating their root absorption and translocation to the shoot (Yao et al., 2010), slight but significant decreases were observed in all the cultivars particularly 48 h after the strongest salt treatments, independently of their sensitiveness. For each cultivar, despite the different treatments, the values of Mg^{++} levels in the shoots resulted very close to the range considered optimal for plant growth (1500-3500 mg kg^{-1}; Marschner, 2012). In greater detail, within the first 48 of the salt treatments, excluding in Vialone nano where they slightly increased, the levels of Mg^{++} in the shoot did not markedly change with respect to those measured in control plants (Table 4).

Because the ability of plants to maintain a low intracellular Na^+-to-K^+ ratio is considered a key determinant for plant salt tolerance (Maathuis and Amtmann, 1999), this parameter was also calculated. Results (Fig. 2) showed similar values, with higher ratios for the salt-sensitive cultivar Thaibonnet and the more tolerant cultivar Volano. On the whole, data therefore suggest that a higher ability to grow under salt stress conditions is not due to a higher capacity in limiting Na^+ translocation to the aerial part of the plant.

3.3. Homeostatic levels of cations in leaves of seedlings grown under stress conditions do not correlate with the differential tolerance to salt

The results up here described did not rule out the possibility that among the rice varieties some defense mechanism might be differentially activated later than 48 h after the exposure to stress conditions, resulting in lower Na^+ (or higher K^+) homeostatic levels in tolerant varieties. To verify this possibility, the level of the cations was determined in the shoots of seedlings at the three-leaf stage directly sown and grown in salt-containing medium. Also in this case Na^+ concentration increased as a function of its concentration in the substrate (Table 5), reaching values 2 to 3-fold higher than those found 48 h after the

exposure to salt stress (Table 4). The levels of Mg^{++} in the shoots tendentially increased as a function of its concentration in the nutrient solutions; however, these increases were not proportional to the treatment (Table 5). Interestingly, more remarkable variations were evident with respect to K^+ content. Contrary to what found early after the exposure to salt stress, a strong reduction was shown that was proportional to the severity of the stress. In the case of the salt-sensitive variety Thaibonnet, a 3-fold decrease at the highest salt treatment caused Na^+ levels to be higher than those of K^+ (Table 5). Indeed, when the Na^+-to-K^+ ratio was calculated (Fig. 3), for all treatments the value found for this cultivar was strikingly higher than that of the other genotypes. However, values for the other 5 rice varieties were very similar, being only those for the cultivar Volano slightly (even if significantly) lower. Since in the previous experiment Volano had on the contrary shown the highest Na^+-to-K^+ ratios early following the exposure to salt (Fig. 2), results suggest that, despite the difference found in the rate of Na^+ translocation from the roots to the shoots, in most cultivars similar Na^+-to-K^+ ratios are attained at the steady-state. Pairwise Pearson's coefficients of correlation among the shoot concentrations of Na^+, K^+ and Mg^{++} were calculated for each cultivar grown at increasing conductivity values and, as expected, significant correlations ($p < 0,05$) resulted (Fig. 4). In detail: a) excluding Vialone nano, the Na^+ concentrations in the shoots resulted always negatively correlated ($p < 0.05$) with those of K^+ (*r* in the range -0.91 to -0.98); b) a positive correlation was observed between the Mg^{++} and Na^+ shoot concentrations in the case of Baldo, Loto, Vialone nano e Volano, but not for Thaibonnet and Carnaroli; however, in the latter cultivar a general positive trend between the concentrations of the two cations exists ($r = 0.83$); c) only in the cases of Baldo and Loto, the existence of a significant negative correlation ($r = -0.95$ and -0.99, respectively) between the shoot concentrations of Mg^{++} and K^+ was observed. In any case, differences appeared once again unrelated to growth susceptibility to salt stress, making unlikely the occurrence of resistance mechanisms either mediating Na^+ exclusion from leaves, or based upon K^+ accumulation in leaf blades.

3.4. Increased TBARS levels and proline accumulation in salt-grown seedlings strengthen the possibility that similar stress conditions arise in leaves of tolerant and sensitive varieties

The experimental approach used does not distinguish between ion content in the cytosol and in the vacuole. Na^+ compartmentalization in the vacuole could strongly reduce ionic toxicity and a higher rate of vacuole loading may provide the basis for a higher tolerance to excess salt, resulting in lower cell damages. To investigate this hypothesis, the occurrence of oxidative stress conditions was measured as the amount of thiobarbituric-reactive substances (TBARS) in cell extracts. Indeed, around 2-fold higher TBARS levels were found in shoots of seedlings grown in the presence of salts at 15.64 dS m^{-1} than in control plants grown under normo-osmotic conditions (Fig. 5A). The most remarkable increase was observed for the two varieties, Thaibonnet and Volano, which after the exposure to salt stress had shown the highest rate of Na^+ accumulation in the shoots (Fig. 2). Although indirect, such an evidence strengthens the possibility that a significant fraction of the Na^+ transported to the shoots is not confined to the apoplast or sequestered in the vacuole, and that it is indeed able to cause oxidative damages inside the cells. Significant differences were evident among varieties concerning TBARS increase ($P < 0.0001$). Anyhow, also in this case data showed no plain relationship with the susceptibility of seedling growth to salt stress.

Because the presence of excess salts in the apoplast decreases the extracellular water potential thereby causing water withdrawal from the cell, also a better ability for osmotic compensation at cellular level may provide the basis for increased salt tolerance. Being proline the most widely adopted compatible osmolyte among higher plants, the intracellular level of this amino acid was therefore measured in stressed and non-stressed seedlings. A significant increase ($P < 0.0001$) was evident in salt-grown seedlings (Fig. 5B). However, the concentrations found appear well below those reported in other species and actually needed to counteract the osmotic component of ionic stress (*e.g.* Verslues and Sharp, 1999). It is known that the osmo-induced accumulation of relatively low proline level may contribute to ROS scavenging and/or enzyme and membrane protection from Na^+ detrimental effects

(Signorelli et al., 2014). Thus, the increased proline concentration further strengthens the occurrence of damages inside the cell in all the rice varieties tested, ruling out the possibility that the differential tolerance may arise from different ion homeostasis in leaves.

4. Discussion

In order to deal with the expected increase of the frequency of stress events (like heat, drought and salinity), future rice breeding programs should target blast resistance, grain quality, adaptation to water-saving strategies and salinity tolerance. The understanding of the genetic basis of some of these traits lately made significant progresses with mapping populations for quantitative trait loci detection (Courtois et al., 2011; Faivre-Rampant et al., 2011). However, quite surprisingly, no study focused to date on the occurrence of a natural variability among the considered temperate japonica rice genotypes with respect to the susceptibility to excess salt. Two hundred selected rice accessions were phenotyped for tolerance to salinity with the aim to identify rice varieties achieving Na^+/K^+ homeostasis through a targeted association analysis (Ahamadi et al., 2011). However, the effect of only a single, quite high (12 dS m^{-1}) conductivity value was assessed, using NaCl alone as the stressor. Based on the results herein presented (Table 3), this approach can underestimate the occurrence of a significant variability in the tolerance to lower, more realistic salt concentrations.

Since in most European countries public disfavor against DNA recombinant technologies makes the approach to obtain salt-tolerant varieties through genetic transformation with exogenous genes (Zhao et al., 2006; Negrão et al., 2011) unfeasible, the availability of genotypes with contrasting ability to cope with salt stress conditions is needed for the future selection of rice cultivars to be sown in salt-vulnerable areas.

Field trials would represent the best approach to screen for increased tolerance to abiotic stress conditions, in that they yield realistic results and avoid the risk that data

obtained under controlled growth conditions would be inconsistent with the behaviour of plants in natural environments. However, also field trials face major issues. Unpredictable climatic conditions or pathogen infections may alter results, and influence plant growth more than the selected trait does. In the case of salinity tolerance, further constraints rely on how to experimentally impose the stress conditions, and how to control them: irregularities in time and space of salinity due to pedoclimatic effects on irrigation waters and/or water table often make results unreliable and difficult to repeat (Negrão et al., 2011).

Taking into account that under field conditions rice plants are more often subjected to mild stress during all their development than episodically exposed to more severe conditions, in the present study seeds were sown directly in the presence of a given salt level that was maintained thereafter during seedling growth. A nonlinear regression analysis of the results led to the estimation of damage thresholds, showing that a remarkable variability does exist at conductivity values near those in the field. In all cases, on the contrary, germination was not affected up to 12 dS m^{-1} (data not shown). Results are in a good agreement with the current trend of rice cultivar use in salt-affected regions of Northern Italy. According to the available statistics, two genotypes herein showing a high damage threshold, Baldo and Volano, are in fact the most used cultivars in the Delta Po area, accounting for two thirds of the overall production (http://www.enterisi.it/servizi/bilanci/bilanci_fase02.aspx?ID=414). The other genotype whose growth appeared less affected by salt, Loto, is on the contrary scarcely cultivated, but this may depend upon its highest susceptibility to the blast pathogen (Faivre-Rampant et al., 2011).

Salinity may be considered a complex syndrome in which ionic unbalance, besides exerting osmotic effects and direct ionic toxicity, perturbs the acquisition of mineral nutrients. Reciprocal interactions of ions inside the plant tissues still await full elucidation, but several aspects have been already characterized. For instance, it is well known that in many species external Ca^{2+} can ameliorate salinity stress symptoms, and that the ability of plants to maintain a high cytosolic K^+/Na^+ ratio is one of the key determinants of plant salt tolerance (Maathuis and Amtmann, 1999). This notwithstanding, the need to study salinity effects

under conditions mimicking the composition of the soil solution has been often underemphasized. Since an exclusive presence of NaCl in salt-affected environments is quite uncommon, this implies that a NaCl-based screening for tolerant cultivars, adopted in several previous studies (*e.g.* Gregorio et al., 1997; Ahmadi et al., 2011; Karan et al., 2012), could lead to an over- or underestimation of the ability of a given genotype to grow in the presence of high soil conductivity.

The significant variability found among the tested genotypes concerning threshold for growth reduction at realistic salt concentrations represents the first step toward the identification of genotypes/genes suited for introduction of resistant traits into new varieties. However, understanding the molecular determinants of salt tolerance would greatly facilitate this goal. Two main mechanisms have been reported in plants to explain reduced sensitivity to excess salt, namely ion exclusion and osmotic compensation (Zhu 2001; Zhang et al., 2012). Ion exclusion may be achieved by limiting ion uptake at the root level, by compartmentalizing/extruding ions in the vacuole/apoplast, or by retrieving Na^+ from the xylem, the latter having been identified in several species as a primary controller of Na^+ concentration in shoots (Munns and Tester, 2008). To shed some light on these aspects, cation levels were determined in shoots from individual plantlets either at increasing time after salt treatment, or directly grown in the presence of inhibitory, yet sublethal salt levels. Results allowed us to compare cation concentrations in genotypes differing in salt susceptibility under the same conditions in which their ability to withstand salt stress had been evaluated. No striking differences were evident with respect to Na^+ content that increased markedly in all rice cultivars. Statistically significant variations were found among genotypes, but these were unrelated to their relative tolerance. The experimental approach employed does not allow us to distinguish between cations present in the cytoplasm from those confined into the vacuole, where for instance the replacement of K^+ by Na^+ does not induce toxic effects (Rodrìguez-Navarro and Rubio, 2006). In any case, results suggest that the cultivars tested do not owe their differential ability to cope with excess salt to mechanism(s) for reduced Na^+ translocation to the aerial part, or for its increased transport

back to the roots mediating Na^+ exclusion from leaves.

Salt tolerance is not exclusively correlated with adaptation to Na^+ toxicity *per se*, but can also rely upon adaptations to secondary effects of salinity, such as water withdrawal from the apoplast, or impaired nutrient acquisition (Maathuis and Amtmann, 1999). Due to the physicochemical similarities between Na^+ and K^+, the latter possibility is particularly pertinent where the acquisition of K^+ is concerned. Having plants an absolute requirement for K^+, their ability to counteract salinity stress could therefore strongly depend on K^+ availability. Two transporter/channel classes have been characterized that mediate either Na^+ transport or Na^+ and K^+ transport. Members of class 1 have a relatively higher Na^+-to-K^+ selectivity, whereas class 2 transporters were found to mediate Na^+-K^+ cotransport and Na^+ influx at high Na^+ concentrations (Yao et al., 2010). As a consequence, increased sodium content usually causes a significant decrease of the intracellular potassium concentration. This effect clearly resulted by the correlation analyses performed between the concentration of the two elements in the shoots of the six rice cultivars. Nevertheless, once again neither this pattern, nor the consequent sodium-to-potassium ratio showed a plain relationship with the relative salinity tolerance of rice cultivar evaluated as IC_{10} values, as demonstrated by a Principal Component Analysis of data (results not shown).

In rice several members of *HKT* gene subfamily (*OsHKT1;1*, *OsHKT1;3*, *OsHKT1;4*, and *OsHKT1;5*) are involved in salt tolerance (Hamamoto et al., 2015; Chen, 2017). These genes codify for plasmalemma Na^+ transporters retrieving the cation from leaf blade (Wang et al., 2015) or from root xylem reducing its accumulation into the shoot (Deinlein et al., 2014). The transport activity of the OsHKT proteins seems to be Mg^{++}-dependent, thus a different capability to maintain Mg homeostasis under stress could explain differences among cultivars tolerance to salt. Nevertheless, our results do not support this hypothesis. Indeed, regardless the salt treatment, the concentration of Mg in the shoots of all the six cultivars (Table 4 and 5) resulted in the optimal range for plant growth (Mareschner, 2012). Reasonably the low, if any, effect of the progressively higher Mg concentration present in the salt solutions on its concentration in the shoot (Table 4 and 5) suggests that Mg nutritional

status of the plants was already optimal at the concentration presents in the control growth solution (0,69 mmol L^{-1}), however largely higher than the lowest usually sufficient to sustain plant growth requirements (Karley and White, 2009).

In the absence of effective mechanisms for ion exclusion, increased Na^+ concentrations inside the cell lead to oxidative stress conditions. Indeed, a significant increase of TBARS was found in salt-treated seedlings of all genotypes (Fig. 5A). Moreover, the same was true for the intracellular levels of the amino acid proline (Fig. 5B). However, the extent of the latter seems not enough to ensure osmotic compensation and counteract water withdrawal that may derive from the presence of high salt levels in the extracellular space. The osmotic role of proline has been debated, suggesting that proline homeostasis more than its accumulation might play a protective function under stress conditions (Kavi Kishor and Sreenivasulu, 2014). However, other protective mechanisms have been hypothesized that may explain a beneficial effect under drought and salt stress of high proline concentrations in cytosol and organelles, such as ROS scavenging that reduces lipid peroxidation (Signorelli et al., 2014), and protecting protein integrity and enzyme activity as a molecular chaperone (Szabados and Savouré, 2010). Whatever the mechanism, the absence of distinctive patterns for TBARS and proline increase between salt-tolerant and salt-sensitive genotypes implies that in both cases the cells in the leaf are experimenting the effect of salt stress, making unlikely that differences in rates of Na^+ compartimentalization provide the basis of the differential susceptibility.

To overcome damages of salt-induced ROS, plants up-regulate antioxidative enzymes. The essential role of antioxidative systems to maintain a balance between ROS overproduction and their scavenging to keep them at signaling level for reinstating metabolic homeostasis has been well established (Türkana and Demiral, 2009). Differences in salt-induced levels of ROS-detoxifying enzymes may contribute significantly to the differential ability of coping with excess salt in the substrate (Vaidyanathan et al., 2003).

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References

Ahmadi, N., Negrão, S., Katsantonis, D., Frouin, J., Ploux, J., Letourmy, P., Droc, G., Babo, P., Trindade, H., Bruschi, G., Greco, R., Oliveira, M.M., Piffanelli, P., Courtois, B., 2011. Targeted association analysis identified *japonica* rice varieties achieving Na^+/K^+ homeostasis without the allelic make-up of the salt tolerant indica variety Nona Bokra. Theor. Appl. Genet. 123, 881–895.

Antonellini, M., Mollema, P., Giambastiani, B., Bishop, K., Caruso, L., Minchio, A., Pellegrini, L., Sabia, M., Ulazzi, E., Gabbianelli, G., 2008. Salt water intrusion in the coastal aquifer of the southern Po Plain, Italy. Hydrogeol. J. 16, 1541–1556.

Chaves, M.M., Flexas, J., Pinheiro, C., 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. Ann. Bot. 103, 551–560.

Chen, Z.C., Yamaji, N., Horie, T., Che, J., Li, J., An, G., Ma, J.F., 2017. A Magnesium transporter OsMGT1 plays a critical role in salt tolerance in rice. Plant Physiol. 174, 1837–1849.

Courtois, B., Greco,, R., Bruschi, G., Frouin, J., Ahmadi, N., Droc, G., Hamelin, C., Ruiz, M., Evrard, J.-C., Katsantonis, D., Oliveira, M., Negrao, S., Cavigiolo, S., Lupotto, E., Piffanelli, P., 2011. Molecular characterization of the European rice collection in view of association mapping. Plant Gen. Res. 9, 233–235.

Deinlein, U., Stephan, A.B., Horie, T., Luo, W., Xu, G.H., Schroeder, J.I., 2014. Plant salt-tolerance mechanisms. Trends Plant Sci. 19, 371–379.

Faivre-Rampant, O., Bruschi, G., Abbruscato, P., Cavigliolo, S., Picco, A.M., Borgo, L., Lupotto, E., Piffanelli, P., 2011. Assessment of genetic diversity in Italian rice germplasm related to agronomic traits and blast resistance (*Magnaporthe oryzae*). Mol. Breed. 27, 233–246.

Forlani, G., Berlicki, Ł., Duò, M., Dziędzioła, G., Giberti, S., Bertazzini, M., Kafarski, P., 2013. Synthesis and evaluation of effective inhibitors of plant δ^1-pyrroline-5-carboxylate reductase. J. Agric. Food Chem. 61, 6792−6798.

Grattan, S.R., Zeng, L., Shannon, M. C., Roberts, S.R., 2002. Rice is more sensitive to

salinity than previously thought. Calif. Agric. 56, 189–195.

Gregorio, G.B., Senadhira, D., Mendoza, R.D., 1997. Screening rice for salinity tolerance. IRRI Disc. Paper Ser. 22.

Hamamoto, S., Horie, T., Hauser, F., Deinlein, U., Schroeder, J.I., Uozumi, N., 2015. HKT transporters mediate salt stress resistance in plants: from structure and function to the field. Curr. Opin. Biotechnol. 32, 113–120.

Karan, R., DeLeon, T., Biradar, H., Subudhi, P.K., 2012. Salt stress induced variation in DNA methylation pattern and its influence on gene expression in contrasting rice genotypes. PLoS One 7, e40203.

Karley, A.J., White, P.J., 2009. Moving cationic minerals to edible tissues: potassium, magnesium, calcium. Curr. Opin. Plant Biol. 12, 291–298.

Kavi Kishor, P. B., Sreenivasulu, N., 2014. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? Plant Cell Environ. 37, 300–311.

Maas, E. V., 1990. Agricultural salinity assessment and management. In: Tanji, K. K. (Ed), ASCE Manuals and Reports on Engineering, ASCE, New York, pp. 262–304.

Maathuis, F.J.M., Amtmann, A., 1999. K^+ nutrition and Na^+ toxicity: the basis of cellular K^+/Na^+ ratios. Ann. Bot. 84, 123–133.

Marschner, H. Mineral Nutrition of Higher Plants, third ed. Academic Press, London, 2012.

Munns, R., Gilliham, M., 2015. Salinity tolerance of crops – what is the cost? New Phytol. 208, 668–673.

Munns, R., Tester, M., 2008. Mechanisms of salinity tolerance. Ann. Rev. Plant. Biol. 59, 651–681.

Negrão, S., Courtois, B., Ahmadi, N., Abreu, I., Saibo, N., Oliveira, M.M., 2011. Recent updates on salinity stress in rice: from physiological to molecular responses. Critical Rev. Plant Sci. 30, 329–377.

Pitman, M., Läuchli, A., 2004. Global Impact of Salinity and Agricultural Ecosystems In: Läuchli, A., Lüttge, U. (Eds) Salinity: Environment - Plants - Molecules, Springer Netherlands, pp. 3–20.

Reddy, I.N.B.L., Kim, B.-K., Yoon I.-S., Kim K.-H., Kwon T.-R., 2017. Salt tolerance in rice: focus on mechanisms and approaches. Rice Sci. 24, 123–144.

Rengasamy, P., 2010. Soil processes affecting crop production in salt-affected soils. Funct. Plant Biol. 37, 613–620.

Rodrìguez-Navarro, A., Rubio, F., 2006. High-affinity potassium and sodium transport systems in plants. J. Exp. Bot. 57, 1149–1160.

Signorelli, S., Coitiño, E.L., Borsani, O., Monza, J., 2014. Molecular mechanisms for the reaction between (˙)OH radicals and proline: insights on the role as reactive oxygen species scavenger in plant stress. J. Phys. Chem. B 118, 37–47.

Szabados, L., Savouré, A., 2010. Proline: a multifunctional amino acid. Trends Plant Sci. 15, 89–97.

Türkana, I., Demiral, T., 2009. Recent developments in understanding salinity tolerance. Environ. Exp. Bot. 67, 2–9.

Vaidyanathan, H., Sivakumar, P., Chakrabarty, R., Thomas, G., 2003. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.)—differential response in salt-tolerant and sensitive varieties. Plant Sci. 165, 1411–1418.

Verslues, P.E., Sharp, R.E., 1999. Proline accumulation in maize (*Zea mays* L.) primary roots at low water potentials. II. Metabolic source of increased proline deposition in the elongation zone. Plant Physiol. 119, 1349–1360.

Wang, R., Jing, W., Xiao, L., Jin, Y., Shen, L., Zhang, W., 2015. The OsHKT1;1 transporter is involved in salt tolerance and regulated by an MYB-type transcription factor. Plant Physiol. 168, 1076–1090.

Yao, X., Horie, T., Xue, S., Leung, H.Y., Katsuhara, M., Brodsky, D.E., Wu, Y., Schroeder, J.I., 2010. Differential Na and K transport selectivities of the rice OsHKT2;1 and OsHKT2;2 transporters in plant cells. Plant Physiol. 152, 341–355.

Zeng, L., Shannon, M.C., 2000. Salinity effects on seedling growth and yield components of rice. Crop Sci. 40, 996–1003.

Zhang, H., Han, B., Wang, T., Chen, S., Li, H., Zhang, Y., Dai, S., 2012. Mechanisms of plant salt response: insights from proteomics. J. Proteome Res. 11, 49–67.

Zhao, F., Guo, S., Zhang, H., Zhao, Y., 2006. Expression of yeast SOD2 in transgenic rice results in increased salt tolerance. Plant Sci. 170, 216–224.

Zhu, J. K., 2001. Plant salt tolerance. Trends Plant Sci. 6, 66–71.

Legends to figures

Fig. 1. Differential susceptibility to salt of a group of six Italian temperate japonica rice genotypes. Panel A. Effect of increasing concentrations of salts on the growth of the most sensitive and the most tolerant variety. Seedling dry weight was measured when untreated controls reached the third leaf stage (about two weeks after sowing). Data were expressed as percent of values for untreated controls grown at 0.72 dS m^{-1}, and are mean ± SE over 40 to 60 replications, depending on germination rate. Panel B. Data reported in Table 3 were used to calculate the concentrations able to inhibit growth by 50% and the thresholds over which seedling growth starts to be inhibited, and their confidence limits.

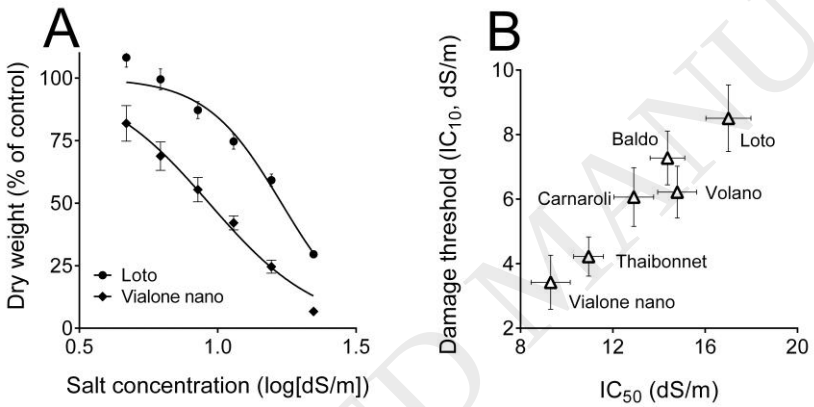

Fig. 2. Sodium-to-potassium ratio in leaves of six Italian rice genotypes showing high, medium and low sensitivity to excess salt 24 and 48 h after the exposure to stress. Seedlings grown under normo-osmotic conditions (0.72 dS m^{-1}) were treated at the three-leaf stage with a salt mixture (NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 in a 10:1:2:1 molar ratio) so as to obtain increasing conductivity values, as indicated. Potassium and sodium content was determined by inductively coupled plasma - mass spectrometry. Data are means ± SE over 10 biological replicates.

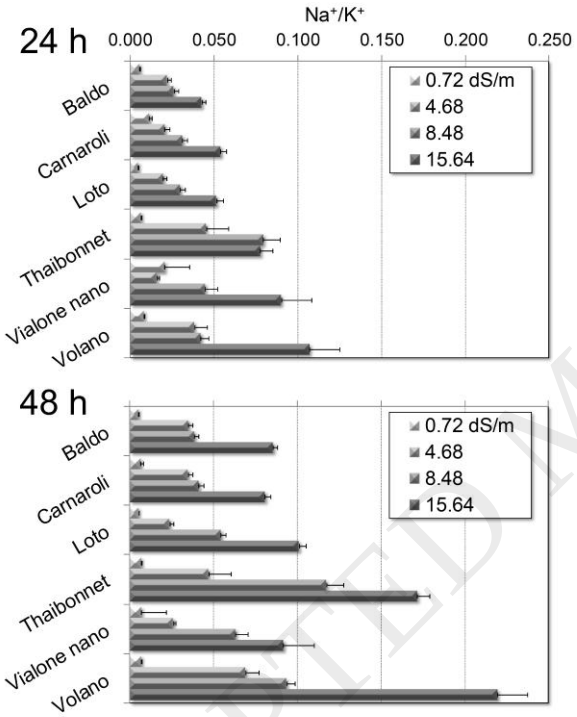

Fig. 3. Sodium-to-potassium ratio in leaves of six Italian rice genotypes showing high, medium and low sensitivity to excess salt grown under salt stress conditions. Seedlings were sown under normo-osmotic conditions (0.72 dS/m) or in the presence of a salt mixture (NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 in a 10:1:2:1 molar ratio) so as to obtain increasing conductivity values, as indicated. Potassium and sodium content in shoots was determined by inductively coupled plasma - mass spectrometry 2 weeks after sowing. Data are means ± SE over 10 biological replicates.

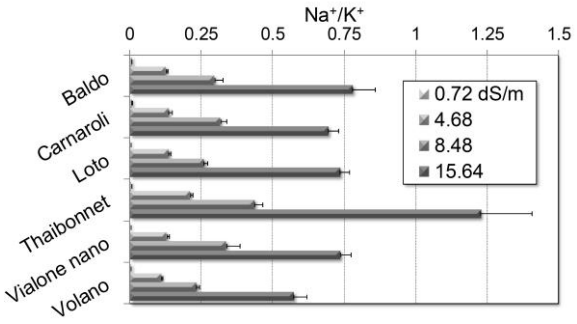

Fig. 4. Pearson correlations among the concentrations of Na^{+}, K^{+} and Mg^{++} in the shoot of plants of each cultivar grown for 14 days at increasing conductivity values. Circle area is proportional to coefficient values. Significant correlations are marked with an asterisk.

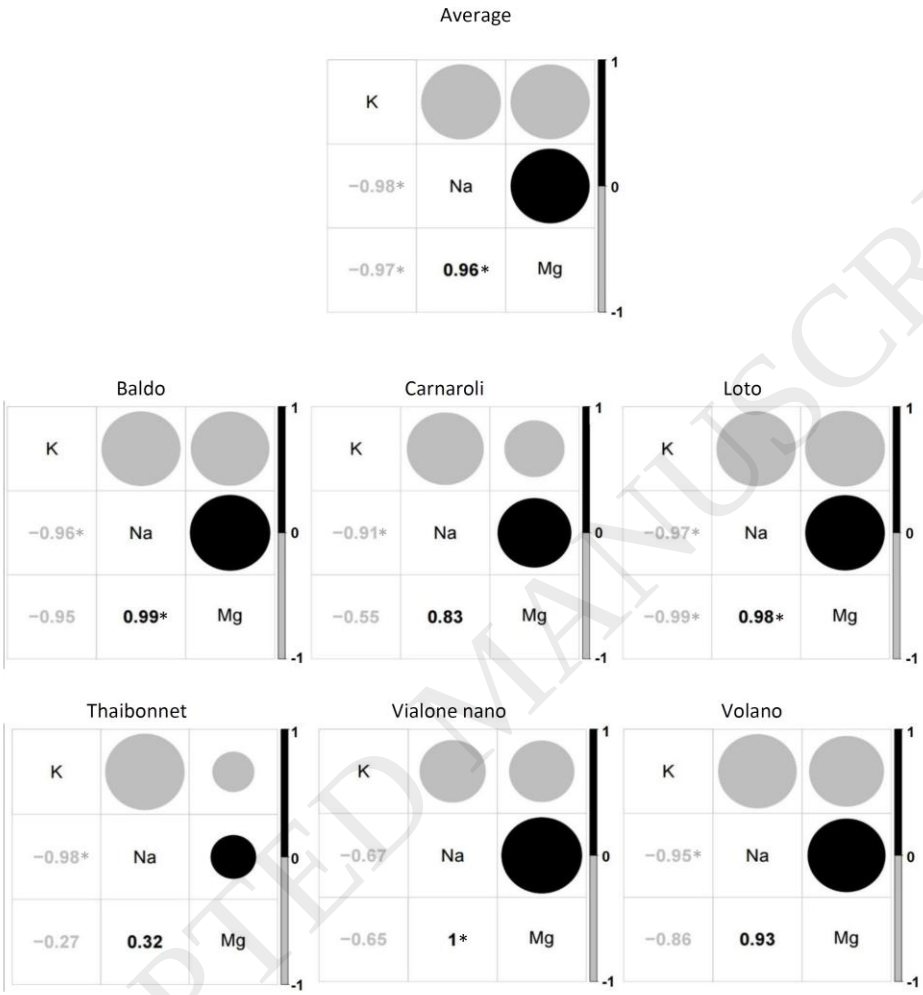

Fig. 5. TBARS (panel A) and proline (panel B) levels in shoots of salt-treated seedlings. Seeds of six Italian rice genotypes showing high, medium and low sensitivity to excess salt were sown in the absence or in the presence of a salt mixture (NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 in a 10:1:2:1 molar ratio) so as to obtain a conductivity value of 15.64 dS m^{-1}. When control plants grown at 0.72 dS m^{-1} reached the three leaf stage, shoots were extracted and used for analysis. In both cases data obtained with 10 biological replicates are shown as box-and-whisker plots.

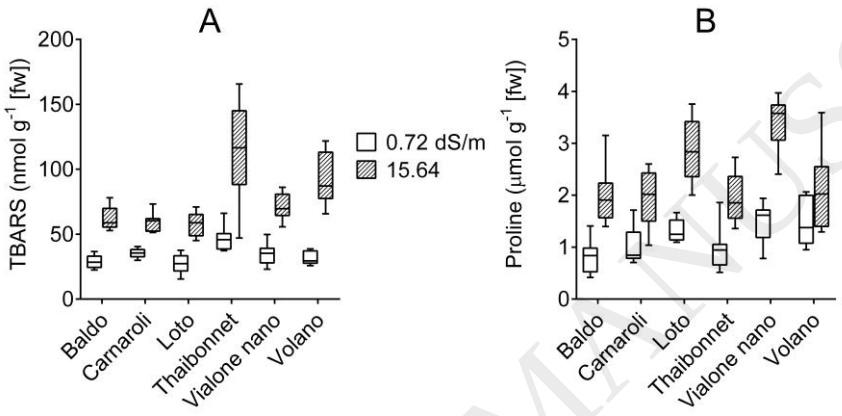

Table 1

Media composition, and corresponding values of electrical conductivity. The effect of increasing conductivity upon seed germination and seedling growth was assessed by complementing the basal nutrient solution with a salt mixture (NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 in a 10:1:2:1 molar ratio). The actual value of conductivity in a given sample was measured with a conductivity-meter. Mean values are reported, with SD never exceeding 5%

	compound	mg L^{-1}	element	mmol L^{-1}
Nutrient solution	NH_4NO_3	206	N	7.50
	KNO_3	238	P	0.16
	KH_2PO_4	21	K	2.51
	$CaCl_2$	42	Ca	0.37
	$MgSO_4$	83	Mg	0.69
	Fe-NaEDTA	4.6	Mn	0.013
	H_3BO_3	0.78	Mo	0.00013
	$MnSO_4 \cdot H_2O$	2.1	B	0.013
	$ZnSO_4 \cdot 7H_2O$	1.1	Zn	0.0038
	KI	0.1	Cu	0.000013
	$Na_2MoO_4 \cdot 2H_2O$	0.03	Co	0.000014
	$CuSO_4 \cdot 5H_2O$	0.003	Fe	0.013
	$CoSO_4 \cdot 7H_2O$	0.004	I	0.00063
	pH 5.8			

Salt mixture supplement (mmol L^{-1})

NaCl	0	25	37.5	50	70	100	150
$CaCl_2$	0	2.5	3.75	5	7	10	15
$MgSO_4$	0	5	7.5	10	14	20	30
Na_2SO_4	0	2.5	3.75	5	7	10	15
dS m^{-1}	0.72	4.68	6.60	8.48	11.15	15.64	22.20

Table 2

Operating conditions for inductively coupled plasma - quantitative mass spectrometry

ICP-QMS instrument	Varian 820-MS	
Generator frequency	27.12 MHz	
Flow Parameters (L min^{-1})	Plasma Flow	18.5
	Auxiliary Flow	1.80
	Sheath Gas	0.24
	Nebulizer Flow	0.86
Torch Alignment (mm)	Sampling Depth	6.5
Other	RF Power (kW)	1.34
	Pump Rate (rpm)	2
	Stabilization delay (s)	40
Ion Optics (volts)	First Extraction Lens	-2
	Second Extraction Lens	-59
	Third Extraction Lens	-373
	Corner Lens	-276
	Mirror Lens Left	51
	Mirror Lens Right	18
	Mirror Lens Bottom	38
	Entrance Lens	2
	Fringe Bias	-2.5
	Entrance Plate	-35
	Pole bias	0.0
CRI (mL min^{-1})	Skimmer Gas Source	OFF
	Sampler Gas Source	OFF
	Skimmer Flow	0
	Sampler Flow	0
Sampling parameters	Sampling mode	autosampler
	Scan Time	1.141 ms
	Replicate Time	11.41 s
Isotopes monitored	^{23}Na, ^{24}Mg, ^{39}K	

Table 3

Effect of increasing salt concentrations on seedling growth. The effect of salts (NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 in a 10:1:2:1 molar ratio) on seedling dry weight was measured when untreated controls had fully developed the third leaf (about two weeks after sowing). Data were expressed as percent of values for untreated controls grown at 0.72 dS m^{-1}, and are mean ± SE over 40 to 60 replications, depending on germination rate.

	Conductivity (dS m^{-1})					
Cultivar	4.68	6.60	8.48	11.42	15.64	22.20
Baldo	103.5 ± 4.7	90.0 ± 4.6	83.7 ± 3.3	68.3 ± 2.8	44.0 ± 2.1	18.8 ± 1.0
Carnaroli	97.0 ± 5.3	78.0 ± 5.0	82.8 ± 4.3	59.4 ± 3.3	39.0 ± 2.4	10.7 ± 0.8
Loto	108.2 ± 3.8	99.5 ± 4.2	87.2 ± 3.4	74.7 ± 3.1	59.2 ± 2.5	29.6 ± 1.5
Thaibonnet	80.8 ± 3.2	76.4 ± 4.0	68.2 ± 3.6	54.3 ± 3.0	29.9 ± 1.9	5.5 ± 1.1
Vialone nano	81.9 ± 7.1	68.8 ± 5.7	55.4 ± 4.9	42.1 ± 2.8	24.6 ± 2.6	6.7 ± 0.5
Volano	95.9 ± 4.6	82.9 ± 3.9	80.5 ± 2.9	69.4 ± 2.6	48.3 ± 1.5	22.4 ± 1.1

Two-way ANOVA	variation	SS	DF	MS	F (DFn, DFd)	*P*
	cultivar x treatment	17365	25	694.6	F (25, 1805) = 1.043	= 0.4053
	treatment	1262000	5	252418	F (5, 1805) = 378.9	< 0.0001
	cultivar	174551	5	34910	F (5, 1805) = 52.40	< 0.0001
	error	1203000	1805	666.2		

Table 4

Cation content in leaves of three leaf-stage seedlings at increasing time after the exposure to high salt levels. Potassium, sodium and magnesium contents were determined by ICP-MS in shoots of rice plantlets grown under normo-osmotic conditions (0.72 dS m^{-1}) 24 and 48 h after the addition of increasing concentrations of salts to the culture medium. The data, expressed as mg kg^{-1} DW, are mean ± SE over 10 biological replicates.

	Conductivity (dS m^{-1})							
	0.72		4.68		8.48		15.64	
Cultivar	T_{24}	T_{48}	T_{24}	T_{48}	T_{24}	T_{48}	T_{24}	T_{48}
K								
Baldo	58788 ± 937	60611 ± 1293	62689 ± 2239	67017 ± 1286	63294 ± 1052	58013 ± 1165	60654 ± 1526	62633 ± 1503
Carnaroli	61413 ± 819	72546 ± 1777	70536 ± 2151	66350 ± 2556	64961 ± 1414	66102 ± 1538	68427 ± 1441	63978 ± 1235
Loto	64355 ± 1492	60850 ± 2483	58516 ± 6021	63691 ± 1087	64551 ± 1947	57002 ± 1022	64500 ± 2047	56560 ± 1475
Thaibonnet	63911 ± 174	68509 ± 2357	58059 ± 2088	59278 ± 705	57989 ± 1732	57990 ± 2639	57132 ± 1011	57056 ± 2089
Vialone nano	64055 ± 6133	64225 ± 1116	68960 ± 2088	68693 ± 1240	61340 ± 1100	58730 ± 850	57103 ± 3112	61355 ± 809
Volano	54781 ± 846	64975 ± 1298	54569 ± 1361	56980 ± 1602	57160 ± 1754	53818 ± 2240	53358 ± 1275	50055 ± 3721
Na								
Baldo	335 ± 25	320 ± 15	1341 ± 104	2345 ± 136	1641 ± 154	2227 ± 95	2579 ± 126	5343 ± 436
Carnaroli	695 ± 104	475 ± 72	1416 ± 163	2255 ± 262	2005 ± 150	2740 ± 170	3667 ± 189	5144 ± 329
Loto	305 ± 14	320 ± 24	1076 ± 149	1541 ± 118	1926 ± 223	3081 ± 180	3281 ± 223	5678 ± 225
Thaibonnet	428 ± 14	495 ± 49	2410 ± 590	2777 ± 335	4476 ± 460	6343 ± 1041	4245 ± 432	9606 ± 1362
Vialone nano	507 ± 115	442 ± 59	1112 ± 99	1812 ± 210	2678 ± 412	3691 ± 265	4663 ± 609	5626 ± 381
Volano	450 ± 23	451 ± 36	2020 ± 337	3830 ± 544	2347 ± 223	4830 ± 891	5564 ± 750	8517 ± 2108
Mg								
Baldo	1612 ± 44	1620 ± 42	1646 ± 29	2092 ± 58	1723 ± 36	2000 ± 75	1742 ± 85	2013 ± 55
Carnaroli	2106 ± 74	1793 ± 89	1924 ± 44	2277 ± 185	2359 ± 152	2209 ± 55	2009 ± 55	2273 ± 48
Loto	1911 ± 54	2358 ± 111	2063 ± 206	2268 ± 83	2507 ± 80	2367 ± 47	2186 ± 82	2134 ± 79
Thaibonnet	2934 ± 5	2797 ± 155	2442 ± 112	2563 ± 100	2206 ± 297	2983 ± 158	3343 ± 755	3151 ± 191
Vialone nano	1845 ± 160	1984 ± 71	2121 ± 83	2355 ± 80	2448 ± 177	2537 ± 93	2215 ± 93	2477 ± 113
Volano	2416 ± 128	2370 ± 107	2857 ± 149	3770 ± 160	2663 ± 79	3104 ± 123	3358 ± 95	3029 ± 115

Two-way ANOVA	24 h after salt addition					48 h after salt addition				
K										
variation	SS	DF	MS	F (DFn, DFd)	*P*	SS	DF	MS	F (DFn, DFd)	*P*
cultivar	2.99×10^9	5	5.99×10^8	F (5, 216) = 10.75	< 0.0001	2.65×10^9	5	5.31×10^8	F (5, 216) = 16.81	< 0.0001
treatment	1.28×10^8	3	4.28×10^7	F (3, 216) = 0.7672	0.5135	2.15×10^9	3	7.15×10^8	F (3, 216) = 22.66	< 0.0001
cultivar x treatment	1.85×10^9	15	1.24×10^8	F (15, 216) = 2.219	0.0067	1.63×10^9	15	1.09×10^8	F (15, 216) = 3.442	< 0.0001
error	1.20×10^{10}	216	5.57×10^7			6.82×10^9	216	3.16×10^7		
Na										
variation	SS	DF	MS	F (DFn, DFd)	*P*	SS	DF	MS	F (DFn, DFd)	*P*
cultivar	6.01×10^7	5	1.20×10^7	F (5, 216) = 12.52	< 0.0001	2.01×10^8	5	4.03×10^7	F (5, 216) = 10.31	< 0.0001
treatment	4.07×10^8	3	1.36×10^8	F (3, 216) = 141.1	< 0.0001	1.24×10^9	3	4.12×10^8	F (3, 216) = 105.3	< 0.0001
cultivar x treatment	6.39×10^7	15	4.26×10^6	F (15, 216) = 4.434	< 0.0001	1.30×10^8	15	8.68×10^6	F (15, 216) = 2.222	0.0066
error	2.07×10^8	216	9.60×10^5			8.44×10^8	216	3.91×10^6		
Mg										
variation	SS	DF	MS	F (DFn, DFd)	*P*	SS	DF	MS	F (DFn, DFd)	*P*
cultivar	3.67×10^7	5	7.35×10^6	F (5, 216) = 19.57	< 0.0001	3.87×10^7	5	7.74×10^6	F (5, 216) = 65.76	< 0.0001
treatment	4.25×10^6	3	1.42×10^6	F (3, 216) = 3.776	0.0114	6.55×10^6	3	2.18×10^6	F (3, 216) = 18.53	< 0.0001
cultivar x treatment	1.33×10^7	15	8.84×10^5	F (15, 216) = 2.355	0.0038	1.04×10^7	15	6.90×10^5	F (15, 216) = 5.861	< 0.0001
error	8.11×10^7	216	3.75×10^5			2.54×10^7	216	1.18×10^5		

Table 5

Cation content in leaves of three leaf-stage seedlings grown in the presence of increasing salt levels. Potassium, sodium and magnesium content was determined by inductively coupled plasma - mass spectrometry in shoots of rice plantlets grown in the presence of increasing concentrations of salts (NaCl, $CaCl_2$, $MgSO_4$ and Na_2SO_4 in a 10:1:2:1 molar ratio). Data, expressed as mg kg^{-1} DW, are mean ± SE over 10 biological replicates.

	---------- Conductivity (dS m^{-1}) ----------				----------Two-way ANOVA----------				
Cultivar	0.72	4.68	8.48	15.64	SS	DF	MS	F (DFn, DFd)	*P*
K									
Baldo	62182 ± 973	50808 ± 1441	37817 ± 1806	29339 ± 1574	variation				
Carnaroli	51600 ± 3286	54476 ± 3089	40790 ± 1394	30309 ± 815	cultivar	1.30×10^9	5	2.60×10^8	F (5, 216) = 11.15	< 0.0001
Loto	62648 ± 1319	45058 ± 1098	37705 ± 1124	25783 ± 738	treatment	3.31×10^{10}	3	1.10×10^{10}	F (3, 216) = 473.8	< 0.0001
Thaibonnet	59398 ± 1341	39515 ± 1093	34020 ± 1036	18094 ± 1486	cv x treat	3.32×10^9	15	2.21×10^8	F (15, 216) = 9.501	< 0.0001
Vialone nano	57907 ± 1036	44136 ± 1042	31065 ± 1087	35893 ± 2354	error	5.03×10^9	216	2.33×10^7		
Volano	59420 ± 1240	50611 ± 909	34720 ± 1011	26977 ± 961						
Na									
Baldo	463 ± 24	6423 ± 250	10928 ± 692	21947 ± 754	variation				
Carnaroli	424 ± 22	7107 ± 262	12784 ± 674	20796 ± 979	cultivar	3.66×10^8	5	7.32×10^7	F (5, 216) = 17.08	< 0.0001
Loto	256 ± 12	6095 ± 240	9739 ± 340	18921 ± 812	treatment	1.30×10^{10}	3	4.35×10^9	F (3, 216) = 1014	< 0.0001
Thaibonnet	393 ± 42	8321 ± 217	14699 ± 648	20150 ± 941	cv x treat	6.17×10^8	15	4.11×10^7	F (15, 216) = 9.590	< 0.0001
Vialone nano	240 ± 9	5729 ± 327	10107 ± 794	26266 ± 1933	error	9.26×10^8	216	4.29×10^6		
Volano	279 ± 10	5611 ± 182	8087 ± 248	15279 ± 970						
Mg									
Baldo	1671 ± 44	2271 ± 56	2422 ± 86	3253 ± 127	variation				
Carnaroli	1783 ± 61	3169 ± 117	3356 ± 145	3410 ± 105	cultivar	1.25×10^7	5	2.49×10^6	F (5, 216) = 23.65	< 0.0001
Loto	1910 ± 56	2460 ± 119	2518 ± 91	3016 ± 136	treatment	6.23×10^7	3	2.08×10^7	F (3, 216) = 196.9	< 0.0001
Thaibonnet	2440 ± 80	3097 ± 50	3325 ± 112	2621 ± 179	cv x treat	2.74×10^7	15	1.83×10^6	F (15, 216) = 17.31	< 0.0001
Vialone nano	1471 ± 34	2052 ± 58	2393 ± 49	4079 ± 208	error	2.28×10^7	216	1.05×10^5		
Volano	1532 ± 57	2483 ± 71	2424 ± 56	2926 ± 126						