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Comparative effects of chloride and sulfate salinities on two contrasting rice cultivars (*Oryza sativa* L.) at the seedling stage

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ABSTRACT

In field conditions, soil salinity may be due to an excess of different soluble salts. In order to compare the impact of chloride and sulfate salinities on rice, two contrasted cultivars (IKP: salt-sensitive and Pokkali: salt-resistant) were exposed to iso-strength Na^+ nutrient solutions (NaCl 50 mM or Na_2SO_4 25 mM; EC: 5.31 dS m^{-1}) for 2 weeks under controlled environmental conditions. It was found that NaCl was more toxic than Na_2SO_4, especially for the salt-sensitive IKP. Sodium and proline accumulation were higher while shoot osmotic potential was lower in NaCl-treated plants than in those exposed to Na_2SO_4. Chloride-treated plants exhibited a higher shoot malondialdehyde concentration, suggesting a higher level of lipid peroxidation while Na_2SO_4-treated plants presented a slightly higher total antioxidant activity. Pokkali was more tolerant than IKP to both types of toxicities although it accumulated similar concentration of toxic ions. Pokkali was able to reduce the root osmotic potential and to quickly recycle oxidized glutathione to reduced glutathione, which may help the plant to more efficiently control its oxidative status in stress conditions. It is concluded that different salts may have distinct impacts on the plant physiology and that differences may vary according to the considered cultivar.

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chloride; ion-specific toxicity; *Oryza sativa*; osmotic adjustment; salinity; sulfate

Introduction

Salinity is one of the major abiotic stresses limiting agricultural production in many areas of the world with adverse effects on germination, plant vigor, and crop yield. More than 900 million hectares of land have been damaged by salt in the word (including 45 million hectares of irrigated lands) and 1.5 million hectares are taken out of production each year as a result of high salinity levels in the soil (Rengasamy 2006).

A given soil is considered as salty if it exhibits an apparent electrical conductivity higher than 4 dS m^{-1} (Daliakopoulos et al. 2016). The major cations issued from soluble salts in saline soils comprise sodium (Na^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}), whereas the most prevalent anions are chloride (Cl^-), sulfate (SO_4^{2-}) and carbonates (including bicarbonates). Among those, sodium chloride is predominant worldwide and much of the research studying the plant physiological responses to salinity rely on experiments focusing on NaCl stress only (Chinnusamy,

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Jagendorf, and Zhu 2005). Some areas, however, may also be affected by an excess of sulfate salts. This is especially the case in the San Joachim valley in California and in Dakota (Rhoades 1989, Thapa, Wick, and Chatterjee 2017), in large parts of India, Egypt, and Argentina (Bowman, Cramer, and Devitt 2006, Devinar et al. 2013), and in Burundi where the lower valley of the Rusizi plain is affected by sodium sulfate from volcanic origin (Nijimbere 2014).

Salinity induces several types of constraints on plants: the low osmotic potential of soil solution compromises water uptake and induces a physiological drought while accumulation of toxic ions results in a wide range of metabolic disorders. Salinity leads to the overproduction of reactive oxygen species (ROS) which are highly toxic and may cause damages to proteins, lipids, carbohydrates and DNA (Ahanger et al. 2017). In order to cope with those deleterious impacts of salinity, some plants are able to (i) perform osmotic adjustment through the accumulation of organic compatible osmoprotectants such as proline and carbohydrates allowing a decrease of plant water potential and the protection of enzymes and cellular structures (Bohnert and Jensen 1996) (ii) efficiently regulate ion absorption and translocation in order to limit toxic ions accumulation and salt-induced deficiencies in essential elements (Hasegawa 2013, Maathuis, Ahmad, and Patishtan 2014) (iii) scavenge reactive oxygen species by triggering antioxidants synthesis (glutathione, ascorbate, tocopherol) and antioxidative enzymes activation (superoxide dismutase, catalase, peroxidases, etc.) (Bose, Rodrigo-Moreno, and Shabala 2014, Ozgur et al. 2013). Endogenous antioxidants are hydrophilic (ascorbate, glutathione, flavonoids, anthocyanins) or lipophilic (α-, δ-, and γ- tocopherol, γ-oryzanol, carotenoids) compounds which may be differently affected by contrasting environmental constraints but data regarding ion-specific impact of salinity on those two classes of compounds remain scanty.

Despite its economic importance which has been evidenced by several authors (Samson et al. 2016, Thapa, Wick, and Chatterjee 2017), plant responses to Na_2SO_4 salinity only received minor attention. The type of salinity may not only influence quantitative yield-related parameters but also the quality of harvested products (Navarro et al. 2002). In the cultivated halophyte *Chenopodium quinoa*, Wu et al. (2016) recently reported that Na_2SO_4 increased grain protein content and seed densities whereas NaCl did not exhibit significant effects. Sodium sulfate was shown to be more toxic than NaCl in potato (Bilski, Nelson, and Conlon 1988), wheat (Datta et al. 1995), tall fescue (Gao, Li, and Chen 2012), and *Brassica rapa* (Reich et al. 2017) while an opposite trend was reported for pea (Hasson-Porath, Kahana, and Poljakoff-Mayber 1972, Mor and Manchanda 1992), soybean (Gupta and Gupta 1984), barley (Curtin, Steppuhn, and Selles 1993), and pepper (Navarro et al. 2002). Such discrepancies may be at least partly explained by the relative proportions of the other mineral components present in the media. Indeed, tolerance to chloride salts was reported to be directly influenced by phosphorous nutrition to a higher extent than tolerance to sulfate salinity (Manchanda, Sharma, and Bhandari 1982, Mor and Manchanda 1992). In contrast, sulfate salinity has a more obvious deleterious impact on Ca^{2+} absorption and translocation than NaCl stress (Bilski, Nelson, and Conlon 1988, Curtin, Steppuhn, and Selles 1993, Han, Gao, and Li 2014). The fact that different authors used different nutrient solution may partly explain contradictory data. Genetic differences between tested species may also be involved in the different behavior observed by different authors. Even cultivars from a given plant species are expected to differ in their relative sensitivities to NaCl and Na_2SO_4 (Rogers, Grieve, and Shannon 1998).

Rice (*Oryza sativa* L.) is considered as a salt-sensitive species but data related to the comparative behavior of plants facing NaCl and Na_2SO_4 toxicities are surprisingly rare. Kimura, Okumura, and Yamasaki (2004) demonstrated that both chloride and sulfate ions provided root growth stimulation when applied in small amounts as ammonium salts. At higher concentrations such as those prevailing in saline conditions, chloride was reported to be more toxic than sulfate in wild rice (*Ziziana palustris*) (Fort et al. 2014). According to Khare, Kumar, and Kishor (2015), Na^+ and Cl^- assume distinct negative impacts on rice and have additive effects when added as

NaCl salts. Khare, Kumar, and Kishor (2015) also considered that salt treatment caused an imbalance in non-enzymatic antioxidants which was greater under NaCl than Na^+ and Cl^- separately. According to Lefèvre, Gratia, and Lutts (2001), the ionic component of salt stress may trigger plant response even on a short term basis and these authors noticed that KCl was more toxic than NaCl which could be related to a higher accumulation of Cl^- in the former case than in the latter. A similar trend was observed in mature embryo-derived calli where Na_2SO_4 was clearly less toxic than NaCl (Lutts, Kinet, and Bouharmont 1996) but no data are available for the whole plant behavior.

The paucity of data regarding rice response to both types of salinity prompted us to compare the behavior of plants from two contrasted rice cultivars (I Kong Pao: salt-sensitive and Pokkali: salt-resistant) exposed to iso-strength Na^+ nutrient solutions at the young seedling stage, which is commonly considered as one of the most salt-sensitive developmental stage in *Oryza sativa* (Lutts, Kinet, and Bouharmont 1995).

Material and methods

Plant material and growing conditions

Seeds of the rice cultivars I Kong Pao (IKP; salt-sensitive) and Pokkali (salt-resistant) were obtained from IRRI (International Rice Research Institute; Philippines). Seeds were germinated on two layers of filter paper (Whatman No. 2) moistened with 10 ml of sterile deionized water in a growth chamber at 25 °C under a 12 hr daylight period (120 μmol m^{-2} s^{-1}). Ten-day-old seedlings of the two cultivars were transferred into a phytotron and fixed on polystyrene plates floating on Yoshida nutritive solution (Yoshida et al. 1976). Illumination was provided by Sylvania fluorescent tubes (F96T12/CW/VHO) for 12 hr day^{-1} at a photon flux density of 300 µmoles m^{-2} s^{-1}. Daytime humidity was between 60% and 80%, and the temperature was maintained at 29 °C during the day and 26 °C during the night. For each treatment, seedlings were distributed among three tanks (20 seedlings per tank) containing 50 L of Yoshida nutritive solution (Yoshida et al. 1976). The solutions were renewed weekly and tanks were randomly rearranged in the phytotron. After 2 weeks of acclimatization in control conditions, seedlings were exposed for two weeks to 25 mM Na_2SO_4 or 50 mM NaCl (electrical conductivity of *c.a.* 5.31 dS m^{-1} for both solutions). At the end of the experiment, 10 plants per treatment were harvested for mineral analysis. Roots were rinsed for 30 s in deionized water to remove ions from the free spaces. Roots and shoots were separated and weighed. Samples were then dried during 72 h at 70 °C in an oven and dry weight was estimated. The remaining plants were quickly frozen in liquid nitrogen and stored at −80 °C until subsequent biochemical analysis.

Estimation of ion content and osmotic potential

For shoots and roots, 20 mg DW was digested with nitric acid (68%) at 80 °C. After complete evaporation, residues were dissolved with HNO_3 (68%) + HCl_{cc} (1:3, v/v). Solution was filtered using a layer of Whatman (85 mm, Grade 1). The filtrate was used to determine the cations concentration (Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{2+}, K^+, and Na^+) by flame emission using Atomic Absorption Spectrometer (Thermo scientific S series model AAS4). Chloride was specifically extracted according to Hamrouni et al. (2011). Anions (PO_4^{3-}, S^{2-}, and Cl^-) were quantified by liquid chromatography (HPLC-Dionex ICS2000).

For the determination of osmotic potential (Ψ_S), the last fully expanded leaf was removed from the plant and cut in small segments. The sample was then introduced in an Eppendorff tube perforated with four small holes, frozen in liquid nitrogen for 30 s and warmed again at room temperature (three cycles of freeze/thaw) in order to break the cellular membrane (Lutts,

Bouharmont, and Kinet 1999). Tubes were then encased in a second intact Eppendorff tube and centrifuged at 8000 *g* during 15 min at 4 °C. The osmolarity of the collected tissular sap was assessed with a Wescor vapor pressure osmometer and converted to Ψ_S according to Van't Hoff equation.

Quantification of proline and total soluble sugars

For proline analysis (Bates, Waldren, and Teare 1973), 200 mg FW of shoots was frozen in liquid nitrogen, ground in a pre-chilled mortar, homogenized in 3 ml of sulfosalicylic acid and then centrifuged at 5000 *g* for 15 min at ambient temperature. An aliquot of 1 ml of ninhydrin solution (2.5 g of ninhydrin dissolved in a mixture of 60 ml glacial acetic acid and 40 ml 6 M phosphoric acid) and 1 ml of concentrated acetic acid were added to the supernatant and the mixture was heated one hour at 90 °C before stopping the reaction in the ice. The reaction mixture was extracted with 2 ml of toluene, mixed vigorously with a test tubes stirrer for 15 s. The chromophore-containing toluene was warmed to room temperature and absorbance was read at 520 nm using toluene as a blank. The proline concentration was determined from standard curve.

Total soluble sugars were quantified according to Yemm and Willis (1954): 500 mg FW of shoots was ground in a pre-chilled mortar with liquid nitrogen. An aliquot of 4 ml of ethanol (70%) was added to the homogeneous powder and the mixture incubated on ice for 5 min. After three successive centrifugations at 8000 *g*, the pooled supernatants were mixed with 1 ml of the anthrone (0.5 g anthrone dissolved in 12.5 ml H_2O and 250 ml H_2SO_4), heated at 100 °C for 10 min and then incubated on ice. Absorbance was measured at 625 nm and the calibration curve was performed via glucose.

Oxidative damages and total antioxidant activity and glutathione content

Lipid peroxidation was estimated as the amount of the thiobarbituric acid-reactive substances [mainly malondialdehyde (MDA)] determined by the thiobarbituric acid reaction (Heath and Packer 1968): 250 mg FW was frozen with liquid nitrogen, crushed in a pre-chilled mortar and homogenized in 5 ml of trichloroacetic acid (5% w/v). The homogenate was centrifuged at 12,000 *g* for 10 min and filtered on Whathman filter Paper No. 1. An aliquot of 2 ml of thiobarbituric acid 0.67% (w/v) was added to 2 ml of supernatants and samples heated during 30 min at 100 °C, cooled on ice and then centrifuged for one min at 2000 *g* to eliminate turbidity. The absorbance was read at 532 nm and the non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using a molar extinction coefficient of 155 $mM^{-1}\,cm^{-1}$.

Total antioxidant was estimated on the basis of α,α-diphenyl-2-picrylhydrazyl (DPPH) method (Al-Temimi and Choudhary 2013). One gram FW of shoots was frozen in liquid nitrogen, ground in a pre-chilled mortar and 10 ml methanol were added to the homogenous powder and then centrifuged at 10,000 *g* for 20 min at 4 °C. The recovered supernatant corresponded to the antioxidant activity measured in methanol extract (AOAM) fraction. The pellet was re-dissolved in 10 ml of dichloromethane, centrifuged at 10,000 *g* for 12 min at 4 °C and this second supernatant corresponded to antioxidant activity measured in dichloromethane extract (AOAD) fraction. AOAM and AOAD fractions were respectively used for determining hydrophilic and lipophilic antioxidant activities (Arnao, Cano, and Acosta 2001): 2.85 ml of DPPH solution (24 mg DPPH in 100 ml methanol) was added to 150 µl sample, the mixture incubated in the dark for 24 hr at room temperature and the absorbance measured at 515 nm.

For reduced (GSH) and total (GSHt) glutathione quantification, 200 mg of frozen samples were extracted and derivatized by orthophthalaldehyde (OPA) according to Cereser et al. (2001). GSHt was quantified after a reduction step of oxidized glutathione (GSSG) by dithiotreitol (DTT) 25 mM. Extracts were filtered through 0.45 µm microfilters (Chromafil PES-45/15, Macherey-

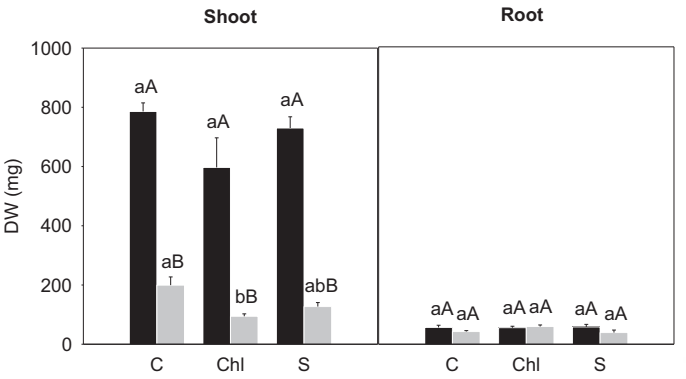

Figure 1. Dry weight of shoot and roots of rice seedlings from cv. Pokkali (black bars) and IKP (gray bars) cultivated during 2 weeks in control (C) conditions or in the presence of 50 mM NaCl [chloride salinity (Chl)] or 25 mM Na_2SO_4 [sulfate salinity (S)]. Each value is the mean of three replicates per treatment and vertical bars are standard errors of the mean. Treatments followed by the same lowercase letter for a particular cultivar do not differ statistically. Cultivars followed by the same uppercase letter in a particular treatment do not differ statistically.

Nagel, Düren, Germany) prior to injection and OPA derivatives were separated on a reversed-phase HPLC column with an acetonitrile-sodium acetate gradient system and detected fluorimetrically. Five microliters of sample was injected into a Shimadzu HPLC system (Shimadzu, 's-Hertogenbosch, The Netherlands) equipped with a Nucleodur C18 Pyramid column (125×4.6 mm internal diameter; 5 μm particle size; Macherey-Nagel). Derivatives were eluted in acetonitril gradient in a 50 mM sodium acetate buffer pH 6.2 at 30 °C at a flow rate of 0.7 ml min^{-1}. Fluorimetric detection was performed with a spectra system Shimadzu RF-20A fluorescence detector at 420 nm after excitation at 340 nm. GSH was quantified using 9-point calibration curves with custom-made external standard solutions ranging from 0.0625 to 50 µM and every 10 injections, a check standard solution was used to confirm calibration of the system. The recovery was determined using GSH as an internal standard and was always found to be higher than 98%.

Statistical treatment

Statistical analyses were performed using JMP Pro 13 software. Data were treated by variance analysis and means were compared using Tukey's HSD all-pairwise comparisons at the $p = .05$ level as a post-hoc test. The graphs were plotted using Sigma Plot 10.0 software.

Results

The tall indica landrace Pokkali always exhibited a higher shoot biomass than IKP, whatever the treatment (Figure 1) while the root biomass was the same for the two cultivars. Chloride and sulfate salinities had no detrimental impact on the shoot DW of Pokkali. In contrast, chloride salinity reduced shoot DW in IKP comparatively to control, while plants exposed to sulfate salinity exhibited an intermediate behavior. Salinities had no impact on the root DW.

For both cultivars, water content was higher in the roots than in the shoots (Table 1). The two types of salinity had no impact on Pokkali shoot and root water content. Chloride salinity drastically decreased water content in IKP while sulfate salinity had no impact on this parameter (Table 1).

The two types of salinity induced a strong increase in shoot and root Na^+ content (Figure 2). As far as shoots are concerned, Na^+ accumulation was obviously higher in plants exposed to NaCl salinity than in those exposed to Na_2SO_4 while an opposite trend was observed for roots.

Table 1. Water content (%) in shoots and roots of rice seedlings from cv. Pokkali and IKP cultivated during 2 weeks in control (C) conditions or in the presence of 50 mM NaCl [chloride salinity (Chl)] or 25 mM Na_2SO_4 [sulfate salinity (S)].

	Pokkali		IKP	
Treatment	Shoot	Root	Shoot	Root
C	84.67 ± 2^{aA}	91.9 ± 0.9^{aA}	83.45 ± 0.4^{aA}	87.6 ± 2^{aA}
Chl	83.25 ± 0.7^{aA}	92.3 ± 2^{aA}	70.7 ± 3.4^{bB}	72.9 ± 1.6^{bB}
S	83.38 ± 1^{aA}	89.76 ± 0.6^{aA}	83 ± 2.7^{aA}	90.6 ± 3.9^{aA}

Treatments followed by the same lower case letter for a particular cultivar do not differ statistically. Cultivars followed by the same upper case letter in a particular treatment do not differ statistically. Each value is the mean of three replicates ± standard errors of the mean.

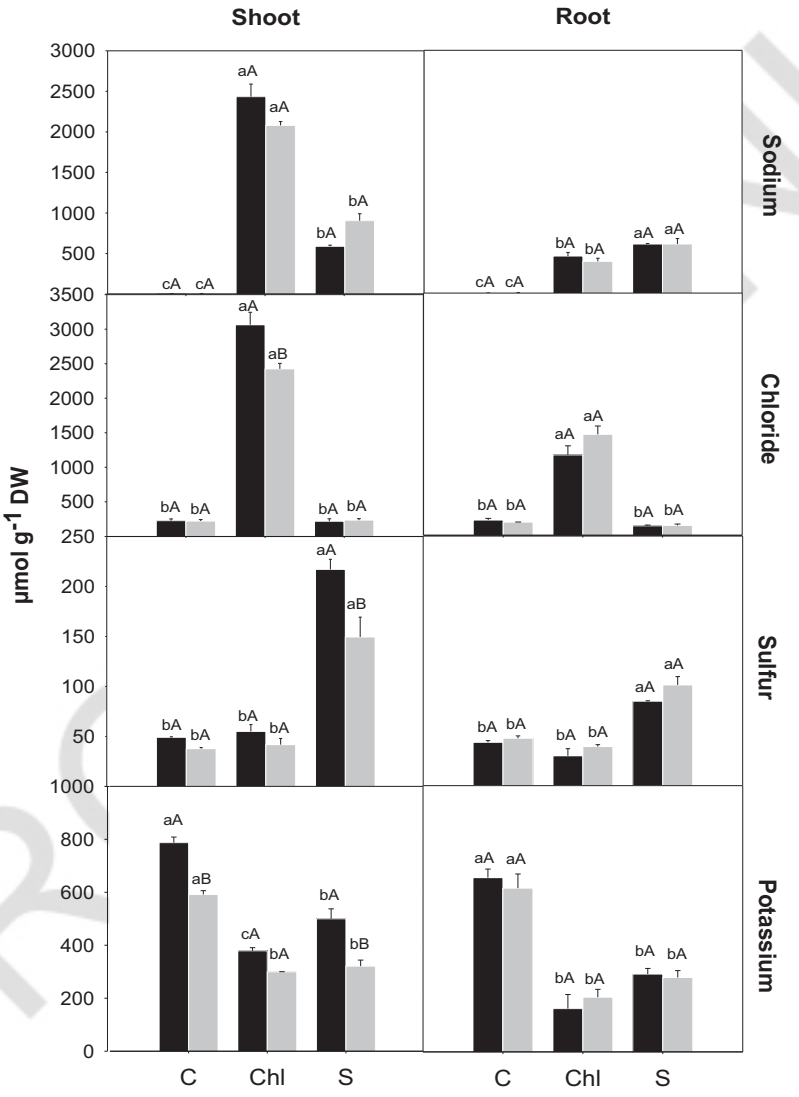

Figure 2. Mineral nutrient concentration in shoot and roots of rice seedlings from cvs. Pokkali (black bars) and IKP (gray bars) cultivated during 2 weeks in control (C) conditions or in the presence of 50 mM NaCl [chloride salinity (Chl)] or 25 mM Na_2SO_4 [sulfate salinity (S)]. Each value is the mean of three replicates per treatment and vertical bars are standard errors of the mean. Treatments followed by the same lowercase letter for a given cultivar do not differ statistically. Cultivars followed by the same uppercase letter in a treatment do not differ statistically.

The two considered cultivars accumulated similar amounts of Na^+ under our experimental conditions. As expected, chloride accumulation was observed mainly in NaCl-treated plants. Pokkali accumulated more Cl^- than IKP in the shoot while the two cultivars did not differ for root Cl^- content. Sulfur accumulation was observed for Na_2SO_4-treated plants. Shoot S concentration was higher in Pokkali than in IKP and once again, no difference between cultivars was recorded for root S concentration. The two types of salinity induced a decrease in K^+ concentration. At the shoot level of Pokkali, the recorded decrease was higher for chloride than for sulfate salinity while the two treatments induced a similar decrease in K^+ content in cv. IKP.

Sulfate salinity induced a significant decrease in the shoot Ca^{2+} and Mg^{2+} concentration in IKP but not in Pokkali (Table 2). Conversely, sulfate salinity induced a significant decrease in the shoot P content of Pokkali while such a decrease was recorded in response to chloride salinity in IKP. In the two studied cultivars, both types of salinity induced an increase in the shoot Mn^{++} content and in the root Fe and P concentration.

The shoot Ψ_s (Figure 3(A)) decreased in response to chloride salinity in the two considered cultivars while it significantly decreased in response to sulfate salinity in cv. Pokkali, only. Whatever the treatment, shoot Ψ_s remained however similar in the two considered cultivars. In contrast, the root Ψ_s of salt-treated plants decreased in cv. Pokkali only and was always lower than in IKP (Figure 3(B)).

The shoot proline concentration drastically increased in response to chloride salinity (Figure 3(C)) and was slightly higher in Pokkali than in IKP. Proline concentration also significantly increased in shoot of sulfate-treated plants but remained lower than values recorded for Cl-treated ones. In sulfate-treated plants, proline concentration was higher in IKP than in Pokkali. The total soluble sugar concentrations (Figure 3(D)) also increased in salt-treated plants but no difference was recorded neither between cultivars nor between the two types of salinity.

The shoot MDA concentration obviously increased in plants exposed to chloride salinity and was higher in IKP than in Pokkali (Figure 4(A)). Malondialdehyde concentration was only slightly higher in sulfate-treated plants than in controls, but the difference between treatments was not significant. As far as total antioxidant activity is concerned, both AOAM (Figure 4(B)) and AOAD fractions (Figure 4(C)) increased in salt-treated plants. Salt-induced increase was more marked for AOAM than for AOAD fraction, and for sulfate than for chloride salinity. Pokkali tended to have slightly higher AOAM activities than IKP but the difference between cultivars was not significant.

Table 3 indicates that chloride salinity reduced the GSH concentration in Pokkali but the recorded decrease was not significant in IKP. In plants exposed to sulfate salinity, shoot GSH concentration was higher in Pokkali than in IKP. No difference was recorded between treatments for shoot GSSG concentration which also remained similar in the two considered cultivars. The GSH/GSSG ratio was always slightly lower in IKP than in Pokkali, whatever the considered treatment but difference was not significant. Chloride salinity reduced GSH/GSSG ratio in Pokkali but not in IKP.

Discussion

A non-negligible proportion of salinization in irrigated lands may be due to Na_2SO_4 and because rice is extremely important for human nutrition and rather salt-sensitive, there is an evident interest to compare the rice response to NaCl and Na_2SO_4 salinities. The present study revealed a higher toxicity of NaCl over Na_2SO_4, as found previously in studies dealing with other species (Curtin, Steppuhn, and Selles 1993, Gupta and Gupta 1984, Hasson-Porath, Kahana, and Poljakoff-Mayber 1972, Mor and Manchanda 1992, Navarro et al. 2002). The sodium ion accumulation in shoots was almost five times higher in the case of chloride stress compared to sulfate stress (Figure 2). This observation suggests that Na^+ uptake is influenced by the nature of anion

Table 2. Mineral content (Ca, Fe, P, Mg, and Mn) in shoots and roots of rice seedlings from cv. Pokkali and IKP cultivated during 2 weeks in control (C) conditions or in the presence of 50 mM NaCl [chloride salinity (Chl)] or 25 mM Na_2SO_4 [sulfate salinity (S)].

(μmol g^{-1} DW)

Ca		Fe		P		Mg		Mn		
Cultivar Treatment	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Pokkali C	137 ± 4^{aA}	11.4 ± 0.9^{aA}	2.3 ± 0.1^{bA}	37 ± 7^{bA}	379 ± 28^{aA}	145 ± 8.3^{bA}	338 ± 45^{aA}	50.6 ± 4.8^{aA}	29 ± 4^{bA}	1.8 ± 0.5^{aA}
Chl	151 ± 7^{aA}	26.0 ± 13.6^{aA}	5.0 ± 0.6^{aA}	274 ± 47^{aA}	307 ± 19^{abA}	362 ± 65^{aA}	307 ± 17^{aA}	54.6 ± 12^{aA}	40 ± 1^{abB}	0.81 ± 0.3^{aA}
S	192 ± 36^{aA}	19.8 ± 3.0^{aA}	2.2 ± 0.3^{bA}	150 ± 17^{abA}	278 ± 12^{bA}	253 ± 16^{abA}	333 ± 30^{aA}	44.7 ± 1.3^{aA}	78 ± 19^{aA}	1.6 ± 0.05^{aA}
IKP C	156 ± 2^{aA}	12.3 ± 3.3^{aA}	1.9 ± 0.2^{aA}	36 ± 4^{cA}	387 ± 13^{aA}	190 ± 13^{bA}	307 ± 12^{aA}	50.3 ± 3.4^{aA}	32.2 ± 1.7^{bA}	2.5 ± 0.5^{aA}
Chl	123 ± 17^{abA}	20.3 ± 4.4^{aA}	4.3 ± 0.8^{aA}	307 ± 18^{aA}	257 ± 36^{bA}	460 ± 34^{aA}	245 ± 45^{abA}	47.6 ± 5.7^{aA}	62.9 ± 7.4^{aA}	1.9 ± 0.2^{aA}
S	85 ± 3^{bB}	11.0 ± 1.0^{aA}	3.6 ± 1^{aA}	143 ± 27^{bA}	276 ± 26^{abA}	286 ± 12^{bA}	172 ± 6^{bB}	38 ± 2.3^{aA}	47.7 ± 0.8^{abA}	3 ± 0.4^{aA}

Treatments followed by the same lower case letter for a particular cultivar do not differ statistically. Cultivars followed by the same upper case letter in a particular treatment do not differ statistically. Each value is the mean of three replicates ± standard errors of the mean.

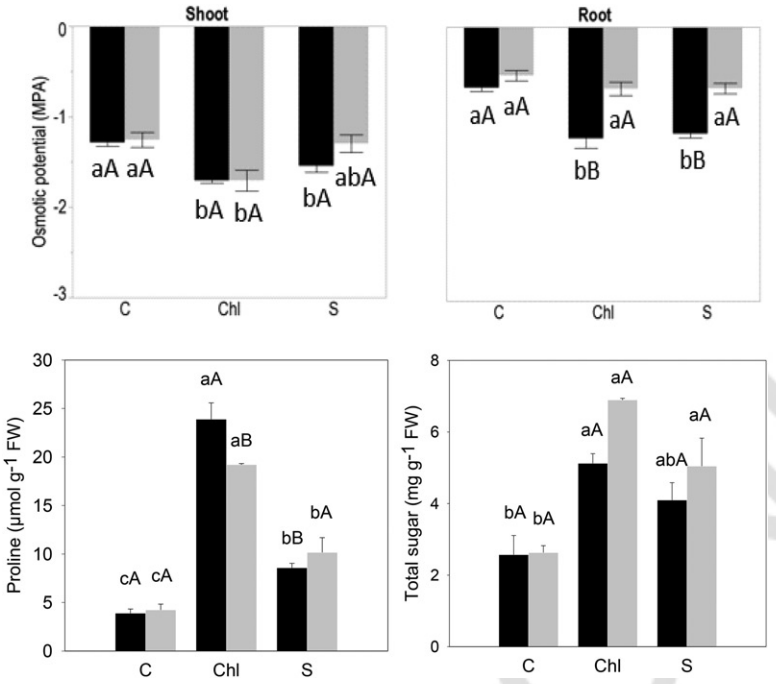

Figure 3. Shoot and root osmotic potential (Ψs), shoot proline and shoot total soluble sugar concentration in rice seedlings from cvs. Pokkali (black bars) and IKP (gray bars) cultivated during 2 weeks in control conditions (C) or in the presence of 50 mM NaCl [chloride salinity (Chl)] or 25 mM Na_2SO_4 [sulfate salinity (S)]. Each value is the mean of three replicates per treatment and vertical bars are standard error of the mean. Treatments followed by the same lowercase letter for a given cultivar do not differ statistically. Cultivars followed by the same uppercase letter in a treatment do not differ statistically.

excess present in nutrient solution. Distinct transporter proteins located in the plasma membrane are involved in Na^+ absorption but the majority of Na^+ influx into the plant cell is thought to occur via nonselective cation channels while Na^+ efflux appears to be mediated by Na^+/H^+ exchange (Hasegawa 2013, Maathuis, Ahmad, and Patishtan 2014). In the present study, salt-treated plants were exposed to similar Na^+ concentration and accumulated anions thus appear to influence Na^+ translocation in the plant. This view is supported by the fact that accumulated Cl^- in NaCl-treated plants was by far higher than sulfate accumulation in Na_2SO_4 treated ones. Since small amounts of Cl^- are required for normal metabolism, plants possess complex molecular mechanisms for chloride transport across root cell membrane and long distance transport of this element from the root to photosynthetic tissues (White and Broadley 2001). Regulation of these mechanisms probably plays an important role in salinity resistance but has been neglected up to now and this question needs to be addressed in the future.

In the present study, root growth estimated on a dry weight basis was not affected by salinity but the morphology of the root system was not studied. Zhou et al. (2011) clearly demonstrated that net Na^+ fluxes change significantly at anatomically distinct root zones of rice seedlings, mainly in relation to the development of exodermis and endodermis conditioning the importance of apoplastic by-pass flow. Local disruption of exodermis during secondary root development from the pericycle could also facilitate Na^+ transport. A differential impact of NaCl and Na_2SO_4 on anatomical features of rice root could not be ruled out and also requires further investigations.

Shoot Ca^{2+} content was affected by salinities in IKP while K^+ decreased in both cultivars. According to Choi et al. (2014) roots may elicit a calcium wave signal that propagates throughout the plant and may be responsible for the adaptative response of salt-treated plants. A decrease in Ca^{2+} concentration of Na_2SO_4-treated plants in IKP may reflect the inability of this cultivar to

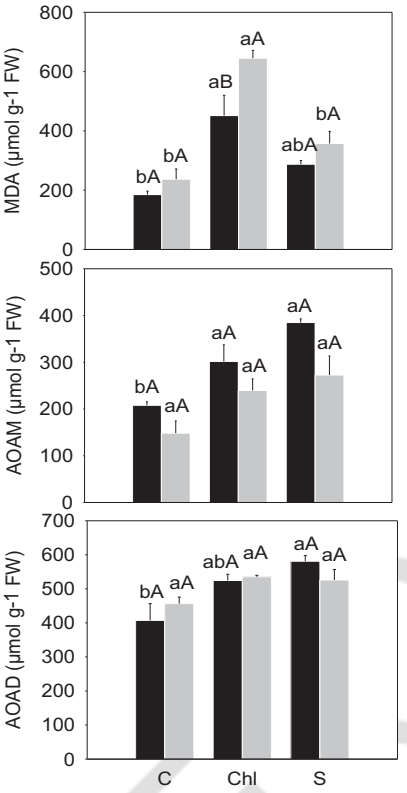

Figure 4. Malondialdehyde (MDA), hydrophilic (AOAM) and lipophilic (AOAD) antioxidants in the shoots of rice seedlings from cv. Pokkali (black bars) and IKP (gray bars) cultivated during two weeks in control (C) conditions or in the presence of 50 mM NaCl [chloride salinity (Chl)] or 25 mM Na_2SO_4 [sulfate salinity (S)]. Each value is the mean of three replicates per treatment and vertical bars are standard errors of the mean. Treatments followed by the same lowercase letter for a particular cultivar do not differ statistically. Cultivars followed by the same uppercase letter in a particular treatment do not differ statistically.

Table 3. Oxidized (GSSG) and reduced (GSH) glutathione content in shoots of rice seedlings from cv. Pokkali and IKP cultivated during 2 weeks in control (C) conditions or in the presence of 50 mM NaCl [chloride salinity (Chl)] or 25 mM Na_2SO_4 [sulfate salinity (S)].

µmol g^{-1} FW				
Cultivar	Treatment	GSH	GSSG	GSH/GSSG
Pokkali	C	0.148 ± 0.008^{aA}	0.206 ± 0.007^{aA}	0.719 ± 0.03^{aA}
	Chl	0.089 ± 0.01^{bA}	0.211 ± 0.01^{aA}	0.429 ± 0.07^{bA}
	S	0.139 ± 0.01^{aA}	0.223 ± 0.02^{aA}	0.627 ± 0.02^{abA}
IKP	C	0.107 ± 0.01^{aA}	0.215 ± 0.02^{aA}	0.515 ± 0.04^{aA}
	Chl	0.080 ± 0.01^{aA}	0.226 ± 0.01^{aA}	0.349 ± 0.03^{aA}
	S	0.087 ± 0.004^{aB}	0.224 ± 0.02^{aA}	0.39 ± 0.01^{aA}

Treatments followed by the same lower case letter for a particular cultivar do not differ statistically. Cultivars followed by the same upper case letter in a particular treatment do not differ statistical. Each value is the mean of three replicates ± standard errors of the mean.

trigger a long-distance Ca^{2+}-signaling process. Calcium has a strong impact on the regulation of plasma membrane H^+-ATPase activity involved in Na^+ compartmentation preventing excessive accumulation of Na^+ in the cytosol and a decrease in Ca^{2+} may thus have a deleterious impact on cell metabolism (Morgan et al. 2014). Calcium also helps to maintain cell growth through its positive impact on cell wall composition (An et al. 2014). Frouin et al. (2018) recently confirmed that several quantitative trait loci for salt tolerance in rice are closed to those of genes involved in

calcium signaling and metabolism and that Pokkali is able to maintain an efficient Na^+-Ca^{2+} selectivity.

Manganese is also thought to assume positive functions in salt-treated plants. Rahman et al. (2016) provided evidences that Mn^{2+} improves ionic and osmotic homeostasis through decreasing Na^+ influx and increasing water status, and increasing ROS detoxification by increasing flavonoids, phenolics and ascorbate content. Aktas et al. (2005) even hypothesized that manganese itself exerts antioxidative effects in plant tissues by being oxidized from Mn^{2+} to Mn^{3+}. Both rice cultivars were able to increase shoot Mn content in response to NaCl and to Na_2SO_4 which should be regarded as an adaptative strategy to cope with salt stress and also suggest that roots did not retain absorbed Mn. In contrast, to Mn, shoot P content was reduced in response to NaCl in IKP and in response to Na_2SO_4 in Pokkali. A myriad of physiological properties rely on an adequate presence of P. It is unlikely that $H_2PO_4^-$ and Cl^- ions are competitive in terms of plant uptake. In the present work using hydroponic culture and which does not take into account the poor P mobility in the soil, it seems that P translocation from the root to the shoot was adversely affected since a decrease in the shoot P content occurred concomitantly with an increase in the root P content. The two types of salinity may thus act on P loading at the xylem level, but in contrast to Mn, the effect was cultivar-dependent.

Beside ion accumulation, plants exposed to salt stress are also exposed to a decrease in the external water potential (Chinnusamy, Jagendorf, and Zhu 2005, Lefèvre, Gratia, and Lutts, 2001, Lutts, Bouharmont, and Kinet 1999). Plant responses classically involve osmotic adjustment allowing the organism to maintain the water potential gradient required for water absorption. Although this osmotic constraint has been regarded as a shoot-ion independent process (Pires et al. 2015), it is noteworthy that in our study, shoot Ψ_S was lower for NaCl-treated plants than for Na_2SO_4-stressed ones. This observation might be related to higher accumulation of proline and soluble sugar which are well known compatible organic compounds putatively involved in cytosolic osmotic adjustment (Bohnert and Jensen 1996). The fact that these compounds increased to a higher extent in response to NaCl than to iso-osmotic Na_2SO_4 suggests that the accumulated ions and not only the external osmotic potential may somewhat influence over synthesis of these organic compounds. It is well-known that K^+ is clearly involved in osmotic potential leading to turgor maintenance in unstressed plants but in our study, the recorded salt-induced decrease in K^+ makes it unlikely to assume this function in the presence of NaCl or Na_2SO_4.

Oxidative stress due to synthesis of ROS is an important component of saline toxicity (Ahanger et al. 2017, Bose, Rodrigo-Moreno, and Shabala 2014). A higher MDA concentration in response to NaCl than Na_2SO_4 also confirms a higher toxicity of the former salt comparatively to the latter. This observation could be related to the fact that Na_2SO_4-treated plants more efficiently trigger antioxidant response than plants exposed to chloride toxicity, as evidenced by the recorded response for AOAM fraction. Glutathione is a multifunctional water soluble tripeptide containing a sulfhydryl group and assumes important role as a major antioxidant in all aerobic organisms, especially in stressed plants where ROS generation is accelerated (Gill et al. 2013). This important compound should be recovered in the AOAM fraction. However, the total glutathione (GSH + 2 × GSSG) content only slightly varied in Pokkali and remained remarkably constant in IKP (0.534 µmol g^{-1} FW). Sulfur uptake and assimilation are essential for GSH synthesis. Despite an increase in sulfur content in sulfate-treated plants, no concomitant increase was recorded for glutathione content although this peptide contains a central cysteine with a thiol group. Conversely, GSH is also involved in sulfur assimilation pathway since it acts as a co-factor for the adenosine-5-phosphosulfate reductase, a key enzyme in sulfite synthesis (Khan et al. 2012). Since chloride salinity did not decrease sulfur content, and since sulfate salinity did not increase GSH synthesis, one may assume that under our experimental conditions glutathione metabolism remain constant. Hence, other antioxidant such as ascorbate may account for the

recorded increase in the total antioxidant activities of the AOAM fraction in stressed tissues. Similarly, lipohilic antioxidant of the AOAD fraction are membrane-bound hydrophobic scavengers where they interrupt chain reaction of lipid peroxidation leading the MDA synthesis and compounds such as α-tocopherol might accumulate in sulfate-treated plants and thus account for their lower MDA content comparatively to chloride-treated ones.

The tall indica landrace Pokkali is frequently used as a salt-tolerant reference in studies devoted to rice response to NaCl (Frouin et al. 2018, Lefèvre, Gratia, and Lutts, 2001, Lutts, Kinet, and Bouharmont 1995). Although this cultivar displayed a quite higher shoot biomass than the semi dwarf salt-sensitive IKP, our study suggested that resistance of Pokkali was not due to a dilution effect of absorbed Na^+ since shoot Na^+ concentration estimated on a dry weight basis was similar in the two cultivars, even if shoot growth was affected in IKP and not in Pokkali. The NaCl-induced decrease in shoot K^+ was higher in Pokkali than in IKP, which confirms the recent analysis of Pires et al. (2015) assuming that K^+ content is not a sufficient trait to assess rice salinity resistance. Similarly, the two cultivars exhibited similar shoot Ψ_S in NaCl-treated plants but WC was drastically decreased in IKP, confirming that osmotic adjustment is necessary but not sufficient to ensure the maintenance of an optimal plant water status. A poor stomatal regulation in salt-sensitive cultivars (Lutts, Bouharmont, and Kinet 1999) might explain the low WC recorded in IKP. Since WC was not affected in IKP in response to Na_2SO_4, one may assume that this salt was less toxic than NaCl in terms of plant water status alteration. Roots from Pokkali displayed osmotic adjustment properties that were not observed in IKP since no root Ψ_S decrease has been observed in this cultivar. This confirms that the roots of the salt-resistant cultivar have a distinct metabolic status comparatively to those of the salt-sensitive one. Our data also suggest that IKP less efficiently cope with salt-induced antioxidative stress comparatively to Pokkali: this is especially the case in NaCl-exposed plants where MDA was higher in IKP than in Pokkali. The total glutathione content remained statistically similar in the two cultivars but the GSH/GSSG ratio slightly differed. It appeared to be lower in IKP than in Pokkali, suggesting that GSSG recycling through glutathione reductase may be less efficient in the former than in the latter.

Conclusion

In conclusion, NaCl appeared more toxic than Na_2SO_4 and lead to a higher Na^+ accumulation in the shoot of rice. Calcium nutrition was impaired by Na_2SO_4 in the salt-sensitive cultivar while phosphorus translocation was affected by NaCl. Increase in the shoot Mn may be regarded as an attempt of adaptation to both types of salinity. The salt-resistant cultivar Pokkali displayed a higher capacity of osmotic adjustment and maintenance of antioxidant status compared to the salt-sensitive IKP in response to both types of salinity.

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Disclosure statement

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