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RESEARCH ARTICLE

Impact of salinity on yield-related parameters in two contrasting cultivars of *Oryza glaberrima* Steud. in Benin

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Summary

Salinity is one of the major environmental stresses limiting growth and yield of rice. The objective of the present study was to analyze the impact of NaCl on yield-related parameters of *Oryza glaberrima*. Two contrasted cultivars of *Oryza glaberrima* previously tested for salt resistance at the vegetative stage [salt-resistant (TOG5307) and salt-sensitive (TOG5949)] were irrigated with a saline solution containing 30 mM NaCl (EC: 3 dS.m^{-1} NaCl). After 6 months of treatments, mineral nutrient and yield-related parameters were assessed. Proline was quantified in the panicle leaf at the start of the grain filling stage. NaCl treatment affected most yield-related parameters: panicle length, panicle leaf dry weight, number of branches per panicle, panicle leaf length, days to 50% heading, straw fresh weight per plant, grain yield per plant, number of spikelets per panicle, and number of filled grains per panicle. The weight of 1,000 grains decreased in the salt-sensitive cultivar only, suggesting that grain filling processes were compromised. The salt-resistant cultivar TOG5307 was less affected than the salt-sensitive TOG5949 and accumulated lower amounts of Na$^+$ in the grains. For both cultivars, hulls contained higher concentration of Na$^+$ and K$^+$ than grains. TOG5307 also contained more proline in the panicle leaf than TOG5949, suggesting that TOG5307 can cope with the osmotic component of salt stress. The cultivar exhibiting the highest salt resistance at the vegetative stage also exhibited the highest resistance at the reproductive one.

Introduction

Salinity is one of the major environmental stresses limiting growth and yield of rice. More than 5% of agricultural lands and 20% of irrigated cultivated areas are affected by an excess of soluble salts (Zörb *et al.*, 2019). In salt stress condition, plants are exposed to i) an osmotic stress due to the reduction of the osmotic potential of the root medium and ii) an ionic stress caused by the progressive accumulation of large amounts of toxic ions into plant tissues (Castillo *et al.*, 2007; Ben Hassine and Lutts, 2010). These major constraints compromise water uptake and nutrient acquisition causing nutritional imbalances and alter a wide range of physiological functions (Zhao *et al.*, 2020). Rice is considered as one of the most salt-sensitive crop. Mohammadi *et al.* (2013) reported that above a threshold soil electrical conductivity (EC) of 3 dS/m, there is a 12 % yield reduction per dS/m. Salinity affects most seriously seedling and reproductive stages of rice (Lutts *et al.*, 1995; Zeng and Shannon, 2000; Singh *et al.*, 2021). Surekha Rao *et al.* (2013) argued that screening for salinity resistance potential at reproductive stage is crucial to identify resistant rice cultivars, since salt resistance at distinct developmental stages may be under the control of different sets of genes

(Zhu *et al.*, 2001; 2004). Salt stress negatively impacts the number of tillers per plant, number of grains per panicle, 1,000-grain weight, and grain yield (Hasamuzzaman *et al.,* 2009; Mohammadi-Nejad *et al.,* 2010; Zhu *et al.*, 2004; Mohammadi *et al.*, 2013). Moreover, Razzaq *et al.* (2020) reported that salt stress not only substantially reduces the rice yield but also adversely affects grain quality.

Besides the well-known Asian rice species *Oryza sativa* L., the African rice species *Oryza glaberrima* Steud. is the second cultivated rice species. While *Oryza sativa* is cultivated in most continents, *Oryza glaberrima* is only cultivated in Africa (Linares, 2002). This marginal species is considered as able to tolerate various biotic and abiotic stresses (Futakuchi *et al.*, 2012; Wambugu *et al.*, 2019), but its capacity to cope with salinity remains poorly documented. Prodjinoto *et al.* (2018) screened 25 cultivars for salinity resistance at the seedling stage. They identified three groups for salinity resistance among the tested material: a salt-resistant group, a salt-sensitive group, and a « medium range » group. Moreover, some cultivars such as TOG5307 can maintain a high net photosynthesis under salt conditions despite high concentration of accumulated Na^+ ions, while other cultivars such as TOG5949 are unable to cope with high concentration of internal Na^+ and are therefore classified as "salt-sensitive" (Prodjinoto *et al.,* 2021a).

The information available for these two contrasting cultivars are issued from experiments performed at the vegetative stage only and for plants cultivated in nutrient solutions under fully controlled environmental conditions. It is now clearly established that the mechanisms involved in salinity resistance may vary depending on the plant phenological stage (Liu *et al.*, 2022). Moreover, roots are the first organs exposed to salinity but root behavior may be drastically different in nutrient solution on the one hand and in a natural solid substrate such as soil on the other hand (Qin *et al.*, 2020). To the best of our knowledge, no data are available regarding the impact of salinity on yield-related parameters of *Oryza glaberrima*. The objective of the present study was therefore to analyze the impact of NaCl on yield-related parameters of two contrasting cultivars of *Oryza glaberrima* steud (TOG5307 and TOG5949) cultivated in salt-affected soil in Benin.

Material and Methods

Soil characterization

Soil samples were randomly collected from a surface layer (0.20 m depth) in a farmer's field of Djègbadji (2°4'36" E, 6°19'27" N) (Republic of Benin). Soil EC (mS cm^{-1}) and pH-$_{H2O}$ were determined in a 1:5 soil/water suspension ratio with a shaking time of about 5 min using glass electrodes. Cation exchangeable capacity (CEC) was assessed using percolation columns and KCl 1.0 M pH 3.0. Exchangeable base concentrations (Mg^{2+}, Ca^{2+}, Na^+, and K^+) were measured using the same percolation columns and ammonium acetate 1.0 M pH 7.0 (Page *et al.*, 1982) through inductively coupled plasma atomic emission spectrometry (ICP-AES; Thermo Jarrell Ash Iris Advantage). Total N and organic C were determined according to Kjeldahl and Walkley–Black methods, respectively. The content of the soil solution in soluble cations was determined in 1:2 soil/water suspension ratio. Cations concentration (Mg^{2+}, Ca^{2+}, Na^+, and K^+) were quantified by ICP-AES (Thermo Jarrell Ash Iris Advantage) (Page *et al.*, 1982). Soil analyses were carried out at Soil Science Laboratory of Earth and Life Institute in Belgium. The main properties of analyzed soil are listed in Table 1.

Experimental setup

This study was carried out in the greenhouses of the International Institute of Tropical Agriculture (IITA) Benin Republic station. The temperature in the greenhouse with natural light varied between 25–30 °C (day time) and 20–25 °C (night time). The soil brought from Djégbadji was sterilized at 180 °C for 72 hours in order to avoid any microbial contamination and nematodes

Table 1. Djègbadji soil chemical properties

	pH-$_{H2O}$	6.81 ± 0.10
	pH-$_{KCl}$	6.23 ± 0.03
	EC (dS/m)	0.268 ± 0.002
	CEC (meq/100 g soil)	13.59 ± 0.27
	Total N [%]	0.04 ± 0.0008
	Organic C [%]	0.43 ± 0.006
	C/N ratio	12.17 ± 0.14
	NH_4 (mg/kg)	520.6 ± 10.87
Exchangeable Cations (meq/100g Soil)	Mg^{2+}	12.9 ± 0.9
	Ca^{2+}	19.3 ± 0.4
	Na^+	0.74 ± 0.01
	K^+	2.83 ± 0.05
Water-soluble ions (mmol/L)	Mg^{2+}	1.64 ± 0.09
	Ca^{2+}	3.29 ± 0.08
	Na^+	2.86 ± 0.06
	K^+	3.32 ± 0.14

and poured in 4-kg pots. The pots were fertilized according to the protocol of Agnoun *et al.* (2012). Fertilizers were used to provide the equivalent of NPK 15–15–15 at the rate of 100 kg ha^{-1} and were uniformly mixed in soil before filling the pots. In order to determine the water-holding capacity of the soil, three "test" pots were watered until saturation (considering that saturation corresponds to a light layer of water stagnating on the surface of the soil). The soils in the pots encountered water drainage through three holes at the bottom of the pot to allow removal of the water excess until measurement of field capacity. Twenty pots per cultivar and per treatment were randomly arranged in the greenhouse and randomly redistributed each week.

Plant material, growth conditions, and stress application

Two contrasted cultivars of *Oryza glaberrima* [salt-resistant (TOG5307; AccNumber WAB0021855) and salt-sensitive (TOG5949; AccNumber WAB0020144)] as described by Prodjinoto *et al.* (2018; 2021a) were used. Seeds were obtained from Africa Rice (Bouaké, Ivory Coast). For each cultivar, non-dehusked seeds were surface-sterilized and germinated on two layers of filter papers (Whatman 85 mm, Grade 1) moistened with sterile deionized water in glass vessels at 25 °C and under 12-h daylight period. After 10 days, equally grown seedlings were transferred into pots containing sterilized and fertilized soil. Four seedlings were transplanted into each pot and afterward thinned to two plants per pot. After two weeks, half of the rice seedlings were subjected only once to saline irrigation treatments by using a saline solution equivalent to 30 mM NaCl (EC = 3 dS.m^{-1}). The salinity was applied after dissolving NaCl in tap water; 1,500 ml of the saline solution or salt-free tap water (containing initial Na$^+$ concentration lower than 50 mg.L^{-1}) were added to the soil depending on the treatment provided (control or NaCl stress). EC of NaCl-treated pots reached 3.4 ± 0.2 dS.m^{-1}. Each treatment was replicated three times in a complete randomized block design. Thereafter, irrigation was applied manually to pot water-holding capacity using tap water. At the end of the treatment, soil EC of NaCl-treated pots was 3.1 ± 0.2 dS.m^{-1}, confirming that salt stress remained constant during the treatment.

Yield parameters estimation

At the end of the experiment, the following growth and yield-related parameters were measured considering the main stem: plant height at maturity, panicle length, panicle leaf dry weight, panicle leaf length, panicle leaf width, and number of branches per panicle. We also quantified the mean number of tillers per plant, fertile tillers per plant, days to 50% heading, straw fresh weight

per plant, grain weight per plant, number of grains per panicle, number of filled grains per panicle, and 1,000 grains weight.

Plant height was assessed at maturity as the distance from the ground to the tip of the tallest panicle of five plants randomly selected per cultivar and per treatment. Days to 50% heading was considered as days required to observe 50% of plants with panicle emergence starting from transplantation. Weight of 1,000 grains were recorded from filled grain collected from random plants. All other yield-related parameters were assessed at maturity stage (6-month experiment).

Estimation of ion and proline concentrations

Ion determination was performed as described by Gouveitcha *et al.* (2021). Hulls were manually separated from the grains. Dried matter (leaf, grain, or grain hull) was individually ground in a mortar, and the powder was dried for 24 h. To determine the concentrations of Na^+ and K^+, *c.a* 20 mg of the obtained powders were placed in 10 mL jars and digested in nitric acid (68%) at room temperature. The solutions were filtered through Whatman paper (85 mm, Grade 1). The filtrate was used for the determination of cations (Na^+ and K^+) using a flame spectrophotometer (Sherwood Model 360).

Proline concentration was quantified on a distinct set of plants not used for yield estimation. Panicle leaf was collected at the start of the grain gilling stage, cut in small segments, quickly frozen in liquid nitrogen, and stored at $-80\,°C$ until analysis. Proline was determined spectrophotometrically using the method of Bates *et al.* (1973), and results were expressed as $\mu mol\ g^{-1}$ FW (fresh weight).

Statistical analysis

All the data in this research were calculated with JMP Pro 15 software. The analysis of the main effects of cultivars and stresses was based on the variance analysis. At $P < 0.05$ a *post hoc* test Tukey's HSD all-pairwise comparisons were used to compare means.

Results

Physiological parameters

All plants remained alive and set seeds during the time course of our experiment. In control condition, plants from the two cultivars had similar sodium content (Figure 1.A). Salt stress increased sodium content in shoots of both studied cultivars of *Oryza glaberrima*. Salt-sensitive cultivar TOG5949 accumulated about 1.3 time more Na^+ in the shoots than TOG5307. An opposite trend was observed for K^+ as indicated in Figure 1.B. Indeed, potassium content decreased almost by half when salt stress was applied, but TOG5307 had significantly higher content of K^+ in the shoots than TOG5949 in the presence of NaCl. Na^+/K^+ ratio was lower in control condition than in salt-treated plants. In plants exposed to NaCl, however, Na^+/K^+ ratio was significantly higher (1.8 time greater) in TOG5949 than in TOG5307 (Figure 1.C).

Proline concentration in the panicle leaf did not vary between studied cultivars in control conditions. However, proline concentration increased in response to NaCl. The salt-resistant cultivar TOG5307 had significantly higher proline concentrations in the panicle leaf than TOG5949 (Figure 1.D).

Figure 2 shows sodium and potassium concentrations in grain and grain hull. Under control conditions, the sodium concentration in the grain and in the hull was negligible in both cultivars. However, in the presence of salt stress, the sodium content increased significantly and higher sodium content was measured in grain hull than in the grain itself. As far as grain is concerned, Na^+ accumulation was obviously higher in TOG5949 than TOG5307. Even if grain hull had higher content of Na^+, variety TOG5949 still had higher content of Na^+ than TOG5307 in
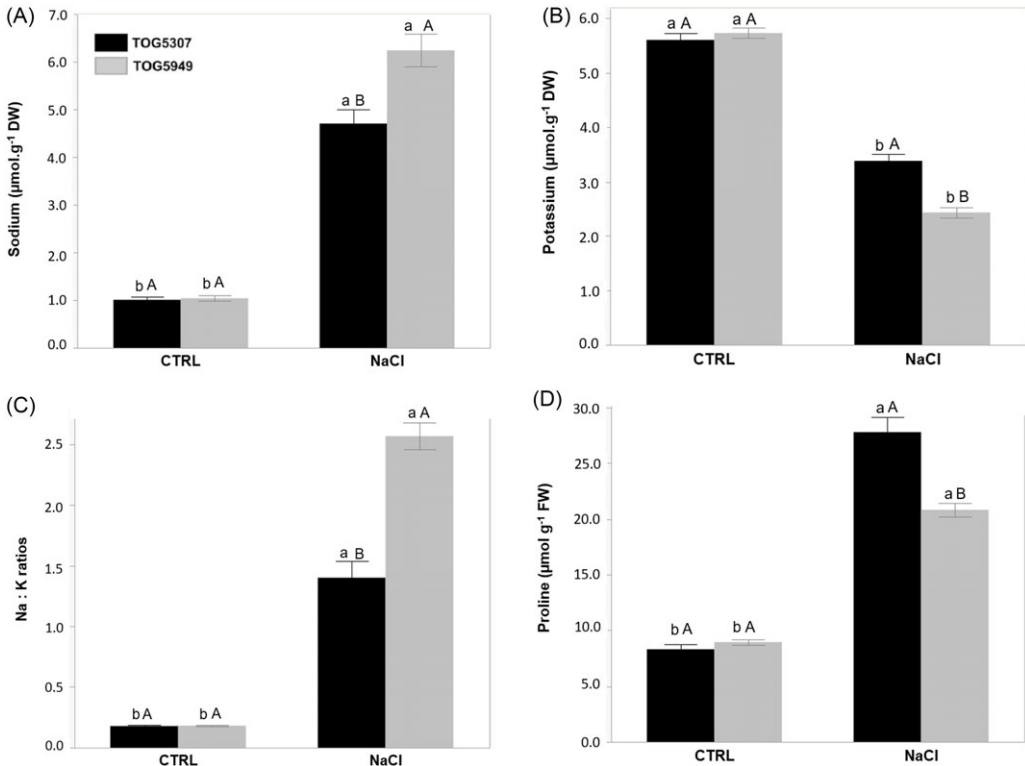
Figure 1. (A) Sodium, (B) potassium, (C) Na/K ratios, and (D) proline concentration in shoots of African rice (*Oryza glaberrima* Steud.) cultivars TOG5307 and TOG5949 cultivated during 25 weeks in control (CTRL) conditions or in the presence of 30 mM NaCl. Treatments followed by the same lowercase latter for a particular cultivar do not differ statistically. Cultivars followed by the same uppercase letter in a particular condition do not differ statistically.

response to salt stress. The sodium concentration in the grain corresponds to 8.7% of the Na^+ concentration in the hull in TOG5307 while this proportion reached 32.8% in TOG5949.

Grain had significantly lower content of K^+ than grain hull whatever the cultivars and conditions. However, plants exposed to control condition had higher potassium contents than those exposed to NaCl. As far grain is concerned, TOG5307 significantly accumulated more potassium than TOG5949 whatever the applied treatment. When salt stress was applied, the grain K^+ concentration decreased by 36% and 75% for TOG5307 and TOG5949, respectively. In the absence of salt, no difference between cultivars was recorded for potassium concentration in the hulls. However, salt stress decreased potassium concentration in grain hull by 40% and 53% for TOG5307 and TOG5949, respectively (Figure 2).

Na^+/K^+ ratio for grain and grain hull were closed to zero for both varieties in control condition. In TOG5307, Na^+/K^+ was higher in grain hull than in grain during salt stress. An opposite trend was recorded for TOG5949, since the Na^+/K^+ was extremely high in grains of TOG5949 exposed to salinity.

Reproductive development under salt stress

Table 2 presented yield-related parameters of *Oryza glaberrima* Steud. cultivars TOG5307 and TOG5949 cultivated in control and saline conditions. As far as control condition is concerned, plant height at maturity, panicle leaf dry weight, panicle leaf width, number of tillers per plant,
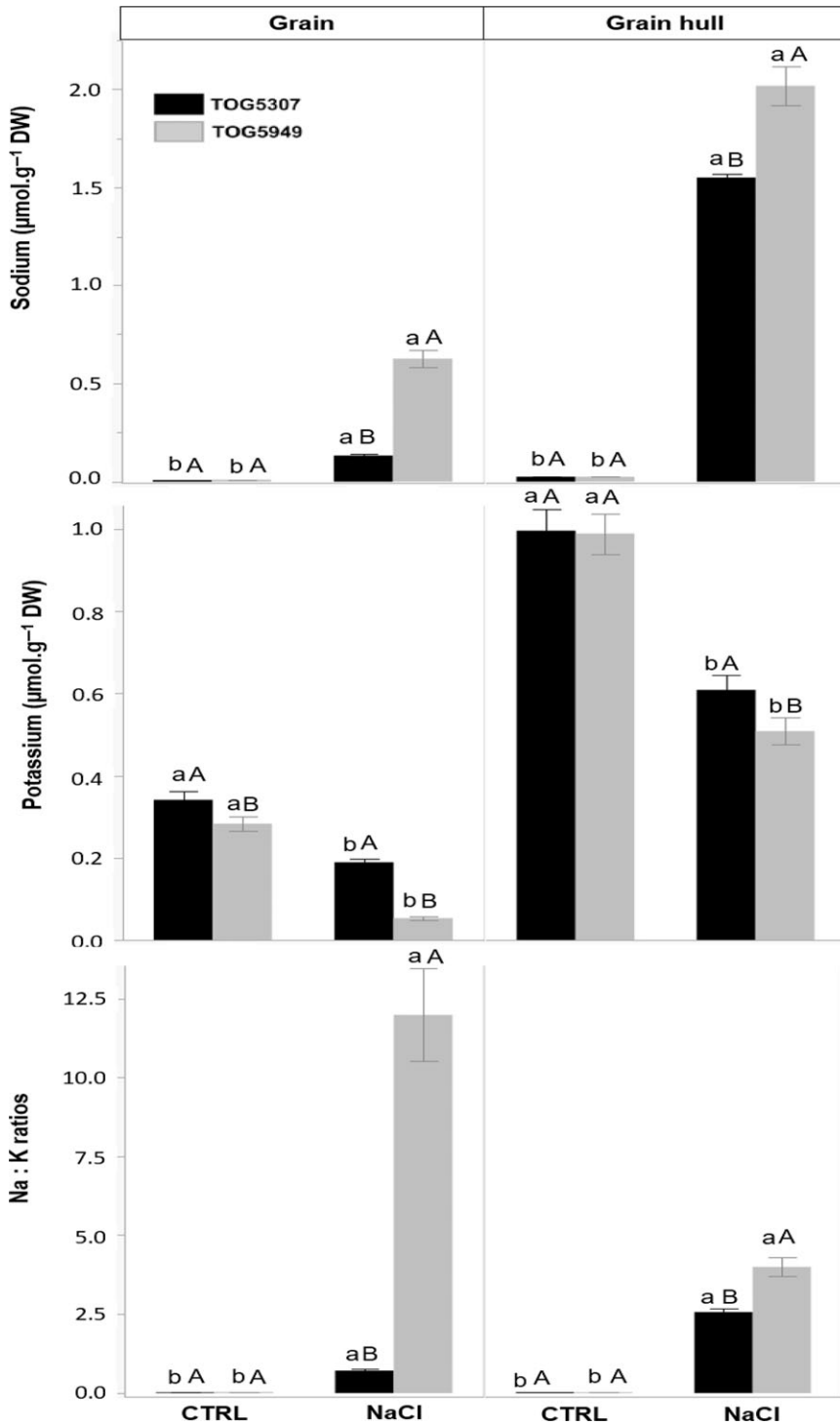
Figure 2. Mineral nutrients concentration in grain and grain hull of African rice (*Oryza glaberrima* Steud.) cultivars TOG5307 and TOG5949 cultivated during 25 weeks in control (CTRL) conditions or in the presence of 30 mM NaCl. Treatments followed by the same lowercase latter for a particular cultivar do not differ statistically. Cultivars followed by the same uppercase letter in a particular condition do not differ statistically.

Table 2. Yield parameters of African rice (*Oryza glaberrima* Steud.) cultivars TOG5307 and TOG5949 cultivated during 25 weeks in control (CTRL) conditions or in the presence of 30 mM NaCl. Treatments followed by the same lowercase latter for a particular cultivar do not differ statistically. Cultivars followed by the same uppercase latter in a particular condition do not differ statistically

Yield parameters	TOG5307		TOG5949	
	CTRL	NaCl	CTRL	NaCl
Plant height at maturity (cm)	176 ± 4.2 aA	172 ± 2.6 bA	178 ± 3.4 aA	166 ± 3.2 bB
Panicle length (cm)	30 ± 1.3 aA	28 ± 2.1 aA	26 ± 2.2 aB	23 ± 3.1 aB
Panicle leaf dry weight (g)	0.12 ± 0.004 aA	0.09 ± 0.003 bA	0.12 ± 0.006 aA	0.06 ± 0.003 bB
Branches/panicle	7.47 ± 0.04 aA	6.34 ± 0.04 bA	7.32 ± 0.03 aB	6.27 ± 0.03 bB
Panicle leaf length	44 ± 0.1 aA	39 ± 0.4 bA	42 ± 0.5 aB	38 ± 0.2 bB
Panicle leaf width	1.5 ± 0.2 aA	1.4 ± 0.1 aA	1.5 ± 0.1 aA	1.4 ± 0.1 bA
Fertile tillers/plant	32 ± 0.5 aA	27 ± 1.4 bA	34 ± 1.3 aA	26 ± 0.8 bA
Days to 50% heading	114	123	117	133
Straw fresh weight (g)/plant	92 ± 1.4 aA	78 ± 2.1 bA	81 ± 1.6 aB	75 ± 1.3 bB
Grain yield/plant (g)	29 ± 0.5 aA	23 ± 0.6 bA	27 ± 0.4 aB	19 ± 0.4 bB
Spikelets /panicle	130 ± 2 aA	120 ± 1 bA	131 ± 1 aA	115 ± 2 bB
Filled grain/panicle	95 ± 1 aB	81 ± 2 bA	98 ± 2 aA	63 ± 2 bB
Weight of 1,000 grains (g)	24 ± 0.5 aA	23.5 ± 0.8 aA	25 ± 0.4 aA	21 ± 0.5 bB

number of fertile tillers per plant, number of spikelets per panicle, and weight of 1,000 grains were similar for both cultivars. However, contrasting properties were recorded for some parameters: TOG5307 showed a higher panicle length, branches per panicle, panicle leaf length, straw fresh weight per plant, and grain yield per plant than TOG5949, while an opposite trend was observed for the number of filled grains per panicle and days to 50% heading. NaCl treatment affected most yield-related parameters. Nevertheless, the deleterious impacts of salt stress were significantly lower in TOG5307 than in TOG5949 for plant height at maturity, panicle leaf dry weight, number of branches per panicle, panicle leaf length, days to 50% heading, straw fresh weight per plant, grain yield per plant, number of spikelets per panicle, and number of filled grain per panicle. This means that TOG5307 was more salt-resistant than TOG5949 at the reproductive stage and was able to ensure a higher yield than TOG5949 when growing on salt-affected soils. Salt stress delayed panicle emergence (flowering) and reduced filled grain by 15% and 36% for TOG5307 and TOG5949, respectively. Salt-induced reduction of filled grain mainly concerned the grains that are located at the basal part of the panicle in TOG5949, while the decrease was more evenly distributed in TOG5307 (detailed data not shown). Salinity had no impact on the weight of 1,000 grains in TOG5307, while it significantly reduced the grain weight in TOG5949.

Discussion

Salt stress induces a double constraint on plants: i) an osmotic constraint related to the decrease in the external osmotic potential and ii) an ionic constraint related to the accumulation of Na^+ and Cl^- ions (Castillo *et al.,* 2007; Ben Hassine and Lutts, 2010; Qin *et al.*, 2020). Although nutrient solutions are frequently used to study the impact of NaCl on plants, the present work was conducted on soil substrate assuming that soil properties may have an impact on root behavior in relation to ion bioavailability, but also that salinity may affect the soil structure as recently demonstrated in *O. sativa* (Irakoze *et al.,* 2021). The chemical analysis of the selected soil demonstrated that it is a neutral soil with low organic carbon and total nitrogen content (Table 1). The soil we used had a very low EC (0.268 dS/m) and a medium CEC (13.59 meq /100 g soil) with a majority of Ca^{2+} as exchangeable bases. Consequently, the Ca^{2+}/Mg^{2+} ratio is 5.05. Djègbadji therefore has a calci-magnesic soil (Table 1). This might explain why local people till crops (and especially rice) in this area. After soil salinization, the EC value rose to 3.4 $dS.m^{-1}$, which should be considered as a moderate salinity for rice plants (Singh *et al.*, 2021).

We found that applied saline stress significantly affected mineral nutrients and yield-related parameters of *Oryza glaberrima* cultivars. Even if all plants remained alive and set seeds during the time course of the experiment, we found that TOG5307 was more salt-resistant than TOG5949. This confirmed the report of Prodjinoto *et al.* (2018) who demonstrated that TOG5307 and TOG5949 displayed contrasting levels of salt-resistance at the seedling stage. Compared to Asian rice species *Oryza sativa*, the African rice species *Oryza glaberrima* is considered as low yielding due to lodging, grain shattering, and low spikelet number (Linares, 2002; Montcho *et al.*, 2013). In this study, we recorded that both studied cultivars produced at least 50% of filled grains during salt stress, but that an obvious decrease in spikelet fertility was recorded at the basal part of the panicle in the salt-sensitive TOG5949.

In *O. sativa* cv. Aiwu exposed to 30 mM NaCl during the reproductive phase of development, Zhu *et al.* (2004) reported that ionic component of salt stress was the main constraint and that the osmotic component had only a minor impact. According to Negrão *et al.* (2017), sodium appears to accumulate to toxic levels before chloride in most species. Moreover, the discrimination of the impact of Na^+ and Cl^- in the deleterious effects of salt stress on the African rice (*Oryza glaberrima*) demonstrated that Na^+ is more toxic than Cl^- for this species (Prodjinoto *et al.* 2021a). Our results revealed that plants of both cultivars accumulated high amounts of Na^+ in shoots, grains, and grains hulls when exposed to NaCl, but that the salt-resistant TOG5307 accumulated less Na^+ than the salt-sensitive TOG5949. This was already reported for plants at the vegetative stage (Prodjinoto *et al.*, 2021a). It implies that TOG5307 displayed an avoidance strategy and reduces Na^+ absorption at this stage of development. The present data demonstrate that this is also valid at the reproductive stage: sodium accumulation was always higher in TOG5949 than in TOG5307, not only in shoots but also in grain and hulls. This means that Na^+ ions toxicity should play a key role in NaCl adverse effects on yield-related parameters of African rice species. Beside Na^+ accumulation, a decrease in K^+ concentration is a major deleterious effect of salt stress and may be explained by the poor selectivity of some ion transporters and channels and the similar chemical properties of Na^+ and K^+ ions. According to Lee *et al.* (2003), Na^+/K^+ ratio in leaves of crops can be used as an important indicator of salinity resistance. Once again, the trend previously observed for plants exposed to NaCl at the vegetative stage is still valid at the reproductive one, and the higher ability of TOG5307 to maintain K^+ homeostasis clearly appeared at the grain level with a very low Na^+/K^+ ratio comparatively to salt-sensitive TOG5949. Mineral content was quantified for the whole shoot, but one should keep in mind that salt-resistant cultivars frequently accumulate Na^+ in old leaves in order to protect the young photosynthetically active leaves from deleterious effect of salinity (Yeo and Flowers, 1982; Lutts *et al.*, 1999). This is of specific interest for the panicle leaf which assumes a key role during grain filling (Parida *et al.*, 2022). Proline is an efficient osmoprotectant involved in both osmotic adjustment, free radicals scavenging, and protection of cellular structure in plants exposed to salinity (Ben Hassine and Lutts, 2010; Mansour and Ali, 2017; Abdelaal *et al.*, 2020). This compound accumulated to higher concentration in panicle leaf of salt-resistant TOG5307 than in salt-sensitive TOG5949, suggesting that this leaf which contributes for more than 50% to grain filling (Zörb *et al.*, 2019; Parida *et al.*, 2022) was more physiologically active in salt-treated TOG5307 than in TOG5949. This is partly supported by a lower salt-induced decrease in panicle leaf dry weight in the former than in the later.

Growth reduction due to salinity has been implicated as a causative factor for yield losses (Zeng and Shannon, 2000). In our experiment, however, NaCl only slightly reduced plant height, but it had no impact on panicle length. From a relative point of view, the salt impact on the number of filled grains per panicle was higher than the salt impact on the number of spikelets per panicle. Although salt stress was applied during the whole plant cycle, this suggests that pre-anthesis parameters were less affected by salinity than post-anthesis parameters. The length of the panicle depends on how quick the rachi get transformed in a panicle (Parida *et al.*, 2022). The formation of the entire panicle, including spikelets, occurred inside the boot leaf, a long time before the macroscopic appearance of the panicle. A decrease in number of spikelets without effect on panicle

length should thus lead to an increase in the inter-grain space, resulting in a less compact panicle. In both cultivars, salinity reduced the number of branches per panicle, thus showing that salinity may induce a change in panicle morphology differentiating at the booting stage.

In the present work, salinity was applied already at the vegetative stage. This strategy is relevant to what occurs in field conditions. However, it did not allow us to specifically discriminate the impact of salinity at the reproductive stage. Indeed, it might be argued that the salt-induced decrease in vigor occurring at the vegetative stage influences the capacity of the plant to efficiently conduct a reproductive phase. In that sense, salinity impact at the seedling stage has 'a delayed' effect on the reproductive phase. Hence, the fact that TOG5949 had a lower yield than TOG5307 is not an absolute proof that it is more sensitive than TOG5307 at the reproductive stage but could be the consequence that it was weaker when it initiated the reproduction phase. Applying salinity at the heading stage may provide useful information in that respect (Lutts *et al.*, 1995). Similarly, Khatun *et al.* (1995) applied salt stress to *O. sativa* at the panicle initiation stage, which is considered to occur *c.a.* 60 days after germination based on dissection approaches.

Our data suggest that the main deleterious impact of salinity on grain yield of *O. glaberrima* is related to an alteration of grain filling process leading to a lower number of filled grain per panicle in both cultivars and to a decrease in 1,000 grains weight in salt-sensitive TOG5949. In TOG5307 exposed to NaCl, 67.5% of the spikelets set seeds, while the percentage dropped to 54.8% in TOG5949. A decrease in spikelets fertility may be due to an increase in Na^+ pollen concentration leading to sterility (Khatun *et al.*, 1995; Zhu *et al.*, 2004) and to Na^+ accumulation on the stigma decreasing its receptivity (Khatun *et al.*, 1995). According to Parida *et al.* (2022), the poor grain filling at the basal part of the panicle may also be due to ethylene oversynthesis. Ethylene is a stress hormone commonly overproduced in response to salinity (Ben Hassine and Lutts, 2010), and a higher ethylene production by the salt-sensitive cultivar TOG5949 could thus not be ruled out.

Our data provide evidences that the 1,000-grain weight was reduced in salt-sensitive TOG5949 but not in salt-resistant TOG5307. The grain weight is positively associated with grain size which is coordinately controlled by cell proliferation and cell expansion of the grain hull (Li *et al.*, 2018). Hulls from TOG5949 accumulated higher Na^+ concentration and lower K^+ concentrations than hulls of TOG5307; the resulting higher Na^+/K^+ ratio in the hulls of the sensitive cultivars may decrease cell proliferation in the lemma and palea leading to small grain size (Shi *et al.*, 2020). Beside Na^+ content in the hulls, TOG5949 also exhibited higher Na^+ content in the grain. A decrease in endosperm cell division has also been reported to induce poor grain filling in *O. sativa* (Sahu *et al.*, 2021) and such a decrease may be related to breakdown of cytokinins through activation of cytokinin oxidase (Parida *et al.*, 2022). It must be mentioned that hulls, as transpiring organs, receive Na^+ through the xylem sap, while the Na^+ present inside the grains is provided by the phloem and depends on the capacity of the plant to mobilize ion accumulated in leaves (and especially in panicle leaf). It is noteworthy that Prodjinoto *et al.* (2021b) previously suggested that the higher Na^+ tolerance of TOG5307 at the leaf level might be related to a preferential accumulation of Na^+ in the apoplasm, while TOG5949 displays a higher symplasmic content: if this hypothesis is valid, it would imply that apoplasmic Na^+ is not necessarily more easily remobilized to the grain than the symplasmic one, which could perhaps be related to Na^+ fixation on the negatively charged polymers of the cell wall.

Although this aspect was not considered in the present study, it must be mentioned that salt stress induces physiochemical alteration in rice grain composition and quality. Razzaq *et al.* (2020) recently reported that salinity decreased the chain length of amylopectin, modified the concentration of gluten, strongly decreased vitamin A, B_1, B_3, and B_6 and adversely affected fat content, the global consequences being an obvious decrease in the nutritional value of rice grain. There is no obvious proof that those modifications directly result from Na^+ accumulation in endosperm; nevertheless, it may be suspected that a higher Na^+/K^+ would have strong negative effect on grain maturation, and that Na^+ has a deleterious effect on enzyme activities involved in reserves storage. Sucrose synthase involved in phloem unloading may be impacted by salinity, while ethylene

biosynthesis lead CN^- as a byproduct: CN^- is a well-known inhibitor of the mitochondrial electron transport, which results in a poor generation of ATP, thus compromising starch synthesis which is an ATP-consuming process (Sekhar *et al.*, 2015).

Conclusion

In the tested cultivars of *Oryza glaberrima*, salinity resistance at the reproductive stage corresponds to salinity resistance previously recorded at the seedling stage and is mainly related to a lower Na^+ accumulation in both vegetative and reproductive organs. Salinity induced a decrease in numerous yield-related parameters and had higher impact on post-anthesis parameters such as the number of filled grains per panicle than on pre-anthesis parameters such as the number of spikelets per panicle or panicle length. Salinity had a detrimental impact on grain filling mainly in the salt-sensitive cultivar, leading to a lower grain weight.

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