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Screening for salinity tolerance of *Oryza glaberrima* Steud. seedlings

Hermann Prodjinoto[1,2], Christophe Gandonou[2] and Stanley Lutts[1*]

[1]Groupe de Recherche en Physiologie végétale – Earth and Life Institute – Université catholique de Louvain, Belgium.
[2]Laboratoire de Physiologie Végétale et d'Etude des Stress Environnementaux, Faculté des Sciences et Techniques, Université d'Abomey-Calavi, République du Cotonou, Bénin.

Rice (*Oryza sativa*) is a salt-sensitive species and improvement of salt resistance is a major goal for plant breeders. Some species of *Oryza* genus may constitute an interesting source of genes involved in stress resistance for cultivated rice improvement. The African rice *Oryza glaberrima* is poorly described for its response to salt stress. Twenty-five accessions of *O. glaberrima* were exposed during 2 weeks to 0 or 60 mM NaCl in nutrient solution. Morphological and physiological parameters were recorded and used to perform principal component analysis allowing us to consider three contrasting groups (salt-resistant, medium, and salt-sensitive). Most of the tested lines appeared more salt-sensitive than the moderately salt-resistant cultivar I Kong Pao from *O. sativa*. Salt-sensitivity index was higher for roots than for shoots and *O. glaberrima* was poorly efficient for regulation of Na$^+$ translocation from the root to the shoot. Some accessions such as TOG5307 however were able to maintain a high net photosynthesis under salt conditions and exhibited a high level of tolerance to accumulated Na$^+$ ions and a high capacity for osmotic adjustment. It is concluded that these salt-tolerant accessions constitute a promising material for rice improvement through inter-specific crosses with *O. sativa*.

Key words: African rice, NaCl, *Oryza glaberrima*, salinity, salt stress.

INTRODUCTION

Rice is an important staple food for more than half of the human population. It provides 50% of the calories consumed in several areas of Asia and Africa (Khush, 2005). In numerous African countries, however, rice production is still not sufficient and the estimated rice import in Africa accounts for several millions of tones each year which represent more than one-fourth of its requirements (Nhamo et al., 2014). There is consequently an urgent need to increase rice production,

especially considering that the world's population is predicted to reach around 10 billion people by 2050 (Hoang et al., 2016). Because of a very limited potential for future expansion of arable lands, such a goal implies to extend rice culture to marginal lands which are not used at the moment for rice culture.

Numerous environmental constraints are limiting rice production. Among them, drought and soil salinity are probably the most prevalent abiotic stresses hampering

plant growth and development. Salinity affects more than 830×10^6 ha in the world. Of the 230×10^6 ha of the world's irrigated lands, 45×10^6 ha (20%) have already been affected by salt, and the problem is increasing due to sea level rise and to erratic irrigation (Munns, 2005). Salinity imposes a double constraint to plants: an osmotic stress and an ionic toxicity. Osmotic stress is related to the presence of a high external salt concentration which decreases the external water potential and thus compromises water uptake by the plant. The ionic component of salt stress is due to progressive accumulation of toxic ions such as Na^+, excess of Cl^- and salt-induced decrease in essential elements, mainly K^+ (Acosta-Motos et al., 2017).

Rice is very sensitive to salt stress (Hoang et al., 2016) and a NaCl dose as low as 50 mM in nutrient solution is considered to be lethal for salt-sensitive cultivars (Yeo and Flowers, 1986; Zhu et al., 2001). Salt-sensitivity in rice varies depending on the phenological stage with young seedlings and plants at the flowering stages being considered as the most sensitive ones (Lutts et al., 1995; Hakim et al., 2010). Despite a high number of available cultivars, Oryza sativa L. still performs poorly under salt stress conditions (Singh and Sengar, 2014). The most salt-tolerant genotypes are tall indica landraces which suffer from major agronomic drawback under West African conditions. Numerous evidences are now available regarding the loss of genetic diversity encountered by O. sativa since its domestication (Caicedo et al., 2007).

Some biotechnological tools may be used to improve salt-tolerance of existing cultivar (Lutts et al., 1999; Singh and Sengar, 2014) but both in vitro selection and transgenic approaches suffer from technical and/or social limitations. A promising alternative is to use other species of Oryza genus for breeding purposes in order to improve abiotic stress resistance in Oryza sativa (Atwell et al., 2014). The cultivated African rice Oryza glaberrima Steud. is receiving a considerable attention since several years. This species was domesticated 3000 years ago. Although it was progressively replaced by the high-yielding Asian rice O. sativa, this hardy species has qualities that make it superior to Asian rice as a subsistence crop (Linares, 2002). This species suffers from easy shedding of grains, resistance to milling, greater breaking of grains, red pericarp and lower yield (Nayar, 2010). However, it also presents a greater resistance to various biotic and abiotic stresses. Resistance to yellow mottle virus (Pidon et al., 2017) and to the nematode Meloidgyne graminicola (Cabasan et al., 2015) has been identified in this species. O. glaberrima produces extra-tillers allowing it to efficiently compete with weeds (Sarla and Mallikarjuna Swamy, 2005). It displays interesting properties for resistance to iron toxicity (Majerus et al., 1999; Dufey et al., 2015) and to submergence (Sakagami et al., 2009). It also possesses promising characters for drought resistance (Bimpong et

al., 2011; Bocco et al., 2012; Ndjiondjop et al., 2012; Kijoji et al., 2013).

Numerous strategies have been efficiently used by breeder to overcome hybrid sterility between O. sativa and O. glaberrima (Shen et al., 2015). Several varieties issued from selected recombined plants obtained after interspecific crosses between the two species are now available and known as New Rice for Africa (NERICA) varieties. Most of them resemble O. glaberrima during early growth, displaying weed competitive ability and with O. sativa at the reproductive stage, allowing high yielding capacities (Jones et al., 1997; Sarla and Mallikarjuma Swamy, 2005). Despite the large set of data available for water stress resistance in O. glaberrima, information regarding salinity resistance in this species remains poorly documented. Awala et al. (2010) reported that O. glaberrima CG14 appeared rather salt-sensitive but the obtained hybrids after crossing this line with the O. sativa WAB56-104 cultivar produced hybrids exhibiting a high level of salinity resistance. Platten et al. (2013) quantified Na^+ accumulation in several salt-exposed lines of O. glaberrima and identified a specific gene (OgHKT1;5) which partly contribute to regulate Na^+ absorption and translocation.

Screening for salinity resistance in O. glaberrima is still required in order to identify the most promising material to integrate in interspecific crosses with O. sativa. The present study therefore screened 25 lines of O. glaberrima exposed to salinity at the seedling stage, and analyzed their overall behavior in terms of growth in relation to physiological properties influencing salt-stress resistance.

MATERIALS AND METHODS

Plant material and growing conditions

Twenty-five accessions of O. glaberrima Steud. and one genotype of O. sativa L. were used in the present study. Seeds of O. glaberrima were obtained from Africa Rice (Abomey-Calavi, Benin) (Table 1). The cultivar I Kong Pao (IKP) of O. sativa was used as a reference since this genotype exhibited a medium level of salt resistance and is well adapted to environmental conditions where O. glaberrima usually occurs (Lutts et al., 1995, 1999; Zhu et al., 2001). Seeds were germinated in glass vessels on 2 layers of Whatman (85 mm, Grade 1) filter paper moistened with 10 ml of deionized water. They were placed in a growth chamber at 25 to 21°C (day/night) under a 16 h daylight period (150 to 220 µmole m^{-2} s^{-1}). Illumination was provided by SYLVANIA fluorescent tubes (F36W/840-T8, cool white). For each genotype, 25 seeds were placed in each glass vessel.

Eleven-days old seedlings were transferred to a phytotron and maintained at 24°C/21°C (day/night). They were fixed on polystyrene plates floating on Yoshida et al. (1976) nutritive solution. For each genotype, seedlings were distributed among tanks containing 1.5 L of nutrient solution. Illumination was provided by PHILIPS metal iodide lamp (HPIT/400W) for 16 h d^{-1} at a photon flux density (PFD) of 180 to 200 µmoles m^{-2} s^{-1}. Daytime humidity was 65%. The nutrient solution was renewed every week and tanks were randomly rearranged in the phytotron. Salt stress was applied when plants were 33 days-old: sodium chloride (NaCl) was added

Table 1. List of accessions from *Oryza glaberrima* used on the current study and their corresponding salt-sensitivity index estimated for roots. shoots and a whole plant basis. I Kong Pao is a moderately salt-resitant variety from *Oryza sativa*.

Variety	Provenance	SI Root	SI Shoot	SI Total
IKP (I KONG PAO)	Thailand	67.1	34.8	38.8
CG17	Senegal	73.0	50.0	54.1
CG20	Senegal	77.7	49.6	55.7
TOG5293	Nigeria	84.9	47.9	55.1
TOG5307	Nigeria	47.0	9.7	16.1
TOG5385	Nigeria	74.6	44.2	49.6
TOG5390	Nigeria	85.7	56.0	62.0
TOG5420	Nigeria	75.7	48.6	53.2
TOG5440	Nigeria	82.0	42.1	49.3
TOG5442	Nigeria	72.6	52.4	55.7
TOG5456	Nigeria	78.8	50.1	55.2
TOG5479	Nigeria	86.1	52.1	58.3
TOG5482	Nigeria	65.3	46.1	49.8
TOG5500	Nigeria	72.3	43.2	46.3
TOG5566	Nigeria	76.3	60.1	62.8
TOG5588	Ghana	77.1	38.6	46.2
TOG5641	Nigeria	73.6	48.5	51.2
TOG5666	Nigeria	88.5	56.2	63.7
TOG5672	Nigeria	70.8	57.6	59.3
TOG5681	Nigeria	69.7	45.9	48.7
TOG5685	Nigeria	82.3	68.2	70.7
TOG5775	Libéria	35.6	25.4	26.9
TOG5885	Liberia	77.1	64.3	66.5
TOG5949	Nigeria	91.3	79.9	82.3
TOG5969	Nigeria	82.7	54.3	60.1
TOG5979	Nigeria	75.4	62.6	64.8

to nutrient solution in order to reach a final concentration of 60 mM to one half of the tanks, the other half being used as unstressed controls. Salt stress was applied for 2 weeks.

Morphological and physiological analysis

The length of the longest leaf (LHL), the number of tillers (NT) and the number of leaves (NL) were determined. The stomatal conductance (g_s) was estimated using porometer (type AP4-UM-3) (Delta T-devices, IK) on 6 plants per treatment. The net photosynthesis (A; net carbon assimilation rate; in mmoles CO_2 m^{-2} s^{-1}) was estimated under constant photosynthetic photon flux (500 µmoles m^{-2} s^{-1}), the instantaneous transpiration (E) (in mmoles H_2O m^{-2} s^{-1}) and the intercellular CO_2 content (Ci; µmoles $mole^{-1}$) were measured on the youngest fully expanded leaf of 6 plants per treatment using a water vapor analyzer (LCA 2 8.7, ADC, Great Amwell, England) and an air supply unit (ASU 10.87, ADC, Hertfordshire, UK) mounted in series in an open system. All these measurements were performed at the time of stress imposition and after 2 weeks of treatment. Plants were then harvested at the end of stress exposure. Roots and shoots were separated, and roots were quickly rinsed for 30 s in deionized water to remove ions from the root surface and the free spaces. Shoots and roots were weighed for fresh weight determination (in g), then incubated in an oven at 72°C for 48 h until constant dry weight were reached. Water content (mL.g^{-1} WC) was estimated using the equation:

$$WC = (FW - DW)/DW$$

The sensitivity index (SI) that is, the difference between dry matter production of salt-treated plants and the control, expressed in % of the matter, was calculated according to the following expression:

$$SI = (100 \times (DW_{control} - DW_{treatment})) / DW_{control}$$

The mean tolerance index (TI_{Na}) to endogenous Na^+ was estimated for each physiological parameter as the ratio between the relative value of this parameter recorded in stress conditions expressed as a % of the mean value recorded in control conditions divided by the concentration of accumulated Na^+ in the considered organ:

$$TI_{Na} = ((X_{NaCl}/X_{control}) \times 100)/Na^+ \text{ content}$$

Ions measurement

For ion content determination, *c.a.* 100 mg DW was weighed. Samples were placed in flask of 10 ml and digested with nitric acid (68%) at 80°C. After complete evaporation, residues were dissolved with nitric acid (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 (K, Na, Mg, Ca and Fe) by flame emission using atomic absorption spectrometry (Thermo scientific S series model AAS4). The analysis was performed on 3

plants per treatment and each sample was analyzed in triplicate. Results are expressed in mg g^{-1} DW.

Osmotic potential measurement

For osmotic potential (Ψs) measurement, roots and leaves of 3 plants per treatment were frozen in liquid nitrogen at harvest. After 3 cycles of frozen/thaw, samples were centrifuged at 15,000 g during 15 min at 4°C. The supernatant corresponding to the extracted sap was used to measure the osmolality (c) using a Wescor 5500 vapor pressure osmometer as previously detailed (Lutts et al., 1999). The Ψs was then calculated according to:

$$Ψs \ (MPa) = - c \ (mosmoles.Kg^{-1}) \times 2.58 \times 10^{-3}$$ according to the Van't Hoff equation.

Malondialdehyde and proline concentrations

Malondialdehyde (MDA) is a common indicator of oxidative stress. It was quantified on roots and leaves of 3 plants per treatment using the method of Heath and Packer (1968). Frozen 250 mg were homogenized in pre-chilled mortar with a solution of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid (TCA) and were heated to 95°C for 30 min. Then samples were cooled at room temperature. After centrifugation at 3000 rpm for 5 min the absorbance of supernatant was read at 532 nm, and the values of the non-specific absorbance were taken at 600 nm and subtracted from the original (532 nm). The MDA concentrations were calculated using the molar extinction coefficient of 155 mM cm^{-1}. Results are expressed as moles g^{-1} FW.

Proline content was measured as described by Bates et al. (1973). Frozen tissue (0.5 g) were homogenized in 10 ml of 3% sulphosalicylic acid and then centrifuged at 10,000 × g. The supernatant (0.5 ml) was mixed with 1 ml of glacial acetic acid and 1 ml of 2.5% acid ninhydrin (2.5 g of ninhydrin dissolved in a mixture of 60 ml glacial acetic acid and 40 ml 6 M phosphoric acid). The mixture was incubated for 1 h at 100°C and then the reaction was terminated by cooling in an ice bath. The reaction mixture was extracted with 2 ml of toluene, mixed vigorously with the 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. Proline concentration was estimated on the basis of a standard curve. Results are expressed as moles g^{-1} FW.

Statistical analysis

The statistical analyses were performed with the "JMP Pro 12" software. Mean values and standard error (SE) were obtained from at least 3 replicates for genotypes. A *P-value* of < 0.05 was considered to be statistically significant. A two-way ANOVA was performed to detect cultivar, treatment, and interaction effects, a *P-value* lower than 0.05 was considered statistically significant. Screenings among accessions and treatments were displayed using principal component analysis (PCA) with R 3.3.2 Statistics software ('FactoMineR' package). Pearson correlation between analyzed parameters were also performed for 3 contrasting groups (salt-resistant, medium, salt-sensitive) using the 'corrplot' package in R 3.3.2 Statistics software.

RESULTS

In the absence of salt, TOG5685 had the highest shoot and total biomass (expressed as dry weight) and

difference was significant when compared with IKP (Figure 1A). In the presence of 60 mM NaCl, TOG5307, TOG5385 and TOG5588 exhibited the highest total biomass while TOG5885, TOG5949 and TOG5672 presented the lowest values. The mean sensitivity index was estimated for roots, shoots and whole plants (Table 1). Mean sensitivity remained low for TOG5307 and TOG5775, suggesting that these accessions displayed a similar level of tolerance comparatively to IKP. In contrast, SI values were especially high for TOG5949 and TOG5685. The mean leaf water content was similar in all genotypes under control conditions with a mean value of 83.7% in shoots and 89.4% in roots. Although the mean shoot WC decreased in response to salinity (78.4%), no significant difference was recorded among the considered accessions (detailed data not shown). The number of leaves was reduced in response to 60 mM NaCl and was the highest in CG17 and the lowest in TOG5949 and TOG5672 (Figure 1B).

Stomatal conductance in numerous varieties of *O. glaberrima* cultivated under control conditions was clearly higher than in IKP (Figure 2A). Salinity decreased stomatal conductance except in one single variety of *O. glaberrima* (TOG5979) where stomatal conductance remained unaffected by NaCl. In the absence of salt, instantaneous transpiration (*E;* Figure 2B) was higher in TOG5775 and TOG5500 than in other genotypes. Salinity reduced *E* values which however remained higher in TOG5420, TOG5775, TOG5307 and TOG5588 than in other genotypes.

Net photosynthesis (*A*; Figure 2C) slightly varied among genotypes under control conditions. From a relative point of view, differences among accessions appeared higher in NaCl-treated plants than in control conditions: while *A* values recorded in TOG5307 remained low affected by NaCl, salinity almost completely inhibited photosynthesis in TG5949 and strongly decreased it in CG20 and TOG5390. In the absence of salt, *C*i values were higher in numerous *O. glaberrima* varieties than in IKP (detailed data not shown). Salinity only had a limited impact on *C*i values, the highest concentration being recorded for TOG5442, TOG5482, TOG5775 and TOG 5979.

The root osmotic potential (Figure 3A) was statistically similar in all accessions in the absence of salt, but salinity decreased root Ψs, mainly in TOG5775, TOG5969, CG17, TOG5979, TOG5456, TOG5672, and TOG5500. In the presence of NaCl, these accessions displayed significant lower root Ψs values than IKP. An important decrease in the shoot water potential was also observed (Figure 3B), with recorded values being minimal for TOG5456, TOG5500 and GC17.

Numerous accessions of *O. glaberrima* accumulated more Na$^+$ in roots and shoots than IKP (Figures 3C and 3D). Some of them, such as TOG5307 presented high concentration in the roots but was able to restrict Na$^+$ accumulation in the shoot, at least to some extent. In
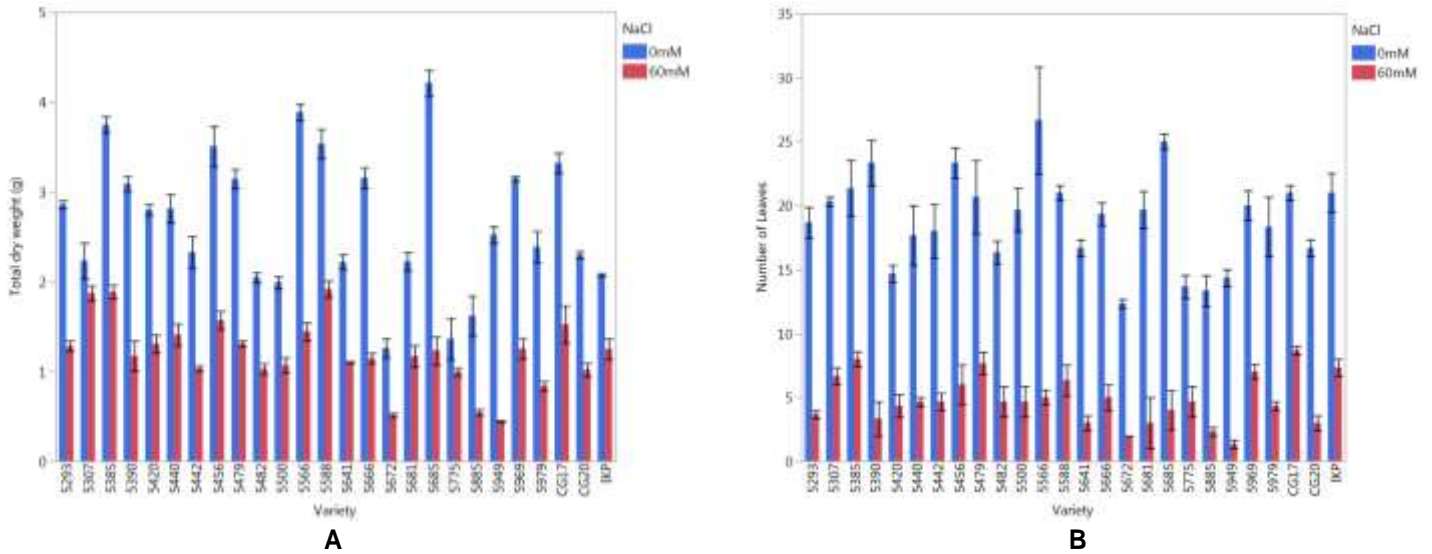
Figure 1. Total dry weight (A) and mean number of leaves (B) in 25 accessions of *Oryza glaberrima* and one moderately salt-tolerant cultivar of *Oryza sativa* (IKP). Seedlings were exposed for 2 weeks to 0 (control) or 60 mM NaCl (stress) in hydroponic culture under controlled environmental conditions. Each value is the mean of 3 replicates and vertical bars are standard errors.

contrast, TOG5681 exhibited high Na^+ concentration in the roots and in the shoots, suggesting that both Na^+ absorption and translocation were not efficiently regulated in this accession. Salt stress reduced the K^+ concentrations in all plant organs (Figures 3C and 3D). The mean root K^+ concentration was especially low for TOG5390, TOG5420, TOG5949, CG20 and TOG5385 in plants exposed to NaCl (detailed data not shown). For the main morphological parameter and net photosynthesis, the mean tolerance index was estimated to accumulate Na^+ (Table 2). The TI_{Na} values were globally higher for roots (root DW and root length) and varied depending on the cultivar. However, for almost all considered parameters the TI_{Na} value was higher for IKP than for *O. glaberrima,* whatever the accession. Some accessions of *O. glaberrima*, however presented high TI_{Na} values for some parameter, as it was the case for TO5307 (shoot DW, root DW and net photosynthesis), TOG 5566 (net photosynthesis) and TOG5775 (shoot and root DW).

The shoot MDA content was similar in all accessions for control plants (Figure 4A) but salt stress obviously increased MDA in all tested accessions, indicating the occurrence of a secondary oxidative stress. However, MDA remained low in TOG5440 and high in TOG5672. Proline (Figure 4B) also accumulated in shoots as a result of salt exposure. While IKP, TOG5666 and TOG5420 exhibited the highest concentration of proline, TOG5307, TOG5385 and TOG5479 presented the lowest concentration in salt-treated shoots.

A first principal component analysis (PCA) was performed in order to reveal the global impact of NaCl on the whole tested material in relation to the set of analyzed parameters, (except proline and MDA since data were not available for 3 accessions). PCA revealed that 77.49% of variance was explained by the principal component 1 (Dim 1) and the principal component 2 (Dim 2) (Figure 5). Dim 1 alone displayed 68.65% of variance. Parameters that have the highest value factor coordinate for the Dim 1, with the highest variable contribution, based on correlations, were, at left, toxic ion (Na^+) and sub-stomatal cavity CO_2 concentration (Ci). At right, they were potassium content, stomatal conductance (g_s), water content, instantaneous transpiration (E), net photosynthesis (A), height of plant, number of leaves, number of tillers, plant dry weight, root length, root dry weight and shoot dry weight.

The second plot showed the classification of seedlings in response to salt treatment in multivariate space of the first PCA (Figure 5B). Dim 1 displayed a clear opposition between the two groups: at the left, the salt-stressed seedlings and the controls seedlings at right. Salt-stressed seedlings showed positive correlation along the left side of Dim 1 which is linked to toxic ion (Na^+) and sub-stomatal cavity CO_2 concentration (Ci). So the left side Dim 1 revealed the seedlings that were severely affected by salt stress.

In order to discriminate salt-tolerant and salt-sensitive accessions, a second PCA on salt-stressed seedlings was performed. This PCA showed that 42.02% of variance was explained by the principal component 1 (Dim 1) and the principal component 2 (Dim 2) (Figure 6). Parameters that have the highest value factor coordinate for the Dim 1, with the highest variable contribution, based on correlations, were K content, water content, instantaneous transpiration (E), net photosynthesis (A),
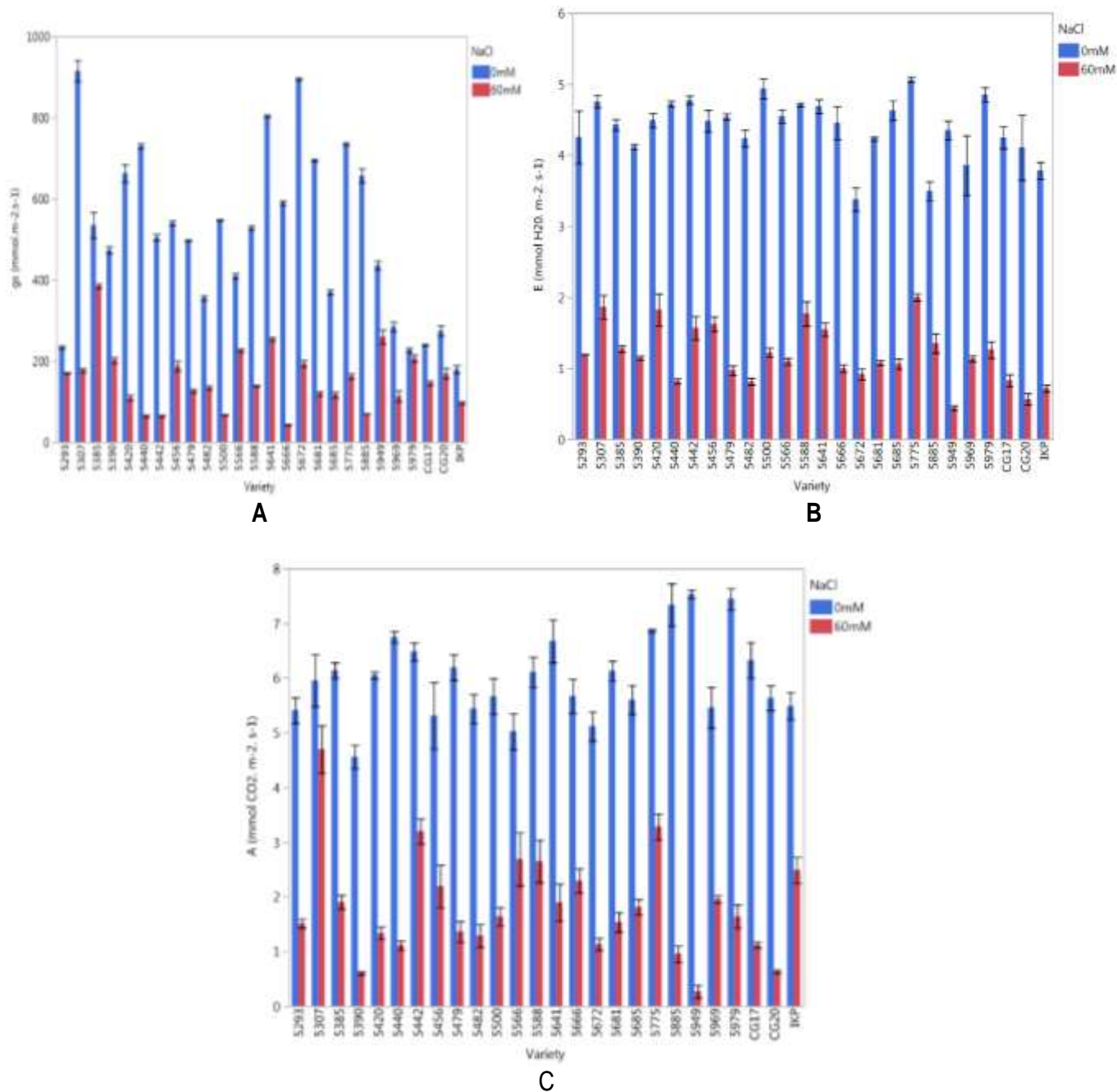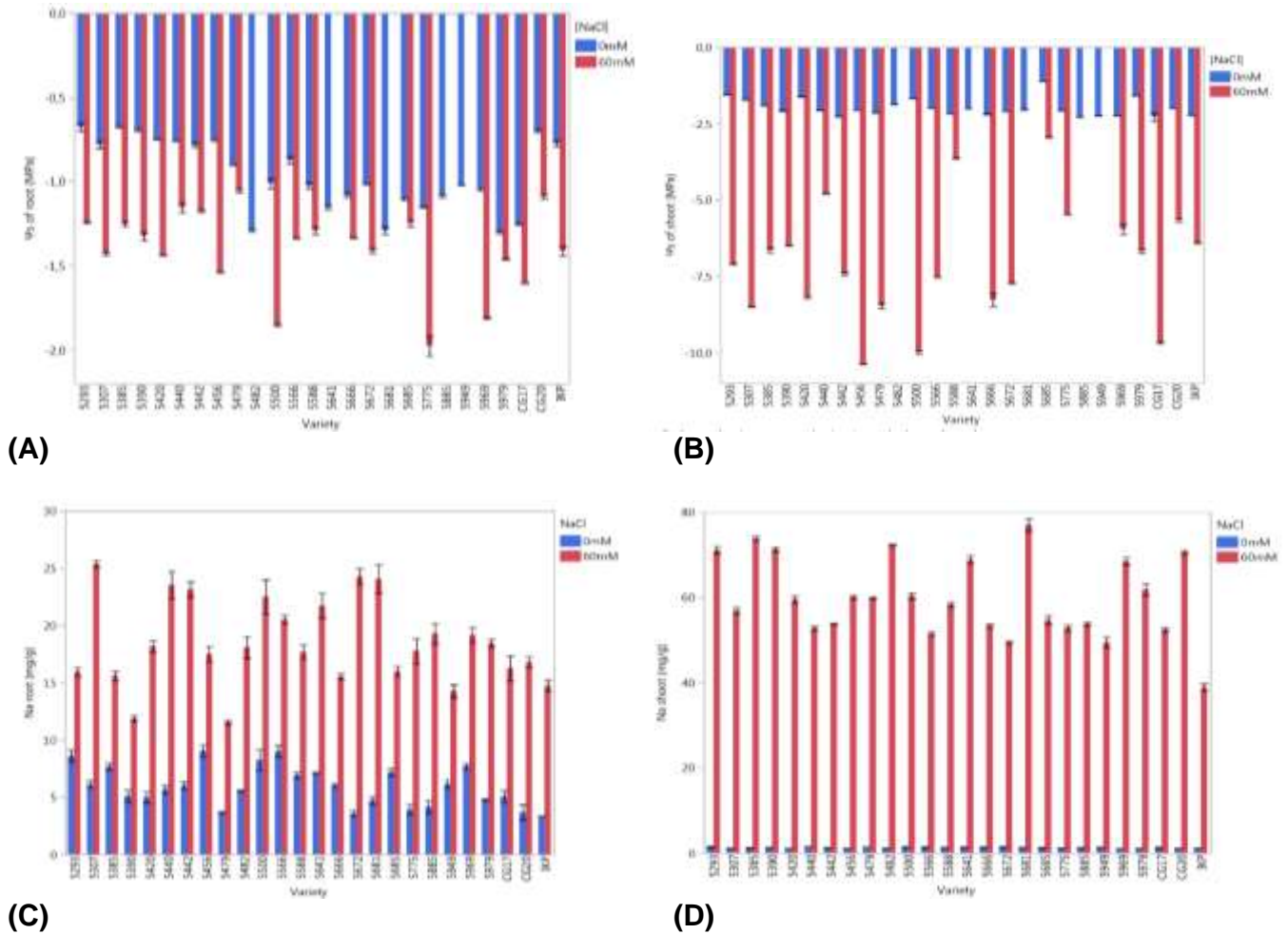
Figure 2. Stomatal conductance (A), instantaneous transpiration (B) and net photosynthesis (C) in 25 accessions of *Oryza glaberrima* and one moderately salt-tolerant cultivar of *Oryza sativa* (IKP). Seedlings were exposed for 2 weeks to 0 (control) or 60 mM NaCl (stress) in hydroponic culture under controlled environmental conditions. Each value is the mean of 3 replicates and vertical bars are standard errors.

height of plant, number of leaves, number of tillers, plant dry weight, root dry weight and shoot dry weight. The Dim 2 had high positive loading for root sodium concentration, root K concentration, instantaneous transpiration, net photosynthesis, height of plant, water content and high negative loading for plant dry weight, root dry weight, shoot dry weight, stomatal conductance (g_s), root length

and number of leaves (Figure 6A). Figure 6B shows the position of all tested accessions under salt stress in the multivariate space of the Figure 6A. Dim 1 highlighted on the opposition among 3 groups species under salt stress which could be related to salt-tolerant, salt-sensitive and "medium".

Under salt stress the salt-tolerant lines showed a strong

(A)

(B)

(C)

(D)

Figure 3. Root osmotic potential (A), shoot osmotic potential (B), root Na$^+$ concentration (C) and shoot Na$^+$ concentration (D) in 25 accessions of *Oryza glaberrima* and one moderately salt-tolerant cultivar of *Oryza sativa* (IKP). Seedlings were exposed for 2 weeks to 0 (control) or 60 mM NaCl (stress) in hydroponic culture under controlled environmental conditions. Each value is the mean of 3 replicates and vertical bars are standard errors. Pay attention that no values are available for shoot Ψs in the case of salt stressed plants of TOG5482, TOG5885 and TOG5949 as a consequence of a lack of available material.

positive correlation along the Dim 1 and this part of the plot was characterized by K content, stomatal conductance (g_s), water content, instantaneous transpiration (E), net photosynthesis (A), height of plant, number of leaves, number of tillers, plant dry weight, root dry weight and shoot dry weight. Lines TOG5307, TOG5456, TOG5588, TOG5385 and CG17 belong to the salt-tolerant group. In contrast, the salt-sensitive species displayed a strong negative correlation along the Dim 1. Species TOG5885, TOG5672, TOG5949, TOG5390 and CG20 belong to salt-sensitive group. The "center-reaction" group contains the other lines which have a weak correlation with analyzed parameters by PCA.

DISCUSSION

The present work confirms that *O. glaberrima* displays

high variability in terms of salinity resistance at the seedling stage. Most accessions of *O. glaberrima* appear more salt sensitive than the moderately-resistant cv. I Kong Pao from *O. sativa*. In the absence of salt, numerous accessions of *O. glaberrima* displayed a high vegetative growth leading to a high total plant biomass. Such a property might be, at least partly, related to a high net photosynthesis (Figure 2). Since the mean Ci value was usually high in *O. glaberrima*, it could be postulated that such a high photosynthesis may be linked to a high level of gas exchange which is confirmed by the high values recorded for stomatal conductance (Figure 2).

Indeed a positive correlation between A and g_s values under control conditions was found. The fast vegetative growth of *O. glaberrima* at the seedling stage is frequently considered as an advantage in terms of weed competition (Sarla and Mallikarjuna Swamy, 2005; Nayar, 2010).

Table 2. Tolerance index (TI) of accessions from *Oryza glaberrima* estimated for shoot dry weight (TI_Shoot DW), roots dry weight (TI_Root DW), plant height (TI_plant height), number of tillers (TI_Tillers), number of leaves (TI_Leaves), net photosynthesis (TI_Net.photo) and root length (TI_Root.length). I Kong Pao is a moderately salt-resistant variety from *Oryza sativa*.

| Variety	TI_Shoot DW	TI_Root DW	TI_Plant height	TI_Tillers	TI_Leaves	TI_Net.photo	TI_Root.length
IKP (I KONG PAO)	**1.68**	**2.23**	**1.91**	**1.49**	**0.90**	**1.17**	**4.96**
CG17	0.95	1.66	1.15	1.00	0.79	0.34	4.81
CG20	0.71	1.33	0.54	0.35	0.26	0.16	4.85
TOG5293	0.73	0.94	0.83	0.50	0.28	0.39	5.16
TOG5307	**1.59**	2.09	1.30	0.78	0.58	**1.39**	3.19
TOG5385	0.76	1.63	0.78	0.85	0.51	0.42	4.03
TOG5390	0.62	1.20	0.65	0.31	0.20	0.19	6.76
TOG5420	0.86	1.34	1.10	0.84	0.50	0.37	4.11
TOG5440	1.10	0.77	1.03	1.00	0.50	0.31	2.24
TOG5442	0.89	1.19	1.02	0.87	0.48	0.92	2.80
TOG5456	0.83	1.21	1.19	0.67	0.43	0.69	4.65
TOG5479	0.80	1.20	1.27	0.98	0.62	0.37	5.86
TOG5482	0.66	1.69	1.01	0.58	0.40	0.33	4.81
TOG5500	0.94	1.23	0.84	0.52	0.39	0.48	3.57
TOG5566	0.80	1.16	1.21	0.51	0.36	**1.04**	4.23
TOG5588	1.05	1.29	0.98	0.82	0.52	0.75	3.77
TOG5641	0.64	1.15	0.97	0.58	0.26	0.41	3.22
TOG5666	0.82	0.74	1.11	0.69	0.49	0.76	4.33
TOG5672	0.86	1.20	1.16	0.78	0.33	0.45	2.87
TOG5681	0.73	1.35	0.86	0.56	0.20	0.33	3.26
TOG5685	0.58	1.11	0.87	0.58	0.29	0.59	4.55
TOG5775	**1.41**	**3.62**	1.03	0.86	0.65	0.90	5.11
TOG5885	0.60	1.19	1.01	0.40	0.33	0.24	4.39
TOG5949	0.41	0.61	1.09	0.47	0.19	0.07	5.85
TOG5969	0.67	0.90	0.88	0.78	0.51	0.52	3.88
TOG5979	0.60	1.33	0.91	0.61	0.38	0.36	4.43
Mean	**0.86**	**1.36**	**1.03**	**0.71**	**0.44**	**0.54**	**4.30**
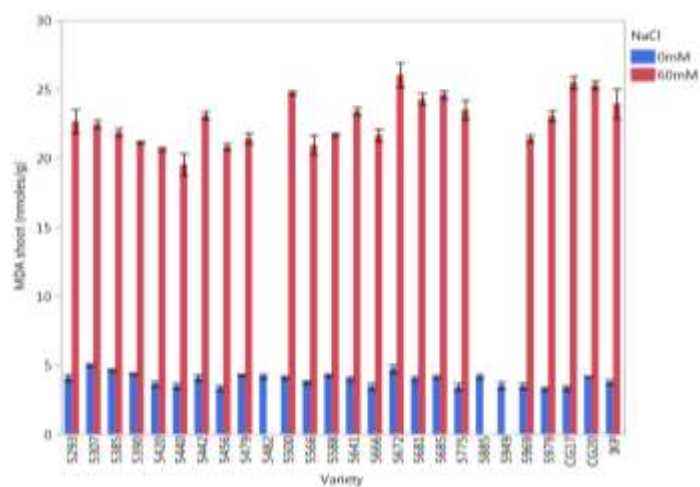
(A)
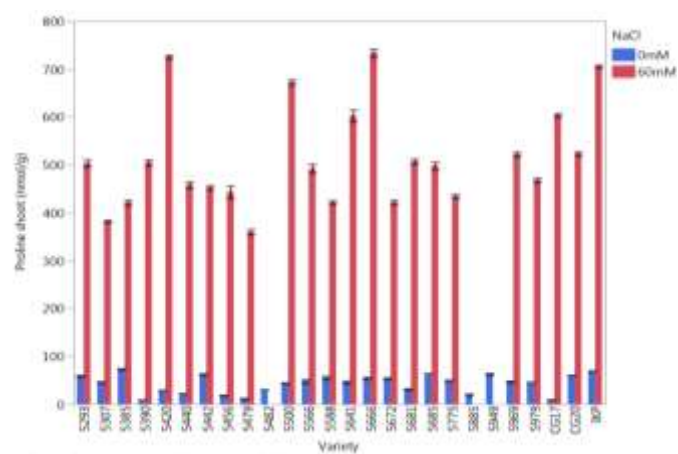
(B)

Figure 4. Shoot malondialdehyde (A) and shoot proline concentration (B) in 25 accessions of *Oryza glaberrima* and one moderately salt-tolerant cultivar of *Oryza sativa* (IKP). Seedlings were exposed for 2 weeks to 0 (control) or 60 mM NaCl (stress) in hydroponic culture under controlled environmental conditions. Each value is the mean of 3 replicates and vertical bars are standard errors. Pay attention that no values are available for salt stressed plants of TOG5482, TOG5885 and TOG5949 as a consequence of a lack of available material.

(A)
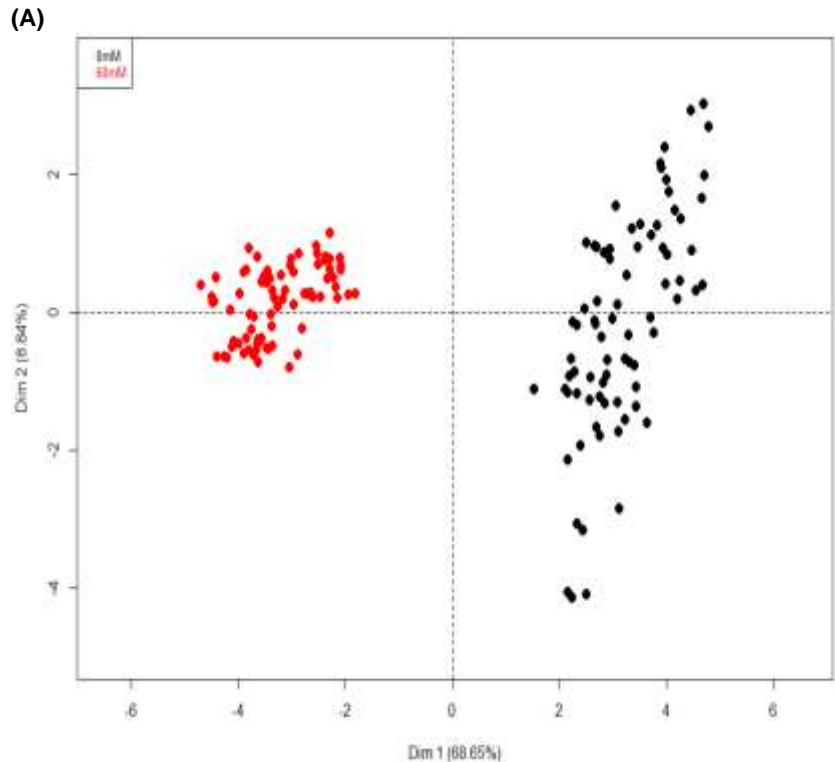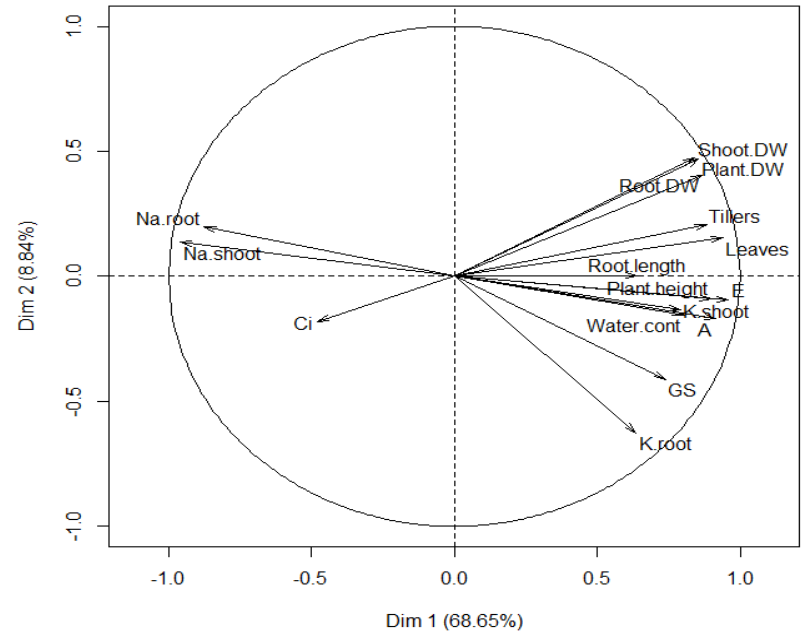
(B)

Figure 5. Principal Component Analysis (A and B) of growth and physiological parameters in the seedlings rice (25 accessions of *O. glaberrima* and one moderately salt-resistant cv of *O. sativa*) cultivated for 2 weeks in the presence of 0 (control) or 60 mM NaCl (stress). (A) Variable graph and (B) individual graph of PCA showing the control and salt-stressed seedlings groups. Only significant parameters were shown ($P < 0.05$) in (A). Plant.DW, plant dry weight; Shoot.DW, shoot dry weight; Root.DW, root dry weight; Water.cont, total water content; GS, stomatal conductance; K.shoot, shoot potassium concentration; K.root, root potassium concentration; Na.shoot, shoot Na concentration; Na.root, root Na concentration; *C*i, sub-stomatal cavity CO_2 concentration; *E*, instantaneous transpiration; A, net photosynthesis; Plant.height, height of plant; Leaves, number of leaves; Tillers, number of tillers; Root.length, root length.

(A)

(B)
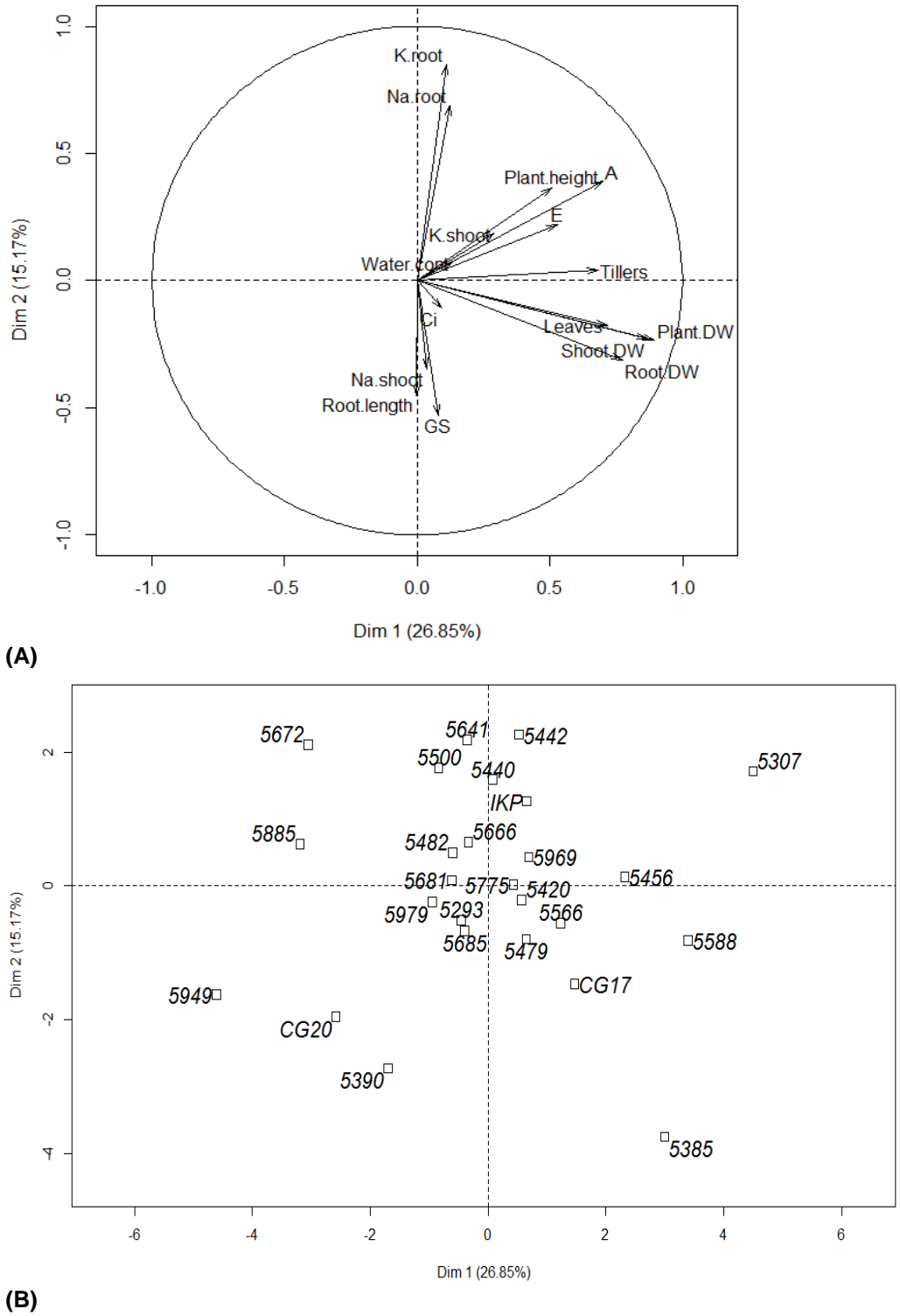
Figure 6. Principal Component Analysis (A and B) of growth and physiological parameters in salt-stressed seedlings of rice (25 accessions of *O. glaberrima* and one moderately salt-resistant cv of *O. sativa* (IKP)) cultivated for 2 weeks in 60 mM NaCl. (A) Variable graph and (B) individual graph of PCA showing the impact of salt stress in seedlings. Only significant parameters were shown ($P < 0.05$) in (A). Plant.DW, plant dry weight; Shoot.DW, shoot dry weight; Root.DW, root dry weight; Water.cont, total water content; GS, stomatal conductance; K.shoot, shoot K concentration; K.root, root K concentration; Na.shoot, shoot Na concentration; Na.root, root Na concentration; Ci, sub-stomatal cavity CO_2 concentration; E, instantaneous transpiration; A, net photosynthesis; Plant.height, height of plant; Leaves, number of leaves; Tillers, number of tillers; Root.length, root length.

Salt stress induces both an osmotic and an ionic constraint in stressed plants (Acosta-Motos et al., 2017; Munns, 2005). Salinity resistance is considered to rely on avoidance mechanisms, allowing the plant to limit Na and Cl absorption and accumulation, and tolerance mechanisms allowing the plant to maintain efficient metabolism despite toxic ion accumulation. Under current experimental conditions, the tested materials appeared to be able to efficiently manage with physiological drought since no obvious decrease was recorded for the leaf water content. In contrast, salt-treated plants accumulated high Na concentration, suggesting that the ionic constraint is the major problem for O. glaberrima. Although the osmotic component is frequently considered as the first component acting on salt-treated plants (Munns, 2005), it has been previously demonstrated that Na^+ may reach high toxic concentration in a short term basis in rice and could be toxic even before modification of the plant water status (Lefèvre et al., 2001). The high transpiration rate recorded in some accessions of O. glaberrima (Figure 2) should probably contribute to increase Na^+ concentration on a short term basis. Total Na^+ concentration was indeed higher in the shoots than in the roots: although roots are commonly acting as a barrier sequestering toxic ions and avoiding their accumulation in photosynthetic tissues, the obtained results suggest that this accumulation was not efficient in O. glaberrima which could be related to the fact that at the young seedling stage, endoderm is not completely differentiated in the young seedling rice plant (Yeo and Flowers, 1986; Zhu et al., 2001). Despite the lower accumulation of Na^+ in the root system, it is noteworthy that under experimental conditions, the mean sensitivity index was higher for root than for shoots (Table 1), suggesting that root metabolism could be highly sensitive to salinity in O. glaberrima.

Beside restriction of Na^+ absorption and translocation, tolerance of photosynthetic tissues to the accumulated toxic ions is an important component of salinity resistance in plants (Roshandel and Flowers, 2009). It implies that biochemical protecting compounds have to be synthesized and/or that compartmentation processes leading to Na^+ accumulation in apoplasm or vacuoles must be operating to limit the deleterious impact of toxic ion on cytoplasm where the major steps of cell metabolism occur. In the current study, a highly significant negative correlation was found between mean TI_{Na} and the overall plant sensitivity index (r = -0.88; P < 0.01). This observation confirms that in O. glaberrima, tolerance mechanisms to accumulated ions are of paramount importance for the overall plant performance. Because salinity resistance is a highly complex property, it poses serious challenge to plant breeders (Flowers and Flowers, 2005). The ability of O. glaberrima to display tolerance mechanisms may be a promising aspect for further breeding schemes which confirms the putative interest of the African rice for crop improvement after

inter-specific crosses with O. sativa (Adedze et al., 2016). Proline has often been regarded as an osmo-protecting compound positively involved in salinity resistance. Proline is thought to be involved in osmotic adjustment but it may also directly act to protect cellular structures and enzymes or scavenge reactive oxygen species (Mansour and Ali, 2017). It is noteworthy, however, that in O. glaberrima, the most salt-resistant accessions such as TOG5307, TOG5588 and TOG5456 accumulated lower proline concentrations than salt-sensitive one. This suggests that proline does not assume key functions in salinity resistance in this species or that the signaling pathway leading to proline over-synthesis is still not triggered in these salt-resistant accessions. A similar situation was reported in O. sativa where salt-resistant cultivars accumulated lower proline concentrations than salt-sensitive ones (Lutts et al., 1996). According to Lutts et al. (1999), proline accumulation in this species might be due to over-accumulation of putatively toxic ammonium which induces over-synthesis of glutamine through activation of the GS/GOGAT cycle. Independently of proline synthesis, some accessions of O. glaberrima exhibited a fascinating ability to perform osmotic adjustment at the shoot level (Figure 3) and the identification of compounds involved in this process could be extremely useful for further improvement of salinity resistance in rice.

Principal component analysis discriminate 3 groups among the tested accessions: i) a salt-resistant group which comprises TOG5307, TOG5456, TOG5588, TOG5385 and CG17; ii) a salt sensitive group which includes TOG5949, TOG5390, CG20, TOG5885 and TOG5672 and; iii) all other accessions were classified as « medium range » for salinity resistance. A correlative analysis was performed among tested parameters in salt-stressed material within each group. Proline and MDA, however, were not included since data are not available for some accessions. Figure 7 demonstrate that the correlation profile is clearly different in each group for stressed plants. While A was negatively correlated with gs and Ci in the salt-sensitive group, this was not anymore the case in the salt-resistant one. Similarly, in the salt-sensitive group, the shoot DW was positively correlated with the root DW but this correlation disappeared in the salt-resistant accession, suggesting that root and shoot behavior were not so directly linked in this material. This hypothesis is supported by the fact that under experimental conditions, salt-sensitivity index was frequently higher for roots than for shoots (Table 1). These observations, however, are based on biomass production but maintenance of metabolic processes in stressed conditions is not always devoted to growing processes. Root metabolism may be involved in root-to-shoot signaling, mainly in relation to hormonal translocation and play a key role in salinity resistance (Ghanem et al., 2011). While the salt-resistant and the salt-sensitive group differed for correlation profile (Figure
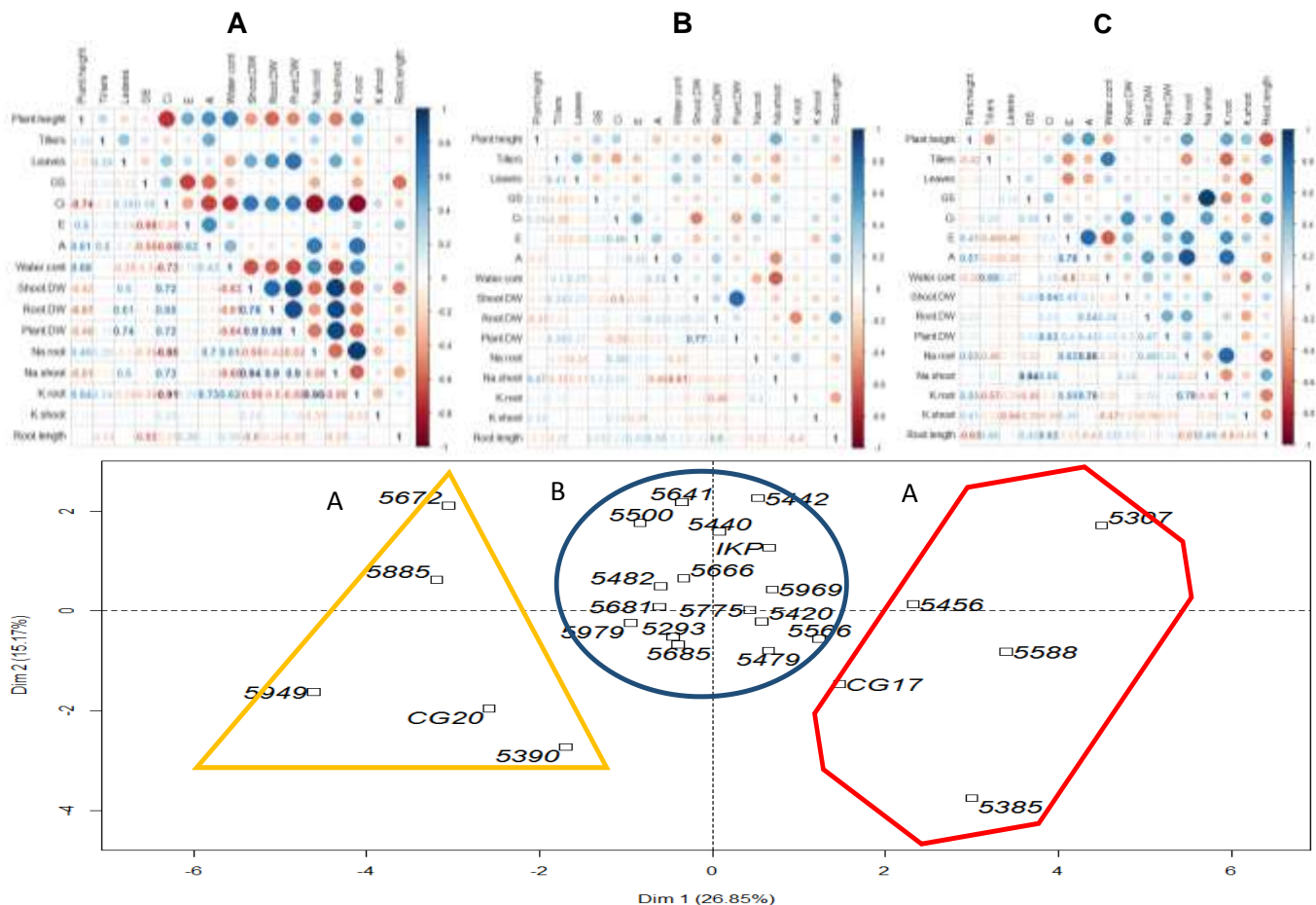
Figure 7. Correlative analysis performed separately for three distinct groups of accessions exhibiting a salt-sensitive type (yellow triangle), medium-range reaction (blue circle) and resistant type (red hexagone). For each group, correlations among morpho-physiological parameters were estimated for stressed plants, a blue circle indicating a positive correlation and a brown circle indicating a negative one. The diameter of the circle and the intensity of the colors are directly relevant of the importance and the nature of correlation.

7), the «medium-range» cultivars exhibited an intermediate behavior characterized by a rather poor level of correlations among recorded parameters.

It is concluded from the present study that *O. glaberrima* exhibit some variability for salinity resistance and that some accessions, such as TOG5307, exhibits interesting properties such as a high capacity of osmotic adjustment, maintenance of photosynthesis and high level of tolerance to accumulated Na^+ ions.

CONFLICT OF INTERESTS

There are no conflicts of interest between the authors.

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