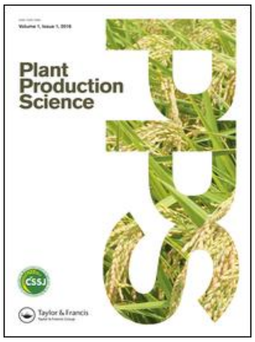

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Abstract: Salinity tolerance has been extensively studied in *Oryza sativa*, but little is known about the salt tolerance levels in *Oryza glaberrima* and the interspecific progenies including New Rice for Africa (NERICA). In this study, the salinity tolerance of the three cultivated rice species, *O. glaberrima* (54 genotypes), the interspecific progenies (21) including NERICA (7) and *O. sativa* (41) mainly grown in West Africa were examined comparatively. At 10 days after sowing (DAS) 80 mM NaCl was added to the culture solution, and the plants were grown for 10 more d. The ratio of shoot biomass in the 80 mM NaCl solution to that in the control was significantly higher in the interspecific progenies than in the other two species, and the relative root biomass was significantly lower in *O. glaberrima* than in the others. The vegetative growth of six genotypes including the salt tolerant Pokkali, and NERICA4 and its parents were evaluated further in pot experiments irrigated with 80 mM NaCl solution from 22 to 52 d after sowing. At 30 d of the salt stress, CG14 and Mala noir IV (*O. glaberrima*) were killed by salt, while WAB56-104 and NERICA4 survived; Pokkali maintained the highest relative shoot biomass growth at all sampling times of 10 d intervals. These results indicate that *O. glaberrima* is relatively weaker to NaCl salinity, while the interspecific progenies are fairly tolerant during the seedling stage, and that the relatively high salt stress tolerance of NERICA4 is derived from the *O. sativa* parent, WAB56-104.

Key words: African rice, Interspecific progenies, New Rice for Africa, Salinity tolerance, Screening.

Oryza glaberrima Steud., African rice, is cultivated on a small scale along the Niger River in West Africa (Chang, 1976; Sano, 1983), where it has been domesticated for more than 3,500 years (Linares, 2002; Semon et al., 2005). Some farmers in the region prefer this indigenous species for its unique characters such as weed competitiveness, low input responsiveness, drought tolerance and resistance to African gall midge and yellow mottled virus (Jones et al., 1997; Jones, 2004; Sarla and Mallikarjuna Swamy, 2005). However, because of its high grain shattering and lodging susceptibility, *O. glaberrima* yields are relatively low, and most varieties have been replaced by high yielding, non-lodging *Oryza sativa* L. (Dingkuhn et al., 1998; Dingkuhn et al., 1999b), though it has poor tolerance to common pests in Africa (Dingkuhn et al., 1999a; Semagn et al., 2007). Recent crosses between *O. glaberrima* and *O. sativa* species by Africa Rice Center have resulted in the development of interspecific progenies called NERICA (New Rice for Africa) which possess the best traits of both parents (Jones et al., 1997). NERICA was developed to increase the rice yield and income of rural households in sub-Saharan Africa, so its cultivation has been spreading rapidly across the continent. This interspecific species is now cultivated in 13 countries in West Africa, Central Africa, and East Africa (Rodenburg et al., 2006).

In arid and semiarid regions, salt often accumulates within surface soil layers due to less precipitation for leaching. Salinity imposes ionic and osmotic stresses on plants thereby reducing the plant photosynthetic rate, growth and yield. Decreased photosynthetic rate result from the closure of stomata, that is, induced by osmotic stress, or from salt-induced damage to the photosynthetic apparatus (Moradi and Ismail, 2007).

Rice is an important grain crop worldwide but is sensitive to salinity. However, there exist genotypic differences among rice genotypes in the response to salinity (Zeng, 2005). Seedling and flowering stages are most sensitive to salinity stress (Lutts et al., 1996; Gregorio et al., 1997).

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Abbreviation: ANOVA, analysis of variance; DAS, days after sowing; *i*WUE, instantaneous water use efficiency; NERICA, New Rice for Africa.

Table 1. Origins and ecotypes of 116 rice genotypes evaluated for salt tolerance in Exp. 1.

Genotype / cultivar	Sp*	Origin	Ecotype[#]	Genotype / cultivar	Sp	Origin	Ecotype
Aawba	G	Guinea	U	NERICA 6	I	Cote d'Ivoire	U
Alfassa noir	G	Mali	D	NERICA 7	I	Cote d'Ivoire	U
Bagua-kandié	G	Mali	D	WAB1159-2-12-11-2-1	I	Cote d'Ivoire	L
Barkaneye bero	G	Mali	D	WAB1159-2-12-11-2-10	I	Cote d'Ivoire	L
Barkaneye blanc	G	Mali	D	WAB1159-2-12-11-2-4	I	Cote d'Ivoire	L
Barkaneye noir	G	Mali	D	WAB1159-2-12-11-5-1	I	Cote d'Ivoire	L
Barkaneye tetera blanc	G	Mali	D	WAB1159-2-12-11-5-3	I	Cote d'Ivoire	L
Barkaneye tetera noir	G	Mali	D	WAB1159-2-12-11-6-8	I	Cote d'Ivoire	L
Barkaneye Thirca blanc	G	Mali	D	WAB1159-4-10-15-1-2	I	Cote d'Ivoire	L
Bodewel nènè	G	Mali	D	WAB1159-4-10-15-1-3	I	Cote d'Ivoire	L
Bouyadian	G	Mali	D	WAB1159-4-10-15-1-5	I	Cote d'Ivoire	L
Bringua hignè noir	G	Mali	D	WAS122-IDSA-10-WAS-1-1-FKR-1	I		
C0440	G	Guinea	D				
CG14	G	Senegal	RL/U	WAS122-IDSA-10-WAS-3	I	Senegal	L
Daye kossa blanc	G	Mali	D	WAS127-B-5-2	I	Senegal	L
Dembou bourawana noir	G	Mali	D	WAS127-B-5-B-1	I	Senegal	L
Djéifata	G	Mali	D	WAS161-B-6-B-3-1B	I	Senegal	L
Djéifata blanc	G	Mali	D	ARC 10352	S	India	
Gbagaye	G	Guinea	RL	AZUCENA	S	Philippines	U
Guayaba	G	Mali	D	BARAN BORO	S	Bangladesh	
Haïra thireye	G	Mali	D	BENGIZA	S	Madagascar	U
Hamala blanc	G	Mali	D	BLACK GORA	S	India	
Kaïna koreye kossa No 1	G	Mali	D	Bokra	S	India	L
Koïréao	G	Mali	D	CK21	S	Guinea	L
Kossa filo blanc	G	Mali	D	CK40	S	Guinea	U
Kossa filo noir	G	Mali	D	CK43	S	Guinea	
Kossa koreye	G	Mali	D	CK72	S	Guinea	U
Kossa No 3	G	Mali	D	CK803	S	Guinea	IL
Laminibougou	G	Mali	D	Ei Boutica	S	Mali	D
Mala Noir III	G	Niger	D	IR24	S	Philippines	
Mala Noir IV	G	Niger	D	IR64	S	Philippines	IL
Mala Noir V	G	Niger	D	IRAT 144	S	Ghana	
Maloba	G	Mali	D	Jagli Boro	S	Bangladesh	
Mokori	G	Mali	D	Ken Chiao Ju Hsiao Li	S	China	
Piekono	G	Mali	D	Nona Bokra	S	India	
Pièpi	G	Mali	D	PEH-KUH	S	Taiwan	
Saligbeli	G	Guinea	RL	Pokkali	S	India	
Salikutaforé	G	Guinea	RL	Rathal	S		U
Salmahali koreye	G	Mali	D	Shai-Kuh	S	China	
Sarbaria blanc	G	Mali	D	Short Grain	S	Thailand	U
Saresare	G	Mali	D	SR26B	S	India	
Saron-saron	G	Mali	D	Ta Hung Ku	S	China	
Simobaleo	G	Mali	D	Tanda	S	Niger	L
Simon Blanc	G	Mali	D	Tatsumimochi	S	Japan	L
Sjiefata	G	Mali	L	Trembese	S	Indonesia	RL
Tataro	G	Mali	RL	Tumo-Tumo	S	Malaysia	U
Thirma	G	Mali	D	WAB56-104	S	Cote d'Ivoire	U
Tombobökéri	G	Guinea	RL	WAS173-B-2-1	S	Senegal	U
Tombobökéri II	G	Guinea	RL	WAS197-B-4-1	S	Senegal	U
W0492	G	Guinea	RL	WAS20-B-B-5-10-1	S		
Walladé	G	Mali	RL	WAS33-B-B-15-1-4-5	S	Senegal	U
Yele	G	Mali	D	WAS47-B-B-194-4-2	S	Senegal	U
Yélé 1 A	G	Mali	D	WAS57-B-B-17-3-3-6	S	Senegal	U
Youssouwel	G	Mali	D	WAS62-B-B-17-1-1-1	S	Senegal	U
NERICA 1	I	Cote d'Ivoir	U	WAS62-B-B-17-1-1-3	S	Senegal	U
NERICA 2	I	Cote d'Ivoir	U	Yamabiko	S	Japan	L
NERICA 3	I	Cote d'Ivoir	U	Yamahikari	S	Japan	L
NERICA 4	I	Cote d'Ivoir	U	Yamahoushi	S	Japan	L
NERICA 5	I	Cote d'Ivoir	U				

* Sp, Species; G, *O. glaberrima*; I, Interspecific progenies; S, *O. sativa*; [#]Ecotype: D, Deepwater; L, Lowland; RL / U, Rainfed lowland/upland; U, Upland; IL, Irrigated lowland.

Under saline conditions, seedling growth is inhibited, resulting in reduced biomass production (Aslam et al., 1993) or even in complete death. Although salinity tolerance has been extensively studied in *O. sativa* (Yeo et al., 1990; Shannon et al., 1998), scientific literature concerning the potential salt tolerance of *O. glaberrima* and NERICA is not reported so far.

The objectives of this study were therefore to elucidate the levels of salinity tolerance of *O. glaberrima* and NERICA (the interspecific progenies) in comparison with *O. sativa* based on biomass production under salt condition at the seedling stage, with special emphasis placed on the response of NERICA and its parent cultivars.

Materials and Methods

Salinity tolerance of the rice species was evaluated three different experiments conducted in 2007 at Nagoya University, Japan. The first experiment (Exp. 1) was designed to assess biomass production in a NaCl-salinized solution at the seedling stage in three rice species. A total of 116 genotypes of *O. glaberrima*, interspecific progenies and *O. sativa* were used and the majority were those grown in West African region, especially in Guinea, although some salt tolerant cultivars from Asia were included (Table 1). The second experiment (Exp. 2), was also conducted in a culture solution,and was designed to assess the growth of NERICA4, as the representative cultivar of the interspecific progeny, and the parent cultivars of all the NERICA lines tested in this paper, CG14 (*O. glaberrima* parent) and WAB56-104 (*O. sativa* parent), under different salinity conditions. These genotypes plus three another genotypes were evaluated further in the third experiment (Exp. 3) conducted in pot soil to assess their responses to salinity in terms of biomass production and gas exchange at the vegetative growth stage. The plants were grown in a growth chamber for Exp. 1 and Exp. 2 and greenhouse for Exp. 3.

1. Three species comparison (Exp. 1)

In Exp. 1, 54 genotypes of *O. glaberrima*, 21 genotypes of interspecific progenies and 41 genotypes of *O. sativa* were grown in half-strength Kimura B solution supplemented with 0 (control) or 80 mM NaCl in three replicate experiments. Seeds were soaked in water for 24 hr, planted in Petri dishes and incubated at 30ºC for 48 hr in darkness to enhance germination and the development of the radicle and plumule. Prior to soaking, the seeds of *O. glaberrima* genotypes were kept in an oven at 49ºC for five days to break seed dormancy, and then peeled to facilitate seed imbibition. Pre-germinated seeds were sown by inserting the radicle in each hole of a nylon mesh floated on tap water culture in 12 L (35 cm length×25 cm width×12 cm height) plastic trays arranged in pairs, one for NaCl treatment and the other as a control. Seven plants each of 12 genotypes were grown in each tray. At 3 d after sowing (DAS), when the seedlings had fully established, the water was replaced with the solution culture. Salt stress was imposed at 10 DAS by adding 80 mM NaCl to the culture solution. The culture solution was renewed every three days and the pH was adjusted to 5.5 by adding hydrochloric acid or sodium hydroxide. The experiment was conducted in the growth chamber with air temperature set at 30/25°C day/night, relative humidity between 50 and 75%, photoperiod of 12 hr, and average photosynthetically active radiation of 430 μmol m^{-2} s^{-1}. At 20 DAS (10 d after stress imposition), the plants were harvested by sampling three plants from each replicate experiment. They were separated into shoots and roots, and each were oven-dried at 80ºC for 72 hr to determine the shoot and root dry weights. The root dry weight of *O. Sativa* was measured only for representative 12 cultivars because the measurement was time-consuming. The 12 cultivars were regarded as the representatives from the results of our former study on tolerance to soil compaction by Nakamura et al. (2006).

2. Comparison between NERICA and parents (Exp. 2)

NERICA4 and its parents CG14 and WAB56-104 were grown under different NaCl at different concentrations to compare salt tolerance between the progeny and parents. In this experiment, NERICA4 was selected to represent the NERICA group because it measured the highest relative shoot biomass among the seven NERICA genotypes evaluated in Exp.1. The genotypes were evaluated using the same nutrient solution culture as for exp. 1 containing NaCl at five concentrations (0, 25, 50, 75, or 100 mM), to compare in detail the growth response of the three genotypes. Seedlings were cultured in 6 L (25 cm length×19 cm width×15 cm height) plastic trays. As in Exp. 1, the seedlings were grown in NaCl-free culture solution for 10 d and then in the solution with NaCl under normal culture and the last half under the different NaCl concentrations. The growth conditions and data collection procedures were similar to those for Exp. 1.

3. Responses to salt stress in the the vegetative stage (Exp. 3)

Growth and physiological responses to NaCl of six rice genotypes were assessed du the vegetative stage from 32 to 52 DAS in the pot experiment under greenhouse conditions. The genotypes included CG14 and Mala noir IV (*O. glaberrima*), NERICA4 and WAS161-B-6-B-3-1B (interspecific progenies), and WAB56-104 and Pokkali (*O. sativa*). The two interspecific progenies had the highest relative shoot biomass in their group in Exp. 1, therefore we selected them for evaluation along with their parents cultivars CG14 and WAB56-104. Mala noir IV is one of the famous *O. glaberrima* genotypes cultivated in inundated areas of Niger River in Niger; while Pokkali was used as the

Table 2. Relative shoot dry weight (DW, Ratio of 80 to 0 mM NaCl treatment) and root dry weight in Exp. 1. Values are the means of three replicate experiments. As for the root dry weight measurement of *O. sativa*, only representative 12 cultivars are measured.

Genotype / cultivar	Sp*	Relative Shoot DW (80 mM/0 mM)	Relative Root DW (80 mM/0 mM)	Genotype / cultivar	Sp	Relative Shoot DW (80 mM/0 mM)	Relative Root DW (80 mM/0 mM)
Nona Bokra	S	0.980	0.536	IR24	S	0.756	0.502
WAS161-B-6-B-3-1B	I	0.947	0.625	WAS47-B-B-194-4-2	S	0.755	–
Saligbeli	G	0.936	0.377	Sarbaria blanc	G	0.754	0.422
Barkaneye bero	G	0.929	0.495	Ken Chiao Ju Hsiao Li	S	0.750	–
Bringua hignè noir	G	0.908	0.510	Salikutaforé	G	0.742	0.311
Barkaneye tetera noir	G	0.904	0.414	Djéifata blanc	G	0.740	0.342
Tataro	G	0.903	0.472	Jagli Boro	S	0.739	–
CK803	S	0.902	–	Ei Boutica	S	0.734	–
NERICA 4	I	0.896	0.492	Yamahoushi	S	0.727	0.375
NERICA 3	I	0.895	0.572	Kossa filo blanc	G	0.721	0.397
WAB1159-4-10-15-1-3	I	0.886	0.389	Piekono	G	0.721	0.363
WAS122-IDSA-10-WAS-1-1-FKR-1	I	0.881	0.614	IRAT 144	S	0.720	–
				Rathal	S	0.718	–
Thirma	G	0.880	0.417	Mala Noir	G	0.712	0.311
WAS20-B-B-5-10-1	S	0.873	–	WAB1159-2-12-11-2-4	I	0.710	0.394
WAB1159-2-12-11-5-3	I	0.866	0.511	NERICA 2	I	0.704	0.446
WAB1159-4-10-15-1-5	I	0.861	0.410	Barkaneye tetera blanc	G	0.704	0.371
Gbagaye	G	0.860	0.340	Mala Noir IV	G	0.703	0.367
WAS122-IDSA-10-WAS-3	I	0.857	0.551	C0440	G	0.702	0.291
PEH-KUH	S	0.853	–	Yamahikari	S	0.698	0.410
Ta Hung Ku	S	0.843	–	Mala Noir III	G	0.697	0.319
Walladé	G	0.842	0.454	WAS62-B-B-17-1-1-3	S	0.688	–
Tanda	S	0.837	–	Bagua-kandié	G	0.687	0.440
WAB1159-2-12-11-5-1	I	0.833	0.399	WAS57-B-B-17-3-3-6	S	0.684	–
Hamala blanc	G	0.829	0.482	Saron-saron	G	0.683	0.278
SR26B	S	0.827	0.519	Kossa No 3	G	0.680	0.446
WAB1159-2-12-11-6-8	I	0.822	0.520	NERICA 7	I	0.677	0.569
WAS127-B-5-2	I	0.821	0.398	Aawba	G	0.674	0.278
Bokra	S	0.818	0.504	Yélé 1A	G	0.673	0.377
Pokkali	S	0.818	0.659	Yele	G	0.669	0.314
Saresare	G	0.817	0.470	Bouyadian	G	0.663	0.339
NERICA 1	I	0.812	0.436	Barkaneye Thirca blanc	G	0.654	0.401
Djéifata	G	0.810	0.386	Trembese	S	0.653	–
Simon Blanc	G	0.809	0.423	Tombobökéri	G	0.653	0.289
WAS173-B-2-1	S	0.806	–	AZUCENA	S	0.653	0.378
WAS127-B-5-B-1	I	0.803	0.376	Salmahali koreye	G	0.651	0.290
WAB56-104	S	0.802	–	Dembou bourawana noir	G	0.647	0.341
Guayaba	G	0.801	0.391	Tatsumimochi	S	0.643	0.302
Koïréao	G	0.797	0.294	Laminibougou	G	0.643	0.301
WAB1159-4-10-15-1-2	I	0.796	0.393	Short Grain	S	0.625	–
CK72	S	0.793	–	CG14	G	0.612	0.331
Sjiefata	G	0.790	0.354	Tombobökéri II	G	0.608	0.250
WAB1159-2-12-11-2-10	I	0.789	0.439	WAB1159-2-12-11-2-1	I	0.606	0.348
CK40	S	0.787	–	NERICA 6	I	0.599	0.401
Yamabiko	S	0.782	0.445	W0492	G	0.596	0.284
Maloba	G	0.781	0.344	BARAN BORO	S	0.587	0.393
WAS197-B-4-1	S	0.780	–	CK21	S	0.578	–
NERICA 5	I	0.780	0.552	Daye kossa blanc	G	0.563	0.440
CK43	S	0.779	–	Bodewel nènè	G	0.559	0.269
Haïra thireye	G	0.777	0.510	Kaïna koreye kossa No 1	G	0.557	0.258
WAS62-B-B-17-1-1-1	S	0.776	–	IR64	S	0.547	–
Simobaleo	G	0.776	0.305	Kossa filo noir	G	0.533	0.275
Barkaneye noir	G	0.775	0.503	BENGIZA	S	0.531	–
Mokori	G	0.770	0.456	Alfassa noir	G	0.510	0.347
WAS33-B-B-15-1-4-5	S	0.769	–	BLACK GORA	S	0.396	–
Shai-Kuh	S	0.768	–	Tumo-Tumo	S	0.359	–
Pièpi	G	0.766	0.382	Barkaneye blanc	G	0.351	0.258
Youssouwel	G	0.760	0.356	Kossa koreye	G	0.281	0.160

* Sp, Species: G, *O. glaberrima*; I, Interspecific progenies; S, *O. sativa*. –, Missing data.

Table 3. Comparison among *O. glaberrima*, interspecific progenies and *O. sativa* for shoot and root growth as affected by 80 mM NaCl stress for 10 d (Exp. 1).

Species	Shoot dry weight ($g\ plant^{-1}$)		Relative shoot dry weight (80 mM / 0 mM)	Root dry weight ($g\ plant^{-1}$)		Relative root dry weight (80 mM / 0 mM)
	0 mM NaCl	80 mM NaCl		0 mM NaCl	80 mM NaCl	
O. glaberrima (n=54)	0.1360	0.097	0.715 b	0.0652	0.0245	0.376 b
Interspecifics (n=21)	0.1260	0.101	0.804 a	0.0577	0.0280	0.485 a
O. sativa (n=41#)	0.1430	0.106	0.738 b	0.0710	0.0337	0.474 a

Values are the means of three replicate experiments. Means followed by the same letters within column are not significantly different ($P<0.05$) by Tukey's multiple range test. #As for the root dry weight measurement of *O. sativa*, only representative 12 cultivars are measured.

salt tolerant check cultivar.

Plants were grown in pots 19.5 cm in height and 16.0 cm in diameter, each filled with 4 kg of soil. The soil was classified as sandy loam with 6.8% clay, 20.9% silt and 72.3% sand, and had a neutral pH value of 7.02. The experiment was a factorial design with 108 pots, 2 salt levels × 3 stress durations × 6 genotypes × 3 replicates. Pre-germinated seeds were sown in moist soil in pots on 1 July 2007. Six seeds of each genotype were sown in three hills (with two seeds per hill) per pot and seedlings were thinned at 7 DAS to leave one plant per hill (three plants per pot). Salt stress was given from 22 DAS, and the water levels in the pots were maintained at approximately 5 cm above the soil surface until the plants were harvested. Before sowing, 83, 111 and 97 mg kg^{-1} soil of N, P and K, respectively, were applied mixed with the soil in each pot. The same amount of fertilizer was applied as topdressing at 22 DAS. These low rates were considered safe for the *O. glaberrima* genotypes which, in other earlier experiments and seed multiplication pots, appeared sensitive to fertilizer burn at the seedling stage. The average maximum and minimum temperatures in the greenhouse during plant growth were 37°C and 25°C, respectively.

Leaf photosynthetic rates and transpiration rates were measured with a portable photosynthesis system (LI-6400, Li-Cor Biosciences, Lincoln, NE, USA). The measurements were taken between 0900 and 1200 on the youngest, fully expanded leaf, measuring three plants per replicate. Data were collected at 20 d after stress initiation and, immediately after the collection, shoot samples were harvested for dry matter determination. The harvested samples were oven-dried at 80°C for 72 hr. Subsequently, the shoot dry weights were measured. Instantaneous water use efficiency (*i*WUE) was calculated as the ratio of the instantaneous rate of CO_2 assimilation to transpiration at the stomata (Condon et al., 2002).

4. Statistical analysis

Tukey's multiple range test was used for the comparisons of the growth parameters measured among the treatments in Exp. 1. Two or Three-way analyses of variance (ANOVA) were performed for Exps. 2 , 3.

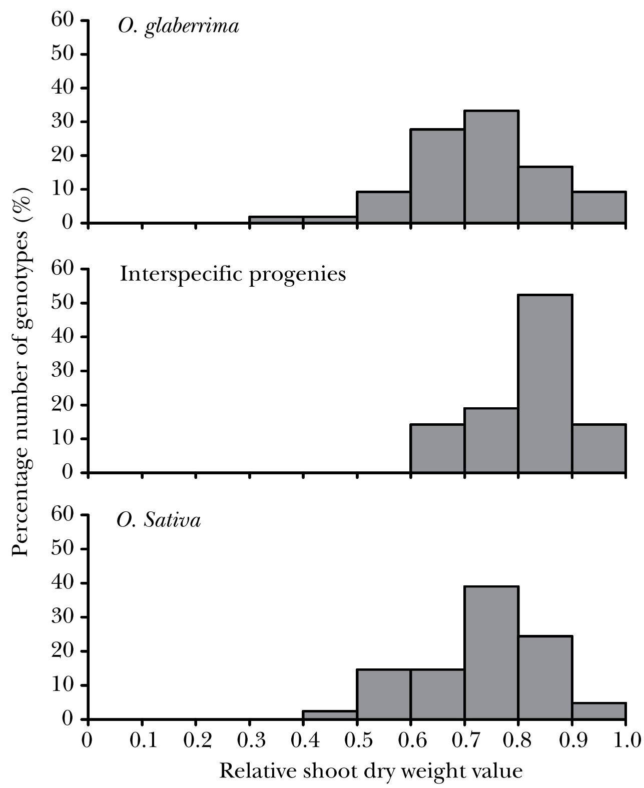

Fig. 1. Distribution of the relative shoot dry weight value in the three rice species. The relative shoot dry weight value is the ratio of 80 mM to 0 mM NaCl treatment.

Results

1. Three species comparison (Exp. 1)

Table 1 shows the origin and the ecotypes of 116 rice genotypes evaluated in Exp. 1. The relative shoot and root dry weights of each genotype were listed in Table 2. The range of the relative shoot dry weight were 0.30–0.94 in *O. glaberrima*, 0.60–0.95 in the interspecific progenies, and 0.40–0.98, in *O. sativa* (Table 2). The shoot and root growth of the three rice species as affected by NaCl stress are summarised in Table 3. The relative shoot dry weight of the interspecific progenies was significantly higher than that of *O. glaberrima* and *O. sativa* species. However, the relative root dry weight of the interspecific progenies was

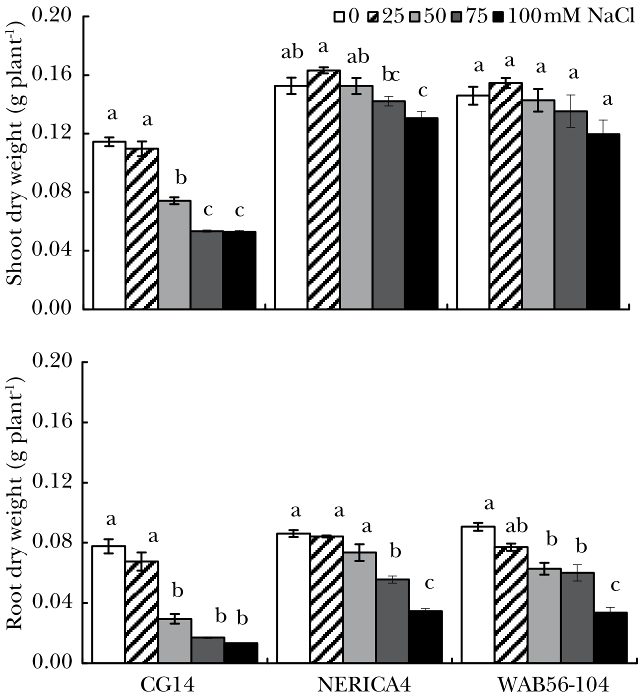

Fig. 2. Shoot (upper) and root (lower) growth of NERICA4 and parents as affected by increasing salt stress (Exp. 2). The same letters within species are not significantly different at the 5% level by Tukey test. Values are means of three replicate experiments ± SE, with four sub-samples per experiment.

Table 4. Shoot dry weight of six rice genotypes as affected by 80 mM NaCl stress for 10, 20 or 30 d (Exp. 3).

Genotype	Stress days	Shoot dw (g pot^{-1}) 0 mM	Shoot dw (g pot^{-1}) 80 mM	Ratio# 80 mM / 0 mM
CG14	10	9.3	2.1	0.23
	20	19.5	2.7	0.14
	30	25.8	1.8	0.07
Mala noir IV	10	9.8	3.7	0.38
	20	22.0	4.4	0.20
	30	30.7	2.5	0.08
NERICA4	10	8.0	2.7	0.34
	20	18.5	3.3	0.18
	30	31.5	4.4	0.14
WAS161-B-6-B-3-1B	10	9.5	2.7	0.28
	20	21.3	4.5	0.21
	30	33.8	4.1	0.12
WAB56-104	10	5.7	1.8	0.31
	20	17.0	3.7	0.22
	30	26.4	3.2	0.12
Pokkali	10	8.8	3.3	0.37
	20	23.1	5.1	0.22
	30	30.3	6.1	0.20
3-way ANOVA				
Salt (S)			***	
Duration (D)			***	
Genotype (G)			***	
S×D			***	
S×G			**	
S×D×G			*	

Shoot dry weight values are the means of 3 replications. # Ratio of 80 mM to 0 mM NaCl treatment. ***, **, * significant at $P<0.001$, <0.01 and <0.05, respectively.

similar to that of *O. sativa*, while that of *O. glaberrima* was the lowest. These results showed that the interspecific progenies maintained higher relative growth rates under the salt stress condition as compared with *O. sativa* and *O. glaberrima*. In fact, *O. glaberrima* had the lowest relative shoot and root biomass.

The distribution of the relative shoot dry weight value in the three rice species evaluated in Exp. 1 is presented in Fig. 1. The data indicated a high variation in the relative shoot dry weight values especially in *O. glaberrima*, and to some extent in *O. sativa*. The interspecific progenies, however, exhibited the least variation, and virtually clustered on the right, indicating a higher degree of tolerance to the NaCl salinity.

2. Comparison between NERICA and parents (Exp. 2)

Fig. 2 shows the shoot and root growth of NERICA4 and its parents CG14 (male) and WAB56-104 (female) in the solution with NaCl at various concentrations. Based on the analysis of variance, the effects of salinity and genotype, and their interaction were highly significant for both the shoot and root growths. At each NaCl concentration, the shoot dry weights of NERICA4 and WAB56-104 were similar to and significantly higher than that of CG14. No statistically significant reduction in the shoot dry weight of WAB56-104 was observed by Turkey's multiple range test even at the highest salt concentration of 100 mM, although there was a tendency of reduction. At the lowest concentration of 25 mM, the shoot dry weights of NERICA4 and WAB56-104 were even higher than that under the respective controls (0 mM). To the contrary, CG14 showed progressive reduction in the shoot dry weight with the increase in NaCl concentration, except at 70 and 100 mM concentrations where the growth was poor and nearly constant. Under the highest NaCl concentration, the shoot growth in CG14, WAB56-104 and NERICA4 was reduced to 47, 82 and 85% of the control, respectively.

The root dry weight generally showed the same trend as that of the shoot dry weight (Fig. 2), decreasing in all the genotypes with increases in the NaCl concentrations, except in NERICA4 at 25 mM concentration. The growth reduction by the salt stress in the root was much higher than that in the shoot. Overall, these results showed that the salt tolerance of NERICA4 was similar to that of WAB56-104, and higher than that of CG14.

3. Salt stress at the vegetative stage (Exp. 3)

Table 4 shows the shoot dry weight of the six rice

Table 5. Photosynthetic rate (Pr) (μmol m^{-2} s^{-1}), transpiration rate (Tr) (mmol m^{-2} s^{-1}) and instantaneous water use efficiency (*i*WUE) (μmol/mmol) of six rice genotypes as affected by 80 mM NaCl stress for 20 d (Exp. 3).

Genotype	Pr			Tr			*i*WUE		
	0 mM	80 mM	Ratio#	0 mM	80 mM	Ratio	0 mM	80 mM	Ratio
CG14	22.5	8.6	0.38	9.8	2.7	0.3	2.3	3.1	1.37
Mala noir IV	23.8	8.1	0.34	10.9	2.2	0.2	2.2	3.1	1.43
NERICA4	29.5	13.6	0.46	14.0	3.9	0.3	2.1	3.5	1.66
WAS161-B-6-B-3-1B	24.4	13.4	0.55	11.10	3.69	0.33	2.21	3.67	1.66
WAB56-104	30.9	16.7	0.54	14.2	4.9	0.3	2.2	3.4	1.56
Pokkali	30.0	15.9	0.53	10.4	3.3	0.3	2.8	4.7	1.66
2-way ANOVA									
Salt (S)		***			***			***	
Genotype (G)		**			***			ns	
S×G		ns			**			ns	

Ratio of 80 mM to 0 mM NaCl treatment. ***, ** significant at $P<0.001$ and <0.01, respectively. ns, not significant.

genotypes grown under 80 mM NaCl stress for 10, 20 or 30 d. Based on the analysis of variance, the overall effects of salinity, salinity stress duration, genotype and their interactions were significant on the shoot dry weight. At 10 d after exposure, the relative shoot dry weight was the highest in Mala noir IV (38%) and Pokkali (37%), and lowest in CG14 (23%). WAB56-104 and NERICA4 exhibited an intermediate performance. At 20 d, the relative shoot dry weight was the lowest in CG14 (14%), and was between 18 and 22% in the other genotypes. At 30 d, at the end of the experiment, CG14 and Mala noir IV, were severely affected and eventually killed after being exposed to the longest salt stress duration. Pokkali, at this stage, had the highest relative shoot dry weight (20%), almost three times higher than that of CG14 and Mala noir IV.

The responses of photosynthetic rate, transpiration rate and *i*WUE of rice genotypes to 20-d NaCl salt stress are presented in Table 5. Salt stress significantly reduced the photosynthetic and transpiration rates, but increased *i*WUE in all the genotypes. The interaction between salinity and genotype was significant for the transpiration rate, but not for the other two parameters. The rice genotypes CG14 and Mala noir IV were most affected by the salinity stress compared with the other genotypes, WAB56-104, NERICA4, WAS161-B-6-B-B-3-1B, and the salt tolerant Pokkali.

Discussion

This study is the first comparative study on salt tolerance of the three cultivated rice species. The results indicated higher shoot and root growth rates in the interspecific progenies as compared with *O. sativa* and *O. glaberrima* (Table 3), which indicates a high salt tolerance of the interspecific progenies. Salt tolerance is generally defined as the fraction of growth under saline conditions as compared with growth under nonsaline conditions. The high relative shoot and root biomass values of the interspecific progenies indicates that this species sustained satisfactory growth under salinity stress, suggesting that the species has some mechanisms for salt stress tolerance. The results also revealed that the salt tolerance level of NERICA4, an interspecific progeny, was similar to that of its *O. sativa* parent WAB56-104 and higher than that of its *O. glaberrima* parent CG14 (Fig. 2). Since the interspecific group is the product of the crosses between the *O. sativa* and *O. glaberrima* species (Jones et al., 1997), some of the genotypes in this group may have acquired mechanisms for salt tolerance from the *O. sativa* parent. There exist genotypic differences in salt tolerance among *O. sativa* genotypes (Zeng, 2005). Our result suggested that some salt-tolerant interspecific progeny produced from the crosses between CG14 and WAB56-104 have acquired its salt tolerance from the *O. sativa* parent. There is currently little information on the salt tolerance of the interspecific progenies and *O. glaberrima*. Further studies are needed to generate sufficient evidence regarding the salt stress tolerance of these two species.

In Exp. 2, increases in NaCl stress duration significantly reduced the growth of the six rice genotypes tested (Table 4). The reduction in plant biomass was attributed to retard plant growth due to the combined effects of ionic and osmotic stresses imposed by NaCl salt (Nakamura et al., 2004; Moradi and Ismail, 2007). In fact, plants of *O. glaberrima* genotypes, CG14 and Mala noir IV, were severely affected and eventually killed after being exposed to the longest salt stress duration of 30 d. Other genotypes including NERICA4 and WAB56-104 were also affected but survived the prolonged salt stress treatment. As such, biomass production under the salt treatment was very low compared to that under the control treatment. Pokkali being the salt tolerant genotype was expected to perform better than the other genotypes. This genotype is well-known for salt tolerance and has been used as check cultivar in many studies on rice salt tolerance (Flowers et al., 1988; Lutts et al., 1995).

Salt stress significantly reduced photosynthetic and transpiration rates, but increased *i*WUE in all the genotypes evaluated for physiological responses (Table 5). The relatively high photosynthetic rate in Pokkali under the salt conditions is a reflection of the genotype ability to withstand salt stress. WAB56-104 and NERICA4 displayed some degree of salt tolerance by sustaining relatively higher relative photosynthetic rates as compared with the *O. glaberrima* genotypes. As stated above, CG14 and Mala noir IV were completely killed by salt by the end of the experiment. These *O. glaberrima* genotypes appear to be more sensitive to NaCl salt thus could not withstand the prolonged stress period. Conclusively, *O. glaberrima* was sensitive to NaCl salt stress, while the interspecific group was relatively tolerant.

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* In Japanese.