Effect of salt stress on physiological response and leaf polyamine content in NERICA rice seedlings

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ABSTRACT

NERICA is a new African rice variety, developed by the West African Rice Development Association (WARDA) in 1990s. NERICA rice shows both vigorous growth and tolerance of stressors such as drought and disease. The purpose of this study was to clarify the physiological and biochemical responses to salt stress of NERICA rice seedlings. The degree of growth inhibition caused by salt stress was small in NERICA rice varieties as compared with japonica Nipponbare. Na accumulation in leaf blades was high in salt-sensitive varieties. Accumulation of proline, a known compatible solute, was also induced by salt stress, especially in salt-sensitive varieties; it was thought that this accumulation was brought on salt-stress injury. The contents of polyamines, especially spermidine, were high in the pre-stressed leaf blades of NERICA rice seedlings. After the salt-stress treatment, the polyamine content of leaf blades differed with the degree of salt tolerance of the NERICA rice seedlings. These results suggested that the salt tolerance of NERICA rice seedlings might be associated not only with the regulation of Na absorption and translocation but also with their ability to maintain leaf polyamine levels under salt-stress conditions.

Keywords: salt tolerance; chlorophyll fluorescence; Na; proline; spermidine

NERICA (New Rice for Africa) is new rice, developed at the West Africa Rice Development Association (WARDA) in the 1990s, and is a cross of *Oryza sativa* L., a high-yield rice, and *Oryza glaberrima* Steud., a west African rice variety with drought, disease and insect tolerance (Jones et al. 1997a). NERICA rice has the property of a short growth period, drought tolerance, disease and insect tolerance, high competitiveness with weeds and resultant decrease of weeding, and high protein contents (Jones et al. 1997b, Dingkuhn et al. 1998, 1999). These properties are very interesting from the viewpoint of plant stress research. Salt stress generally causes osmotic stress, similar to drought stress, and inhibition of plant growth. NERICA rice, which is supposed to be drought resistant, is hypothesized to also have salt tolerance, because of the common mechanism of drought and salt stress. Recently, Awala et al. (2010) reported that WAB56-104 (*Oryza glaberrima* Steud.), one of the parents of NERICA 4, was sensitive to salt stress, while CG14 (*Oryza sativa* L.), the other parents, was tolerant of salt stress, and it was shown clearly that NERICA 4 has a character midway between the two. However, physiological and biochemical research of NERICA rice under salt-stress condition is relatively lacking.

In this study, we investigated the physiological responses to salt stress of NERICA rice seedlings in contrast to those of japonica Nipponbare (*Oryza sativa* L.) seedlings. Specifically, we compared the contents of Na, free amino acids and polyamines with an examination of responses to salt stress in NERICA rice seedlings.

MATERIAL AND METHODS

Plant materials and NaCl treatment. We used seeds of NERICA 1–7 provided by Dr. Hiroshi Tsunematsu (JIRCAS, Japan). Seeds of NERICA rice and japonica Nipponbare (*Oryza sativa* L. cv. Nipponbare) were germinated at 30°C in an incubator for 48 h after disinfection and transferred to a growth chamber (25/20°C; day/night, light intensity 270 μmol/m^2/s, 14-h photoperiod, 60% relative humidity). These seedlings were grown in modified Kasugai's nutrient solution (Kasugai 1939). After the appearance of the 2nd leaf, these seedlings (about

2-week-old plants) were further treated with diluted (1/2-strength) Kasugai's nutrient solution containing 100 mmol/L NaCl for 3 days. Seedlings without NaCl treatment were used as control plants. The plants were harvested and weighed at the onset (0 day) and 3 days after NaCl treatment. The second leaf blades of intact seedlings were immediately used for the measurement of chlorophyll fluorescence. Other seedlings were frozen in liquid nitrogen and lyophilized with a freeze dryer (FD-550R, Tokyo Rikakikai Co, Ltd, Tokyo, Japan). Freeze-dried tissue was used for the extraction and determination of Na, amino acid and polyamine contents.

Analysis of each parameters. The effective quantum yield of photosynthetic energy conversion ($\Phi II = \Delta F/Fm'$) was monitored by a chlorophyll fluorometer (MINI-PAM, WALZ, Effeltrich, Bayern, Germany). Na content was determined according to Kim et al. (1999). Analysis of free amino acids and proline was carried out according to Desmaison et al. (1984). Polyamines were analyzed by high performance liquid chromatography (HPLC) according to Flores and Galston (1982). More details of analytic methods were given in a previous report (Yamamoto et al. 2004).

All the data were based on two independent experiments with three replications. The difference in each value between treatments was analyzed by the Tukey's HSD test ($P < 0.05$).

RESULTS

Effect of salt stress on plant growth and chlorophyll fluorescence. Primary growth of NERICA rice, especially NERICA 1 and NERICA 7, was greater than that of japonica Nipponbare (data not shown). NERICA 6 was inferior to other NERICA rice varieties in primary and final growth (Figure 1a). Growth of all rice seedlings was inhibited by 15–25% under 100 mmol/L NaCl treatment (Figure 1a).

Figure 1b shows chlorophyll fluorescence (ΦII) in the 2nd leaf blades under the salt-stress condition. The ΦII value of all varieties were the same under the non-stress condition, but decreased variably under the salt-stress condition. The decrement rates of ΦII of NERICA 2 and Nipponbare, 40% and 46%, respectively, were larger than those of other varieties. The ΦII value of NERICA 6 was inhibited only by 10% by salt stress, whereas its growth was significant inhibition by salt stress. Thus plant growth and ΦII levels show different responses to salt stress.

Effect of salt stress on Na content. Figure 2 shows the Na content in the leaf blades and roots of NERICA rice varieties and Nipponbare under the salt-stress condition. Leaf-blade Na accumulations in rice seedlings differed between varieties. In NERICA 2 and NERICA 6, leaf Na accumulation was higher, as in japonica Nipponbare, reflecting the growth and ΦII reduction under the salt-stress condition. Other NERICA rice varieties accumulated Na in leaf blades at rates of 44–63% of Nipponbare. Root Na accumulation was lower than that in leaf blades, and no clear differences were observed.

Effect of salt stress on amino acids. Proline contents, known to be compatible solutes (Yancey et al. 1982, Rajendrakumar et al. 1997, Magdy and Mansour 1998), increased in leaf blades of

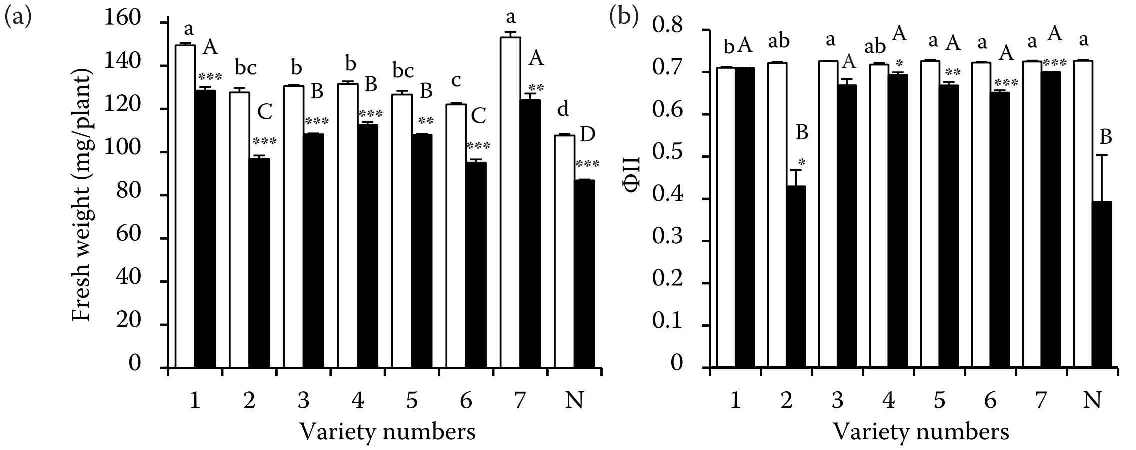

Figure 1. Effect of NaCl treatment on plant growth (a) and ΦII value (b) of rice varieties. Symbols indicate control plants (□) and salt-stressed plants (■). 1–7 – NERICA 1–7; N – Nipponbare. Vertical bars represent ± standard error ($n = 3$). Asterisks indicate a significant difference between control and salt stress ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). Bars labeled with the same lowercase letters on open square bars or uppercase letters on closed square bars are not significantly different ($P = 0.05$)

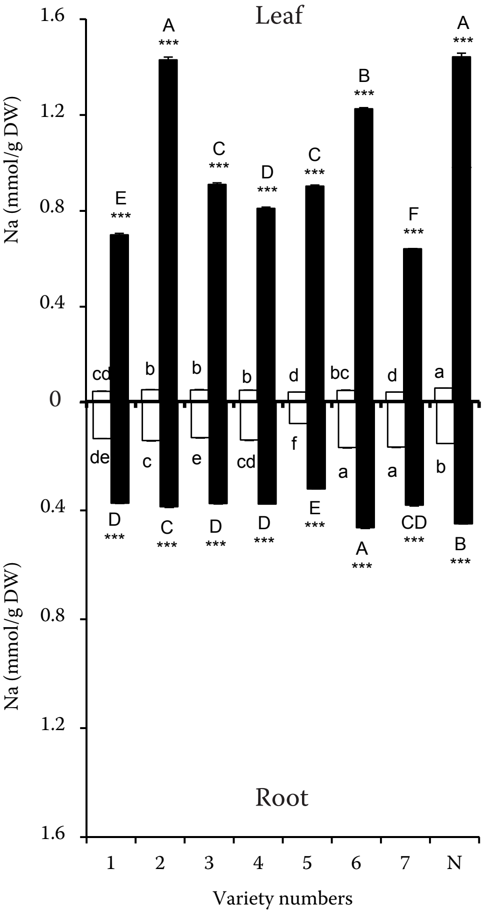

Figure 2. Effect of NaCl treatment on the contents of Na in the 2nd leaf blades and roots of rice varieties. Details of symbols, variety numbers and statistical indicators are the same as for Figure 1

salt sensitive cultivar NERICA 2 by the salt stress (Table 1). Meanwhile, proline accumulation of the salt-tolerant cultivar NERICA 1 was 35% of NERICA 2. As for the other varieties, NERICA 3, NERICA 4, NERICA 6 and Nipponbare accumulated 50% of the amino acids accumulated in NERICA 2 after the salt-stress treatment. Total amino acids increased 1.5–2.8 times with the salt stress.

Effect of salt stress on polyamines. Contents of three polyamines, putrescine, spermidine and spermine, in the 2nd leaf blades were more than twice higher in NERICA rice varieties than in Nipponbare (Figure 3a). Especially, spermidine content was 2.4–3.6 times higher in NERICA rice varieties.

Changes of the polyamines under salt stress differed among varieties, though the pattern of decreases in putrescine and spermidine and an increase in spermine was most common (Figure 3b). However, the decrements of putrescine and spermidine were more severe in salt-sensitive cultivar NERICA 2 than in salt-tolerant cultivar NERICA 1. NERICA 6, which did not show a decrease of ΦII despite having the same Na accumulation in the leaf blades as NERICA 2, showed a high concentration of polyamines after NaCl treatment together with salt-tolerant NERICA 1.

DISCUSSION

NERICA 1 was the most salt-tolerant among the NERICA rice varieties, based on growth inhibition and decrease of ΦII value in the 2nd leaf blades; by contrast, NERICA 2 was the most salt sensitive (Figure 1). The trend in growth inhibition

Table 1. Effects of salt stress on the content of proline and total amino acids in the 2nd leaf blade of NERICA rice seedlings

Varieties	Proline (μmol/g DW)		Total amino acids (μmol/g DW)	
	control	salt stressed	control	salt stressed
NERICA 1	0.42 ± 0.01^{b}	$3.29 \pm 0.08^{CD,***}$	52.67 ± 3.15^{a}	$80.94 \pm 5.91^{B,*}$
NERICA 2	0.51 ± 0.01^{ab}	$11.47 \pm 0.71^{A,**}$	58.72 ± 7.19^{a}	$163.20 \pm 8.47^{A,***}$
NERICA 3	0.43 ± 0.00^{b}	$5.73 \pm 0.44^{BC,**}$	43.49 ± 4.78^{a}	$110.46 \pm 4.52^{B,***}$
NERICA 4	0.43 ± 0.01^{b}	$5.68 \pm 0.88^{BC,*}$	55.01 ± 6.74^{a}	$97.14 \pm 2.53^{B,**}$
NERICA 5	0.45 ± 0.00^{b}	$3.75 \pm 0.51^{BCD,*}$	58.40 ± 8.24^{a}	$121.73 \pm 6.88^{AB,**}$
NERICA 6	0.46 ± 0.01^{b}	$6.30 \pm 1.05^{BC,*}$	52.38 ± 2.80^{a}	$109.81 \pm 17.36^{B,*}$
NERICA 7	0.44 ± 0.03^{b}	$2.45 \pm 0.25^{D,*}$	49.04 ± 0.12^{a}	$97.64 \pm 7.40^{B,*}$
Nipponbare	0.59 ± 0.04^{a}	$6.40 \pm 0.36^{B,**}$	57.96 ± 3.92^{a}	$164.38 \pm 8.54^{A,***}$

Data are means ± SE ($n = 3$). Asterisks indicate a significant difference between control and salt-stressed plants ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$). Values labeled with same lowercase letters on control columns or uppercase letters on salt-stressed columns are not significantly different ($P = 0.05$)

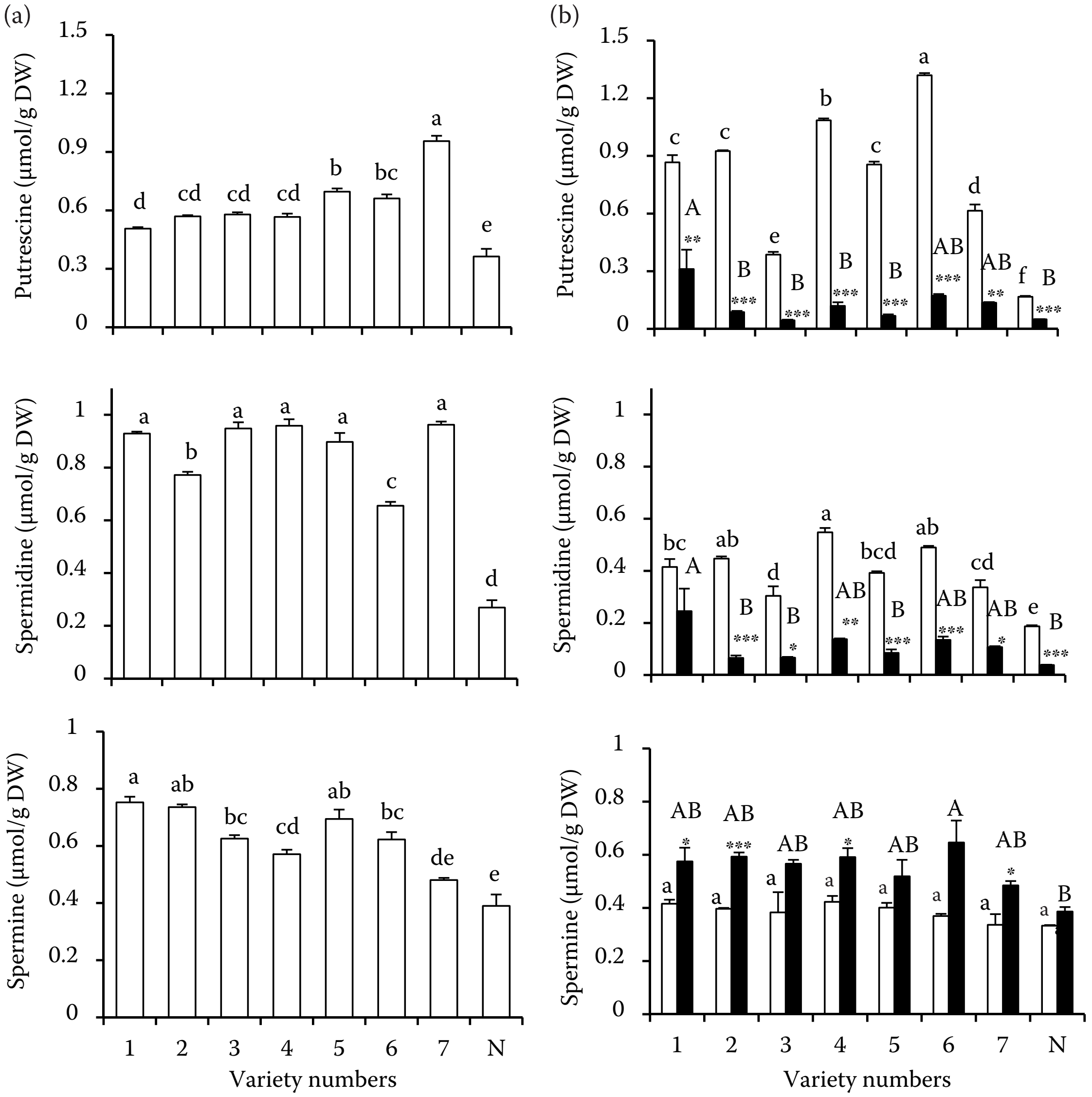

Figure 3. Effect of NaCl treatment on the polyamine contents in the 2nd leaf blades of rice varieties. (a) Polyamine contents at 0 day; (b) Polyamine contents at 3 days. Details of symbols, variety numbers and statistical indicators are the same as for Figure 1

of NERICA 2 at 3 days after NaCl treatment was higher than that of Nipponbare (Figure 1a). Salt tolerance indicated by maintenance of ΦII was markedly larger among these varieties; no decrease of ΦII was observed in NERICA 1 whereas in NERICA 2 and Nipponbare, ΦII decreased by 40% and 46% respectively under the salt-stress condition (Figure 1b). Furthermore, marked reduction was shown in Nipponbare under the 1.6-times light-intensity condition (data not shown). These results suggest that NERICA rice is not only drought-tolerant but also salt-tolerant, and certainly more salt-tolerant than japonica Nipponbare. This is in agreement also with previous report that NERICA 1 is more tolerant to salt stress than NERICA 2 (Awala et al. 2010). Additionally, NERICA 1 and NERICA 7 showed superior growth at the 2nd leaf stage (Figure 1a). It seems that a short growing period and early initial growth of NERICA rice were brought on by these varieties' high potential of polyamine biosynthesis, because spermidine is known to be an essential component of growth and development in polyamines (Feirer et al. 1985).

It is clear that the difference in salt tolerance between Nipponbare and NERICA rice is related to accumulation of Na in the leaf blade (Figure 2). The relation of salt tolerance to absorbed Na was reported by many previous researchers (Yeo and Flowers 1986, Lin et al. 2002, Hoai et al. 2003); the present study supports their results. Salt tolerance of Leguminosae *Lotus* species was correlated with not only high responses of antioxidant system but also low Na absorption and accumulation in leaves (Melchiorre et al. 2009). Moreover, it was suggested that the difference of Na content in the leaf blade of NERICA rice varieties and Nipponbare were caused by Na absorption and translocation from the roots to leaf blades. Total amino acid content in leaf blades under NaCl treatment was high in NERICA 2 and low in NERICA 1 (Table 1), which might be caused by proteolysis, a great accumulation of protein amino acids, and disturbance of nitrogen metabolisms, and accumulation of non-protein amino acids or ammonia (data not shown). A previous study reported a positive accumulation of amino acids by proteolysis for osmoregulation related to salt tolerance of rice growing under different nitrogen media (Yamamoto et al. 2004). A close correlation between salt tolerance and amino acid accumulation was not considered for NERICA rice at this stage. Krishnamurthy and Bhagwat (1989) and many researchers reported changes in the polyamine content in rice under salt stress conditions. Lin and Kao (1995) observed a decrease in putrescine in shoots and roots of rice under salt stress, and an increase in polyamines and recovery of growth achieved by exogenous treatment of the putrescine substrates, arginine and ornithine. Exogenous putrescine was shown to inhibit Na absorption and decrease in rice yields (Prakash and Prathapasenan 1988). We also reported that the metabolism of polyamines was correlated with the salt-stress response in rice seedlings (Yamamoto et al. 2004). It was reported that the salt, drought and freezing tolerance changed by control of polyamine metabolic pathway in *Arabidopsis thaliana* (Urano et al. 2004, Yamaguchi et al. 2006, Cuevas et al. 2008, Alcázar et al. 2010). Our present results suggest that the difference in salt tolerance between NERICA rice and japonica Nipponbare not only involves translocation and accumulation of Na in shoots, absorbed from roots, but a difference in the biosynthesis and metabolic activity of polyamines in the leaf blades (Figure 3). In the future, it will be necessary to perform a more detailed analysis of polyamine metabolism and gene expression in NERICA rice seedlings, and the responses and yield under salt stress conditions introduced at a later growth stage.

Acknowledgment

The authors are grateful to Dr. Hiroshi Tsunematsu of JIRCAS for providing them with NERICA seeds, and the Chemical Analysis Center, University of Tsukuba, for Na and amino acid analysis data. The authors also thank emeritus Professor Tadakatsu Yoneyama of the University of Tokyo for instructive comments on the manuscript.

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Received on July 21, 2011

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