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Responses of different rice (*Oryza sativa* L.) genotypes to salt stress and relation to carbohydrate metabolism and chlorophyll content

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The study aims to investigate the physiological mechanisms associated with salt tolerance of different rice genotypes; with emphasis on carbohydrate metabolism and chlorophyll concentration. Studies were conducted in a greenhouse and fields at the International Rice Research Institute (IRRI) during the dry season (November 2008 to March 2009) and the wet season (April 2009 to October 2010). Salt stress increased chlorophyll concentration in leaves of a tolerant (IR651) and a moderately tolerant (IR64) rice genotypes, but significantly decreased chlorophyll *a*/*b* ratio. Chlorophyll *a* concentration and chlorophyll *a*/*b* ratio were higher in the leaves of IR651 than in the leaves of IR64 under salt stress, and this is probably one of the reasons for the higher tolerance of IR651 compared with IR64. Differences between genotypes in dry weight and leaf area were not significant under control condition; however, higher soluble sugars and starch concentrations in plant tissues were observed under control conditions than under salt stress. Conversion of soluble sugars into starch seems to be partially inhibited by salt stress as suggested by the higher concentrations of soluble sugars compared with starch under salt stress. Apparently, the salt tolerant genotype maintained higher soluble sugars, higher chlorophyll *a* and chlorophyll *a*/*b* ratio under salt stress, and these traits could have partially contributed to its salt tolerance.

Key words: Carbohydrate metabolism, chlorophyll concentration, rice, salt stress, salt tolerance.

INTRODUCTION

Large amount of soluble sodium ions accumulates in soil and water because of the combined effects of natural and human factors; and this seriously affects plant growth and yield (Sahin et al., 2002). Salt stress is becoming one of the key factors that restrict agricultural productivity, especially in irrigated areas and in rainfed coastal areas (Neue, 1991; Castillo et al., 2000). Because of the

increasing need for enhancing productivity of salt affected areas, more interest is being devoted in recent years on studies of the adaptive physiological and metabolic processes associated with salt tolerance of crop plants (Ismail et al., 2007). Numerous physiological responses of plants to salt stress were observed before, including cellular and whole plant responses (Chen and Filippis, 2001). The seedling stage is one of the most sensitive stages to salt stress in rice, and studies on salt tolerance during this stage could probably provide insights for enhancing tolerance throughout the plant life cycle

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Table 1. Characteristics of the soil used in the studies.

Soil style	pH (H$_2$O,1:1)	Organic carbon (g kg^{-1})	CEC (cmol kg^{-1})	Total N (g kg^{-1})	Available P (mg kg^{-1})	Active iron (g kg^{-1})	Clay (g kg^{-1})
Paddy soil	6.1	22.7	25.3	2.26	80.0	25.0	300

(Munns and Tester, 2008). Moreover, the relation between sodium concentration in plant tissue and growth and yield were observed to be negative, and with greater effects on shoot growth than on root growth (Eschie et al., 2002). Percentage survival of transplanted seedlings correlated positively with the dry weight of seedling at transplanting, as well as with biomass accumulation during stress (Maiale et al., 2004).

Carbohydrates produced by photosynthetic tissues is either transported to other organs as soluble sugars, or accumulated in leaves as soluble sugars and starch during the different growth stages. Under most abiotic stresses, the ability of plants to recover from stress normally increase with increasing concentrations of photosynthetic assimilates in plant tissues during or after stress (Bagheri and Sadeghipour, 2009; Naureen and Naqvi, 2010). Soluble carbohydrates and starch, which accumulates under normal conditions before the stress commonly constitute the main resources for plants to supply energy during stress condition, as well as during recovery (Khelil et al., 2007). Therefore, higher concentrations of carbohydrates in plant tissue is one of the important adaptive mechanisms as observed under submergence (Chaturvedi et al., 1996; Dkhil and Denden, 2010). Reduction in plant biomass is sometimes observed under severe salt stress, and this is possibly because of the decrease in carbohydrate accumulation caused by reduction in carbon assimilation (Moradi and Ismail, 2007; Pattanagul and Thitisaksakul, 2008).

Potassium uptake is usually inhibited under salt stress, because of its molecular similarity to sodium ions, causing competition during active uptake. This could affect the rate of conversion of soluble sugars into starch when the uptake of K$^+$ and its concentration in plant tissues is reduced, as K$^+$ is needed for the catalytic activities of starch biosynthesis enzymes (Chartzoulakis et al., 2006; Dkhil and Denden, 2010). Previous reports attributed the decrease of starch content in shoot tissues to the decrease in the amount of K$^+$ absorbed under salt stress (Cakmak et al., 1994; Jenci and Natarajan, 2009). Another reason for reduction in starch concentration in plant tissue is the direct effects of decreased CO$_2$ assimilation caused by reduction in stomatal conductance and content of chlorophyll in plant tissues under salt stress (Moradi and Ismail, 2007). The effects of salinity on chlorophyll synthesis and integrity seems to vary with the level of salt stress, as few reports suggested an accelerated rate of biosynthesis and higher concentrations during vegetative growth (Asch et al., 2000; Santo, 2004), however, significant differences

between genotypes were sometimes observed regarding the effects of salt stress on chlorophyll concentration in leaves (Rout et al., 1997; Datta et al., 2009).

Apparently, previous studies considered changes in carbohydrate metabolism as important physiological responses for adaptation to abiotic stress in plants, and various metabolic changes were documented (Asch et al., 2000; Santo, 2004; Pattanagul and Thitisaksakul, 2008; Naureen and Naqvi, 2010). However, detailed studies on genetic differences in these traits are still scanty. Here we evaluated the variation in carbohydrate concentration in rice genotypes known to contrast in their tolerance of salt stress, to further investigate whether these changes are associated with tolerance. We compared changes in non-structural carbohydrate (NSC) and chlorophyll concentrations in one salt tolerant (IR651) and one moderately tolerant (IR64) rice genotypes grown under normal and saline (EC 9.8 dS/m) conditions. The data associated the ability to accumulate higher concentrations of starch in plant tissues and chlorophyll *a* in leaves to tolerance of salt stress.

MATERIALS AND METHODS

Plant materials

Two rice genotypes were used in this study, IR65192-4B-10-3 (IR651 hereafter), a salt tolerant genotype, and IR64, a widely grown variety with moderate tolerance of salt stress (Moradi et al., 2003). The experiment was conducted in a greenhouse and a field at the international Rice Research Institute (IRRI), Philippines, during the dry (November 2008 to March 2009) and wet (April 2009 to October 2010) seasons. The soil used was taken from the experimental farm of IRRI, a heavy clay soil developed from volcanic ash. The basic physical and chemical characteristics of this soil are summarized in Table 1.

Experimental design

Pre-germinated seeds of the two genotypes were sown in the greenhouse in seeding trays filled with field soil (Table 1). Two rice genotypes, the tolerant IR651 and the moderately tolerant IR64 were sown in a greenhouse in seeding trays of 1 x 0.5 m, filled with 6 kg of sieved field soil fertilized with 0.40 g N, 0.25 g P and 0.25 g K. Two seeds were sown per hole with a total of 240 holes per entry using pre-germinated seeds. After 30 days, seedlings were transplanted in the field either under control or in saline soil with an average electrical conductivity (EC) of about 9.8 dS m^{-1}, with 2 seedlings per hill in a 9 m^2 plots. A factorial RCBD was used with three replications. The trial was conducted two times once during the dry season and the second during the wet season on IRRI experimental farm in the Philippines.

Sampling and growth analyses

Leaf area, dry weight per seedling and concentrations of starch, soluble sugars and total non-structural carbohydrates (NSC) in plant tissues were determined using whole seedlings at transplanting. Percentage survival was determined after 6, 10 and 15 days following transplanting. The modified standard evaluation system (SES) of IRRI (Moradi and Ismail, 2007) was used for evaluating salinity tolerance of the two genotypes at 20 days after transplanting. Whole plants were then sampled after 20 and 30 days following transplanting, and used for measuring the concentrations of starch, soluble sugars, and chlorophyll in plant tissues. Correlations between different growth attributes (dry weight, leaf area, root/shoot ratio and carbohydrate content) determined at transplanting and percentage survival determined 15 days after transplanting were calculated.

Measurements of growth and physiological attributes

Concentration of starch and soluble sugars

Carbohydrate concentrations in plant tissues were determined at transplanting and at intervals after treatment. A modified colorimetric method was used for analysis of starch and soluble sugar concentrations (Thakur and Sharma, 2005; Dkhil and Denden, 2010). For starch concentration, plant tissues were homogenized in an ice-cold mortar and pestle in a volume of 16 ml 80% (v/v) ethanol. The homogenates were centrifuged at 3000×g, for 10 min at 4°C, and then perchloric acid ($HClO_4$, 6 ml, 30%, v/v) was added to dissolve starch from the pellet. The slurry was left at room temperature for 6 h, and starch was detected with I_2-KI reagent prepared by diluting 0.1 ml stock solution (0.06 g I_2 and 0.60 g KI in 10 ml deionized water) with 0.05 M HCl just prior to the assay. Samples of 0.5 ml starch solution were mixed with 0.5 ml I_2-KI reagent, 1 ml 30% (v/v) perchloric acid and vortexed, then left standing at room temperature. The absorbance of the samples at 620 nm wavelength was then determined using a spectrophotometer, and the concentration determined using a standard carve. For soluble sugars, plant tissues were suspended in test tubes with 3 ml of 80% ethanol, the extract was evaporated to dryness and the residue was dissolved in 20 ml distilled water. Total soluble sugars were determined by the phenol-sulfuric acid method, using glucose as standard. The total NSC was determined as the sum of starch and soluble sugar concentrations.

Standard evaluation system (SES) scores

The modified standard evaluation system (SES) of IRRI was used for evaluation of the visual symptoms of salt damage during seedling stage. A scoring system of 1 to 9 was used, with 1 indicates normal growth with no symptoms of injury; 3 indicates near normal growth with leaf tips or few leaves whitish and rolled; 5 indicates intermediate tolerance with growth severely retarded, most leaves rolled and few are elongating; 7 indicates susceptibility with complete cessation of growth, most leaves dry, some plants dying; and 9 indicates high susceptibility with most plants dead or dying (Zhang et al., 2010).

Growth and leaf area measurements

Green leaf area measurements were made each morning of the sampling dates. Plants were randomly chosen and gently uprooted. All leaves were detached and the senescing portions removed. A total of 4 plants were harvested per replicate and total green leaf area measured using a LiCor-3100 leaf area meter (LiCor, Lincoln, Nebraska, USA). Shoot and root growth were assessed on the same 4 plants from each replicate. Roots and shoots were gently separately and rinsed for few times with distilled water and then with NanoPure quality water for three additional times to remove adhering salt. Dry weights (mg) were determined with a top loading balance after drying the samples to a constant weight in an oven set at 70°C.

Chlorophyll concentration

About 20 mg of freeze-dried plant material were heated at 80°C for 10 min in 10 mL aqueous ethanol (80% v/v) in extraction vials, then cooled to room temperature and the volume adjusted to 10 mL with 80% v/v ethanol. After extraction, the leaf sections looks white, grayish or light brown, and sink to the bottom of the extraction vial allowing the extract to be decanted into a glass cuvette without filtration or centrifugation. Absorption readings were determined using a spectrophotometer, with the optical density determined at 649, 652 and 665 nm (Santo, 2004). Chlorophyll concentration in mg/ml was then calculated using the following formula:

$$C_a = 13.95 D_{665} - 6.88 D_{649}$$

$$C_b = 24.96 D_{649} - 7.32 D_{665}$$

$$C_{a+b} = 27.8 A_{652}$$

Chlorophyll (mg/g) = C (mg/ml) × V (ml) / fresh weight (g),

with C = absorption reading and V = solution volume.

Statistical analysis

Data analyses were carried out using SAS software 8.0 (SAS institute, 2001). Differences between means with $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ were considered significant based on LSD values. Correlations of seedling survival with other parameters were calculated using Microsoft Excel 2003 software. The data was presented as averages across the dry and wet seasons as the responses of the genotypes were similar across both seasons.

RESULTS

Variation in visual injury and survival

Effect of salinity on both genotypes was apparent from the higher SES values after 20 days of exposure to salt stress (Table 2). The SES score of IR651 was significantly lower than that of IR64, confirming the higher tolerance of this genotype. Salinity also caused considerable reduction in survival across the two genotypes, where it decreased by about 35, 44 and 53% points after 6, 10 and 15 days, respectively, following transplanting in saline soil. However, the survival of the tolerant genotype IR651 was significantly higher than the moderately tolerant variety IR64 at all sampling dates.

Seedling growth at transplanting and correlation with survival

No significant differences were observed between the

Table 2. Variation in SES scores at 20 days after transplanting (DAT) and survival percentages at 6, 10, 15 DAT under control and saline conditions.

Treatments	SES score 20 DAT	Survival (%)		
		6 DAT	10 DAT	15 DAT
Genotype				
IR651	5.1	77.7*	69.8**	55.9**
IR64	7.8***	52.8	43.1	37.7
Salinity				
Saline	6.4***	65.2	56.5	46.9
Normal	1.0	100.0***	100.0***	100.0***

*, **, *** Significant at P ≤ 0.05, 0.01 and 0.001, respectively.

Table 3. Variation between IR651 and IR64 in leaf area, dry weight and root:shoot ratio at transplanting.

Genotype	Leaf area $(cm^2\ plant^{-1})$	Root dry weight (g)	Shoot dry weight DW(g)	Root: shoot ratio
IR651	22.2	0.118	0.475	0.269***
IR64	25.5	0.119	0.481	0.227
Mean	23.8	0.119	0.477	0.256

*** Significant at P ≤ 0.001.

Table 4. Correlation coefficients for the association of survival at 15 days after transplanting with seedling growth and dry weights at transplanting.

Growth parameter	Survival (%)	Growth parameter	Survival (%)
Leaf dry weight	0.64**	Shoot dry weight	0.62*
Stem dry weight	0.61*	Leaf area	0.60*
Root dry weight	0.68**	Root: shoot ratio	-0.04[ns]

*, ** significant at P ⩽ 0.05, 0.01 respectively; ns, not significant.

two genotypes in leaf area and root and shoot dry weights at transplanting, however, the root:shoot ratio of IR651 was significantly higher than that of IR64 (Table 3). Seedling survival determined 15 days after transplanting in saline soil correlated significantly with shoot and root dry weights as well as with dry weights of leaves and stems and with leaf area (Table 4). However, the correlation with root:shoot ratio was insignificant, suggesting that variation in this trait during early seedling growth might not affect survival after transplanting in saline soils.

Variation in carbohydrate concentration in seedlings at transplanting and relation with survival

Starch concentrations in stems, leaves and roots of the tolerant genotype IR651 at transplanting were higher, respectively, by about 22, 8 and 14%, than the moderately tolerant genotype IR64. However, the differences are significant only for stem starch concentrations. Conversely, soluble sugar concentrations were lower in the stems (12%), leaves (10%) and roots (11%) of IR651, though the differences are not statistically significant (Table 5).

Starch concentration in stems and leaves of seedlings measured at transplanting correlated positively with survival at 15 days of transplanting in saline soils. However, correlation of survival with soluble sugar concentrations was negative (Table 6). This suggests that maintaining conditions in the nursery that maximize the conversion of soluble sugars into starch in the shoot before transplanting could contribute to survival of seedlings when transplanted in saline soils. Correlations of both soluble sugars and starch in roots with seedling survival were not significant.

Table 5. Variation in starch and sugar concentrations in plant tissues in two rice genotypes at transplanting.

Genotype	Starch concentration (%)			Sugar concentration (%)		
	Stem	Leaf	Root	Stem	Leaf	Root
IR651	16.1*	5.3	5.6	9.4	4.3	5.4
IR64	13.2	4.9	4.9	10.7	4.8	6.1
Mean	14.7	5.1	5.3	10.1	4.6	5.8

* Significant at $P \leq 0.05$.

Table 6. Correlation coefficients for the association of survival at 15 days after transplanting in saline soils and carbohydrate concentrations in the seedlings at transplanting.

Starch concentration	Survival (%)	Sugar concentration (%)	Survival (%)
Stems	0.60*	Stems	-0.54*
Leaves	0.67**	Leaves	-0.55*
Roots	0.39^{ns}	Roots	-0.48^{ns}

*,** Significant at $P \leq 0.05$ and 0.01, respectively; ns, not significant.

Table 7. Variation in carbohydrate concentration in shoots and roots at 20 and 30 days after transplanting (DAT).
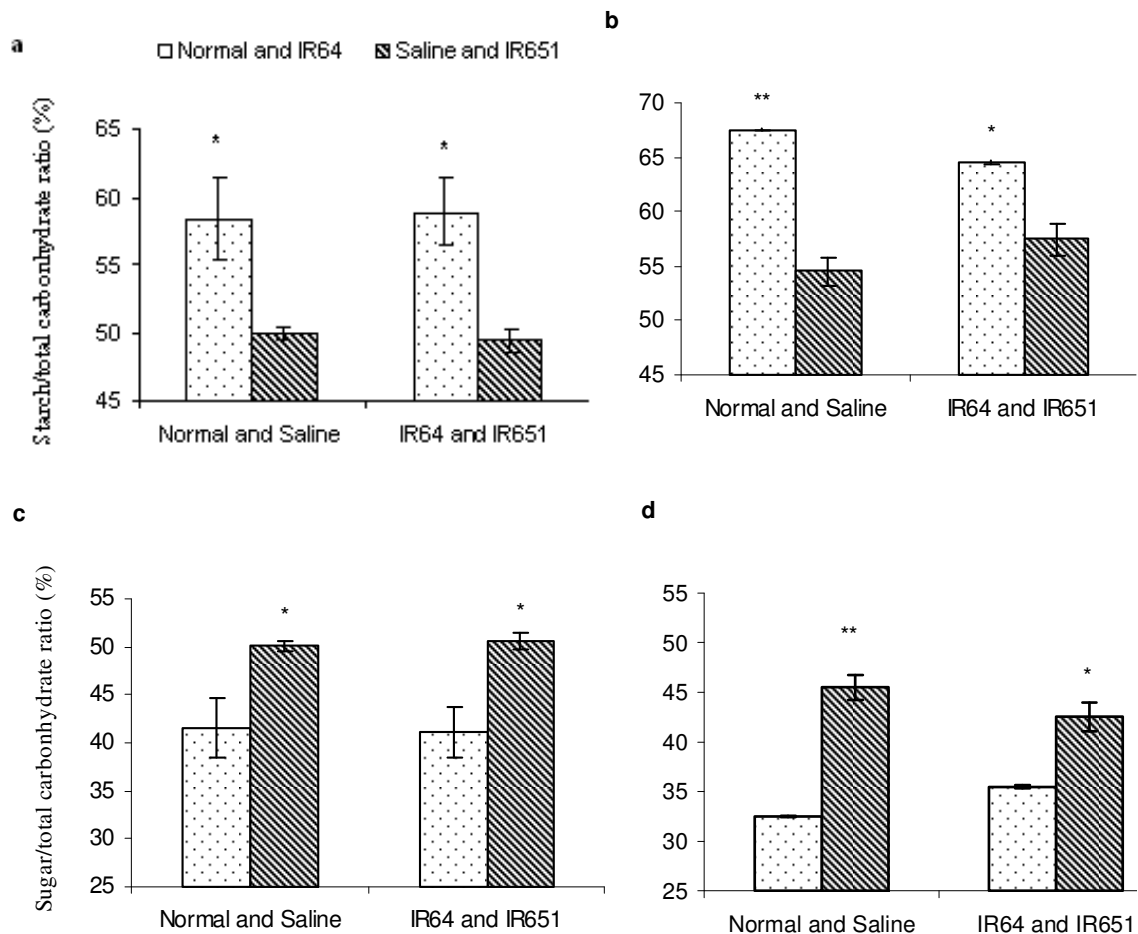
Treatments	Carbohydrate concentration 20 DAT (%)			Carbohydrate concentration 30 DAT (%)		
	Soluble sugars	Starch	Total	Soluble sugars	Starch	Total
Shoot						
IR651	15.8*	17.4	33.2	13.5*	18.2	31.6
IR64	14.5	19.9*	34.4	11.9	18.1	30.0
Saline	14.7	14.5	29.2	15.2*	15.2	30.4
Normal	15.6	22.8**	36.0**	10.2	21.1**	31.2
Mean	15.1	18.6	33.2	12.7	18.2	30.8
Root						
IR651	9.8*	8.8	18.6	6.5*	8.7	15.2*
IR64	7.5	9.9	17.4	4.2	9.2	13.4
Saline	7.7	7.7	15.4	6.2*	8.9	15.1*
Normal	9.6*	11.1**	20.7**	4.4	8.9	13.3
Mean	8.6	9.4	18.0	5.3	8.9	14.2

*,** Significant at $P \leq 0.05$ and 0.01, respectively.

Variation in carbohydrate concentration between genotypes and treatments after 20 and 30 days of transplanting

After 20 days of transplanting, starch and total carbohydrate concentrations in the shoot were considerably lower under saline condition than under control condition (Table 7), but with no significant differences in total soluble sugar concentration, suggesting effects of salt stress on conversion of soluble sugars into starch. This becomes clearer after 30 days of salt stress where soluble sugars accumulated to significantly higher concentrations in shoots compared to that under control conditions. Differences between genotypes were greater 20 DAT, where the tolerant genotype accumulated higher soluble sugars, but lower amount of starch, and after prolonged stress of 30 days, the tolerant genotype still maintained higher soluble sugars than IR64. However, total NSC concentration was statistically similar in both genotypes under both saline and control conditions. This suggests that the higher tolerance of IR651 is probably not associated with its

Figure 1. Ratios of starch (a and b) and soluble sugars (c and d) to total carbohydrate concentrations at 20 days (a and c) and 30 days (b and d) after transplanting. *, ** indicate significance at P ≤ 0.05, and 0.01, respectively.

ability to accumulate starch or NSC in its shoots under stress. Trend in carbohydrate accumulation in roots seem to be similar to that in shoots, where soluble sugars, starch and total NSC were significantly less under salt stress. But this effect seem to revert as the seedlings age, where both soluble sugars and total carbohydrates became higher under salt stress, possibly suggesting inhibition of growth through effects other than photosynthesis and carbohydrate translocation to roots. IR651 maintained higher soluble sugar concentration in roots both at 20 and 30 days following transplanting in saline soils (Table 7). For both shoot and root NSC concentrations, interactions between genotypes and salinity were not significant.

After 20 days of transplanting, the ratios of starch to total carbohydrate in plant tissues was significantly higher under control (normal) than under saline conditions (Figure 1a), and the difference was even higher at 30 DAT (Figure 1b), which suggests that starch formation is probably hindered under salt stress. The ratios of soluble sugars to total carbohydrates showed reverse trends, they are significantly higher under saline than under

control conditions (Figure 1c and d). This also suggests that conversion of soluble sugars into starch is probably limited under saline conditions. The tolerant genotype IR651 maintained substantially lower ratio of starch to total carbohydrates in its tissues (Figure 1a and b), but conversely higher ratio of soluble sugars to total carbohydrates (Figure 1c and d). The higher proportion of soluble sugars in the tissue of the tolerant genotype could probably be advantageous for direct use during growth and maintenance under salt stress conditions.

Variation in chlorophyll concentration and ratios at 20 and 30 days after transplanting
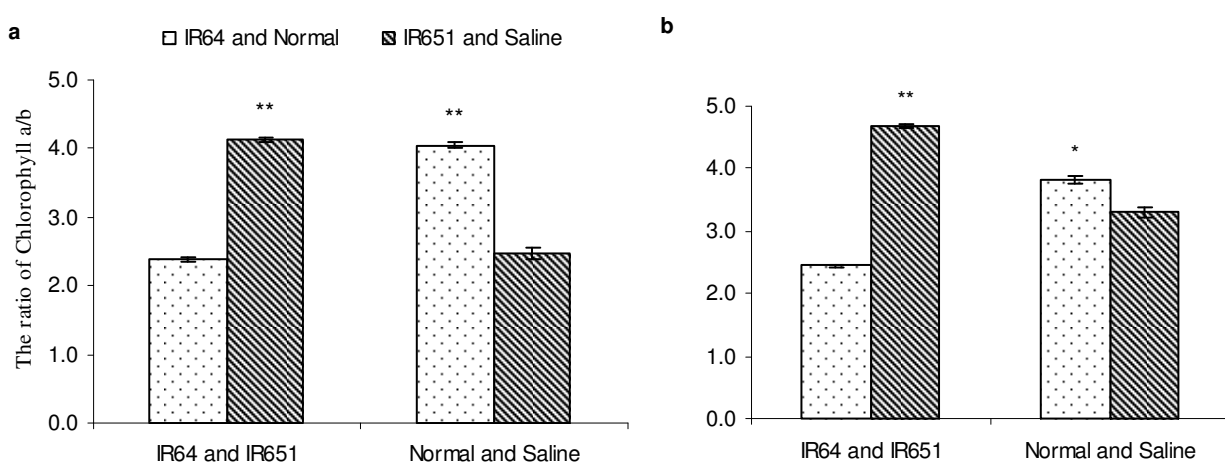
After 20 and 30 days of transplanting in saline soil, chlorophyll *a* concentrations in leaves of IR651 were 12.9 and 23.6% higher than in IR64, while chlorophyll *b* concentrations were 34.4 and 34.8% lower than in IR64 at 20 and 30 DAT, respectively (Table 8).

However, no significant differences were observed in total chlorophyll (*a+b*) concentration of the two genotypes.

Table 8. Variation in chlorophyll concentration in leaves of the two rice genotypes measured at 20 and 30 days after transplanting (DAT) in saline and normal soils.

Treatments	Chlorophyll concentration 20 DAT (%)			Chlorophyll concentration 30 DAT (%)		
	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a+b*	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a+b*
IR651	7.9	2.1	10.0	6.8**	1.5	8.2
IR64	7.0	3.2*	10.2	5.5	2.3**	7.8
Saline	8.1*	3.5**	11.5**	6.4	2.1	8.4
Normal	6.9	1.8	8.7	5.9	1.7	7.6
Mean	7.5	2.7	10.1	6.2	1.9	8.0

*,** Significant at P ≤ 0.05 and 0.01, respectively.

Figure 2. Variation in chlorophyll *a/b* ratios in leaves at (a) 20 and (b) 30 days after transplanting. *, ** Significant at P ≤ 0.05 and 0.01, respectively.

After 20 days of transplanting, the concentrations of chlorophyll *a*, chlorophyll *b* and chlorophyll *a+b* in plant tissues under salt stress were greater than under control conditions, by about 17, 94 and 32%, respectively, and similar trends were observed at 30 DAT, though the differences were not significant. The data showed that the chlorophyll concentration in leaves increased under salt stress, with the tolerant genotype maintaining higher concentration of Chlorophyll *a*, but lower concentration of chlorophyll *b*. Consequently, chlorophyll *a/b* ratio decreased substantially under salt stress, both at 20 and 30 DAT, and the tolerant genotype IR651 maintained significantly higher chlorophyll *a/b* ratio than the moderately tolerant genotype IR64 (Figure 2) at both sampling dates.

DISCUSSION

Variation in chlorophyll concentration and growth as affected by salinity and genotype

Significant differences were observed between the tolerant IR651 and the moderately tolerant IR64 in their responses to salt stress (Table 2). Genetic variation in salinity tolerance in rice as well as in other crop species has been frequently documented in the past (Moradi et al., 2003; Eschie et al., 2002; Moud and Maghsoudi, 2008). Seedling survival measured 15 days after exposure to salt stress correlated positively with different growth attributes at transplanting, including root and shoot biomass, and leaf area and dry weight, suggesting a direct effect of seedling biomass at transplanting on survival of salt stress when seedlings are transplanted in saline soils. This has been observed in previous studies (Maiale et al., 2004; Sunnart et al., 2010), and older rice seedlings are mostly recommended when transplanting in saline soils. Eschie et al. (2002) and Ozturk et al. (2004) observed a negative association of sodium concentration in culture solution with plant growth rate and root-shoot ratio. No significant differences in shoot and root dry weights and leaf area were observed between the tolerant IR651 and the moderately tolerant genotype IR64 (Table 3), but the root-shoot ratio of IR651 was significantly greater than that of IR64. However, the

insignificant correlation between root-shoot ratio and survival percentage under salt stress (Table 4) suggested that the higher root: shoot ratio of the tolerant genotype is probably not involved in salt tolerance in rice.

Concentrations of chlorophyll *a*, chlorophyll *b* and total chlorophyll were higher under salt stress than under control condition, which agreed with previous observations (Asch et al., 2000; Santo, 2004). However, chlorophyll *a*/*b* ratio was significantly lower (Figure 2), suggesting greater effects of salt stress in reducing chlorophyll *a* than chlorophyll *b*. Considering that chlorophyll *a* is the main photosynthetic pigment (Daiz et al., 2002; Santo, 2004), this reduction in ratio could probably be one of the main reasons for reduced photosynthesis under salt stress as reported in rice before (Moradi and Ismail, 2007). Significant differences in chlorophyll concentrations under salt stress were also observed between genotypes, with the tolerant genotype having higher chlorophyll *a*, but lower chlorophyll *b*, resulting in substantially higher chlorophyll *a*/*b* ratio than the moderately tolerant genotype (Table 8; Figure 2). Ability of the tolerant genotype to maintain higher concentration of chlorophyll *a* is probably one of the important mechanisms contributing to salinity tolerance in this genotype, which could consequently result in higher photosynthetic capacity and carbohydrate formation (Moradi and Ismail, 2007; Rout et al., 1997; Datta et al., 2009).

Responses of different rice genotypes to salt stress and its relation to carbohydrate metabolisms

Higher non-structural carbohydrate concentration in plant tissue under abiotic stresses was known to have positive effects on plant survival of stress and recovery afterwards (Bagheri and Sadeghipour, 2009; Naureen and Naqvi, 2010). These carbohydrates could provide important resources for energy supply under abiotic stresses when carbon assimilation is reduced, (Khelil et al., 2007), and is considered an important adaptation strategies under unfavorable growth conditions as the case with complete submergence in rice (Das et al., 2005; Dkhil and Denden, 2010). In this study, we observed that starch concentration was significantly higher in the tissue of the tolerant genotype at transplanting (Table 5); and there was a significant positive correlation between survival percentage under salt stress and starch concentration in stems and leaves at transplanting. However, seedling survival under salt stress correlated negatively with soluble sugar concentration at transplanting (Table 6). This suggests that accumulation of starch in plant tissue before transplanting could improve seedling survival when transplanted in saline soil, and this can be enhanced both through breeding as well as proper management of seedlings in the nursery before transplanting.

Under salt stress, consumption of metabolic energy increased while the amount of carbohydrate accumulation decreased because of reduced photosynthetic capacity; which will then cause slower growth and biomass accumulation (Pattanagul and Thitisaksakul, 2008). Besides its direct effects on carbon assimilation, salt stress could also hinder other important metabolic processes. For example, the activity of several starch biosynthesis enzymes can be affected by the concentration of potassium in plant tissues, and salinity was known to cause considerable reduction in the uptake of potassium and its concentration in plant tissue (Chartzoulakis et al., 2006; Ismail et al., 2007; Moradi and Ismail, 2007; Dkhil and Denden, 2010). The reduction in starch concentration in plant tissue observed in this study could be due, in part, to the decrease in K^+ absorption under salt stress (Cakmak et al., 1994; Jenci and Natarajan, 2009). Contrary to starch concentration, soluble sugar concentration was higher in the tolerant genotype (Table 7); and the ratio of soluble sugars to total carbohydrate was higher than in the moderately tolerant genotype under salt stress (Figure 1). This can probably be due to lesser effects of salt stress on carbon assimilation and soluble sugar formation than on conversion of soluble sugars to starch.

This effect could be mediated by the lower K^+ and the unfavorable ratio of Na^+ to K^+ in plant tissue under salt stress (Ismail et al., 2007; Zhang et al., 2010), with the consequent effects on the activity of the enzymes involved in the translocation and conversion of soluble sugars into starch. Maintaining greater ability to convert soluble sugars into starch before and during salt stress could potentially play an important role in rice tolerance of salinity. Further studies are needed to substantiate this relation using a wider range of contrasting rice genotypes, and also to investigate the extent of genetic variation to be explored in gene discovery as well as in breeding.

Conclusions

Salinity causes substantial decrease in seedling survival and considerable reduction in growth of surviving plants as indicated by the higher SES scores. Greater root and shoot biomass, leaf area and starch concentration in plant tissue at transplanting correlated positively with seedling survival after transplanting in saline soils. Salinity causes substantial reduction in total non-structural carbohydrate concentrations in plant tissue, basically through greater effects on reducing starch formation, and this is possibly mediated through mechanisms associated with conversion of soluble sugars into starch caused by the unfavorable sodium: potassium ratio and homeostasis. The salt tolerant genotype IR651 had higher soluble sugars and lower starch concentration during exposure to salt stress, suggesting that the tolerance of this genotype is probably

mediated through mechanisms other than maintenance of higher starch/total carbohydrate ratio under salt stress. Tolerance of salt stress is related to the ability to maintain higher concentration of chlorophyll *a* and greater chlorophyll *a*/*b* ratio in plant tissues under salt stress. Further studies using a large and more diverse set of rice genotypes are needed to evaluate the potential use of these traits in breeding salt tolerant rice varieties.

ACKNOWLEDGEMENTS

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