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Full Length Research Paper

Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (*Oryza sativa* L. spp. *indica)* roots under iso-osmotic conditions

Kongake Siringam[1], Niran Juntawong[1,2*], Suriyan Cha-um[3] and Chalermpol Kirdmanee[3]

[1]Department of Botany, Faculty of Science, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand.
[2]Center for Advanced Studies in Tropical Natural Resources, National Research University-Kasetsart University, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand (CASTNAR, NRU-KU, Thailand).
[3]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA), Klong Luang, Pathumthani, 12120, Thailand.

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Excess salt induced ionic and osmotic stresses that disturbed metabolism and led to reduction of plant development. Previous studies reported that sugars in stressed plants were involved in stress tolerance. However, the role of sugars in salt-stressed plants against only ionic effects is still unclear. The objective of this research was to investigate accumulation and homeostasis of ions, membrane injury, water content, growth characters and sugar contents in roots, in-response to salt stress under iso-osmotic conditions. Salt-sensitive rice, Pathumthani1 (PT1) was grown on MS culture medium for 7 days and was adjusted to salt stress under iso-osmotic conditions (-1.75 ± 0.20 MPa) by mannitol for 4 days. An increase in NaCl increased Na^+ and Na^+:K^+ in PT1 roots leading to increased membrane injury, while the water content was decreased. Additionally, growth characters, including number, length, fresh weight and dry weight of roots, were inhibited. Sugar accumulations in PT1 roots were enhanced by increases in NaCl. The increase in Na^+ was positively related to total soluble sugars, resulting in an osmotic adjustment of the membrane that maintained water availability. The accumulation of sugars in PT1 roots may be a primary salt-defense mechanism and may function as an osmotic control.

Key words: Mannitol, membrane injury, oligosaccharides, sodium ion, potassium ion, sodium chloride.

INTRODUCTION

Salt-affected soil is one of the serious abiotic stresses that cause reduced plant growth, development and productivity worldwide (Qadir et al., 2008). In salt-affected soil, there are many salt contaminants, especially NaCl which readily dissolves in water to yield the toxic ions, sodium ion (Na^+) and chloride ion (Cl^-). Also, the water available in the salt-contaminated soil is restricted, inducing osmotic stress (Castillo et al., 2007; Pagter et al., 2009). Na^+ is a small molecule that is easily absorbed into root tissues of higher plants and transported throughout plant organs, leading to toxic ion damage, osmotic stress and nutritional imbalance (Cha-um et al., 2007; Siringam et al., 2009). Root tissues are the first barrier which not only select nutrient ions but also protect against toxic ions. Excess Na^+ in plant cells directly damages membrane systems and organelles, resulting in plant growth reduction and abnormal development prior to plant death (Essah et al., 2003; Tester and Davenport, 2003; Davenport et al., 2005; Quintero et al., 2007). In halophyte species, there are many salt-defense mechanisms including ion homeostasis, osmoregulation, antioxidant and hormonal regulation (Hasegawa et al., 2000; Sairam and Tyagi, 2004). Sugars are compatible solutes which accumulate in plant tissues that are exposed to abiotic stresses, such as, water deficit, ex-

*Corresponding author. E-mail: juntawongn@yahoo.com. Tel: 662-562 5444. Fax: 662-9405627.

treme temperatures and salt stress. The accumulation of sugars may play an important role in the plant defensive mechanisms of osmoregulation and energy preservation (Norwood et al., 2003; Minorsky, 2003; Morsy et al., 2007).

Rice (*Oryza sativa* L. spp. *indica*) is a top five world carbohydrate crop (Khush, 1997), especially in Asia. It has been previously classified as being salt-susceptible in both the vegetative and reproductive stages (Zeng et al., 2001; Moradi and Ismail, 2007), leading to a reduction in productivity of more than 50% when exposed to 6.65 dS m^{-1} electrical conductivity (EC) of salinity (Zeng and Shannon, 2000). In Thailand, the Pathumthani1 (PT1) cultivar is an aromatic rice which has high cooking quality (long grain and soft texture) and high export value (Laohakunjit and Kerdchoechuen, 2007). It is a major cultivar widely grown in irrigated paddy fields and reported as being salt susceptible (Cha-um et al., 2007; Siringam et al., 2009). Previous studies showed that the response of rice included the effects of osmotic stress and ionic stress. However, the previous results could not separate osmotic effects from ionic effects (Radic et al., 2006; Ahmad et al., 2007). A rapid response in the root tissues of the salt-sensitive rice cultivar PT1 exposed to salt stress under iso-osmotic conditions is an attractive issue that needs to be clarified. Therefore, for this study we hypothesized that an increase of sugar contents could be a salt-defense mechanism. The objective of this research was to investigate the responses of physiological characteristics and sugar contents in PT1 salt-sensitive rice roots to salt stress under iso-osmotic conditions.

MATERIALS AND METHODS

Plant materials and salt stress treatments

Seeds of the PT1 rice cultivar (*O. sativa* L. spp. *indica*) were obtained from the Rice Research Institute, Pathumthani Rice Research Center, Pathumthani, Thailand. Rice seeds were dehusked, disinfected once in 5% (v/v) Clorox® [5.25% (w/v) sodium hypochlorite solution, Clorox Co. Ltd., USA] for 12 h, once in 25% (v/v) Clorox® for 30 min, and then rinsed with sterile distilled water. Surface-disinfected seeds were germinated on 25 ml 0.25% Phytagel®-solidified MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose (w/v) (photomixotrophic condition) in a 250 ml glass vessel. The medium pH was adjusted to 5.7 before autoclaving. *In vitro* rice seedlings were cultured in a culture room under conditions of 25 ± 2°C air temperature, 60 ± 5% relative humidity (RH), 60 ± 5 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) provided by fluorescent lamps (TLD 36 W/84 Cool White 3350 lm, Philips, Thailand) with a 16 h d^{-1} photoperiod. Fourteen-day-old seedlings were aseptically transferred to MS sugar-free liquid medium (photoautotrophic condition) using vermiculite as a supporting material in NUAIRE™ Biological Safety Cabinets (Model NU-440-400E Series 9, NuAire Inc., USA) and left on the shelf in the culture room for 7 days. Air-exchange in the glass vessels was adjusted to 2.32 μmol CO_2 h^{-1} by punching a hole in the plastic cap (∅ 1 cm) and covering the hole with a gas-permeable microporous polypropylene film (0.22 μm pore size, Nihon Millipore Ltd., Japan). To adjust iso-osmotic conditions to -1.75 ± 0.20 MPa in the culture medium, 548.9, 329.4, 219.6, 109.8 or 0.0 mM mannitol were added to 0.0, 85.5, 171.0, 256.5 or 342.0 mM NaCl, respectively and plants were cultured for 4 days. Sodium ions (Na^+), potassium ions (K^+), ion homeostasis (Na^+:K^+), membrane injury, water content, growth characters and sugar contents in PT1 rice roots were evaluated.

Data measurements

Ion contents

One hundred milligrams of rice roots were ground in liquid nitrogen and extracted by the acidic method (Cha-um et al., 2007). Sodium ions (Na^+) and potassium ions (K^+) in the roots were determined according to Dionisio-Sese and Tobita (1998) by using an atomic absorption spectrophotometer (AA, Model M6, Thermo Elemental, MA, USA). In addition, Na^+:K^+ was calculated following Lee et al. (2003).

Membrane injury

The membrane injury in roots was determined by the modified method of Shalata and Neumann (2001). The rice roots were cut into 5.0 ± 0.2 mm in lengths and placed in glass vessels (Opticlear®; KIMBLE, Vineland, New Jersey, USA) containing 10 ml deionized water. The glass vessels were capped and maintained at room temperature (25°C) for 2 h. The initial electrical conductivity (EC_1) was measured by using an electrical conductivity meter (Model ID1010, INDEX, Kuala Lumpur, Malaysia). The root tissues were incubated at 100°C in a water bath for 30 min, cooled down to 25°C and then the electrical conductivity (EC_2) was measured. The membrane injury was calculated following Shalata and Neumann (2001).

Growth parameters

Root number, root length and root fresh weight were measured after exposure to salt stress under iso-osmotic conditions for 4 days. The rice roots were dried in a hot-air oven (Memmert, Model 500, Germany) at 110°C for 48 h and then incubated in a desiccator before measuring the dry weight. Water content was calculated following Bonnet et al. (2000).

Sugar determinations

Sugar extractions

Sugar contents in rice roots were extracted by the modified method of Karkacier et al. (2003). Fifty-milligrams of rice roots were ground in liquid nitrogen with a pestle in a pre-cooled eppendorf tube. One milliliter nanopure water was added and then sonicated for 15 min. The aliquot was centrifuged at 12,000 rpm for 15 min. Supernatant was collected and filtered through a 0.45 μm millipore filter (VertiClean™; NYLON Syringe, Vertical Chromatography Co., Ltd., Thailand) and stored at -20°C prior to sugar content determinations.

Sugar analysis

Stachyose and raffinose were analyzed by high performance liquid chromatography (HPLC) integrated with a 410 differential refractometer (RI) detector that consisted of a Waters 600 gradient controller pump (Water, Milford, MA, USA) and on-line detection monitored by a RI detector. The stachyose and raffinose were analyzed by Empower software. Chromatography of stachyose and raffinose was performed by a VertiSep PRP-NH_2 column (4.6×250

Table 1. Sodium ions (Na^+), potassium ions (K^+), Na^+:K^+ and membrane injury in fourteen-day-old PT1 salt-sensitive rice roots cultured on MS medium and subsequently exposed to salt stress under iso-osmotic conditions (-1.75 ± 0.2 MPa) for 4 days (n = 4).

NaCl (mM)	Mannitol (mM)	Na^+ (mg g^{-1} FW)	K^+ (mg g^{-1} FW)	Na^+:K^+	Membrane injury (%)
0.0	548.9	0.75 d	13.85	0.058 c	45.1 c
85.5	329.4	3.55 c	10.05	0.473 bc	45.2 c
171.0	219.6	11.29 b	15.13	0.749 ab	66.3 b
256.5	109.8	14.41 a	13.99	1.043 a	71.5 ab
342.0	0.0	13.38 a	11.80	1.138 a	79.2 a
ANOVA		**	ns	*	**

Means with different letters in the same column are significantly different at P ≤ 0.01 (**), P ≤ 0.05 (*) and non-significant (ns) by Duncan's new multiple range test. ANOVA, Analysis of variance.

mm) (Ligand Scientific Co., Ltd., Thailand) equipped with a guard column. Acetonitrile:nanopure water (75:25; v/v) was used as the mobile phase. An internal standard of stachyose and raffinose was added to the sample. The injection volume was 40 µl and the flow rate was set at 1.0 ml min^{-1}. In addition, sucrose, glucose and fructose were analyzed by Metacarb 87C (7.8×300 mm) (Varian Inc., USA) equipped with guard column. Nanopure water was used as the mobile phase. The injection volume was 40 µl and the flow rate was set at 0.4 ml min^{-1}. Quantification of sugar contents were performed by comparing the peak areas with the sugar standard solutions. Stachyose, raffinose, sucrose, glucose and fructose (Sigma, Germany) were used as standards and sugar contents were calculated using a standard curve equation.

Experimental design and data analysis

The experiment was arranged as a completely randomized design (CRD) with four replications per treatment, five seedlings per replicate. One-way analysis of variance (ANOVA) was performed using SPSS software. Mean values in each treatment were compared by Duncan's new multiple range test (DMRT).

RESULTS AND DISCUSSION

Effect of NaCl on ion contents, ion homeostasis and membrane injury

Sodium ions (Na^+), ion homeostasis (Na^+:K^+) and membrane injury in PT1 salt-sensitive rice roots progressively increased with increasing NaCl concentrations in the culture medium (Table 1) while potassium ion (K^+) levels were not significantly related to increasing NaCl concentrations (Table 1). In this study, Na^+ accumulation in PT1 salt-sensitive rice roots showed a positive relationship to NaCl concentrations in the culture medium. The Na^+ in PT1 roots exposed to 85.5, 171.0, 256.5 and 342.0 mM NaCl accumulated 4.7, 15.0, 19.2 and 17.8 folds, respectively, when compared to the control (without salt-stress induction) (Table 1). In addition, the Na^+:K^+ was enriched 8.2, 12.9, 18.0 and 19.6 folds, respectively, when compared to the control (Table 1). The membrane injury in PT1 roots was 1.0, 1.5, 1.6 and 1.8 folds at 85.5, 171.0, 256.5 and 342.0 mM NaCl, respectively, when compared to the control (Table 1). The Na^+ in the salt-sensitive rice roots was increased, while the K^+ was unchanged, leading to an increased Na^+: K^+ ($P < 0.05$, $r = 0.96$) (Figure 1A), leading to increased membrane injury ($P < 0.05$, $r = 0.96$) (Figure 1B), resulting in growth inhibition (Table 2). Moreover, the Na^+ was negatively related to the water content ($P < 0.05$, $r = 0.98$) (Figure 1C).

Generally, Na^+ is accumulated overall in plant tissues of salt-sensitive cultivars of rice such as IR 28 (Dionisio-Sese and Tobita, 1998; Nakamura et al., 2002), PT1 (Cha-um et al., 2007; Siringam et al., 2009), IR20 (Krishnamurthy et al., 2009), Khao Dawk Mali 105 (KDML105) (Summart et al., 2010) and I Kong Pao (IKP) (Lefevre et al., 2001), relative to salt stress levels. In this study, an increase in Na^+ in PT1 roots was related to increasing salt stress under iso-osmotic conditions (Table 1). Similar to Nakamura et al. (2002), who reported that in IR28 salt-sensitive rice cultivar, Na^+ was increased 3.0 folds over the control (0 mM NaCl) when exposed to 113 mM NaCl for 14 days. In addition, the Na^+ in PT1 leaves was increased 28.0 folds over the control when exposed to 342 mM NaCl for 4 days (Siringam et al., 2009). The Na^+ accumulation was increased while K^+ was unchanged. In contrast, the previous studies showed that the salt stress enhanced Na^+ accumulation while K^+ was decreased in salt-sensitive rice varieties such as IR28 (Dionisio-Sese and Tobita, 1998; Nakamura et al., 2002), IR20 (Krishnamurthy et al., 2009) and I Kong Pao (IKP) (Lefevre et al., 2001). It might be possible that the PT1 salt-sensitive rice variety exposed to NaCl may use other salt-defense mechanisms for survival. An increase in Na^+ in the salt-stressed rice roots directly increased the Na^+:K^+, especially in salt-sensitive cultivars. Damaging of cell membranes was identified by electrolyte leakage (Dionisio-Sese and Tobita, 1998) and water balance (Lefevre et al., 2001; Nakamura et al., 2002). At 342 mM NaCl, PT1 roots lost the membrane function of conserving ion homeostasis involved in controlling the Na^+ intake and K^+ conservation. An increase in Na^+:K^+ in IR29, GZ5310-20-3-2, GZ177, Sakha101 and IR70074-AC14 rice cultivars cultured under 57 mM NaCl for 34

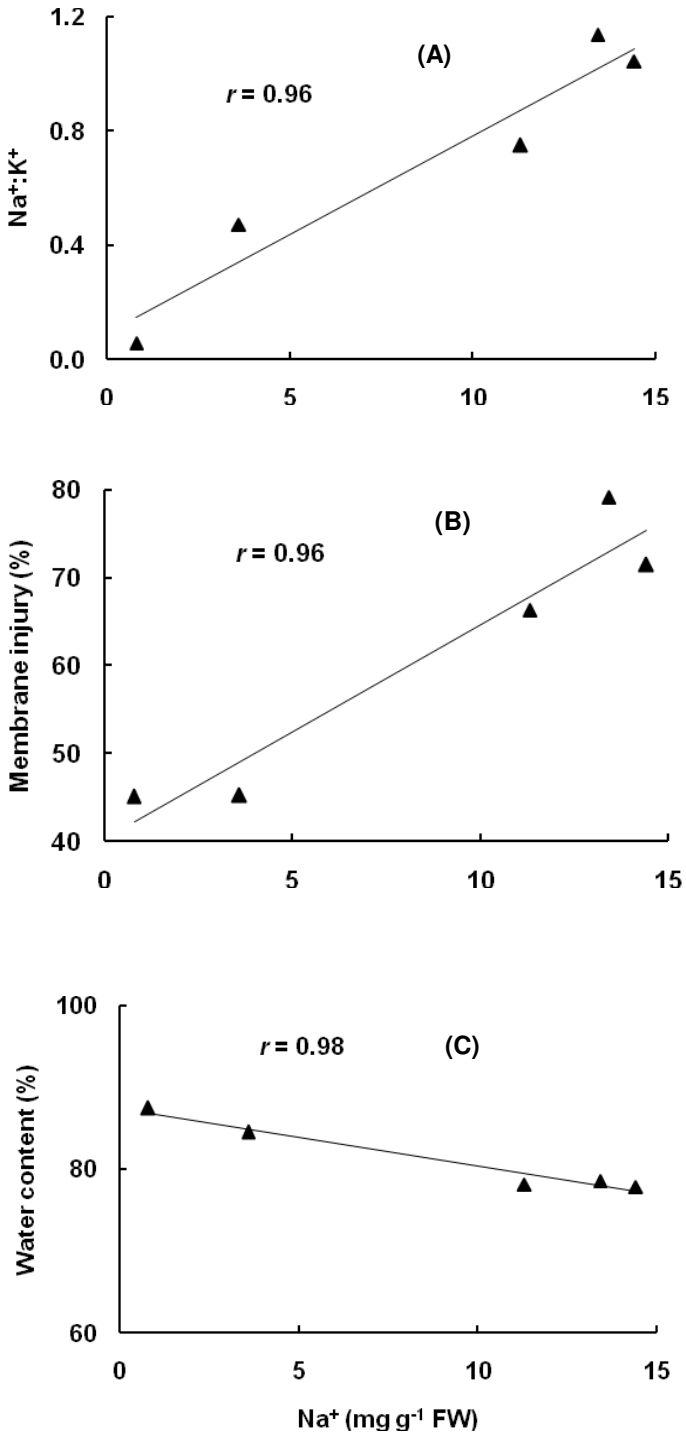

Figure 1. Na^+ induced Na^+:K^+ (A) and membrane injury (B) while water content was decreased (C) in fourteen-day-old PT1 salt-sensitive rice roots cultured on MS medium and subsequently exposed to salt stress under iso-osmotic conditions (-1.75 ± 0.2 MPa) for 4 days (n = 4).

days was reported (Zeng, 2005). Dionisio-Sese and Tobita (1998) reported that at 120 mM NaCl, the membrane injury was increased 8.7 and 12.0 folds in Hitomebore and IR28 salt-sensitive cultivars, respectively in 7 days. In rice plants, the Na^+ accumulation, Na^+:K^+ and membrane injury has been utilized to identify salt-

Table 2. Root number, root length, fresh weight (FW) and dry weight (DW) in fourteen-day-old PT1 salt-sensitive rice roots cultured on MS medium and subsequently exposed to salt stress under iso-osmotic conditions (-1.75 ± 0.2 MPa) for 4 days (n = 4).

NaCl (mM)	Mannitol (mM)	Root number	Root length (cm)	FW (mg)	DW (mg)
0.0	548.9	11 a	5.2 a	26.7 a	3.3 a
85.5	329.4	7 b	3.7 b	21.9 b	3.3 a
171.0	219.6	6 c	3.3 c	12.5 c	2.3 b
256.5	109.8	6 c	2.9 d	11.2 c	2.2 b
342.0	0.0	6 c	2.6 e	11.0 c	1.8 c
ANOVA		**	**	**	**

Means with different letters in the same column are significantly different at P ≤ 0.01 (**) by Duncan's new multiple range test. ANOVA, Analysis of variance.

Table 3. Sugar contents in fourteen-day-old PT1 salt-sensitive rice roots cultured on MS medium and subsequently exposed to salt stress under iso-osmotic conditions (-1.75 ± 0.2 MPa) for 4 days. (n = 4).

NaCl (mM)	Mannitol (mM)	Contents (μmol g^{-1} FW)				
		Stachyose	Raffinose	Sucrose	Glucose	Fructose
0.0	548.9	7.8 d	6.0 c	37.8 e	94.1 e	-
85.5	329.4	19.5 c	14.5 c	39.2 d	98.1 d	-
171.0	219.6	46.8 a	42.7 a	48.4 a	119.3 a	-
256.5	109.8	39.7 b	28.0 b	43.4 b	110.0 b	-
342.0	0.0	38.9 b	33.1 b	43.1 c	109.0 c	-
ANOVA		**	**	**	**	ND

Means with different letters in the same column are significantly different at P ≤ 0.01 (**) and non-detected (ND) by Duncan's new multiple range test. ANOVA, Analysis of variance.

tolerant or salt-sensitive varieties (Dionisio-Sese and Tobita, 1998; Zeng et al., 2004; Zeng, 2005).

Effect of NaCl on sugar accumulations

The sugar contents including stachyose, raffinose, sucrose and glucose in PT1 roots were enhanced by increasing NaCl concentrations, while a fructose profile did not appear (Table 3). At 171 mM NaCl, the stachyose, raffinose, sucrose and glucose in PT1 roots peaked at 46.8, 42.7, 48.4 and 119.3 μmol g^{-1} FW, respectively and were 6.0, 7.1, 1.3 and 1.3 folds, respectively, when compared to the control (Table 3). The Na^+ levels in PT1 roots were positively related to total soluble sugars ($P < 0.05$, $r = 0.87$) (Figure 2A). Furthermore, sucrose induced oligosaccharide (stachyose and raffinose) accumulations ($P < 0.05$, $r = 0.96$) (Figure 2B).

Sugar accumulations in salt-stressed plants have salt tolerant ability and play an important role in a salt-defense mechanism when plants are exposed to salt stress (Bohnert and Jensen, 1996). Salt stress induced sugar accumulations in many species, such as barley (Ahmad et al., 2006), sorghum (Almodares et al., 2008a), tomato (Chookhampaeng et al., 2008), rice (Pattanagul and Thitisaksakul, 2008) and eggplant (Abbas et al., 2010). The increase of stachyose, raffinose, sucrose and glucose in PT1 roots (Table 3) may function as an osmotic adjustment to prevent water loss in the plant cells during salt stress (Cushman, 2001; Norwood et al., 2003; Sairam and Tyagi, 2004). This relationship was similar to *Arabidopsis* (Taji et al., 2002), algarrobo (Meloni et al., 2004), poplar (Jouve et al., 2004), tomato (Khelil et al., 2007), rice (Morsy et al., 2007; Cha-um et al., 2008), sweet sorghum (Almodares et al., 2008b) and maize (Hajlaoui et al., 2010). Furthermore, an increase in oligosaccharides (stachyose and raffinose) resulted from the increased sucrose content (Figure 2B). This result agreed with Karner et al. (2004) who reported that sucrose was the secondary substrate for raffinose, which played a key role in the raffinose family oligosaccharides (RFOs) biosynthesis. Therefore, variation of sucrose levels may affect the formation of RFOs which may play a crucial role in maintaining membrane stability *via* interaction with phospholipid headgroups and may scavenge reactive oxygen species when exposed to salt stress (Bohnert and Jensen, 1996; Bentsink et al., 2000; Roy et al., 2005).

In conclusion, the Na^+ in PT1 salt-sensitive rice roots was directly increased with higher NaCl concentrations. The Na^+:K^+ and membrane injury derived from Na^+ toxicity was demonstrated, leading to lower water content

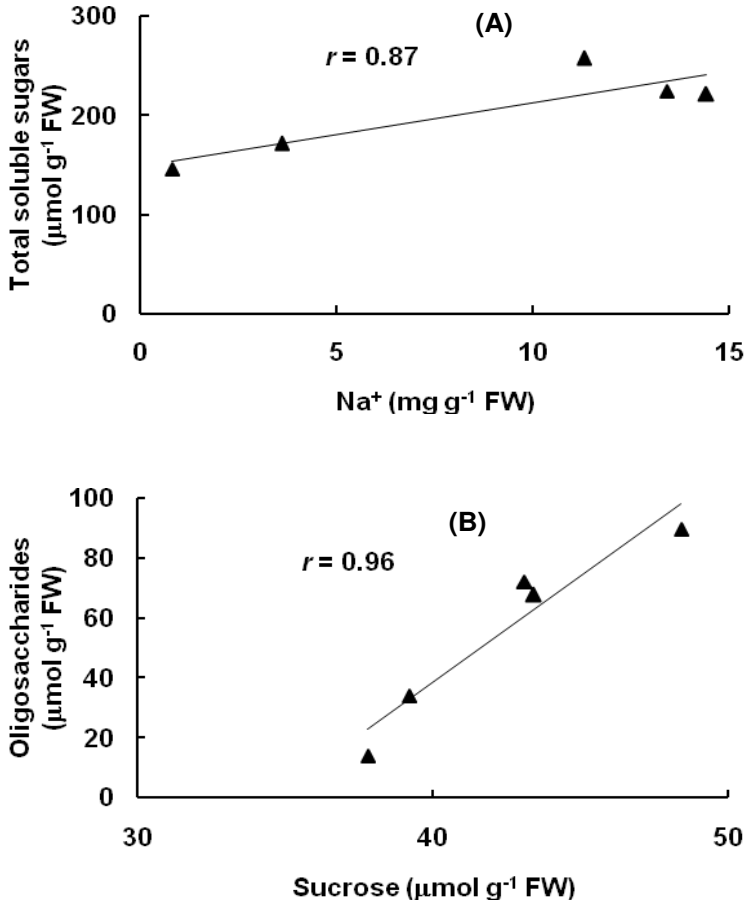

Figure 2. Total soluble sugars (A) and oligosaccharides (B) were induced by Na^+ and sucrose, respectively, in fourteen-day-old PT1 salt-sensitive rice roots cultured on MS medium and subsequently exposed to salt stress under iso-osmotic conditions (-1.75 ± 0.2 MPa) for 4 days (n = 4).

and growth inhibition. In addition, accumulation of soluble sugar contents was related to the increase of Na^+ and may play a role as an osmotic adjustment to maintain the water use efficiency in the root cells when exposed to salt stress.

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REFERENCES

Abbas W, Ashraf M, Akram NA (2010). Alleviation of salt-induced adverse effects in eggplant (*Solanum melongena* L.) by glycinebetaine and sugarbeet extracts. Sci. Hort. 125: 188-195.

Ahmad MSA, Ali Q, Bashir R, Javed F, Alvi AK (2006). Time course changes in ionic composition and total soluble carbohydrates in two barley cultivars at seedling stage under salt stress. Pak. J. Bot. 38: 1457-1466.

Ahmad MSA, Javed F, Ashraf M (2007). Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two indica rice (*Oryza sativa* L.) genotypes. Plant Growth Regul. 53: 53-63.

Almodares A, Hadi MR, Ahmadpour H (2008a). Sorghum stem yield and soluble carbohydrates under different salinity levels. Afr. J. Biotechnol. 7: 4051-4055.

Almodares A, Hadi MR, Dosti B (2008b). The effects of salt stress on growth parameters and carbohydrates contents in sweet sorghum. Res. J. Environ. Sci. 2: 298-304.

Bentsink L, Alonso-Blanco C, Vreugdenhil D, Tesnier K, Groot SPC, Koornneef M (2000). Genetic analysis of seed soluble oligosaccharides in relation to seed storability of *Arabidopsis*. Plant Physiol. 124: 1595-1604.

Bohnert HJ, Jensen RG (1996). Strategies for engineering water stress tolerance in plants. Trend Plant Sci. 14: 89-97.

Bonnet M, Camares O, Veisseire P (2000). Effect of zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll *a* fluorescence and antioxidant enzyme activities of ryegrass (*Lolium perenne* L. cv. Apollo). J. Exp. Bot. 51: 945-953.

Castillo EG, Tuong TP, Ismail AM, Inubushi K (2007). Response to

salinity in rice: Comparative effects of osmotic and ionic stresses. Plant Prod. Sci. 10: 159-170.
Cha-um S, Vejchasarn P, Kirdmanee C (2007). An effective defensive response in Thai aromatic rice varieties (*Oryza sativa* L. spp. *indica*) to salinity. J. Crop Sci. Biotechnol. 10: 257-264.
Cha-um S, Charoenpanich A, Roytrakul S, Kirdmanee C (2008). Sugar accumulation, photosynthesis and growth of two indica rice varieties in response to salt stress. Acta Physiol. Plant, 31: 477-486.
Chookhampaeng S, Pattanagul W, Theerakulpisut P (2008). Effects of salinity on growth, activity of antioxidant enzymes and sucrose content in tomato (L*ycopersicon esculentum* Mill.) at the reproductive Stage. Sci. Asia, 34: 69-75.
Cushman JC (2001). Osmoregulation in plants: implications for agriculture. Am. Zool. 41: 758-769.
Davenport R, James RA, Zakrisson-Plogander A, Tester M, Munns R (2005). Control of sodium transport in durum wheat. Plant Physiol. 137: 807-818.
Dionisio-Sese ML, Tobita S (1998). Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135: 1-9.
Essah PA, Davenport R, Tester M (2003). Sodium influx and accumulation in Arabidopsis. Plant Physiol. 133: 307-318.
Hajlaoui H, El Ayeb N, Garrec JP, Denden M (2010). Differential effects of salt stress on osmotic adjustment and solutes allocation on the basis of root and leaf tissue senescence of two silage maize (*Zea mays* L.) varieties. Ind. Crops Prod. 31: 122-130.
Hasegawa PM, Bressan RA, Zhu J, Bohnert HJ (2000). Plant cellular and molecular responses to high salinity. Annu. Rev. Plant Mol. Biol. 51: 463-499.
Jouve L, Hoffmann L, Hausman JF (2004). Polyamine, carbohydrate, and proline content changes during salt stress exposure of Aspen (*Populus tremula* L.): involvement of oxidation and osmoregulation metabolism. Plant Biol. 6: 74-80.
Karkacier M, Erbas M, Uslu MK, Aksu M (2003). Comparison of Different Extraction and Detection Methods for Sugars Using Amino-Bonded Phase HPLC. J. Chromatogr. Sci. 41: 331-333.
Karner U, Peterbauer T, Raboy V, Jones DA, Hedley CL, Richter A (2004). Myo-inositol and sucrose concentrations affect the accumulation of raffinose family oligosaccharides in seeds. J. Exp. Bot. 55: 1981-1987.
Khelil A, Menu T, Ricard B (2007). Adaptive response to salt involving carbohydrate metabolism in leaves of a salt-sensitive tomato cultivar. Plant Physiol. Biochem. 45: 551-559.
Khush GS (1997). Origin, dispersal, cultivation and variation of rice. Plant Mol. Biol. 35: 25-34.
Krishnamurthy P, Ranathunge K, Franke R, Prakash HS, Schreiber L, Mathew MK (2009). The role of root apoplastic transport barriers in salt tolerance of rice (*Oryza sativa* L.). Planta, 230: 119-134.
Laohakunjit N, Kerdchoechuen O (2007). Aroma enrichment and the change during storage of non-aromatic milled rice coated with extracted natural flavor. Food Chem. 101: 339-344.
Lee KS, Choi WY, Ko JC, Kim TS, Gregorio GB (2003). Salinity tolerance of japonica and indica rice (*Oryza sativa* L.) at the seedling stage. Planta, 216: 1043-1046.
Lefevre I, Gratia E, Lutts S (2001). Discrimination between the ionic and osmotic components of salt stress in relation to free polyamine level in rice (*Oryza sativa*). Plant Sci. 161: 943-952.
Meloni DA, Gulotta MR, Mart-nez CA, Oliva MA (2004). The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in *Prosopis alba*. Braz. J. Plant Physiol. 16: 39-46.
Minorsky PV (2003). Raffinose oligosaccharides. Plant Physiol. 131: 1159-1160.
Moradi F, Ismail AM (2007). Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann. Bot. 99: 1161-1173.
Morsy MR, Jouve L, Hausman JF, Hoffmann L, Stewart JD (2007). Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. J. Plant Physiol. 164: 157-167.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473-479.
Nakamura I, Murayama S, Tobita S, Bong BB, Yanagihara S, Ishimine Y, Kawamitsu Y (2002). Effect of NaCl on the photosynthesis, water relations and free proline accumulation in the wild *Oryza* species. Plant Prod. Sci. 5: 305-310.
Norwood M, Toldi O, Richter A, Scott P (2003). Investigation into the ability of roots of the poikilohydric plant *Craterostigma plantagenium* to survive dehydration stress. J. Exp. Bot. 54: 2313-2321.
Pagter M, Bragato C, Malagori M, Brix H (2009). Osmotic and ionic effects of NaCl and Na_2SO_4 salinity on *Phragmites australis*. Aquat. Bot. 90: 43-51.
Pattanagul W, Thitisaksakul M (2008). Effect of salinity stress on growth and carbohydrate metabolism in three rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. Indian J. Exp. Biol. 46: 736-742.
Qadir M, Tubeileh A, Akhtar J, Larbi A, Minhas PS, Khan MA (2008). Productivity enhancement of salt-affected environments through crop diversification. Land Degradr. Dev. 19: 429-453.
Quintero JM, Fournier JM, Benlloch M (2007). Na^+ accumulation in shoot is related to water transport in K^+-starved sunflower plants but not in plants with a normal K^+ status. J. Plant Physiol. 164: 60-67.
Radic S, Radic-Stojkovic M, Pevalek-Kozlina B (2006). Influence of NaCl and mannitol on peroxidase activity and lipid peroxidation in *Centaurea ragusina* L. roots and shoots. J. Plant Physiol. 163: 1284-1292.
Roy P, Niyogi K, Gupta DNS, Ghosh B (2005). Spermidine treatment to rice seedlings recovers salinity stress-induced damage of plasma membrane and PM-bound H^+-ATPase in salt-tolerant and salt-sensitive rice cultivars. Plant Sci. 168: 583-591.
Sairam RK, Tyagi A (2004). Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci. 86: 407-421.
Shalata A, Neumann PM (2001). Exogenous ascorbic acid (vitamin C) increases resistance to salt stress and reduces lipid peroxidation. J. Exp. Bot. 52: 2207-2211.
Siringam K, Juntawong N, Cha-um S, Kirdmanee C (2009). Relationships between sodium ion accumulation and physiological characteristics in rice (*Oryza sativa* L. spp. *indica*) seedlings grown under iso-osmotic salinity stress. Pak. J. Bot. 41: 1837-1850.
Summart J, Thanonkeo P, Panichajakul S, Prathepha P, McManus MT (2010). Effect of salt stress on growth, inorganic ion and proline accumulation in Thai aromatic rice, Khao Dawk Mali 105, callus culture. Afr. J. Biotechnol. 9: 145-152.
Taji T, Ohsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Shinozaki KY, Shinozaki K (2002). Important roles of drought-and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. Plant J. 29: 417-426.
Tester M, Davenport R (2003). Na^+ tolerance and Na^+ transport in higher plants. Ann. Bot. 91: 503-527.
Zeng L, Shannon MC (2000). Salinity effects on seedling growth and yield components of rice. Crop Sci. 40: 996-1003.
Zeng L, Shannon MC, Lesch SM (2001). Timing of salinity stress affects rice growth and yield components. Agric. Water Manage. 48: 191-206.
Zeng L, Kwon TR, Liu X, Wilson C, Grieve CM, Gregorio GB (2004). Genetic diversity analyzed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptations to saline soils. Plant Sci. 166: 1275-1285.
Zeng L (2005). Exploration of relationships between physiological parameters and growth performance of rice (*Oryza sativa* L.) seedlings under salinity stress using multivariate analysis. Plant Soil, 268: 51-59.