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Salt tolerance in two rice cultivars differing salt tolerant abilities in responses to iso-osmotic stress

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Abstract

The aim of this investigation was to study on the salt tolerant ability of Thai jasmine (KDML105) salt sensitive and Homjan (HJ) salt tolerant cultivars grown under iso-osmotic stresses. Growth, ion contents, relative electrolyte leakage (REL), photosynthetic pigments and net photosynthetic rate (P_n) in iso-osmotic stressed seedlings were collected. Growth characters including shoot height, fresh weight, dry weight and leaf area of salt-stressed rice seedlings were inhibited, depending on NaCl concentrations and rice genotypes. Sodium ion (Na^+) in salt-stressed tissues was rapidly accumulated, especially in KDML105, while potassium ion (K^+) was quickly decreased. Na:K ratio and proline content in salt-stressed leaves were increased, relating to salt concentrations. The Na^+ accumulation in the salt stressed seedlings was positively related to osmolarity, causing to membrane injury or high REL with pigment degradation. The degradation of total chlorophyll (TC) and total carotenoids (C_{x+c}) in salt-stressed seedlings grown under osmotic stresses were positively correlated with P_n, leading to growth retardation. The salt tolerant mechanisms in HJ salt tolerance should be further investigated as well as utilized as parental line for salt-tolerant breeding program.

Keywords: aromatic rice; net photosynthetic rate; *Oryza sativa* L. spp. *indica*; pigments; relative electrolyte leakage; salt stress.

Abbreviations: Chl_a_chlorophyll a; Chl_b_chlorophyll b; CRD_completely randomized design; C_{x+c}_total carotenoids; DMRT_Duncan's new multiple range test; E_transpiration rate; EL_0_initial electro conductivity; EL_1_final electro conductivity; g_s_stomatal conductance; HJ_Homjan rice; IRGA_infra red gas analyser; KDML105_Jasmine rice; MS_Murashige and Skoog; P_n_net photosynthetic rate; PEG_polyethylene glycol; PPFD_photosynthetic photon flux density; REL_ relative electrolyte leakage; RH_relative humidity; TC_total chlorophyll; WUE_water use efficiency.

Introduction

Saline soil is one of the most important abiotic stresses to directly reduce on plant growth and developments (Mühling and Läuchli, 2001; Maathuis, 2006; Galvani, 2007; Läuchli and Grattan, 2007). It widely distributes in irrigated and non-irrigated agricultural areas. Na^+ and Cl^- derived from NaCl salts are contaminated in the saline soil, which are well known as the toxic ions to damage the plant cells in both ionic and osmotic effects. Plant growth and development are directly inhibited, leading to low yield prior to plant death (Mansour and Salama, 2004; Chinnusamy et al., 2005; Läuchli and Grattan, 2007). Salt tolerant mechanism in higher plants including osmoregulation, ion homeostasis, and hormonal regulation is a fruitful topic to be discovered by many scientists (Hasegawa et al., 2000; Munns et al., 2002; Mansour and Salama, 2004; Chinnusamy et al., 2005; Zuther et al., 2007). Salt-tolerant defense mechanisms have been identified in many plant species, especially halophyte species (Hasegawa et al., 2000; Breckle, 2002; Lüttge, 2002). However, the salt defense mechanisms in

Table 1. Shoot height, shoot fresh weight, shoot dry weight and leaf area in Homjan (HJ) and Jasmine (KDML 105) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

Rice variety	NaCl (mM)	Shoot height (cm)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Leaf area (cm^2)
HJ	0	9.80ab	68.53a	9.90a	296ab
	85	10.95a	61.90ab	8.68a	269ab
	171	9.55ab	59.98ab	8.63a	227b
	256	9.08ab	56.37ab	7.55ab	175bc
	342	8.98ab	52.63ab	7.25ab	166bc
KDML105	0	7.18ab	58.48ab	9.03a	326a
	85	6.98b	48.50ab	8.70a	268ab
	171	6.25b	47.50ab	8.65a	253ab
	256	6.13b	45.63ab	7.00ab	179bc
	342	5.70b	37.80b	6.00b	159c
Significant level					
Variety		**	**	*	**
NaCl		*	**	NS	**
Variety × NaCl		NS	NS	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) or $p \leq 0.05$ (*) by Duncan's New Multiple Range Test (DMRT). Non significant in statistical analysis is represented by ^{NS}.

crop species are still need to investigate for crop improvement through breeding program.

Rice is a major crop in many regions of the world, especially Asian countries. It is a staple food to feed more than 3 billion people and to provide 50-80% daily calorie intake (Khush, 2005). Rice crop has been identified as salt-susceptible, which is showed the negative effects on both seedling and reproductive stages (Shannon et al., 1998; Zeng and Shannon, 2000; Khan and Abdullah, 2003; Zeng et al., 2003). Salt-tolerant breeding program in rice is a profitable issue for plant breeders (Gregorio et al., 2002; Senadhira et al., 2002; Flowers and Flowers, 2005). For examples, salt-tolerant landrace, cultivars and breeding lines of rice are Pokkali, Nona-Bokra, Agami, Daeyabyeo, GZ5310-20-2-1, GZ5310-20-3-2, GZ5310-20-3-3 and IR4630-22-2-2-5-1-3. These varieties have been identified and utilized as the parental lines in salt-tolerant breeding programs. Whereas, IR 26, M-104, M-202, M-205, L-205, S-102, GZ177, Sakha101, GZ5121-5-2-1, GZ5291-7-1-2 and IR63352-AC202 rice cultivars are defined as salt susceptible (Zeng et al., 2004; Zeng, 2005). In Thailand, Homjan (HJ) rice variety has been identified as salt tolerance using multivariate parameters (Cha-um et al., 2007a). In addition, KDML105 or jasmine rice is widely cultivated in northeastern Thailand which is a large area of salinity problem. The jasmine rice paddy field in this area is a high cooking quality including long grain, softness texture and high jasmine flavor. The aim of this investigation was to study on the salt tolerant ability of KDML105 and HJ rice varieties grown under iso-osmotic salt stress.

Materials and methods

Plant materials and stress treatments

Jasmine salt-sensitive and Homjan (HJ) salt-tolerant (GS No. 4371) rice seeds were obtained from the Pathumthani Rice Research Center (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand). Seeds were dehusked by hand, sterilized once in 5% Clorox® (5.25% sodium hypochlorite, The Clorox Co, Oakland, CA, USA) for 60 min, once in 30% Clorox® for 30 min, and then rinsed thrice by sterile distilled-water. Surface-sterilized seeds were germinated on 0.25% Phytagel®-solidified MS media (Murashige and Skoog, 1962) in a 250-mL glass jar vessel. The media were adjusted to pH 5.7 before autoclaving. Seedlings were cultured *in vitro* under condition of 25±2°C ambient temperature, 60±5% relative humidity (RH) and 60±5 µmol m^{-2} s^{-1} photosynthetic proton flux density (PPFD) provided by fluorescence lamps (TDL 36 W/84 Cool White 3350 Im, Phillips, Bangkok, Thailand) with 16 hd^{-1} photoperiod. Fourteen-day-old rice seedlings were aseptically transferred to MS sugar-free liquid media (photoautotrophic condition) using vermiculite as supporting material. The number of air-exchanges in the glass vessels was adjusted to 2.32 h^{-1} by punching a hole on plastic cap (∅ 1 cm) and covering the hole with a microporous filter (0.20 µm of pore size; Nihon Millipore Ltd., Tokyo, Japan). All seedlings were continuously cultured under the same conditions as during the seed germination and subsequently

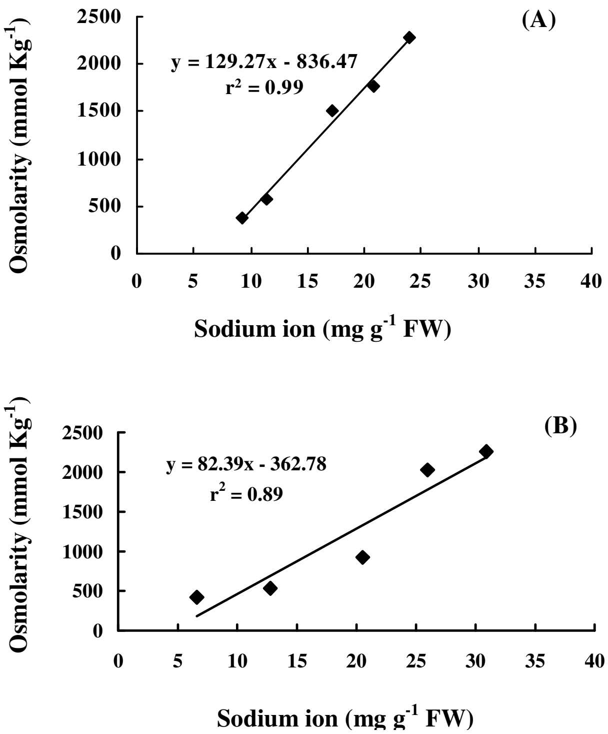


Fig 1. Relationship between sodium ion and osmolarity in Homjan (A) and Jasmine (B) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

exposed to 0, 85, 171, 256 or 342 mM NaCl with iso-osmotic mannitol adjustment (600±50 mmol Kg^{-1}) for 7 days. Sodium ion, potassium ion, osmolarity, proline, membrane leakage, photosynthetic pigments, water relation, P_n and growth parameters were measured.

Data collection

One hundred milligrams of whole plant materials were ground in liquid nitrogen. Sodium and potassium ions in plant materials were extracted using acidic methods (HNO_3 and $HClO_4$) and assayed according to Dionisio-Sese and Tobita (1998) using Atomic Absorption Spectrophotometer (AA, Model M6, Thermo Elemental, MA, USA).

Leaf osmolarities of rice seedlings was measured, according to Lanfermeijer et al. (1991). Fresh leaf tissues (100 mg) were cut into small pieces, transferred to 1.5 mL micro tube, and then debris by glass rod. A twenty micro liter of extracted solution was directly dropped on a disc-shaped filter paper in an osmometer chamber (Wescor, Utah, USA). The osmolarity was then measured.

Relative electrolyte leakage percentage was analyzed according to Dionisio-Sese and Tobita (1998). Fresh leaf tissues (100 mg) were cut into small pieces, transferred to glass vial containing 10 mL deionized-water, covering with plastic cap and then incubated at 32°C in the water bath for 2h. Initial electro conductivity (EL_0) of solution was collected and measured using electrical conductivity meter (Model ID1010, INDEX, Kuala Lumpur, Malaysia). Then, the sample was boiled at 100°C for 30 min, cooled down at 25°C, and then the final electro conductivity (EL_1) was determined. The relative electrolyte leakage (%) was calculated, following the formula REL= $[(EL_0/EL_1) \times 100]$.

Proline content from leaves was extracted according to the method of Bates et al. (1973). Fifty-milligram fresh weights were grounded in a mortar with liquid nitrogen. The homogenate powder was mixed with 1 mL aqueous sulfosalicylic acid (3 % w/v) and filtered through filter paper (Whatman #1, England). Extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25 mg ninhydrin in 30 mL of glacial acetic acid and 20 ml 6 M H_3PO_4) and incubated at 95°C for 1 h. The reaction was terminated placing on an ice bath. The reaction mixture was vigorously mixed with 2 mL toluene. After warming at 25°C, the chromophore was measured by Spectrophotometer DR/4000 (DR/4000, HACH, Loveland, Colorado, USA) at 520 nm as well as L-proline (Fluka, Switzerland) used as a standard.

Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll, and total carotenoid (C_{x+c}) concentrations were analyzed following the methods of Shabala et al. (1998) and Lichtenthaler (1987), respectively. One hundred milligrams of leaf material were collected from the second and third nodes of the shoot tip. The leaf samples were placed in a 25 mL glass vial (Opticlear® KIMBLE, Vineland, New Jersey, USA), added with 10 mL of 95.5% acetone, and blended with a homoginizer (T25 basic ULTRA-TURRAX®, IKA, Kuala Lumpur, Malaysia). The glass vials were sealed with parafilm to prevent evaporation and then stored at 4°C for 48 h. The Chl_a and Chl_b concentrations were measured using an UV-visible Spectrophotometer (DR/4000, HACH, Loveland, Colorado, USA) at 662 nm and 644 nm wavelengths. As well as, the C_{x+c} concentration was measured by Spectrophotometer at 470 nm. A solution of 95.5% acetone was used as a blank. The Chl_a, Chl_b, total chlorophyll and C_{x+c} (μg g^{-1} FW) concentrations in the leaf tissues were calculated according to the following equations.

$$[Chl_a] = 9.784D_{662} - 0.99D_{644}$$

$$[Chl_b] = 21.42D_{644} - 4.65D_{662}$$

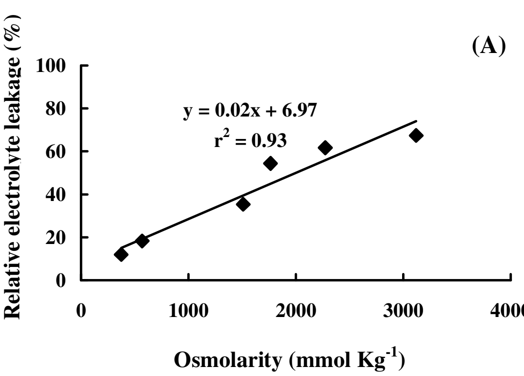
$$\text{Total chlorophyll} = [Chl_a] + [Chl_b]$$

$$[C_{x+c}] = \frac{1000D_{470} - 1.90[Chl_a] - 63.14\,[Chl_b]}{214}$$

Table 2. Sodium (Na), potassium (K), Na:K ratio and proline content in Homjan (HJ) and Jasmine (KDML 105) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

Rice variety	NaCl (mM)	Na ion (mg g^{-1}DW)	K ion (mg g^{-1}DW)	Na:K ratio	Proline content (μg g^{-1} FW)
HJ	0	9.20e	22.36ab	0.41e	169.53e
	85	11.41de	21.57bc	0.53d	153.85e
	171	17.21cd	21.47bc	0.80c	213.00de
	256	20.85bc	21.19bc	0.98bc	456.59c
	342	23.93b	17.67cd	1.16b	586.46bc
KDML105	0	6.60e	27.24a	0.24f	59.22f
	85	12.78de	23.56ab	0.54d	248.42d
	171	20.52bc	23.14ab	0.89bc	288.94d
	256	25.98ab	19.95bc	1.30b	616.01b
	342	30.91a	14.75d	2.10a	774.50a
Significant level					
Variety		**	**	**	**
NaCl		**	*	**	**
Variety × NaCl		**	*	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) or $p \leq 0.05$ (*) by Duncan's New Multiple Range Test (DMRT).



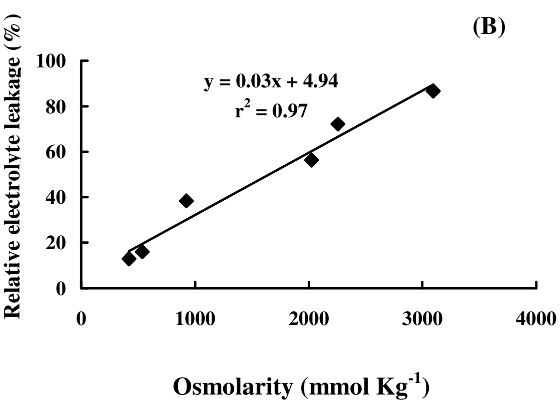


Fig 2. Relationship between osmolarity and relative electrolyte leakage in Homjan (A) and Jasmine (B) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

where D_i is the optical density at wavelength i.

The net-photosynthetic rate (P_n), transpiration rate (E; mmol m^{-2} s^{-1}), stomata conductance (g_s; μmol H_2O m^{-2} s^{-1}) and water use efficiency (WUE; %) of rice seedlings were measured on the leaf using Infra-red Gas Analyser (IRGA; Model Portable Photosynthesis System LI 6400, LI-COR® Inc, Lincoln, Nebraska, USA). The E and g_s were measured continuously monitoring H_2O of the air entering and existing in the IRGA headspace chamber. The flow rate of air in sample line was adjusted to 500 μmol s^{-1}. The microchamber temperature was set at 25°C. The light intensity was fluxed by 6400-02B red-blue LEDs light source at 1,000 μmol m^{-2} s^{-1} PPFD (Cha-um et al., 2007b).

Shoot height, shoot fresh weight, shoot dry weight and leaf area of rice seedlings were measured as described by Cha-um et al. (2006). Rice seedlings were dried at 110 °C in a hot-air oven (Model 500, Memmert, Buchenbach, Germany) for 2 days, and then incubated in desiccators before measurement of the dry weight. Leaf area of rice seedlings was measured using a Leaf Area Meter DT-scan (Delta-Scan Version 2.03, Delta-T Devices, Ltd., Burwell, Cambridge, UK).

Experimental design

The experiment was designed as 2×5 factorials in Completely Randomized Design (CRD) with six replicates and four plantlets per replication. The mean in each treatment was compared by Dancan's New Multiple Range Test (DMRT) at p ≤ 0.01 and analyzed by SPSS software (SPSS for Windows, SPSS Inc., Chicago, Illinois, USA). Relationships

Table 3. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) in Homjan (HJ) and Jasmine (KDML 105) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

Rice variety	NaCl (mM)	Chl_a ($\mu g\ g^{-1}$ FW)	Chl_b ($\mu g\ g^{-1}$ FW)	TC ($\mu g\ g^{-1}$ FW)	C_{x+c} ($\mu g\ g^{-1}$ FW)
HJ	0	314.25cd	148.46cd	462.71cd	105.69c
	85	288.27d	138.29d	426.56cd	102.08c
	171	272.49d	114.46d	386.95d	96.47c
	256	232.75d	104.36d	337.11d	95.88c
	342	224.25d	103.11d	327.36d	89.05c
KDML105	0	519.55a	336.99a	856.54a	208.67a
	85	458.37b	262.76b	721.13b	207.08a
	171	449.06bc	205.86bc	654.92b	198.59ab
	256	433.62bc	200.57bc	634.19b	179.78ab
	342	361.06c	195.95bc	557.01b	164.23b
Significant level					
Variety		**	**	**	**
NaCl		**	**	**	**
Variety × NaCl		**	**	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT).

between sodium ion and osmolarity, osmolarity and membrane leakage, membrane leakage and chlorophyll degradation, chlorophyll degradation and P_n as well as P_n and dry weight were evaluated.

Results and Discussion

Shoot height, fresh-, dry-weight and leaf area in both Homjan (HJ) salt tolerance and Jasmine rice (KDML105) salt sensitivity grown under NaCl stress were significantly decreased when compared to those under 0 mM NaCl or control condition. The results showed that the growth performances in salt stressed HJ seedlings were evidently maintained better than those in KDML105 (Table 1). The growth reduction percentage in 342 mM NaCl stressed HJ seedlings compared to control was less expressed than that in KDML105 rice for 2.46, 1.52, 1.25 and 1.17 folds, respectively. It should be concluded that overall growth performances in salt-stressed HJ seedlings were better than those in salt-stressed KDML105 seedlings (Table 1).

In rice both japonica and indica, there are many research topic to compare the defense mechanisms as well as overall growth responses when expose to sodium chloride salt stress. In recent study, the results showed that the growth performances of HJ salt tolerant seedlings were better than those KDML105 seedlings (Table 1). Similarly, the growth characters in salt tolerant rice cultivars, Bankat, Pokkali, Bhoora rata, Lunishree and Dongjin, are less toxic damages to against with salt stress when compared to those salt sensitive cultivars, Hitomebore (Dionisio-Sese and Tobita, 1998), IR28 (Demiral and Türkan, 2005), GR_{11} (Chauhan and Prathapasenan, 2000), Begunbitchi (Khan and Panda, 2008) and Kumnam (Sohn et al., 2005). The most research articles are generally applied the NaCl as a stressor with the moderate salt stress (≤ 200 mM NaCl). In the recently study, the rice seedlings grown under photoautotrophic conditions of plant tissue culture were treated with extreme salt stress (≥ 200 mM NaCl) as a salt stress shock. In addition, the osmolarity of the culture is adjusted to 600±50 mmol Kg^{-1} or using mannitol (sugar alcohol) or polyethylene glycol 6000 (PEG) or -0.52 MPa (water deficit), which is limited on the water availability (Castillo et al., 2007). Osmotic and ionic stresses generated from NaCl salt and mannitol or PEG has been successfully applied to study on IR64 (Castillo et al., 2007), Basmati-Kashmir and Basmati 370 (Ahmad et al., 2007) rice cultivars in callus, seedlings, vegetative and reproductive stages.

In the cellular level, sodium ion (Na^+) contents in seedlings were continuously accumulated, relating to increase NaCl concentrations in the culture media as well as salt tolerant ability of rice cultivars (Table 2). The Na^+ in salt-stressed KDML105 seedlings was collected higher than that in salt-stressed HJ seedlings, especially in the high salt treatments (Table 2). The ion reaching in the plant cells was directly correlated with osmolarity parameter [r^2=0.99 and r^2=0.91 in KDML105 (Fig. 1A) and HJ (Fig. 1B), respectively]. In contrast, potassium ion in salt-stressed seedlings were significantly reduced, leading to increase the Na:K ratio in the plant tissues. In addition, the proline biosynthesis and gathering in both HJ and KDML105 were increased, involving with salt concentration in the media (Table 1). The proline content in salt-stressed KDML105 seedlings was enriched and higher than those in salt-stressed HJ

Table 4. Net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (E), and water use efficiency (WUE) in Homjan (HJ) and Jasmine (KDML 105) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

Rice variety	NaCl (mM)	P_n (μmol $m^{-2}s^{-1}$)	g_s (mmol H_2O m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	WUE (%)
HJ	0	3.17b	1.0c	0.030d	10.57a
	85	2.49c	1.0c	0.030d	8.30b
	171	2.30c	1.0c	0.034d	6.77c
	256	1.43d	1.3c	0.040cd	3.58d
	342	1.21d	2.1b	0.054c	2.24e
KDML105	0	4.74a	0.9c	0.060bc	7.90bc
	85	3.64b	1.1c	0.060bc	6.07c
	171	3.16b	2.3b	0.083b	3.81d
	256	2.31c	2.7b	0.103a	2.24e
	342	1.45d	3.2a	0.132a	1.10f
Significant level					
Variety		**	**	**	**
NaCl		**	*	**	**
Variety × NaCl		**	*	**	**

Different letters in each column show significant difference at $p \leq 0.01$ (**) or $p \leq 0.05$ (*) by Duncan's New Multiple Range Test (DMRT).

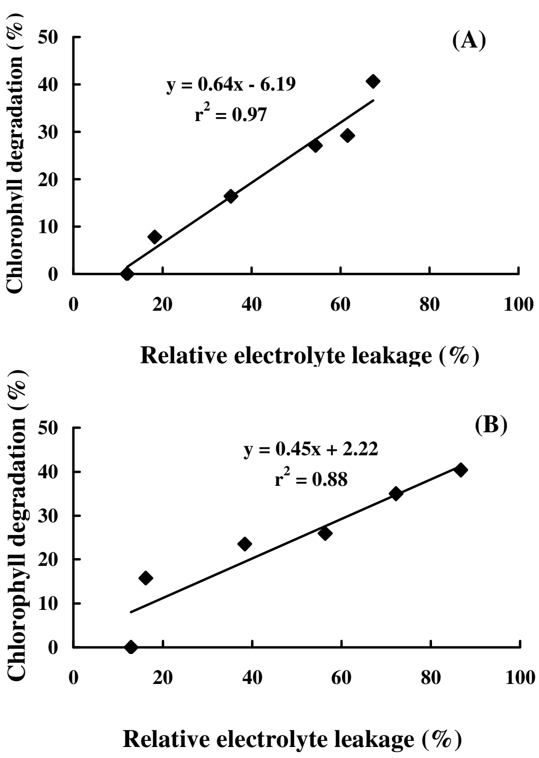


Fig 3. Relationship between relative electrolyte leakage and total chlorophyll degradation in Homjan (A) and Jasmine (B) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

seedlings. It was possible that the proline accumulation may not a major defense mechanism in HJ salt tolerance.

Na^+ in salt-stressed rice is generally accumulated, depending on salt concentrations (Djanaguiraman et al., 2006; Ahmad et al., 2007), developmental stages (Castillo et al., 2007), salt exposure times (Chauhan and Prathapasenan, 2000; Golldack et al., 2003; Sohn et al., 2005) and salt tolerant abilities (Gregorio and Senadhira, 1993; Dionisio-Sese and Tobita, 1998; Hoai et al., 2003; Ahmad et al., 2007; Khan and Panda, 2008). In recent study, the Na^+ accumulation, K^+ reduction and the increased Na:K ratio in HJ salt tolerance was lower than those in KDML105 salt sensitive cultivar. Similarly, the Na^+ limitation, maintained K^+ with low Na:K ratio in the salt tolerant cultivars, Nona Bokra, Pokkali, SR26B and Lunishree, are better than those in salt sensitive cultivars, IR28, IR29, M1-48 and Begunbuchi, respectively (Gregorio and Senadhira, 1993; Ahmad et al., 2007; Khan and Panda, 2008). On the other hand, the proline contents in salt sensitive cultivars (IR28, Kumnam, Basmati-370, GZ-1368, Samcheon Nipponbare and DT271) are accumulated and higher than those in salt tolerant cultivars (Pokalli, Dongjin, Basmati-Kashmir, Keowha, Anapurna and T. hatamochi) (Hoai et al., 2003; Demiral and Türkan, 2005; Sohn et al., 2005; Ahmad et al., 2007) as well as related to salt concentrations (Lin and Kao, 1996; Sultana et al., 1999; Demiral and Türkan, 2005; Ahmad et al., 2007). In the previous studies, glycine betaine has been recently reported to accumulate in HJ rice salt-stressed seedlings, relating to salt exposure

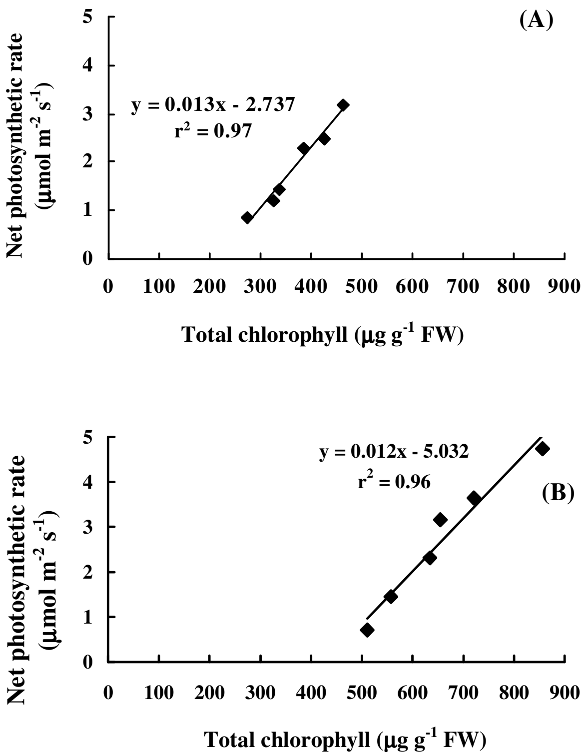


Fig 4. Relationship between total chlorophyll degradation and net photosynthetic rate (P_n) in Homjan (A) and Jasmine (B) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

times (Cha-um et al., 2007b). In addition, the glycine betaine osmolyte accumulation in HJ rice is reach higher than that in KDML105 (Cha-um et al., 2004). It may play an important role as the major defense mechanism in HJ salt tolerant rice when expose to salt stress.

An increasing osmolarity in the salt-stressed tissues was related to relative electrolyte leakage (REL) in both salt tolerant and salt sensitive cultivars with r^2=0.93 (Fig. 2A) and r^2=0.97 (Fig. 2B), respectively. The REL in salt-stressed KDML 105 seedlings was increased following the salt concentrations and higher than those in salt-stressed HJ seedlings, linking to chlorophyll degradation percentage [r^2=0.97 and r^2=0.88 in KDML105 (Fig. 3A) and HJ (Fig. 3B), respectively]. Chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid damages in the leaf tissues were trend to correlate with salt treatments and salt tolerant ability of rice cultivars (Table 3). The degradation percentage of pigments in the salt-stressed seedlings was evidently expressed in the extreme salt treatments (342-427 mM NaCl). The chlorophyll degradation was positively related to net-photosynthetic rate (P_n) reduction in both salt tolerant and salt sensitive cultivars with r^2=0.97 (Fig. 4A) and r^2=0.96 (Fig. 4B), respectively. The P_n and water use efficiency (WUE) in salt stressed seedlings were significantly decreased relating to salt concentrations in the media as well as salt tolerant cultivars (Table 4). Controversially, stomatal conductance (g_s) and transpiration rate (E) in both KDML105 and HJ seedlings were exhibited when expose to salt stresses, especially in the high salt concentrations (342-427 mM NaCl). In addition, the P_n reduction was directly inhibited on overall growth, especially plant dry weight (Fig. 5).

Osmotic potential reduction in salt stressed rice is one of the most sensitive parameters to identify the salt tolerant ability. For example, the osmotic- and water-potential in salt tolerant cultivars, Basmati-Kashmir and IR31785, grown under salt stress are gradually dropped, while those parameters in salt sensitive cultivars, Basmati370 and I Kong Pao, are sharply declined (Zhu et al., 2001; Ahmad et al., 2007). Pokkali cultivar is well known as salt tolerant rice, which is a membrane stabilization when expose to salt stress. On the other hand, the membrane leakage in Hitomebore and IR28 salt sensitive is evidently enhanced, relating to salt concentrations (Dionisio-Sese and Tobita, 1998).

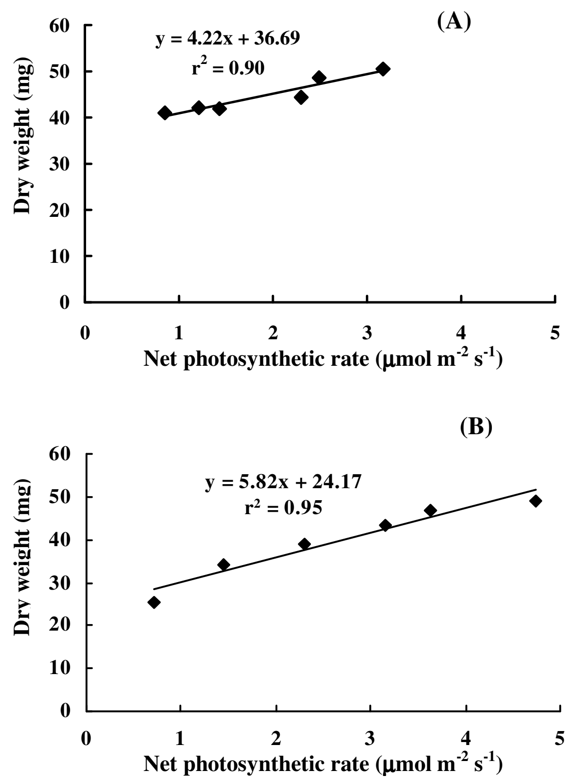


Fig 5. Relationship between total net photosynthetic rate (P_n) and dry weight in Homjan (A) and Jasmine (B) rice cultivars grown in photoautotrophic culture supplemented with sodium chloride (NaCl) concentrations for 7 days.

The chlorophyll and carotenoid contents in Koshihikari and Taipei 309 cultivars cultivated on soil salinity are commonly damaged (Sultana et al., 1999; Bahaji et al., 2002). The chlorophyll pigment in rice is very sensitive to salt stress (Bahaji et al., 2002; Ali et al., 2004; Wanitchananan et al., 2004), especially in salt susceptible variety (I Kong Pao) (Asch et al., 2000). Chlorophyll degradation in salt stressed rice, Koshihikari and KDML105, is positively related to net photosynthetic rate (P_n) reduction or CO_2 assimilation as well as stomatal conductance and transpiration rate (Sultana et al., 1999; Cha-um et al., 2004). In addition, the chlorophyll and P_n reduction percentages in GS 4371 salt tolerant rice are lower than those in GS 7032 salt sensitive rice (Cha-um et al., 2007b). The physiological changes in salt stressed rice are directly impaired on overall growth i.e. shoot height, fresh weight and dry weight (Aslam et al., 1993; Chowdhury et al., 1995; Sohn et al., 2005; Khan and Panda, 2008).

Conclusion

Sodium ion in HJ salt tolerant and KDML105 seedlings was relatively accumulated with salt concentrations in the culture media. Osmolarity in salt sensitive seedlings grown under salinity stress was reach and related to membrane injury and pigment degradation, causing on P_n and growth reduction. In contrast, the water relation, membrane stability and pigment content in salt tolerant seedlings cultured on salt stresses were maintained better than those in salt sensitive seedlings, leading to high P_n and growth performances.

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