RELATIONSHIPS BETWEEN SODIUM ION ACCUMULATION AND PHYSIOLOGICAL CHARACTERISTICS IN RICE (*ORYZA SATIVA* L. SPP. *INDICA*) SEEDLINGS GROWN UNDER ISO-OSMOTIC SALINITY STRESS

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Abstract

The objective of this research was to elucidate the role of sodium ion (Na^+) on photosynthetic machinery and growth characteristics in both salt-tolerant (HJ) and salt-sensitive (PT1) rice varieties grown under iso-osmotic salinity stress. The Na^+ and Na^+/K^+ in HJ and PT1 rice seedlings were increased with increasing salt concentrations in the culture media. K^+ in salt-stressed HJ seedlings was increased but that in salt-stressed PT1 seedlings was unchanged. The Na^+ accumulation in salt-stressed seedlings was negatively related to the water potential in HJ ($r^2 = 0.78$) and PT1 ($r^2 = 0.72$), leading to pigment degradation i.e. chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total carotenoids (C_{x+c}). The water potential in both HJ and PT1 salt-stressed seedlings was positively related to the Chl_a concentration ($r^2 = 0.78$ and $r^2 = 0.58$). Furthermore, Chl_a and total chlorophyll (TC) concentrations in HJ and PT1 salt-stressed seedlings were positively related to water oxidation in PSII (PSII) as maximum quantum yield of PSII (F_v/F_m) ($r^2 = 0.73$ and $r^2 = 0.68$) and quantum efficiency of PSII (Φ_{PSII}) ($r^2 = 0.92$ and $r^2 = 0.91$), respectively. The Φ_{PSII} in HJ and PT1 salt-stressed seedlings was positively related to the dry matter ($r^2 = 0.86$ and $r^2 = 0.67$). The K^+ accumulation in HJ salt-stressed seedlings may play a major role in salt defense mechanisms, leading to enhance on photosynthesis capacity, water oxidation in photosystem II (PSII) and growth abilities.

Introduction

Salinity stress is the major factor to limit plant growth and productivity in many areas of the world (Allakhverdiev *et al*., 2000; Ashraf, 2009). Excessive amounts of salts in the soil solution cause both osmotic and ionic stresses (Khelil *et al*., 2007). These stresses are generated from the major salt contaminated soil, namely NaCl, which is readily dissolved in the water to give rise Na^+ and Cl^- (Taiz & Zeiger, 2002). The Na^+ is generally reported as a strong cation to interact with other cations, especially K^+ (Hu *et al*., 2007; Aziz & Khan, 2001; Ashraf, 2004) in the membrane system, since Na^+/K^+ exchanges for intracellular influx to increase the Na^+/K^+ selectivity (Parida & Das, 2005). Enhanced membrane permeability in salt stressed plants has been well documented (Hasegawa *et al*., 2000), resulting in Na^+ accumulation and increasing Na^+/K^+, conversely reducing K^+ in many plant species i.e. rice (Dionisio-Sese & Tobita, 1998), green bean (Yasar *et al*., 2006), winter wheat (Zheng *et al*., 2008), umbu plant (da Silva *et al*., 2008).

Furthermore, a deteriorated membrane affects on the reduction of the physiological and biochemical processes (Taiz & Zeiger, 2002; Noreen & Ashraf, 2008), especially photosynthesis. Photosynthetic pigments are necessary for photosynthesis mechanism

including; chlorophyll a (Chl$_a$), chlorophyll b (Chl$_b$), total chlorophyll (TC) and total carotenoids (C$_{x+c}$) which play a role in the light-harvesting complex of photosystem II (PSII). Chl$_a$, Chl$_b$ and TC in higher plants play an important role on the water oxidation and electron transport in photosystem II. In addition, C$_{x+c}$ play a key role for antioxidative functions to protect the plants from the reactive oxygen species (ROS), which generally generate by salinity stress. Under salinity stress, the plasma membrane and activities of antioxidant enzymes are regulated by Na$^+$ accumulation to prevent the damage of chloroplasts, which contain photosynthetic pigments such as Chl$_a$, Chl$_b$, TC and C$_{x+c}$ (Demiral & Türkan, 2005). The chloroplast is very sensitive to damage both the properties and efficiencies in the light-harvesting complexes, leading to disturb in the chloroplast ultrastructure (Zheng *et al.*, 2008). Many reports show salt induced reduction in photosynthetic pigments in many plant species i.e. rice (Cha-um *et al.*, 2007a), castor bean (Pinheiro *et al.*, 2008), raspberry (Neocleous & Vasilakakis, 2007).

The efficiency of photosynthetic pigments is indicated by the function of chlorophyll pigments or chlorophyll a fluorescence, which demonstrate the functioning of water oxidation and photosynthetic electron transport in photosystem II (PSII) under stress condition (Gray *et al.*, 1997). The photosynthetic pigments in the chloroplast are composed of light harvesting complexes namely photosynthetic apparatus. Chlorophyll a fluorescence parameters such as maximum quantum yield (F$_v$/F$_m$), photosystem II quantum efficiency (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ) have been investigated (Maxwell & Johnson, 2000) and applied as effective indices for abiotic stress screening. There are many reports which show the reduction of chlorophyll a fluorescence in salt-stressed plant species i.e., rice (Cha-um *et al.*, 2007a), sunflower (Santos, 2004), Rumex (Chen *et al.*, 2004) and citrus (Lopez-Climent *et al.*, 2008).

Rice (*Oryza sativa* L. spp. *indica*) is a staple food for feeding and providing the necessary daily calories for three billions of people in the world (Kush, 1997). However, the growth and yield are dramatically reduced by abiotic extreme conditions, especially salinity stress, since the rice has been reported as salinity susceptible crop (Yokoi *et al.*, 2002; Flowers & Yeo, 1995). From some previous studies, the results showed that rice growth and development are reduced when exposed to the salinity stress (Morsy *et al.*, 2007; Cha-um *et al.*, 2007a; Dionisio-Sese & Tobita, 1998; Shannon *et al.*, 1998). However, the response of rice to salt stress depends on its salt tolerance abilities. The objective of this study was to investigate the role of Na$^+$/K$^+$ on physiological responses of salt-tolerant and salt-sensitive rice varieties grown under iso-osmotic salinity stress.

Materials and Methods

Plant materials and treatments: Seeds of salt-tolerant (HJ) and salt-sensitive (PT1) varieties (Cha-um *et al.*, 2007b) of indica rice (*Oryza sativa* L. spp. *indica*) were obtained from 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 thrice with sterilized distilled water. Surface sterilized seeds were germinated on 25 mL MS-gelled (Murashige & Skoog, 1962) with 3% sucrose (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 density (PPFD) provided by fluorescent lamps (TLD 36 W/84 Cool White 3350 Im, Philips, Thailand) for 16 h d^{-1} photoperiod. Fourteen-day-old

seedlings were aseptically transferred to MS sugar-free liquid media (photoautotrophic condition) by using vermiculite as a supporting material for 7 days. Air-exchange rate in the glass vessels was adjusted to 2.32 h^{-1} by punching a hole in the plastic cap ($\varnothing$ 1 cm) and covering the hole with a gas-permeable microporous polypropylene film (0.22 μm pore size, Nihon Millipore Ltd., Japan). The culture medium was adjusted to 0.0, 85.5, 171.0, 256.5 or 342.0 mM NaCl with iso-osmotic level using mannitol solution for controlling the range of water potential in the medium (-1.4±0.2 MPa) for 4 days.

Ion contents: One-hundred-milligrams fresh weight of leaf tissues were ground in liquid-nitrogen and extracted by acidic method. Na^+ and K^+ concentrations were measured by an atomic absorption spectrophotometer (AA; Model M6 Thermo Elemental, MA, USA) according to Dionisio-Sese & Tobita method (1998).

Photosynthetic pigment concentrations: Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) concentrations were determined following Shabala *et al.,* (1998) and Lichtenthaler (1987) methods, respectively. One-hundred-milligram leaf tissues were placed in a glass vessel, added 10 mL of 95.5% acetone and blended with a homogenizer (T25 Basic ULTRA-TURRAX®; IKA, Kuala Lumpur, Malaysia). The glass vessels were sealed with parafilm to prevent evaporation and then stored at 4ºC for 48 h. The Chl_a, Chl_b, TC and C_{x+c} concentrations were measured using a UV-visible spectrophotometer (DR/4000; HACH, Loveland, Colorado, USA) at 662, 644 and 470 nm. A solution 95.5% acetone was used as a blank. The Chl_a, Chl_b, TC and C_{x+c} (μg g^{-1} FW) concentrations in the leaf tissues were calculated according to the following equations:

$$Chl_a = 9.784D_{662} - 0.99D_{644}$$
$$Chl_b = 21.42D_{644} - 4.65D_{662}$$
$$TC = Chl_a + Chl_b$$
$$C_{X+C} = \frac{1000D_{470} - 1.90Chl_a - 63.14Chl_b}{214}$$

where D_i is an optical density at the wavelength i.

Chlorophyll a fluorescence measurement: Chlorophyll a fluorescence emission of adaxial leaf surface was monitored by a Fluorescence Monitoring System (FMS 2; Hansatech Instruments Ltd, Norfolk, UK) in the pulse amplitude modulation mode, as previously described by Loggini *et al.*, (1999). The maximum quantum yield of PSII photochemistry (F_v/F_m), quantum efficiency of PSII (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ) were evaluated by FMS software for Windows (Fluorescence Monitoring System Software; Hansatech Instruments Ltd), and were calculated as described by Maxwell & Johnson (2000).

Growth abilities: Fresh weight was measured after exposing salt stress for 4 days. The plant sample was then dried at 110 ºC in a hot-air oven (Memmert, Model 500, Germany) for 48 h and then incubated in a desiccator before measuring the dry weight. Dry matter was calculated with the following equation:

$$Dry\ matter\ (\%) = \frac{Dry\ weight}{Fresh\ weight} \times 100$$

Statistical analysis of data: The experiment was designed as 2×5 factorials in a Completely Randomized Design (CRD) with four replications and five seedlings per replicate. Significant level was determined by one-way analysis of variance (ANOVA) using the SPSS software (SPSS for Windows, SPSS Inc., USA). Differences between means were compared by the Duncan's Multiple Range Test (DMRT). The relations between Na^+ accumulation and water potential, water potential and Chl_a, Chl_a and F_v/F_m, TC and Φ_{PSII}, Φ_{PSII} and dry matter were evaluated.

Results and Discussions

Sodium ion (Na^+) accumulation in salt-tolerant (HJ) and salt-sensitive (PT1) rice seedlings was directly enhanced, relating to NaCl salt concentrations (85.5, 171.0, 256.5 and 342.0 mM NaCl) in the culture medium (Table 1). The Na^+ in HJ seedlings cultured on 85.5, 171.0, 256.5 and 342.0 mM NaCl enhanced for 23.1, 68.0, 88.5 and 146.9 folds when compared to those on without NaCl. In a parallel way, the Na^+ in PT1 salt-stressed seedlings was accumulated 6.5, 21.3, 24.0 and 28.4 folds when compared to those cultured on that without NaCl (Table 1). Moreover, the Na^+ accumulation in HJ and PT1 salt-stressed seedlings was negatively related to water potential ($r^2 = 0.78$ and $r^2 = 0.72$) (Fig. 1A and 1B). In contrast, potassium ion (K^+) in HJ salt-stressed seedlings was significantly accumulated, depending on NaCl salt concentrations in the media. The K^+ in HJ seedlings cultured on 85.5, 171.0, 256.5 and 342.0 mM was 2.8, 2.7, 3.3 and 4.4 folds higher than those cultured on without NaCl. On the other hand, K^+ accumulation in PT1 seedlings was not significantly changed when exposed to NaCl stress (Table 1). In addition, Na^+/K^+ ratio in HJ and PT1 salt-stressed was continuously increased with increasing the NaCl concentrations (Table 1). The Na^+/K^+ ratio in HJ seedlings cultured on 85.5, 171.0, 256.5 and 342.0 mM NaCl was higher than those cultured on without NaCl for 8.4, 25.3, 27.3 and 35.2 folds, respectively. As well as the Na^+/K^+ ratio in PT1 salt-stressed seedlings was higher than those cultured on without NaCl for 7.2, 18.9, 26.7 and 25.8 folds (Table 1).

Na^+ accumulation and Na^+/K^+ ratio in salt-stressed plants depend on salt stress treatments, while K^+ accumulation is generally decreased (Parida & Das, 2005; Parida *et al*., 2004; Levigneron *et al*., 1995; Sabir & Ashraf, 2007). The Na^+ and K^+ enrichment and Na^+/K^+ ratio have been utilized to identify the salt tolerant ability in many plant species such as rice (Zeng, 2005; Nakamura *et al*., 2002; Dionisio-Sese & Tobita, 1998), winter wheat (Zheng *et al*., 2008) umbu plant (da Silva *et al*., 2008), *Arbutus unedo* (Navarro *et al*., 2007) and maize (Çiçek & Cakirlar, 2002). In this study, the Na^+ and K^+ accumulation and Na^+/K^+ ratio in salt-tolerant HJ seedlings was increased with increasing iso-osmotic salinity stress, while the K^+ in PT1 salt-stressed seedlings remained unchanged. The K^+ accumulation in HJ rice seedlings grown under iso-osmotic salinity stress may play a role as salt defense mechanism when compared to that in PT1 salt sensitive seedlings. There are many reports, which show an important role of K^+ in different physiological processes such as osmotic adjustment, ionic balance and ion homeostasis (Rejili *et al*., 2007; Nguyen *et al*., 2005; Serrano & Navarro, 2001). In addition, an increase in K^+ is well established (Yasar *et al*., 2006; Chow *et al*., 1990), which leads to reduced Na^+ uptake in the cells (Parida & Das, 2005; Saqib *et al*., 2005) thereby maintaining low [Na^+/K^+] ratio in plant tissues exposed to salt stress (Rejili *et al*., 2007; Nguyen *et al*., 2005).

Table 1. Sodium ion (Na$^+$), potassium ion (K$^+$) and Na$^+$:K$^+$ ratio in leaves of salt-tolerant (HJ) and salt-sensitive (PT1) rice varieties cultured on MS medium and exposed to 0.0, 85.5, 171.0, 256.5 and 342.0 mM NaCl with iso-osmotic level using mannitol application for 4 days.

Rice varieties	NaCl (mM)	Na$^+$(mg g^{-1} FW)	K$^+$(mg g^{-1} FW)	Na$^+$:K$^+$
HJ	0.0	0.30 f	22.82 e	0.013 f
	85.5	6.92 de	64.15 bc	0.109 ef
	171.0	20.36 c	62.00 bc	0.328 cde
	256.5	26.51 c	74.72 b	0.355 cd
	342.0	44.01 a	99.27 a	0.456 bc
PT1	0.0	1.17 ef	43.04 d	0.027 f
	85.5	7.58 d	39.26 de	0.193 def
	171.0	24.71 c	48.44 cd	0.511 abc
	256.5	22.85 c	37.37 de	0.630 ab
	342.0	33.04 b	48.01 cd	0.696 a
Significant level				
Rice varieties		ns	**	**
NaCl		**	**	**
Rice varieties × NaCl		*	**	ns

Means with the different letters are significantly different at p≤ 0.01 (**), p≤ 0.05 (*) and non-significant (ns) by Duncan's New Multiple Range Test.
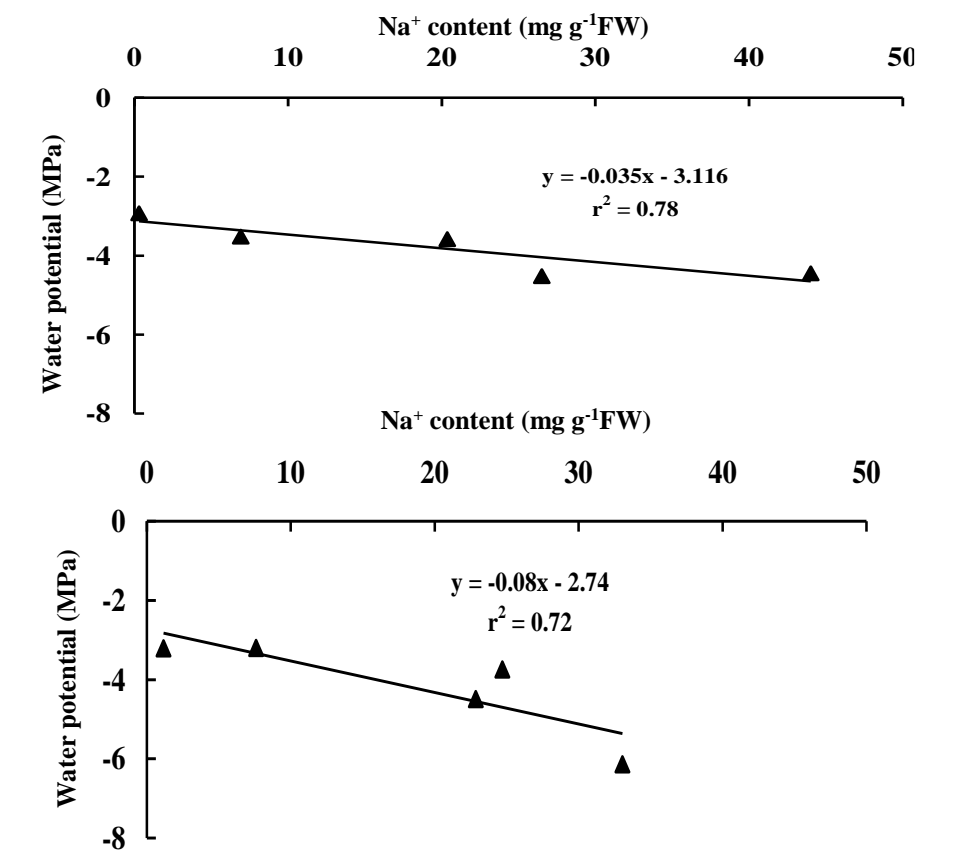

Fig. 1. Relationships between Na$^+$ accumulation (mg g^{-1} FW) and water potential (MPa) of salt-tolerant (A) and salt-sensitive (B) rice seedlings cultured *In vitro* and exposed to salt stress with iso-osmotic level using mannitol application for 4 days.

Table 2. Chlorophyll a (Chl$_a$), chlorophyll b (Chl$_b$), total chlorophyll (TC) and total carotenoid (C$_{x+c}$) concentrations of salt-tolerant (HJ) and salt-sensitive (PT1) rice varieties cultured on MS medium and exposed to 0.0, 85.5, 171.0, 256.5 or 342.0 mM NaCl with iso-osmotic level using mannitol application for 4 days.

Rice varieties	NaCl (mM)	Chl$_a$ (µg g^{-1} FW)	Chl$_b$ (µg g^{-1} FW)	TC (µg g^{-1} FW)	C$_{x+c}$ (µg g^{-1} FW)
HJ	0.0	320 b	661 b	905 b	40.1 a
	85.5	260 d	529 c	802 c	36.4 b
	171.0	239 e	451 e	702 d	29.7 d
	256.5	232 e	440 ef	700 d	29.2 d
	342.0	213 f	413 f	632 e	26.5 e
PT1	0.0	375 a	698 a	967 a	37.2 b
	85.5	289 c	493 d	779 c	31.4 c
	171.0	192 g	467 de	629 e	23.2 f
	256.5	178 g	412 f	558 f	21.6 g
	342.0	154 h	170 g	425 g	21.4 g
Significant level					
Rice varieties		**	**	**	**
NaCl		**	**	**	**
Rice varieties × NaCl		**	**	**	**

Means with the different letters are significantly different at p≤ 0.01 (**) by Duncan's New Multiple Range Test

Photosynthetic pigment concentrations in both salt tolerant and salt sensitive varieties were sharply dropped when exposed to high salinity stress (Table 2). Chl$_a$, Chl$_b$, TC and C$_{x+c}$ concentrations in salt-stressed seedlings were significantly decreased, depending on rice varieties, NaCl concentration and their interaction (Table 2). The Chl$_a$ in HJ salt-stressed seedlings cultured on 85.5, 171.0, 256.5 and 342.0 mM NaCl was lower than those cultured on without (Table 2). The Chl$_a$ degradation in salt-stressed seedlings was positively related to water potential in both HJ salt tolerant ($r^2 = 0.78$) (Fig. 2A) and PT1 salt sensitive plants ($r^2 = 0.58$) (Fig. 2B). The Chl$_a$ degradation percentage of salt stressed HJ was gradually increased from 18.75-33.43% whereas that of salt stressed PT1 was sharply exhibited (22.9-58.9%), relating to salt concentration in the culture media. In parallel way, the Chl$_b$, TC and C$_{x+c}$ in HJ rice seedlings were maintained better than those in PT1 seedlings when exposed to salt stress, especially in 342 mM NaCl. The Chl$_a$ contents in salt stressed seedlings of rice were positively correlated with maximum quantum yield of PSII (F$_v$/F$_m$) in both HJ ($r^2 = 0.73$) (Fig. 3A) and PT1 ($r^2 = 0.68$) (Fig. 3B). Chlorophyll a fluorescence parameters, F$_v$/F$_m$, Φ$_{PSII}$, qP and NPQ in salt stressed seedlings were decreased significantly, depending on rice varieties, salt concentration in the media and their interactions (Table 3). In addition to, a positive relationship was found between Φ$_{PSII}$ and TC in HJ ($r^2 = 0.92$) (Fig. 4A) and PT1 ($r^2 = 0.91$) (Fig. 4B). Diminishing of Φ$_{PSII}$ in salt stressed rice seedlings was positive related to plant dry matter of HJ ($r^2 = 0.92$) (Fig. 4A) and PT1 ($r^2 = 0.88$) (Fig. 4B). Growth characteristics, fresh weight, dry weight and dry matter percentage, in rice seedlings was decreased by the factors of rice varieties, salt concentration and their interaction, except in the dry matter parameter was unchanged by rice varieties (Table 4). As well as, the growth reduction percentage of HJ salt tolerance was extensively maintained better than those in PT1 salt susceptible.
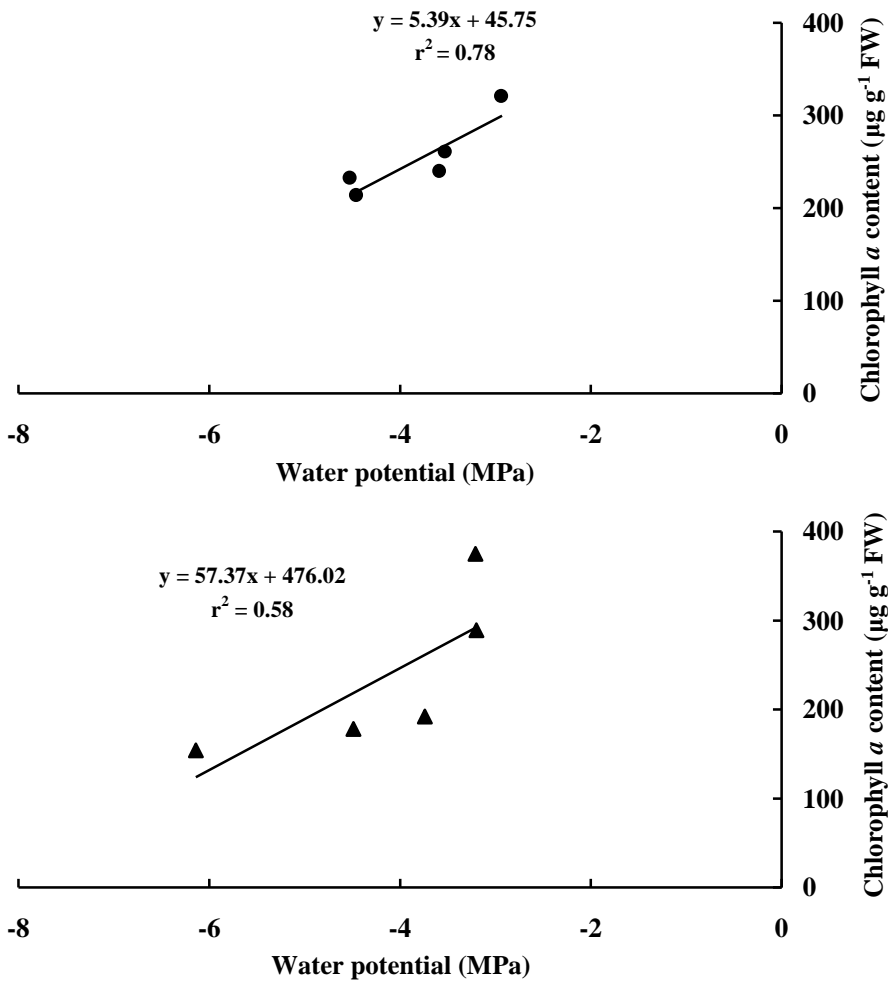
Fig. 2. Relationships between water potential (MPa) and chlorophyll *a* concentration ($\mu g \; g^{-1}$ FW) of salt-tolerant (A) and salt-sensitive (B) rice seedlings cultured *In vitro* and exposed to salt stress with iso-osmotic level using mannitol application for 4 days.

The photosynthetic pigments in both rice seedlings exposed to iso-osmotic salinity stress decreased significantly (Table 2). There are many reports which show the reduction of photosynthetic pigments in the salt-stressed leaves of different plants such as bean (Parida & Das, 2005; Hernandez *et al.*, 1995), sunflower (Santos, 2004), castor bean (Pinheiro *et al.*, 2008), winter wheat (Sairam & Srivastava, 2002; Zheng *et al.*, 2008), rice (Cha-um *et al.*, 2007a), tomato (Khavarinejad & Mostofi, 1998) and cotton (Meloni *et al.*, 2003). In this study, the photosynthetic pigments including Chl_a, Chl_b, TC and C_{x+c} in salt-stressed leaves of HJ were stabilized better than those in PT1. Under salinity stress, the photosynthetic apparatus, especially chloroplast may be damaged by toxic ion, especially sodium ion (Na^+). Since, the Na^+ can generate the lipid peroxidation in the

Table 3. Maximum quantum yield (F$_v$/F$_m$), photosystem II quantum efficiency (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ) in the leaf tissues of salt-tolerant (HJ) and salt-sensitive (PT1) rice varieties cultured on MS medium and exposed to 0.0, 85.5, 171.0, 256.5 or 342.0 mM NaCl with iso-osmotic level using mannitol application for 4 days

Rice varieties	NaCl (mM)	Fv/F$_m$	Φ_{PSII}	qP	NPQ
HJ	0.0	0.824 a	0.618 a	0.817 a	0.072 a
	85.5	0.771 b	0.526 b	0.678 b	0.051 b
	171.0	0.729 cd	0.466 d	0.622 d	0.037 d
	256.5	0.620 e	0.414 f	0.581 f	0.026 e
	342.0	0.514 f	0.297 g	0.527 g	0.017 f
PT1	0.0	0.737 c	0.507 c	0.659 c	0.044 c
	85.5	0.728 cd	0.497 c	0.594 e	0.037 d
	171.0	0.709 d	0.446 e	0.528 g	0.011 g
	256.5	0.627 e	0.411 f	0.516 h	0.010 g
	342.0	0.620 e	0.403 f	0.508 h	0.010 g
Significant level					
Rice varieties		**	**	**	**
NaCl		**	**	**	**
Rice varieties × NaCl		**	**	**	**

Means with the different letters are significantly different at p≤ 0.01 (**) by Duncan's New Multiple Range Test.

chloroplast to damage the ultrastructure and chloroplast functions (Hernandez *et al.*, 1995) as well as produce reactive oxygen species (ROSs) (Vaidyanathan *et al.*, 2003) to disturb the function of photosynthetic pigments (Demiral & Türkan, 2005). The Na ions generated by salt stress have been reported as the major toxicity to be damaged by the photosynthetic pigments. The function of photosynthetic pigments has been well established as light harvesting and water oxidation in photosystem II (PSII) which is identified by chlorophyll a fluorescence. It is a simple, rapid and sensitive procedure as physiological changes in plant responses to abiotic stresses (Gray *et al.*, 1997; Sudhir *et al.*, 2005; Cha-um *et al.*, 2009). In present study, the chlorophyll a fluorescence including; F$_v$/F$_m$, Φ_{PSII}, qP and NPQ in salt-stressed seedlings were significantly reduced (Table 3). The reduction of chlorophyll a fluorescence in higher plant species such as citrus (Lopez-Climent *et al.*, 2008), red raspberry (Neocleous & Vasilakakis, 2007), sunflower (Santos, 2004), rice (Cha-um *et al.*, 2007a) and Rumex (Chen *et al.*, 2004) has been reported. Decrease in chlorophyll a fluorescence is indicated as the loss of photosynthetic pigment function to gain the energy from light in the light reaction of photosynthesis, leading to growth inhibition (Neocleous & Vasilakakis, 2007). There are many documents to report on growth reduction of plant species when exposed to salt stress such as rice (Morsy *et al.*, 2007; Cha-um *et al.*, 2007a; Nguyen *et al.*, 2005; Dionisio-Sese & Tobita, 1998), guava (Ali-Dinar *et al.*, 1999), pea (Hernandez *et al.*, 1995), citrus (Lopez-Climent *et al.*, 2008), safflower (Siddiqi *et al.*, 2007), cotton (Chachar *et al.*, 2008), red raspberry (Neocleous & Vasilakakis, 2007), sunflower (Santos, 2004) and *Rumex* (Chen *et al.*, 2004).
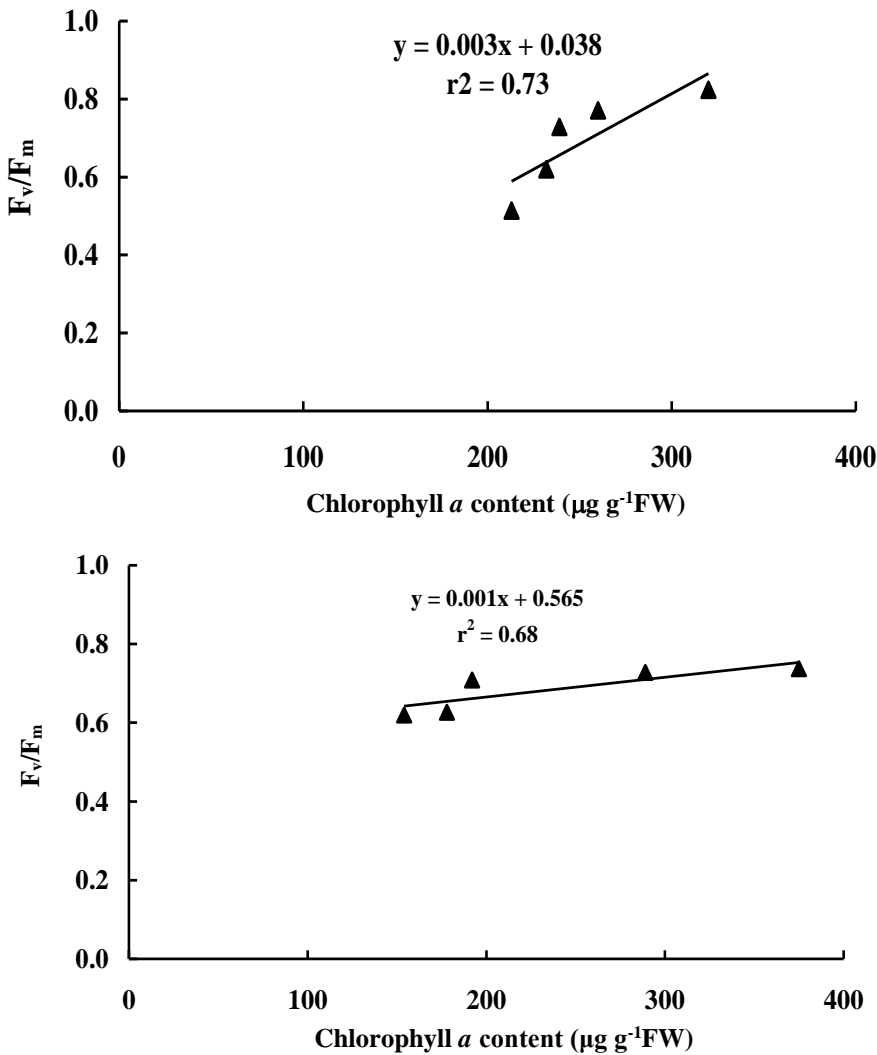
$$y = 0.003x + 0.038$$
$$r2 = 0.73$$

$$y = 0.001x + 0.565$$
$$r^2 = 0.68$$

Fig. 3. Relationships between chlorophyll *a* concentration (µg g^{-1} FW) and F$_v$/F$_m$ of salt-tolerant (A) and salt-sensitive (B) seedlings cultured *In vitro* and exposed to salt stress with iso-osmotic level using mannitol application for 4 days.

Conclusions

Na$^+$ ions in the culture media enriched into root tissues of salt tolerant HJ and salt sensitive PT1 genotypes and subsequently accumulated in the leaf tissues whereas K$^+$ markedly reduced, especially in HJ rice. The K$^+$ homeostasis in salt tolerant HJ rice may play a key role as salt defense mechanism. The Na$^+$ enrichment in the salt-stressed leaf tissues caused to low water potential, pigment degradation and chlorophyll a fluorescence diminishing, leading to growth reduction, especially in PT 1 salt sensitive genotype.

Table 4. Fresh weight, dry weight and dry matter of salt-tolerant (HJ) and salt-sensitive (PT1) rice varieties cultured on MS medium and subjected to 0.0, 85.5, 171.0, 256.5 or 342.0 mM NaCl with iso-osmotic level using mannitol application for 4 days.

Rice varieties	NaCl (mM)	Fresh weight (mg)	Dry weight (mg)	Dry matter (%)
HJ	0.0	145.2 a	47.0 a	35.8 a
	85.5	132.1 b	33.7 b	26.5 b
	171.0	116.4 c	25.4 cd	20.3 c
	256.5	112.4 cd	23.9 cde	19.0 cd
	342.0	110.4 cd	20.4 def	16.6 d
PT1	0.0	108.8 d	28.4 c	35.7 a
	85.5	77.1 e	19.6 ef	22.2 c
	171.0	72.7 ef	17.4 f	21.9 c
	256.5	69.3 f	16.3 f	20.6 c
	342.0	68.6 f	16.0 f	19.3 cd
Significant level				
Rice varieties		**	**	ns
NaCl		**	**	**
Rice varieties × NaCl		**	**	*

Means with the different letters are significantly different at $p \le 0.01$ (**), $p \le 0.05$ (*) and non-significant (ns) by Duncan's New Multiple Range Test
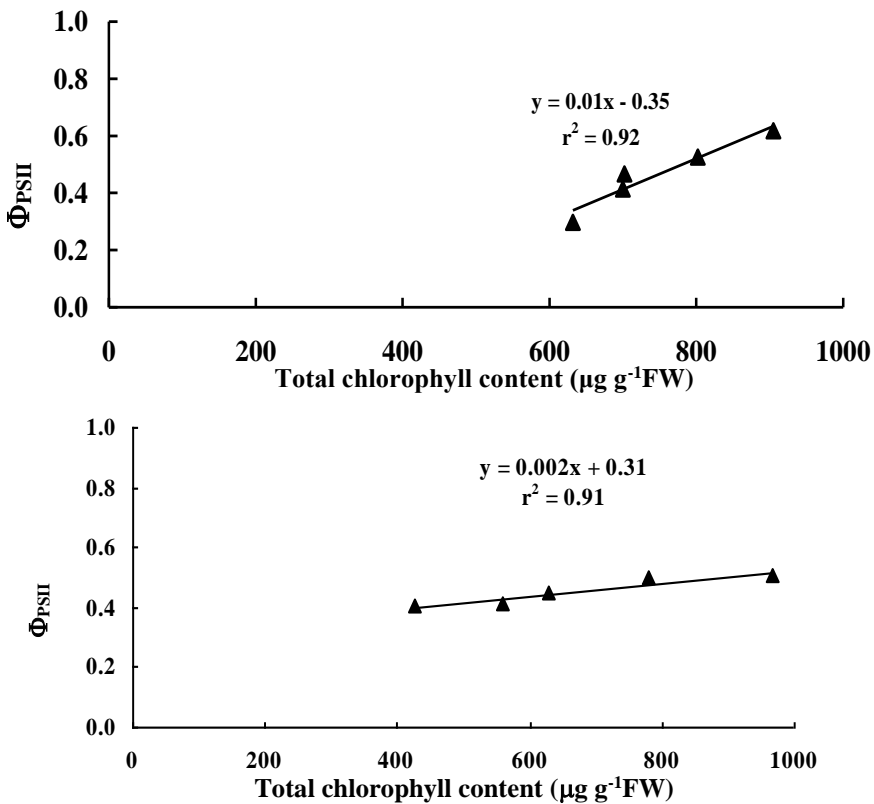

Fig. 4. Relationships between total chlorophyll concentration (µg g^{-1} FW) and Φ_{PSII} of salt-tolerant (A) and salt-sensitive (B) rice seedlings cultured *In vitro* and exposed to salt stress with iso-osmotic level using mannitol application for 4 days.
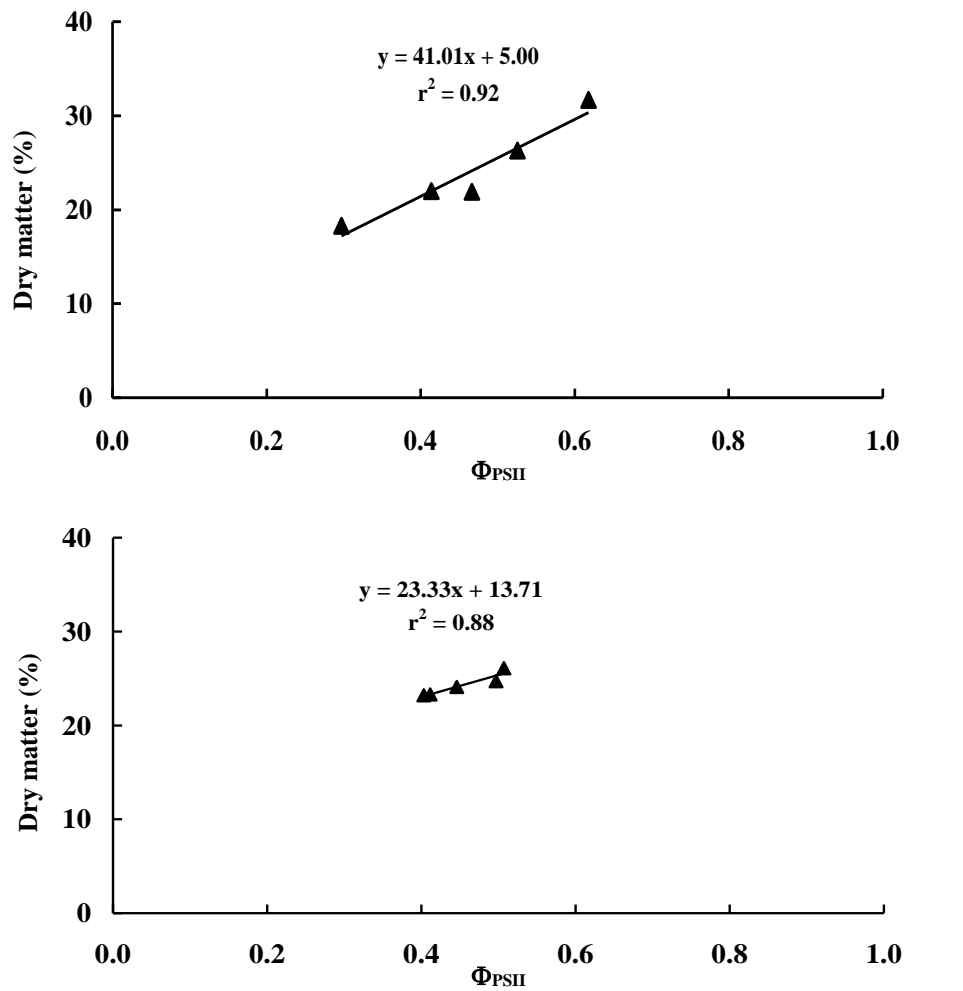
Fig. 5. Relationships between Φ_{PSII} and dry matter (%) of salt-tolerant (A) and salt-sensitive (B) rice seedlings cultured *In vitro* and exposed to salt stress with iso-osmotic level using mannitol application for 4 days.

Acknowledgements

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