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Survival percentage, photosynthetic abilities and growth characters of two indica rice (*Oryza sativa* L. spp. *indica*) cultivars in response to iso-osmotic stress

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Abstract

The aim of this study was to investigate pigment degradation, chlorophyll fluorescence diminution, photosynthetic ability and growth reduction in two rice cultivars, in response to either iso-osmotic salt stress or water-deficit stress. Seedlings of rice cultivars RD6 and KDML105 were photo-autotrophically grown in MS media and subsequently exposed to –0.23 (control), –0.40 or –0.67 MPa iso-osmotic NaCl (salt stress) or mannitol (water-deficit stress) for 14 days. The survival percentage of the two rice cultivars reduced dramatically when subjected to –0.67 MPa NaCl treatment. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total carotenoids (C_{x+c}), maximum quantum yield of PSII (F_v/F_m) and photon yield of PSII (Φ_{PSII}) in the stressed seedlings were significantly lower when compared to seedlings in the control group (without mannitol or NaCl), leading to low net-photosynthetic rate (P_n) and growth reduction. In addition, the growth characters of plantlets in the salt stress conditions were more sharply reduced, and the physiological changes greater than those in water-deficit stress conditions. On the other hand, non-photochemical quenching in the leaves of stressed plantlets increased significantly, especially in response to iso-osmotic salt stress. In the present study, overall growth performance and physiological characters of KDML105 grown under iso-osmotic stress were better than those of RD6.

Additional key words: net-photosynthetic rate, photosynthetic pigments, salt stress, water deficit.

Resumen

Porcentaje de supervivencia, capacidad fotosintética y crecimiento de dos cultivares de arroz tipo índica (*Oryza sativa* L. spp. *indica*) en respuesta al estrés iso-osmótico

El objetivo de este estudio fue investigar la degradación de pigmentos, la disminución de la fluorescencia de la clorofila, la capacidad fotosintética y la reducción en el crecimiento de dos cultivares de arroz, en respuesta al estrés salino iso-osmótico o hídrico. Se cultivaron de forma foto-autotrófica en medio MS plántulas de los cultivares RD6 y KDML105 y posteriormente se expusieron durante 14 días a NaCl (estrés salino) o manitol (estrés hídrico) iso-osmótico –0,23 (control), –0,40 ó –0,67 MPa. El porcentaje de supervivencia de los dos cultivares de arroz se redujo drásticamente con el tratamiento de NaCl 0,67 MPa. La clorofila a (Chl_a), clorofila b (Chl_b), carotenoides totales (C_{x+c}), rendimiento cuántico máximo del PSII (F_v/F_m) y el rendimiento de fotones del PSII (Φ_{PSII}) fueron significativamente menores en las plántulas estresadas respecto a las plántulas del grupo control (sin manitol o NaCl), lo que les llevó a una baja tasa de fotosíntesis neta (P_n) y a una reducción del crecimiento. Además, el crecimiento de las plántulas bajo estrés salino se redujo drásticamente y los cambios fisiológicos fueron mayores que bajo estrés hídrico. Por otra parte, el amortiguamiento no fotoquímico (NPQ) en las hojas de las plántulas estresadas aumentó considerablemente, especialmente en respuesta al estrés salino iso-osmótico. En este estudio, los caracteres de crecimiento y fisiológicos en conjunto de KDML105 cultivados bajo estrés iso-osmótico fueron mejores que los de RD6.

Palabras clave adicionales: estrés hídrico, estrés salino, pigmentos fotosintéticos, tasa de fotosíntesis neta.

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Introduction

Rice is a major source of carbohydrates in many regions of the world, especially Asian countries, feeding more than 3 billion people and providing 50-80% of their daily calorie intake (Khush, 2005). Rice has been identified as a salt susceptible and water deficit susceptible plant species, demonstrating negative effects, including, leaf necrosis, burning, stunting, flower sterility and yield loss (Shannon *et al.,* 1998; Zeng and Shannon, 2000; Khan and Abdullah, 2003; Zeng *et al.,* 2003). The trait of salt tolerance is a major issue, and has been a large factor in implementing breeding programs in rice for the development of novel crops, in order to provide food security (Gregorio *et al.,* 2002; Senadhira *et al.,* 2002; Flowers and Flowers, 2005). In Thailand, jasmine rice or KDML105 is a premium quality grain, which is well known worldwide for its cooking quality, long grains, aroma, flavor and soft texture (Ariyaphanphitak *et al.,* 2005; Laohakunjit and Kerdchoechuen, 2007). In addition, RD6, a gamma-irradiation mutant variety derived from KDML105, is a sticky or glutinous rice with high cooking quality, long grains, sticky texture and enriched aroma and flavor (Keeratipibul *et al.,* 2008). These cultivars are widely cultivated in northeastern Thailand, which is a large area of rain fed lowland, affected by inland salinity and water deficit problems (Wongpokkhom *et al.,* 2008). Hydroponic system has been well established for salt tolerant screening in large population of rice crop especially in IRRI (International Rice Research Institute) (Gregorio *et al.,* 1997). Environmental control in plant tissue culture is an alternative protocol, which is a rapid, simple and reliable method to be screened more than 100 tree species (Kirdmanee and Mosaleeyanon, 2000), 1,000 lines of rice, 50 cultivars of sugarcanes and 40 clones of eucalypt for salt tolerant trait in one and a half months. A positive correlation between the data in the environmental control and salinity field trials has been investigated (Cha-um and Kirdmanee, 2008).

Water-deficit, and salt affected soil are two major abiotic stresses which reduce crop productivity, especially that of rice, by more than 50% world-wide (Mahajan and Tuteja, 2005). In salt-susceptible (glycophyte) plant species, biochemical, physiological and morphological characteristics are negatively affected, leading to abnormal growth and development, and eventual plant death (Hasegawa *et al.,* 2000; Wang *et al.,* 2001; Parida and Das, 2005). The effects of water-deficit stress, caused by excessive salts or osmotic agents added to the media (sugar alcohol and polyethylene glycol), are well established in many crop species (Lutts *et al.,* 2004; Wahid, 2004; Luo *et al.,* 2005; Castillo *et al.,* 2007). In halophyte, or tolerant plants, there are several defense mechanisms, such as osmoregulation, ion homeostasis, antioxidant and hormonal systems, which help plants to stay alive and continue developing, prior to their reproductive stages (Hasegawa *et al.,* 2000; Wang *et al.,* 2003; Reddy *et al.,* 2004; Sairam and Tyagi, 2004; Mahajan and Tuteja, 2005). Mannitol is a member of the sugar alcohols which has been used to control the osmotic potential in nutrient solutions in order to induce water deficit conditions, especially in the root zone (Zang and Komatsu, 2007). It has been reported as an effective osmoticum which only controls the osmotic potential, whereas polyethylene glycol (PEG) has been established as a candidate osmoticum, being a membrane injury agent (Ahmad *et al.,* 2007). Sodium chloride salt is a small molecule, quickly oxidized by water into Na^+ and Cl^-, which cause damage to plant cells (Dionisio-Sese and Tobita, 1998; Sultana *et al.,* 1999; Vaidyanathan *et al.,* 2003; Cha-um *et al.,* 2007a). Sodium chloride salts are quickly dissolved in the water and play as ionic effects in higher plant including rice crop. In contrast, the mannitol-induced osmotic stress is only functioned as osmoticum or water deficit effects (Castillo *et al.,* 2007).

The aim of this work was to investigate pigment degradation, chlorophyll fluorescence diminution, photosynthetic ability and growth reduction in two rice cultivars, in response to either iso-osmotic salt stress or water-deficit stress.

Material and methods

Plant materials

Seeds of two rice cultivars, glutinous rice (cv. RD6 a KDML105-gamma irradiation mutant) and non-

Abbreviations used: Chl_a (chlorophyll a), Chl_b (chlorophyll b), C_{x+c} (total carotenoids), DW (dry weight), F_m (maximum fluorescence yields), F_0 (original fluorescence yields), F_v (variable fluorescence yields), FW (fresh weight), LA (leaf area), NPQ (non photochemical quenching), PEG (polyethylene glycol), P_n (net photosynthetic rate), PPFD (photosynthetic photon flux density), RH (relative humidity), RL (root length), SH (shoot height), TC (total chlorophyll), Φ_{PSII} (photon yield of PSII).

glutinous rice (cv. KDML105), were obtained from the germplasm bank, Rice Research Center, Thailand. The seeds were manually dehusked, sterilized once in 5% Clorox® for 60 min, once in 30% Clorox® for 30 min, then rinsed three times with sterile distilled-water. Surface-sterilized seeds were germinated on 0.25% Phytagel®-solidified MS media (Murashige and Skoog, 1962) with 3% sucrose (photomixotrophic condition) in a 250 mL glass vessel. The media were adjusted to pH 5.7 before autoclaving. Rice seedlings were cultured *in vitro* under conditions of 25 ± 2°C ambient temperature, 60 ± 5% relative humidity (RH) and 60 ± 5 µmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) provided by fluorescent lamps with 16 h d^{-1} photoperiod. Fourteen-day-old seedlings were aseptically transferred to MS-liquid sugar-free media (photoautotrophic conditions). The uncovered vessels containing photoautotrophic seedlings were transferred aseptically to a culture box chamber (Carry Box Model P-850, size 26 × 36 × 19 cm, Japan) with RH controlled at 65 ± 5% by 1.5 L saturated NaCl solution, in order to enhance realistic phenotypic expression in the aerial parts of the rice seedlings. The number of air exchanges in the culture box chambers was increased to 5.1 ± 0.3 µmol CO_2 h^{-1} by perforating the sides of the plastic chambers with 32 holes and placing gas-permeable microporous polypropylene film (0.22 µm pore size) over the holes (Cha-um *et al*., 2007a). The chambers containing the rice seedlings were acclimated for 14 days in a plant growth incubator under a temperature shift of 28± 2°C/ 25 ± 2°C (light/dark), 500± 100 µmol mol^{-1} CO_2 concentration, 60 ± 5% RH, 120 ± 5 µmol m^{-2} s^{-1} PPFD provided by fluorescent lamps with 16 h d^{-1} photoperiod. Mannitol (water-deficit stress) and sodium chloride (salt stress) in the culture media were adjusted to –0.23 (control), –0.40 (50 mM NaCl or 100 mM mannitol) or –0.67 MPa (100 mM NaCl or 200 mM mannitol) iso-osmotic pressure for 14 days. Survival percentage, photosynthetic pigments, chlorophyll fluorescence, net-photosynthetic rate (P_n) and growth characters were measured to determine physiological and biochemical changes.

Data collection

Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll (TC) concentrations were determined following the method of Shabala *et al.* (1998) and the total carotenoids (C_{x+c}) concentration was measured according to Lichtenthaler (1987). One hundred milligrams of leaf material was collected, placed in a 25 mL glass vial along with 10 mL 95.5% acetone, and blended using a homogeniser. The Chl_a, Chl_b, and C_{x+c} concentrations were measured using an UV-visible spectrophotometer. A solution of 95.5% acetone was used as a blank.

Chlorophyll fluorescence emission from the adaxial surface of the leaf was measured using a fluorescence monitoring system in the pulse amplitude modulation mode, as previously described by Loggini *et al.* (1999). A leaf, adapted to dark conditions for 30 min using leaf-clips, was initially exposed to the modulated measuring beam of far–red light (LED source with typical peak at wavelength 735 nm). Original (F_0) and maximum (F_m) fluorescence yields were measured under weak modulated red light (< 0.5 µmol m^{-2} s^{-1}) with 1.6 s pulses of saturating light (> 6.8 µmol m^{-2} s^{-1} PAR) and calculated using FMS software for Windows®. The variable fluorescence yield (F_v) was calculated by the equation $F_m - F_0$. The ratio of variable to maximum fluorescence (F_v/F_m) was calculated as maximum quantum yield of PSII photochemistry. The photon yield of PSII (Φ_{PSII}) in the light was calculated by $\Phi_{PSII} = (F_m' - F)/F_m'$ after 45 s illumination, when steady state was achieved (Maxwell and Johnson (2000).

Net-photosynthetic rate (P_n) was calculated by measuring the different concentrations of CO_2 inside and outside the glass vessel containing the seedlings. The CO_2 concentrations (C_{in} and C_{out}) at steady state were measured by gas chromatography (GC; Model GC-17A, Shimadzu Co. Ltd., Japan). The detector (TCD; thermal conductivity detector) and injector were set at 250°C. The temperature program of a GC capillary column (GS-Q, J&W Scientific®, Germany) was set at 30°C for 1 min in the initial state and increased to 100°C at a rate of 20°C min^{-1} and held for 1 min (Fujiwara *et al.,* 1987). The P_n of *in vitro* cultivated seedlings was calculated according to the method of Fujiwara *et al.* (1987).

Survival percentage, fresh weight (FW), dry weight (DW), shoot height (SH), root length (RL) and leaf area (LA) of rice seedlings were measured, as described by Cha-um *et al.* (2006). Seedlings were dried at 80°C in a hot-air oven for 2 days, and then incubated in desiccators before the measurement of dry weight. The leaf area of rice seedlings was measured using a leaf area meter DT-scan.

Table 1. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC), total carotenoid content (C_{x+c}) and Chl_a:Chl_b ratio of two rice cultivars grown under iso-osmotic water deficit (mannitol) and salt (NaCl) stress for 14 days. Different letters in each column show significant differences at $p \leq 0.01$ by Tukey's HSD

Osmotic potential (MPa)	Chl_a (μg g^{-1} FW)	Chl_b (μg g^{-1} FW)	TC (μg g^{-1} FW)	C_{x+c} (μg g^{-1} FW)	Chl_a:Chl_b
RD6					
–0.23 (Control)	488.9^a	271.1^a	760.0^a	168.1^a	1.80^a
–0.40 Mannitol	406.1^b	255.9^a	662.0^b	138.0^b	1.59^a
–0.40 NaCl	335.9^c	268.9^a	604.8^b	99.5^c	1.25^b
–0.67 Mannitol	278.2^d	299.2^a	577.4^b	71.3^d	0.93^{bc}
–0.67 NaCl	137.6^e	180.0^b	317.6^c	32.5^e	0.76^c
KDML105					
–0.23 (Control)	406.0^a	206.1^b	612.1^a	135.7^a	1.97^a
–0.40 Mannitol	365.5^a	231.7^b	597.2^a	107.2^b	1.58^a
–0.40 NaCl	370.4^a	201.8^b	572.2^a	94.5^{bc}	1.84^a
–0.67 Mannitol	253.9^b	294.6^a	548.5^a	70.7^c	0.86^b
–0.67 NaCl	186.3^c	207.1^b	393.4^b	42.5^d	0.90^b

Experiment design and data analysis

The experiment was arranged as completely randomized design with ten replicates and four plantlets per replicate. The mean values obtained were compared by Tukey's HSD (honestly significant difference) test and analyzed using SPSS software. The correlations between biochemical, physiological and growth characters were evaluated using Pearson's correlation coefficients.

Results

RD6 rice cultivar

The survival percentage of RD6 rice seedlings declined to 66.7 and 33.3% when exposed to -0.67 MPa mannitol induced stress and salt induced osmotic stress, respectively (Fig. 1). The level of photosynthetic pigments in the leaf tissues of rice seedlings cultivated under osmotic stress conditions decreased, and was correlated with the decrease in osmotic potential in the culture media (Table 1). Chl_a, TC and C_{x+c} in the osmotically stressed leaves of RD6 rice seedlings decreased significantly, related to the decrease in osmotic pressure in the culture media (Table 1). The Chl_b content in the leaf tissues showed a dramatic decrease only under –0.67 MPa NaCl treatment. The Chl_a:Chl_b ratio began to decrease following exposure to –0.40 MPa NaCl. Those pigments were extensively damaged, by 71.9, 33.6, 58.2 and 80.7% respectively, when subjected to –0.67 MPa NaCl (Table 1). Moreover, the Chl_a content in relation to osmotic stress was positively related to maximum quantum yield of PSII (F_v/F_m) ($R^2 = 0.65$) (Fig. 2a). The F_v/F_m and photon yield of PSII (Φ_{PSII}) in RD6 rice seedlings grown under –067 MPa NaCl were significantly reduced when compared to the plantlets of the control group (–0.23MPa), while the same measurements in plantlets grown in –0.67 MPa mannitol were unchanged (Table 2). On the other hand, non-photochemical quenching (NPQ) in osmotically stressed seedlings increased, especially in response to –0.67 MPa NaCl. The reduction of Φ_{PSII} in response to osmotic stress was positively correlated with net-photosynthetic rate (P_n) ($R^2 = 0.52$) (Fig. 2c). P_n reduced sharply in osmotically stressed seedlings exposed both

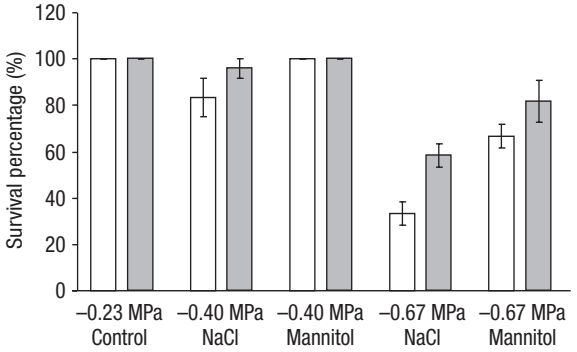

Figure 1. Survival percentage of RD6 (white bar) and KDML105 (gray bar) rice seedlings grown under iso-osmotic water deficit (mannitol) and salt (NaCl) stress for 14 days.

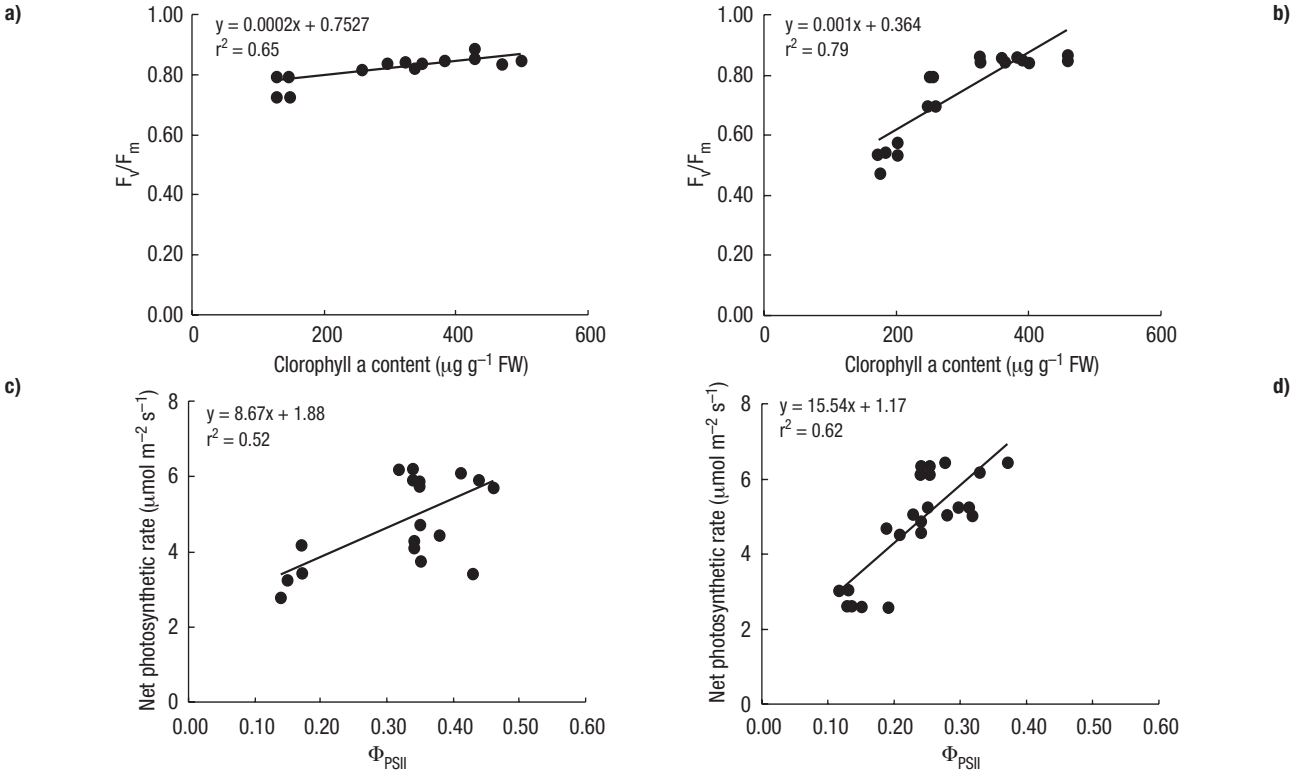

Figure 2. Relationship between chlorophyll a content and maximum quantum yield of PSII (F_v/F_m) of RD6 (a) and KDML105 (b) as well as photon yield of PSII (Φ_{PSII}) and net-photosynthetic rate (P_n) of RD6 (c) and KDML105 (d).

to extreme salt stress and extreme water-deficit stress (Table 2), leading to growth reduction. FW, DW, SH, RL and LA in osmotically stressed seedlings were significantly reduced, related to the osmotic pressure in the culture media (Table 3). In addition, biochemical, physiological and growth characters, including Chl_a, TC, C_{x+c}, F_v/F_m, Φ_{PSII}, P_n and DW were positively correlated with each other to a highly significant ($p \leq 0.01$) level in statistical analysis (Table 4). In this study, iso-osmotic salt stress was found to inhibit the growth and development of RD6 rice seedlings to a greater extent than water-deficit stress.

Table 2. Maximum quantum yield of PSII (F_v/F_m), photon yield of PSII (Φ_{PSII}), non photochemical quenching (NPQ) and net photosynthetic rate (P_n) of two rice cultivars grown under iso-osmotic water deficit (mannitol) and salt (NaCl) stress for 14 days. Different letters in each column show significant difference at $p \leq 0.01$ by Tukey's HSD

Osmotic potential (MPa)	F_v/F_m	Φ_{PSII}	NPQ	P_n (μmol m^{-2} s^{-1})
RD6				
–0.23 (Control)	0.846[a]	0.369[a]	0.050[d]	6.08[a]
–0.40 Mannitol	0.859[a]	0.383[a]	0.087[c]	5.84[a]
–0.40 NaCl	0.839[a]	0.352[a]	0.089[bc]	4.32[b]
–0.67 Mannitol	0.827[a]	0.347[a]	0.112[b]	4.14[b]
–0.67 NaCl	0.776[b]	0.154[b]	0.147[a]	3.16[c]
KDML105				
–0.23 (Control)	0.847[a]	0.286[a]	0.044[c]	6.34[a]
–0.40 Mannitol	0.854[a]	0.282[a]	0.044[c]	5.57[b]
–0.40 NaCl	0.847[a]	0.258[a]	0.048[c]	4.91[c]
–0.67 Mannitol	0.746[b]	0.224[a]	0.072[b]	4.73[c]
–0.67 NaCl	0.539[c]	0.142[b]	0.124[a]	2.76[d]

KDML105 rice cultivar

In the case of KDML105, the survival percentage of rice seedlings declined to 81.7% and 58.3% when subjected to –0.67 MPa mannitol-induced stress and salt-induced osmotic stress, respectively (Fig. 1). The survival percentage of KDML105 rice under osmotic stress was maintained better than that of RD6 rice. Photosynthetic pigments in the leaf tissues of rice seedlings grown under osmotic stress were damaged, positively related to the decrease in osmotic potential in the culture media (Table 1). Chlorophyll a (Chl_a) and

Table 3. Growth characters, fresh weight (FW), dry weight (DW), shoot height (SH), root length (RL) and leaf area (LA) of two rice cultivars grown under iso-osmotic water deficit (mannitol) and salt (NaCl) stress for 14 days. Different letters in each column show significant difference at $p \leq 0.01$ by Tukey's HSD

Osmotic pressure (MPa)	FW (mg)	DW (mg)	SH (cm)	RL (cm)	LA (mm^2)
RD6					
–0.23 (Control)	281.2^{a}	77.0^{a}	42.2^{a}	10.2^{a}	$1{,}058^{a}$
–0.40 Mannitol	265.5^{ab}	60.0^{b}	38.5^{b}	8.8^{b}	852^{b}
–0.40 NaCl	202.6^{c}	57.4^{b}	37.8^{b}	8.2^{b}	616^{c}
–0.67 Mannitol	236.6^{bc}	55.2^{b}	35.8^{b}	6.7^{c}	548^{c}
–0.67 NaCl	151.9^{d}	39.4^{c}	20.7^{c}	5.4^{d}	352^{d}
KDML105					
–0.23 (Control)	305.5^{a}	76.9^{a}	43.2^{a}	9.3^{a}	$1{,}210^{a}$
–0.40 Mannitol	248.5^{ab}	61.6^{b}	38.2^{ab}	9.2^{a}	$1{,}132^{a}$
–0.40 NaCl	260.9^{abc}	55.8^{bc}	37.5^{b}	7.6^{b}	994^{b}
–0.67 Mannitol	250.4^{bc}	52.8^{bc}	38.0^{b}	6.2^{c}	919^{b}
–0.67 NaCl	209.1^{c}	46.6^{c}	31.1^{c}	5.1^{d}	534^{c}

the Chl_a:Chl_b ratio in the leaves of osmotically stressed KDML105 rice seedlings dropped significantly under –0.67 MPa osmotic pressure, whereas chlorophyll b (Chl_b) and total chlorophyll (TC) decreased dramatically only in –0.67 MPa NaCl. The total carotenoid (C_{x+c}) content in the leaf tissues dropped sharply, depending on the degree of mannitol-induced stress or salt-induced stress (Table 1). Chl_a, TC and C_{x+c} were significantly degraded, by 54.9, 35.7 and 68.7%, respectively when subjected to –0.67 MPa NaCl. The exception was Chl_b, which was maintained (Table 1). In addition, the Chl_a content in response to osmotic stress was positively related to maximum quantum yield of PSII (F_v/F_m) ($R^2 = 0.79$) (Fig. 2b). The F_v/F_m and photon yield of PSII (Φ_{PSII}) in KDML105 seedlings grown under –0.67 MPa NaCl decreased significantly when compared to plantlets in the control group (–0.23MPa), while the Φ_{PSII} in plantlets grown in –0.67 MPa mannitol were unchanged (Table 2). In contrast, NPQ in osmotically stressed seedlings increased, especially in response to –0.67 MPa NaCl. The reduction of Φ_{PSII} in response to osmotic stress was positively correlated with net-photosynthetic rate (P_n) ($R^2 = 0.62$) (Fig. 2d). The P_n in osmotically stressed seedlings reduced sharply during exposure to salt stress and water-deficit stress (Table 2), leading to growth reduction. FW, DW, SH, RL and LA in osmotically stressed seedlings were significantly reduced, related to osmotic pressure in the culture media (Table 3). The Chl_a, TC, C_{x+c}, F_v/F_m, Φ_{PSII}, P_n and DW showed a positive correlation with a highly significant level ($p \leq 0.01$) (Table 4).

Table 4. Relationship between biochemical, physiological and growth characters of two rice cultivars grown under iso-osmotic water deficit (mannitol) and salt (NaCl) stress for 14 days. Highly significant levels at $p \leq 0.01$ of all relationships are represented by Pearson's correlation coefficients

Variables	Chl_a	TC	C_{x+c}	F_v/F_m	Φ_{PSII}	P_n	DW
RD6							
Chl_a	—	—	—	—	—	—	—
TC	0.950	—	—	—	—	—	—
C_{x+c}	0.984	0.910	—	—	—	—	—
F_v/F_m	0.798	0.777	0.766	—	—	—	—
Φ_{PSII}	0.816	0.881	0.739	0.824	—	—	—
P_n	0.949	0.875	0.954	0.734	0.704	—	—
DW	0.803	0.873	0.735	0.572	0.678	0.736	—
KDML105							
Chl_a	—	—	—	—	—	—	—
TC	0.876	—	—	—	—	—	—
C_{x+c}	0.847	0.675	—	—	—	—	—
F_v/F_m	0.888	0.852	0.818	—	—	—	—
Φ_{PSII}	0.822	0.754	0.797	0.836	—	—	—
P_n	0.865	0.823	0.908	0.883	0.820	—	—
DW	0.748	0.561	0.761	0.624	0.637	0.777	—

Discussion

The survival percentage of rice seedlings cultivated in salt-induced osmotic stress was greatly reduced, and to a higher degree than those under mannitol-induced stress, related to the genotype and degree of salt tolerance. RD6 and KDML105 rice cultivars have been reported as salt susceptible, showing toxic symptoms, including pigment degradation and growth reduction, in 171 mM NaCl or –0.42 MPa osmotic potential (Cha-um *et al.,* 2007b, 2010). The survival percentage of INIAP12 salt tolerant rice grown under iso-osmotic water deficit stress (200 mM mannitol) and salt stress (100 mM NaCl) was higher than that of CT6748 -8-CA-17, a salt sensitive cultivar (Morsy *et al.,* 2007). Na^+ in culture media supplemented with NaCl may not only play a role as a toxic ion, but also have osmotic effects for the rice plant, especially in the seedling stage. In this study, KDML105 was found to be more tolerant to salt stress than RD 6. It is possible that Na^+ uptake and accumulation in KDML105 is lower than in RD6. Under 12 dS m^{-1} NaCl treatment, the Na^+ level in Pokkali rice (salt tolerant) was lower than that in IR28 (salt susceptible) (Dionisio-Sese and Tobita, 1998). The low Na^+ accumulation in salt tolerant cultivars of rice may depend on the activity of H^+-ATPase, H^+-PPase, Na^+/H^+ antiporters and Na^+/K^+ pumping (Fukuda *et al.,* 2004; Martínez-Atienza *et al.,* 2007). The photosynthetic pigments in osmotically stressed rice seedlings of different genotypes were damaged, depending on the increase in osmotic pressure. The degradation of TC and C_{x+c} in the leaves of Koshihikari, a moderately salt tolerant genotype, when grown under salt stress, was demonstrated (Sultana *et al.,* 1999). In addition, Chl_a and Chl_b in the Taipei 309 rice cultivar grown under sorbitol-induced water deficit were enriched, whereas under NaCl induced stress they dropped significantly (Bahaji *et al.,* 2002). Enriched Na^+ in salt-stressed seedlings badly damages the cells, organelles and tissues, prior to cell death (Ahmad *et al.,* 2007; Castillo *et al.,* 2007). Photosynthetic pigments and their activities are very sensitive to enriched Na^+ in the cells, identified by pigment degradation and chlorophyll fluorescence diminution, respectively (Dionisio-Sese and Tobita, 1998; Castillo *et al.,* 2007). Pigment degradation in osmotically-stressed rice seedlings was a major factor in limiting photosynthetic activity, including low F_v/F_m, Φ_{PSII} and P_n, leading to overall growth inhibition. P_n in salt-stressed Koshihikari rice (100 mM NaCl) dropped significantly, by 2.33 times, when compared to the control (0 mM NaCl) (Sultana *et al.,* 1999). In most cases, the physiological characteristics, including levels of photosynthetic pigments, chlorophyll fluorescence and P_n in salt stressed seedlings were higher than in seedlings grown under mannitol-induced stress and were directly related to growth inhibition and decreased productivity (Bahaji *et al.,* 2002; Cha-um *et al.,* 2010). Na^+ toxicity from the salt stress treatment is one of the most important factors in damaging the plant cells, and is greater than is found under mannitol-induced osmotic stress (Castillo *et al.,* 2007). Growth characters, including SH, RL, LA, FW and DW, of rice grown under iso-osmotic water deficit and salt stress were reduced, related to the osmotic pressure in the culture media. This was especially the case under salt-induced osmotic stress and is similar to previous reports on the reduction of growth in rice exposed to iso-osmotic stress (Bahaji *et al.,* 2002; Hien *et al.,* 2003; Ahmad *et al.,* 2007). In addition, fresh weight and dry weight in tolerant cultivars, including Basmati-370 and DR2, were maintained better than in the sensitive genotypes, Basmati-Kashmir and Cuom, when subjected to iso-osmotic stress derived from mannitol/PEG (polyethylene glycol) and NaCl salt (Hien *et al.,* 2003; Ahmad *et al.,* 2007). In this study, RD6 (waxy mutant line) was more sensitive to iso-osmotic stress than the original, KDML105. It is possible that the mutant line of RD6 rice is more susceptible to both salt and water deficit stress than the KDML105 cultivar. In contrast, salt tolerance in the Drew (Uddin *et al.,* 2007), Dongjinbyeo (Lee *et al.,* 2004) and IR-8 (Shereen *et al.,* 2009) mutant lines of rice, derived using gamma ray irradiation, was superior to the original cultivars. The Homjan (HJ) rice variety has been classified as salt tolerant and water deficit tolerant, using *in vitro* rapid screening (Cha-um *et al.,* 2010).

In conclusion, survival percentage, chlorophyll pigment levels and the photosynthetic abilities of rice seedlings grown under iso-osmotic salt stress, declined to a greater degree than in seedlings grown under mannitol-induced iso-osmotic stress, leading to a greater reduction in growth rate. Also, the RD6 rice cultivar was more sensitive to osmotic stress than the KDML105 cultivar when subjected to extreme conditions.

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References

AHMAD M.S.A., JAVED F., ASHRAF M., 2007. Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissues of two indica rice (*Oryza sativa* L.) genotypes. Plant Growth Regul 53, 53-63.

ARIYAPHANPHITAK W., CHIDTHAISONG A., SAROBOL E., BASHKIN V.N., TOWPRAYOON S., 2005. Effects of elevated ozone concentrations on Thai jasmine rice cultivars (*Oryza sativa* L). Water Air Soil Poll 167, 179-200.

BAHAJI A., MATEU I., SANZ A., CORNEJO M.J., 2002. Common and distinctive responses of rice seedlings to saline- and osmotically-generated stress. Plant Growth Regul 38, 83-94.

CASTILLO E.G., TUONG T.P., ISMAIL A.M., INUBUSHI K., 2007. Response to salinity in rice: comparative effects of osmotic and ionic stresses. Plant Prod Sci 10, 159-170.

CHA-UM S., SUPAIBULWATANA K., KIRDMANEE C., 2006. Water relation, photosynthetic ability, and growth of Thai jasmine rice (*Oryza sativa* L. ssp. *indica* cv. KDML105) to salt stress by application of exogenous glycinebetaine and choline. J Agron Crop Sci 192, 25-36.

CHA-UM S., SUPAIBULWATANA K., KIRDMANEE C., 2007a. Glycinebetaine accumulation, physiological characterizations, and growth efficiency in salt tolerant and salt sensitive lines of indica rice (*Oryza sativa* L. spp. *indica*) response to salt stress. J Agron Crop Sci 193, 157-166.

CHA-UM S., VEJCHASARN P., KIRDMANEE C., 2007b. An effective defensive response in Thai aromatic rice variwties (*Oryza sativa* L. spp. *indica*) to salinity. J Crop Sci Biotechnol 10, 257-264.

CHA-UM S., KIRDMANEE C., 2008. Assessment of salt tolerance in *Eucalyptus*, rain tree and Thai neem under laboratory and the field conditions. Pak J Bot 40, 2041-2051.

CHA-UM S., NHUNG N.T.H., KIRDMANEE C., 2010. Effect of mannitol- and salt-induced iso-osmotic stress on proline accumulation, photosynthetic abilities and growth characters of rice cultivars (*Oryza sativa* L. spp. *indica*). Pak J Bot 42, 927-941.

DIONISIO-SESE M.L., TOBITA S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci 135, 1-9.

FLOWERS T.J., FLOWERS S.A., 2005. Why dose salinity pose such a difficult problem for plant breeders? Agric Water Manage 78, 15-24.

FUJIWARA K., KOZAI T., WATANABE I., 1987. Fundamental studies on environment in plant tissue culture vessels. (3) Measurements of carbon dioxide gas concentration in closed vessels containing tissue cultured plantlets and estimates of net-photosynthetic rates of the plantlets. J Agric Method 4, 21-30.

FUKUDA A., NAKAMURA A., TAGIRI A., TANAKA H., MIYAO A., HIROCHIKA H., TANAKA Y., 2004. Function, intracellular localization, and the importance in salt tolerance of a vacuolar Na^+/H^+ antiporter from rice. Plant Cell Physiol 45, 146-159.

GREGORIO G.B., SENADHIRA D., MENDOZA R.D., 1997. Screening rice for salinity tolerance. IRRI Discussion Paper Series No. 22, International Rice Research, pp. 1-34.

GREGORIO G.B., SENADHIRA D., MENDOZA R.D., MANIGBAS N.L., ROXAS J.P., GUERTA C.Q., 2002. Progress in breeding for salinity tolerance and associated abiotic stresses in rice. Field Crops Res 76, 91-101.

HASEGAWA P.M., BRESSAN R.A., ZHU J.K., BOHNERT H.J., 2000. Plant cellular and molecular responses to high salinity. Ann Rev Plant Physiol Mol Biol 51, 463-499.

HIEN D.T., JACOBS M., ANGENON G., HERMANS C., THU T.T., VAN SON L., ROOSENS H., 2003. Proline accumulation and Δ-1-pyrroline-5-carboxylate synthetase gene properties in three rice cultivars differing in salinity and drought tolerance. Plant Sci 165, 1059-1068.

KEERATIPIBUL S., LUANGSAKUL N., LERTSATCHAYARN T., 2008. The effect of Thai glutinous rice cultivars, grain length and cultivating locations on the quality of rice cracker (arare). LWT-Food Sci Technol 41, 1934-1943.

KHAN M.A., ABDULLAH Z., 2003. Salinity-sodicity induced changes in reproductive physiology of rice (*Oryza sativa*) under dense soil conditions. Environ Exp Bot 49, 145-157.

KHUSH G.S., 2005. What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol Biol 59, 1-6.

KIRDMANEE C., MOSALEEYANON K., 2000. Environmental engineering for transplant production. In: Transplant production in the 21st century (Kubota C., Chun C., eds). Kluwer Academic Publishers, Netherlands. pp. 78-81.

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 I.S., KIM D.S., KANG S.Y., WI S.G., JIN H., YUN P.Y., LIM Y.P., LEE Y.I., 2004. Characterizing salt stress response in a rice variety and its salt tolerant lines derived from *in vitro* mutagenesis. J Plant Biotechnol 6, 205-212.

LICHTENTHALER H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148, 350-380.

LOGGINI B., SCARTAZZA A., BRUGNOLI E., NAVARI-IZZO F., 1999. Antioxidant defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. Plant Physiol 119, 1091-1099.

LUO Q., YU B., LIU Y., 2005. Differential sensitivity to chloride and sodium ions in seedlings of *Glycine max* and *G. soja* under NaCl stress. J Plant Physiol 162, 1003-1012.

LUTTS S., ALMANSOURI M., KINET J.M., 2004. Salinity and water stress have contrasting effects on the relationship between growth and cell viability during and after stress exposure in durum wheat callus. Plant Sci 167, 9-18.

MAHAJAN S., TUTEJA N., 2005. Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444, 139-158.

MARTÍNEZ-ATIENZA J., JIANG X., GARCIADEBLAS B., MENDOZA I., ZHU J.K., PARDO J.M., QUINTERO F.J., 2007. Conservation of the salt overlay sensitive pathway in rice. Plant Physiol 143, 1001-1012.

MAXWELL K., JOHNSON G.N., 2000. Chlorophyll fluorescence - a practical guide. J Exp Bot 51, 659-668.

MORSY M.R., JOUVE L., HAUSMAN J.F., HOFFMANN L., STEWARD J.M., 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.

PARIDA A.K., DAS A.B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Safe 60, 324-349.

REDDY A.R., CHITANYA K.V., VIVEKANANDAN M., 2004. Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol 161, 1189-1202.

SAIRAM R.K., TYAGI A., 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci 86, 407-421.

SENADHIRA D., ZAPATA-ARIAS F.J., GREGORIO G.B., ALEJAR M.S., DE LA CRUZ H.C., PADOLINA T.F., GÁLVEZ A.M., 2002. Development of the first salt-tolerant rice cultivar through indica/indica anther culture. Field Crops Res 76, 103-110.

SHABALA S.N., SHABALA S.I., MARTYNENKO A.I., BABOURINA O., NEWMAN I.A., 1998. Salinity effect on bioelectric activity, growth, Na^+ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. Aust J Plant Physiol 25, 609-616.

SHANNON M.C., RHOADES J.D., DRAPER J.H., SCARDACI S.C., SPYRES M.D., 1998. Assessment of salt tolerance in rice cultivars in response to salinity problems in California. Crop Sci 38, 394-398.

SHEREEN A., ANSARI R., MUMTAZ S., BUGHIO H.R., MUJTABA S.M., SHIRAZI M.U., KHAN M.A., 2009. Impact of gamma irradiation induced changes on growth and physiological responses of rice under saline conditions. Pak J Bot 41, 2487-2495.

SULTANA N., IKEDA T., ITOH R., 1999. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environ Exp Bot 42, 211-220.

UDDIN M.I., RASHID M.H., KHAN N., PERVEEN M.F., TAI T.H., TANAKA K., 2007. Selection of promising salt tolerant rice mutants derived from cultivar 'Drew' and their antioxidant enzymes activity under salt stress. SABRAO J Breed Genet 39, 89-98.

VAIDYNATHAN H., SIVAKUMAR P., CHAKRABARTY R., THOMAS G., 2003. Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) -differential response in salt-tolerant and sensitive varieties. Plant Sci 165, 1411-1418.

WAHID A., 2004. Analysis of toxic and osmotic effects of sodium chloride on leaf growth and economic yield of sugarcane. Bot Bull Acad Sin 45, 133-141.

WANG W., VINOCUR B., SHOSEYOV O., ALTMAN A., 2001. Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. Acta Hort 560, 285-292.

WANG W., VINOCUR B., ALTMAN A., 2003. Plant responses to drought, salinity and extreme temperature: towards genetic engineering for stress tolerance. Planta 218, 1-14.

WONGPOKKHOM N., KHEORUENROMNE I., SUDDHIPRAKARN A., GILKES R.J., 2008. Micromorphological properties of salt affected soils in Northeast Thailand. Geoderma 144, 158-170.

ZANG X., KOMATSU S., 2007. A proteomics approach for identifying osmotic-stress-related proteins in rice. Phytochem 68, 426-437.

ZENG L., SHANNON M.C., 2000. Salinity effects on seedling growth and yield components of rice. Crop Sci 40, 996-1003.

ZENG L., LESCH S.M., GRIEVE C.M., 2003. Rice growth and yield respond to changes in water depth and salinity stress. Agric Water Manage 59, 67-75.