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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*)

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Abstract

The aim of this study was to investigate the biochemical, physiological and morphological responses of rice cultivars to iso-osmotic water deficit and salt stress. Seedlings of three rice cultivars were photoautotrophically grown in MS media and subsequently exposed to -0.23 (control), -0.42 or -0.94 MPa iso-osmotic mannitol (water-deficit stress) or NaCl (salt stress). 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 osmotically-stressed seedlings were significantly reduced when compared to those of the control group (without mannitol or NaCl), leading to net-photosynthetic rate (P_n) and growth reduction with positive correlation. In addition, physiological changes and growth parameters of salt stressed seedlings were more sharply reduced than those of water-deficit stressed seedlings, especially in PT1 salt susceptible. On the other hand, the proline contents in the root and leaf tissues of osmotically-stressed seedlings increased significantly, especially in response to iso-osmotic salt stress. The chlorophyll pigments in iso-osmotically-stressed leaves were significantly degraded, related to low water oxidation, low P_n and growth reduction. Those multivariate parameters were subjected to classify the salt tolerance, HJ and salt susceptible, PT1 and RD6 as well as the water deficit tolerance, HJ and RD6 and water deficit susceptible, PT1 using Hierarchical cluster analysis.

Introduction

Water-deficit and salt affected soil are the major abiotic stresses, reducing rice crop productivity by more than 50% world-wide (Mahajan & Tuteja, 2005). Plant growth and developmental processes in terms of biochemical, physiological and morphological characteristics are inhibited by both water deficit and salt stresses (Hasegawa *et al*., 2000; Wang *et al*., 2001; Parida & Das, 2005). Osmotic stresses derived from salt affected soil and water deficit conditions are well established in crop species (Lutts *et al*., 2004; Wahid, 2004; Luo *et al*., 2005; Castillo *et al*., 2007). In tolerant plants, there are many defense mechanisms such as osmoregulation, ion homeostasis, antioxidant and hormonal systems, helping plants to stay alive and development prior to their reproductive stages (Hasegawa *et al*., 2000; Wang *et al*., 2003; Reddy *et al*., 2004; Sairam & Tyagi, 2004; Mahajan & Tuteja, 2005; Ashraf, 2010).

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Biochemical [proline, glycine betaine, soluble sugars, photosynthetic pigments and defensive proteins] and physiological [water use efficiency, osmotic adjustment, chlorophyll a fluorescence, and net-photosynthetic rate (P_n)] changes in plants growing under salt or water-deficit conditions have been broadly investigated in many crop species such as rice (Castillo *et al*., 2007; Cha-um *et al*., 2007a), maize (Hu *et al*., 2007; Wang *et al*., 2008) and potatoes (Teixeira & Pereira, 2007). Those parameters in crop species cultivated in water deficit or salt stresses have been developed as effective indices for tolerant selection in breeding programs (Ashraf & Harris, 2004; Parida & Das, 2005; Ashraf & Foolad, 2007). Mannitol, a member of sugar alcohols, is an osmotic adjustment chemical to control osmotic potential in the culture media or nutrient solutions in order to induce water deficit conditions for protein expression or proteomic studies (Zang & Komatsu, 2007). In addition to, sodium chloride salt, a small molecule with rapid dissolving and oxidizing by water into Na^+ and Cl^-, is generally selected as salt stress pressure (Dionisio-Sese & Tobita, 1998; Sultana *et al*., 1999; Vaidyanathan *et al*., 2003; Cha-um *et al*., 2004; Cha-um *et al*., 2007a). As well as, the mannitol and NaCl induced iso-osmotic stress in rice crop has been established (Hien *et al*., 2003; Morsy *et al*., 2007).

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 and water deficit susceptible, which has showed the negative effects on both seedling and reproductive stages (Shannon *et al*., 1998; Zeng & Shannon, 2000; Khan & Abdullah, 2003; Zeng *et al*., 2003a). Abiotic stressed tolerance in rice breeding program is a profitable issue for plant breeders (Gregorio *et al*., 2002; Senadhira *et al*., 2002; Flowers & Flowers, 2005). In Thailand, Homjan (HJ) salt tolerance and Pathumthani 1 (PT1) salt susceptible varieties have been identified using multivariate parameters (Cha-um *et al*., 2007b). In addition to, RD6, a gamma-irradiation mutant variety derived from Thai jasmine rice (KDML105), is sticky or glutinous rice with high cooking quality including long grain, sticky texture and enriched aroma flavor and widely cultivated in northeastern Thailand, which is a large area of salinity and water deficit problems. The objective of this research was to determine the physiological changes and the growth parameters of three rice cultivars in response to iso-osmotic salt, or water-deficit stresses as well as to classify the salt or water-deficit tolerance using multivariate cluster analysis.

Materials and Method

Plant materials: Rice seeds of three cultivars including glutinous rice (RD6) and non glutinous rice [Homjan (HJ; salt tolerance) and Pathumthani 1 (PT1; salt susceptible)] were obtained from a germplasm bank. Seeds were manually dehusked, sterilized once in 5% Clorox® for 60 min, once in 30% Clorox® for 30 min, and then rinsed three times with sterile distilled-water. Surface-sterilized seeds were germinated on 0.25% Phytagel®-solidified MS media 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 hd^{-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 controlled RH at 65±5% by 1.5 L saturated NaCl solution. The number of air exchanges in the culture box chambers was increased to 5.1±0.3 h^{-1} by punching the side of the plastic chambers with 32 holes and placing gas-permeable microporous polypropylene film (0.22 μm pore size) over each hole

(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 hd^{-1} photoperiod. Mannitol (water-deficit stress) and sodium chloride (salt stress) in the culture media were adjusted to -0.23 (control), -0.42 or -0.94 MPa iso-osmotic pressures for 7 days. Photosynthetic pigments, proline contents, chlorophyll a fluorescence, net-photosynthetic rate (P_n) and growth characters were measured as physiological and biochemical changes.

Data measurement: Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll concentrations were analyzed following the methods of Shabala *et al*., (1998) and total carotenoid (C_{x+c}) concentrations were measured according to Lichtenthaler (1987). One hundred milligrams of leaf material were collected, placed in a 25mL glass vial, added with 10 mL of 95.5% acetone, and blended using a homogenizer. The Chl_a and Chl_b concentrations were measured using a UV-visible spectrophotometer at 662 nm and 644 nm wavelengths. The C_{x+c} concentration was also measured by spectrophotometer at 470 nm. A solution of 95.5% acetone was used as a blank.

The proline content of the root and leaf tissues was extracted and analyzed according to the method of Bates *et al*., (1973). Fifty-milligrams of fresh weight material were ground with liquid nitrogen in a mortar. The homogenate powder was mixed with 1 mL aqueous sulfosalicylic acid (3% w/v) and filtered through filter paper (Whatman #1, England). The extracted solution was reacted with an equal volume of glacial acetic acid and ninhydrin reagent (1.25mg 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 by placing the container in 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 at 520 nm using L-proline as a standard.

Chlorophyll *a* fluorescence emission from the adaxial surface on the third leaf from the shoot tip was monitored with 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 735nm). 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 autocalculated using FMS software for Windows®. The variable fluorescence yield (F_v) was calculated by the equation of 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 45secs of illumination, when steady state was achieved. In addition, non-photochemical quenching (NPQ) was calculated as described by Maxwell & Johnson (2000).

Net photosynthetic rate (P_n) was calculated by comparing the different concentrations of CO_2 inside and outside of glass vessel containing with rice seedlings. The CO_2 concentrations inside and outside the glass vessel (C_{in} and C_{out}) at steady state were measured by gas chromatography (GC; Model GC-17A, Shimadzu Co. Ltd., Japan). The P_n of *in vitro* cultivated seedlings was calculated according to the method of Fujiwara *et al*., (1986).

Shoot height (SH), root length (RL), leaf area (LA), fresh weight (FW) and dry weight (DW) 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 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. Growth inhibition, P_n reduction, chlorophyll a fluorescence diminishing and pigment degradation were calculated following Cha-um & Kirdmanee (2008).

Experiment design: The experiment was arranged as 3×5 factorial in completely randomized design (CRD) with eight replicates (n=8). The mean values obtained were compared by Duncan's new multiple ranges test (DMRT) and analyzed using SPSS software. The correlations between physiological, biochemical and growth parameters were evaluated with Pearson's correlation coefficients. Pigment degradation, chlorophyll a fluorescence and P_n reduction, growth inhibition and proline accumulation in rice cultivars were subjected to classify group as tolerance and susceptible using Hierarchical cluster analysis in SPSS software.

Results and Discussion

Salt-stressed rice seedlings of PT1 salt susceptible grown under -0.94 MPa osmotic potential were found a 100% death and the data of all parameters were undetected. Photosynthetic pigments, including chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoid (C_{x+c}) in the osmotically stressed leaves of rice seedlings were sharply reduced, related to the decrease in osmotic pressure in the culture media using mannitol or salt adding media (Table 1). Pigment degradation in the leaf tissues of stressed seedlings was a rapid indicator of plant responses to osmotic stress and was negatively related to the osmotic pressure derived from mannitol ($r^2 = 0.92$) and salt ($r^2 = 0.47$) in the culture media (Fig. 1). Pigment contents in the HJ salt tolerant genotype were better maintained than those in RD6 and PT1 when exposed to osmotic stress (Table 1). The Chl_a, Chl_b, TC and C_{x+c} contents in salt-stressed RD6 seedlings (-0.94 MPa) were significantly reduced by 2.31, 2.25, 2.28 and 1.97 times respectively, when compared to those in water-deficit stressed seedlings (Table 1). The TC degradation due to osmotic stresses derived from mannitol and salt stress was inversely related to maximum quantum yield of PSII (F_v/F_m) ($r^2 = 0.52$ and $r^2 = 0.47$, respectively) (Fig. 2). The chlorophyll *a* fluorescence parameters, F_v/F_m and photon yield of PSII (Φ_{PSII}) in rice seedlings grown under -0.94 MPa salt-stress were significantly diminished when compared to those of seedlings of the control group (-0.23MPa), while non photochemical quenching (NPQ) was enriched (Table 2). The reduction of F_v/F_m in response to osmotic stress generated by mannitol and salt treatments was positively correlated with net-photosynthetic rate (P_n) ($r^2 = 0.38$ and $r^2 = 0.40$) (Fig. 3). The P_n in PT1 susceptible cultivar cultured under osmotic stress was sharply reduced when exposed to both water-deficit and salt stresses, whereas that in HJ salt tolerance was retained (Table 2). The P_n reduction in osmotically stressed seedlings by mannitol and salt stress was positively related to biomass production, which was represented by dry weight (DW) ($r^2 = 0.73$ and $r^2 = 0.58$) (Fig. 4). Shoot height (SH), root length (RL) leaf area (LA), fresh weight (FW), and dDW in osmotically stressed seedlings were significantly reduced, relating to osmotic pressure in the culture media (Table 3). In contrast, proline contents in the osmotic stressed root and leaf tissues of rice cultivars were increased, positively relating to the osmotic stress, especially salt induced osmotic stress (Fig. 5). Proline content in osmotically-stressed rice seedlings was rapidly accumulated in HJ and RD6 root and leaf tissues to be played a key role as osmoregulation defense mechanisms. The biochemical, physiological and growth parameters were subjected to analysis using SPSS software to determine the Pearson's correlation coefficients, which are demonstrated in Table 4. In addition to, pigment degradation, chlorophyll a fluorescence diminishing, P_n reduction, proline accumulation and growth inhibition were input to classify the group salt tolerance, HJ and salt susceptible, PT1 and RD6 as well as the water deficit tolerance, HJ and RD6 and water deficit susceptible, PT1 using Hierarchical cluster analysis (Fig. 6). From the result, RD6 cultivar was defined as water deficit tolerance nevertheless sensitive to salt stress.

Table 1. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) contents of rice cultivars grown under iso-osmotic mannitol and salt stress for 7 days.

Rice cultivars	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)
RD6	-0.23 (Control)	177.1bc	174.3ab	351.4abc	103.6a
	-0.42 Mannitol	168.2c	170.1ab	338.3bcd	95.4ab
	-0.42 NaCl	96.2d	97.1bc	193.3ef	75.5bc
	-0.94 Mannitol	106.8d	110.5bc	217.3ef	68.9c
	-0.94 NaCl	46.3e	49.2c	95.5f	34.9d
HJ	-0.23 (Control)	250.0a	261.3a	511.3a	75.6bc
	-0.42 Mannitol	249.8a	212.6ab	462.4bc	72.6c
	-0.42 NaCl	218.6ab	136.9bc	355.5abc	71.8c
	-0.94 Mannitol	214.1abc	136.6bc	350.7bc	66.1c
	-0.94 NaCl	214.8abc	122.9bc	337.7bcd	63.9c
PT1	-0.23 (Control)	229.3a	174.9ab	404.2ab	73.9bc
	-0.42 Mannitol	206.0abc	128.5bc	334.5bcd	63.1c
	-0.42 NaCl	105.9d	47.5c	153.4ef	29.8d
	-0.94 Mannitol	201.1abc	88.6bc	289.7cde	61.5c
	-0.94 NaCl	ND	ND	ND	ND
Significant level					
Cultivar		**	**	**	**
Osmotic		**	**	**	**
Cultivar × Osmotic		**	*	**	**

Different letters in each column show significant difference at *p≤0.01* (**) by Duncan's New Multiple Range Test (DMRT) and ND represents as non detection.

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 rice cultivars grown under iso-osmotic mannitol and salt stress for 7 days.

Rice cultivars	Osmotic potential (MPa)	F_v/F_m	Φ_{PSII}	NPQ	P_n (μmol m^{-2} s^{-1})
RD6	-0.23 (Control)	0.864a	0.330a	0.028d	2.11de
	-0.42 Mannitol	0.852ab	0.250a	0.032d	2.06de
	-0.42 NaCl	0.836ab	0.291a	0.039cd	1.53ef
	-0.94 Mannitol	0.846ab	0.250a	0.052cd	1.84de
	-0.94 NaCl	0.788ab	0.162b	0.054cd	1.50ef
HJ	-0.23 (Control)	0.835ab	0.291a	0.043cd	4.45a
	-0.42 Mannitol	0.833ab	0.289a	0.051cd	3.69ab
	-0.42 NaCl	0.831ab	0.249a	0.067cd	3.29bc
	-0.94 Mannitol	0.798ab	0.250a	0.073cd	3.58b
	-0.94 NaCl	0.794ab	0.245a	0.075cd	2.33de
PT1	-0.23 (Control)	0.851ab	0.327a	0.096bc	2.57cd
	-0.42 Mannitol	0.671bc	0.311a	0.139ab	1.85de
	-0.42 NaCl	0.648bc	0.254a	0.156a	1.13f
	-0.94 Mannitol	0.540d	0.304a	0.189a	1.55ef
	-0.94 NaCl	ND	ND	ND	ND
Significant level					
Cultivar		**	*	**	**
Osmotic		**	**	*	**
Cultivar × Osmotic		**	*	*	*

Different letters in each column show significant difference at *p≤0.01* (**) by Duncan's New Multiple Range Test (DMRT) and ND represents as non detection.

Table 3. Relationship between physiological and biochemical parameters of rice seedlings grown under iso-osmotic mannitol and salt stress for 7 days.

Parameters	Chl_a	Chl_b	C_{x+c}	PRO	F_v/F_m	Φ_{PSII}	P_n	DW	LA
Chl_a	-	-	-	-	-	-	-	-	-
Chl_b	0.666**	-	-	-	-	-	-	-	-
C_{x+c}	0.455**	0.506**	-	-	-	-	-	-	-
PRO	-0.676**	-0.502**	-0.524**	-	-	-	-	-	-
F_v/F_m	0.695**	0.387**	0.353**	-0.589**	-	-	-	-	-
Φ_{PSII}	0.400**	0.274**	0.388**	-0.458**	0.701**	-	-	-	-
P_n	0.616**	0.416**	0.221	-0.474**	0.315*	0.108	-	-	-
DW	0.377**	0.280*	0.188	-0.273*	0.191	0.169	0.486**	-	-
LA	0.421**	0.091	0.132	-0.494**	0.583**	0.475**	0.235	0.385**	-

Highly significant and significant levels at $p \leq 0.01$ and $p \leq 0.05$ are represented by ** and *, respectively using Pearson's correlation coefficients.

Table 4. Growth characters, shoot height (SH), root length (RL), leaf area (LA), fresh weight (FW) and dry weight (DW) of rice cultivars grown under iso-osmotic mannitol and salt stress for 7 days.

Rice cultivars	Osmotic pressure (MPa)	SH (cm)	RL (cm)	LA (cm^2)	FW (mg)	DW (mg)
RD6	-0.23 (Control)	35.0a	7.6cde	16.18ab	315.1bc	61.4ab
	-0.42 Mannitol	31.3b	7.5cde	8.33d	256.7de	54.1cd
	-0.42 NaCl	28.1c	6.2de	8.51d	236.6de	52.7cd
	-0.94 Mannitol	28.5c	6.9de	4.74f	246.7de	53.2cd
	-0.94 NaCl	27.8c	5.9e	2.72f	219.6e	50.4cd
HJ	-0.23 (Control)	32.4ab	10.2ab	18.71a	437.3a	77.4a
	-0.42 Mannitol	31.9b	9.9ab	13.13bc	390.1ab	65.4ab
	-0.42 NaCl	30.2bc	8.5bcd	9.84cd	315.8bc	55.0cd
	-0.94 Mannitol	30.1bc	8.5bcd	7.45de	296.3cd	57.2bc
	-0.94 NaCl	28.6c	7.4cde	6.53ef	219.4e	48.9d
PT1	-0.23 (Control)	35.1a	12.1a	21.24a	452.5a	70.8a
	-0.42 Mannitol	33.2ab	11.9a	18.81a	440.5a	61.4ab
	-0.42 NaCl	30.9bc	9.2ab	11.09c	288.4cd	47.7d
	-0.94 Mannitol	28.1c	8.7bcd	13.44bc	326.4bc	47.0d
	-0.94 NaCl	ND	ND	ND	ND	ND
Significant level						
Cultivar		*	**	**	**	*
Osmotic		**	**	**	**	**
Cultivar × Osmotic		*	*	**	**	*

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

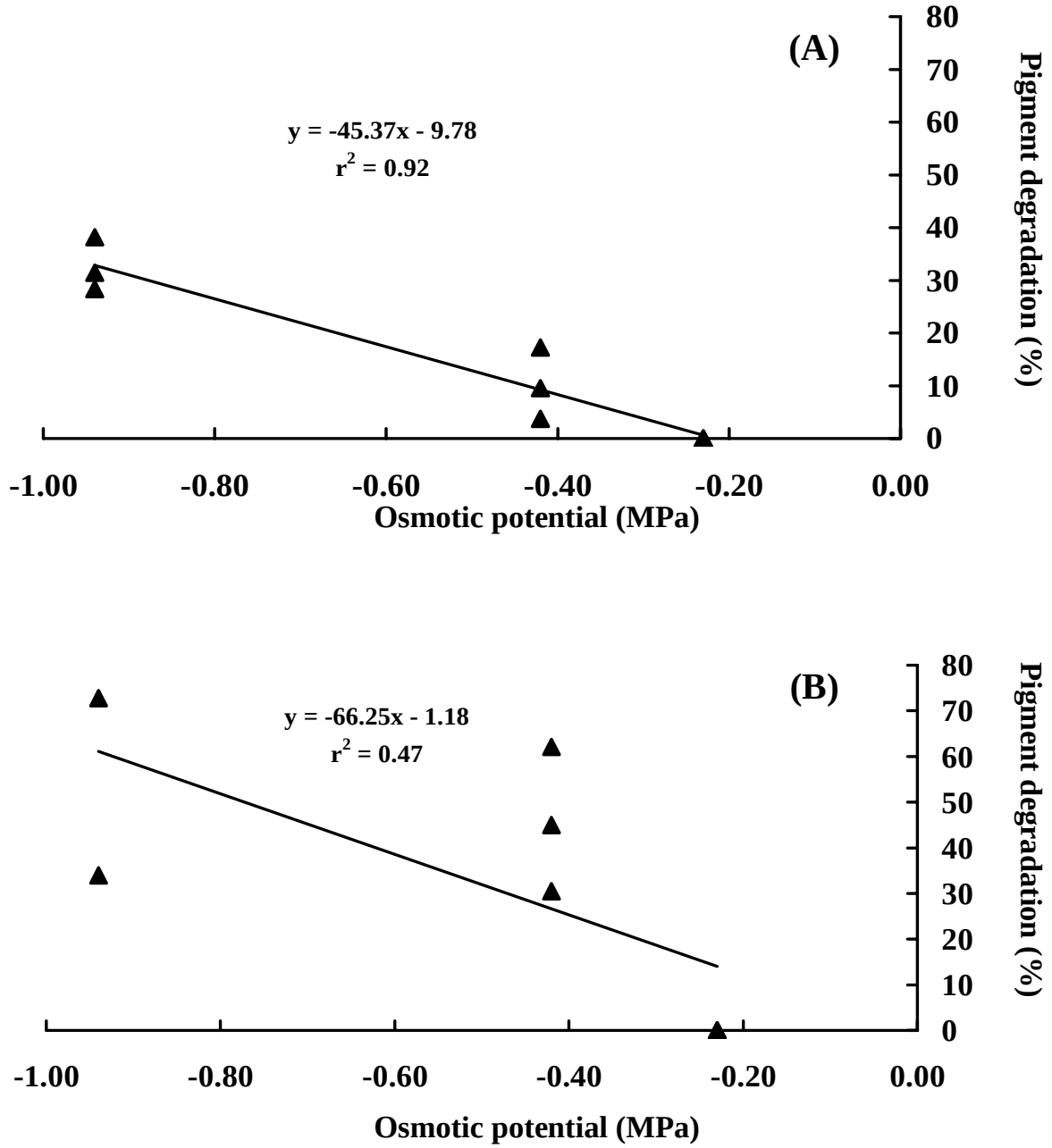

Fig. 1. Relationship between osmotic potential in the culture media and pigment degradation of rice seedlings grown under iso-osmotic mannitol (A) and salt stress (B) for 7 days.

In the -0.94 MPa salt-induced osmotic stress, PT1 salt sensitive seedlings was 100% death and the data were undetected. Survival percentage of rice seedlings cultivated in salt-induced osmotic stress was strongly reduced more than that in mannitol-induced stress as well as the different genotypes, salt tolerance or susceptible. For example, the survival percentage of INIAP12 salt tolerant rice grown under iso-osmotic water deficit (200 mM mannitol) and salt stress (100 mM NaCl) is better than that of CT6748 -8-CA-17 salt sensitive cultivar (Morsy *et al*., 2007). Photosynthetic pigments in osmotically stressed seedling of rice genotypes were degraded, relating to increase osmotic pressure (mannitol or NaCl) in the culture media. It is quite similar to the degradation of TC and C_{x+c} in the salt stressed leaves of Koshihigari (japonica rice), which is identified as moderately salt tolerant genotype (Sultana *et al*., 1999). In Taipei 309 rice cultivar, Chl_a and Chl_b in water deficit stress (0.4 M sorbitol) are enriched, whereas those in salt stress

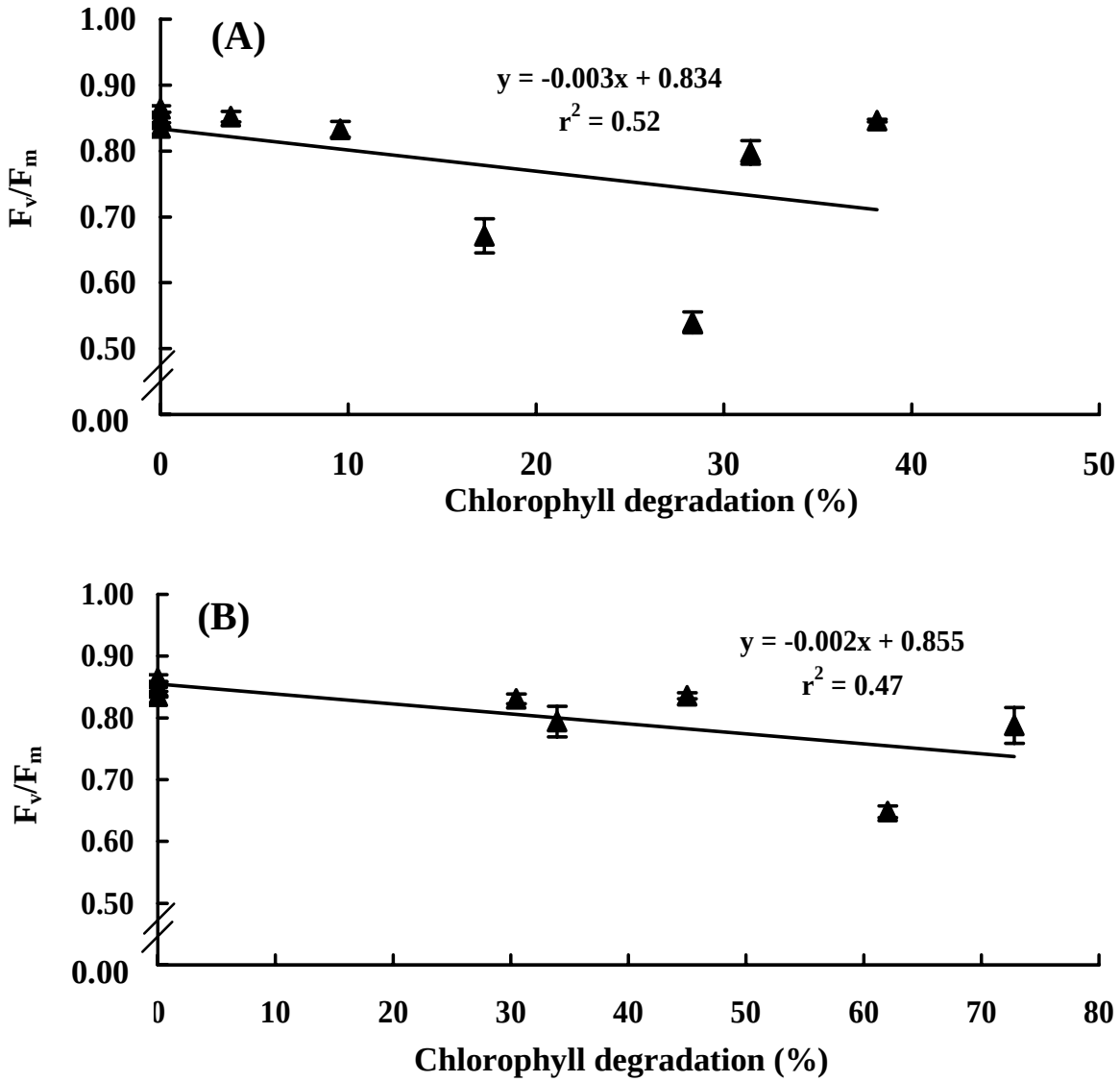

Fig. 2. Relationship between pigment degradation and maximum quantum yield of PSII (F_v/F_m) of rice seedlings grown under iso-osmotic mannitol (A) and salt stress (B) for 7 days. Error bars represented by ± SE.

(0.15 M NaCl) are significantly dropped (Bahaji *et al*., 2002). The pigment degradation in osmotically-stressed rice seedling was a major factor to limit photosynthetic abilities, defining by low efficient F_v/F_m, Φ_{PSII} and P_n, leading to overall growth inhibition. The reduction on the photosynthetic abilities in response to water deficit or salt induced osmotic stresses has been widely investigated in rice (Sultana *et al*., 1999; Cha-um *et al*., 2004; Cha-um *et al*., 2007a), sugarcane (Wahid &Ghazanfar 2006; Cha-um & Kirdmanee, 2009a) and maize (Cha-um *et al*., 2009b). The P_n in both flowering and milking stages of Koshihigari rice cultivar grown under 100 mM NaCl is significantly decreased for 1.57 and 2.33 folds, respectively when compared to control condition (0 mM NaCl) (Sultana *et al*., 1999). In sugarcane (cv. K84-200), the F_v/F_m, Φ_{PSII} and P_n parameters in -1.2 MPa salt-induced stresses are significantly dropped for 1.04, 1.02 and 3.36 folds, respectively when compared to those in iso-osmotic mannitol (Cha-um & Kirdmanee, 2009a). Proline contents in salt or water deficit stress of rice seedlings were accumulated in root and leaf tissues. There are many documents in rice crop varieties to demonstrate the proline

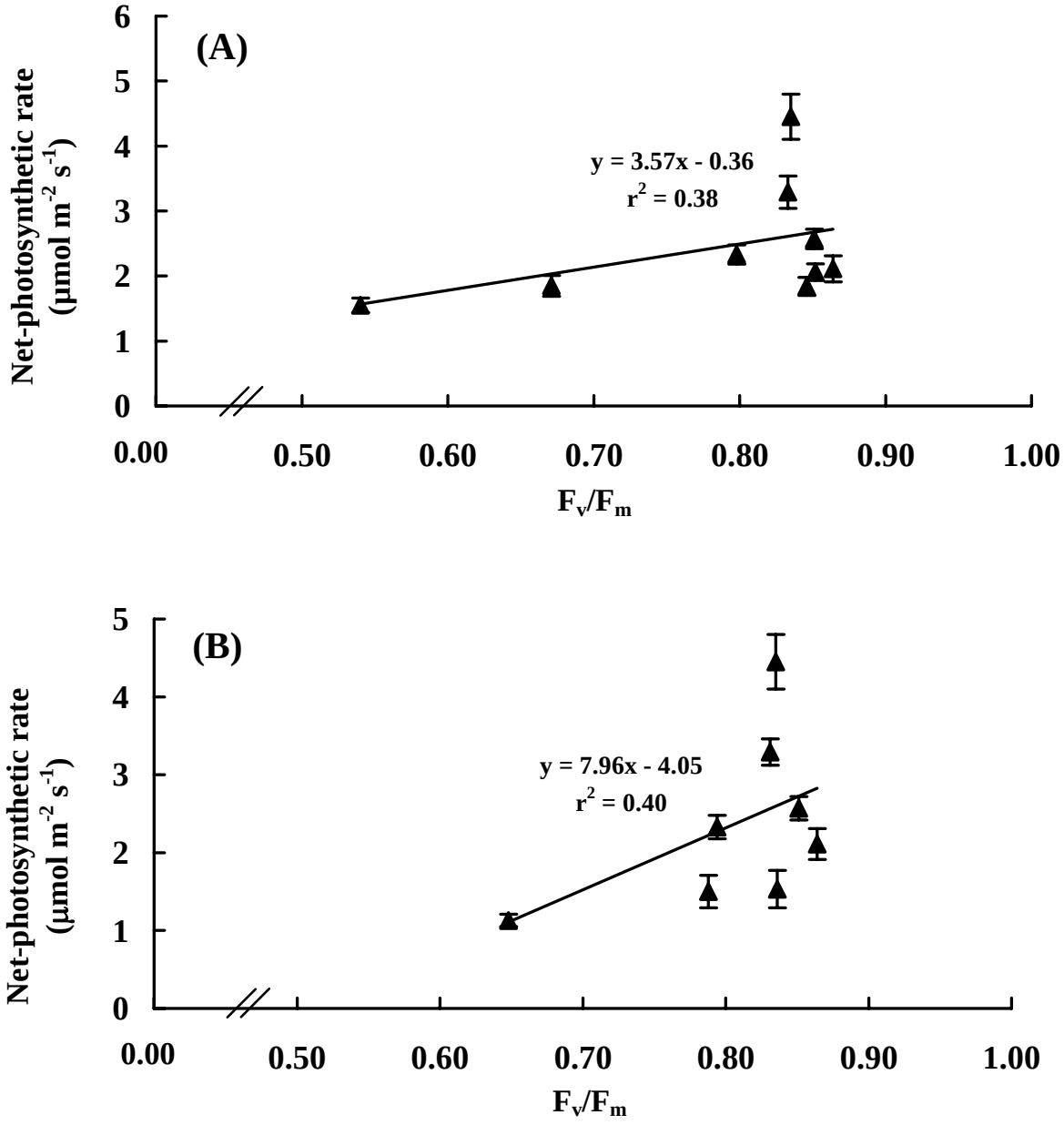

Fig. 3. Relationship between maximum quantum yield of PSII (F_v/F_m) and net-photosynthetic rate (P_n) of rice seedlings grown under iso-osmotic mannitol (A) and salt stress (B) for 7 days. Error bars represented by ±SE.

accumulation when exposed to water deficit or salt stresses *i.e.* Koshihikari (Sultana *et al*., 1999), Taichung Native 1 (Lin &Kao, 1996), six inbred line (2, 12, 43, 64, 96 and 104) (Shereen *et al*., 2007), Basmati-370, Basmati-Kashmir (Ahmad *et al*., 2007), eight rice cultivars (GZ-1368, Samcheon, Nipponbare, DT-271, Dongjin, Keowha, Anapurna and T. hatamochi) (Hoai *et al*., 2003), Pokkali, IR28 (Demiral & Türkan, 2005), Pusa Basmati 1 (Vaidyanathan *et al*., 2003), Cuom, DR2 and CR203 (Hien *et al*., 2003). In case of iso-osmotic stress, the proline content in Basmati-370 salt tolerance is enriched higher than that of Basmati-Kashmir salt susceptible, especially in polyethylene glycol (PEG) induced osmotic stress (Ahmad *et al*., 2007). Moreover, the proline accumulation in the root tissues of salt or water deficit induced iso-osmotic stress in Cuom, DR2 and

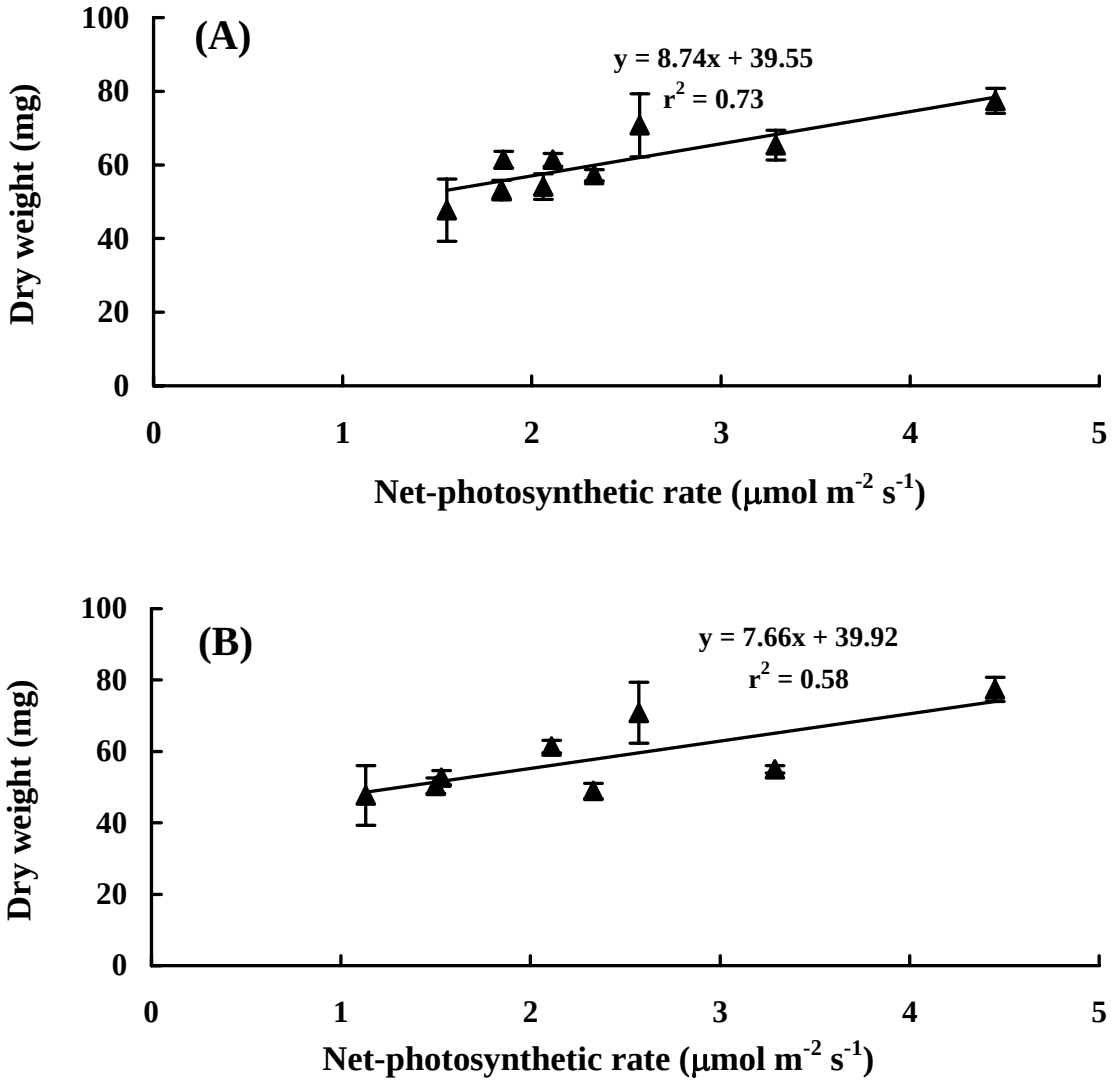

Fig. 4. Relationship between net-photosynthetic rate (P_n) and dry weight of rice seedlings grown under iso-osmotic mannitol (A) and salt stress (B) for 7 days. Error bars represented by ±SE.

CR203 rice genotypes is more than that in the leaf tissues (Hien *et al.*, 2003). It quite differs from present study that showed the higher proline in the osmotically stressed leaf tissues of RD6, HJ and PT1 than that in the root tissues. The proline enrichment in the tolerant cultivars of rice may that played a role as osmotic adjustment or water used efficiency as well as non-enzymatic antioxidant compound in the plant cell when exposed to osmotic stresses (Vaidyanathan *et al*., 2003; Demiral & Türkan, 2005; Ahmad *et al*., 2007). Growth parameters including SH, RL, LA, FW and DW of rice grown under iso-osmotic water deficit and salt stress were decreased relating to osmotic pressure in the culture media, especially salt-induced osmotic stress. It is similar to previous documents in rice growth reduction when expose to iso-osmotic stress (Bahaji *et al*., 2002; Hien *et al*., 2003; Ahmad *et al*., 2007). In addition to, the growth characters in osmotic stress tolerance are maintained better than those in sensitive genotypes (Hien *et al*., 2003; Ahmad *et al*., 2007). The physiological, biochemical and morphological changes as well as yield reduction has been played an important role as indices in osmotic stress tolerant screening of rice breeding programs, which are widely investigated for the cluster

ranking as tolerant or susceptible (Cabuslay *et al*., 2002; Zeng *et al*., 2002; Zeng *et al*., 2003b; Zeng, 2005). In this study, the multivariate parameters of pigment degradation, chlorophyll a fluorescence diminishing, P_n reduction and growth inhibition in osmotic stressed seedlings are developed as rapid indices for osmotic tolerant classification of rice genotypes.

In conclusion, chlorophyll pigments and photosynthetic abilities of rice seedlings grown under iso-osmotic salt stress declined to a greater degree than those of plants grown under iso-osmotic water-deficit stress, leading to a greater reduction in growth rate. The physiological and growth characters of susceptible rice cultivars were more sensitive to both soil salinity and water deficit than those of tolerant cultivars. The salt tolerance, HJ and salt susceptible, PT1 and RD6 as well as the water deficit tolerance, HJ and RD6 and water deficit susceptible, PT1 were identified using Hierarchical cluster analysis.

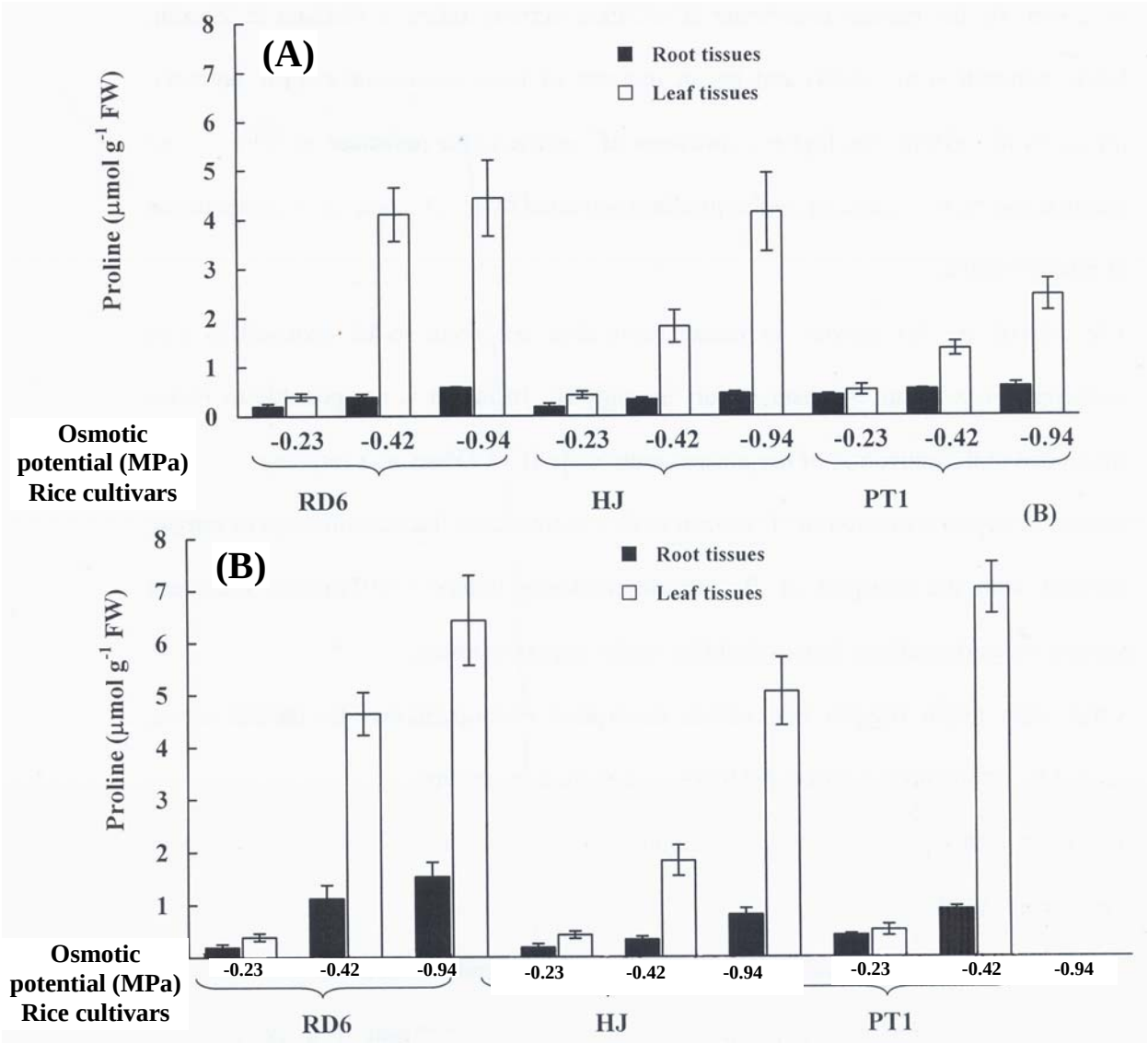

Fig. 5 Proline contents in root and leaf tissues of rice cultivars grown under iso-osmotic mannitol (A) and salt stress (B) for 7 days. Error bars represented by ± SE.

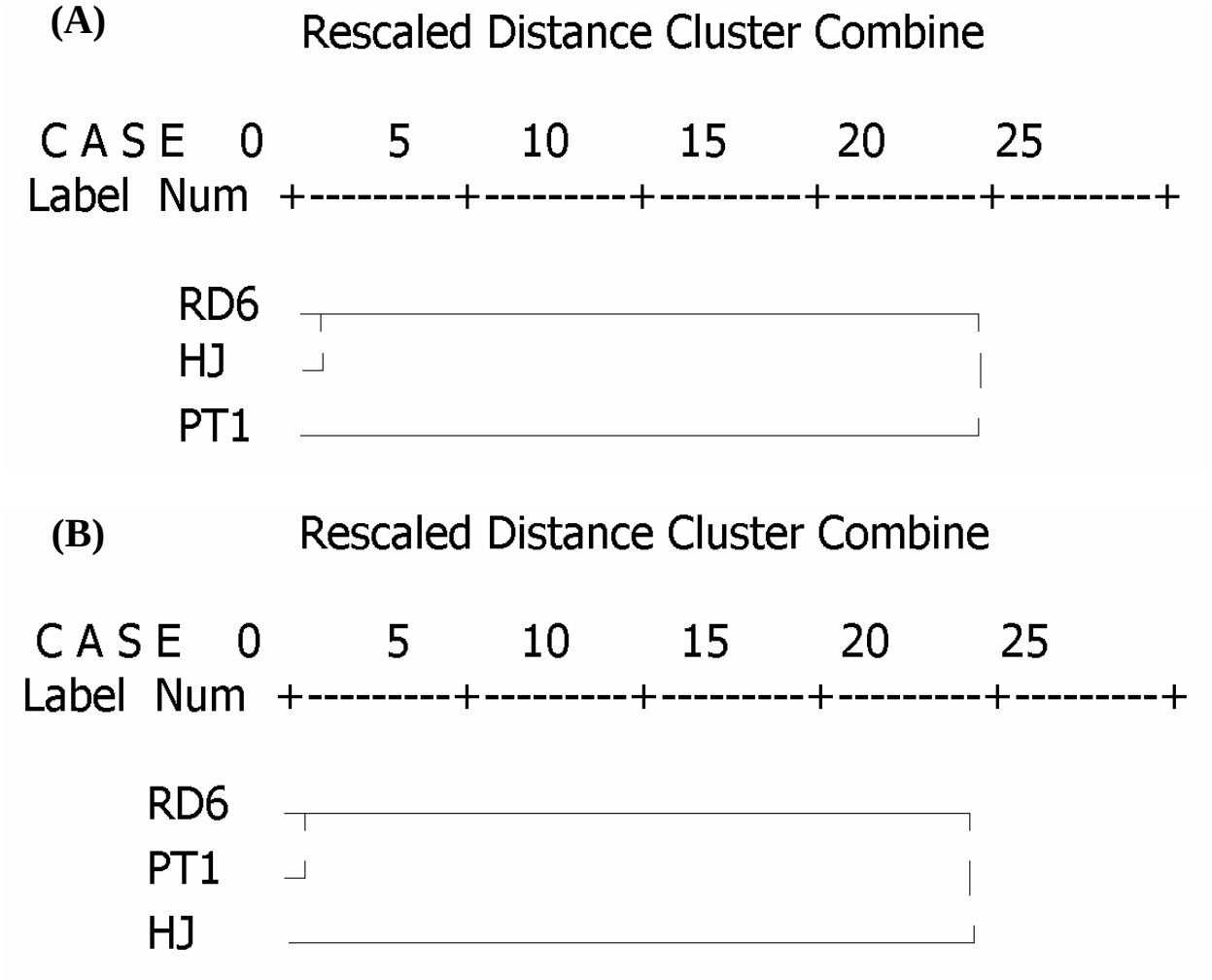

Fig. 6. Hierarchical cluster analysis of three rice cultivars to classify as water-deficit susceptible, PT1 and water-deficit tolerance, RD6 and HJ (A) as well as salt susceptible, PT1 and RD6, and salt tolerance, HJ (B), using pigment degradation, chlorophyll a fluorescence and net photosynthetic rate reduction, proline accumulation, and growth inhibition.

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