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MULTIVARIATE PHYSIOLOGICAL INDICES FOR SALT TOLERANCE CLASSIFICATION IN INDICA RICE (*ORYZA SATIVA* L. SPP. *INDICA*)

Suriyan Cha-um[1]*, Thanapol Boriboonkaset[2], Aussanee Pichakum[2], Chalermpol Kirdmanee[1]

[1]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, 113 Thailand Science Park, Paholyothin Rd, Klong 1, Klong Luang, Pathu mthani 12120, Thailand

[2]Department of Plant Science, Faculty of Science, Mahidol University, Rama VI Road, Payathai, Bangkok 10400, Thailand

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Summary. The aim of this investigation was to identity effective criteria for classification of salt tolerance and salt sensitivity in rice cultivars by using multivariate characters. Osmolarity in salt-stressed leaves decreased, and was positively related to low water potential (Ψ_w) (r^2=0.99 and r^2=1), leading to chlorophyll a degradation (r^2=0.65 and r^2=0.77) in both salt-tolerant (HJ and Pok), and salt-sensitive cultivars (PT1 and IR29). Chl_a, Chl_b, TC and C_{x+c} in salt-stressed seedlings decreased by 0, 8.88, 1.37 and 0% in the salt-tolerant lines, respectively, whereas the same pigments in the salt-sensitive lines were reduced by 41.06, 20.74, 35.96 and 41.40%, respectively. Multivariate parameters of pigment degradation and chlorophyll a fluorescence diminution were used to classify salt-tolerant (HJ and Pok) and salt-susceptible (PT1 and IR29) rice cultivars. In addition, salt-tolerant HJ, 21 and Pok (positive control), moderately salt-tolerant 26, 409 and 306, and salt-susceptible PT1, 31, 20, IR29 (negative control), 598, 18 and 2 were classified using the multivariate parameter indices.

***Keywords*:** backcross population, chlorophyll degradation, chlorophyll *a* fluorescence quenching, multivariate parameters, osmolarity, water potential.

Abbreviations: Chl_a – chlorophyll a; Chl_b – chlorophyll b; CRD – Completely Randomized Design; DMRT – Duncan's New Multiple Range Test; FMS – Fluorescence Mornitoring System; F_v/F_m – maximum quantum yield; MS – Murashige and Skoog medium; NPQ – non-photochemical quenching; Pok – Pokkali; PPFD – photosynthetic photon flux density; PT1 – Pathumthani 1; qP – photochemical quenching; (Φ_{PSII}) – quantum efficiency of PSII; RH – relative humidity; NaCl – sodium chloride; C_{x+c} – total carotenoid; TC – total chlorophyll; HJ; Homjan; Ψ_w – water potential.

*Corresponding author: suriyanc@biotec.or.th

INTRODUCTION

Rice is one of the top five major crops in the world, providing one-third of total dietary carbohydrate, especially in Asian countries (> 60% world population). Rice is a staple food for more than 3 billion people, supplying 50% to 80% of their daily calorie intake (Khush, 2005). A major limitation to rice crop production is abiotic stress, which can be induced by salinity, drought, extreme temperature, submergence, and heavy metal contamination. Salinity, affecting arable land, is one of the most important factors in retarding rice growth and development at both vegetative and reproductive stages (Shannon et al., 1998; Zeng and Shannon, 2000; Khan and Abdullah, 2003; Zeng et al., 2003).

Saline soil is enriched with salts which are readily water-soluble, i.e. sodium chloride (NaCl), sodium sulfate (Na_2SO_4), calcium chloride ($CaCl_2$) and magnesium chloride ($MgCl_2$). NaCl is a small molecule which, and when oxidized to sodium ions (Na^+) and chloride ions (Cl^-), is easily absorbed by root cells and transferred to the plant overall through the xylem vascular tissues. Na^+ ions are well known as causing toxic damage to plant cells by both ionic and osmotic effects, causing growth retardation, low productivity and eventually, cell death (Taiz and Zeiger, 1995; Hasegawa et al., 2000; Munns et al., 2002; Mansour and Salama, 2004; Chinnusamy et al., 2005). Glycophyte species are susceptible to salt stress, resulting in a reduction of leaf expansion and chlorophyll synthesis prior to plant death. In addition, glycophyte species cultivated in salt affected soil show toxic symptoms, such as wilting, chlorosis, necrosis, burn and senescence, causing slow growth and loss of productivity (Akita and Cabuslay, 1990; Lutts et al., 1999). Chlorophyll *a* fluorescence is one of the most powerful and widely applied parameters for assaying the PSII photochemistry of salt stressed plants (Maxwell and Johnson, 2000).

The development of breeding programs for salt-tolerant rice is a fruitful topic for plant breeders in finding a solution of the problem of soil salinity (Gregorio et al., 2002; Senadhira et al., 2002; Flowers and Flowers, 2005). There are many research groups working to identify salt-tolerant rice from genetic resources, using multivariate criteria at both vegetative and reproductive stages. For examples, 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 are classified as salt tolerant and have played a role as parental lines in salt-tolerant breeding programs worldwide. In contrast, 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 cultivars are reported as being salt-sensitive (Zeng et al., 2004; Zeng, 2005). However, effective criteria for the identification of salt tolerance still need to be investigated, especially for mass population breeding programs.

An *in vitro* environmental control system has been successfully applied to screen for salt-tolerance in many plant species (Kirdmanee et al., 1997; Cha-um et al., 2004a, Cha-um et al., 2004b; Cha-um et al., 2005). In addition, chlorophyll degradation and net photosynthetic rate reduction in salt-stressed plants have been developed as effective indices for classification of salt tolerance (Kirdmanee and Mosaleeyanon, 2000; Wanichananan et al., 2003). Therefore, the best criteria for the identification of salt tolerance have been recommended as multiple indices in crop species such as rice (Zeng, 2005),

green gram (Ahmad et al., 2005), wheat (El-Hendawy et al., 2005) and tomato (Juan et al., 2005). The aim of this investigation was to develop rapidly applicable indices for salt tolerance using multivariate parameters, and the effective utilization of the indices for classification of salt tolerance in rice breeding populations.

MATERIALS AND METHODS

Establishment of salt tolerance indices

Seeds of rice (*Oryza sativa* L. spp. *indica*) including both salt-tolerant cultivars, Homjan (HJ; GS.No. 4371) and Pokkali (Pok; GS.No. 17905) and salt-sensitive cultivars, IR29 (IR29; GS.No. 2818) and Pathumthani 1 (PT1), were obtained from the Pathumthani Rice Research Center (Rice Research Institute, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand). The rice seeds were de-husked by hand, rinsed with 70% ethanol and surface-sterilized by immersing in 5% (v/v) Clorox® 5.25% (w/v) sodium hypochlorite, Clorox Co, USA) overnight. They were then soaked in 25% Clorox® for 25 min, and rinsed three times with sterile distilled water. Surface sterilized seeds were germinated on MS-solidified media (Murashige and Skoog, 1962). The rice seedlings were cultured under 25±2°C air temperature, 60±5% relative humidity (RH), and 60±10 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) at a 16/8 h (day/night) photoperiod provided by fluorescent lamps (TLD 36W/84, Cool White, Philips, Thailand). Fourteen-day-old rice seedlings were aseptically transferred to a culture vessel containing 50 ml sugar-free liquid MS media and supported by 20 g vermiculite, for 7 days. The air exchange rate in the culture vessel was increased to 2.32 μmol CO_2 h^{-1} by punching a hole in the plastic cap and placing gas permeable microporous polypropylene film (0.22 μm pore size, Nihon Millipore Ltd., Japan) over the hole. Sodium chloride (NaCl) in the culture media was adjusted to 0 (control) or 342 mM (salt stress). Leaf osmolarity, water potential (Ψ_w), pigment concentration and chlorophyll *a* fluorescence, including maximum quantum yield (F_v/F_m), photon yield of photosystem II (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ), parameters were measured, after exposure to salt stress for 4 days.

The leaf osmolarity of rice seedlings was measured according to Lanfermeijer et al. (1991). In addition, water potential (Ψ_w) in the leaf tissues was measured according to the method of Warne et al. (1990).

Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll concentrations were analyzed following the methods of Shabala et al. (1998). Total carotenoid (C_{x+c}) concentrations were assayed according to Lichtenthaler (1987). One hundred milligrams of leaf material was collected from the second and third nodes of the shoot tip. Leaf samples were placed in 25 ml glass vials together with 10 ml 95.5% acetone, and blended using a homogenizer. The glass vials were sealed with parafilm to prevent evaporation and then stored at 4°C for 48 h. Chl_a and Chl_b concentrations were measured using an UV-visible Spectrophotometer at 662 nm and 644 nm wavelengths. Also, the C_{x+c} concentration was measured using a Spectrophotometer at 470 nm. A solution of 95.5% acetone was used as a blank. 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:

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

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

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

Total chlorophyll (TC) = $[Chl_a] + [Chl_b]$ where A_i is an absorbance at the wavelength i.

Chlorophyll a fluorescence emissions from the adaxial surface of the leaf were monitored using 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). A leaf, light-adapted for 120 min in a growth chamber at 120±5 μmol m^{-2} s^{-1} photosynthetic proton flux (PPF) provided by fluorescent lamps, and dark adaptated for 30 min, was initially exposed to a modulated measuring beam of far-red light (LED source with typical peak at a wavelength of 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 pulse of saturating light (>6.8 mmol m^{-2} s^{-1} PAR) and autocalculated by FMS software for Windows® (Fluorescence Monitoring System Software; Hansatech Instruments Ltd). The variable fluorescence yield (F_v) was calculated according to the equation: $F_m - F_0$. The ratio of variable to maximum fluorescence (F_v/F_m) was calculated. The photon yield of photosystem II (Φ_{PSII}) in the light was calculated as $\Phi_{PSII} = (F_m' - F_t)/F_m'$ after 145 sec of illumination. In addition, the hotochemical quenching of photosystem II (qP) and non-photochemical quenching (NPQ) were calculated as described by Maxwell and Johnson (2000). Pigment degradation and chlorophyll a fluorescence quenching were calculated according to the equation:

$$\text{Degradation (\%)} = \left[1 - \frac{342 \text{ mM NaCl}}{0 \text{ mM NaCl}}\right] \times 100$$

The experiment was arranged as 4×2 factorials in Completely Randomized Design (CRD) with four replications and five plantlets per replication. The mean values were compared using Duncan's New Multiple Range Test (DMRT) and analyzed with SPSS software (SPSS for Windows version 11, SPSS Inc., USA). The correlations between osmolarity and water potential, water potential and chlorophyll a, chlorophyll a and F_v/F_m were evaluated by Pearson's correlation coefficients. Multivariate parameters including pigment degradation and chlorophyll a fluorescence quenching were used to classify the cluster groups as salt-tolerant or salt-susceptible, using Hierarchical cluster analysis in SPSS software.

Salt tolerance classification in backcross rice populations

Nine lines: 2, 18, 20, 21, 26, 31, 306, 409 and 598, of BC_1F_2 [(PT1×HJ)×PT1] population from a salt-tolerant rice breeding program, were used as a plant material. Fourteen-day-old rice seedlings were aseptically transferred to a culture vessel containing 50 ml sugar-free liquid MS media, and supported by 20 g vermiculite, for 7 days. The air exchange rate in the culture vessel was increased to 2.32 μmol CO_2 h^{-1} by punching a hole in the plastic cap and covering the hole with gas permeable microporous polypropylene film (0.22 μm pore size). NaCl in the culture media was adjusted to 0 (control) or 342 mM (salt stress). The osmolarity of the culture media of the control group was adjusted to 684 mmol kg^{-1}, using mannitol application for iso-osmolarity among treatments. Pigment degradation and chlorophyll *a* fluorescence quenching in salt-stressed plantlets were calculated. The experiment was designed as CRD with four replications

and five plantlets per replication. The mean values were compared by DMRT and analyzed using SPSS software. Multivariate parameters, including pigment degradation and chlorophyll a fluorescence quenching, were used in order to classify the cluster groups as salt-tolerant, moderately salt-tolerant or salt-susceptible using Hierarchical cluster analysis in SPSS software.

RESULTS AND DISCUSSION

Establishment of salt tolerance indices

Osmolarity, in both salt-tolerant and salt-sensitive cultivars, increased 1.70 and 2.19 times respectively upon exposure to salt stress (342 mM NaCl) for 4 days. Osmolarity in the leaf tissues was related to water potential (Ψ_w) in both salt-tolerant (r^2=0.99) (Fig. 1A) and salt-sensitive

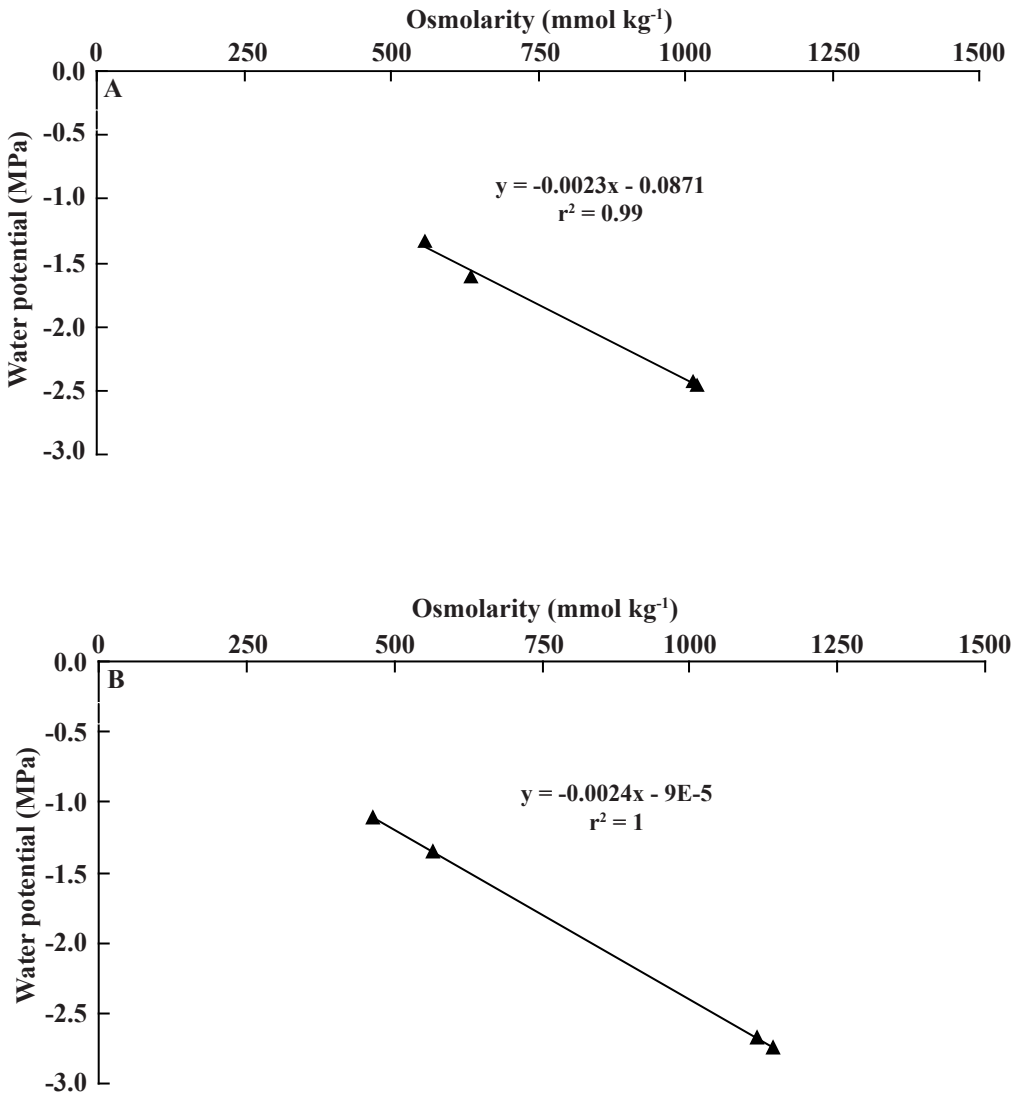

Fig. 1. Relationship between osmolarity and water potential (Ψ_w) in salt-tolerant (Pok and HJ) (A) and salt-sensitive (IR29 and PT1) (B) seedlings grown *in vitro* under photoautotrophic conditions when exposed to 0 or 342 mM NaCl for 4 days.

cultivars (r^2=1) (Fig. 1B). Ψ_w remained constant in the salt-tolerant cultivars, while in the salt-sensitive cultivars it dropped. It correlated positively with Chl_a degradation (r^2=0.77) (Fig. 2). Chl_a, Chl_b, TC and C_{x+c} content in salt-stressed seedlings were degraded, depending on the cultivar, the level of salt stress and the interactions of these factors (Table 1). Chl_a, Chl_b, TC and C_{x+c} content in the salt-sensitive cultivars grown under salt stress were reduced by 41.06, 20.74, 35.96 and 41.40 %, respectively, while in the salt-tolerant cultivars they remained unchanged (Table 1). The Chl_a:Ch_b and C_{x+c}:TC ratios in all rice cultivars grown under salt

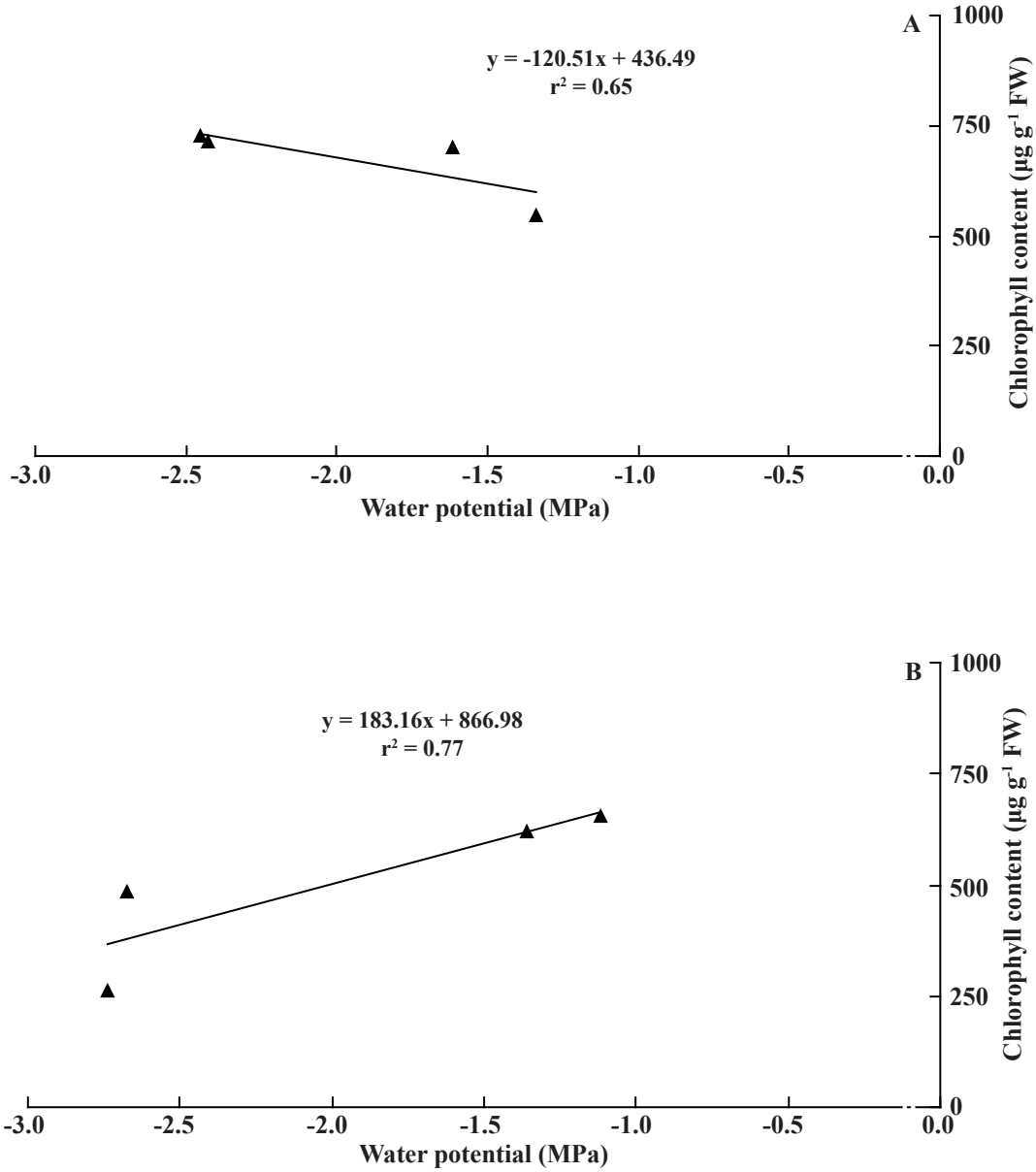

Fig. 2. Relationship between water potential (Ψ_w) and chlorophyll a content in salt-tolerant (Pok and HJ) (A) and salt-sensitive (IR29 and PT1) (B) seedlings grown *in vitro* under photoautotrophic conditions when exposed to 0 or 342 mM NaCl for 4 days.

Table 1. Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC), total carotenoid concentrations (C_{x+c}), Chl_a:Chl_b and C_{x+c}:TC in salt-tolerant (Pok and HJ) and salt-sensitive (IR29 and PT1) seedlings grown *in vitro* under photoautotrophic conditions, subsequently exposed to 0 or 342 mM NaCl for 4 days.

Cultivars	Salt stress [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]	Chl_a:Chl_b	C_{x+c}:TC
Pok	0	700.3^{c}	230.2^{a}	930.5^{a}	170.2^{c}	3.04	0.18
	342	715.4^{b}	189.5^{c}	904.9^{b}	212.0^{a}	3.78	0.23
HJ	0	546.1^{f}	143.5^{d}	689.6^{e}	154.8^{f}	3.81	0.23
	342	728.2^{a}	212.4^{b}	940.6^{a}	207.4^{b}	3.43	0.22
IR29	0	620.3^{e}	179.5^{c}	799.7^{d}	163.9^{d}	3.46	0.21
	342	484.5^{g}	131.1^{d}	615.6^{f}	145.0^{g}	3.70	0.24
PT1	0	657.1^{d}	215.9^{b}	873.0^{c}	169.8^{c}	3.04	0.20
	342	261.4^{h}	184.5^{c}	445.9^{g}	48.8^{h}	1.42	0.11
Significant level							
Cultivars		**	**	**	**		
Salt stress		**	*	**	**		
Cultivars × Salt stress		**	**	**	**		

Different letters in each column show significant difference at $P \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT). Significant and highly significant in statistics are represented by * and **, respectively.

Table 2. Maximum quantum yield (F_v/F_m), quantum efficiency of PSII (Φ_{PSII}), photochemical quenching (qP) and non-photochemical quenching (NPQ) in salt-tolerant (Pokkali and HJ) and salt-sensitive (IR29 and PT1) seedlings grown *in vitro* under photoautotrophic conditions, subsequently exposed to 0 or 342 mM NaCl for 4 days.

Cultivars	Salt stress [mM]	F_v/F_m	Φ_{PSII}	qP	NPQ
Pok	0	0.866^{a}	0.740^{a}	0.859^{a}	0.051
	342	0.859^{a}	0.647^{b}	0.760^{bc}	0.066
HJ	0	0.886^{a}	0.602^{bc}	0.705^{bcd}	0.045
	342	0.856^{a}	0.629^{bc}	0.719^{bcd}	0.111
IR29	0	0.875^{a}	0.595^{bc}	0.693^{cd}	0.036
	342	0.757^{b}	0.564^{c}	0.701^{bcd}	0.071
PT1	0	0.875^{a}	0.669^{ab}	0.770^{b}	0.047
	342	0.794^{b}	0.594^{bc}	0.682^{d}	0.109
Significant level					
Cultivars		**	**	**	NS
Salt stress		**	**	**	NS
Cultivars × Salt stress		**	NS	**	NS

Different letters in each column show significant difference at $P \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT). Non-significant and highly significant in statistics are represented by ^{NS} and **, respectively.

stress were stabilized, except in PT1 salt-sensitive rice (Table 1). The F_v/F_m ratio in the salt-tolerant cultivars was unchanged when exposed to salt stress (Table 2, Fig. 3A). In the case of salt-sensitive rice, the Chl_a degradation percentage was related to low F_v/F_m ratio (Fig. 3B). It should be concluded that pigment degradation and chlorophyll a fluorescence diminution should serve as effective indices to identify salt-tolerant rice cultivars.

The leaf color of the salt-stressed rice seedlings changed from green to light green/yellow within four days of exposure to salt treatment. It was shown that upon exposure to salt stress photosynthetic

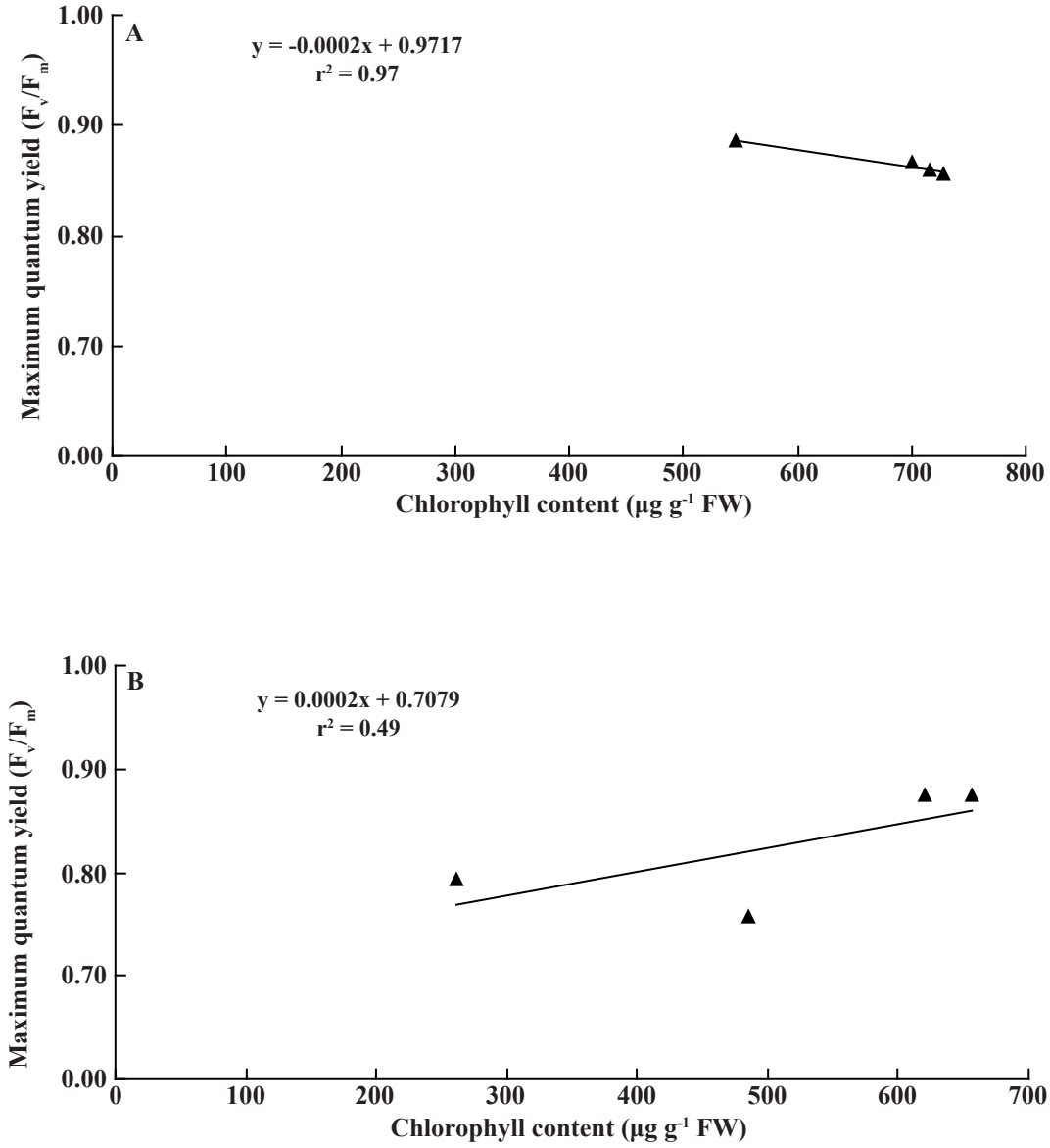

Fig. 3. Relationship between chlorophyll a and maximum quantum yield (F_v/F_m) in salt-tolerant (Pok and HJ) (A) and salt-sensitive (IR29 and PT1) (B) seedlings grown *in vitro* under photoautotrophic conditions when exposed to 0 or 342 mM NaCl for 4 days.

pigments in green gram (Ahmad et al., 2005), wheat (Sairam et al., 2002), cotton (Meloni et al., 2003) and sorghum (Netondo et al., 2004) were reduced 1.96, 1.44, 1.48 and 1.83 times, respectively. The levels of photosynthetic pigments, Chl_a, Chl_b and C_{x+c} in the salt-sensitive cultivars grown under salt-stress were significantly decreased when compared to the salt-tolerant lines (Table 1). In tomatoes, Chl_a, Chl_b and C_{x+c} content in the salt-sensitive cultivar Royesta declined to a greater degree than in the salt-tolerant cultivar Brillante (1.50, 1.16 and 2.14 times, respectively) after exposure to salt stress (Juan et al., 2005). The activities of photosynthetic pigments in terms of water oxidation and light harvesting were measured, using chlorophyll a fluorescence parameters, which have been reported as being sensitive criteria for plant response to salt stress. Our results showed that the F_v/F_m ratio, Φ_{PSII} and qP in salt-stressed rice seedlings decreased, depending on the cultivar and the degree of salt-stress (Table 2). In barley (Lee et al., 2004) and sorghum (Netondo et al., 2004), F_v/F_m dropped significantly depending on the degree of salt stress and the cultivar. Upon exposure to salt stress, F_v/F_m, Φ_{PSII} and qP in salt-tolerant sorghum cv. Seredo and barley cv. AZ-8501 were shown to maintain better than in the salt-sensitive cvrs. Serena (Netondo et al., 2004) and Morex (Jiang et al., 2006). Photosynthetic performance, growth characters and survival percentage in rice (Yeo et al., 1990; Zeng, 2005), wheat (Sairam et al., 2002; El-Hendawy et al., 2005), sorghum (Netondo et al., 2004), barley (Jiang et al., 2006), and green gram (Ahmad et al., 2005) were sensitive to salt stress. This is especially the case in salt-sensitive cultivars.

Salt-tolerance classification in backcross rice populations

Chl_a, Chl_b, TC and C_{x+c} content in backcross (BC_1F_2) populations of rice grown under salt stress were degraded in parallel with the reduction of F_v/F_m, Φ_{PSII} and qP. Data related to the reduction of photosynthetic pigments and chlorophyll *a* fluorescence were subjected to Hierarchical cluster analysis in SPSS software. Salt-tolerant HJ, 21 and Pok, moderately salt-tolerant, 26, 409 and 306, and salt-susceptible, PT1, 31, 20, IR29, 598, 18 and 2 (Fig. 4) were classified. Photosynthetic pigment degradation and chlorophyll a fluorescence diminution should serve as effective criteria to classify salt-tolerant cultivars in rice breeding programs.

Multivariate parameters in BC_1F_2 rice populations were used to classify salt tolerance (HJ, 21 and Pok), moderate salt tolerance (26, 409 and 306) and salt susceptibility (PT1, 31, 20, IR29, 598, 18 and 2) using Hierarchical cluster analysis (Fig. 4). Multivariate criteria of biochemical, physiological and morphological characters are the most effective way to classify salt-tolerant and salt-sensitive species. For example, four classes of sugarcane variety, high salt tolerance (CP-4333 and S-86-US-699), moderate salt tolerance (CO-1148, L118, BL-4 and Triton), moderate salt sensitivity (BF-162 and COL-54) and high salt sensitivity (CP-71-3002), have been identified using EC_{50} values of germination percentage, plant dry weight, number of green leaves, leaf area and number of tillers (Wahid et al., 1997). In barley varieties, the salinity susceptibility index (SSI), in terms of efficiency of light harvesting of PSII (F'_v/F'_m), internal CO_2 concentration (C_i) and stomatal conductance (g_s), have been utilized to classify salt-tolerant (AZ-

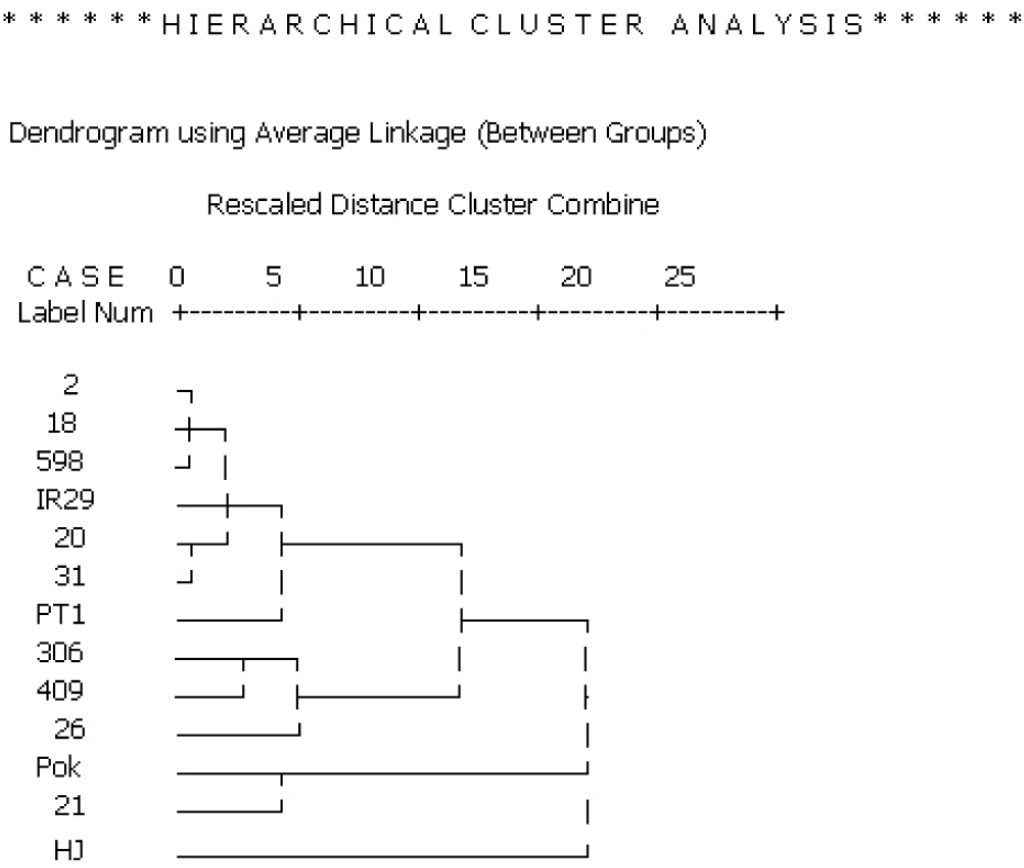

Fig. 4. Cluster analysis of BC1F2 population in term of salt tolerant class, HJ, 21 and Pok, moderate class, 26, 409 and 306, and salt sensitive class, PT1, 31, 20, IR29, 598, 18 and 2, in rice seedlings using multivariate parameters of pigment degradation and chlorophyll a fluorescence quenching by Hierarchical cluster analysis of SPSS software.

8501 and Giza125), and salt-sensitive varieties (Morex and TR306) (Jiang et al., 2006). Wheat genotypes have been identified in three clusters, salt-tolerant (Kharchia, Sakha 8, Sakha 93 and Sakha 69), moderately salt-tolerant (Drysdale, Thassos, Westonia and Triso) and salt-sensitive (Gemmeza 7, Giza 168, Sehel 1, Sids 1 and Sakha 61) by Ward's minimum variance cluster analysis based on tiller number, leaf number, and leaf area per plant (El-Hendawy et al., 2005). In rice genotypes, four clusters, high salt tolerance (IR63352-AC202), moderate salt tolerance (Daeyabyeo, GZ5385-29-3-3, GZ5121-5-2-1, Nonabokra, IR29, IR63731-1-1-4-3-2, S-102, Pokkali, IR4630-22-2-2-5-1-3, IR50184-3B18-2B-1 and IR51490-AC10), moderate salt sensitivity (AC26, GZ5310-20-3-2, Agami, GZ1368-5-4, GZ5385-29-3-2, Sakha 101, IR70074-AC14 and IR70074-AC1) and high salt sensitivity (IR61920-3B-15-2-2, GZ178, GZ5310-20-3-3, GZ177, M-205, GZ5385-3-2-3-1, GZ5310-20-2-1, M-104, GZ5291-7-1-2, M-202 and L-205), have been identified by Ward's minimum variance cluster analysis based on tiller number, leaf area and shoot dry weight (Zeng, 2005).

In conclusion, pigment concentrations in salt-stressed seedlings of rice cultivars were reduced and were found to correlate positively with the PSII light reaction. Pigment degradation and chlorophyll a fluorescence diminution were effectively developed as multivariate salt tolerance indices. In addition, the multivariate salt tolerance indices were applied to classify salt tolerance, moderate salt tolerance and salt susceptibility in backcross (BC_1F_2) population lines. The biochemical and molecular defense mechanisms in isogenic populations of salt-tolerant and salt-sensitive rice cultivars should be further investigated.

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