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A Rapid Method for Identifying Salt Tolerant Water Convolvulus (*Ipomoea aquatica* Forsk) under *In Vitro* Photoautotrophic Conditions

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ABSTRACT

Water convolvulus is an aquatic plant capable of growing in a low nutrient solution and poor water quality. There are many reports on utilization of this plant for the efficient remediation of salt contaminated wastewater. The aim of this investigation was to identify the criteria that could be used to classify the salt tolerance and salt sensitivity of water convolvulus using multivariate characters. Six lines of water convolvulus plantlets were photoautotrophically grown in a controlled environmental system and then treated with 0 (control) or 342 mM NaCl (salt stress) for a week. Pigment levels, chlorophyll *a* fluorescence and growth reduction were measured as potential multivariate parameters to group plants into two classes: salt tolerant (WC083, SR739 and SR716) and salt sensitive (MK98, WC001 and WC092). Total chlorophyll and carotenoid pigments in salt-stressed plantlets were reduced by 80.0% and 68.6% in salt tolerant lines. In salt-sensitive lines these pigments were degraded by 88.0% and 79.8%, respectively. This suggests that the major pigments, total chlorophyll and carotenoids in salt-tolerant lines were more stable than those in salt-sensitive lines. The function of both major pigments in salt-tolerant lines was strongly related to light harvesting (Φ_{PSII}) ($r^2 = 0.81$) and photooxidative damage (NPQ) defenses ($r^2 = 0.81$), respectively. Further, several growth parameters (plant height, number of leaves, root length, number of roots, fresh weight) progressively decreased when exposed to salt stress, especially in salt-sensitive lines. The salt-tolerant lines of water convolvulus can be further utilized for NaCl-contaminated wastewater phytoremediation, while the salt-sensitive lines may be applied as effective indicators of salt contamination in the water.

Keywords: carotenoids, chlorophylls, chlorophyll a fluorescence, growth, phytoremediation

Abbreviations: BA, N^6-benzyl adenine; **C_{x+c}**, total carotenoids; **Cl^-**, chloride ions, **Chl_a**, chlorophyll a; **Chl_b**, chlorophyll b; **CRD**, Completely Randomized Design; **DMRT**, Duncan's New Multiple Range Test; **F_v/F_m**, maximum quantum yield of PSII; **MS**, Murashige and Skoog medium; **Na^+**, sodium ions; **NaCl**, sodium chloride; **NPQ**, non photochemical quenching; **PPF**, photosynthetic photon flux density; **Φ_{PSII}**, quantum efficiency of PSII; **RH**, relative humidity

INTRODUCTION

Wastewater, released from urban, agricultural and industrial zones is a critical problem (Oron 2003). It has increased salt concentrations considerably (El-Fadel *et al.* 1997; Bowman *et al*. 2002) and includes domestic wastewater (345 mg L^{-1} Na^+) (Patterson 2004), leached waste landfill (1,442 mgL^{-1} Cl^-) (Aluko *et al*. 2003), sewage sludge [0.8% Na^+ (% total solids)] and horticultural waste [1.27% Na^+ (% total solids)] (Stabnikova *et al*. 2005). The contaminant salts in the wastewater pond are readily oxidized to toxic ions, especially sodium ions (Na^+) and chloride ions (Cl^-), which damage aquatic plant species (Hootsmans and Wiegman 1998; Rout and Shaw 2001; Klomjek and Nitisoravut 2005) in terms of biochemical, physiological and morphological characters. Aquatic plant species have a high potential phytoremediation capacity for removing salt contamination and filtering sediments (Karnchanawong and Sanjitt 1995; Jing *et al.* 2002; Kyambadde *et al.* 2004; Klomjek and Nitisoravut 2005; Chen *et al.* 2006). Phytoremediation of salt-contaminated waste water using aquatic plant species is an important topic for investigation. Lack of information regarding the emerging plant species and those adapted to flooding is a major impediment for practical application of this method for reclaiming salt-contaminated waste water.

Water convolvulus (*Ipomoea aquatica* Forsk) belonging to Convolvulaceae family is an effective aquatic species, which grows well in fresh water marshes and ponds (Sharma 1994). It has been used as vegetable crop in Asian countries and is rich in antioxidant compounds, namely carotenoid and vitamin A (Chen and Chen 1992; Wills and Rangga 1996; Tofern *et al.* 1999; Malalavidhane *et al.* 2000; Huang *et al.* 2005). Several studies utilizing water convolvulus in wastewater phytoremediation in terms of heavy metals i.e. cadmium (Cd), cupper (Cu), zinc (Zn), lead (Pb), mercuric (Hg) and nickel (Ni) have been reported (Sun and Wu 1998; Fonkou *et al.* 2002; Gothberg *et al.* 2004), bisphenol A (Noureddin *et al.* 2004), sulfate (Sakulkoo *et al.* 2005; Meerak *et al.* 2006), sewage sludge and horticultural waste (Stabnikova *et al.* 2005) as well as salt contaminant removal (Jing *et al.* 2002; Klomjek and Nitisoravut 2005; Stabnikova *et al.* 2005; Kirdmanee *et al*. 2006).

In this study, water convolvulus is utilized as a model plant to investigate the multivariate parameters for the identification of salt tolerance. As previous studies have shown, mechanisms of salt tolerance are composed of multiplex defenses or quantitative traits such as membrane systems, osmoregulation systems, antioxidant systems and hormonal systems (Hasegawa *et al.* 2000; Mansour and Salama 2004; Ashraf 2004; Parida and Das 2005). There are many reports on *in vitro* culture systems as a tool for selection of stress-tolerant clones (Lee *et al.* 2003; Misra and Dwivedi 2004;

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Original Research Paper

Houshmand *et al.* 2005), gene expression for stress resistance (Kumria and Rajam 2002), and plant responses to extreme environmental conditions (Ekanayake and Dodds 1993; Wahome *et al.* 2001; Lin *et al.* 2002). However, the natural environment is quite different from the conventional *in vitro* culture (Kozai *et al.* 1997; Mills and Tal 2004). An *in vitro* environmental control system of photoautotrophic condition has been successfully applied to simulate realistic phenotypic responses to salt stress in woody plants (Kirdmanee and Cha-um 1997; Cha-um *et al.* 2004a) and crop species (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 indices to classify the salt tolerant clones of forest tree and crop species (Kirdmanee and Mosaleeyanon 2000; Wanichananan *et al.* 2003). Salt tolerance is recommended for multiple indices in 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 rapid indicators of salt tolerance in water convolvulus lines using an *in vitro* photoautotrophic system.

MATERIALS AND METHODS

Plant materials

Seeds of six water convolvulus lines (MK98, SR716, SR739, WC001, WC092, and WC083) were obtained from the Asian Vegetable Research and Development Center (AVRDC), Thailand and the Faculty of Horticulture, Chiba University, Japan. Seeds were bark-peeled to approximately 0.25 cm diameter, and then washed for 2-3 min in 70% ethanol. The whole seeds were sterilized once in 5% Clorox® [5.25% active ingredient sodium hypochlorite (w/v), Clorox Co., Ltd., USA] for 12 h and once in 30% Clorox® for 30 min, then washed thrice in sterilized distilled water. The surface-sterilized seeds were germinated in 25 ml vials (Opticlear® KIMBLE, USA) on hormone-free-MS media (Murashige and Skoog 1962) supplemented with 87.60 mM sucrose (photomixotrophic condition) and solidified with 0.24% (w/v) Phytagel® (Sigma, USA). The pH of the culture media was adjusted to 5.7 before autoclaving at 120ºC for 15 min. All of the cultures were incubated under 25 ± 2ºC temperature, 60 ± 5% relative humidity (RH) and 60 ± 5 μmol m^{-2} s^{-1} photosynthetic photon flux density (PPF) provided by fluorescence lamps (TLD 36W/84 3350 lm Philips Thailand) with a 16 hd^{-1} photoperiod for 10 days. Nodes of seedlings were propagated on MS media supplemented with 13.32 μM N^6-benzyl adenine (BA) with a monthly subculture interval. A single shoot was dissected and induced to root on MS hormone-free media for 14 days. Plantlets, 5 ± 0.5 cm in height, were selected as initial plant material, then aseptically transferred to sugar-free liquid MS media (photoautotrophic condition) in glass vessels using vermiculite as supporting material (**Fig. 1A**) and incubated in plastic culture chambers (W×L×H; 26×36×19 cm). The air-exchange rate was adjusted to 5.1 ± 0.3 h^{-1} by perforating the sides of the plastic chambers with 32 holes and placing filters over them and reducing relative humidity (65 ± 5 %RH) by adding sodium chloride saturated salt solution in the chamber (**Fig. 1B**). After incubation for a week, the culture media were adjusted to 0 (control) or 342 mM NaCl (salt-stress) for 7 days (**Fig. 2**). Chlorophyll content, chlorophyll a fluorescence, shoot height, number of leaves, root length, number of roots, fresh weight and dry weight of plantlets were measured.

Data collections

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

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

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

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

$$[C_{X+C}] = \frac{1000D_{470} - 1.90[Chl_a] - 63.14[Chl_b]}{214}$$

where D_i is an absorbance at the wavelength i.

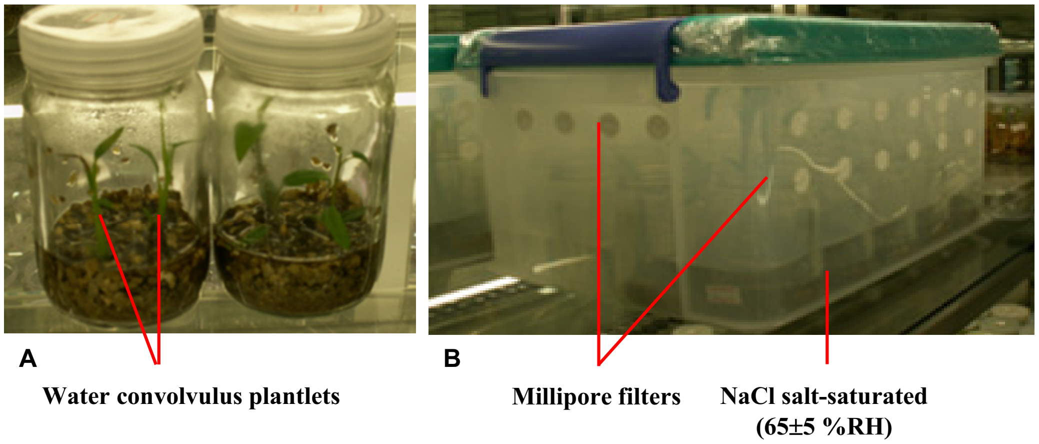

Fig. 1 *In vitro* culture system of water convolvulus grown in liquid MS media using vermiculite as support material (**A**). Plastic culture chambers covered with Millipore filters. The chamber sides were perforated for aeration (**B**). Relative humidity was maintained at 65 ± 5% using saturated sodium chloride solution.

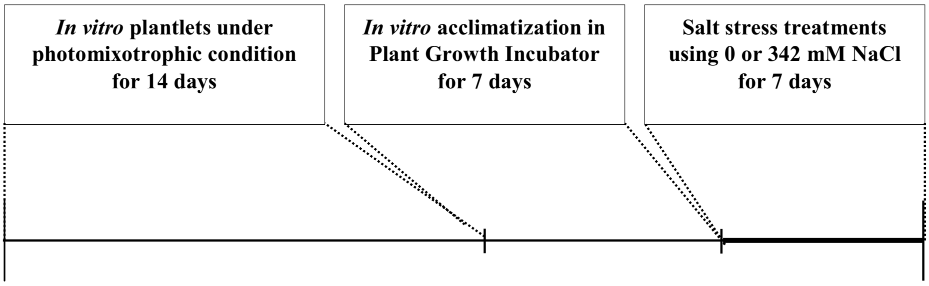

Fig. 2 Schematic of screening for salt tolerance in water convolvulus.

Chlorophyll *a* fluorescence emission from the adaxial surface on the third leaf from the shoot tip was monitored with a Fluorescence Monitoring System (*FMS* 2; Hansatech Instruments Ltd., UK) 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 (PEA/LC, Hansatech Instrument Ltd., UK) 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 auto-calculated by FMS software for Windows® (Fluorescence Monitoring System Software, Hansatech Instrument Ltd., UK). 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 45 sec of illumination, when steady state was achieved. In addition, non-photochemical quenching (NPQ) was calculated as described by Maxwell and Johnson (2000).

The shoot height, leaf number, root length, root number, the fresh weight and dry weight of plantlets were measured as growth characteristics by the methodology of Lutts *et al.* (1996). The plantlets were dried at 110°C in a hot-air oven (Memmert, Model 500, Germany) for 2 days and then incubated in desiccators before the measurement of their dry weight. The pigment degradation, chlorophyll a fluorescence diminution and growth reduction percentages were calculated according to equation:

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

Experimental design

The experiment was designed as 2×6 factorial in Completely Randomized Design (CRD) with five replicates and four plantlets per replicate. The mean values obtained were compared by Duncan's New Multiple Range Test (DMRT) and analyzed by SPSS software (SPSS for Windows, SPSS Inc., USA). The correlations between chlorophyll a and F_v/F_m, total chlorophyll concentration and Φ_{PSII}, total carotenoid concentration and NPQ, as well as Φ_{PSII} and plant height, were evaluated by Pearson's correlation coefficients. Multivariate parameters associated with significant difference in statistical analysis of lines were input to classify groups as salt-tolerant and salt-sensitive using Hierarchical cluster analysis in SPSS software.

RESULTS AND DISCUSSION

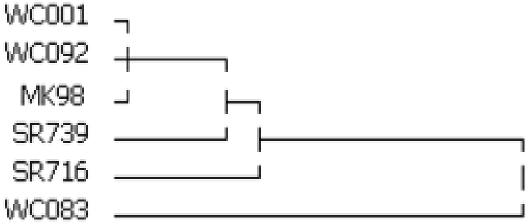

Fig. 3 Cluster analysis of water convolvulus lines using multivariate parameters following salt stress.

Chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll and total carotenoid (C_{x+c}) levels in all water convolvulus lines sharply decreased when exposed to extreme salt stress (342 mM NaCl), leading to low quantum efficiency of PSII (Φ_{PSII}) as well as a reduction in root length and fresh weight. Multivariate parameters of pigment degradation, chlorophyll a fluorescence diminution and growth reduction in salt-stressed water convolvulus were subjected to Hierarchical cluster analysis in SPSS software for salt tolerant or salt sensitive classification. The results were significant and showed that there were two district classes, salt-tolerant lines – SR739, SR716 and WC083, and salt-sensitive lines – MK98, WC001 and WC092 (**Fig. 3**). The Chl_a, Chl_b, total chlorophyll and C_{x+c} levels in both salt-tolerant and salt-sensitive lines decreased significantly when exposed to 342 mM NaCl or salt stress (**Table 1**). Those pigments in salt-tolerant line were degraded for 77.9, 82.5, 80.0 and 68.6%, and were lower than those in salt-sensitive lines by 1.13, 1.06, 1.10 and 1.16 times, respectively. The pigment degradation in salt-stressed water convolvulus was strongly affected by both the choice of water convolvulus lines or salt-stress (**Table 1**). The total chlorophyll degradation in salt-sensitive and salt-tolerant lines was closely related to Φ_{PSII} ($r^2 = 0.64$ and $r^2 = 0.81$, respectively) (**Fig. 4**). In addition, the C_{x+c} degradation in both lines was inversely related to non-photochemical quenching (NPQ) ($r^2 = 0.66$ and $r^2 = 0.81$, respectively) (**Fig. 5**). In the case of chlorophyll a fluorescence parameters, the salt stress conditions significantly affected on Φ_{PSII} and NPQ, while F_v/F_m did not change (**Table 2**). The Φ_{PSII} activity in salt-tolerant lines was two-fold higher than that in salt-sensitive lines. Conversely, the NPQ parameter, antioxidant defensive activity, in salt-tolerant lines was lower than that in salt-sensitive lines by 1.20 times. This means that the chlorophyll pigments in salt-tolerant lines of salt-stressed water convolvulus have a high potential to harvest the light energy, represented by Φ_{PSII} as well as enriched C_{x+c} pigments to function as an antioxidant system with low NPQ activity. The Φ_{PSII} diminution in salt-stressed plantlets was positively related to plant height ($r^2 = 0.81$ and $r^2 = 0.83$) (**Fig. 6**). Growth characters, such as plant height, number of leaves, root length, number of roots

Table 1 Analysis of pigments in water convolvulus lines subjected to salt stress.

Lines	Salt-stress (mM)	Chlorophyll a (µg g^{-1} FW)	Chlorophyll b (µg g^{-1} FW)	Total chlorophyll (µg g^{-1} FW)	Total carotenoid (µg g^{-1} FW)
WC001	0	393.29 bc	139.23 bc	532.52 bc	162.15 ab
	342	64.25 d	26.20 c	90.45 e	42.66 d
WC092	0	493.90 ab	239.40 ab	733.30 ab	180.51 ab
	342	45.31 d	15.93 c	61.24 e	34.03 d
MK98	0	359.35 bc	108.34 bc	467.69 bc	146.64 b
	342	37.67 d	12.40 c	50.07 e	22.67 d
SR716	0	425.73 b	131.74 bc	557.47 bc	170.41 ab
	342	69.79 d	20.52 c	90.31 e	50.27 d
SR739	0	675.16 a	331.30 a	1006.46 a	238.76 a
	342	185.41 cd	58.55 c	243.96 de	91.98 c
WC083	0	468.16 ab	161.61 bc	692.77 b	172.93 ab
	342	105.29 d	30.93 c	136.22 de	45.19 d
Significant level					
Line		**	**	**	**
Salt stress		**	**	**	**
Line×Salt stress		NS	NS	NS	NS

Highly significance at $P \leq 0.01$ and non-significant are represented by ** and ^{NS}, respectively. Means followed by different letters are significantly different at $P \leq 0.01$ by Duncan's New Multiple Range Test.

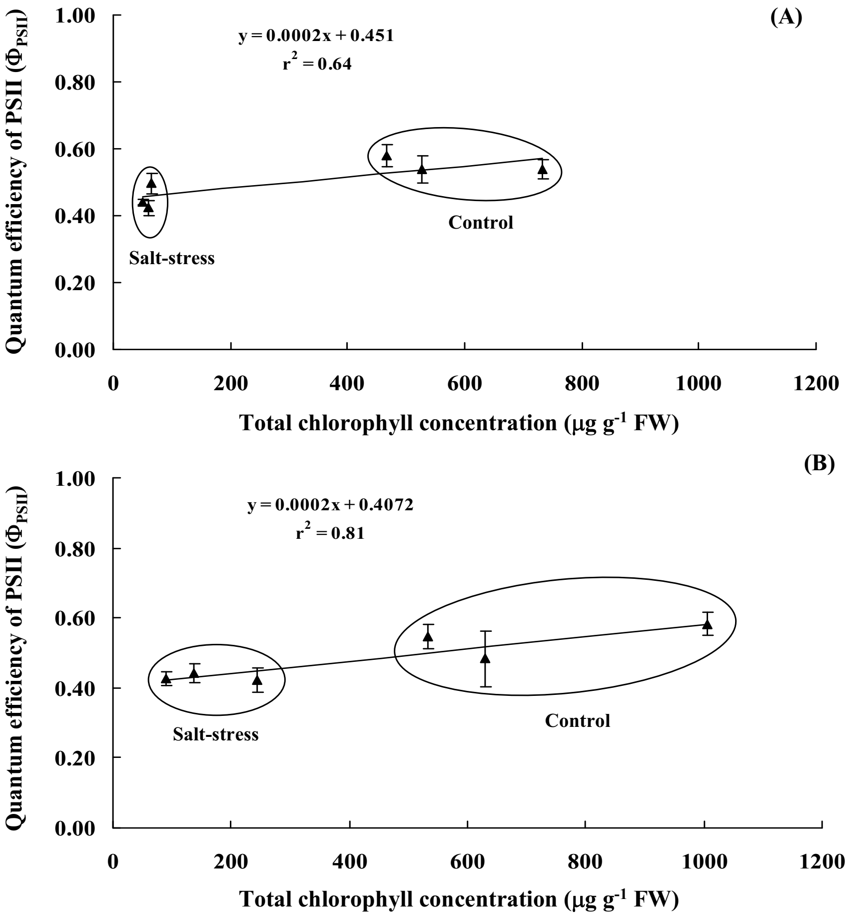

Fig. 4 Relationship between total chlorophyll concentration and quantum efficiency of PSII (Φ_{PSII}) of salt-sensitive (**A**) and salt-tolerant (**B**) water convolvulus lines.

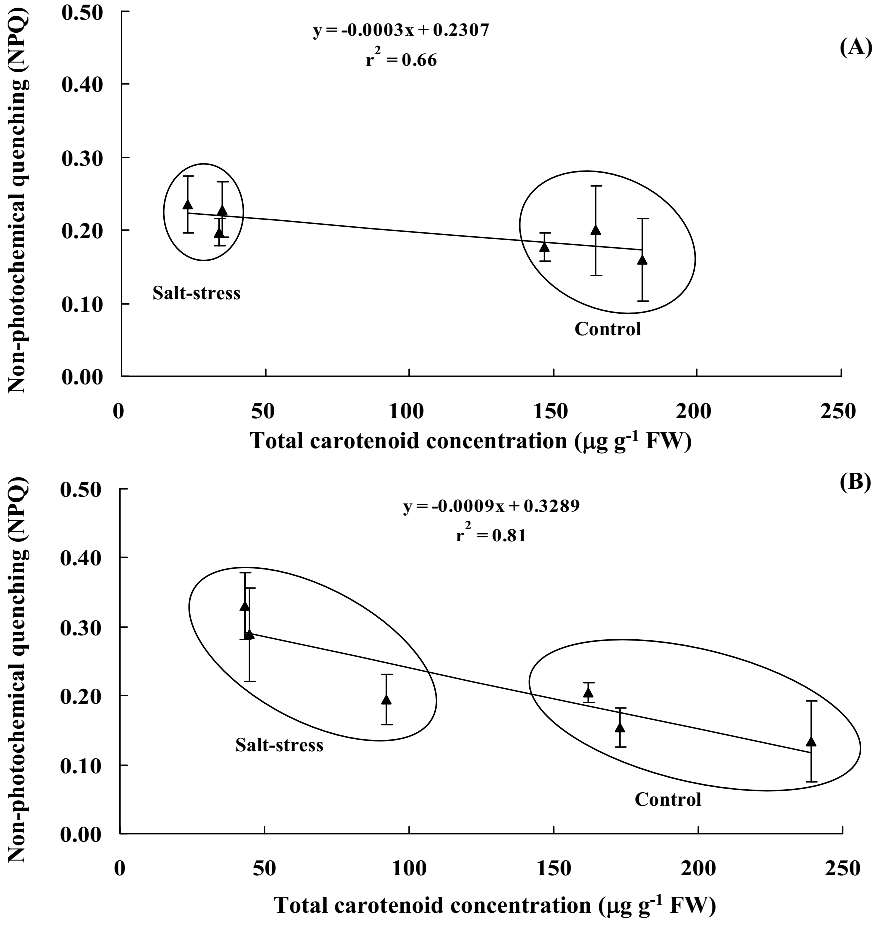

Fig. 5 Relationship between total carotenoid concentration and non-photochemical quenching (NPQ) of salt-sensitive (**A**) and salt-tolerant (**B**) water convolvulus lines.

Table 2 Analysis of photosynthesis parameters in response to salt stress in water convolvulus.

Lines	Salt-stress (mM)	Maximum quantum yield of PSII (F_v/F_m)	Quantum efficiency of PSII (Φ_{PSII})	Non-photochemical quenching (NPQ)
WC001	0	0.794	0.548 ab	0.154 c
	342	0.793	0.425 b	0.289 ab
WC092	0	0.817	0.580 a	0.176 bc
	342	0.794	0.442 b	0.236 ab
MK98	0	0.824	0.582 a	0.134 c
	342	0.772	0.422 b	0.195 bc
SR716	0	0.820	0.483 ab	0.204 abc
	342	0.814	0.440 b	0.330 a
SR739	0	0.801	0.577 a	0.219 abc
	342	0.788	0.461 ab	0.251 ab
WC083	0	0.800	0.539 ab	0.200 bc
	342	0.700	0.496 ab	0.223 abc
Significant level				
Line		^{NS}	^{NS}	^{NS}
Salt stress		^{NS}	**	*
Line×Salt stress		^{NS}	^{NS}	^{NS}

Highly significance at $P \leq 0.01$, significant at $P \leq 0.05$ and non-significant are represented by **, * and ^{NS}, respectively. Means followed by different letters are significantly different at $P \leq 0.01$ by Duncan's New Multiple Range Test.

and fresh weight, in salt-stressed water convolvulus were reduced in both salt-tolerant and salt-sensitive lines (**Table 3**). The reduction in percentages of root length and fresh weight in salt-sensitive lines were greater than those of salt-tolerant lines by 1.33 and 1.44 times, respectively. Moreover, all growth parameters were strongly reduced by salt stress condition, except dry weight (**Table 3**). The different classes were significantly affected in terms of number of leaves, root length and fresh weight, but there was no variation in plant height and numbers of roots.

Green/dark green leaves of salt-stressed water convolvulus were changed to light green/yellow leaves within a week of salt treatment. The photosynthetic pigments, chlorophyll a, chlorophyll b and total carotenoid contents in salt-sensitive water convolvulus lines cultured under salt-stress were significantly degraded when compared to salt-tolerant lines (**Table 1**). It has been showed that in olive, the chlorophyll b and total carotenoid contents in the leaves of salt-stressed plants (200 mM NaCl) decrease by 1.30 and 1.48 times respectively, compared with unstressed wild species and by 1.33 and 1.73 times respectively, compared with unstressed hybrids (Ma *et al.* 1997). Other research has also shown that there are significant degradations of chlorophyll pigment in salt sensitive varieties of green gram by 1.96 times (Ahmad *et al.* 2005), the 50% anthesis stage of wheat by 1.44 times (Sairam *et al.* 2002), cotton by 1.48 times (Meloni *et al.* 2003) and sorghum by 1.83 times (Netondo *et al.* 2004) when grown in conditions of salt stress, compared to plant grown without salt stress. It should be noted that salt stress strongly affected chlorophyll degradation and disturbed the chlorophyll biosynthetic partway, especially in glycophyte species (Bohnert *et al.* 1995; Santos 2004). On the other hand, the chlorophyll *a*, chlorophyll *b* and total carotenoid concentrations in the salt-tolerant tomato cultivars "Brillante" stabilized at higher levels than those in the salt-sensitive cultivars "Royesta" by 1.50, 1.16 and 2.14 times, respectively (Juan *et al.* 2005) when grown in the conditions of salt stress, compared to plants grown without salt stress. The activities of pigments in water oxidation and light harvesting were measured using chlorophyll a fluorescence parameters, which are reported as highly sensitive in plants' responses to salt stress (Maxwell and Johnson 2000; Netondo *et al.* 2004). In the present study, the results showed that the water oxidation or maximum quantum yield of PSII (F_v/F_m) of water convolvulus were unaffected by both salt stress and the lines chosen (**Table 2**). It is a similar pattern to the previous studies in cotton (Meloni *et al.* 2003), olive (Ma *et al.* 1997) and sorghum (Netondo *et al.* 2004). In those studies, the F_v/F_m values in both salt stress and cultivars are not significantly different. Alternatively, the quan-

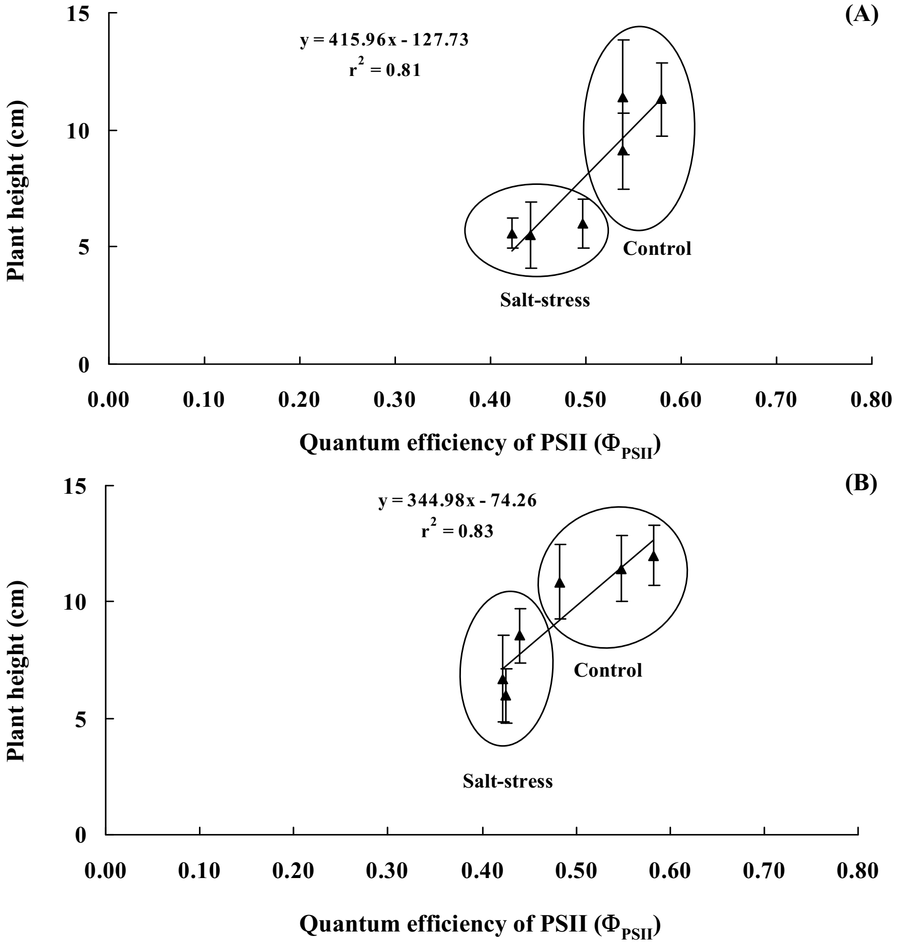

Fig. 6 Relationship between quantum efficiency of PSII (Φ_{PSII}) and plant height of salt-sensitive (**A**) and salt-tolerant (**B**) water convolvulus.

Table 3 Analysis of growth parameters in response to salt stress in water convolvulus.

Lines	Salt-stress (mM)	Plant height (cm)	Number of leaves	Root length (cm)	Number of roots	FW (g)	DW (mg)
WC001	0	11.27 a	6.0 abc	8.1 a	4.8 ab	0.59 bc	38.2
	342	5.48 b	4.4 def	5.4 bcd	2.8 c	0.39 c	33.8
WC092	0	11.43 a	7.6 ab	7.4 ab	4.4 ab	1.01 a	55.0
	342	5.96 b	5.0 def	6.1 bc	2.8 c	0.64 bc	59.9
MK98	0	9.13 ab	6.4 abc	6.5 abc	5.0 ab	0.62 bc	32.4
	342	5.60 b	2.6 f	4.6 cd	3.0 bc	0.40 c	35.4
SR716	0	12.02 a	6.8 abc	4.6 cd	6.0 a	0.71 ab	47.1
	342	6.99 b	3.6 ef	3.9 d	4.0 ab	0.63 bc	51.6
SR739	0	13.47 a	8.0 a	6.3 abc	5.2 a	0.88 ab	47.0
	342	10.68 ab	5.6 bcde	4.9 cd	4.6 ab	0.69 ab	53.0
WC083	0	10.86 ab	5.0 de	5.7 bc	4.0 ab	0.66 abc	23.8
	342	8.54 ab	2.6 f	4.4 cd	3.0 bc	0.39 c	38.6
Significant level							
Line		^{NS}	**	**	^{NS}	*	^{NS}
Salt stress		**	**	**	**	**	^{NS}
Line×Salt stress		^{NS}	^{NS}	^{NS}	^{NS}	^{NS}	^{NS}

Highly significance at $P \le 0.01$, significant at $P \le 0.05$ and non-significant are represented by **, * and ^{NS}, respectively. Means followed by different letters are significantly different at $P \le 0.01$ by Duncan's New Multiple Range Test.

tum yield efficiency (Φ_{PSII}) and non-photochemical quenching (NPQ) in salt-sensitive lines significantly decreased when compared to those in salt-tolerant lines (**Table 2**). The Φ_{PSII} and NPQ in salt-tolerant sorghum 'Seredo' and wheat 'AZ-8501' are more highly expressed than those in salt-sensitive sorghum 'Serena' (Netondo *et al.* 2004) and barley 'Morex' (Jiang *et al.* 2006), respectively. The pigment degradation and activity as well as growth reduction were used effectively to identify salt tolerance in water convolvulus lines (**Fig. 3**). The growth characters and survival percentage of salt-stressed cowpea genotypes are applied to classify the salt-tolerant abilities into four groups namely, tolerant, moderately tolerant, moderately sensitive and sensitive (Murillo-Amador *et al.* 2006). Thus, multivariate criteria or parameters in biochemical, physiological and morphological characters provide effective means to classify the salt-tolerant or salt-sensitive species. Similarly, sugarcane varieties have been grouped into four classes – highly tolerant, tolerant, sensitive and highly sensitive – 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 salt tolerant (AZ-8501 and Giza125) and salt sensitive varieties (Morex and TR306) have been classified using salinity susceptibility index (SSI) in terms of physiological characters, including efficiency of light harvesting of PSII (F'_v/F'_m), internal CO_2 concentration (C_i) and stomatal conductance (g_s) (Jiang *et al.* 2006). In addition, wheat genotypes have been clustered into three groups – tolerant, moderately tolerant and sensitive – based on growth performances, biomass and grain yield using Ward's minimum cluster analysis (El-Hendawy *et al.* 2005). Rice genotypes have been identified into four clusters by Ward's minimum variance cluster analysis based on ion contents, ion selectivity and growth performances (Zeng 2005). This study shows that photosynthetic pigments, chlorophyll a fluorescence and growth characters in salt-tolerant lines are significantly different compared to those in salt-sensitive lines and can be reliably used in multiple parameter evaluation.

CONCLUSION

Pigment degradation and PSII function in salt tolerant lines were positively correlated with overall growth performances and were effectively developed as multivariate salt-tolerant parameters to rapidly screen water convolvulus populations for salt tolerance. In addition, pigment degradation or yellow leaf color, the activity of pigments and growth performances in salt-sensitive lines in response to salt-stress can be utilized as bio-monitors in detecting salt-contaminated wastewater, released-out from urban or industrial zones.

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REFERENCES

Ahmad S, Wahid A, Rasul E, Wahid A (2005) Comparative morphological and physiological responses of green gram genotypes to salinity applied at different growth stages. *Botanical Bulletin of Academia Sinica* **46**, 135-142

Aluko OO, Sridhar MKC, Oluwande PA (2003) Characterization of leachates from a municipal solid waste landfill site in Ibadan, Nigeria. *Journal of Environmental Health Research* **2**, 32-36

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. *Flora* **199**, 361-376

Bohnert HJ, Nelson DE, Jensen RG (1995) Adaptations to environmental stresses. *The Plant Cell* **7**, 1099-1111

Bowman MS, Clune TS, Sutton BG (2002) Sustainable management of landfill leachate by irrigation. *Water Air and Soil Pollution* **134**, 81-96

Cha-um S, Mosaleeyanon K, Supaibulwatana K, Kirdmanee C (2004a) Physiological responses of Thai neem (*Azadirachta siamensis* Val.) to salt stress for salt-tolerance screening program. *Science Asia* **30**, 17-23

Cha-um S, Kirdmanee C, Supaibulwatana K (2004b) Biochemical and physiological responses of Thai jasmine rice (*Oryza sativa* L. ssp. *indica* cv. KDML105) to salt-stress. *Science Asia* **30**, 247-253

Cha-um S, Supaibulwatana K, Kirdmanee C (2005) Phenotypic responses of Thai jasmine rice to salt-stress under environmental control of *in-vitro* photoautotrophic system. *Asian Journal of Plant Science* **4**, 85-89

Chen BH, Chen YY (1992) Determination of carotenoids and chlorophylls in water convolvulus (*Ipomoea aquatica*) by liquid chromatography. *Food Chemistry* **45**, 129-134

Chen TY, Kao CM, Yeh TY, Chien HY, Chao AC (2006) Application of a constructed wetland for industrial wastewater treatment: A pilot-scale study. *Chemosphere* **64**, 497-502

El-Fadel M, Findikakis AN, Leckie JO (1997) Environmental impacts of solid waste landfilling. *Journal of Environmental Management* **50**, 1-25

El-Hendawy SE, Hu Y, Yakout GM, Awad AM, Hafiz SE, Schmidhalter U (2005) Evaluating salt tolerance of wheat genotypes using multiple parameters. *European Journal of Agronomy* **22**, 2243-253

Ekanayake IJ, Dodds JH (1993) *In-vitro* testing for the effects of salt stress on growth and survival of sweet potato. *Scientia Horticulturae* **55**, 239-248

Fonkou T, Agendia P, Kengne I, Akoa A, Nya J (2002) The accumulation of heavy metals in biotic and abiotic components of the Olezoa wetland complex in Yaounde-Cameroon (West Africa). In: *Proceedings of International Symposium on Environmental Pollution Control and Waste Management*, 7-10 January 2002. Tunis (EPCOWM 2002), pp 29-33

Gothberg A, Greger M, Holm K, Bengtsson BE (2004) Influence of nutrient levels on uptake and effects of mercury, cadmium and lead in water convolvulus. *Journal of Environmental Quality* **33**, 1247-1255

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Molecular Biology* **51**, 463-499

Hootsmans MJM, Wiegman F (1998) Four halophyte species growing under salt stress: their salt of life? *Aquatic Botany* **62**, 81-94

Houshmand S, Arzani A, Maibody SAM, Feizi M (2005) Evaluation of salt-tolerant genotypes of durum wheat derived from *in-vitro* and field experiments. *Field Crops Research* **91**, 345-354

Huang DJ, Chen HJ, Lin CD, Lin YH (2005) Antioxidant and antiproliferative activities of water convolvulus (*Ipomoea aquatica* Forsk) constituents. *Botanical Bulletin of Academia Sinica* **46**, 99-106

Jiang Q, Roche D, Monaco TA, Durham S (2006) Gas exchange, chlorophyll fluorescence parameters and carbon isotope discrimination of 14 barley genetic lines in response to salinity. *Field Crop Research* **96**, 269-278

Jing SR, Lin YF, Wang TW, Lee DY (2002) Microcosm wetlands for wastewater treatment with different hydraulic loading rate and macrophytes. *Journal of Environmental Quality* **31**, 690-696

Juan M, Rivero RM, Romero L, Ruiz JM (2005) Evaluation of some nutritional and biochemical indicators in selecting salt-resistant tomato cultivars. *Environmental and Experimental Botany* **54**, 193-201

Karnchanawong S, Sanjitt J (1995) Comparative study of domestic wastewater treatment efficiencies between facultative pond and water convolvulus pond. *Water Science and Technology* **32**, 263-270

Kirdmanee C, Cha-um S (1997) Morphological, and physiological comparisons of plantlets *in-vitro*: responses to salinity. *Acta Horticulturae* **457**, 181-186

Kirdmanee C, Mosaleeyanon K (2000) Environmental engineering for transplant production. In: Kubota C, Chun C (Eds) *Transplant Production in the 21st Century*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 78-82

Kirdmanee C, Phaephun W, Teerakathiti T, Cha-um S, Takagaki M (2006) An effective *in-vitro* selection of water convolvulus (*Ipomoea aquatica* Forsk.) for NaCl-, KH_2PO_4- and temperature-stresses. *Environmental Control in Biology* **44**, 265-277

Klomjek P, Nitisoravut S (2005) Constructed treatment wetland: a study of eight plant species under saline conditions. *Chemosphere* **58**, 585-593

Kozai T, Kubota C, Jeong BR (1997) Environmental control for the large-scale production of plants through *in-vitro* techniques. *Plant Cell, Tissue and Organ Culture* **51**, 49-56

Kumria R, Rajam MV (2002) Ornithine decarboxylase transgene in tobacco affects polyamines, *in-vitro* morphogenesis and response to salt stress. *Journal of Plant Physiology* **159**, 983-990

Kyambadde J, Kansiime F, Gumaelius L, Dalhammar G (2004) A comparative study of *Cyperus papyrus* and *Miscanthidium violaceum*-based constructed wetlands for wastewater treatment in a tropical climate. *Water Research* **38**, 475-485

Lee SY, Lee JH, Kwon TO (2003) Selection of salt-tolerant doubled haploids in rice anther culture. *Plant Cell, Tissue and Organ Culture* **74**, 143-149

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology* **148**, 350-380

Lin CC, Hsu YT, Kao CH (2002) The effect of NaCl on proline accumulation in rice leaves. *Plant Growth Regulation* **36**, 275-285

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 Physiology* **119**, 1091-1099

Lutts S, Kinet JM, Bouharmont J (1996) NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany* **78**, 389-398

Ma HC, Fung L, Wang SS, Altman A, Huttermann A (1997) Photosynthetic response of *Populus euphratica* to salt stress. *Forest Ecology and Management* **93**, 55-61

Malalavidhane TS, Wickramasinghe SMDN, Jansz ER (2000) Oral hypoglycaemic activity of *Ipomoea aquatica*. *Journal of Ethnophamacology* **72**, 293-298

Mansour MMF, Salama KHA (2004) Cellular basis of salinity tolerance in plants. *Environmental and Experimental Botany* **52**, 113-122

Maxwell K, Johnson GN (2000) Chlorophyll fluorescence – a practical guide. *Journal of Experimental Botany* **51**, 659-668

Meerak J, Akaracharanya A, Leepipatpiboon N, Chadchawan S, Kaothien-Nakayama P, Shinmyo A, Sano H (2006) Simultaneous expression of serine acetyltransferase and cysteine synthase results in enhanced sulfate uptake and increased biomass in *Ipomoea aquatica*. *Plant Biotechnology* **23**, 185-189

Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. *Environmental and Experimental Botany* **49**, 69-76

Mills D, Tal M (2004) The effect of ventilation on *in-vitro* response of seedlings of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii* to salt-stress. *Plant Cell, Tissue and Organ Culture* **78**, 209-216

Misra N, Dwivedi UN (2004) Genotypic difference in salinity tolerance of green gram cultivars. *Plant Science* **166**, 1135-1142

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Murillo-Amador B, Troyo-Dieguez E, Garcia-Hernandez JL, Lopez-Aguilar R, Avila-Serrano NY, Zamora-Salgado S, Rueda-Puente EO, Kaya C (2006) Effect of NaCl salinity in the genotypic variation of cowpea (*Vigna unguiculata*) during early vegetative growth. *Scientia Horticulturae* **108**, 423-431

Netondo GW, Onyango JC, Beck E (2004) Sorghum and salinity: Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Science* **44**, 806-811

Noureddin MI, Furumoto T, Ishida Y, Fukui H (2004) Absorption and metabolism of bisphenol A, a possible endocrine disruptor, in the aquatic edible plant, water convolvulus (*Ipomoea aquatica*). *Bioscience, Biotechnology and Biochemistry* **68**, 1398-1402

Oron G (2003) Agriculture, water and the environment: future challenges. *Water Science and Technology: Water Supply* **3**, 51-57

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. *Ecotoxicology and Environmental Safety* **60**, 324-349

Patterson R.A. (2004). A Resident's role in minimizing nitrogen, phosphorus and salt in domestic wastewater. In: Mankin KR (Ed), *10th National Symposium on Individual and Small Community Sewage Systems Proceedings*, Sacramento, California March 21-24, 2004, American Society of Agricultural Engineers, pp 740-749

Rout NP, Shaw BP (2001) Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. *Plant Science* **160**, 415-423

Sairam RK, Rao KV, Srivastava GC (2002) Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidative stress, antioxidant activity and osmolyte concentration. *Plant Science* **163**, 1037-1046

Santos CV (2004) Regulation of chlorophyll biosynthesis and degradation by salt stress in sun flower leaves. *Scientia Horticulturae* **103**, 93-99

Shabala SN, Shabala SI, Martynenko AI, Babourina O, Newman IA (1998) Salinity effect on bioelectric activity, growth, Na^+ accumulation and chlorophyll fluorescence of maize leaves: a comparative survey and prospects for screening. *Australian Journal of Plant Physiology* **25**, 609-616

Sharma M (1994) Taxonomic notes on north Indian plants. *Journal of Economic and Taxonomic Botany* **18**, 387-394

Stabnikova O, Goh WK, Ding HB, Ding HB, Wang JY (2005) The use of sewage and horticultural waste to develop artificial soil for plant cultivation in Singapore. *Bioresource Technology* **96**, 1073-1080

Sakulkoo N, Akaracharanya A, Chareonpornwattana S, Leepipatpiboon N, Nakamura T, Yamaguchi Y, Shinmyo A, Sano H (2005) Hyper-assimilation of sulfate and tolerance to sulfide and cadmium in transgenic water convolvulus expressing an *Arabidopsis* adenosine phosphosulfate reductase. *Plant Biotechnology* **22**, 27-32

Sun EJ, Wu FY (1998) Along-vein necrosis as indicator symptom on water convolvulus caused by nickel in water culture. *Botanical Bulletin of Academia Sinica* **39**, 255-259

Tofern B, Mann P, Kaloga M, Jenett-Siems K, Witte L, Eich E (1999) Aliphatic pyrrolidine amides from two tropical convolvulaceous species. *Phytochemistry* **52**, 1437-1441

Wahid A, Rao AR, Rasul E (1997) Identification of salt tolerance traits in sugarcane lines. *Field Crops Research* **54**, 9-17

Wahome PK, Jesch HH, Pinker I (2001) Effect of sodium chloride stress on *Rosa* plants growing *in-vitro*. *Scientia Horticulturae* **90**, 187-191

Wanichananan P, Kirdmanee C, Vutiyano C (2003) Effect of salinity on biochemical and physiological characteristics in correlation to selection of salt-tolerant ability in aromatic rice (*Oryza sativa* L.). *Science Asia* **29**, 333-339

Wills RBH, Rangga A (1996) Determination of carotenoids in Chinese vegetables. *Food Chemistry* **56**, 451-455

Zeng L (2005) Exploration of relationships between physiological parameters and growth performance of rice (*Oryza sativa* L.) seedlings under salinity stress using multivariate analysis. *Plant and Soil* **268**, 51-59