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Screening upland rice (*Oryza sativa* L. ssp. *indica*) genotypes for salt-tolerance using multivariate cluster analysis

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Seedlings of thirteen genotypes of rice were photoautotrophically grown on MS medium and subsequently exposed to 0 (control) or 200 mM NaCl (salt stress) for 14 days. Chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total carotenoids (C_{x+c}), in the salt stressed leaves of all genotypes decreased significantly, but the extent of the decrease varied among different genotypes. Maximum quantum yield of photosystem II (PSII) (F_v/F_m), photon yield of PSII (Φ_{PSII}) and net photosynthetic rate (P_n) in salt stressed seedlings of all genotypes dropped significantly, whereas Φ_{PSII} in cv. Homjan (HJ), Dokpayom (DPY), Chewmaejan 2 (CMJ2) and upland rice 1 (UR1) were alleviated. Moreover, growth parameters including shoot height, root length, fresh weight, dry weight and leaf area in salt stressed plantlets of all genotypes were significantly inhibited. The pigment degradation, photosynthetic abilities and growth inhibition in saline regimes were subjected to hierarchical cluster analysis, which lead to the classification of Kumuanguang (KML), Khao Dawk Mali (KDML), Pokkali (POK), HJ, DPY, Chewmaejan 1 (CMJ1), CMJ2, UR1 and Chowho (CH) as salt tolerant and R258, Pathumthani 1 (PT1), IR29 and upland rice 2 (UR2) as salt sensitive.

Key words: Chlorophyll a fluorescence, growth reduction, net-photosynthetic rate, pigment degradation, salt-tolerant classification.

INTRODUCTION

Salt affected soils are enriched with salts that is, sodium

chloride (NaCl), sodium sulfate (Na₂SO₄), calcium chloride (CaCl₂) and magnesium chloride (MgCl₂). Sodium chloride is a major salt contaminant in most saline soils. The effects of Na⁺ ions are well established as this ion can cause damage to plant cells by both ionic and osmotic effects, leading to growth retardation, low productivity and eventually cell death (Hasegawa et al., 2000; Munns et al., 2002; Mansour and Salama, 2004; Chinnusamy et al., 2005). Glycophytic or salt susceptible species including rice are sensitive to salt stress (Lutts et al., 1999). Rice is a major carbohydrate crop, providing one-third of the world population, especially in Asian countries. It plays an important role as a staple food and is used to feed more than 3 billion people on a daily calorie intake of 50 to 80% (Khush, 2005). Abiotic stresses including salinity, drought and extreme temperatures are large

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Abbreviations: SES, Standard evaluation score; LAI, leaf area index; UR1, upland rice 1, UR2, upland rice 2; DPY, Dokpayom; CMJ1, Chewmaejan 1; CMJ2, Chewmaejan 2; CH, Chowho; KML, Kumuanguang, KDML105, Khao Dawk Mali 105; POK, Pokkali; HJ, Homjan; PT1, Pathumthani 1; RH, relative humidity; Chl_a, chlorophyll a, Chl_b, chlorophyll b TC, total chlorophyll; F_v, variable fluorescence; PSII, photosystem II; Φ_{PSII}, photon yield of photosystem II; P_n, net-photosynthetic rate; SH, shoot height; RL, root length; FW, fresh weight; DW, dry weight; LA, leaf area; NPQ, non-photochemical quenching.

Table 1. Names, abbreviations and accession number of upland rice cultivars.

Genotypes	Abbreviations	Accession number	Salt tolerance abilities
Pokkali	POK	17905	Salt tolerant
IR29	IR29	2818	Salt susceptible
Homjan	HJ	4321	Salt tolerant
Pathumthani 1	PT1	Inbred cultivar	Salt susceptible
R258	R258	9761	Unknown
Upland rice 1	UR1	3462	Unknown
Upland rice 2	UR2	3474	Unknown
Dokpayom	DPY	1947	Unknown
Chewmaejan 1	CMJ1	4001	Unknown
Chewmaejan 2	CMJ2	9111	Unknown
Chowho	CH	7933	Unknown
Kumuanguang	KML	4002	Unknown
Khao Dawk Mali 105	KDML	Inbred cultivar	Unknown

barriers to the limit of rice crop production. Salt stress is known to induce abnormal growth and development in rice crop (Shannon et al., 1998; Zeng and Shannon, 2000; Khan and Abdullah, 2003; Zeng et al., 2003). Certain rice varieties have been reported as being salt sensitive at their seedling and reproductive stages (Zeng et al., 2002; Moradi and Ismail, 2007), leading to reduced crop productivity of more than 50% when exposed to soil salinity of 6.65 dS m⁻¹ (Zeng and Shannon, 2000). Breeding programs for enhanced salt tolerance in rice crop are meaningful means of overcoming the Salinity problem (Gregorio et al., 2002; Senadhira et al., 2002; Flowers and Flowers, 2005).

In some reports published earlier, multivariate analysis has been successfully used to identify salt tolerant rice germplasm. For example, Pokkali, Nona-Bokra, Agami, Daeyabyeo, GZ5310-20-2-1, GZ5310-20-3-2, GZ5310-20-3-3, IR65192-4B-10-3, IR63295-AC209-7 and IR4630-22-2-2-5-1-3, have been categorized as salt tolerant and have been utilized as the parental lines in different rice breeding programs worldwide (Zeng et al., 2004; Zeng, 2005; Moradi and Ismail, 2007). 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 were reported to be salt sensitive (Zeng et al., 2004; Zeng, 2005). In some other studies, screening of rice germplasm has been done using the standard evaluation score (SES) (Haq et al., 2009; Quijano-Guerta and Kirk, 2002; Ali et al., 2004; Bhowmik et al., 2009) and leaf area index (LAI) (Zeng et al., 2003; Alamgir and Ali, 2006) as selection criteria. However, the effective criteria for identification of salt tolerant genotypes still need to be investigated, especially in mass population of a breeding program. However, some researchers have recommended multiple indices for the identification of salt tolerant genotypes in different crop species, especially in rice (Zeng et al., 2004; Zeng, 2005). Thus the aim of this investigation

was to develop and apply the multivariate indices for the identification of salt tolerant rice genotypes.

MATERIALS AND METHODS

Plant materials

Seeds of upland rice cultivars including R258, upland rice 1 (UR1), upland rice 2 (UR2), Dokpayom (DPY), Chewmaejan 1 (CMJ1), Chewmaejan 2 (CMJ2), Chowho (CH) and Kumuanguang (KML), Khao Dawk Mali 105 (KDML105), known salt tolerant, Pokkali (POK) and Homjan (HJ), and salt-susceptible, IR29 and Pathumthani 1 (PT1) (Table 1) were hand-dehusked, rinsed with 70% ethanol, surface-sterilized once overnight, in 5% (v/v) Clorox[®] (5.25% w/v sodium hypochlorite, Clorox Co, USA), soaked once in 25% Clorox[®] for 25 min, and then rinsed thrice with sterile distilled water. Surface sterilized seeds of all cultivars were germinated on MS-solidified media (Murashige and Skoog, 1962). 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 density (PPFD) with 16 h d⁻¹ photoperiod provided by fluorescent lamps (TLD 36W/84, Cool White, Philips, Thailand). Fourteen-day-old rice seedlings were aseptically transferred to 50 mL liquid sugar-free MS media, supported by 20 g vermiculite for 7 days. The amount of air-exchange in the glass vessels was adjusted to 2.32 μmol CO₂ h⁻¹ by punching a hole over the plastic cap (Ø 1 cm) and covering with a gas-permeable microporous polypropylene film (0.22 μm pore size, Nihon Millipore Ltd., Japan).

The open-cap vessels containing photoautotrophic seedlings were aseptically transferred to culture chamber boxes (Carry Box Model P-850, size 26 × 36 × 19 cm, Japan) controlled at 65 ± 5% RH in 1.5 L NaCl-saturated solution. The amount of air exchange in the culture chambers was increased to 5.1 ± 0.3 μmol CO₂ h⁻¹ by perforating the side of the plastic chambers with 32 holes and covering each hole with gas-permeable microporous polypropylene film (0.22 μm of pore size). These chambers were incubated in an EYELA Plant Growth incubator at a temperature of 28 ± 2/25 ± 2°C (12 h photoperiod/12 h dark-period), 500 ± 100 μmol mol⁻¹ CO₂ concentration, 60 ± 5% RH, and 120 ± 5 μmol m⁻² s⁻¹ PPFD by fluorescent lamps, for 14 days. Sodium chloride (NaCl) in the culture media was adjusted to 0 (control) or 200 mM (salt-stress) for

14 days. Photosynthetic pigments (chlorophyll a and b, and total carotenoids), chlorophyll a fluorescence, net-photosynthetic rate (P_n) and some key growth characters were measured.

Data measurements

Concentrations of chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total chlorophyll (TC) were determined following the methods of Shabala et al. (1998) and total carotenoids (C_{x+c}) were measured according to 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, Vineland, New Jersey, USA), 10 mL of 95.5% acetone was added to it, and the mixture was blended with a homogenizer (T25 basic ULTRA-TURRAX®, IKA, Kuala Lumpur, Malaysia). The glass vials were sealed with parafilm to prevent evaporation and then stored at 4°C for 48 h. The Chl_a, Chl_b and C_{x+c} concentrations were measured using a UV-visible spectrophotometer (DR/4000, HACH, Loveland, Colorado, USA). A solution of 95.5% acetone was used as blank. 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., Norfolk, 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., Norfolk, 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 ($<0.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) with 1.6 s pulses of saturating light ($>6.8 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) and autocalculated by FMS software for Windows® (Fluorescence Monitoring System Software, Hansatech Instrument Ltd., Norfolk, UK). The variable fluorescence (F_v) yield 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 photosystem II (PSII) photochemistry. The photon yield of PSII (Φ_{PSII}) in the light was calculated by $\Phi_{\text{PSII}} = (F_m' - F)/F_m'$ after 45 sec of illumination, when a steady state was achieved. In addition, non-photochemical quenching (NPQ) were calculated as described by Maxwell and Johnson (2000).

The net-photosynthetic rate (P_n) of rice seedlings was calculated by comparing the different concentrations of CO₂ inside and outside of a glass vessel containing rice seedlings. The CO₂ concentrations inside and outside the glass vessel (C_{in} and C_{out}) were measured at steady state using a gas chromatograph (GC; Model GC-17A, Shimadzu Co. Ltd., Japan). The P_n of *in vitro* cultivated plantlets was calculated according to the method of Fujiwara et al. (1987). Shoot height (SH), root length (RL), fresh weight (FW), dry weight (DW) and leaf area (LA) of rice seedlings were measured as growth characters. Rice seedlings were dried at 80°C in a hot-air oven (Model 500, Memmert, Buchenbach, Germany) for 2 days, and then incubated in desiccators before measuring the dry weight. Leaf area of rice seedlings was measured using a leaf area meter (Delta-Scan Version 2.03, Delta-T Devices, Ltd., Burwell, Cambridge, UK). Degradation percentages of photosynthetic pigments, chlorophyll fluorescence and growth parameters were calculated using the following equation:

$$\text{Degradation (\%)} = 1 - \left[\frac{\text{Salt treatment}}{\text{Control}} \right] \times 100$$

Experimental designs

The experiment was arranged as 2×13 factorials in a completely

randomized design (CRD) with ten replicates ($n = 10$). The mean values were compared by Duncan's New Multiple Range Test (DMRT) and analyzed by statistical package for the social sciences (SPSS) software (SPSS for Windows, SPSS Inc., Chicago, USA). Pigment degradation, chlorophyll a fluorescence and P_n reduction and growth inhibition in rice genotypes were subjected to classified groups as tolerant and susceptible using hierarchical cluster analysis in SPSS software.

RESULTS AND DISCUSSION

Membrane electrolyte leakage in salt stressed leaves was significantly increased when compared to that in control seedlings (Table 1) leading to TC damages (Figure 1A). Chl_a, Chl_b, TC and total carotenoid (C_{x+c}) contents in the salt stressed leaves of 13 rice genotypes decreased significantly, and showed a large variation among genotypes (Table 1). In KML and POK genotypes, the Chl_b content in salt stressed leaves was maintained. The Chl_a and Chl_b contents in rice seedlings showed a positive relation with maximum quantum yield of PSII (F_v/F_m) (Figure 1B) and photon yield of PSII (Φ_{PSII}) (Figure 1C), respectively. The degradation percentages of Chl_a, Chl_b, TC and C_{x+c} were found maximum in the leaf tissues of UR2, while those in KML and POK were lowest (Figure 2A-D). In the case of chlorophyll a fluorescence parameters, F_v/F_m and Φ_{PSII} in salt stressed leaves decreased significantly, except for Φ_{PSII} in HJ, DPY, CMJ2 and UR1 (Table 2). The decline in F_v/F_m and Φ_{PSII} in PT1 genotypes was considerably high (Figure 3A-B), which might have resulted in decreased net photosynthetic rate (P_n) (Figure 1D and 3C). Non-photochemical quenching (NPQ) in the leaf tissues of all rice genotypes was enhanced by salt stress (Table 3). The growth characters such as shoot height, root length, fresh weight, dry weight and leaf area of salt stressed seedlings reduced significantly in all genotypes (Table 4). Percent growth reduction in terms of leaf area in salt-stressed rice genotypes ranged from 34 to 73% with respect to control (Figure 3D). In UR2 genotype, percent reduction in leaf area was peaked. The pigment degradation, photosynthetic abilities and growth inhibition in rice genotypes under saline conditions were subjected to hierarchical cluster analysis of rice genotypes. This analysis led us to group the genotypes into two categories such as salt tolerant, KML, KDML, POK, HJ, DPY, CMJ1, CMJ2, UR1 and CH and salt susceptible (SS), R258, PT1, IR29 and UR2 (Figure 4).

Membrane electrolyte leakage in salt stressed leaves of rice genotypes was higher than that under control conditions, especially in the salt sensitive cultivars. Similar results have been reported in Hitomebore, IR28 and I Kong Pao salt susceptible rice grown in hydroponics culture salinized with 50 and 120 mM NaCl hydroponic culture in the greenhouse (Lutts et al., 1995; Dionisio-Sese and Tobita, 1998). The pigment concentration in salt-stressed plantlets of 13 rice genotypes was measured and the function of those pigments in terms of light

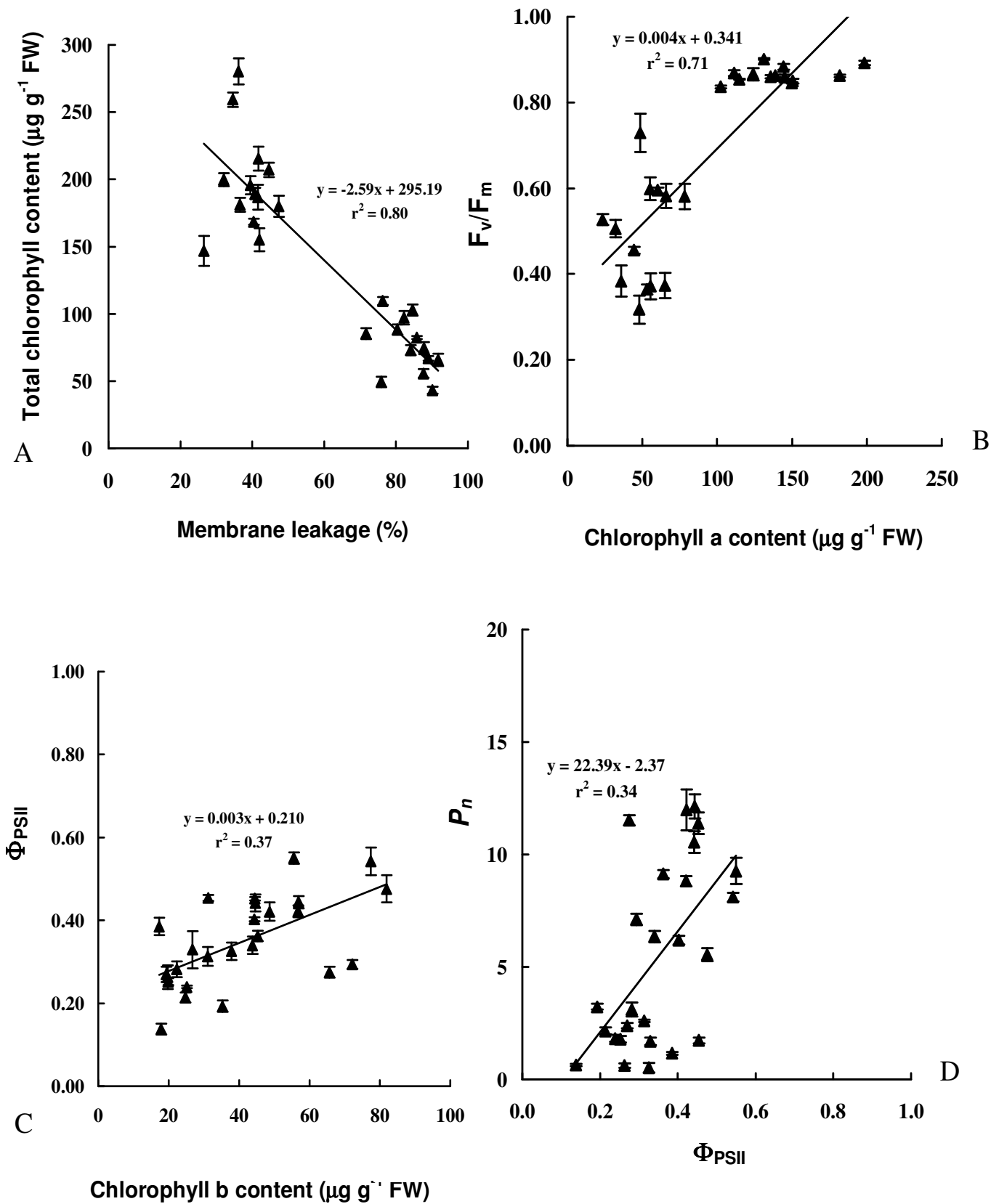


Figure 1. Relationships between membrane leakage and total chlorophyll content (A), chlorophyll a content and maximum quantum yield of PSII (F_v/F_m) (B), chlorophyll b content and quantum efficiency of PSII (Φ_{PSII}) (C), Φ_{PSII} and net photosynthetic rate (P_n) (D), in the leaf tissues of 13 rice genotypes treated with 0 or 200 mM NaCl for 14 days. Error bars represent SE values.

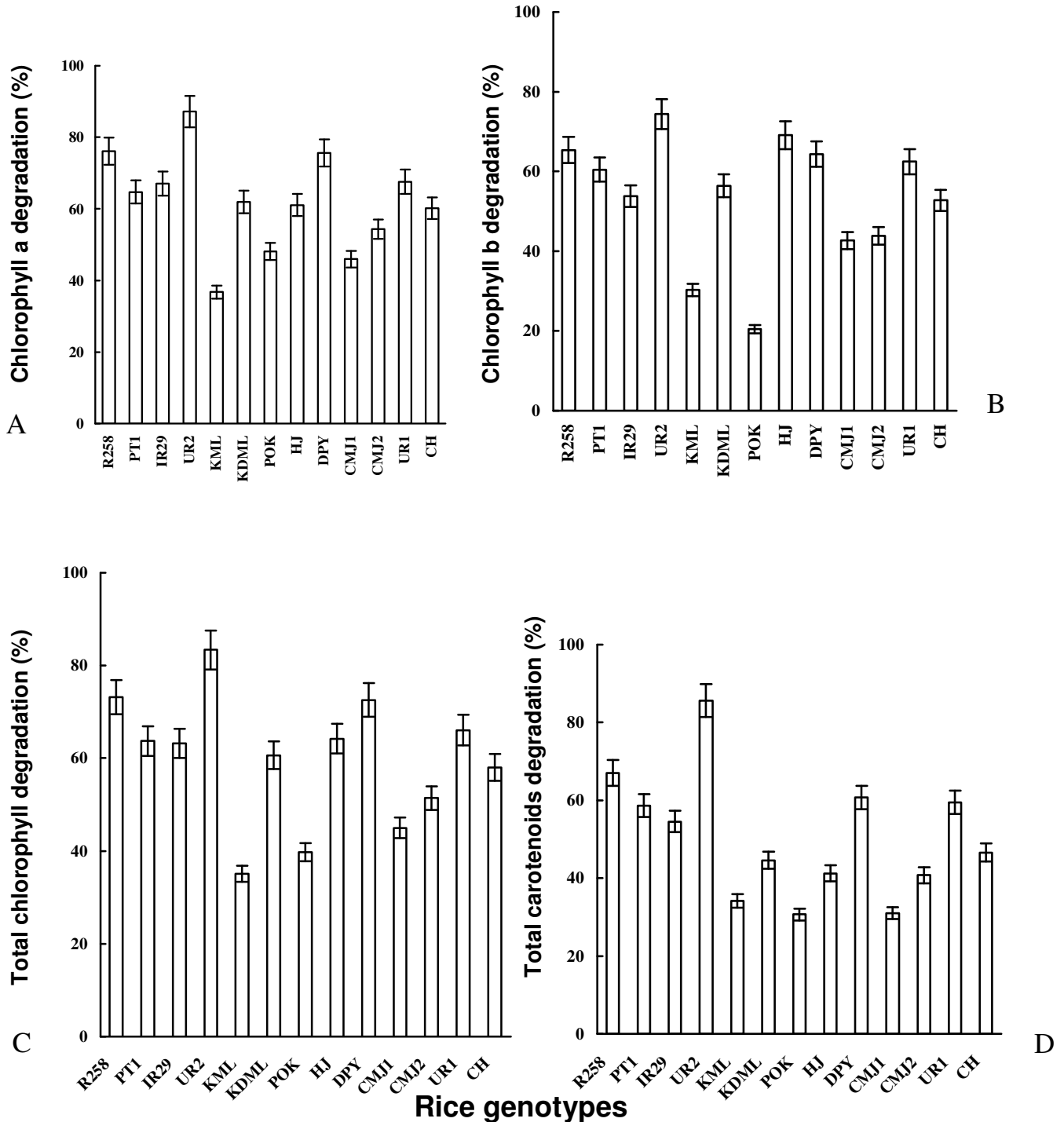


Figure 2. Degradation percentages of chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and total carotenoids (D) of 13 rice genotypes treated with 200 mM NaCl for 14 days. Error bars represent SE values.

harvesting and electron transport system in photosystem II prior to photosynthesis was also investigated. The photosynthetic pigments including TC in salt tolerance, Nona Bokra and IR4630, were reasonably high leading to high efficiency of PSII including F_0 and F_m values under salt stress and these were better than those reported in

salt susceptible I Kong Pao and IR31785 (Lutts et al., 1996). The Φ_{PSII} in HJ, DPY, CMJ2 and UR1 rice cultivars was significantly maintained when exposed to 200 mM NaCl for 14 days. The P_n ratios in rice seedlings (80 mM NaCl/0 mM NaCl) of salt tolerant Pokkali, WAS161-B-6-B-3-1B, and WAB56-104 are maintained better than

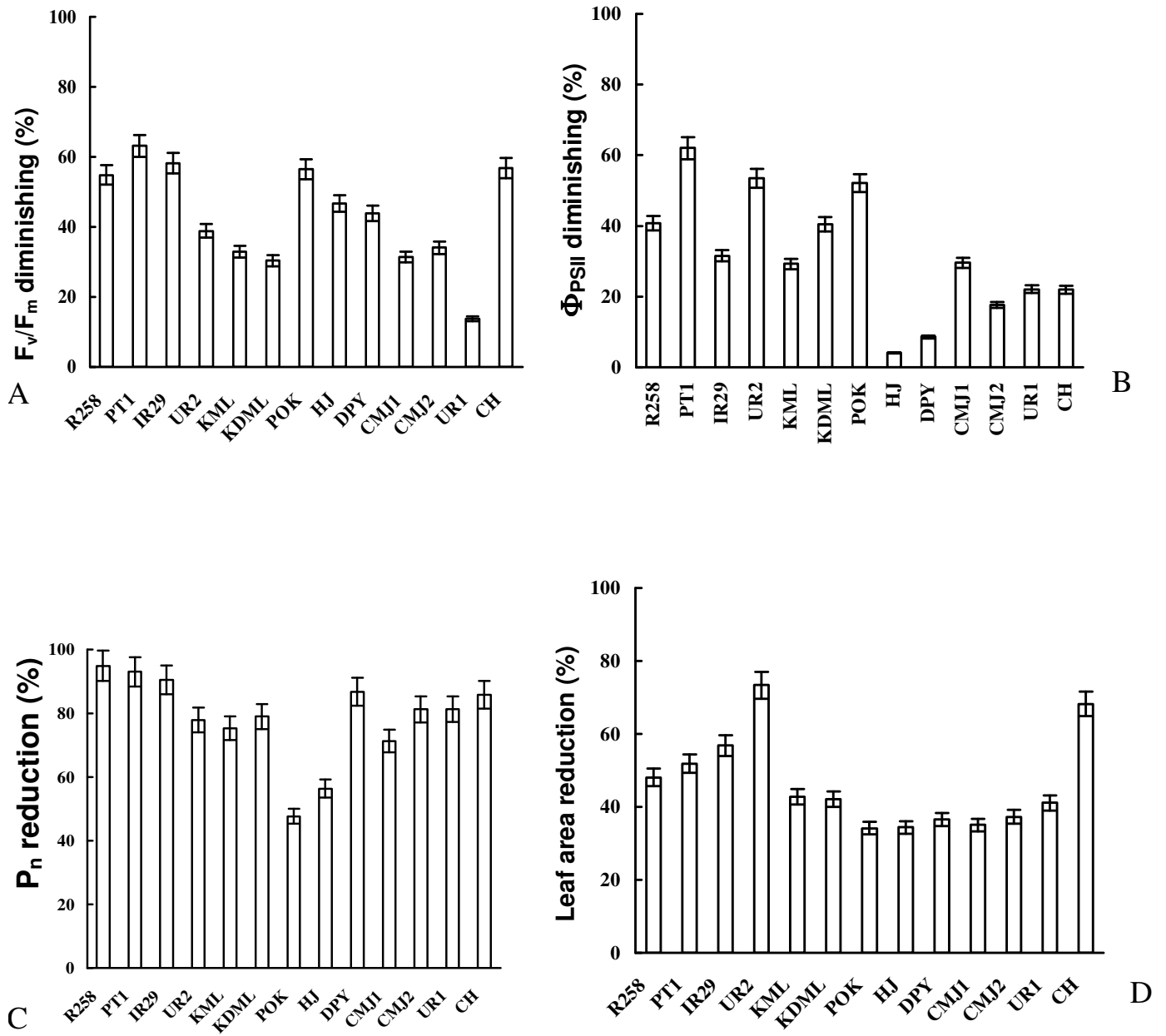
Table 2. Membrane electrolyte leakage (EL), chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophyll (TC) and total carotenoids (C_{x+c}) in 13 rice genotypes treated with 0 or 200 mM NaCl for 14 days. Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's new multiple range test (DMRT).

Genotypes	NaCl (mM)	EL (%)	Chl _a (mg g ⁻¹ FW)	Chl _b (mg g ⁻¹ FW)	TC (mg g ⁻¹ FW)	C _{x+c} (mg g ⁻¹ FW)
R258	0	47.3e	150.3b	56.8cd	207.1bc	42.9b
	200	75.9cd	35.9jkl	19.7hi	55.6lmn	14.1j
PT1	0	39.5efg	135.8bc	45.3def	181.1cde	39.8bc
	200	85.8abc	47.9ijk	17.9i	65.8lmn	16.5ij
IR29	0	36.5fgh	198.3a	81.9a	280.2a	52.3a
	200	91.7a	65.2gh	37.8fg	103.0hi	23.8efg
UR2	0	41.6efg	181.7a	77.5ab	259.2a	50.5a
	200	88.9ab	23.3l	19.9hi	43.2n	7.2k
KML	0	32.1gh	123.8cde	44.6def	168.4efg	34.1cd
	200	82.2abc	78.3g	31.1gh	109.4h	22.4fgh
KDML	0	41.7efg	144.9b	44.5def	189.3cde	41.9b
	200	84.0abc	55.1hi	19.4hi	74.5kl	23.2efg
POK	0	44.6efg	102.4f	44.3def	146.7g	26.5ef
	200	87.6ab	53.2hij	35.2fgh	88.4hij	18.4hij
HJ	0	36.1fgh	114.6def	72.2ab	176.8e	33.7cd
	200	84.5abc	44.6ijk	22.3hi	66.9lmn	19.8hij
DPY	0	34.5fgh	131.3ef	48.7de	180cde	37.0bc
	200	90.1ab	32.0kl	17.4i	49.4mn	14.5j
CMJ1	0	40.4efg	111.3ef	43.8def	155.1fg	29.0de
	200	76.3cd	60.2ghi	25.1ghi	85.3hij	20.0ghi
CMJ2	0	40.7efg	144.3def	55.5cd	199.8cd	37.9bc
	200	87.7ab	65.9gh	31.2gh	97.1hij	22.5fgh
UR1	0	26.5h	149.7b	65.7bc	215.4b	42.1b
	200	80.3bcd	48.5ijk	24.7ghi	73.2klm	17.0hij
CH	0	42.0efg	138.9bc	56.7cd	195.6cde	39.7bc
	200	71.6d	55.4hi	26.8ghi	82.2ijk	21.2ghi
Significance level						
Genotype		**	**	**	**	**
NaCl		**	**	**	**	**
Genotype × NaCl		**	**	**	**	**

those in salt susceptible CG14 and Mala noir IV (Awala et al., 2010). Relative growth rate in salt susceptible Hito-mebore and IR28, grown under 12 dS m⁻¹ salt stress was significantly inhibited when compared to that in salt tolerant Bankat and Pokkali (Dionisio-Sese and Tobita, 1998). In addition, this parameter in salt sensitive I Kong Pao, Aiwu and Tainung 67 was declined extensively when compared with that in salt tolerant Nona Bonkra, Buhra Rata, Panwell and Pokkali (Lutts et al., 1995).

In the present study, the data for pigment degradation, and reduction in chlorophyll *a* fluorescence, P_n and growth in salt stressed seedlings of 13 rice genotypes

were subjected to multivariate analysis which resulted into the categorization of the genotypes into salt tolerant, (KML, KDML, POK, HJ, DPY, CMJ1, CMJ2, UR1 and CH) and salt susceptible, (R258, PT1, IR29 and UR2). Multivariate cluster analysis was investigated and implemented to identify the salt-tolerant genotypes in various breeding programs of rice (Zeng et al., 2004; Cha-um et al., 2009). For example, other rice genotypes have already been identified and classified into four clusters, namely; highly salt tolerant (IR63352-AC202), moderately salt tolerant (Daeyabyeo, GZ5385-29-3-3, GZ5121-5-2-1, Nonabokra, IR29, IR63731-1-1-4-3-2, S-102, Pokkali,



Rice genotypes

Figure 3. Percent reduction in maximum quantum yield of PSII (F_v/F_m) (A), photon yield of PSII (Φ_{PSII}) (B), net photosynthetic rate (P_n) (C) and percent reduction in leaf area (D) of 13 rice genotypes treated with 200 mM NaCl for 14 days. Error bars represent SE values.

IR4630-22-2-2-5-1-3, IR50184-3B18-2B-1 and IR51490-AC10), moderately salt sensitive (AC26, GZ5310-20-3-2, Agami, GZ1368-5-4, GZ5385-29-3-2, Sakha 101, IR70074-AC14 and IR70074-AC1) and highly salt sensitive (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) using Ward's minimum variance cluster analysis based on growth performance, including

tiller number, leaf area and shoot dry weight (Zeng, 2005).

In conclusion, on the basis of multivariate cluster analysis using the data for pigment degradation, chlorophyll *a* fluorescence decline, photosynthetic abilities and growth reduction, it was possible to group the 13 rice cultivars in two categories with respect to salt tolerance, that is, KML, KDML, POK, HJ, DPY, CMJ1, CMJ2, UR1 and CH as salt tolerant and R258, PT1, IR29 and UR2 as salt sensitive

Table 3. Maximum quantum yield of PSII (F_v/F_m), quantum efficiency of PSII (qP), photon yield of PSII (Φ_{PSII}) and net-photosynthetic rate (P_n) of rice genotypes treated with 0 or 200 mM NaCl for 14 days. Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's new multiple range test (DMRT).

Genotypes	NaCl (mM)	F_v/F_m	Φ_{PSII}	NPQ	P_n (mmol m ⁻² s ⁻¹)
R258	0	0.850a	0.444abc	0.064efg	12.14a
	200	0.384ef	0.263hij	0.116b	0.62jk
PT1	0	0.860a	0.362def	0.042gh	9.13cd
	200	0.317f	0.138k	0.098bcd	0.64jk
IR29	0	0.893a	0.476ab	0.025h	5.55g
	200	0.373ef	0.326fg	0.088cde	0.53k
UR2	0	0.862a	0.542a	0.040gh	8.11de
	200	0.527cd	0.253hij	0.106bc	1.79hij
KML	0	0.866a	0.442abc	0.036h	10.54bc
	200	0.581c	0.313fgh	0.068ef	2.60hi
KDML	0	0.860a	0.452ab	0.065efg	11.39ab
	200	0.599c	0.270hij	0.157a	2.40hi
POK	0	0.836a	0.402cde	0.068ef	6.19fg
	200	0.364ef	0.313fgh	0.101bc	3.24h
HJ	0	0.854a	0.294ghi	0.037h	7.12ef
	200	0.456de	0.282ghi	0.074de	3.11h
DPY	0	0.901a	0.421cd	0.033h	8.82d
	200	0.506cd	0.385def	0.086cde	1.17ijk
CMJ1	0	0.868a	0.339efg	0.031h	6.36fg
	200	0.595c	0.239hij	0.090cde	1.83hij
CMJ2	0	0.883a	0.550a	0.035h	9.27cd
	200	0.583c	0.454ab	0.068ef	1.74ijk
UR1	0	0.846a	0.275ghi	0.034h	11.52ab
	200	0.729b	0.214ijk	0.046fgh	2.16hij
CH	0	0.862a	0.422cd	0.040gh	12.00a
	200	0.372ef	0.329fg	0.084cde	1.70ijk
Significant level					
Genotype		**	**	**	**
NaCl		**	**	**	**
Genotype × NaCl		**	**	**	**

Table 4. Growth characters, shoot height (SH), root length (RL), fresh weight (FW), dry weight (DW) and leaf area (LA) of 13 rice genotypes treated with 0 or 200 mM NaCl for 14 days. Different letters in each column show significant difference at $p \leq 0.01$ (**) by Duncan's New Multiple Range Test (DMRT).

Genotypes	NaCl (mM)	SH (cm)	RL (cm)	FW (mg plant ⁻¹)	DW (mg plant ⁻¹)	LA (mm ² plant ⁻¹)
R258	0	35.2de	10.6ab	421.4b	86.9ab	1536d
	200	33.0efg	9.4bc	309.5cd	78.6bcd	797ij
PT1	0	31.1fgh	4.5jk	276.3de	67.0def	1391de
	200	27.3i	4.1k	195.2fgh	53.0ghi	669k
IR29	0	26.6i	8.0efg	132.2ijk	52.5ghi	1656cd
	200	20.1j	4.5jk	90.8k	20.9o	715jk
UR2	0	28.0hi	11.4a	225.1efg	49.2hij	4547a
	200	26.6i	5.6hij	158.2hij	38.7klm	1210def
KML	0	33.4ef	4.4jk	409.5b	84.7bc	2308b
	200	31.0fgh	4.3k	348.0c	74.2cde	1322de

Table 4. Contd.

KDML	0	32.6efg	3.8k	169.4ghi	36.7lmn	969ghi
	200	28.6hi	3.8k	131.6ijk	27.3mno	560k
POK	0	44.8a	7.1fgh	279.0de	57.7fgh	1268def
	200	38.7bc	5.6hij	199.2fgh	42.9jkl	835hij
HJ	0	38.4cd	10.2ab	158.8hij	32.4mno	1144fgh
	200	26.9i	8.8cde	98.8jk	22.1o	751jk
DPY	0	39.0bc	8.8cde	144.2ijk	33.1mno	985ghi
	200	32.1efg	7.7fg	96.6k	21.7o	625k
CMJ1	0	40.8bc	9.7bc	173.6ghi	44.2jkl	1185fgh
	200	34.3ef	7.2fgh	100.7jk	26.2no	769jk
CMJ2	0	42.1ab	10.9ab	202.3fgh	51.3hij	1242def
	200	32.2efg	6.3ghi	141.2ijk	40.1jkl	779ijk
UR1	0	33.9ef	10.3ab	303.0cd	64.0efg	1982bc
	200	27.3i	5.3ij	231.1ef	53.6ghi	1167fgh
CH	0	33.8ef	8.9cde	579.5a	97.0a	2036bc
	200	29.4ghi	8.8cde	322.1cd	77.3bcd	646k
Significant level						
Genotype		**	**	**	**	**
NaCl		**	**	**	**	**
Genotype × NaCl		**	**	**	**	**

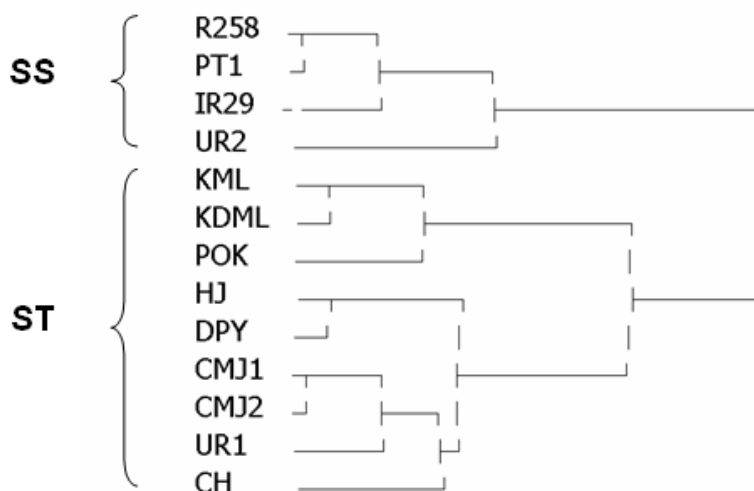


Figure 4. Cluster analysis of 13 rice genotypes using data for physiological and morphological attributes in Hierarchical cluster analysis: salt tolerant (ST), KML, KDML, POK, HJ, DPY, CMJ1, CMJ2, UR1 and CH, and salt susceptible (SS), R258, PT1, IR29 and UR2.

genotypes.

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