

Evaluation and classification of selected rice varieties for salinity tolerance at seedling stage

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ABSTRACT: To identify the new source of breeding materials for rice salt tolerance, the salinity tolerance of thirty-four varieties was evaluated under 0.5% saline condition at seedling stage. The salinity score showed highly significant correlations to dry weight and dead leaf ratio. The tested varieties were classified into three groups by visual score, reduction ratio of dry weight, and dead leaf ratio. Eighteen varieties were classified as the highly tolerant group (salinity scores of 1.3-3.7), seven varieties were fallen into the tolerant group (salinity scores of 4.2-5.8), and others were susceptible (salinity scores of 6.7-9.0). In highly tolerant group, most indica varieties including Getu, Dikwee and Kuatic Putic, didn't exert a panicle under the Korean climate. But six varieties, Xiangcho V, Annapuruna, HP 3319-2wx-6-3-1, Giza 175, and GZ 2447-S-17, GZ 4255-6-3 were suitable to the Korean climate, and their heading date (6-16, August) and culm length (65-78cm) were similar to the Korean varieties. Accordingly, these varieties can be utilized as crossing materials for the salt tolerance in japonica rice.

Key words: Evaluation, *Oryza sativa*, Salt-tolerance, Seedling stage.

The soil salinity of reclaimed paddy field is one of the important stresses to limit rice (*Oryza sativa* L.) growth and yield in Asia and Africa. The problem is more serious in reclaimed soils where the rainfall is poorly distributed at transplanting time of rice. Fortunately, rice is a hydrophilous plant, and it is adapted to flood condition (Yeo *et al.*, 1990). Most salts in surface of reclaimed soils can be diluted by abundant irrigation water. Therefore, if the fresh-water can be abundantly irrigated, rice is a good crop for early cultivation in reclaimed soils. However, most rice varieties are sensitive to salt except some traditional indica varieties such as Pokkali, Nona Bokra, Annapuruna (Yeo *et al.* 1990). The salinity tolerance has been improved in indica rice, but not in japonica rice (Zapata *et al.* 1991; Lee, 1995; Miha *et al.* 1996; Sathish *et al.* 1997). In East Asia, rice is the most important crop for farmer's income, and japonica varieties are mainly cultivated in the paddy

field of temperate regions. Therefore, the development of salt-tolerant variety is an important breeding goal for the early cultivation in reclaimed paddy fields. However, salt-tolerant varieties have hardly been identified in japonica rice of East Asia (Lee, 1995). In the case of salt-tolerant indica varieties, the inheritance of salinity tolerance was reported to be governed by polygenes, and the heritability values for characteristics concerned with salinity tolerance were also high (Akbar *et al.*, 1986, Gregorio and Senadhira 1993). The problem is genetically distant for crossing between japonica and indica varieties. It is also difficult to transfer salt-tolerant trait into japonica varieties from indica varieties such as Pokkali, Nona Bokra, because salt tolerance is coinherited with non-desirable agronomic characters in their progenies, or the disappearance of salt-tolerant genes often happened in the process for selection of progenies. In Korea, few varieties with moderately tolerance have been bred from japonica rice, but they are not compatible for cultivation in the reclaimed soils containing high salts. For many years, yields of Korean rice varieties had been reduced by salt stress during reproductive phase in reclaimed paddy fields. Accordingly, the development of new variety with high salt-tolerance is necessary for farmers in the new reclaimed paddy fields. In a salt-tolerant breeding program, identification and utilization of salt-tolerant parents with the good characteristics will have a large impact on the level of salinity tolerance. The rapid and simple screening methods are also required for the selection of salt-tolerant plants in large populations of breeding process.

The objectives of this work were: 1) to evaluate breeding materials to salt tolerance, 2) to identify relationships among some traits concerned with salt tolerance, 3) to classify their tolerant levels to salt, and 4) to select breeding materials adaptable to some agricultural traits under the temperate climate.

MATERIALS AND METHODS

Thirty-four rice varieties were collected from International Rice Research Institute (IRRI; Manila, Philippines) and National Honam Agricultural Experiment Station (NHAES; Iksan, Korea). Seeds were soaked in distilled water for 3 days at room temperature of about 25°C, and then germi-

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<Received August 16, 2003>

nated for 24 hours at 25°C. Twelve seeds per variety were sown one seed per cell on 72 plug cell trays filled with the commercial soil for machine transplanting (Punongsangto Co., Korea). The seeded plug tray was placed in a plastic box (42×65×12 cm) and seedlings were grown until 3 rd to 4th leaf stages, and then were treated with the nutrient solution containing 0.5% NaCl (Yoshida *et al.*, 1976) for 15 days. Seedling were cultured by bottom watering every 4 day in greenhouse of 28/21°C (day/night). The salinity symptoms of ten plants were scored by the standard investigation system of Rural Development Administration of Korea at 15 days after the treatment (RDA, 1993) as the following; 1 : normal growth of seedling, 3 : leaf tips discol-

ored slightly and old leaves dry, 5 : leaves discolored mostly, and growth inhibited, 7 : leaves drooped mostly or dead partially, and growth stopped, 9 : leaves dead or dry mostly.

All treatments were tested in a complete randomized design with four replications. Ten seedlings in a replication were measured for plant height, and they were oven-dried for 72 hrs. at 70°C and weighed. The reduction ratio of plant height and dry weight were computed for each replication as;

$$\text{Reduction ratio} = \frac{\text{Value at control} - \text{Value at saline condition}}{\text{Value at control}} \times 100$$

Dead leaf ratio was estimated the length of dead leaf to

Table 1. Growth reduction and visual evaluation of seedling tested under saline-soil condition treated with 0.5% NaCl.

Variety	Reduction ratio (%)		Dead leaf ratio	Visual salinity score [†]
	Seedling height	Dry weight		
Dikwee	25.7±1.6	23.6±2.4	47.6±4.6	5.4±0.2
Gaw Diaw Bow	22.1±1.5	12.0±1.7	37.8±5.1	3.7±0.3
Getu	7.3±2.2	20.8±1.8	43.6±5.2	4.8±0.4
Mahsuri	25.7±1.6	42.2±2.4	76.2±6.1	9.0±0.1
Madhuka	22.9±0.9	37.6±2.8	68.9±9.5	7.6±0.3
Kuatic Putik	6.4±2.2	8.4±2.8	21.2±3.5	2.1±0.2
Kombili	15.2±1.4	13.0±3.6	38.8±4.1	3.6±0.2
Engatec	10.1±1.4	23.1±2.4	46.4±7.2	5.7±0.3
Cheriviruppu	16.1±1.9	8.5±2.7	35.3±2.5	3.3±0.3
Dular	19.8±2.7	39.2±5.0	68.2±7.1	7.5±0.4
Damodar	22.1±1.1	24.9±3.1	39.9±2.9	4.4±0.3
Dahanala	25.1±2.2	23.8±3.8	41.4±5.1	4.2±0.2
Xiangcho V	14.2±1.8	14.0±3.2	35.5±3.5	3.4±0.4
Kalarata	8.8±1.9	6.5±3.0	32.6±3.5	3.2±0.4
Annapuruna	25.7±1.9	20.2±3.2	36.6±5.2	3.3±0.3
Nabatat Asmer	23.5±2.0	19.5±3.2	28.6±3.5	1.9±0.2
Agami-M ₁	18.3±1.2	15.8±2.8	26.8±3.3	1.7±0.2
Giza 159	17.5±1.3	21.3±3.6	30.2±3.8	1.9±0.3
Giza 175	13.8±1.2	18.5±3.0	34.3±4.9	3.3±0.3
Giza 176	15.0±1.9	16.3±2.7	36.1±5.2	3.4±0.4
GZ 1368-S-5-4	10.9±2.0	12.5±0.9	24.8±2.4	1.4±0.3
GZ 2447-S-17	9.5±1.5	13.8±3.3	27.3±4.8	1.7±0.3
GZ 2611-1-6-4	11.3±1.8	15.8±2.9	29.3±5.8	1.4±0.3
GZ 4255-6-3	13.5±1.3	10.5±2.4	33.8±5.6	3.2±0.4
GZ 4565-S-10	15.3±1.5	23.2±3.5	27.5±4.4	1.8±0.4
Gyehwa 5	14.1±2.4	25.6±2.1	48.4±4.1	5.8±0.4
Gancheokbyeo	21.7±4.5	37.5±3.6	63.4±6.7	6.8±0.4
Dongjinbyeo	22.9±2.7	37.8±3.1	61.7±5.8	6.8±0.3
Seomjinbyeo	26.8±3.0	38.9±3.5	62.0±9.8	6.7±0.3
Sinseonchalbyeo	16.4±2.3	28.6±3.0	50.4±5.4	5.8±0.2
HP3319-2wx-6-3-1	16.8±3.5	14.6±2.9	23.0±3.5	1.3±0.1
Jangseongbyeo	9.5±2.3	37.0±2.1	74.7±7.2	8.8±0.1
Chilseongbyeo	21.2±3.0	38.2±3.3	79.5±5.7	8.6±0.2
Taebaegbyeo	17.8±2.6	40.9±3.9	82.2±3.9	8.4±0.2
LSD (5%)	3.0	4.2	7.5	
(1%)	4.0	5.6	10.0	

[†]Salt tolerance was evaluated by the standard investigation system of Rural Development Administration of Korea (RDA 1995) at 15 days after the treatment of 0.5% NaCl. 1, normal growth of seedling; 3, leaf tips discolored slightly, old leaves dry; 5, leaves discolored mostly, and growth inhibited; 7, leaves drooped mostly or dead partially, and growth stopped; 9, leaves dead or dry mostly.

viable leaf from each seedling. Correlation coefficients were calculated to determine the highest relationship between salinity score and these parameters. The tested varieties were classified into highly tolerant, ordinary tolerant and sensitive based on reduction of dry weight, dead leaf ratio and salinity score. Data analyses were performed using the SAS statistical software (V 6.12, SAS Institute Inc.). Averages were compared by Analysis of variance (ANOVA). The salinity tolerance of tested varieties was classified by average linkage cluster analysis using squared Euclidean distance for similarity measure.

Twenty-six varieties from IRRI were investigated for the same agronomic characteristics in the paddy field. Each variety was transplanted with spacing 30×15 cm with three replications, and heading date, culm length, and panicle length were investigated in 20 plants per replication. Nitrogen, phosphorous (P₂O₅) and potassium (K₂O) were applied at 110, 70 and 80 kg/ha, respectively. Nitrogen fertilizer was split-applied with urea as basal 50%, 25% at tillering stage and 25% at panicle initiation stage.

RESULTS AND DISCUSSION

Evaluation for salt tolerance

Thirty-four varieties were evaluated for salt tolerance at seedling stage (Table 1). Phenotypic variation was found among genotypes for salinity symptoms. The reduction in plant height showed the trends increased in taller varieties than shorter ones. The reduction of plant height is a index for estimation of salt tolerance (Gregorio and Senadhira, 1993; Lee *et al.*, 1996). However, the correlation between salinity score and reduction in plant height wasn't significant in this study (Fig. 1). The reason was regarded as the differ-

ences of salt-tolerant mechanism or early-growing potential according to varieties (Yeo and Flowers, 1984; Lee and Senadhira, 1998). The variation in dry weight was also found among varieties according to salinity score. Mahsuri, Madhuka, Dular, Seomjinbyeo, Gancheokbyeo, Dongjinbyeo, Jangseongbyeo, Chilseongbyeo, Taebagbyeo and GZ 4255-6-3 showed the large reduction in dry weight compared to the control, but Gaw Diaw Bow, Kuatic Putik, Cheriviruppu, Xiang Cho V, Kalarata, GZ 1368-S-5-4 and HP3319-2wx-6-3-1 showed the low reduction in dry weight. The reduction in dry weight was positively associated with salinity score under 0.5% NaCl treatment (Fig. 2). Most rice varieties were largely affected by saline condition in plant height and dry weight. Lee *et al.* (1996) reported that the relationships between the salinity score and the reduction ratio in dry weight and seedling height were linearly corre-

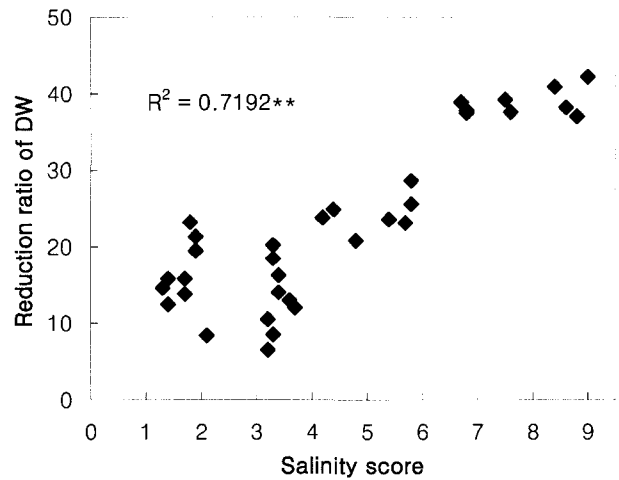


Fig. 2. Relationship between salinity score and reduction ratio of dry weight (DW) for salt tolerance in thirty-four rice varieties.

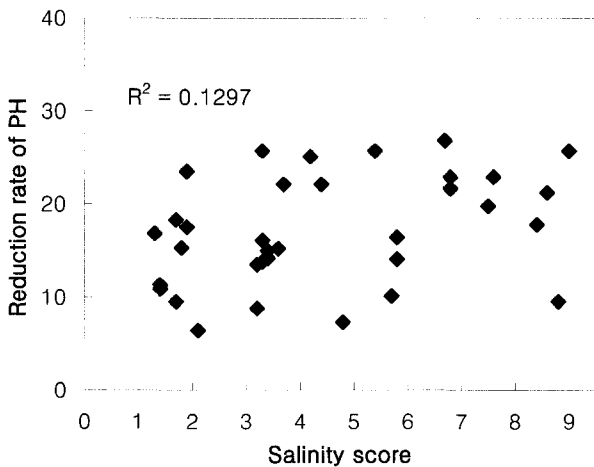


Fig. 1. Relationship between salinity score and reduction ratio of plant height (PH) for salt tolerance in thirty-four rice varieties.

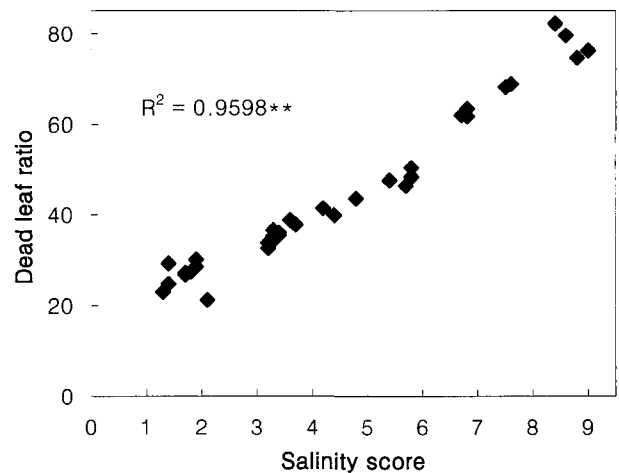


Fig. 3. Relationship between salinity score and dead leaf ratio for salt tolerance in thirty-four rice varieties.

lated at the seedling stage in japonica rice. Especially, the reduction ratio in dry weight is one of the general indices to estimate salt tolerance (Akita and Cabsulay, 1990; Gregorio and Senadhira, 1993). In this experiment, the reduction ratio in dry weight can be an index for salt.

The relationships between salinity score and dead leaf ratio also showed linearly significant correlations (Fig. 3). The cause of dead leaf is due to the excessive sodium uptake and osmotic imbalance (Akbar, 1975; Ponnampereuma, 1984; Munns, 1993). Dead leaf ratio at panicle formation stage is also important as parameter of salt tolerance (Choung *et al.*, 1995). In this experiment, the visual salinity score at the seedling stage showed the highest correlation coefficient with dead leaf ratio. This result showed that visual salinity score based on dead leaf ratio is a simple and rapid method for screening of salt tolerance at seedling stage in a rice breeding program. Recently, it had also been demonstrated that Na^+ - K^+ ratio in shoot, which are known as a important index of salt tolerance, was closely correlated with salt tolerance on the basis of salinity score at seedling stage (Gregorio and Senadhira, 1993; Lee *et al.*, 1996). However, this method was not adequate for a mass screening of breeding materials, because it requires a long time, much labor, and expenses for Na^+ - K^+ analysis. Accordingly, for the evaluation of salt tolerance, the visual salinity score based on dead leaf ratio can be used as a rapid and simple method for mass screen in the early generation of breeding populations.

Varietal classification for salt tolerance

The tested varieties were classified into three groups by visual salinity score, reduction ratio of dry weight, and dead leaf ratio (Fig. 4). Eighteen varieties, including Kuantik Putik, Agami M₁, Nabatat Asmer, HP3319-2wx-6-3-1, Giza 159, and Xiangcho V, were classified as the highly tolerant group (salinity scores of 1.3-3.7), and seven varieties, including Gaw Diaw Bow, Kombili, Gyehwa 5, and Sinseonchalbyeo,

fell into the tolerant group (salinity scores of 4.2-5.8), whereas nine varieties, including Seomjinbyeo, Gancheogbyeo, Madhukar, Taebaegbyeo, and Jangseongbyeo, were classified as sensitive (salinity scores of 6.7-9.0). The varieties in Group I could be subdivide into two groups, highly tolerant (Group I-1) and tolerant (Group I-2). Tested varieties were classified into three degrees basis on the visual salinity score (Table 2). Of Korean varieties, most Tongil varieties crossed between indica and japonica were susceptible to salinity except HP3319-2wx-6-3-1, and the japonica varieties, Gyehwa 5 and Sinseonchalbyeo were moderately

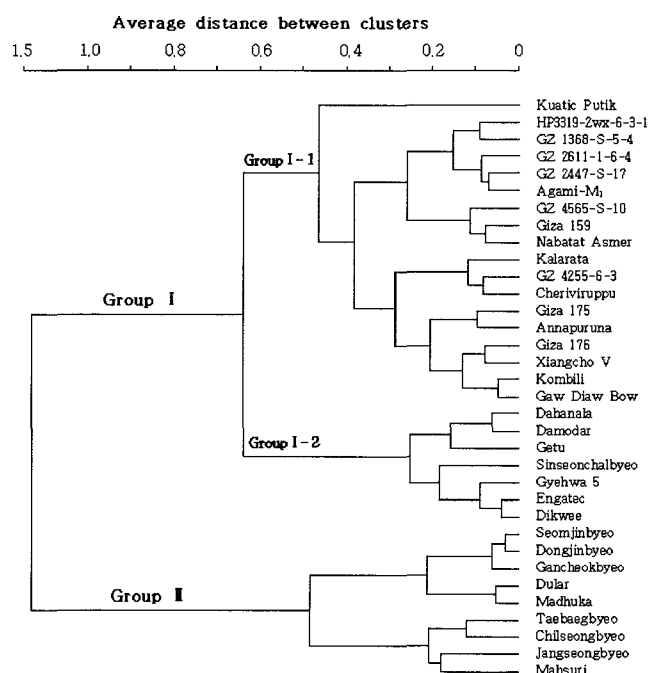


Fig. 4. Classification of rice varieties according to the reduction ratio of seedling height, reduction ratio of dry weight and salinity score under saline soil condition treated with 0.5% NaCl by average linkage cluster analysis (SAS statistical software; V 6.12). Group, tolerant (Group-, highly tolerant ; Group-, tolerant); Group , susceptible.

Table 2. Classification of tested materials for salt tolerance under saline-soil condition treated with 0.5% NaCl.

Degree of salt tolerance	Visual score (1-9) [†]	Material	Number of variety
Highly tolerant	1	Kuantik Putik, Nabatat Asmer, Agami-M ₁ , Giza 159, GZ1368-S-5-4, GZ2447-S-17, GZ2611-1-6-4, GZ4565-S-10, HP3319-2wx-6-3-1	9
	3	Cheriviruppu, Xiangcho V, Kalarata, Annapurna, Giza 175, Giza 176, GZ 4255-6-3	7
Tolerant	4	Gaw Diaw Bow, Kombili, Damodar, Dahanala	4
	5	Dikwee, Getu, Engatec, Gyehwa 5, Sinseonchalbyeo	5
Susceptible	7	Madhukar, Dular, Gancheogbyeo, Dongjinbyeo, Seomjinbyeo	5
	9	Mahsuri, Jangseongbyeo, Chilseongbyeo, Taebaegbyeo	4

[†]Salinity symptoms see Table 1.

tolerant under 0.5% NaCl. HP3319-2wx-6-3-1, a Tongil variety showed the highest tolerance to salt in Korean varieties, and Gyehwa 5, a japonica semi-dwarf variety was favorable as a female parent for introducing salt-tolerant genes of indica varieties.

Agronomic characteristics of rice varieties

The development of salt-tolerant variety is very difficult, because most rice varieties are sensitive to salt, the environmental effects were large, and the heritability of the trait was low in some varieties (Skriver and Mundy, 1990; Yeo *et al.*, 1990; Lee, 1995). To solve these was very important problem for developing the salt-tolerant variety. To select salt-tolerant genetic resources with good traits, twenty-six varieties were investigated for some agronomic characteristics following

the evaluation of salt tolerance at seedling stage under the Korean climate (Table 3). Most varieties introduced from foreign countries showed the undesirable plant type with tall culm and long-drooped leaf compared to the Korean varieties. Heading date was also later compared to the Korean varieties, and 10 varieties including Dikwee, Gaw Diaw Bow, Getu, Mahsuri, Madhuka, Kuatic Putik, Kombili, Engatec, Cheriviruppu and Damodar didn't exert a panicle until harvesting stage under the Korean climate because of different ecotype. Pokkali and Nona Bokra, indica varieties is well-known donors for salt-tolerant breeding, but it didn't also exert a panicle as the same reason (Lee and Senadhira, 1998). In most Giza lines (Egyptian varieties), heading date and plant type were similar to those of the Korean varieties. Six varieties Annapuruna, Dular, Xiangcho V, Giza 175, Giza 176, GZ 4255-6-3 and GZ 2447-S-17 had the similar

Table 3. Agronomic characteristics of breeding materials introduced for improving salt-tolerance under the Korean climate.

Varieties	Heading date	Culm length (cm)	Panicle length (cm)	No. of panicle/hill	Plant type (Source)
Kalarata	Sept. 22	115	28	14	Indica (IRRI)
Annapuruna	Aug. 8	65	24	13	Indica (IRRI)
Dikwee	—	(138)	—	(13)	Indica (IRRI)
Gaw Diaw Bow	—	(131)	—	(17)	Indica (IRRI)
Getu	—	(132)	—	(12)	Indica (IRRI)
Mahsuri	—	(128)	—	(12)	Indica (IRRI)
Madhuka	—	(158)	—	(12)	Indica (IRRI)
Kuatic Putik	—	(92)	—	(16)	Indica (IRRI)
Kombili	—	(142)	—	(9)	Indica (IRRI)
Engatec	—	(112)	—	(10)	Indica (IRRI)
Cheriviruppu	—	(165)	—	(12)	Indica (IRRI)
Damodar	—	(133)	—	(15)	Indica (IRRI)
Dahanala	Aug. 24	109	26	7	Indica (IRRI)
Dular	Aug. 14	89	24	13	Indica (IRRI)
Xiangcho V	Aug. 16	65	28	14	Indica (IRRI)
Nabatat Asmer	July 30	115	22	10	Japonica (Egypt)
Agami-M ₁	Sept. 2	97	21	8	Japonica (Egypt)
Giza 159	Aug. 30	99	22	8	Japonica (Egypt)
Giza 175	Aug. 10	78	23	15	Japonica (Egypt)
Giza 176	Sept. 5	78	21	8	Japonica (Egypt)
GZ 1368-S-5-4	Aug. 10	91	24	13	Japonica (Egypt)
GZ 2447-S-17	Aug. 9	77	22	10	Japonica (Egypt)
GZ 2611-1-6-4	Aug. 30	79	21	11	Japonica (Egypt)
GZ 4255-6-3	Aug. 6	71	20	12	Japonica (Egypt)
GZ 4565-S-10	Sept. 2	86	21	11	Japonica (Egypt)
Gyehwa 5	Aug. 15	73	19	14	Japonica (Korea)
Gancheokbyeo	Aug. 10	82	20	12	Japonica (Korea)
Dongjinbyeo	Aug. 17	87	20	12	Japonica (Korea)
Seomjinbyeo	Aug. 16	85	20	13	Japonica (Korea)
Sinseonchalbyeo	Aug. 11	86	21	11	Japonica (Korea)
HP 3319-2wx-6-3-1	Aug. 8	70	22	13	Jap/Ind (Korea)
Jangseongbyeo	Aug. 13	74	23	15	Jap/Ind (Korea)
Chilseongbyeo	Aug. 12	66	21	14	Jap/Ind (Korea)
Taebaegbyeo	Aug. 7	68	22	15	Jap/Ind (Korea)

Data were recored at Iksan, Korea. —: Panicle didn't exert until September, (): Plant height or tiller per hill.

heading date (6 to 16, August) and culm length (65-78 cm) to Korean varieties. These varieties can be used as mid-parents for salt tolerance of japonica rice in Korea.

ACKNOWLEDGEMENTS

This work was supported by the Biogreen 21 R&D program of Rural Development Administration, Republic of Korea (02-A-2).

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