



Exploration of relationships between physiological parameters and growth performance of rice (*Oryza sativa* L.) seedlings under salinity stress using multivariate analysis

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Abstract

Knowledge of relationships between physiological parameters and growth performance of seedlings and respective genotypic differences would permit selection of salt tolerance at early growth stages. The goals of this study were to investigate the relationships between physiological parameters and growth performance and quantify the respective genotypic differences using multivariate analysis. Plants of thirty-one genotypes were grown in sand tanks in a greenhouse and irrigated with Yoshida nutrient solution. Two salinity treatments were imposed at 0.9 dS m^{-1} (control) and 6.4 dS m^{-1} with sodium chloride and calcium chloride ($\sim 6:1$ molar ratio). Seedlings were sampled 34 days after planting (7th to 8th leaf stage). The characters of Na^+ , K^+ , Ca^{2+} , K-Na selectivity ($S_{\text{K,Na}}$) and Na-Ca selectivity ($S_{\text{Na,Ca}}$) were measured as physiological parameters. The characters of tiller number, leaf area, plant height and shoot dry weight were measured as growth performance. Under salinity stress, $S_{\text{K,Na}}$ increased whereas $S_{\text{Na,Ca}}$ decreased compared to the controls. Canonical correlation analysis indicates a strong relationship between physiological parameters and growth performance. Tiller number is a desirable parameter among the growth parameters analyzed to predict seedling growth under salinity stress. Genotypes grouped into four clusters based on ion contents and ion selectivity using Ward's minimum-variance cluster analysis. $S_{\text{K,Na}}$ and shoot Na^+ content contributed the most to the cluster formation. Similarly, genotypes grouped into four clusters based on growth performance. Genotypes were classified into three categories based on ion cluster rankings: Category 1 with high $S_{\text{K,Na}}$ and low shoot Na^+ content; Category 2 with intermediate $S_{\text{K,Na}}$ and shoot Na^+ content; Category 3 with low $S_{\text{K,Na}}$ and high shoot Na^+ content. The classification of the genotypes into Categories 1 and 3 based on their high or low $S_{\text{K,Na}}$ was generally consistent with their growth performance under salt stress. In contrast, ion selectivity was a less dominant mechanism controlling salt tolerance in Category 2 with intermediate $S_{\text{K,Na}}$. It was concluded that ion selectivity was a relatively dominant mechanism controlling salt tolerance among rice genotypes although multiple mechanisms may be involved under moderate salt stress. The results also provide the first example of the effectiveness of cluster analysis for physiological responses to salinity stress.

Abbreviations: $S_{\text{K,Na}}$ – K-Na selectivity; $S_{\text{Na,Ca}}$ – Na-Ca selectivity; DAP – days after planting.

Introduction

Rice seedlings are very sensitive to salinity. Salinity in rice paddy fields affects seedling establishment and reduces plant density, especially in the regions where

direct water-seeded cultural system is dominant (Grattan et al., 2002). Growth parameters such as seedling biomass, leaf area, tiller number, and plant height are reduced by salinity (Flowers and Yeo, 1981; Lutts et al., 1995; Zeng et al., 2000).

Knowledge of physiological mechanisms controlling salt tolerance at seedling stages is important for

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prediction of agronomic performance of rice seedlings under salt stress. There are three types of salinity effects on plants, osmotic stress, specific ion toxicity, and nutritional imbalance. Generally, osmotic stress is the primary effect in short-term salinity treatment while specific ion toxicity and nutritional imbalance are the main effects in long-term salinity treatment (Munns, 2002). For long-term experiments, high salinity in the root zone increased sodium content in rice shoots (Flowers and Yeo, 1981), affected potassium and phosphate nutrition, and further reduced growth in rice (Aslam et al., 1996; Khan et al., 1992; Qadar, 1995). The concentration of Na^+ and K^+/Na^+ in rice plants was correlated with seedling growth and grain yield under salt stress (Flowers and Yeo, 1981; Khatun et al., 1995; Lutts et al., 1995). The reports on the interactive effects between Na^+ and Ca^{2+} were inconsistent. Yeo and Flowers (1985) observed a lack of response in rice growth and shoot ion content to a change of Na/Ca (in a range between 5 and 100) in growth medium. In contrast, ameliorating effects of external Ca^{2+} on plant growth were observed in rice (Aslam et al., 2003). In a previous study of rice salt tolerance, K/Na selectivity increased while Na/Ca selectivity decreased with increases of Na^+ in growth medium (Zeng et al., 2003). In that study, the change of Na/Ca selectivity at panicle initiation stage was significantly correlated with the scores among rice genotypes for grain yield under salt stress. These results indicate that the preferential uptake of K^+ and Ca^{2+} could be measured as selection criterion for salt tolerance.

Physiological parameters, e.g., ion contents, are suggested as selection criteria for salt tolerance (Yeo and Flowers, 1986). The related genetic resources are abundant with a large number of breeding lines developed in salt tolerance breeding programs worldwide (Gregorio et al., 2002; Quijano-Guerta and Kirk, 2002). Although genotypic variation exists for salt tolerance in terms of physiological characters (Asch et al., 2000; Yeo et al., 1990), rice germplasm has yet to be classified in a way that will allow quantification of these genotypic differences.

Traditionally, rice cultivars or accessions are classified into two major categories, japonica and indica subspecies, based on the distinguishable morphological characters between the two categories. The simple classification into the two subspecies does not always provide enough guidance to rice breeders to use germplasm in dealing with complicated traits such as salt tolerance. Although salt tolerance may be higher

in indica rice than that in japonica rice (Lee et al., 2003), genotypic comparison of salt tolerance beyond the subspecies level is necessary for practical purposes because traits from either japonica or indica rice could dominate in commercial cultivars at a specific region due to climate or consumer preference. Furthermore, salt tolerance often has to be assessed among breeding lines that are derived from multiple crosses between indica and japonica rice and the parentage of these breeding lines is usually difficult to trace.

The quantification of genotypic differences for salt tolerance is difficult because of the continuous variation among genotypes when physiological and growth parameters are evaluated. Rankings of genotypes based on the artificial ranges of score boundaries in traditional breeding are often inaccurate and cumbersome when multiple characters and large number of genotypes are screened. The difficulty could be exacerbated when the ranges of genotypic differences are relatively small, especially for some physiological characters such as K^+ and Ca^{2+} contents (Zeng et al., 2003). Cluster analysis was suggested as an effective method for comparing cultivars (Jolliffe et al., 1989). Cluster methods have been used to characterize plant germplasm and group genotypes into homogeneous clusters when trials were conducted under non-saline conditions at different locations (Crossa et al., 1995; Franco et al., 1999, 2003). Traditional Philippine upland rice cultivars have been classified by clusters analysis on growth characters under non-saline conditions (Schlösser et al., 2000). The cluster method was effective in screening for salt tolerance among the genotypes of potato (Khrais et al., 1998) and rice (Zeng et al., 2002) when agronomic parameters were analyzed. Using cluster methods, genotypes can be assessed by multiple parameters simultaneously. There is no need to set scoring boundaries because genotypes group on the basis of variances of the characters analyzed.

This study was designed to investigate the relationships between ion contents and growth performance and quantify the respective genotypic differences using multivariate analysis.

Materials and methods

Plant materials

Seeds of thirty-one rice (*Oryza sativa* L.) genotypes were received from three locations: Field Crop Research Institute at Giza, Egypt; International Rice Research Institute (IRRI), Philippines; and California Rice Experiment Station at Biggs, CA. This collection of genotypes represents germplasm originating in Egypt, Philippines, India, and USA. These genotypes consist of breeding lines, commercial cultivars, and two salt-tolerant landraces, 'Pokkali' and 'Nona Bokra'.

Plant culture

The trial was conducted in a greenhouse at Riverside, CA [33°58'24" N latitude, 117°19'12" W longitude] during June and August 2001. The plants were cultured in tanks (122 × 61 × 46 cm deep) filled with sand irrigated with nutrient solution (Yoshida et al., 1971). The irrigation and nutrient solutions were maintained as previously described (Zeng et al., 2003). Seeds were planted in two rows per genotype with eight genotypes per tank. The rows were spaced 6 to 7 cm apart with 15 seeds per row. Water depth was controlled at 6 to 8 cm during the growing season. Air temperature ranged from 23 to 37 °C during the day and 17 to 23 °C during the night. Humidity ranged from 40 to 60%. Photosynthetically active radiation averaged 577 $\mu\text{mol m}^{-2} \text{s}^{-1}$ with a minimum of 35 and a maximum 1300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ during the day. The experiment was designed as a randomized block in a split-plot with three replicates. Salt level was a main plot factor and genotype was a sub-plot factor.

Salinity treatments

NaCl (57 mM) and CaCl₂ (10 mM) (~6:1 molar concentration) were added to the nutrient solutions on the sixth day after planting. Salinity was maintained continuously until the harvest. Over the duration of stress, the salt level was maintained at an electrical conductivity (ECw) of 6.4 dS m⁻¹. The control, i.e., nutrient solution without added salts, was maintained at 0.9 dS m⁻¹ during the trial.

Measurements of growth parameters

Twelve seedlings of each genotype from each replicate were randomly sampled at 34 days after planting, i.e.,

7th to 8th leaf stage. Four growth parameters, tiller number, plant height, leaf area, and shoot dry weight were measured from the collected seedlings. Tiller number was measured as the number of primary tillers per plant. Plant height was measured from the base of the shoot to the ligule of the youngest fully extended leaf on the main stem. Leaf area was measured as the total leaf area per plant using a LI-3100 Area Meter (LI-COR, Inc., Lincoln, NE)¹. The same plants were washed with deionized water, and dried in a forced-air oven (70 °C) until weights became constant. The samples were then measured for shoot dry weight, i.e., above ground biomass. Data were averaged over the sub-samples.

Ion analysis

The same seedlings collected for growth measurements were used for ion analysis. Shoots, i.e., above ground biomass, were weighed and ground to pass a 60-mesh screen. Dead leaves were also included to the shoots in order to accurately estimate ion selectivity. The concentrations of Na⁺, K⁺ and Ca²⁺ in shoot were determined on nitric-perchloric acid digests by inductively coupled plasma optical emission spectrometry (ICP atomic emission spectrometer, Perkin-Elmer Co., Norwalk, CT, USA)¹. The K-Na selectivity was calculated using the equation described by Pitman (1976):

$$S_{K,Na} = (\text{K content}/[\text{K}] \text{ medium}) / (\text{Na content}/[\text{Na}] \text{ medium}),$$

where $S_{K,Na}$ represents K-Na selectivity; K content and Na content represent the concentrations (mmoles kg⁻¹ dry wt.) of K⁺ and Na⁺ in shoot. Na-Ca selectivity ($S_{Na,Ca}$) was calculated using Gapon selectivity constant (K_g) (Sposito, 1981):

$$K_g = (E_{Na}^* (A_{Ca}) 0.5) / (E_{Ca}^* A_{Na}),$$

where E represents the equivalent fraction of a given cation and A represents the activity of the ion in solution. In this way, K_g relates the equivalent fractions of the exchange ions to the activities of the ions in solution.

Statistical analysis

The variables analyzed in the study were divided into two sets, physiological parameters (i.e., Na⁺, K⁺, Ca²⁺, K-Na selectivity, and Na-Ca selectivity) and growth performance (i.e., tiller number, leaf area,

Table 1. The indexes of relative salt tolerance and cluster rankings

Genotypes	Na ⁺	K ⁺	Ca ²⁺	S _{K,Na} ^a	S _{Na,Ca} ^b	Tiller no.	Leaf Area	Plant height	Shoot wt	Clusters ^c ranking (ion)	Clusters ^d ranking (growth)
IR61920-3B-15-2-2	4.71	0.88	1.09	41.8	0.08	0.64	0.82	0.98	0.79	1	4
IR63352-AC202	5.98	0.80	1.39	25.4	0.09	0.94	1.61	1.05	1.65	2	1
Daeyabyeo	5.37	0.82	1.38	25.5	0.08	0.65	0.95	0.97	0.95	2	2
GZ5385-29-3-3	7.61	0.81	1.43	23.6	0.08	0.75	0.73	1.02	1.08	2	2
GZ5121-5-2-1	5.40	0.74	1.32	25.5	0.08	0.80	0.80	0.91	0.84	2	2
Nona Bokra	7.04	0.89	1.56	24.0	0.09	0.85	0.75	0.89	0.68	2	2
IR29	5.47	0.85	1.11	29.0	0.09	1.04	0.96	0.89	0.86	2	2
IR63731-1-1-4-3-2	6.60	0.97	1.15	26.4	0.11	0.90	0.99	1.01	0.96	2	2
AC26	7.01	0.91	1.58	24.8	0.09	0.88	0.71	1.01	0.75	2	3
GZ5310-20-3-2	7.56	0.89	1.71	22.4	0.09	1.11	0.80	1.00	0.77	2	3
Agami	6.82	0.82	1.39	24.2	0.10	1.05	0.75	0.96	0.72	2	3
GZ1368-5-4	6.24	0.77	1.24	24.3	0.10	0.95	0.62	0.84	0.57	2	3
GZ178	6.75	0.92	1.17	25.8	0.12	0.60	0.78	0.96	0.62	2	4
S-102	8.16	0.90	1.51	20.7	0.11	0.85	0.95	0.94	0.87	3	2
Pokkali	6.69	0.61	1.09	16.8	0.12	0.79	1.08	0.99	0.89	3	2
IR4630-22-2-2-5-1-3	8.33	0.69	1.10	17.3	0.18	0.77	0.87	0.91	0.80	3	2
IR50184-3B18-2B-1	7.28	0.73	1.22	20.4	0.12	1.02	1.12	0.96	1.01	3	2
IR51490-AC10	7.74	0.77	1.23	18.9	0.13	0.72	1.20	1.02	1.17	3	2
GZ5385-29-3-2	9.46	0.85	1.90	17.5	0.10	0.88	0.55	0.91	0.54	3	3
GZ5310-20-3-3	8.44	0.74	1.39	15.8	0.12	0.68	0.73	0.92	0.68	3	4
GZ177	9.71	0.84	1.67	17.7	0.12	0.42	0.68	1.12	0.61	3	4
M-205	9.98	0.83	1.58	15.7	0.13	0.77	0.55	0.93	0.64	3	4
GZ5385-3-2-3-1	8.74	0.80	1.17	17.3	0.15	0.71	0.57	0.98	0.58	3	4
GZ5310-20-2-1	8.72	0.79	1.30	17.0	0.14	0.78	0.66	1.02	0.53	3	4
Sakha101	11.25	0.75	1.88	12.6	0.12	1.00	0.62	1.00	0.76	4	3
IR70074-AC14	11.7	0.93	1.85	14.7	0.13	1.08	0.72	0.97	0.74	4	3
IR70074-AC1	12.3	0.77	2.22	12.6	0.11	0.89	0.40	0.91	0.38	4	3
M-104	16.1	0.93	1.69	10.9	0.19	0.49	0.44	0.98	0.40	4	4
GZ5291-7-1-2	10.8	0.82	1.68	14.6	0.13	0.57	0.47	0.93	0.42	4	4
M-202	10.4	0.59	1.30	10.5	0.16	0.56	0.65	0.99	0.53	4	4
L-205	10.4	0.80	1.58	14.2	0.13	0.67	0.66	0.89	0.70	4	4

^aS_{K,Na}, K-Na selectivity.

^bS_{Na,Ca}, Na-Ca selectivity.

^cCluster rankings were obtained from the cluster means of K-Na selectivity from the highest to the lowest and the cluster means of Na content from the lowest to the highest.

^dCluster rankings were obtained from the cluster means of tiller number, leaf area, and shoot dry weight from the highest to the lowest.

plant height, and shoot dry weight). The relationships between ion parameters and growth performance were analyzed by canonical correlations. Canonical correlation analysis was performed using CANCELL procedures (SAS Institute, 1994).

In order to remove the arbitrary effects due to different scales in the measurements of different parameters analyzed, all the data were converted to relative salt tolerance, i.e., the indexes derived from the observations of each character under salt stress divided

by the mean of the same character under non-saline condition, before clustering.

The methods for cluster analysis have been described in previous report (Zeng et al., 2002). Cluster groups were obtained based on Ward's minimum-variance cluster analysis on the means of the relative salt tolerance indexes for five physiological parameters (Na⁺, K⁺, Ca²⁺, S_{K,Na} and S_{Na,Ca}) and four growth parameters (tiller number, leaf area, plant height and shoot dry weight) at seedling stage, separately. The distance between two clusters was calcu-

Table 2. Canonical correlation between ion parameters and growth performance

Canonical pair variate	Canonical R	Canonical R ²	Contributed variation (%)	Significance (<i>P</i> > <i>F</i>)
1	0.55	0.31	0.56	0.000
2	0.45	0.21	0.33	0.000
3	0.25	0.06	0.08	0.020
4	0.15	0.02	0.03	0.130

lated as the ANOVA sum of squares between the two clusters in all the parameters analyzed. The procedures are described in the SAS User's Guide (SAS Institute, 1994). The cluster groups were identified in dendrograms. The number of cluster groups was determined by calculating the pseudo *F* and *t*² that reached a local maximum.

The contribution of each parameter to the cluster formation was calculated based on pseudo-*F* values, i.e., the ratios of between-cluster sum of squares and within-cluster sum of squares (Schlösser et al., 2000). The cluster rankings were obtained based on the most important parameters contributing to the cluster formation.

The genotypes were further classified into different categories based on the cluster rankings of physiological parameters. The distances between the categories were measured by Mahalanobis distance. Mahalanobis distance was calculated using CANDISC procedures in canonical discriminant analysis (SAS Institute, 1994). The significance of non-nullity for each distance was determined by *F*-tests.

Results

All the data were analyzed using relative salt tolerance indexes. In all genotypes, the indexes of K⁺ content and Na-Ca selectivity were less than 1 whereas those of Na⁺ content, Ca²⁺ content, and K-Na selectivity were greater than 1 (Table 1). The indexes of all growth parameters were smaller than 1 in most genotypes. There are five genotypes, IR63352-AC202, GZ5385-29-3-3, IR63731-1-1-4-3-2, IR50184-3B18-2B-1, and IR51490-AC10, are tolerant under moderate salt level. The index of shoot dry weight in these genotypes was either greater than 1 or close to 1. The extreme index (i.e., 1.65) of shoot dry weight in IR63352-AC202 was considered as an experiment error because this genotype was only moderately tol-

Table 3. Coefficients of canonical correlation

Variables	C1 ^a	C2 ^b
Ion parameters (set 1)		
Na ⁺	-1.46	0.28
K ⁺	-0.07	0.80
Ca ²⁺	0.22	0.82
S _{K,Na}	0.06	0.61
S _{Na,Ca}	-0.35	0.73
Growth performance (set 2)		
Tiller no.	0.71	0.24
Leaf area	-0.01	1.91
Plant height	-0.39	-0.00
Shoot wt	0.61	-2.06

^a C1, coefficients of canonical correlation for the first canonical variate pair.

^b C2, coefficients of canonical correlation for the second canonical variate pair.

erant in previous trials (unpublished data). There were a few other genotypes, IR29, GZ5310-20-3-2, GZ177, Sakha101, and IR70074-AC14, which were not affected in one to two growth parameters. However, these genotypes were considered as sensitive under moderate salinity since their indexes of shoot dry weight were less than 1.

Canonical analysis showed that the first and the second canonical variate pairs were highly significant (*P* < 0.000) and contributed most of the variations, 55% and 45%, respectively, between physiological parameters and growth performance (Table 2). For the first canonical variate pair, tiller number and shoot dry weight were the main factors with canonical correlation coefficients, 0.71 and 0.61, respectively (Table 3). Among ion parameters, Na⁺ was the main factor in the first pair with highest canonical correlation coefficient (-1.46, Table 3). For the second canonical variate pair, all ion parameters except Na⁺ affected growth performance with most effects on shoot dry weight and leaf area (Table 3).

Table 4. Cluster means of indexes in ion parameters

Cluster rankings	Genotype (number)	$S_{K,Na}$ ^a pF = 82 ^c	Na^+ pF = 20	$S_{Na,Ca}$ ^b pF = 4.4	Ca^{2+} pF = 1.0	K^+ pF = 0.0
1	1	41.8	4.71	0.08	1.09	0.88
2	12	25.1	6.49	0.09	1.37	0.86
3	11	17.7	8.48	0.13	1.38	0.78
4	7	12.9	11.84	0.15	1.74	0.80

^a $S_{K,Na}$, K-Na selectivity.

^b $S_{Na,Ca}$, Na-Ca selectivity.

^cpF, pseudo-F values (ratio of between-cluster sum of squares and within-cluster sum of squares).

Genotypes grouped into four clusters based on physiological parameters using Ward's minimum-variance cluster analysis (Table 4). The pseudo-F tests showed that Ca^{2+} and K^+ contents contributed the least whereas K-Na selectivity and Na^+ content contributed the most to the formation of clusters. The clusters were ranked based on the indexes of K-Na selectivity from the highest to the lowest and the indexes of Na^+ content from the lowest to the highest. Similarly, genotypes grouped into four clusters based on growth parameters using Ward's minimum-variance cluster analysis (Table 5). The pseudo-F tests showed that all growth parameters except plant height contributed to the formation of clusters. The clusters were ranked based on the averages of the growth parameters, tiller number, leaf area and shoot dry weight, from the highest to the lowest.

Genotypes were classified into three categories based on K-Na selectivity and Na^+ content: Category 1 with high K-Na selectivity, and low Na^+ content; Category 2 with intermediate ion selectivity and Na^+ content; and Category 3 with low K-Na selectivity and high Na^+ content (Table 6). Highly significant ($P < 0.001$) non-nullity was identified for each Mahalanobis distance between categories (Table 6). The classification based on ion parameters was generally consistent with their growth performance (Table 6). In Category 1, most genotypes were ranked either as 'good' or 'intermediate' except IR61920-3B-15-2-2 and GZ178 ranked as 'poor' in terms of growth parameters. The cause of this inconsistency is unknown. In Category 3, genotypes were ranked either as 'poor' or 'intermediate' with none as 'good' in terms of growth parameters.

Unexpectedly, most genotypes in Category 2 were ranked either as 'good' or 'poor' with only one as 'intermediate' in terms of growth parameters.

Discussion

This trial was conducted to evaluate a subset of rice germplasm for salt tolerance and classify the genotypes based on physiological response to salinity. Compared with earlier trials conducted at the same location to evaluate salt tolerance of twelve rice genotypes based on seedling growth parameters (Zeng et al., 2002), the rankings of the same genotypes for salt tolerance using the cluster method were generally consistent across different trials. Only one genotype, GZ5291-7-1-2, that we ranked as tolerant in earlier trials, ranked as 'poor' for growth performance in this trial. Compared with another earlier report on evaluating salt tolerance among twelve rice genotypes based on physiological parameters (Zeng et al., 2003), only one genotype, AC26, showed extreme inconsistency across different trials. This genotype was ranked as high Na^+ content in earlier trial, but ranked as low Na^+ content in this trial. These two outliers, GZ5291-7-1-2 and AC26, were considered as experimental errors during the trials. Overall, the results of this trial are consistent with our earlier evaluation trials conducted in greenhouses during different growing seasons.

The strength of relationships between physiological parameters and growth performance was analyzed using canonical correlations in multivariate analysis. Compared with univariate analysis that only analyzes simple correlations between two variables at a time, all variable pairs can be analyzed simultaneously using canonical correlations. In this study, Na^+ was identified as the most significant parameter affecting growth performance in the first multivariate pair. The negative value of the coefficient for Na^+ indicates that the genotypes with less shoot Na^+ generally perform better under salinity stress. This result is consistent with previous findings based on simple correlations in uni-

Table 5. Cluster means of the indexes in growth parameters

Cluster rankings	Genotype (number)	Tillers per plant pF = 35 ^a	Leaf area pF = 18	Shoot dry wt pF = 13	Plant height pF = 1	Average	Growth performance
1	1	0.94	1.61	1.66	1.05	1.40	Good
2	9	0.83	0.95	0.92	0.96	0.90	Good
3	5	0.98	0.65	0.65	0.95	0.76	Intermediate
4	16	0.63	0.64	0.59	0.97	0.62	Poor

^apF, pseudo-F values (ratio of between-cluster sum of squares and within-cluster sum of squares).

Table 6. Classification of rice genotypes based on ion parameters and their growth performance under salinity

Categories	Cluster Ranking	Mahalanobis distance ^b	K-Na selectivity	Na content	Growth performance ^c		
					Good	Intermediate	Poor
					Number of genotypes		
1	1, 2	6.73*** ^d	High	Low	7	4	2
2	3	7.27***	Intermediate	Intermediate	5	1	5
3	4	24.1***	Low	High	0	3	4

^aThe categories were classified based on cluster rankings of ion parameters.

^bMahalanobis distance between categories was calculated based on K-Na selectivity and Na⁺.

^cGrowth performance under salinity was defined based on the cluster rankings of growth parameter: good – cluster ranking 1 and 2; intermediate – cluster ranking 3; poor – cluster ranking 4.

^dThe values in this column represent the distances between Categories 1 and 2, Categories 2 and 3, and Categories 1 and 3, respectively.

***Significant at $P = 0.001$.

variate analysis (Flowers and Yeo, 1981; Lutts et al., 1995). The rest of the physiological parameters, K⁺, Ca²⁺, K-Na selectivity, and Na-Ca selectivity, were identified with similar effects on growth performance in the second multivariate pair. Shoot dry weight and tiller number were the main growth factors affected by ions in the first multivariate pair (Table 3). In contrast, more complicated relationships between ion parameters and growth performance were identified in the second multivariate pair. Negative relationships between K⁺ and shoot dry weight and between K-Na selectivity and shoot dry weight (Table 3) were not consistent with the previous findings by univariate analysis (Zeng et al. 2003). This complexity may be due to confounding effects of leaf area, transpiration, and ion uptake on biomass accumulation. Therefore, tiller number is a more desirable parameter than the others analyzed to predict seedling growth under salinity stress.

Standardization of data, i.e., the means of respective character divided by their standard errors, have been used when different types of responses are involved in cluster formation (Jolliffe et al., 1989). It would be worthwhile to mention this type of data treatment was not appropriate for relative salt tolerance

indexes because genotypes failed to group into clusters with double coded data. Instead, the two types of measurements, physiological parameters and growth parameters, were analyzed separately during the cluster analysis to avoid the substantial difference in error variances between the two different measurements.

Two physiological parameters, K-Na selectivity and Na⁺ content, were identified as significant contributors for cluster formation. This is because of the relatively wide ranges of genotypic differences and homogeneity of the variances for the characters that were the basis for grouping genotypes into homogeneous clusters. The general consistency between physiological response and seedling growth performance under salt stress in Category 1 and Category 3 indicates the importance of K-Na selectivity in controlling salt tolerance of rice seedlings. The results suggest the effectiveness of K-Na selectivity as a criterion in classification of rice germplasm for salt tolerance.

Salt tolerance may be controlled by diverse mechanisms among genotypes under moderate salt stress. Salt tolerance may be influenced by discontinuous distribution of ions within or among leaves (Yeo and Flowers, 1986) or even within cells (Gorham and

Johns, 2002). Partitioning of ions between old and young leaves or between the symplast and apoplast significantly contributes to salt tolerance in rice (Yeo et al., 1990). Direct apoplastic leakage (Yeo et al., 1987) or growth vigor (Yeo et al., 1990) may cause variations of shoot K^+ and Na^+ contents among genotypes. Salt tolerance was different among genotypes with similar shoot Na^+ concentrations at whole plant level (Zeng et al., 2003). 'Intermediate' growth response among genotypes in Category 2 with intermediate ion selectivity was not observed as expected. Instead, seedling growth responses were either 'good' or 'poor' among the genotypes in this category. It is obvious that salt tolerance among the genotypes of Category 2 is more influenced by mechanisms other than ion selectivity. It was concluded that ion selectivity was the relatively dominant mechanism controlling salt tolerance in rice. Other mechanisms were important only when ion selectivity was intermediate. These results clearly indicate that the complexity of multiple mechanisms could only be simplified when the mechanisms were analyzed among the classified genotypes. Therefore, the classification of genotypes, i.e., the 'categories', allows us to identify the mechanisms controlling salt tolerance by analyzing the relationships between physiological parameters and growth performance between the classifications.

Salinity effects on plants were easily modulated by environmental factors such humidity and temperature (Flowers and Yeo, 1981). It would be very difficult to determine critical parameters, especially the interrelationships between the physiological parameters and growth performance, at field conditions. In this study, the relationships between ion parameters and growth performance were analyzed under controlled conditions. The results provide the first evidence that cluster analysis is an effective method in classification of rice germplasm for their physiological responses to salinity. There are alternative approaches to analyze the relationships between physiological parameters and agronomic performance under salinity stress. For example, Zeng et al. (2004) have classified rice germplasm using microsatellite markers and identified significant differences in ion contents and agronomic performance between the classifications.

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Endnote

¹Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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