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 (SK\(_{Na}\)) and Na-Ca selectivity (S\(_{Na,Ca}\)) 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\(_{K,Na}\) increased whereas S\(_{Na,Ca}\) 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. SK\(_{Na}\) 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 SK\(_{Na}\) and low shoot Na\(^+\) content; Category 2 with intermediate SK\(_{Na}\) and shoot Na\(^+\) content; Category 3 with low SK\(_{Na}\) and high shoot Na\(^+\) content. The classification of the genotypes into Categories 1 and 3 based on their high or low SK\(_{Na}\) 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 SK\(_{Na}\). 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: SK\(_{Na}\) – K-Na selectivity; S\(_{Na,Ca}\) – 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 (Grat-tan 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
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.