

Morphological, physiological and biochemical evaluation of IR64 mutant lines under saline and normal conditions

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Salinity is one of the most important environmental hazards to rice production. Nearly 20% of the world's cultivated area and about half of the world's irrigated lands are currently affected by salinity. Soil salinity affects plant growth and development by way of osmotic stress, ion toxicity, and nutrient imbalances. Since rice is salt-sensitive and its growth and yield can drastically be reduced by salt stress, identifying the key mechanisms and genes involved in salinity tolerance will help speed the breeding efforts to develop salt-tolerant varieties.

Materials and methods

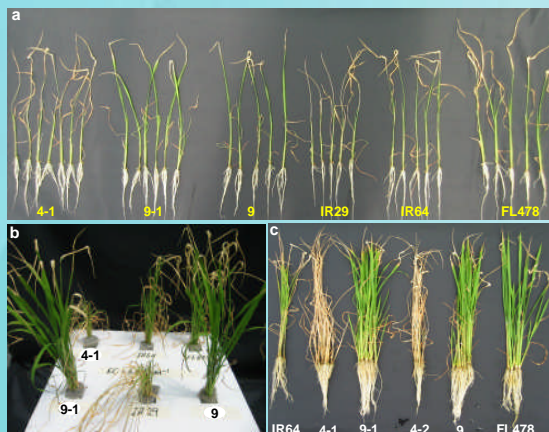
About 5,000 IR64 mutant lines were screened for seedling-stage salinity tolerance to identify mutants with altered responses compared with IR64 parent. Entries were scored based on visual symptoms using IRR's standard evaluation system (SES) scores. Putative sensitive and tolerant individual seedlings were rescued and advanced to M7 in the greenhouse.

Twenty-eight selected IR64 mutant lines with better tolerance, or higher sensitivity to salt stress, along with three check varieties; IR29 (sensitive), IR64 (parent, intermediate), and FL478 (highly tolerant), were evaluated for salinity tolerance using hydroponics under controlled conditions (29/21 °C day/night temperature and ~70% RH) in a RCBD experiment with four replications. Pre-germinated seeds were sown on styrofoam floats with a net bottom suspended on trays filled with distilled water. Salt stress was imposed 3 d after germination by adding NaCl to an EC of 12 dS m⁻¹ in Yoshida nutrient solution. The pH of the nutrient solution was adjusted daily to ~5.0 and culture solutions were changed once a week.

Results

Physiological responses of selected mutants to salt stress

Significant differences between control and salt stress treatments were observed for all traits studied, except for root length, chlorophyll a/b, and root soluble sugars. We identified eight lines with altered response to salinity stress: five with higher tolerance (# 9, 15, 19, 25, 27) and three with greater sensitivity (# 1, 3, 4) relative to IR64. Sensitive and tolerant IR64 mutant lines showed significant differences in the measured characteristics when compared to the parental line IR64. Sensitive mutants had higher SES, Na-K ratio, Na⁺ translocation, lower survival rate, SPAD reading, biomass, leaf area, total and osmotic leaf water potentials, plant height, and carbohydrate content in comparison with IR64. In contrast, the tolerant lines showed lower SES, Na-K ratio, Na⁺ translocation, higher survival rate, SPAD reading, biomass, leaf area, total and osmotic potentials, plant height, and carbohydrate content compared with IR64.



Comparison between most sensitive (4-1, 4-2) and tolerant (9, 9-1) IR64 mutant lines at different EC levels. a & b) 1 wk at EC=24 dS m⁻¹; c) 3 wk at EC=18 dS m⁻¹.

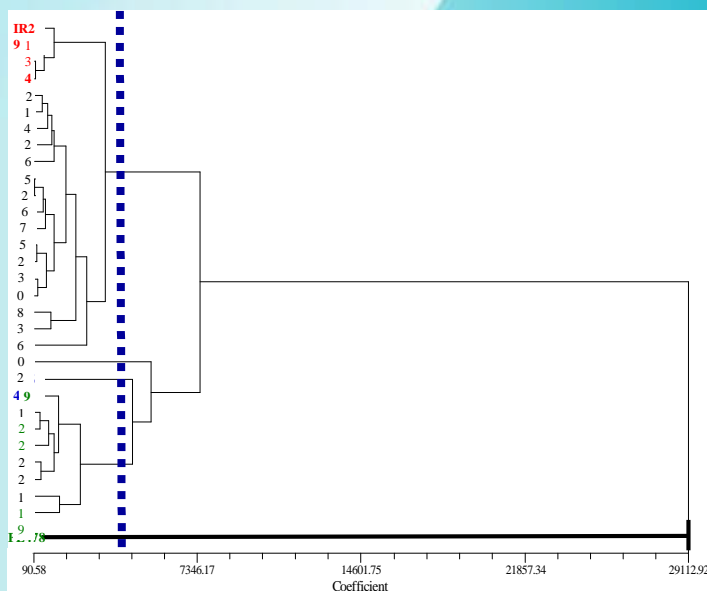
Clear differences between sensitive and tolerant lines were observed under salt stress in all measured characteristics, except for root-shoot ratio, chlorophyll a-b ratio, and root Na⁺ content. Sensitive lines showed higher values of SES scores, Na⁺ uptake, Na-K, and osmotic potential but lower survival rate, SPAD readings, chlorophyll content, and biomass compared with tolerant lines. Sensitive lines also showed higher root to shoot Na⁺ translocation and they seemed to absorb more Na⁺ and transport higher amounts to the shoot than the tolerant mutants. Conversely, tolerant mutants seemed to absorb less Na⁺, retain most of it in the roots and maintaining higher growth rate. Their uptake of K⁺ was also higher, resulting in lower Na-K ratio. FL478 showed the best performance under salinity stress, with lowest SES scores, Na-K ratio and Na translocation, while IR29 and a selected mutant line (#4), were identified as the most sensitive ones.

Phenotypic variability in rice mutant lines under salt stress

Correlation analysis showed that under salt stress, SES scores had significant correlations with all traits studied, except with root length, root/shoot, and root Na⁺ content. SES had a strong and significant correlation with survival rate (r=0.87). Both SES and survival rate correlated significantly with leaf area (r=0.74 & 0.70), plant height (r=0.73 & 0.75), osmotic potential (r=0.72 & 0.71), and chlorophyll a (r=0.71 & 0.64). Chlorophyll a had a bigger effect on SES and survival rate (r=0.71) than chlorophyll b (r=0.58). Interestingly, shoot Na⁺, K⁺, and Na-K ratio showed stronger correlations (r=0.52, 0.58, & 0.56) with SES than Na⁺, K⁺, and Na-K ratio in the roots (r=0.14, 0.40, & 0.15). Similarly, shoot carbohydrate content showed better correlation (r=0.59) with SES than with root carbohydrate (r=0.41). These results suggest that lower concentrations of Na⁺, K⁺, and Na-K ratio, and higher soluble carbohydrates in shoots are more important than their contents in the roots for salinity tolerance and plant survival.

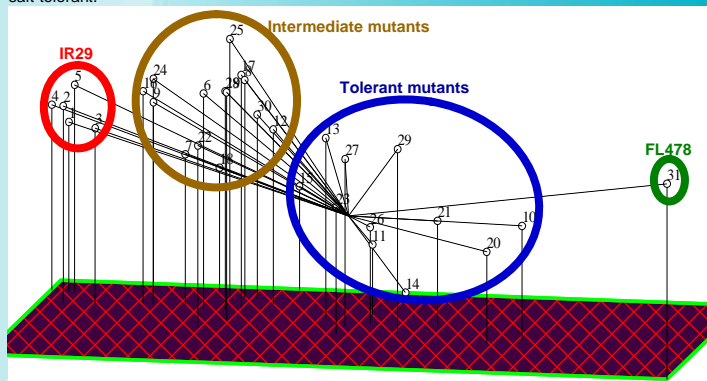
Assessing the linear response of all the measured traits under salinity and control conditions using stepwise multiple linear regression approach showed the great effect of K⁺ on dry weight and biomass production under both conditions. K⁺ also had a significant effect on SES scores and survival rate under salinity after osmotic potential and root to shoot Na⁺ translocation.

Results also showed that changes in total chlorophyll content were mainly affected by total carbohydrate then Na⁺ content of the plant. Furthermore, the changes in SPAD readings were mainly governed by root K⁺ and total chlorophyll contents. Plant height was controlled by root K⁺ content, shoot Na⁺, and water potential.



Clusters of 28 IR64 mutant lines along with three check varieties based on Ward method calculated from a matrix of phenotypic data.

Evaluation of genotypes for all the traits under salt stress showed that all genotypes can be classified into four distinct phenotypic groups. These groups were significantly different in root and total K⁺ content, root dry weight, shoot dry weight, leaf area, survival rate, and SES scores but did not differ significantly in root carbohydrate, root Na⁺, and chlorophyll a-b ratio, as well as root to shoot Na⁺ translocation. IR29 and the sensitive mutant lines (# 1, 3, and 4) were classified in the same group whereas the most tolerant lines (# 9, 15, 19, 25, and 27) were grouped together in a different group. FL478 was classified alone in a unique group as highly salt-tolerant.



Triplot derived from pattern analysis based on the significant factors, which revealed by factor analysis, confirmed the results of above cluster analysis; showing that all sensitive genotypes shares the same group with IR29, whereas the intermediate and tolerant lines fall between the checks IR29 and FL478, with the same distribution as in cluster analysis.

Conclusions

- IR64 mutants with contrasting response to salt stress were successfully isolated. These mutants provide an ideal system for further analysis and for gene discovery. Tolerant mutants are potential varieties for salt affected areas provided that other agronomic and quality traits of IR64 were not altered.
- Salinity significantly affect growth and ionic relations of all mutant genotypes as well as between the different genotypic groups in most of the traits studied.
- Under salt stress, Na⁺ and K⁺ contents, Na-K ratio, and carbohydrate content in the shoot are more important than in the roots for salinity tolerance and plant survival.
- Further biochemical and genetic analysis of these mutants is in progress to further enhance our understanding of the bases of salinity tolerance and help in the discovery of essential pathways and genes involved.