Responses of tolerant and sensitive rice mutants to salt stress at seedling stage

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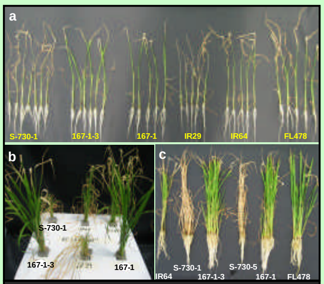
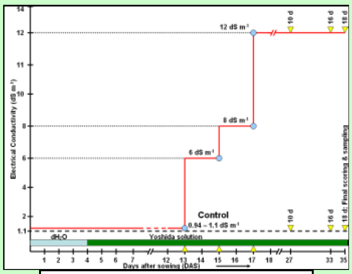
Salt stress occurs when excessive salts in soil solution causes a reduction in plant growth or yield. Soil salinity is a major abiotic stress that affects crop productivity. Currently, more than 800 million ha of land worldwide are affected either by salinity (397 million ha) or sodicity (434 million ha). This constitutes nearly 20% of the world's cultivated land area and about half of the world's irrigated land. Salinity affects plant growth and development through ion toxicity, osmotic stress, 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 facilitate the breeding efforts to develop salt-tolerant varieties. In this regard, mutant lines with altered responses to salinity can be effectively used to elucidate the mechanisms of salt tolerance and to identify candidate genes involved in these mechanisms.

Materials and methods

Six mutant lines, four of them with better tolerance for and two with higher sensitivity to salt stress compared with wild type IR64, were selected. This was achieved through high-throughput screening of more than 5,000 diepoxybotane IR64 mutant lines under controlled greenhouse conditions (29/21 ºC day/night temperature and ~70% RH) using hydroponics.



Performance of sensitive (S-730-1, S-730-5) and tolerant (167-1, 167-1-3) 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}.



Process of imposing stress, scoring and sampling

To decipher the key mechanisms contributing to salinity tolerance or sensitivity during the early seedling stage, selected mutants with contrasting responses to salt stress, along with three checks (IR29 [sensitive], IR64 [parent, intermediate], and FL478 [tolerant], were phenotypically analyzed. Morphological, physiological, and biochemical parameters were measured in a set of experiments under controlled greenhouse conditions. Pregerminated seeds were sown on styrofoam floats with a net bottom suspended on trays filled with distilled water. On the 4th day, the distilled water was replaced with Yoshida nutrient solution. It was subsequently renewed every 5-7 d and the pH maintained at 5.0 daily. Plants were grown on nutrient solution for 10 d and weak plants were removed before imposing the stress. Salt stress was then imposed 13 d after sowing by adding NaCl to the nutrient solution. Electrical conductivity (EC) of the nutrient solution was gradually increased in three steps (6, 8, and 12 dS m^{-1}) over a period of 5 d with 2-d intervals for a final EC of 12 dS m^{-1}. The EC of the nutrient solution in the stress treatment was monitored and maintained at 12 dS m^{-1} during the experiment. The non-saline control treatment with Yoshida solution had an EC of 0.94-1.1 dS m^{-1}. The chart above summarizes the gradual process of imposing stress, scoring, and sampling times during the experiment.

Results

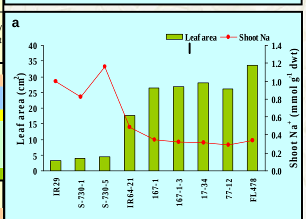
Growth and morphological responses of selected mutants to salt stress

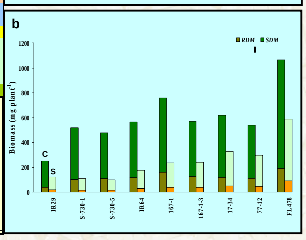
Highly significant differences were observed between control and stress treatments for almost all traits studied. Similarly, salinity caused significant differences between salt-tolerant and salt-sensitive mutants and their parent line. Tolerant mutants had lower SES scores, lower Na^+-K^+ ratio, smaller root to shoot Na^+ translocation, higher chlorophyll contents, greater root and shoot biomass, greater green leaf area, higher osmotic potential, taller plants, and higher carbohydrate concentrations than IR64 and their sensitive counterparts (see Table). Tolerant mutants are more efficient in upregulating their antioxidant system, which helps in scavenging radical oxygen species generated during stress. These advantages allow tolerant mutants to survive and grow better under saline conditions compared with wild type and sensitive mutants.

SES scores, growth, and morphological parameters of salt-sensitive and salt-tolerant IR64 mutants under salt stress. Salinity imposed 2 wk after sowing at EC = 12 dS m^{-1}. Final scoring and sampling were done when IR29 scored 7 based on SES. Data are mean values of four replications with five subsamples per replication.

Genotype	SES scores	Root volume (cm^3)	Root length (cm)	Plant height (cm)	Leaf area (cm^2)	Root fresh weight (mg plant^{-1})	Shoot fresh weight (mg plant^{-1})	Total fresh weight (mg plant^{-1})	Root dry weight (mg plant^{-1})	Shoot dry weight (mg plant^{-1})	Total dry weight (mg plant^{-1})	Root/shoot
Saline												
IR29	7.00	0.24	15.0	27.0	3.2	221.1	494.0	715.2	16.5	105.1	121.6	0.16
S-730-1	7.00	0.24	21.3	30.5	4.0	214.7	492.6	707.3	15.3	90.9	106.3	0.17
S-730-5	7.00	0.27	19.1	29.3	4.4	221.7	456.6	678.3	15.1	82.8	98.0	0.18
IR64-21	5.00	0.46	26.9	32.2	17.7	416.9	742.0	1158.9	28.6	146.8	175.4	0.19
167-1	4.00	0.46	28.5	32.0	26.3	529.4	1002.3	1531.7	38.8	196.0	234.7	0.20
167-1-3	4.00	0.51	26.6	35.8	26.8	554.9	1009.4	1564.3	37.5	202.3	239.8	0.19
17-34	5.00	0.55	29.1	37.4	27.9	744.0	1412.5	2156.5	47.8	279.0	326.8	0.17
77-12	5.00	0.54	29.2	36.0	26.1	686.4	1240.4	1926.8	46.1	249.2	295.3	0.18
FL478	4.00	1.16	23.2	48.3	33.6	1206.0	2429.1	3635.1	91.6	495.2	586.8	0.18
Mean	**5.33**	**0.49**	**24.3**	**34.3**	**18.9**	**532.8**	**1031.0**	**1563.8**	**37.5**	**205.3**	**242.7**	**0.18**
Normal												
IR29	1.00	0.50	24.8	41.1	16.9	355.0	983.3	1305.5	37.9	211.0	248.9	0.18
S-730-1	1.00	1.52	26.5	53.8	38.6	923.7	1843.2	2768.9	101.6	415.2	516.8	0.24
S-730-5	1.00	1.57	27.1	54.2	38.9	977.5	1631.2	2618.5	107.5	369.2	476.7	0.29
IR64-21	1.00	1.64	28.9	53.9	40.8	1010.1	1981.6	2986.3	116.0	448.0	564.0	0.26
167-1	1.00	2.15	31.7	53.5	66.9	2056.6	2800.0	4684.1	160.1	597.6	757.7	0.27
167-1-3	1.00	1.75	29.0	55.9	51.2	1138.9	1956.9	3116.7	125.3	445.0	570.3	0.28
17-34	1.00	1.67	30.0	54.6	45.6	1048.5	2241.4	3244.5	116.6	502.4	619.0	0.23
77-12	1.00	1.99	26.6	57.5	51.8	1371.9	1852.5	3264.1	110.5	429.3	539.9	0.26
FL478	1.00	2.17	22.9	59.2	66.9	1474.3	3947.4	5351.5	191.9	871.3	1063.3	0.22
Mean	**1.00**	**1.66**	**27.5**	**53.7**	**46.4**	**1150.7**	**2137.5**	**3260.0**	**118.6**	**476.6**	**595.2**	**0.25**
Significance												
Salinity(S)	***	***	ns	**	**	***	**	***	***	**	***	**
Genotype(G)	***	***	***	***	***	***	***	***	***	***	***	***
SxG	***	***	***	***	***	***	***	***	***	***	***	***
$LSD_{0.05}$												
Salinity(S)	0.00	0.24	–	7.9	46.4	1150.7	2137.5	3260.0	118.6	476.6	595.2	0.25
Genotype(G)	0.00	0.09	1.5	1.6	3.3	95.9	122.6	190.3	6.2	23.6	28.0	0.02
SxG	0.00	0.1	2.1	2.3	4.7	135.6	173.3	269.1	8.8	33.3	39.5	0.02
CV	**0.0**	**8.8**	**5.7**	**3.6**	**10.2**	**11.3**	**7.7**	**7.8**	**8**	**6.9**	**6.6**	**0.0**

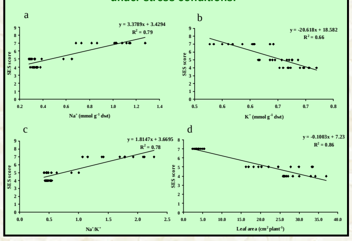
(a) Relationship between leaf area and shoot Na^+ concentration of selected mutant lines and checks under stress conditions. (b) Root and shoot dry matter and total biomass of selected mutant lines and checks under normal and stress conditions.

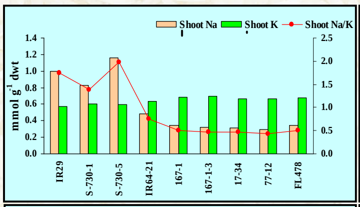


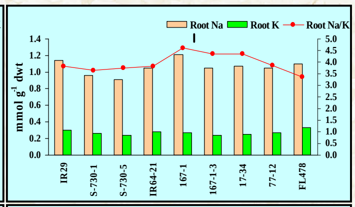


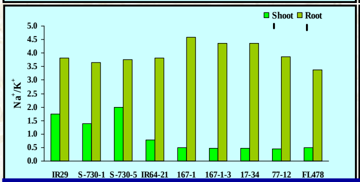
- Salinity considerably reduced leaf area and root, shoot, and total biomass in all genotypes, with greater effects on sensitive mutants.
- Sensitive mutants had higher shoot Na^+ concentrations and lower leaf area under salt stress compared with tolerant mutants and wild type.
- The SES scores had significant and positive correlation with shoot Na^+ and shoot Na^+/K^+.
- The SES scores showed significant and negative correlation with shoot K^+ and green leaf area.

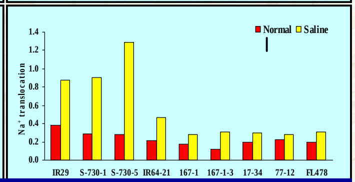
Correlations between SES with shoot Na^+ (a), shoot K^+ (b), shoot Na^+-K^+ ratio (c), and leaf area (d) across all genotypes under stress conditions.





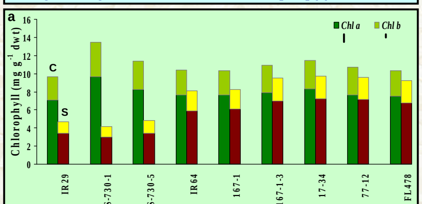


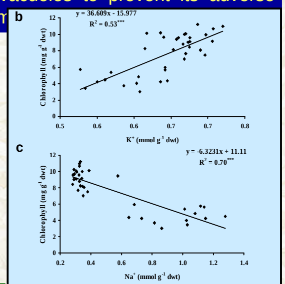


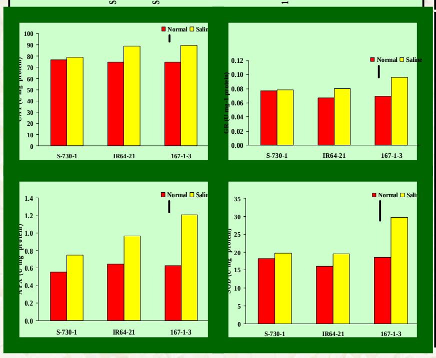


- Tolerant mutants absorbed less Na^+ and kept most of the absorbed Na^+ in their roots.
- Sensitive mutants showed significantly higher Na^+ concentrations in their shoots and higher root to shoot Na^+ translocation compared with tolerant mutants and wild type.
- Tolerant mutants maintained higher ratios of K^+ to Na^+ in their shoots under saline conditions.
- Tolerant mutants are probably more efficient in excluding Na^+ from the transpiration stream and in retrieving Na^+ from the xylem to the roots. They might be able to sequester Na^+ to the root vacuoles to prevent its adverse

Effect of salinity on chlorophyll 'a', chlorophyll 'b', and total (a+b) chlorophyll (a). Chlorophyll concentration showed a significant and positive correlation with shoot $[K^+]$ (b) but a strong and negative correlation with shoot $[Na^+]$ (c).







- Effects of salinity on antioxidant enzymes under stress and non-stress conditions were evaluated in IR64 and its mutants.
- Activities of catalase (a), glutathione reductase (b), ascorbate peroxidase (c), and superoxide dismutase (d), were significantly upregulated under stress conditions in tolerant mutant.
- The sensitive mutant did not show any significant differences in the levels of enzyme activities studied.

Conclusions

- **Tolerant mutants take less Na^+ and are more efficient in excluding Na^+ from their shoots. They probably sequester Na^+ in vacuoles of their roots to help in osmotic regulation and to maintain water uptake.**
- **Sensitive mutants take more Na^+ and transfer most of it to their shoots.**
- **Sensitive mutants are less efficient in upregulating their radical oxygen scavenging system. However, tolerant mutants are more efficient in this respect, which could help in scavenging radical oxygen species generated during stress.**

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