

Isolation and characterization of rice knockout mutant lines with altered responses to salt stress

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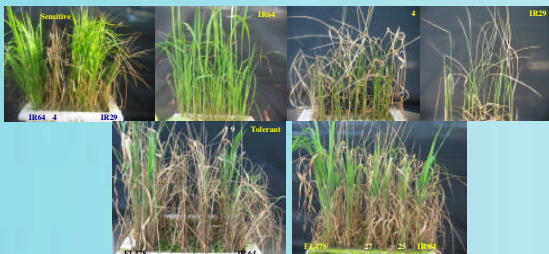
High soil and water salinity is a major problem for rice production around the world. Nearly 20% of the world's cultivated area and about half of the world's irrigated lands are affected by salinity. Tolerance for salinity is expressed as the ability to survive and grow in a saline medium. It is conferred by genes that control salt uptake through roots and its distribution within different plant organs, genes that affect ionic and osmotic balance of cells in roots and shoots, as well as genes that regulate leaf development and the onset of senescence.

Rice mutants with altered response to salinity can effectively be used to elucidate the biochemical and genetic basis of tolerance and to identify candidate genes involved. We screened more than 5000 IR64 mutant lines and sublines under salinity stress imposed at the seedling stage. Putative tolerant and sensitive lines were selected and phenotypically evaluated to confirm their responses under salt stress. Few lines were subsequently identified as being either substantially tolerant or more sensitive than the parental line, IR64.

Materials and methods

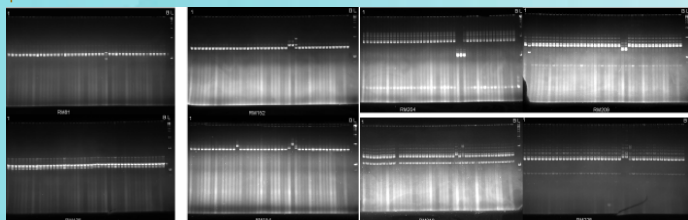
More than 4000 IR64 M4D mutant lines and about 1000 of their respective progeny sublines were evaluated for salinity tolerance under controlled conditions (29/21 °C day/night temperature and ~70% RH). Pregerminated seeds were sown on styrofoam floats with a net bottom suspended on trays filled with distilled water. Salt stress was imposed 3 d after germination by adding NaCl to an EC of 12 dS m⁻¹ in Yoshida nutrient solution. IR29 (sensitive), IR64 (parent, intermediate), and FL478 (highly tolerant) were used as checks. The pH of the nutrient solution was adjusted daily at 5.5 and culture solutions were changed weekly. Entries were scored based on visual symptoms using IRR's Standard Evaluation System (SES) scores. Putative sensitive lines were rescued at 10 d and 16 d after salinization and transferred to pots in a greenhouse for seed increase. After 16 d of salt stress, the EC of the nutrient solution was raised to 18 dS m⁻¹ and the most tolerant plants were selected after the check parent, IR64, scored 9 (dead). Selected plants were transferred to pots for seed increase. Seeds of selected tolerant and sensitive mutants were then further characterized in replicated trials.

Results



Confirmation of genetic background of selected IR64 mutants

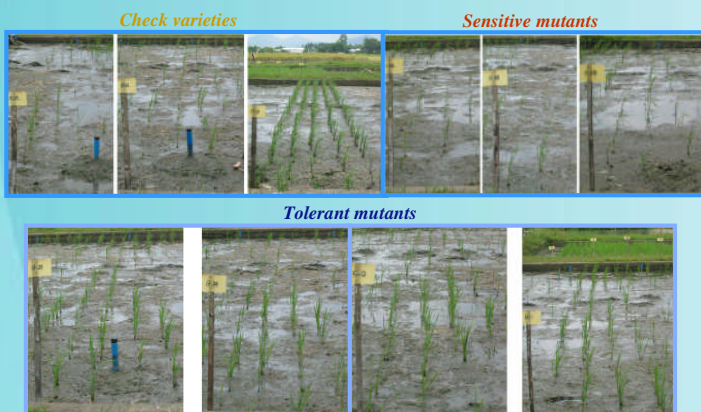
About 45 of the selected tolerant and sensitive IR64 mutants were surveyed with 36 SSR markers specific for IR64.



All selected mutants had the same banding pattern as IR64, confirming that they are true IR64 mutants.

Performance of selected mutants under field condition

Four sensitive and 10 tolerant mutants as well as the three checks (IR29, IR64, and FL478) were transplanted in artificially salinized and normal field plots during the dry season of 2006. The EC of the soil in saline plots was adjusted to 6 dS m⁻¹ by adding saline water. Three-week-old seedlings were transplanted and the EC at the root zone was monitored daily using perforated pizometers installed in the field and a portable EC meter. Water in the pizometers was sucked out daily after readings. The EC is maintaining between 6-7 dS m⁻¹ by adding either saline or normal water.



Genotype	IR29	1	3	4	IR64	9	15	19	25	27	FL478
Survival rate (%)	17.5	18.1	6.9	28.8	33.1	52.5	48.8	46.9	36.9	36.3	96.0
SES score	7.0	7.0	9.0	7.0	6.0	3.0	3.0	4.0	4.0	5.0	2.0

Survival of the sensitive lines was substantially lower than that of parental line IR64 and their SES scores were also higher. However, selected tolerant lines showed much better survival and lower SES scores than IR64 under field conditions.

Physiological responses of selected mutants to salt stress

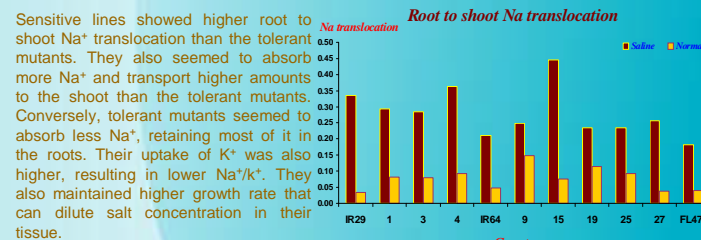
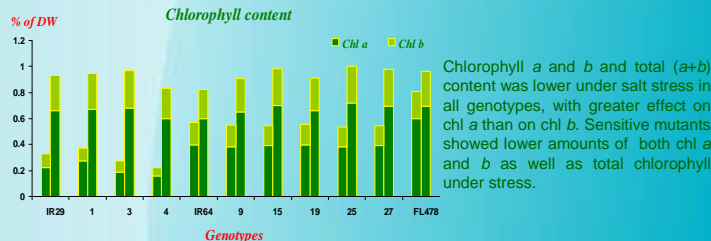
Based on the results of screening trials, 20 tolerant and eight sensitive mutants were selected for further analysis along with the three checks. The experiment was laid in a RCBD with four replications and several traits were monitored during the seedling stage. Salt uptake of the mutant lines was examined to elucidate the physiological and biochemical bases of their tolerance at the early vegetative stage. Subsequently, three lines (# 1, 3, 4) were selected as most sensitive and five as most tolerant (# 9, 15, 19, 25, 27) based on their consistent response to salinity.

	SES ^a		Survival (%)		SPAD		Osmotic potential (Mpa)		TDW ^b		Root Na (%)	
	Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control
IR29	7.5	1	33.8	100	28.5	34.7	3.935	0.836	32.82	84.13	1.443	0.430
1	5.0	1	58.1	100	29.8	34.8	3.149	0.842	34.79	93.77	1.499	0.401
3	5.3	1	68.8	100	27.8	33.7	3.174	0.819	31.60	96.43	1.735	0.397
4	5.8	1	57.5	100	26.4	33.6	3.504	0.867	37.36	92.20	1.442	0.410
IR64	4.8	1	80.3	100	33.8	34.3	1.980	0.881	57.42	113.70	1.828	0.438
9	3.6	1	78.3	100	32.3	34.5	2.092	0.840	41.20	106.53	1.944	0.367
15	3.8	1	90.0	100	32.7	34.1	2.025	0.833	56.83	90.33	1.444	0.409
19	4.3	1	83.8	100	32.5	32.8	1.793	0.874	61.01	93.77	1.774	0.455
25	3.8	1	85.2	100	32.2	33	1.978	0.858	47.14	101.60	1.709	0.490
27	3.8	1	85.0	100	31.2	35	2.293	0.844	57.06	101.77	1.781	0.418
FL478	1.6	1	97.5	100	40.7	38.3	1.467	0.824	113.66	160.80	1.472	0.550

	Root K (%)		Shoot Na (%)		Shoot K (%)		Root Na/K		Shoot Na/K		Total Na/K	
	Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control	Salinity	Control
IR29	0.998	2.148	2.259	0.072	1.698	3.286	2.03	0.20	1.53	0.02	1.57	0.04
1	1.029	2.688	2.376	0.139	1.836	3.600	2.61	0.15	1.60	0.04	1.64	0.06
3	1.185	2.541	2.435	0.112	1.793	3.377	2.72	0.16	1.60	0.03	1.65	0.05
4	1.014	2.342	2.612	0.141	1.932	3.100	2.78	0.18	1.67	0.05	1.70	0.07
IR64	1.328	2.239	1.701	0.087	2.091	3.357	1.42	0.20	0.82	0.03	0.88	0.05
9	1.379	2.625	2.138	0.202	1.992	4.460	1.52	0.14	1.09	0.05	1.14	0.06
15	1.484	2.301	1.995	0.167	1.980	3.815	0.93	0.18	1.08	0.04	1.06	0.06
19	1.494	2.152	1.846	0.214	2.263	3.400	1.35	0.21	0.82	0.06	0.88	0.08
25	1.495	2.324	2.106	0.186	1.985	3.344	1.23	0.21	1.07	0.06	1.09	0.08
27	1.262	2.366	2.334	0.071	2.061	3.707	1.84	0.18	1.23	0.02	1.28	0.04
FL478	1.476	2.340	1.494	0.098	2.288	3.815	1.15	0.24	0.68	0.03	0.71	0.05

^aSES: Standard Evaluation System. ^bTDW: Total Dry Weight.

All genotypes were adversely affected by salt stress with significantly higher SES score, Na⁺ uptake, Na⁺/K⁺, osmotic potential, but lower survival rate, SPAD readings, and biomass than the control. Clear differences between sensitive and tolerant lines in almost all measured characteristics were observed. 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.



Conclusions

- Mutant lines with higher tolerance (# 9, 15, 19, 25, 27) or greater sensitivity (# 1, 3, 4) than the parental line IR64 were successfully isolated and confirmed to have the same genetic background as IR64.
- Partial characterization of isolated mutants showed that these lines differ in a number of traits known to be associated with response to salt stress however, the apparent differences in Na⁺ and K⁺ uptake and Na⁺/K⁺ could be one of the major mechanisms of salinity tolerance/sensitivity in IR64 mutant lines.
- Further physiological and genetic analysis of these mutants is under way to understand the bases of altered response and for use in gene discovery.
- The tolerant mutants are potential new cultivars for salt-affected areas, considering that other traits of IR64 are not significantly altered. Biochemical and genetic analysis of these mutants could further enhance our understanding of the bases of salinity tolerance and help in the discovery of essential pathways and genes involved.