

Novel IR64 Mutant Lines with Contrasting Phenotypes Under Salt Stress

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Salinity is one of the major environmental constraints to rice production. Rice is salt-sensitive and its growth and yield can drastically be reduced by salt stress. Rice mutants with altered responses to salt stress can effectively be used to elucidate the biochemical and genetic basis of tolerance and to identify the candidate genes involved, which, once validated, can substantially speed efforts to breed salt-tolerant varieties.

Materials and methods

IR64 Mutant Bank @ IRR

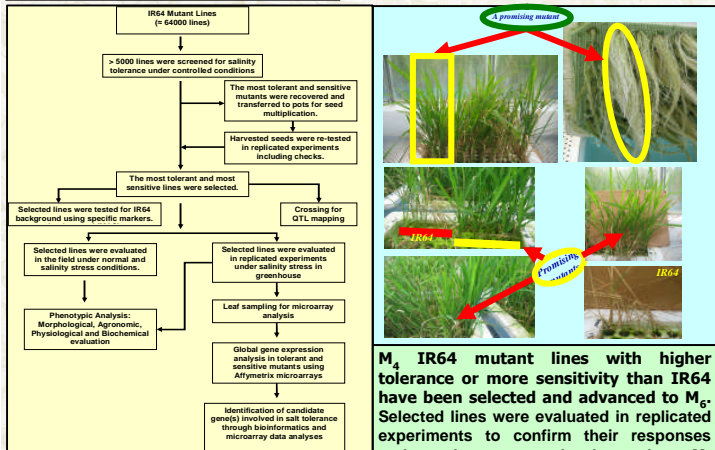
Materials: Gamma ray, Fast neutron, EMS, DSB

Forward genetics: Conditional phenotypes

Reverse genetics: Candidate-gene knockouts/variation

Currently > 60,000 M4 lines available

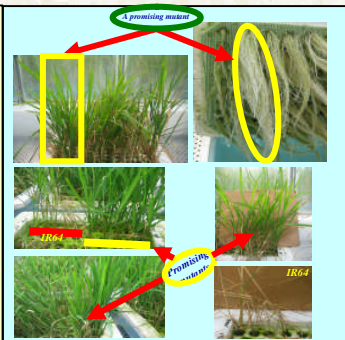
High-throughput screening of more than 5,000 diepoxibotane IR64 mutant lines in saline hydroponics at the seedling stage.



Flowchart showing the steps followed during the present study.

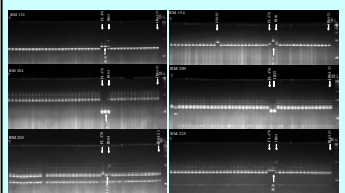


Selecting putative susceptible and tolerant IR64 mutants for seed increase.



M₆ IR64 mutant lines with higher tolerance or more sensitivity than IR64 have been selected and advanced to M₈. Selected lines were evaluated in replicated experiments to confirm their responses under salt stress and advanced to M₉ generation for detailed phenotypic analysis and gene expression profiling.

Results of SSR ID test confirmed that all the selected lines were true IR64 mutants.



The banding patterns of the selected mutants and three checks were compared using 37 SSR markers specific for IR64. All the selected mutants showed the same banding pattern as the wild type (IR64-21).

Phenotypic analysis

Several morphological, agronomical, physiological, and biochemical traits were assessed on 20 tolerant and 8 sensitive mutants, together with parental line IR64-21, and checks IR29 (sensitive) and FL 478 (tolerant).

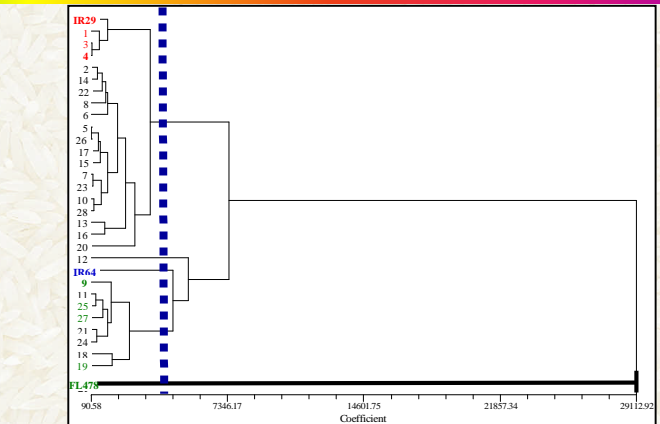
Chlorophyll content was measured using a SPAD meter.

Leaf stomatal conductance and transpiration were measured using ΔT-AP4 - Porometer.

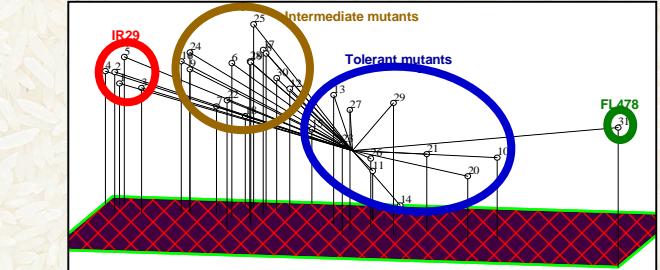
Leaf water potential was measured using the Scholander pressure bomb.

Plant height, root length, and leaf area were measured.

Time chart of stress imposition, scoring, and sampling.



Clusters of 28 IR64 mutants along with three checks based on Ward method; calculated from a matrix of phenotypic data.



Triplot derived from pattern analysis based on significant factors revealed by factor analysis confirmed the results of cluster analysis above. It showed that all sensitive genotypes share the same group with IR29, whereas the intermediate and tolerant lines fall between checks IR29 and FL 478, with the same distribution as that in cluster analysis.

Subsequently, six mutant lines, four with greater tolerance for and two with more sensitivity to salt stress than IR64, were selected. To decipher the key mechanisms contributing to salinity tolerance or sensitivity during the early seedling stage, selected mutants, along with three checks (sensitive IR29, parent IR64, tolerant FL478), were further evaluated for morphological, physiological, and biochemical traits in a set of experiments under greenhouse conditions.

TOLERANT

- Higher K⁺ uptake
- Lower Na⁺:K⁺ ratio
- Lower Na⁺ translocation
- Na⁺ retained mainly in roots
- Greater biomass, leaf area, osmotic potential, plant height, chlorophyll, and carbohydrate content
- Upregulation of ROS system

Physiological characteristics of mutants

SENSITIVE

- Higher Na⁺ uptake
- Higher Na⁺:K⁺ ratio
- Higher root to shoot Na⁺ translocation
- Lower biomass, leaf area, osmotic potential, plant height, chlorophyll, and carbohydrate content

Performance of sensitive (S730-1, S730-5) and tolerant (T67-1, T67-3) 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.

Genotype	SES score	Root volume (cm ³)	Root length (cm)	Plant height (cm)	Leaf area (cm ²)	Root fresh weight (mg)	Root dry weight (mg)	Shoot fresh weight (mg)	Shoot dry weight (mg)	Root:Shoot ratio		
Normal												
IR29	1.00	0.50	24.8	41.1	16.9	355.0	96.3	1305.5	37.9	2110	248.9	0.18
S730-1	1.00	1.52	26.5	53.8	36.6	223.7	184.2	2768.9	101.6	415.2	516.8	0.24
S730-5	1.00	1.57	27.1	54.2	38.9	97.5	163.2	2618.5	107.5	369.2	476.7	0.29
IR64-21	1.00	1.64	28.9	53.9	30.8	1010.1	1981.6	2986.3	116.0	448.0	564.0	0.26
T67-1	1.00	2.15	31.7	53.4	46.9	2056.6	2800.0	4654.1	160.1	597.6	757.7	0.27
T67-3	1.00	1.75	29.0	55.9	31.2	1138.9	1956.9	3167.7	125.3	445.0	570.3	0.28
T7-34	1.00	1.67	30.0	54.6	45.6	1048.5	2241.4	3244.5	116.6	502.4	619.0	0.23
T7-12	1.00	1.99	26.6	57.2	31.8	1371.9	1852.4	3264.1	116.5	429.3	539.7	0.26
T7-12	5.00	0.54	29.2	36.0	26.1	686.4	1240.4	1926.8	46.1	249.2	295.3	0.19
FL478	1.00	1.16	27.2	49.3	31.6	1259.0	2429.1	3635.1	91.6	495.2	586.8	0.28
Mean	5.33	0.49	24.3	54.3	38.9	532.8	1031.0	1563.8	37.5	265.3	242.7	0.18
Normal												
IR29	1.00	0.50	24.8	41.1	16.9	355.0	96.3	1305.5	37.9	2110	248.9	0.18
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Significance												
Salinity(S)	***	***	***	***	***	***	***	***	***	***	***	***
Genotype(G)	***	***	***	***	***	***	***	***	***	***	***	***
StG	***	***	***	***	***	***	***	***	***	***	***	***
LSDis												
Salinity(S)	0.00	0.24	-	7.9	46.4	1150.7	2137.5	3260.0	118.6	476.6	592.3	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
Genotype(G)	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	6.9	6.6	6.6	0.0

- 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 seem more efficient in excluding Na⁺ from the transpiration stream, possibly through exclusion or retrieval from the xylem to the roots. They might be able to sequester Na⁺ in the root vacuoles to prevent its adverse effects on cytosolic enzymes and to use it to help in osmoregulation and water uptake.