

# A knockout mutant population for the discovery of genes associated with salt tolerance in rice

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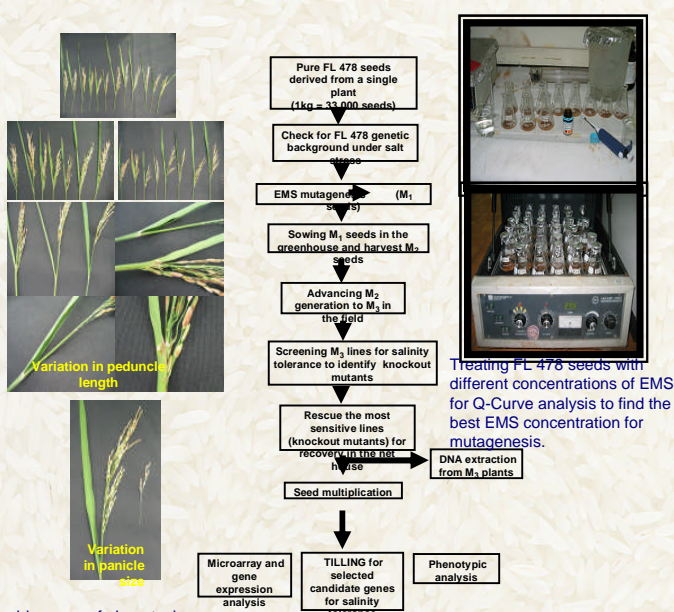
Salt stress is a major problem that limits rice production in vast areas worldwide. About 20% of the world's cultivated area is affected by salinity. Tolerance for salinity stress is controlled by genes that regulate salt uptake through roots and its distribution within different plant organs, genes that affect ionic and osmotic balance of cells in roots and shoots, as well as genes that regulate leaf development and onset of senescence. Rice mutants with altered responses to salinity can effectively be used to elucidate the biochemical and genetic basis of tolerance and to identify putative candidate genes involved. FL 478 (IR66946-3R-178-1-1), a recombinant inbred line (RIL) from a cross between IR29 (sensitive) and Pokkali (tolerant), was identified as being highly tolerant of salt stress at the early seedling stage and was selected for this study.

A large mutant population was developed by treating about 1 kg of pure FL 478 seeds with 1% of the mutagen ethyl methanesulfonate (EMS). Percentage germination of  $M_1$  seeds was about 69%. A wide range of phenotypic variations was observed among  $M_1$  mutants. About 11,350  $M_1$  plants were harvested and multiplied in the field using single seed descent to produce the  $M_2$  generation. In each  $M_2$  family, five to six normal  $M_2$  plants were individually harvested.

The  $M_2$  seeds of each individual plant were screened for salinity tolerance in hydroponics using Yoshida nutrient solution with a salinity level of 8 dS  $m^{-1}$  for 1 week which was raised to 12 for another week after rescuing putative knockout mutants at a salinity level of 8 dS  $m^{-1}$ . About 4,000 mutant lines from 775 families were evaluated under salt stress.

## Experimental setup

Any point mutation in genes involved in salinity tolerance may result in loss-of-function mutants. These mutants can be identified through phenotypic screening under salt stress and control conditions. Gene discovery can then be achieved through various methods, including techniques such as positional cloning after mapping of the mutant loci, or through Targeting Induced Local Lesions in Genomes (TILLING) technique. The steps involved in the development of the mutant population is outlined below.



A wide range of phenotypic variation was observed among  $M_1$  mutants.

Flowchart of the steps followed during the experiment.

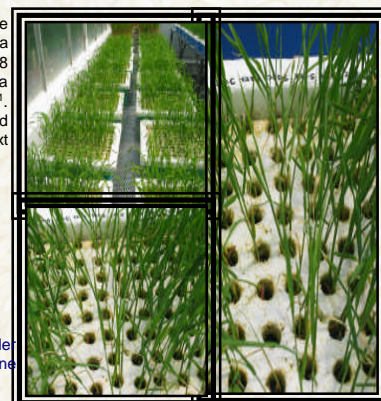
Seeds used for mutagenesis were produced from a single healthy FL 478 plant selected in the greenhouse. About 3 kg of seeds was produced in the nethouse, cleaned manually, and all the defective and infected seeds were removed. The seeds were then fumigated and stored in a cold room at 4 °C until use. The Q-curve for FL 478 was constructed using variable concentrations of EMS (0-2.0%) to determine the ideal concentration of EMS needed for effective mutagenesis (50% survival).

## Results

Of these, 448 sensitive mutants (from 354 families) with clear symptoms of salinity damage were rescued and advanced to the  $M_3$  generation in pots in the nethouse. About 132 individuals selected from 101 families of the  $M_3$  generation were evaluated in a replicated experiment under saline and non saline conditions to confirm the knockout phenotype of selected mutant lines.



Four individual plants were selected as most sensitive under a salinity level of 8 dS  $m^{-1}$ , while 18 individuals were recovered from a salinity level of 12 dS  $m^{-1}$ . Selected plants were rescued and used to produce seeds of the next generation.



The selected knockout FL 478 mutant lines were transplanted into pots and transferred to the net house. These lines will further be analyzed phenotypically. Stable mutants will then be used for further evaluation of the mechanisms involved and for gene discovery using different molecular techniques.

## Conclusions

- A novel mutant population from a highly salt-tolerant line FL478 was developed. This population will serve as a useful resource for understanding the physiological mechanisms associated with salinity tolerance and for gene discovery.
- Few plants with greater sensitivity to salt stress were identified as putative knockout mutants and rescued for seed production. This material will further be advanced and evaluated in replicated trials and to find mutated gene(s)/regions using molecular techniques.
- This approach might help speed up the identification of useful genes involved in salinity tolerance. These genes can then be developed into markers for breeding for salt tolerance.

