

## Trait based assessment in seedling stage of rice for salinity tolerance

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Rice is one of the major staple food crop of the globe (Dawe *et al.* 2010). Rise in population along with reduction in arable land and climate change poses serious threat to rice cultivation. Among different abiotic stress, salinity stress is one of the key abiotic stress and rice is most sensitive to salinity stress at seedling and early vegetative stage (Lutts *et al.* 1995). Salinity stress affect plant growth and development by affecting water relation and mineral acquisition. Salinity stress has shown to affect the process like photosynthesis, tiller number, panicle length, grain filling and plant biomass and ultimately reducing the yield and quality (Baker 2008; Ismail *et al.* 2007; Rao *et al.* 2013). Rice response to salinity stress include ion exclusion, tissue tolerance and osmotic tolerance (Munns and Tester, 2008; Roy *et al.*, 2014).

Salinity stress at seedling stage leads to poor establishment of crop due to early death of seedlings and thereby reducing plant population by unit area and ultimately yield (Awaji *et al.*, 2019; Manohara *et al.*, 2020). Hence, seedling stage salinity tolerance is crucial for better crop establishment and ultimately the yield. The huge genetic diversity in rice genotypes can be exploited to produce new varieties to challenging environment condition. Certain landrace been shown to have tolerance to salinity stress, but these cannot be commercially cultivated due to

less yield. These cultivars, especially Pokkali, Nona Bokali and Kalaratta has been used as source for prospecting genes for salinity tolerance. One of the key seedling stage salinity tolerance QTL, *Saltol* was derived from a cross between IR29/Pokkali, which was mapped on chromosome 1 (Bonilla *et al.*, 2002). FL478 (IR66946-3R-178-1-1), a seedling-stage salt tolerant recombinant inbred line from this cross (IR29/Pokkali), has been widely used as a donor for enhancing salt tolerance in rice. Introgression of *Saltol* QTL has shown to enhance Na<sup>+</sup>/K<sup>+</sup> homeostasis and ion levels in the cells thereby enhancing salinity tolerance.

The present investigation on screening of rice lines introgressed with *Saltol* QTL, for salinity tolerance was conducted in the Crop Physiology unit of Department of Plant Breeding and Genetics, Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, UT of Puducherry. 12 genotypes were screened to identify the saline tolerant line. FL478 was used as salt tolerant check (donor for *Saltol* QTL) and IR29 was used as salt susceptible check. Other genotypes used in the study were N22, IR 64. One line, IR87707 – 445-B-B/IR64 with two drought QTLs viz., 2.2 & 4.1 QTLs introgressed IR 64 lines was also evaluated for its tolerance (Vijayalakshmi *et al.*, 2014; Vishnu Prasanth *et al.*, 2017). Seven entries



used for screening are, KR15002, KR15005, KR15007, KR15104, KR15101, KR15081 and KR15058. These entries are generated from the cross of ADT45 x FL478 which was developed as part of project “From QTL to variety: Marker- assisted breeding of abiotic stress tolerant rice varieties with major QTLs for drought, submergence and salt tolerance” sponsored by DBT, New Delhi. Entries are part of 20 NILs (KR15001 to KR15020) of *Saltol* introgressed lines in background of ADT45 and 55 RILs (KR15051 to KR15105). These seven entries were selected based on the performance of station trials and multilocation trial (MLT).

Screening of these rice genotype was carried out in hydroponic system under greenhouse condition (Fig 1).



Figure 1: Hydroponic based experimental setup for screening of rice genotypes/ lines for seedling stage salinity stress tolerance

Root to shoot ratio is one of the important physiological traits which governing tolerance to abiotic stress. Root to shoot ratio on weight basis was calculated from control and stress samples. Other physiological parameters like Relative Water Content (RWC) and Cell membrane stability were also recorded. RWC was calculated using the formula proposed by Turner (1986). Cell membrane injury was calculated using Sullivan (1971) and membrane stability was calculated based on membrane injury. Biochemical parameters like chlorophyll and carotenoid were also estimated. Chlorophyll was estimated from the stress and control plants using DMSO and the total chlorophylls were calculated by the formulae as given by Arnon (1949). Carotenoid was estimated by method given by Porra *et al.* (1989). The genotypes/ lines were then ranked based on the performance under salinity stress

Tubs (0.45 x 0.30 x 0.12 meter) were used for the setup and control and salinity conditions were maintained separately. Pregerminated seeds were transferred to holes on styrofoam floats with a net bottom and these were suspended on tray filled with nutrient solution. pH of the solution was maintained to 5.0 to 5.1 daily and the solutions were replaced weekly. Salinity stress was imposed by raising EC of solution to 12 dSm<sup>-1</sup> by adding sodium chloride (Gregoria *et al.*, 1997). Stress was imposed from 14 DAS for a period of 14 days and physiological and biochemical observation were recorded at the end of 14 days stress period. Observations were recorded from salinity and control treatment. For all the physiological and biochemical parameters percent reduction over control was calculated.

in respect to the above traits and the tolerant genotypes were selected based on their rankings.

The experiment was laid in completely randomized design and mean values were mentioned at the significant level of  $\alpha = 0.05$  (Gomez and Gomez, 1984). MSTATC software was used to perform the analysis.

When root to shoot ratio on weight basis was analyzed, an increase in mean root to shoot ratio under salinity stress was observed (Fig 2). Salt tolerant genotypes has generally higher root to shoot ratio compared to susceptible one and this trait can be used for screening genotypes for tolerance to abiotic stress (Amouri, 2015; Thomas *et al.*, 2020). Increase of root to shoot ratio is adaptation strategy to mine for water and for active absorption of water. Under salinity stress, among the genotypes, KR15104, had the highest root to shoot ratio of 0.69, while IR 29 had the least of 0.1.

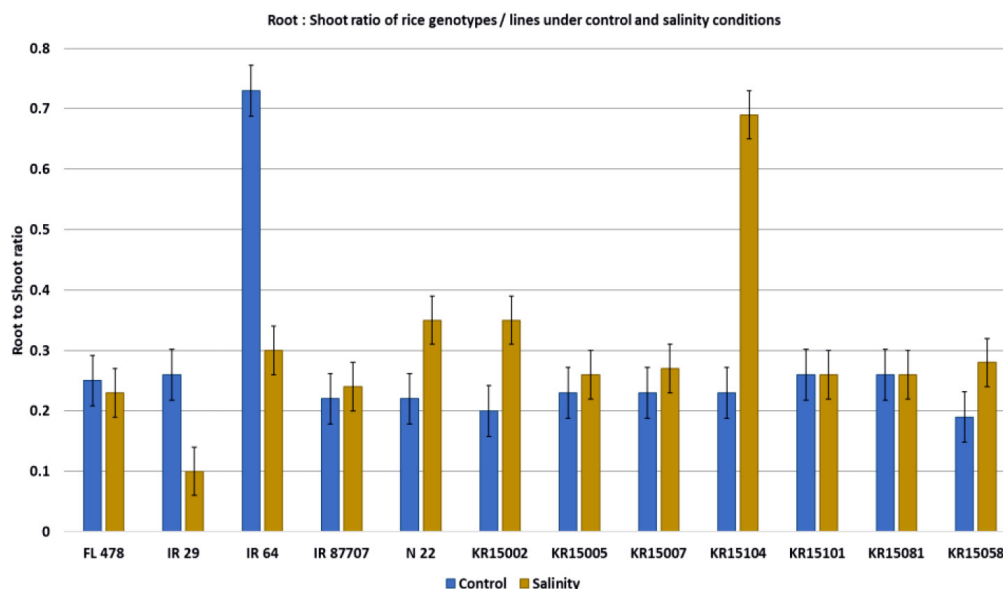


Figure 2: Root: Shoot ratio on weight basis of rice genotypes / lines under control and salinity conditions

The relative water content of the seedlings decreased when exposed to salinity stress (Fig 3). Reduction in RWC in salinity stress is due to the disturbed water relation of the plant (Yan *et al.*, 2020). Under salinity stress condition,

KR15058 had the highest RWC (84.09 %), while IR29 had the least. Membrane stability index was also affected under exposure to salinity stress (Fig 4). N22 (68.63) had the highest membrane stability index while IR 29 (6.85) had least membrane stability under stress.

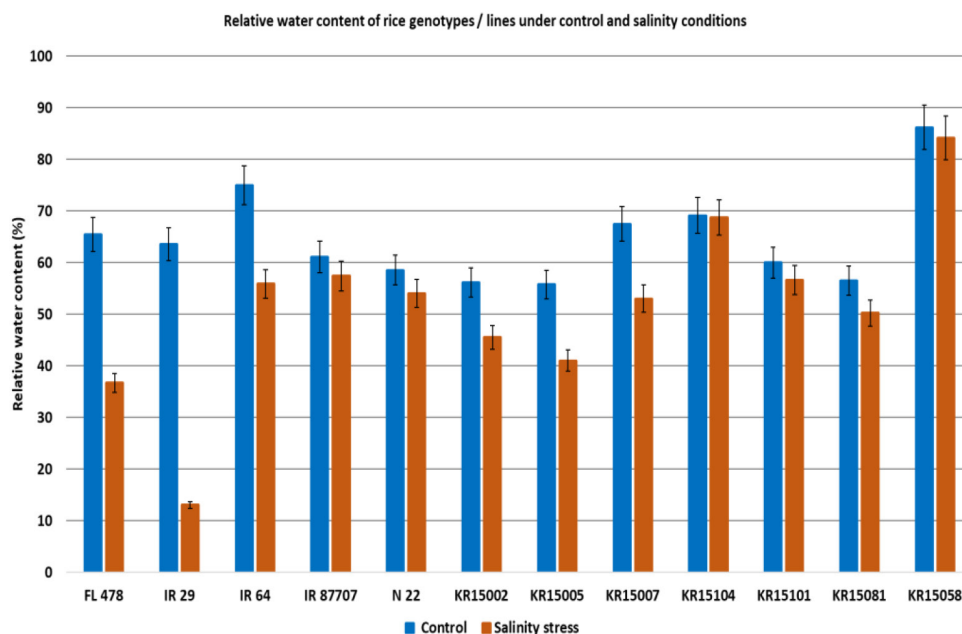


Figure 3: Relative water content (in percentage) of rice genotypes / lines under control and salinity conditions



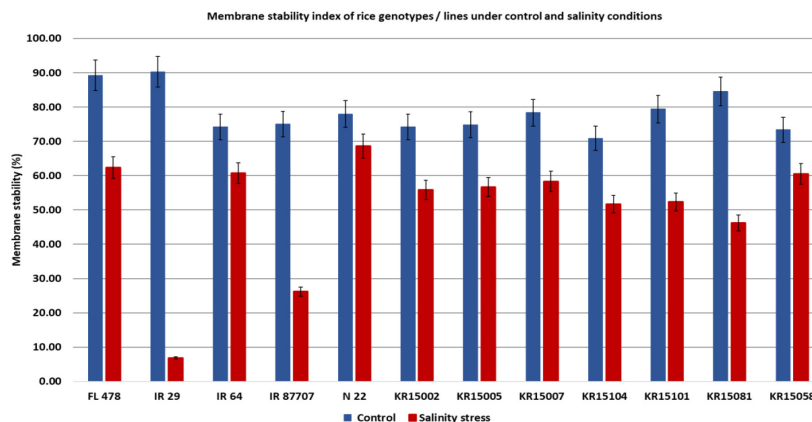


Figure 4: Membrane stability (in percentage) of rice genotypes / lines under control and salinity conditions

Carotenoid is one of the important pigments in plant photosynthetic machinery and in addition it acts as a strong antioxidant (Bouvier *et al.*, 2005). Increase in carotenoid content under stress is an adaptive mechanism to protect photochemical machinery and the cell. Among the screened lines/ genotypes, N22 had the highest

carotenoid content in salinity stress while IR29 had the least (Fig 5). Chlorophyll content decreased during salinity stress (Fig 6). KR15005 (1.60 mg g<sup>-1</sup> fw) had the highest chlorophyll content while least was recorded in IR 29 (0.45 mg g<sup>-1</sup> fw).

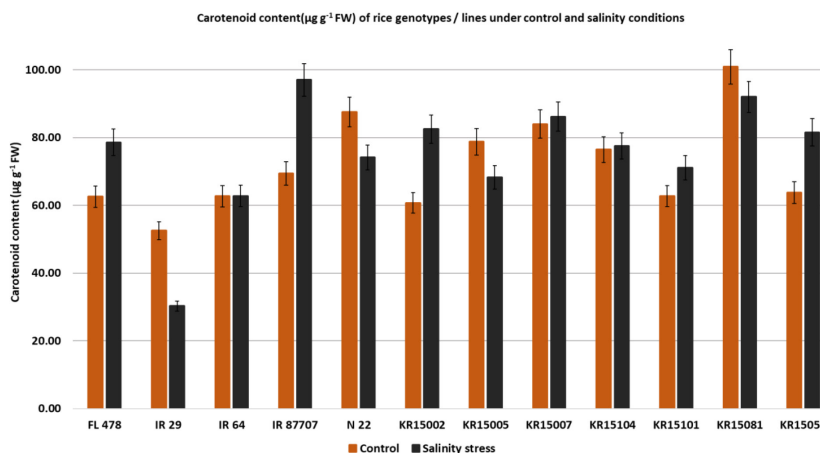


Figure 5: Carotenoid content (µg g<sup>-1</sup> FW) of rice genotypes / lines under control and salinity conditions

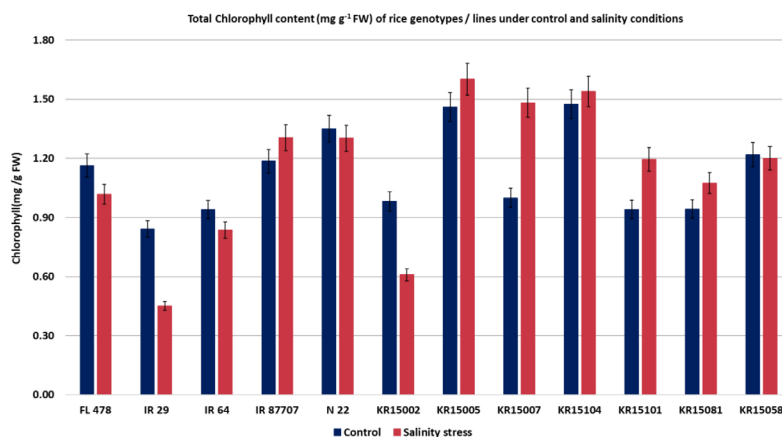


Figure 6: Total Chlorophyll content (mg g<sup>-1</sup> FW) of rice genotypes / lines under control and salinity conditions



The physiological and biochemical traits investigated are crucial for salinity stress tolerance and hence genotypes were ranked for their performance in different traits to

select the tolerant genotypes (Table 1). Based on the ranking, KR15058 was selected as the tolerant, followed by IR 87707 and KR15104.

Table 1: Ranking of rice genotypes/ lines based on different traits

Genotypes/ lines	Root to shoot ratio	Membrane stability	RWC	Carotenoid content	Chlorophyll content	Mean Rank
FL 478	11	2	11	6	5	7
IR 29	12	4	12	12	12	10
IR 64	4	8	5	11	10	8
IR 87707	10	1	3	1	4	4
N 22	2	12	6	8	1	6
KR15002	3	10	9	4	11	7
KR15005	7	9	10	10	9	9
KR15007	6	7	7	3	6	6
KR15104	1	11	2	7	3	5
KR15101	8	5	4	9	8	7
KR15081	9	6	8	2	7	6
KR15058	5	3	1	5	2	3

## Summary

Breeding for salinity tolerance is the need of the hour and identifying the potential donors and sources is very crucial. Hence, screening for the genotypes or lines by assessing the physiological and morphological traits are vital for identifying the tolerant genotypes. The lines identified in this study can be used for trait-based breeding for improving salinity tolerance in rice.

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## Declarations/ disclosures

**Compliance with ethical standards:** NA

## Author Contribution

S. Nadaradjan and S. Thirumeni conceptualized and designed the experiments. Tinu Thomas, J. Purushothaman, R. Janarthanan, N. Anusuya, and J. Karthick performed experiment. Manuscript was drafted by S Nadaradjan, Tinu Thomas, N. Anusuya and J.

Purushothaman. **Conflict of Interest:** Authors declare that they have no conflict of interest

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