



## Morphological Evaluation Of Rice Genotypes For Salinity Tolerance Under Hydroponics

Mathankumar P<sup>1</sup>, S Geetha<sup>2\*</sup>, R. Saraswathi<sup>3</sup>, M. Raveendran<sup>4</sup>, D. Vijayalakshmi<sup>5</sup>, R. Suresh<sup>6</sup>

<sup>1</sup> Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore - 641003, India

<sup>2\*</sup> Department of Pulses, Tamil Nadu Agricultural University, Coimbatore - 641003, India

<sup>3</sup> Department of Plant Genetic Resources, Tamil Nadu Agricultural University, Coimbatore - 641003, India

<sup>4</sup> Directorate of Research, Tamil Nadu Agricultural University, Coimbatore - 641003, India

<sup>5</sup> Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore - 641003, India

<sup>6</sup> Department of Rice, Tamil Nadu Agricultural University, Coimbatore - 641003, India

**\*Corresponding author: S Geetha**

*Department of Pulses, Tamil Nadu Agricultural University, Coimbatore - 641003, India*

*Email: geethagovind1@gmail.com*

### Abstract

Salinity, a prominent abiotic stressor, profoundly influences the growth and development of rice plants. In this study, 26 genotypes, accompanied by two control varieties, underwent screening for salinity tolerance at the seedling stage through hydroponics. Analysis of variance unveiled significant variations among genotypes in the recorded traits. Unlike many studies that assess salt tolerance based on individual trait means, our approach utilized membership function values, encapsulating cumulative tolerance across all traits. The identification of highly tolerant and tolerant genotypes in this study suggests their potential for enhancing salt tolerance during the seedling stage in rice through targeted breeding efforts.

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**Keywords: Rice, Salinity, Membership function values, Regression**

### Introduction

To meet the projected food demands of a burgeoning population expected to reach 9 billion by 2050, rice production must surge by 160 million tons (Muthu *et al.*, 2020). However, the escalating incidence of abiotic stresses, exacerbated by climate change, poses a significant threat to rice cultivation (Raza *et al.*, 2018). Salinity, in particular, has emerged as a major constraint on rice production worldwide, affecting nearly 950 million hectares of arable land, including 250 million hectares of irrigated land (Raja *et al.*, 2022). Rice, being a salt-sensitive glycophyte, exhibits varying degrees of salt tolerance across different growth stages (Chen *et al.*, 2021; Radha *et al.*, 2023).

Salinity imposes detrimental effects on rice plants through osmotic stress, ion toxicity, and nutrient imbalances (Khare *et al.*, 2020; Ljaz *et al.*, 2023). At the seedling stage, salinity-induced osmotic stress impedes root and shoot growth, reduces leaf size, and eventually leads to premature plant death (Krishnamurthy *et al.*, 2020). The hydroponic system offers a robust platform for screening rice genotypes under precisely controlled conditions, circumventing the complexities associated with soil and environmental stressors (Jayabalan, 2022).

Here, membership function value-based classification system was used to classify the salinity tolerance among rice genotypes (Ding *et al.*, 2018; Wu *et al.*, 2019).

## Materials and methods

Twenty- six rice genotypes, along with two well-known salt-tolerant checks, Pokkali and IR29, were evaluated for their salt tolerance. A completely randomized block design with three replicates was used for this experiment. Pre-germinated seedlings were transferred to hydroponic systems containing Yoshida nutrient solution. The solution was refreshed weekly to ensure proper nutrient delivery. Daily monitoring of the solution's pH ensured a consistent range of 5.1 to 5.5. After growing in the base nutrient solution for 21days, genotypes were exposed to a salinity stress of 12 dS/m. The control group continued to grow in the base nutrient solution without added salt.

## Morphological Traits

After 7 days of exposure to salinity, measurements were taken for eight morphological traits: shoot length (SL) (cm), root length (RL) (cm), shoot fresh weight (SFW) (g), root fresh weight (RFW) (g), shoot dry weight (SDW) (g), root dry weight (RDW) (g), total fresh weight (TFW) (g), and total dry weight (TDW) (g). To determine dry weights, root and shoot tissues underwent oven drying at 60°C for a period of 3 days.

## Salinity tolerance index

Salt tolerance index (STI) values were calculated for each trait using the following formula:

$$\text{Salt tolerance index (STI)} = \frac{\text{Trait value of NaCl treated plant}}{\text{Trait value of control plant}}$$

## Membership Function Values

The salt tolerance of rice genotypes was then assessed using the fuzzy comprehensive evaluation method employing membership function values (MFV) derived from the STI of each trait, calculated through the formula:

$$X_i = \frac{X - X_{min}}{X_{max} - X_{min}}$$

Were,

$X_i$  = Membership function value of each trait for individual genotype

$X$  = Value of STI for individual genotype

$X_{min}$  = Minimum STI value recorded in all genotypes

$X_{max}$  = Maximum STI value recorded in all genotypes

## Assessment of salinity tolerance

Chen (2012) employed a refined method to categorize salt tolerance in rice genotypes by utilizing the average Membership Function Value (MFV) and Standard Deviation (SD) values, leading to the classification of these genotypes into five distinct classes.

## Statistical analysis

Analysis of variance was performed using R software, STI and MFV values were calculated using Microsoft Excel.

## Results and Discussion

### Effect of Salinity on Morphological Traits

Significant reductions in growth due to salinity were observed for all recorded traits (Table 1). Under salinity, STI of shoot length ranged from 0.26 to 0.80, with the highest values observed in Vytilla10, Pokkali, and Jothi (0.80, 0.69, and 0.65, respectively). Conversely, the lowest STI values were recorded in IR29, CO55, and ADT55 (0.26, 0.35, and 0.39, respectively). Larger STI values directly correlate to reduced negative impact on plant growth under salt stress conditions. Shoot length suffers significant reduction under salinity, likely due to its effect on cell growth (Munns, 2002; Ravikiran, 2017) and hormone levels (Mazher *et al.*, 2007).

These findings align with previous studies by Arif *et al.*, (2017), Ravikiran *et al.*, (2017), and Banumathy *et al.*, (2020).

Root length STI values recorded a range from 0.33 to 0.84. Vytilla8, Vytilla10 and CSR23, exhibited the highest STI values (0.84, 0.83 and 0.82 respectively), while IR29, IR64, and ADT55 had the lowest STI values (0.33, 0.36, and 0.40 respectively). The observed results could be attributed to the elevated salinity levels, leading to an increase in H<sub>2</sub>O<sub>2</sub> accumulation, thereby impeding root growth (Demiral *et al.*, 2005). Salinity impacts primary root growth by altering cell proliferation in the root apical meristem via modifications in the ethylene biosynthesis pathway (Qin, 2019). Similar reductions in root length under salinity have been reported by Arif *et al.* (2017), Ravikiran *et al.* (2017) and Banumathy *et al.* (2020).

High shoot fresh weight and shoot dry weight stress tolerance index (STI) values were recorded for Pokkali and Vytilla10, whereas IR29, TKM13, and CR1009sub1 exhibited lower STI values for both these traits. Additionally, for root fresh weight (RFW), CSR43, Pokkali, and TRY3 demonstrated high STI values (0.87, 0.84 and 0.75 respectively), while CSR27, IR29 and TKM13 exhibited low STI values (0.11, 0.21, and 0.23, respectively). In terms of root dry weight (RDW), V10, Pokkali, and CSR43 displayed high STI values (0.56, 0.50, and 0.50, respectively), while CSR27, IR29 and TKM13 showed low STI values (0.13, 0.10, and 0.14, respectively). Moreover, TFW and TDW exhibited high STI values in Pokkali, Vytilla10, and Vytilla8, whereas IR29, TKM13 and CR1009sub1 displayed lower STI values (0.23, 0.10, and 0.26 for TFW, and 0.10, 0.16, and 0.15 for TDW, respectively). The reduction in fresh and dry weight is attributed to the accumulation of sodium and chloride ions in plant tissues, resulting in cell wall solidification, damage to cell ultrastructure, and impaired metabolic activities (Pauk and Jansco *et al.*, 2017). Decreased root dry weight may be caused by inadequate nutrient availability to roots and the detrimental effects of salt and chloride ions (Rasel *et al.*, 2020). This observation is consistent with findings by Chanthabhuree *et al.* (2016), Pongprayoon *et al.* (2018), Arif *et al.* (2018), Rasel *et al.* (2020), and Rasel *et al.* (2021).

### Salt tolerance evaluation

To classify the salt tolerance of 28 rice genotypes, the membership function values (MFV) for each trait of each genotype and the average MFV were calculated (see Table 2). The mean and standard deviation (SD) of the average MFV were 0.50 and 0.22, respectively, with the range of average MFV varying between 0.02 to 0.94. Based on the average MFV, 26 rice genotypes and 2 checks were categorized into five salt tolerance groups. Specifically, two genotypes (Pokkali and Vytilla 10) were grouped as highly salt tolerant ( $\bar{X} \geq 0.87$ ), 4 genotypes (Jothi, V8, CSR56, and CSR43) as tolerant ( $0.73 \leq \bar{X} < 0.87$ ), 18 genotypes as moderately tolerant ( $0.28 \leq \bar{X} < 0.73$ ), 3 genotypes (CR1009sub1, IR64, and TKM13) as susceptible ( $0.13 \leq \bar{X} < 0.28$ ), and one genotype (IR29) as highly susceptible ( $\bar{X} < 0.13$ ). Salinity exerted deleterious effects on all morphological traits (Munns and Tester, 2008; Pongprayoon *et al.*, 2018), consistent with observations in the present study. This effect may be attributed to the accumulation of excess salt in older leaves, leading to leaf senescence (Arif *et al.*, 2017). Consequently, the reduction in the number of photosynthetically active leaves decreased photosynthesis rates, limiting the supply of carbohydrates and growth hormones to meristematic tissues, ultimately impeding plant growth (Munns and Tester, 2008). Salinity-induced growth reduction varied between tolerant and susceptible genotypes, with highly tolerant and tolerant genotypes exhibiting less reduction in growth parameters compared to highly susceptible and susceptible genotypes. Similar findings have been reported by Hariadi *et al.* (2015), Chanthabhuree *et al.* (2016) and Arif *et al.* (2018), Rasel *et al.* (2020), and Rasel *et al.* (2021). This may be attributed to the genotypes' capability to adopt tolerance mechanisms through physiological and biochemical changes (Rasel *et al.*, 2019), including salt exclusion, ion compartmentation (Chanthabhuree *et al.*, 2016).

### Conclusion

Utilizing a membership function value (MFV)-based system, rice genotypes were effectively classified for salt tolerance. This categorization identified highly tolerant and tolerant genotypes suitable for integration into breeding programs aimed at enhancing salt tolerance during the seedling stage in rice.

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**Table 1. Stress Tolerance Index (STI) Values of Eight Traits for Twenty-Six Genotypes and Two Control genotypes**

Genotypes	SL STI	RL STI	SFW STI	RFW STI	SDW STI	RDW STI	TFW STI	TDW STI
ADT55	0.37	0.4	0.49	0.59	0.26	0.22	0.51	0.22
CO56	0.49	0.67	0.49	0.43	0.37	0.34	0.46	0.36
C055	0.35	0.5	0.41	0.52	0.28	0.27	0.46	0.28
CO52	0.49	0.54	0.43	0.5	0.32	0.36	0.47	0.34
CR1009SUB1	0.47	0.68	0.24	0.26	0.16	0.16	0.26	0.15
FR13a	0.42	0.51	0.44	0.39	0.31	0.31	0.42	0.31
ADT36	0.42	0.71	0.58	0.51	0.37	0.34	0.55	0.36
CSR27	0.49	0.4	0.4	0.11	0.33	0.13	0.28	0.24
CSR30	0.58	0.45	0.44	0.41	0.27	0.34	0.42	0.29
CSR36	0.43	0.6	0.55	0.62	0.34	0.38	0.58	0.37
CSR43	0.55	0.82	0.5	0.87	0.38	0.5	0.6	0.43
IG12	0.55	0.64	0.54	0.6	0.28	0.26	0.57	0.28
IG13	0.49	0.8	0.39	0.56	0.23	0.29	0.45	0.26
IG44	0.47	0.77	0.35	0.44	0.24	0.28	0.4	0.25
IR29	0.26	0.33	0.24	0.21	0.09	0.13	0.23	0.1
IR64	0.37	0.36	0.31	0.46	0.19	0.24	0.38	0.22
ASD18	0.48	0.42	0.37	0.36	0.32	0.32	0.37	0.32
Jothi	0.65	0.74	0.61	0.75	0.39	0.45	0.66	0.42
TRY2	0.58	0.43	0.35	0.49	0.25	0.26	0.4	0.25
Paiyaur 1	0.41	0.5	0.57	0.58	0.35	0.31	0.57	0.33
Pokkali	0.69	0.8	0.68	0.84	0.53	0.5	0.74	0.52
CSR56	0.58	0.73	0.63	0.68	0.42	0.38	0.65	0.41
TKM13	0.51	0.68	0.24	0.23	0.18	0.14	0.24	0.16
TRY3	0.53	0.66	0.59	0.75	0.39	0.38	0.66	0.39
TRY4	0.48	0.48	0.58	0.51	0.32	0.36	0.54	0.34

<b>VYTILLA 4</b>	0.47	0.52	0.56	0.61	0.29	0.29	0.59	0.29
<b>VYTILLA 8</b>	0.64	0.84	0.64	0.72	0.39	0.48	0.68	0.43
<b>VYTILLA10</b>	0.8	0.83	0.65	0.71	0.44	0.56	0.68	0.49
<b>Minimum</b>	0.26	0.33	0.24	0.11	0.09	0.13	0.23	0.10
<b>Maximum</b>	0.80	0.84	0.68	0.87	0.53	0.56	0.74	0.52

**Table 2. Membership Function Values (MFV) and Average MFV Traits for 26 Genotypes and Two Control genotypes**

Genotypes	SL MFV	RL MFV	SFW MFV	RFW MFV	SDW MFV	RDW MFV	TFW MFV	TDW MFV	AVG MFV	ST Grades
<b>ADT55</b>	0.20	0.14	0.57	0.63	0.39	0.21	0.55	0.29	0.37	MT
<b>CO56</b>	0.43	0.67	0.57	0.42	0.64	0.49	0.45	0.62	0.53	MT
<b>C055</b>	0.17	0.33	0.39	0.54	0.43	0.33	0.45	0.43	0.38	MT
<b>CO52</b>	0.43	0.41	0.43	0.51	0.52	0.53	0.47	0.57	0.49	MT
<b>CR1009SUB1</b>	0.39	0.69	0.00	0.20	0.16	0.07	0.06	0.12	0.21	S
<b>fr13a</b>	0.30	0.35	0.45	0.37	0.50	0.42	0.37	0.50	0.41	MT
<b>ADT36</b>	0.30	0.75	0.77	0.53	0.64	0.49	0.63	0.62	0.59	MT
<b>CSR27</b>	0.43	0.14	0.36	0.00	0.55	0.00	0.10	0.33	0.24	MT
<b>CSR30</b>	0.59	0.24	0.45	0.39	0.41	0.49	0.37	0.45	0.42	MT
<b>CSR36</b>	0.31	0.53	0.70	0.67	0.57	0.58	0.69	0.64	0.59	MT
<b>CSR43</b>	0.54	0.96	0.59	1.00	0.66	0.86	0.73	0.79	0.76	T
<b>IG12</b>	0.54	0.61	0.68	0.64	0.43	0.30	0.67	0.43	0.54	MT
<b>IG13</b>	0.43	0.92	0.34	0.59	0.32	0.37	0.43	0.38	0.47	MT
<b>IG44</b>	0.39	0.86	0.25	0.43	0.34	0.35	0.33	0.36	0.41	MT
<b>IR29</b>	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.02	HS
<b>IR64</b>	0.20	0.06	0.16	0.46	0.23	0.26	0.29	0.29	0.24	S
<b>ASD18</b>	0.41	0.18	0.30	0.33	0.52	0.44	0.27	0.52	0.37	MT
<b>Jothi</b>	0.72	0.80	0.84	0.84	0.68	0.74	0.84	0.76	0.78	T
<b>TRY2</b>	0.59	0.20	0.25	0.50	0.36	0.30	0.33	0.36	0.36	MT
<b>Paiyaur 1</b>	0.28	0.33	0.75	0.62	0.59	0.42	0.67	0.55	0.53	MT
<b>Pokkali</b>	0.80	0.92	1.00	0.96	1.00	0.86	1.00	1.00	0.94	HT
<b>CSR56</b>	0.59	0.78	0.89	0.75	0.75	0.58	0.82	0.74	0.74	T
<b>TKM13</b>	0.46	0.69	0.00	0.16	0.20	0.02	0.02	0.14	0.21	S
<b>TRY3</b>	0.50	0.65	0.80	0.84	0.68	0.58	0.84	0.69	0.70	MT
<b>TRY4</b>	0.41	0.29	0.77	0.53	0.52	0.53	0.61	0.57	0.53	MT
<b>VYTILLA 4</b>	0.39	0.37	0.73	0.66	0.45	0.37	0.71	0.45	0.52	MT
<b>VYTILLA 8</b>	0.70	1.00	0.91	0.80	0.68	0.81	0.88	0.79	0.82	T
<b>VYTILLA 10</b>	1.00	0.98	0.93	0.79	0.80	1.00	0.88	0.93	0.91	HT