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RESEARCH ARTICLE

Screening for salinity tolerance in rice landraces through morphological and molecular markers

Juliet Hepziba S[1], Anisha Parveen R[2], Aashika N[3], Aathilakshmi S[3], Kavitha Pushpam A[4] & Arumugachamy S[1*]

[1]Department of Genetics and Plant Breeding, V.O. Chidambaranar Agricultural College & Research Institute, Killikulam 628 252, Tamil Nadu, India
[2]Department of Genetics and Plant Breeding, The Directorate of Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[3] V.O. Chidambaranar Agricultural College & Research Institute, Killikulam 628 252, Tamil Nadu, India
[4]Department of Crop Physiology and Biochemistry, V.O. Chidambaranar Agricultural College & Research Institute, Killikulam 628 252, Tamil Nadu, India

*Correspondence email - arumugachamys@tnau.ac.in, juliethepziba.s@tnau.ac.in

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Abstract

Salinity stress is a major challenge to global rice production and agricultural sustainability, especially as climate change intensifies its impact. This study highlights the need for salinity-tolerant rice genotypes to support climate-resilient agriculture. Seventeen traditional rice landraces were evaluated alongside a salt-tolerant check (PTB 33) and a susceptible check (IR 64) using phenotypic and molecular approaches. Phenotypic assessments included germination percentage, salt tolerance index, seedling growth and growth reduction under NaCl-induced salinity levels of 2500 ppm, 5000 ppm, 7500 ppm and 10000 ppm. Molecular characterization involved 20 simple sequence repeat (SSR) markers, of which six (RM 8094, RM 237, RM 314, RM 3412, RM 562 and RM 284) were identified as polymorphic. The polymorphic information content (PIC) values were calculated for all six markers to assess marker informativeness. Genetic diversity analysis using DARwin 6.0 software grouped genotypes into four distinct clusters, demonstrating substantial variability. Among the genotypes, Kothamalli Samba showed strong salinity tolerance with high germination rates, robust seedling growth and minimal growth reduction across all salinity levels. Maikuruvai and Nellaiyappar exhibited moderate tolerance, whereas Kallundai was highly susceptible. The molecular findings corroborated the phenotypic results, offering valuable insights into the genetic basis of salinity tolerance. These findings provide a foundation for developing mapping populations and precision breeding strategies aimed at enhancing salinity resilience in rice. By leveraging genetic diversity, breeders can optimize heterosis, accelerate the development of high-yielding, salt-tolerant rice varieties and strengthen climate-resilient agriculture.

Keywords: landraces; salinity; salt tolerant index

Introduction

Rice (*Oryza sativa* L.) is a vital food crop providing sustenance to over half of the global population, which underscores its importance for food security. However, salinity stress poses a significant barrier to rice production, particularly in coastal and irrigated regions where soils are often affected by high salt levels (1). Globally, out of 380 million hectares (M ha) of saline soils, 28.2 M ha of coastal saline soils are located in South and Southeast Asia. In India alone, 3.1 M ha of salt-affected soils are distributed across coastal states such as Gujarat, Maharashtra, Kerala, Tamil Nadu, Andhra Pradesh, Odisha, West Bengal and the Andaman and Nicobar Islands (2). This abiotic stress disrupts critical physiological and molecular functions including ionic homeostasis, osmotic regulation and energy metabolism, ultimately hampering plant growth, yield and survival (3). Traditional rice varieties known for their genetic diversity and unique adaptations to specific environments often display variable levels of tolerance to salinity (4). As a result, significant efforts have been directed toward investigating the phenotypic traits and molecular mechanisms associated with salinity resilience (1). Studies have combined phenotypic evaluations such as germination rates, seedling vigour and biomass production with molecular research identifying key quantitative trait loci (QTLs) such as Saltol along with genes and regulatory pathways involved in ion transport and osmotic adjustments (3). These efforts aim to uncover the genetic and physiological strategies employed by salt-tolerant rice varieties to maintain photosynthetic efficiency, nutrient uptake and cellular integrity under saline conditions. Integrating phenotypic and molecular approaches offers a robust framework for breeding and genetic engineering initiatives to enhance salinity tolerance in rice.

Materials and Methods

Seventeen traditional rice varieties (Table S1), along with a tolerant check (PTB 33) and a susceptible check (IR 64), were screened at varying salinity levels under laboratory conditions at V.O. Chidambaranar Agricultural College and Research Institute, Killikulam, Tamil Nadu, India. Seeds from the selected rice varieties were surface sterilized with 1 % (v/v) sodium hypochlorite for 10 minutes, rinsed four times with distilled water and blotted dry on germination paper. Ten seeds per replication for each variety were placed in petri dishes containing 10 mL of NaCl solution at concentrations of 0, 2500, 5000, 7500 and 10000 ppm. The experiment was conducted using a Completely Randomized Block Design (CRBD) with two replications for each variety. Observations on shoot length and root length were recorded on the 14th day by measuring the average height of five seedlings per replication and expressing the results in centimetres.

Germination Percentage

The percentage of germinated seeds was calculated using the formula:

$$GE(\%) = \left(\frac{\text{Number of seeds germinated}}{\text{Number of seeds tested}} \right) \times 100$$

Shoot Length

Shoot length was measured from the collar region to the tip of the plumule for the same five seedlings used for root length measurements. The mean value was calculated and expressed in centimetres.

Root Length

Root length was measured from the collar region to the tip of the primary root in five randomly selected seedlings. The mean value was calculated and expressed in centimetres.

Fresh and Dry Weight of Shoots and Roots

Shoots and roots were separated using a blade and their fresh weights were recorded using a weighing balance. For dry weight, the plant samples were exposed to 60 °C for 5 minutes in a hot air oven before weighing.

Salt tolerant Index

The Salt Tolerance Index (STI) is calculated as the ratio of the value of a trait under salt stress to the value of the same trait under control (non-stressed) conditions. It is a way to quantify the ability of a plant to tolerate salt stress.

STI = Value under salt stress / Value under control conditions.

Molecular Analysis

Genomic DNA Extraction

Genomic DNA was extracted from plant tissues grown using the roll-towel germination method for 14 days, following a modified CTAB protocol (5). A 0.5 g leaf sample was finely chopped, ground in a sterilized mortar with 200 µL of 2X CTAB extraction buffer and homogenized into a smooth paste. The mixture was transferred to 2 mL microcentrifuge tubes and an additional 400 µL of extraction buffer was added. The samples were incubated at 65 °C for 20 min, followed by the addition of 600 µL chloroform: isoamyl alcohol (24:1, v/v) and shaken for 10 min. After centrifugation at 12000 rpm for 15 min, the upper aqueous phase was transferred to fresh tubes and DNA was precipitated by adding an equal volume of ice-cold isopropanol and the samples were stored at 4 °C overnight. The following day, DNA was pelleted by centrifugation, washed with 70 % ethanol, air-dried and resuspended in 50-100 µL of 1X TE buffer or Milli-Q water. To prevent RNA contamination, 10 µL of RNase was added per 200 µL of DNA sample before storage at -20 °C.

DNA Fingerprinting Using PCR

Genetic fingerprinting was performed using 20 SSR markers, out of which 6 markers were identified as polymorphic. PCR reactions were conducted in a 25 µL reaction volume containing genomic DNA (1 µL), dNTPs (1 µL), primers (2 µL), 10× PCR buffer (2.5 µL), Taq DNA polymerase (0.5 µL) and sterile water. The PCR profile included an initial denaturation at 95 °C for 1 min, followed by 35 cycles at 95 °C for 45 sec, 50-58 °C for 45 sec (depending on primer Tm) and 72 °C for 45 sec, with a final extension at 72 °C for 5 min. PCR products were resolved on 1.5-3 % agarose gels, visualized under UV light and compared against DNA markers.

Cluster analysis

Cluster analysis was performed using marker data obtained from SSR-based DNA fingerprinting. The data were analysed using DARwin 6 software to generate dendrograms and determine the genetic relationships among the traditional rice varieties. The software utilized the neighbour-joining method and genetic distances were calculated to group varieties based on their salinity tolerance and genetic diversity. The results provided insights into the clustering of salt-tolerant and susceptible varieties, aiding in the identification of promising genotypes for breeding programs.

Polymorphic Information Content (PIC) calculation

The PIC value for each SSR marker was calculated using the formula:

$$PIC = 1 - \sum Pi2$$

Where:

P_i is the frequency of the i^{th} allele detected for a given marker. Markers with higher PIC values were considered more informative for assessing genetic diversity.

Results and Discussion

The salinity tolerance of 17 traditional landraces was assessed alongside the tolerant check (PTB 33) and the susceptible check (IR 64) under salinity levels of 0, 2500, 5000, 7500 and 10000 ppm, based on key parameters such as germination percentage, shoot length, root length, salinity tolerance index (STI) and biomass measurements. PTB 33 exhibited strong tolerance, maintaining high germination rates, with only a slight decline from 85 % at 0 ppm to 75 % at 10000 ppm, whereas IR 64 showed extreme sensitivity, with germination dropping from 50 % to 0 % under the same conditions. Among the traditional varieties, Neelam Samba, Maikuruvai and Ketki Joha (Assam) demonstrated superior salinity tolerance, maintaining 100 % germination even at 10000 ppm. Kothamalli Samba and Karunkuruvai also performed well, retaining 100 % germination up to 5000 ppm and 90 % at 10000 ppm. In contrast, Athur Kichili Samba and Kallundai showed significant reductions in germination, closely resembling the response of IR 64. These findings align with previous studies, which reported similar declines in germination percentage, germination speed and fresh and dry weight of plumule and radicle in rice cultivars under increasing NaCl stress (6). The inhibitory effects of higher NaCl concentrations (120 and 150 mM) on seed germination has been corroborated by a previous study (7). The adverse effects of salinity on germination are largely attributed to osmotic stress, which limits water uptake and disrupts essential metabolic processes necessary for seedling establishment (8). Similar observations have been reported, demonstrating the impact of salt stress on seedling vigor through inhibiting enzymatic activity and early growth dynamics in susceptible rice varieties (9).

PTB 33, the tolerant check, exhibited the highest resilience, maintaining nearly stable shoot and root growth (Fig. 1) across all salinity levels, with only a minor reduction of 5.33 % in shoot and -3.13 % in root growth at 10000 ppm. Similarly, Ketki Joha (Assam) and Kothamalli Samba demonstrated strong tolerance, with minimal shoot and root growth reductions (Table 1), making them potential candidates for cultivation in saline environments. Neelam Samba and Navara showed moderate tolerance, handling salinity stress effectively up to 7500 ppm before experiencing significant declines. In contrast, IR 64, the susceptible check, exhibited extreme sensitivity, with 100 % reduction in both shoot and root growth at 10000 ppm, confirming its unsuitability for saline conditions. Other varieties, such as Kallundai and Chakoambi, also showed drastic reductions, with over 50 % loss in shoot and root growth at higher salinity levels. Maikuruvai and Kamban Samba displayed intermediate tolerance, showing stability up to 5000 ppm but experiencing gradual declines thereafter. Overall, the results highlight the exceptional performance of PTB 33, Ketki Joha (Assam) and Kothamalli Samba in maintaining growth under high salinity, while confirming the vulnerability of IR 64 and other similarly sensitive varieties. Root growth is more adversely affected than shoot growth under salinity stress (10). The salt-sensitive rice genotypes exhibit greater shoot length reductions due to NaCl-induced ionic imbalances (11). The salt tolerance in rice is strongly correlated with root architecture, where tolerant genotypes maintain root elongation to sustain nutrient uptake despite high salinity levels (12). Additionally, plant height and leaf area index were significantly impacted by saline conditions, further corroborating these results (13).

The salinity tolerance index (STI) (Table 2) further underscored the exceptional performance of certain traditional varieties. PTB 33 maintained an average STI of 95.59, while IR 64 recorded a low average of 30. Traditional varieties like Kothamalli Samba consistently exceeded STI values of 100 across all salinity levels, surpassing PTB 33. Neelam Samba

Table 1. Growth reduction percentage under varying salinity levels

Varieties	Control	Growth Reduction (%) Shoot Growth 2500 ppm	5000 ppm	7500 ppm	10000 ppm	Control	Growth Reduction (%) Root Growth 2500 ppm	5000 ppm	7500 ppm	10000 ppm
Kuruvaikalayan	8.3	-15.6	-4.58	-2.41	-2.41	12.1	0.00	-4.96	1.24	19.83
Neelam samba	12.8	8.59	34.38	34.77	46.88	14.3	4.90	7.69	14.34	36.36
Maikuruvai	6.6	0.00	0.00	2.17	-1.45	10.2	-13.7	-14.7	-10.7	0.00
Bardhamal	7.4	4.05	18.24	17.57	22.30	7.9	5.70	0.95	1.27	8.23
Nellaiyappar	7.8	-1.91	1.47	4.41	10.29	13.05	32.18	36.40	37.93	38.70
Iluppaipoo samba	6.4	6.25	9.38	7.81	35.94	10.75	6.05	4.65	2.33	48.84
Kothamalli samba	6.325	-3.56	0.40	-1.98	2.77	12.38	18.42	0.65	15.59	17.61
Athur kichili samba	5.5	-1.82	9.09	9.09	2.73	6.5	13.85	13.46	7.69	11.54
Karunkuruvai	8.7	5.17	13.79	20.69	24.71	10.6	0.00	-29.2	-12.7	13.21
Kallundai	7.25	13.79	41.38	37.93	55.86	7	14.29	14.29	0.00	7.14
Navara	9.2	33.70	36.96	35.87	39.95	16.3	13.50	8.59	19.33	44.48
Kundhal	9.6	13.02	11.46	24.22	54.17	17	10.00	46.32	40.00	48.82
Ketki joha (Assam)	9.7	7.22	8.25	9.28	10.31	13.1	27.48	32.06	7.63	27.48
Kottara samba	8.5	-11.7	32.35	50.00	52.94	15.5	-3.23	22.58	27.42	22.58
Kalanamak	9.1	0.00	3.85	6.59	8.79	10.3	-21.3	-24.2	-3.88	-12.62
Kamban samba	7.4	17.57	16.22	28.38	32.43	6.6	3.03	9.09	12.12	16.67
Chakoambi	10.6	7.74	27.36	34.43	56.60	14	25.71	22.86	20.71	40.00
IR 64	6.8	70.59	63.24	70.59	100.00	9.75	28.21	43.59	48.72	100.00
PTB 33	8.45	-1.18	0.95	-0.59	5.33	8	-8.75	-1.25	0.62	-3.13

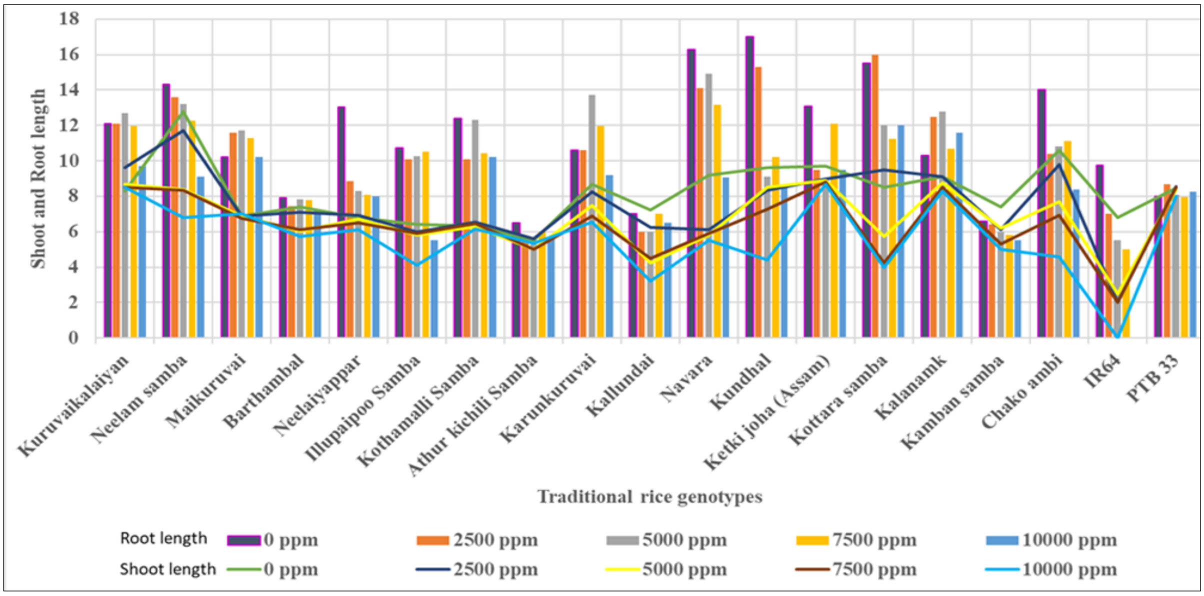

Fig. 1. Shoot and Root growth of genotypes under different salinity levels.

(average STI: 94) and Ketki Joha (Assam) (average STI: 100) also displayed strong performance. In contrast, Athur Kichili Samba (average STI: 56.25) and Kallundai (average STI: 45) had low STI values, like IR 64. This reduction in salinity tolerance is consistent with previous findings, which demonstrated that different rice genotypes exhibit varying degrees of salinity tolerance, affecting morpho-physiological attributes and yield components (14, 15). These findings also align with previous reports indicating that high STI values are indicative of strong salinity resilience in rice landraces, which tend to accumulate less Na^+ in leaf tissues compared to commercial hybrids (16).

Biomass measurements (Table 3) further highlighted the tolerance of PTB 33, which retained over 80 % of its control fresh and dry weights even at 10000 ppm, while IR 64 exhibited a complete loss of biomass under the same salinity conditions. Among the traditional varieties, Kothamalli Samba (0.47 g shoot, 0.48 g root fresh weight) and Ketki Joha (Assam) (0.43 g shoot, 0.33 g root fresh weight) maintained biomass levels comparable to PTB 33, demonstrating strong tolerance. However, Athur Kichili Samba and Kallundai showed significant reductions in biomass, recording shoot fresh weights of 0.34 g and 0.33 g, respectively, at 10000 ppm, resembling the response of IR 64. These variations in biomass retention under salinity stress among different genotypes align with previous research. Previous studies have reported a 30 % reduction in yield and a 24 % loss in dry matter weight under salt stress (17). Moreover, high Na^+ accumulation in leaves induces osmotic stress, which adversely affects growth and accelerates senescence, potentially leading to reduced biomass in susceptible varieties (18). Salt-tolerant rice varieties effectively regulate K^+/Na^+ ratios to maintain higher biomass production, whereas susceptible varieties suffer rapid biomass loss due to ion toxicity and osmotic stress (19).

Genetic diversity analysis using 20 SSR markers revealed varying levels of polymorphism, with 6 markers (Fig. 2) identified as polymorphic, as indicated by the polymorphic information content (PIC) values (Table 4). The PIC values ranged from 0.55 to 0.65, with RM 8094 recording the highest PIC value of 0.65, followed by RM 3412 at 0.61. Other polymorphic markers, such as RM 314 and RM 237, recorded values of 0.60 and 0.56, respectively, while RM 562 and RM 284 had slightly lower values of 0.55 and 0.57. The high PIC values suggest that these polymorphic markers were highly informative in differentiating salt-tolerant and susceptible varieties, making them valuable for further genetic studies and marker-assisted selection.

Cluster analysis based on SSR marker data grouped the seventeen traditional rice varieties into four distinct clusters (Table 5), reflecting differences in salinity tolerance. Cluster 1 (Fig. 3) included Kottara Samba, Kamban Samba, Kundhal and Navara, which demonstrated strong salinity tolerance, maintaining high germination rates and biomass retention. Cluster 2 comprised Maikuruvai, Iluppaipoo Samba and Nellaiyappar, which exhibited moderate salinity tolerance, showing a balanced response to increasing salinity levels. Cluster 3 grouped together Karunkuruvai, Athur Kichili Samba, Kothamalli Samba, Chakoambi, Kalanamak and Ketki Joha (Assam), displaying varying degrees of tolerance, with Kothamalli Samba and Ketki Joha (Assam) performing significantly better than Athur Kichili Samba. Cluster 4 included Kallundai, Kuruvaikalayan, Neelam Samba and Bardhamal, which were highly sensitive to salinity stress, showing sharp declines in germination and growth parameters.

This study highlights the potential of traditional rice varieties like Kothamalli Samba, Neelam Samba and Ketki Joha (Assam) as genetic resources for breeding programs aimed at improving salinity resilience in rice. Their adaptability to saline environments positions them as ideal candidates for introgressing stress-tolerant traits into high-yielding but sensitive varieties like IR 64, contributing to sustainable rice production in salinity-affected regions.

Conclusion

This study comprehensively evaluated the salinity tolerance of 17 traditional rice varieties compared to the tolerant check (PTB 33) and the susceptible check (IR 64) using germination percentage, shoot and root lengths, (STI) and biomass measurements across varying salinity levels. Traditional varieties such as Kothamalli Samba, Neelam Samba and Ketki Joha

Table 2. Salinity Tolerant Index (STI) of varieties on the basis of germination percentage under varying salt stress

Variety	Germination percentage on different salinity levels					STI on different salinity levels (dSm^{-1})				Mean STI
	0 ppm	2500 ppm	5000 ppm	7500 ppm	10000 ppm	2500 ppm	5000 ppm	7500 ppm	10000 ppm	
Kuruvaikalayan	100	100	90	90	90	100	90	90	90	92.5
Neelam samba	100	100	100	100	100	100	100	100	100	100
Maikuruvai	100	100	100	100	100	100	100	100	100	100
Bardhamal	100	90	90	85	80	60	90	60	80	72.5
Nellaiyappar	90	90	90	90	90	100	100	100	100	100
Iluppaipoo samba	100	90	90	90	90	70	80	90	90	82.5
Kothamalli samba	100	100	100	100	100	111.1	111.1	111.1	111.1	111.1
Athur kichili samba	40	35	35	35	30	75	75	50	25	56.25
Karunkuruvai	100	100	100	100	80	100	100	100	80	95
Kallundai	50	30	30	20	10	60	60	40	20	45
Navara	100	100	100	90	90	100	100	90	90	95
Kundhal	100	90	90	90	80	90	90	90	80	87.5
Ketki joha (Assam)	100	100	100	100	100	100	100	100	100	100
Kottara samba	60	50	40	30	10	83.3	66.6	50	16.67	54.17
Kalanamak	100	90	90	90	90	90	90	90	90	90
Kamban samba	100	100	100	100	100	100	100	100	100	100
Chakoambi	100	100	90	90	90	100	90	90	100	95
IR 64	50	20	15	10	0	40	40	40	0	30
PTB 33	85	80	80	80	75	94.1	82.35	100	105.88	95.59

Table 3. Seedling growth under varying salinity levels

Varieties	Salinity level	Seedling growth							
		Shoot			Root			Salinity Tolerance Index (STI)	
		Fresh weight (g)	Dry weight (g)	STI	Fresh weight (g)	Dry weight (g)	STI	Average STI	Mean STI
Kuruvaikalayan	0	0.38	0.3		0.4	0.3		0	104.00
	2500 ppm	0.53	0.31	103	0.56	0.31	103	103	
	5000 ppm	0.46	0.31	103	0.39	0.3	100	101.5	
	7500 ppm	0.58	0.33	110	0.4	0.4	103	106.5	
	10000 ppm	0.48	0.33	110	0.39	0.3	100	105	
Neelam samba	0	0.5	0.36		0.37	0.33		0	94
	2500 ppm	0.54	0.34	94	0.38	0.31	94	94	
	5000 ppm	0.48	0.34	94	0.47	0.32	97	97	
	7500 ppm	0.54	0.36	106	0.48	0.32	97	97	
	10000 ppm	0.51	0.35	103	0.35	0.31	94	94	
Maikuruvai	0	0.34	0.31		0.33	0.3		0.00	102.25
	2500 ppm	0.48	0.32	103	0.48	0.31	103	103	
	5000 ppm	0.48	0.33	106	0.47	0.3	100	103	
	7500 ppm	0.43	0.32	103	0.38	0.3	100	101.50	
	10000 ppm	0.48	0.33	106	0.5	0.29	97	101.50	
Bardhamal	0	0.39	0.32		0.38	0.31		0	100
	2500 ppm	0.37	0.32	100	0.33	0.31	100	100	
	5000 ppm	0.37	0.31	103	0.33	0.31	100	101.50	
	7500 ppm	0.37	0.32	100	0.32	0.3	97	98.50	
	10000 ppm	0.36	0.31	103	0.32	0.3	97	100	
Nellaiyappar	0	0.4	0.31		0.32	0.3		0	101
	2500 ppm	0.38	0.31	100	0.33	0.3	100	100	
	5000 ppm	0.42	0.32	103	0.33	0.31	103	103	
	7500 ppm	0.4	0.32	103	0.32	0.31	103	103	
	10000 ppm	0.4	0.3	96	0.32	0.3	100	98	
Iluppaipoo samba	0	0.44	0.32		0.37	0.31		0	97.38
	2500 ppm	0.46	0.32	100	0.4	0.24	77	88.50	
	5000 ppm	0.44	0.31	96	0.45	0.31	100.	98.00	
	7500 ppm	0.48	0.3	93	0.42	0.34	110	101.50	
	10000 ppm	0.4	0.34	106	0.42	0.3	97	101.50	
Kothamalli samba	0	0.39	0.32		0.41	0.31		0	104.50
	2500 ppm	0.44	0.33	103	0.41	0.32	103	103	
	5000 ppm	0.43	0.34	106	0.53	0.32	103	104.50	
	7500 ppm	0.43	0.34	106	0.46	0.32	103	104.50	
	10000 ppm	0.47	0.33	103	0.48	0.34	109	106	
Athur kichili samba	0	0.34	0.3		0.32	0.3		0.00	95.50
	2500 ppm	0.32	0.2	67	0.34	0.3	100	83.50	
	5000 ppm	0.34	0.29	97	0.32	0.31	103	100.00	
	7500 ppm	0.32	0.3	100	0.31	0.3	100	100.00	
	10000 ppm	0.34	0.3	100	0.32	0.29	97	98.50	
Karunkuruvai	0	0.4	0.31		0.44	0.31		0.00	100
	2500 ppm	0.39	0.31	100	0.47	0.32	103	101.50	
	5000 ppm	0.46	0.31	100	0.37	0.31	100	100	
	7500 ppm	0.48	0.3	97	0.44	0.32	103	100	
	10000 ppm	0.41	0.32	103	0.37	0.29	94	98.50	
Kallundai	0	0.32	0.3		0.31	0.3		0.00	101.50
	2500 ppm	0.35	0.31	103	0.33	0.3	100	101.50	
	5000 ppm	0.33	0.3	100	0.32	0.31	103	101.50	
	7500 ppm	0.34	0.31	103	0.32	0.3	100	101.50	
	10000 ppm	0.33	0.31	103	0.31	0.3	100	101.50	
Navara	0	0.41	0.33		0.35	0.34		0.00	98.5
	2500 ppm	0.39	0.33	100	0.36	0.33	97	98.50	
	5000 ppm	0.34	0.33	100	0.39	0.33	97	98.50	
	7500 ppm	0.37	0.32	97	0.34	0.33	97	97.00	
	10000 ppm	0.39	0.34	103	0.33	0.33	97	100.00	
Kundhal	0	0.5	0.32		0.6	0.3		0.00	105.88
	2500 ppm	0.49	0.33	103	0.43	0.32	107	105.00	
	5000 ppm	0.5	0.35	109	0.44	0.32	107	108.00	
	7500 ppm	0.56	0.35	109	0.52	0.31	103	106.00	
	10000 ppm	0.49	0.33	109	0.41	0.3	100	104.50	

Ketki joha (Assam)	0	0.44	0.31		0.45	0.3		0.00	103.13
	2500 ppm	0.46	0.32	103	0.48	0.31	103	103.00	
	5000 ppm	0.41	0.31	100	0.38	0.33	110	105.00	
	7500 ppm	0.39	0.32	103	0.49	0.31	103	103.00	
	10000 ppm	0.43	0.31	100	0.33	0.31	103	101.50	
Kottara samba	0	0.32	0.31		0.3	0.31		0.00	102.00
	2500 ppm	0.33	0.31	100	0.3	0.31	100	100.00	
	5000 ppm	0.44	0.34	110	0.39	0.33	106	108.00	
	7500 ppm	0.42	0.31	100	0.36	0.32	103	101.50	
	10000 ppm	0.34	0.31	100	0.3	0.3	97	98.50	
Kalanamak	0	0.38	0.32		0.32	0.32		0.00	103.13
	2500 ppm	0.39	0.31	97	0.37	0.31	97	97.00	
	5000 ppm	0.41	0.34	106	0.38	0.34	106.	106.	
	7500 ppm	0.51	0.36	113	0.3	0.31	97	105.00	
	10000 ppm	0.4	0.34	106	0.34	0.33	103	104.50	
Kamban samba	0	0.45	0.32		0.44	0.3		0.00	99.63
	2500 ppm	0.45	0.31	97	0.42	0.31	103	100.00	
	5000 ppm	0.42	0.31	97	0.35	0.3	100	98.50	
	7500 ppm	0.42	0.31	97	0.37	0.3	100	98.50	
	10000 ppm	0.44	0.33	103	0.34	0.3	100	101.50	
Chakoambi	0	0.44	0.33		0.33	0.31		0.00	102.38
	2500 ppm	0.54	0.34	103	0.33	0.3	97	100.00	
	5000 ppm	0.48	0.34	103	0.35	0.32	103	103.00	
	7500 ppm	0.32	0.31	94	0.42	0.34	110	102.00	
	10000 ppm	0.46	0.35	106	0.35	0.32	103	104.50	
IR 64	0	0.39	0.35		0.33	0.3		0	66.63
	2500 ppm	0.31	0.27	77	0.31	0.25	83	80	
	5000 ppm	0.31	0.28	80	0.31	0.26	87	83.50	
	7500 ppm	0.34	0.32	119	0.31	0.26	87	103	
	10000 ppm	0	0	0	0	0	0	0	
PTB 33	0	0.41	0.36		0.34	0.33		0	96.25
	2500 ppm	0.41	0.33	92	0.42	0.4	121	106.50	
	5000 ppm	0.41	0.4	111	0.31	0.24	73	92	
	7500 ppm	0.3	0.24	67	0.31	0.26	79	73	
	10000 ppm	0.46	0.38	106	0.4	0.4	121	113.50	

Table 4. List of primers with PIC values

Sr.No	Name of the primer	Base Pair Size	Chromosome Number	PIC value
1	RM 8094	209	1	0.65
2	RM 237	130	1	0.56
3	RM 314	118	6	0.60
4	RM 3412	211	1	0.61
5	RM 562	243	1	0.55
6	RM 284	141	8	0.57

Table 5. Clustering pattern among 17 genotypes

Clusters	No. of genotypes	Details of genotypes
I	4	Kottara samba, Kamban samba, Kundhal, Navara
II	3	Maikuruvai, Iluppaipoo samba, Nellaiyappar
III	6	Karunkuruvai, Athur kichili samba, Kothamalli samba, Chakoambi, Kalanamak, Ketki joha (Assam)
IV	4	Kallundai, Kuruvaikalayan, Neelam samba, Bardhamal

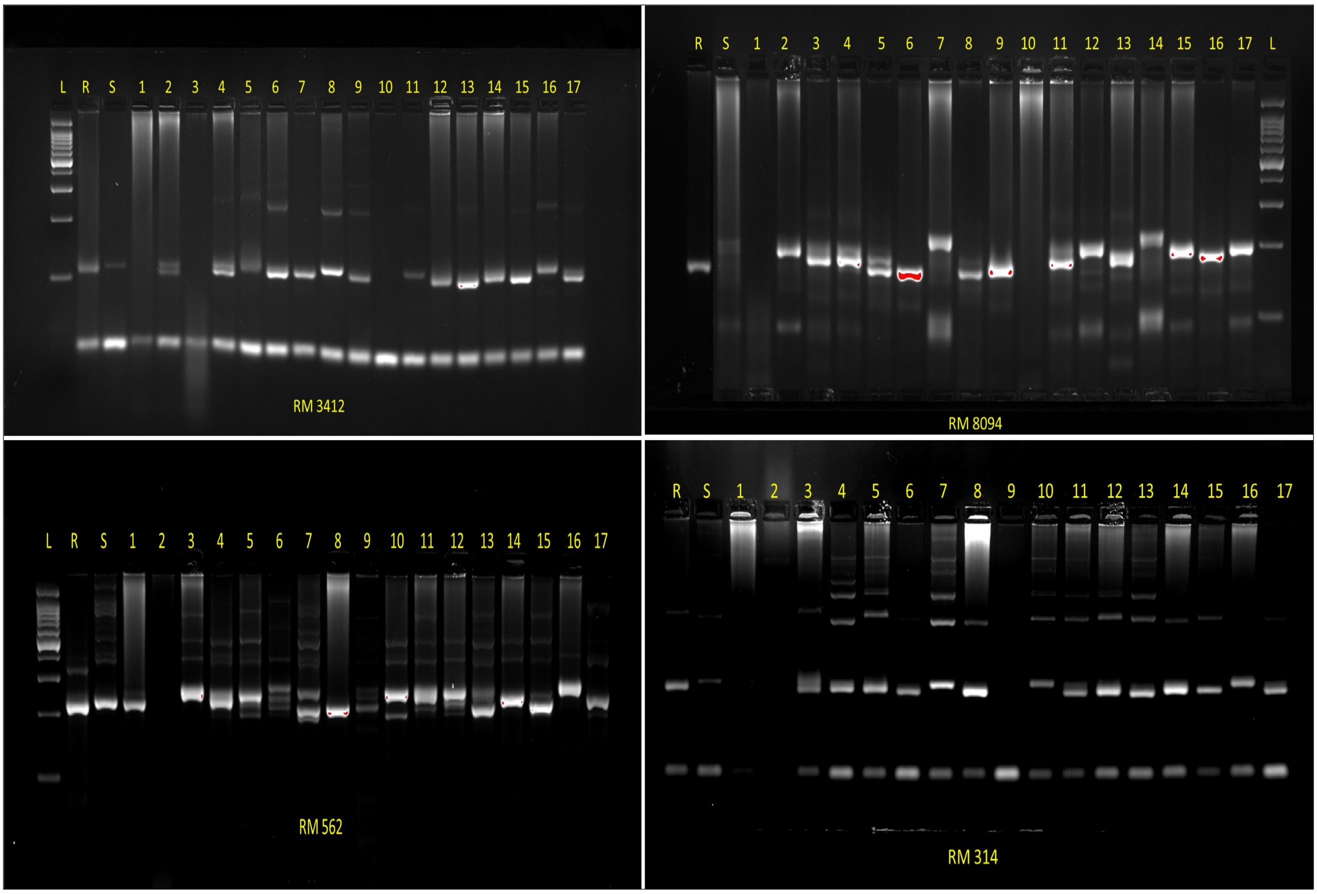

Fig. 2. Gel electrophoresis image showing DNA amplification using SSR markers.

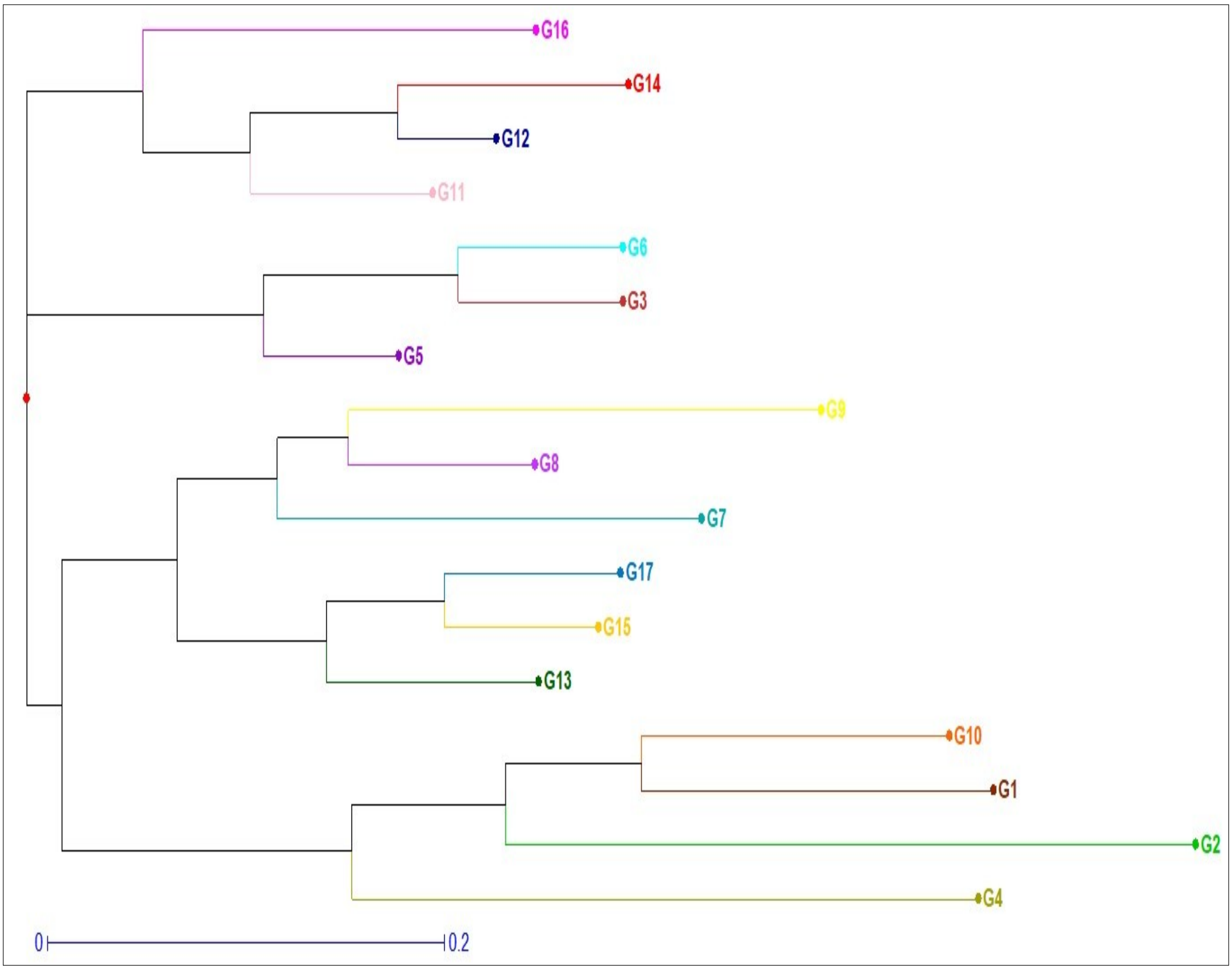

Fig. 3. Dendrogram of 17 genotypes using DARwin software.

(Assam) exhibited exceptional salinity tolerance, outperforming PTB 33 in key parameters, including germination and STI, even at the highest salinity levels (10000 ppm). These varieties, classified under Cluster 1 and Cluster 3, maintained high germination rates, superior shoot and root growth,and strong biomass retention, reinforcing their genetic adaptability to salinity stress. Their higher STI values that exceeds 100 along with the presence of genetic markers on chromosomes 1, 6 and 8 suggest their ability to regulate ion homeostasis and maintain a favorable K^+/ Na^+ balance. Conversely, susceptible varieties such as Athur Kichili Samba and Kallundai, which clustered closer to IR 64, exhibited significant reductions in all parameters, aligning with low STI values (56.25 and 45, respectively), poor germination and reduced biomass retention at increasing salinity levels. These findings indicated their weaker genetic basis for salinity adaptation, necessitating targeted genetic improvement through breeding programs or marker-assisted selection. The results underscore the genetic diversity and resilience of traditional rice varieties, with high PIC values in markers RM 8094 (0.65) and RM 3412 (0.61) further confirming their genotypic variation linked to salinity tolerance. This study highlights the potential of these traditional varieties as valuable genetic resources for breeding salt-tolerant rice cultivars, contributing to the development of improved rice varieties suited for salinity-affected regions. The findings provide a strong foundation for enhancing salinity resilience in rice, ensuring sustainable production and food security in regions facing salinity stress.

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Authors' contributions

JHS conceived the concept of research. APR carried out the molecular genetic studies, drafted the manuscript. SJH reviewed and revised the manuscript. AN and AS² carried out the phenotypic characterisation of the genotypes. KPA and AS¹ conceived of the study and participated in its design and coordination. All authors read and approved the final manuscript. [AS¹ - Aathilakshmi S; AS² - Arumugachamy S]

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