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Evaluation of morphological and molecular characterization of rice (*Oryza sativa* L.) through SSR marker under saline stress condition

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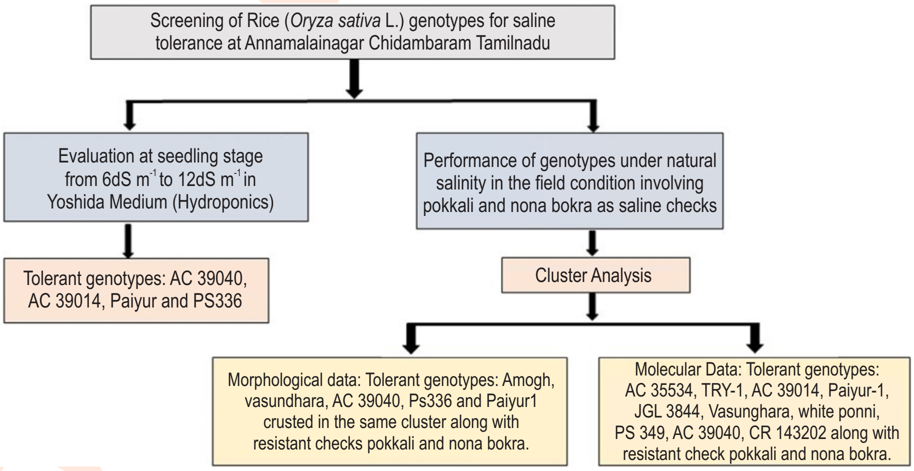
Abstract

Aim: To assess 43 rice genotypes for their resistance to salinity and to assess the hereditary variety by 10 quantitative characteristics just as 40 SSR markers.



Methodology: The seedlings were scored on the basis of 1-9 scale of SES following 10 days of 12 dS/m EC. The morphological traits (10 quantitative traits) were evaluated under natural saline soils (EC: 3.1; pH 7.8) to assess the diversity. At molecular level, 40 Simple Sequence Repeat (SSR) markers were also used to evaluate the diversity in different rice genotypes.

Results: The cluster analysis of genotypes based on the morphological traits, identified the Amogh, Vasundhara, AC39040, PS336, and Paiyur-1 clustered in the Pokkali and Nona Bokra clusters. A total of 111 alleles were produced from 26 polymorphic SSR markers, with an average of 4.26 alleles per locus. The PIC value ranged from 0.314 (RM 13) to 0.824 (RM 6283) with an average of 0.633 and the expected heterozygosity varied from 0.498 to 0.844 with a mean of 0.690. Clustering of genotypes based on the molecular data clubbed Pokkali, AC35534, TRY-1, AC39014, Paiyur-1, JGL3844, Vasundhara, White Ponni, PS 349, AC39040, CR143202, and Nona Bokra in one cluster.

Interpretation: The combination of screening at the seedling stage, morphological diversity, and molecular diversity clearly show the Vasundhara, AC39040, and Paiyur-1 as new sources of salinity. These genotypes may be further explored to identify novel genes/(QTLs) Quantitative Trait Loci, which could increase the production under salt-affected soils.

Key words: Morphological diversity, Molecular diversity, Saline stress, Salinity screening

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Introduction

Rice is the major staple food crop for more than 3.5 billion people all over the globe (Xu *et al.,* 2016). Provides 50-70% of daily calorie intake among poor people. Due to unpredictable changes in the global climate, rice production is severely affected by various abiotic stresses such as salinity, flood, drought, high temperature and heavy metals (Bagdi *et al.,* 2015; Fusi *et al.,* 2014). In India, nearly 8.4 million ha of land has been affected by salinity (Ali *et al.,* 2014). Around the world, consistently 2,000 ha of watered land are being destroyed by undeniable degrees of salt (Machado and Serralheiro, 2017). To satisfy the dietary necessities of a steadily expanding populace, it is quite difficult for raisers to foster salt-open-minded assortments.(Negrao *et al.,* 2017). The most proficient approach to resolve the issue is by improving salt-lenient assortments. Saltiness is one of the most massive abiotic stress and is regarded as a real threat to growth. It reduces the yield or plant death by increasing the salt compartmentalization in the cytoplasm. Excessive accumulation of salt in the roots increases osmotic stress, which affects plant growth, development, and productivity (Ma *et al.,* 2020).

Osmotic stress increases ROS production that causes oxidative damage to biomolecules (Hasanuzzaman *et al.,* 2020). The effect of salinity on growth and development of rice depends on ion concentration, exposure time, growth stage, variety, and soil physical properties. At seedling stage, rice is extremely sensitive to salt stress while at vegetative stage it is moderately sensitive and at reproductive stage, it is highly sensitive (Emon *et al.,* 2015). Typical symptoms at seedling stage include burning or whitening of leaf tips, poor root growth and stunted plant growth (Mohammadi *et al.,* 2014). Plants adopt several stress-responsive mechanisms to mitigate the effects of salinity by changing their cellular, physiological, biochemical, and molecular events. Salt tolerant genotypes evolve several tolerance mechanisms include changes in the structure of membrane, ion excretion, compartmentation of ions in the vacuole, and reducing the concentration of ions in the cytoplasm (Li *et al.,* 2008). Salinity is a polygenic quality that is exceptionally affected by natural factors and shows persistent variety. Qin and Huang (2020) also stated that tolerance to salinity at gemination stage is one of the important breeding objectives.

To evaluate the appropriate response of genotype, a proper screening method should be followed. Rice being more sensitive to salt stress at seedling stage and regenerative stage; screening should be done at both stages for accurate response of a genotype towards salt stress. (Sing *et al.,* 2017) Though a majority of improved cultivars and varieties are sensitive to salt stress, several landraces provide opportunities to improve salt tolerance by exploring them (Rasel *et al.,* 2021). Genetic diversity is generally predicted based on the morphological differences of economically important quantitative traits (Begna, 2021). Due to their quantitative nature, most of these characteristics are profoundly affected by natural conditions and veiled their phenotypic articulation. Because of interactions with the environment, selection based on morphological traits is expected to be less efficient for assessing genetic diversity. Nonetheless, the new advances in atomic science helps to evaluate the hereditary variety with the assistance of DNA markers, which are not affected by climate and phase of the plant, and they give profoundly solid data (Reynolds *et al.,* 2020).

Molecular markers have been used in different genetic studies like marker-assisted selection (MAS), genome-wide association studies (GWAS), QTL mapping, phylogenetic studies, and assessing variability in germplasm. Simple sequence repeats (SSRs) are one such marker widely used to determine genetic diversity in many species including rice (Salgotra and Stewart, 2020). SSRs are co-dominant, highly polymorphic, widely distributed throughout the genome, accurate, multi-allelic, and high throughput in nature, highly advantageous in various plant breeding studies and genetic research. (Vieira *et al.,* 2016; Amiteye, 2021). With this background, the present investigation was undertaken to study the genotypes based on screening at seedling stage, morphological, and molecular diversity to discover a new tolerant variety to salinity to assess the hereditary variety with the assistance of SSR markers.

Materials and Methods

Plant materials: A total of 43 rice genotypes consisted of released varieties, cultures and landraces. They were evaluated for their tolerance under salinity stress. Out of these, 21 genotypes (ADT 36, ADT 37, ADT 39, ADT 40, ADT 41, ADT 42, ADT 43, ADT 44, ADT 45, ADT 46, ADT 47, ADT 48, ADT 49, ADT 50, ADT 51, White Ponni, JGL 3844, CR1009, CR1009 SUB1, TRY-1 and CO-50) were collected from Tamil Nadu Rice Research Institute, Aduthurai; 14 varieties (PS349, Krishna ponni, PS336, PS307, CR143202, AC39040, Vasundhara, AC35534, PS379, AC39000, JBT37/128, PS226, Hankarsonam, BPT 5204) were collected from Acharya N.G. Ranga Agricultural University,Andhra Pradesh. The varieties viz., Dhanisika and Amogh were collected from Agrisis Kurnool Seeds Pvt. Ltd, Andhra Pradesh. The other collected genotypes included Amman rice variety (from Ambasamudram, Tamil Nadu), Paiyur-1 (from Regional Research Station, Paiyur), AC 390149 (from University of Agriculture Science, Mandya), Annapoorna (from Regional Agricultural Research Station, Pattambi, Kerala). Two unique landraces of Kerala viz., Pokkali and NonoBokra were also included in the present study. These two landraces *viz.,* Pokkali and Nono Bokra were highly resistant to salinity and were used as resistant checks. The genotypes *viz.,* CR1009, and ADT 37 were used as susceptible checks. The experiment was conducted at the Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Chidambaram, India.

Evaluation at seedling stage: To evaluate salinity stress at seedling stage, the seeds were surface sterilized with the 1% sodium hypochlorite, washed thoroughly, and imbibed for 48 hrs. Ten seeds per genotype were grown under hydroponics with two replicates. Seedlings were grown in Yoshida nutrient solution for 7 days without NaCl. Following 7 days, the supplement arrangement was supplanted with salinized supplement arrangement at 6 dS m^{-1} (NaCl) EC for 4 days. The salinized arrangement was then changed to 12 dS m^{-1} EC for the following 7 days and the arrangement was maintained at 5.0 to 5.5 pH during whole cycle. (Gosh *et al.,* 2009). The plants grown in non-salinized environment were also kept for comparison that served as. After 10 days of treatment 12 dS m^{-1} EC, the plants were scored based on 1-9 scale of the modified standard evaluation system (Mondal *et al.,* 2016).

Evaluation of morphological traits: The experimental plot was arranged in Randomized Block Design (RBD) with three blocks, and three replications were maintained in each block. Thirty-day old seedlings were transplanted in the experimental plot at a spacing of 25 × 15 cm with the following of all recommended practices to raise the healthy crop. The agro-morphological observations of all genotypes were recorded on ten randomly selected plants of each genotype from each replication under naturally salt-affected soil conditions (EC: 3.1; pH 7.8).A total of 10 key agronomic characters *viz.,* days to 50% flowering (DFF), plant height (PH) (cm), number of productive tillers (NPT), panicle length (PL) (cm), number of grains per panicle (NGP), grain length (GL) (mm), grain breadth (GB)(mm),grain length/breadth ratio (L/B), 100-grain weight (100-GW) (g), and single plant yield (SPY) (g) were evaluated.

Molecular analyses: The genomic DNA was extracted from 21-day-old seedlings by CTAB method (Doyle and Doyle, 1987). The quality of extracted DNA was checked with 0.8% agarose gel and also by spectrophotometer method (ND-1000, Nano Drop Inc., USA). A total of 40 random SSR markers covering all the chromosomes were used to evaluate the diversity of selected rice genotypes. Gradient PCR protocol was followed to find the annealing temperature of selected primers. Ten microliter of PCR reaction mixture contain 50 ng of genomic DNA, 30 ng of each forward and reverse primer, 4 µl of 2× PCR master mix (Amplicon, Denmark), and 4 µl of water. The amplified PCR products were separated on 3% agarose gel, stained with EtBr (ethidium bromide), and observed under UV light for banding pattern.

Data analyses: The morphological data were subjected to cluster analysis with the help of R software (Shah *et al.*, 2018). The coefficient of genetic distance based on 10 morphological traits were calculated to find the similarities among the genotypes. Analysis of variance (ANOVA) was also calculated to find significant variation among the genotypes and the dissimilarity coefficients were calculated using DARwin software (Akohoue *et al.*, 2020) by utilizing Neighbor-Joining method (NJ).

Results and Discussion

Crop breeding programs always aim to produce new varieties with improved tolerance to biotic and abiotic stresses. To develop salt-tolerant varieties, breeding programs are conducting evaluation trials in various genotypes to increase their utility for salt tolerance (Yadav *et al.,* 2011). Many traditional landraces and genotypes can withstand high levels of salinity and may be used as donors in breeding salt tolerant varieties (Leon *et al.,* 2015). This study was conducted to assess 43 rice genotypes for their resilience to salinity both laboratory and field conditions. A total of 10 quantitative traits were evaluated to understand the morphological diversity and 40 molecular markers were used to assess genetic diversity in 43 rice genotypes. The combination of both morphological and molecular diversity would help to find the new saline tolerant cultivars. Early-stage evaluation of rice genotypes is crucial to identify the genotypes with saline tolerance since rice is more sensitive to salinity at seedling stage (Kakar *et al.,* 2019).

All the genotypes showed varied response at seedling stage towards saline stress. The genotypes were scored from 1 (highly tolerant) to 9 (highly susceptible) (RajabiDehnavi *et al.,* 2020). The resistant checks, Pokkali and Nona Bokradid not show any symptoms under saline conditions and had normal growth and development (Rahman *et al.,* 2016). While, the susceptible checks CR1009 and ADT 37 showed whitish leaf tips, drying and rolling of leaves, and death of seedlings (Negrao *et al.,* 2017). The genotypes like AC39040, AC39014, Paiyur 1, and PS336 (score of 1 and highly tolerant) also showed normal growth and development as resistant checks at seedling stage. Besides these genotypes, JGL3844, Annapoorna, Krishna Ponni, Vasundhara, PS379, Hankar Sonam, and Amman showed SES score of 3, indicating their tolerance level to salinity. The tolerant genotypes maintain ion homeostasis by ion uptake and compartmentalization of excess ions in the vacuole or older tissues (Hasegawa, 2013). In addition to ion homeostasis, the tolerant genotypes accumulate large amount of osmolytes like proline, glycine betaine, polyols, and sugars to maintain the osmotic balance (Saxena *et al.,* 2013).

Analysis of variation for all the characters under study was calculated and all the characters showed significant variation. All the genotypes under the investigation showed variation for different characters. Days to 50% flowering ranged from 67.67 days (ADT 36) to 113.33 days (JGL3844) with a mean of 92.12 days. Hankar Sonam (72.67) and PS379 (76.33) showed earliness in 50% flowering with tolerance to salinity (score of 3), these genotypes may be useful to breed short duration varieties under saline conditions. The pH ranged from 78 cm (ADT 36) to 116 cm (Paiyur-1) with a mean of 93.22 cm. The panicle length ranged from 16.67 cm (Amman) to 23.67 cm (ADT 48) with a mean of 20.35 cm. The resistant checks, Pokkali and Nona Bokra showed a panicle length of 20 and 22.33 cm while susceptible checks, ADT 37 and CR1009 showed a panicle length of 19.33 and 20.33 cm, respectively (Table 2). The number of productive tillers ranged from 7.67 (HankarSonam) to 11 (Krishna Ponni) with a mean of 8.9. The highest single plant yield under salinity was recorded in Paiyur-1 (27.59 g) and the lowest was recorded in the susceptible check, ADT 37 (17.84 g).

The genotypes like PS336, AC39040, AC39014, and Paiyur-1 showed the tolerance level (highly tolerant) and SES score (1) as resistant checks under saline conditions. The genotypes, AC39040 and Paiyur-1 produced relatively higher

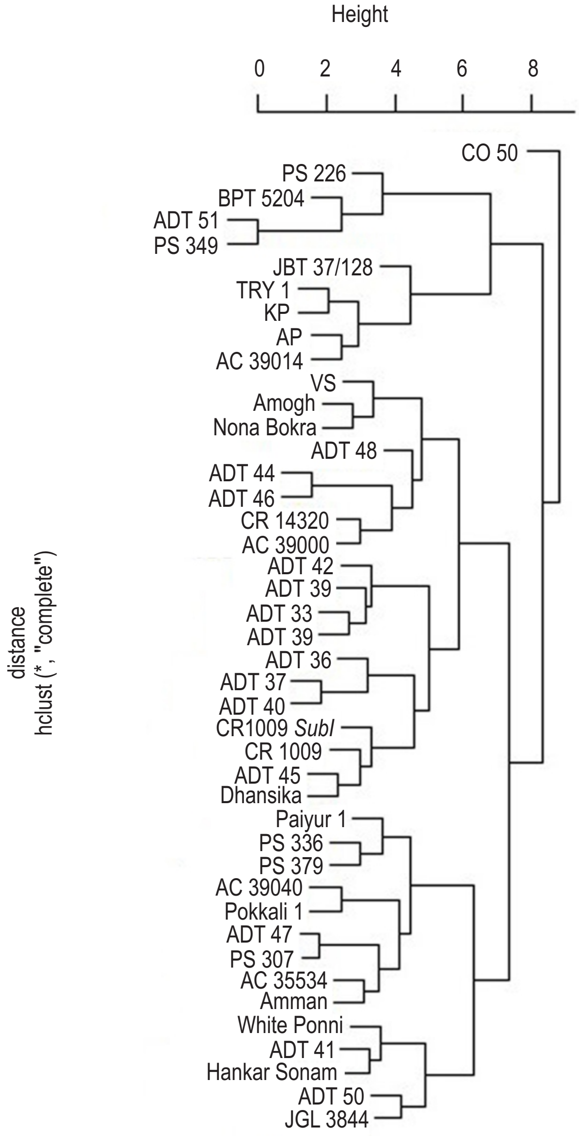

Fig. 1: Cluster diagram representing genotypic distance for 10 quantitative traits under salinity stress condition in rice field.

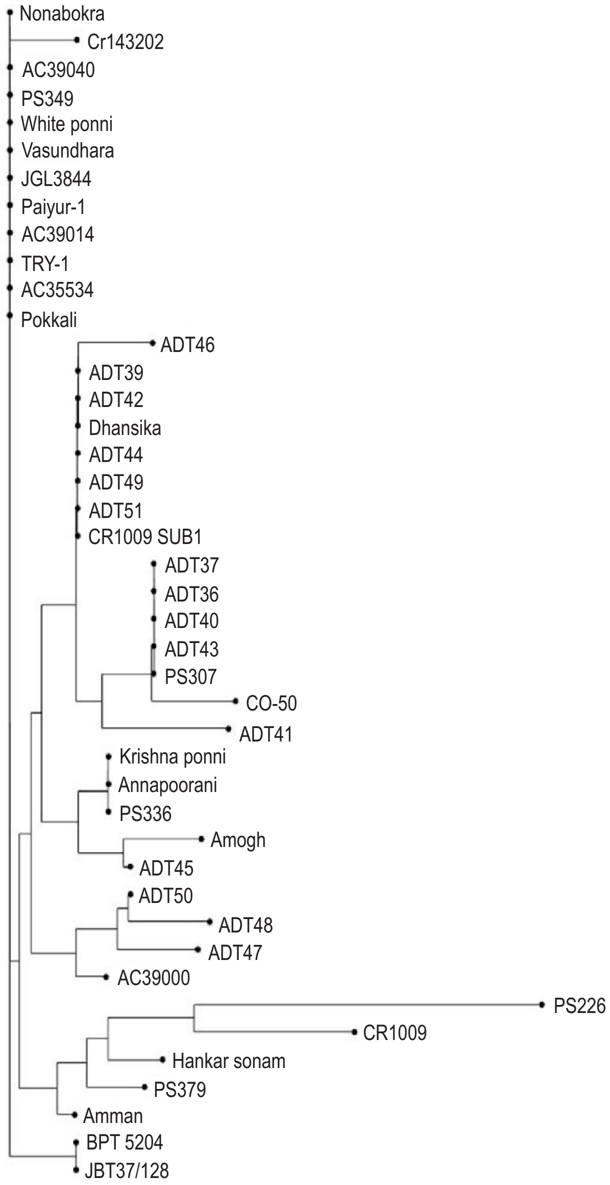

Fig. 2: Dendrogram showing Unweighted pair group method arithmetic averages among 43 rice genotypes based on 26 polymorphic SSR markers under saline situation.

number of NGP and high SPY than other genotypes. These genotypes may be used as potential donors to transfer the traits to develop salt-tolerant cultivars (Table 2). Carvalho *et al.* (2019) studied the package NbClust which was used to determine the best number of clusters, based on 24 indexes that establish the criteria of compactness and separation between clusters. The data was analyzed using R software version 3.4.1. In this study, the morphological data of genotypes were subjected to cluster analysis to find the similarities among the genotypes using R software. Mainly two major clusters were formed, one with CO 50 alone other with the remaining genotypes. The resistant checks, Pokkali and Nona Bokra were clustered in different sub-clusters. In the Nona Bokra sub-cluster, Amogh and Vasundhara were clustered, while in the Pokkali sub-cluster, AC39040 clustered (Fig. 1). PS 336, and Paiyur-1 were also clustered near the Pokkali sub-cluster. The majority of ADT varieties and susceptible checks were clustered in the same cluster, indicating their susceptibility towards salinity stress (Fig. 1). An investigation on correlation was completed to discover the relationship among the characters under salinity. A solid and positive connection was found between the 100-GW and SPY (r = 0.81), NGP and 100-GW (r = 0.65) trailed by NGP and SPY (r = 0.61). A strong and

Table 1: Details of allelic diversity information of 26 polymorphic simple sequence repeats markers with polymorphism information content and expected heterozygosity values in rice

Markers	Chr. No.	No. of alleles	PIC	Expected heterozygosity
RM 488	1	5	0.716	0.754
RM 3412	1	3	0.481	0.547
RM 3268	2	7	0.786	0.811
RM 279	2	3	0.584	0.659
RM 15080	3	4	0.674	0.724
RM 13	3	2	0.314	0.498
RM 1153	4	3	0.471	0.566
RM 6283	4	8	0.824	0.844
RM 307	5	6	0.792	0.818
RM 153	5	5	0.743	0.779
RM 400	6	4	0.662	0.713
RM 276	6	5	0.750	0.785
RM 248	7	4	0.649	0.702
RM 469	7	3	0.549	0.621
RM 310	8	3	0.528	0.595
RM 25	8	3	0.442	0.544
RM 1328	9	5	0.711	0.752
RM 3025	9	6	0.791	0.817
RM 461	9	3	0.504	0.592
RM 228	10	3	0.524	0.598
RM 222	10	3	0.572	0.645
RM 1233	11	5	0.727	0.766
RM 224	11	3	0.530	0.599
RM 336	12	7	0.817	0.837
RM 302	12	3	0.524	0.59
RM 26	12	5	0.745	0.7805
Total no. of alleles	111			
Mean	4.269	0.633	0.690	

negative correlation was found between PH and PL (r = -0.17), followed by PH and DFF (r = -0.19). These results revealed highly significant genotypic variations among the genotypes for all these traits. The highest PCV and GCV were observed as 20.24 and 20.19% whereas the lowest PCV values for plant height and number of productive tillers per plant was 10.79% and 4.36%, respectively. The lowest PCV (10.79%) was noted in plant height and number of productive tillers (4.36%) in GCV. Results also showed narrow differences between PCV and GCV for most traits.

All the characters exhibited high heritability which ranged from 99% in days to fifty percent flowering, number of grains per panicle, and 100 seed weight. Among the traits, only some productive tillers per plant had relatively low heritability. The genetic advances as a percent of mean (GA%) ranged from 3.25% in the number of productive tillers per plant to 40.34% Number of grains per panicle. In addition, PCV, GCV, and heritability were calculated to understand variation and inheritance of characters. Evaluation of genetic diversity among the germplasm utilizing only morphological data cannot define true relationship, due to expression of additive gene action on agronomically important traits (Jasim Aljumaili *et al.*, 2018). The environmental factors may mask the effect of true phenotypic performance, thus selection based only on phenotypic performance cannot be reliable. Molecular markers have proved to be effective in studying genetic diversity, as these are not influenced by environmental factors (Ishii *et al.,* 2001).

Lately, microsatellite markers have been exceptionally famous for examining variety in many yield species (Miah *et al.,* 2013). In this study, 40 SSR markers were utilized, 26 of them were polymorphic and were utilized to discover the variety of genotypes (Table 1). The number of alleles ranged from 2 to 8 with an average of 4.26 alleles per locus. RM 6283 had created the most noteworthy number of alleles (8), while RM 13 had delivered the least (2) (Table 1). The number of alleles produced in this study was higher than the previous reports, where the average alleles ranged from 2.40 to 3.35 per locus (Singh *et al.,* 2000; Gowda *et al.,* 2012). Usually, the number of alleles per locus indicates the richness of population, and 2 to 8 alleles per locus are considered as a good amount of diversity, which was observed in this study. The information of a molecular marker is based on the PIC value and less than 0.4 PIC value indicates, moderate information and >0.7 PIC value indicates, highly informative marker (Hildebrand *et al.,* 1992). The PIC value of the

Table 2: Mean performance and SES score for yield and its contributing traits for forty-three genotypes under saline stress condition in rice

Name of cultivars	DFF	PH	PL	NPT	NGP	GL	GB	GL/GB	100-GW	SPY	SES	Tolerance
ADT36	67.67	78.00	19.33	9.00	77.00	0.73	0.20	3.62	1.98	20.69	5	M
ADT37	74.67	92.00	19.33	10.00	73.67	0.79	0.26	3.02	2.00	17.84	9	HS
ADT39	94.33	107.00	21.33	10.00	86.33	0.75	0.21	3.52	2.04	20.00	5	M
ADT40	82.67	87.33	19.00	9.33	88.33	0.73	0.23	3.19	2.09	18.56	5	M
ADT41	99.67	103.33	20.33	8.67	95.33	0.75	0.26	4.00	2.12	16.24	5	M
ADT42	88.67	85.33	22.33	9.33	117.00	0.81	0.20	3.38	2.12	19.81	9	HS
ADT43	94.00	89.33	21.33	9.00	79.67	0.83	0.25	4.14	2.17	19.41	5	M
ADT44	104.33	78.33	19.00	9.00	94.00	0.92	0.22	2.96	2.19	19.78	5	M
ADT45	96.00	86.33	21.33	10.00	116.33	0.83	0.28	3.49	2.24	22.44	5	M
ADT46	105.67	84.33	19.00	9.00	85.67	0.89	0.26	3.15	2.28	20.13	5	M
ADT47	76.33	105.33	18.00	8.33	125.33	0.77	0.28	3.96	2.32	22.83	5	M
ADT48	100.67	80.33	23.67	9.33	120.33	0.74	0.29	3.78	2.36	21.65	5	M
ADT49	97.33	92.33	21.67	10.00	85.33	0.86	0.24	4.23	2.40	21.27	9	HS
ADT50	87.00	112.67	17.33	9.33	98.00	0.97	0.23	2.67	2.08	19.46	5	M
ADT51	99.67	97.33	18.67	9.00	145.67	0.96	0.29	3.91	3.07	20.86	7	S
White ponni	83.00	84.33	17.67	8.00	106.67	0.74	0.28	3.87	2.40	19.21	3	T
JGL3844	108.33	106.67	21.33	8.00	136.33	0.92	0.26	2.95	2.17	18.40	3	T
CR1009	77.67	98.33	20.33	9.67	87.67	0.87	0.28	3.30	2.30	20.71	9	HS
CR1009 SUB1	75.33	79.67	18.00	9.33	128.33	0.94	0.26	3.58	2.36	20.28	7	S
TRY-1	90.33	90.33	22.00	8.67	139.00	0.85	0.20	3.25	2.65	22.98	1	HT
CO-50	106.33	87.00	20.33	8.00	108.33	0.37	0.23	1.68	3.28	21.22	9	HS
Annapoorna	102.00	85.00	22.67	8.67	119.67	0.87	0.20	4.49	2.45	21.33	3	T
PS349	99.67	97.33	18.67	9.00	145.67	0.96	0.29	3.91	3.07	20.86	7	S
Krishna ponni	90.00	93.33	21.67	9.33	130.33	0.80	0.24	3.62	2.46	23.01	3	T
PS336	84.00	100.33	21.67	11.00	108.00	0.97	0.29	4.28	2.63	26.27	1	HT
PS307	81.33	107.67	20.33	8.67	130.33	0.78	0.28	3.63	2.58	23.24	5	M
CR143202	108.00	95.00	22.33	8.67	129.33	0.83	0.24	3.06	2.63	22.82	9	HS
AC39040	93.67	93.67	19.33	9.00	160.00	0.81	0.30	3.96	2.67	25.65	1	HT
Vasundhara	84.33	98.67	22.33	8.00	84.33	0.93	0.30	3.12	2.42	18.95	3	T
AC35534	85.33	96.67	17.67	9.33	139.67	0.92	0.27	4.98	2.70	19.81	5	M
PS379	76.33	109.67	21.33	8.67	120.33	1.00	0.24	4.38	2.74	25.15	3	T
AC39000	105.00	92.33	19.33	8.33	107.67	0.92	0.24	4.28	2.77	19.02	9	HS
JBT37/128	113.33	82.33	22.33	8.33	128.00	1.02	0.20	5.08	2.81	23.12	5	M
PS226	97.67	83.00	21.67	9.33	153.33	0.99	0.30	3.21	3.49	18.63	9	HS
Hankarsonam	72.67	102.00	21.67	7.67	118.33	0.77	0.23	3.49	2.46	20.56	3	T
BPT 5204	108.67	84.00	19.67	8.33	149.33	0.99	0.25	3.50	3.15	24.62	7	S
Dhansika	94.67	82.67	19.00	9.67	84.33	0.88	0.27	3.54	2.53	20.26	5	M
Amogh	84.67	94.33	20.33	8.00	117.67	0.92	0.27	3.20	2.48	24.33	5	M
Amman	99.00	103.00	16.67	9.33	106.33	0.92	0.29	3.92	2.45	22.90	3	T
Paiyur-1	92.67	116.00	19.33	9.67	155.67	0.93	0.29	3.57	2.85	27.59	1	HT
Ac39014	83.33	87.00	23.00	8.00	127.33	0.94	0.22	4.21	2.54	23.80	1	HT
Nona Bokra	85.67	83.00	22.33	8.00	121.33	0.85	0.27	3.08	2.98	23.43	1	HT
Pokkali	103.67	91.33	20.00	8.67	149.00	0.88	0.26	3.26	2.56	22.25	1	HT
Mean	92.12	93.22	20.35	8.9	114.9	0.86	0.26	3.53	2.50	21.96		
CD (P=0.05)	0.78	3.3	1.7	0.7	2.7	0.10	0.01	0.69	0.02	4.3		

DFF- Days to fifty percent flowering; PH- Plant height; PL- Panicle length; NPT- Number of productive tillers per plant; NGP- Number of grains per panicle; GL- Grain length; GB- Grain breadth; 100-GW- 100-grain weight; SPY-Seed per yield; SES-Standard evolution score

present study ranged from 0.314 to 0.824 with an average of 0.633. This indicates, the markers used in this study are highly informative and have required properties for genetic diversity studies. Nirubana *et al.* (2020) has also reported, a total of 101 alleles produced from 24 polymorphic markers with a mean of 4.21 alleles per locus and a PIC value ranging from 0.238 to 0.799 with an average of 0.6070, which supports the results of the present study (Table 1). The expected heterozygosity among the SSR's was greater than 0.5 (0.690), reflecting the heterozygous nature of the population. The molecular data were subjected to cluster analysis by their dissimilarity coefficients using NJ method. (Pandey *et al.,* 2015) The total genotypes were divided to form two main clusters, where the resistant checks, Pokkali and Nona Bokra were clubbed in one cluster. Similar results were obtained by Rahman *et al.* (2016) where three major clusters corresponding to indica, aus and aromatic subgroups were identified. The largest group was indica, with salt-tolerant Pokkali accessions in one sub-cluster, while a set of Bangladeshi land races were in a different sub-cluster. Recently, (Singh *et al.* (2021) reported that Nona Bokra, Koshihikari and Pokkali possessed the same haplotype, while other salt-tolerant genotypes exhibited diferent OsHKT1;5 haplotypes, although all of them were highly salt tolerant at seedling stage. In this study, the other relevant genotypes like AC35534, TRY-1, AC39014, Paiyur-1, JGL3844, Vasundhara, White Ponni, PS349, AC39040, CR143202, and Nano Bokra were also clubbed in the resistant checks cluster.

The susceptible checks, CR1009 and ADT 37 were clustered in different sub-clusters. (Fig. 2). This indicates that genotypes in their respective cluster have a similar molecular pattern as resistant or susceptible checks. Based on the morphological response of genotypes, Amogh and Vasundhara, and AC39040 were clubbed in the resistant check's clusters. Screening at seedling stage revealed that AC39040, AC39014, Paiyur 1, and PS336 were tolerant to salinity (SES score of 1). In view of the above, the present research identified Vasundhara, AC39040, and Paiyur-1 as new tolerant sources for salinity. These genotypes may be further explored to find the novel genes/QTLs for saline tolerance to develop salt-tolerant cultivars.

The present research has successfully identified the new saline tolerant genotypes using both morphological and molecular diversity. The new tolerant sources could be used as potential donors to develop the introgression lines to be used as pre-breeding material in hybridization programmes. This research also highlights the importance of conserving the landraces or wild relatives of rice, which possesses valuable genes for abiotic and biotic stresses.

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Add-on Information

Authors' contribution: J. Johnny Subakar Ivin: Preparation and implementation of research work, field data collection, wrote the manuscript; **S. Kayathri:** Assisted in manuscript editing; **Ch.Raveendra:** Performed Statistical analysis, Data interpretation; **M. Anbarasu:** Helped for grammatical corrections; **Y. Anbuselvam:** Supervision, validation, formal analyses, data correction, review, editing.

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