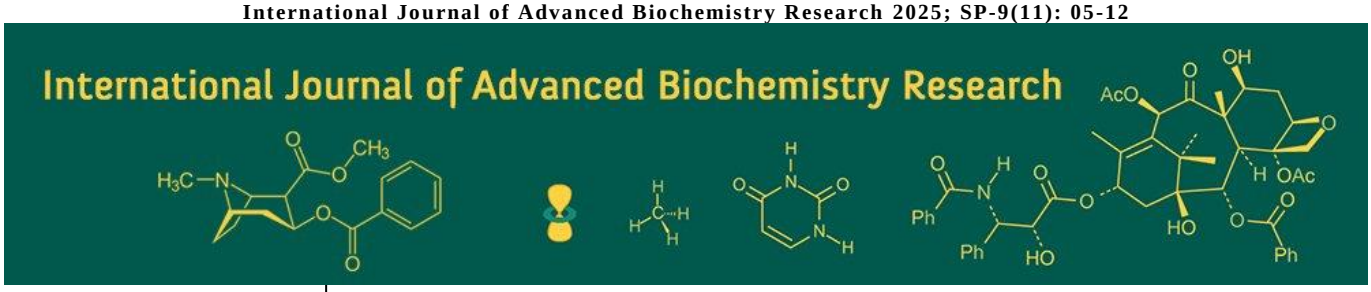

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In vitro molecular evaluation of rice (*Oryza sativa* L.) genotypes and validation of SSR markers for salinity tolerance

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Abstract
A set of twenty rice genotypes including four tolerant (CSR-10, CSR-36, FL-478 and CSR-27) and two susceptible (IR-29 and IR-64) checks, were evaluated in *in vitro* conditions for salinity tolerance by molecular analysis using SSR markers. Genetic profiling of entries with a panel of 20 SSR primer pairs, a total of 78 alleles were reported, with an average of 3.9 alleles per marker. 2 to 8 distinct alleles were found for each marker pair. The lowest alleles 2.0 were produced by primer RM 3412, while the highest number of alleles 8 was produced by primer RM-1089 respectively. Among the primer pairs utilized, three SSR primer pairs, namely RM 140, RM 1287 and RM 3412, were validated based on their efficiency in distinguishing salt tolerant genotypes from susceptible genotypes. These three primers can be utilized for the purpose of genetic differentiation and discrimination in relation to salt stress responsiveness of the rice genotypes. Overall, the study demonstrates that screening with SSR-based validation is effective for rapidly identifying salt tolerant rice genotypes, such as CSR-36, CSR-27, FL-478, CSR-10, Panvel-1, Panvel-3, Konkan Khara and Panvel-2 which emerge as promising donors for breeding programs.

Keywords: SSR markers, rice, salt stress

Introduction
Rice (*Oryza sativa* L.), with a chromosome number of 2n = 24, is a self-pollinating monocotyledonous crop classified under the genus Oryza within the Poaceae (Graminae) family and the Oryzoidea subfamily. The genus Oryza comprises 27 species, of which 25 are wild and two, *Oryza sativa* L. and *Oryza glaberrima* L. are cultivated. These two cultivated species, *Oryza sativa* L. and *Oryza glaberrima* L., possess AA genomes (Vaughan *et al.*, 2003) [48]. Rice, with its diploid genetics (2n = 24) and an estimated genome size of 430 Mb, is considered a model plant for studying other cereal crops due to its relatively small genome (Kurata *et al.*, 1994) [34] and high degree of genetic polymorphism (Tanksley, 1983; Wang *et al.*, 1992; McCouch *et al.*, 2002) [45, 50, 12]. Compared to crops like maize, wheat and potatoes, raw, long grain white rice is a relatively rich source of energy, carbohydrates, calcium, iron, thiamine, pantothenic acid and vitamin E. Additionally, due to its diploid genetic structure and well understood genome organization, rice serves as an ideal model plant for studying the genetics and genome architecture of grasses. Rice is thought to have been first domesticated in China around 10,000 years ago, developing into three primary subspecies: indica, japonica and javanica, which are adapted to tropical, temperate and mixed climatic regions, respectively. As the global population surges from 7 billion to an estimated 9 billion by 2050, agricultural output must increase by 50% or more compared to current yields to meet escalating demands. This urgency applies particularly to staple crops like rice, wheat, soybeans and corn, which are critical for global food security (Kromdijk & Long, 2016) [29]. More than half of the global population relies on rice (*Oryza sativa* L.) as a primary food source. This vital cereal crop is cultivated in over 95 countries worldwide. Currently, approximately 163.5 million hectares are dedicated to rice farming, yielding around 758.9 million tons annually, with an average productivity of 4,641.5 kg per hectare (Chaudhary *et al.*, 2023) [11]. India stands out as one of the top rice producers, with nearly 51.4 million hectares under cultivation and an expected production of around 149.07 million metric tons

in 2024-25 (Anonymous, 2025) [6]. India ranks as the world's second largest rice producer, just after China and holds the top position in rice exports among major producing countries. Major rice growing states in India include Punjab, Haryana, Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Bihar and West Bengal, with West Bengal being the largest contributor in terms of both production and productivity.

In Maharashtra, rice is the second most important crop after sugarcane. The state grows rice on about 1.69 million hectares, producing around 4.0 million metric tons, with an average yield of 2.37 tons per hectare (Anonymous, 2025) [6]. Within Maharashtra, the Konkan region plays a key role in rice cultivation. About 3.42 lakh hectares of land in Konkan are used for rice farming, yielding roughly 8.5 lakh tons of paddy. The region has an average productivity 1 of 2495.8 kg per hectare. Among the five major rice growing districts in Konkan, Raigad leads with the largest area under cultivation (0.89 lakh hectares) and the highest production (2.25 lakh tons), followed by Palghar (0.77 lakh hectares, 1.83 lakh tons), Ratnagiri (0.62 lakh hectares, 1.66 lakh tons) and Sindhudurg (0.59 lakh hectares, 1.55 lakh tons). Thane has the smallest area and production, with 0.54 lakh hectares and 1.23 lakh tons. However, Ratnagiri district records the highest rice productivity in the region, averaging 2700 kg per hectare (Anonymous, 2024) [5]. The productivity of many commercial crops is significantly hindered by various abiotic stresses, such as salinity, drought, waterlogging and heat. These stresses cause substantial damage, leading to significant economic losses worldwide. Among these, salinity is a major abiotic stress that negatively impacts crop growth. Salinity refers to a soil condition marked by high levels of soluble salts. Soils are considered saline when the electrical conductivity of the soil extract (EC) reaches 4 dS m^{-1} or higher, equivalent to approximately 40 mM NaCl, creating an osmotic pressure of about 0.2 MPa (Munns and Tester, 2008; Kranto *et al.*, 2016) [5, 28]. This condition arises due to factors such as rock weathering, excessive irrigation with saline water, seawater intrusion into freshwater areas, poor water management, high evaporation rates and the overuse of chemical fertilizers (Kumari *et al.*, 2016b; Kumari *et al.*, 2018) [31, 32, 33]. Salinity is a major constraint in rice growing regions globally (Senadhira, 1987) [42], with approximately 1.4 billion hectares of salt affected land worldwide, around 10.7 percent of global land (FAO, 2024) [17]. In India, 6.72 million hectares are salt affected; the affected area includes both saline and sodic soils. Saline soils cover 2.95 million hectares, while sodic soils cover 3.77 million hectares (FAO, 2024) [17], with 1.2 million hectares being coastal saline soils (CSSRI, 2010). FAO's global map of salt affected soils reveals that 10% of irrigated cropland and 10% of rainfed cropland are currently impacted by salinity. This poses a significant threat to global food security (FAO, 2024) [17]. The projections suggest that salinization could reduce arable land by 30% in the next 25 years and up to 50% by 2050 (Wang *et al.*, 2003; Kumari *et al.*, 2016b) [49, 31, 32]. Rice plants exhibit various responses to salinity stress, ranging from physiological, biochemical adaptations, to changes in gene expression. Salinity triggers physiological disruptions in plants, including to ion imbalance, mineral deficiency, water stress, phosphate deficiency, reactive oxygen species (ROS) production and altered phytohormone levels, osmotic stress, ion toxicity and oxidative stress (Munns and Tester, 2008; Kumari *et al.*, 2016b) [38, 31, 32]. Normally, plant cells maintain higher osmotic pressure than the soil, facilitating water and nutrient uptake. However, under salt stress, the soil's osmotic pressure surpasses plant cells due to high salt concentration, impairing water absorption and uptake of essential minerals like K^+ and Ca^{2+} (Kader and Lindberg, 2010) [26]. Salinity effects damage cellular components like DNA, proteins, lipids and pigments (Zhu, 2002; Kumari *et al.*, 2016b) [53, 31, 32], hindering crop growth and development. Based on their response to salt, plants are categorized into two groups: glycophytes, which are salt sensitive crop species and halophytes, which can thrive in high salinity environments or tolerate elevated salt concentrations (Tuteja *et al.*, 2011; Reddy *et al.*, 2017) [47, 41].

There are different techniques for the reclamation of salt affected soils but cultivation of salinity tolerant crop varieties is considered economical as these reclamation techniques are costly and time consuming. Selection of breeding tolerant genotypes and varieties is necessary for steady sustainable food production with the increasing abiotic (salinity) stress conditions. but, progress of conventional breeding programs for improving salinity tolerant rice varieties is limited. The screening of salinity tolerant genotypes based on molecular parameters for enhancing crop production even under salinity conditions is essential for future food production (Kumar and Sharma, 2020) [30]. To improve salinity tolerance in rice, identifying sufficient genetic variation and establishing reliable screening techniques are crucial (Kranto *et al.*, 2016) [28]. Effective screening methods, including *in vitro* molecular studies, are essential for developing salt tolerant rice genotypes. *in vitro* molecular screening offers advantages over field screening, enabling precise evaluation of salinity tolerance (Ali *et al.*, 2014) [1]. Additionally, molecular markers such as Simple 3 Sequence Repeat (SSR), are highly effective tools for identifying genetic differences and distinguishing between genotypes from diverse germplasm sources. They are even capable of detecting subtle variations among closely related genetic materials (Lapitan *et al.*, 2007) [35]. They are highly effective for characterizing salt tolerant genotypes due to their polymorphism, reproducibility, co-dominance and multi-allelic nature. SSR markers are widely used in rice for varietal identification, diversity analysis, gene mapping and marker assisted selection (Wong *et al.*, 2009) [51]. Their utility is well documented in studies on landraces (Thomson *et al.*, 2007) [46], cultivars (Garris *et al.*, 2005) [19] and wild relatives (Brondani *et al.*, 2003) [9], highlighting their importance in genetic differentiation and diversity analysis at the molecular level.

Importance of the study

Maharashtra's coastal region, which stretches from north to south, encompassing a 720 km coastline and a width of about 50 km, covering districts like Palghar, Thane, Raigad, Ratnagiri and Sindhudurg, has approximately 65,465 hectares of coastal saline soils. These soils become unproductive due to periodic inundation by seawater during high tides, leading to salinity issues that severely impact agriculture and farmers' livelihoods. Salinity disrupts physiological and biochemical processes, causing ion imbalance, mineral deficiency, osmotic stress, ion toxicity and oxidative stress, damaging cellular components like DNA, proteins, lipids and pigments. Plants combat salt

stress through antioxidative enzyme induction, ion homeostasis and synthesis of compatible solutes. Therefore, developing salt tolerant crops is a practical solution to mitigate these challenges. *in vitro* molecular studies, are essential for developing salt tolerant rice genotypes. *in vitro* molecular screening offers advantages over field screening, enabling precise evaluation of salinity tolerance. Molecular evaluation identifies and validates genetic markers linked to salinity tolerance, facilitating marker-assisted selection (MAS) for faster breeding of tolerant varieties. Molecular studies also elucidate the genetic mechanisms of tolerance, aiding targeted breeding. Validating these markers ensures their reliability across diverse genetic backgrounds and environments, making them practical tools for breeding programs. Based on the available information, it is evident that selecting salinity tolerant and high yielding genotypes is the most efficient way to improve crop production under salinity stress conditions. Therefore, there is a need to understand the various molecular mechanisms involved in the salinity stress tolerance mechanism in crop plants. Keeping these facts in view the present investigation, "In vitro molecular evaluation of rice genotypes and validation of markers for salinity tolerance" was undertaken with the following objectives of molecular characterization of rice (*Oryza sativa* L.) genotypes and validation of SSR markers concerning salt tolerance.

Materials and Methods

Experimental materials

Seeds of 20 rice genotypes along with four tolerant (CSR-10, CSR-36, FL478 and CSR 27) and two susceptible (IR-29, IR-64) checks were obtained from the Indian Institute of Rice Research, Hyderabad, ICAR-Central Coastal Agricultural Research Institute (CCARI) Ela, Old Goa and DBSKKV University released rice genotypes for salinity tolerance. The study was conducted in the Plant Biotechnology Centre, College of Agriculture, Dapoli. The details of rice genotypes are given in Table 1.

Table 1: List of rice genotypes

Sr. No.	Name of rice genotype	Source	Purpose
1	CSR-10	ICAR-CCARI, Goa	Salt tolerant
2	CSR-36	ICAR-CCARI, Goa	Salt tolerant
3	FL478	RARS, Karjat	Salt-tolerant QTL
4	CSR-27	IIRR, Hyderabad	Salt tolerant
5	IR-29	ICAR-CCARI, Goa	Salt susceptible
6	IR-64	IIRR, Hyderabad	Salt susceptible
7	Karjat-3	RARS, Karjat	*In vitro* evaluation of the university released rice genotypes for salinity tolerance
8	Karjat-5	RARS, Karjat	
9	Karjat-6	RARS, Karjat	
10	Karjat-7	RARS, Karjat	
11	Karjat-184	RARS, Karjat	
12	Panvel-1	RARS, Karjat	
13	Panvel-2	RARS, Karjat	
14	Panvel-3	RARS, Karjat	
15	Ratnagiri-1	RARS, Karjat	
16	Ratnagiri-7	RARS, Karjat	
17	Ratnagiri-8	RARS, Karjat	
18	Ratnagiri-24	RARS, Karjat	
19	Kokan khara	RARS, Karjat	
20	Ratnagiri purple	RARS, Karjat	

Screening for salinity tolerance

The plants grown in the greenhouse under natural light conditions. A preliminary test for salt tolerance was carried out using Gregorio *et al.* (1997) [21] method. The seed samples were heated for 5 days in a convection oven set at 50 °C to break seed dormancy. Proper breaking of seed dormancy was essential for effective screening. Seedling vigor provided a significant advantage when salinization occurred at a very early seedling stage. After breaking dormancy, seeds were surface sterilized with fungicide and rinsed thoroughly with distilled water. The sterilized seeds were then placed in germination paper and incubated at 30 °C for 48 hours to allow germination. The preparation of seedling float, stock and nutrient solution was carried out using Yoshida *et al.* (1976) [52]. Pregerminated seeds were sown per hole on the Styrofoam seedling float (Each genotype having 4 replications), ensuring that the radicle was inserted through the nylon mesh. During this process, the radicle could be damaged, though the damage might not have been visible. Any such damage could have compromised the main salt tolerance mechanism in rice. Therefore, sufficient time was allowed for the seedlings to repair any root damage. For this reason, the pregerminated seeds were first seeded in a tray filled with distilled water instead of a salinized nutrient solution. The Styrofoam seedling float was suspended on the tray filled with distilled water. The endosperm provided adequate nutrients for the seedlings to grow normally for 3-4 days. After 3 days, once the seedlings were well established, the distilled water was replaced with a salinized nutrient solution at different EC levels, 4, 8 and 12 dSm^{-1} along with control (0 dSm^{-1}). The solution was renewed every 8 days and the pH was maintained at 5.0 daily. Test entries were rated at 10 and 16 days after the initial salinization.

DNA extraction and SSR analysis

The fresh leaves were used for DNA extraction according to Doyle and Doyle (1990) with the slight modifications. The quality and quantity of the extracted DNA were evaluated by electrophoresis on a 0.8% agarose gel and A total of 20 SSR DNA markers, all of them were in different rice chromosomes (Table 2).

Table 2: List of SSR primers used for present study

Sr. No	Primer	Chromosome No.	Primer Sequence (5 → 3)	Annealing Temp
1	RM 14	1	F-CCGAGGAGAGGAGTTCGAC	55 °C
			R-GTGCCAATTTCCTCGAAAAA	
2	RM 39	5	F-GCCTCTCTCGTCTCCTTCCT	60 °C
			R-AATTCAAACTGCGGTGGC	
3	RM 140	1	F-T GCCTCTTCCCTGGCTCCCCTG	62 °C
			R-GGCATGCCGAATGAAATGCATG	
4	RM 162	6	F-GCCAGCAAAACCAGGGATCCGG	61 °C
			R-CAAGGTCTTGTGCGGCTTGCGG	
5	RM 220	1	F-GGAAGGTAACTGTTTCCAAC	55 °C
			R-GAAATGCTTCCCACATGTCT	
6	RM 302	1	R-TCATGTCATCTACCATCACAC	55 °C
			F-ATGGAGAAGATGGAATACTTGC	
7	RM 310	8	F-CCAAAACATTTAAAATATCATG	55 °C
			R-GCTTGTTGGTCATTACCATTC	
8	RM 332	11	F-GCGAAGGCGAAGGTGAAG	58 °C
			R-GCGAAGGCGAAGGTGAAG	
9	RM 493	1	F-TAGCTCCAACAGGATCGACC	59 °C
			R-GTACGTAAACGCGGAAGGTG	
10	RM 550	2	F-CTGAGCTCTGGTCCGAAGTC	61 °C
			R-GGTGGTGGAAGAACAGGAAG	
11	RM 562	1	F-CACAACCCACAAACAGCAAG	62 °C
			R-CTTCCCCCAAAGTTTTAGCC	
12	RM 588	6	F-GTTGCTCTGCCTCACTCTTG	58 °C
			R-AACGAGCCAACGAAGCAG	
13	RM 1089	5	F-CAGAAGGATTATCTCGATACC	55 °C
			R-AATAGGGCTTGAAATAAATTG	
14	RM 1287	1	F-GTGAAGAAAGCATGGTAAATG	58 °C
			R-CTCAGCTTGCTTGTGGTTAG	
15	RM 3412	1	F-AAAGCAGGTTTTCCTCCTCC	62.5 °C
			R-CCCATGTGCAATGTGTCTTC	
16	RM 8094	1	F-AAGTTTGTACACATCGTATACA	58 °C
			R-CGCGACCAGTACTACTACTA	
17	RM 10665	1	F-CCTGCTGCAATTGATGACAAGC	57 °C
			R-TGGACAGAATGAAGCATCTGTGG	
18	RM 10694	1	F-TTTCCCTGGTTTCAAGCTTACG	56 °C
			R-AGTACGGTACCTTGATGGTAGAAAGG	
19	RM 10748	1	F-CATCGGTGACCACCTTCTCC	60 °C
			R-CCTGTCATCTATCTCCCTCAAGC	
20	RM 10825	1	F-GGACACAAGTCCATGATCCTATCC	61 °C
			R-GTTTCCTTTCCATCCTTGTTGC	

The amplification was carried out in a thermo-cycler using a 10 µl reaction mixture prepared by varying the components involved in the composition of the reaction mixture in the cases of each of the primer pairs used in the study. Negative and positive controls were incorporated in PCR amplification conducted to verify the absence of contamination and the efficiency of the amplification reaction.

The annealing temperature for different primer pairs was kept approximately 5 °C less than their melting temperature (Tm). For different primer pairs used in the study, the annealing temperature varied depending on the AT and GC content of the primer pair in question. The gel loading buffer was added to the DNA samples. An appropriate amount of loading buffer was always added. Once the samples were loaded in the gel, the power supply was switched on and the gel was run at 80 volts. The amplified DNA fragments were visible as the orange band because the ethidium bromide intercalated into DNA and fluorescenced under UV light.

The amplified products were documented with the help of a gel documentation system (Bio-Era) and the size of amplified fragments was estimated with the help of a 50 bp ladder and 100bp DNA ladder (Fermentas). The different bands produced by each one of the primer pairs were compared and classified into the two different categories of shared and unique bands and then expressed in percentage. The data on polymorphism information content (PIC) of the SSR was obtained by calculating the value according to the formula as described by Anderson *et al.* (1993) [4]. The data were entered into a binary matrix as discrete variables and this data matrix was subjected to further analysis. Genetic

similarities among genotypes were calculated based on the presence and absence of common bands. The genetic associations among genotypes were analysed by calculating the similarity coefficient (Dice, 1945) [15] for pair wise comparisons based on the proportions of shared bands produced by primers.

Validation of markers for salinity tolerance

SSR markers were validated based on their efficiency to distinguish salt tolerant genotypes from the salt susceptible ones.

Results and Discussion

An *in vitro* experiment was conducted using a set of 20 rice genotypes presented in Table 1, including 4 tolerant (CSR-1, CSR-36, FL-478 and CSR-27) and two susceptible (IR-29 and IR-64) checks for molecular studies using SSR markers for evaluation and analysis of genetic polymorphism related to salt tolerance.

SSR Molecular marker studies

Utilising a panel of 20 SSR primer pairs of different chromosomes known to harbour the salt stress response related locus, molecular profiling of 20 rice genotypes was carried out and amplification of simple sequence repeat loci was successfully achieved with different primer pairs. A total of 78 alleles were reported, with an average of 3.9 alleles per marker. 2 to 8 distinct alleles were found for each marker pair. The smaller number of alleles (2) were produced by primer RM 3412, while the greatest number of alleles (8) was produced by primer RM-1089 (Table 14), respectively. In general, marker detecting greater number of alleles per locus detected a greater number of alleles following the earlier reports (Bajracharya *et al.*, 2006; Joshi and Behera, 2007; Lapitan *et al.*, 2007; Ghneim *et al.*, 2008; Bhowmik *et al.*, 2009; Rabbani *et al.*, 2010; Singh *et al.*, 2011; Kumari *et al.*, 2016a; Kumar *et al.*, 2018) [7, 25, 35, 20, 8, 40, 43, 31, 32, 33].

The overall size of the amplified products generated using 20 SSR primer pairs ranged from 94 bps (RM 310) to 480 bps (RM 10665). The molecular size difference between the smallest and the largest alleles for a given SSR locus varied from 33 bps (RM 3412) to 149 bps (RM 1089) (Table 14) (Kumari *et al.*, 2016a; Kumari *et al.*, 2016b; Kumar *et al.*, 2018) [31, 32, 33].

The polymorphism percentage, expressed as the percentage of unique alleles, was recorded as the maximum in the case of RM 1089 (75.0%) and the minimum in the case of RM 10825 (45.0%), with an average value of 59.0% (Table 14). Among the primer pairs utilised, RM 14, RM 39, RM 140, RM 220, RM 302, RM 310, RM 332, RM 1089, RM 8094 and RM 10665 recorded noticeably greater polymorphism percentages. The above results correlate with Kumari *et al.*, 2016a; Kumar *et al.*, 2018 [31, 32, 33]. The markers (RM 7075, RM 336 and RM 253) showed polymorphism and were able to discriminate salt-tolerant genotypes from susceptible (Bhowmik *et al.*, 2009) [8].

Polymorphism information content (PIC) of each of the primers (Table 14), indicated that the values revealing allele diversity and frequency among the entries varied from 0.455 (RM 3412) to 0.840 (RM 1089) with an average of 0.649 across the primer. Comparable polymorphic information content (PIC) values ranging from 0.21 to 0.76 with an average of 0.57 in 14 genotypes of stress tolerant rice with 40 SSR markers (Islam *et al.*, 2012) [23], 0.845 (RM 336) to 0.864 (RM 510) with an average of 0.855 (Islam *et al.*, 2015) [24], 0.745 in the case of RM 152 to 0.890 in the case of RM 10701 (Dhar *et al.*, 2012) [14], values ranging from 0.564 (RM 220 and RM 562) to 0.901 (RM 10665) with an average of 0.763 in 18 genotypes of rice with 24 SSR markers for salinity tolerance in different levels (Kumar *et al.*, 2018) have also been reported.

Similarity coefficients calculated amongst 20 entries on the basis of presence and absence of the amplified products generated by using 20 primers specific to the unique flanking sequences of microsatellites indicated maximum similarity between CSR-10 and CSR-36 (0.818) amongst pair wise combinations of entries. This was followed by a remarkably higher magnitude of similarity coefficient between Karjat-184 and Panvel-2 (0.667). Similar inference has been derived in the studies conducted on the molecular markers, including SSR marker based divergence analysis in rice by earlier researchers (Aliyu *et al.*, 2011; Kanawapee *et al.*, 2011; Dhar *et al.*, 2012; Sudharani *et al.*, 2013; Kumari *et al.*, 2018; Priyadarshini *et al.*, 2018 and Hossain *et al.*, 2024) [3, 27, 14, 44, 33, 39, 22]. Further, Sudharani *et al.* (2013) [44] found 100 per cent similarity between the rice genotypes RP Bio-226 and CSR-27.

Considering the broad classification of entries, as indicated by the dendrogram, basically, the entries were divided into three groups, which were further divided into clusters, sub-clusters and sub-sub-clusters. The first multi genotypic group consisted of six entries, namely, Ratnagiri-1, Ratnagiri-7, Ratnagiri-8, Ratnagiri-24, Ratnagiri purple and Konkan Khara, whereas the second multi-genotypic group consisted of six entries, namely, Karjat-3, Karjat-5, Karjat-6, Karjat-7, IR-29 and IR-64. The third multi-genotypic group consisted of eight entries, namely Panvel-1, Panvel 2, Panvel-3, Karjat-184, FL-478, CSR-10, CSR-36 and CSR-27. The results revealing the remarkable potential of microsatellite markers characterized by their polymorphic nature and allelic diversity, as noticed in the present study and also reported earlier by several research workers (Mohammadi *et al.*, 2008 and Ganie *et al.*, 2016) [37, 18].

Validation of microsatellite markers for salinity tolerance

A gradual exclusion of primer pairs in the analysis of differentiation and divergence, as well as interrelationship among the entries, finally led to the selection and validation of 3 SSR primer pairs, namely, RM 140, RM 1287 and RM 3412, based on their efficiency to distinguish salt tolerant genotypes from susceptible genotypes. These 3 primers appeared to be efficient and sufficient for genetic differentiation and discrimination concerning salt stress responsiveness of the rice genotypes. A specific combination of either three (RM 1287, RM 3412 and RM 140) or even two markers (RM 1287 and RM 3412) from this panel was also found equally effective in the discrimination of entries according to their salt stress responsiveness.

The result of the present study supports the previous reports of several investigators (Islam *et al.*, 2012; Aliyu *et al.*, 2013; Chattopadhyay *et al.*, 2014; Meghana *et al.*, 2015; Ganie *et al.*, 2016; Kumari *et al.*, 2018; Dewi, 2022) [23, 2, 10, 36, 18, 33, 13].

Table 14: Analysis of 20 SSR primer pairs used for the amplification of genomic DNA extracted from 20 rice genotypes

Sr. No.	Primer	Total no. of polymorphic bands	Polymorphism (%)	No. of alleles	PIC	Range of amplified products
1	RM 14	13	65	6	0.79	206-320
2	RM 39	13	65	4	0.665	108-173
3	RM 140	13	65	3	0.605	228-280
4	RM 162	11	55	3	0.635	196-240
5	RM 220	12	60	4	0.73	305-368
6	RM 302	13	65	3	0.665	181-224
7	RM 310	13	65	4	0.675	94-163
8	RM 332	13	65	4	0.6375	144-210
9	RM 493	10	50	3	0.545	291-333
10	RM 550	12	60	3	0.595	219-268
11	RM 562	11	55	4	0.615	142-205
12	RM 588	11	55	3	0.505	167-204
13	RM 1089	15	75	8	0.84	235-384
14	RM 1287	12	60	3	0.635	255-307
15	RM 3412	10	50	2	0.455	277-310
16	RM 8094	12	60	5	0.715	219-310
17	RM 10665	13	65	5	0.7225	394-480
18	RM 10694	10	50	3	0.62	222-279
19	RM 10748	10	50	4	0.695	125-181
20	RM 10825	9	45	4	0.645	136-206
	Average	11.8	59	3.9	0.6495	

Conclusion

The present investigation was on "In vitro molecular evaluation of rice (*Oryza sativa* L.) genotypes and validation of SSR markers for salinity tolerance". From the ongoing discussion experiment concluded that molecular evaluation of 20 rice genotypes under different salinity levels revealed significant genotypic variation for salt tolerance. Molecular analysis using SSR markers confirmed the genetic distinctness between tolerant and susceptible genotypes and provides a basis for marker assisted selection (MAS).

Promising salt tolerant genotypes identified in this study include the genotypes like CSR-36, CSR-27, FL-478, CSR-10, Panvel-3, Panvel-1, Konkan Khara and Panvel-2, all of which consistently exhibited high salinity tolerance across molecular screening. The molecular analysis done by using SSR markers, which have validated the same genotypes. Thus, understanding the salinity tolerance mechanism at the molecular levels can help in efficient screening and producing salinity tolerant rice genotypes. These genotypes can be utilized as potential donors in rice improvement programs targeting salt affected areas. Furthermore, the study validated that *in vitro* screening methods combined with molecular tools offer a rapid and reliable approach for early stage identification of salt tolerant rice cultivars.

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