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Assessing Genetic Diversity and Population Structure of Rice Genotypes using ISSR Markers

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ABSTRACT

Background: The realm of genetic diversity within rice is immense and undermining it provides the opportunity to utilize them in rice improvement programs. Hence in our study, we aimed to undermine the genetic composition and structure of the selected rice accessions utilizing ISSR marker systems.

Methods: ISSR analysis encompassed a set of thirty genotypes, comprising 16 cultivated varieties and 14 landraces. The screening was performed with a total of 49 ISSR primers. The consensus tree constructed from banding patterns generated by ISSR-PCR clustered 30 genotypes according to their respective genomes. The Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering was employed with the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. The grouping of the 30 accessions was carried out through data analysis using NTSYSpc 2.02.

Result: Utilizing 49 ISSR markers, the cluster analysis produced three clusters. These clusters displayed pronounced separation and exhibited evident patterns. The Cluster I encompassed Bharathi, BG367-2, PTB33, ASD9, ASD16, ASD20, Rathu Heenati and Columbia-2. Notably, the largest cluster was Cluster II comprising 20 accessions, while Cluster III contained only Jeeraga Samba and Basmati 370. The study validates the efficacy of ISSR markers in detecting polymorphism within and among rice populations and/or species. The resulting DNA profiles hold potential for serving as diagnostic fingerprints of both cultivated and wild rice germplasm, aiding in comprehending evolutionary relationships.

Key words: Genetic diversity, ISSR markers, Population structure, Rice.

INTRODUCTION

Rice serves as the primary dietary staple for the majority of the global population and its worldwide production is 517 million tonnes during 2022-2023 (FAO 2023). The remarkable genetic diversity of rice is evident from the wide range climatic conditions under which it is cultivated. However, the productivity of rice is limited by various biotic and abiotic stresses which may affect the global production and the livelihood of rice farmers in the under-developed countries (Kumar *et al*., 2021). Hence it is crucial to investigate the wide range of stress-resistant genes from the extensive gene reservoirs of rice and its indigenous counterparts. The initial step in this process is to assess the genetic diversity of various rice germplasm to understand the similarities and differences in their genetic composition (Dale and Schantz, 2002). Comparison of complete genome sequences of germplasm lines enables the detection of a comprehensive spectrum of potential variations. However, considering the extent of time and effort, for regular investigations, the methods focusing on discrete genomic alterations are favoured (Dale and Schantz, 2002). In this context, molecular markers stand out as a valuable tool, facilitating the estimation of genetic variability across and within numerous species. The technique of ISSR-PCR is swiftly employed by the research community across multiple domains of plant improvement (Godwin *et al*., 1997).

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MATERIALS AND METHODS

Plant materials

A set of 30 rice accessions including 16 cultivated varieties and 14 landraces were collected from Tamil Nadu Agricultural University. The details of the materials are furnished in Table 1.

ISSR primers

For this study, a set of 49 ISSR primers, selected randomly to encompass the repeats of di-nucleotide, tri-nucleotide and tetra-nucleotide were subjected to screening. Within this set, 22 ISSR markers which displayed polymorphism and distinctive patterns were selected to determine the genetic diversity (Table 2).

DNA extraction

The Genomic DNA from the leaf samples was extracted using the protocol detailed by McCouch *et al*. (1988).

PCR and determination of polymorphism information content

The DNA amplification was conducted within a reaction volume of10 µl with 20-30 ng of genomic DNA, 0.5 µM each of forward and reverse primers, 1.0mM dNTPs, 1.0 mM assay buffer and 0.03 units of Taq DNA polymerase. The PTC Thermal Cycler (MJ Research Inc.,) was employed with the program involving initial denaturation at 94°C for 1.5 min, 35 cycles of 40-seconds denaturation at 94°C, primer annealing at 45°C for 45 s, final extension at 72°C for 1.5 min. and then hold at 4°C. The computation of the Polymorphic Information Content (PIC) value for each ISSR marker was accomplished using the formula Hn = 1 - Spi 2, where pi represents the allele frequency of the ith allele, as outlined by Nei in 1973.

Cluster Analysis

The Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering technique was applied to a similarity matrix, with the dice co-efficient for binary data generated by the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) method. The clustering of the 60 accessions was done with NTSYSpc version 2.02 (Rohlf, 1994).

RESULTS AND DISCUSSION

The landraces and cultivars used in this study are regularly employed as one of the parents in the rice breeding programmes.

Determination of PIC values

The polymorphic potential of 49 ISSR markers was evaluated among the set of 30 rice genotypes. Out of these,

Table 1: List of accessions used in the study.

Entry name	Parentage	Source/Origin
ADT38	IR1529-680-3-2/IR4432-52-6/IR7963-30-2	Tamil Nadu, India
ADT39	IR8/ IR20	Tamil Nadu, India
ASD9	Avasara samba	Tamil Nadu, India
ASD16	ADT31/CO39	Tamil Nadu, India
ASD20	IR18348-38-3/IR25863-61-32 /IR58	Tamil Nadu, India
Basmati 370	Selection from Punjab local Basmati	Punjab, India
BG367-2	Sri Lankan cultivar	Sri Lanka
Bharathi	Landrace	Tamil Nadu, India
BPT5204	GEB-24/TN 1/Mahsuri	Andhra Pradesh
CO43	Dasal × IR20	Tamil Nadu, India
CO50	CO43 × ADT38	Tamil Nadu, India
Columbia-2	Columbian *indica* cultivar	Columbia
GEB24	Spontaneous mutant	Tamil Nadu, India
IR50	IR2153-14-1-6-2/IR28//IR 36	Philippines
Jeeraga samba	Landrace	Tamil Nadu, India
Kallurundaikar	Landrace	Tamil Nadu, India
Kathanellu	Landrace	Tamil Nadu, India
Mattaikar	Landrace	Tamil Nadu, India
Nagina22 (N22)	Seletion from landrace Rajbhog	Uttar Pradesh, India
Nootripathu	Landrace	Tamil Nadu, India
PTB33	Pure line selection from Arikkirai	Kerala, India
Purple puttu	Landrace	Tamil Nadu, India
Pusa basmati	Pusa167/Karnal local	New Delhi, India
Rascadam	Landrace	Tamil Nadu, India
Rathu heenati	Sri Lankan local variety	Sri Lanka
Sivappu chithiraikar	Landrace	Tamil Nadu, India
SR26B	Local variety	Odisha, India
TN1	Chow-Woo-Gen/Tsai-Yuan-Chung	Taiwan
Veeradangan	Landrace	Tamil Nadu, India
White ponni	Taichung 65/2/Mayang Ebos-80	Tamil Nadu, India

22 markers exhibited polymorphism. On the average, there were 16.36 alleles ranging from 7 to 28 for ISSR 829 and ISSR 808 respectively. In cases where an amplification product could not be detected for a specific genotype-marker combination, a variety was designated to have a null allele at the corresponding ISSR locus. The PIC values, which are indicative of allele diversity and frequency among the varieties, displayed variations among the tested ISSR loci. With an average of 0.666, the PIC value ranged from 0.359 (ISSR 890) to 0.846 (ISSR 826). The most informative marker based on the PIC value was ISSR 826 which is composed of $(AC)_n$ repeats. ISSR 808 wih $(AG)_n$ recorded the second maximum PIC value. The higher number of alleles were detected from primers ISSR 808, 840, 885 and 807 which comprised of either $(AG)_n$ or $(GA)_n$ repeats. This is in correspondence with reports of Sarla *et al*. (2005) and Reddy *et al*. (2009) wherein the primers comprising $(AG)_n$ or $(GA)_n$ repeats demonstrated the highest PIC and Rp values in distinguishing rice germplasm lines. The polymorphism percentage and the PIC values obtained are similar to those observed by Khumbar *et al*. (2015) and Zayed *et al*. (2023) in their study on the landraces and improved varieties of rice and on hybrids respectively. Details regarding the ISSR primers employed in the determination of genetic diversity including the allele count for each ISSR locus and their corresponding PIC values are presented in Table 2. The allele distribution ISSR loci *viz*. 889 and 842 across a section of 30 rice cultivars is shown in Fig 1.

Table 2: Details of ISSR primers used in the study.

Primer	Sequence	Alleles	PIC value
ISSR 807	(AG) 8T	24	0.716898
ISSR 808	(AG) 8C	28	0.838254
ISSR 809	(AG) 8G	18	0.647716
ISSR 810	(GA) 8T	20	0.627389
ISSR 817	(CA) 8A	11	0.576263
ISSR 826	(AC) 8C	11	0.846465
ISSR 829	(TG) 8C	7	0.726825
ISSR 834	(AG) 8YT	11	0.609293
ISSR 840	(GA) 8YT	28	0.748333
ISSR 841	(GA) 8YC	17	0.68902
ISSR 842	(GA) 8YG	12	0.705926
ISSR 848	(CA) 8RG	16	0.624097
ISSR 855	(AC) 8YT	10	0.542889
ISSR 856	(AC) 8YA	10	0.678111
ISSR 859	(TG) 8RC	15	0.623111
ISSR 864	(ATG) 6	14	0.794127
ISSR 869	(GTT) 6	12	0.763148
ISSR 872	(GATA) 4	19	0.581287
ISSR 880	(GGAGA) 3	22	0.805051
ISSR 885	BHB (GA) 7	25	0.636711
ISSR 889	DBD (AC) 7	20	0.520611
ISSR 890	VHV (GT) 7	10	0.359667

DNA marker-based diversity across rice accessions

Genetic relationships among the thirty genotypes were determined by computing Dice's similarity coefficient through the assessment of shared bands proportions generated by the primers (Dice, 1945) and the dendrogram is represented in Fig 2. All 30 rice accessions were classified using 22 primers. Three distinct groups emerged from the analysis, characterized by the similarity coefficient of 0.66. The Cluster I contained Bharathi, BG367-2, PTB33, ASD9, ASD16, ASD20, Rathu Heenati and Columbia-2. Notably, Cluster II appeared to be the largest cluster with 20 accessions. Most of the cultivated genotypes used in the study were grouped together in this cluster indicating the common genetic background of their parents. Cluster III was found to possess two accessions, Jeeraga Samba and Basmati370. Major cluster I have three sub-clusters, in which Rathu Heenati and Columbia-2 formed separate group. Cluster II had four sub-clusters. Among four sub-clusters, Pusa basmati fell in

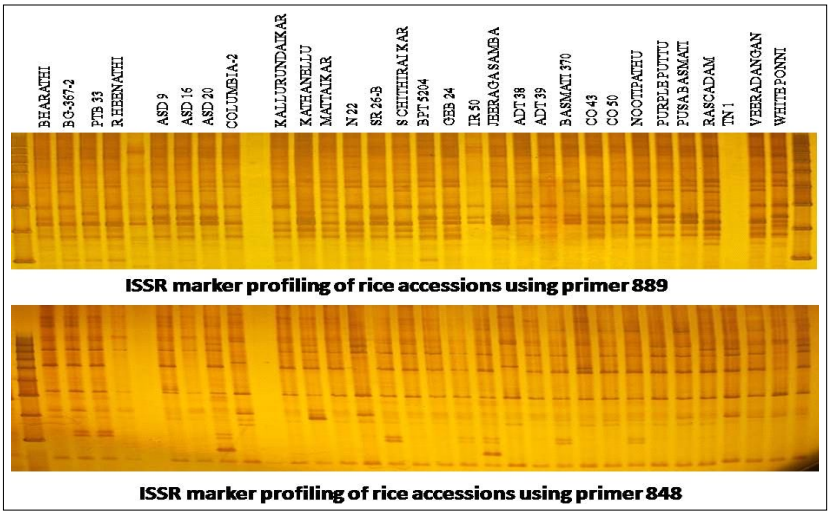

Fig 1: ISSR profiling of rice accessions.

separate sub-cluster and the set of accessions comprising TN1, Veerdangan, White Ponni and N22, Rascadam grouped into separate sub-clusters. Table 3. Similarly, the traditional rice varieties were divided into three major clusters

Table 3: Clustering of 30 rice accessions based on similarity co-efficients.

Cluster	Sub-cluster	Entry name
I	A	Bharathi
		BG367-2
		PTB33
		ASD9
		ASD16
		ASD20
	B	Rathu Heenati
	C	Columbia -2
II	D	Kallurundaikar
		Sivappu Chithiraikar
		Mattaikar
		SR26B
		GEB24
		IR50
		ADT38
		ADT39
		BPT5204
		CO43
		CO50
		Nootripathu
		Purple Puttu
	E	TN1
		Veeradangan
		White Ponni
	F	Pusa Basmati
	G	Kathanellu
		Nagina22 (N22)
		Rascadam
III	H	Jeeraga Samba
		Basmati 370

using ISSR markers by Nahar *et al*. 2020, indicating moderate genetic diversity among the genotypes studied. The efficiency of ISSR markers in determining the genetic diversity of rice was evidenced in the study by Taratima *et al*. (2019) as compared to the RAPD markers.

Population structure

In rice, the population structure analysis is carried out in different panels of genotypes and under various growth and stress conditions like salinity and drought using various molecular markers and genomic tools (Bhattarai *et al*., 2019; Warraich *et al*., 2021). In this study, the population structure of 30 germplasm lines was determined using the Bayesian approach which has been used to identify sub-populations in various crops including rice, wheat, chickpea, carrot and bamboo (Seyedimoradi *et al*., 2020; Chaitra *et al.,* 2020; Li *et al*., 2019). The ideal number of populations was determined through the examination of correlated allele frequencies as 3 (K = 3). Likewise, the maximum of *adhoc* measure ΔK was also found to be K = 3 (Fig 3), thereby indicating the possibility of categorizing the entire population into three sub-groups denoted as SG1, SG2 and SG3. It is interesting to note that the cluster analysis based on the similarity coefficients, also divided the genotypes into three major clusters. Similar coherence among the grouping of genotypes using clustering and population structure analysis has been reported in many crops. Khumbar *et al*. (2015) reported similar results in the genetic diversity analysis of rice using SSR and ISSR markers. The ability of structure analysis to determine the extent of admixture and the unique genotypes within and among various races and species of rice has been reported in a multitude of research investigations (Haritha *et al*., 2016; Zhou *et al*., 2020). The membership fractions were utilized to assign the accessions to different sub-gruops. Those with the probability of ≥70 per cent were designated to the respective subgroups while the rest were labelled as admixture (Table 4). Within SG1, there were 5 accessions including 3 landraces and 2 varieties of Indian origin and SG2 was constituted by 12

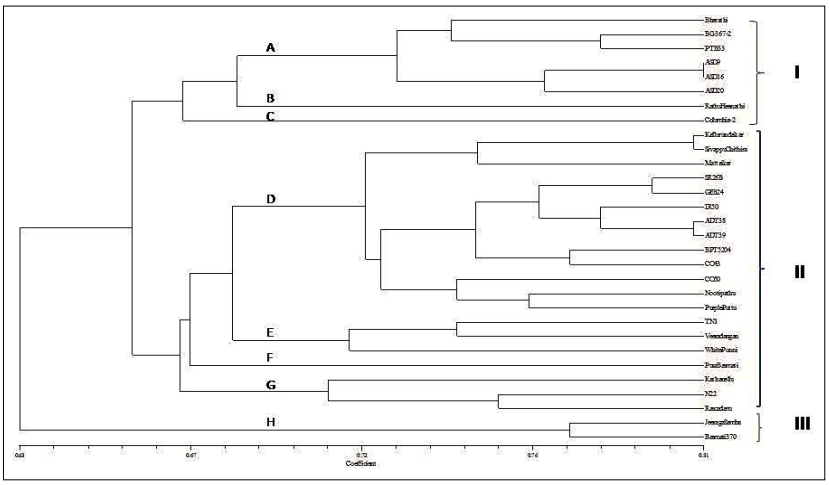

Fig 2: Dendrogram of rice accessions based on ISSR markers, using UPGMA based on Dice similarity coefficients.

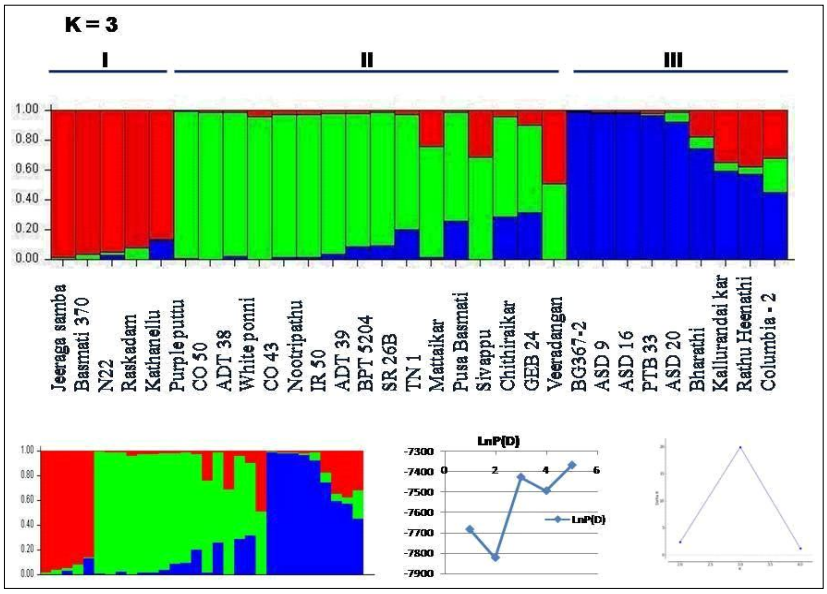

Fig 3: Population structure of rice accessions.

Table 4: Inferred ancestry of rice accessions based on structure analysis.

Genotype	Population 1 Q value	Population 2 Q value	Population 3 Q value	Inferred subpopulation
N22	0.946	0.025	0.029	1
Jeeraga Samba	0.979	0.014	0.007	1
Basmati370	0.96	0.037	0.003	1
Rascadam	0.918	0.075	0.007	1
Kathanellu	0.864	0.007	0.129	1
BPT5204	0.013	0.891	0.095	2
IR50	0.016	0.943	0.041	2
ADT38	0.013	0.964	0.023	2
ADT39	0.015	0.895	0.089	2
CO43	0.025	0.954	0.021	2
Mattaikar	0.014	0.722	0.265	2
SR26B	0.023	0.772	0.205	2
CO50	0.013	0.982	0.005	2
Nootripathu	0.028	0.953	0.019	2
Purple Puttu	0.006	0.984	0.011	2
TN1	0.24	0.745	0.015	2
White Ponni	0.04	0.956	0.004	2
Bharathi	0.177	0.08	0.743	3
BG-367-2	0.006	0.006	0.989	3
PTB33	0.018	0.014	0.968	3
ASD9	0.01	0.005	0.985	3
ASD16	0.008	0.007	0.984	3
ASD20	0.012	0.062	0.926	3
Pusa Basmati	0.309	0.685	0.005	2, 1
Veeradangan	0.489	0.504	0.007	2,1
Sivappu Chithiraikar	0.041	0.67	0.289	2, 3
GEB24	0.094	0.588	0.318	2, 3
Rathu Heenati	0.374	0.048	0.578	3,1
Kallurundaikar	0.343	0.062	0.595	3,1
Columbia-2	0.321	0.225	0.454	3, 1, 2

accessions, of which 4 were landraces and 8 were varieties of Indian origin. SG3 contained 6 accessions *viz*.,1 landrace and 5 varieties of Indian and Sri Lankan origin respectively. The grouping of landraces and the commonly cultivated varieties together in a group denotes the extent of shared alleles between them. Hence the landraces which are grouped with the cultivars can be used as donors in breeding schemes as they might result in lesser linkage drag (Mazumder *et al.,* 2020). Seven accessions were retained to be admixture. It is observed that the genotypes were placed in distinct groups based on their membership fractions and there are only fewer admixture genotypes. This could be attributed to the autogamous nature of the crop resulting in restricted gene flow and allele sharing (Gao and Innan, 2008; Choudhry *et al*., 2013). Rice genotypes including Basmati 370, Jeeraga Samba, N22, Kathanellu and Rascadam formed the components of SG1. SG2 possessed BPT5204, IR50, ADT38, ADT39, CO43, CO50, Nootripathu, Purple Puttu, TN 1, Mattaikar, SR26B and White Ponni. SG 3 comprised Bharathi, BG 367-2, PTB33, ASD9, ASD16 and ASD20. The ancestry values inferred from the structure analysis were utilized for the clustering of rice accessions. Rhathu Heenati, Colambia-2, Kallurandaikar, Sivappu Chithraikar, GEB24, Pusa Basmati and Veeradangan were found to be not in any of the distinct populations based on their inferred ancestry values. Despite the incomplete genome coverage, the allele frequencies derived from these markers yielded valuable insights into relationships among accessions, reflecting the allele sharing pattern. Owing to the limitations posed by the marker's scope and the number of genotypes examined, the additional parameters associated with the structure analysis were disregarded, yet the analysis notably highlighted the presence of three distinct sub-populations within the cohort of 30 accessions of the present investigation.

CONCLUSION

The genetic diversity assessment of the set of rice accessions comprising landraces and cultivars using ISSR markers classified them into three groups. The ISSR markers with (GA) and parentheses (AG) repeats were highly informative in determining the genetic composition. The genetic relatedness among the genotypes was well characterized using similarity co-efficient-based clustering and Bayesian approach-based population structure analysis and were coherent with each other. It is observed from the study that this set of genotypes possess moderate to high genetic diversity and the landraces and cultivars analysed in this study sharing similar alleles can be crossed to produce improved cultivars.

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Conflict of interest: None.

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