

RESEARCH ARTICLE

Molecular characterization of Indian paddy varieties using SSR markers and unveiling G × E interactions of paddy varieties under the Cauvery Delta region

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Abstract

Twenty-eight popular paddy varieties released and notified throughout India between 1976 and 2015 were molecularly characterized using SSR markers. The lab analysis was conducted at the Biotechnology Laboratory, Faculty of Agriculture, Annamalai University and stability study over three locations under the Cauvery Delta region during December 2022 to May 2023. The results showed that nine SSR primers were polymorphic and PIC values varied from 0.363 to 0.551, while the marker RM 118 had the highest PIC value (0.551). The range of the detected heterozygosity was 0.304 to 0.622. Neis' genetic distance created the dendrogram, which divided the varieties into seven clusters. According to Neis' genetic measure, variety G8 (ADT 39) from cluster I and G15 (TKM 9) from cluster VII confirmed the degree of genetic diversity among the varieties studied. From molecular genetic diversity, nine high-yielding varieties were chosen based on grain yield per plant and subjected to stability analysis in three different environments. Significant genotype × environment interaction was observed for all the characters; hence, these varieties' stability was assessed. Variety, G9 (ADT 48) showed non-significant S²d_i and regression coefficient value around unity with high mean for days to first flowering, grain length and grain yield per plant and plotted under group I in genotype grouping technique. Varieties viz., G8 (ADT 37), G9 (ADT 48), G15 (TKM 9) and G16 (MDU 6) are the most stable and acceptable for both favourable and unfavourable environments. It showed stability factor around unity for grain yield per plant and they will be selected as parents to develop stress-tolerant varieties.

Keywords

Eberhart and Russell model; rice; SSR markers; stability

Introduction

Rice is the bread and butter for over half of the worlds' population. About 90 % of the population in Asia consumes rice as a staple food, which is referred to as the grain of life (1). Global rice production is projected at a record 520.5 million tons. In India, rice production from 2023-2024 was about 138.00 M mt respectively. In Tamil Nadu, rice production in 2023-2024 was about 3354 kg/ha respectively (2). Light, temperature, relative humidity and soil condition are vital during the germination, flowering and grain maturity stages (3). In the Cauvery Delta region,

unexpected rainfall, alternate temperatures and salinity affect crop growth, resulting in a yield loss compared to the previous year (4). Identifying successful varieties over seasons will pave the way to utilize that variety in crop improvement and hybridization programmes (5).

The success of any plant breeding programme largely depends on genetic variability among the genotypes/cultivars studied (6). Hence, estimating genetic diversity for yield and its components among the geographically diversified varieties is essential in planning the future hybridization programme (7). Morphological assessment is not enough to identify the superior varieties; it is crucial to characterize them genetically. It has gained momentum with the advent of PCR-based molecular markers. Nowadays, the SSR marker is a choice for molecular characterization as it is a co-dominant marker distributed throughout the genome, highly reproducible, variable, reliable, easily scorable, abundant and multi-allelic (8). SSR markers can give a better genetic diversity spectrum even in fewer numbers due to their multi-allelic and highly polymorphic. Molecularly characterized varieties were chosen based on the genetic distance and applied to stability analysis to check their performances. It is necessary to undertake a $G \times E$ interaction to determine stability in which $G \times E$ significantly affects and causes changes in the genotype ranking across different environments, either in general adaptability or specialized adaptation (9). This study uses the parametric stability analysis to reveal the genotype \times environment interactions. The least sensitive to environmental fluctuation genotype is considered stable and most preferred in varietal selection (10). The popular paddy varieties across four states and ruling over two decades were taken for current research with an objective of molecular characterization and genotype \times environment over three locations of the Cauvery Delta region.

Materials and Methods

Location

Twenty-eight popular Indian paddy varieties were used in the present scrutiny. Seeds of rice varieties were obtained from various research institutions all over India. The details of the varieties are shown in Table 1. The contemporary research was carried out in the Experimental field and molecular laboratory, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India, during December 2022 and March 2023 and stability studies carried over three different locations (Table 2).

Quantitative traits

Each variety was sown in a raised bed and 25-day-old seedlings were transplanted with spacing of 20 cm \times 15 cm. Recommended cultural practices were followed throughout the crop growth and need-based plant protection measures were observed. The experiment was carried out in a randomized block design with three replications, with twenty plants maintained in a row per replication during December 2022 and March 2023. Observations were recorded from five randomly selected plants in each genotype per replication for thirteen characters viz., days to first flowering, plant height, number of productive tillers, panicle length, number of grains per panicle, hundred-grain weight, grain length, grain breadth, grain L/B ratio, kernel length, kernel breadth, kernel L/B ratio and grain yield per plant.

Data analysis

Data was analyzed by using TNAU STAT (11).

Molecular diversity analysis

The genomic DNA was extracted from 20-25 days old seedlings and DNA isolation was carried out following the

Table 1. List of rice varieties taken for the study

Genotypes	Varieties	Source	Parentage	Year of Release/Notification
G1	ADT 36	Tamil Nadu Rice Research Institute, Aduthurai	Triveni \times IR20	1982
G2	ADT 37	Tamil Nadu Rice Research Institute, Aduthurai	BG280-12 \times PTB33	1987
G3	ADT 47	Tamil Nadu Rice Research Institute, Aduthurai	ADT 43 \times Jeeragasamba	2005
G4	ADT 41	Tamil Nadu Rice Research Institute, Aduthurai	Dwarf mutant of Basmati 370	1993
G5	JGL 1798	A.N.G.R.A.U Research station Andhra Pradesh	-	2002
G6	ADT 43	Tamil Nadu Rice Research Institute, Aduthurai	IR 50 \times White Ponni	1998
G7	ADT 45	Tamil Nadu Rice Research Institute, Aduthurai	IR 50 \times ADT 37	2001
G8	ADT 39	Tamil Nadu Rice Research Institute, Aduthurai	IR 8 \times IR 20	1988
G9	ADT 48	Tamil Nadu Rice Research Institute, Aduthurai	IET11412 \times IR 64	2005
G10	ADT 49	Tamil Nadu Rice Research Institute, Aduthurai	CR1009 \times Seeraga Samba	2011
G11	ADT 50	Tamil Nadu Rice Research Institute, Aduthurai	BPT 5204 \times CR1009	2012
G12	CO 48	Tamil Nadu Agricultural University, Coimbatore	CO43 \times ASD19	2007
G13	CO 51 BS	Tamil Nadu Agricultural University, Coimbatore	ADT43 \times RR272-1745	2013
G14	TRY 3	Tamil Nadu Agricultural University, Trichy	ADT42 \times Seeraga samba	2010
G15	TKM 9	Rice Research Station, Tirurkuppam	TKM 7 \times IR 8	1978
G16	MDU 6	Agricultural College & Research Institute, Madurai	MDU5 \times ACM 96136	2015
G17	BPT 5204	Agricultural college, Bapatla Andhra Pradesh	GEB24 \times (TN1) \times Mahsuri	1976
G18	NLR 34449	A.N.G.R.A.U Research station Andhra Pradesh	-	-
G19	I.white ponni	Tamil Nadu Rice Research Institute, Aduthurai	Selection from white ponni	1989
G20	ASD 20	Rice Research Station, Ambasamudram	IR18348 \times IR25863 \times IR58	1997
G21	Amogh	Agris Kurnool Seeds Pvt. Ltd andhra Pradesh	-	-
G22	Annapurna	Regional Agricultural Station, Pattambi, Kerala	Taichng(Native1) \times PTB10	1977
G23	Vasundhara	A.N.G.R.A.U Research station Andhra Pradesh	RGL2538	1996
G24	Paiyur 1	Regional Research Station, Paiyur	IR1721-14 \times IR1330-3-3-2	1984
G25	CO 43	Tamil Nadu Agricultural University, Coimbatore	Dasal \times IR20	1982
G26	RNR-1446	A.N.G.R.A.U Research station Andhra Pradesh	-	-
G27	NDR 359	Narendra Deva University of Agriculture & Technology, U. P	BG-90-2-4 \times 08677	1994
G28	NDR 325	Narendra Deva University of Agriculture & Technology, U. P	-	-

Table 2. Details of three different environments selected for study

Particulars	E ₁	E ₂	E ₃
Location	Keerapalayam Bhuvanagiri Block, Tamilnadu	Plant Breeding Farm, Annamalai University, Tamilnadu	Puthur Sirkazhi Block, Tamilnadu
Season	June 2023-Sep 2023 (Kuruwai)	June 2023-Sep 2023 (Kuruwai)	June 2023-Sep 2023 (Kuruwai)
Soil Type	Clay	Clay	Clay
Soil pH	7.4	6.3	6.6
Soil status			
N	High	Medium	Medium
P	Medium	Medium	Low
K	High	Medium	Medium
Temperature			
Maximum °C	30.36	35.6	31.1
Minimum °C	23.5	25.53	22.7
Relative humidity (%)	75	72.25	79.3
Average Sunshine Hours	6.46	6.13	5.43
Evaporation (%)	3.62	4.68	2.95

standard procedures during May 2023.

SSR marker and PCR amplification

A total of 9 SSR markers were used for the diversity analysis. The PCR assay mixture contains 15 picomols of primer pairs, 200 µM of deoxy nucleotides, 2.5 µL of 1X buffer with 0.1 mM MgCl₂, 1 unit of Taq polymerase and 25-50 ng of DNA template. The PCR assay technique for the SSR marker is given below.

PCR Steps

An initial denaturation step for 3 min at 94 °C followed by 35 cycles of denaturation (94 °C), annealing (55-67 °C), primer elongation (72 °C) for 2 min and final extension at 72 °C for 7 min. Amplified products were stored at 4 °C until further use.

Gel electrophoresis and PCR

Preceding electrophoresis, 3 µL of loading dye was added to the 25 µL of PCR product, mixed thoroughly and the samples were loaded into the well. 5 µL of marker DNA (100 bp DNA ladder) was loaded in the first well for reference. Electrophoresis was carried out at 70 V for the separation of products. The DNA fragments were visualized and documented in gel documentation system.

Data analysis

The bands were scored and represented by their allele sizes as allelic data. Using software package POPGENE ver. 3.2 software, the cluster analysis was performed to obtain a dendrogram. Neis' unbiased measures of genetic identity, genetic distance and Neis' heterozygosity was also calculated (12). Polymorphic Information Content (PIC) values were calculated for SSR markers to characterize the capacity of each primer to reveal or detect polymorphic loci among the genotypes. It is the total of polymorphism information content values of all the markers produced by a particular primer. PIC value was calculated using the Equation 1 formula,

$$PIC = 1 - \sum p_i^2 \quad (\text{Eqn. 1})$$

where p_i is the frequency of the i 'th allele (13).

Stability analysis

The analysis of variance for each environment was escorted. The mean genotypic values for each environment were taken to analyze the data over the environment. The characters that recorded significant $G \times E$ were examined for stability (14). By molecular diversity analysis, seven varieties, namely

G8, G10, G15, G16, G22, G24 and G27, were selected to account for hypervariability, better genomic coverage and high reproducibility. Many studies revealed that genetic diversity had been reported to use both morphological and molecular markers simultaneously, decided that nine varieties, namely G8, G9, G10, G15, G 16, G22, G23, G24 and G27, were selected for stability analysis out of twenty-eight varieties. Biometric observations were recorded for the thirteen characters already studied in genetic diversity. Following the methodology, three parameters, namely (i) the overall mean of each genotype over a range of environments, (ii) the regression of each genotype on the environmental index and (iii) a function of the squared deviation from the regression, were estimated and used to study the stability of genotypes under different environments (14) (Table 2).

Genotype grouping analysis

An alternative approach for grouping the genotypes for stability performance based on mean and coefficient of variation across environments. The genotypes were grouped into four categories, namely

- Group I - High mean and low variation
- Group II - High mean and high variation
- Group III - Low mean and low variation
- Group IV - Low mean and high variation

Results and Discussion

Mean performance

The analysis of variance revealed significant differences for all the Thirteen traits indicate the existence of high genetic variability among the varieties (Table 3). Among the twenty-eight varieties studied, the highest and most significant mean values were found in the varieties viz., G9, G16, G4 and G1 for most of the characters observed. Variety G9 exhibited significant by superior mean values for the characters viz., days to first flowering, plant height, number of productive tillers, panicle length, number of grains per panicle, hundred-grain weight, grain length, grain L/B ratio, kernel length, kernel L/B ratio and grain yield per plant except grain breadth and kernel breadth (Table 4). Out of twenty-eight varieties, G8 (ADT 39), G9 (ADT 48), G16 (MDU 6), G17 (BPT 5204), G2 (Annapurna) and G7 (NDR 359) had high significant mean values for yield and yield attributing traits (Table 4). Similar

Table 3. Analysis of variance of 28 varieties for various morphological characters

Sources	df	MSS												
		DFF	PH (cm)	NPP	PL (cm)	NGP	HGW (g)	GL (mm)	GB (mm)	GLB ratio	KL (mm)	KB (mm)	KLB ratio	GYP (g)
Replication	2	0.16	1.10	0.19	1.07	31.46	0.003	0.02	0.02	0.02	0.08	0.004	0.08	0.16
Genotype	27	100.	542.	9.	12.	278.	0.	1.	0.	0.	1.	0.	0.	43.
Error	54	33**	10**	59**	41**	68**	30**	58**	30**	96**	63**	12**	67**	55**
		0.70	11.03	0.74	4.86	11.86	0.003	0.04	0.02	0.04	0.08	0.03	0.07	3.11

*Significant at 5 % level; **Significant at 1 % level; DFF-Days to first flowering; PH-Plant height; NPP-No. of productive tillers per plant; PL-Panicle length; NGP-No. of grains per panicle; HGW-Hundred grain weight; GL-Grain length; GB-Grain breadth; GLB-Grain LB ratio; KL-Kernel length; KB-Kernel breadth; KLB-Kernel LB ratio; GYP-Grain yield per plant

reports were observed in rice by (16). G8 (ADT 39), G9 (ADT 48) and G17 (BPT 5204) were the promising varieties cultivated in southern parts of Tamil Nadu. Research indicates a similar results in rice (5).

Molecular diversity using SSR markers

All the 9 SSR markers showed polymorphism (Table 5). A total of 24 alleles were generated. The number of alleles ranged from two to three, with an average of 2.67 alleles per primer. Six rice markers viz., RM 118, RM 181, RM 153, RM 171, RM 570 and RM 179 recorded the highest number of three alleles and three primers, RM 278, RM 126 and RM 119 recorded lowest number of two alleles (Fig. 1 & 2). Heterozygosity ranges from 0.304 to 0.622. Highest heterozygosity was recorded by primer RM 118 (Table 6). In the present study, markers RM 118 (0.551) and RM 181 (0.528) recorded high PIC values, which could be used for the identification and diversity estimation of rice varieties and in the taxonomical and genetic resource management field. The varieties with high heterozygosity might have high genetic variability and this has proved to be an efficient tool for assessing genetic diversity and identifying the varieties (17-19). High genetic variability between varieties G15 and G8 indicates rich genetic material of a species; hence, it

Table 5. List of SSR primers used for the study

Primer	Sequence	Annealing Temperature (°C)
RM 118	F - CCAATCGGAGCCACCGAGAGC R - CACATCCTCCAGCGACGCCGAG	67
RM 181	F - ACGGGAGCTTCTCCGACAGCGC R - TATGCTTTTGCCGTGTGCCGCG	67
RM 179	F - CCCATTAGTCCACTCCACCACC R - CCAATCAGCCTCATGCTCCCC	61
RM 153	F - GCCTCGAGCATCATCATCAG R - ATCAACCTGCACTTGCTTGG	55
RM 278	F - GTAGTGAGCCTAACAATAATC R - TCAACTCAGCATCTCTGTCC	55
RM 126	F - CGCGTCCGCGATAAACACAGGG R - TCGCACAGGTGAGGCCATGTGC	55
RM 171	F - AACGCGAGGACACGTACTTAC R - ACGAGATACGTACGCCTTTG	55
RM 570	F - GTTCTTCAACTCCAGTGC R - TGACGATGTGGAAGAGCAAG	55
RM 119	F - CATCCCCCTGCTGCTGCTGCTG R - CGCCGGATGTGTGGACTAGCG	67

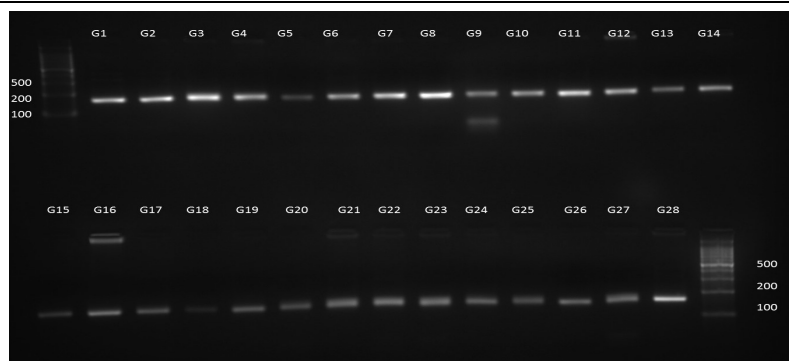
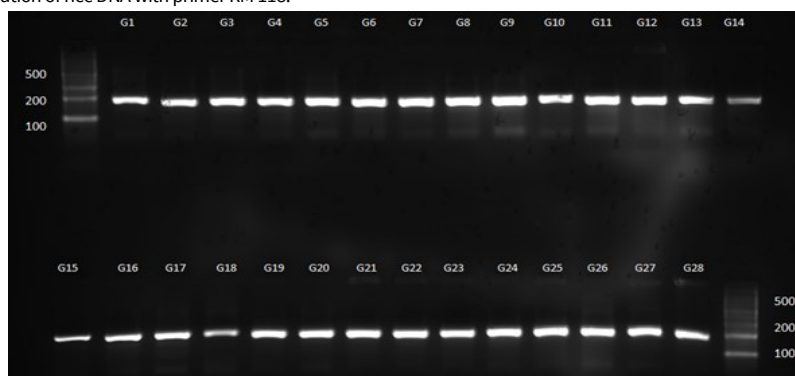
Table 4. Ranking of 28 varieties based on mean performance for various traits

Characters Genotypes	DFF	PH (cm)	NPP	PL (cm)	NGP	HGW (g)	GL (mm)	GB (mm)	GLB ratio	KL (mm)	KB (mm)	KLB ratio	GYP (g)	Total score
G1	**	**	**	-	-	**	-	**	-	-	-	-	-	5
G2	**	*	-	-	-	-	-	**	-	-	**	-	-	4
G3	-	-	-	-	**	-	-	**	-	-	-	-	-	2
G4	**	-	-	**	-	-	**	-	**	**	-	**	-	6
G5	*	-	-	-	-	**	**	**	-	**	-	-	-	5
G6	*	**	**	-	-	-	-	-	**	-	-	-	-	4
G7	-	**	-	-	-	-	-	-	-	-	-	-	-	1
G8	-	**	-	-	**	**	-	-	-	-	-	-	**	4
G9	**	**	**	**	**	**	**	-	**	**	-	**	**	11
G10	-	-	**	-	-	-	-	-	-	-	-	-	-	1
G11	-	-	-	-	-	-	-	-	-	-	-	-	-	0
G12	**	**	-	-	-	-	-	-	-	-	-	-	-	2
G13	-	**	-	-	-	-	-	-	-	-	-	-	-	1
G14	-	-	-	-	-	**	-	-	-	-	-	-	-	1
G15	-	-	-	-	**	**	-	**	-	-	**	-	-	4
G16	**	**	-	-	**	**	**	**	-	**	-	-	**	8
G17	-	-	**	-	-	**	-	-	-	-	-	-	**	3
G18	**	-	-	-	-	-	-	-	*	-	-	-	-	2
G19	**	-	-	-	-	-	-	-	-	-	-	-	-	1
G20	-	**	*	-	-	**	-	-	-	-	-	-	-	3
G21	**	-	-	**	-	-	-	-	**	-	-	**	-	4
G22	**	**	-	-	-	-	-	-	**	-	-	-	**	4
G23	**	**	-	-	-	-	-	-	-	-	-	-	-	2
G24	*	-	-	-	-	-	-	-	-	-	-	-	-	1
G25	**	-	-	-	-	**	-	-	-	-	-	-	-	2
G26	-	**	-	-	-	-	-	-	-	-	-	-	-	1
G27	-	**	-	-	**	**	-	-	-	-	-	-	**	4
G28	-	-	-	-	-	-	-	-	-	-	-	-	-	0

**Significant at 1 % level; DFF-Days to first flowering; PH-Plant height; NPP-No. of productive tillers per plant; PL-Panicle length; NGP-No. of grains per panicle; HGW-Hundred grain weight; GL-Grain length; GB-Grain breadth; GLB-Grain LB ratio; KL-Kernel length; KB-Kernel breadth; KLB-Kernel LB ratio; GYP-Grain yield per plant

Table 6. SSR markers, including allele size range, number of alleles, polymorphic information content (PIC) and heterozygosity

S. No.	Marker	Allele size (bp)	Number of alleles	Heterozygosity	PIC value
1.	RM 118	180-195	3	0.622	0.551
2.	RM 181	180-200	3	0.610	0.528
3.	RM 179	180-250	3	0.528	0.459
4.	RM 153	170-200	3	0.304	0.274
5.	RM 278	140-160	2	0.494	0.372
6.	RM 126	170-180	2	0.497	0.374
7.	RM 171	300-350	3	0.523	0.442
8.	RM 570	250-270	3	0.528	0.421
9.	RM 119	170-180	2	0.477	0.363

**Fig. 1.** Gel picture of PCR amplification of rice DNA with primer RM 118.**Fig. 2.** Gel picture of PCR amplification of rice DNA with primer RM 181.

can be used as a parental source for breeding line to improve rice varieties.

Polymorphic Information Content revealed the amount of information that can be obtained from a particular marker. In the present study, PIC value of the SSR markers ranged from 0.363 to 0.551 with an average of 0.42. The PIC value ranged from 0.142 to 0.792. The higher PIC value indicates the presence of high genetic diversity among the rice varieties. Neis' genetic distance value identified the highest genetic distance among the varieties G15 and G8 (Table 7). The lowest genetic distance (0.00) was found between the varieties G22 and G21, G24 and G22 which shows lesser genetic diversity among the studied rice varieties. A dendrogram based on Neis' matrix revealed that the twenty-eight varieties were grouped into seven clusters (Fig. 3). Among the seven clusters, cluster I was the largest with eight varieties, followed by clusters II, III and IV with five varieties each, clusters V and VI with two varieties and the cluster VII showed single variety (Table 8). The diverse genotypes' grouping pattern suggested no parallelism between the different clusters (20). Therefore, the extensive diversity at both molecular and morphological levels of genotypes will help the rice breeders find suitable parents containing economically important field crop traits for future hybridization.

Stability analysis

Genotype \times Environment

Considering Eberhart and Russells' model of analysis, significant differences were revealed by a pooled variance analysis for the main effects, genotypes and environments and interaction effects. The mean sum of squares due to environment (linear) was highly significant for all the characters studied (Table 9). The $E + (G \times E)$ interaction was highly significant for all the characters against pooled error and indicated the distinct nature of environments and $G \times E$ interactions in the phenotypic expression (17). The genotype \times environment (linear) interaction component showed significance for all the characters studied, indicating that significant differences among the varieties existed for linear response to environments (b) (21). The predominance of linear components noticed would help predict the varieties' performance across various environments (22).

Eberhart and Russell model

According to this model, varieties G9 and G16 exhibited a more desirable value than the population mean for grain yield per plant. For the regression coefficient, all the varieties showed non-significant values except G10 and G15 (Table 10). Varieties G8 (0.90) and G9 (1.02) recorded closer to unity. Four varieties viz., G22, G23, G24 and G27 registered greater than unity and the remaining three varieties G10, G15 and G16 showed values

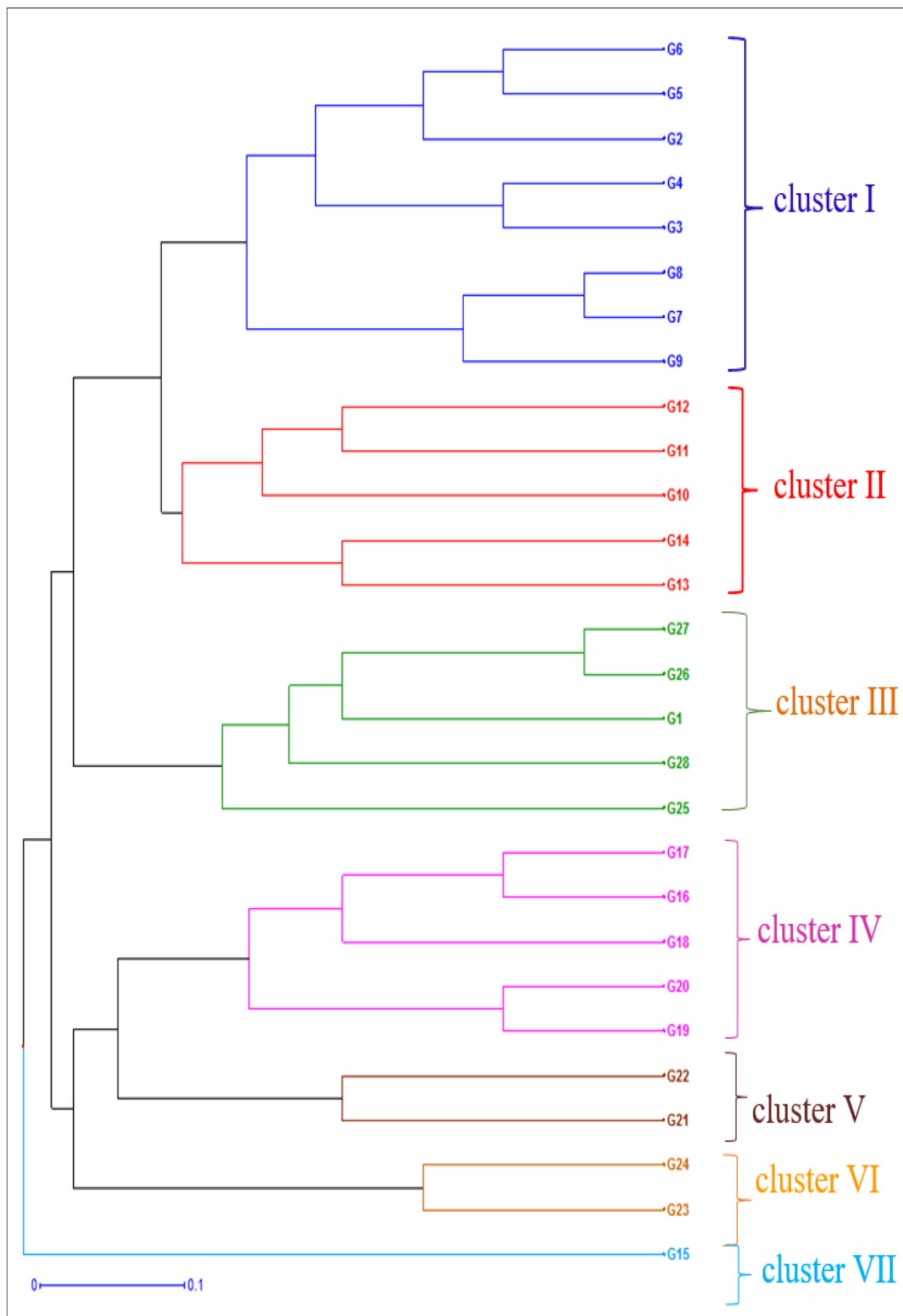


Fig. 3. Dendrogram showing genetic relationship using SSR Markers.

Table 7. Neis' unbiased measure of genetic identity and genetic distance

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	****																											
2	0.51	****																										
3	0.51	0.22	****																									
4	0.40	0.32	0.13	****																								
5	0.46	0.30	0.17	0.02	****																							
6	0.51	0.36	0.22	0.02	0.053	****																						
7	0.51	0.22	0.22	0.07	0.05	0.11	****																					
8	0.56	0.56	0.40	0.15	0.20	0.13	0.25	****																				
9	0.36	0.36	0.11	0.02	0.05	0.11	0.11	0.25	****																			
10	0.46	0.30	0.17	0.02	0.00	0.05	0.05	0.20	0.05	****																		
11	0.36	0.36	0.11	0.02	0.053	0.11	0.11	0.25	0.00	0.05	****																	
12	0.76	0.56	0.40	0.15	0.20	0.13	0.25	0.29	0.25	0.20	0.25	****																
13	0.36	0.36	0.11	0.02	0.05	0.11	0.11	0.25	0.00	0.05	0.00	0.25	****															
14	0.62	0.53	0.29	0.12	0.16	0.15	0.22	0.32	0.15	0.16	0.15	0.15	0.15	****														
15	0.40	0.40	0.40	0.29	0.34	0.40	0.40	0.62	0.25	0.34	0.25	0.22	0.25	0.29	****													
16	0.22	0.51	0.51	0.40	0.46	0.51	0.51	0.56	0.36	0.46	0.36	0.40	0.36	0.44	0.13	****												
17	0.22	0.36	0.51	0.25	0.30	0.22	0.36	0.25	0.36	0.30	0.36	0.40	0.36	0.44	0.40	0.22	****											
18	0.17	0.30	0.46	0.27	0.25	0.30	0.30	0.34	0.30	0.25	0.30	0.51	0.30	0.47	0.34	0.17	0.05	****										
19	0.36	0.11	0.36	0.19	0.17	0.22	0.11	0.40	0.22	0.17	0.22	0.40	0.22	0.36	0.25	0.36	0.22	0.17	****									
20	0.51	0.22	0.51	0.32	0.30	0.36	0.22	0.56	0.36	0.30	0.36	0.56	0.36	0.53	0.40	0.51	0.36	0.30	0.11	****								
21	0.22	0.22	0.22	0.13	0.17	0.22	0.22	0.40	0.11	0.17	0.11	0.40	0.11	0.29	0.13	0.22	0.22	0.17	0.11	0.22	****							
22	0.22	0.22	0.22	0.13	0.17	0.22	0.22	0.40	0.11	0.17	0.11	0.40	0.11	0.29	0.13	0.22	0.22	0.17	0.11	0.22	0.00	****						
23	0.22	0.22	0.22	0.13	0.17	0.22	0.22	0.40	0.11	0.17	0.11	0.40	0.11	0.29	0.13	0.22	0.22	0.17	0.11	0.22	0.11	0.11	****					
24	0.22	0.22	0.22	0.13	0.17	0.22	0.22	0.40	0.11	0.17	0.11	0.40	0.11	0.29	0.13	0.22	0.22	0.17	0.11	0.22	0.11	0.00	0.11	****				
25	0.22	0.22	0.22	0.13	0.17	0.22	0.22	0.40	0.11	0.17	0.11	0.40	0.11	0.29	0.13	0.22	0.22	0.17	0.11	0.22	0.11	0.11	0.11	0.11	****			
26	0.36	0.36	0.36	0.25	0.30	0.36	0.36	0.56	0.22	0.30	0.22	0.56	0.22	0.44	0.25	0.36	0.36	0.30	0.22	0.11	0.11	0.11	0.11	0.11	0.11	****		
27	0.36	0.36	0.36	0.25	0.30	0.36	0.36	0.56	0.22	0.30	0.22	0.40	0.22	0.15	0.13	0.22	0.36	0.30	0.22	0.36	0.11	0.11	0.11	0.11	0.11	0.22	****	
28	0.36	0.36	0.60	0.48	0.46	0.51	0.36	0.56	0.51	0.46	0.51	0.56	0.52	0.36	0.40	0.22	0.22	0.17	0.22	0.36	0.36	0.36	0.36	0.36	0.36	0.51	0.22	****

[Neis' genetic identity (above diagonal) and genetic distance (below diagonal)]

Table 8. Composition of molecular clusters for 28 rice varieties

Clusters	Number of genotypes	Name of genotypes
I	8	ADT 37, ADT 39, ADT 41, JGL 1789, ADT 43, ADT 45, ADT 47, ADT 48.
II	5	ADT 49, ADT 50, CO 48, CO 51 BS, TRY 3.
III	5	ADT 36, CO 43, RNR 1446, NDR 325, NDR 359.
IV	5	MDU 6, BPT 5204, NLR 34449, I. white ponni, ASD 20.
V	2	Amogh, Annapurna.
VI	2	Vasundhara, Paiyur 1.
VII	1	TKM 9

Table 9. Analysis of variance for Eberhart and Russell model

Sources	df	MSS												
		DFF	PH (cm)	NPP	PL (cm)	NGP	HGW (g)	GL (mm)	GB (mm)	GLB ratio	KL (mm)	KB (mm)	KLB ratio	GYP (g)
Genotypes	8	152.43**	257.97**	11.20**	13.93**	226.89**	0.46**	1.44**	0.28**	0.70**	2.12**	0.205**	0.76**	53.62
Environments	2	7.60**	69.22**	2.48**	3.02**	4.18**	0.15**	0.10**	0.02**	0.02**	0.20**	0.03**	0.02**	13.33**
G × E	16	0.90**	10.71**	1.97**	3.72**	6.82**	0.01**	0.02**	0.02**	0.04**	0.03**	0.01**	0.03**	12.27**
E + (G × E)	18	1.64**	17.21**	2.03**	3.64**	6.53**	0.03**	0.03**	0.02**	0.04**	0.05**	0.01**	0.03**	1.21**
Environment (Linear)	1	15.20**	138.44**	4.95**	6.04**	8.36**	0.031**	0.10**	0.03**	0.04**	0.39**	0.1**	0.04**	2.60**
Genotype × Environment (Linear)	8	0.46**	9.26**	1.58**	2.80**	4.79**	0.02**	0.02**	0.01**	0.04**	0.01**	0.01**	0.04**	1.27**
Pooled deviation	9	1.18**	10.81**	2.10**	4.12**	7.87**	0.01**	0.03**	0.03**	0.04**	0.1**	0.01**	0.02**	10.27**
Pooled error	48	0.44	4.90	0.47	3.01	1.21	0.001	0.02	0.01	0.03	0.04	0.01	0.04	3.03

**Significant at 1 % level; DFF-Days to first flowering; PH-Plant height; NPP-No. of productive tillers per plant; PL-Panicle length; NGP-No. of grains per panicle; HGW-Hundred grain weight; GL-Grain length; GB-Grain breadth; GLB-Grain LB ratio; KL-Kernel length; KB-Kernel breadth; KLB-Kernel LB ratio; GYP-Grain yield per plant

Table 10. Mean performance and stability parameters of rice varieties for grain yield per plant

Genotypes	Mean performance over three environments				Stability parameter		
	E1	E2	E3	Pooled	Mean	b_i	S^2d_i
G 8	29.05	27.72	26.00	27.59	27.59	0.90	1.35
G 9	27.59	35.51**	29.93**	31.01**	31.01**	1.01	0.33
G 10	24.67	21.19	30.61**	25.49	25.49	-3.91*	-0.96
G 15	20.45	20.49	20.14	20.36	20.36	0.15*	-1.01
G 16	29.96*	35.89**	33.74**	33.20**	33.20**	0.45	6.40*
G 22	32.31**	26.76	20.92	26.66	26.66	2.08	5.65*
G 23	19.85	23.85	19.82	21.17	21.17	1.46	3.45
G 24	24.18	25.61	19.86	23.22	23.22	2.45	-0.83
G 27	28.73	27.11	21.57	25.80	25.80	2.62	6.90*
Grand mean	26.31	27.13	24.73	26.06			
SE	1.12	1.65	0.65	1.05			
CV (%)	7.34	7.45	4.56	6.68			
CD (P=0.05)	3.34	3.50	1.95	2.86			
CD (P=0.01)	4.60	4.82	2.69	3.81			

*Significant at 5 % level; **Significant at 1 % level

that were less than unity. Three varieties viz., G16, G22 and G 27 were significant concerning squared deviation (Fig. 4). The results revealed under the stability model that G 9 recorded regression coefficient is almost equal to unity, variety is considered to have average stability (similar performance in all the environments). Variety G8 registered regression coefficient is more than unity ($d_i=1.35$), G8 is supposed to have below average stability ($S^2d_i=0.90$) (good performance in unfavourable environment). Varieties viz., G10, G15 and G16 showed moderate mean, regression coefficient value lesser than unity with non-significant deviation from regression, indicating that the varieties found to be above average stability performance in poor environment. Research suggests that the regression coefficient is less than unity (23, 24). The genotype is considered to have above-average stability (good performance in poor environment) (25, 26).

Genotype grouping analysis

An alternative approach for grouping the varieties for stability performance based on mean and coefficient of variation across environments (15). The characters viz., days to first flowering recorded the lowest coefficient of variation (1.66 %) followed by several grains per panicle (1.79 %), grain length (2.11 %), plant height (3.48 %), kernel length (4.19 %), kernel breadth (5.25 %), grain L/B ratio (5.73 %), grain breadth (6.05 %), number of productive tillers (6.5 %), hundred-grain weight

(7.02 %) which indicates that these characters were least influenced by environment. Grain yield per plant (12.15 %), panicle length (8.45 %) and kernel L/B ratio (7.09 %) recorded the highest coefficient of variation, indicating a more significant influence by the environment. The genotypic mean value was plotted against each variety's coefficient of variation (CV). A relatively high coefficient of variation was found in characters viz., grain yield per plant, panicle length, kernel L/B ratio, hundred-grain weight, number of productive tillers, grain breadth and grain L/B ratio. Researchers observed similar results (27, 28). Table 11 revealed that variety 9 was plotted in group I for six characters, namely panicle length, number of grains per panicle, hundred-grain weight, kernel length, kernel L/B ratio and grain yield per plant, exhibited high mean with low variation, followed by G16 for six characters, G10 for five characters and G8 for four characters. The scatter plot of genotypes into different groups across environments indicates a variety having high mean and low variation was considered stable, while genotypes that do not perform consistently across environments with high variation (29, 30). Hence, prediction of the performance of genotypes based on stability parameters would be feasible and reliable. The existence of linear and non-linear components of genotype \times environment interaction for different characters in rice has also been emphasized by various researchers (31, 32).

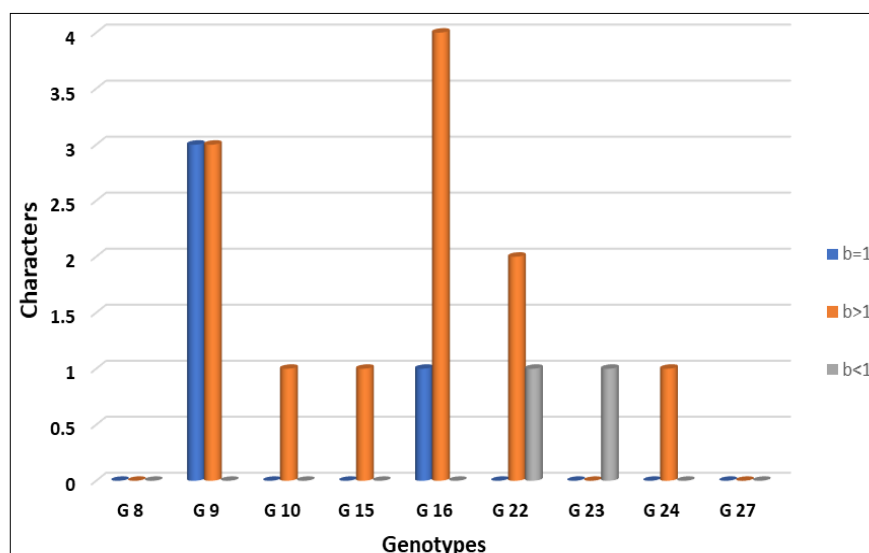


Fig. 4. Grouping of varieties based on stability parameter.

Table 11. Relationship between genotypic mean and coefficient of variation across environments

S. No.	Characters	Group I	Group II	Group III	Group IV
1.	Days to first flowering	G 8, G 10, G 15, G 27	Nil	G 9	G 16, G 22, G 23, G 24
2.	Plant height (cm)	G 10, G 15, G 24	Nil	G 22, G 23	G 8, G 9, G 16, G 27
3.	Number of productive tillers	G 10	G 9, G 22	G 8, G 15, G 16, G 24, G 27	G 23
4.	Panicle length (cm)	G 8, G 9, G 10	Nil	G 15, G 23, G 27	G 16, G 22, G 24
5.	Number of grains per panicle	G 9, G 15, G 27	G 8, G 9	G 24	G 10, G 22, G 23
6.	Hundred grain weight (g)	G 9, G 16, G 27	G 15	G 8, G 24	G 10, G 22, G 23
7.	Grain length (mm)	G 16, G 22	G 9	G 8, G 10, G 23, G 27	G 15, G 24
8.	Grain breadth (mm)	G 15, G 16	Nil	G 8, G 10, G 24, G 27	G 9, G 22, G 23
9.	Grain L/B ratio	G 10, G 16, G 24, G 27	G 9, G 22, G 23	G 8, G 15	Nil
10.	Kernel length (mm)	G 9, G 16	Nil	G 15, G 22, G 23, G 24	G 8, G 10, G 27
11.	Kernel breadth (mm)	G 8	G 15, G 16	G 9, G 10, G 22, G 24, G 27	G 23
12.	Kernel L/B ratio	G 9	G 16, G 22	G 8, G 23, G 24, G 27	G 10, G 15
13.	Grain yield per plant (g)	G 8, G 9, G 16	Nil	G 15, G 23, G 24, G 27	G 10, G 22

Conclusion

Of the twenty-eight varieties, the yield and yield-attributing features of G 8 (ADT 39), G 9 (ADT 48), G 16 (MDU 6), G 17 (BPT 5204), G 22 (Annapurna) and G 27 (NDR 359) exhibited high significant mean values. Among the nine SSR diversity markers studied, high PIC values were reported by markers RM 118 (0.551) and RM 181 (0.528), which might be used to identify and estimate the diversity of rice types. A dendrogram constructed on Neis' matrix revealed that the twenty-eight varieties were grouped into seven clusters. The genetic distance between varieties G15 and G8 was the largest based on Neis' genetic distance value. Based on Eberhart and Russels' stability model, the varieties G9 (ADT 48), G15 (TKM 9) and G16 (MDU 6) were the most stable in terms of grain yield per plant. They also had higher mean, non-significant S^2di and regression coefficient values around unity, which suggested stability across the three environments. Genotype grouping analysis revealed that traits viz., days to first flowering, number of grains per panicle, grain length, plant height, kernel length, kernel breadth, grain L/B ratio, grain breadth, number of productive tillers per plant, hundred-grain weight were least influenced by the environment. Thus, the scatter plot of varieties viz., G9, G16, G8 recorded high mean and low variation for most of the characters studied, which are recorded as stable varieties across environments.

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Authors' contributions

SV and SR carried out the molecular genetics and stability studies. SS¹ and SS² was involved in reviewing and formatting while KR, RE and SS³ was involved in data analysis and interpretation of the results. [SS¹ stands for S Sudhasha and SS² stands for S Sanjaygandhi and SS³ stands for S Suganthi]

Compliance with ethical standards

Conflict of interest: All the authors declare no conflict of interest

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