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Molecular and Morphological Profiling of Rice Cultivars Using Hypervariable Microsatellite Markers and DUS Descriptors

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Authors' contributions

This work was carried out in collaboration among all authors. Authors RV, TM and BK designed and conceived the experiment. Authors RV and TM performed the experiments. Authors PJ, SK and RC analyzed the data. Author RV wrote the manuscript. Authors RV and BK critically revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Identification and protection of varieties is one of the most important tasks in the plant breeding programme. As the number of new varieties increasing year by year, there is a need to protect and maintain the purity of the varieties. DNA fingerprinting studies allows us to identify the plant varieties with high precision as it is not influenced by environment and other factors. In this context,

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the present study has been taken up for morphological and molecular profiling of 20 rice genotypes using 39 DUS descriptors and SSR markers. The field experiment was conducted during the year Kharif 2021 and Kharif 2022 at Agricultural Research Station, Bapatla and molecular profiling studies were conducted at RARS, Lam, Guntur. Significant variations were observed for 16 morphological traits at different growth stages, which are relatively more informative in the identification and characterization of rice genotypes. A total of 45 polymorphic SSR markers were used to screen 20 rice genotypes, which produced 336 alleles. The number of alleles produced by these markers ranged from 3 (RM1048) to 15 (RM124) with an average number of alleles of 7.46 per marker. The PIC values of these markers ranged from 0.54 (RM1048) to 0.92 (RM124) with an average PIC value of 0.75. SSR based molecular profiles were developed using 12 highly polymorphic SSR markers viz., RM495, RM6933, RM489, RM6006, RM3351, RM510, RM418, RM3215, RM105, RM6364, RM144 and RM2972 for discrimination of each genotype as well as easy identification.

Keywords: Molecular profiling; DUS (Distinctness, Uniformity and Stability) testing; SSR markers; varietal identification; rice genotypes.

1. INTRODUCTION

Rice is one of the world's most important food crop and a primary food source for more than one third of world's population. The purity of the seed and authenticity of the variety is extremely important for breeding of crops and to meet the global food demand. Molecular profiling and DUS characterization is essential for varietal identification, registration and certification. It is also helpful to identify narrow genetic base, different quality parameters and product adulteration.

Protection of rice varieties through Distinctiveness Uniformity Stability (DUS) characters as well as DNA finger printing is necessary to avoid unauthorized commercial exploitation. But DUS testing done based on only morphological characters is not very effective and it is selective to environmental influence. Hence, Polymerase chain reaction (PCR) based molecular markers, especially simple sequence repeats (SSRs), are very helpful in varietal profiling, purity analysis (Kuleung et al. 2014) and in development of unambiguous DNA fingerprints (Chakravarthi et al. [1] and Zhu et al. [2].

Usage of agro-morphological markers in the characterization of rice has been reported by Rao et al., [3]. Similarly, Sherina et al., 2023 studied genetic diversity among 42 rice genotypes for 15 quantitative and 11 qualitative traits and grouped them into eight clusters. The days to 50% flowering and apiculus colour were found to be the major contributing characters towards genetic diversity. Dinesh et al., [4] assessed 41 genotypes and grouped them into 8 different clusters. The character, grain breadth contributed maximum towards divergence. Roy et al. [5], conducted agro-morphological diversity study among 78 Nagaland accessions and found significant variations in the majority of the traits. Among them, grains per panicle and panicle length showed high phenotypic coefficient of variation indicating that selection for trait improvement is possible in this germplasm. Principal Component Analysis is one of the important tools used for identifying the plant characters that categorize the distinctiveness among the promising genotypes. Asish et al., 2022 conducted principal component analysis in 55 indigenous rice germplasm to estimate the relative contribution of various traits for total variability. Principal component analysis revealed that 1st two component with eigen value greater than 1 accounted 65.38% of total variation. Genetic diversity among 95 rice germplasm lines was estimated by Ravi et al., 2018 and identified that six axes accounted for 71.37% cumulative variance of the total variability for twenty agro-morphological and quality traits. Principal Component Analysis (PCA) was used by Mulsanti et al., [6] to study the genetic diversity of the rice germplasm accession. Most of the morphological characters showed variation in different accessions. Identified that PC1 and PC2 explained about 32.5% and 22.1% of the variability, mostly related with traits such as productive tiller number plant height, and culm length.

Hence, in view of the above-mentioned scenario, the present study was taken up to characterize rice genotypes based on both morphological characters and at molecular level for varietal

identification and development of SSR based DNA barcodes/molecular fingerprints for identification of rice genotypes umambiguously.

2. MATERIALS AND METHODS

2.1 Plant Materials and Experimental Design

The experimental material utilized in the present study comprised of 20 rice advanced breeding lines, minikit and few released verities developed from Agricultural Research station (ARS), Bapatla. Andhra Pradesh, India. The field experiment was conducted during the year *Kharif* 2021 and *Kharif* 2022 at Agricultural Research Station, Bapatla. Molecular profiling studies were conducted at RARS, Lam, Guntur during 2022. Details of the varieties studied in the present investigation are provided in Supplementary Table 1. Thirty-day-old seedlings of each genotype was transplanted in 4 rows of 4 meters length with a spacing of 30 cm between each row and 20 cm between each plant (as per DUS guidelines given by PPV and FR Act (PPV&FRA, 2007) in a randomized block design with three replications. Crop was maintained by employing standard cultural and management practices.

Table 1. Frequency distribution of 20rice genotypes for various DUS traits

S. No.	Name of the Descriptor	Descriptor state	No. of accessions	Frequency (%)
1	Coleoptile: colour	Colourless	20	100
2	Basal leaf: sheath colour	Green	20	100
3	Leaf: Anthocyanin colouration	Absent	20	100
4	Leaf sheath: Anthocyanin colour	Absent	20	100
5	Leaf: Pubescence of blade surface	Absent	20	100
6	Leaf: Auricles	Absent	20	100
7	Leaf: Length of blade	Medium	13	65
		Long	7	35
8	Leaf: Width of blade	Medium	18	90
		Broad	2	10
9	Time of Heading	Early	4	20
		Medium	9	45
		Late	7	35
10	Flag leaf: Attitude of blade (Early observation)	Erect	20	100
11	Spikelet: density of pubescence of lemma	Absent	12	60
		Week	4	20
		Medium	4	20
12	Male sterility	Absent	20	100
13	Lemma: Anthocyanin colouration of keel	Absent	20	100
14	Lemma: Anthocyanin colouration of area below apex	Absent	20	100
15	Lemma: Anthocyanin colouration of apex	Absent	20	100
16	Spikelet: colour of stigma	White	20	100
17	Stem: length	Medium	20	100
18	Stem: Anthocyanin colouration of nodes	Absent	20	100
19	Stem: Anthocyanin colouration of internodes	Absent	20	100
20	Panicle: Length of main axis	Medium	14	70
		Long	6	30
21	Flag leaf: Attitude of blade (Late observation)	Erect	8	40
		Semi erect	11	55
		Horizontal	1	5
22	Panicle: Curvature of main axis	Deflexed	6	30
		Drooping	14	70

S. No.	Name of the Descriptor	Descriptor state	No. of accessions	Frequency (%)
23	Panicle: Number per plant	Few	3	15
		Medium	17	85
24	Spikelet: Colour of tip of lemma	White	20	100
25	Lemma and Palea colour	Straw	20	100
26	Panicle: Awns	Absent	20	100
27	Panicle: Exertion	Mostly exerted	10	50
		Well exerted	10	50
28	Time of Maturity	Early	5	25
		Medium	9	45
		Late	6	30
29	Sterile Lemma colur	Straw	20	100
30	Grain weight of 1000 fully developed grains	Very low	5	25
		Low	12	60
		Medium	3	15
31	Grain Length	Medium	20	100
32	Grain Width	Narrow	10	50
		Medium	10	50
33	Decorticated grain: Length	Medium	19	95
		Long	1	5
34	Decorticated grain: Width	Narrow	11	55
		Medium	9	45
35	Decorticated grain: Shape	Short slender	1	5
		Short bold	1	5
		Medium slender	15	75
		Long slender	3	15
36	Decorticated grain: Colour	White	9	45
		Red	3	15
		Variegated Purple	3	15
		Dark purple	5	25
37	Endosperm presence of amylose	Present	20	100
38	Endosperm of content of amylose	Medium	20	100
39	Decorticated grain: Aroma	Absent	20	100

2.2 Characterization of Rice Genotypes for DUS Descriptors

"The data was recorded for 39 DUS descriptors in all rice genotypes used in the present study (Supplementary Table 2). Visual observations were recorded on ten arbitrarily chosen and tagged plants of each genotype per replication as per DUS test guidelines issued by PPV&FR Authority". (PPV&FRA, 2007).

2.3 Statistical Analysis

Cluster analysis was done using complete linkage method using Minitab software. Principal component analysis was performed using General R-shiny based Analysis Platform empowered by Statistics **(**GRAPES) software [7]. Shannon diversity indices (Hl) were calculated as described by Perry and McIntosh [8]. Diversity indices was adapted from Rabara et al. (2014) to categorize the computed indices into high (H' = 0.76–0.99), moderate (H' = 0.46–0.75), and low diversity (0.01–0.45).

2.4 Molecular Characterization

The genomic DNA was isolated from leaves of 20–25 days old seedlings using Cetyl Trimethyl Ammonium Bromide (CTAB) method developed by Murray and Thompson [9]. The isolated DNA was quantified using Nanodrop (ND1000, Thermo Scientific, Nanodrop Technologies, U.S.A). The PCR reaction mixture consisting of 2 µl of template DNA (50 ng/µl) from each genotype and 8 µl of master mixture comprising of 0.5 µl of both 5µM forward and reverse primers, 1 µl of 1µM deoxy nucleotide tri phosphate (dNTPs), 1 µl of 10X PCR buffer, 0.1 µl of (5 U/µl) Taq DNA polymerase (Genie) and 4.9 µl of autoclaved distilled water. The PCR reaction was performed with the following

conditions of initial denaturation 94 °C for 5 min, denaturation 94 °C for 30s, annealing temperature 55 for 30s, extension 72 °C for 1 min and final extension of 72 °C for 10 min. Electrophoresis was carried out with a 3% agarose gel along with the 100 base pair DNA ladder. The sizes of the amplified fragments were then visualized under gel documentation system (Thermo fisher scientific, USA).

2.5 Microsatellite Markers and DNA Profiling

"Molecular characterization of the 20 rice varieties was done by using 43 hyper variable microsatellite markers selected from http:// www.gramene.org/markers/microsat/ distributed across all the 12 chromosomes of rice" [10]. (Supplementary Table 3). Gels were scored for presence of band as 1 and absent as 0 for particular allele for diversity analysis utilizing Darwin v 5.0 [11]. The Polymorphism Information Content (PIC) was calculated according to the formula of Anderson et al. [12]. Molecular diversity analysis was done based on the genetic distance with respect to their genetic dissimilarity and constructed dendrograms with DARwin software using Unweighted Neighbor Joining method.

3. RESULTS AND DISCUSSION

3.1 Morphological Characterization of Rice Varieties

The 20 rice genotypes were characterized for 39 DUS characters at different growth stages of the crop. Out of these 39 visually assessed DUS characters, 23 characters were monomorphic. Nine characters were dimorphic and the remaining seven were polymorphic. Among the studied traits, 16 were differentiating and found to be more advantageous in the characterization of the studied varieties. Similarly, Harisha et al. [13] observed that 25 traits were monomorphic, 18 were dimorphic, 3 were polymorphic among 46 characters studied in 18 rice varieties. Previously, a few studies reported the different polymorphic status of the traits among the genotypes studied (Rao et al. [3], Bhargavi et al. [14]. The details on the characters studied and their frequency distributions were presented in the Table 1.

Among the 16 differentiating traits, nine characters were dimorphic. The remaining seven traits were found to be polymorphic. Similar findings of variable flowering time was also reported by Rawte and Saxena (2018) and Aravind et al. [15] and variable panicle lengths by Rao et al. [3], and Aravind et al. (2019). Further similar reports on variability with respect to panicle exertion by Rao et al. [3] and Islam et al. [16]. Curvature of panicle main axis, number of panicles, panicle exertion and time of maturity were found to be more useful in the characterization of the varieties during the grain maturity and reproductive stages [17]. In case of decorticated grain length Komala et al. [18] have recorded the similar results. Variation in the decorticated grain color in the genotypes was presented in Fig 1. The characters, grain size and grain shape are important criteria for grain quality that usually breeders consider while development of new varieties for commercial production.

3.2 Principal Component Analysis

The results of Principal Component analysis revealed that (Supplementary Table 4) first five principal components showed eigen values more than 1 and accounted for about 77.5% of the total variation. The first, second, third, fourth and fifth PC's accounted for 23.39, 17.85%, 5.42%, 12.71% and 8.12% of total variability respectively. The characters time of heading, density of pubescence of lemma, curvature of panicle main axis, number of panicles per plant, 1000 grain weight and grain width have contributed positively towards the variability respectively. Similarly, Islam et al. [16] reported that "the first five components with vector values > 1 contributed 76.51% of the total variations". On the other hand, Sohrabi et al. [19] and Chakravorty et al. [20] reported "contribution of 76.7 and 75.9% of the first six and four components, respectively to the total variation in their study".

3.3 Shannon Diversity Index

The 39 characters were categorized into three groups based on shannon weaver diversity Indices. Among them, none displayed high diversity index. Only 16 traits exhibited moderate to low levels of phenotypic diversity, while the remaining traits were categorized as invariants (Supplementary Table 5). The findings indicated that nine traits exhibited moderate diversity index values ranging from 0.66 to 0.58. The remaining seven traits

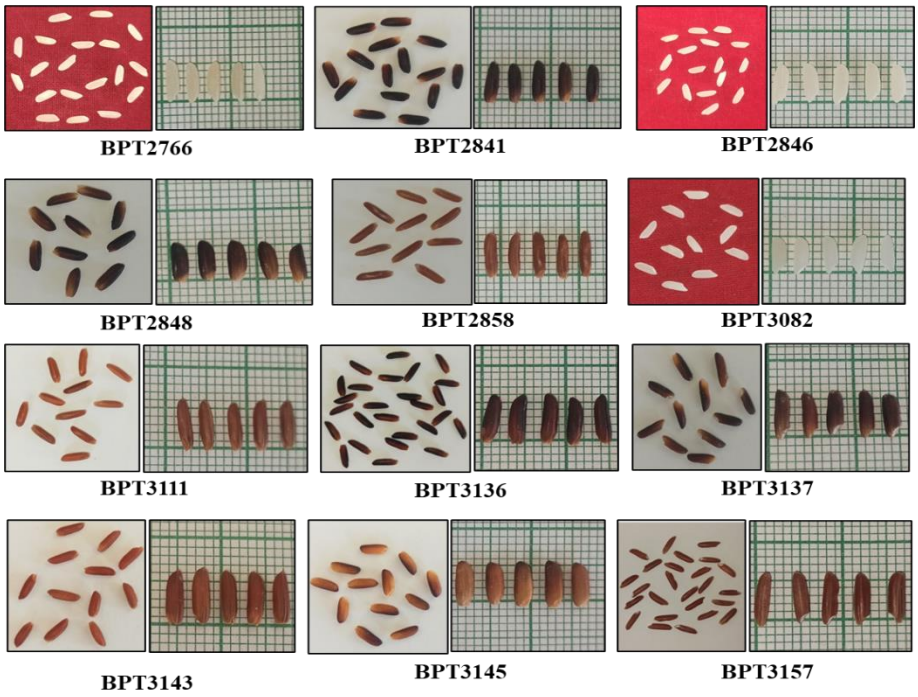

Fig. 1. Variation in decorticated grain color

Table 2. Polymorphism information content and number of alleles per 45 SSR markers

S. No.	Marker	Chr. No.	PIC	No. of Alleles	S. No.	Marker	Chr. No.	PIC	No. of Alleles
1	RM10344	1	0.90	7	24	RM418	7	0.81	9
2	RM495	1	0.69	5	25	RM455	7	0.73	6
3	RM3865	2	0.74	7	26	RM264	8	0.84	7
4	RM12569	2	0.81	9	27	RM2910	8	0.89	9
5	RM6933	2	0.85	10	28	RM3215	8	0.84	14
6	RM5430	2	0.81	10	29	RM1235	8	0.85	10
7	RM338	3	0.76	6	30	RM1099	9	0.78	9
8	RM231	3	0.76	5	31	RM219	9	0.71	6
9	RM5924	3	0.78	6	32	RM23865	9	0.69	7
10	RM489	3	0.82	10	33	RM105	9	0.78	11
11	RM124	4	0.92	15	34	RM8017	10	0.83	8
12	RM2530	4	0.60	5	35	RM6364	10	0.69	6
13	RM6006	4	0.81	7	36	RM271	10	0.60	6
14	RM163	4	0.73	6	37	RM484	10	0.76	7
15	RM2010	5	0.85	8	38	RM144	11	0.59	7
16	RM3351	5	0.59	4	39	RM206	11	0.70	6
17	RM6024	5	0.75	6	40	RM224	11	0.73	6
18	RM8107	6	0.68	4	41	RM552	11	0.74	6
19	RM2229	6	0.72	4	42	RM2972	12	0.81	8
20	RM510	6	0.78	10	43	RM2529	12	0.87	10
21	RM8101	6	0.82	10	44	RM309	12	0.80	8
22	RM1048	7	0.54	3	45	RM19	12	0.70	6
23	RM1335	7	0.66	7					

demonstrated low phenotypic diversity, with values spanning from 0.44 to 0.18. In contrast, Tushara et al. [21] studied shannon weaver diversity Indices in coloured rice genotypes and reported high to low diversity indices ranged from 0.81 to 0.16. Similarly, Rao et al [5] also

reported high to low diversity indices ranged from 0.21 to 0.90 for 14 qualitative characters in their study.

3.4 Identification of Rice Genotypes Using Molecular Markers

3.4.1 Molecular marker analysis

In the present study, 45 polymorphic molecular markers spanning across 12 chromosomes were used to screen 20 rice genotypes. A total of 336 alleles were produced by 45 SSR markers. The details of number of alleles amplified and PIC values are presented in the Table 2.

The number of alleles produced by the markers in the present study ranged from 3 (RM1048) to 15 (RM124) with an average number of alleles of 7.46 per marker. Similarly, Rani et al. [10] reported 4 to 20 alleles per marker in her study. In contrast, 3 to 7 alleles with an average of 3.6 alleles [22], two to three alleles with an average of 2.05 alleles (Bhargavi et al, 2021) 2 to 4 alleles with an average of 2.84 alleles [13].

The PIC value is the reflection of allelic diversity and their frequency among genotypes. Markers with higher PIC value about more than 0.5 are considered to be informative and will be useful for molecular breeding and germplasm evaluation studies. The PIC value of the markers in the present study ranged from 0.54 (RM1048) to 0.92 (RM124) with an average PIC value of 0.75. Similarly, Rani et al. [10] reported the PIC values ranged from 0.370 to 0.890 with mean of 0.762. Choudhary et al. [22] reported PIC values of 0.67 to 0.97 with an average of 0.87 using 52 hypervariable SSR markers. In contrast, lesser PIC value ranging from 0.0312 to 0.3684 with an average of 0.2128 (Bhargavi et al. [14], 0.03 to 0.64 with an average of 0.40 [13], 0.14 to 0.99 [23].

Based on the polymorphism exhibited by the markers, and PIC value 12 markers (RM495, RM6933, RM489, RM6006, RM3351, RM510, RM418, RM3215, RM105, RM6364, RM144 and RM2972) representing 1 marker per chromosome were selected for establishment of barcode for each genotype. The number alleles produced by the selected 12 markers were ranged from 7 (RM495 and RM6364) to 15 (RM3215). Genotype specific DNA bar code was developed by selecting the clearly distinguishable and polymorphic allele for each marker for all 20 genotypes [24-26].

The 12 selected polymorphic markers were given with different codes from A to L. The alleles generated from each of the polymorphic markers were labelled as A1, A2, A3…. based on their allele sizes in ascending order (Table 3a). The differences in the pattern with respect to the allele code could distinguish one genotype from the other. Some genotypes may look similar with respect to the morphological and grain physical characters, but they can easily be differentiated with respect to the DNA barcode. For instance, the genotypes BPT2766 and BPT2824, both are having similar plant type and white kernel with medium slender grain type. Hence, it is difficult to identify them phenotypically. In the present study, BPT2766 was assigned with an allelic bar code generated from the 12 polymorphic markers (A2/NA/C11/D10/E4/F1/G6/H13/I7/J6/K4/L8),whil e, another genotype BPT2824 was assigned with a different allelic bar code (A2/B9/C10/D10/E1/F1/G7/H2/I9/J6/K3/NA).Now it is easy to distinguish these two varieties using the DNA barcode. Utilizing these codes the 20 rice genotypes could be unambiguously distinguished. The diagrammatic representation of genotype-specific molecular profile is represented in the Table 3b. Similar pattern of DNA barcodes was developed earlier for identification of 111 rice cultivars by Rani et al. [10] and Harisha et al. [13].

3.4.2 Cluster analysis of rice genotypes based on Molecular markers

The twenty genotypes were separated into three major clusters I, II and III (Fig 2). Cluster I comprised of 3 genotypes, which was again separated into two sub clusters IA and IB with two (BPT3152 and BPT3143) and one genotype (BPT2766) respectively. Cluster II comprised of seven genotypes, which was again separated into two sub clusters IIA and IIB comprising of two (BPT 3145 and BPT 3137) and five genotypes (BPT 2824, BPT 3136, BPT3157, BPT2858 and BPT3082) respectively. Further cluster III was largest cluster comprised of 10 genotypes, with again two sub clusters of IIIA and IIIB with three (BPT2841, BPT2846 and BPT3164) and seven genotypes (BPT2808, BPT3151, BPT3178, BPT3391, BPT3111, BPT3113 and BPT3140) respectively. The genotypes falling in different clusters are said to be diverse and hybridization between the genotypes of different clusters are predicted to result in desirable transgressive segregants [27-29].

Table 3a. Coding of alleles produced by 12 polymorphic primers studied in 20 rice genotypes

Marker	Chr. No.	No. of Alleles	Alleles														
RM495	1	7	A1	A2	A3	A4	A5	A6	A7								
Amplicon size bp			150	160	165	170	172	180	350								
RM6933	2	11	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11				
Amplicon size bp			380	390	400	410	445	450	457	472	478	490	500				
RM489	3	14	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15
Amplicon size bp			155	180	200	210	220	225	230	238	240	246	250	260	270	320	500
RM6006	4	13	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12	D13		
Amplicon size bp			276	280	284	290	300	305	410	500	503	510	520	530	552		
RM3351	5	8	E1	E2	E3	E4	E5	E6	E7	E8							
Amplicon size bp			120	130	140	150	154	155	158	160							
RM510	6	9	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10					
Amplicon size bp			100	110	120	124	135	150	300	310	320	340					
RM418	7	8	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10					
Amplicon size bp			210	220	230	246	250	260	280	290	300	317					
RM3215	8	15	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15
Amplicon size bp			120	140	150	153	160	170	172	175	180	185	190	200	300	430	800
RM105	9	11	I1	I2	I3	I4	I5	I6	I7	I8	I9	I10	I11				
Amplicon size bp			100	137	140	150	160	170	350	650	700	730	872				
RM6364	10	7	J1	J2	J3	J4	J5	J6									
Amplicon size bp			150	160	180	181	190	200									
RM144	11	10	K1	K2	K3	K4	K5	K6	K7	K8	K9	K10					
Amplicon size bp			70	160	200	210	220	240	250	300	900	1100					
RM2972	12	11	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11				
Amplicon size bp			100	150	180	190	200	210	220	250	280	300	450				

Table 3b. Allelic profiles produced by 12 polymorphic markers in 20 rice genotypes

S. No.	Genotype	Allele Code
1	BPT 2846	A1/B6/C3/D4/E4/F7/G1/H6/I8/J2/K4/L11
2	BPT 2841	A7/B11/C11/D8/E1/F2/G7/H1/I8/J1/K2/L10
3	BPT 2766	A2/NA/C11/D10/E4/F1/G6/H13/I7/J6/K4/L8
4	BPT 3157	A1/B4/C8/D3/E5/F4/G4/H4/I2/J4/K4/L7
5	BPT 2858	A3/B6/C7/D5/E7/F10/G8/H10/I11/J6/K5/L5
6	BPT 2824	A2/B9/C10/D10/E1/F1/G7/H2/I9/J6/K3/NA
7	BPT 3164	A1/B6/C2/D4/E4/F8/G5/H6/I4/J1/K5/NA
8	BPT 3136	A5/B9/C9/D13/E6/F5/G10/H8/I3/J3/K4/L5
9	BPT 3111	A4/B7/C11/D12/E2/F1/G3/H3/I9/J1/K4/L2
10	BPT 3391	A1/B8/C6/D9/E1/F1/G5/H9/I10/J5/K6/NA
11	BPT 3140	A2/B5/C4/D5/E4/F1/G5/H12/I4/J6/K8/L2
12	BPT 3137	A2/B11/C5/D4/E8/NA/G9/H5/NA/J6/K9/L5
13	BPT 3143	A2/B10/C12/D10/E4/F6/G9/H11/I9/J6/K4/NA
14	BPT 3151	A1/B10/C13/D4/E4/F9/G7/H13/I9/J5/K4/L5
15	BPT 3178	NA/NA/NA/NA/E4/F6/G1/H3/I5/J6/K4/L1
16	BPT2808	A2/B4/C11/D4/E4/F3/G9/H9/I1/J6/K4/L3
17	BPT 3113	A1/B6/C15/D8/E4/F7/G9/H14/I6/J6/K7/NA
18	BPT 3082	A2/B6/C5/D2/E3/F1/G9/H5/NA/J5/K4/NA
19	BPT 3152	A6/B11/C5/D10/E4/F8/G8/H3/I4/J3/K4/L4
20	BPT 3145	A6/B6/C5/D5/E4/F3/G9/H2/I4/J6/K1/L6

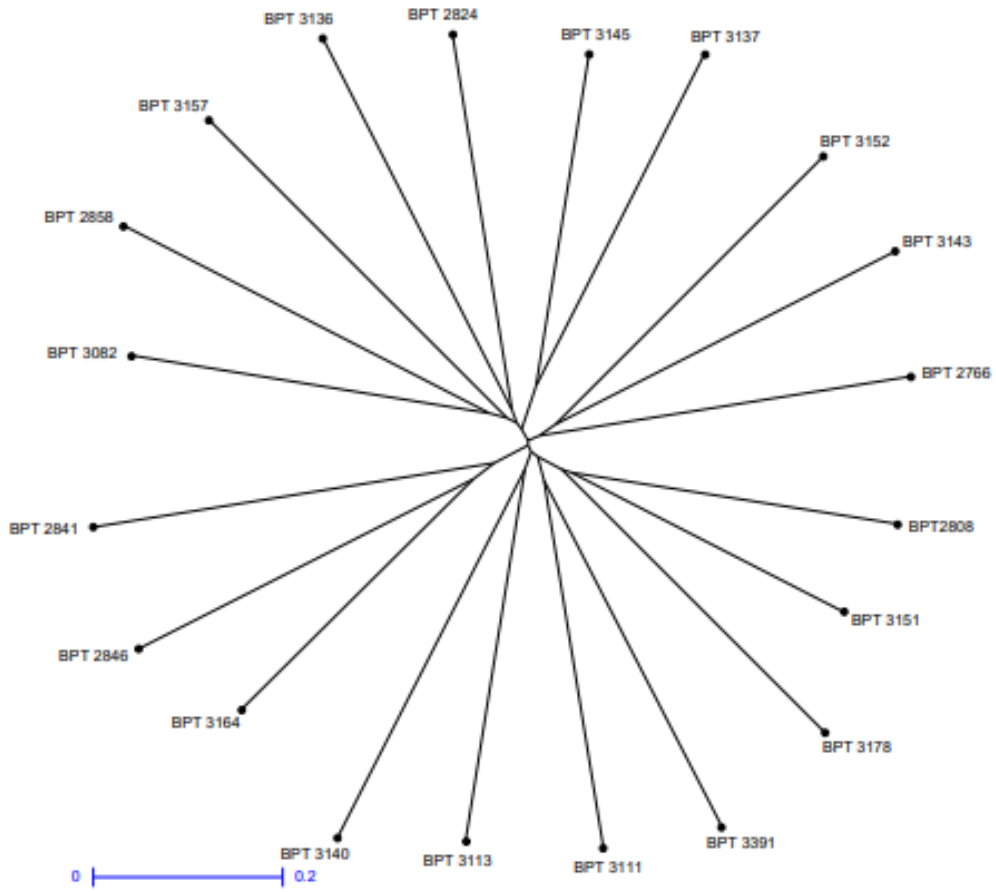

Fig. 2. Dendrogram of 20 rice genotypes based on molecular diversity

4. CONCLUSION

The present study demonstrated that the combination of DUS traits and molecular markers can be used to develop DNA fingerprints. Among the morphological traits, 16 characters showed variation among the 20 genotypes and these characters were more useful in characterizing the rice genotypes. Molecular profiles of 20 rice genotypes established in the present study were unique and able to distinguish the genotypes from each other. This study assists in varietal differentiation and identification to assess the genetic purity to address the problem of admixtures and solving the adulteration disputes in commercial seed lots. The markers used in the study are highly informative and useful in cultivar identification.

FUTURE SCOPE

This study will be useful for breeders, researchers and farmers to identify, and protection of the varieties.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Supplementary Table 1. Twenty rice genotypes used in the study, parentage and the grain type

S. No.	Genotype	Cross combination	Grain type
1	BPT 2846	MTU 1061/IR-78585-64-2-4-3-1	MS
2	BPT 2841	Swarna/IRGC 18195/MTU 1081	MS
3	BPT 2766	BPT 2270/NLR 145	MS
4	BPT 3157	MTU 7029/IRGC 18195/MTU 1081	LB
5	BPT 2858	RP Bio 226*1/IRGC 48493	SS
6	BPT 2824	BPT 2231/NLR 145	MS
7	BPT 3164	B-95-1/RPHR 1005//B-95-1	MS
8	BPT 3136	RP Bio 226/IRGC 48493	LB
9	BPT 3111	MTU7029/IRGC18195/MTU1081	MS
10	BPT 3391	Cult.01120305/cult.0910025-7	LB
11	BPT 3140	MTU 7029/IRGC 18195/MTU 1081	LS
12	BPT 3137	RP Bio 226/IRGC 48493	MS
13	BPT 3143	RP Bio 226*1/IRGC 48493	LB
14	BPT 3151	RP Bio226/Jarava	MS
15	BPT 3178	Cult.01120305/cult.0910025-7	LB
16	BPT 2808	BPT 2270/NLR 145	MS
17	BPT 3113	BPT 2270/NLR 145	LS
18	BPT 3082	BPT 5204/MTU 1075	MS
19	BPT 3152	BPT 5204*2/*O.longistaminata*//B-95-1/Swarna Sub-1	LS
20	BPT 3145	RP Bio 226/IRGC 48493	LB

MS: Medium Slender; LB: Long Bold; SS: Short Slender; LB: Long Bold; LS: Long Slender

Supplementary Table 2. DUS characteristics recorded in rice genotypes

S. No.	Characteristics	States	Note	Stage of observation	Type of assessment
1	Coleoptile: colour	Colourless	20	After germination	VS
		Green	0		
		Purple	0		
2	Basal leaf: sheath colour	Green	20	Booting	VS
		Light purple	0		
		uniform purple	0		
3	Leaf: Anthocyanin colouration	Absent	20	Booting	VG
		Present	0		
4	Leaf sheath: Anthocyanin colour	Absent	20	Booting	VG
		Present	0		
5	Leaf: Pubescence of blade surface	Absent	20	Booting	VS
		week	0		
		Medium	0		
		Strong	0		
6	Leaf: Auricles	Absent	20	Booting	VS
		Present	0		
7	Leaf: Length of blade	Short	0	Booting	MS
		Medium	13		
		Long	7		
8	Leaf: Width of blade	Narrow	0	Booting	MS
		Medium	18		
		Broad	2		
9	Time of Heading	Very early	0	½ of inflorescence emerged	VG
		Early	4		
		Medium	9		
		Late	7		
10	Flag leaf: Attitude of blade (Early observation)	Erect	20	Beginning of anthesis	VG
		Semi erect	0		
		Horizontal	0		
11	Spikelet: density of pubescence of lemma	Absent	12	Beginning of anthesis	VS
		week	4		
		Medium	4		

S. No.	Characteristics	States	Note	Stage of observation	Type of assessment
		Strong	0		
12	Male sterility	Absent	20	Anthesis half way	VG
		Present	0		
13	Lemma: Anthocyanin colouration of keel	Absent	20	Anthesis half way	VS
		week	0		
		Medium	0		
		Strong	0		
14	Lemma: Anthocyanin colouration of area below apex	Absent	20	Anthesis half way	VS
		week	0		
		Medium	0		
		Strong	0		
		Very strong	0		
15	Lemma: Anthocyanin colouration of apex	Absent	20	Anthesis half way	VS
		week	0		
		Medium	0		
		Strong	0		
16	Spikelet: colour of stigma	white	20	Anthesis half way	VS
		light green	0		
		yellow	0		
		light purple	0		
		purple	0		
17	Stem: length	Very short	0	Milk development stage	MS
		short	0		
		medium	20		
		long	0		
		Very long	0		
18	Stem: Anthocyanin colouration of nodes	Absent	20	Milk development stage	VS
		Present	0		
19	Stem: Anthocyanin colouration of internodes	Absent	20	Milk development stage	VS
		Present	0		
20	Panicle: Length of main axis	Very short	0	Milk development stage	MS
		short	0		
		medium	14		
		long	6		

S. No.	Characteristics	States	Note	Stage of observation	Type of assessment
		Very long	0		
21	Flag leaf: Attitude of blade (Late observation)	Erect	8	Ripening	VG
		Semi erect	11		
		horizontal	1		
		deflexed	0		
22	Panicle: Curvature of main axis	Erect	0	Ripening	VG
		Semi striaght	0		
		deflexed	6		
		Drooping	14		
23	Panicle: Number per plant	Few	3	Dough development-Ripening	MS
		Medium	17		
		Many	0		
24	Spikelet: Colour of tip of lemma	White	20	Dough development-Ripening	VS
		yellowish	0		
		brown	0		
		red	0		
		Black	0		
25	Lemma and Palea colour	straw	20	Dough development-Ripening	VG
		Gold and gold furrows on straw background	0		
		brown spots on straw	0		
26	Panicle: Awns	Absent	20	Ripening	VG
		Present	0		
27	Panicle: Exertion	partly exerted	0	Ripening	VG
		mostly exerted	10		
		well exerted	10		
28	Time of Maturity	Very early	0	Ripening	VG
		early	5		
		medium	9		
		late	6		
		Very late	0		

S. No.	Characteristics	States	Note	Stage of observation	Type of assessment
29	Sterile Lemma colur	Straw	20	Caryopsis hard	VS
		Gold	0		
		Red	0		
		Purple	0		
30	Grain weight of 1000 fully developed grains	Very low	5	Caryopsis hard	MG
		low	12		
		medium	3		
		high	0		
		very high	0		
31	Grain Length	Very short	0	Caryopsis hard	MS
		short	0		
		medium	20		
		long	0		
		very long	0		
32	Grain Width	very narrow	0	Caryopsis hard	MS
		narrow	10		
		medium	10		
		broad	0		
33	Decorticated grain: Length	Short	0	Caryopsis hard	MS
		Medium	19		
		Long	1		
		Long*	0		
34	Decorticated grain: Width	Narrow	11	Caryopsis hard	MS
		Medium	9		
		Broad	0		
35	Decorticated grain: Shape	Short slender	1	Caryopsis hard	MS
		short bold	1		
		Medium slender	15		
		long bold	0		
		short slender	0		
		long slender	3		
		Extra long slender	0		

S. No.	Characteristics	States	Note	Stage of observation	Type of assessment
36	Decorticated grain: Colour	white	9	Caryopsis hard	VG
		Light brown	0		
		variegated brown	0		
		dark brown	0		
		light red	0		
		red	3		
		variegated purple	3		
		purple	0		
		dark purple	5		
37	Endosperm presence of amylose	Absent	0	Caryopsis hard	MG
		Present	20		
38	Endosperm of content of amylose	Very low	0	Caryopsis hard	MG
		low	0		
		medium	20		
		high	0		
		very high	0		
39	Decorticated grain: Aroma	Absent	20	Caryopsis hard	MG
		Present	0		

VS: Visual assessment by observation of individual plant or parts of plants
VG: Visual assessment by a single observation of a group of plants or parts of plants
MS: Measurement of a number of individual plants or parts of plants
MG: Measurement by a single observation of a group of plants or parts of plant

Supplementary Table 3. List of primers included under study

S. No.	Marker	Chr. No.	Forward sequence	Reverse sequence	Annealing temperature
1.	RM10344	1	GAACAATAAGGCCGGCTAAGAGC	TTTCAGCCGTTTCTTGTGTCTAGC	55°C
2.	RM495	1	ATGATGATGGACGACGACAACG	TGAATCCAAGGTGCAGAGATGG	55°C
3.	RM12569	2	GCTCATCATCATCATCGCAGTGG	ATCCATGTGGCAGACACACTTGC	55°C
4.	RM6933	2	AATGCCTAGCACTCATCCTTGC	AGGCACCCTACGATGAAATAGTGG	55°C
5.	RM3865	2	CTTGATCTCATCCACCCTGTTCC	GCCAGGTACAACAAACCACAACC	55°C
6.	RM5430	2	TAAAAACTGAGCCGTGAGCC	ACCATGGGGAGCTGCTTC	61°C

S. No.	Marker	Chr. No.	Forward sequence	Reverse sequence	Annealing temperature
7.	RM8017	2	CTTACATTATGAAACGGATG	ATAACAAAACCACACTTTGA	55°C
8.	RM338	3	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	55°C
9.	RM231	3	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	55°C
10.	RM5924	3	GCTCAACTGCTGTTTAGAGGATTACC	AGCTCTCCCAAGAAACTGAACC	55°C
11.	RM489	3	ACTTGAGACGATCGGACACC	TCACCCATGGATGTTGTCAG	55°C
12.	RM124	4	ATCGTCTGCGTTGCGGCTGCTG	CATGGATCACCGAGCTCCCCCC	67°C
13.	RM2530	4	GAACCTCTAGTATATAACCG	ATCTATTTAGGAGTTAACCA	55°C
14.	RM6006	4	CTCGGCGATGAACAGCTC	AGAAGATCATGAAGCGGTCG	55°C
15.	RM163	5	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT	55°C
16.	RM2010	5	ATCTTCTAGGAAATCGAGGA	GTTGGCAACTTGTAGTCTTG	55°C
17.	RM3351	5	ATGGAAGGAATGGAGGTGAG	TACCCCTACGTCGATCGATC	55°C
18.	RM6024	5	ACATTCGTCCAGGGATTCAC	TTGTGGTTGCTCACCTCTTG	50°C
19.	RM8107	6	ATTGACCTGATGTATGTAATATATCAAG	AGAACAAGAAAGCCTATCACTATATATC	55°C
20.	RM2229	6	AGCACCTAAGCATCTAGCAC	CATGTCACCCAAAACAATTA	55°C
21.	RM510	6	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC	55°C
22.	RM8101	6	CACTGACATAGCTAAGGTCTCATGTCTTAT	TGGTTAACTCGCTATTATAATGAGTTCG	55°C
23.	RM1335	7	GCATGCATGAATATGATGG	AGATCGAACAAGAAGAGTGG	55°C
24.	RM418	7	TCGCGTATCGTCATGCATAG	GAGCACATATGCCACGTACG	55°C
25.	RM455	7	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC	55°C
26.	RM1048	7	CAAGCCTATAATGTGAATTG	AATTTTTAGTTTGGGGTAGA	55°C
27.	RM1235	8	AGCAGAGGAGGAGATGATGG	GGACCAAAACGAAGCTATCC	55°C
28.	RM3215	8	CGGCGTAGCTAAATTTGGAC	ATGGCGAGCAAGGAAGTAAG	55°C
29.	RM2910	8	CAGCTGCTCATATTCATATA	ATAAGGTACTTCATCCGTTA	55°C
30.	RM264	8	GTTGCGTCCTACTGCTACTTC	GATCCGTGTCGATGATTAGC	55°C
31.	RM105	9	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	55°C
32.	RM23865	9	TCATCCCATTCTCTTCCTCACC	CATACGGCCATACAAATGAACC	55°C
33.	RM219	9	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	55°C
34.	RM1099	9	CTCGGCGAATCAGAGAAGAC	ATCCTAACGTGCCTATCCCC	50°C
35.	RM271	10	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	55°C
36.	RM6364	10	GTTCATTTCGTCCTTCTCGG	TCTCGATTCTTCCTTCTCCG	55°C
37.	RM484	10	TCTCCCTCCTCACCATTGTC	TGCTGCCCTCTCTCTCTCTC	55°C
38.	RM144	11	TGCCCTGGCGCAAATTTGATCC	GCTAGAGGAGATCAGATGGTAGTGCATG	55°C

S. No.	Marker	Chr. No.	Forward sequence	Reverse sequence	Annealing temperature
39..	RM206	11	CCCATGCGTTTAACTATTCT	CGTTCCATCGATCCGTATGG	55°C
40.	RM224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG	55°C
41.	RM552	11	CGCAGTTGTGGATTTCAGTG	TGCTCAACGTTTGACTGTCC	55°C
42.	RM2972	12	GAGCCAATATGTTGTCTTGA	GTTCAGATCATGATGCCTAC	55°C
43.	RM2529	12	CATTAAAATCAGTGGGACTG	AGGCATTTCCTGATATGATC	55°C
44.	RM309	12	CACGCACCTTTCTGGCTTTCAGC	AGCAACCTCCGACGGGAGAAGG	55°C
45.	RM19	12	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	55°C

Supplementary Table 4. Contribution of different morphological characters to total variability

Qualitative characters	Component					
	PC1	PC2	PC3	PC4	PC5	PC6
Eigen value	3.743	2.856	2.468	2.034	1.299	0.947
% of Variance	23.394	17.85	15.426	12.714	8.121	5.921
Cumulative %	23.394	41.243	56.67	69.384	77.505	83.426
Leaf: Length of blade	-0.184	-0.434	0.119	-0.007	0.189	-0.064
Leaf: Width of blade	-0.132	-0.384	-0.262	-0.031	-0.109	-0.2
Time of Heading	0.113	-0.017	-0.541	0.11	0.183	0.148
Spikelet: density of pubescence of lemma	0.128	-0.259	0.038	-0.377	-0.327	-0.188
Panicle: Length of main axis	-0.378	-0.025	-0.034	0.075	0.053	0.234
Flag leaf: Attitude of blade (Late observation)	-0.328	-0.332	-0.001	-0.003	0.18	-0.345
Panicle: Curvature of main axis	0.204	-0.176	-0.161	-0.414	-0.05	-0.318
Panicle: Number per plant	0.17	0.209	0.075	-0.561	-0.005	0.234
Panicle: Exertion	0.104	-0.381	-0.015	-0.159	0.027	0.631
Time of Maturity	0.12	-0.015	-0.568	0.111	0.256	0.075
Grain weight of 1000 fully developed grains	0.34	0.015	0.125	0.444	-0.074	-0.245
Grain Width	0.354	-0.288	0.185	0.209	-0.164	0.107
Decorticated grain: Length	0.118	-0.189	0.2	-0.117	0.663	-0.02
Decorticated grain: Width	0.35	-0.34	0.111	0.223	-0.077	0.16
Decorticated grain: Shape	0.28	0.167	0.232	-0.074	0.481	-0.16
Decorticated grain: Colour	-0.343	-0.057	0.335	0.079	0.013	0.201

Supplementary Table 5. Estimates of Shannon weaver diversity indices for morphological descriptors studied

S. No.	Character	Shannon Weaver Diversity Index
High Diversity (0.76-0.99): Nil		
Moderate Diversity (0.46-0.75)		
1	Time to Maturity	0.66
2	Time of Heading	0.65
3	Panicle: Exertion	0.63
4	Decorticated grain: Width	0.62
5	Flag leaf: Attitude of blade (Late observation)	0.6
6	Leaf: Length of blade	0.59
7	Spikelet: density of pubescence of lemma	0.59
8	Grain weight of 1000 fully developed grains	0.58
9	Decorticated grain: Colour	0.58
Low Diversity (0.01-0.45)		
10	Panicle: Curvature of main axis	0.44
11	Grain Width	0.43
12	Decorticated grain: Shape	0.41
13	Panicle: Number per plant	0.38
14	Panicle: Length of main axis	0.37
15	Leaf: Width of blade	0.30
16	Decorticated grain: Length	0.18

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