

Molecular and morphological profiling of rice cultivars using hypervariable microsatellite markers and DUS descriptors

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, 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. SSR based molecular profiles using 12 polymorphic SSR markers were established for discrimination of each genotype as well as easy identification. The combination of morphological descriptors and molecular markers is very useful approach for distinguishing the rice genotypes easily and unambiguously.

Key words: Molecular profiling, DUS testing, SSR markers, varietal identification, rice genotypes

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. Rapid development and release of improved rice varieties during the present days, leads to narrow genetic base and difficult to identify the varieties based on only morphological characters. The morphological descriptors are very limited in their utility, when too closely related cultivars are to be distinguished from one other (Sattur *et al.* 2018). Unambiguous identification of varieties is extremely important for registration and certification of newly released and notified varieties. Further, many rice varieties are difficult to identify based on their appearance and cooking quality and hence, there is a chance of adulteration with poor quality seed to get benefit out of it because of the price variation existing among the rice varieties (Bhargavi *et al.* 2021). 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

(Kuleunget *et al.* 2014) and in development of unambiguous DNA fingerprints (Chakravarthi *et al.* 2006 and Zhu *et al.* 2012).

The combination of both SSR and DUS based fingerprinting aids rice breeders in varietal registration and protection of intellectual property rights. It is very important for identification of adulterated seeds at field and lab level. 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 unambiguously.

Materials and Methods

Plant material and experimental design

The experimental material utilized in the present study comprised of 20 rice advanced breeding lines, minikit and few released varieties 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.

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).

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 (Gopinath *et al.* 2020). Shannon diversity indices (H₁) were calculated as described by Perry and McIntosh (1991). 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).

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 (1980). 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).

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/](http://www.gramene.org/markers/microsat/) distributed across all the 12 chromosomes of rice (Rani *et al.* 2021).(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 (Perrier and Jaquimond, 2006). The Polymorphism Information Content (PIC) was calculated according to the formula of Anderson et al. (1993). 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.

Results and discussion

Morphological characterization of rice varieties

Identification of rice varieties is vital for protecting Plant Breeders' Rights and also during the trade and seed production. There are two strategies commonly used for identification such as morphological descriptors and molecular or DNA markers. Morphological descriptors used for DUS testing were less expensive and easy to measure, stage-specific and inconsistent expression due to the environmental influence, furthermore, they failed to distinguish large number of varieties with slight variation in agronomic traits, which is a common phenomenon in recently released varieties. These problems of the morphological descriptors can be solved efficiently using molecular markers as they are stable, abundant with high discriminating power.

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.* (2021) 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.* 2021, Bhargavi *et al.* 2021, Aravind *et al.* 2019, Rawte and Saxena (2018) and Islam *et al.* (2018). The details on the characters studied and their frequency distributions were presented in the Table 1.

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
		Weak	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
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 colour	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

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 Kalyan *et al.* (2017), Umarani *et al.*(2017), Rawte and Saxena (2018) and Aravind *et al.*(2019) and variable panicle lengths by Rao *et al.*(2021), Roy *et al.* (2016); Islam *et*

al.(2018), Kalyan *et al.*(2017), Umarani *et al.* (2017), Rawte and Saxena (2018) and Aravind *et al.* (2019). Further similar reports on variability with respect to panicle exertion by Rao *et al.* (2021), Roy *et al.*(2016); Islam *et al.* (2018). 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. Gour *et al.* (2019) and Sharma *et al.* (2020). In case of decorticated grain length Mondal *et al.* (2014), Komala *et al.*(2017) and Umarani *et al.* (2017) have recorded the similar results. Variation in the decorticated grain color in the genotypes was presented in figure 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.

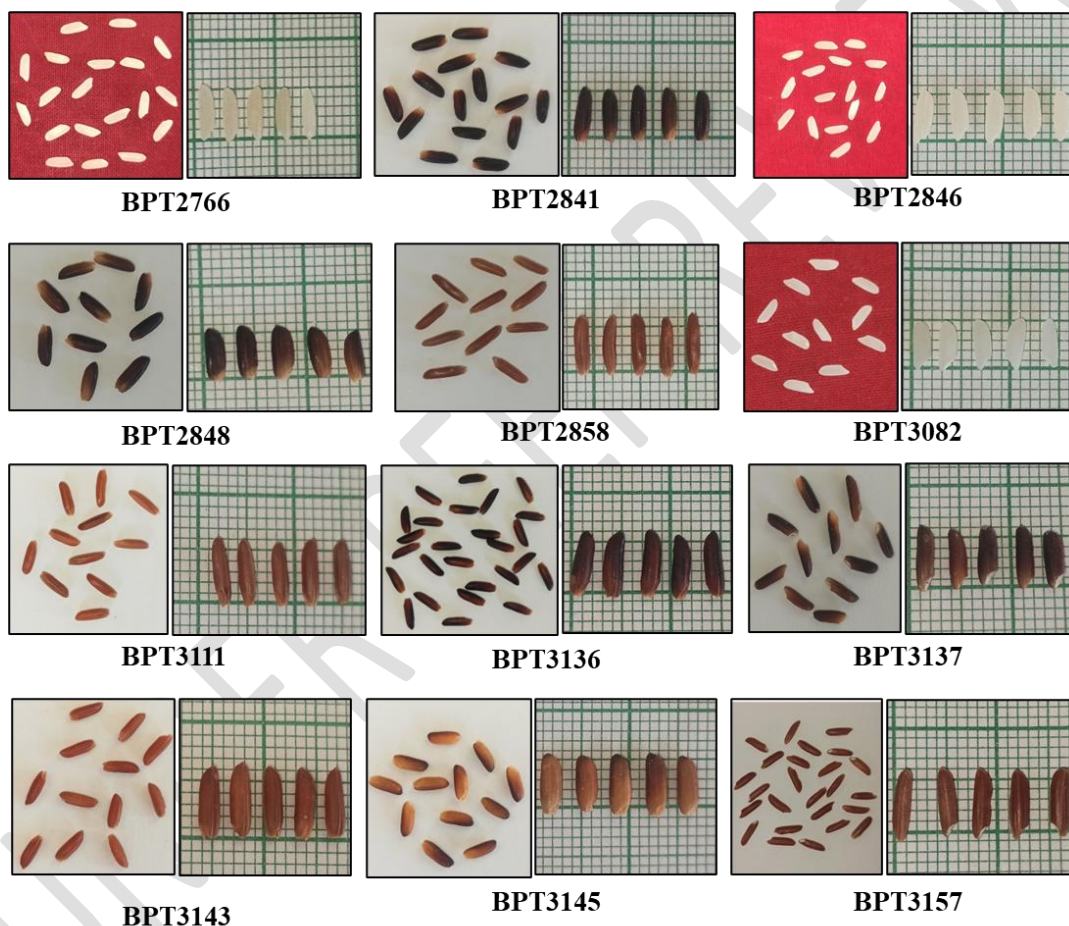


Fig. 1 Variation in decorticated grain color

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.* (2018) reported that the first five components with vector values > 1 contributed 76.51% of the total variations. On the other hand, Sohrabi *et al.* (2012) and Chakravorty *et al.* (2013) reported contribution of 76.7 and 75.9% of the first six and four components, respectively to the total variation in their study.

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 demonstrated low phenotypic diversity, with values spanning from 0.44 to 0.18. In contrast, Tushara *et al.* (2022) studied Shannon-Weaver diversity indices in coloured rice genotypes and reported high to low diversity indices ranging from 0.81 to 0.16. Similarly, Rao *et al.* (2021) also reported high to low diversity indices ranging from 0.21 to 0.90 for 14 qualitative characters in their study.

Identification of rice genotypes using molecular markers

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 markers used in present study were able to discriminate among the genotypes. Hence, these markers could be of great value for cultivar identification. The details of number of alleles amplified and PIC values are presented in the Table 2

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					

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.* (2021) reported 4 to 20 alleles per marker in her study. In contrast, 3 to 7 alleles with an average of 3.6 alleles (Choudhary *et al.* 2013), 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 (Harisha *et al.* 2021).

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.* (2021) reported the PIC values ranged from 0.370 to 0.890 with mean of 0.762. Choudhary *et al.* (2013) 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.* 2021), 0.03 to 0.64 with an average of 0.40 (Harisha *et al.*, 2021), 0.14 to 0.99 (Satturuet *et al.* 2018).

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.

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), while, 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 profiles is represented in the Table 3b. Similar pattern of DNA barcodes was developed earlier for identification of 111 rice cultivars by Rani *et al.* (2021), Vemireddy *et al.* (2015), Bhargavi *et al.* (2021), Satturuet *et al.* (2018) and Harisha *et al.* (2021).

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
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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

Cluster analysis of rice genotypes based on Molecular markers:

The twenty genotypes were separated into three major clusters I, II and III (Figure 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.



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

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.

References:

Anderson J A, Churchill G A, Autrique J E, Tanksley S D and Sorrels M E. 1993. Optimizing parental selection for genetic maps. *Genome***36**:181-186.

- Aravind K, Banumathy S, Vanniarajan C, Arunachalam P, Illamaran M and Kalpana K. 2019. DUS characterization and genetic variability studies of rice mutants. *Electronic Journal of Plant Breeding*. **10(2)**:451–461.
- Bhargavi M, Maneesha K, Withanawasam D M, Kavitha R, Aratikatla, Himabindu S, Prashanth M, Shanthi P, Madhavi L, Kommana, Mohan Reddy D, Ravindra Reddy B and Vemireddy L R. 2021. A novel barcode system for rapid identification of rice (*Oryza sativa* L.) varieties using agro-morphological descriptors and molecular markers. *Molecular Biology Reports***48**:2209–2221.
- Chakravarthi B K and Naravaneni R. 2006. SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). *African Journal of Biotechnology***5**:684–688.
- Chakravorty A, Ghosh P D and Sahu P K. 2013. Multivariate analysis of phenotypic diversity of landraces of rice of West Bengal. *American Journal of Experimental Agriculture*.**3(1)**: 110-23.
- Choudhary G, kumar N R, Surapaneni M, Deborah D A, Vipparla A, Anuradha G, Siddiq E A and Vemireddy L N. 2013. Molecular genetic diversity of major Indian rice cultivars over decadal periods. *PLoS One***8(6)**:1–12.
- Gopinath PP, Parsad R, Joseph B and Adarsh V S. 2020. GRAPES: General Rshiny Based Analysis Platform Empowered by Statistics. <https://www.kaugrapes.com/home>. version 1.0.0.
- Gour L, Koutu G K and Singh Y. 2019. Characterization and grouping of indigenous rice lines based on DUS test for different qualitative differentials. *The Pharma Innovation Journal***8**:1162–1168.
- Harisha R, Bhadru D, Vanisri, S, Gourishanakar, V and Satish, L. 2021. SSR and morphological traits based fingerprints and DNA barcodes for varietal identification in rice. *Biotechnology & Biotechnological Equipment***35(1)**:1461-1473
- Islam M Z, Akter N, Chakrabarty T, Bhuiya A, Siddique M A and Khalequzzaman M. 2018. Agro-morphological characterization and genetic diversity of similar named aromatic Rice (*Oryza sativa* L.) landraces of Bangladesh. *Bangladesh Rice Journal*. **22(1)**: 45-56.
- Kalyan B, Krishna K V R and Rao L V S. 2017. DUS Characterization for germplasm of rice. *International Journal of Current Microbiology and Applied Sciences***6(10)**:3480–3487 13.
- Komala N Y, Gurumurthy R and Surendra P. 2017. Morphological characterization of popular rice varieties of zone VIII and zone IX of Karnataka state. *Bulletin of Environment, Pharmacology and Life Sciences***6(3)**:515–519.
- Kuleung C, Baenziger P S and Dweikat I. 2004. Transferability of SSR markers among wheat, rye, and triticale. *Theoretical and Applied Genetics***108(6)**:1147–1150.
- Mondal B, Singh S P and Joshi D C. 2014. DUS characterization of rice (*Oryza sativa* L.) using morphological descriptors and quality parameters. *Outlook on Agriculture***43(2)**:131–137.
- Murray M G and Thompson W F. 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research***8(19)**:4321–4325
- Perrier X and Jacquemoud-Collet J P. 2006. Darwin software. <http://darwin.cirad.fr/darwin>
- Perry M C and McIntosh M S. 1991. Geographical patterns of variation in the USDA soybean germplasm collection: International Morphological traits. *Crop Science***31**: 1350– 1355.
- Rabara R C, Ferrer M, Diaz C L, Newingham M C V and Romero G O .2014. Phenotypic diversity of farmers' traditional rice varieties in the Philippines. *Agronomy***4**: 217-241.

- Rani M G, Rao P V R, Ravikumar B N V S R, Chamundeswari N, Satyanarayana P V, Uma K and Kalpana K. 2021. Molecular Profiling of Rice Varieties To Assess Genetic Purity And Exploring Genes For Bacterial Leaf Blight, Blast Resistance And Phosphorus Uptake Plant. *Cell Biotechnology and Molecular Biology***22(71&72)**: 642-657.
- Rao N N D, Roja V, Tushara M, Satyanarayana Rao V and Srinivasa Rao V. 2021.Characterization and diversity analysis of Rice germplasm. *Biological Forum – An International Journal***13**: 170-179.
- Rawte S, and Saxena R. 2018. Phenotypic diversity and correlation analysis for agromorphological traits in 100 landraces of rice from Chhattisgarh. *International Journal of Pure and Applied Biosciences***6(6)**: 345 – 353.
- Roy PS, Samal R, Narasimha Rao GJ, Patnaik SSC, Jambhulkar NN, Patnaik A and Mohapatra T. 2016. Differentiation and description of aromatic short grain rice landraces of eastern Indian state of Odisha based on qualitative phenotypic descriptors. *BMC Ecology***16 (36)**: 3-14.
- Satturu V, Durga Rani Ch, Swathi G, Jamal Md, Sreedhar M, Ranjit Kumar N, Ramprasad E, Raviteja Y, 2018. DNA Fingerprinting for Identification of Rice Varieties and Seed Genetic Purity Assessment. *Agric Res*. <https://doi.org/10.1007/s40003-018-0324-8>
- Sharma S, Pokhrel A and Dhakal A. 2020. Agro-morphological characterization of rice (*Oryza sativa* L.) landraces of lamjung and tanahun district. *Nepal Annual Plant Sciences* :**1**–3741.
- Sohrabi M, Rafii M Y, Hanafi M M, Akmar A S N and Latif M A. 2012. Genetic diversity of upland rice germplasm in Malaysia based on quantitative traits. *The Scientific World Journal* 416291: 1-9.
- Tushara M, Veni B K and Rao N S. 2022. Morphological Characterization of Advanced Coloured Rice Genotypes. *Journal of Rice Research***15(2)**.
- Umarani E, Radhika K, Padma V and Subbarao L V. 2017. Agro morphological characterization of rice (*Oryza sativa* L.) landraces based on DUS descriptors. *International Journal of Pure and Applied Biosciences***5(4)**:466–475.
- Vemireddy L R, Kumar N R, Vipparla A, Surapaneni M, Choudary G, Sudhakararo K V and Siddiq EA. 2015. Molecular profiling of major Indian rice cultivars using a set of eighty hypervariable microsatellite markers. *Cereal Research Communications***43(2)**:189–203
- Zhu Y F, Hu J and Han R. 2012. Fingerprinting and identification of closely related wheat (*Triticum aestivum* L.) cultivars using ISSR and fluorescence-labelled TP-M13- SSR markers. *Australian Journal of Crop Sciences***5**:846–850.