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Molecular and Morphological Genetic Diversity for Yield and Yield Attributing Traits in Rice (*Oryza sativa* L.)

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ABSTRACT: The present study was carried out with 40 genotypes of rice at wetland farm, S.V. Agricultural College, Acharya N.G. Ranga Agricultural University, Tirupati, Andhra Pradesh during *Rabi*, 2022 in a randomized block design with three replications to estimate genetic divergence at both morphological and molecular level. Morphological diversity was estimated by using Mahalanobis D^2 statistics for 14 yield and yield attributing traits and grouped the 40 rice genotypes into eight different clusters. Among the eight clusters, Cluster I was the largest comprising maximum of 17 genotypes followed by Cluster III with 13 genotypes, cluster II and Cluster IV comprising of 3 genotypes each. Whereas, clusters V, VI, VII and VIII were observed to be monogenotypic clusters with one genotype each. Maximum inter-cluster distance was observed between clusters VI and VIII followed by clusters VII and VIII, cluster III and IV and cluster V and VIII which indicated that the genotypes in these clusters have maximum genetic diversity. Whereas, Intra-cluster distance was observed maximum in cluster III. Among all the characters studied, days to 50% flowering, grain length and 1000 grain weight contributed maximum towards genetic divergence. Molecular diversity among 40 rice genotypes was estimated by using gene-specific markers related to yield revealed that out of 13 markers studied, seven markers showed polymorphism. A total of 15 alleles were detected by using seven polymorphic markers with an average of 2.143 alleles per locus. PIC values were ranged from 0.524 (RGS-1) to 0.134 (Dep1-S9) with an average of 0.289. Cluster analysis by using Unweighted Neighbour Joining method revealed that all the 40 genotypes were grouped into three clusters. Cluster I is the largest comprising of 25 genotypes followed by cluster II with 9 genotypes and cluster III with 6 genotypes.

Keywords: Rice, genetic divergence, D^2 statistics, molecular diversity, gene-specific markers.

INTRODUCTION

Rice (*Oryza sativa* L.) is an important cereal and staple food crop for more than half of the population in the world. Over the world, Asia alone produces 90% of the world's rice and consumed by over 2 billion people to derive 80% of their energy needs and it contains 80% carbohydrates, 7-8% protein, 3% fat and 3% fibre (Chaudhari *et al.,* 2018).

Rice is encoded with phenomenal genetic diversity also with hundreds and thousands of germplasms in gene banks. India, as a part of the centre of origin, rice is endowed with a wide variety of germplasm, which includes landraces, obsolete cultivars, wild or weedy species, improved varieties, and so on, resulting in immense genetic diversity. The genus *Oryza* comprising of 24 species, of them only 2 species such as *Oryza sativa* and *Oryza glaberrima* are cultivated in Asia and Africa, respectively. The most cultivated species *Oryza sativa* is again sub-divided into *indica*, *japonica*, *javanica*, *aus* (deep-water rice) and *aromatic* (Basmati, Jasmine, Joha etc.) (Garris *et al.,* 2005).

The knowledge of genetic divergence is very important in the selection of suitable parents for hybridization under varying conditions. The wider the diversity among the parents, the greater will be the chance of obtaining heterotic combinations. A hybridization programme combining genetically varied parents from distinct clusters would give a chance to bring together gene constellations of heterogeneous type, with promising hybrid descendants resulting from the complimentary interaction of divergent genes in parents.

Conventionally, genetic diversity can be estimated by means of D^2 analysis, developed by Mahalanobis (1936), which is a technique based on multivariate

analysis and is found to be an efficient tool in quantifying the degree of divergence in germplasm and determining the relative contribution of each character towards total divergence.

However, the selection of parents based on phenotype is more effective and precise when it is combined with selection based on genotype since the phenotype also includes environmental factors. Diversity in genes of rice accessions creates an option for the breeder to select desirable traits and use them in making new combinations (Garris *et al.,* 2005). At the genotypic level, one of the best methods for analysing diversity in rice is by molecular markers which can tell huge differences among accessions at the DNA level, providing a more reliable and well-planned aid in accession characterization and genetic make-up.

The recent improvements and advances in high throughput sequencing techniques facilitated high-quality reference genomes, which led to numerous molecular markers, high-density genetic/physical maps, several QTLs and candidate genes for key traits. Further, several gene-specific markers have been developed and deployed for the improvement of several rice popular varieties like BPT5204, Pusa Basmati, Swarna, Tellahamsa, Tetep, etc. Thus, the present study aimed the assessment of genetic diversity at both morphological and molecular levels for yield and yield-attributing traits among the rice genotypes. This would help in the identifying diverse parents in the population for the development of new varieties having heterotic combinations.

MATERIAL AND METHODS

The present experiment was carried out using 40 rice genotypes during *Rabi*, 2022 at Wetland farm, Sri Venkateswara Agricultural College, Tirupati. Each genotype was grown in two rows of two meters length with a spacing of 20 cm between rows 15 cm between plants in a Randomized Block Design with three replications. All the recommended cultural practices were followed for raising a good and healthy crop. The data was recorded on 14 yield and yield attributing traits *viz.,* days to 50% flowering**,** days to maturity, plant height (cm), number of tillers plant^{-1}, number of panicles plant^{-1}, panicle length (cm), total number of grains panicle^{-1}, number of chaffy grains panicle^{-1}, number of filled grains panicle^{-1}, grain length (mm), grain width (mm), length/width ratio of rice grain, 1000 grain weight (g) and grain yield plant^{-1} (g). Observations were collected on five randomly selected plants from each genotype in each replication, with the exception of days to 50% flowering and days to maturity, which were recorded on a plot basis. The recorded data was subjected to analysis of variance and Mahalanobis D^2 statistics were used for genetic divergence analysis. The genotypes were clustered by using Tocher's method. The intra- and inter-cluster distances were calculated and were used to describe the genotype relationship with the help of the formula proposed by Singh *et al.* (2016). To perform the statistical analysis INDOSTAT software was used.

Table 1: List of 40 rice genotypes and their pedigree.

Sr. No.	Genotype	Pedigree	Sr. No.	Genotype	Pedigree
1.	NLR 33892	NLR 27999 × MTU 4870	21.	ND44	NLR 34449 × DRR Dhan 42
2.	MTU 1001	MTU 5249 × MTU 7014	22.	ND3	NLR 34449 × DRR Dhan 42
3.	Pusa Basmati	Pusa-167 × Karnal Local	23.	MD5	MTU 1010 × DRR Dhan 42
4.	WGL 1142	WGL 32100 × RP-1	24.	MM152	MTU 1010 MUTANT
5.	Udayagiri	IRAT-138 × IR-13543-66	25.	81C	N22 × IR64
6.	Warangal Samba	(BPT 5204 × ARC5566) × BPT 3291	26.	MM11	MTU 1010 MUTANT
7.	RNR 19186	BPT5204 × Tellahamsa	27.	LRG-5	(BPT 5204 × MTU 3626) × NLR 33892
8.	NLR 34242	Selection from NLR 30491	28.	187-3	BPT 5204 × NLR 33894
9.	Anjali	Sneha × RR 149-1129	29.	SM227	Swarna mutant
10.	Heera	CR-404-48 × CR-289-1208	30.	MTU 1010	Krishnaveni × IR-64
11.	WGL 32100	Divya × BPT 5204	31.	NLR 34449	IR72 × BPT 5204
12.	NLR 2422	Selection from ARS, Nellore	32.	MTU 1061	PLA1100 × MTU 1010
13.	BPT 1235	Sabarmati × W12708	33.	MTU 7029	Vasista × Mahsuri
14.	DRR Dhan 38	BPT 5204 × KMR-3	34.	BPT 5204	GEB24 × TN1 × Mahsuri
15.	BPT 3006	BPT 2274 × NLR 145	35.	MTU 1153	MTU 1010 × MTU 1081
16.	Sharbati	Local selection from Uttar Pradesh	36.	MTU 3626	IR8 × MTU3
17.	Vikramaya	RPW 6-13 × PTB 2	37.	MTU 1210	MTU 1001 × KMP 150
18.	Aditya	M-63-83 × Cauvery	38.	MTU 1318	MTU 1064 × MTU 7029
19.	Pant dhan 12	Govind × UPR 201	39.	MTU 1217	MTU 1001 × CR1081-1-14-3-1
20.	Navara	Landrace	40.	MTU 1224	(JGL 3844 × NLR 34449) × BPT 5204

DNA isolation and PCR assay. The genomic DNA was isolated from leaves of 20-25 days old seedlings of 40 rice genotypes using Cetyl Trimethyl Ammonium Bromide (CTAB) method (Murray and Thompson 1980). The isolated DNA was quantified using Nano drop spectrophotometer (ND-1000, Thermo Scientific, Nanodrop Technologies, U.S.A). 10µl of PCR mixture consists of 1µl of PCR buffer with Mg^{2+}, 0.5 µl of dNTPs, forward and reverse primers of 0.5 µl each, 0.1 µl of *Taq* DNA polymerase, 5.4 µl of autoclaved

double distilled water and 2 µl of template DNA. Amplification was done using an Eppendorf thermo cycler, with the temperature profiles of initial denaturation at 94°C for 5 min, denaturation at 94 °C for 30 sec, annealing (55-65 °C) based on primer for 30 sec, extension at 72 °C for 1.0 min, final extension for 10 min at 72°C for 35 cycles and storage at 4 °C for 10-15 min. The amplified PCR products were electrophoresed on a 3% agarose gel stained with ethidium bromide (10mg/ml) at 100 volts for 1.5 hours in 1X TBE buffer. A 50 bp or 100 bp ladder (Genei) was used to determine the optimal product size. The gel pictures were taken under UV light with the Syngene Ingenius geldoc system.

Table 2: List of gene-specific markers related to yield included in the study.

Sr. No.	Primer	Primer sequence	Trait	Gene	Chromosome No.
1.	HY2-4	F-TTGATACTCGTCTTCGGATAGC R-GACTGACCTGACACACAAGGT	grain number	*GN2*	2
2.	RGS-1	F-TCCACCTGCAGATTTCTTCC R-GCTGGTCTTGCACATCTCTCT	grain length	*GS3*	3
3.	RM16942	F-CCAGTACTCTCGCTCCACTCTCC R-ATCGCTTTCACGTCACCAAGG	grain incomplete filling	*GIF1*	4
4.	RMw513	F-GTATTTGTTTGTCGCATTC R-TAGGACCATAGATGTGAGTTA	grain width and grain length	*gw5*	5
5.	Dep1S7	F-AGTTTCTTGGTTTCCGATCA R-CATATTGGAATGCTCCCTCCT	grain number	*DEP1*	7
6.	RID-711	F-GCACATGCATGCTAGGACAT R-AGCCGGTAAATTTCTTGCAC	grain length	*qGL7*	7
7.	RM5499	F-TGGAGTACGACGTGATCGTG R-CAGAAACGGGAGGGGATC	grain length	*Ghd7*	7
8.	RM21945	F-CTACACAAGTGAACGCCATCAGG R-GTTCTAGGGTGTCCTTTCATGAGC	grain length	*qGL7-2*	7
9.	PAY1SP6	F-TTGGATGAAAGGGAGATTTT R-GTCAAAGAACAGCACACCAG	grain yield	*PAY1*	8
10.	RM502	F-GCGATCGATGGCTACGAC R-ACAACCCAACAAGAAGGACG	grain size	*OsSPL16*	8
11.	SPIKE indel3	F-GGAGAGACATGGACGGCT R-TGGTGGCGATCATGCTGC	grain number per panicle	*SPIKE*	8
12.	Dep1S9	F-TGGACACTTGTTATCTTCTCAT R-AACTGGAAGTTTGTAACACTCA	grain number	*DEP1*	9
13.	RM7289	F-GGCCCACGACTTAATAGACATCG F-GGCAATATGATATGACCAGCAC	panicle length	*LP1*	9

Molecular data analysis. The amplified products for marker analysis were scored visually based on the presence (taken as '1') or absence (taken as '0') of band for each primer. Each marker fragment was treated as a unit character and only clear and unambiguous bands were scored. Genetic diversity parameters like number of alleles per locus, major allele frequency and heterozygosity were estimated by using markers data. The allele frequency represents the frequency of a particular allele for each marker; while heterozygosity is the proportion of heterozygous individuals in the population. Polymorphic information content (PIC) was estimated using the following formula (Hwang *et al.,* 2009; Barik *et al.,* 2019):

$$PIC_i = 1 - \sum P_{ij}^2$$

where i = 1 to n and P_{ij} is the frequency of j^{th} allele for the i^{th} band scored for a particular marker.

Molecular diversity analysis for the genotypes was according to unweighted neighbour joining method and a dendrogram was constructed using Jaccard's dissimilarity coefficient in DARwin software version 6.0.21 (Perrier, 2006).

RESULTS AND DISCUSSION

Morphological diversity by using D^2 statistics

Estimation of D^2 Values. The mean values of 40 genotypes [(X1)-(X2)] were converted into standardized uncorrelated mean values [(Y1)-(Y2)] using the pivotal condensation technique. D^2 values were calculated for all possible [40 (40-1)/2] 780 pairs of genotypes.

Grouping of Genotypes into Clusters. All the 40 genotypes were clustered into eight distinct clusters using Tocher's method (Rao, 1952). The genotypes present within the cluster had smaller D^2 values than those with different clusters. Table 3 and Fig. 1 shows how genotypes are distributed into distinct clusters. Out of eight clusters, clusters I was the largest one comprising of 17 genotypes followed by cluster III with 13 genotypes, clusters II and IV had 3 genotypes each. Clusters V, VI, VII and VIII are monogenotypic clusters. The genotypes present in the monogenotypic clusters were unique and very useful for breeding purpose.

Table 3: Distribution of 40 rice genotypes into eight clusters based on Tocher's method.

Cluster No.	No. of Genotypes	Genotypes
I	17	BPT3006, SM227, MTU1061, Sharbati, MTU1001, NLR2422, ND44, WGL32100, MTU1224, Pusa Basmati, MM152, Vikramarya, MTU1153, MTU1010, NLR34242, Aditya, MD5
II	3	Heera, Pant dhan 12, 81C
III	13	LRG-5, MTU1318, MTU7029, MTU1217, Warangal Samba, BPT5204, BPT1235, 187-3, ND3, NLR33892, MTU1210, MM11, NLR34449
IV	3	Udayagiri, Navara, Anjali
V	1	DRR Dhan 38
VI	1	RNR19186
VII	1	WGL1142
VIII	1	MTU3626

Average Intra and Inter Cluster D^2 Values. The average intra and inter cluster D^2 values among the eight clusters were represented in Table 4 and the cluster diagram was furnished in Fig. 2. The average intra-cluster distance ranged from 0.00 to 219.32. Maximum intra-cluster distance was reported in cluster III (219.32) followed by cluster II (147.28), cluster I (133.82) and cluster IV (105.22) which manifested that some divergence still existed among the genotypes of the same cluster, which could be used in the yield improvement whereas no intra-cluster distance was observed in clusters V, VI, VII and VIII because of monogenic clusters.

The inter-cluster D^2 values ranged from 231.47 to 1853.65. The maximum inter-cluster distance (1853.65) was recorded between cluster VI and VIII followed by cluster VII and VIII (1278.73), cluster III and IV (1246.12) and cluster V and VIII (1179.41). It may be extrapolated that the genotypes in these clusters have the maximum genetic diversity. Hence, the genotypes from these clusters could be utilized in the crossing programme to develop promising genotypes. Whereas, minimum inter-cluster distance of 231.47 was recorded between cluster V and VII, followed by cluster I and II (277.38), cluster I and V (279.94) and cluster VI and VII (296.07) which denoted that genotypes of these clusters were genetically close. Inter-cluster distances were greater than intra-cluster distances, indicating that there is more genetic diversity between clusters rather than within cluster performance.

Table 4: Average Inter (above diagonal) and Intra (diagonal) cluster distances (D^2 values) for eight clusters of 40 rice genotypes.

Cluster	I	II	III	IV	V	VI	VII	VIII
I	133.82 (11.57)	277.38 (16.65)	380.54 (19.51)	592.72 (24.35)	279.94 (16.73)	425.26 (20.62)	369.03 (19.21)	724.23 (26.91)
II		147.28 (12.14)	754.63 (27.47)	650.7 (25.51)	358.79 (18.94)	918.35 (30.30)	679.49 (26.07)	542.43 (23.29)
III			219.32 (14.81)	1246.12 (35.30)	632.67 (25.15)	675.72 (25.99)	473.87 (21.77)	846.24 (29.09)
IV				105.22 (10.26)	934.56 (30.57)	848.27 (29.13)	1060.67 (32.57)	1070.9 (32.72)
V					0.00 (0.00)	408.45 (20.21)	231.47 (15.21)	1179.41 (34.34)
VI						0.00 (0.00)	296.07 (17.21)	1853.65 (43.05)
VII							0.00 (0.00)	1278.73 (35.76)
VIII								0.00 (0.00)

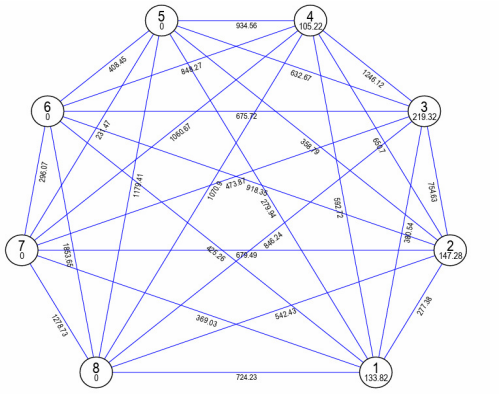

Fig. 1. Cluster diagram representing inter and intra-cluster distances among eight clusters of 40 rice genotypes.

Mahalanobis Euclidean Distance (Not to the Scale)

Cluster Means for Yield and Yield Attributes. The cluster means for 14 yield and yield attributing traits were presented in Table 5. Considerable variation among the cluster means for all the characters indicated the divergent nature of clusters formed.

Table 5: Cluster means for yield and yield attributing traits among 40 rice genotypes.

Cluster	DFF	DM	PH	TP	PP	PL	TG	CG	FG	GL	GW	LWR	TGW	GYP
I	102.1	132.82	84.63	10.68	9.34	21.9	187.95	15.09	172.87	8.2	2.57	3.2	17.86	27.15
II	100.89	131.89	84.03	10.04	8.76	21.97	137.36	14.4	123	9.14	2.75	3.33	22.58	23.64
III	113.64	145.18	91.05	10.5	9.21	22.62	268.69	25.29	243.39	7.5	2.42	3.11	14.59	30.66
IV	89.67	120.67	81.64	16.4	12.04	21.09	101.42	5.76	95.67	7.58	3.08	2.46	21.34	23.44
V	100	130.67	95.6	11.67	10.53	26.77	184	15.87	168.13	9.6	2.45	3.92	16.2	28.18
VI	97	127.67	70.63	14.8	12.27	20.37	266.07	19.53	246.6	7.87	2.19	3.58	12.52	37.3
VII	104	135	96.47	9.73	8.6	27.03	290.87	39.4	251.47	8.83	2.17	4.08	16.15	34.23
VIII	113.67	144.67	82.43	10.53	9.33	21.4	143.4	12.93	130.47	8.43	3.19	2.64	25.22	29.72
Mean	102.62	133.57	85.81	11.79	10.01	22.89	197.47	18.53	178.95	8.39	2.6	3.29	18.31	29.29

DFF: Days to 50% flowering; **DM:**Days to maturity; **PH:** Plant height (cm); **TP:** Number of tillers plant^{-1}; **PP:** Number of panicles plant^{-1}; **PL:** Panicle length (cm); **TG:** Total number of grains panicle^{-1}; **CG:** Number of chaffy grains panicle^{-1}; **FG:** Number of filled grains panicle^{-1}; **GL:** Grain length (mm); **GW:** Grain width (mm); **LWR:** Length/width ratio of rice grain; **TGW:** 1000 grain weight (g); **GYP:** Grain yield plant^{-1} (g)

Early flowering was recorded in the genotypes of cluster IV (89.67 days), while late flowering was recorded in the genotypes of cluster VIII (113.64 days) with the general mean of 102.62 days. Cluster I, II, IV, V and VI had lower values than the cluster mean. Days to maturity ranged from 120.67 days in cluster IV to 145.18 days in cluster III and cluster I, cluster II, cluster IV, cluster V and cluster VI recorded lower cluster means for days to maturity than the general mean (133.57 days).

The genotypes of cluster VI were shorter in plant height (70.63 cm), while genotypes of cluster VII were taller in plant height (96.47 cm). The clusters that recorded lower values than the general mean (85.81 cm) for plant height were cluster I, II, IV, VI and VIII. Cluster mean for number of tillers plant^{-1} was highest in cluster IV (16.40) and lowest in cluster VII (9.73), higher cluster mean than the general mean (11.79) was recorded in clusters IV and VI. Cluster means for number of panicles plant^{-1} was highest in cluster VI (12.27) and lowest in cluster VII (8.60), cluster means that exceeded the general mean (10.01) were clusters IV, V and VI. Cluster means for panicle length ranged from 20.37 cm (cluster VI) to 27.03 cm (cluster VII). The superior clusters for panicle length that exceeded the general mean (22.89 cm) were clusters V and VII.

The cluster means for total number of grains panicle^{-1} varied from 101.42 (cluster IV) to 290.87 (cluster VII). The clusters III, VI and VII recorded a higher number of grains panicle^{-1} than the general mean (197.47). Number of chaffy grains panicle^{-1} exhibited an overall mean value of 18.53 with cluster means ranging from 5.76 (cluster IV) to 39.40 (cluster VII), while the clusters that were below the general mean value were I, II, IV, V and VIII. The cluster means for number of filled grains panicle^{-1} varied from 95.67 (cluster IV) to 251.47 (cluster VII). The clusters III, VI and VII recorded a higher number of filled grains panicle^{-1} than the general mean (178.95).

The maximum and minimum cluster means observed for grain length were 9.60 mm in cluster V and 7.50 mm in cluster III, respectively. The general mean of 8.39 mm was exceeded by the clusters II, V, VII and VIII. The cluster mean for grain width was ranged from 2.17 mm in cluster VII to 3.19 mm in cluster VIII, cluster means that were lower than the general mean (2.60 mm) was recorded in clusters I, III, V, VI and VII. The cluster mean for length/width ratio of rice grain was ranged from 2.46 in cluster IV to 4.08 in cluster VII, the cluster means that were superior than the general mean (3.29) was recorded in clusters II, V, VI and VII.

The cluster means for 1000 grain weight ranged from 12.52 g (cluster VI) to 25.22 g (cluster VIII), higher values than the general mean for 1000 grain weight (18.31 g) were recorded in clusters II, IV and VIII. Cluster means for grain yield plant^{-1} ranged from 23.44 g (cluster IV) to 37.30g (cluster VI) and the clusters III, VI, VII and VIII were superior with higher values than the general mean of 29.29 g.

Cluster VI comprised of RNR19186 recorded desirable values for plant height, number of panicles plant^{-1} and grain yield plant^{-1}.Cluster V comprised of DRR Dhan 38 recorded desirable value for grain length. Cluster VII comprised of WGL1142 recorded desirable values for panicle length, grain width, length/width ratio of rice grain, total number of grains panicle^{-1} and number of filled grains panicle^{-1}. Cluster IV registered a desirable value for days to 50% flowering, days to maturity, number of tillers plant^{-1} and number of chaffy grains panicle^{-1}. Cluster VIII consisted MTU3626 which recorded desirable value for 1000 grain weight. Hence it is inferred that crossing between the genotypes from these clusters could be advised to generate a wide spectrum of variability followed by effective selection for these characters in later generations.

Relative Contribution of Each Character towards Diversity. The number of times each of the 14 characters appeared in first rank and its respective percent contribution towards diversity was presented in Table 6.

Table 6: Percent contribution of 14 yield and yield attributing traits towards genetic divergence in 40 rice genotypes.

Sr. No.	Character	No. of times ranked 1st	Contribution (%)
1.	Days to 50% flowering	375	48.08
2.	Days to maturity	0	0.00
3.	Plant height (cm)	5	0.64
4.	Number of tillers plant^{-1}	0	0.00
5.	Number of panicles plant^{-1}	1	0.13
6.	Panicle length (cm)	5	0.64
7.	Total number of grains panicle^{-1}	7	0.90
8.	Number of chaffy grains panicle^{-1}	1	0.13
9.	Number of filled grains panicle^{-1}	17	2.18
10.	Grain length (mm)	132	16.92
11.	Grain width (mm)	77	9.87
12.	Length/width ratio of rice grain	19	2.44
13.	1000 grain weight (g)	131	16.79
14.	Grain yield plant^{-1} (g)	10	1.28

Among all the characters studied, maximum contribution towards genetic divergence was recorded by days to 50% flowering (48.08%) by obtaining the first rank which was followed by grain length (16.92%), 1000 grain weight (16.79%), grain width (9.87%), length/width ratio of rice grain (2.44%), number of filled grains panicle^{-1} (2.18%) and grain yield plant^{-1} (1.28%). The characters *viz.,* total number of grains panicle^{-1} (0.90%), plant height (0.64%), panicle length (0.64%), number of panicles plant^{-1} (0.13%) and number of chaffy grains panicle^{-1} (0.13%) contributed least towards genetic divergence.

In the present study, days to 50% flowering, grain length and 1000 grain weight were found the best discriminatory characters for selection of diverse genotypes. So, these characters could be exploited maximum to get varieties with a higher yield.

The results with respect to the relative contribution of each character towards diversity were supported by earlier findings of Vanlalrinngama *et al.* (2023); Lakshmi *et al.* (2022); Roy *et al.* (2022); Singhal *et al.* (2022); Naik *et al.* (2021); Chandramohan *et al.* (2016) for days to 50% flowering; Devi *et al.* (2022); Sujitha *et al.* (2020) ; Singh *et al.* (2008) for grain length; Gayathri *et al.* (2023); Lakshmi *et al.* (2022); Singhal *et al.* (2022); Guru *et al.* (2017); Chandramohan *et al.* (2016) for 1000 grain weight.

Molecular diversity by using UPGMA method

Molecular Marker Analysis. A total of 13 gene-specific markers related to yield (Table 2) were used for the assessment of molecular diversity among 40 rice genotypes. These 13 gene-specific markers were spread on seven chromosomes 2, 3, 4, 5, 7, 8 and 9 of rice genome. Out of 13 markers, seven markers *viz.,* Dep1-S7, Dep1-S9, RM7289, PAY1SP6, RGS-1, RMw513 and Spike-Indel3 produced clear polymorphic amplicons in 40 rice genotypes and the remaining six markers HY2-4, RM16942, RID-711, RM5499, RM21945 and RM502 were found to be monomorphic. A total of 15 alleles were identified with seven gene-specific markers across 40 rice genotypes. The number of alleles per locus observed maximum of three (Spike-Indel3) and the remaining markers with two alleles each with an average of 2.143 alleles per locus. The Polymorphic Information Content (PIC) values for the used gene-specific markers ranged from 0.134 to 0.524 with an average of 0.289. The highest PIC value was obtained for the marker RGS-1 (0.524) followed by Dep1-S7 (0.414), PAY1SP6 (0.375), Spike-Indel3 (0.296), RM7289 (0.139), RMw513 (0.139) and Dep1-S9 (0.134).

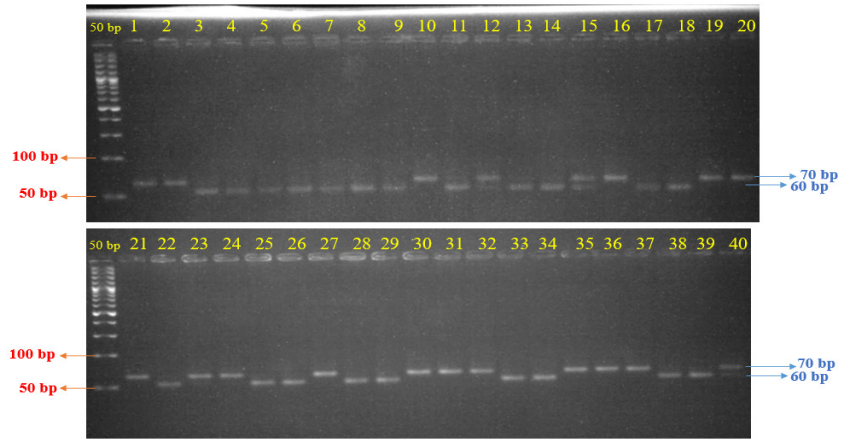

Fig. 2. Gel picture showing the allelic pattern in 40 rice genotypes with Dep1-S7.

Table 7: Number of alleles, allele size and PIC value of the gene-specific markers.

Sr. No.	Marker	No. of alleles	Allele size (bp)	PIC value
1.	Dep1-S7	2	60 and 70	0.414
2.	Dep1-S9	2	195 and 460	0.134
3.	RM7289	2	150 and 170	0.139
4.	PAY1SP6	2	200 and 220	0.375
5.	RGS-1	2	190 and 200	0.524
6.	RMw513	2	180 and 200	0.139
7.	Spike-Indel3	3	151, 160 and 171	0.296
	Mean	**2.143**		**0.289**

Markers with PIC values of 0.5 or above are very informative for genetic investigations and especially effective in differentiating the polymorphism rate of a marker at a specific locus (Virk *et al.,* 1995). In this study, marker with PIC value ≥ 0.5 is only one *i.e.*, RGS-1 indicating this marker has higher discriminating power when compared to other markers.

As we have selected gene-specific markers, that are most favoured by breeders during their selection. The number of alleles and PIC values of these gene-specific markers were significantly lower than the frequently utilized SSR markers from prior studies. For example, Choudhary *et al.* (2013) found an average of 3.6 alleles per locus with a PIC of 0.87 using 52 SSR markers in 100 rice genotypes, whereas Vigneshwari *et al.* (2017) found an average of 4.2 alleles per locus with a PIC of 0.453 using 15 SSR markers. However, in accordance with the current study, Ngangkham *et al.* (2018) reported a total of 21 alleles with an average of 2.1 allele per locus and a PIC value ranging from 0.13 to 0.58 with an average value of 0.31 using 10 gene-specific markers regulating grain size in 89 rice genotypes.

Molecular Diversity Analysis. The data from gene-specific markers related to yield was used to analyse the genetic diversity among the 40 rice genotypes. The dissimilarity matrix was estimated using seven gene-specific markers based on Jaccard's dissimilarity coefficient using DARwin version 6.0.21 (Perrier, 2006). The calculated dissimilarity matrix was used for the clustering of 40 rice genotypes. An un-weighted neighbour-joining (UNJ) method was used for the dendrogram construction with 1000 permutations to determine the bootstrap values. The dendrogram was presented in Fig. 3.

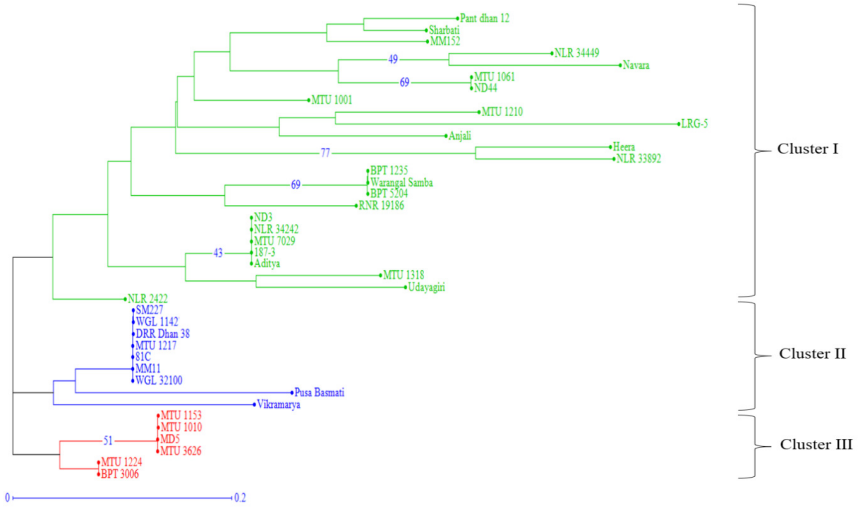

Fig. 3. Dendrogram of 40 rice genotypes based on molecular data of gene-specific markers using Jaccard's dissimilarity coefficient by UPGMA method.

Table 8: Grouping of 40 rice genotypes into three clusters by using gene-specific markers data based on Jaccard's dissimilarity coefficient by UPGMA method.

Cluster No.	No. of Genotypes	Genotypes
I	25	Pant dhan 12, Sharbati, MM152, NLR34449, Navara, MTU1061, ND44, MTU1001, MTU1210, LRG-5, Anjali, Heera, NLR33892, BPT1235, Warangal Samba, BPT5204, RNR19186, ND3, NLR34242, MTU7029, 187-3, Aditya, MTU1318, Udayagiri and NLR2422
II	9	SM227, WGL1142, DRR Dhan 38, MTU1217, 81C, MM11, WGL32100, Pusa Basmati and Vikramarya
III	6	MTU1153, MTU1010, MD5, MTU3626, MTU1224 and BPT3006

The results led to the clustering of 40 rice genotypes into three clusters, cluster I, cluster II and cluster III. Among the three clusters, cluster I is the largest comprising of 25 genotypes followed by cluster II with 9 genotypes and cluster III with 6 genotypes. Genotypes *viz.,* Warangal samba, RNR19186 and 187-3 in Cluster I were having one common parent BPT5204; MTU1061 and MTU1210 were derived from one common parent MTU1001; ND3 and ND44 were derived from one common parent NLR34449; MTU1318 and LRG-5 were grouped in cluster I along with their parents MTU7029 and NLR33892, respectively. The remaining genotypes *viz.,* Pant dhan 12, Sharbati, MM152, Navara, Anjali, Heera, BPT1235, NLR34242, Aditya, Udayagiri and NLR2422 were grouped under the cluster I may be due to the common ancestral origin. In Cluster II, one genotype *i.e.,* WGL1142 along with its parent WGL32100 grouped together. Out of the six genotypes in cluster III, two genotypes *i.e.*, MTU1153 and MD5 along with their common parent, MTU1010 were clustered together.

Upadhyay *et al.* (2012) have previously documented genotype grouping based on the effect of the pedigree, which is consistent with the current study, categorized the 25 popular rice varieties into two major clusters and showed that varieties with at least one common parent were grouped in one cluster; Choudhary *et al.* (2013) showed that varieties released during different decades were also grouped together due to the presence of common parents in their pedigree; Singh *et al.* (2016) showed that varieties sharing common parentage were grouped in the same cluster; and Vigneshwari *et al.* (2017) showed the relationship between the clustering pattern of the thirteen rice varieties was obviously due to the pedigree. There were a few cases where genotypes with shared parentage were not grouped in the same cluster. The two mutants from MTU1010, MM11 and MM152, fell into distinct groups.

CONCLUSIONS

Mahalanobis D^2 analysis grouped the 40 genotypes into eight clusters. Cluster I was observed to be largest with 17 genotypes followed by cluster III with 13 genotypes. Maximum inter-cluster distance was observed between cluster VI and VIII followed by cluster VII and VIII, cluster III and IV and cluster V and VIII. While, maximum intra-cluster distance was observed in cluster III. Cluster VI comprised of RNR19186 recorded desirable values for plant height, number of panicles plant^{-1} and grain yield plant^{-1}. Among all the characters studied, maximum contribution towards genetic divergence was recorded by days to 50% flowering. Cluster analysis by molecular diversity grouped 40 genotypes into three clusters. Cluster I is the largest cluster with 25 genotypes followed by cluster II with 9 genotypes and cluster III with 6 genotypes. By comparing both morphological and molecular diversity studies, crossing between the genotypes from diverse clusters *viz.,* RNR19186 × MTU3626, WGL1142 × MTU3626 and DRR Dhan 38 × MTU3626 could be suggested in hybridization programme to generate a broad spectrum of variability in segregating generations.

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