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Genetic divergence studies for yield and quality traits in coloured rice

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Abstract
The present investigation was undertaken with 33 coloured and white rice genotypes to estimate genetic divergence of the genotypes for yield and quality traits. The study involved seven red pericarp, eight black pericarp and 17 white rice genotypes, in addition to the check, BPT 5204. The diversity was evaluated using multivariate analysis technique of Mahalanobis [4] D^2. The 33 rice genotypes studied were grouped into seven clusters. Cluster I was observed to be the largest with 18 genotypes, followed by Cluster III with nine genotypes and Cluster II with two genotypes. The clusters, IV, V, VI and VII were monogenotypic. The pattern of distribution of genotypes into various clusters was observed to be at random with no relation to geographical diversity. Results on inter-cluster distances revealed maximum diversity between genotypes of cluster I and cluster VII, while intra-cluster distance was noticed to be maximum for cluster III. Cluster II had recorded maximum grain yield per plant, in addition to test weight and plant height along with hulling percentage. The cluster II had also recorded intermediate amylose content and alkali spreading value (ASV) in addition to protein content more than 10 per cent and zinc more than 20ppm along with iron more than 10ppm. Further, maximum contribution towards genetic divergence was noticed by total phenol content (28.45%), followed by Total antioxidant activity (16.67%) and minimum by length/breadth ratio (0.10%).

Keywords: Coloured rice, genetic divergence, grain yield and nutritional quality

Introduction
Rice is a major source of food for about three million people worldwide and accounts for about 20 per cent of calorie consumption. In Asia, nearly two billion people depend on rice for their 60-70 percent calories. Further, rice is the only cereal, cooked and consumed as a whole grain and quality considerations in rice are much more important than for any other food crop (Hossain *et al.*, 2009) [3]. Whole grain pigmented rice has been categorized as one of the potent functional foods since it contains high amounts of phenolic compounds (Yawadio *et al.*, 2007) [11]. In addition, coloured rice contains higher levels of proteins, vitamins and minerals than common white rice, while red rice is good source of fibre, antioxidants, zinc and iron (Sridevi *et al.*, 2019) [8]. Therefore, increased health consciousness among the rice consumers in the recent years has resulted in greater attention to rice genotypes with red and black pericarp color containing high levels of antioxidants (Tian *et al.*, 2004) [10] and these genotypes are in increasing demand. However, the yields of coloured rice need to be improved and high yielding slender grain coloured rice varieties with good nutritional quality are required to meet the increasing demand for coloured rice. In this context, information on genetic divergence in coloured rice genotypes plays a key role in analyzing of diversity among the genotypes and aids in the selection of parents towards realization of enhanced levels of heterosis in addition to wide range of variability for effective selection. Multivariate analysis like Mahalanobis [4] D^2 statistic provides a useful tool for measuring the genetic diversity in a given population with respect to different characters considered together. The present investigation was undertaken in this direction to estimate genetic diversity of different coloured genotypes for their utilization in crop improvement programs aimed at the development of high yielding coloured rice varieties with good nutritional quality.

Material and methods
The experimental material consisted of 33 white and coloured rice genotypes obtained from Agricultural Research Station, Bapatla, Andhra Pradesh state in addition to collections from Telangana and Tamil Nadu states.

Among the 33 genotypes, 15 genotypes were coloured, of which, seven were with red pericarp and eight genotypes were with black pericarp, while remaining 18 genotypes had brown pericarp and were white rice genotypes including, BPT 5204, BPT 5204, a popular high yielding white rice genotype with excellent cooking quality traits, which was used as check variety in the present study. Details of the genotypes studied in the present investigation are presented in Table 1 and Plates 1-2.

All the 33 genotypes were sown at Agricultural College Farm, Bapatla during *Kharif* 2019 on separate raised nursery beds. All recommended package of practices were adopted to raise a healthy nursery and thirty days old seedlings were transplanted in the main field laid out in Randomized Block Design (RBD) with three replications. Each genotype was transplanted separately in 5 rows of 4.5 m length by adopting a spacing of 20 cm between rows and 15 cm between plants. All the recommended package of practices was adopted throughout the crop growth period and need based plant protection measures were taken up to raise a healthy crop. Observations were recorded on five randomly selected plants for grain yield per plant; days to 50 per cent flowering; and the quality characters, namely, head rice recovery per cent, amylose content, alkali spreading value, protein content, total phenol content, Total antioxidant activity, zinc and iron content in addition to grain type were recorded. However, days to 50 per cent flowering was recorded on plot basis. In contrast, observations for the quality traits studied were obtained from a random grain sample drawn from each plot in each genotype and replication using standard procedures. The data collected was subjected to standard statistical procedures given by Panse and Sukhatme (1967) [5]. Genetic divergence analysis was done following the D^2 statistics proposed by Mahalanobis (1928) [4] and described by Rao (1952) [7]. The analysis was carried out using the software Window Stat Version 8.5.

Results and discussion

The results on genetic divergence of 33 rice genotypes including red, black and white coloured rice for yield, yield components and quality characters are presented in Tables 2-6 and Figs.1-3.

Test with Wilk's criterion '∧'

Univariate analysis of variance (Table 2) revealed the significant difference for all the 21 characters under study in the 33 rice genotypes. This significance of difference among 33 genotypes for all characters justified further calculation of D^2 values. However, pooled differences were determined by Wilk's criterion. Wilk's statistic '∧' which follows v-statistic representing χ2-distribution at 672 degree of freedom was calculated and found to be highly significant with a magnitude of 2974.3320, justifying the need to calculate D^2 values.

Grouping of genotypes into various clusters

The 33 rice genotypes were grouped into seven clusters using Tocher's method based on D^2 value such that the genotypes belonging to the same cluster (Intra-cluster) had an average smaller D^2 value than those belonging to different clusters (Inter-cluster). The distribution of 33 genotypes into seven clusters is presented in Table 3 and Fig. 1. A perusal of the results revealed Cluster I to be the largest comprising of 18 genotypes (WGL 14, BPT 5204, BPT 2507, PHI 17108, JKRH 3333, 27 P 63, BPT 2411, BPT 2846, US 301, BPT 2615, BPT 2782, BPT 2660, BPT 2595, BPT 3173, BPT 2776, BPT 2766, ADT 49, MTU 1281), representing white rice genotypes collected from the states of Andhra Pradesh, Telengana and Tamil Nadu. Cluster III consisted of nine genotypes (BPT 3111, Apputhokal, BPT3178, BPT 3139, BPT 2848, BPT 3145, BPT 2841, BPT 3136 and BPT 3165), including red and black pericarp genotypes, collected from Andhra Pradesh and Telengana states. However, Cluster II consisted of only two genotypes (Asandi and Hallabhatta), collected from the state of Telengana and are landraces with red pericarp colour. Cluster IV (BPT 3141), Cluster V (BPT 3140), Cluster VI (Chittiga) and Cluster VII (Kakirekalu) were observed to be monogenotypic clusters, with one genotype each. The mode of distribution of genotypes from different geographical regions into various clusters was thus observed to be at random indicating no relation of geographic and genetic diversity. Genotypes chosen from the same eco-geographical region were observed to be present in different clusters as well as in same cluster, while genotypes from diverse geographical regions were also included in different clusters as well as the same cluster. The findings are in conformity with the reports of (Ashok *et al.*, 2017) [2]. This random grouping may be attributed to the exchange of breeding material over locations and further intensive natural and human selection for diverse and adaptable gene complexes resulting in genetic drift and consequently increased genetic diversity (Arunachalam and Ram, 1967) [1].

Average intra and inter-cluster D^2 value

The results are presented in Table 4 and Fig. 2. A perusal of these results on intra-cluster distances, indicative of the diversity among the genotypes grouped in that cluster revealed intra-cluster D^2 values to range from 0.000 (Clusters IV, V, VI and VII) to 166.07 (Cluster III). Maximum intra-cluster D^2 value was 166.07 for cluster III, followed by 103.10 for cluster I and 43.05 for cluster II, indicating that genotypes from these clusters were relatively highly divergent meriting their consideration in selection of parents for hybridization. However, the intra-cluster distance was zero for the monogenotypic clusters, IV, V, VI and VII. Further, greater the distance between two clusters, wider is the genetic diversity expected between genotypes of the two clusters. In the present study, maximum inter-cluster distance was observed between Cluster I and VII (2004.83). Therefore, hybridization between the genotypes of Cluster I (WGL 14, BPT 5204, BPT 2507, PHI 17108, JKRH 3333, 27 P 63, BPT 2411, BPT 2846, US 301, BPT 2615, BPT 2782, BPT 2660, BPT 2595, BPT 3173, BPT 2776, BPT 2766, ADT 49, MTU 1281) with Kakirekalu genotype of cluster VII is expected to result in greater variability and transgressive segregants. Minimum inter-cluster distance was observed between Cluster IV and Cluster V (92.57), indicating their relatively closer relationship and similarity with regards to the characters studied for most of the genotypes in the two clusters.

Cluster means

Cluster means indicate average performance of all genotypes present in a particular cluster. Estimate of cluster means provides information on suitable donors for improvement of particular traits. The cluster means for grain yield, yield components and quality characters for the 33 genotypes studied in the present investigation are presented in Table 5. The results revealed considerable differences between the clusters for all characters under study.

The cluster means ranged from 96.33 days (Cluster VII) to

117.00 (Cluster IV) for days to 50 per cent flowering; 131.33 days (Cluster VII) to 151.67 days (Cluster IV) for days to maturity; 99.16cm (Cluster I) to 143.77cm (Cluster II) for plant height; 11.93 (Cluster II and Cluster VI) to 14.47 (Cluster IV) for productive tillers per plant; 21.81cm (Cluster VII) to 27.17cm (Cluster IV) for panicle length; 109.00 (Cluster VI) to 271.00 (Cluster V) for grains per panicle; 16.75g (Cluster I) to 26.28g (Cluster II) for test weight; 16.00g (Cluster VI) to 28.39g (Cluster II) for grain yield per plant; 5.27mm (Cluster II) to 6.71mm (Cluster VII) for kernel length; 1.80mm (Cluster IV) to 2.73mm (Cluster II) for kernel breadth; 1.93 (Cluster II) to 3.50 (Cluster IV) for length/breadth ratio; 61.88 per cent (Cluster VI) to 84.96 per cent (Cluster II) for hulling per cent; 60.83 per cent (Cluster VI) to 70.91 per cent (Cluster IV) for milling per cent; 42.33 per cent (Cluster VI) to 63.12 per cent (Cluster I) for head rice recovery; 19.53 per cent (Cluster VII) to 23.96 per cent (Cluster II) for amylose content; 3.00 (Cluster IV) to 4.34 (Cluster III) for ASV; 7.88 per cent (Cluster I) to 13.50 per cent (Cluster VII) for protein content; 59.14mg/100g (Cluster I) to 267.14mg/100g (Cluster VII) for total phenol content;36.40mgAAE/100g (Cluster I) to 103.87mgAAE/100g (Cluster IV) for Total antioxidant activity; 16.27ppm (Cluster I) to 28.00ppm (Cluster VII) for zinc content; and 8.57ppm (Cluster I) to 14.67ppm (Cluster VII) for iron content in the present study.

Cluster II had recorded maximum grain yield per plant, in addition to test weight and plant height along with hulling percentage, while Cluster I had recorded maximum head rice recovery percent; Cluster IV, maximum Total antioxidant activity; and Cluster VII, maximum protein, total phenol content, zinc and iron contents. There was no single cluster with all the desirable traits, which ruled out the possibility of direct selection of genotypes for immediate use. The results are in broad agreement with the reports of Sudeepthi *et al.* (2020) [9]. Selection of genotypes from clusters with high mean for the respective traits is suggested for utilization in hybridization programmes aimed at improvement of the respective traits. Further, hybridization between the selected genotypes from divergent clusters is suggested for judicious combination of all the targeted traits. In this direction, selection of genotypes from the clusters, II and VII is suggested for utilization in hybridization programmes aimed at the development of high yielding coloured rice genotypes with good nutritional quality.

Relative contribution of individual characters towards divergence

Information on the relative contribution of various characters towards divergence was reported to aid the breeder in choice of parents for hybridization and effective selection (Prasad *et al.*, 2018) [6]. A perusal of the results on per cent contribution towards genetic divergence by the yield, yield component and quality characters studied in the present investigation is presented in Table 6 and Fig. 3. A perusal of these results revealed maximum contribution towards genetic divergence by total phenol content (28.45%), followed by Total antioxidant activity (16.67%), kernel breadth (7.77%), protein content (7.39 %), zinc content (7.00%), amylose content (6.82%), alkali spreading value (6.25 %), iron content (4.55%), kernel length (4.36%), test weight (3.41%), grain yield per plant (2.46 %), plant height (1.89 %), grains per panicle (0.57%), head rice recovery (0.57%), productive tillers per plant (0.50%), panicle length (0.30%), days to maturity (0.30%), days to 50 per cent flowering (0.24%), milling percentage (0.22%), hulling percentage (0.19 %) and length/breadth ratio (0.10%). Hence, selection for divergent parents based on total phenol content and total antioxidant activity would be useful for increasing scope of isolating desirable recombinants in breeding of high yielding coloured rice genotypes with good nutritional quality.

Table 1: Details of the rice genotypes studied in the present investigation

S. No.	Genotype	Cross combination/ Pedigree	Origin
Red pericarp genotypes			
1.	Apputhokal	Landraces	Telangana
2.	Asandi	Landraces	Telangana
3.	Chittiga	Landraces	Telangana
4.	BPT 3111	Swarna/ IRGC 18195// MTU 1081	Andhra Pradesh
5.	BPT 3139	Cult. 01120305/ cult. 0910025-7	Andhra Pradesh
6.	BPT 3178	Cult. 01120305/ cult. 0910025-7	Andhra Pradesh
7.	Hallabhatta	Landraces	Telangana
Black pericarp genotypes			
8.	BPT 2841	MTU 7029/IRGC 18195/MTU 1081	Andhra Pradesh
9.	BPT 2848	RP Bio 226*1/1RGC 48493	Andhra Pradesh
10.	BPT 3136	RP Bio 226*1/1RGC 18195	Andhra Pradesh
11.	BPT 3140	Swarna/1RGC 18195 /MTU 1081	Andhra Pradesh
12.	BPT 3141	RP Bio 226*1/ 1RGC 30938	Andhra Pradesh
13.	BPT 3145	RP Bio 226/ IRGC26940// MTU 1081	Andhra Pradesh
14.	BPT 3165	BPT 3291/BPT 2411	Andhra Pradesh
15.	Kakirekalu	Landraces	Telangana
Brown pericarp white rice genotypes			
16.	ADT 49	CR 1009/Jeeragasambha	Tamil Nadu
17.	BPT 2411	BPT 5204/BPT 4358	Andhra Pradesh
18.	BPT 2507	BPT 1235/BPT 5204//BPT 5204	Andhra Pradesh
19.	BPT 2595	Mutant of BPT 2270	Andhra Pradesh
20.	BPT 2615	IR 8/Tulasi	Andhra Pradesh
21.	BPT 2660	BPT 1768/ NLR 145	Andhra Pradesh
22.	BPT 2766	BPT 2270/NLR 145	Andhra Pradesh
23.	BPT 2776	BPT 2231/ NLR 145	Andhra Pradesh
24.	BPT 2782	NLR 145/ MTU 2077	Andhra Pradesh

25.	BPT 2846	MTU 1061/IR 78585-64-2-4-3-1	Andhra Pradesh
26.	BPT 3173	BPT 3291/JGL 3844	Andhra Pradesh
27.	BPT 5204	GEB24/TN1/ Mahsuri	Andhra Pradesh
28.	JKRH 3333	Pvt. Research Hybrid	Telangana
29.	PHI 17108	Pvt. Research Hybrid	Telangana
30.	WGL 14	BPT 5204/ARC 5984//BPT 3291	Andhra Pradesh
31.	MTU 1281	MTU 1075/MTU 1081/MTU 1121	Andhra Pradesh
32.	27 P 63	Pvt. Research Hybrid	Telangana
33.	US 301	Pvt. Research Hybrid	Telangana

Table 2: Analysis of variance for yield, yield components and quality characters in rice

Source of variation	d.f.	Days to 50 per cent flowering	Days to maturity	Plant height	Productive tillers per plant	Panicle length	Grains per panicle	Test Weight	Grain yield per plant	Kernel length	Kernel breadth	L/ B ratio
		Mean sum of squares										
Replications	2	52.16	44.64	42.38	7.51	22.21	1898.27	0.63	9.11	0.021	0.031	0.014
Genotypes	32	133.54**	124.04**	768.50**	6.46**	28.50**	12002.75**	37.48**	99.61**	0.67**	0.21**	0.49**
Error	64	13.32	20.15	12.41	1.17	2.74	665.65	0.52	5.22	0.015	0.0078	0.03

** Significant at 1 per cent level of probability

Table 2: Contd…

Source of variations	d.f.	Hulling percentage	Milling percentage	Head Rice Recovery	Amylose Content	Alkali Spreading Value	Protein Content	Total Phenol Content	Total Antioxidant Activity	Zinc content	Iron content
		Mean sum of squares									
Replications	2	3.77	2.66	15.98	1.93	0.050	0.13	21.78	47.27	2.89	2.71
Genotypes	32	68.59**	39.06**	118.60**	18.55**	3.77**	13.12**	7154.33**	2709.38**	95.66**	25.74**
Error	64	5.001	2.39	6.16	0.56	0.13	0.10	18.58	15.74	1.23	0.96

** Significant at 1 per cent level of probability

Table 3: Clustering pattern of 33 genotypes for yield, yield components and quality characters in rice

Cluster No.	Number of genotypes	Genotypes
I	18	WGL 14, BPT 5204, BPT 2507, PHI 17108, JKRH 3333, 27 P 63, BPT 2411, BPT 2846, US 301, BPT 2615, BPT 2782, BPT 2660, BPT 2595, BPT 3173, BPT 2776, BPT 2766, ADT 49, MTU 1281
II	2	Asandi, Hallabhatta
III	9	BPT 3111, Apputhokal, BPT 3178, BPT 3139, BPT 2848, BPT 3145 BPT 2841, BPT 3136, BPT 3165
IV	1	BPT 3141
V	1	BPT 3140
VI	1	Chittiga
VII	1	Kakirekalu

Table 4: Average intra-and inter-cluster D^2 values among seven clusters of rice genotypes for yield, yield component and quality characters

Cluster No.	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	103.10	516.46	451.84	805.27	903.14	734.65	2004.83
Cluster II		43.05	368.73	718.29	784.85	250.08	1588.04
Cluster III			166.07	250.66	336.06	366.19	1072.66
Cluster IV				0.00	92.57	481.24	511.73
Cluster V					0.00	599.32	381.93
Cluster VI						0.00	951.19
Cluster VII							0.00

Diagonal bold values indicate intra-cluster distances

Table 5: Cluster means of 33 rice genotypes for yield, yield component and quality characters

CLUSTER	DFF	DM	PH	PTPP	PL	GPP	TW	GYPP	KL	KB	L/B	HULL	MILL	HRR	AMY	ASV	PRO	TPC	TAA	Zn	Fe
I	111.69	144.11	99.16	13.22	23.13	212.52	16.75	20.22	5.56	1.87	2.97	81.97	70.08	63.12	22.69	4.27	7.88	59.14	36.40	16.27	8.57
II	97.67	133.00	143.77	11.93	22.86	116.50	26.28	28.39	5.27	2.73	1.93	84.96	68.52	50.92	23.96	4.25	10.78	84.80	95.48	23.82	11.26
III	109.56	143.22	114.52	12.44	26.56	253.19	18.25	24.85	5.63	1.84	3.07	78.47	64.97	58.17	22.89	4.34	11.14	114.28	93.59	26.74	13.59
IV	117.00	151.67	114.67	14.47	27.17	244.00	18.50	18.67	6.30	1.80	3.50	75.36	70.91	60.91	20.58	3.00	13.00	146.10	103.87	27.98	14.33
V	111.33	142.33	122.33	14.13	24.33	271.00	18.85	20.33	6.20	1.82	3.2	81.49	68.67	58.69	22.72	3.61	10.35	200.23	102.88	26.42	13.22
VI	98.00	133.00	123.53	11.93	22.45	109.00	26.20	16.00	5.31	2.67	1.98	61.88	60.83	42.33	21.75	3.33	11.50	112.68	76.25	25.67	12.33
VII	96.33	131.33	115.73	13.40	21.81	133.00	23.80	23.80	6.71	2.15	3.12	76.50	63.43	50.83	19.53	3.44	13.50	267.14	85.22	28.00	14.67

DFF = Days to 50 per cent flowering, **DM** = Days to maturity, **PH** = Plant height (cm), **PTPP** = Productive tillers per plant, **PL** = Panicle length (cm), **GPP** = Grains per panicle, **TW** = Test Weight (g), **KL** = Kernel length (mm), **KB** = Kernel breadth (mm), **L/B** = Length/Breadth ratio, **HULL** = Hulling percentage, **MILL** = Milling percentage, **HRR** = Head Rice Recovery (%), **AMY** = Amylose Content (%), **ASV** =Alkali Spreading Value, **PRO** =Protein Content (%), **TPC** = Total Phenol Content (mg/100g), **TAA** = Total Antioxidant Activity (mg AAE/100g), **Zn** = Zinc content (ppm), **Fe** = Iron content (ppm)

Table 6: Contribution of different characters towards genetic divergence in rice

S. No.	Character	%Contribution towards divergence
1	Days to 50 per cent flowering	0.24
2	Days to maturity	0.30
3	Plant height	1.89
4	Productive tillers per plant	0.50
5	Panicle length	0.30
6	Grains per panicle	0.57
7	Test Weight	3.41
8	Grain yield per plant	2.46
9	Kernel length	4.36
10	Kernel breadth	7.77
11	Length/Breadth ratio	0.10
12	Hulling percentage	0.19
13	Milling percentage	0.22
14	Head Rice Recovery	0.57
15	Amylose Content	6.82
15	Alkali Spreading Value	6.25
17	Protein Content	7.39
18	Total Phenol Content	28.45
19	Total Antioxidant Activity	16.67
20	Zinc content	7.00
21	Iron content	4.55

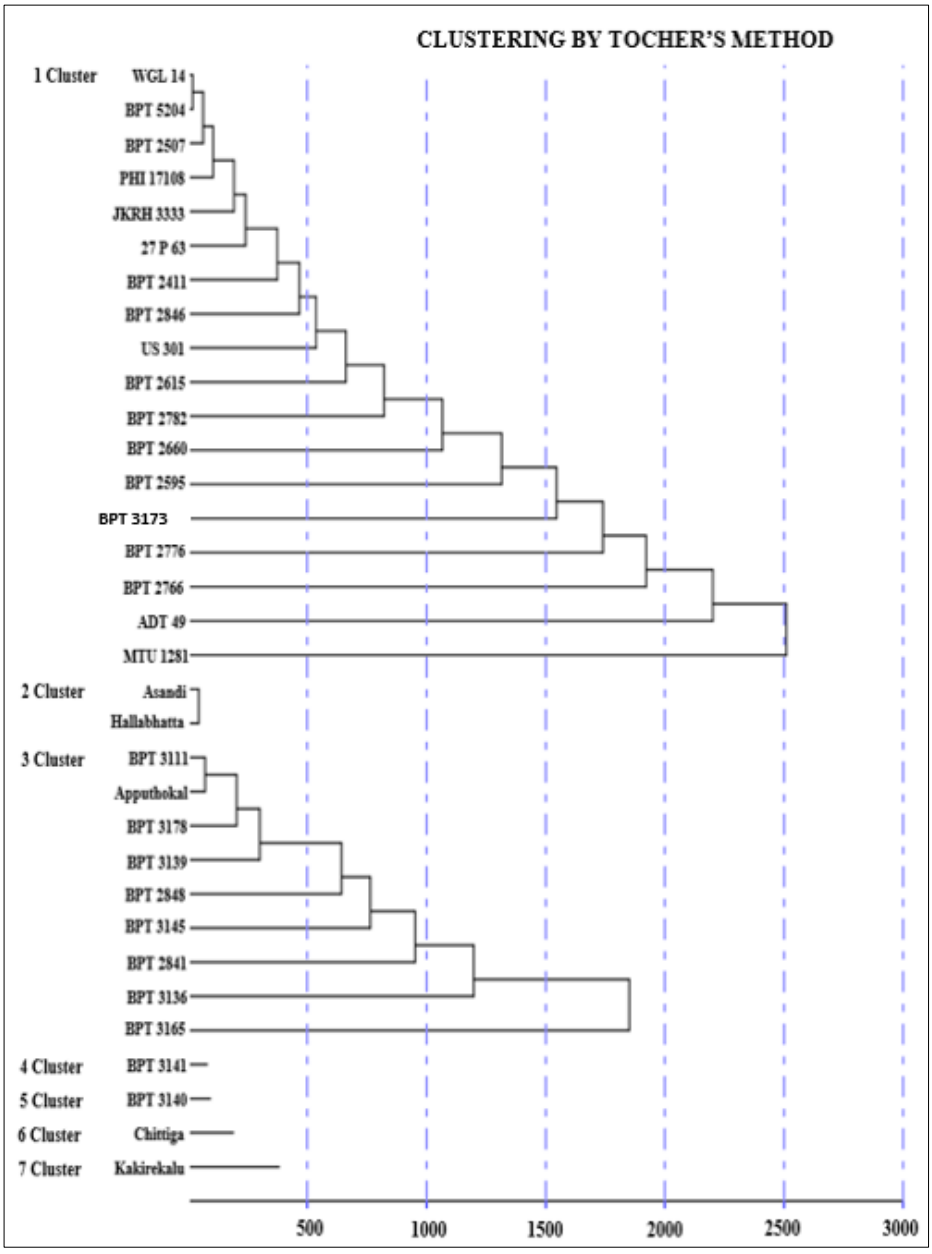

Fig 1: Dendrogram showing relationship among 33 rice genotypes in seven clusters based on Mahalanobis D^2 values.

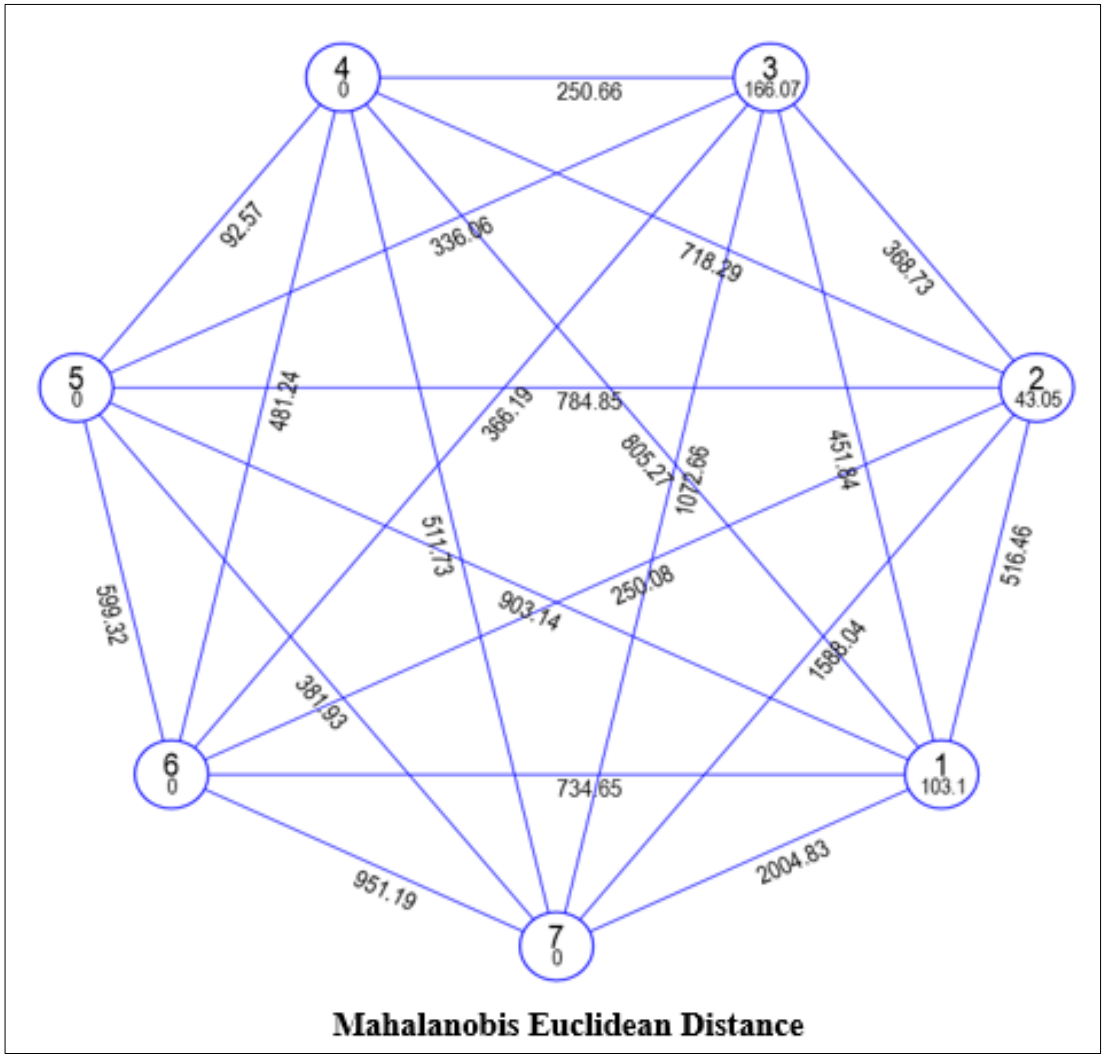

Fig 2: Intra and inter-cluster distance of 33 rice genotypes in seven clusters

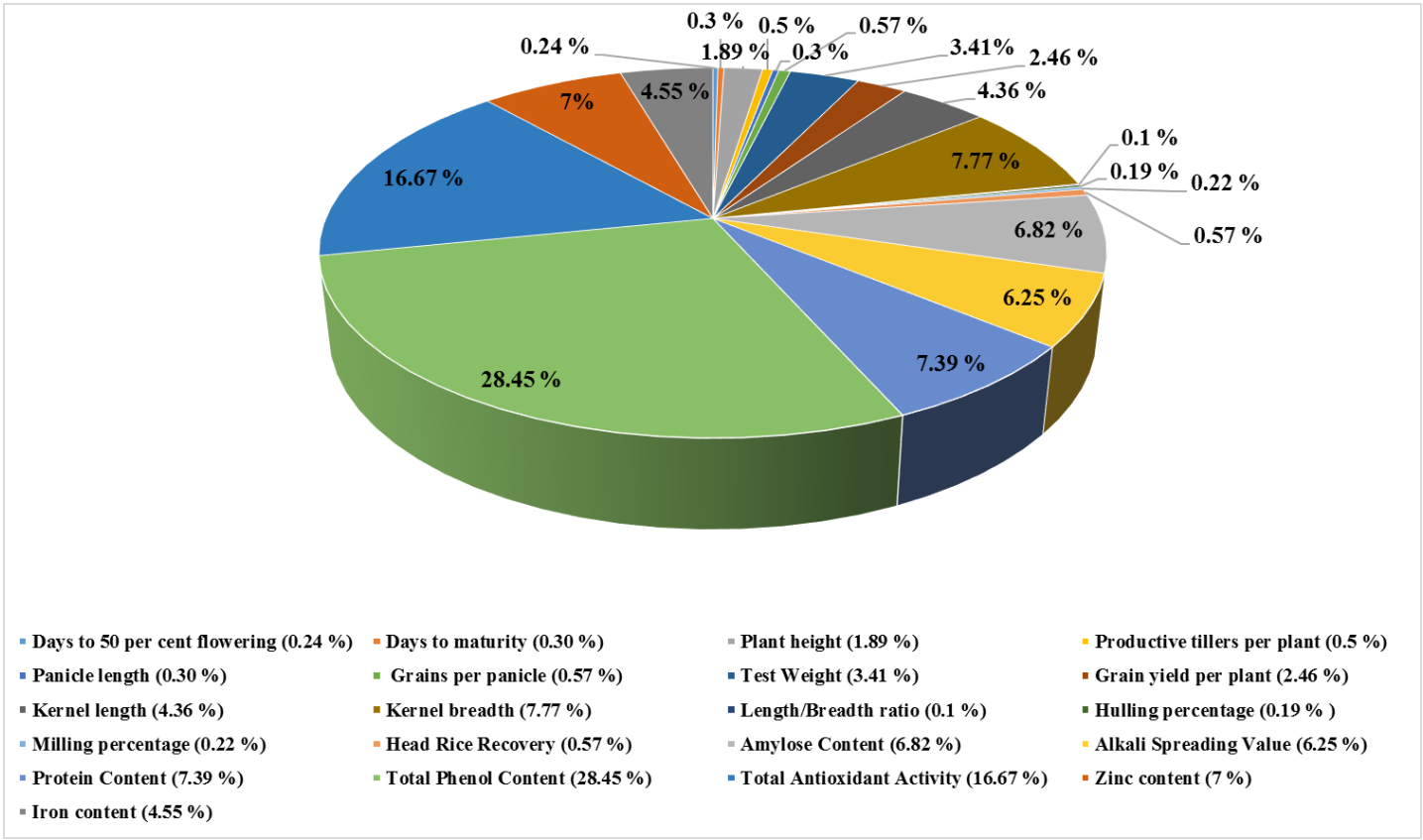

Fig 3: Contribution of yield, yield components and quality characters toward divergence

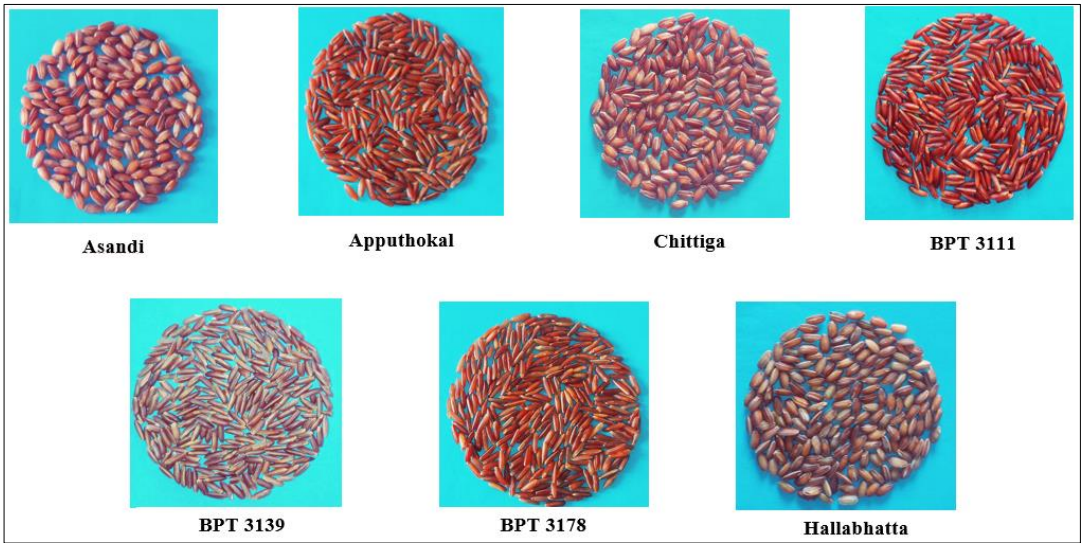

Plate 1: Red pericarp rice genotypes studied in the present investigation

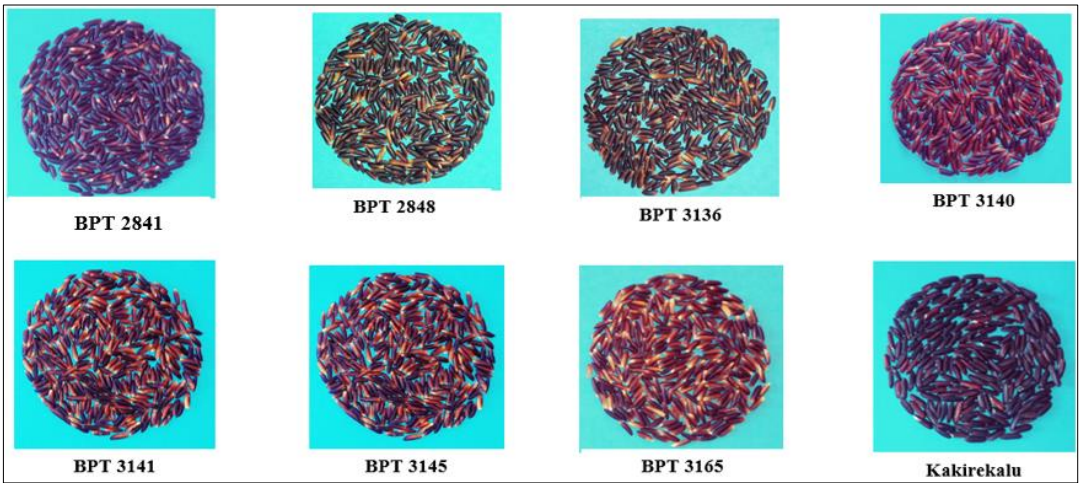

Plate 2: Black pericarp rice genotypes studied in the present investigation

Conclusions

The results suggest hybridization between the black pericarp genotype, Kakirekalu of Cluster VII with the genotypes of Cluster I for realization of transgressive coloured segregants towards development of high yielding coloured genotypes with good nutritional quality. Further, total phenol content and antioxidant activity were identified as important traits contributing maximum for genetic divergence in coloured rice genotypes.

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