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Research Note

Genetic divergence studies for yield and yield components in landraces of rice (*Oryza sativa* L.)

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Abstract
The present investigation was carried out with 36 rice landraces along with four checks to estimate genetic divergence for yield and yield component. Principal component analysis showed that first four principal components *viz.,* PC_1, PC_2, PC_3 and PC_4 with eigen value more than one and contributed 86.74% of cumulative variance. Hierarchical cluster analysis by ward's minimum variance method grouped 40 genotypes including checks into six clusters. Of all the six clusters, cluster III was the largest comprising 14 genotypes followed by cluster I with 11 genotypes, cluster II with seven genotypes, cluster IV with four genotypes, cluster V with three genotypes and cluster VI with one genotype *i.e.,* monogenotypic cluster. The 2D and 3D plots from principal component analysis and clustering pattern from hierarchical cluster analysis indicated that genotypes namely *Arakuloya*, *Narayanakami*, *Kujipataliya*, *Tandasagar* and *Chintaluri sannalu* were most divergence for most of the yield and yield components. Molecular diversity analysis using 15 SSR markers revealed that six markers *viz.,* RM154, RM404, RM402, RM85 and RM21 showed polymorphism. Among these, the markers namely RM404 and RM21 recorded higher PIC values. These markers can be considered as more informative and capable of discriminating the genotypes more effectively.

Keywords: Genetic divergence, PCA, Hierarchical cluster analysis, Molecular diversity.

Rice is a predominant staple food crop of Asia, being cultivated globally in over 100 countries. Selection of genetically diverse parents for hybridization requires evaluation of a large number of germplasm to produce heterotic hybrids. Rice is grown in more than 154 million hectares throughout the world in a wide range of ecosystems under varying temperatures and water regimes (Sellammal *et al.,* 2013). The success of any plant breeding programme largely depends on the existence of genetic diversity among the genotypes selected (Allard, 1960). Principal component analysis (PCA) is a multivariate technique that produces canonical vectors or roots representing different axes of differentiation and the percentage of variation accounted by each of such axis, respectively, or derived (Rao, 1952). PCA can be used to find similarities between variables and categorize the genotypes (Kaufman and Rousseeuw, 2009). It reflects the importance of the highest contributor to the total variation at each axis of differentiation (Sharma, 1998).

Genetic characterization of crop plants has gained momentum with the advent of PCR based molecular markers. The SSR is a marker of choice for molecular characterization. It is co-dominant, distributed throughout the genome, highly reproducible, variable, reliable, easily scorable, abundant and multi-allelic in nature. Over the centuries, rice has faced a significant decline in genetic diversity particularly after the green revolution due to replacement of many native varieties by high yielding ones. Despite the continuous development of new rice varieties, molecular show the genetic diversity within these newly released varieties is limited suggesting a

narrow genetic base. With this background, the present study was under taken to assess the genetic diversity of landraces using SSR markers and the results will improve the selection of diverse parents and to select appropriate parental genotypes for future breeding programmes.

The experimental material comprised of 36 rice landraces and four checks grown in alpha lattice design with two replications at Agricultural College Farm, Bapatla during *kharif* 2023 season. Each genotype was planted in three rows with row length of 3.0 m adapting a spacing of 20 x 15 cm. The details of landraces are presented in **Table 1**. Principal component analysis was carried using INDOSTAT software. Agglomerative hierarchical clustering technique was done as per Anderberg (1993).

The molecular characterization among the rice genotypes was studied by using Simple Sequence Repeats (SSR) markers. Leaf samples were collected randomly from 30 days old seedlings from each genotype. DNA extraction was done using modified CTAB (Cetyl Tri Methyl Ammonium Bromide) method (Doyle and Doyle, 1990). The purity and concentration of DNA was estimated by Nano drop (Jenway Genova Nano). PCR reaction was carried out using 10 µl PCR reaction mixture consisting of 2 µl of 100 ng template DNA and 8µl of master mix. Further, preparation

Table 1. List of landraces used in the present study

S.No.	Landrace	S.No.	Landrace	S.No.	Landrace
1.	*Doodeswar*	13.	*Doddiga*	25.	*Tarang*
2.	*Haladichudi*	14.	*Jeeragasamba*	26.	*Illapusamba*
3.	*Dalamalipuli*	15.	*Gandasaali*	27.	*Kauligauti*
4.	*Kukudamunde*	16.	*Narayanakami*	28.	*Kundadam-4*
5.	NMS -2	17.	*Ranikanda*	29.	*Ramsri* **(Bold grain)**
6.	*Bhajana*	18.	*Sunsugandha*	30.	*Jeeramani*
7.	Ramlocal	19.	*Kujipataliya*	31.	*Kishtampetasa*
8.	*Kaligejaarali*	20.	*Chandragand*	32.	*Kalalangad*
9.	*Kaalajeera*	21.	*Ambemohar2*	33.	*Sonagatara*
10.	*Arakuloya*	22.	*Nikko*	34.	*Chintaluri sannalu*
11.	*Ghani*	23.	*Kavsandi 2*	35.	DRK 2 **(Long slender grain)**
12.	*Ratnachodi*	24.	*Eesakarvaad*	36.	*Tandasagar*
Checks					
1.	BPT 5204	3.	NLR 34242 (Blast Susceptible check)		
2.	TN 1	4.	NLR 145 (Blast Resistant check)		

Table 2. Details of SSR markers used in the present study for molecular diversity analysis

S.No.	Marker	Forward Sequence	Reverse Sequence	Reference
1	RM85	CCAAAGATGAAACCTGGATTG	GCACAAGGTGAGCAGTCC	Sharma *et al*. (2021)
2	RM168	TGCTGCTTGCCTGCTTCCTTT	GAAACGAATCAATCCACGGC	Donde *et al*. (2020)
3	RM6	GTCCCCTCCACCCAATTC	TCGTCTACTGTTGGCTGCAC	Panaud *et al*. (1996)
4	RM204	GTGACTGACTTGGTCATAGGG	GCTAGCCATGCTCTCGTACC	Donde *et al*. (2020)
5	RM263	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG	Donde *et al*. (2020)
6	RM31	GATCACGATCCACTGGAGCT	AAGTCCATTACTCTCCTCCC	Raza *et al*. (2020)
7	RM225	TGCCCATATGGTCTGGATG	GAAAGTGGATCAGGAAGGC	Lakshmi *et al*. (2021)
8	RM21	ACAGTATTCCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG	Zulfiqar *et al*. (2023)
9	RM154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCCTGCGACCGCTCC	Donde *et al*. (2020)
10	RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG	Borale and Kumbhar (2021)
11	RM404	CCAATCATTAACCCCTGAGC	GCCTTCATGCTTCAGAAGAC	Donde *et al*. (2020)
12	RM402	GAGCCATGGAAAGATGCATG	TCAGCTGGCCTATGACAATG	Zulfiqar *et al*. (2023)
13	RM437	ACACCAACCAGATCAGGGAG	TGCTCGTCAATGGTGAGTTC	Zulfiqar *et al*. (2023)
14	RM5711	GTCCATGCATCCATCTCTAG	ACGGAAGGAATACGTCTGTA	Donde *et al*. (2020)
15	RM13331	CACCAGCTTCATGCATGC	AGCACTCAACTGATGCAGTG	IRGSP and Sasaki (2005)

of gels, loading and running of gels for genotyping of SSR markers using Agarose gel electrophoresis was done to know the marker polymorphism. The details of markers used for the molecular diversity are described in **Table 2**. Cluster analysis and dendrogram construction was done by UPGMA (Unweighted Pair Group Method with Arithmetic Averages) clustering algorithm using DARwin software ver. 6.0.12.

In the current study, principal component analysis (PCA) was carried out to study the genetic divergence of rice landraces. PCA is used to transform large data set into smaller principal components (PCs) without any loss of details, by considering the interdependence among the characters (Christina *et al*., 2021). Eigen values, variance (%) and cumulative variance (%) of the PCs of rice landraces are given in **Table 3 and Fig 1.** The results indicated that the eigen value of first four PCs were greater than or equal to one in discriminating the germplasm collection.

The results revealed that four canonical roots accounted for 86.74 per cent of total divergence. PC_1 contributed maximum towards total divergence (35.77%) followed by the second (21.44%), third (18.92%) and fourth (10.61%) canonical vectors respectively (**Table 3**). The study indicated that the number of tillers per plant, and ear bearing tillers per plant were maximum yield contributing traits in PC_1. The maximum contributing traits are days to 50 per cent flowering and test weight in PC2; days to 50 per cent flowering, number of filled grains per panicle and L/B ratio in PC_3; plant height, panicle length and test weight in PC_4 respectively.

Table 3. Canonical vectors for nine characters in 40 rice genotypes

S.No.	Parameter	PC_1	PC_2	PC_3	PC_4
1.	Eigen Value (Root)	3.2197	1.9293	1.7031	0.95
2.	% Var. Exp.	35.77	21.44	18.92	10.61
3.	Cum. Var. Exp.	35.77	57.21	76.13	86.74
S.No.	**Character**				
1.	Days to 50 per cent flowering	0.087	0.1457	0.6013	0.4620
2.	Plant height (cm)	-0.4594	-0.2667	-0.2323	0.1103
3.	Number of tillers per plant	0.4078	-0.3403	-0.1729	-0.1672
4.	Ear bearing tillers per plant (Productive tillers)	0.4782	-0.1458	-0.2198	-0.2816
5.	Panicle length (cm)	-0.3815	-0.3439	-0.2976	0.2695
6.	Test weight (g)	0.2897	0.149	-0.4656	0.3812
7.	Number of filled grains per panicle	0.2518	-0.3928	0.3655	0.0176
8.	L/B ratio	-0.2457	-0.4058	0.2552	-0.4513
9.	Grain yield / plant (g)	0.1826	-0.5577	0.0472	0.4952

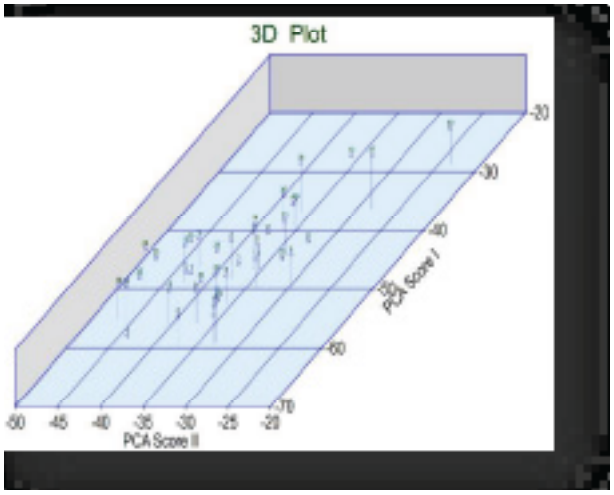

Fig.1. Three-dimensional (3D) graph showing relative positions based on PCA scores in 40 genotypes of rice (*Oryza sativa* L.)

The 2D and 3D plots indicated that the genotypes *Arakuloya, Ghani, Narayanakami, Kujipataliya, Eesakarvaad, Kundadam-4* and *Tandasagar* were divergent for most of the yield and component traits like number of tillers per plant, ear bearing tillers per plant and number of filled grains per panicle. These genotypes can be exploited for improvement of above traits (Pachauri *et al*., 2020 and Bekis *et al*., 2021).

*Hierarchical Cluster Analysis:*The 36 genotypes along with four checks were grouped into six clusters using Ward's minimum variance method (**Table 4**). Among all the clusters, cluster III was the largest comprising 14 genotypes followed by cluster I with 11 genotypes, cluster II with seven genotypes, cluster IV with four genotypes, cluster V with three genotypes and cluster VI with one genotype.

Table 4. Clustering pattern estimated by Ward's minimum variance method in 40 genotypes of rice (*Oryza sativa* L.)

Cluster. No	No. of genotypes	Name of genotype(s)
I	11	*Doodeswar, Ghani, Doddiga, Narayanakami, Ranikanda, Kausandi- 2, Tarang, Kundadam-4. Jeeramani, Kalalangad*, TN-1
II	7	*Haladichudi, Arakuloya,* NMS-2,*Jeeragasamba, Sonagatara, Chintaluri sannalu, Tandasagar*
III	14	*Dalamalipuli, Kukudamunde, Bhajana, Ramlocal, Kaligejarali, Kaalajeera, Ratnachodi, Ambe Mohar-2, Nikko, Eesakarvaad, Illapusamba, Kauligauti, Ramsri, Kishtampetasa*
IV	4	*Gandasaali, Sunsugandha, Chandragand*, DRK-2
V	3	*Kujipataliya*, BPT 5204, NLR 145
VI	1	NLR 34242

Table 5. Average intra and inter-cluster Eucledian^2 values among the six clusters in 40 genotypes of rice (*Oryza sativa* L.)

Cluster No.	I	II	III	IV	V	VI
I	**15.911**	54.654	38.581	32.047	61.193	79.820
II		**17.105**	91.854	34.952	51.968	118.170
III			**19.130**	64.453	92.093	74.067
IV				**11.649**	68.407	109.617
V					**22.710**	83.169
VI						**0**

The mutual relationship between clusters is represented diagrammatically by taking average intra- and inter-cluster squared euclidean distances (**Table 5**). Intra cluster distance values were recorded for cluster I (15.911), cluster II (17.105), cluster III (19.130), cluster IV (11.649), V (22.710) and VI (0) respectively. The maximum intra cluster distance was observed in cluster V which contains maximum yield contributing characters like number of filled grains per panicle followed by cluster III. The inter cluster squared euclidean distances varied from 32.047 (between cluster I and IV) to 118.170 (between cluster II and VI). The inter cluster distance was maximum between cluster II and VI (118.170) followed by cluster IV and VI (109.617). The genotypes selected from these clusters for hybridization can result in good heterotic hybrids (Talekar *et al*., 2022).

Cluster means were computed for all nine characters on a pooled basis and are presented in **Table 6**. The cluster II recorded highest mean values for number of filled grains per panicle, cluster IV s for plant height, number of tillers per plant, panicle length and L/B ratio, cluster V for days to 50 per cent flowering and grain yield per plant, cluster VI for ear bearing tillers per plant and test weight. The genotypes from cluster II (*Haladichudi*, NMS-2, *Jeeragasamba, Sonagatara, Chintaluri sannalu,*

Table 6. Cluster means of six clusters estimated by Ward's minimum variance method in 40 genotypes of rice (*Oryza sativa* L.)

Character	Cluster number					
	I	II	III	IV	V	VI
Days to 50 per cent flowering	86.45	88.93	87.36	86.25	102.00	90.00
Plant height (cm)	144.31	139.27	153.21	160.01	95.40	81.00
Number of tillers per plant	18.23	16.36	14.82	20.63	15.83	20.50
Ear bearing tillers per plant	16.59	13.14	12.46	17.25	13.33	17.50
Panicle length (cm)	21.88	23.05	22.23	23.96	20.74	20.20
Test weight (g)	20.11	17.68	20.55	16.24	17.95	23.30
Number of filled grains per panicle	118.73	172.87	82.74	145.95	150.13	70.50
L/B ratio	3.34	3.77	3.31	4.41	3.45	3.14
Grain yield / plant (g)	30.62	31.65	21.45	27.04	40.73	28.24

Tandasagar), cluster IV (*Gandasaali*, *Sunsugandha, Chandragand*, DRK-2) and cluster V (*Kujipataliya*, BPT 5204, NLR 145) can be used in future crop improvement programmes as these clusters are having maximum yield attributing traits pertaining to their *per se* performance

Molecular diverstiy studies using ssr markers: A total of 15 SSR markers were used to screen the diversity among the 40 genotypes (36 rice landraces and four checks). These 15 markers were spread on chromosomes 2,4,6,7 and 11 and six markers only exhibited polymorphism. The amplification pattern of six polymorphic markers is presented in **Table 7** where the number of alleles, number of effective alleles, major allelic frequency, Shannon's information index, polymorphic information content (PIC) and genetic diversity index are presented. The highest PIC value was recorded for the marker RM404 (0.71) among the six polymorphic markers.

Cluster analysis based on UPGMA: The genetic similarity scores for the six polymorphic markers between the rice genotypes included in the study were used to build the dendrogram (**Fig.3**). The similarity matrix was computed using the UPGMA method based on Jaccard's coefficient. The 40 genotypes were grouped into two major groups *viz*., cluster I and II. The subcluster IIA was the largest with

Table 7. Number of alleles and PIC value of the six polymorphic SSR markers used in the present study

S.No.	SSR Marker	Chr.No.	Na	Ne	MAF	I	PIC	Nei	Amplicon size range (bp)
1.	RM154	2	3	2.07	0.62	0.860	0.58	0.51	180-220
2.	RM404	4	4	3.80	0.31	1.359	0.71	0.73	50-270
3.	RM204	6	3	2.59	0.52	1.025	0.59	0.60	100-200
4.	RM402	6	4	1.68	0.75	0.803	0.46	0.40	100-200
5.	RM85	7	3	2.44	0.51	0.973	0.61	0.59	100-200
6.	RM21	11	3	2.41	0.56	0.985	0.62	0.61	100-180
Minimum			3	1.68	0.31	0.803	0.46	0.40	50
Maximum			4	3.80	0.75	1.359	0.71	0.73	270
Total			20	14.99	3.27	6.01	3.57	3.42	
Mean			3.33	2.50	0.54	1.001	0.59	0.57	

Na = Number of alleles; **Ne** = Number of effective alleles; **MAF** = Major allelic frequency; **I** = Shannon's information index; **PIC** = Polymorphic information content; **Nei** = Genetic diversity index; **bp**=Base pairs.

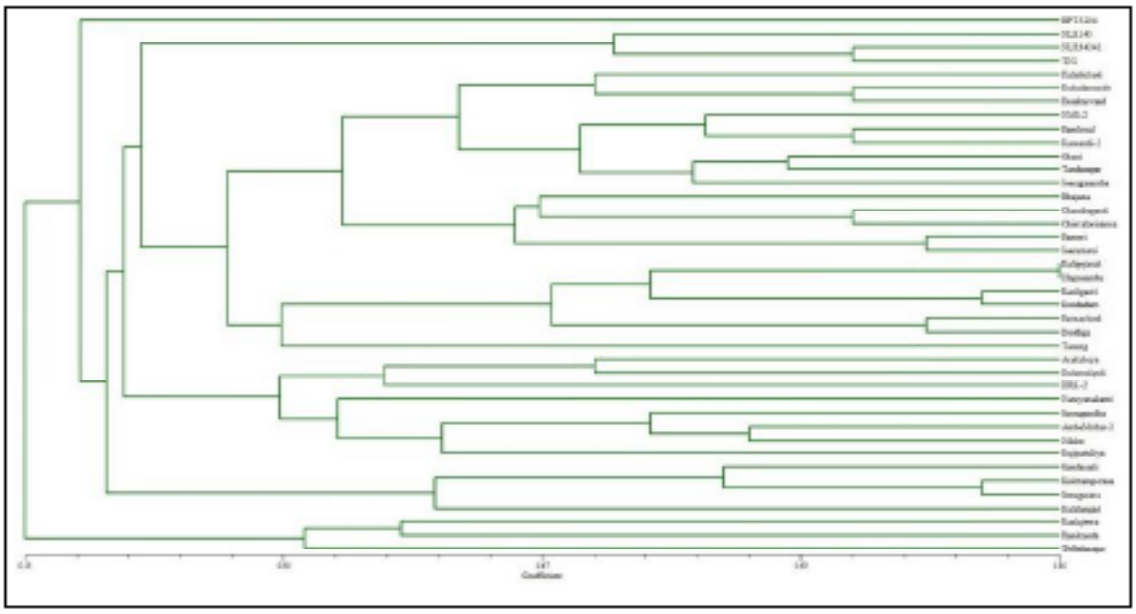

Fig.3. UPGMA cluster dendrogram showing the genetic relationships among 40 genotypes based on the alleles detected by six SSR markers in rice (*Oryza sativa* L.)

Table 8. Grouping into different clusters based on Jaccard's similarity coefficient using UPGMA method in 40 rice genotypes

S.No.	Cluster	Sub cluster	Number of genotypes	Name of the genotypes
1.	I	IA	1	*Doodeswar*
		IB	2	*Kaalajeera, Ranikanda*
2.	II	IIA	36	*Haladichudi, Kukudamunde, Eesakarvaad*, NMS-2, *Ramlocal, Kausandi-2, Bhajana, Ghani, Tandasagar, Jeeragasamba, Arakuloya*, DRK-2, *Kaligejarali, Illapusmba, Kauligauti, Kundadam-4, Ratnachodi, Doddiga*, *Tarang, Ganadasaali, Kishtampetasa, Sonagatara, Chandragand, Chintaliuri sannalu, Ramsri, Jeeramani, Kalalangad, Dalamalipuli, Sunsugandha, Ambe Mohar-2, Nikko, Kujipataliya, Narayanakami*, TN 1, NLR 34242, NLR 145
		IIB	1	BPT 5204

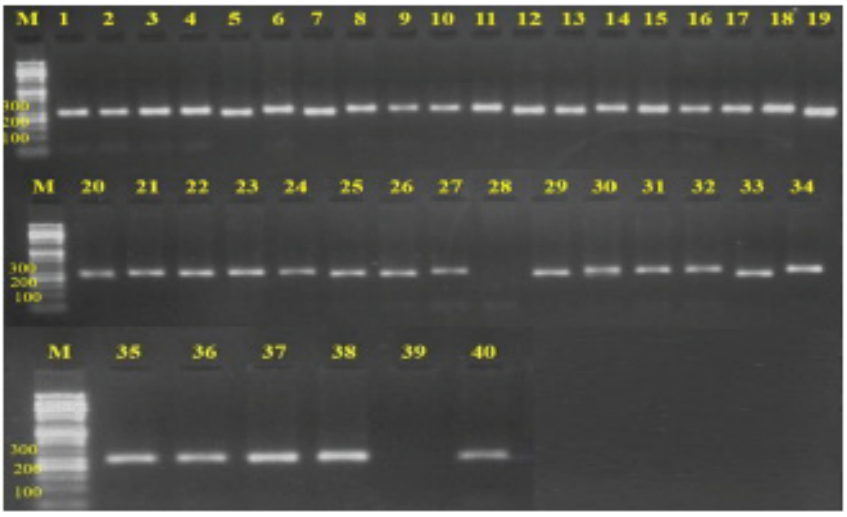

Plate 1. Amplification profile of the DNA using the primer RM404 M: 50bp ladder

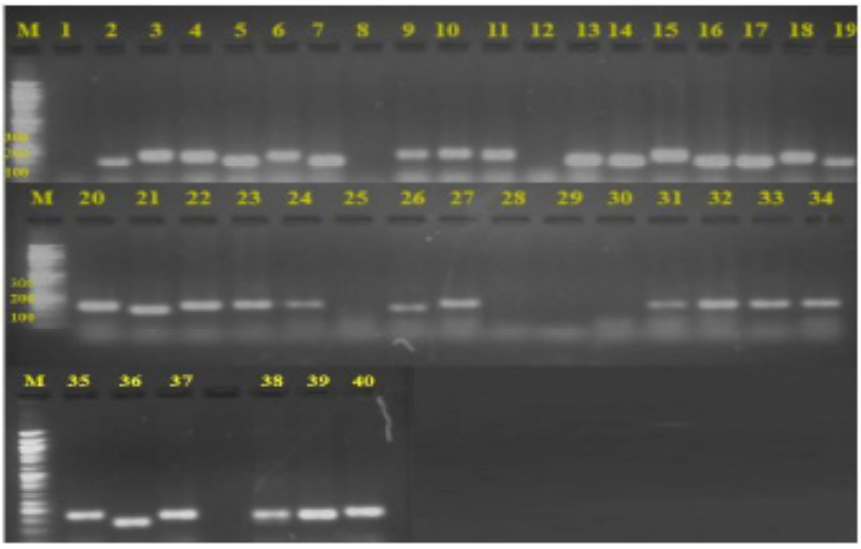

Plate 2. Amplification profile of the DNA using the primer RM21 M: 50bp ladder

36 genotypes, followed by IB with two genotypes while IA and IIB were solitary clusters (**Table 8**).

Among these six polymorphic markers, RM404 and RM21 recorded higher PIC values along with higher values of Nei's genetic diversity index and Shannon's information index. The range of similarity values exhibited by them is evidence for the assessment of genetic diversity and relationship between them for future breeding programmes (**Table 7**). Amplification profile of the DNA using the primers RM404 and RM21 were presented in plates 1 and 2 respectively.

Genetic divergence studies using principal component analysis, observed the first four principal components *viz.,* PC_1, PC_2, PC_3 and PC_4 with eigen values more than one and 86.74% of cumulative variance. Hierarchical cluster analysis by Ward's minimum variance method grouped the 40 genotypes into six clusters. The cluster III was the largest comprising 14 genotypes followed by cluster I with 11 genotypes, cluster II with seven genotypes, cluster IV with four genotypes, cluster V with three genotypes and cluster VI with single genotype. The random distribution of genotypes indicated the absence of parallelism between geographical and genetic diversity. The inter cluster distance between cluster II and VI (118.170) was maximum followed by cluster IV and VI (109.617), therefore hybridization between the genotypes of these two clusters can result in good heterotic hybrids. The 2D and 3D plots from principal component analysis and clustering pattern from hierarchical cluster analysis indicated that genotypes *Arakuloya, Narayanakami, Kujipataliya, Tandasagar* and *Chintaluri sannalu* were showing genetic diversity along with mean *per se* performance of yield traits. Hence, these genotypes can be suggested for future crop improvement programmes for obtaining higher yield.

Molecular diversity studies using 15 SSR markers revealed that six markers exhibited polymorphism *viz.,* RM154, RM404, RM204, RM402, RM85 and RM21. But among the six polymorphic markers, only RM404 and RM21 recorded higher PIC values along with higher values of Nei's genetic diversity index and Shannon's information index. These markers can be considered more informative and are capable of discriminating the genotypes more effectively. Additionally, these markers can serve as valuable tools for diversity studies, as they reveal variations in banding patterns and DNA fragment sizes across different genotypes, thereby confirming polymorphism. Therefore, the information generated from this study will enable better selection of diverse parents in future breeding programmes.

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