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Assessment of Genetic Variability, Correlation Coefficient, Path Analysis and Diversity Using D^2 Statistics and Molecular Markers in Rice (*Oryza sativa* L.) Genotypes

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Abstract: ***The present experiment was executed to gather information on genetic variability, phenotypic and genotypic correlation coefficients, path analysis and genetic divergence using D2 statistics and molecular markers among fifty genotypes of rice (Oryza sativa L.) for fourteen quantitative and qualitative characters. The analysis of variance revealed significant differences among genotypes for all the characters under study. Number of grains per panicle showed the shighest estimates of GCV and PCV. High heritability coupled with high genetic advance as percent of mean was observed for numbers of grains per panicle, seed index, head rice recovery %, panicle weight, grain yield per plant, harvest index and plant height. Correlation coefficient analysis showed positive and significant correlation of grain yield with seed index, harvest index, hulling % and milling %. Path analysis revealed that productive tillers per plant exerted maximum direct effect on grain yield per plant. Based on D2 statistics and SSR marker analysis fifty genotypes were grouped in four clusters. SSR marker RM7075 (0.66) reported for having the highest PIC value.***

Keywords: Genetic variability, Heritability, Genetic advance as percent of mean, Correlation coefficient, Path analysis, D2 statistics, Molecular markers, Grain yield per plant, Rice

1. Introduction

Rice (*Oryza sativa* L.) is a staple food of more than 60 % of the world's population. It belongs to the family *Poaceae*, genus *Oryza* and subfamily *Bambusoidea*e and has 22 wild and 2 cultivated species. The numbers of tetraploid (4n=48) species are nine among wild species, while the group of diploid (2n = 24) species included remaining wild species and two cultivated species. The rice genome is made up of 12 chromosomes (2n=24) and its overall length is 430Mb corresponding to about 1500 cM. [20]. It is grown across all states and in a wider range of environments, including lowlands, saline coastal regions and high mountains [41].

The primary centres of origin of *Oryza sativa* are believed to be river valleys of the Yangtze, and Mekon Rivers while, for *Oryza glaberrima*, it is Delta of Niger River in Africa [37]. The Asian AA genome diploid species *Oryza rufipogon* is the progenitor of *Oryza sativa* L. [14], while The African AA genome diploid species *Oryza barthii* is the progenitor of the cultivar *Oryza glaberrima* [43]. Rice (*Oryza sativa* L.) is planted on 164.19 million hectares worldwide, with a production of around 509.87 million tonnes [31]. China is the major producer of paddy rice followed by India, Bangladesh, Indonesia, Vietnam and Thailand.

Cooking is the most popular way of eating rice. It is mainly consumed with other nutrient-rich foods like legumes, seafood, nuts *etc*. Other by-products are rice bran, rice husk and rice straw. Along with a major source of complex carbohydrates, it is also an excellent food which can be included in a balanced diet as it has no cholesterol, no fat and is sodium-free [9]. Rice grain contains eight percent of protein with a high proportion of lysine as well as the B-Complex proteins *viz.*, thiamine, riboflavin and niacin [5]. Red and black rice are reported for their richness in iron (Fe) and zinc (Zn) content, which in turn, are essential for enzymatic processes and haemoglobin production, respectively [2].

Variability has a huge role in sucessful plant breeding program. It is desirable to understand the nature and magnitude of genetic variability present in a particular material and its use. In total variability, only heritable genetic components are important. So, it is necessary to partition the phenotypic variations into its genetic and environmental components. Estimation of the Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV), helps in selecting diverse parents for improvement in rice.

Yield being a complex character is affected by various interconnected traits. A study of the correlation between different quantitative features can reveal patterns of association that can be used to design and assess the relative size of correlation of various features with yield in any effective selection method.

Divergent parents are equally important for a successful programme. The D^2 analysis is enables measurement of the

degree of diversification and does determine the relative portion of each component trait to total divergence. D^2 enables the selection of genotypes as suitable parental lines for heterosis breeding (Mahalanobis, 1936) [21]. For precise genetic manipulation of complex quantitative traits understanding the genetic and molecular basis of target traits needs to be investigated thoroughly. The assessment of genetic diversity by DNA molecular markers is highly precise and least affected by the environment.

2. Materials and Methods

The present study was carried out at Main Rice Research Station, Nawagam comprising of fifty rice genotypes. The field trial was evaluated in Randomized Block Design (RBD) along with three replications in 0.6 m x 1.5 m sized gross plot. The inter and intra row distance was maintained 20 x 15 cm^2 respectively. To raise a good crop package of practices and plant protection measures were adopted as per recommendation during experimentation.

The total of 15 quantitative and qualitative characters *viz.*, days to 50 % flowering, plant height (cm), productive tillers per plant, panicle length (cm), panicle weight (g), numbers of grains per panicle, seed index (g), grain yield per plant (g), harvest index (%), hulling (%), milling (%), grain L: B ratio, head rice recovery (%) and amylose content (%) were considered for analysis of variability traits, genotypic and phenotypic correlation coefficient and path analysis. Five randomly selected plants of each genotype in every replication were considered for observations of various characters except one phenological character *viz.*, days to 50 percent flowering. This character was recorded on plot basis.

2.1 Genetic variability

Analysis of variance (ANOVA) was carried out based on the model proposed by by Panse and Sukhatme (1978) [28]. Genotypic, phenotypic and environmental variance were calculated as per the formulae suggested by Johnson *et al.* (1955) [15]. The formula given by Hanson and Weber (1956) [12] was used to estimate heritability in broad sense.

2.2 Correlation coefficient

The estimates of covariance worked out as per Singh and Chaudhary (1985) [36].

2.3 Path analysis

The approach proposed by Dewey and Lu (1959) [6] was used to calculate the path coefficient.

2.4 Genetic divergence using D2 statistics

After testing the difference in regard to individual characters through ANOVA, a simultaneous test of significance for difference of mean values in regard to the pooled effect of 16 characters was carried out using Wilk's criterion (Wilk, 1932; Rao, 1952) [44][30] Grouping of genotypes in different clusters was carried out using Tocher's method (Rao, 1952). [30].

2.5 Genetic diversity using molecular markers

Genomic DNA extraction from leaf samples was carried out at the Department of Agricultural Biotechnology Anand Agricultural University, AAU, Anand. Isolation of DNA was performed using modified Cetyl Trimethyl Ammonium Bromide (CTAB) protocol (Doyle and Doyle, 1987) [7].

A total of 32 Simple Sequence Repeats (SSR) primer pairs were used to screen the extracted DNA samples, the list is provided in Table 1. All primers were rice specific. and were procured from the Department of Agricultural Biotechnology, Anand Agricultural University, Anand.

Table 1: List of primers found polymorphic for the experimental material

Sr. No.	SSR loci	F/R	Sequence (5'-3')	T_m
1	RM3252 F	F	GGTAACTTTGTTCCCATGCC	64.8
	RM3252 R	R	GGTCAATCATGCATGCAAGC	
2	RM8068 F	F	AAACCTCTCGCTGTAATTAG	55.3
	RM8068_R	R	TGAACATTTATTGATATGGTAAA	
3	RM493_F	F	TAGCTCCAACAGGATCGACC	64.2
	RM493_R	R	GTACGTAAACGCGGAAGGTG	
4	RM7075_F	F	TATGGACTGGAGCAAACCTC	63.1
	RM7075_R	R	GGCACAGCACCAATGTCTC	
5	RM8004 F	F	TTGACCAAAGGTGATTGTAAT	58.1
	RM8004 R	R	CTTGATGAGTTTCATGAGCA	
6	RM129_F	F	TCTCTCCGGAGCCAAGGCGAGG	76.5
	RM129 R	R	CGAGCCACGACGCGATGTACCC	
7	RM3341 F	F	AGGACAGTCCACTCCCACTG	65
	RM3341 R	R	TCGTCGCCATCATTGGTATC	
8	RM1196 F	F	AGCTGCCGTGAGCCTCAAG	66.3
	RM1196 R	R	TCCAAAACGCTCTCTTCGTC	
9	RM5 F	F	TGCAACTTCTAGCTGCTCGA	64.1
	RM5 R	R	GCATCCGATCTTGATGGG	
10	RM246 F	F	GAGCTCCATCAGCCATTCAG	65.9
	RM246 R	R	CTGAGTGCTGCTGCGACT	
11	RM128 F	F	AGCTTGGGTGATTTCTTGGAAGCG	74.2
	RM128 R	R	ACGACGAGGAGTCGCCGTGCAG	
12	RM3285 F	F	AGAGATGACAGCCGCGTC	64.1

	RM3285_R	R	GCTCCACACCTCTCGTTTTC	
13	RM212_F	F	CCACTTTCAGCTACTACCAG	58.7
	RM212_R	R	CACCCATTTGTCTCTCATTATG	
14	RM5794 F	F	AGCTAGCTGAGCTCGTCGTC	64.1
	RM5794 R	R	CAGACTCATGGACACATGGG	
15	HvSSR05-12_F	F	TCCTCTACAGTTGTCTGCCT	56.28
	HvSSR05-12_R	R	CATTCCTCTCCACTTTCTTG	
16	HvSSR06-13_F	F	CCCATCTGCACTACCATAAT	55.2
	HvSSR06-13_R	R	AGATGTGCTTTGCTACCAGT	

F: Forward, R: Reverse, Tm: Melting temperature (ºC)

The data generated from PCR amplification of rmSSR and hvSSR markers were further analysed. Alpha EASE software (Alpha Innotech, San Leandro, California., USA) was used to determine size (bp) of amplified bands while PHYLIP software (v 3.5) (Felsenstein 1993)) [8]. used to calculate Major allele frequency, Observed heterozygosity (Ho), expected heterozygosity (He), Effective number of allele (Ae), Polymorphism information content (PIC), Nei's gene diversity (D) and Genetic distance (d). Pairwise dissimilarity (genetic distance) matrix was used for constructing a dendrogram using modified Neighbor-Joining method, proposed by Nei (1978) [26] as implemented in PHYLIP v 3.5.

3. Results and Discussion

3.1 Genetic variability parameters

The results from analysis of variance for 14 characters are presented below in Table 2. The results indicated that mean sum of square was highly significant for all characters. The highly significant sum of square indicated the presence of high variability among traits.

Estimation of component of variability for various traits was carried out and data are represented in Table 3. A wide range of mean values was demonstrated by different characters. The lower to higher values of GCV and PCV was recorded for different traits. A high estimate of GCV and PCV was recorded for numbers of grains per panicle (51.79 and 51.90), plant height (36.78 and 37.12), productive tillers per plant (33.05 and 34.07), panicle length (27.96 and 29.51) and panicle weight (22.63 and 28.56) indicating presence of high variability among genotypes for these characters. the characters numbers of grains per panicle, (17.58 and 19.23), seed index (16.39 and 18.34), grain yield per plant (12.02 and 13.53), harvest index (11.66 and 14.12) revealed moderate estimates of GCV and PCV. However, the remaining characters hulling %, milling %, grain L: B ratio, head rice recovery and amylose content indicated low estimates of GCV and PCV. The similar results were recorded by Srujana *et al.* (2017) [40], Singh *et al.* (2020) [33], Ayyenar *et al.* (2021) [4], Aravind *et al.* (2022) [3], Manivelan *et al.* (2022) [22].

The characters which showed high heritability coupled with high genetic advance as percent of mean was observed for seed index, head rice recovery, panicle weight, grain yield per plant, harvest index, milling and plant height. The findings are in accordance with Srujana *et al.* (2017) [40], Girma *et al.* (2018) [10], Kalpana *et al.* (2018) [16], Kumar *et al.* (2020) [13], Manju *et al.* (2021) [17] and Aravind *et al.* (2022) [3].

3.2 Correlation coefficient

The analysis of genotypic and phenotypic correlation coefficient is described in the table 4. Correlation coefficient analysis had revealed that the grain yield per plant showed significant and positive relation with seed index (r_g = 0.371 and r_p = 0.295), harvest index (r_g = 0.380 and r_p = 0.462), hulling % (r_g = 0.421 and r_p = 0.301), milling % (r_g = 0.400 and r_p = 0.304), however the negative significant correlation was recorded for grain L: B ratio (r_g = -0.535 and r_p = -0.327). The increase or decrease in these characters can directly influence the grain yield per plant. The significant and positive correlation was also recorded between panicle weight and numbers of grains per panicle (r_g = 0.345 and r_p = 0.329) as well as plant height and (r_g = 0. 389 and r_p = 0.267). The correlation between numbers of grains per panicle and seed index was found negative and significant (r_g = -0. 531 and r_p = -0.523). Similar results were recorded by Kumar *et al.* (2018a) [17], Shobhana *et al.* (2018) [32], Singh *et al.* (2018) [35] and Manivelan *et al.* (2022) [22].

3.3 Path analysis

In the experiment under study, all the 14 quantitative and qualitative characters were considered as causal variables of grain yield per plant. The direct and indirect contribution of each component character towards grain yield per plant in rice is presented in table 5.

High positive direct effect on the grain yield per plant was accelerated by productive tillers per plant (0.390). However, grain L: B Ratio (-0.682) indicated high but negative direct effect. The association of these traits with grain yield per plant indicated the significant relation of respected traits for grain yield improvement in the present material. The moderate positive direct effect was recorded for days to 50% flowering (0.255), Plant Height (0.175), Panicle Length (0.248), Harvest Index (0.211). The remaining characters showed low to negligible direct effect on grain yield per plant. Similar findings were recorded by Sowmiya and Venkatesan (2017) [38], Kumar *et al.* (2018a) [17], Shobhana *et al.* (2018) [32], Singh *et al.* (2018) [35] and Manivelan *et al.* (2022) [22].

Table 2: Analysis of variance for different characters

Sr. No.	Character / DF	Mean sum of square		
		Replication	genotypes	Error
		2	49	98
1	Days to 50 % flowering	9.147	162.005**	5.766
2	Plant height (cm)	17.250	603.270**	49.470
3	Productive tillers per plant	0.895	1.548**	0.444
4	Panicle length (cm)	0.899	18.274**	3.802
5	Panicle weight (g)	0.207	2.601**	0.095
6	Numbers of grains per panicle	107.000	25883.800**	36.300
7	Seed index (g)	0.032	1.72734**	0.010
8	Grain yield per plant (g)	0.878	40.192**	6.628
9	Harvest index (%)	1.149	93.057**	5.723
10	Hulling (%)	40.240*	34.200**	1.252
11	Milling (%)	451.420*	354.730**	27.570
12	L: B ratio	0.313	0.756**	0.102
13	Head rice recovery (%)	23.730	376.900**	7.720
14	Amylose content (%)	1.113	5.882**	0.364
**- Significant at 1% and 5% level of significance, respectively				

Table 3: Estimates of different genetic parameters for rice genotypes

Sr. No.	Character	Range		σ^2_g	σ^2_p	σ^2_e	GCV (%)	PCV (%)	h^2_b (%)	GAM
		Min.	Max.							
1	Days to 50 % flowering	84	106.33	52.08	57.85	5.77	7.79	8.21	90.03	15.23
2	Plant height (cm)	83.72	148.22	184.6	234.07	15.09	12.02	13.53	78.86	21.98
3	Productive tillers per plant	6.13	8.97	0.37	0.81	0.44	8.47	12.59	45.3	11.74
4	Panicle length (cm)	19.68	31.51	4.82	8.63	3.8	8.76	11.72	55.93	13.5
5	Panicle weight (g)	1.03	6.39	0.84	0.93	0.09	27.96	29.51	89.81	54.59
6	Numbers of grains per panicle	56.9	571.3	8615.82	8652.13	36.31	51.79	51.9	99.58	106.46
7	Seed index (g)	0.82	4.85	0.57	0.58	0.01	36.78	37.12	98.2	75.08
8	Grain yield per plant (g)	7.37	22.54	11.92	17.82	6.63	22.63	28.56	62.8	36.95
9	Harvest index (%)	15.8	41.26	29.11	34.83	5.72	17.58	19.23	83.57	33.11
10	Hulling (%)	70.03	88.4	10.98	12.23	1.25	4.32	4.56	89.77	8.42
11	Milling (%)	22.16	73.93	109.05	136.62	27.57	16.39	18.34	79.82	30.16
12	Grain L: B ratio	3.11	5.1	0.22	0.32	0.1	11.66	14.12	68.12	19.82
13	Head rice recovery (%)	10.37	53.61	123.06	130.78	7.72	33.05	34.07	94.1	66.05
14	Amylose content (%)	22.91	29.43	1.84	2.2	0.36	5.42	5.93	83.49	10.2

Table 4: Genotypic and phenotypic correlation coefficients among different characters in rice

		DFF	PH	PTP	PL	PW	NGP	SI	HI	HULL	MILL	L/B	HRR	AC	GYP
DFF	r_g	1													
	r_p	1													
PH	r_g	-0.062	1												
	r_p	-0.055	1												
PTP	r_g	-0.025	-0.204	1											
	r_p	0.002	-0.114	1											
PL	r_g	-0.049	0.389**	-0.19	1										
	r_p	-0.075	0.267**	-0.057	1										
PW	r_g	0.218	-0.059	0.004	-0.062	1									
	r_p	0.199 *	-0.043	-0.018	-0.042	1									
NGP	r_g	0.183	0.062	0.255	-0.119	0.345*	1								
	r_p	0.173*	0.054	0.169*	-0.088	0.329**	1								
SI	r_g	-0.131	0.13	-0.164	0.229	-0.101	-0.531**	1							
	r_p	-0.119	0.119	-0.109	0.172*	-0.088	-0.523**	1							
HI	r_g	-0.133	-0.03	0.216	-0.315*	-0.065	0.196	-0.137	1						
	r_p	-0.116	-0.041	0.091	-0.165*	-0.027	0.180*	-0.12	1						
HULL	r_g	-0.171	0.222	-0.213	-0.077	-0.099	-0.195	0.385**	0.289 *	1					
	r_p	-0.148	0.200*	-0.163 *	-0.032	-0.088	-0.181*	0.360**	0.259**	1					
MILL	r_g	-0.13	0.360 *	-0.044	-0.136	0.121	-0.005	0.1406	0.306 *	0.524**	1				
	r_p	-0.073	0.305**	-0.019	-0.047	0.089	-0.005	0.125	0.270**	0.504**	1				
L/B	r_g	0.345 *	0.126	0.243	0.211	-0.047	0.088	-0.324 *	-0.277	-0.405**	-0.354*	1			
	r_p	0.293**	0.098	0.101	0.165*	-0.04	0.07	-0.268 **	-0.197*	-0.256**	-0.134	1			
HRR	r_g	0.276	0.179	0.099	-0.131	0.163	0.277	-0.128	0.172	-0.029	0.488**	0.211	1		
	r_p	0.249**	0.152	0.038	-0.09	0.136	0.266**	-0.126	0.145	-0.02	0.410**	0.189*	1		
AC	r_g	-0.064	-0.127	-0.006	0.095	0.05	-0.216	-0.044	0.093	-0.005	0.089	-0.164	-0.38**	1	
	r_p	-0.058	-0.049	-0.015	0.056	0.041	-0.194 *	-0.041	0.066	0.022	0.079	-0.107	-0.34**	1	

GYP	r_g	-0.105	0.126	0.098	0.036	-0.139	-0.183	0.371**	0.380**	0.421**	0.400**	-0.535**	0.017	0.124	1
	r_p	-0.087	0.059	-0.023	0.042	-0.051	-0.143	0.295**	0.462**	0.301**	0.304**	-0.327**	0.006	0.063	1

rg= genotypic correlation, rp= genotypic correlation DFF =Days to 50 % flowering, PH = Plant height (cm), PTP = Productive tillers per plant, PL = Panicle length (cm), NGP = Numbers of grains per panicle, SI = Seed Index (g), GYP = Grain yield per plant (g), HI = Harvest index (%), HULL = Hulling (%) MILL = Milling(%), L/B = Grain L: B ratio, HRR = Head rice recovery (%), AC = Amylose content (%).

Table 5: Direct and indirect effect of different characters on grain yield in rice

Cha.	DFF	PH	PTP	PL	PW	NGP	SI	HI	HULL	MILL	L/B	HRR	AC	Correlation with GYPP
DFF	**0.255**	-0.011	-0.01	-0.012	-0.024	-0.046	-0.002	-0.028	-0.021	-0.001	-0.235	0.03	-0.0004	-0.105
PH	-0.016	**0.175**	-0.079	0.097	0.007	-0.016	0.002	-0.006	0.028	0.001	-0.086	0.02	-0.001	0.126
PTP	-0.006	-0.036	**0.39**	-0.047	-0.0005	-0.064	-0.003	0.046	-0.026	-0.0002	-0.166	0.011	-0.00004	0.098
PL	-0.013	0.068	-0.074	**0.248**	0.007	0.03	0.004	-0.066	-0.01	-0.001	-0.144	-0.014	0.001	0.036
PW	0.056	-0.01	0.002	-0.015	**-0.109**	-0.086	-0.002	-0.014	-0.012	0.001	0.032	0.018	0.0003	-0.139
NGP	0.047	0.011	0.099	-0.03	-0.038	**-0.25**	-0.009	0.041	-0.024	0.00002	-0.06	0.031	-0.001	-0.183
SI	-0.034	0.023	-0.064	0.057	0.011	0.133	**0.018**	-0.029	0.048	0.001	0.221	-0.014	0.0002	0.371**
HI	-0.034	-0.005	0.084	-0.078	0.007	-0.049	-0.002	**0.211**	0.036	0.001	0.189	0.019	0.001	0.380**
HULL	-0.044	0.039	-0.083	-0.019	0.011	0.049	0.007	0.061	**0.124**	0.002	0.277	-0.003	-0.00003	0.421**
MILL	-0.033	0.063	-0.017	-0.034	-0.013	0.001	0.002	0.065	0.065	**0.004**	0.242	0.054	0.0005	0.400**
L/B	0.088	0.022	0.095	0.052	0.005	-0.022	-0.006	-0.058	-0.05	-0.001	**-0.682**	0.023	-0.001	-0.535**
HRR	0.07	0.031	0.039	-0.032	-0.018	-0.069	-0.002	0.036	-0.004	0.002	-0.144	**0.11**	-0.002	0.017
AC	-0.016	-0.022	-0.003	0.024	-0.006	0.054	-0.001	0.02	-0.001	0.0003	0.112	-0.042	**0.005**	0.124

*, ** significant at 5% and 1% level of significance respectively

DFF =Days to 50 % flowering, PH = Plant height (cm), PTP = Productive tillers per plant, PL = Panicle length (cm), NGP = Numbers of grains per panicle, SI = Seed Index (g), GYP = Grain yield per plant (g), HI = Harvest index (%), HULL = Hulling (%) MILL = Milling(%), L/B = Grain L: B ratio, HRR = Head rice recovery (%), AC = Amylose content (%) Cha.= Character

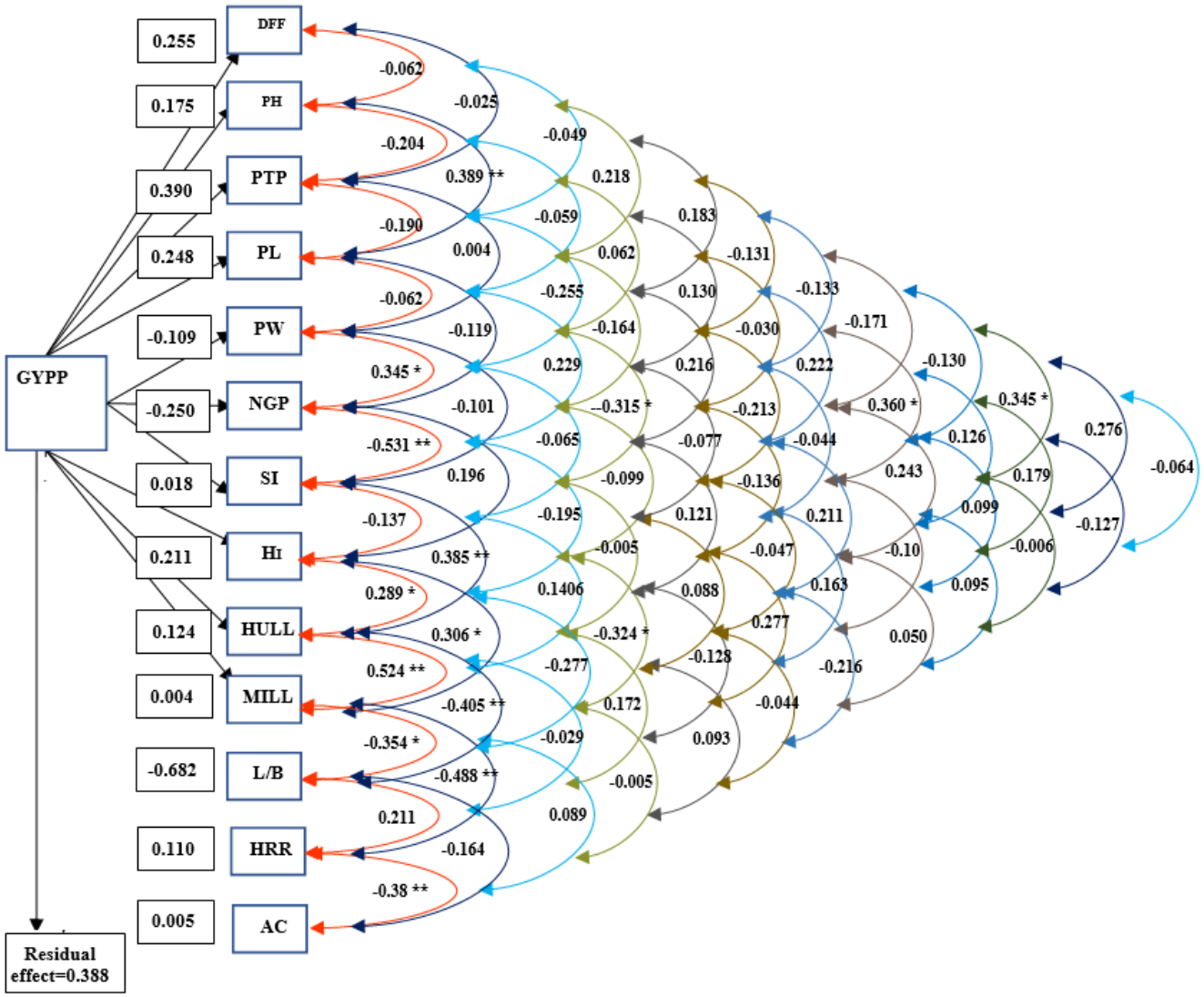


Figure 1: Path diagram showing direct effect on grain yield and genotypic correlation between yield contributing characte

3.4 Genetic divergence using D2 statistics

Analysis based on Mahalanobis D^2 statistics (1936) [21] grouped fifty genotypes in four clusters. The cluster I found to be largest cluster with 46 rice genotypes. Cluster II had two genotypes. While, the remaining clusters III and IV had only single genotype each. The results were in accordance with Netam *et al.* (2021) [27] and Mohamud *et al.* (2022) [24] in which five clusters were formed from 40 and 18 rice genotypes, respectively. The clusters formed in present study were lower than the results of previous study of Guru *et al.* (2017) [11] and Tejaswini *et al.* (2018) [42] as they recorded 13 and 10 clusters, respectively.

The average D^2 values of intra and inter clusters distances are presented in Table 9 [supporting material]. The highest intra cluster distance was recorded for cluster I (613.67) while, Cluster III and IV had values of zero as they had only single genotype. cluster III and cluster IV showed the maximum inter cluster distance was observed between (11670.56) followed by cluster I and cluster IV (6232.20), cluster II and cluster III (5458.81) indicating that genotypes in these clusters were highly diverse. The minimum values for inter cluster distance were recorded between clusters II and IV (1766.34), representing lower genetic diversity of the genotypes from respective clusters.

The genotypes of Custer IV recorded the highest cluster mean for traits plant height (124.38), productive tillers per plant (8.67), panicle length (27.02), grains per panicle (571.33), harvest index (38.83) and head rice recovery (39). The Cluster II had showed maximum cluster mean for days to 50 % flowering (96.33), panicle weight (3.28), L: B ratio (4.60) and amylose content (25.24). The mean values of Cluster III were also found to be highest in seed index (4.85), hulling % (88.40), milling % (64.28) and grain yield per plant (22.37). However mean values of genotype of Cluster I were recorded to be moderate.

Contribution of individual characters towards total genetic divergence is given in Table 10[supporting material]. The trait numbers of grains per panicle contributed maximum (60.80%) towards total genetic divergence followed by seed index (16.31%), head rice recovery (5.12%), hulling % (3.23%) and days to 50 % flowering (2.44%). These five characters collectively contributed 87.92% of genetic divergence, whereas other 10 characters contributed only 12.08% cumulatively. Lowest contribution was made by productive tillers per plant.

3.5 Genetic diversity using molecular markers

The overall allele frequency observed among the rice genotypes are presented in the Table 11[supporting material]. The allele frequency represented as allele A, B and C shows an average of 0.61, 0.25 and 0.13, respectively.

The result presented in Table 7 revealed that these 16 SSR primers generated 38 alleles with band size ranging from 102 bp (HvSSR05-12) to 250 bp (RM493).

The effective number of alleles varied from 1.13 (HvSSR06-13) to 2.97 (RM7075) with an average of 1.62. The average number of alleles per locus was 2.38 with a range of 2.00 (RM8068, RM493, RM129, RM3341, RM5, RM246, RM128, RM3285, RM212 and HvSSR06-13) to 3.00 (RM3252, RM7075, RM8004, RM1196, RM5794 and HvSSR05-12). The average number of alleles in current experiment was lower than Singh *et al.* (2016) [34], Kumar *et al.* (2018b) [19] and Hassan and Emad (2021) [13]. This may be due to use of different set of SSR markers on genotypes with more or less variability.

Major allelic frequency ranged from 0.38 (RM7075) to 0.94 (RM129) with a mean of 0.77. The highest level of gene diversity was observed for SSR marker RM7075 (0.66); while, it was minimum (0.11) for RM129 and HvSSR06-13, with mean gene diversity of 0.33 (Table 7). Naaz *et al.* (2022) [25] and Pradhan *et al.* (2023) [29] recorded average of gene diversity was 0.48 and 0.51, respectively, which was lower than the present study.

Observed heterozygosity varied widely from 0.00 (RM128) to 0.40 (RM7075) with an average of 0.13; while expected heterozygosity ranged from 0.11 (RM129) to 0.67 (HvSSR06-13) with average of 0.33. Rice is self-pollinated crop which prevent of gene flow between two populations which can also lead to prevent combination of the two gene pools, decreasing the genetic variation and increasing the homozygosity.

PIC values for SSR markers under study ranged from 0.14 (HvSSR06-13) to 0.66 (RM7075) with an average of 0.36. It found to be higher than the results of previous study of Singh *et al.* (2016) [34] and Aboulila *et al.* (2019) [1] as well as lower than Kumar *et al.* (2018b), Srivastava *et al.* and Pradhan *et al.* (2023) [29]. This difference in PIC value showed that in present population various allelic diversity was present which was reflected in the value of genetic diversity.

Fifty rice genotypes were grouped into four main clusters (Fig. 4.4). Maximum number of genotypes (46) were grouped in cluster A, indicating high genetic similarity among the grouped genotypes. Cluster B had only single genotype *viz.*, IET-29142. Cluster C too had two sub clusters C_1 (NWGR-15050) and C_2 (NWGR-16024). Cluster D also had only one genotype (GR-21). GR-21 was found to be the most diverse genotype.

Table 6: Cluster mean for 14 different characters

Cluster	Characters													
	DFF	**PH**	**PTP**	**PL**	**PW**	**NGP**	**SI**	**HI**	**HULL**	**MILL**	**L/B**	**HRR**	**AC**	**GYP**
I	92.13	111.98	7.10	24.96	3.27	164.14	2.05	30.31	76.09	63.28	3.99	33.56	24.91	14.58
II	**96.33**	115.91	7.00	**21.21**	**3.28**	348.50	**1.06**	**29.22**	**74.65**	63.50	**4.06**	31.29	**25.24**	13.64
III	**84.00**	**111.01**	**6.66**	26.28	**2.50**	**95.33**	**4.85**	31.28	**88.40**	**64.28**	**3.11**	**22.58**	**22.91**	**22.37**
IV	87.33	**124.38**	**8.67**	**27.02**	3.25	**571.33**	1.28	**38.83**	75.59	**61.27**	4.01	**39.00**	23.89	**13.42**
GM	92.04	112.37	7.13	24.87	3.26	178.28	2.05	30.45	76.27	63.27	3.98	33.36	24.86	14.68
S.Em	4.89	8.19	0.51	1.60	0.46	29.81	0.29	2.89	3.24	6.00	0.30	5.35	1.16	1.95
CD@ 5%	NS	NS	NS	4.44	NS	83.622	0.802	NS	8.968	NS	NS	NS	NS	5.41
CV%	11.60	15.90	15.61	14.05	30.51	36.47	30.80	20.73	9.25	20.67	16.27	35.00	10.16	29.01
R^2	0.02	-	0.19	0.27	-	0.92	0.84	0.16	0.31	-	0.47	0.03	-	0.34
CV_b %	1.58	-	7.59	8.54	-	121.78	70.73	8.91	6.15	-	9.19	6.44	-	21.01

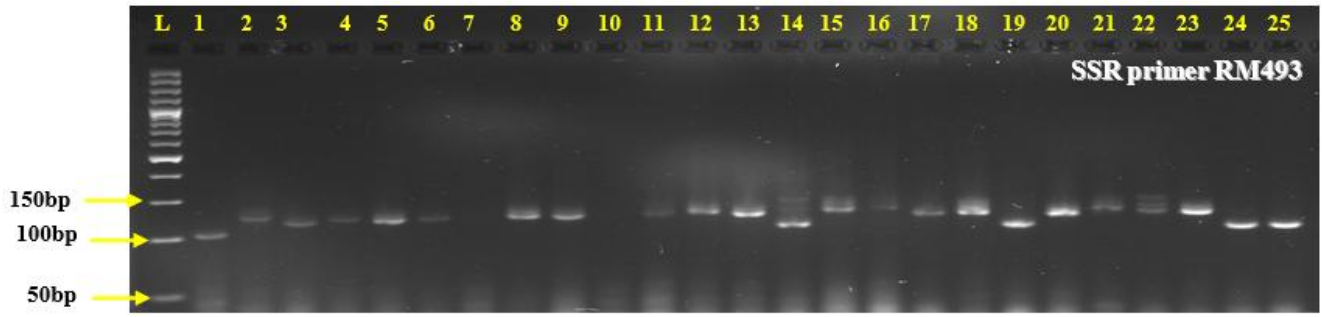
DFF = Days to 50 % flowering
PH = Plant height (cm)
PTP = Productive tillers per plant
PL = Panicle length (cm)
PW = Panicle weight (g)

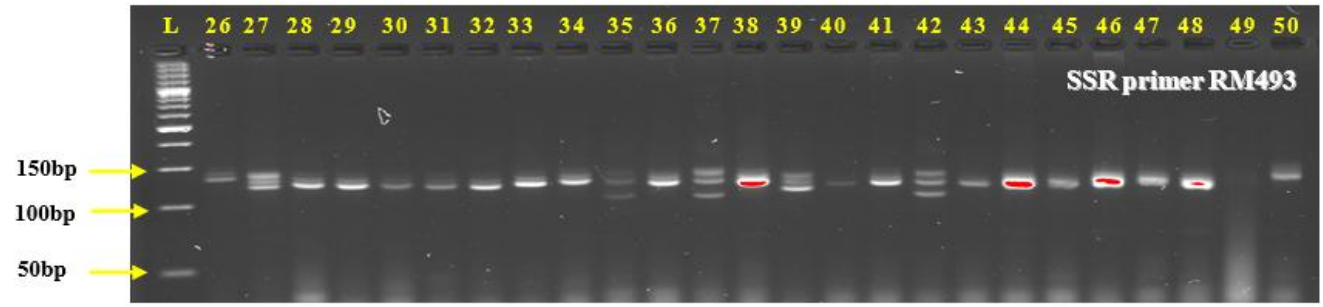
NGP = Numbers of grains per panicle
SI = Seed index (g)
GYP = Grain yield per plant (g)
HI = Harvest index (%)
HULL = Hulling (%)

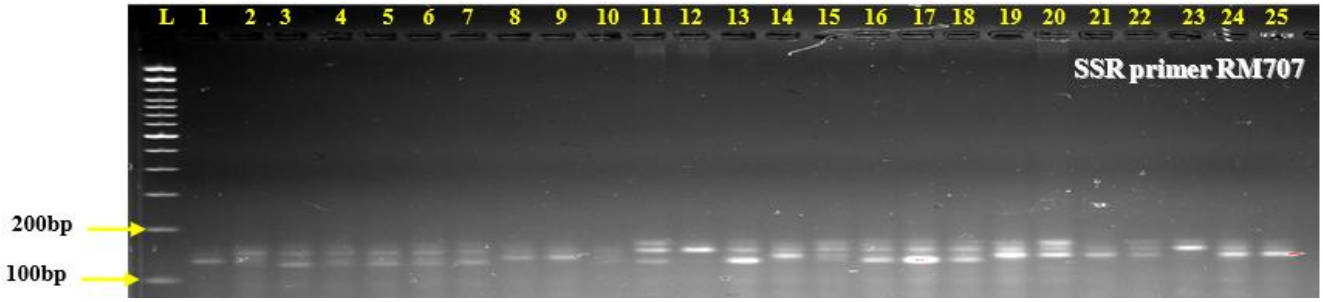
MILL = Milling (%)
L/B = Grain L: B ratio
HRR = Head rice recovery (%)
AC = Amylose content (%)

Table 7: Results of polymorphic SSR marker analysis

S. No.	Locus	No. of allele(s)	Effective number of allele(s)	Major Allele Frequency	Observed heterozygosity	Expected heterozygosity	Nei's Gene diversity	PIC
1	RM3252	3.00	1.60	0.77	0.07	0.38	0.38	0.40
2	RM8068	2.00	1.34	0.85	0.30	0.26	0.25	0.35
3	RM493	2.00	1.47	0.80	0.06	0.32	0.32	0.34
4	RM7075	3.00	2.97	0.38	0.40	0.67	0.66	0.66
5	RM8004	3.00	2.72	0.47	0.12	0.64	0.63	0.64
6	RM129	2.00	1.13	0.94	0.08	0.11	0.11	0.14
7	RM3341	2.00	1.22	0.90	0.12	0.19	0.18	0.25
8	RM1196	3.00	1.54	0.79	0.18	0.35	0.35	0.41
9	RM5	2.00	1.63	0.74	0.08	0.39	0.38	0.39
10	RM246	2.00	1.71	0.70	0.10	0.42	0.42	0.43
11	RM128	2.00	1.27	0.88	0.00	0.22	0.21	0.21
12	RM3285	2.00	1.24	0.89	0.22	0.20	0.20	0.30
13	RM212	2.00	1.98	0.55	0.14	0.50	0.50	0.50
14	RM5794	3.00	1.19	0.92	0.08	0.16	0.16	0.24
15	HvSSR05-12	3.00	1.71	0.72	0.08	0.42	0.41	0.43
16	HvSSR06-13	2.00	1.13	0.94	0.04	0.11	0.11	0.14
Total		38.00	-	-	-	-	-	-
Min		2.00	1.13	0.38	0.00	0.11	0.11	0.14
Max		3.00	2.97	0.94	0.40	0.67	0.66	0.66
Average		**2.38**	**1.62**	**0.77**	**0.13**	**0.33**	**0.33**	**0.36**







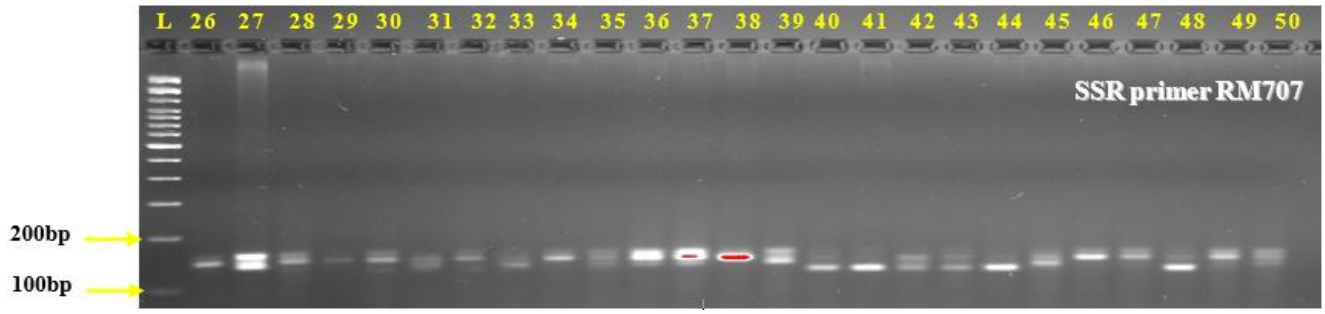


Figure 2: Gel photograph of SSR primer RM493 and RM707 profile

The highest pair wise comparison values of Nei's genetic distance (1.30) was observed between parental genotype NWGR-19200 and GR-21 indicating vast difference at genotypic level between this pair of genotypes and can further exploited in order to develop biparental mapping populations and to wider the genetic background of various rice genotypes in rice improvement program. The lowest genetic distance (0.16) was observed between NWGR-16033 and NWGR-16035 which showing maximum similarity at genomic level between this pair of genotypes.

4. Conclusion

Magnitudes of phenotypic variances were higher as compared to genotypic variances for all the traits as well as estimates of GCV were lower than PCV for entire set the characters under study, which indicated interaction of genotypes with environments. The traits panicle weight, number of grains per panicle and harvest index were revealed as the major yield components. Also, the positive association amongst themselves indicated that selection for one character or the other would be benefited in improvement of the above-mentioned characters and overall yield. Direct selection based on productive tillers per plant, days to 50 % flowering, panicle length, harvest index and plant height can help in increasing grain yield in rice. Genetic divergence between most of the genotypes were not distinct as evident from the clustering composition of cluster I, while the single genotypes formed separate clusters (cluster III, IV) indicated their unique genetic constitution. Grains per panicle contributed maximum towards total genetic divergence followed by seed index, head rice recovery and days to 50 % flowering. Five markers *viz.*, RM3252, RM7075, RM8004, RM1196 and HvSSR05-12 were found to offer three polymorphic loci each, hence could be exploited further for crop improvement in rice. Marker RM7075 showed highest PIC (0.66) value suggesting the most informative marker among all the markers used in present study. The genotype GR-21 from Cluster D found to be the most diverse genotype among all the 50 rice genotypes under study.

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