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ESTIMATION OF GENETIC VARIABILITY PARAMETERS FOR YIELD, YIELD-RELATED ATTRIBUTES AND BIOCHEMICAL PARAMETERS AND MOLECULAR MARKER-BASED GENETIC DIVERSITY IN RICE GERMPLASM

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ABSTRACT

The thorough examination of genetic variability is prerequisite to formulate an appropriate breeding strategy for improvement and development of traits along with yield. The present investigation was carried out with 49 genotypes of rice in a randomized complete block design with three replications at, Main Rice Research Station, Nawagam Agricultural University, Anand during *kharif* 2020. The data for eighteen characters was recorded to estimate the nature and magnitude of variability present in rice germplasm with respect to yield, quality and yield attributing traits. For all of the traits, the analysis of variance found significant differences across genotypes. The genotypic variance contributed major proportion of total variance for plant height, productive tillers per plant, flag leaf length, panicle length, panicle weight, test weight, hulling, milling, head rice recovery, grain L:B ratio, water uptake / 100 g kernels, amylose content and alkali spreading value. The high values of genotypic and phenotypic coefficient of variation were observed for grains per panicle, alkali spreading value, test weight, grain yield per plant, productive tillers per plant, grain L:B ratio and panicle weight. High heritability was found in all the characters studied while high genetic advance was observed for grains per panicle, alkali spreading value, test weight, grain L:B ratio, grain yield per plant, productive tillers per plant, panicle weight, plant height, panicle index, water uptake / 100 g kernels, flag leaf length and panicle length. Mean value-based phenotypic diversity analysis *via* Euclidean distance showed that the genotypes were segregated into four main clusters. Molecular screening of 49 genotypes with 12 SSR markers revealed a total of 60 alleles with mean major allele frequency of 0.35 and mean PIC of 0.74. The UPGMA approach used for elucidating molecular relationships revealed a total of five main clusters where the cluster A was found to be the biggest one with a total of 32 genotypes while the cluster D comprised of only one genotype.

Key words : Variability, Selection, Heritability, SSR, Cluster.

Introduction

Rice (*Oryza sativa* L.) is the most important crop of the world which is widely grown in the tropical as well as subtropical regions (Ezuka and Kaku, 2000). The genus *Oryza* is comprised of 24 species where two of them are cultivated worldwide *i.e.*, African rice (*Oryza glaberrima*, 2n=24=AA) and Asian rice (*Oryza sativa*, 2n-=24=AA). Asian rice dominates the total cultivated area and production and it has genome size of about 565 Mb (Brown, 1999). Asia is popularly termed as “Rice bowl of the world” as it has major part in the production as well as consumption *i.e.*, around 90% of the world rice.

Contributing to the 40 per cent of the overall food grain production, rice is unequivocally the principal staple food crop in India. With 49 million hectares under cultivation, India has the largest area among the rice-producing countries (Kharif rice accounts for 43.6 M ha

and Rabi rice 5.4 Mha), followed by China. India is the second-largest producer of rice, producing 118.9 million tonnes. (Ministry of Agriculture and Farmers Welfare, 2020- 21).

Emphasization on the quality parameters in rice is one of the most important aspect as the whole grain has to be cooked and consumed (Hossain *et al*., 2009). Focus should be given on maintaining the yield of the crop when the improvement in the quality of the grain is being carried out which will benefit the farmers as well as consumers (Dhanwani *et al*., 2013). Size of the grain and its dimensions are of great importance and considered as target for improvement in rice in recent years (Xing and Zhang, 2010).

Selection of a breeding plan with respect to the improvement or development of rice varieties for higher yield is based on the extent of the variability of the germplasm. Higher estimates of the variability in a germplasm will be helpful as it will provide greater opportunities to carry out selection for developing a variety enriched with desirable traits (Jalandhar *et al.* 2017). Robinson *et al*. (1949) stated that the heritability of a particular character should be considered by a breeder as it reveals its possibility of transferability in further generation *via* selection. Heritability coupled with higher estimates of genetic advance can give us better genetic gain from the selection (Johnson *et al*., 1955).

For every crop improvement programme, genetic diversity among genotypes is the pre-requisite factor (Manomani and Khan, 2003). It is essential to study several components of the germplasm at molecular level. Molecular analysis can be utilized to investigate the genetic relationships within and between species. DNA-based markers can be a crucial and promising technique for assessment of genetic diversity underlying the germplasm and also to identify the evolutionary relationships for this purpose. As the Simple Sequence Repeats (SSR) are co-dominant markers, they are capable of marking the difference between the heterozygote and homozygote as well as it shows multiallelic nature and higher allelic diversity than RFLP. Hence, they are of great use (Mc Couch *et al*., 1997). SSR shows good reproducibility and hence can be efficiently exploited to create a consistent data through different laboratories (Saghai *et al*., 1994).

Materials and Methods

Plant Material and observations

Field trial consisted of 49 genotypes which were sown at the Main Rice Research Station, AAU, Nawagam. The gross plot area was 2.55 × 0.8 m and the spacing of 20 × 15 cm was followed. The experimental design used for the study was the Randomized Complete Block Design (RBD), which included three replications during *kharif* 2020. Random selection of five plants from the middle three rows of each genotype was performed in each replication and accordingly, observations were recorded for all characters except grain yield and days to 50% flowering. Data for these traits were taken on the plot basis. Five plants were selected from randomly selected five plants from the three middle rows. Alkali spreading value was assessed as per the procedure elaborated by Jennings *et al*. (1979).

Molecular Marker study

Young leaves of 10-15 days old seedlings of forty-nine rice genotypes were used for the extraction of DNA. The protocol of Doyle and Doyle (1987) was followed for the extraction of the DNA. Genotyping was carried out using SSR markers. The PCR reaction conditions were an initial denaturation of 94°C for 5 min., followed by 35 cycles of 94°C for 45 s, ΔT°C (primer specific) for 45 s, 72°C for 45 s, and a final extension at 72°C for 7 min. For separation and visualization of PCR products, agarose gel (3.5%) was used.DNA polymorphism among the genotypes was observed based on length of amplified fragments in terms of number of base pairs by comparing with a 50 bp ladder.

Statistical analysis

Computation of analysis of variance as well as genetic parameters such as genotypic and phenotypic variance, genotypic and phenotypic coefficient of variation, heritability in broad sense (H^2_{bs}) (%) and genetic advance (%) mean was performed using package variability 0.1.0 (Popat *et al*., 2020) through R-software (ver. 4.2.1). Using mean values of phenotypic data, assessment of Euclidean distance was carried out and accordingly, a dendrogram was constructed by using XLSTAT 2021 (version 3.1.1172). Further genetic diversity studies were carried out based upon the summary statistics for all the markers derived using PowerMarker v 3.25 software (Liu and Muse, 2005). A similarity matrix was created using processed allele calls for all markers. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis approach implemented in XLSTAT 2021 (version 3.1.1172) was utilized to create dendrograms using the Jaccard's similarity matrix.

Results and Discussion

Analysis of Variance

The analysis of variance estimated for eighteen yield and yield related traits showed highly significant

differences among genotypes for all the characters under study at 5% level of significance (Table 1). This clearly reveals that an inherent genetic difference is present among the genotypes under the investigation for traits studied. These findings were in accordance with Devi *et al.* (2017) and Rashmi *et al.* (2017).

Variance and coefficient of variation

All 18 characters showed wide range of genotypic as well as phenotypic variance (Table 2). Highest genotypic and phenotypic variance was observed for grains per panicle (4380.3 and 4583.34) followed by water uptake/100 g kernels (1867.93 and 1885.63) and plant height (276.92 and 281.66) and the varied contribution of the genotypic variance to the phenotypic variance observed which indicates the influence of environmental parameters on the expression of traits. The estimates of genotypic and phenotypic variances of the characters such as plant height, productive tillers per plant, flag leaf length, panicle length, panicle weight, test weight, hulling, milling, head rice recovery, grain L: B ratio, water uptake / 100g kernel, amylose content and alkali spreading value revealed that genotypic variance have a large contribution in the phenotypic variance, which implies a less influence of environment on the expression of these characters.

Table 1 : ANOVA for various characters of rice.

Sources of variation		Replications	Genotypes	Error
Degrees of freedom		2	48	96
Mean sum of squares	DTFF	21.76	154.09**	10.19
	PH	11.93	835.51**	4.74
	PTPP	0.89	16.95**	0.65
	FLL	0.89	46.11**	0.89
	PL	0.81	23.82**	1.19
	PW	0.11	1.52**	0.06
	GPP	527.4	13344**	203
	PI	35.47	177.36**	18.29
	HI	8.21	40.13**	5.25
	TW	1.04	71.51**	0.75
	HL	2.57	67.31**	0.85
	ML	1.5	53.38**	1.08
	HRR	0.19	38.57**	0.53
	LB	0.04	1.92**	0.01
	WU	0.2	5621.5**	17.7
	AC	0.78	9.16**	0.26
	ASV	0.006	4.60**	0.02
	GYP	10.76	46.51**	3.93

** Significant at 5% levels.

DTFF: Days to 50% flowering
PH: Plant Height (cm)
PTPP: Productive tillers per plant
FLL: Flag leaf length (cm)
PL: Panicle Length (cm)
PW: Panicle weight (g)
WU: Water uptake / 100 g kernels (ml)
GPP: Grains per panicle
PI: Panicle index (%)
ASV: Alkali spreading value
HI: Harvest Index (%)
GYP: Grain yield per plant (g)
TW: Test weight (g)
HL: Hulling (%)
ML: Milling (%)
HRR: Head rice recovery
LB: Grain L: B ratio
AC: Amylose content

The high values of genotypic and phenotypic coefficient of variation were observed for grains per panicle (37.68 and 38.54), alkali spreading value (34.45 and 34.76), test weight (27.58 and 28.01), grain yield per plant (25.2 and 28.48), productive tillers per plant (23.15 and 24.51), grain L:B ratio (23.01 and 23.29) and panicle weight (20.04 and 21.32); Moderate GCV and PCV exhibited by panicle index (14.93 and 17.32), plant height (14.61 and 14.73), flag leaf length (12.77 and 13.14), water uptake / 100 g kernels (12.87 and 12.93) and panicle length (11.76 and 12.65) indicating considerable variation for these characters (Table 2). Narrow range between GCV and PCV traits reveals the less influence on environment on characters. The similar results were reported by Nandeshwar *et al.* (2010), Veni *et al.* (2013), Kumar *et al.* (2015), Nirmaladevi *et al.* (2015), Shrivastava *et al.* (2015), Ashok *et al.* (2016), Devi *et al.* (2016), Devi *et al.* (2017) and Rashmi *et al.* (2017).

Heritability and genetic advance as percent of mean

High heritability estimates were observed for all characters (Table 2). High heritability (%) coupled with high genetic advance as percent of mean was observed in characters grains per panicle (95.57% and 75.89%), alkali spreading value (98.22% and 70.34%), test weight (96.00% and 55.93%), grain L:B ratio (97.59% and 46.82%), grain yield per plant (78.29% and 45.93%), productive tillers per plant (89.00% and 45.06%), panicle weight (88.00% and 38.82%), plant height (98.32% and 29.84%), panicle index (74.00% and 26.52%), water uptake / 100 g kernels (99.06% and 26.39%), flag leaf length (94.39% and 25.56%) and panicle length (86.36% and 22.51%) indicated that these characters were predominantly governed by additive gene action. Hence, there would be better scope for improvement of these characters by selection. High heritability coupled with moderate genetic advance as percent of mean was observed for harvest index (68.88% and 16.91%),

Table 2 : Estimates of variability and genetic parameters for yield and quality characters in rice.

S. no.	Character	General Mean	Range of mean		Variance (%)		Coefficient of variance (%)		Heritability in broadsense (H^2_{bs}) (%)	Genetic advance (%) mean
			Min.	Max.	Genotypic	Phenotypic	Genotypic	Phenotypic		
1	**Days to 50% flowering**	92.72	77	110	47.96	58.16	7.46	8.22	82.00	13.97
2	**Plant height**	113.88	77.4	146.8	276.92	281.66	14.61	14.73	98.32	29.84
3	**Productive tillers per plant**	10.06	5.9	15.90	5.43	6.08	23.15	24.51	89.00	45.06
4	**Flag leaf length**	30.38	21.2	38.8	15.07	15.96	12.77	13.14	94.39	25.56
5	**Panicle length**	23.35	16.9	31.9	7.54	8.73	11.76	12.65	86.36	22.51
6	**Panicle weight**	3.48	1.84	5.78	0.48	0.55	20.04	21.32	88.00	38.82
7	**Grains per panicle**	175.61	61.15	422.39	4380.3	4583.34	37.68	38.54	95.57	75.89
8	**Panicle index**	48.75	31.28	73.33	53.02	71.31	14.93	17.32	74.00	26.52
9	**Harvest index**	34.47	26.77	47.68	11.62	16.88	9.89	11.91	68.88	16.91
10	**Test weight**	17.60	9.1	32.7	23.58	24.34	27.58	28.01	96.00	55.93
11	**Hulling (%)**	77.99	64.2	87.2	22.15	23	6.03	6.14	96.30	12.19
12	**Milling (%)**	71.33	58.67	80.66	17.43	18.51	5.85	6.03	94.12	11.69
13	**Head rice recovery**	59.75	43.69	64.38	12.67	13.21	5.95	6.08	95.95	12.02
14	**Grain L:B ratio**	3.46	2.11	7.1	0.63	0.65	23.01	23.29	97.59	46.82
15	**Water uptake/100 g kernels**	335.68	260	440	1867.93	1885.63	12.87	12.93	99.06	26.39
16	**Amylose content**	23.69	19.4	28.66	2.96	3.23	7.27	7.58	91.84	14.35
17	**Alkali spreading value**	3.58	2	7	1.52	1.55	34.45	34.76	98.22	70.34
18	**Grain yield per plant**	14.94	7.14	28.84	14.19	18.12	25.2	28.48	78.29	45.93

amylose content (91.84% and 14.35%), days to 50% flowering (82.00% and 13.97%), hulling (96.30% and 12.19%), head rice recovery (95.95% and 12.02%) and milling (94.12% and 11.69%) indicated that the influence of both additive and non-additive gene effects in control of these characters. The results were in accordance with the estimates reported by Nandeshwar *et al.* (2010), Babu *et al.* (2012), Veni *et al.* (2013), Kumar *et al.* (2015), Nirmaladevi et al. (2015), Roy *et al.* (2015), Shrivastava *et al.* (2015), Ashok *et al.* (2016), Devi *et al.* (2016), Devi *et al.* (2017), Rashmi *et al.* (2017), Sumanth *et al.* (2017) and Hasan *et al.* (2020).

Phenotypic Diversity analysis

A dendrogram was constructed based upon the Euclidean distance which revealed that all 49 genotypes of rice were divided into 4 main clusters *viz.*, A, B, C and D comprised of 33, 13, 1 and 2 genotypes, respectively (Fig. 1).

Main cluster A was found to be the largest with a total of 24 genotypes and it was further subdivided into two subclusters A1 and A2. Subcluster A1 consisted of 25 genotypes while, remaining 8 genotypes were categorized under cluster A2. Genotypes IC-426126, IC-346892, IC-346252, IC-277338, IC-346855, IC-283226, IC-283251, GP-612, NWGR-19253, IR 64, NWGR-19247, NWGR-19252, NWGR-19244, GP-594, EC-496935, EC-497093, GP-609, EC-497157, GP-615, GP-610, NWGR-19246, GP-614, NWGR-19249, GP-589 and NWGR-19251 were under cluster A1. Subcluster A2 consisted of genotypes IC-438540, IC-426149, IC-438643, IC-346890, IC-438639, IC-283249, IC-426139 and IC-346899. Main cluster B consisted of a total of 13 genotypes. It was further bifurcated into two subclusters. Subcluster B1 consisted of 9 genotypes *viz.*, GP-587, IC-426148, GP-595, GP-592, IC-277274, GP-613, NWGR-19245, NWGR-19250, and GP-588. Subcluster B2 was comprised of a total 4 genotypes *viz.*, NWGR-19242, IC-343499, IC-280502 and GP-593. Main cluster C was the smallest among all of 4 clusters

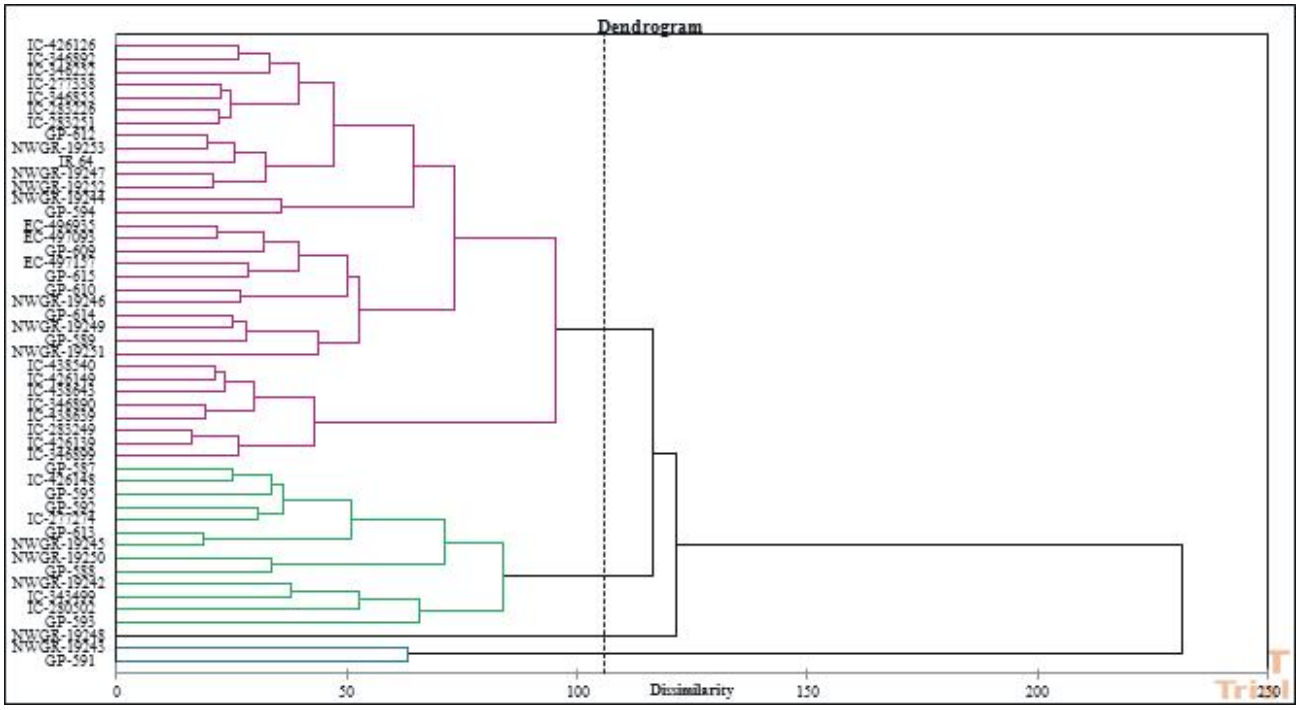

Fig. 1 : Dendrogram constructed by Euclidean distance values showing relationship among 49 rice genotypes generated by XLSTAT based on mean values of phenotypic data.

Table 3 : Results of SSR marker analysis.

S. no.	Primer Name	Molecular weight (bp)	No. of alleles	Major allele frequency	H_e	H_1	PIC
1	RGNMS167	173-207	9	0.34	0.76	0.00	0.72
2	RM10	175-194	6	0.44	0.70	0.00	0.66
3	RM149	216-250	7	0.44	0.72	0.00	0.68
4	RM171	304-356	10	0.28	0.84	0.00	0.82
5	RM346	134-161	7	0.28	0.80	0.00	0.77
6	RM144	209-254	9	0.32	0.78	0.00	0.75
7	RM140	183-246	12	0.34	0.82	0.00	0.80
	Total	-	60	2.49	5.44	0.00	5.24
	Mean	-	8.57	0.35	0.77	0.00	0.74

which was comprised of only one genotype, NWGR-19248. Main Cluster D consisted of two genotypes, NWGR-19243 and GP-591.

Molecular diversity analysis

To assess the genetic diversity, microsatellite (SSR) marker was used to perform molecular profiling of 49 rice genotypes. Twelve primer pairs were chosen for PCR amplification. Out of 12 primers, 7 were amplified successfully, and they all exhibited polymorphism. Finally, a PCR reaction was performed to analyze the genetic diversity of 49 rice genotypes using 7 polymorphic primers. The different amplified PCR products showed molecular weights ranged from 134 bp (RM346) to 356 bp (RM171), indicating a significant variation in the number of repeats between alleles. A total of 60 alleles were found distributed across 49 rice genotypes. With a range of 6 (RM10) to 12 (RM140), the average number of allele per locus was 8.57 (Table 3). Similar observation was reported by Hossain *et al*. (2020) where they reported alleles per locus ranged from 4 (RM536) to 20 (RM209) with high mean of 9.48 for number of allele.

With an average of 0.35, the major allele frequency ranged from 0.28 (RM171 and RM346) to 0.44 (RM10 and RM149). Because there is greater allelic diversity, higher values were more informative. The highest amount of gene diversity, 0.84, was found in RM171, while, the lowest level (0.70) was found in RM10. Hossain *et al*. (2020) reported mean major allele frequency of 0.30.The PIC values for SSR markers in this investigation varied from 0.66 (RM10) to 0.82 (RM171) with a mean of 0.74. It was in confirmatory with the results reported by Brar *et al*. (2012) where they obtained a mean PIC value of

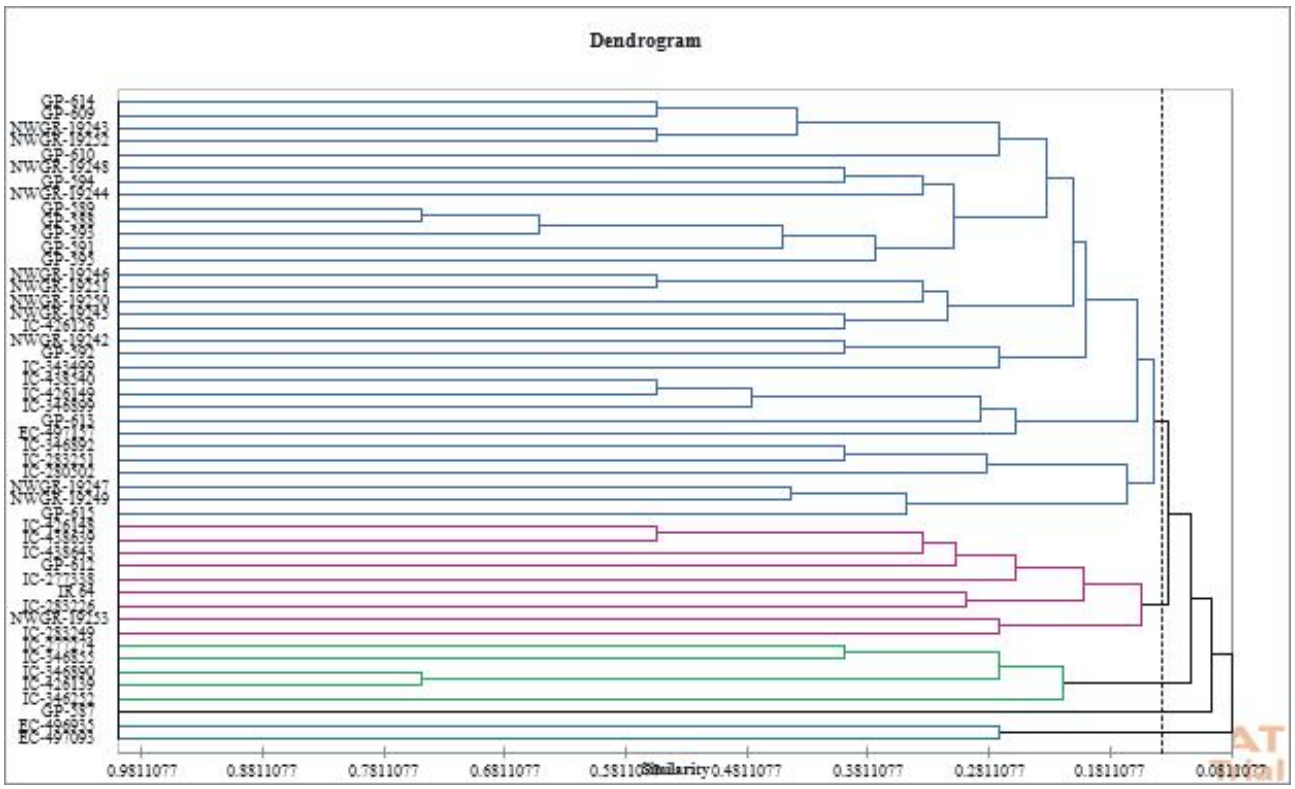

Fig. 2 : Dendrogram showing relationships among 49 rice genotypes generated by XLSTAT using molecular marker data.

0.66. All markers were shown to be informative, with a PIC greater than 0.5. SSR markers had an average PIC value of 0.74.

Genetic relationship among the Genotypes and Cluster analysis

All 49 rice genotypes were shown to be classified into five main clusters A, B, C, D and E with 32, 9, 5, 1 and 2 genotypes, respectively (Fig. 2). Main cluster A was the largest and it was further subdivided in to two sub clusters A1 (26 genotypes) and A2 (6 genotypes). The sub clusters A1 the consisted of highest number of accessions, indicating a high genetic similarity between genotypes of this group. Out of 32, 26 genotypes *viz*., GP-614, GP-609, NWGR-19243, NWGR-19252, GP-610, NWGR-19248, GP-595, NWGR-19244, GP-589, GP-588, GP-593, GP-591, GP-596, NWGR-19246, NWGR-19251, NWGR-19250, NWGR-19245, IC-426126, NWGR-19242, GP-592, IC-343499, IC-438540, IC-426149, IC-346899, GP-613 and EC-497157 were grouped in sub cluster A1. 6 genotypes *viz*., IC-346892, IC-283251, IC-280502, NWGR-19247, NWGR-19249 and GP-615 were grouped in sub cluster A2.

Main cluster B comprised of 9 genotypes. It was divided into two sub clusters as B1 and B2. The sub cluster B1 contained 7 genotypes *viz*., IC-426148, IC-438639, IC-438643, GP-612, IC-277338, IR 64 and IC-283226. Similarly, other 2 genotypes *viz*., NWGR-19253 and IC-283249 were grouped in Cluster B2.Main cluster C included 5 genotypes and it was divided into two sub clusters as C1 and C2. The sub cluster C1 contained 4 genotypes *viz*., IC-277274, IC-346855, IC-346890 and IC-426139 while, IC-346252 was included in sub cluster C2.Main cluster D was comprised of only 1 genotype *i.e*., GP-587 which indicates its distinct evolutionary divergence from other rice accessions included in this study. While, main cluster E consisted of 2 genotypes namely EC-496935 and EC-497093.

The pair wise comparison estimates of genetic distance among 49 genotypes were ranging from 0.00 to 0.75 (Tables 3). The highest genetic distance (0.75) was observed between IC-346855/IC-426139, GP-591/GP-588 and GP-595/GP-589 genotypes which indicated that this pair of genotypes were highly variable at molecular level.

Conclusion

In the current experiment, the study of genetic parameters on yield and yield related traits was carried out on 49 rice genotypes. Analysis of variance among the genotypes indicated the significant difference for all the studied characters. The estimates on genetic parameters revealed that the high GCV coupled with high heritability and high genetic advance indicates the major role of additive gene action and such traits could be improved *via* selection. With the current experimental

material, crossing of elite genotypes followed by selection may be beneficial for genetic improvement of grain production per plant and its components.SSR markers had an average PIC value of 0.74. Because RM171 had the greatest PIC value, it was determined to be superior for future genetic diversity investigation. The lines which shown significantly higher distances based upon molecular diversity as well as phenotypic diversity studies may contain diverse set of alleles and may be relevant for constructing biparental mapping populations as well as rice improvement programmes to broaden the genetic background of different rice genotypes.

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