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Genetic diversity analysis in radiation induced small grain mutants of rice (*Oryza sativa*)

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Rice (*Oryza sativa* L.) is the world's most important cereal crop for food security. Asia is known as the Rice bowl of the world, about 90% of the world's rice is produced in Asia and 50% of the people depends on rice for food (Tenorio *et al.* 2013). The grain size and appearance decides market value and play an important role in the adoption of new rice varieties. However, grain quality traits in rice differ with different local cultures and cuisines (Fitzgerald *et al.* 2009). Grain size is a key breeding target, as it plays a vital role in both yield and quality. Therefore, the study of grain size is important to improve rice yield and quality (Shomura *et al.* 2008). The Mahalanobis D^2 analysis helps to measure the quantum of genetic diversity in a given population and assesses the relative contribution of different traits to the total divergence (Zahan *et al.* 2008). Principal component analysis (PCA) reveal the patterns and eliminate redundancy in data sets as variations regularly arise in crop genotypes (Maji and Shaibu 2012). This study aimed to understand extent of genetic diversity among small grain mutants and group them by multivariate analysis to excise further selection for grain quality and grain yield improvement.

Present study was carried out during rainy (*kharif*) 2018 and winter (*rabi*) seasons of 2018–19 at Agricultural College and Research Institute (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu), Madurai, Tamil Nadu. The mutants were generated from long slender (7.0 mm) Anna (R) 4 rice variety by irradiating gamma rays (^{60}Co) and electron beam (10 MeV). The doses at which seeds were exposed, viz. 200 Gy, 250 Gy, 300 Gy and 350 Gy of each radiation. The diverse spectrum of variations was generated in different quantitative traits in M_2 generation. The selection was excised for small-sized grain mutants with kernel length of less than 6.6 mm and short duration (<120 days) in M_2 and M_3 generations. The 56 small grain mutants were isolated from gamma rays (12 no.) and electron beam (44 no.) were advanced to M_4 generation. The mutant families namely E1, E2–E4, E5–E39, and E40–E44 are generated from electron beam irradiation of 200 Gy, 250 Gy, 300 Gy and 350 Gy respectively; G45 to G51 and G52 to G56 have generated from gamma rays irradiation of 200 Gy and 250 Gy respectively along with wild type Anna (R) 4 cultivar (G57) were used for this study.

These mutant families were raised in the plant to rows of progenies in two replications along with Anna (R) 4 rice. The field evaluation was conducted during rainy (*kharif*) season of 2019 at Agricultural College and Research Institute (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu), Madurai, Tamil Nadu. From each mutant 5 plants were randomly selected for recording yield and yield attributing traits. The characters measured were plant height at maturity (cm), days to maturity, flag leaf length (cm), flag leaf breadth (cm), total number of tillers, number of productive tillers, panicle length (cm), panicle weight (g), number of grains/panicle, number of filled grains/panicle, kernel length (mm), kernel breadth (mm), 100-grain weight (g) and grain yield/plant (g). The genetic divergence of mutants was estimated using Mahalanobis D^2 analysis. The grouping of mutant families was done by Toucher's method of clustering (Rao 1952) and principal component analysis (PCA). The statistical analysis was carried out using software STAR nebula 2.0.1 (2013) and GENRES version 7.01.

The small grain mutants were grouped into 10 clusters, their inter and intra cluster D^2 values are given in Table 1. The maximum cluster distance estimated between cluster IX

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Table 1 Inter and intra cluster D^2 value of small grain mutants of rice

	I	II	III	IV	V	VI	VII	VIII	IX	X
I	123.665	179.379	175.477	152.135	218.588	219.317	172.733	274.161	270.737	227.733
II		221.016	215.341	178.463	318.906	254.653	195.481	319.339	384.408	320.530
III			221.482	156.869	243.605	217.391	186.152	250.051	425.978	260.631
IV				61.861	249.935	107.381	108.927	176.759	507.942	226.671
V					64.653	264.690	211.321	185.421	366.427	87.616
VI						69.170	117.149	166.104	514.883	214.335
VII							99.980	141.221	426.660	203.397
VIII								107.110	638.950	233.590
IX									0	382.293
X										0

and cluster VIII (638.95), followed by cluster VI (514.88), cluster IV (507.94), cluster VII (426.66), cluster II (384.40), cluster X (382.29), cluster V (366.42) and cluster I (270.73). The cluster IX is a solidary cluster of E44 mutant having less number of grains/panicle, filled grains/panicle, less panicle weight resulted low grain yield/plant (16.32 g) compared to other clusters. The genetic diversity analysis of 36 advanced breeding lines in rice by Palaniyappan *et al.* (2020) reported that the rice genotypes in cluster I and VI exhibited higher mean values for grain length, grain breadth, and seed weight. However, the genotypes from cluster VII exhibited a short slender grain type. Suggested that the selection from these clusters will help to improve the traits namely earliness, grain yield, and grain qualities of rice.

Interestingly the most of small grain mutants generated from electron beam up to 300 Gy are grouped in adjacent clusters of I, II and III. Moreover, the genotypes in cluster I and II are originated from few pedigrees. Whereas, five grain mutants generated 350 Gy of electron beam is scattered in clusters II, III and IX. This indicates beyond 300 Gy of electron beam generates more morphological variations in many traits. The mutants generated from gamma rays falls in clusters IV, V, VI, VII, VIII and X. The least inter cluster distance obtained between cluster V and cluster X (87.61) from three gamma rays generated mutants of G46, G54 and G53 are having reduced plant height and less grain yield/plant. More intra cluster distance is obtained in cluster II and III, indicates there is a possibility of selecting high grain

Table 2 Proportion of variance, cumulative proportion, eigen values and eigen vectors of small grain mutants in rice

Statistics	PC1	PC2	PC3	PC4	PC5	PC6
Proportion of variance	0.2036	0.1701	0.1599	0.1215	0.075	0.0674
Cumulative proportion	0.2036	0.3737	0.5336	0.6551	0.7301	0.7975
Eigen value	3.0534	2.5518	2.3985	1.8231	1.1254	1.0107
Trait			Eigen vectors			
Plant height at maturity	0.352	0.238	0.230	0.044	0.004	0.121
Days to maturity	0.058	0.252	-0.127	-0.336	0.060	0.498
Flag leaf length	0.221	0.275	0.033	0.014	0.507	0.011
Flag leaf breadth	-0.255	0.160	-0.290	-0.140	0.477	-0.112
Total number of tillers	0.431	0.209	-0.126	-0.272	-0.159	-0.043
No. of productive tillers	0.428	0.242	-0.118	-0.265	-0.169	0.010
Panicle length	-0.179	0.384	-0.168	-0.060	0.335	-0.267
Panicle weight	0.053	0.066	-0.485	0.348	0.032	-0.006
No. of grains/panicle	0.315	-0.258	-0.359	0.247	0.097	0.041
No. of filled grains/panicle	0.309	-0.273	-0.344	0.269	0.118	0.090
Kernel length	-0.225	0.388	-0.155	0.280	-0.263	0.305
Kernel breadth	-0.148	-0.157	-0.357	-0.357	-0.191	0.189
Kernel length to breadth ratio	-0.003	0.385	0.148	0.503	-0.135	0.148
100-grain weight	-0.283	0.119	-0.324	-0.065	-0.274	0.076
Grain yield/plant	0.083	0.214	-0.164	-0.001	-0.350	-0.697

yield/plant with long flag leaf length, more number of productive tillers/plant and more filled grains/panicle.

Among small grain mutants, the traits contributing towards genetic diversity are grain yield per plant (60.02%), plant height (10.58%) and 100-grain weight (8.08%). This is due to the selection pressure exerted on reduced kernel length and early maturity from M_2 and M_3 generations, resulting in fewer variations in all the growth and grain traits except grain yield/plant and plant height. The variation in grain yield indicates there is a possibility of selecting mutants with high grain yield/plant.

The principal component analysis (PCA) of 56 small grain mutants assembling 15 quantitative traits was done and their eigen value and coefficient of vector are presented in Table 2. The eigen value estimates of grain mutants revealed that first six principal components with more than 1 and contributes 79.75% of cumulative variance. The principal component 1 contributes 20.36% of the variance. The high positive values of co-efficient of the vector are obtained from the number of productive tillers, total number of tillers/plant, plant height at maturity, number of grains/panicle and filled grains/panicle. Rice grain yield is determined by three traits viz. number of grains/panicle, grain weight and number of panicles/plant (Shomura *et al.* 2008). Pachauri *et al.* (2017) observed a maximum cumulative percentage of variation within four PCs of about 72.48% with eigen value of more than 1 from 124 rice accessions. The traits such as days to 50% of flowering, plant height, grain yield/plant, and days to maturity accounted for maximum variability which is classified as the first component. Grain length breadth ratio which is highly correlated with second component and it had 13.56% of variance to the total variability. Whereas, Sathish and Senapati (2017) explained the genetic diversity among rice cultivars with five principal components which had 72.58% of total variability. The PC 1 had the contribution from the characters namely grain length/breadth ratio, grain length, and harvest index.

The second principal component exploits 17.01% of total variation. Maximum contribution of traits is from kernel length, kernel length to width ratio and panicle length. The principal component 3 extract 15.995% of variation, but none of the traits added maximum positive effect of more than 0.3 to this component. Fourth principal component contributes 12.15% of variation and mainly loaded from panicle weight with high positive effect. The principal component 5 loaded mainly from flag leaf length, flag leaf breadth and panicle length to the variance of 7.5%. The principal component 6 explores 6.74% of variance and maximum vector contributed from days to maturity and kernel length.

The grouping of genotypes based on estimates of PC 1 and PC 2 is represented in the biplot (Fig. 1). The mutants derived from the electron beam are mainly grouped in quadrant I, these mutants are highly influenced by the variance of traits such as plant height, total number of tillers, number of productive tillers, length of flag leaf, and to some extent days to maturity and single plant yield. The genotypes grouped in quadrant II are loaded from the variance of the

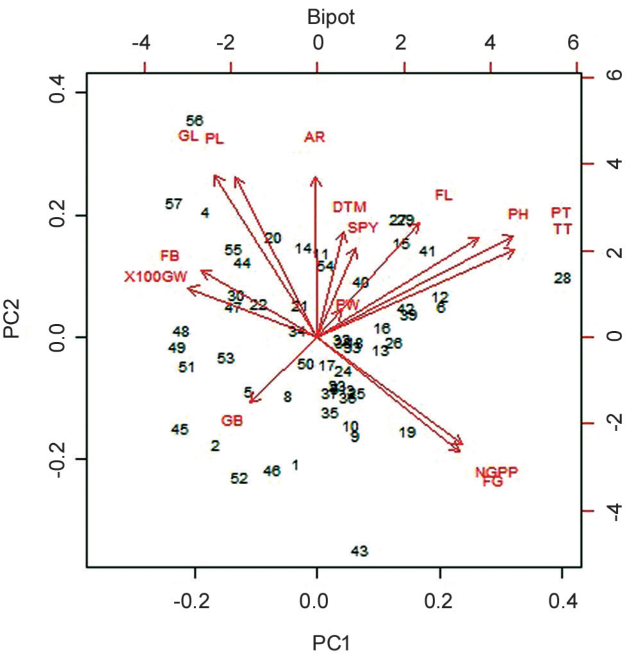

Fig. 1 Biplot from first two principal components (PC1: 30.36%, PC 2: 17.01%) of small grain mutants in rice.
Genotypes: 1–44 electron beam mutants, 45–56 gamma rays mutants, 57 long grain wild type [Anna (R) 4 cultivar].
Traits: PH, Plant height at maturity; DTM, Days to maturity; FL, Flag leaf length; FB, Flag leaf breadth; TT, Total number of tillers; PT, Productive tillers; PL, Panicle length; PW, Panicle weight; NGPP, Number of grains/panicle; FG, Number of filled grains/panicle; GL, Kernel length; GB, Kernel breadth; AR, Kernel length to breadth ratio; 100-GW, 100-grain weight; SPY, Grain yield/plant.

number of grains/panicle and number of filled grains/panicle. The mutants generated from gamma rays fall in quadrant III, these genotypes are highly influenced by the variance of kernel width. This indicates, gamma rays mutants were relatively more kernel width than electron beam mutants. Among top 5% small grain mutants, the kernel length was ranged from 5.4–6.0 mm as compared to 7.1 mm in parental type [Anna (R) 4]. Most of reduced grain length mutants were generated from 300 Gy of electron beam irradiation.

SUMMARY

Present study was carried out during rainy (*kharif*) 2018 and winter (*rabi*) seasons of 2018–19 at Agricultural College and Research Institute (Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu), Madurai, Tamil Nadu aimed to understand extent of genetic diversity among small grain mutants and group them by multivariate analysis to excise further selection for grain quality and grain yield improvement. The small grain mutants were grouped into 10 clusters based on genetic diversity by D^2 values. The maximum cluster distance was estimated between cluster IX and cluster VIII and least inter cluster distance was obtained between cluster V and cluster X. After early generation selection of mutants for small grain size and

early maturity; from remaining variability, the trait grain yield/plant contributed maximum to genetic diversity. Which offers scope for selecting high yielding genotypes with reduced grain size. The high positive values of the co-efficient of the vector were obtained from the total tillers/plant, number of productive tillers, plant height at maturity, number of grains/panicle, and number of filled grains/panicle from PC 1 and PC 2. Grouping based on PC 1 and PC 2 biplots helps to understand the contribution of traits to each group of genotypes. The information derived from this study will help to develop rice cultivars with improved grain quality and grain yield.

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