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GENETIC STRUCTURE AND ASSOCIATION ANALYSIS OF YIELD AND GRAIN LENGTH TRAITS IN RICE GERMPLASM ACCESSIONS

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Association mapping

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ABSTRACT

Rice is a primary staple food for more than half of the world's population and more than 70% of Indian population. Chhattisgarh state is one of the major parts of India having richest rice biodiversity with wide genetic diversity. To make advances in rice molecular design breeding, it is important to understand the genetic background and phenotypes of germplasm lines, to know superior alleles of the breeding material. An attempt was made to identify the marker trait association for grain features with SSR markers and to identify the genetic diversity and population structure in rice germplasm lines of Chhattisgarh. In this study, 59 SSR markers were used to analyze differentiation pattern in 48 rice germplasm lines. The genetic structure showed that 48 *indica* lines were divided into two subgroups based on grain morphology. Sub population-1 and sub-population-2, consisted 23-23 genotypes respectively whereas two genotypes were of admixture type. Results of neighbour-joining tree (NJ tree) and PCoA analysis showed similar results of population structure analysis. GWAS analysis identified total 25 significant marker-trait associations. Of which five for grain length, four for grain length: breadth ratio, twelve for grain yield and four for thousand-grain weights were recorded. RM135 and RM201 were significantly associated with three traits *viz.*, grain length, grain L: B ratio and total grain weight. We do hope that the results obtained through this study will be useful for future breeding programmes.

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1 Introduction

Rice (*Oryza sativa* L.) is one of the major staple foods belonging to more than half of the world's population. Increasing the productivity of rice is essential to feed the burgeoning population. Moreover, demand of quality rice is increasing as living standard of people has improved (Sahu et al., 2019). Preference for rice with different grain size (long and short) varies region to region. Hence, there is a need to breed high-quality rice with higher yield potential. (Huang et al., 2013; Li et al., 2020).

Since rice grain length is quantitatively inherited (McKenzie & Rutger, 1983), it is difficult for breeders to efficiently improve grain appearance using conventional selection methods. Thus, it should be particularly helpful for enhancing breeding efficiency to use markers closely linked to genes or major quantitative trait loci (QTLs) for grain length to screen target genotypes directly in early generations (Weng et al., 2008). In rice, numerous studies have been conducted to genetically map QTLs for grain yield traits, and thousands of QTLs have been detected during the past several decades (Li et al., 2020). Grain size and shape are important determinants of grain yield and grain quality, which are usually controlled by QTLs. More than 400 QTLs responsible for grain shape and size were reported by various scientists (Shao et al., 2012; Huang et al., 2013).

Many QTLs for rice grain size traits have been reported in the last decade (Huang et al., 2013). Among them, *qGL3* (Zhang et al., 2012), *GW8* (Wang et al., 2012), *GS5* (Li et al., 2011), *GS3* (Mao et al., 2010), *GW5* (Weng et al., 2008) and *qSW5* (Shomura et al., 2008) have been cloned, and *gw3.1* (Li et al., 2004), *qGL7* (Bai et al., 2010), *GS7* (Shao et al., 2012) and *gw9.1* (Xie et al., 2008) have been fine mapped. Thus, understanding the genetic and molecular basis of grain size is extremely important for rice improvement programs. Molecular marker technique has proved valuable in identification of loci involved in quantitative traits related to grain quality characters and has provided insight into its complex relationship with associated factors and their underlying genes are now far more accessible (Weng et al., 2008).

Association mapping has proven significance for identification and mapping of QTLs for various desired in rice germplasm populations. In this study, total 48 germplasm lines of rice with high and low grain length and 59 SSR markers were used to assess the genetic diversity and the same were applied to population structure. Moreover, association analysis was undertaken between SSR markers and four grain parameters. Aim of present study was to distinguish the population structure of selected rice varieties and to identify SSR markers,

associated significantly with rice grain yield, grain length, and grain length: breadth and thousand-grain weight.

2 Materials and Methods

2.1 Plant materials

For current study, total 48 rice germplasm lines including 24 short-grain length lines ranging from 5.5 to 6.0 mm and 24 long grain lines ranging from 11.0 to 12.6 mm were procured from Rice Biodiversity Division of Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.), India (Table 1).

Table 1 List of rice landraces used in the study and their origin

S. No.	Accession Name	S. No.	Accession Name
1	Lokti Machhi	25	Farsa phool
2	Atma Sital	26	Jay Bajrang
3	Lokti Machhi	27	Gilas
4	ADT:27	28	Khatia pati
5	Anjanina	29	Mani
6	Kanak Jira	30	Khatriya pati
7	Jhumera	31	Girmit
8	Kakeda (I)	32	Lanji
9	Dubraj II	33	Banreg
10	Bhulau	34	Ruchi
11	Rani kajar	35	Safed luchai
12	Sundar mani	36	Kanthi deshi
13	Bhado kanker	37	Piso III
14	Jhumarwa	38	Kakdi
15	Bishnu	39	Gajpati
16	Basa Bhog	40	Gadur sela
17	Krishna Bhog	41	Aadan chilpa
18	Hira Nakhi	42	Unknown
19	Lokti Maudi	43	Saja chhilau
20	Kariya bodela bija	44	Parmal Safri
21	Ganja Kali	45	Safri
22	Banas KupiII	46	Narved
23	Dhangari Khusha	47	Nagbel
24	Bhaniya	48	Mudariya

2.2 Field experiments

Experimental materials were grown under field condition by following the Randomized Complete Block Design (RCBD) with three replications during the wet season of 2015. The crop was maintained under irrigated condition. Standard agronomical practices were adopted for all the treatments. Five random plants from each of the plot were taken for recording data on agro-morphological and yield characters.

2.3 Phenotyping of rice germplasm lines for grain yield and grain characteristics

The average length of randomly selected ten hulled spikelets was measured in terms of millimetres. This is grouped into the very short, short, medium, long and very long grain. The grain length/breadth ratio of randomly selected ten hulled spikelets was calculated by dividing respective length with breadth. A thousand seeds of each of the entry were taken randomly and weighed in gram. The grain yield per plant for total five plants was recorded individually after drying the seeds for 5-8 days after harvesting and averaged value were used for further analysis.

2.4 Genotyping of rice landraces based on SSR markers

Total 59 SSR markers distributed on 12 chromosomes were used in this study (<https://archive.gramene.org/markers/>) (Table 2). DNA was extracted using the CTAB method (Murray & Thompson, 1980). The reaction volume for PCR was kept 10µl. The PCR reaction was carried out on a 96-well plate with temperature profile of 94°C for 5 min (denaturation); 35 cycles of 94°C for the 50s (denaturation), 55°C for 50s (annealing) and 72°C for 1 min (extension); and 7 min of final extension at 72°C. The amplified products were size-separated by using 5% polyacrylamide gel electrophoresis and detected by Ethidium bromide stain (10µl/ 100ml) and visualized in BIO RAD Gel Doc XR+ (Sahu et al., 2017).

2.5 Statistical Analysis

The morphological data were managed using Excel 2007 (MS Excel, 2007), and the descriptive statistics and variance analysis were produced using SPSS 16.0 (IBM SPSS). Broad sense heritability was calculated by using OPSTAT. Informativeness of molecular markers and their diversity parameters viz., average number of alleles per locus, gene diversity (He), and the Polymorphic information content (PIC) values were calculated using Power Marker v3.25 (Liu & Muse, 2005).

Table 2 List of 59 microsatellite markers with their chromosome locations, number of alleles, allele size and PIC value found among 48 rice accessions.

S. No.	Marker	Amplicon Size	No. of Alleles	Chromosome No. #	PIC Value
1	RM 1	67-119	11	1	0.87
2	RM 5	94-138	6	1	0.74
3	RM11	118-151	3	7	0.49
4	RM 19	192-250	10	12	0.85
5	RM 25	121-159	6	8	0.79
6	RM 30	100-140	1	6	0.00
7	RM 104	222-238	1	1	0.00
8	RM 105	100-141	4	9	0.59
9	RM 125	105-147	3	7	0.12
10	RM 130	73-81	2	3	0.22
11	RM 132	70-85	2	3	0.50
12	RM 134	92-94	2	7	0.04
13	RM 135	100-150	7	3	0.81
14	RM 148	190-210	4	3	0.65
15	RM 152	133-157	3	8	0.64
16	RM 154	148-230	3	2	0.55
17	RM 161	154-187	3	5	0.28
18	RM 168	96-116	2	3	0.64
19	RM 171	307-347	5	10	0.77
20	RM 172	159-165	2	7	0.49
21	RM 175	80-95	2	3	0.15
22	RM 186	115-132	3	3	0.51
23	RM 201	155-350	2	9	0.48
24	RM 215	126-161	3	9	0.38
25	RM 218	100-120	4	3	0.64
26	RM 231	157-182	3	3	0.55
27	RM 234	133-163	3	7	0.26
28	RM 242	200-290	4	9	0.32
29	RM 248	75-100	4	7	0.53
30	RM 287	82-118	3	11	0.46
31	RM 316	194-216	3	9	0.55
32	RM 338	178-184	2	3	0.35
33	RM 408	112-128	2	8	0.50
34	RM 422	385-450	5	3	0.76
35	RM 431	233-261	2	1	0.25

S. No.	Marker	Amplicon Size	No. of Alleles	Chromosome No. #	PIC Value
36	RM 432	150-187	5	7	0.76
37	RM 433	216-248	2	8	0.49
38	RM 436	83-134	4	7	0.66
39	RM 447	95-146	4	8	0.72
40	RM 455	127-144	6	7	0.79
31	RM 316	194-216	3	9	0.55
32	RM 338	178-184	2	3	0.35
33	RM 408	112-128	2	8	0.50
34	RM 422	385-450	5	3	0.76
35	RM 431	233-261	2	1	0.25
36	RM 432	150-187	5	7	0.76
37	RM 433	216-248	2	8	0.49
38	RM 436	83-134	4	7	0.66
39	RM 447	95-146	4	8	0.72
40	RM 455	127-144	6	7	0.79
41	RM 468	260-350	4	3	0.72
42	RM 481	95-200	6	7	0.79
43	RM 489	248-314	3	3	0.29
44	RM 501	130-179	3	7	0.53
45	RM 517	260-287	3	3	0.57
46	RM 520	200-290	4	3	0.69
47	RM 523	130-150	3	3	0.55
48	RM 527	200-233	2	6	0.33
49	RM 545	150-230	3	3	0.53
50	RM 546	115-150	1	3	0.00
51	RM 560	237-368	3	7	0.52
52	RM 569	170-185	2	3	0.15
53	RM 22565	200-280	5	8	0.55
54	RM 22710	150-180	1	8	0.44
55	RM 3825	147-200	3	1	0.70
56	OSR-13	85-122	2	8	0.00
57	Xa- 5 S	300	1	5	0.00
58	Xa-13 Pro	290-610	1	8	0.00
59	Xa-21	800-1200	3	11	0.23

The population structure analysis was performed in STRUCTURE v2.3.4 (Earl & Von Holdt, 2012) using a burn-in period of 100,000 and MCMC replicates of 100,000. The number of groups (K) was set from 1 to 10, with five iterations. Probable structure number (K) was estimated by Evanno's method at Structure Harvester software (Evanno et al., 2005). The rice lines with membership probability of 0.80 or above were considered in a specific group whereas genotypes with membership probability of $Q < 0.80$ were considered as Admixture type (AD) (Zhang et al., 2011). TASSEL v4.3 was also used to calculate Jaccard's similarity-based Neighbour-Joining tree (Liu & Muse, 2005). XLSTAT was used for PCoA analysis based on the molecular markers.

The association mapping analysis was performed by TASSEL v2.3 (Bradbury et al., 2007) using the mixed linear model (MLM). The model MLM_Q+K, using kinship matrix and Q-matrix as the concomitant variable, was used to identify the marker-trait association. Marker trait association at $P < 0.01$ were considered significant.

3 Results

3.1 Variation parameters of yield and grain related traits

Descriptive statistics of the four yield and grain related traits are given in Table 3. Results of the study showed that grain length ranges from 5.11 mm to 11.80 mm with an average of 8.21 mm and standard deviation of 2.54 mm whereas grain L: B ratio ranges from 1.91 to 5.84 with mean 3.32 and standard deviation 1.11. Based on the grain length, rice landraces were categorized into two broad categories *i.e.*, short-grain rice and long grain rice (Table 4) whereas mean value of grain length and grain L: B ratio is given in Table 5. Grain yield per plant ranges from 15 (g) to 35.5 (g) with an average of 25.75(g) and standard deviation 7.65(g) whereas thousand-grain weight ranges from 10.35(g) to 33.65(g) with mean 23.3 (g) and standard deviation 7.65(g). Descriptive statistics revealed sufficient variation for all four traits within the studied genotypes. Coefficient of variations ranges from 30.85 to 38.58%. Analysis of variance revealed that treatments were highly significant for all four traits which showed the presence of sufficient variation for these traits (Table 6). The broad-sense heritability of the traits was $>90\%$ except for grain yield, which was only 78.61%, suggesting that there is less environmental impact on these traits.

Table 3 Descriptive statistics for GL, GL: B, GY and TSW in 48 rice germplasm accessions

Trait	Mini mum	Maxi mum	Mean	Std. Error	Std. Deviation	CV (%)	Heritability (h^2_{bs}) (%)
Grain length (GL),	5.15	11.80	8.21	0.37	2.53	30.85	99.57
Grain length:breadth ratio (GL: B)	1.91	5.84	3.32	0.16	1.11	33.41	97.71
Grain yield (GY)	15.00	35.50	25.75	1.12	7.65	36.89	78.61
Thousand seed weight (TSW)	10.35	33.65	22.03	1.23	8.50	38.58	99.93

Table 4 Germplasm categorized into very short, short, medium and long grain length

Category	Name of accession
Short grain rice germplasm	
Very short	Lokti Machhi; Atma Sital; Lokti Machhi; Anjanika; Jhumera; Kakeda (I); Dubraj II; Bhulau; Rani kajar; Bhado kanker; Jhumarwa; Bishnu; Basa Bhog; Krishna Bhog; Hira Nakhi; Lokti Maudi; Gganja Kali; Dhangari Khusha;
Short	ADT:27; Kanak Jira; Sundar mani; Kariya bodela bija; Banas KupiII; Bhaniya
Long grain rice germplasm	
Medium	Gilas; Khatia pati; Mani; Girmmit; Lanji; Safed luchi; Gajpati; Saja chhilau; Safri; Narved
Long	Farsa phool; Jay Bajrang; Khatriya pati; Banreg; Ruchi; Kanthi deshi; Piso III; Kakdi; Gadur sela; Aadan chilpa; Unknown; Parmal Safri; Nagbel; Mudariya

Table 5 Grain length and grain L:B ratio of various germplasm lines

Name of Germplasm	Grain length (mm)	Name of Germplasm	Grain shape (L: B ratio)
Jhumarwa	5.15	Basa Bhog	1.91
Rani kajar	5.20	Dubraj II	1.97
Basa Bhog	5.25	Bhulau	2.02
Dhangari Khusha	5.25	Bhado kanker	2.08
Krishna Bhog	5.25	Krishna Bhog	2.14
Lokti Machhi	5.25	Jhumarwa	2.15
Bhado kanker	5.50	Ganja Kali	2.15
Bhulau	5.55	Rani kajar	2.17
Lokti Machhi	5.65	Hira Nakhi	2.19
Atma Sital	5.70	Lokti Maudi	2.25
Ganja Kali	5.70	ADT:27	2.27
Bishnu	5.75	Kanak Jira	2.28
Jhumera	5.75	Lokti Machhi	2.33
Dubraj II	5.80	Jhumera	2.35
Kakeda (I)	5.80	Kakeda (I)	2.37
Anjanika	5.85	Anjanika	2.44
Lokti Maudi	5.85	Sundar mani	2.45
Hira Nakhi	5.90	Lokti Machhi	2.46

Name of Germplasm	Grain length (mm)	Name of Germplasm	Grain shape (L: B ratio)
Sundar mani	6.00	Bishnu	2.56
Kanak Jira	6.05	Atma Sital	2.65
Banas KupiII	6.10	Kariya bodela bija	2.73
ADT:27	6.25	Dhangari Khusha	2.76
Bhaniya	6.50	Banas KupiII	2.77
Kariya bodela bija	6.70	Saja chhilau	2.93
Khatia pati	9.70	Unknown	3.31
Mani	9.85	Farsa phool	3.44
Lanji	10.05	Bhaniya	3.51
Narved	10.10	Aadan chilpa	3.59
Gilas	10.35	Nagbel	3.63
Saja chhilau	10.40	Lanji	3.72
Girmit	10.45	Mudariya	3.81
Safed luchai	10.45	Girmit	3.87
Safri	10.45	Gadur sela	3.89
Gadur sela	10.50	Gajpati	3.98
Gajpati	10.55	Ruchi	4.04
Kakdi	10.70	Khatia pati	4.04
Ruchi	10.70	Gilas	4.06
Unknown	10.75	Safri	4.35
Parmal Safri	10.80	Kakdi	4.46
Piso III	10.80	Narved	4.49
Farsa phool	10.85	Mani	4.58
Kanthi deshi	10.90	Banreg	4.72
Aadan chilpa	10.95	Piso III	4.91
Mudariya	11.05	Jay Bajrang	4.92
Banreg	11.10	Khatriya pati	4.96
Khatriya pati	11.40	Safed luchai	5.23
Nagbel	11.45	Kanthi deshi	5.59
Jay Bajrang	11.80	Parmal Safri	5.84

Table 6 Analysis of variance (ANOVA) based on Randomized Block Design (RBD) for grain length, grain length: breadth ratio, thousand grain weight and grain yield per plant

SV	DF	Mean sum of squares			
		GL	GL: B	TGW	GY
Replication	1	0.003	37**	0.07	101465.01**
Treatment	47	12.82**	58.65**	144.58**	8,927.39**
Error	47	0.028	0.68	0.05	6130.45

Table 7 Genetic diversity parameters of molecular markers

Germplasm	Major Allele Frequency	Allele No.	Gene Diversity (H_e)	PIC
Entire collection	0.62	3.39	0.47	0.42

Figure 1 Estimation of K and population structure. Changes in ΔK value with the number of subpopulations. Figure 2 Bar plot showing the population structure of 48 rice landraces based on SSR markers at $K=2$.

3.2 Informativeness of molecular markers and their diversity parameters:

By using 59 SSR markers, 202 alleles were detected in the 48 rice lines in total. The allele number ranged from 1 to 11 per locus, with an average of 3.39. The average major allele frequency was 0.62 whereas the gene diversity (H_e) was in the range of 0.00 to 0.88 with an average of 0.47. The average PIC was 0.42 (Table 7). The polymorphic markers appear to be adequate in delineating accessions according to their lineage.

3.3 Population Structure analysis

Population structure of the 48 rice germplasm lines based on 59 molecular markers was analyzed by Bayesian model based approach. By comparing $\ln P(D)$ and Evanno's ΔK values by increasing K from 1 to 10. It was reported that $\ln P(D)$ values increased with K , with the highest log likelihood score at $K = 2$, while ΔK value was also highest at $K = 2$ (Figure 1). This indicated that 48 rice germplasm had a genetic structure of two sub-populations as shown in bar plot (Figure 2). Further, based on

the membership fractions, genotypes under different populations were categorized as pure and admixtures. The genotypes with the probability more than ≥ 0.80 score was considered as pure and less than 0.80 score as an admixture. With population inferred ancestry, 23 lines were assigned to subgroup POP1 whereas 23 lines were assigned to subgroup POP2 and two lines were assigned to admixture (AD) (Figure 2). It was observed that the genotypes of subgroup POP1 have long grain type whereas the genotypes of subgroup POP2 have short grain type.

A neighbour-joining tree was constructed based on the Jaccard's similarity coefficient and UPGMA algorithm (Figure 3). The 48 rice lines were grouped in two clusters *viz.*, cluster I and II which corresponded to POP 1 and POP 2, respectively. A scatter plot was generated based on principal coordinate analysis (PCoA) of the binary data matrix developed from the 48 rice genotypes. It demonstrated a clear pattern of two major sub-group (SG-1 and SG-2) differentiations as shown in Figure 4 based on PC1 and PC2 variability. PCoA grouped the 48 germplasm lines into two major groups (Figure 4). First two coordinates *viz.*, PCoA 1 and PCoA 2 explains 75.07% and 21.86 % variance respectively of the total variation. The result of neighbour joining tree and principal coordinate analysis was similar to result of population structure analysis which indicated that total genotypes structured into two main subgroups.

3.4 Identification of marker-trait association (MTAs) through association mapping:

Association analysis between SSR markers and four agronomic traits was carried out using the MLM model over the 48 rice germplasm lines. Marker trait association by mixed linear model (MLM) were analyzed by using molecular marker data matrix, phenotypic data, population inferred ancestry (Q-matrix) and kinship (K) matrix. Total 25 significant marker trait associations (MTAs) were recorded for four traits (Table 8) at the FDR correction level in the entire population explaining 1.02-5.27% of the phenotypic variance. Total five markers were significantly associated with grain length; four markers were associated with grain L: B ratio, 12 markers were associated with grain yield per plant and four markers were significantly associated with thousand-grain weight.

Two markers *viz.*, RM135 and RM 201 were significantly associated with three traits namely grain length, grain length: breadth ratio and thousand-grain weight. The range of phenotypic variation explained by RM135 is 65.72% (grain length: breadth ratio) to 80.19% (grain length) whereas RM201 showed 55.59% (thousand grain weight) to 68.20% (grain length). Moreover, RM 168 was significantly associated with two traits *viz.*, grain length and grain length breadth ratio whereas RM218 was significantly

Figure 3 Neighbour-joining tree for the 48 lines based on UPGMA algorithm and Jaccard's similarity coefficient

The ability to provide genetic distance measures between the genotypes that reflect pedigree relatedness ensures a more stringent evaluation of the adequacy of a marker profile data. Population of rice having high genetic variation may be precious genetic materials for augmenting the genetic base of any breeding program. Genetic diversity and variation available in the population may be quantified through molecular markers, which can be exploited in rice breeding programs. Based on grain shape used rice germplasm lines were divided in two subgroups. Most of the genotypes having long grains were come into subgroup-1

Molecular markers are promising and effective tools for measuring genetic diversity in germplasm collection and elucidating their evolutionary relationships. Using molecular marker technology, it

Table 8 Significant marker-trait associations based on MLM model

S. No.	Trait	Locus	Chr. No.	p_Marker	Rsq_Marker
1	Grain Length	RM11	3	0.0402	0.3418
2	Grain Length	RM 105	9	0.0496	0.4231
3	Grain Length	RM 135	3	2.86E-07	0.8019
4	Grain Length	RM 168	3	0.0282	0.4582
5	Grain Length	RM 201	9	1.32E-04	0.682
6	Grain L:B ratio	RM 135	3	5.27E-04	0.6572
7	Grain L:B ratio	RM 168	3	0.0116	0.4481
8	Grain L:B ratio	RM 201	9	7.34E-05	0.6056
9	Grain L:B ratio	Xa-21	3	0.0084	0.0686
10	Grain Yield per plant	RM 125	7	0.0066	0.2037
11	Grain Yield per plant	RM 132	3	0.0121	0.1317
12	Grain Yield per plant	RM 148	3	0.0339	0.1808
13	Grain Yield per plant	RM 218	3	0.0291	0.187
14	Grain Yield per plant	RM 234	7	0.043	0.1331
15	Grain Yield per plant	RM 338	3	0.048	0.0859
16	Grain Yield per plant	RM 431	1	0.0154	0.1235
17	Grain Yield per plant	RM 481	7	0.0354	0.2452
18	Grain Yield per plant	RM 517	3	0.0437	0.1325
19	Grain Yield per plant	RM 523	3	0.0127	0.1799
20	Grain Yield per plant	RM 527	6	0.0331	0.0969
21	Grain Yield per plant	RM 560	7	0.0443	0.1319
22	Thousand grain weight	RM 1	1	0.0407	0.7042
23	Thousand grain weight	RM 135	3	9.93E-06	0.6786
24	Thousand grain weight	RM 201	9	4.38E-04	0.5559
25	Thousand grain weight	RM 218	3	0.0239	0.2184

whereas short grain genotypes were present in subgroup-2. Courtois et al. (2012) has also detected two subgroups in their study population and assigned rice varieties into two groups with few admixture lines. A model-based approach by structure was implemented frequently for studying population structure analysis in rice germplasm lines by various researchers Courtois et al. (2012); Surapaneni et al. (2016) and Li et al. (2020). Neighbour joining tree and principal coordinate analysis were strongly supported the results of population structure analysis and grouped the germplasm lines into two clusters based on the grain shape. The result of the current study was supported by Zhang et al. (2011); Upadhyay et al. (2012) and Li et al. (2020), as they

were found two distinct subgroups (*i.e.* SG 1 and SG 2) within the entire rice germplasm. The overall results indicated that the whole population was divided into two different subgroups.

Understanding the basis of complex quantitative traits of economic importance is a major tactic behind the whole progress in plant breeding. Phenotypic variation in the form of changes in DNA sequence and their location in the genome have been traditionally performed using linkage mapping but during the past decade association mapping has shown great promise and power of mapping complex quantitative traits in plants as compared to traditional linkage mapping. Total 25 significant marker-trait associations were detected

for four different traits. The results indicated that genome-wide association mapping may help to better understand the architecture of these traits and to discover novel trait-related genes. Agrama et al. (2007) identified 25 marker-trait associations of which, seven were identified as explaining 20% or more of the total variation (R^2) for grain yield (RM261, RM228), kernel length (RM284), kernel length-width ratio (RM7, RM228), and total kernel weight (RM440, RM122). Association mapping in rice germplasm lines was successfully applied by Huang et al. (2011); Zhang et al. (2011); Li et al. (2011); Upadhyay et al. (2012); Surapaneni et al. (2016) and Li et al. (2020). Marker-trait associations detected through this study could be further validated and used in marker-assisted breeding for improving particular trait in any rice variety.

In this study, ample amount of variability was present which support their suitability for population structure and association mapping analysis. Rice germplasm were subdivided into two subgroups according to their grain shape which were further confirmed by neighbour joining tree and PCoA analysis. Germplasm lines which are distantly related or belongs different subgroups can be further used in the breeding program for developing hybrids. Total 25 significant marker-trait associations were detected for four different traits in which total 5 markers were significantly associated with grain length, 4 markers were associated with grain L:B ratio, 12 markers were associated with grain yield per plant and 4 markers were significantly associated with thousand grain weight, which can be further confirmed in new set of population as well as in bi-parental mapping population.

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Conflict of interest

The authors declare that they have no conflict of interest.

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