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ORIGINAL ARTICLE

Genome-wide association mapping revealed numerous novel genomic loci for grain nutritional and yield-related traits in rice (*Oryza sativa* L.) landraces

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Abstract

A core set of 190 rice landraces were used to decipher the genetic structure and to discover the chromosomal regions containing QTLs, affecting the grain micro-nutrients, fatty acids, and yield-related traits by using 148 molecular markers in this study. Landraces were categorized into three sub-groups based on population stratification study and followed by neighbor-joining tree and principal component analysis. Analysis of variance revealed abundant variations among the landraces for studied traits with less influence of environmental factors. Genome Wide Association Studies (GWAS) revealed 22 significant and consistent QTLs through marker trait association (MTAs) for 12 traits based on 2 years and pooled analysis. Out of 22 QTLs, three have been reported earlier while 19 QTLs are novel. Interestingly, 13 QTLs out of 22 were explained more than 10% phenotypic variance. Association of RM1148 and RM205 with Days to 50% flowering was comparable with flowering control genes *Ghd8/qDTH8* and *qDTH9*, respectively. Similarly, Zn content was associated with RM44, which is situated within the QTL *qZn8-1*. Moreover, significant association of RM25 with oleic acid content was closely positioned with QTL *qOle8.* Association of RM7434 with grain yield/plant; RM184 with spikelet fertility %; R3M10, R9M42 with hundred seed weight; RM536, RM17467, RM484, RM26063 with Fe content; RM44, RM6839 with Zn content are the major outcomes of this study. In addition, association of R11M23 with days to 50% flowering, panicle length and total spikelets per panicle are explained the possible occurrence of pleiotropism among these traits. Prominent rice landraces viz., Anjani (early maturity); Sihar (extra dwarf); Gangabaru (highest grain yield/plant); Karhani (highest iron content); Byalo-2 (highest zinc content) and Kadamphool (highest oleic acid) were identified through this study. The present study will open many avenues towards utilization of these QTLs and superior landraces in rice breeding for developing nutrition-rich high yielding varieties.

Keywords Association mapping · Fatty acids · Micronutrients · Qtls · Rice landraces

Introduction

Rice (*Oryza sativa* L.) has been considered as an important cereal crop for the food security of over half of the world's population (~ 3.5 billion), with Asia accounting for 90% of global rice consumption. In lower and middle-income countries, rice serves as a means of livelihood for millions of rural households and source of dietary energy and nourishment for more than 70% people (FAOSTAT 2020). However, rice is a rich source of carbohydrate but poor in some essential micro-nutrients, proteins, fats and vitamins therefore, heavy dependency on it increases the possibilities of hidden hunger among the rice consuming children and people (Descalsota et al. 2018; Zhang et al. 2014; Garcia-Oliveira et al. 2009). According to the latest estimates, about 2 billion people (26.4%) of the world are suffering from moderate to severe levels of food insecurity and hidden hunger (FAOSTAT 2020). Constant increment in the world population with limiting land resource, gives intimation to us for improvising the yield potential and nutritional quality of

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rice consequently. Therefore, efforts to combat the food deficiency and malnutrition at global level is one of the prime goals for the rice breeders. Elevating the nutritional quality of food crops with maintaining yield potential through traditional breeding, modern biotechnology and advanced genomic approaches, is one of the vital and balanced approach to overcome the problems (Descalsota et al. 2018; Norton et al. 2018; Juan et al. 2018). As rice being the most consumed food crop throughout the world, attentiveness for enhancement in their nutritive value is as much essential as yield (Descalsota et al. 2018; Huang et al. 2015). Some nutritionally enriched and high yielding rice varieties viz., CR Dhan-311, DRR Dhan-45, Chhattishgarh Zinc Rice-1, Chhattisgarh Zinc Rice-2, Zinco Rice-MS, Protezein, CG Madhuraj-55, ARC10063 were recently developed in India through traditional breeding strategies (Sarawgi et al. 2019; Pradhan et al. 2019, 2020; Sanjeeva Rao et al. 2020).

Availability of abundant genetic variability and diversity in the gene pool is the fundamental need for genetic enhancement of rice. Traditional rice landraces best adapted in their inhabitant environment possess vast genetic diversity, variability and huge number of valuable genes for nutritional, agronomic traits along with resistance to biotic and abiotic stresses (Agrama et al. 2007; Sahu et al. 2017). Several rice landraces viz., Laicha, Gathuvan, Maharaji, Bhejri, Danwar, Bora, Karangi, Chapti Gurmatiya, Red Kavuni, Kaivara Samba, Kuruvi Kar, Poongar, Kattu Yanam, Koliyal, Maappillai Samba etc. have been reported and are very popular for their high nutritional and medicinal properties in India (Rathna Priya et al. 2019; Rahman et al. 2006). The indigenous rice landraces have long been served as a source of several valuable genes, often used in genetic improvement of rice. They have also played a significant role in maintaining the food security in the light of changing climate (Del Cruz and Khush 2000).

Rice, being a model plant with fully sequenced genome provides an opportunity to use genomic approaches for studying the genetic control of traits, exploring new genes, its domestication, adaptive selection and the genetic basis of adaptation across the varying environments (Zhang et al. 2014; Agrama et al. 2007). Advancement in molecular technologies, availability of genome sequences, accessibility of genome-wide molecular markers, economical genotyping facilities, and advances in statistical analysis have made it possible to unravel the genetic basis and linkages of complex traits. In rice, mapping of grain nutritional and yield-related traits have been done with the help of linkage based mapping method (Zhang et al. 2019; Zhao et al. 2011). However, linkage mapping approach does not sample the larger pool of genetic variation that may contribute to phenotypic variation within a species. In addition to the bi-parental mapping strategies used in QTL mapping of important agronomic and nutritional traits, GWAS is widely used in the recent times (Suman et al. 2020; Haritha et al. 2020; Donde et al. 2020; Pradhan et al. 2019, 2020; Zhang et al. 2014, 2019; Norton et al. 2018; Kadam et al. 2018; Juan et al. 2018; Prasanth et al. 2017; Swamy et al. 2017; Xu et al. 2016; Lu et al. 2015; Zhao et al. 2011; Borba et al. 2010; Agrama et al. 2007). GWAS maximizes the utilization of genetic variation of unrelated individuals in the natural population for large and more representative set of loci without the additional requirement of mapping population development (Kadam et al. 2018; Swamy et al. 2017). It has been considered as a feasible approach to decipher and map the multiple loci for many traits simultaneously with high accuracy and fine resolution in crop plants. Identifying the QTLs in multi-environments may possibly provide an accurate information for gene cloning and molecular breeding (Norton et al. 2018; Pradhan et al. 2020).

In this study, we employed GWAS in a large sample of cultivated rice landraces (190 accessions) with following major objectives (1) To dissect the genetic structure and diversity of traditional rice landraces, (2) to identify the chromosomal regions containing QTLs affecting the grain micro-nutrients (Fe content and Zn content), fatty acids (palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic) and yield-related traits, (3) to select the landraces with high nutritional value and yield potential for further breeding use. Our results revealed a number of important donor landraces and novel QTLs for grain nutritional and yield-related traits that can serve as foci of future studies to characterize the molecular basis of the observed variations and for developing nutritional rich, high yielding rice varieties.

Materials and methods

Experimental plant materials

The experimental materials comprised of 188 traditional rice landraces and two improved check varieties viz., Rajeshwari (IGKV R-1) and Mahamaya (Supplementary Table 1) of Chhattisgarh, India. Rice landraces were procured from R.H. Richharia Rice Gene Bank, Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur-492012 (Chhattisgarh), India for the study.

Field layout and experimental design

Rice genotype were evaluated in field for two consecutive years (2015 and 2016) during wet season (June–September) at research farm, college of agriculture, IGKV, Raipur to acquire the precise morphological data. Plant materials were transplanted in field by following the Augmented Block Design (ABD) (Federer 1956) during both the years.

Two improved cultivars viz., Rajeshwari (IGKV R-1) and Mahamaya were used as repeated checks in each block and randomized within the blocks. Field was divided into 13 blocks and each block had 17 genotypes (15 test genotypes and two check varieties) except for block 13 which had total 10 genotypes (eight test genotypes + two checks). Each genotype was transplanted in two rows of two meter length at spacing of 0.2 m between rows and 0.15 m between plants. Distance between entries was kept 0.3 m and block to block was maintained at 0.5 m. Each block had 9.50 m in length and 2 m width (Supplementary Fig. S1). Standard agronomic practices were adopted for normal crop growth and proper expression of genotypes throughout the crop season during both years.

Phenotyping for yield-related traits and grain nutritional traits

Eleven morphological traits viz., days to 50% flowering (DFF), plant height (PH) (cm), total tillers/plant (TTP), productive tillers/plant (PTP), panicle length (PL), fertile spikelets/panicle (FSP), sterile spikelets/panicle (SSP), total spikelets/panicle (TSP), spikelet fertility percent (SF%), 100 seed weight (g) (HSW) and grain yield (g/plant) (GY) were recorded by following the Standard Evaluation System of International Rice Research Institute (IRRI), Philipines (SES IRRI 2013). The observations for grain yield and its contributing traits were recorded on ten random plants in each genotype at specific stage.

Seven nutritional traits (micronutrients and fatty acids) were also recorded in this study. Micronutrient content viz., iron (Fe), zinc (Zn) were estimated from hulled rice (brown rice) by energy dispersive x-ray fluorescence (ED-XRF) (OXFORD Instruments X-Supreme 8000, Abingdon, UK) at Indian Institute of Rice Research (IIRR), Hyderabad, India (Babu et al. 2014; Rao et al. 2014). Rough rice samples were hulled by a single pass through standard rubber roll huller (Satake Engineering Co. Ltd. Tokyo, Japan) to eliminate the mineral contamination from huller machine. Similarly, milling of hulled rice was done in a standard miller to extract the bran fractions (Satake Engineering Co. Ltd. Tokyo, Japan). Components of five fatty acids viz., palmitic acid (PA), stearic acid (SA), oleic acid (OA), linoleic acid (LA) and linolenic acid (LnA) were estimated from bran fraction by base-catalyzed trans-esterification method (Mondal et al. 2018) in Gas Chromatography (Shimadzu, Kyoto, Japan) at Nuclear Agriculture and Biotechnology Division (NA&BTD), Bhabha Atomic Research Centre (BARC), Mumbai, India. Bran of each sample was collected after milling of hulled rice in a standard miller (Satake Engineering Co. Ltd. Tokyo, Japan). Rice bran of each genotype was packed into zipper polythene bag, properly labelled and kept immediately at 4^0C to avoid the harmful activities of lipase enzyme. The machine was cleaned after every sample run to avoid sample contamination.

Analysis of statistical parameters and variation components

Analysis of Augmented Randomized Block Design was done with the help of SAS v9.4 software by following GLM procedure (SAS, Inc., Cary, NC, USA). Shapiro–Wilk test for normal distribution of observations, descriptive statistics and heritability estimates were analyzed by software WINDOSTAT v9.3. Phenotypic correlations among studied traits were estimated with the help of PAST v3.14 software (Hammer et al. 2001). Mean data (adjusted mean) of all the traits for both the years along with the pooled data were used in bio-statistical analysis and association mapping study.

Genomic DNA extraction and marker locus selection

The total genomic DNA was isolated from young leaf tissue using GenElute™ Plant Genomic DNA miniprep kit (Sigma-Aldrich, Missouri, USA) as following the manufacturer's protocol. Young leaf tissues from 10 plants of each genotype were used for DNA extraction to capture the landrace heterogeneity. The quality and quantity of DNA were estimated with a NanoDrop system (ND-1000, Thermo Scientific, USA). Total 148 molecular markers including 107 SSR markers and 41 InDel markers were used for genotyping of 190 rice landraces (Table 1). Total 106 SSR markers were adopted from '*Gramene*: a genomics and genetics resource for rice marker database' (https://www.gramene.org/markers/microsat/) in which, the panel of 50 standard SSR markers of Generation Challenge Program for rice diversity analysis was included (Table 1) and one SSR marker was manually designed for this study (Supplementary Table 2). InDel markers were taken from the published data (Shen et al. 2004).

PCR amplification and electrophoresis analysis

PCR reaction was performed in a 10 µl reaction mixture containing 4 µl (2.5 ηg/µl) template DNA, 2 µl of 5 × assay buffer, 2 mM $MgCl_2$ (Promega, Madison, USA), 0.2 µM of each forward and reverse SSR/InDel primer, 200 µM dNTPs (Roche, Indianapolis, USA) and 1.5 U of *Taq* DNA polymerase (BRIT, Mumbai, India). PCR reactions were performed in a thermal-cycler (Eppendorf, Hamburg, Germany). The amplification profile consisted of initial denaturation for 5 min at 95 °C; 10 cycles of 40 s denaturation at 95 °C, 30 s annealing at 60 °C followed by a decrement of temperature @ − 0.5 °C per cycle (for 10 cycles) and 40 s extension at 72 °C. Remaining 25 cycles were used to amplify DNA with denaturation at 94 °C for the 40 s, 40 s annealing at

Table 1 List of molecular markers (SSRs and InDels) used in the study along with their map positions and genetic diversity parameters

S. no	Marker name	Chromosome No	Map position (bp)[b]	Major allele frequency	Allele No	Gene diversity (He)	Landraces (proportion) showing > 1 alleles	PIC[a]	Marker type[c]	Availability[d]
1	RM495	1	2,15,956	0.56	8	0.64	0.01	0.6	SSR	GDB
2	RM1	1	46,35,793	0.18	17	0.9	0	0.89	SSR	GDB
3	RM283	1	48,85,915	0.43	9	0.68	0	0.63	SSR	GDB
4	RM259	1	74,45,627	0.22	10	0.84	0	0.82	SSR	GDB
5	RM312	1	91,06,659	0.42	5	0.67	0	0.62	SSR	GDB
6	RM23	1	91,06,659	0.32	6	0.77	0	0.73	SSR	GDB
7	R1M7	1	1,05,18,002	0.98	2	0.05	0.01	0.05	InDel	PBS
8	RM229	1	1,84,07,879	0.41	8	0.72	0	0.67	SSR	GDB
9	RM5	1	2,39,71,321	0.28	10	0.8	0	0.77	SSR	GDB
10	R1M30	1	2,46,60,853	0.7	2	0.42	0.05	0.33	InDel	PBS
11	RM237	1	2,83,39,373	0.37	9	0.76	0	0.73	SSR	GDB
12	RM212	1	3,30,53,493	0.64	6	0.53	0	0.49	SSR	GDB
13	RM1003	1	3,34,75,908	0.52	6	0.61	0	0.54	SSR	GDB
14	RM3403	1	3,49,95,620	0.3	22	0.85	0	0.84	SSR	GDB
15	R1M47	1	3,68,28,634	0.89	2	0.2	0.01	0.18	InDel	PBS
16	RM431	1	3,88,93,890	0.3	8	0.79	0.01	0.76	SSR	GDB
17	RM154	2	10,83,820	0.18	13	0.88	0.01	0.87	SSR	GDB
18	RM279	2	28,82,052	0.16	15	0.91	0.03	0.9	SSR	GDB
19	R2M10	2	71,07,574	0.99	2	0.01	0	0.01	InDel	PBS
20	RM53	2	77,06,705	0.28	15	0.84	0	0.82	SSR	GDB
21	RM452	2	95,63,257	0.37	7	0.73	0.01	0.69	SSR	GDB
22	RM521	2	1,08,04,792	0.49	12	0.72	0	0.7	SSR	GDB
23	R2M24	2	1,12,09,429	0.89	2	0.2	0.01	0.18	InDel	PBS
24	RM300	2	1,31,91,380	0.37	12	0.75	0.01	0.72	SSR	GDB
25	R2M26	2	1,51,04,418	0.84	2	0.27	0.01	0.23	InDel	PBS
26	RM29	2	1,74,84,665	0.5	9	0.67	0	0.62	SSR	GDB
27	R2M37	2	2,35,63,428	0.51	2	0.5	0.05	0.37	InDel	PBS
28	RM263	2	2,58,65,334	0.4	13	0.8	0	0.78	SSR	GDB
29	RM221	2	2,76,09,877	0.23	11	0.86	0	0.84	SSR	GDB
30	R2M50	2	3,04,06,330	0.88	2	0.21	0.01	0.19	InDel	PBS
31	RM60	3	1,05,852	0.37	11	0.73	0	0.69	SSR	GDB
32	RM81	3	19,45,837	0.29	9	0.78	0	0.75	SSR	GDB
33	RM489	3	43,33,680	0.48	14	0.73	0	0.71	SSR	GDB
34	R3M10	3	60,44,614	0.99	2	0.02	0	0.02	InDel	PBS
35	RM7	3	1,24,07,382	0.39	8	0.72	0	0.68	SSR	GDB
36	RM338	3	1,32,21,482	0.68	7	0.5	0	0.46	SSR	GDB

Table 1 (continued)

S. no	Marker name	Chromosome No	Map position (bp)[b]	Major allele frequency	Allele No	Gene diversity (He)	Landraces (proportion) showing > 1 alleles	PIC[a]	Marker type[c]	Availability[d]
37	R3M23	3	1,56,78,406	0.89	2	0.2	0.01	0.18	InDel	PBS
38	R3M30	3	1,98,88,471	0.96	2	0.07	0	0.07	InDel	PBS
39	RM6832	3	2,24,00,917	0.66	11	0.53	0	0.5	SSR	GDB
40	R3M37	3	2,64,74,984	0.63	3	0.51	0.03	0.44	InDel	PBS
41	RM55	3	2,82,31,692	0.39	9	0.75	0	0.72	SSR	GDB
42	R3M53	3	3,24,85,490	0.64	2	0.46	0.02	0.36	InDel	PBS
43	RM514	3	3,52,81,232	0.27	12	0.84	0	0.83	SSR	GDB
44	R4M13	4	81,15,751	0.89	2	0.2	0	0.18	InDel	PBS
45	RM307	4	86,10,617	0.25	12	0.84	0	0.82	SSR	GDB
46	R4M17	4	1,16,36,507	0.54	2	0.5	0.04	0.37	InDel	PBS
47	RM16623	4	1,24,31,839	0.09	30	0.95	0	0.95	SSR	GDB
48	R4M30	4	1,81,72,382	0.56	2	0.49	0.01	0.37	InDel	PBS
49	RM471	4	1,88,24,746	0.28	10	0.79	0	0.75	SSR	GDB
50	R4M43	4	2,45,94,268	0.87	2	0.23	0	0.2	InDel	PBS
51	RM1388	4	2,50,35,173	0.09	28	0.95	0	0.95	SSR	GDB
52	RM241	4	2,68,57,374	0.16	19	0.92	0.01	0.91	SSR	GDB
53	R4M50	4	2,89,89,250	0.74	2	0.39	0.02	0.31	InDel	PBS
54	RM17467	4	3,06,74,329	0.39	24	0.8	0.04	0.79	SSR	GDB
55	RM124	4	3,47,39,636	0.49	5	0.64	0.01	0.57	SSR	GDB
56	RM507	5	1,02,742	0.39	6	0.73	0	0.69	SSR	GDB
57	RM413	5	22,12,736	0.28	13	0.86	0	0.84	SSR	GDB
58	RM3322	5	42,62,731	0.27	14	0.83	0	0.81	SSR	GDB
59	R5M13	5	59,50,163	0.87	2	0.22	0	0.2	InDel	PBS
60	R5M30	5	2,14,47,905	0.64	2	0.46	0.01	0.35	InDel	PBS
61	RM178	5	2,51,01,829	0.55	6	0.64	0	0.6	SSR	GDB
62	RM87	5	2,68,48,154	0.59	7	0.61	0	0.58	SSR	GDB
63	RM161	5	2,68,48,154	0.5	8	0.68	0.01	0.64	SSR	GDB
64	RM334	5	2,73,42,022	0.15	13	0.89	0	0.88	SSR	GDB
65	RM133	6	4,41,616	0.75	4	0.41	0	0.38	SSR	GDB
66	RM510	6	28,31,443	0.61	5	0.56	0	0.5	SSR	GDB
67	RM585	6	37,22,966	0.43	9	0.76	0.01	0.74	SSR	GDB
68	RM217	6	42,12,289	0.14	21	0.92	0.01	0.91	SSR	GDB
69	RM253	6	54,25,408	0.23	15	0.88	0	0.87	SSR	GDB
70	RM2126	6	59,05,238	0.28	15	0.8	0	0.77	SSR	GDB
71	RM7488	6	69,98,987	0.27	19	0.84	0.01	0.82	SSR	GDB

Table 1 (continued)

S. no	Marker name	Chromosome No	Map position (bp)[b]	Major allele frequency	Allele No	Gene diversity (He)	Landraces (proportion) showing > 1 alleles	PIC[a]	Marker type[c]	Availability[d]
72	R6M14	6	74,36,963	0.68	2	0.43	0.01	0.34	InDel	PBS
73	RM3330	6	1,10,64,125	0.22	10	0.87	0	0.86	SSR	GDB
74	RM7434	6	2,39,34,663	0.37	7	0.71	0	0.66	SSR	GDB
75	RM7434	6	2,39,34,663	0.25	8	0.79	0.01	0.76	SSR	GDB
76	RM162	6	2,40,35,491	0.41	8	0.71	0.01	0.66	SSR	GDB
77	R6M44	6	2,57,35,724	0.89	2	0.2	0.01	0.18	InDel	PBS
78	RM454	6	2,85,32,453	0.44	5	0.65	0	0.58	SSR	GDB
79	RM481	7	57,35,196	0.26	16	0.88	0.04	0.87	SSR	GDB
80	R7M7	7	65,81,196	0.76	2	0.36	0	0.3	InDel	PBS
81	RM501	7	67,79,215	0.57	14	0.61	0	0.56	SSR	GDB
82	RM125	7	67,79,215	0.46	7	0.68	0	0.64	SSR	GDB
83	R7M20	7	1,12,79,013	0.5	2	0.5	0.01	0.38	InDel	PBS
84	RM418	7	1,81,32,231	0.37	14	0.79	0	0.77	SSR	GDB
85	RM11	7	1,92,56,914	0.38	9	0.75	0	0.71	SSR	GDB
86	RM455	7	2,23,50,593	0.65	3	0.48	0	0.39	SSR	GDB
87	R7M37	7	2,36,24,859	0.99	2	0.03	0.01	0.03	InDel	PBS
88	RM505	7	2,51,01,758	0.42	4	0.65	0	0.58	SSR	GDB
89	RM234	7	2,54,72,688	0.52	10	0.66	0	0.62	SSR	GDB
90	RM118	7	2,93,39,845	0.36	7	0.73	0	0.69	SSR	GDB
91	RM408	8	1,25,275	0.42	6	0.67	0	0.61	SSR	GDB
92	RM407	8	5,22,394	0.45	12	0.74	0	0.71	SSR	GDB
93	RM152	8	6,82,963	0.36	8	0.78	0	0.75	SSR	GDB
94	RM22254	8	6,91,385	0.44	22	0.69	0	0.64	SSR	GDB
95	RM44	8	21,14,843	0.45	9	0.67	0	0.62	SSR	GDB
96	RM25	8	21,14,843	0.38	9	0.78	0	0.75	SSR	GDB
97	RM1148	8	37,39,008	0.39	8	0.74	0	0.7	SSR	GDB
98	RM6999	8	39,84,260	0.14	19	0.91	0	0.91	SSR	GDB
99	RM3481	8	91,35,139	0.22	12	0.88	0	0.86	SSR	GDB
100	RM3395	8	1,02,93,664	0.58	6	0.59	0	0.54	SSR	GDB
101	R8M23	8	1,34,82,749	0.95	2	0.2	0	0.01	InDel	PBS
102	R8M33	8	2,07,97,429	0.65	2	0.45	0.01	0.35	InDel	PBS
103	RM284	8	2,11,42,348	0.62	5	0.57	0	0.53	SSR	GDB
104	RM433	8	2,55,90,576	0.29	11	0.8	0	0.77	SSR	GDB
105	RM447	8	2,65,46,992	0.41	10	0.66	0	0.6	SSR	GDB
106	RM316	9	12,71,123	0.32	11	0.82	0	0.8	SSR	GDB

Table 1 (continued)

S. no	Marker name	Chromosome No	Map position (bp)[b]	Major allele frequency	Allele No	Gene diversity (He)	Landraces (proportion) showing > 1 alleles	PIC[a]	Marker type[c]	Availability[d]
107	R9M10	9	45,15,585	0.74	2	0.38	0	0.31	InDel	PBS
108	R9M20	9	93,78,728	0.89	2	0.2	0.01	0.18	InDel	PBS
109	RM105	9	1,18,07,446	0.58	5	0.58	0	0.53	SSR	GDB
110	RM6839	9	1,45,66,026	0.7	5	0.46	0	0.42	SSR	GDB
111	R9M30	9	1,46,47,925	0.84	2	0.27	0	0.23	InDel	PBS
112	RM257	9	1,77,19,660	0.21	12	0.85	0.01	0.83	SSR	GDB
113	R9M42	9	1,91,15,701	0.99	2	0.02	0	0.02	InDel	PBS
114	RM215	9	2,11,89,110	0.35	7	0.75	0	0.71	SSR	GDB
115	RM205	9	2,27,20,624	0.39	18	0.77	0.01	0.74	SSR	GDB
116	RM474	10	18,18,800	0.42	19	0.78	0	0.77	SSR	GDB
117	R10M10	10	52,07,502	0.52	2	0.5	0.06	0.37	InDel	PBS
118	RM271	10	53,52,766	0.32	10	0.81	0	0.79	SSR	GDB
119	R10M17	10	90,04,292	0.97	2	0.06	0	0.06	InDel	PBS
120	RM184	10	1,63,58,895	0.32	7	0.75	0	0.7	SSR	GDB
121	R10M30	10	1,71,91,366	0.54	2	0.5	0.04	0.37	InDel	PBS
122	RM171	10	1,90,48,795	0.3	12	0.81	0	0.78	SSR	GDB
123	R10M40	10	1,96,17,284	0.15	2	0.1	0	0.01	InDel	PBS
124	RM484	10	2,10,66,729	0.46	5	0.61	0	0.53	SSR	GDB
125	RM228	10	2,22,43,157	0.33	21	0.85	0.01	0.84	SSR	GDB
126	RM591	10	2,28,99,617	0.17	27	0.91	0	0.9	SSR	GDB
127	RM590	10	2,30,43,156	0.33	7	0.78	0	0.75	SSR	GDB
128	RM26063	11	22,56,991	0.18	20	0.9	0.01	0.89	SSR	GDB
129	RM552	11	48,43,013	0.12	17	0.91	0	0.91	SSR	GDB
130	RM536	11	89,85,132	0.27	9	0.82	0	0.8	SSR	GDB
131	RM287	11	1,67,67,319	0.29	9	0.82	0	0.8	SSR	GDB
132	RM26771	11	1,73,59,392	0.41	18	0.75	0.01	0.72	SSR	GDB
133	RM21	11	1,83,18,826	0.2	16	0.88	0	0.87	SSR	GDB
134	R11M23	11	2,01,48,709	0.89	2	0.2	0	0.18	InDel	PBS
135	RM26981	11	2,16,89,935	0.09	38	0.96	0	0.96	SSR	GDB
136	R11M40	11	2,48,11,386	0.71	2	0.41	0.04	0.33	InDel	PBS
137	RM144	11	2,82,81,693	0.31	13	0.84	0	0.83	SSR	GDB
138	RiceSSR PODIP	12	6,23,664	0.26	6	0.81	0	0.78	SSR	MD
139	RM19	12	31,85,384	0.23	11	0.86	0	0.85	SSR	GDB
140	RM27601	12	35,53,566	0.48	16	0.71	0	0.68	SSR	GDB
141	R12M10	12	42,89,075	0.99	2	0.02	0.02	0.02	InDel	PBS

Table 1 (continued)

S. no	Marker name	Chromosome No	Map position (bp)[b]	Major allele frequency	Allele No	Gene diversity (He)	Landraces (proportion) showing > 1 alleles	PIC[a]	Marker type[c]	Availability[d]
142	RM277	12	88,26,555	0.67	6	0.5	0	0.45	SSR	GDB
143	RM7102	12	1,32,11,325	0.38	8	0.72	0.01	0.68	SSR	GDB
144	R12M27	12	1,73,79,856	0.77	2	0.35	0	0.29	InDel	PBS
145	RM2972	12	1,91,38,384	0.45	7	0.71	0	0.67	SSR	GDB
146	RM519	12	1,99,03,791	0.36	7	0.78	0	0.75	SSR	GDB
147	R12M33	12	2,00,06,587	0.55	2	0.5	0	0.37	InDel	PBS
148	R12M43	12	2,61,02,497	0.89	2	0.19	0.01	0.17	InDel	PBS
	Mean			0.49	8.75	0.62	0.01	0.58		

[a]Polymorphic Information Content

[b]Physical locations of SSR markers were taken from *Gramene*: a genomics and genetics resource for rice marker database' (https://www.gramene.org/markers/microsat/) whereas physical locations of InDel markers were accessed from MSU Rice Genome Annotation Project (https://rice.plantbiology.msu.edu/analyses_search_blast.shtml) based on the BAC accession ID given in the published study of Shen et al. (2004)

[c]SSR: Simple Sequence Repeats, InDel: Insertion Deletion,

[d]GDB: adopted from '*Gramene*: a genomics and genetics resource for rice marker database' (https://www.gramene.org/markers/microsat/), PBS: adopted from the published study of Shen et al. (2004), MD: manually designed for this study

55 °C and amplification for 40 s at 72 °C. After that, the final extension was carried out at 72 °C for 7 min. PCR products of SSR markers were resolved on a capillary gel electrophoresis system (Qiagen, Hamburg, Germany). Whereas PCR products of InDel markers were resolved on 2.5% agarose gel (containing 1.75% Methaphor® agarose + 0.75% normal agarose) (Sigma-Aldrich, Missouri, USA). Initially, allele size of all the InDel primer pairs was checked on capillary gel electrophoresis (Qiagen, Hamburg, Germany).

Genetic diversity parameters and informativeness of the molecular markers

Marker utility information and genetic diversity parameters such as the number of alleles per locus, Landraces (proportion) showing > 1 alleles, major allele frequency, gene diversity and the polymorphic information content (PIC) for each marker were estimated by using software POWERMARKER v3.25 (Liu and Muse 2005).

Analysis of population structure and genetic differentiation among the rice landraces

Population structure of the 190 rice landraces based on 148 molecular markers was analyzed by following the Bayesian statistical model in the program STRUCTURE v2.3.4 (Pritchard et al. 2000). The run-length was specified as 100,000 burning period followed by 100,000 MCMC replication with an admixture model and correlated allele frequencies for inferring the number (K) of subpopulations, ranging from $K = 1$ to 10. The analysis was performed using five replicates per K value. The result generated from structure analysis was used to identify optimum K value based on Ln$P(D)$ and Evanno's ΔK method in STRUCTURE HARVESTER (Earl 2012). The genotypes with the probability of more than ≥ 0.80 scores (inferred ancestry) were considered as pure and less than 0.80 scores (inferred ancestry) as an admixture (Supplementary Table 1).

Genetic differentiation among subpopulations was estimated by analysis of molecular variance (AMOVA) using software GenAlEx 6.503 (Smouse et al. 2015). Unrooted neighbor joining (NJ) tree based on Jaccard similarity coefficient and UPGMA algorithm was constructed by SAS v9.4 software (SAS, Inc., Cary, NC, USA). A principal component analysis (PCA) for assessing genetic diversity among landraces was conducted based on variance–covariance matrix using PAST v3.15 software (Hammer et al. 2001). The principal component axes were drawn according to eigenvalues. The coefficients of kinship between pairs of accessions were determined using the data from above, 148 markers in TASSEL v3.0 software (Bradbury et al. 2007).

Genome-wide association studies (GWAS) for grain nutritional and yield-related traits

Genome-wide association mapping was performed using the TASSEL v3.0 software (Bradbury et al. 2007) by following the mixed linear model (MLM) using 50,000-time permutations for the correction of multiple testing (Pritchard et al. 2000). MLM analysis was performed with molecular marker data, phenotypic data, population inferred ancestry (*Q* matrix) and kinship (*K*) matrix (Yu et al. 2006). Significant marker-trait associations were identified by considering the Bonferroni threshold and it was calculate by standard method in MSExcel (Moran, 2003). Bonferroni threshold level for this study was 1/148 = 0.0067, where 148 is the number of markers used for association tests for each trait in this study. Therefore, QTLs with the probability *p* value 0.0067 or less than 0.0067 were considered as significant.

Results

Phenotypic variation in grain yield and nutritional traits among the rice landraces

The factors imparting variance in the population for studied nutritional and yield-related traits laid out in augmented block design were duly analyzed under unidirectional elimination of heterogeneity separately for blocks and treatments respectively during both years (Supplementary Table 3a, b). The critical differences (CD) per se briefed the response of the augmented treatments for all the studied traits. The CD of controls confirms the uniform contrast expression of traits across the blocks. CD of augmented treatment between the blocks, being higher with respect to within the blocks for all the traits during both years, implicitly inferred that environmental heterogeneity was insignificant within the blocks for all the traits. Comparable CD for the augmented treatments within block and between control and augmented treatment for all the traits during both years also infer lesser microenvironment effect within the blocks.

Under block adjustment, variance due to augmentation, control versus augmentation and block per se were found to be significant for most of the traits during both years. It implies that considerable environmental interaction effect contributes to the overall variation for studied traits. Under heterogeneity elimination for the treatments and control + control versus augmentation showed significant variance for most of the traits during both the years. The controls were found to be significant and equal mean sum of squares (MSS) under both cases heterogeneity elimination (adjustment) and non-adjustment for most of the traits during both years. It implied that some fixed effect was making the controls to stabilize the performance. The MSS of the treatments was found to be significant and comparable both under adjustment of blocks and treatment per se for most of the traits during both years, which was an intriguing result. Significant variation in the population for most of the studied traits due to genotypic effect (treatment) under both the cases of heterogeneity elimination and equivalent MSS of the treatments indicated towards the sound genetic component of the respective trait (Supplementary Table 3a, b). The residual MSS was observed fewer for all the traits during both years, indicated the minor difference in phenotypic variance and genotypic variance of the traits. It implied the less environmental contribution in the total variance. Lower value of the residual MSS under both systems of heterogeneity elimination during both the years confirmed the precision of the experimental set-up, methodology and research findings.

Testing of normal distribution by Shapiro–Wilk test of pooled data revealed that panicle length ($W = 0.995$, $p = 0.804$), grain yield/plant ($W = 0.988$, $p = 0.117$), stearic acid ($W = 0.986$, $p = 0.075$) and linolenic acid ($W = 0.991$, $p = 0.327$) were following the normal distribution pattern (Supplementary Fig. 2). This indicated the quantitative nature of traits which are controlled by many genes. Mean, range and standard deviation of all rice landraces for all the traits revealed a wide range of phenotypic variation. More than 10% coefficient of variation among the landraces for all the traits were recorded except for palmitic acid, oleic acid, and linoleic acid. Heritability in the broad sense was recorded very high (> 70%) for all the traits except the oleic acid and linoleic acid which showed moderate heritability (Table 2).

Phenotypic correlation analysis

Present study revealed significant ($p < 0.05$) positive correlations of DFF with PH, PL, FSP, SSP, TSP and GY; PH with PL, FSP, SSP, TSP and GY; TTP with Fe and Zn; PL with FSP, SSP, TSP and GY; TSP with GY; Fe content with Zn content during both years and pooled analysis. Furthermore, significant ($P < 0.05$) negative correlations were found between DFF with TTP, PTP, Fe, Zn and SA; PH with Fe content; PL with PA; FSP with HSP, Fe and Zn content; SSP with SF%, Fe and Zn content; GY with Fe and Zn content; PA with OA and LA; OA with LA (Fig. 1).

Genetic diversity parameters and informativeness of the molecular markers

Total 190 rice landraces were used for genotyping with 148 molecular markers (107 SSRs and 41 InDels markers). A total of 1294 bands (alleles) were amplified among tested landraces. The number of alleles per locus varied from 2 to 38 (RM26981) with an average of 8.7 alleles per locus.

Table 2 Summary statistics and genetic parameters of variation for grain nutritional and yield attributing traits in rice landraces

Traits	Year	Mean	Range		Standard deviation	Coefficient of variation (%)	Heritability (bs)
			Minimum	Maximum			
Days to 50% Flowering (DFF)	2015–2016	112.41	75.45	131.25	13.56	12.06	0.97
	2016–2017	111.94	72.00	135.00	12.52	11.18	0.98
	Pooled	112.18	73.73	133.63	12.81	11.42	0.98
Plant height (cm) (PH)	2015–2016	148.66	82.20	175.46	21.91	14.74	0.96
	2016–2017	143.84	86.00	172.00	19.76	13.74	0.94
	Pooled	146.25	85.10	170.26	20.42	13.96	0.95
Total Tiller/plant (TTP)	2015–2016	9.87	5.60	19.80	2.81	28.47	0.79
	2016–2017	10.06	5.00	19.00	2.56	25.45	0.74
	Pooled	9.97	6.20	18.50	2.46	24.67	0.77
Prod Tiller/plant (PTP)	2015–2016	9.52	4.96	17.86	2.52	26.47	0.75
	2016–2017	9.33	4.00	18.00	2.60	27.87	0.75
	Pooled	9.43	5.38	16.83	2.29	24.28	0.75
Panicle length (cm) (PL)	2015–2016	25.60	15.59	35.35	3.06	11.95	0.92
	2016–2017	25.01	17.00	34.05	3.09	12.36	0.91
	Pooled	25.31	16.97	34.70	2.92	11.54	0.92
Fertile spikelets/plant (FSP)	2015–2016	133.18	28.31	353.72	53.64	40.28	0.99
	2016–2017	132.98	23.31	359.72	54.47	40.96	0.99
	Pooled	133.08	25.81	356.72	53.90	40.50	0.99
Sterile spikelets/plant (SSP)	2015–2016	44.25	5.91	114.65	21.90	49.49	0.98
	2016–2017	45.83	4.58	120.65	23.53	51.34	0.99
	Pooled	45.04	6.58	117.65	22.49	49.93	0.99
Total spikelets/plant (TSP)	2015–2016	177.43	48.82	468.37	65.15	36.72	0.99
	2016–2017	178.81	39.82	480.37	66.79	37.35	0.98
	Pooled	178.12	44.32	474.37	65.71	36.89	0.99
Spikelet fertility % (SF%)	2015–2016	74.68	40.65	92.30	9.69	12.98	0.96
	2016–17	74.03	35.49	94.68	10.59	14.31	0.95
	Pooled	74.36	39.16	92.19	9.99	13.43	0.96
100 seed weight (g) (HSP)	2015–2016	2.49	1.25	3.67	0.58	23.29	0.98
	2016–2017	2.50	1.03	3.67	0.58	23.20	0.98
	Pooled	2.50	1.14	3.59	0.57	22.80	0.98
Grain Yield (g/plant)(GY)	2015–2016	30.01	10.63	43.83	6.08	20.26	0.95
	2016–2017	29.84	10.78	43.46	6.19	20.74	0.96
	Pooled	29.92	12.24	41.83	5.85	19.55	0.96
Iron content (ppm) (Fe)	2015–2016	11.31	7.25	22.30	2.43	21.49	0.95
	2016–2017	11.26	7.00	23.21	2.44	21.67	0.95
	Pooled	11.28	7.13	22.76	2.43	21.54	0.95
Zinc content (ppm)(Zn)	2015–2016	25.19	14.80	44.05	4.34	17.23	0.94
	2016–2017	25.14	14.70	44.30	4.36	17.34	0.95
	Pooled	25.16	14.75	44.18	4.35	17.29	0.95
Palmitic acid (%) (PA)	2015–2016	16.59	12.49	20.44	1.29	7.78	0.71
	2016–2017	18.46	11.14	23.25	2.32	12.57	0.93
	Pooled	17.53	12.59	20.25	1.34	7.64	0.82
Stearic acid (%) (SA)	2015–2016	1.68	0.56	2.81	0.36	21.43	0.88
	2016–2017	1.91	1.05	2.09	0.43	22.51	0.93
	Pooled	1.80	1.01	2.90	0.29	16.11	0.91
Oleic acid (%) (OA)	2015–2016	44.33	40.29	50.25	1.62	3.65	0.35
	2016–2017	43.39	38.22	52.66	2.86	6.59	0.67
	Pooled	43.86	37.60	49.17	1.83	4.17	0.51

Table 2 (continued)

Traits	Year	Mean	Range		Standard deviation	Coefficient of variation (%)	Heritability (bs)
			Minimum	Maximum			
Linoleic acid (%) (LA)	2015–2016	36.11	32.33	41.28	1.33	3.68	0.31
	2016–2017	35.12	28.00	43.47	3.46	9.85	0.69
	Pooled	35.61	31.55	44.67	1.88	5.28	0.50
Linolenic acid (%) (LnA)	2015–2016	1.28	0.49	2.07	0.21	16.41	0.98
	2016–2017	1.07	0.42	1.70	0.25	23.36	0.98
	Pooled	1.18	0.55	1.83	0.17	14.41	0.98

Polymorphic Information Content (PIC) value ranged from 0.01 (R2M10, R8M23, R10M40) to 0.96 (RM26981) with an average of 0.58. The percentage of landraces that were found to exhibit more than one allele ranged from 0 to 0.06 (R10M10) with an average of 0.01 which showed that several loci did not detect any level of heterozygosity. Expected heterozygosity or gene diversity (H_e) computed according to Nei (1973) varied from 0 to 0.96 (RM26981) with an average of 0.62. Genotype number ranged from 1 to 41 (RM26981) with an average of 9.77. The major allele frequency ranged from 0.09 (RM1388) to 1 (R10M40) with an average of 0.49 (Table 1).

Population structure and genetic diversity analysis of rice landraces

Based on both Ln$P(D)$ and Evanno's ΔK values, optimum K value was found as three (Supplementary Fig. 3). This indicated that 190 rice landraces had a genetic structure of three sub-populations viz., SG-1, SG-2 and SG-3. The SG1, SG2 and SG3 had total 77 (40.52%), 64 (33.68%) and 22 (11.57%) pure genotypes whereas had eight, five and 14 admixture genotypes (14.21%), respectively (Supplementary Table 1 and Fig. 2).

Population-specific mean *Fst* value of the three subgroups SG-1, SG-2 and SG-3 was recorded 0.10, 0.22 and 0.17, respectively, with an average of 0.165. The mean alpha value of the population was 0.043, indicating the less number of admixture type genotypes. The allele frequencies (divergence among sub-populations based on net nucleotide distance) were 0.09 between SG-1 and SG-2; 0.16 between SG-1 and SG-3 and 0.20 between SG-2 and SG-3. Further, analysis of molecular variance (AMOVA) detected a significant genetic differentiation ($\phi_{PT} = 0.162$, $p = 0.000$) between three sub-populations. Total molecular variance was partitioned into two, of which, 16% explained variation among populations and the remaining 84% explained variation within the populations (Supplementary Table 4). Furthermore, the results of the unrooted neighbor-joining tree (NJ tree) and principal component analysis (PCA) were coinciding with the result of model based population structure analysis. NJ tree (Fig. 3) and PC scatter diagram (Fig. 4) grouped the rice landraces into three main clusters/groups. Genotypes of different clusters/groups are well matched with the genotypes of different sub groups of population structure analysis.

Effect of Mixed Linear Model (MLM) for controlling type-1 error

Observed versus expected p values for each marker-trait association were plotted in the quantile–quantile plot (Q–Q plot) to assess the control of type-I errors. Uniform distributions of the observed and expected p values for all traits were observed during both the years. The false positives were well controlled by MLM model which indicates the robustness of results obtained through statistical analysis (Fig. 5a–c).

Identification and mapping of QTLs for grain yield and nutritional traits through genome-wide association studies

Genome-wide association studies (GWAS) revealed total 22 consistent and significant QTLs for 12 traits (out of 18 traits investigated) based on 2 years and pooled analysis (Table 3). Of which, total 14 significant and consistent QTLs were detected for grain yield and attributing traits during both years and pooled analysis which are further explained here. DFF was significantly associated with RM1148 (chr-8), RM205 (chr-9), and R11M23(chr-11) in all three conditions. Association of RM118 (chr-7) with TTP was significant and consistent. Two InDel markers R11M23 (chr-11) and R12M43 (chr-12) were significantly linked with PL. TSP, an important yield contributing traits was significantly associated with three markers, RM234 (chr-7), R11M23 (chr-11) and R1M47 (chr-1). Furthermore, it was found that SSR markers RM234 (chr-7) significantly associated with fertile spikelets/panicle during both years and pooled analysis. Another marker RM184(chr-10) was also found to have association with spikelet fertility%. HSW was

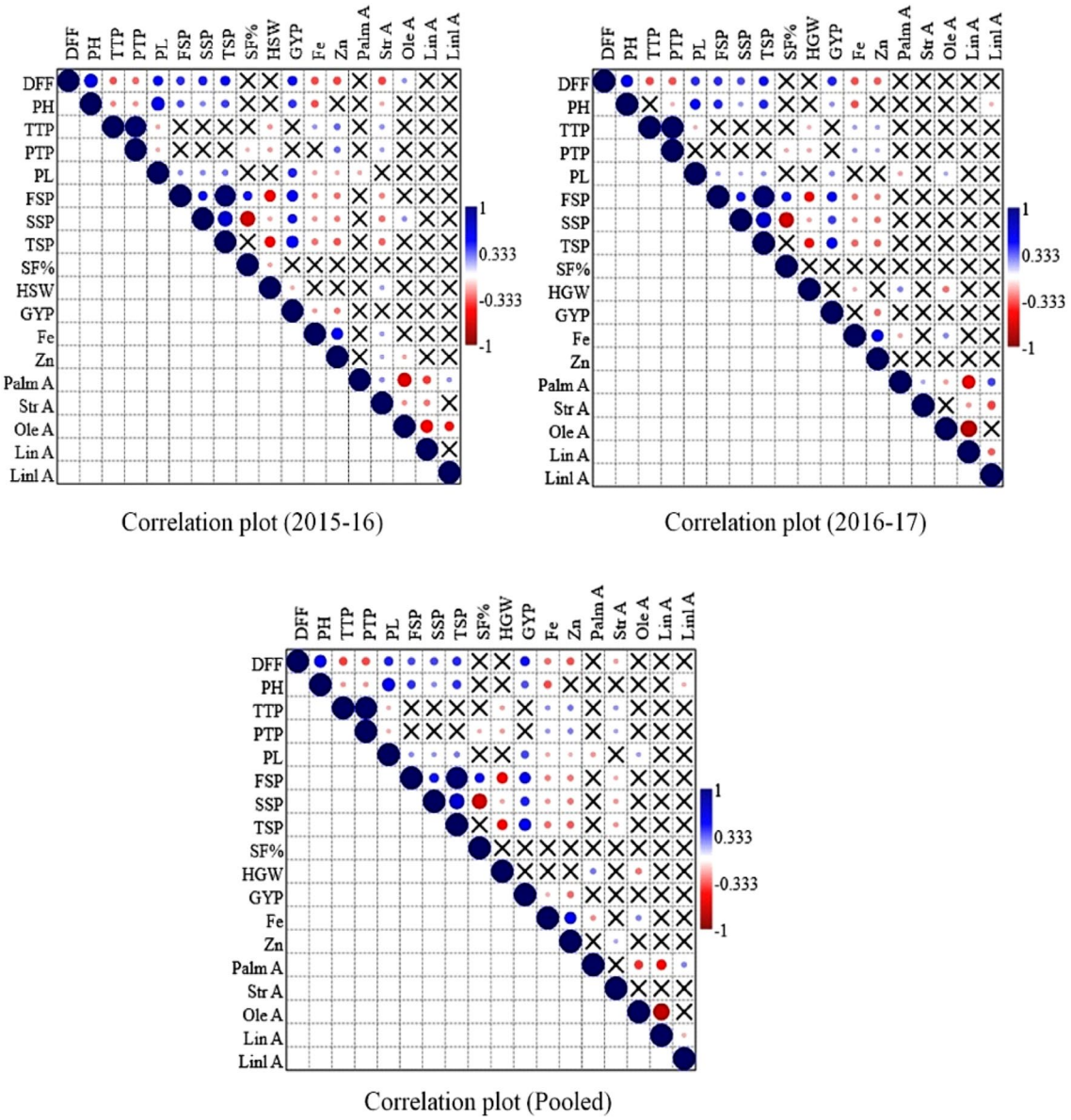


Fig. 1 Correlations among the grain nutritional and yield related traits in rice landraces evaluated in two years along with pooled analysis. The blue colored circle shows the positive association, red colored circle indicates the negative association and the intensity of the color indicates degree of association (as explained in the right side of the each diagram). Cross sign shows no correlation between traits. The test of significance was calculated based on 0.05 probability level. *DFF* days to 50% flowering, *PH* plant height, *TTP* total tillers/plant, *PTP* productive tillers/plant, *PL* panicle length, *FSP* fertile spikelets/panicle, *SSP* sterile spikelets/panicle, *TSP* total spikelets/panicle, *SF%* spikelet fertility percent, *HSW-100* seed weight, *GY* grain yield, *Fe* iron content, *Zn* zinc content, *PA* palmitic acid, *SA* stearic acid, *OA* oleic acid, *LA* linoleic acid and *LnA* linolenic acid

significantly associated with two markers R3M10 (chr-3) and R9M42 (chr-9) in all the three tested conditions. It was found that grain yield per plant was significantly associated with RM7434 (chr-6) with more than 10% explained phenotypic variance (R^2-value). No significant and consistent associations were detected for PH and PTP. Interestingly, the InDel marker R11M23 was significantly associated with three traits viz., DFF, PL and TSP in this study.

Similarly, totally eight significant and consistent QTLs were detected for studied grain nutritional traits. GWAS based marker-trait association analysis revealed four common markers (RM536 at chr-11, RM17467 at chr-4, RM484 at chr-10 and RM26063 at chr-11) for grain Fe content; two (RM44 at chr-8 and RM6839 at chr-9) for grain Zn content; one (RM25 at chr-8) for oleic acid content and one (RM495 at chr-1) for linolenic acid content based on 2 years and

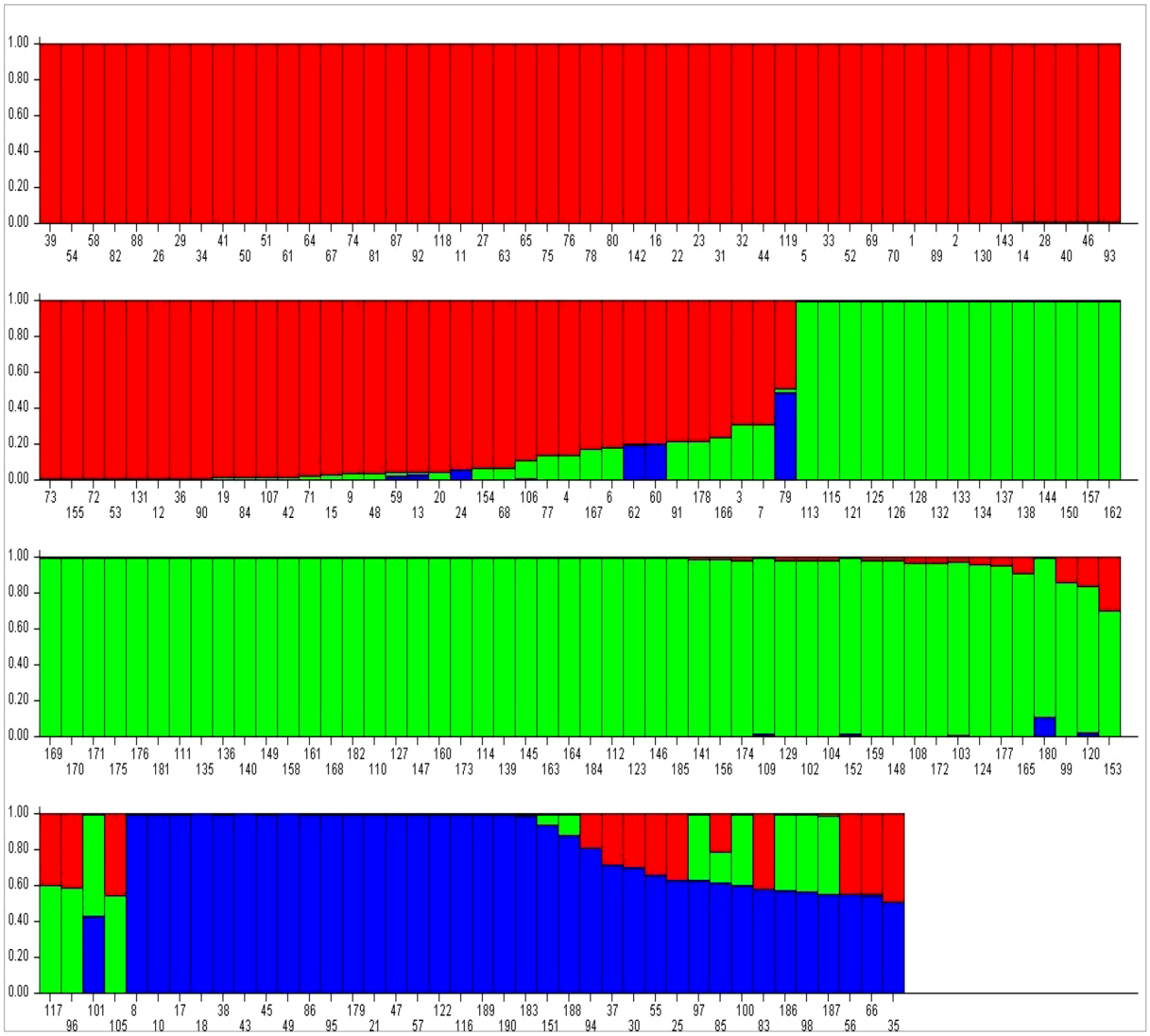


Fig. 2 The bar plot presentation the genetic structure of 190 rice landraces based on population stratification at three sub-population level ($K=3$). Red colored bar shows the genotypes of Sub Group-1, green colored bar shows the genotypes of Sub Group-2 and Blue colored bar shows the genotypes of Sub Group-3. Bars with mixed colored represent the mixed ancestry of genotypes (admixtures). *X* axis shows the serial number of genotypes and *Y* axis shows the membership proportion of genotype on respective sub-group

pooled analysis. Two markers RM536 and RM26063 linked with Fe content on chromosome-11 were closely linked and were 6.73 Mb apart from each other. Similarly, Zn content was associated with RM44, which is closely spaced with QTL *qZn8-1*. Moreover, significant QTL for oleic acid content was found around RM25 that was closely located with QTL *qOle8* in chromosome 8. None of the markers were significantly and consistently associated with linoleic acid, palmitic acid and stearic acid.

Interestingly, 13 QTLs out of 22, explained more than 10% phenotypic variation (R^2 value) for respective traits. Of which, three (RM536, RM1746, RM484) for Fe content; two (RM1148 and RM205) for DFF and one for PL(R12M43), FSP(RM234), TSP (RM234), SF% (RM184), GY (RM7434), Zn(RM44), OL(RM25) and LnL (RM495) were found major QTLs in the present study (Table 3). Of the 22 identified associated QTLs, 19 are novel and reported first time in this study.

Discussion

Since rice is the staple food crop of the developing world, a lot of efforts are being made to develop nutritionally enriched high yielding genotypes. The first pre-requisite for such breeding program is to explore the genetic variation

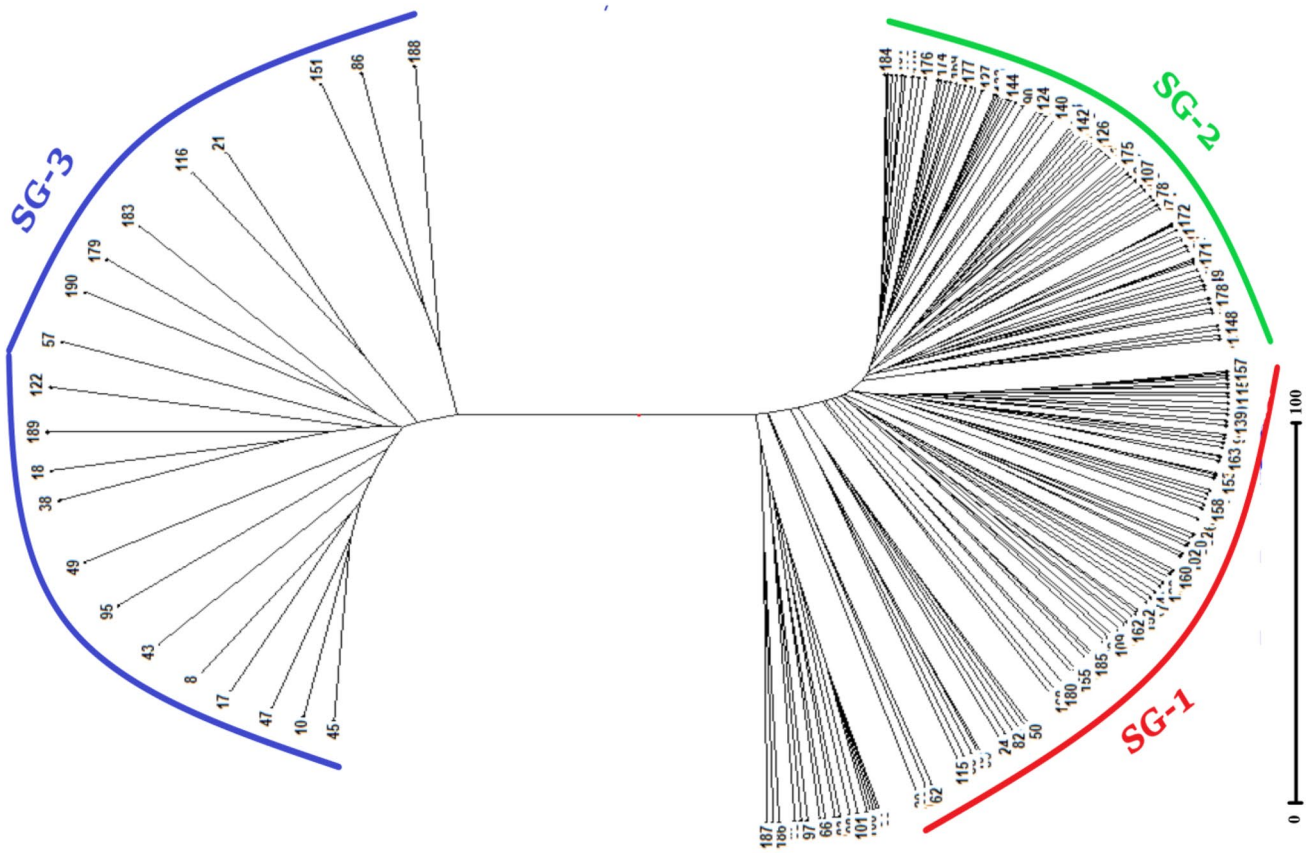


Fig. 3 Neighbor joining (NJ) tree based on UPGMA algorithm and Jaccard's similarity coefficient showing the pattern of grouping of 190 rice landraces. Rice landraces were grouped into three clusters as marked in NJ tree. SG-1, SG-2 and SG-3 represent the genotypes of cluster-1, cluster-2 and cluster-3, respectively. Genotypes of cluster-3 (SG-3) are clearly distinguished from cluster-1 (SG-1) and cluster-2 (SG-2). The serial number against each spoke of the figure represents genotype as per the supplementary Table 1

and diversity in available germplasm, which can be used later in hybridization, genetic studies, marker development, and marker-trait association studies. Natural population with high phenotypic variation is crucial for the success of GWAS study. Sufficient genetic variation among the augmented landraces for all the traits were recorded through ANOVA, making of population appropriate for this study. Minor differences in phenotypic variance and genotypic variance for all the traits during both the years indicated the less influence of environment. CD values for controls over the blocks, CD of augmented treatment between the blocks, CD for augmented treatment within the blocks and CD for control and augmented treatment for all the studied traits during both years revealed the insignificant environmental heterogeneity and lesser micro-environment effect (Supplementary Table 3a, b). These all indicated the precision of the experimental methodology and research finding. Mondal et al. (2017) also implied augmented block design in 300 Recombinant Inbred Lines (RILs) for QTL mapping for early ground cover in wheat (*Triticum aestivum* L.) under drought stress condition and reported significant and reliable informations.

Presence of abundant variability for studied traits might be due to diverse landraces possessing several unknown natural recombinations and mutations accumulated over many generations. Coefficient of variation was also higher for most of the agronomic (Agrama et al. 2007; Swamy et al. 2017; Zhang et al. 2019) and nutritional traits (Norton et al. 2014; Descalsota et al. 2018; Huang et al. 2015).This provides a good opportunity to select the superior genotypes for grain yield and nutritional traits to be utilized in breeding programs. Most of the traits showed high heritability, indicates the less influence of environmental factors. Direct selections in breeding program would be rewarding for improvement of these traits. Present study revealed significant positive correlation of GY with most of the yield attributing traits. However, GY showed negative or no correlation with nutritional traits. This negative or no correlation may be even more likely due to the increased starch content in seed that dilute concentration of nutritive compounds (Rabson et al.

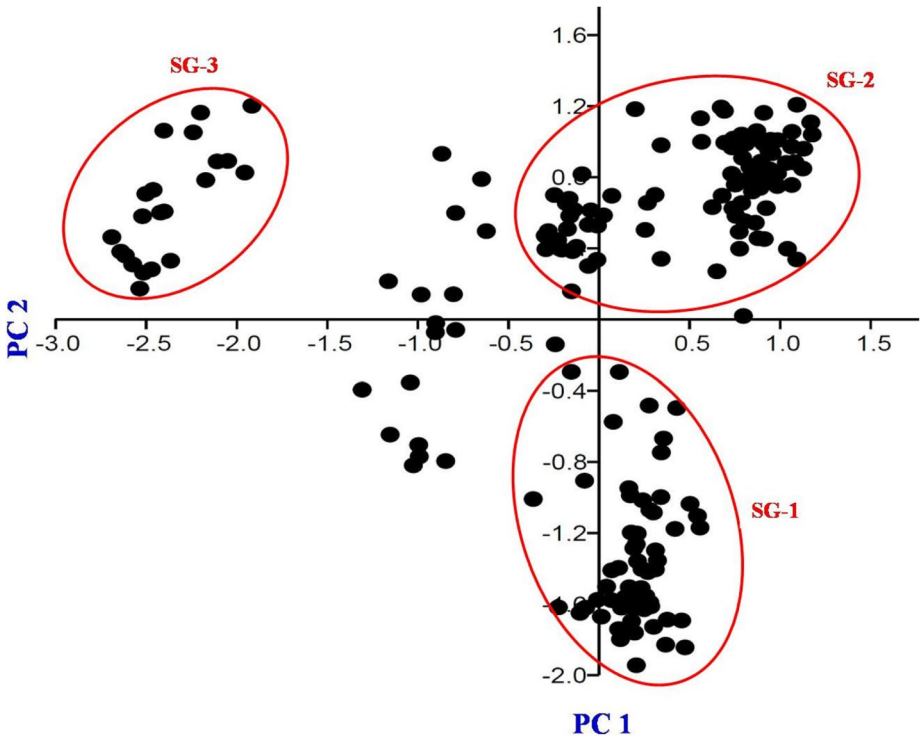


Fig. 4 Principal Component (PC) scatter diagram showing the grouping pattern of 190 rice landraces based on the PC1 and PC2. *X* axis represents the PC1 and *Y* axis represents the PC2. Rice landraces were grouped into three sub-groups as marked with red coloured circle. SG-1, SG-2 and SG-3 represent the genotypes of subgroup-1, subgroup-2 and subgroup-3, respectively. Genotypes of subgroup-3 (SG-3) are clearly distinguished from subgroup-1 (SG-1) and subgroup-2 (SG-2)

1978). The higher negative correlation between oleic acid and linoleic acid exist due to possible natural mutation in oleyl phosphatidyl choline desaturase gene which converts oleic acid to linoleic acid (Mondal et al. 2018).

Some prominent rice landraces have been identified for important traits through this study. Based on the observations for two years, landraces, Anjani and Maran Dhan (early maturity); Sihar and Kanakbhog (extra dwarf); Korma, Manmohan and Raja Banga (longest panicles); Sichar and Chhindmauri (highest spikelet fertility%); Gangabaru and Bhusu (highest grain yield per plant); Karhani and Bathrash (highest Fe content); Byalo-2 and Petgadi (highest Zn content); Kadamphool and Rudra Dhan (highest oleic acid content) and Gangachur and Antarved (highest linoleic acid content) were recognized in this study (Supplementary Table 5). Landraces identified for various traits may play an important role in rice breeding program for nurturing desirable genotypes.

SSR and InDel markers used in this study were distributed in entire 12 chromosomes, with an average of 12 markers per chromosome (ranging from 10 to 15 markers per chromosome). Average allele per marker or locus was high due to diverse landraces and usage of capillary gel electrophoresis to resolve the SSR alleles. Heterozygosity was found to be very low due to the autogamous nature of rice. Total 66 highly polymorphic, useful and informative markers were identified by considering the parameters of PIC value (> 0.70), gene diversity (> 0.7) and the number of polymorphic alleles (> 0.6) (Table 1). Amount of molecular diversity existing within the current population panel is comparable with the earlier reports in rice germplasm (Agrama et al. 2007; Swamy et al. 2017; Sahu et al. 2017; Descalsota et al. 2018; Norton et al. 2018; Zhang et al. 2019). The informative markers identified in this study may be further effectively used for various genetical studies in rice genotypes.

It is essential to determine the genetic structure of the population to reduce the possibilities of spurious associations in GWAS (Pritchard et al, 2000). Population stratification revealed three sub-populations (SG-1, SG-2, and SG-3) with few admixtures among the 190 landraces. Total 86% (163) genotypes separated into any one of the three subgroups, while 14% (27) genotypes showed allelic reshuffling or allelic sharing with genotypes of different subgroups. Few admixtures detected due to accumulation of spontaneous mutations in the genotypes from different geographical areas (Sahu et al. 2017; Swamy et al. 2017; Agrama et al. 2007). Based on the results of our previous experiment (Sahu et al. 2017), genotypes appeared in SG-3 contain some *japonica* oriented alleles while genotype of SG-1 and SG-2, carried *indica*-oriented alleles (Sahu et al. 2017). Genotypes of SG-3 were differentiated as '*close to japonica*' type whereas genotypes of SG-1 and SG-2 were categorized under '*indica type*' and '*close to indica type*' category based on 'InDel Molecular Index' (Sahu et al. 2017). Richharia et al. (1960) also reported the allelic reshuffling between *indica* and *japonica* genotypes due to previous hybridization and migration phenomena in the landraces of Chhattisgarh state, which is very well depicted in the findings of present study. However, genotypes of SG-1 and SG-2 were not

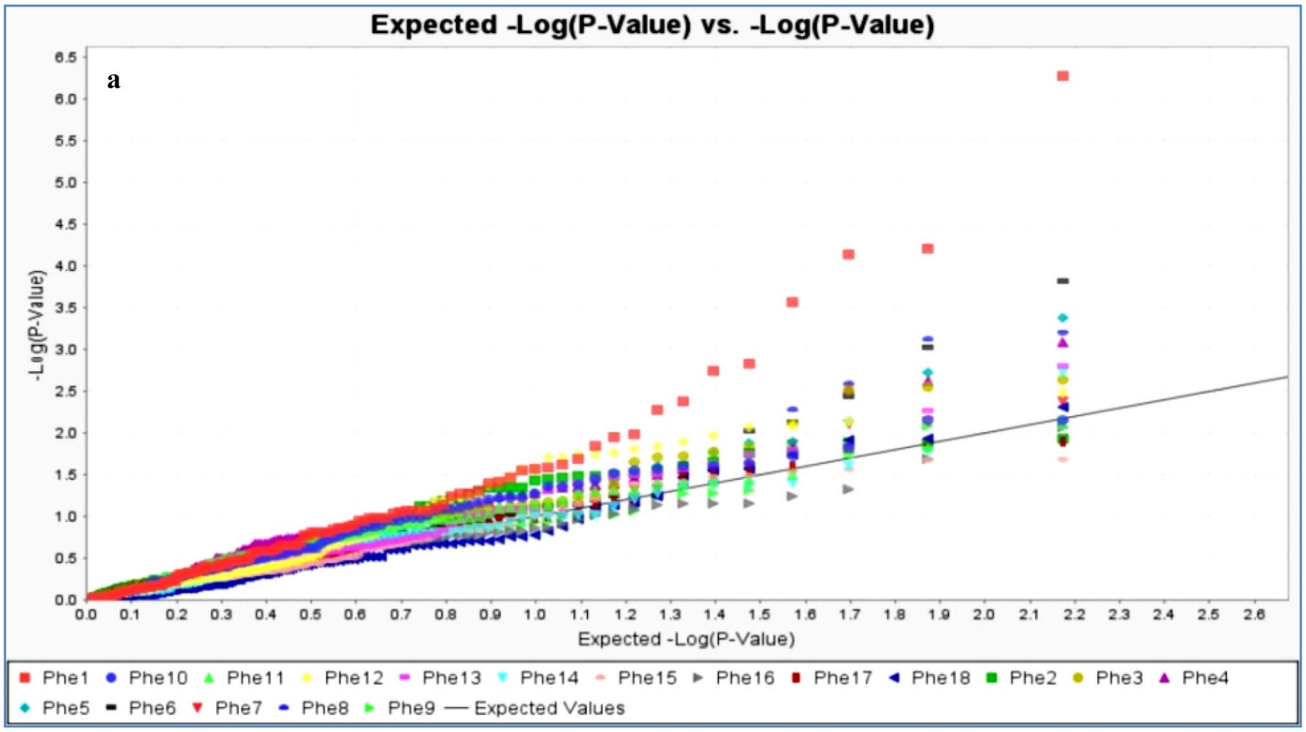
clearly distinguishable based on plant or grain morphology. Therefore, it is assumed that they may fall in two subgroups due to their different evolutionary pattern and adaptation behavior in the environment. Moderate to high genetic variation (based on *Fst* statistics) among sub-populations indicated the possibilities of generating super rice hybrids with better adaptability while crossing the genotypes of SG-3 with genotypes of SG-1 and SG-2. Earlier, several scientists also reported two to five sub-groups in rice population panel and assumed the evolutionary pattern and adaptation behavior to environment as main reason for differentiation (Agrama et al. 2007; Zhao et al. 2011; Swamy et al. 2017; Sahu et al. 2017; Juan et al. 2018; Kadam et al. 2018; Zhang et al. 2019). Interestingly, neighbor joining tree and principal component based scatter diagram showed the similar distribution of landraces in three sub-groups as explained in population structure analysis, indicating the robustness of population stratification.

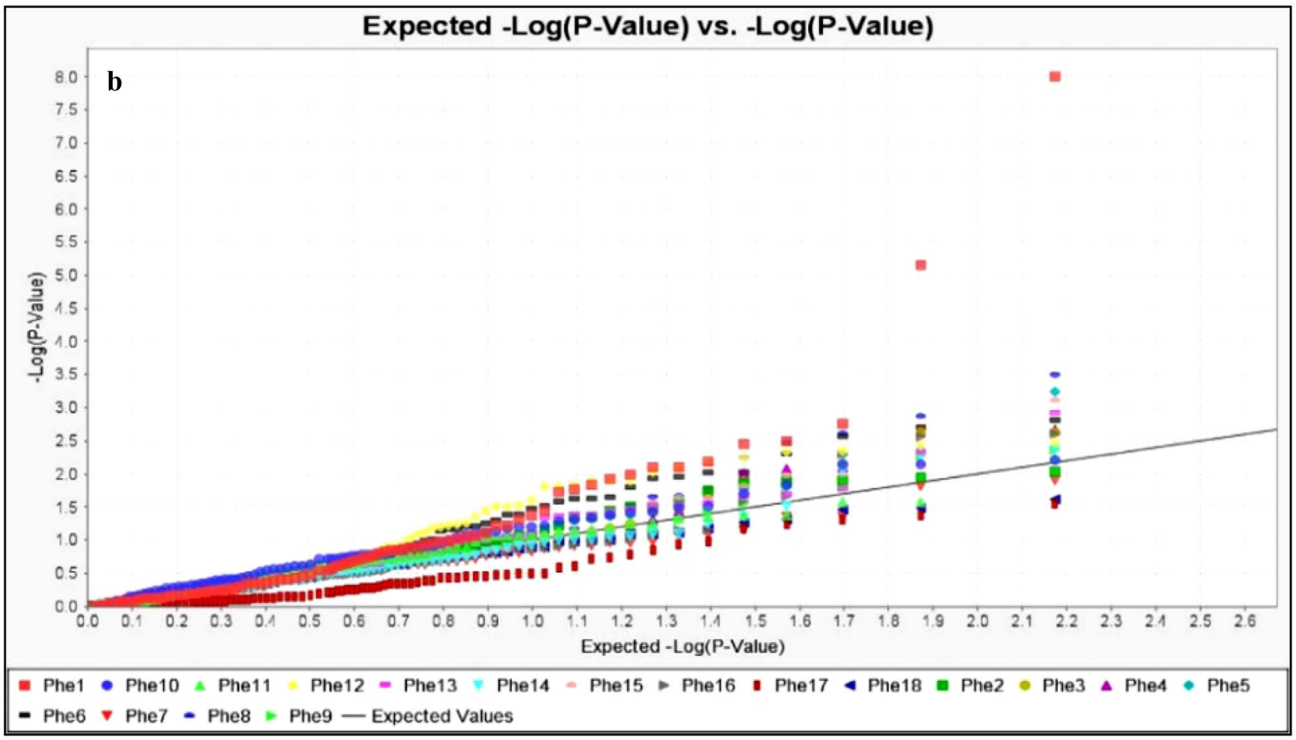
Association mapping has shown great promise and power of mapping the complex quantitative traits in plants as compared to traditional bi-parental mapping. Several scientists employed GWAS in the rice population panel for various traits and reported novel QTLs (Suman et al. 2020; Haritha et al. 2020; Donde et al. 2020; Pradhan et al. 2019, 2020; Zhang et al. 2014, 2019; Norton et al. 2018; Kadam et al. 2018; Juan et al. 2018; Prasanth et al. 2017; Swamy et al. 2017; Xu et al. 2016; Lu et al. 2015; Zhao et al. 2011; Borba et al. 2010; Agrama et al. 2007). But such study in diverse rice landrace is very meager. Present study reported total 22 consistent and significant QTLs for 12 different grain yield and nutritional trait in those studied landraces. Out of 22 QTLs, association of three markers (RM205 with DFF; RM234 with FSP and RM234 with TSP) were previously reported in rice. While rest 19 QTLs are novel and reported first time in this study. Marker RM205 was significantly associated with QTL *qDTH9* (days to heading) at position 173.5–177.7 cM in chromosome-9 (Suh et al. 2005). Similarly, Hua et al. (2002) reported the significant association of RM234 with QTL *gp7b* (grain/panicle) at 129 cM genomic positions of chromosome-7 which is comparable to present reports. Xing et al. (2001) and Feng et al. (2015) also reported the association of RM234 with QTLs *qsp7b and qsp7c* (total spikelets/panicle) and *qSP-7c* (spikelets/plant), respectively on chromosome-7. These four QTLs for three above traits have been validated co-incidentally through the present investigation.

Apart from these, some noteworthy results were also found in this study. SSR marker RM1148 (physical position: 3.739 Mb) consistently associated with DFF, is closely positioned with RM22475 (physical position: 4.288 Mb) in chromosome 8. RM22475 is linked to the *Ghd8/DTH8* (days to heading) gene in rice (Wei et al. 2010). This study revealed an association of RM44 (chr-8) with Zn content in rice. Moreover, Xiang-Dong et al. (2016) reported the close linkage between markers RM44 and RM152 (chr-8) during the QTL mapping for cold tolerance in *Oryza rufipogon* Griff. at early seedling stage. Interestingly, marker RM152 was also reported to be associated with QTL *qZn8-1* (Zn content) in another QTL analysis (Garcia-Oliveira et al. 2009). Until now, no attempts have been made to map QTLs for fatty acid components in rice by GWAS. Ying et al. (2012) identified a QTL *qOle8* for oleic acid in the proximity of RM1148 on chromosome-8 by analyzing an F_2 population. Later close linkage (4.4 cM) of RM25 and RM1148 was established by Xing et al. (2015) during the tagging of a new rice blast resistance gene in a RIL population. Thus, identification of RM25 as a significantly associated marker for oleic acid content in these rice landrace has immense opportunity to use this marker for the MAS program in the future breeding for high oleic acid content in rice bran. In addition, association of R11M23 with DFF, PL and TSP explained the possible occurrence of pleiotropism among these traits. Moreover, significant and positive correlations among these traits were also observed in the study (Fig. 1).

In GWAS, importance of QTLs depends on the explained phenotypic variance (R^2 value) by the marker. Wide range (3.4–36.7%) of R^2 value was obtained by the associated markers/QTLs. Of 22, 13 QTLs explained more than 10% phenotypic variation and considered as major QTLs. The wider range of phenotypic variance explained for most of the

Fig. 5 **a** Quantile–Quantile (QQ) plot of observed versus expected *p* value for all the marker-trait association (MTA) identified by GWAS from the experimental year 2015. *Y* axis shows the observed − log10(*p* values) and *X* axis shows the expected − log10(*p* values) for all the identified MTAs. Each trait has assigned a colored shape (different for each trait). **b** Quantile–Quantile (QQ) plot of observed versus expected *p* value for all the marker-trait association (MTA) identified by GWAS from the experimental year 2016. *Y* axis shows the observed − log10(*p* values) and *X* axis shows the expected − log10(*p* values) for all the identified MTAs. Each trait has assigned a colored shape (different for each trait). **c** Quantile–Quantile (QQ) plot of observed versus expected *p* value for all the marker-trait association (MTA) identified by GWAS from the pooled analysis. *Y* axis shows the observed − log10(*p* values) and *X* axis shows the expected − log10(*p* values) for all the identified MTAs. Each trait has assigned a coloured shape (different for each trait). *Phe1* days to 50% flowering, *Phe2* plant height, *Phe3* total tillers/plant, *Phe4* productive tillers/plant, *Phe5* panicle length, *Phe6* fertile spikelets/panicle, *Phe7* sterile spikelets/panicle, *Phe8* total spikelets/panicle, *Phe9* spikelet fertility percent, *Phe10* hundred seed weight, *Phe11* grain yield, *Phe12* iron content, *Phe13* zinc content, *Phe14* palmitic acid, *Phe15* stearic acid, *Phe16* oleic acid, *Phe17* linoleic acid, *Phe18* linolenic acid


Expected -Log(P-Value) vs. -Log(P-Value)
a
-Log(P-Value)
Expected -Log(P-Value)
Phe1 Phe10 Phe11 Phe12 Phe13 Phe14 Phe15 Phe16 Phe17 Phe18 Phe2 Phe3 Phe4
Phe5 Phe6 Phe7 Phe8 Phe9 Expected Values


Expected -Log(P-Value) vs. -Log(P-Value)
b
-Log(P-Value)
Expected -Log(P-Value)
Phe1 Phe10 Phe11 Phe12 Phe13 Phe14 Phe15 Phe16 Phe17 Phe18 Phe2 Phe3 Phe4 Phe5
Phe6 Phe7 Phe8 Phe9 Expected Values

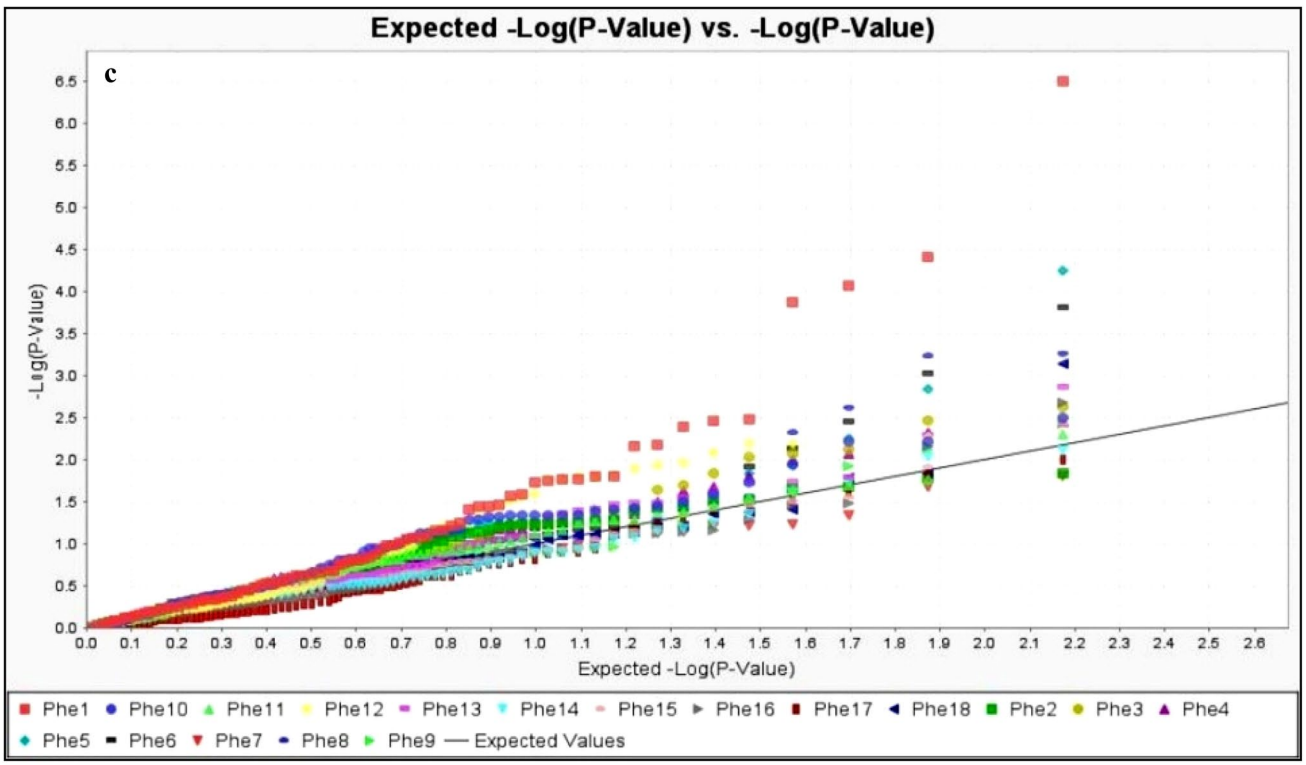


Fig. 5 (continued)

traits entail genome-wide significance and high statistical power of the current panel of 190 rice landraces.

Conclusion

Landraces, a reservoir of unique and important genes, are the most imperative component in the crop improvement program. Therefore, landraces identified for grain yield and nutritional traits can be utilized as donors in cross breeding, construction of mapping population or can be used to breed new cultivars through conventional breeding methods. Genetic dissection of rice landraces revealed abundant genetic variation and diversity for the grain micronutrients, fatty acids and yield-related traits with less influence of environment on their inheritance. Population stratification based grouping of rice panel offers a way to breed the super hybrids by crossing the divergent genotypes among and within different sub-groups (Fig. 2). This study reported 19 novel QTLs for 12 grain yield-related traits, micronutrients and fatty acid components while three earlier reported QTLs were also validated. We do hope that the present research work will open many avenues towards utilization of these QTLs and superior landraces in rice breeding for developing nutrition-rich high yielding varieties.

Table 3 List of Significant Marker Trait Associations (MTAs) identified under the study during both the years and pooled analysis along with reports of previous findings

Traits	Year	Marker name	Chromosome number	*p*_marker value	R^2 _ Marker	Reports of QTLs for same or similar traits
Days to fifty % flowering (DFF)	2015–2016	RM1148	8	5.264E–07	0.260	–
		RM205	9	6.15E–05	0.318	–
		R11M23	11	7.23E–05	0.087	–
	2016–2017	RM1148	8	1.01E–08	0.323	–
		RM205	9	6.88E–06	0.367	–
		R11M23	11	0.0066	0.040	–
	Pooled	RM1148	8	3.16E–07	0.267	–
		R11M23	11	3.91E–05	0.094	–
		RM205	9	8.63E–05	0.311	Suh et al. (2005) (*qDTH9*)
Total tillers/plant (TTP)	2015–2016	RM118	7	0.00148	0.085	–
	2016–17	RM118	7	0.0061	0.097	–
	Pooled	RM118	7	0.0024	0.112	–
Panicle length (PL)	2015–2016	R12M43	12	4.22E–04	0.107	–
		R11M23	11	0.00190	0.053	–
	2016–2017	R12M43	12	5.73E–04	0.103	–
		R11M23	11	0.0060	0.131	–
	Pooled	R12M43	12	5.75E–05	0.110	–
		R11M23	11	0.0015	0.056	–
Fertile spikelets/Plant (FSP)	2015–2016	RM234	7	1.50E–04	0.197	Hua et al. (2002) (*gp7b*)
	2016–2017	RM234	7	0.0015	0.160	
	Pooled	RM234	7	1.54E–04	0.196	
Total spikelets/Plant (TSP)	2015–2016	R11M23	11	6.28E–04	0.064	–
		RM234	7	7.62E–04	0.172	–
		R1M47	1	0.00261	0.065	–
	2016–2017	RM234	7	3.14E–04	0.187	–
		R11M23	11	0.0013	0.057	–
		R1M47	1	0.0056	0.057	–
	Pooled	R11M23	11	5.53E–04	0.066	–
		RM234	7	5.84E–04	0.176	Xing et al. (2001) (*sp7b & sp7c*) Feng et al. (2015) (qSP-7c)
		R1M47	1	0.0024	0.066	–
Spikelet fertility % (SF%)	2015–2016	RM184	10	0.00643	0.108	–
	2016–2017	RM184	10	0.0043	0.105	–
	Pooled	RM184	10	0.0038	0.107	–
Hundred seed weight (HSW)	2015–2016	R3M10	3	0.00610	0.040	–
		R9M42	9	0.00610	0.040	–
	2016–17	R3M10	3	0.0061	0.140	–
		R9M42	9	0.0061	0.140	–
	Pooled	R3M10	3	0.0061	0.041	–
		R9M42	9	0.0061	0.041	–
Grain yield/Plant (GY)	2015–2016	RM7434	6	0.00179	0.102	–
	2016–17	RM7434	6	0.0058	0.108	–
	Pooled	RM7434	6	0.0050	0.110	–

Table 3 (continued)

Traits	Year	Marker name	Chromosome number	*p*_marker value	R^2 _ Marker	Reports of QTLs for same or similar traits
Iron content (Fe)	2015–2016	RM536	11	0.00327	0.138	–
		RM17467	4	0.00601	0.304	–
		RM484	10	0.00639	0.076	–
		RM26063	11	0.00637	0.244	–
	2016–2017	RM536	11	0.0033	0.138	–
		RM17467	4	0.0045	0.316	–
		RM26063	11	0.0047	0.258	–
		RM484	10	0.0057	0.079	–
	Pooled	RM536	11	0.0033	0.138	–
		RM17467	4	0.0056	0.310	–
		RM26063	11	0.0062	0.251	–
		RM484	10	0.0065	0.077	–
Zinc content (Zn)	2015–2016	RM44	8	0.00158	0.148	–
		RM6839	9	0.00541	0.090	–
	2016–2017	RM44	8	0.0012	0.153	–
		RM6839	9	0.0047	0.092	–
	Pooled	RM44	8	0.0014	0.150	–
		RM6839	9	0.0051	0.091	–
Oleic acid (OA)	2015–2016	RM25	8	0.00595	0.134	–
	2016–2017	RM25	8	0.0024	0.129	–
	Pooled	RM25	8	0.0021	0.131	–
Linolenic acid (LnA)	2015–2016	RM495	1	0.00503	0.122	–
	2016–2017	RM495	1	0.0015	0.104	–
	Pooled	RM495	1	7.43E–04	0.151	–

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Author contributions Conceptualization of work: DS, SM, BKD; Data curation: PKS, SM, GV, RS, VK; Data analysis: PKS, SM; Funding acquisition: DS, BKD; Investigation: PKS, SM; Methodology: PKS, SM, GV, VK; Resources: DS, SM, BKD, VK; Supervision: DS, BKD; Writing ± original draft: PKS, SM, RS; Review and editing: SM, DS, BKD, VK, GV. All authors read and approved the final manuscript.

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Compliance with ethical standards

Conflict of interest statement The authors declare that they have no conflict of interest in the publication.

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