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Research article

IDENTIFICATION OF ELITE RICE GERMPLASM LINES FOR GRAIN PROTEIN CONTENT, SSR BASED GENOTYPING AND DNA FINGERPRINTING

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ABSTRACT: The present investigation was aimed at characterizing the rice germplasm lines of Chhattisgarh core collection for grain protein content and their morphological and molecular analysis. The protein content analysis resulted in identification of five high protein germplasm lines namely, CGR-436 (11.2%), GP-145-48 (10.68%), CGR-446 (10.43%), CGR-52 (10.35%) and CGR-77 (9.92%). Morphological analysis for the same revealed a wide range of diversity for eight quantitative traits. Further, the genetic diversity was assessed among 58 rice germplasm lines and varieties using 69 alleles generated by 25 SSR primers. DNA fingerprinting of identified high grain protein containing rice lines (≥ 9.0% grain protein) was carried out using 25 polymorphic SSR markers among which SSR marker RM 489 was found to be highly discriminating marker. Graphical genotyping analysis revealed that these markers differed significantly for the genomic constitution contributed by marker alleles A and B. For SSR marker RM215, the genomic constitution was contributed majorly by allele A (99.6%) with little contribution of allele B (0.4%) whereas these alleles contributed almost equally in case of RM447. Marker trait associations by ANOVA single marker analysis revealed six markers to be significantly associated with grain protein content. The high grain protein germplasm lines and associated DNA markers indentified in this study can be used in the breeding program for the improvement of grain protein content in rice. Also the developed DNA fingerprint of the selected elite rice lines generates the base level data and provides the basis as per requirement of Biodiversity act for registration of varieties and landraces.
Keywords: Association, DNA fingerprinting, Graphical genotyping, Protein, Rice.

Abbreviations

GPC-grain protein content, GGT- graphical genotyping tool, PCR-polymerization chain reaction, SSR-simple sequence repeats, MAS-marker assisted selection.

INTRODUCTION

Rice, *Oryza sativa* (2n = 24) belonging to the family *Graminae* and subfamily *Oryzoidea* is the staple food for one third of the world's population and occupies almost one-fifth of the total land area covered under cereals [5]. It is grown under diverse cultural conditions and over wide geographical range. Most of the world's rice is cultivated and consumed in Asia, which constitutes more than half of the global population. Approximately 11% of the world's arable land is planted annually to rice, and it ranks next to wheat. The world's rice production has doubled during last 25 years, largely due to the use of improved technology such as high yielding varieties and better crop management practices [5]. Further scope of crop improvement depends on the conserved use of genetic variability and diversity in plant breeding programs and use of new biotechnological tools. There is wide genetic variability available in rice among and between wild relatives and varieties leaving a wide scope for future crop improvement.

Rice is the important cereal and a source of calories for one-third of the world population. About 800 million people in developing world are undernourished suffering from either protein energy or micro-nutrient (Vitamin A, Vitamin C, Iron and Zinc) deficiency; so there is a serious need to re-design the global food system and change in the way that that will ensure the balance nutrient supply of major staple food for people in adequate and affordable amount. To improve nutritive value of rice the preliminary step is to characterize genetic variability for grain protein content in germplasm and then to use this variability for breeding nutrient rich rice [9].

Characterization and quantification of genetic diversity has long been a major goal in evolutionary biology. Information on the genetic diversity within and among closely related crop varieties is essential for a rational use of genetic resources. The analysis of genetic variation both within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can also be used to predict potential genetic gains. Diversity based on phenological and morphological characters usually vary with environments and evaluation of these traits requires growing the plants to full maturity prior to identification. Protein or isozyme marker studies are also influenced by environment and reveal low polymorphism. Now, the rapid development of biotechnology allows easy analysis of a large number of loci distributed throughout the genome of plants. Molecular markers have proven to be powerful tools in the assessment of genetic variation and in the elucidation of genetic relationships within and among species. Information regarding genetic variability at molecular level could be used to help, identify and develop genetically unique germplasm that compliments existing cultivars.

Association or linkage disequilibrium (LD) mapping has revolutionized genetic mapping in humans [7] and is increasingly being applied to plants [11] and is considered an efficient way of determining the genetic basis of complex traits. In most plant species, the identification of those genomic regions which contribute to important characteristics has been mostly achieved through linkage analysis within segregating populations, the result of crosses between genitors with contrasting phenotypes and genotypes [4, 17]. There is also the potential for discovering the QTL responsible for multiple traits, and examination of a higher proportion of polymorphic molecular markers could provide better genome coverage than any bi-parental population [8, 11]. Rice is a self-pollinating species; it is expected to present high linkage disequilibrium, thereby requiring fewer markers. Association mapping is feasible, and potentially very useful for rice in particular, since rice has been completely sequenced, it has a relatively small genome and is well suited to genome-wide association (GWA). Recently, the association between 152 SSR loci and the eleven important agronomic traits were estimated by [18] in 128 japonica rice varieties. In the present investigation following objective were studied; phenotyping of rice germplasm lines for grain protein content, DNA fingerprinting of high grain protein content rice lines, association mapping for grain protein content using DNA markers. The present investigation was undertaken to identify high grain protein content rice germplasm lines, their DNA fingerprinting and association mapping for this trait using DNA markers.

MATERIALS AND METHODS

Plant material

A total of 58 rice genotypes comprising 40 germplasm lines of Chhattisgarh core collection, some advanced breeding lines and popular varieties of Chhattisgarh were used.

Field experiments and phenotypic evaluation

For field studies, each rice genotypes were planted out in a field at the experimental farm of the College of Agriculture, IGKV, Raipur (Chhattisgarh, India) in experimental design RBD. There were two replicates for each germplasm line and varieties in each phenotypic evaluation experiment during 2012-2013. The random five plants of each plot were sampled in order to examine agronomic traits. Eight quantitative traits of agronomic and economic importance, particularly relating to grain yield, were evaluated**.** These traits included Plant height (PH), Panicle length (PL), Number of tillers (NT), Productive tillers (PT), Flag leaf length (FLL), Flag leaf width (FLW), Grain length (GL), and Grain width (GW). All quantitative traits were obtained from two replicates in the experiment. For each trait, means of the replicates were used in the data analyses.

Grain Protein Estimation

Grain protein was estimated in dehusked, brown rice grain carrying intact embryo. Grain protein content was estimated by modified micro-Kjeldahl method described by [10] and data were statistically analyzed using CRD.

SSR genotyping

Total genomic DNA was extracted from rice leaves using CTAB method. A total of 25 polymorphic microsatellite markers, approximately evenly distributed across 12 chromosomes of rice, were used for genotyping (Table 3). Microsatellite polymorphism was analyzed by PCR. Amplification of DNA was performed in a 20 µL reaction mix consisting of 2 µL template DNA, 2 µL PCR buffer, 2 µL dNTPs, 2 µL forward and reverse primers, autoclaved distilled water 11.5 µL and 0.5 unit of *Taq* DNA polymerase. The PCR products were electrophoresed in a 2.5% agarose gel (for SSRs) at 100 V for 1 hour. The gel was then observed on a Gel documentation system (Biorad). Alternatively the PCR products of SSR primers were also run on QAIxcel gel free system (Qiagen) to achieve better resolution of the bands. The individual bands were scored for further analysis. Primer sequences and PCR amplification conditions for each set of primers and the physical positions of marker loci were worked out based on published Nipponbare sequence databases (http://www.gramene.org).

Data analysis

Marker data was scored for the presence and absence of the SSR bands. Moreover, the data were entered into a binary matrix as discrete variables; A for presence and B for absence of the character and this data matrix were subjected to further analysis. The excel file containing the binary data was prepared as per the requirement of graphical genotyping tool (GGT V2.0). The resultant data file was employed to generate graphical image indicating chromosome wise contribution of A and B alleles amplified by different SSR markers.

Association analysis

Association between trait and markers were calculated using single marker analysis. The significant marker trait associations were indicated by a P-value with corresponding R^2 for each marker as the percent of the total variation explained.

RESULTS

Phenotypic and morphological variations

In this study marked variations were recorded among the rice germplasm lines and varieties for the traits. For example, the value of PH ranged from 66.1 to176.45 cm.

Grain protein content analysis

Protein content analysis was done for all the 58 rice lines to know the difference in protein content. A wide variation was found in protein content of all the 58 lines varying from 6.09 to 11.2 %. Among these, highest protein content (11.2%) was observed for germplasm accession CGR-436 and the lowest protein content (6.09) was observed for rice variety Danteshwari (Table 2). In high grain protein content CGR-436 was followed by GP-145-48, CGR-446, CGR-52 and CGR-72 were identified as donor lines for grain protein content.

Table 1 Genetics parameters of variation for different quantitative character

	PH[a] (cm)	FLL[b] (cm)	FLW[c] (cm)	NT[d]	PT[e]	PL[f] (cm)	GL[g] (cm)	GW[h] (cm)
MEAN	123.14	37.28	0.91	7.95	6.97	23.28	0.84	0.266
MINIMUM	66.1	21.7	0.33	3.5	3.5	18.43	0.55	0.18
MAXIMUM	176.45	62.43	1.6	17.75	26.5	28.1	1.07	0.335
SEM[i]	2.85	2.76	0.16	0.59	0.64	1.17	0.01	0.006
CD[j]	8.07	7.81	0.44	1.67	1.8	3.32	0.04	0.017
CV %[k]	3.28	10.47	24.45	10.53	12.9	7.12	2.22	3.36

a; plant height, b; flag leaf length, c; flag leaf width, d; number of tillers, e; productive tiller, f; panicle length, g; grain length, h; grain width, i; standard error mean, j; critical difference, k; coefficient of variation.

DNA fingerprinting of high grain protein rice germplasm lines and varieties

DNA fingerprinting of high grain protein containing rice germplasm lines and varieties was carried out on randomly selected five individuals of each germplasm lines and varieties using polymorphic SSR markers (Table 3). For comparison some local popular genotypes were also included. It was observed that the majority of the SSR markers tested (RM431, RM13, RM161, RM225, RM11, RM21, RM452, RM447, RM283, RM215, RM154, RM25 and RM552) produced similar banding pattern among the two sets of germplasm line and varieties used. This might be due to similar genotypic constitution of the selected genotypes on those SSR loci. The SSR marker RM 489 showed variation in the banding pattern in which the germplasm line CGR436, GP-145-48 and CGR446 showed different pattern amplifying DNA fragments of 310 bp and 200 bp when compared to other germplasm lines and varieties which generated a single fragment of 220 bp (Fig. 1).

Graphical genotyping of rice germplasm lines and varieties using SSR makers' data

A total of 25 polymorphic SSR markers were used to genotype 58 rice germplasm lines and varieties, generating a total of 69 alleles (Table 3). The graphical genotyping analysis revealed that the two alleles more or less contributed equally in case of markers located on chromosome 2 whereas in case of chromosome 9 spanning SSR markers RM215 the genomic constitution was contributed majorly by allele A (99.6%) with little contribution of allele B (0.4%) (Fig. 2). Similarly the marker located on chromosome 5 (RM161) showed minimum contribution of allele A and maximum contribution of allele B (Table 4).

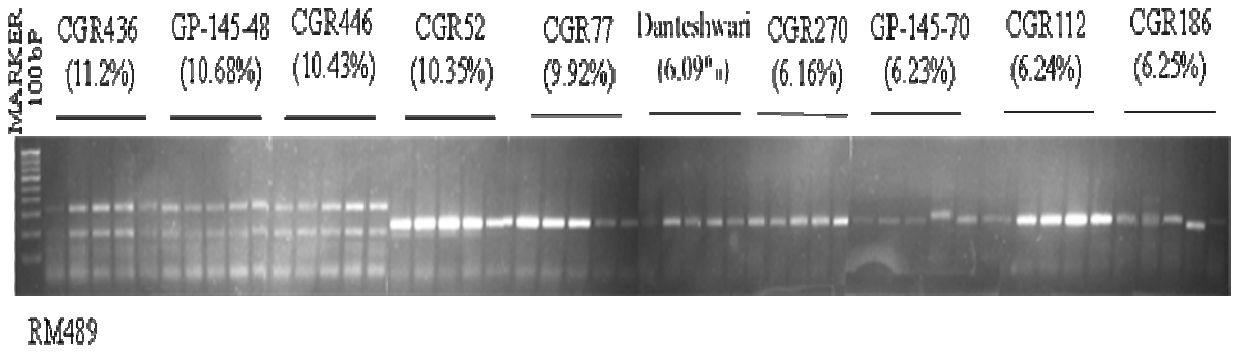


Fig. 1 DNA fingerprinting of top five rice germplasm lines and varieties having high and low grain protein content

Table 2 Grain protein content (%) of 58 rice germplasm lines and varieties used in the study

SN	Accession	Mean (%)	SN	Accession	Mean (%)
1	CGR-1102-19	9.64	30	CGR-5899-204	6.66
2	CGR-1125-21	8.70	31	CGR-7075-270	6.16
3	CGR-1130-24	7.55	32	CGR-7154-276	7.14
4	CGR-1157-30	6.48	33	CGR-7165-278	7.63
5	CGR-1168-32	8.13	34	CGR-91-436	11.20
6	CGR-1207-40	7.68	35	CGR-120-439	9.42
7	CGR-1294-46	8.56	36	CGR-164-446	10.43
8	CGR-1332-52	10.35	37	CGR-166-447	9.42
9	CGR-1361-54	9.36	38	CGR-168-448	8.65
10	CGR-1380-56	8.39	39	CGR-211-452	9.20
11	CGR-1407-62	7.92	40	CGR-306-470	9.41
12	CGR-1529-76	9.83	41	R-RF-31	6.54
13	CGR-1539-77	9.92	42	LALMATI	9.09
14	CGR-1536-87	8.21	43	R-1033-968-2-1	7.04
15	CGR-1601-91	6.86	44	R-RF-25	9.00
16	CGR-1602-92	6.91	45	NAGINA-22	8.44
17	CGR1721-106	6.41	46	SWARNA	7.43
18	CGR-1780-108	6.34	47	IR-68144-3B	9.43
19	CGR-1803-111	6.91	48	IR-64	7.96
20	CGR-1809-112	6.24	49	GP-145-37	9.79
21	CGR-1898-122	8.12	50	GP-145-48	10.68
22	CGR-2190-145	7.21	51	GP-145-66	7.87
23	CGR-2995-147	7.51	52	GP-145-29	6.76
24	CGR-2549-164	6.68	53	GP-145-70	6.22
25	CGR-2552-165	6.74	54	GP-145-128	7.84
26	CGR-2871-174	6.33	55	CHANDRAHASNI	6.82
27	CGR-2978-175	6.47	56	KARMA MASURI	6.98
28	CGR-5681-186	6.24	57	DANTESHWARI	6.09
29	CGR-5816-194	6.47	58	HMT	6.48

Association analysis between agronomic traits and molecular markers

The association between trait and markers were calculated using single marker analysis (SMA) in Microsoft Excel program. The significant marker trait associations were indicated by a P-value (<0.05) with corresponding R^2 for each marker which is the total phenotypic variation for a trait that is accounted by markers. We detected a total of 17 significant marker-trait association (P<0.05) (Table 5). All of the 17 significant SSR loci were indentified for the agronomic traits, with the R^2, percentage of the total variation explained ranging from 6.23to 23.88%.

Table 3 List of SSR markers showing polymorphism used for genotyping.

Marker	Chr	Primer sequence (F & R)	AT	PS (bp)	No. of Alleles	Position in cM
RGNMS190	1	F -GTCGTCCGAATCGCCTGACTAGG R- ACGGCCTCCAGAAGGAAGAGC	54	152	2	45.25
RM431	1	F - TCCTGCGAACTGAAGAGTTG R –AGAGCAAAACCCTGGTTCAC	55	251	2	178.3
RM283	1	F -GGCATGAGAGTCTGTGATGTTGG R –TAGTACTGCTCCATCTGCCTTGG	55	151	4	31.4
RM226	1	F -GAAGCTAAGGTCTGGGAGAAACC R –AATGGCCTTAACCAAGTAGGATGG	55	289	3	154.8
RM452	2	F -GTGGACTTGGCGAGATGCTACG R –GTTAAGGGCAGCCACCAGATCG	55	209	2	58.4
RM154	2	F -GACGGTGACGCACTTTATGAACC R –CGATCTGCGAGAAACCCTCTCC	61	183	5	4.8
RM489	3	F -GAACAGGGACACAATGATGAGG R –GACGATCGGACACCTAATTACAGC	55	271	2	29.2
RM338	3	F -CACAGGAGCAGGAGAAGAGC R –GGCAAACCGATCACTCAGTC	55	183	4	108.4
RM13	5	F -TTGGATTGTTTTGCTGGCTCGC R –GGAACACGGGGTCGGAAGCGAC	55	141	2	28.6-31.4
RM161	5	F -TGCAGATGAGAAGCGGCGCCTC R –TGTGTCATCAGACGGCGCTCCG	61	187	2	96.9
RM334	5	F -GTTCAGTGTTCAGTGCCACC R –GACTTTGATCTTTGGTGGACG	55	182	2	141.8
RGNMS167	6	F -ACCACGCGTCATTGACATCC R –ATGGGATGAAACTGCCACAACC	54	170	2	103.98
RM162	6	F -GCCAGCAAAACCAGGGATCCGG R –CAAGGTCTTGTGCGGCTTGCGG	61	229	2	108.3
RM454	6	F -CTCAAGCTTAGCTGCTGCTG R –GTGATCAGTGCACCATAGCG	55	268	5	99.3
RM225	6	F -TATGTGGTTGGCTTGCCTAGTGG R –TGCCCATATGGTCTGGATGTGC	55	196	2	26.2
RM125	7	F -ATCAGCAGCCATGGCAGCGACC R –AGGGGATCATGTGCCGAAGGCC	55	127	2	24.8
RM11	7	F -ATCGGTGCTTGGCTGGATAGC R –CCACCTTCTTCTCCTCCTCTTCC	55	140	3	47
RM447	8	F -ACGGGCTTCTTCTCCTTCTCTCC R –TCCCTTGTGCTGTCTCCTCTCC	55	111	3	124.6
RM25	8	F -GGAAAGAATGATCTTTTCATGG R –CTACCATCAAAACCAATGTTC	55	146	4	52.2
RM215	9	F -GAGCAGCAAGAGCAGCAGAGG R –CATGCTCGACTTCAGAAGCTTGG	55	148	4	99.4
RM474	10	F -TACACGAGGGAGTACTCGAATGG R –CATGGAGGTATAGAAGAGCATTGG	55	252	4	0
RGNMS28	11	F -GCATGCTAGCTACTAATTGTGTGG R-CTTTAGTTACCCAACGTACTCTCTCC	54	109	2	9.61
RM536	11	F -TACCAGGATCATGTTTCTCTCC R –ACTGTGAGATTGACTGACAGTGG	55	243	2	55.1
RM206	11	F -ATCGATCCGTATGGGTTCTAGC R –GTCCATGTAGCCAATCTTATGTGG	55	157	2	102.9
RM21	11	F -ACAGTATTCCGTAGGCACGG R –GCTCCATGAGGGTGGTAGAG	55	157	2	85.7

AT; annealing temperature, PS (bp); product size in base pair, cM ; centimorgan

Table 4 Chromosome wise allelic contribution of marker alleles A and B

Chromosome	A%	B%	Chromosome	A%	B%
1	85.5	14.5	7	56.9	43.1
2	55.2	44.8	8	63.8	36.2
3	89.7	10.3	9	99.6	0.4
5	37.8	62.2	10	82.8	17.2
6	78.1	21.9	11	75.9	24.1

For the trait grain protein content: We detected six loci with significant association with grain protein content. SSR marker RM-11 located on chromosome 7 had the greatest effect, explaining 23.88% of the phenotypic variation followed by RM-21 on chromosome 11 (23.60%), RM-431 on chromosome 1 (14.16%), RM-13 on chromosome 5 (12.75%), RM-161 on chromosome 5 (6.46%) and RM-225 on chromosome 6 (6.32%) (Fig.3). For the trait number of tiller: We detected six locus with significant association ($P<0.05$); RM338 on chromosome 3 had the effect explaining 17.77% of the total phenotypic variation. For plant height: We detected two loci with significant association; RGNMS167 had the largest effect, explaining 10.64% of the total phenotypic variation. Productive tiller: There are four markers locus significantly associated with PT; RM125 on chromosome 7, which explained 16.70% of the total phenotypic variation. Flag leaf width: There are two markers loci significantly associated with FLW; RM154 on chromosome 2, which explained 11.45% of the total phenotypic variation. Flag leaf length: There are two markers loci significantly associated with FLL; RM226 on chromosome 1, which explained 13.18% of the total phenotypic variation. Grain width: Two loci exhibited significant association; of these, RM215 on chromosome 9 had the largest effect, explaining 8.76% of the total phenotypic variation.

Table 5 Association between SSR markers and eight agronomic traits and grain protein content with P<0.05.

Trait	Chromosome	Position in (cM)	Marker	P-value	%R2
FLL	1	154.8	RM-226	0.005	13.18
NT	1	31.4	RM-283	0.016	9.92
PT	1	31.4	RM-283	0.025	8.62
NT	1	178.3	RM-431	0.004	13.79
PT	1	178.3	RM-431	0.016	9.88
GPC	1	178.3	RM-431	0.003	14.16
FLW	2	4.8	RM-154	0.009	11.45
NT	2	58.4	RM-452	0.054	6.49
NT	3	108.4	RM-338	0.001	17.77
NT	3	29.2	RM-489	0.05	6.23
GW	5	96.9	RM-161	0.051	6.62
GPC	5	96.9	RM-161	0.054	6.46
GPC	5	28.6-31.4	RM-13	0.005	12.75
GPC	6	26.2	RM-225	0.05	6.32
PH	6	103.98	RGNMS-167	0.012	10.64
GPC	7	47	RM-11	$9.92*10^{-5}$	23.88
FLL	7	24.8	RM-125	0.031	8.08
NT	7	24.8	RM-125	0.014	10.31
PT	7	24.8	RM-125	0.001	16.70
PT	8	124.6	RM-447	0.011	10.97
PH	9	99.4	RM-215	0.021	9.12
GW	9	99.4	RM-215	0.024	8.76
GPC	11	85.7	RM-21	0.0001	23.60
FLW	11	9.61	RGNMS-28	0.017	9.83

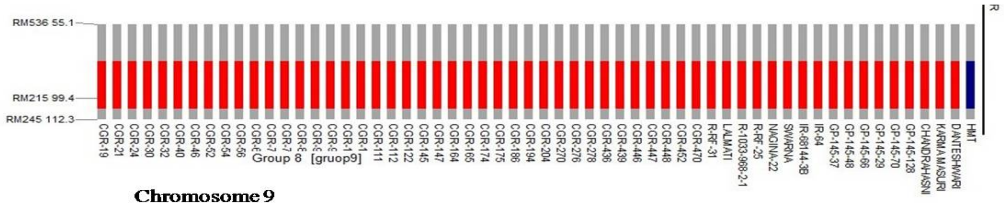


Fig. 2a

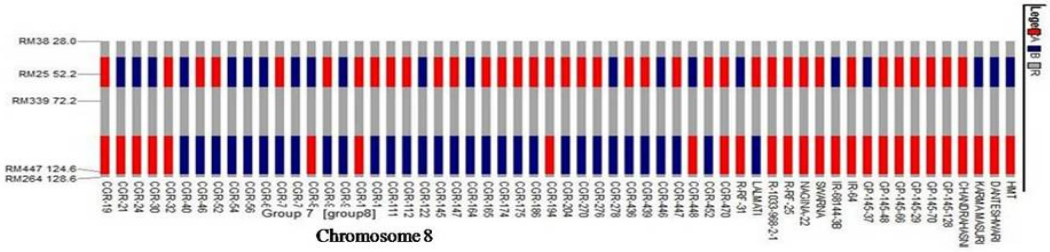
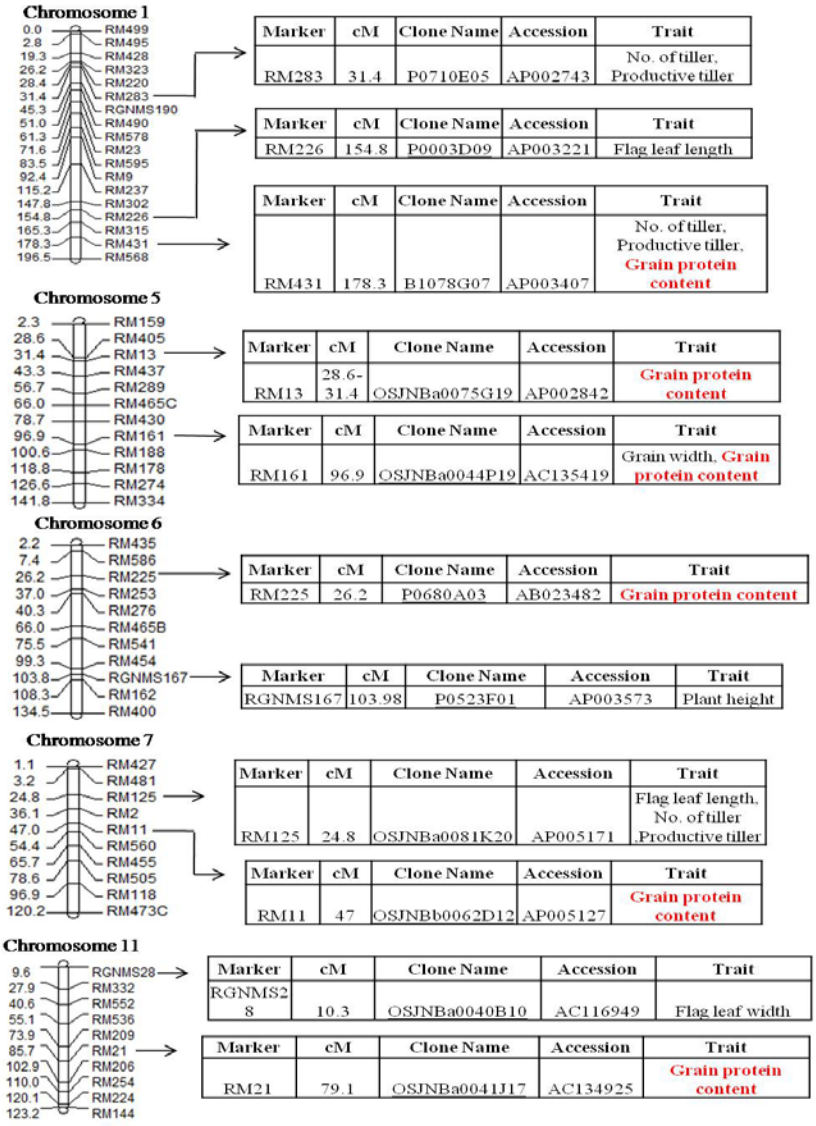


Fig. 2b

Fig. 2 Genomic constitution of 58 rice germplasm lines and varieties based on position of SSR alleles generated by markers RM-215 (a) and RM447 (b). The discriminated allele's types A and B are marked in red and blue colors respectively. Graphical outputs of genotyping data were generated using GGT version 2.0 tool.



Marker	cM	Clone Name	Accession	Trait
RM283	31.4	P0710E05	AP002743	No. of tiller, Productive tiller
RM226	154.8	P0003D09	AP003221	Flag leaf length
RM431	178.3	B1078G07	AP003407	No. of tiller, Productive tiller, Grain protein content
RM13	28.6-31.4	OSJNBa0075G19	AP002842	Grain protein content
RM161	96.9	OSJNBa0044P19	AC135419	Grain width, Grain protein content
RM225	26.2	P0680A03	AB023482	Grain protein content
RGNMS167	103.98	P0523F01	AP003573	Plant height
RM125	24.8	OSJNBa0081K20	AP005171	Flag leaf length, No. of tiller ,Productive tiller
RM11	47	OSJNBb0062D12	AP005127	Grain protein content
RGNMS28	10.3	OSJNBa0040B10	AC116949	Flag leaf width
RM21	79.1	OSJNBa0041J17	AC134925	Grain protein content

Fig.3 The marker loci significantly associated with the traits in this study (P<0.05)

DISCUSSION

Phenotypic and morphological variations

Generally our findings showed that rice cultivars from CG core collection posses wide range of phenotypic and morphological variation. From the present study, a marked variation was recorded among the rice germplasm lines and varieties for various traits like plant height, flag leaf length *etc*. For example, the value of PH ranged from 66.1 to176.45 cm, for FLL value ranged from 21.7 to 37.28 cm *etc*. There are reports showing relatively greater range in plant height than the other characters [2]. Plant height in rice is a multifaceted character and the end product of a number of genetically controlled factors called internodes [6]. Reduction in plant height may develop their resistance to lodging and reduce substantial yield losses associated with this trait [1]. In addition to this, for the traits like PL, NT, PT, FLL, FLW, GL and GW the maximum values were approximately three time larger than the minimum values for the germplasm line and varieties studied. These relatively large levels of phenotypic variability were measured for all the traits among rice germplasm line and varieties, indicating that this collection was appropriate to be used in association studies of agronomic traits. Protein content analysis was done for all the 58 rice lines to know the difference in protein content. A wide variation was found in protein content of all the 58 lines varying from 6.09 to 11.2 %. Grain protein content in the range of 6.3% to 9.1% in a set of 58 rice genotypes have also been reported [16]. Similar results were observed in a study conducted by [3] on estimation of protein content in a set of 12 diverse rice genotypes including both cultivated and wild genotype of rice, results showed a range of 6.19-10.75% protein in whole grains.

DNA fingerprinting of high grain protein rice germplasm lines and varieties

In this study DNA fingerprinting of high grain protein containing rice germplasm lines and varieties was carried out on randomly selected five individuals of each germplasm lines and varieties using polymorphic SSR markers (Table 3). The SSR marker RM 489 showed variation in the banding pattern in which the germplasm line CGR436, GP-145-48 and CGR446 showed different pattern amplifying DNA fragments of 310 bp and 200 bp when compared to other germplasm lines and varieties which generated a single fragment of 220 bp (Fig. 1). Rahman *et al*., used a small set of three previously developed rice microsatellite markers for the identification and discrimination of 17 HYVs and 17 local rice cultivars including two wild rice cultivars [13]. These three markers were able to identify 15 local rice cultivars and 11 HYVs. A total of three variety specific alleles, RM-11/147, RM-151/289 and RM153/178 were identified for BR-11, Badshabhog and BR-19 cultivars respectively. Similarly in a different study hundred SSR markers were used to analyze the genetic diversity among hybrid rice parental lines of rice, out of which sixty two were found to be polymorphic [14]. Most of the primer sets generated unique fingerprints and were reported to be useful in their hybrid detection. For instance, commercial rice hybrid KRH-2 can be detected using its male parent KMR-3 specific markers RM297, RM442, RM541, RM584 and RM107 and female parent IR58025A specific markers RM529, RM489, RM589, RM533 and RM182 identified. The SSR marker identified to produce distinguishable banding pattern among high and low GPC line in our study can be similarly employed.

Graphical genotyping of rice germplasm lines and varieties using SSR maker data

The graphical genotyping analysis revealed that the two alleles more or less contributed equally in case of markers located on chromosome 2 whereas in case of chromosome 9 spanning SSR markers RM215 the genomic constitution was contributed majorly by allele A (99.6%) with little contribution of allele B (0.4%) (Fig.2). Similarly the chromosome 5 showed minimum contribution of allele A and maximum contribution of allele B (Table 4). Parida *et al*., analyzed genomic constitution of rice genotypes based on chromosome-wise physical distribution of variant SNP loci and allele sharing between *indica* and *japonica* [12]. This revealed maximum introgression of *japonica* in chromosome 12 (average 63%) followed by chromosomes 7 (59%) and 1 (57%). In contrast chromosome 6 contained maximum introgression (68%) of *indica* genome.

Association analysis between agronomic traits and molecular markers

In this study association analysis resulted in the identification of 17 significant SSR loci for the agronomic traits, with the R^2, percentage of the total variation explained ranging from 6.23to 23.88%. Zhou *et al*., reported a total of 16 significant marker trait association ($P<0.01$) for eleven agronomic traits using the MLM program in the TASSAL software [18]. All of the 12 significant loci were identified for the agronomic trait, with the R^2, percentage of the total variation explained ranging from 1.99 to 21.58%. Our data therefore demonstrate that using association analysis to examine important agronomic traits in rice varieties is an efficient way of identifying significant loci associated with these traits. Simultaneous associations for other traits can also be identified. This can be potentially useful for plant breeding programs. The information about the genetic diversity will be very useful for proper identification and selection of appropriate parents for breeding programs, including gene mapping, and ultimately for emphasizing the importance of MAS in rice improvement worldwide.

CONCLUSION

Chhattisgarh state is blessed with number of different types of rice and can be considered as foundation of variability where approximately at every 250 ha the germplasm pattern changes due to extreme variation in the topography, altitudes, soil tress coupled with variation in cultural heritage of the inhabitants [15]. Also the present day gene pool accommodate high yielding rice varieties every year which is evolved through breeding programs continuously resulting in difficulty in variety identification and cataloguing. Addressing this issue this research work attempts to unravel the nutritional quality in terms of grain protein content and popularize the rice landraces of CG, develop DNA fingerprint of the selected elite rice lines using SSR markers. This generates the base level data and foundation for genetic profiling of the diverse germplasm collection of the state as well as provides the basis as per requirement of Biodiversity act for registration of varieties/ landraces and patenting of genes. This will further revitalize the plant genetic resource base which will help to improve the economy of rural people with active participation of various stakeholders. In addition to this, the high grain protein germplasm lines and associated DNA markers indentified in this study can be used in the breeding program for the improvement of grain protein content in rice.

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