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Research Article

Validation of SSR markers linked to protein content in F_5 recombinant inbred lines derived from BPT 5204 × HPR 14 rice cross

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Abstract:
Grain protein content is polygenic character in rice and plays very important role in human nutrition. The objective of this study was to validate already mapped markers associated to protein content in F_5 Recombinant Inbred Lines of rice derived from BPT 5204× HPR 14 using Near Infrared Reflectance Spectroscopy. About 17 polymorphic primers were validated on selected F_5 lines. In F_5 generation, about 7 primers showed significant association with total grain protein content. Among 7 primers, RM520 showed 9.1 per cent phenotypic variability (R^2) followed by RM206 with 8.75 per cent R^2 in F_5 generation. Selected 2 lines with high protein content were subjected to Graphical genotyping analysis using 63 microsatellite markers. Line number 73 represented 39.7 per cent of BPT parent, 56.4 per cent HPR parent and 4 per cent of heterozygote. This indicates high protein content was inherited from male parent. It could be due to samples were taken from a large population and also less number of polymorphic markers scanned on chromosomes and it needs to be fine tuned with more number of markers on the whole genome. Lines with high protein content can be used for further improvement programme to develop a suitable variety to feed the human with nutritious diet.

Key words:
Rice, Grain protein content, Polymorphic markers, Phenotypic Variability, Graphical genotyping

Introduction

Protein plays important role in human nutrition. It acts as a catalyst in biological process and a constituent of cell membrane. Protein malnutrition had direct impact on the human growth and development. Rice is the main staple food crop in Asia and Africa. Rice provides 20 per cent of the world's dietary energy supply, while wheat supplies 19 per cent and maize 5 per cent (Kennedy and Burlingame, 2003). Even though rice provides 14 per cent of global protein but it limits in essential amino acids like lysine and threonine. The supplemented rice protein was comparable to milk protein, casein. The genetic variation in protein content provides a basis for breeding protein content. Breeding for improved nutritional character is the one of the major objectives nowadays. However, manipulating this trait using traditional breeding is difficult because such substantial variation is quantitatively inherited (Shi *et al*., 1999). Normally protein content was estimated by Nitrogen content using Kjeldahl method which is laborious. DNA markers linked to protein content help in screening of large number of genotypes within a short span of time. Molecular markers act as tools for the identification of trait of interest in seedling stage. DNA markers are highly polymorphic and not affected by the environment. Microsatellite markers are popular as they are co-dominant and polymorphic. Several recent studies have been undertaken to decipher the genetic basis of protein content in rice by QTL mapping (Tan *et al.*, 2001; Yoshida *et al.*, 2002; Wang *et al.*, 2008; Zhang *et al.*, 2008; Ye *et al.*, 2010) providing useful information for improving protein quality of rice. Utilization of already mapped specific markers linked to protein helps in selection of high protein alleles in the genotypes. The linkage mapping approach to find QTLs is expensive and requires much time to develop mapping populations, such as F_2, back cross (BC), doubled haploids (DH), recombinant inbred lines (RIL), and near isogenic lines (NIL); furthermore, these populations must be evaluated in multiple environments in order to obtain robust phenotype data. In the present study, we used a set of 100 F_5 Recombinant Inbred Lines constructed by single-seed descendent from a cross between BPT 5204 and HPR 14 to validate already mapped markers associated with protein content in our population.

Material and Methods

Plant material:

The F_5 RILs along with parents was planted at the GKVK, UAS, Bangalore district during *Kharif*

2009 under aerobic condition with recommended agronomic practices. The F_5 Recombinant Inbred Lines consisting of 100 lines maintained by a single seed descent from a cross between BPT-5204 and HPR-14 were used in this study. The female parent BPT 5204 is a fine grain variety and high yielder with average protein content of 8 per cent whereas HPR 14 is a local cultivar and14 per cent of protein content with bold grain variety.

Trait measurement: All harvested seeds from single plant were dried and dehusked. The dehusked rice samples from each line were used for estimation of protein content using Near Infrared Reflectance Spectroscopy (NIRS, Foss, Germany) facility available at National Seed Project (NSP), Dharwar.

DNA extraction: Genomic DNA was extracted from leaf tissues of 21 day old seedlings of the 100 lines by modified Cetyl Trimethyl Ammonium Bromide method (Cao and Oard, 1997). The DNA quantified at 260 nm using a UV spectrophotometer.

DNA markers and assays: About 17 simple sequence repeat (SSR) markers were validated on F_5 Recombinant Inbred Lines. These 17 rice microsatellites markers specific to protein were already mapped in different mapping population by various workers (Wang *et al.*, 2008, Zhang *et al.*, 2008, Tan *et al.*, 2001).

Amplification of microsatellites and detection of their polymorphism:The nucleotide sequences of the primer pairs used for each microsatellite are identified. PCR Reaction mixture consists of 2µl of template DNA, 25 ng each of forward and reverse primers, 100 µM each of dNTPs, 1 U of Taq polymerase and 1x RCR buffer(10 mM Tris pH 8.0, 50 mM KCl, 1.8 mM $MgCl_2$ and 0.01 mg/ml gelatin) in a volume of 20µl. One drop of mineral oil was put on reaction mixture. Thirty five PCR cycles were performed with 30 sec of denaturation at 94^{o}C, 1 min of annealing at 56^{o}C and 1 min of polymerization at 72^{o}C. Polymorphism in the PCR products was detected by ethidium bromide staining after electrophoresis in 3% agarose gel and PAGE. Highly polymorphic markers validated on Agarose gel whereas low polymorphic markers validated on Polyacrylamide Gel Electrophoresis (PAGE).

Data analysis: The markers linked to loci affecting protein content were identified using single marker analysis using Microsoft Excel 2007. Linear regression technique used to identify the marker linked to loci affecting protein content (Tinker, 1996). The proportion of the total phenotypic variation explained by each marker was calculated as R^2 value (R^2 = Ratio of the sum of square explained to the QTL to the total sum of square).

For the detection of genomic regions of parents in selected lines, Graphical genotyping was carried out using computer software 'GGT. 2.0' (http://www.spg.wau.nl/pv/pub/GGT) using 63 SSRs primers and graphical genotypes were obtained. Two lines were selected on the basis of protein content with moderate yield.

Results and discussion

Phenotypic analysis:Total grain protein content ranged from 7.39 to 12.81 per cent in F_5 RILs. Moderate phenotypic coefficient of variation was observed for crude grain protein content in RILs. Where as, genotypic coefficient of variation was low indicating significant variability present among the genotypes. The least influence of environmental variables was reflected by higher broad sense heritability estimates. Estimates of heritability and genetic advance suggest protein content to be mainly controlled by additive gene action.

SSR validation: Among 17 primers, eight were showing significant association with protein content using regression method. Primer name, phenotypic variability explained by a marker and additive effects are given in Table 1. Utilization of already mapped specific markers for protein helps in selection of high protein alleles in the genotypes. Before employing the associated markers in marker assisted selection, it is essential to validate their association with the trait of interest. Zhang *et al.*(2008) reported 3 QTL (qCP-2, qCP-7 and qCP-12) for crude protein content with 14.4, 11.2 and 14 per cent of phenotypic variation using RFLP markers. In the present study, RM 555 giving 8.07 per cent phenotypic variation and also the location of RM 555 was very near to a QTL qCP-2 as reported by Zhang *et al.* (2008). Wang *et al.* (2008) reported that RM 555 loci tightly linked to Chorismate mutase family protein, indicating the involvement of quantitative trait loci in amino acid synthesis pathway. In the present study, RM 206 and RM 168 significantly associated with protein content with 8.75 and 6.4 per cent of phenotypic variation whereas Yoshida *et al.* (2002) reported 12.4 per cent and 11 per cent phenotypic variation for protein content in rice. Since parents involved in F5 production were both *indica* parents, so the difference will occur from *indica* X *japonica* cross and also genotype and environment also matters. RM228 significantly associated with total protein content with 5.1 per cent phenotypic variation whereas, (Tan *et al.*, 2001) reported epistasis with the other protein markers for the protein content. RM 253 significantly associated with total protein content with 5.6 per cent phenotypic variability

whereas, (Aluko *et al*., 2004) reported 8.8 per cent phenotypic variability in doubled haploid population. RM 80 and MRG 2702 significantly associated with total grain protein content with 7.6 and 5.43 per cent phenotypic variability. On the other hand, (Wang et al., 2008) reported 5.5 - 11.7 and 6.9-12.9 per cent phenotypic variability in RIL population respectively. The difference from earlier studies and also present studies indicates the already mapped markers were specific to those genotypes derived from the respective parents and indicates that more markers needed to identify the protein specific markers in this population.

In the present investigation, graphical genotyping was carried out using 63 rice microsatellites for two selected lines in F_5 generation. The chromosomal segments distribution in selected lines along with parents is represented in Figure 1. Among the 12 linkage groups, chromosome 2 and 8 anchoring were more of protein specific. This result was supported by (Ye *et.al*., 2010) studies on chromosomal segment substitution lines. Line number 73 represented 39.7 per cent of BPT parent which was a female parent, 56.4 per cent HPR parent and 4 per cent of heterozygote and this was represented in Figure 2. This indicates high protein content inherited from the male parent i.e. HPR 14. Similar results were observed for the line number 82 (58.2 % of HPR genome). It could be due to samples were taken from a large population and also less number of polymorphic markers scanned on chromosomes and it needs to be fine tuned with more number of markers on the whole genome. Graphical genotyping will helps to know the donor segment in the progenies. It also help in direct selection of the individual. And it also gives parent genome content in the progenies.

Conclusion

From the present validation of protein specific markers on F_5 population, it indicate that there is a need of markers to satisfy all the region of chromosome. Selection for protein content is arduous conventionally since it is controlled by polygene and has low heritability. Molecular markers can be used to identify linkage to quantitative trait loci (QTL) for total grain protein content and these can be selected more easily in a breeding programme than the trait themselves. Graphical genotyping acts as indirect tool to select the genotype with high protein content based on genome content. These two lines with high protein content can be used for further crop improvement programme to develop a variety/hybrid with high protein content.

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Table 1. Results of SSR marker analysis in F_5 population with reference to protein content in the cross between BPT 5204 × HPR 14 in rice

Marker name	Chromosome	R^2(%)	F- value	Probability	Additive effect
RM 555	2	8.07**	7.98	0.006	0.255
RM206	11	8.75**	8.1895	0.0038	0.32
RM447	8	3.3	1.3549	0.2474	0.17
RM205	9	1.1	1.0634	0.3052	0.14
RM209	11	1.1	0.1714	0.6799	0.05
RM1369	6	1.5	0.9362	0.3358	0.12
RM204	6	2.8	0.2979	0.5866	0.06
RM520	3	9.1**	9.1972	0.0032	0.73
RM228	10	5.1*	4.696	0.0328	0.23
RM255	4	0.9	1.1254	0.2915	0.13
RM304	10	2.4	0.1634	0.6897	-0.05
RM80	8	7.6**	2.9164	0.0911	0.27
RM253	6	5.6*	5.6806	0.0193	0.3
RM1313	2	1.9	0.0756	0.7834	-0.05
RM168	3	6.4*	6.1543	0.0149	0.3
MRG2702	8	5.43*	5.1639	0.0255	0.2
RM6911	2	2.1	1.9522	0.1657	0.17

*- significant at 5% **- significant at 1%
R^2- Phenotypic variability by the QTL

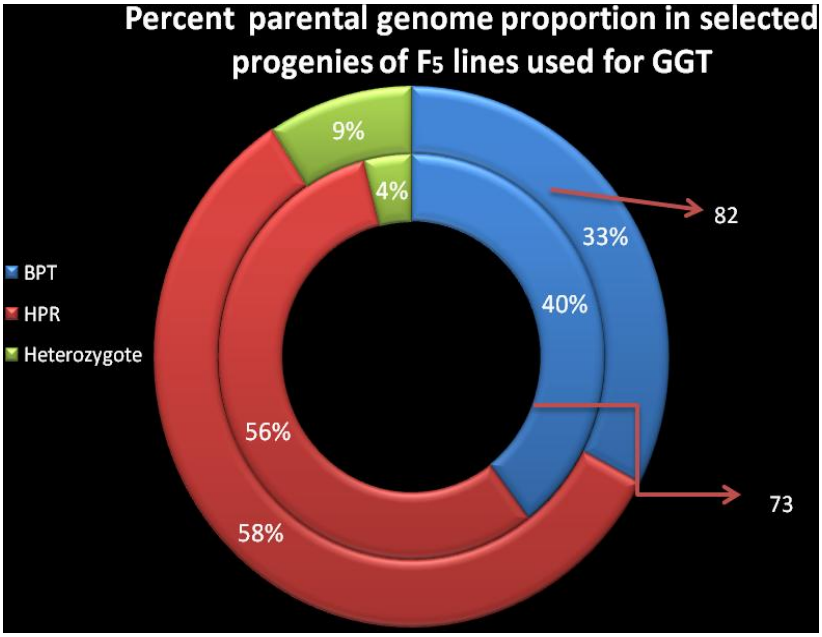

Figure 2. Per cent parental genome proportion as identified by SSR markers in selected lines of F_5 generation using graphical genotyping (GGT)

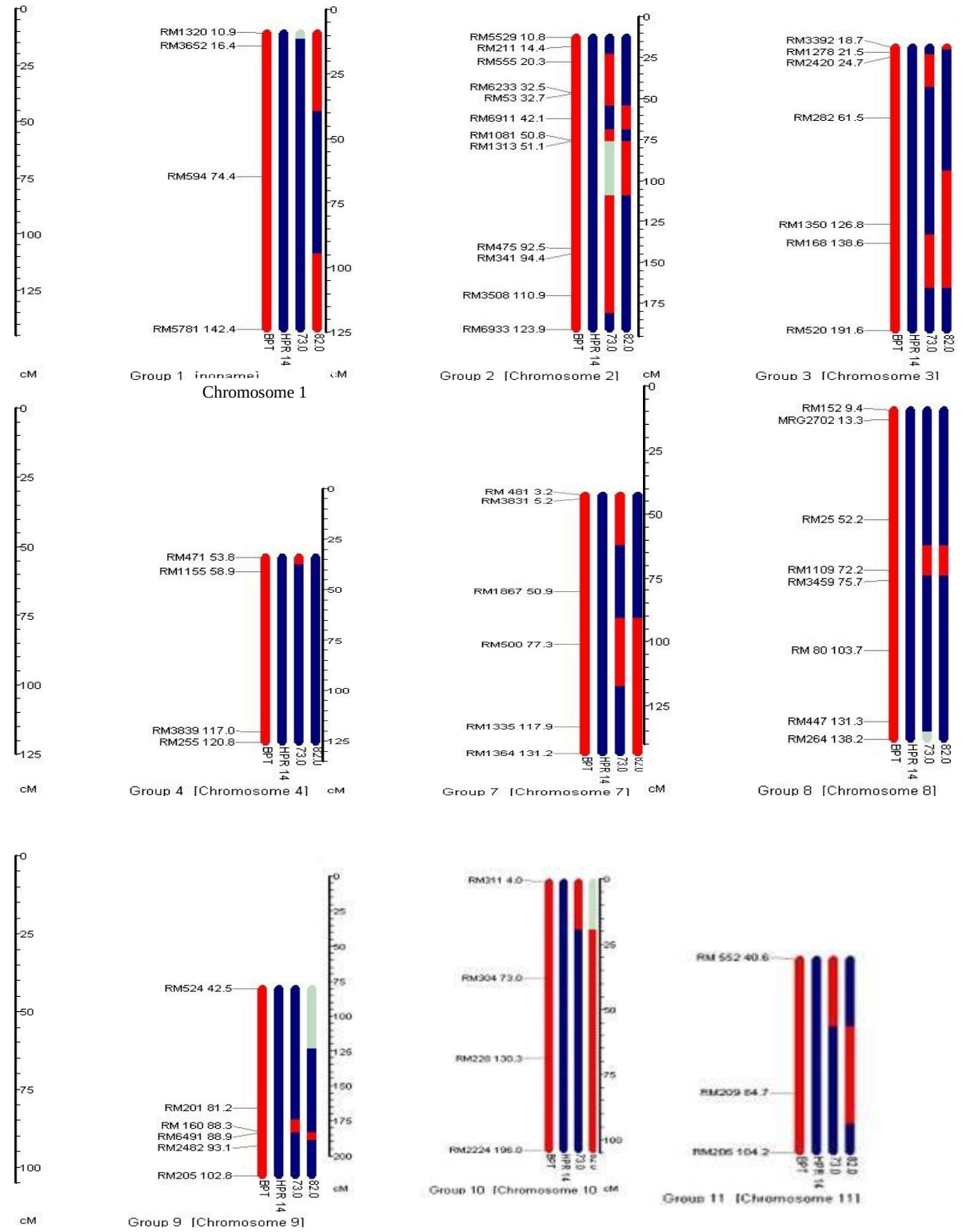

Figure 1. Distribution of parental chromosomal segments in selected two genotypes of F_5 generation and parents