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Identification of SSR Markers Linked to Regions Associated with Protein Content in F2 Population of Rice (*Oryza sativa L.)*

D. Sravani[1*], C. N. Neeraja[2], V. Ravindrababu[2] and M. Rajendar Reddy[3]

[1]Agricultural Research Station, Karimnagar, India.
[2]Indian Institute of Rice Research, Rajendranagar, Hyderabad, India.
[3]Agricultural Research station, Adilabad, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author DS collected, analysed, interpreted the data and prepared the manuscript. Authors CNN and VR conceptualized, designed and executed the experiment. Author MRR prepared the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Improvement of rice proteins is important in rice breeding for high nutritional quality. The objective of this study was to identify protein associated regions in the chromosomes in F2 population of the cross derived from swarna and Mahamaya, Parental polymorphism survey between Mahamaya and Swarna was studied using 24 SSR markers. Out of 24 markers, 4 (16%) were polymorphic, 185 F2 plants were assayed individually for protein content and genotyping. Total grain protein content ranged from 4.12 to 12.0 per cent in F2 population. The strategy of selective genotyping was carried out with the F2 plants. The results showed that these three markers RM1369 on chromosome number 6, RM263 on chromosome number 2 and RM337 on chromosome number 8 were unlinked among themselves. Since for mapping a minimum of two markers per locus are required, the data obtained for this study were analyzed by Recombination frequency. Recombination frequency values revealed the association of markers, RM1369 (Chromosome-6) with high protein content (Recombination frequency=0.41) and low protein content (Recombination frequency=0.25),

**Corresponding author: E-mail: dsravanireddy@gmail.com;*

RM263(Chromosome-2) with high protein content (Recombination frequency =0.37%) and low protein content (Recombination frequency =0.33%) and RM337 (Chromosome-8) with high protein content (Recombination frequency =0.22%) and low protein content (Recombination frequency =0.18). This trial study reveals that the above markers can be utilized for identification of regions associated with protein content in rice.

Keywords: Rice; protein; polymorphic markers; genotyping.

1. INTRODUCTION

Rice is the staple food for majority of the global population. However, rice grain has low protein content. Mapping of QTLs controlling grain protein content is essential for enhancement of the trait through breeding programs. Rice is staple food for more than half of the world population. It has a significant contribution in daily calorie-intake as millions of poor families depend mainly on rice for their nutrition. Rice supplies abundant carbohydrate as its grain constitutes mainly of starch (>80%) in addition to protein (7–8%) which is the source of concern in this work.

The genetic variation in protein content provides a basis for breeding protein content. Breeding for improved nutritional character is one of the major research objectives nowadays. However, manipulating this trait using traditional breeding is difficult because such substantial variation is quantitatively inherited Shi et al. [1]. Lang and Buu [2] found that protein content was controlled by genetic effects with heritability of 25.9%. Inheritance studies carried out earlier reported that difference in grain protein content between two parents was due to two major genes, even though a number of QTLs per se may control the trait. In recent years, the potential of molecular marker-assisted selection in plant breeding has been demonstrated in several crops Ye et al. [3].

The current rice breeding programs enhanced by marker-assisted selection looked to combine desirable traits/genes among the local gene pool into a single variety. Although rice breeding programs have had various achievements in meeting current human demands, even though molecular markers associated with dozens of genes controlling several traits of economic importance have been reported in rice Kenji Fujino et al. [4]. The improvement in grain protein content and its composition in protein has been a major concern of plant breeders. It has been difficult to achieve for effective selection criteria and because selection is expensive and time-consuming.

Normally protein content is estimated by Nitrogen content using Kjeldahl method but this 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. So development of one or more molecular markers to be used for indirect selection for protein content / composition should be a convenient alternative Ashwini Samak et al., [5].

2. MATERIALS AND METHODS

2.1 Experimental Location and Plant Materials

These studies were conducted at the Indian Institute Rice Research (IRRI), Hyderabad. Present study was carried out with the F_2 population of cross between Swarna and Mahamaya. Swarna is one of the most widely adapted and popular rice varieties while Mahamaya is known for its high protein content. Mapping population consisting of 185F_2 progeny derived from above crosses were selected for the mapping purpose. Total numbers of 185 individual F_2 plants seed were analyzed for Nitrogen content of rice was determined by Micro-kjheldahl distillation method Piper [6].

Protein content was calculated by multiplying nitrogen content of seed with factor Sadasivam and Manickam [7].

Protein content (%) = Nitrogen content (%) × 6.25 (Factor)

2.2 Genotyping

Collected leaf samples from 185F_2 progeny and parental lines, were ground after freezing with liquid nitrogen. The genomic DNA was extracted using modified CTAB protocol. The DNA quality was checked by 1.5% of Agarose gel electrophoresis. For the parental polymorphism survey, parents were tested against 24

microsatellites. Total numbers of 185 individual F_2 plants were analyzed through microsatellite markers for the mapping purpose. The genomic DNA of parental lines and the mapping population were subjected to PCR amplification using the procedure described by Chen et al. [8]. PCR was carried out using a programmable thermocycler (Corbett Research, Australia).

2.3 Mapping Analysis

Homozygotes were given a value of 0 or 1 based on their phenotype group. Heterozygotes were given a value of 0.5. Recombination frequency in percentage in relation to the total sample was calculated manually by using below mentioned formulae.

$$\text{Recombination Frequency} = \frac{\text{Number of Recombinant Progeny}}{\text{Total Number of Progeny}} \times 100\%$$

3. RESULTS & DISCUSSION

3.1 Parental Polymorphism Studies

Parental polymorphism survey between Mahamaya and Swarna was studied using 24 markers. Among Swarna and Mahamaya four (representing 16%) were polymorphic, eighteen (18) markers were monomorphic and two (2) were not amplified.

Total grain protein content ranged from 4.12 to 12.0 per cent in F2 population .The strategy of selective genotyping was carried out with the F2 plants showing extreme phenotypes exhibiting high and low protein content in grains individually with all polymorphic markers, to identify specific associated regions of the chromosomes with protein content [9].

The strategy of selective genotyping was attempted and was shown to be effective to

Table 1. Details of SSR markers used and their chromosomal locations

S. No	Markers	Chromosome number
1	RM4A	12
2	RM20A	12
3	RM42	8
4	RM168	3
5	RM206	11
6	RM214	7
7	RM222	10
8	RM234	7
9	RM219	9
10	RM255	4
11	RM263	2
12	RM330A	10
13	RM337	8
14	RM407	8
15	RM427	7
16	RM435	6
17	RM462	2
18	RM464	9
19	RM481	7
20	RM569	3
21	RM1369	6
22	RM1896	9
23	RM2111	5
24	RM5470	6

Table 2. Parental polymorphism survey between Mahamaya and Swarna

Polymorphism	No. of markers	Markers
Polymorphic	4	RM1369, RM263, RM206, RM168

Table 3. Details of selective genotyping for identification of regions associated with Protein content in cross Mahamaya × Swarna and parents

F2 population	Protein percentage	No of individuals
Highest	12.49	12
Lowest	4.12	10
Parents		
Mahamaya	12.00	1
Swarna	7.50	1

identify specific associated regions of the chromosomes as suggested by Nandi et al. [10]. The methodology of selective genotyping could be successfully used to identify the chromosomal regions associated with high protein content in rice grains. In this crossing, unlike in the former, only three markers were polymorphic i.e RM1369, RM263, RM337.)

3.2 Screening of F2 Population with Polymorphic Markers

To identify protein associated regions in the chromosomes, 185 F2 plants were assayed individually with three polymorphic markers identified in selective genotyping. Efficiency of molecular marker technology, for protein content in brown and milled rice were mapped using various rice mapping populations Yun et al. [11]. The results showed that these three markers RM1369, RM263, RM337 were unlinked among themselves. This result apparently seems expected because three of the markers represent three different chromosomes *i.e* if the markers wewre unlinked means they are present in different chromosomes. Though three markers per locus were surveyed for parental polymorphism, polymorphism was detected only for one marker per locus in the two parents. Since for mapping a minimum of two markers per locus are prerequisite, the present data were analyzed by Recombination frequency.

From the three markers, RM1369 on chromosome number 6, RM263 on chromosome number 2 and RM337 on chromosome number 8 and these were unlinked. Due to this Calcuating Recombination frequency is the feasible way to check the association of protein with the marker. Recombination frequency values revealed the association of markers, RM1369 (Chromosome-6) with high protein content (Recombination frequency=0.41) and low protein content (Recombination frequency=0.25), RM263 (Chromosome-2) with high protein content (Recombination frequency =0.37%) and low protein content (Recombination frequency =0.33%) and RM337 (Chromosome-8) with high protein content (Recombination frequency =0.22%) and low protein content (Recombination frequency =0.18). These results are in conformity with study conducted by Ashwini Samak N.R *et al.*(2011). RM1369, RM263 and RM337 were identified to be associated with protein content. Out of these three markers, RM337 showed close association with protein content based on the Recombination frequency value. So further investigation is warranted to identify closely linked markers involving increased population size and number of microsatellite markers, this study indicates that indicate that there is need for markers to satisfy all the region of chromosome. Similarly in addition to the F2 lines studied under selective genotyping, analysis of more F2 lines would increase the stringency of the loci identified for the protein content in the grain Selection for protein content is arduous conventionally since it is controlled by polygene and has more effected by genetic and environment interaction. 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.

Table 4. Recombination frequency for protein with RM1369, RM263 and RM337 markers

Trait	Marker	Recombination frequency
High Protein Content in the grain	RM1369	0.41
	RM263	0.37
	RM337	0.22
Low Protein Content in the grain	RM1369	0.25
	RM263	0.33
	RM337	0.18

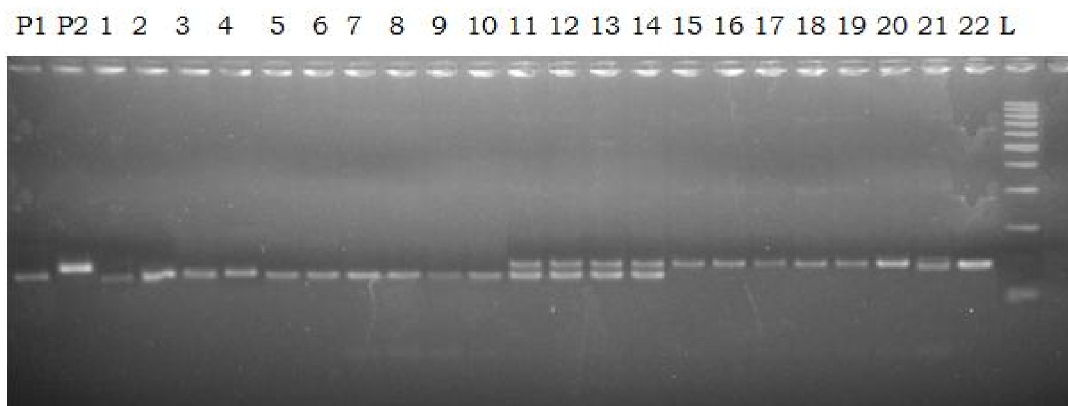


Fig. 1. Selective genotyping of F_2 population with RM 1369

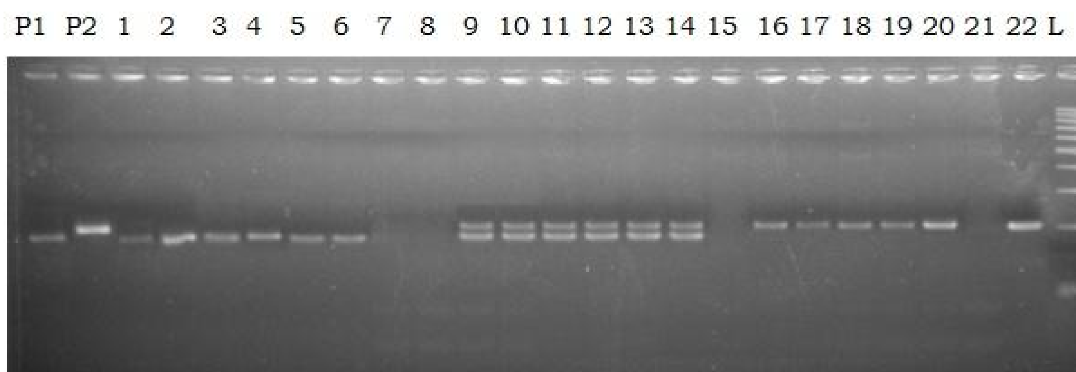
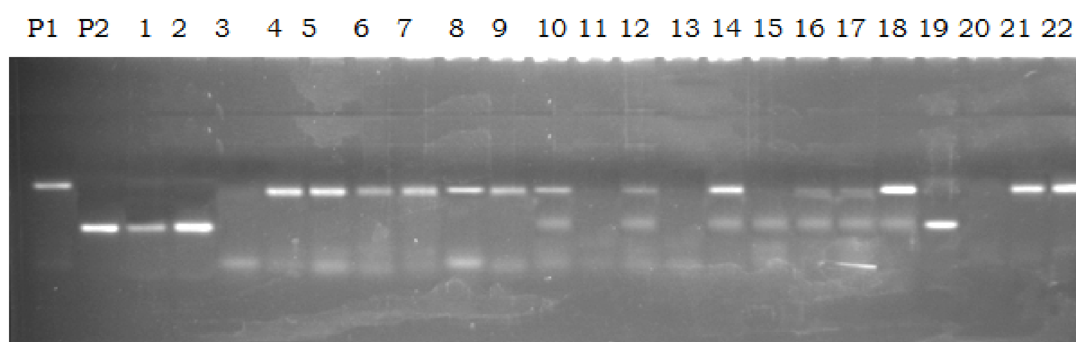


Fig. 2. Selective genotyping of F_2 population with RM 337



P1-Mahamaya P2-Swarna, 1-12, High protein F_2 population 13-22, Low protein F_2 population

Fig. 3. Selective genotyping of F_2 population with RM 263

4. CONCLUSION

Even though it is a preliminary study with limited number of molecular markers, it indicate that there is a need of markers to satisfy all the region of chromosome. In addition to the F2 lines studied under selective genotyping, analysis of more F2 lines would increase the stringency of the loci identified for the protein content in the grain. Selection for protein content is arduous conventionally since it is controlled by polygene and has more effected by genetic and environment interaction. Molecular markers RM1369, RM263 and RM337 may 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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