Use of Simple Sequence Repeat Marker to Assess the Segregating Population of Rice (*Oryza sativa*) in field for Grain Length Quality Parameter

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ABSTRACT

Rice grain quality is of paramount importance in the consumer preference for rice varieties and therefore the development of rice varieties should match to the consumer preference. Assessment of segregating population of breeding material in rice for quality parameter made it possible by the use of simple sequence repeat (SSR) marker. The F₂ mapping population of a cross between a long slender rice genotype VDN 1137-1-1 and a short slender rice landrace Badshabhog was studied for the inheritance of grain length and to identify the marker associated with grain type. The F₂ population showed 3:1 ratio for long slender to short slender grain type. This segregating population was assessed by 24 SSR markers using bulk segregant analysis. Among these 24 SSR Markers, a marker RM 147 reported polymorphism between the long slender and short slender parents and respective bulks. Genetic analysis indicated goodness of fit for single gene model indicating the association of RM147 with grain length gene in the segregating population. Therefore, SSR marker RM 147 is of immense value in the assessment of grain quality parameter in rice during the development of rice varieties.

Keywords: Rice, Grain Length quality, SSR marker, Segregating population, Marker Association.

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repeat marker to assess their use in the detection of grain character in the segregating F₂ population of rice crop.

I. INTRODUCTION

Rice (*Oryza sativa* L.) is the world's most important food crop and a primary source of food for more than half of the world's population as its main staple food. More than 90 % of the world's rice is grown and consumed in Asia where 60% of the earth's people live. It is consumed by more than 3 billion Asians daily. It is planted on about 154 million hectares annually or on about 11% of the world's cultivated land [1]. It is mainly grown in large areas of Asia, Latin America and Africa that are characterized by a semitropical climate with alternating rainy and dry seasons [2]. In India, rice is the main food source for more than 60 per cent of the population and contribute a share of 45 per cent in the Indian cereal production. The consumer preference for the rice varieties varies with the people of the agro-ecological zones. The favorable grain appearance of long and slender rice is preferred by consumers in America, Africa and Southeast Asia, the short and bold rice is preferred by northern China, Japan and Korea, while the long, soft grain rice is preferred in India [3]. In Indian urban population the preference is mostly to the long and slender varieties. Therefore, breeding of rice varieties for this character is an important task to the rice breeders. Large scale screening of rice germplasm and breeding segregating population in the field, is a time consuming and tedious process. Therefore, the use of marker assisted selection is advantageous in this process. In the present studies we used 24 simple sequence

II. MATERIAL AND METHOD

A. Parentage of Crossing Population and Screening for Grain Character

The F₂ mapping population was derived from a cross made between VDN 1137-1-1 and Badshabhog. The line VDN 1137-1-1 developed by Agricultural Research Station, Vadgaon (Maval) has long slender grain type whereas Badshabhog, the local type from Orissa state, India has short slender grains. The F₂ population and the parents were evaluated during the rice growing season for all the quality parameters under natural conditions. The observations on grain length, grain width and length-breadth (L: B) ratio were recorded on parents and 136 randomly selected F₂ plants. Observations were recorded for grain length and grain width on ten randomly selected grains from parents and 136 F₂ plants at the time of harvesting and calculated the L: B ratio. The genotypes with grain length more than 6.00 mm and L: B ratio ≥ 3 was considered as long slender whereas, genotypes with grain length less than 6.00 mm and L: B ratio ≥ 3 are as considered as short slender.

III. GENOMIC DNA EXTRACTION OF PARENTS AND CROSSING POPULATION

Genomic DNA was extracted from the leaves of both the parents and 136 individual F₂ plants by CTAB method described by Doyle and Doyle [4]. The quality and quantity of DNA were estimated spectrophotometrically using a Nano Drop (ND-1000, Wilmington, USA). Bulk segregant analysis (BSA) method as suggested by Michelmore *et al.* [5] was used for quick identification of SSR markers associated with grain length.

Based on phenotypic observations, two bulks *viz.*, long

slender (B1: comprising of ten long slender F₂ plants) and short slender (B2: comprising of ten short slender F₂ plants) were made. These 20 F₂ plants were found homozygous when screened with the SSR markers used in the study. A pooled DNA sample was prepared for each bulk by mixing in equal quantity the DNA of ten respective component F₂s. The parents and the bulks were screened with 24 SSR primers (Table 1) to determine polymorphism and possible association with grain length. These markers were selected based on the information collected from the website www.gramene.org and available with us were used.

TABLE 1: SSR PRIMERS EMPLOYED IN THE IDENTIFICATION OF POLYMORPHISM FOR THE GRAIN TRAIT IN F₂ POPULATION

Sr. No	Primer name	Forward Primer(5'-3')	Reverse Primer(5'-3')
1	RM 304	TCAAACCGGCACATATAAGAC	GATAGGGAGCTGAAGGAGATG
2	RM 147	TACGGCTTCGGCGGTGATTCC	CCCCGAATCCCATCGAAACCC
3	RM 265	CGAGTTCGTCGAAGTGAGC	CATCCACCATTCACCAATC
4	RM 315	GAGGTACTTCTCCGTTTCAC	AGTCAGTCACTGTGCAGTG
5	RM 109	GCCGCCGGAGAGGGAGAGAGAG	CCCCGACGGGATCTCCATCGTC
6	RM 154	ACCCTCTCCGCCTCGCCTCCTC	CTCCTCCTCTGCGACCGCTCC
7	RM 29	CAGGGACCCACCTGTCATAC	AACGTTGGTCATATCGGTGG
8	RM 341	CAAGAAACCTCAATCCGAGC	CTCCTCCCAGTCCCAATC
9	RM 112	GGGAGGAGAGGCAAGCGGAGAG	AGCCGGTGCACTGGACGGTGAC
10	RM 121	ACCGTCGCTTCCACTTTCCCC	TTCCGGGTTGCCGGTGATGTTG
11	RM 285	CTGTGGGCCCAATATGTCAC	GGCGGTGACATGGAGAAAG
12	RM 251	GAATGGCAATGGCGCTAG	ATGCGGTTCAAGATTCGATC
13	RM 338	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC
14	RM 255	TGTTGCGTGTGGAGATGTG	CGAAACCGCTCAGTTCAAC
15	RM 270	GGCCGTTGGTTCTAAAATC	TGCGCAGTATCATCGGCGAG
16	RM 42	ATCCTACCGCTGACCATGAG	TTTGGTCTACGTGGCGTACA
17	RM16	CGCTAGGGCAGCATCTAAA	AACACAGCAGGTACGCGC
18	RM60	AGTCCCATGTTCCACTTCCG	ATGGCTACTGCCTGTACTAC
19	RM21	ACAGTATTCGTAGGCACGG	GCTCCATGAGGGTGGTAGAG
20	RM229	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTAATGT
21	RM515	TAGGACGACCAAGGGTGAG	TGGCCTGCTCTCTCTCTCTC
22	RM72	CCGGCGATAAAACAATGAG	GCATCGGTCCTAACTAAGGG
23	RM125	ATCAGCAGCCATGGCAGCGACC	AGGGGATCATGTGCCGAAGGCC
24	RM481	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG

IV. POLYMERASE CHAIN REACTION

The PCR protocol involved a total volume of 20 µL containing 20 ng genomic DNA, 0.1 µM of each primer, 2.0 µL of 10× Taq DNA polymerase buffer (100 mMTris pH 9.0, 500 mM KCl), 200 µM of each dNTPs and 1 U of Taq DNA polymerase. The reaction profile was 5 min at 95 °C, 40 cycles of 30 s at 94 °C, 30 s at 55 °C/60 °C annealing, 1 min at 72 °C and 10 min at 72 °C for final extension. The PCR products were electrophoresed on agarose gel (2.5%) and visualized on Gel Documentation System (Flour ChemTM Alpha Innotech Corporation, San Leandro, USA). The SSR marker found polymorphic among the parents and the bulks was used for F₂ progeny analysis.

The clearly resolved alleles of SSR were scored manually as homozygote for the allele for short slender (0), homozygote for the allele for long slender parent (1) and homozygote for the allele (2) in the data sheet. Chi-square (χ^2) test was performed to test the goodness of fit of the F₂ population for the phenotypic and marker data by comparing an observed frequency distribution with an expected one and was analysed by Simple linear regression method to estimate association among marker and character in a spread sheet.

V. RESULTS AND DISCUSSION

The rice grain of crossing parent VDN 1137-1-1 was found long slender while that of Badshabhog was found short slender in the field. Among the randomly selected 136 F₂ progeny plants, 105 plants showed long slender grain type and 31 plants showed short slender grain type. The observed frequencies when tested for goodness of fit with chi-square (χ^2) test for single gene model showed goodness of fit ($P = 0.038$) to the expected segregation ratio (3:1) (Table 2).

TABLE 2: PHENOTYPIC EVALUATION OF THE F₂ POPULATION

S. No.	Category	Observed phenotype	Expected phenotypes	χ^2 value	P value	Observed ratio
1	Long slender	105	102	0.602	0.038	3:1
2	Short slender	31	34			
3	Total	136	136			

Among the 24 SSR markers used, only one marker *i.e.*, RM 147 reported polymorphism between long slender and short slender parents and corresponding bulks indicating their possible association with grain length in the mapping population. The F₂ mapping population was assessed with this primer to study its possible association with grain length. Segregation study with marker RM147 recorded an allele of ~154 bp amplified in 98 plants, whereas an alleles

of ~161 bp and ~154 bp were amplified in 38 plants (Table 3; Fig. 1).

TABLE 3: MOLECULAR EVALUATION OF THE F2 POPULATION WITH SSR MARKER RM 147

S. No.	Category	Observed phenotype	Expected phenotypes	χ^2 value	P Value
1	Long slender	98	102	0.627	0.039
2	Short slender	38	34		
3	Total	136	136

Fig. 1. Bulk segregant analysis with SSR marker RM 147.

Genetic analysis with chi-square test indicated goodness of fit to the expected ratio of 3:1 for single gene model indicating the association of RM147 with grain length gene in the present population. This confirmed that the grain length in rice in present investigation was governed by single gene. The simple regression analysis between phenotypic data of grain length and the genotypic data of SSR marker RM147 indicated that the marker was significantly linked with grain length (Table 4).

TABLE 4: ANOVA FOR REGRESSION ANALYSIS FOR SSR MARKER RM147 WITH PHENOTYPIC DATA

S. No.	Source	Degrees of freedom	Sum of squares	Mean sum of squares	F	P
1	Regression	1	8.87	8.87	67.51**	3.911
2	Residual	134	17.60	0.13		
3	Total	135	26.47

Fig. 2. Validation on individual F₂ plants with SSR marker RM 147.

New genetic tools, such as molecular markers provide a new alternative selection approach to enhance genetic improvements in crops. Molecular study in relation with the grain length is very important for the grain quality in rice. In order to identify the new sources of quality traits, there is need for identification of new quality genes in genetically diversified rice material. Independent studies have identified many QTLs associated with rice grain length using primary mapping populations [6]-[8]. During the last few years, genetics of rice grain length has been extensively studied and many genes associated with grain length were mapped on rice chromosomes 1, 3 and 10 [6], [9]-[11]. Chromosome 10 of rice has been reported to contain important genes and QTLs for grain size, especially grain length. In the present study, the SSR marker RM147 which was found associated with grain length (through BSA) segregated in 3:1 ratio confirming the single gene control. The results obtained in this study are in accordance with several earlier reports [6], [12]-[16]. However, it should be noted that the material used in the present study was totally different from the one used in by earlier workers [12]-[16]. The SSR marker RM147 has been used earlier by Pal *et. al.* [17], Jiang *et.al* [18] and Bai *et al.* [19], on the long arm of chromosome 10 explaining 30.7% phenotypic variation.

The genetic makeup of a particular variety dictates to a large extent the grain quality in rice. Plant breeders persistently refine and improve genetic traits of new varieties required to produce. The QTLs related to yield potential and grain length have been detected by using the genetic linkage maps. Independent studies have identified many QTLs associated with rice grain length using primary mapping populations [6], [20], [21]. The identification of QTLs for grain length and identification of their genetic control are necessary for the development of MAS strategies aimed at improving breeding efficiency. Molecular marker technology offers such a possibility and therefore are useful for mapping genes of interest, marker-assisted breeding and cloning genes by map-based cloning strategies. Molecular study in relation with the grain length is very important from the quality point of view as the consumers either prefer long slender / short slender with aroma and other quality characters.

Marker assisted selection (MAS) has been applied for integrating different quality genes into rice cultivars which lacks into them. In order to identify the new sources of quality traits, there is need for identification of new quality genes in genetically diversified rice material. In the present investigation the inheritance of grain length in F₂ mapping population and the genes/QTLs associated with it was studied. The ultimate objective of present study was to create a genetic mapping resource in the form of recombinant inbred lines (RILs) for future genetic studies from the population derived in the present study.

In present investigation, wide variation was observed for the grain length in the parental genotypes as well as in the F₂ population. This indicated that the parental genotypes used in the present study for developing the population was an appropriate choice and a permanent genetic stock in the form of RILs can be developed from the present F₂ population. The phenotypic data on the grain length showed that the grain length is governed by single dominant gene.

VI. CONCLUSION

The present study indicated that the SSR marker RM147 is associated with grain length gene/locus in the rice and also highlight the importance of BSA in establishing marker-trait association in a rapid way. The findings of this study could be directly useful in molecular analysis of segregating generations, breeding lines and varieties of rice for the grain quality trait. This marker can detect the rice grain quality trait in the segregating populations of rice under field at an early growing plant stage and therefore can save lot of time and efforts in the identification of grain quality trait and development of consumer preferred rice varieties.

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