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Research Article

Validating effect of QTLs and identifying breeder friendly markers for manipulating grain length in local rice (*Oryza sativa* L.) genotypes

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Abstract

Rice varieties possessing short slender grains with aroma is highly preferred by the farmers. Most of the non-basmati rice varieties are traditional genotypes possessing poor yield and undesirable characteristics like lodging, susceptibility against pests/diseases/abiotic stresses which necessitates accelerating development of such varieties. The present study was undertaken with an aim of validating SSR markers linked to grain length in an F_2 population derived between Pusa Basmati 1 (long slender grain) and CBMAS1405 (short slender grain). Genotyping and phenotyping of F_2 progenies revealed that two SSR markers *viz.*, RM11 and RM336 were found to be linked to *qGRL7.1* and *qGRL7.2* respectively with a phenotypic effect (R^2) of 7.9% and 16.5%. Genotyping and phenotyping of F2 progenies of CBMAS14065 x Pusa Basmati 1 led to the identification of two agronomically superior lines *viz.,* 224-7 (5.2 mm) and 224-9 (7.7 mm) harboring short grains with aroma and high yield potential.

Key words
Rice, grain length, aroma, marker validation

Introduction

Being the staple food for two third of the population, rice plays an important role in Indian economy. During Green revolution, high yielding varieties were developed and released leading to a quantum jump inproduction and productivity of rice making India one of the leading exporters of rice. Unattractive grain characters and poor cooking quality adversely affect the acceptance and spread of modern varieties. Grain quality in rice is determined by the physical appearance of grain like length, width, shape, chalkiness and cooking quality etc. (Fitzgerald *et al.*, 2009). Regional differences exist in the preferences for grain length and shape in rice (Calingacion *et al.*, 2014). Nearly 40% of people throughout the world especially USA, Canada, Europe, Middle East and South East Asian countries prefer consuming aromatic rice irrespective of grain types. In India, preferences for rice grains are highly varying across states ranging from short slender to long bold.

In South India, short/medium slender rice is most preferred by the people irrespective of aroma in rice. Grain length is a key component determining its quality and market in South India. Efforts on mapping QTLs/genes controlling grain length in rice over past few years resulted in identification of three significant QTLs viz., *qGRL-1.1* (Chromosome 1) and *qGRL-7.1* and *qGRL-7.2* on Chromosome 7 (Amarawathi *et al.*, 2008). These identified QTLs were flanked by marker interval RM431-RM104 for *qGRL-1.1*, RM11-RM505 for *qGRL-7.1*and RM505-RM336 for *qGRL-7.2.* Amarawathi *et al.* (2008) reported that QTLs *qGRL-7.1, qGRL-7.2* are tightly linked to the nearest flanking marker RM11 and RM336 with genetic distance of 0.01cM and 0.49 cM, hence can be suitably used for marker assisted breeding after validation of these linked markers in desired breeding population.These major QTLs *qGRL-1.1, qGRL-7.1,*and *qGRL-7.2*were further fine mapped by Singh *et al.* (2012). In this study efforts were made to validate the effects of the above 3 QTLs and identify breeder friendly SSR markers for monitoring retention of short grain character in the genetic back ground of a locally adapted high yielding and short slender grain genotype CBMAS14065.

Materials and Methods

The present study was carried out with an aim of validating effects of three major effect QTLs viz., qGRL1.1, qGRL7.1 and qGRL7.2 linked to grain length in rice in the genetic background of CBMAS14065, a locally adapted rice culture with short slender grains (4.9 mm length). An F_2population derived between CBMAS14065 x Pusa Basmati 1 (long slender grains; 7.9 mm length) segregating for grain length were used in this study. Genetically pure seeds of these parental lines obtained from Department of Rice, TNAU, Coimbatore. Three major effect QTLs *viz.,qGRL-1.1, qGRL-7.1,* and *qGRL-7.2* reported to be linked to grain length in rice were identified through literature survey (Singh *et al.*, 2012).

QTL	Chromosome	Marker Interval	Physical Position	Reference
qGRL1.1	1	RM226-RM104	34.027Mbp-40.166Mbp	Singh et al., 2012
qGRL7.1	7	RM11-RM505	19.20Mbp-19.44Mbp	
qGRL7.2	7	RM505-RM336	19.44Mbp-21.81Mbp	

Eight SSR markers located in vicinity each grain length QTLs were identified from GRAMENE database (www.gramene.org) and used for polymorphism survey between parents.

Genomic DNA was isolated from leaf samples of CBMAS14065, Pusa Basmati 1 using the modified CTAB protocol as described by Murray and Thompson (1980). Isolated genomic DNA was quantified by Nanodrop spectrophotometer (ND-1000 Spectrophotometer, NanoDrop Technologies, USA) and was diluted to a final concentration of 25 ng/µl and stored at -20°C for further use.Parental polymorphism survey was carried out using eight SSR markers. PCR reactions were carried out in a volume of 10 µl reaction mixtures consisting of 50ng of DNA, 1x PCR master mix (Ampliqon, Denmark) and 2 µM of each primer set. PCR reactions was performed in a thermal cycler (DNA Engine, BioRad, USA) programmed for an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s, extension at 72°C for 30 s and final extension at72°C for 10 min. Amplification products were electrophoresedin 3.5% agarose in 1X TBE buffer (90 mM Tris-borate, 2 mM EDTA, pH 8). The gels were stained with ethidium bromideand documented using gel documentation system (BioRad, USA). Polymorphic markers were identified based on their variation in allele size in both the parents for the respective marker.

SSR markers showing polymorphism between the parents CBMAS14065 and Pusa Basmati 1 *viz.*, RM3403, RM11 and RM336 were used for genotyping of 84 F_2 progenies. SSR genotyping including PCR amplification and gel electrophoresis was carried out as described above.

Grain length of 84 F_2 progenies derived from CBMAS14065 x Pusa Basmati 1 along with parents was measured using a Digital Vernier Calliper. Grains selected from the primary rachis of primary panicles were dehusked and length was measured. For each progeny, 10 grains were used for measurement and mean values were used for estimating market-trait associations.

Mean grain lengths of 84 F_2 progenies were analysed against the genotyping data through single marker analysis using Haldane function in QTL Cartographer v2.5 software (Wang *et al.*, 2001). The F probabilities and R^2 values generated were analyzed for declaring significant association of markers with grain length in the F_2 population.

Results and Discussion

Rice is cultivated in an area of 156 Mha across 124 countries with a worldwide production of 745 million tons (Todaka *et al.*, 2015). In India, it is cultivated in an area of about 43.5 Mha and also remains as one of the staple food crops Singh *et al.* (2016). Significant efforts have been put through various rice research programs which led to continuous release of high yielding varieties. Several reports have pointed out consumer preferences determine a varieties popularity and cultivation. Calingacion *et al.* (2014) conducted an extensive survey on regional preferences of major quality traits in rice viz., length and shape of grains, amylose content and gel consistency and came out with two most preferred combinations *viz.*, both long and medium length with slender grains, while one with low amylose, low gelatinization temperature and aroma and the other with intermediate amylose and without aroma. Grain length is an important quality attribute in rice when it comes to consumer preference. Finding a reliable and consistent marker closely linked to the QTLs associated with grain length will help in accelerating marker assisted breeding program for improving grain length in rice. Several QTLs/genes associated with grain length traits have been mapped in rice including *GW2, GL-3/GS3, qSW5/GW5 and qGRL7.1/qGL7/qGL7-2* etc. Redona and Mackill (1998) identified 7 QTLs on chromosome 2, 3, 4 and 7 for grain length which explain a phenotypic variance of 42% whereas Xu *et al.* (2004) identified 8 QTLs on chromosome 3 and 5 with a phenotypic variance of 65.9%. Singh *et al.* (2012) reported 3 grain length QTLs viz., *qGRL-1.1, qGRL-7.1, qGRL-7.2* in the genetic background of Pusa basmati 1121 located on chromosomes 1 and 7 contributing a phenotypic variance of 10.8%, 15.2 % and 5.4% for grain length. In this study attempts were made to validate the association of the above 3 QTLs viz., *qGRL1.1*, *qGRL7.1* and *qGRL7.2* with grain length in the genetic background of a locally adapted culture CBMAS14065 and also to identify markers for MAS programs. Pusa Basmati 1 is a long grained aromatic rice variety has international importance in rice market where as CBMAS14065 is a derivative of White Ponni having high yield potential, higher number of grains per panicle and having short slender grains. Grain length of CBMAS14065 was found to be 4.9mm whereas grain length of Pusa Basmati 1 was 7.9 mm showing a significant difference in their grain length and their suitability in their application to develop a mapping population to validate the grain length QTLs. Grain length in F_2 population ranged from 4.8 to 6.9 mm with a population mean of 5.83 mm showing continuous variation and their suitability for mapping grain length trait. Parental polymorphism survey using makers located in the vicinity of three QTLs associated with grain length viz., *qGRL1.1*, *qGRL7.1* and *qGRL7.2* led to the identification of three polymorphic SSR markers i.e., RM3403, RM11 and RM336 respectively between CBMAS14065 and Pusa Basmati 1 (Fig. 1).

Genotyping of 84 F_2 progenies using RM3403 (*qGRL1.1*) revealed that 29 progenies were found to possess CBMAS14065 allele, 15 progenies were found to possess Pusa Basmati 1 allele and 40 were found to remain under heterozygous conditions. Genotyping using RM11 resulted in identification of 24 progenies harboring CBMAS14065 allele of RM11, 20 with Pusa Basmati 1 allele and 40 progenies under heterozygous conditions. Genotyping of 84 progenies using RM336 resulted in identification of 20 progenies harboring CBMAS14065 allele, 26 with Pusa Basmati 1 allele and 38 under heterozygous conditions.

Seeds harvested from 84 F_2 progenies were used for recording grain length along with CBMAS14065 (short grain) and Pusa Basmati 1 (long grain). Ten uniform sized seeds were selected from each line and used for measuring grain length (mm). Grain length of CBMAS14065 was found to be 4.9 mm whereas grain length of Pusa Basmati 1 was found to be 7.9mm (Fig. 2). Grain length was found to be ranging from 4.8 mm to 6.9 mm among the F2 progenies and followed a normal pattern of distribution (Fig. 4).

Single marker analysis using the genotyping and phenotyping data of F2 individuals revealed the non-association of RM3403 (qGRL1.1) with grain length in CBMAS14065 x Pusa Basmati 1. Single marker analysis revealed the significant association of RM11 (qGRL7.1) and RM336 (qGRL7.2) to grain length with a phenotypic effect of 7.95% and 16.5% respectively at P = 0.01% (Table 1). The identified markers linked to *qGRL7.1* and *qGRL7.2* i.e. RM11 and RM336 can be used for marker assisted breeding for grain length using genetic background of CBMAS14065 and Pusa Basmati 1.

Success of any breeding by design approach depends mainly on selection of right genotypes for inter-mating and backcrossing. In this study, genotyping and phenotyping of 84 progenies of CBMAS14065 x Pusa Basmati 1 led to the identification of 2 agronomically superior lines viz., 224-7 and 224-9 possessing QTLs of short grain length. Line # 224-7 was found to possess short grains (Grain Length = 5.2 mm) and another line #224-9 was found to possess grain length of 5.7 mm. Continuation of efforts in genotyping of inter-mated F_1s, identification of lines possessing all targeted grain quality traits and further backcrossing with CBMAS14065 will lead to development of elite rice genotypes possessing short slender grains.

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Table 1. Results of single marker analysis showing R^2 value and F value for each marker.

QTL	Chromosome	Marker	Probability value (F)	R^2
qGRL1.1	1	RM3403	0.955882472	0
qGRL7.1	7	RM11	0.009026867 **	7.9%
qGRL7.2	7	RM336	0.000122358 **	16.5%

**Significance at probability value ≥ 0.01%

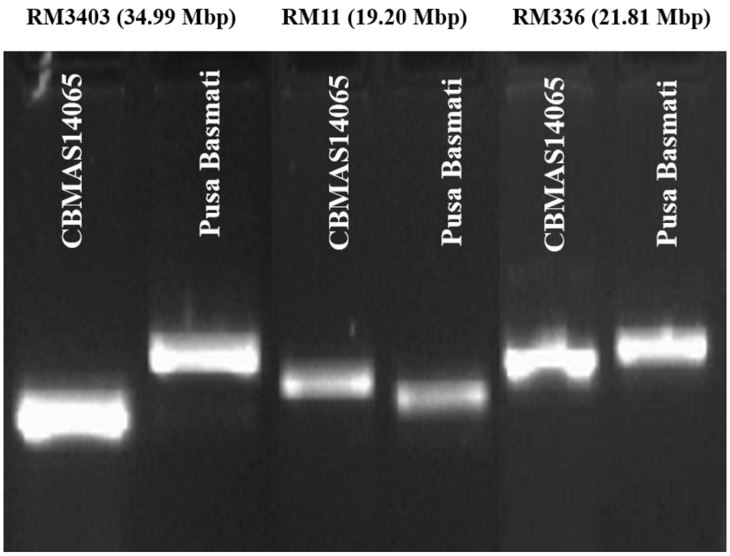

Fig. 1. SSR markers showing polymorphism between CBMAS14065 and Pusa Basmati 1 in the vicinity of *qGRL1.1* (RM3403), *qGRL7.1* (RM11) and *qGRL7.2* (RM336).

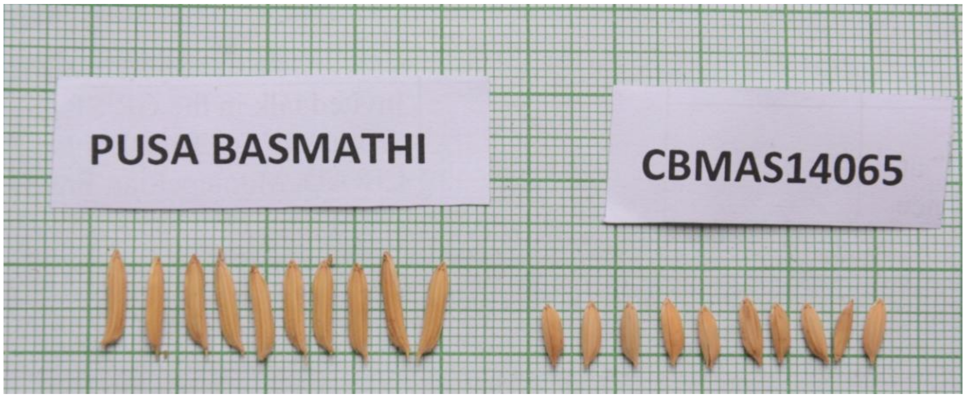

Fig. 2. Rice genotypes CBMAS14065 (short) and Pusa Basmati 1 (long) differing in grain length.

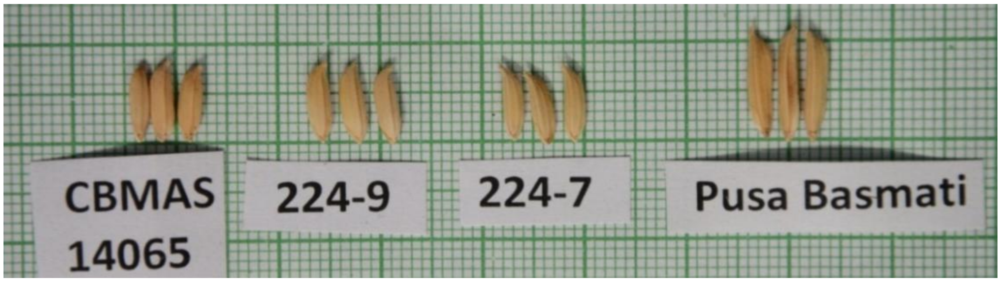

Fig. 3. Grain characteristics of 224-9 and 224-7 (short grains).

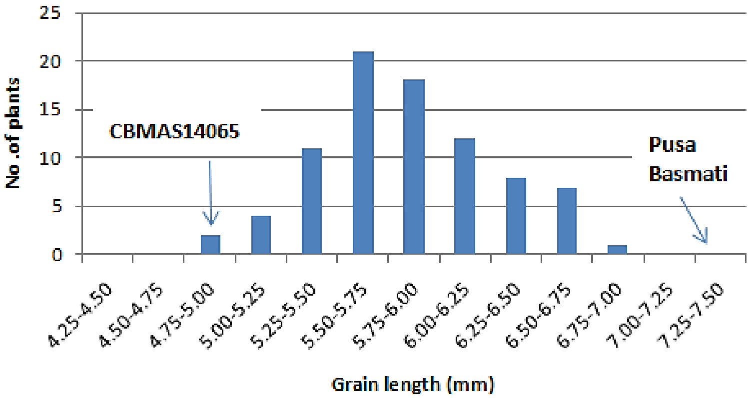

Fig. 4. Frequency distribution of grain length among 84 F2 progenies of CBMAS14065 x Pusa Basmati 1