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Validation of Molecular Markers Linked to Grain Quality Traits in Rice (*Oryza sativa* L.)

Kavurikalpana[1*], S. Thirumeni[1] and N. Shashidhara[2]

[1]Department of Plant Breeding and Genetics, Pandit Jawaharlal Nehru College of Agriculture is an agricultural College (PAJANCOA & RI), Karaikal – 609603, India
[2]Agricultural Research Station (ARS), Bailhongal. University of Agricultural Sciences, Dharwad, Karnataka, India

**Corresponding author*

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A B S T R A C T

Global demand for high-quality rice has grown substantially in recent years and continues to trend upward due to taste preferences and a greater interest in healthy diets. Most rice quality traits are inherited in a complex way and may be affected by multiple genes and environmental factors. Experiment was conducted by using 64 rice accessions at Pandit Jawaharlal Nehru collage of agriculture and research institute, karaikal during *Rabi* 2016. Lattice square design with three replications was followed for phenotypic data and analyzed by using R. software version 3.4.0. Molecular analysis was done by using functional markers for pericarp color and fragrance. Out of 64 rice accessions seven are red seeded and 57 white seeded, which could be easily identified in the seedling stage itself by using functional marker RID 12 developed by Sweeney *et al.,* (2006) that perfectly targets the 14 bp functional nucleotide polymorphism with in *bHLH* gene. However, for fragrance, none of 64 genotypes were scented as reflected from the mono-morphism of the target allele.

Introduction

As a major cereal crop, rice (*Oryza sativa* L.) is crucial to food security for at least half of the world population. Improvement of rice quality has now become a foremost consideration for rice buyers and breeding programs.

Therefore, breeding strategies to attain high grain quality while maintaining high yield are essential to satisfy both consumer needs and preferences (Ni *et al.,* 2011). In recent years, breeders have paid more attention to quality improvement while maintaining the stability of rice production.

One of the most important quality attributes of rice is its typically pleasant aroma. Numerous chemical constituents including different volatile compounds are the major sources of aroma in cooked rice, along with environmental factors. Bergman *et al.,* (2002) established that 2-acetyl-1-pyrroline is the key aroma constituent of fragrant rice. Furthermore, Bradbury *et al.,* (2005) reported

that aroma is controlled by a recessive gene (*fgr*) on chromosome 8; that contains of an 8-bp deletion and three single nucleotide polymorphisms (SNPs). Two types of molecular markers, including simple sequence repeats (SSRs) and SNPs, were identified as promising markers for the selection and identification of fragrance in rice (Yeap *et al.*, 2013). A perfect marker system, namely Allele Specific Amplification (ASA) was developed by Bradbury *et al.*, (2005) to genotype and discriminate aromatic and non-aromatic varieties. The improvement of indigenous small and medium grained aromatic races which possess outstanding quality traits were neglected as they lack export value. With the exception of sporadic reports on germplasm evaluation and the genetics of some quality traits, there was no serious attempt to improve varieties or to arrest the rapid erosion of the germplasm base in the absence of sound conservation programmes (DRR, 2004). Keeping these in view, the present investigation was undertaken, involving indigenous strains or landraces, high yielding and breeding lines.

Materials and Methods

The present investigation was carried out in Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, during *Rabi* 2016. Experimental materials consisted of 64 rice genotypes which include varieties, land races and breeding lines grown in lattice square design with 3 replications. Recommended practices were followed during crop period. Phenotypic data was analysed by using R statistical software R. Version 3.4.0.

Extraction of genomic DNA

Twenty one days after transplanting leaf samples were collected from the each genotype for isolation of DNA for molecular marker studies. Total genomic DNA was extracted after crushing in centrifuge tubes using CTAB extraction buffer (100mM Tis-HClpH 8, 20mM EDTA pH 8, 1.3M NaCl, 2% CTAB) and chloroform-Isoamyl alcohol extraction followed by RNAase treatment, isopropanol precipitation and final wash with ethanol (Murray and Thompson, 1980). Agarose gel electrophoresis was used to estimate DNA concentration, and each sample was then diluted to approximately 30ng/µL.

SSR marker polymorphism

Two SSR markers distributed over chromosomes 7 and 8 were analysed for quality traits. The details of SSR primer sequence are given in Table 1.

Polymerase Chain Reaction (PCR) and visualization of products

DNA amplification reaction was performed in a volume of 20µl containing 1.5mM TrisHCl (pH8.75), 50mM KCl, 2mM $MgCl_2$, 0.1% TritonX-100, 200µM each of dATP, dCTP, dTTp, dGTP, 4pmole of each forward and reverse primers (Table 1), 1 unit of taq polymerase and 30ng of genomic DNA. Amplification was performed in a programmable thermal cycler (Eppendorf, pros).The PCR profile adopted was: (i) initial denaturation at 94^{o}C for 5 mins, followed by (ii) 35 cycles of denaturation at 94^{o}C for 1 min, annealing at respective temperature (TM) for 1 min and extension at 72^{o}C for 2 mins and (iii) final extension at 72^{o}C for 5 mins and at 4^{o}C for cooling.

The amplified products were separated in 3 per cent agarose gel prepared in 1X TBE buffer stained with Ethidium Bromide (0.5 g/ml). The gel was run in 1 X TBE buffer (0.89M Tris borate, 0.02M EDTA, pH 8.0) at constant voltage of 85 V for a period of 2-3 hours. The gel was visualized in UV transilluminator and photographs were taken

using gel documentation system (Model Alpha Imager 1200, Alpha Innotech Corp., USA).

Results and Discussion

All the 64 rice accessions were subjected to functional marker analysis for pericarp color and fragrance, which were resolved in an agarose gel and validated. The ultimate aim of using molecular markers, tightly linked to the trait of interest is to use it for indirect selection or Marker Assisted Selection early in the seedling stage itself. Further, functional markers which form complete linkage with the trait of interest, owing to occurrence of polymorphism within the gene controlling the trait under investigation (Anderson and Lubberstedt, 2003) are superior to random genetic markers. In the present investigation two functional markers: one for the red pericarp colour (RID 12) and other for fragrance (frg1) were used to screen the 64 rice accessions. The RID 12 marker gene produced two alleles of sizes 110 and 124 bp and clearly grouped 57 rice accessions bearing white pericarp together with allele of size 110 bp and seven rice accessions possess red pericarp with allele of size 124 bp (Figure 1). One of the key advantages of molecular marker is ontogeny independent and no influence of environment.

Consumers generally prefer fragrant rice to non-fragrant rice. Functional markers for *fgr* have been developed and successively used to transfer this gene from fragrance rice to the target non-fragrance rice (Yi *et al.,* 2009; Jin *et al.,* 2010; Salgotra *et al.,* 2012; Jantaboon *et al.,* 2011).

Fragrant rice varieties, such as basmati and jasmine, are of great interest to consumers due to their distinctive flavor. In order to assist in the development of aromatic rice varieties suited to particular local environment, a simple and inexpensive method for distinguishing aromatic and non-aromatic races would be highly useful. The flavor and fragrance of basmati have been associated with increased levels of 2-acetly-1-pyroline (2AP). With the advent of rice genome sequencing and positional cloning strategies, the candidate genes of various important traits are being resolved (Sakthivel *et al.,* 2009).

Recently, an 8bp deletion and 3 SNPs in exon of the gene encoding betaine aldehyde dehydrogenase (BAD2) on chromosome 8 was identified as the likely cause of fragrance in jasmine and basmati type rice (Bradbury *et al.,* 2005). Non-fragrant rice possess a copy of the gene encoding BAD2 which contains the deletion and SNPs, resulting in a frame shift that generates a premature stop codon that presumably disables BAD2 enzyme. This polymorphism provides an opportunity for the construction of a perfect marker for fragrance in rice. In the present study, a simple co-dominant, functional marker for fragrance trait was used and validated in 64 rice accessions, in the Athurkichadi and Kichadi samba are suspected to be little fragrant.

Table.1 Details of SSR markers used for PCR amplification

S. No	Annealing Temperature	Marker name and chromosome number	Primer sequence	Position (Mb)
1	51.4	Frg 1 (8)	CATTTATTGGGAATTATGAAAACTGGTA	2.03
			TTAAAAAGAAAAGGATAACATTGAGAATTG	
2	53	RID 12 (7)	TACAGGGGAGCAGAAACACC	6.06
			AAAGGTACCAAAGATCGCAGAA	

Fig.1 Gel profile showing the amplificatin of primer RID 12 for pericarp colour

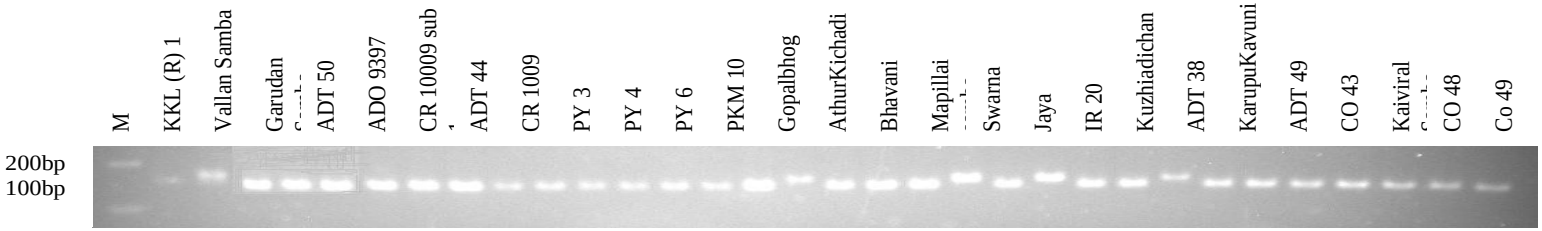
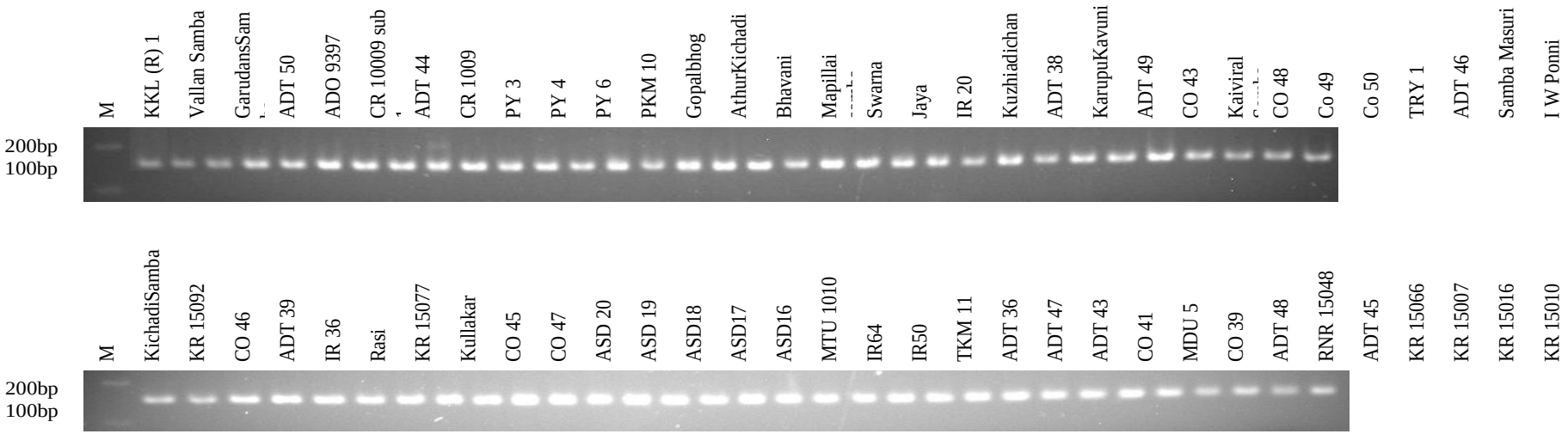


Fig.2 Gel profile showing the amplificatin of primer frg 1 for fragrance



The marker *frg1* (Figure 2) targets the InDel polymorphism in *frg*1 gene and amplifies 110bp fragments in non-fragrance genotypes, marker did not showed any polymorphism due to none of the genotype taken for study were scented (Sakthivel *et al.*, 2009).

Functional markers derived from *fgr* are sufficient to carry out molecular marker assisted breeding to improve the sensory quality of rice (Shi *et al.*, 2008; Chen *et al.*, 2008; Jin *et al.*, 2010). So far as we are aware, there is no genetic report on the other sensory characteristics of rice.

Grain quality of rice as a whole is a complex trait that is comprised of appearance quality, milling quality, eating and cooking quality, nutritional quality and so on. Researches on the genetic control of the quality traits have made a great progress, especially for the appearance quality, cooking and eating quality. More genetic studies are needed for milling quality and nutritional quality. The progress on the molecular genetics on grain quality has allowed MAS to be conducted more efficiently. However, only MAS for cooking and eating quality and genetic engineering for nutritional quality have made some achievements. More molecular breeding practices are needed for improvement of grain quality. With social development and improvement of living standards, cooking and eating quality of brown rice will be a new theme that deserves greater attention from researches. Studies including cooking methods, parameters for cooking and eating, genetics, and molecular breeding are among the top priorities.

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