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Research Article

Studies to Identify Genes for Fragrance in Rice (*Oryza sativa* L.) through Gene Specific Primers

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ABSTRACT

Atrial was conducted at the Crop Research Centre, Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, India. During Kharif season 2014. The plant materials used in the study was consisted of forty genotypes of Rice (Oryza sativa L.). They were obtained from molecular biology laboratory, Deptt. of Genetics and Plant Breeding Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, India. The data were analyzed based on the presence of fragrant and non-fragrant alleles. We can use these marker systems in marker assisted selection for incorporating the fgr gene from fragrant varieties into non fragrant varieties. These results show that mutation in badh2 or fgr gene is not universal to all fragrant rices. It may be possible that mutation elsewhere in badh2 or fgr gene or some other gene could be the likely cause for the fragrance in these genotypes. Based on these results, it can be assumed that fragrance in these genotypes may be controlled by some gene other than fgr.

Out of 40 varieties used, only 16 varieties were determined fragrant by six gene specific primers used. The eleven varieties Basmati-386, Vallabh Basmati-22, CSR-30, PS-2, PUSA-1401, Vallabh Basmati-24, PUSA-1121, Vallabh Basmati-23, IRBB-116, and Vallabh Basmati-21 & HB-1 expressed conspicuous fragrance. All of these 11 varieties were determined fragrant by all the six molecular markers used. It indicated that sequences for fragrance were available at all six loci of fgr gene represented by the corresponding six molecular markers used presently. The varieties Pusa Basmati-1, Punjab Basmati-2, PUSA-1509, Ranbir Basmati, CSR-23 expressed relatively low level of fragrance depending upon the involvement of number of loci containing DNA sequences responsible for development of fragrance. Fragrance of rice varieties PUSA-1509, Ranbir Basmati, CSR-23 was determined by 4, 3 and 1 loci, respectively.

Keywords: *Identification of genes for fragrance in rice genotypes/varieties using gene specific markers and Molecular characterization of rice for fragrance.*

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INTRODUCTION

The word "rice" generally indicates a cereal crop of the species *Oryza sativa* L. The genus *Oryza* L. is classified under the tribe Oryzeae, subfamily Oryzoideae, of the grass family Poaceae (Gramineae) (Lu, 1999). This genus has two cultivated species (*O. sativa*L. *and O. glaberrima* Steud.) and 22 wild species distributed throughout the tropics and subtropics. The so-called Asian cultivated rice (*O. sativa*) is actually cultivated worldwide, while *O. glaberrima* is only cultivated in a few countries in West and Central Africa. Rice is used for food in various forms. Grains are heated in water to become cooked rice. Rice flour is usually kneaded with water, boiled and used for various rice products. The bran is an important source of oil for food and manufacturing. Husks are used for fertilizers and animal feed, and rice straw are used as an important animal feed and for making various wrapping materials and mats. Rice has relatively small genome (430 Mb) compare to other cereal crops, such as maize (2,400 Mb), barley (4,900 Mb) and wheat (16,000 Mb) (Bennetzen, 2002). Rice researchers have developed important tools for genetic analysis, including high-density molecular genetic maps and efficient genetic transformation techniques. Comparative genetic maps within the grass family indicate the existence of conserved gene content and gene order among grasses genomes. On the basis of aroma characteristics rice has been classified into two types, such as aromatic and non-aromatic. Aromatic rice varieties have similar nutritional values and higher amino acid profiles than non aromatic varieties (Sekhar et al., 1982). In India Basmati rice is considered as the queen of aromatic rice and sold at high price (Sakthivel et al., 2009). Almost every state in India possesses numerous aromatic rice varieties (Joshi et al., 2006) and West Bengal (23°00′ N 87° 00′ E) is a rich reservoir of rice biodiversity that derives from a legacy of indigenous farming practices.

MATERIALS AND METHODS

The plant materials used in the study was consisted of forty genotypes of Rice (*Oryza sativa* L.). They were obtained from molecular biology laboratory, Deptt. of Genetics and Plant Breeding Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut, India. The seeds of different varieties were grown in the field of 100 M^2at Meerut (North West Plains Zone, India, 28.99^{O}N and 77.70^{O}E in the year of 2013-14. (Table 3.1).

Table 3.1 Varieties with source of origin and characteristic features

Sr.	Varieties Name	Sources/origin	Characteristic Features
1	Basmati-386	PAU Punjab	Aromatic basmati rice variety
2	VL-31077	VL, Almora	Non-aromatic rice variety
3	Sarbati	Landrace	Aromatic Semi dwarf, short duration
4	Nagina-22	Nagina, U.P	Non-aromatic rice variety
5	CSR-10	CSSRI, Karnal	Non- aromatic rice variety
6	PR 106	PAU, Punjab	Non- aromatic rice variety.
7	Vallabh Bangni	SVPUAT, Meerut	Non- aromatic rice variety
8	Vallabh Basmati-22	SVPUA & T, Meerut	Aromatic, Super fine rice variety
9	Pant Dhan-12	GBPUAT,UK	Non-aromatic rice variety
10	CSR-30	CSSRI, Karnal	Aromatic basmati rice variety
11	VLD-81	VL, Almora	Non-aromatic rice variety
12	CSR-27	CSSRI, Karnal	Semi dwarf, salt tolerant, coarse grain
13	VLD-86	VL, Almora	Semi dwarf, short duration, coarse
14	PS-2	IARI, New Delhi	Basmati rice variety
15	PUSA-1401	IARI, New Delhi	Aromatic basmati rice variety
16	VLD-65	VL, Almora	Semi dwarf, short duration, coarse
17	TETEP	Vietnam	Blast resistant, semi dwarf, coarse grain
18	Vallabh Basmati-24	SVPUAT, Meerut	Basmati rice variety
19	Vivek Dhan-62	VL, Almora	Non-aromatic rice variety
20	Pusa-1121	IARI, Delhi.	Basmati rice variety
21	IR-64	IRRI, The Philippines.	Non-aromatic rice variety
22	Vallabh Basmati-23	SVPUAT, Meerut	Aromatic Basmati rice variety
23	VLD-61	VL, Almora	Non-aromatic rice variety
24	Type-3	Nagina, U.P	Moderate aromatic basmati rice variety
25	Ranbir Basmati	J&K, India	Basmati rice variety
26	Taroari Basmati	HAU, Kaul, Haryana	Aromatic Basmati rice variety
27	Pusa Basmati-2	IARI, Delhi.	Aromatic basmati rice variety
28	Pusa-1509	IARI, New Delhi	Aromatic basmati rice variety
29	Pusa Basmati-1	IARI, Delhi.	Aromatic basmati rice variety
30	Basmati-370	Punjab	Aromatic basmati rice variety
31	NDR-118	NDAUAT, UP	Non-aromatic rice variety

32	IRBB-16	IRRI, Philippines	Semi dwarf, basmati,
33	CSR-36	CSSRI, Karnal	Semi dwarf, salt tolerant, coarse
34	CSR-43	CSSRI, Karnal	Semi dwarf, salt tolerant, coarse
35	CSR-13	CSSRI, Karnal	Semi dwarf, salt tolerant, coarse grain
36	CSR-23	CSSRI, Karnal	Semi dwarf, salt tolerant, coarse grain
37	Vallabh Basmati-21	SVBPUA&T, Meerut	Aromatic basmati rice variety
38	Govind	GBPUAT, UK	Non-aromatic rice variety
39	VD-154	VL, Almora	Non-aromatic rice variety
40	Haryana Basmati-1	HAU, Kaul, Haryana	Aromatic basmati rice variety

RESULTS AND DISCUSSION

Out of the fortyrice genotypes, 16 genotypes has shown fragrant alleles. For BADEX7-1 primer shows 481bp allele which included Basmati-386, Vallabh Basmati-22, CSR-30, PS-2, PUSA-1401, Vallabh Basmati-24, Pusa Basmati-1121, Vallabh Basmati-23, Type-3, Ranbir Basmati, PB-2, PB-1, Basmati-370, IRBB-16, Vallabh Basmati-21, HB-1,the kinds of results were also confirmed by Sakthivel et al. (2009) and Rai et al. (2015) but in different sets of genotypes and with the same & different sets of primers. BADEX7-2 primer shows a 391bp fragmentin only thirteen rice genotypes out of 40 rice genotypes, (Basmati-386, Vallabh Basmati-22, CSR-30, PS-2, PUSA1401, Vallabh Basmati-24, Pusa-1121, Vallabh Basmati-23, Type-3, Basmati-370, IRBB-16, Vallabh-21, and HB-1) the similar results were obtained by Saha et al. (2012), Sakthivel et al. (2009) and Rai et al. (2015) but in different set of genotypes. BADEX7-3 primer gave an amplicon size of 265bp in 17 fragrant rice genotypes, which includes varietiesBasmati-386, Vallabh-22, CSR-30, PS-2, PUSA-1401, Vallabh Basmati-24, Pusa-1121, Vallabh-23, Type-3, PB-2, Pusa-1509, PB-1, Basmati-370, IRBB-16, CSR-23, Vallabh Basmati-21, and HB-1. The similar primers were also used Sakthivel et al. (2009), Kottearachchi et al. (2010) & Bourgis et al. (2008). Pattern of amplification remained the same in both the studies. BADEX7-4 primer gave amplicon of the size187bp in seventeen rice genotypes/varieties Basmati-386, Vallabh-22, CSR-30, PS-2, PUSA-1401, Vallabh Basmati-24, Pusa-1121, Vallabh-23, Type-3, Ranbir Basmati, PB-2, Pusa-1509, PB-1, Basmati-370, IRBB-16, Vallabh-21 and HB-1. Almost the same banding pattern was observed with this primer by Saha et al. (2012), Sakthivel et al. (2009) and Rai et al. (2015). BADEX7-5 primer is amplified (95bp)in seventeen genotypes out of forty rice genotypes, which includes Basmati-386, Vallabh Basmati-22, CSR-30, PS-2, PUSA-1401, Vallabh Basmati-24, Pusa-1121, Vallabh-23, Type-3, Ranbir Basmati, PB-2, Pusa-1509, PB-1, Basmati-370, IRBB-16, Vallabh Basmati-21, and HB-1. The similar results were also reported with this primer by Sakthivel et al. (2009), Kottearachchi et al. (2010), Bourgis et al. (2008) and Rai et al. (2015). BADEX7-6 primer shows 56bp fragment in sixteen out of forty rice genotypes (Basmati-386, Vallabh-22, CSR-30, PS-2, PUSA-1401, Vallabh Basmati -24, Pusa-1121, Vallabh-23, Type-3, PB-2, Pusa-1509, PB-1, Basmati-370, IRBB-16, Vallabh Basmati -21 and HB-1). The same primer was also used by Sakthivel et al. (2009), Kottearachchi et al. (2010), Bourgis et al. (2008). Based on the present study we found that these primerclearly classified all the 40 varieties of rice into fragrant and non-fragrant cultivars, except four varieties which were determined fragrant by traditional physico chemical methods. Contrary to their fragrant phenotype, a non-fragrant banding pattern was observed for two aromatic genotypes viz., Ranbir Basmati, Taroari Basmati. Among all six primers, BADEX7-4 and BADEX7-5 primer could discriminate all the rice varieties into fragrant and non-fragrant varieties very clearly. So we can say that these primers are more reliable for evaluation of fragrance in rice cultivars. Because evaluation of fragrance through sensory method is time consuming and require skill personals. But molecular analysis of fragrance is rapid, cost effective and reliable method. In the present investigation out of 40 varieties only 16 varieties were determined fragrant by six gene specific primers used. The eleven varieties Basmati-386, Vallabh Basmati-22, CSR-30, PS-2, PUSA-1401, Vallabh Basmati-24,

PUSA-1121, Vallabh Basmati-23, IRBB-116, and Vallabh Basmati-21 & HB-1expressed conspicuous fragrance. All of these 11 varieties were determined fragrant by all the six molecular markers used. It indicated that sequences for fragrance were available at all six loci of *fgr* gene represented by the corresponding six molecular markers used presently. The rest five varieties Pusa Basmati-1, Punjab Basmati-2, PUSA-1509, Ranbir Basmati, CSR-23 expressed relatively low level of fragrance depending upon the involvement of number of loci containing DNA sequences responsible for development of fragrance. Fragrance of rice varietiesPUSA-1509, Ranbir Basmati, CSR-23 was determined by 4, 3 and 1 loci, respectively.

Identification and determination of fragrant varieties of rice using six molecularmarkers

S.No.	Numbers of molecular Markers	Genotypes determined fragrant by a number of molecular markers mentioned in column 2
1.	6	Basmati-386, Vallabh Basmati-22, CSR-30, PS-2, PUSA-1401, Vallabh Basmati-24, PUSA-1121, Vallabh Basmati-23, IRBB-116, Vallabh Basmati-21 & HB-1.
2.	5	Pusa Basmati-1, PunjabBasmati-2.
3.	4	PUSA Basmati-1509.
4.	3	Ranbir Basmati

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