Plant Archives Vol. 20, Supplement 2, 2020 pp. 1238-1241 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

THE EFFICIENCY OF BADEX5-7 MARKER IN SCREENING AROMATIC RICE GERMPLASM

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Abstract

Aromatic rice is an important food commodity and fetches high price in international market. Hundreds of rice germplasm shows aroma which is due to more than hundred volatile compounds. Aromatic rice is identified by cooking test (Panel test), volatile profiling by GC-MS and using molecular markers. In this study, 101 rice germplasm that includes 99 aromatic and 02 non aromatic rices were screened using BADEX7-5 marker and the results were compared with presence or absence of 2AP. This marker was prepared based on 8 base pair deletion in exon 7 of badh2 gene and this mutated gene produce a nonfunctional enzyme leading to 2AP accumulation. Later, mutations in other regions of this gene were also reported for the formation of aromatic rices. This maker was able to identify 70% of aromatic germplasm.

Keywords **:** Aromatic rice, BADEX7-5, badh2, 2AP

Introduction

Rice is the major global staple food source for more than half of the world's population. It is mostly consumed in well-polished white rice which supplies more energy than nutrition. Aroma is one of the important grain quality aspect that affects palatability and consumer acceptability. 2-acetyl 1-pyrroline (2AP) is responsible for the popcorn like fragrance (Buttery *et al.*, 1983; Paule *et al*., 1989). 2AP was observed in all parts of the aromatic rice plant except roots (Sood and Siddiq, 1978).

2AP is synthesized in rice plant during cultivation and accumulates in leaves, culm, and kernel (Yoshihashi 2002). Itaccumulates due to a mutated BADH2 gene with 8-bp deletion in exon-7 (Bradbury *et al*., 2005; Pichedsak *et al*., 2008). 2AP content varies among the aromatic varieties (Goufo *et al.,* 2010).

The available methods for identifying aromatic rice includes a) panel test, b) 2AP analysis, c) molecular markers. Panel test which involves tasting of cooked rice is the most acceptable way of identifying the best aromatic rice (Reinke *et al*., 1991). However, this exercise is labor intensive, individual specific and a panel of analysts having the ability to detect fragrance is required (Peddamma *et al.,* 2018).

2AP estimation can be done by GC or GC-MS (Lorieux *et al*., 1996; Widjaja *et al*., 1996) which includes concentration of 2AP in rice by purge and trap (Buttery *et al* 1988), steam distillation or solvent extraction (Lin *et al.,* 1990), solvent extraction followed by direct injection (Bergman *et al.,* 2000), solid-phase microextraction (SPME) (Grimm *et al.,* 2001) and headspace analysis (Sriseadka *et al.,* 2006). 2,4,6-trimethylpyridine (TMP) as an internal standard because of its similar physical properties (basicity, water solubility, volatility, and GC retention time) in comparison with 2AP (Buttery *et al.,* 1986; Peddamma *et al.,* 2018).

Molecular marker analysis is more advantageous than the other two methods. Panel test requires more amount of rice sample, identifying panel examiners with the required skill and all this makes it laborious. 2AP analysis requires gas chromatograph which is very expensive, trained persons are required for operating GC as well as understanding the results and moreover, the procedure requires costly chemicals and consumables.

Molecular markers analysis is advantageous for the following reasons. DNA can be isolated from seed or any part of the plant at any growth stage of the crop, a small amount of tissue (100 mg) is enough for extracting DNA followed by marker analysis. BADEX7-5 marker (Sakthivel *et al.,* 2009) is a functional, co-dominant marker for large scale and routine fragrance genotyping using agarose gel systems. They screened a total of 60 rice germplasm that includes 47 aromatic and 13 non-aromatic in validating the marker. We developed a core set of 101 rice germplasm from a large collection of 552 diverse aromatic accessions (unpublished data). Hence, in this study, this core set (99 aromatic and two non-aromatic rices) was screened to reveal the functional utility of BADEX7-5*marker*in the selection of aromatic rice.

Materials and Methods

DNA extraction and PCR amplification

In this study, 556 genotypes (552 aromatic and 4 non-aromatic) were collected from various network centers (Peddamma *et al* 2018) and core set (Table 1) that were identified by divergence analysis (Unpublished data). DNA was extracted from young leaves of 30 day old seedlings (Dellaporta *et al*., 1983). A co-dominant functional marker BADEX7-5, forward sequence TGTTTTCTGTTAGGTTGCATT and reverse sequence ATCCACAGAAATTTGGAAAC, that can clearly differentiate in an agarose gel was used for validation of aromatic rice varieties of the core set (Shaktivel *et al*., 2009).

The final volume of the PCR reaction system was 10 µl with 10X PCR buffer containing 1.5 mM $MgCl_2$, 2.5 mM dNTPs, 1 unit Taq DNA polymerase and 40-50 ng of

template DNA. PCR program includes an initial denaturation for 5 min at 94°C followed by 35 cycles of denaturation for 1 min at 94°C, 30 s annealing at 54 °C, extension step at 72°C for 1 min and a 5 min of final extension at 72°C. Amplified mixture was separated using 4% Agarose gel and bands were observedin Biorad gel documentation unit. Expected fragment size in aromaticand non-aromatic rice varieties are 95 bp and 103 bp respectively.

Results and Discussion

Out of 101 genotypes (99 aromatic and 2 non aromatic quality rice), 29 aromatic genotypes also showed negative result (Figure 1). Hence, the apparent efficiency of BADEX7-5 marker is nearly 70%. It is due to the other mutations of Badh2 gene leading to the production of non functional enzyme and inevitable accumulation of 2AP. 7-bp deletion (CGGGCGC) in exon 2 (Shi *et al*., 2008), two non-synonymous SNPs in central section of 8thintron (Sun *et al*., 2008), 2-bp deletion (TT) in intron 2 and repeated (AT) insertion in intron 4 discovered (Chen *et al*., 2008), 7-bp insertion in exon 8 (Amaravathi *et al*., 2008), absence of miniature interspersed transposable element (MITE) in promoter region (Bourgis *et al*., 2008), eight badh2 alleles (2.3 to 2.10)of insertion, deletion or SNP in other exons (Kovach *et al*., 2009), 3-bp insertion in exon 13 (Myint *et al*., 2012), 3 alleles- badh2.2 75 bp deletion in exon 2, badh2.4-5 806 base pair deletion between exon 4 and 5 and badh2.10 G/A SNP in exon 10 (Shao *et al*., 2013), 3-bp deletion in 5´-UTR and 8-bp insertion in the promoter region of badh2-P-5´-UTR (Shi *et al*., 2013), a splicing mutation G/A SNP found between 1st exon and 1st intron (Ootsuka *et al*., 2014).

Since 8bp deletion is the major mutation leading to the formation of aromatic germplasm, BADEX7-5 marker is useful to screen large germplasm in identifying aromatic germplasm, instead of more difficult panel test or 2AP estimation by GC-MS. The aromatic germplasm that showed negative result with BADEX7-5 marker can be less in number and they can be confirmed by panel test or 2AP estimation. Considering the identification of other mutations, it is essential to study the sequence variations of badh2 gene of core germplasm to reveal any other unreported variations responsible for the formation of aromatic rices.

Table 1: List of genotypes screened by BADEX7-5 primer

S.No	Accession name	Marker result	S.No	Accession name	Marker result
1	BANSPATRI -1	Negative	51	TARUN BHOG	Negative
2	MOONGPHALI B	Positive	52	NANU -2	Positive
3	DHANIA - B2	Positive	53	RANDHUNI PAGAL -1	Positive
4	RAU 3055	Negative	54	TULASI GHANTI	Positive
5	MAGURAPHULLA	Negative	55	DHAN PRASAD -1	Positive
6	BARIKUNJA	Positive	56	KARNAL LOCAL	Positive
7	KANIKABHOG	Positive	57	IET 15833	Positive
8	HEERAKANI -1	Positive	58	BASMATI KAMON	Positive
9	PIMPUDIBASA	Positive	59	PUSA BASMATI 6	Positive
10	TENDU PHOOL	Positive	60	MUSKAN	Positive
11	R-1462-243-100-7-1-1	Negative	61	BASMATI 37	Negative
12	ADAM CHINI B	Positive	62	BASMATI 6141 -1	Positive
13	KANAK JEER -2	Positive	63	BASMATI 213 -2	Positive
14	BANTHA PHOOL -1	Positive	64	BASMATI 5836	Positive
15	CHAMPARAN BASMATI 3	Positive	65	BASMATI 5853	Positive
16	DUBRAJ (RAIPUR)	Positive	66	BASMATI 6141 -2	Negative
17	KHORIKA JOHA	Positive	67	BASMATI 11	Negative
18	KAMINI JOHA	Positive	68	BASMATI 127A	Positive
19	RAMBHOG B	Positive	69	BASMATI 138	Positive
20	KDML 105	Positive	70	TULSI MANJARI -2	Negative
21	VALLABH BASMATI 22	Positive	71	AYEPYAUNG	Negative
22	VALLABH BASMATI 24	Positive	72	BINIRHEN	Negative
23	BINDLI	Positive	73	BONGCAY -2	Negative
24	PANT SUGANDH DHAN - 15	Positive	74	DAW LEUANG	Negative
25	BINDALI	Positive	75	DU THOM THAI BINH HAI PHONG	Positive
26	IMPROVED SARBATI	Negative	76	GUINATA	Positive
27	SATHI	Positive	77	HUNG-MI-HSIANG-MA-TSAN -1	Negative

28	LAL BASMATI	Negative	78	HAWN JAN	Positive
29	UPRI 1840 - 31-1-1-16	Positive	79	KHAO MALI	Positive
30	TULSI MANJARI -1	Positive	80	LUA NHE DEN	Negative
31	BANSPATRI -2	Negative	81	LONGKU LABAT	Negative
32	SHAMJIRA	Positive	82	NEPALI JOHA	Negative
33	DHAWARA SAWA	Positive	83	NIIAW HAWM	Negative
34	JAI GUNDI	Positive	84	HBC 46	Positive
35	SAMUNDCHINI	Negative	85	IR 841 - 85 - 1-1-2	Negative
36	BANTAPHOOL -3	Negative	86	KALIMOOCH (RAIPUR)	Positive
37	RANBIR BASMATI -1	Positive	87	IR-62871- 138- 5	Positive
38	PUSA 677	Negative	88	IR 62871-549-3-5	Positive
39	CHIMBALATE BASMATI	Positive	89	IR 62873-227-1-16	Positive
40	IET 21959 (PUSA 1509-03-1-7-2)	Positive	90	IR 62873-238-2-3	Positive
41	IET 21953 (UPR 3506-7-1-1)	Positive	91	HASAN SERAI	Positive
42	IET 22290 (1612-07-6-5)	Positive	92	KOLIHA	Positive
43	P 1568 -05-6-4-154	Positive	93	BAS 837	Negative
44	KRISHNA KAMOD -1	Positive	94	BASMATI -3	Positive
45	TARAORI BASMATI	Positive	95	KH SAKANI	Positive
46	PUSA BASMATI 1121 -2	Positive	96	UPR 3565-10-1-1	Positive
47	VASUMATI	Positive	97	IR 74720-13-1-2-2	Positive
48	BASMATI JAMUNA	Negative	98	IET 14131	Positive
49	Sheetal kani	Positive	99	BPT 5204	Negative
50	R-1498-778-388-1-1	Positive	100	IET 22787	Positive
			101	JGL 1798 (JAGITHYAL SANNALU)	Negative

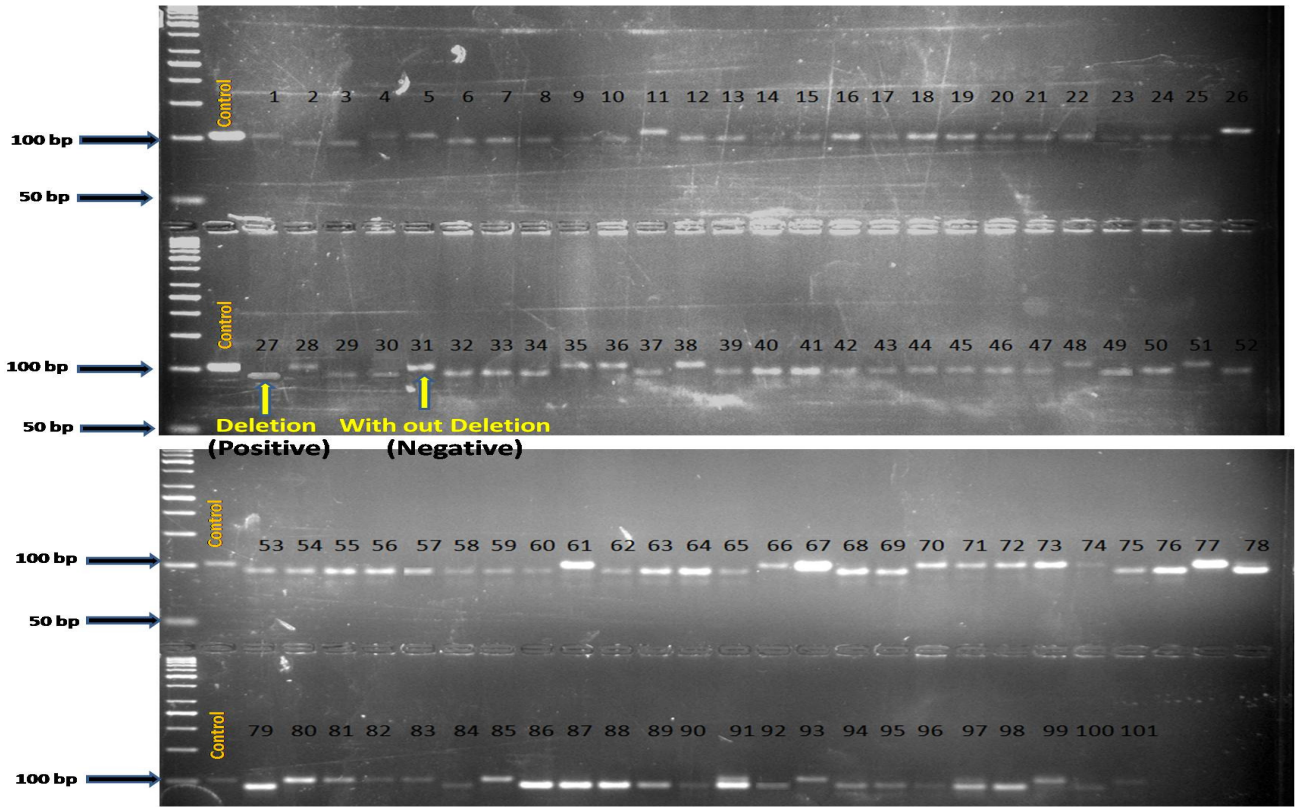

Fig. 1 : Amplification pattern of the marker BADEX7 in 99aromatic and 2 non-aromatic rice accessions

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