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Detection of basmati rice using microsatellite markers

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Abstract

Basmati rice is a fine and aromatic rice grown mainly in certain areas of Punjab province in Pakistan. It has high demand in the international market and a source of earning foreign exchange via export. Adulteration of non-basmati rice grains is a major challenge to secure its export standards. Hence, the development of a simple and cost-effective method is necessary to screen the basmati and non-basmati rice samples. In this study, we have validated the efficiency of different molecular markers by screening seven unknown rice samples. Our results demonstrated that three markers namely RM1, RM19 and RM225 proved to be efficient microsatellite molecular markers that could be used to screen basmati and non-basmati rice samples. Further, these results are validated based on expression pattern of *Badh2* gene among the basmati and non-basmati rice. Thus, this study provides a contribution towards development of a simple and cost-effective method for rapid screening of basmati rice.

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Introduction

Rice is a major staple food crop around the world especially in Asia and Africa (Zafar et al., 2020; Zafar et al., 2021). It feeds at least one half of the human population on earth (Zafar et al., 2017; Ahmed et al., 2021). A huge variation in grain size of rice exists which is not only important for its grain yield but also has the aesthetic value (Chun et al., 2020; Ahmad et al., 2021). Basmati rice is a kind of long grain, slender and aromatic rice which is mainly grown in certain districts of Punjab province in India and Pakistan (Akhter & Haider, 2020). It is considered the top-quality rice in this area mainly due to its aromatic and non-sticky characters. The demand of basmati rice is very high in the international market and thus it is a great source of earning foreign exchange for Pakistan. Basmati rice is prone to adulteration with long grain non-basmati rice due to its high demand and premium quality (Lopez, 2008) and it is often difficult to differentiate the two because of comparable grain size and architecture (Fig. 1). Therefore, it is necessary to check the purity of product to meet the export standards. Traditionally, detection of basmati rice was based on morphological and chemical characters such as grain length or aroma but these methods were not practically feasible and reliable (Nagaraju et al., 2002) (Fig. 1).

Biochemical characteristics were exploited as source for identification of Basmati rice. A recessive gene *badh2* was responsible for the particular fragrance in basmati rice (Bradbury et al., 2005). *Badh2* gene encodes 2- acetyl 1 pyrolline which produce aroma in rice. The 8bp deletions on chromosome 8 of *badh2* gene present in basmati rice is associated with aroma while normal *badh2* is present in non-aromatic rice. Moreover, DNA based microsatellite molecular markers have been developed for the detection of basmati rice (Bligh, 2000; Jain et al., 2004). The present study was conducted with the objective to screen unknown rice samples using molecular markers and to validate the efficiency of these molecular markers for basmati cultivars identification.

Methodology

Genetic material

Seven different types of rice seeds were collected and labeled as AA, BB, CC, DD, EE, FF and GG along with super-basmati (Basmati type rice) and green super rice-3 (GSR-3, non-basmati type rice) rice samples as a control (Table 1). Phenotyping of basmati and non-basmati seeds shown in figure 1 was performed using professional camera (Nikon) with scale.

DNA extraction

Genomic DNA was extracted from rice seeds through a modified CTAB method described previously (Aboul-Maaty and Oraby 2019). Briefly, 10 seeds of each genotype were dehusked and washed with distilled water. After drying, seeds were ground into fine powder in cold mortar and pestle followed by homogenization in hot CTAB buffer. Samples were incubated in a hot water bath at 60–65 °C for 1 h, an equal volume of chloroform : isoamyl alcohol (24:1 v/v) was added and centrifuged at 13000 rpm to collect clean supernatant. After adding half volume of 6 M NaCl, 1/10 volume 3 M potassium acetate and 500 µl ice cold 100% isopropyl alcohol, samples were

incubated at -20 °C for 30 min and then centrifuged at 13000 rpm to get pellet. The concentration and purity of extracted DNA was measured using Nanodrop1000.

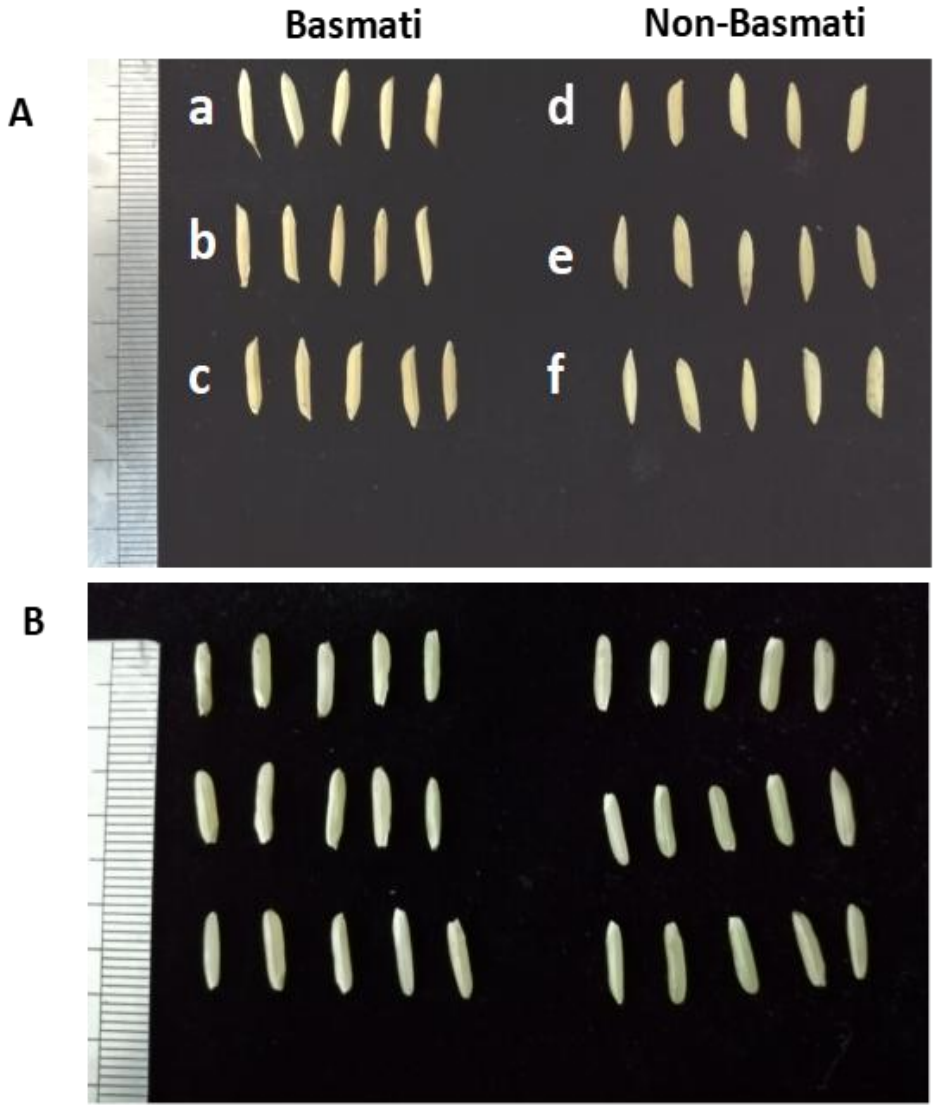

Figure 1. Comparison of rice grains length (with husk A and without husk B) of basamti and non-basamti rice varieties. (a)Super-basamtai, (b) kisan basamti, (c)basamti- pak, (d) GSR-3, (e) GSR-13, (f) GSR-9. Arrangement of genotypes is same for A and B panels. Unit of scale for the distance between indivual lines is milli meter (mm). GSR, green super rice

Table 1. Unknown rice samples along with two checks. PCR product size is given for each marker

Sample Name	Rice Type	PCR Product Size (bp)					
		BADH2	**RM1**	**RM19**	**RM208**	**RM 222**	**RM 225**
AA	unknown		108	211	164	280	120
BB	unknown		108	211	164	280	120
CC	unknown		108	211	164	280	120
DD	unknown		108	211	164	280	120
EE	unknown	80	130	241	179	300	138
FF	unknown	80	130	241	179	300	138
GG	unknown	80	130	241	179	300	138
Super Basmati	Basmati		110	211	164	280	120
GSR-3	Non-Basmati	80	130	241	179	300	138

PCR based marker analysis and expression pattern

Samples were screened through *badh2* gene specific markers using PCR. Total five rice primer sets (RM1, RM19, RM208, RM222 and RM225) were selected to distinguish basmati and non-basmati rice on the basis of published rice microsatellite markers (Table 2). Polymorphism among the tested genotypes was observed on 2% agarose gel. To further confirm these results, expression analysis of the *Badh2* gene was performed among basmati and non-basmati lines.

Table 2. Primer sequences for markers used for detection of rice samples

Marker	Primer Sequence
RM 1	F GCGAAAACACAATGCAAAAA
	R GCGTTGGTTGGACCTGAC
RM19	F CAAAAACAGAGCAGATGAC
	R CTCAAGATGGACGCCAAGA
RM208	F TCTGCAAGCCTTGTCTGATG
	R TAAGTCGATCATTGTGTGGACC
RM 222	F CTTAAATGGGCCACATGCG
	R CAAAGCTTCCGGCCAAAAG
RM 225	F TGCCCATATGGTCTGGATG
	R GAAAGTGGATCAGGAAGGC
BADH2	F CATGGTTTATGTTTTCTGTTAGGTTG
	R TAGGAGCAGCTGAAGCCATAAT

Results and Discussions

Molecular marker assisted breeding has played a significant role in accelerating the selection process in plant breeding (Zafar et al., 2018; Waqas et al., 2021). The ease of use of molecular markers to distinguish basmati from non-basmati rice is of great importance to the exporters, farmers and consumers to promise the legitimacy of the product (Inam et al., 2017). Several molecular markers have been developed that have the potential to differentiate the basmati rice from non-basmati.

30 rice microsatellite markers showed genetic relationship between Indian aromatic and quality rice ((Jain et al., 2004). They reported that RM1, RM5, RM135, RM170, RM171, RM222, RM252 and RM 253 were the most suitable markers to distinguish basmati form non-basmati. Similarly, Bligh (2000) also reported several fluorescent simple sequence repeat markers including RM19, RM208 and RM225 to differentiate basmati and non-basmati rice.

In the present study, RM1, RM 19, RM 208, RM222 and RM225 were selected on the basis of large difference between PCR product sizes and thus having higher efficiency to discriminate basmati and non-basmati (Bligh, 2000). In addition, *badh2* gene specific primer was also tested for the 8-bp deletion in basmati rice (Bradbury et al., 2005).

According to our results all markers showed polymorphic results for basmati and non-basmati rice (Fig. 2), however, level of polymorphism varied. RM1 clearly differentiated the tested rice samples and amplified 108bp PCR product in AA, BB, CC, DD and in basmati check rice, while 130bp PCR product was observed in EE, FF, GG and in non-basmati rice (Fig. 2a). Using RM19 markers, 211 bp PCR products was detected in AA, BB, CC, DD and basmati samples while 241 bp product was detected in EE, FF, GG, HH and in non-basmati samples showing clear difference among the tested samples (Fig. 2b). The RM208 marker showed 164 bp amplification in AA, BB, CC, DD and basmati samples and 179 bp amplification was observed in EE, FF, GG and non-basmati rice (Fig. 2c). This showed a difference of only 15-bp and thus it was difficult to distinguish among the basmati and non-basmati rice. The marker RM222 indicated 280bp PCR product in AA, BB, CC, DD and in basmati check samples while amplification of 300bp was observed in EE, FF,GG and non-basmati samples (Fig. 2d). The marker RM225 also distinguished the tested rice samples and showed amplification of 120 bp in AA, BB, CC, DD and basmati samples and 138 bp amplification in EE, FF, GG and non-basmati samples (Fig. 2e). Next, we tested badh2 gene specific primer and employed the 8-bp deletion in basmati rice. Although, badh2 primer showed some polymorphism, it was not able to clearly distinguish among the basmati and non-basmati rice samples (Figure 2f). For confirmation of these molecular markers results, expression pattern was observed among the basmati and non-basmati rice samples (Figure 3). Expression pattern were compatible with the molecular markers polymorphism.

Conclusion

Our results suggested that sample AA, BB, CC, DD were declared as basmati samples and samples EE, FF and GG were declared as non-basmati samples. Marker amplification results indicated that the markers RM1, RM19 and RM225 were efficient to distinguish among the basmati and non-basmati rice samples due to a large difference in product size. This is probably due to a large genomic mutation in the basmati rice

samples at these markers. Thus, these markers could serve as a simple, reliable and cost-effective tool to screen basmati and non-basmati rice samples.

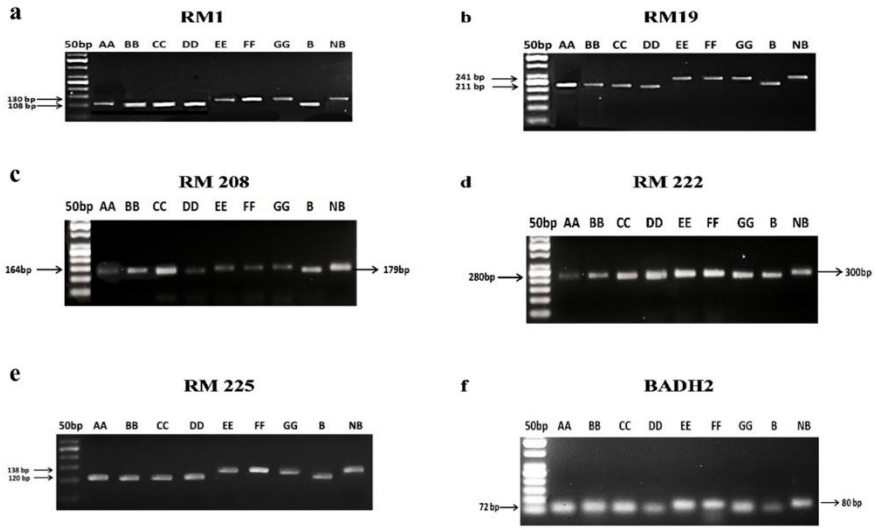

Figure 2. Two percent gel showing the amplification products of rice varieties using 6 primers set. The sizes given are the PCR product sizes. AA,BB,CC,DD,FF,GG are the unknown samples while B is super basmati and NB is for non-basmati (a) RM1(b) RM 19 (c) RM 208 (d) RM 222 (e) RM 225 (f) BADH2 Confirmation of rice type through *badh2* gene primers.

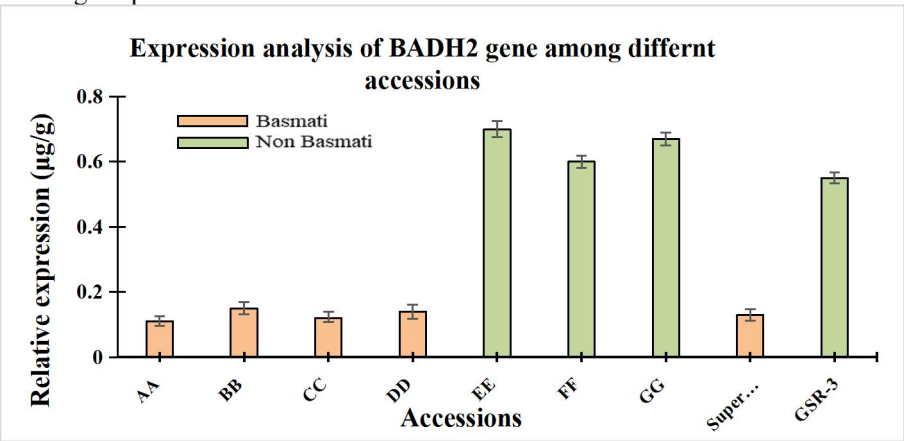

Figure 3. Examination of the Badh2/badh2 expression pattern in various accessions using Real-Time RT-PCR. Expression level was determined in the leaf tissues of each accession. Super Basmati is used as a check for basmati while GSR-3 was used as a check for Non-Basmati accessions. Error bars represent the SD of expression level determined from the three biological replicates.

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