

TaqMan based real time PCR method for quantitative detection of basmati rice adulteration with non-basmati rice

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Abstract A TaqMan based real time PCR method is developed for the first time to check the adulteration in basmati rice. Betaine aldehyde dehydrogenase 2 (BAD2) gene specific primers and probes were used to detect adulterant non-basmati rice content in basmati samples. The results obtained with market samples and validation samples (basmati rice spiked with non-basmati rice in different proportions) clearly revealed that this technique is useful to detect the adulteration very accurately with 1% detection limit.

Keywords Basmati · Real time PCR · Betaine aldehyde dehydrogenase

Introduction

Basmati is considered to be one of the premium aromatic rice varieties in the world. It is grown in foothill of Himalayas spread over Pakistan and India. The main characteristic feature of basmati rice is its typical fragrance and 1- to 1.5-fold elongation of rice during cooking [1]. Aromatic features of basmati rice are regulated by the cultivation practices and environment. There are two major categories of basmati rice, one is traditional basmati and other one is evolved basmati. In evolved basmati, traditional basmati is crossed with non-basmati rice of elite characters. European Union has recognized certain Indian and Pakistan varieties as “basmati” (Table 1). Basmati

costs four times more than the normal rice. Because of the high price, much of the crop is exported to US and Europe. Since it has very high price and demand in the world market, it is subjected to heavy adulteration with non-aromatic rice. In this context, detection of adulteration in basmati rice has importance to ensure the authenticity of product to meet the quality regulations of import countries.

Certain methods are available to detect basmati adulteration such as smelling of grains after boiling in water or treating with potassium hydroxide solution [2]; chromatography analysis of aromatic compounds [3, 4]. But it is found that these techniques are not reliable to detect basmati adulteration properly. Recently SSR marker based molecular detection of basmati adulteration is developed and found to be effective in detection of basmati adulteration [5]. The only limitation of this method is highly expensive to perform the test.

It is already reported that aroma of basmati rice is associated with amount of 2-acetyl-1-pyrroline (2AP) by Buttery et al. [6], Lorieux et al. [3], Widjaja et al. [4] and Yoshihashi [7]. In a recent research work by Bradbury et al. [9] revealed that the level of 2-acetyl-1-pyrroline is controlled by a recessive gene encoding betaine aldehyde dehydrogenase 2 (BAD2) on chromosome 8 of rice. An eight base pair deletion and three single nucleotide polymorphisms (SNP) on BAD2 gene is responsible for aroma in basmati rice where as normal BAD2 gene present in non-basmati rice [8]. Based on the above work, we report that for the first time we have developed a TaqMan based real time PCR method to identify basmati adulteration. This method will detect the quantity of non-basmati rice present in basmati rice. Compared to the present advanced molecular method, this method is fast, reliable and most economical.

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Table 1 List of rice varieties approved by FSA as “basmati”

Indian varieties	Pakistani varieties
Basmati 370	Basmati 370
Dehradun (Type-3)	Kernel (basmati Pakistan)
Basmati 217	Super
Basmati 386	Basmati 385
Taraori (karnal local, HBC-19)	Basmati 198
Ranbir basmati (IET 11348)	
Pusa basmati (duplicate basmati, IET 10364)	
Punjab basmati (bauni basmati)	
Haryana basmati (HKR 228/IET 0367)	
Kasturi (IET 8560)	
Mahi suganda	
FSA Food Standard Agency, UK	

Materials and methods

Basmati rice

Authentic basmati and non-basmati rice grains of Pakistan and Indian origin were collected from various sources. Purity of the grains was checked by physical appearance as well as chemical analysis.

DNA extraction

Extraction of DNA was performed with 0.1 g starting material of fine powder of rice using Nucleon Phytopure (Tepnel Life Sciences plc and Amersham Biosciences, UK) according to the manufacturer's instructions. DNA quality and quantity is checked spectrophotometrically.

Conventional polymerase chain reaction (PCR)

Initial screening of basmati and non-basmati standards were performed using forward (5'tgtttggagcttgctgatg 3') and reverse (5' ctggtaaaaagattatggcttca 3' primers [8, 9] specific to non-basmati rice on a conventional thermal cycler (Bio-Rad, USA). PCR conditions were 94 °C for 2 min; 34 cycles comprised of 94 °C for 14 s, 59.7 °C for 14 s and 72 °C for 14 s. Final extension was carried out at 72 °C for 5 min. The amplified product was subjected to electrophoresis (Bangalore Genei) on 1.5% agarose gel. The gel picture was captured using a gel documentation system (Bio-Rad, USA).

Real time PCR

The primers and fluorescent probe for BAD2 gene of rice (GenBank accession no. AP004463) were designed with Primer Express Software (Perkin Elmer, Applied Biosystems, USA) and were obtained from Eurogentec (Belgium). The nucleotide sequence of forward primer was 5' ctggtaaaaagattatggcttca 3', the sequence of reverse primer was 5' gatattttttgttctc 3' and probe was 5' ctgctcctctggttaaggtttgttccaa 3'. The fluorescent reporter dye at the 5' end of the probe was 6-carboxyfluorescein (FAM); the quencher at 3' end was 6-carboxy-N,N,N',N'-tetramethylrhodamine (TAMRA). The real time PCR amplifications were performed in 25 µl reaction volumes containing 2× TaqMan Universal master mix (Applied Biosystems, USA), which include dUTP and uracil-N-glycosylase, each primer concentrations 300 nM, fluorescent labeled probe at 100 nM and 5 µl of DNA (100 ng). All reactions were performed in triplicate, and for amplification and detection, 7,500 real time PCR system (Applied Biosystems Inc, USA) was used. Real time PCR parameters used were 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of comprised of 95 °C for 15 s and 60 °C for 1 min. Real time data were analysed with sequence detection system software version 1.3.1.

Results and discussion

A conventional PCR amplification of available basmati and non-basmati rice were performed using forward and reverse primers. The results clearly showed only non-basmati rice was amplified (Fig. 1). The primers are for active BAD2 gene which is present only in non-basmati rice. Only presence of inactive BAD2 gene in basmati rice leads to aroma formation [9].

Dilutions of 100, 20, 10, 2 and 1 ng of purified DNA of non-basmati rice was prepared. The dilutions were subjected to real time PCR assay in triplicate and constructed a standard curve. The standard curve showed a linear relationship between log non-basmati DNA concentration and Ct value (Fig. 2a, b). The slope of the curve was -3.51 and the R^2 after linear regression was 0.98.

Specificity of primers and probes were checked by using pure basmati DNA along with non-basmati DNA. None of the basmati BAD2 gene was amplified during real time PCR reaction where as all non-basmati DNA showed amplification (Fig. 3). It indicates that primers and probe were very specific to non-basmati rice where there is no mutation in the BAD2 gene [9].

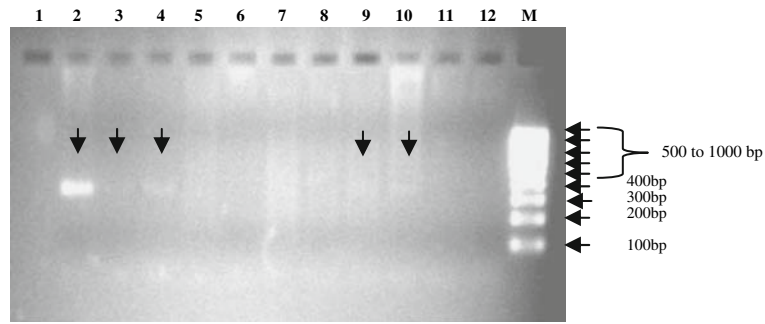
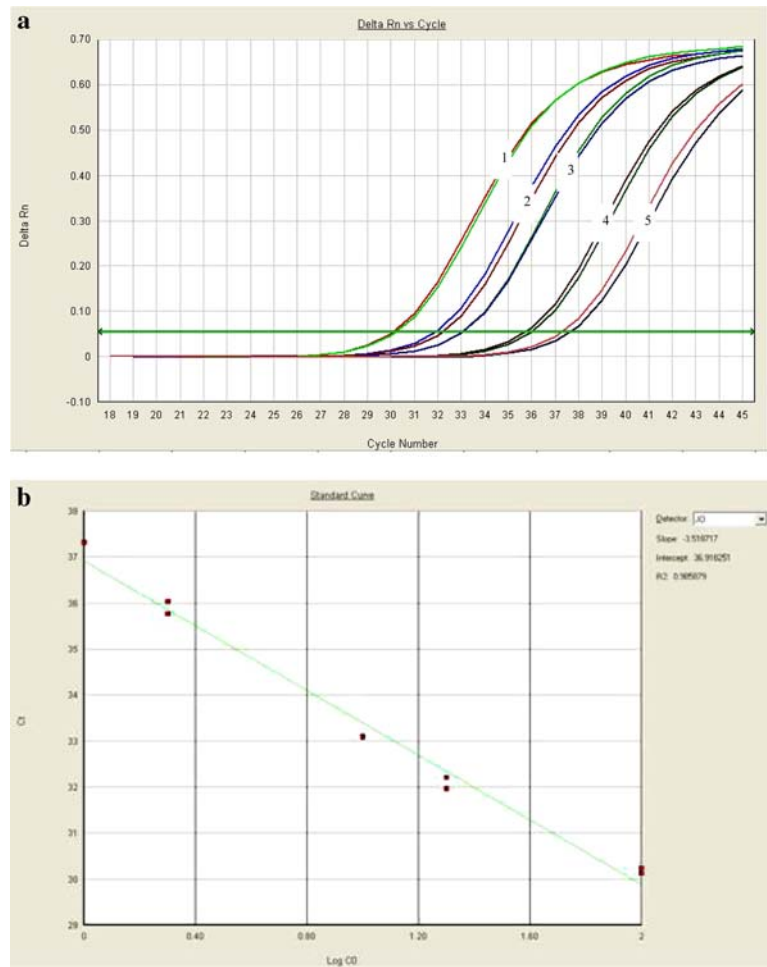


Fig. 1 Specificity of non-basmati primers basmati (Sharbati) DNA. Lane 1, 5, 6, 7, 8 and 11 are different pure basmati samples (no amplification). Lane 2 pure non-basmati sample (355 bp intense amplification product is seen). Lane 3, 4, 9 and 10 are mixture of

basmati and non-basmati samples (355 bp less intense amplification is noted). Lane 12 is negative control (without DNA); Lane M is 100 bp DNA ladder

Fig. 2 a Amplification profile of non-basmati (Sharbati) DNA. Numbers on the figure indicates non-basmati DNA initial concentrations Line 1 is 100 ng; line 2 is 20 ng; line 3 is 10 ng; line 4 is 2 ng and line 5 is 1 ng. **b** Standard curve obtained from amplification of non-basmati (Sharbati) DNA. Non-basmati DNA initial concentrations were 100, 20, 10, 2 and 1 ng per reaction, respectively; Slope is -3.51 ; R^2 is 0.98



The method was validated with non-basmati rice (different varieties) spiked with basmati rice (different varieties) in different proportions and also with commercial market samples (Table 2). DNA was extracted in triplicate from all the validation samples and real time PCR assay was performed. The thresh hold cycle (Ct) value of validation samples were plotted against the standard curve and calculated the amount of non-basmati DNA present in the unknown

validation samples. The data were analysed statistically and calculated the standard deviation of each result (Table 2). Based on the above findings, the newly developed TaqMan based real time PCR method for basmati adulteration analysis will help to find out the non-basmati content in domestic market samples as well as export samples. It is proved to be a very accurate and economical test method for basmati adulteration.

Fig. 3 Comparison of amplification of BAD2 gene in basmati and non-basmati DNA. The arrow mark indicates amplification curve profile of non-basmati DNA while the other three are basmati DNA profile showed no amplification

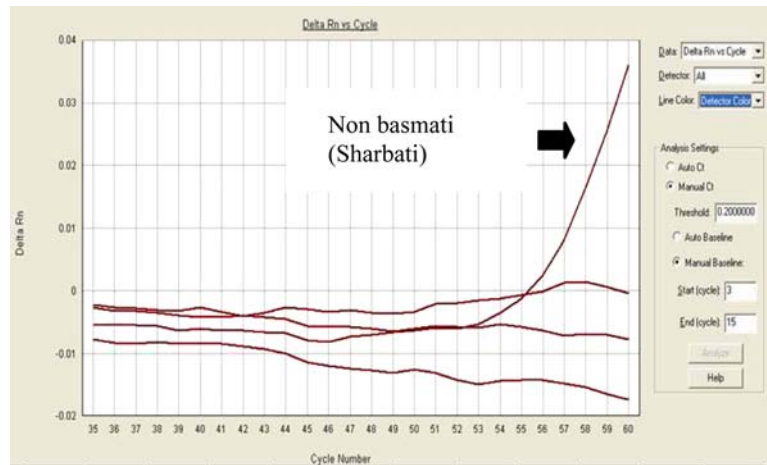


Table 2 Method validations by using basmati samples spiked with non-basmati rice

S. No.	Mixture of rice varieties analysed	Actual concentration (1/100 g)	Observed non-basmati concentration (1/100 g)
1	Non-basmati 1 ^a	2.0	18.24 ± 0.31
	Non-basmati 2 ^a	15.0	
	Basmati 1 ^a	7.0	
	Basmati 2 ^a	25.0	
	Basmati 3 ^a	51.0	
	Basmati 4 ^a	–	
2	Non-basmati 1	4	8.83 ± 0.56
	Non-basmati 2	4	
	Basmati 1	4	
	Basmati 2	77	
	Basmati 3	–	
	Basmati 4	10	
3	Non-basmati 1	2	12.42 ± 0.91
	Non-basmati 2	10	
	Basmati 1	3	
	Basmati 2	25	
	Basmati 3	60	
	Basmati 4	–	
4	Non-basmati 1	5	9.20 ± 0.57
	Non-basmati 2	5	
	Basmati 1	–	
	Basmati 2	90	
	Basmati 3	–	
	Basmati 4	–	
5	Non-basmati 1	–	1.96 ± 0.45
	Non-basmati 2	–	

Table 2 continued

S. No.	Mixture of rice varieties analysed	Actual concentration (1/100 g)	Observed non-basmati concentration (1/100 g)
	Basmati 1	–	
	Basmati 2	50	
	Basmati 3	50	
	Basmati 4	–	
	Basmati 5	–	
6	Non-basmati 2	30	33.03 ± 2.19
	Basmati 3	70	
7	Non-basmati 2	48	54.77 ± 3.30
	Basmati 3	52	

^a Non-basmati 1 is PR type rice; Non-basmati 2 is Sharbati rice
Basmati 1 is Shabnam; Basmati 2 is Pusa basmati, Basmati 3 is Basmati 370; Basmati 4 is Pusa 1121; Basmati 5 is Sugandha

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