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Short Communication

Development of a novel InDel based molecular marker, a potential to differentiate most of the traditional Basmati from non-Basmati rice varieties

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Abstract

Owing to the presence of desirable characteristics like extra-long and slender grains, delicious taste, superior aroma and distinct flavor, Basmati rice varieties are immensely popular are fetching higher prices in the market than non Basmati rice varieties. Basmati rice is commonly adulterated with many look alike, long grain non Basmati rice varieties. Microsatellite markers (SSRs) have proven to be the markers of choice to detect Basmati adulteration. Here, we report the development of a novel InDel based STS marker, which can differentiate most of the traditional Basmati from non-Basmati rice varieties.

Keywords: Basmati, adulteration, promoter, STS, InDel

Basmati rice has been believed to be nature's gift to the Indian sub-continent. What makes this rice superior to the other non-Basmati rices, is its long and slender kernels, exquisite aroma, sweet taste and soft texture (All India Rice Exporters Association). Due to its global popularity, India is a leading exporter of the Basmati Rice. During the year 2016-17, 3.25 million tons of Basmati rice was exported (Department of Commerce,GOI). There are 26 notified varieties of Basmati rice in India (All India Rice Exporters Association, 2016). These have been categorized as traditional Basmati varieties (TB) and evolved Basmati rice varieties (EB). The TB rice varieties like Basmati 370, Basmati 386, Taraori Basmati, Dehraduni Basmati, Ranbir Basmati and Basmati 217, are tall, low yielding, photoperiod sensitive and less fertilizer responsive (Nagaraju et al. 2002). In an attempt to increase the yield of TB, they were crossed with high yielding non-Basmati varieties which resulted in the development of many evolved Basmati rice varieties like Pusa Basmati 1, Haryana Basmati, Punjab Basmati, Super Basmati, Pusa Basmati 1121, Kasturi, MahiSugandha etc.Though higher yielding than TB varieties, EB varieties do not compare with the quality standards defined for true Basmati varieties (Archak et al. 2007). Due to this reason, the EB varieties are given a lesser price grade than TB varieties both in the domestic as well as international markets (Siddiq et al. 2012). Nine Basmati rice varieties are eligible for zero import duty in the European Union (Regulation EC-1549/2004), most of which (six in number) are TB rice varieties (Cassier and Kozulic 2014).The remaining three are Super Basmati, Pusa Basmati 1 and Karnal Basmati (Basmati Pakistan) (British Code of Practice for Basmati Rice, 2017). These steep price differences between TB, EB and non Basmati (NB) varieties, has led to deliberate adulteration of premium traditional Basmati rice varieties with evolved Basmati rice varieties and other look - alike non Basmati rice varieties. Identification and detection of the degree of adulteration in Basmati rice varieties is therefore, important in order to ensure the authenticity of the Basmati rice. Many DNA based methods have been developed and are widely used to detect Basmati rice adulteration due to their repeatability, simplicity and accessibility (Siddiq et al. 2012). These methods

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commonly utilize microsatellite markers (SSRs) which are able to differentiate between TB, EB and NB to various extent. Nagaraju et al. (2002) and Jain et al. (2004) used the fluorescently labeled microsatellite and ISSR (Inter-Simple- Sequence-Repeat) markers to differentiate TB, EB and NB rice varieties. A single tube multiplex assay which included eight microsatellite loci, when analyzed on capillary electrophoretic system has been reported to be able to detect adulteration from 1 % upwards (Archak et al. 2007). More recently, the padlock probe ligation and microarray detection tool, which is a DNA-based multiplex detection tool, has been developed to detect adulteration in Basmati rice (Voorhuijzen et al. 2012). In addition to microsatellite markers, the *fgr* gene encoding for the BADH2 (betaine aldehyde dehydrogenase homologue 2) enzyme, has been reported to have an eight base-pairs deletion in exon 7, which is considered to be the cause of fragrance in many of the fragrant rice varieties like Basmati and Jasmine rice. This deletion has therefore been used for distinction of Basmati from non-Basmati rice (Bradbury et al. 2005; Sakthivel et al. 2009).Since the microsatellite analysis frequently utilizes either fluorescently labeled PCR products separated on polyacrylamide gels followed by visualization by silver staining, or capillary electrophoresis or high resolution melting analysis (Ganopoulos et al. 2011), all of which are laborious and time consuming, efforts should be made to develop simple to use protocols which enable quick and easy detection of adulteration in Basmati rice. In an attempt to achieve this, here we report a single InDel based sequence tagged marker (STS) which is able to differentiate between TB, EB and NB to a large extent.

LAX PANICLE1 (*LAX1*) gene, which codes for a helix-loop-helix transcription factor protein has been shown to be expressed at the site of axillary meristem initiation in rice (Komatsu et al. 2003). The transcription factor controls panicle length in rice. The full length reference gene sequence, TIGR locus ID, LOC_Os01g 61480, of 1078 bps along with an 865 bp upstream promoter sequence, was obtained from TIGR Rice Genome Browser database. A total sequence length of 1943 base pairs was amplified from five varieties, i.e. Swarna, Pusa Basmati1, Pusa Basmati6, Nipponbare and Pusa Basmati1121 using two pairs of sequence specific primers which were designed for end sequencing, using CLC Genomics workbench 6.0.4. Figure 1(a) shows the position in the gene sequence from where the primers were designed for

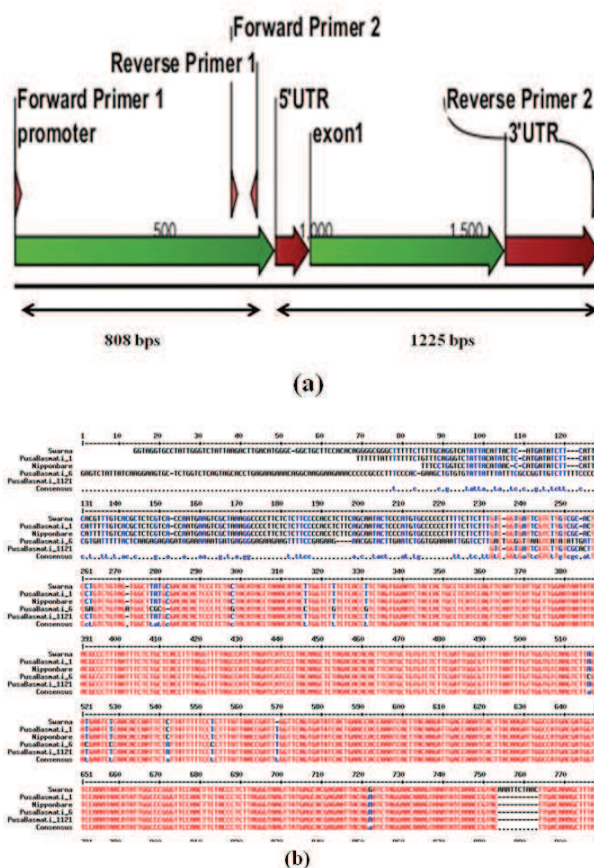

Fig. 1. (a) The position of primers designed for full length gene amplification and sequencing and (b) Part of multiple sequence alignment of the sequences obtained along with the reference sequence

end sequencing. The PCR amplicons were extracted from the gel and end sequenced with the same set of PCR primers. The sequences obtained were assembled into contigs using CLC genomics workbench 6.0.4 and then used for multiple sequence alignment along with the reference sequence by MultAlin (Corpet 1988) (Fig. 1b). A 10 base pair InDel was found at –205 position upstream from the transcription start site. A set of primers (STS_661F' and STS_661R') flanking this indel were designed to further amplify this target sequence. This target sequence containing the InDel was amplified across 245 rice germplasm lines to verify for the presence of InDel. Figure 2(a) shows the position of the InDel and the flanking primers and the subsequent amplification pattern obtained by PCR amplification with the same primers.

In the present study, most EB and NB varieties except Pusa Basmati1, Pusa 1121, Pusa Basmati 6

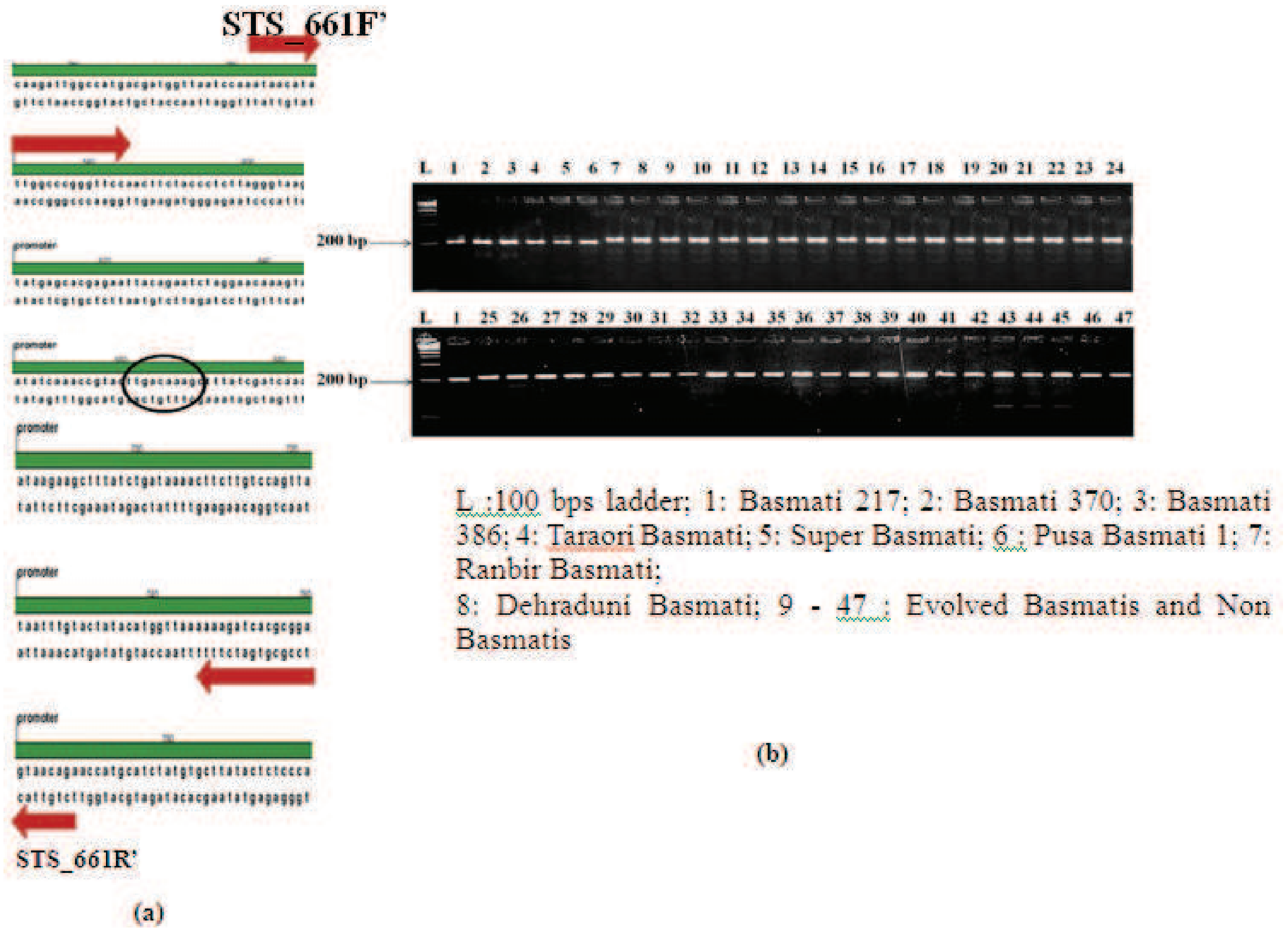

Fig. 2: (a) Position of primers flanking the InDel and (b) Amplification profile of rice varieties on amplification with STS_661

and Super Basmati produced an allele size of 216 bps while most TB varieties except Ranbir Basmati and Dehraduni Basmati produced an allele size of 206 bps, on amplification with the primer STS_661.The plausible explanation for Pusa Basmati 1 and Super Basmati, both EBs, producing an allele of 206bps could be because of the retention of the genomic region from one of the parents, Karnal local and Basmati 320, both traditional varieties, respectively. Since, Pusa 1121 is a sister line of Pusa Basmati 1 and Pusa Basmati 6 is a variety developed from a cross between Pusa Basmati 1 and Pusa 1121, the reason for them producing the same allele as that of Pusa Basmati 1 and Pusa 1121 is understandable. Evolved Basmati variety Pusa Basmati 1 which is the country's first high yielding, semi dwarf Basmati variety accounts for a large proportion of Basmati rice exports, has desirable quality characters which compare well with TBs like Basmati 370 and Taraori Basmati (Siddiq et al. 2012). Hence, this marker can be successfully used to detect adulteration of 6 of the 9 Basmati varieties, on which no import duty is levied. It is difficult to attribute a reason for Ranbir Basmati (TB), which is a selection from Basmati 370 (TB), to be showing an allele size of 216 bps when Basmati 370 produces a fragment size of 206bps. However, a similar observation has been reported by Pal et al. (2004), where it was reported that Basmati 386 (TB) which is a local selection made from Taraori Basmati (TB), had different alleles at as many as 10 out of 35 SSR loci, than Taraori Basmati. *Japonica* rice varieties, Nipponbare and Naggardhan, also produced a fragment size of 206 bps, demonstrating the presence of deletion at the locus. The presence of this deletion in majority of Basmati rice varieties and the *japonica* rice varieties, goes on to further prove the genetic relatedness of Basmati and *japonica* rice varieties. Interestingly, the fragrance trait in rice has been traced back to *japonica* rice (Kovach et al. 2009).

Table 1. Details of allele/band sizes obtained for different rice varieties on PCR amplification with STS_661

S.No.	Variety name	Pedigree	Type	Allelic status (bps)
Varieties exempted from import duty (EC1549/2004)				
1.	Basmati 217	Pure line selection	TB	206
2.	Basmati 370	Pure line selection from traditional basmati	TB	206
3.	Basmati 386	Selection from local material	TB	206
4.	Taraori Basmati/ HBC19/ Karnal Local	Pureline selection from local Basmati	TB	206
5.	Super Basmati	Basmati 320/ IR661	EB	206
6.	Pusa Basmati	Pusa 150/Karnal local	EB	206
7.	Ranbir Basmati	Selection from Basmati 370	TB	216*
8.	Dehraduni Basmati	Selection from Type 3	TB	216*
9.	Karnal Basmati	CM7/Basmati 370	EB	-
Other Basmati varieties				
1.	Haryana Basmati	Sona / Basmati 370	EB	216
2.	Punjab Basmati	Basmati 386/ Super Basmati	EB	216
3.	Basmati 385	TN1/ Basmati 370	EB	216
4.	Mahisugandha	BK-79 /Basmati370	EB	216
5.	Kasturi	Basmati370/CR 88-17-1-5	EB	216
6.	Pusa Basmati 1121	Sister line of Pusa Basmati-1	EB	206
7.	Pusa Basmati 1509	Pusa 1301/ Pusa Basmati 1121	EB	216
8.	Vallabh Basmati	Type 3/ Pusa Basmati 1121	EB	216
9.	Pusa Basmati 6 (Pusa 1401)	Pusa Basmati-l/ Pusa 1121-92-8-2-7-1	EB	206
10.	CSR 30	Buraratha 4-10/ Pak Basmati	EB	216
Common Basmati Rice Adulterants				
1.	Improved Sabarmati	TN1/Bas.370// Bas.370	NB	216
2.	Kalimuch	-	NB	216
3.	Lakra	-	NB	216
4.	Parimal	-	NB	216
5.	Pusa 169	IR28/Pusa 140-56	NB	216
6.	PR 106	(IR-8 x Peta 5)/ Bella Patna	NB	216

TB:Traditional Basmati; EB: Evolved Basmati; NB: Non Basmati

Traditional Basmatis, like Basmati 370, Basmati 386, Taraori Basmati, Basmati 217 and evolved Basmatis, Super Basmati, Pusa Basmati 1, can be differentiated from common adulterants like Improved Sabarmati, Kalimuch, Lakra, Parimal, PR106 and PR169 (Vaingankar et al. 1989) by using this marker in a simple PCR assay. Pusa Basmati 1121 and Pusa Basmati 6 constitute the major exports of Basmati rice to EU. Any adulteration in these varieties with non Basmati rice varieties can be easily detected using this marker. Most EBs and NBs in our study have shown an allele of 216 bps whereas most TBs have shown an allele size of 206bps (Table 1). The presence of a similar band/allele in many EBs and NBs can be explained when the allelic region is present near the genomic regions which control/govern the desirable traits in NBs, which is contributed by the non Basmati rice variety when crossed with a Basmati variety.

Although many reports have reported exclusivity of alleles in TBs, which have not been found in EBs, none of the reports have reported the development of a single marker which is able to provide a clear cut difference between TBs, EBs and NBs, validated across 245 rice accessions, which included the most common Basmati rice adulterants as well.

Author's contribution

Conceptualization of research (SY, RS, CR); Designing of experiments (SY DW); Contribution of materials (RS, AKS, SK); Execution and coordination (SY, DW), analysis of data, quality analysis, compilation and interpretation (SY, RS, DW, CR); Preparation of manuscript (SY, AKS, SK).

Declaration

The authors declare no conflict of interest.

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