

SHORT COMMUNICATION

A Novel Loop-Mediated Isothermal Amplification Assay as a Potential Method for Varietal Discrimination- A Case Study in Rice

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Abstract

A novel loop-mediated isothermal amplification (LAMP) assay was developed to distinguish authentic Basmati rice varieties from non-Basmatis, based on 10 bp insertion-deletion (InDel) identified in the *Lax1* gene. The developed assay demonstrated high specificity and allowed discrimination between Basmati and adulterant non-Basmatis with a detection limit of 10%.

Keywords: Basmati rice, Adulteration, LAMP, InDel, Promoter

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Introduction

India accounts for ~72% of the market share of the global Basmati industry. The code of practice (COP) on Basmati rice, enlists the Basmati rice varieties which are exempted from import duty. This includes Basmati 217, Basmati 370, Basmati 386, Pusa Basmati 1, Ranbir Basmati, Super Basmati, Taraori Basmati and Type 3 (Commission Regulation (EC) 972/2006). Some of these are traditional Basmati (TB) varieties, which are no longer significantly produced in the country due to the presence of undesirable traits like lodging proneness, lower yield and photoperiod insensitivity. These have been replaced with evolved Basmati (EB) varieties which are higher-yielding, short-duration varieties, developed through concerted breeding efforts of rice breeders (Siddiq *et al.*, 2012). The most popular EBs which constitute the major share of Basmati export from India, are Pusa Basmati 1121, which is grown in > 50% of the total area under Basmati rice cultivation in India, followed by Pusa 1509 and PB 1 (Basmati survey report, APEDA, 2019).

Basmati rice is often adulterated with grains of look-alike, low-cost, non-Basmati rice varieties. For instance, Pusa Basmati 1121 is commonly adulterated with Pusa Sugandha 2 and 3 and Pusa Basmati 1 is adulterated with Sharbati (Vemireddy *et al.*, 2015). The detection of adulteration becomes challenging because it is very difficult to differentiate between authentic Basmati and look alike non-Basmati grains visually or by analyzing the grain quality properties like presence or absence of aroma, grain elongation quotient, etc. DNA-based molecular markers have therefore been adopted as a method of choice to do the same. Microsatellite or simple sequence repeat (SSR) markers have been widely used for varietal identification in rice (Nagaraju *et al.*, 2002; Archak *et al.*,

2007; Sundaram *et al.*, 2008; Vemireddy *et al.*, 2015). In the present study, we report, a novel, loop-mediated isothermal amplification (LAMP) assay, which can potentially be used to differentiate between Basmati and non-Basmati rice varieties in a single step without the need for a gel-based resolution of amplified products. LAMP assays have been used for various diagnostic purposes such as species identification, pathogen detection, detection of food spoilage, etc (Panek *et al.* 2019). The advantages of this method over other DNA-based detection methods, lie in its higher sensitivity and specificity, lesser time consumption and no requirement of specialized laboratory facilities, not even the thermocycler.

In a previously published paper from our lab, we reported the presence of a 10 bp (AAATTCTAAC) deletion in the promoter region of the *Lax1* gene (Yadav *et al.*, 2017). An STS marker was used to amplify the region and allowed the differentiation of most of the Basmati rice varieties from non-Basmati rice varieties. The deletion was found in the sequences amplified from the varieties Pusa Basmati 1, Pusa Basmati 6 and Pusa Basmati 1121. A total of 211 germplasm accessions were screened for the presence of deletion by using sequence-specific PCR-based primers (STS) flanking the InDel. Among the accessions screened, the deletion was identified in only 15 varieties namely Basmati 386, Basmati 217, Taraori Basmati, Basmati 370, Pusa Basmati 1, Pusa Basmati 6, Pusa Basmati 1121, Super Basmati, Hassan Sarai, Nipponbare, Naggardhan, Bhalum-1, Bhalum-2, Khonorullo and Mega Rice-1. Commonly used non-Basmati rice adulterants like PR-106, Pusa-169, Improved Sabarmati, Kali-much, Lakra, Parimal, Sharbati, Sugandh 2, Sugandh 3 (Vaingankar and Kulkarni, 1989) were all found to yield an amplicon size of 216 bp indicating the presence of ten bp insertion at the locus (Figure 1). We have exploited this sequence information to develop a LAMP assay that provides a color-based discrimination between these varieties.

Based on the identified 10 bp InDel, a set of four LAMP primers was designed using the Primer explorer V4 program (Eiken Chemical Co., Ltd.) (Table 1).

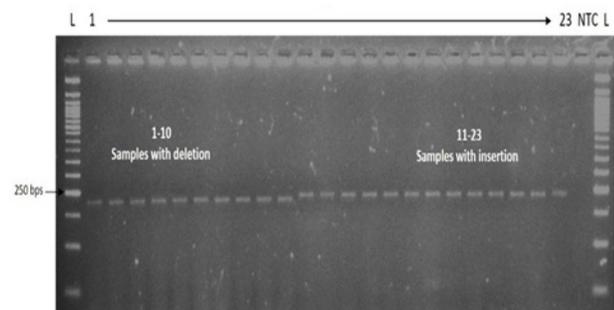


Figure 1: Representative gel pictures depicting the screening of the germplasm accessions for the presence of deletion by using sequence-specific PCR primers flanking the InDel. 1-23: different germplasm accessions; NTC: no template control. Samples with deletion produced an amplicon of 206 bps and samples with insertion produced an amplicon size of 216 bps.

The positions of the LAMP primers on the target DNA sequence are shown in Figure 2a. The forward inner primer (FIP) consisted of sequences F1c and F2, and the backward inner primer (BIP) consisted of sequences B1c and B2. The outer primers F3 and B3 were required for initiation of the

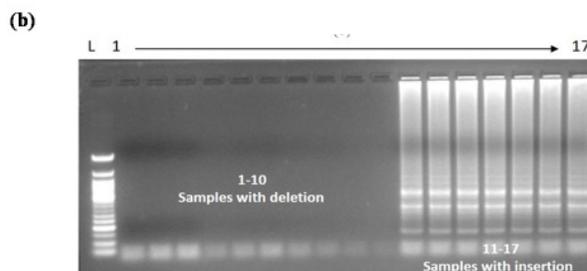
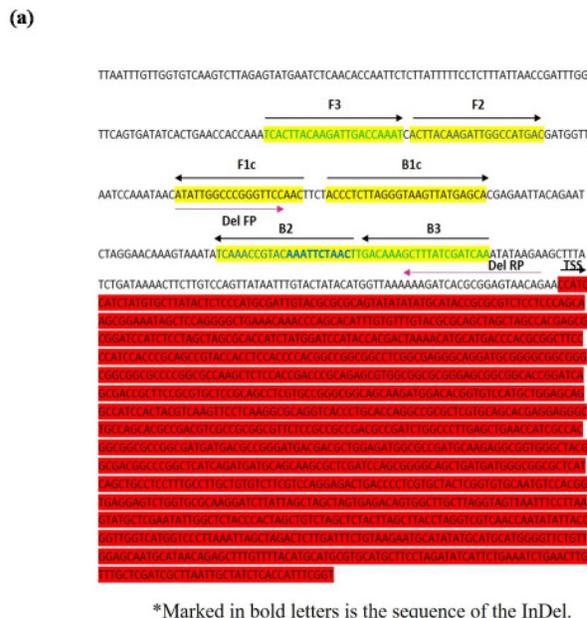


Figure 2: The LAMP assay developed. a) Positions of the primers designed from the genic sequence, which were used in the study. b) Gel-based resolution of LAMP products obtained for accessions with deletion and insertion. L: 100bp DNA ladder; 1-17: different germplasm accessions

Table 1: Details of primers used in the study

PCR-based screening primers		
Primer	Length	Sequence
Del FP	22	ATAACATATTGGCCCGGTTCC
Del RP	22	TCTGTACTCCGCGTGATCTTT
LAMP primers		
Primer	Length	Sequence
F3	22	TCACTTACAAGATTGACCAAAT
FIP (F2 + F1c)	41	GTTGGAACCCGGGCAATAT- ACTTACAAGATTGGCCATGAC
BIP (B2 + B1c)	47	ACCCCTTAGGGTAAGTTATGAGCA- AGTTAGAATTTGTACGGTTTGA
B3	21	TTGATCGATAAAGCTTTGTCA

LAMP reaction. The InDel was located within the sequence region of primer B2. The LAMP assay was carried out in the standard way (Deb *et al.*, 2017; Singh *et al.*, 2020) with standardization performed for amplification temperatures (61–65°C). The highest specificity was obtained at 62°C and therefore the amplification was performed at this temperature. A ladder-like amplification profile was generated when the LAMP products were resolved on an agarose gel in varieties that carried the insertion, whereas no such profile was generated in varieties that carried the deletion (Figure 2b).

The LAMP primers were designed in such a way that the Basmati rice variety (B) which carries the deletion, fails to give an amplification product which can be seen as an absence of a ladder-like amplification pattern when the LAMP product is run on an agarose gel and red/orange color on SYBR Green addition to the tubes, indicating a negative reaction. The non-Basmati or adulterant (A) can be differentiated as it gives a ladder-like amplification pattern when run on an agarose gel and a color change from red/orange to green on SYBR Green addition to the tubes, indicating a positive reaction (Figure 3a).

To detect the sensitivity of the assay, samples of Basmati rice grains with varying amounts of grains of non-Basmati

rice variety were made by progressively increasing the number of non-Basmati rice grains (Pusa Sugandh 2, a long grain, non-Basmati rice variety, a common adulterant) in Basmati rice grain samples (Basmati 370) at various concentrations *viz.*, 1, 5, 7, 10, 25 and 50% (on a number basis) as done previously (Archak *et al.*, 2007). Broken and damaged seeds were discarded. For a given percentage of adulteration, three biological replicates were prepared and DNA was extracted from equal quantities of seeds, using the modified CTAB method (Doyle and Doyle, 1987). The LAMP assay was performed on these samples and it was observed that the intensity of color change and ladder-like bands observed, was found to be proportional to the concentration of DNA of the adulterant in the sample (Figure 3b).

The sensitivity of the LAMP assay when compared to conventional PCR was determined by performing both LAMP reaction and PCR amplification on the same set of DNA templates. While the four LAMP primers were used for the LAMP assay, the external F3 and B3 LAMP primers were used for the conventional PCR (Figure 3c). The sensitivity of the LAMP assay was found to be higher than the conventional PCR assay. Since two of three biological replicates for 10% adulterant concentration showed successful amplification and all three biological replicates of adulterant concentration beyond 25% showed successful amplification, it can be concluded that the limit of detection ranges between 10 to 25%. No such distinctly visible differentiation in terms of the presence of two different bands (codominance), was observed on the gel which was loaded with conventional PCR products. This demonstrates the higher sensitivity of the LAMP assay over the conventional PCR assay.

Though not very sensitive, this assay can be used as a preemptive screening method to screen a large number of samples as its less time-consuming and easier to perform. We have demonstrated its utility in terms of a presence/absence method of detection of adulteration. This study successfully demonstrates how a genomic level difference can be translated into an application for diagnostic purposes. The developed assay provides a cost-effective and user-friendly detection method that can be utilized for the detection of potential adulterants in Basmati rice in a time span of about 1 hour.

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References

- Archak S, V Lakshminarayananreddy and J Nagaraju (2007) High-throughput multiplex microsatellite marker assay for detection and quantification of adulteration in Basmati rice (*Oryza sativa*). *ELECTROPHORESIS*. 28: 2396–2405.
- Deb R, GS Sengar, U Singh, S Kumar, TV Raja, R Alex, RR Alyethodi and B Prakash (2017) LAMP assay for rapid diagnosis of cow

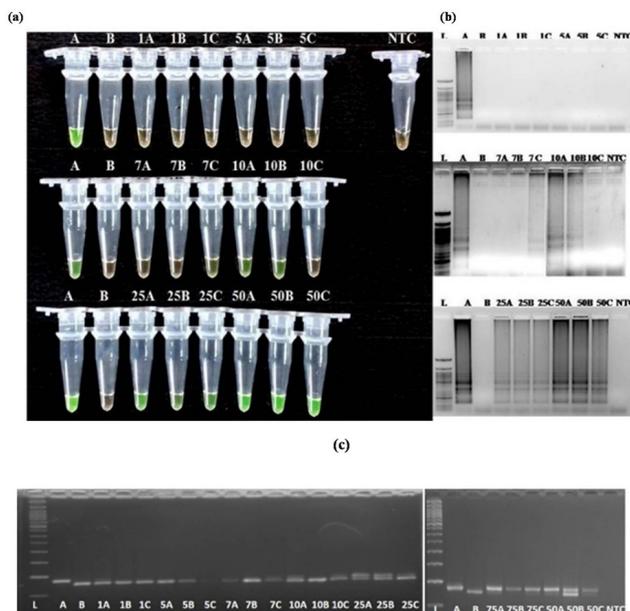


Figure 3: The results of a) SYBR Green-based visualization of LAMP products. b) Gel-based resolution of LAMP products. c) Gel-based resolution of PCR products obtained on amplification with external F3 and B3 LAMP primers. A: Pusa Sugandha 2 (PS-2; a long grain, non-Basmati rice variety, a common adulterant); B: Basmati 370 (a Basmati rice variety); 1A, 1B, 1C: The three biological replicates of Basmati 370 with 1% adulteration with PS-2 (in terms of no. of grains); 5A, 5B, 5C: The three biological replicates of Basmati 370 with 5% adulteration with PS-2 and likewise with 7, 10, 25 and 50% adulterant (A) grain number in Basmati (B) grains; NTC: no template control; L: 50 bp plus ladder

- DNA in goat milk and meat samples. *Iran J. Vet. Res. Spring* 18(2): 134-137.
- Doyle JJ and JL Doyle (1987) A rapid DNA isolation procedure from small quantities of fresh leaf tissues. *Phytochem Bull.* 19: 11–15.
- LR Vemireddy, VV Satyavathi, EA Siddiq and J Nagaraju (2015) Review of methods for the detection and quantification of adulteration of rice: Basmati as a case study. *J Food Sci. Technol.* 52: 3187–3202.
- Nagaraju J, M Kathirvel, RR Kumar, EA Siddiq and SE Hasnain (2002) Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR PCR and SSR markers. *Proc. Natl. Acad. Sci.* 99: 5836–5841.
- Panek J and M Frac (2019) Loop-mediated isothermal amplification (LAMP) approach for detection of heat-resistant *Talaromyces flavus* species. *Sci. Rep.* 9: 5846.
- Siddiq EA, LR Vemireddy and J Nagaraju (2012) Basmati Rices: Genetics, Breeding and Trade. *Agric. Res.* 1: 25–36.
- Singh M, D Pal, P Sood and G Randhawa (2020) Construct-Specific Loop-Mediated Isothermal Amplification: Rapid Detection of Genetically Modified Crops with Insect Resistance or Herbicide Tolerance. *J AOAC Int.* 103(5): 1191-1200.
- Sundaram RM, B Naveenkumar, SK Biradar, SM Balachandran, B Mishra, M Ilyas Ahmed, BC Viraktamath, MS Ramesha and NP Sarma (2008) Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. *EUPHYTICA.* 163: 215–224.
- Vaingankar NM and PR Kulkarni (1989) A cooking quality parameter as an indicator of adulteration of Basmati rice. *J. Sci. Food Agric.* 48: 381-384.
- Yadav S, R Singh, D Wankhede, C Ram, AK Singh and S Kumar (2017) Development of a novel InDel based molecular marker, with a potential to differentiate most of the traditional Basmati from non-Basmati rice varieties. *Indian J. Genet.* 77: 564-568.