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Value-added forms of black pepper (*Piper nigrum* L.) are an important item of trade globally. Adulteration by default or design of the commodity not only leads to economic loss and public health issues but also to self-respect of a nation. DNA barcoding is assuming significance as a quality assurance technique in many agri-food commodities. Three barcoding loci viz*., psbA-trnH, rbcL, rpoC1* were used in the study to detect bio adulteration of traded black pepper powder. PCR amplification of *P. nigrum* and traded black pepper powder was performed for all the three loci. Sequence analysis and BLAST results revealed chilli adulteration in two out of nine market samples, originating probably from exhausted black pepper powder fortified with chilli. Of the three loci, *psbA-trnH* proved to be the best and ideal for detection of chilli adulteration in black pepper yielding amplicons of size 600 bp and 350 bp, respectively. Cloning and sequencing of the adulterant specific band of both market samples were done to confirm the results. It was further validated using simulated samples of chilli and black pepper powders in various proportions. The method proved efficient to detect adulteration even at very low levels (0.5% adulteration). HPLC analysis also supported the chilli adulteration of black pepper powder. The method is easy, reliable and efficient, and can be used by the regulatory agencies for quality assurance of black pepper powder.

Key Words: adulteration; *psbA-trnH*; *rbcL*; *rpoC1*; *Piper* species; market samples

INTRODUCTION

Black pepper (*Piper nigrum* L., Family Piperaceae) is known as King of Spices or Black Gold and is an important spice and medicine besides its cosmetic uses.

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Apart from its use as a spice and flavoring agent, it has antimicrobial, antioxidant, anti-inflammatory, and antitoxic properties (Vijayan and Thampuran, 2000). Black pepper is traded as whole dried corns, white pepper, powder, extracts, spice blends, and mixtures. Quality of the commodity is the first requirement to sustain and boost the trade besides ensuring public health.

Adulteration has been reported in whole black pepper and its products such as white pepper, ground pepper, essential oil, and oleoresins (Madan et al., 1996; Archer, 1987) affecting the quality of the product. Among the reported plant based adulterants, papaya seed (*Carica papaya* L.) is one of the most common adulterants (Dhanya et al., 2009; Paramita et al., 2003; Paradhakar et al., 2001; Pruthi and Kulkarni, 1969), besides wild *Piper* species viz, *P. attenuatum. P. galeatum* (Dhanya and Sasikumar, 2010). Though ginger powder (*Zingiber officinale*) is reported to be adulterated with chilli (*Capsicum annuum*) (Dhanya and Sasikumar), there is no report of adulteration of black pepper with chilli. However, exhausted black pepper fortified with other plant based pungent principles is an adulteration option.

Numerous conventional analytical methods which rely on physical, histochemical, or standard biochemical methods have been reported for detection of plant based adulterant in black pepper (Paramita et al., 2003; Paradhakar et al., 2001; Tremlova, 2001; Madan et al., 1996; Curl and Fenwick., 1983). However, these are of little consequence in detecting the bio-adulterants in black pepper (Dhanya and Sasikumar, 2010).

Nucleic acid analysis is more reliable, quick and easy when compared to conventional analytical methods like microscopy, spectrometry and Thin Layer Chromatography for the detection of plant based adulterants and food authentication (Aida et al., 2007; Lum and Hirsch., 2006; Chang-chai et al., 2005; Sasikumar et al., 2004). DNA based methods such as RAPD, Real-Time PCR, PCR-RFLP-ARMS, SSR and SCAR markers have been used in detecting adulteration of medicinal plants (Hussain and Bedi., 2012; Li et al., 2007) and traded spices like turmeric (Sasikumar et al., 2004), black pepper (Dhanya et al., 2009), chilli (Dhanya et al., 2008, 2011), and oregano (Marieschi et al., 2009). Here we report a robust DNA barcoding based molecular technique for detecting chilli adulteration in traded black pepper powder. DNA barcoding uses sequences of DNA to discriminate species since genetic variation among the species exceeds that within species (Hebert et al., 2003). DNA barcoding is now being used in detecting plant based adulteration in many commodities such as medicinal plants, commercial tea packets, olive oils, etc. (Yuan et al., 2011; Stoeckle et al., 2011; Kumar et al., 2011; Vijayan and Tsou, 2010; Srirama et al., 2010). In spices, there are not many reports barring the ones in Lamiaceae (Mattia et al., 2010; Guo et al., 2011) and saffron (*Crocus sativus* L.) (Gismondi et al., 2013). The technique was also proved to be effective in identification of ancient *Olea europaea* L. and *Cornus mas* L. seeds (Gismondi et al., 2012).

MATERIALS AND METHODS

Sample Preparation

Nine popular powdered branded market samples (MS 1–9) of black pepper procured from the local market at Kozhikode, Kerala, India, were used in the study. Of these, five were tested initially to identify the best loci discriminating the adulterant, and the rest of the samples were used for further validation of the result. Authenticated specimens of black pepper (Accession Nos. 6834, 6857, 6833, 6835, and 6849) obtained from the germplasm repository, Indian Institute of Spices Research (IISR) Experimental Farm, Peruvannamuzhi, Kozhikode, Kerala, India, were used as reference. Model blends were prepared by mixing dried berries of *Piper nigrum* (var. Thevam) and genuine chilli samples procured from the local market in the proportions 1:9, 1:24, 1:49, 1:99, and 1:199 (weight basis). These samples were powdered using a Cyclotec 1093 sample mill. All the samples were preserved in sealed covers and stored at −20°C. Sequences of chilli were retrieved from GenBank (www.ncbi.nlm.nih.gov).

DNA Isolation

Total genomic DNA was isolated from the reference *Piper* accessions, chilli powder, market samples, and model blends using DNA easy plant mini kit (QIAGEN) and stored at −20°C.

PCR Amplification and Sequencing

Three popular barcoding loci viz., *psbA-trnH, rbcL* and *rpoCl* were selected for PCR amplification using Universal primers (Table 1). The reaction mix contained 10–50 ng of DNA, 1X assay buffer with 1.5 mM $MgCl_2$, 1 mM dNTP, 1 picomol μl^{-1} each of forward and reverse primers and 1 U *Taq* DNA polymerase (Banglore, Genei, India). The PCR was performed in an Eppendorf vapo protect thermal cycler as per conditions optimized for each loci (Table 2).

Table 1: Primers used for PCR amplification.

Primer name	Sequence	Reference
rbcL a-f	5′ ATG TCA CCA CAA ACA GAG ACT AAA GC3′	Kress and Erickson, 2007
rbcL a-r	5′ GTA AAA TCA AGT CCA CCG CG 3′	
rpoC1–2	5′ GGC AAA GAG GGA AGA TTT CG3′	Ford et al., 2009
rpoC1–4	5′ CCA TAA GCA TAT CTT GAG TTG G 3′	
trnH-2	5′ CGC GCA TGG TGG ATT CAC AAT CC 3′	Tate and Simpson, 2003; Yang et al., 2011
psbA-F	5′ GTT ATG CAT GAA CGT AAT GCT C 3′	

Table 2: PCR reaction conditions for different barcoding loci.

Reaction condition	Locus		
	rbcL	*psbA-trnH*	*rpoC1*
Initial denaturation	95°C-4 min	92°C -4 min	94°C -4 min
Denaturation	94°C -30 sec	94°C -1 min	94°C -30 sec
Annealing	55°C -1 min	52°C -1 min	50°C -40 sec
Extension	72°C -1 min	64°C -1 min	72°C -40 sec
Final extension	72°C -10 min	64°C -8 min	72°C -5 min
Total cycles	35	35	40

The PCR amplified products were resolved using 1% agarose gel (containing 0.5 μg ml^{-1} Ethidium bromide) in 1X Tris-Borate-Ethylene diamine tetra acetic acid buffer (TBE) along with 100 bp ladder (Fermentas) as a size marker and documented (Syngene). The PCR products of expected size were extracted using QIA quick PCR purification kit (QIAGEN). The products were custom sequenced at Scigenom Labs, Pvt, Ltd (Kerala, India).

Cloning

PCR products corresponding to the adulterant bands were extracted using QIA quick PCR amplification kit (QIAGEN) and cloned using TOPO-TA cloning kit (Invitrogen, Carlsbad, CA, USA). Blue-white selection as well as colony PCR were done for the screening of recombinants. Colony PCR reaction mix contained 1X assay buffer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 5 pmol each of M13 forward and reverse primer (IDT, San Jose, CA, USA) with 1U *Taq* polymerase in a total reaction volume of 25 μl. Thermal cycling conditions were 94°C for 3 min followed by 30 cycles of denaturation at 94°C for 1 min, primer annealing at 48°C for 1 min, primer extension at 72°C for 1 min, and final extension at 72°C for 5 min. Amplified products were resolved on a 1.5% agarose gel. Positive transformants were identified based on the insert size and plasmid DNA was isolated using QIA prep spin miniprep kit (QIAGEN). The recombinant plasmids were bidirectionally sequenced at Scigenom Labs.

Contig generation was accomplished by DNA baser software. Sequences were screened using BLAST algorithm (Atschul et al., 1997) to identify the closest matching sequences in the nucleotide database of GenBank. The sequences were aligned using Clustal W (Larkin et al., 2007) and edited by BioEdit software. Data analysis was done using MEGA 5 software (Tamura et al., 2011).

High Performance Liquid Chromatography (HPLC) Analysis

Samples (25g) were accurately weighed (three market samples–two putative adulterated samples [MS3 & 9] and negative control [MS4]) plus one simulated sample (1:100) in duplicates into 500 ml boiling flask. Added 200 ml

95% ethanol and refluxed gently for 5 h. Cooled and filtered 3 to 4 ml using 0.45 μm syringe filter into stoppered test tubes and injected 10 μl in to HPLC apparatus.

HPLC analysis was performed using Shimadzu LC10A, with photo diode array detector and RP-C-18 column. Acetonitrile: 1% acetic acid (60:40) was used as mobile phase with detector wavelength at 280 nm. Piperine and capsaicin (Sigma Aldrich) were used as authentic standards. 10 μl of sample was injected at a flow rate of 0.8 ml min^{-1}.

RESULTS

DNA Isolation

High molecular weight DNA could be isolated from all the samples. The absorbance ratio (A_{260}/A_{280}) of 1.7-1.8 indicated insignificant levels of contaminating RNA, proteins and polysaccharides in the isolated DNA. The quality of DNA was confirmed on 0.8% Agarose gel.

PCR Amplification and Sequencing

Amplification efficiency of all the three loci was found to be 100%. PCR amplification of *rbcL* and *rpoC1* loci yielded 600 bp and 500 bp sized fragments (Figs. 1 and 2), respectively, in all samples. The locus *psbA-trnH* yielded a 350 bp size fragment in all the *Piper* samples including the market samples and 600 bp fragment in the samples adulterated with chilli (Fig. 3).

BLAST searches revealed that except in case of one market sample, all the others showed maximum (100%) similarity to *Piper nigrum* sequences available in NCBI for all the three loci tested. The two distinct market samples showed maximum similarity to *Capsicum annuum* sequences implying the probable adulteration of the sample with chilli. The Neighbour-Joining tree

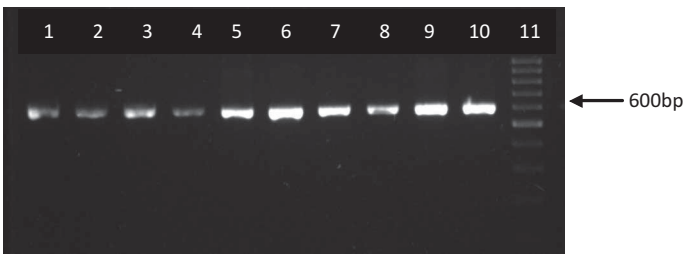

Figure 1: Amplification of *rbcL* locus (Lane 1 – *P. nigrum* Acc. No. 6834, lane 2 – *P. nigrum* Acc. No. 6857, lane 3 – *P. nigrum* Acc. No. 6833, lane 4 – *P. nigrum* Acc. No. 6853, lane 5 – *C. annuum*, lane 6 – MS-1, lane 7 – MS-2, lane 8 – MS-3, lane 9 – MS-4, lane 10 – MS-5, and lane 11 – 100 bp ladder).

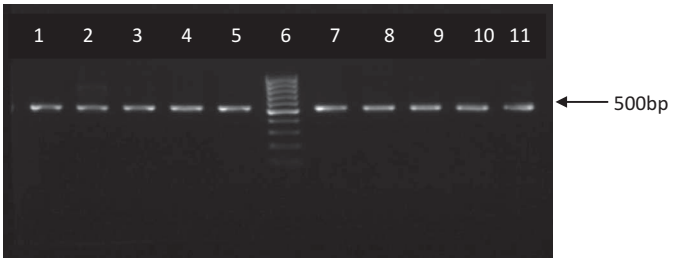

Figure 2: Amplification of *rpoC1* locus (Lane 1 – *P. nigrum* Acc. No. 6834, lane 2 –*P. nigrum* Acc. No. 6857, lane 3 – *P. nigrum* Acc. No. 6833, lane 4 – *P. nigrum* Acc. No. 6835, lane 5 – *C. annuum*, lane 6 – MS-1, lane 7 – MS-2, lane 8 – MS-3, lane 9 – MS-4, lane 10 – MS-5, and lane 11 – 100 bp ladder).

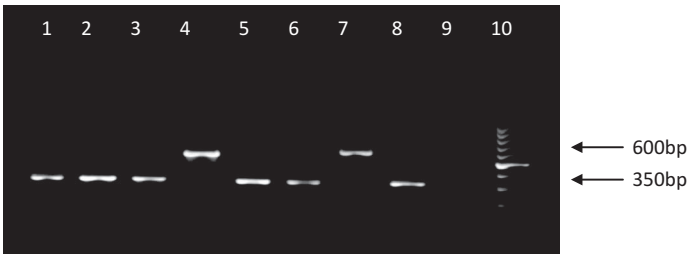

Figure 3: Amplification of *psbA-trnH* locus (Lane 1 – *Piper nigrum* Acc.No.6834, lane 2 – *Piper nigrum* Acc.No.6857, lane 3 – *Piper nigrum* Acc. No. 6833, lane 4 – *Capsicum annum*, lane 5 – MS- 1, lane 6 – MS-2, lane 7 – MS-3, lane 8 – MS-4, lane 9 – MS-5, and lane 10 – 100bp ladder).

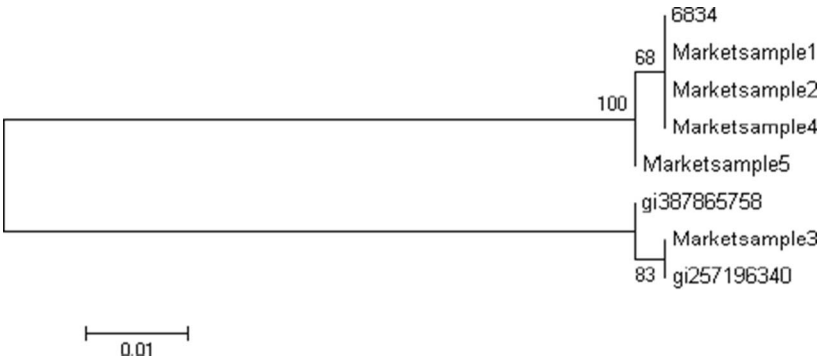

Figure 4: The N-J tree constructed from *rpoC1* sequences based on the K-2-P distance.

constructed on the basis of Kimura-2-Parameter revealed the close similarity of adulterated marketed samples with *Capsicum annuum* for all the three loci, thereby confirming the adulteration of the samples with chilli (Figs. 4–6).

The sequences generated for *Piper nigrum* in the present study are submitted to GenBank (Accession Numbers: KF278650-KF27865).

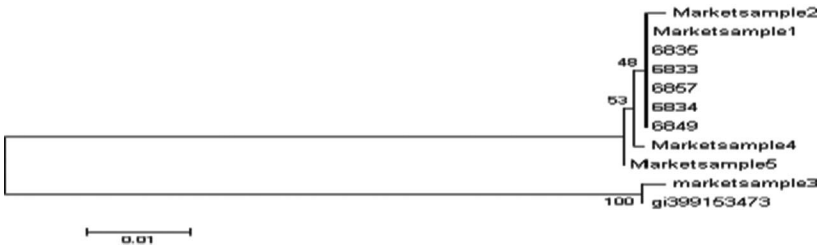

Figure 5: The N-J tree constructed from *rbcL* sequences based on the K-2-P distance.

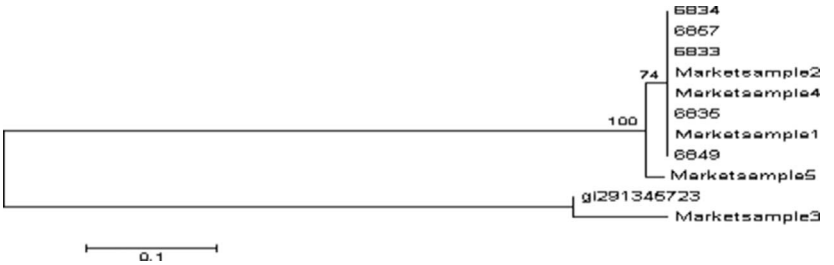

Figure 6: The N-J tree constructed from *psbA-trnH* sequences based on the K-2-P distance.

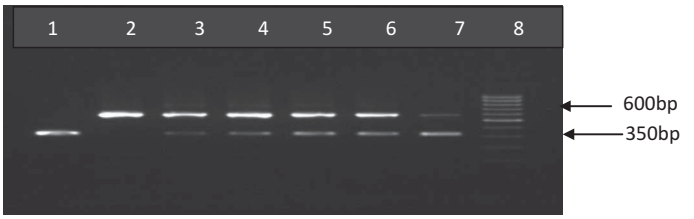

Figure 7: Amplification of adulterant specific bands in simulated samples and controls with *psbA-trnH* locus. (Lane 1 – *Piper nigrum* (negative control), lane 2 – C*apsicum annum* (positive control) lane 3 – simulated sample (10% adulteration), lane 4 – simulated sample (4% adulteration), lane 5 – simulated sample (2% adulteration), lane 6 – simulated sample (1% adulteration), lane 7 – simulated sample (0.5% adulteration), and lane 8 – 100bp ladder.

Locus *psbA-trnH* proved to be best in adulteration detection in market samples of black pepper. Band level detection of adulteration in the market samples was also possible with this locus as pepper yielded a band of 350 bp and chilli yielded a fragment of 600 bp size. This difference in the fragment size was further verified using model blends and clear bands distinguishing black pepper from chilli were observed. Adulteration even at very low level (0.5%, i.e., 1:200) could be detected with this method (Fig. 7).

The efficacy of *psbA-trnH* locus was also validated in the remaining four branded market samples (Fig. 8) along with three different batches of each adulterated brand. The adulterant specific bands from both MS revealed 100%

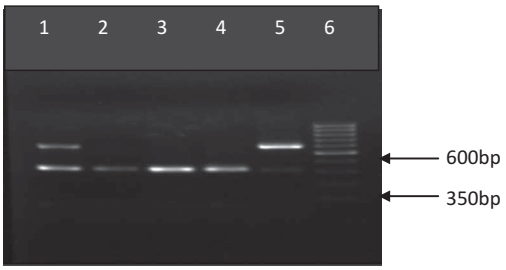

Figure 8: Validation of *psbA-trnH* locus. lane 1 – MS-3 (another batch of first lot), lane 2 – MS-6, lane 3 – MS-7, lane 4 – MS-8, lane 5 – MS-9, and lane 6 – 100bp ladder).

similarity with *Capsicum annuum* (Accession. Numbers JX270811) sequences of GenBank and 99% similarity between them (Fig. 9).

HPLC Analysis

HPLC data supported the adulteration of the samples with chilli. (Table 3). HPLC profile of these samples and standards are given in Figs. 10–15. Adulterated market samples 3 and 9 gave two characteristic peaks corresponding to piperine and capsaicin. No adulteration was detected in the negative control (sample 1) as is evident from Fig. 12. Chromatographic profile of the simulated sample (sample 4) was also consistent with those reported for adulterated samples (Fig. 15). This further confirms the adulteration of the two market samples of black pepper with chilli.

DISCUSSION

Among the different barcoding loci *psbA-trnH* and *rbcL* are reported to have high amplification success with universal primers (Gregory et al., 2005; Kress et al., 2005; Newmaster et al., 2006). Barcoding as a tool to detect adulteration in traded commodities such as commercial tea packets, olive oil, medicinal plants, saffron, etc., is now gaining momentum. (Gismondi et al., 2013; Kool et al., 2012; Kumar et al., 2011; Stoeckle et al., 2011; Yuan et al., 2011; Srirama et al., 2010). Molecular authentication (species identification) studies using DNA barcoding loci such as *psbA-trnH, rbcL, matK, rpoC1, ITS, trnT-trnL, trnF-trnL* have also been getting the attention of the researchers. (Ali et al., 2012; Sun et al., 2012; Rai et al., 2012; Deng et al., 2011; Hollingsworth et al., 2011; Li et al., 2010; Pettengill and Neel, 2010; Ford et al., 2009; Jarret, 2008; Kress and Erickson, 2007; Zuo et al., 2011). Mislabelling/substitution of traded fishery products (Cline, 2011; Cawthorn et al., 2012) as well as ancient DNA plant samples were also identified by barcoding technique (Gismondi et al., 2012).

a)

1 CGCGCATGGTGGATTCACAATCCACTGCCTTGATCCACTTGGCTACATCCGCCCCCTCGC 60

61 CTACTTACATTCCATTTTTACATTATTTAAATTAGAAAACAAAAGATTCAAGTTCGAATA 120

121 TTTCTCTTCTTTCTTATTTGAATGATATTATTATTTCAAAGATGAGAATATGAATCAAAG 180

181 ATCAGAATCTGAAGTAAAAATTAATTTTTTTTTTGAAATGAAATAAAAAAGATATAGTA 240

241 ACATTAGCAAGAAGAGGAACAAGTTATATTTCTATAATTTTCAATAAATACATACAAAA 300

301 TGAAAATAGAATACTCAATCCTGAATAAATGATAAATGCATGCAAATATCCTCTCTTTCTT 360

361 TTTCTATAATGTAAACAAAAAAGTATATGTAAGTAAAATACTAGTAAATTACTAAATAAA 420

421 ATAAAAAGAAAAAAAGAAAGGAGCAATAGCACCCTCTTGATAAAACAAGAAAATGATTA 480

481 TTGCTCCTTTCTTTTCAAAACCTCCTATAGACTAGACTGGGATCTTATCCATTTGTAGATGG 540

541 AGCTTCGATAGCAGCTAAGTCTAGAGGGAAGTTATGAGCATTACGTTCATGCATAAC 597

b)

1 CGCGCATGGTGGATTCACAATCCACTGCCTTGATCCACTTGGCTACATCCGCCCCCTCGC 60

61 CTACTTACATTCCATTTTTACATTATTTAAATTAGAAAACAAAAGATTCAAGTTCGAATA 120

121 TTTCTCTTCTTTCTTATTTGAATGATATTATTATTTCAAAGATAAGAATATGAATCAAAGA 180

181 TCAGAATCTGAAGTAAAAATTAATTTTTTTTTTGAAATGAAATAAAAAAGATATAGTAAC 240

241 ATTAGCAAGAAGAGGAACAAGTTATATTTCTATAATTTTCAATAAATACATACAAAATG 300

301`AAAATAGAATACTCAATCCTGAATAAATGATAAATGCATGCAAATATCCTCTCTTTCTTT 360

361 TTCTATAATGTAAACAAAAAAGTATATGTAAGTAAAATACTAGTAAATTACTAAATAAAA 420

421 TAAAAAGAAAAAAAGAAAGGAGCAATAGCACCCTCTTGATAAAACAAGAAAATGATTAT 480

481 TGCTCCTTTCTTTTCAAAACCTCCTATAGACTAGACTGGGATCTTATCCATTTGTAGATGG 540

541 AGCTTCGATAGCAGCTAAGTCTAGAGGGAAGTTATGAGCATTACGTTCATGCATAAC 597

c)

1 GCGAATCTCTCTCACTGGCTGCTGTCGATCTACTCTGGGTTTGCAGAATGCTTCACCTTA 60

61AGATACGTACCTAAACGATTAAAAAAAATT ATGCCCGCGTCGATTAAATGTAAATAAAAC120

121ACTAGGAAATTCGGGGGAGCAATACCAAAACCTTGAAAAACAAGAAATTGGGTATTGCTC180

181 CTTCAACAACTCCTATACATTAACATAAGG AGGAAGCATTATCCATTTGTAGATGGAGCT240

241 TCAACAGCAGCCAAGTCTAGAGGGAAGTTG TGAGCATTACGTTCATGCATAACCAGA 297

d)

1GGTATCGATCGCTCATGGCTGCTGTCGAGCTCCTCGGGTTTGGAGAATGCTTCTACTAATATG60

61ATACTATACCTAAACGATTAAAAAAAATTATGCCCGCGTCGATTAAATGTAAATAAAACA 120

121CTAGGAAATTCGGGGGAGCAATACCAAAACCTTGAAAAACAAGAAATTGGGTATTGCTCC180

181TTCAACAACTCCTATACATTAACATAAGGA30GGAAGCATTATCCATTTGTAGATGGAGCTT240

241CAACAGCAGCCAAGTCTAGAGGGAAGTTGT30GAGCATTACGTTCATGCATAACAAAA 296

Figure 9: Nucleotide sequences of *psbA-trnH* adulterant specific amplicon of *C. annuum* cloned from (a)MS-3 and (b) MS-9 (c) nucleotide sequence of *psbA-trnH* gene of *Piper* from MS-3 (d) nucleotide sequence of *psbA-trnH* gene of *Piper* from MS-9.

Table 3: Retention time and area of piperine and capsaicin in black pepper powders vis-à-vis standards.

Sample	Retention time	Area %
Piperine (standard)	11.680	100
Capsaicin (standard)	11.136	100
Sample 1-Market(negative control)	11.563	68.77
Sample 2-(Market sample3)	11.296	33.92
	11.712	33.16
Sample 3-(Market sample 9)	11.179	1.60
	11.499	39.10
Sample 4-Simulated sample(1:100)	11.179	1.02
	11.648	46.17

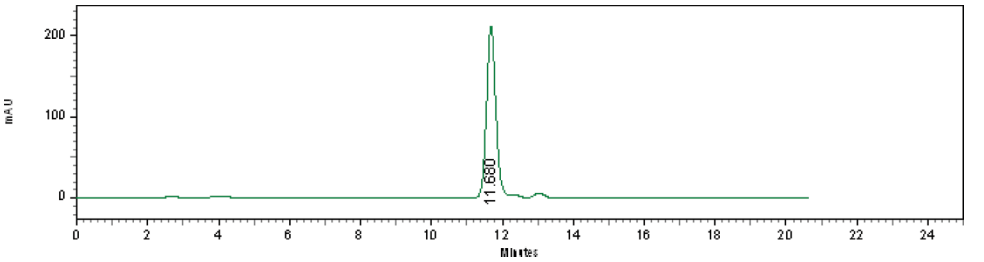

Figure 10: Chromatogram of piperine standard (60 ppm).

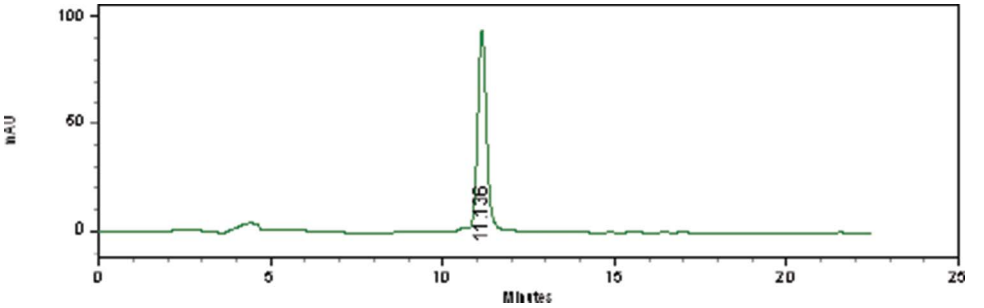

Figure 11: Chromatogram of capsaicin standard (100 ppm).

Authentication of black pepper powder could be done using all three loci, in the present study. Amplicons of *psbA-trnH* yielded an adulterant specific band (600 bp) enabling easy detection thereby reducing the cost of sequencing. Thus *psbA-trnH* proved to be the most promising locus for detection of chilli adulteration in black pepper. Out of the nine market samples analyzed, two were found positive for chilli adulteration, which was supplemented with the HPLC data. HPLC has been used as an analytical tool in quality control and authentication of many agri-food commodities (El Hamdi and El Figza, 1995; di Anibal et al., 2009, 2011).

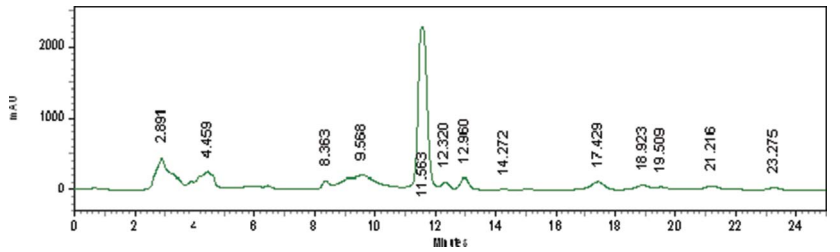

Figure 12: Chromatogram of negative control.

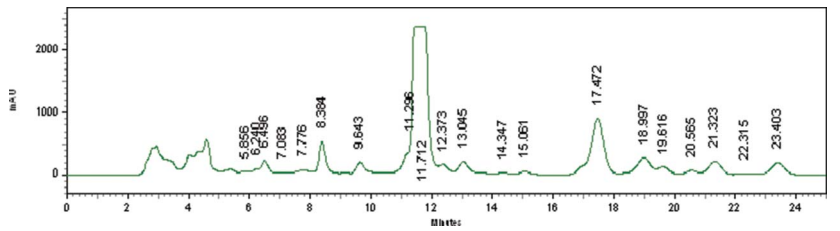

Figure 13: Chromatogram of adulterated sample (MS-3).

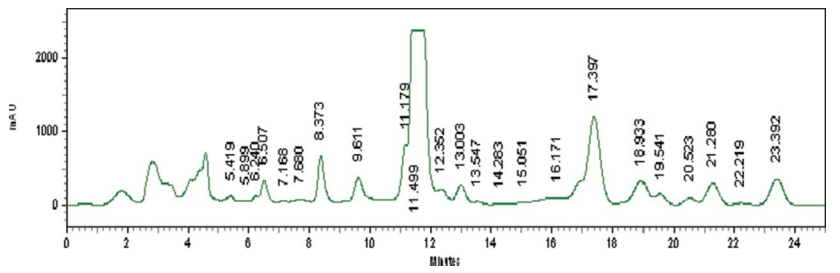

Figure 14: Chromatogram of adulterated sample (MS-9).

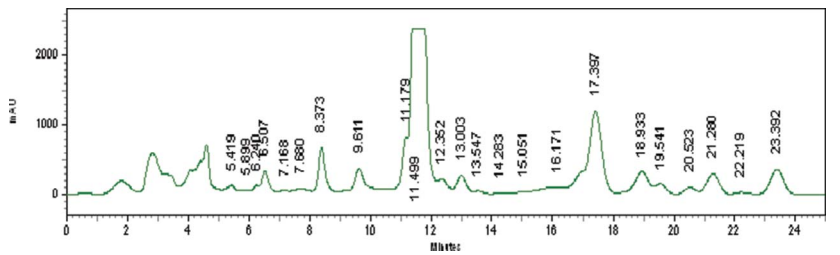

Figure 15: Chromatogram of the simulated sample (1:100).

Traded forms of spice and spice powder are highly subjected to admixing or substitution with inferior substances (Singhal and Kulkarni, 2003). Compared to whole spices, spice powders and paste are more vulnerable to adulteration

because foreign matters go into it visually undetected. Though acceptable levels of many contaminants such as pesticide residues, heavy metals, artificial dyes, synthetic adulterants, natural toxins, etc., and their detection techniques are available, there is a paucity of methods for the detection of plant based adulterants and their acceptable levels in powder forms of traded spices. This assumes significance in view of the Sanitary and Phytosanitary (SPS) regulations of WTO.

As per the latest data available in print, the global trade in black pepper powder is estimated to be 32.4 thousand metric tons worth 99.5 million U.S. dollars, that is about 12% of the total global pepper exports (ITC UNCTAD/WTO, 2006) and India's contribution is 3381 tons. The high cost coupled with low volume of the commodity is an inductive to adulteration (Singhal et al., 1997). Though traded black pepper and its value added forms are reported to be adulterated with papaya seeds (*Carica papaya* L.), wild *Piper* species viz., *P. attenuatum,* and *P. galeatum* (Dhanya and Sasikumar, 2010; Dhanya et al., 2009; Paramita et al., 2003; Paradhakar et al., 2001; Pruthi and Kulkarni, 1969), this is the first report on adulteration of black pepper powder with chilli. Ginger powder, probably from spent ginger, adulteration with chilli is already known (Dhanya and Sasikumar, 2010).

With globalization, there is liberalization of import and many countries are importing black pepper and re-exporting it as whole commodity or value-added products. Unscrupulous elements may find it lucrative to recycle the exhausted black pepper (the black pepper left after the extraction of the pungent principles) as value added black pepper (powder), probably fortified with other pungent substances such as chilli.

The already available DNA based techniques such as RAPD, ISSR, and SCAR to detect adulteration in traded spices and other agri-food commodities are handicapped with limitations such as poor reproducibility, less abundance, low level of polymorphism, genotypic specificity, prior sequence knowledge, and so forth (Kumar et al., 2009; Schierwater and Ender, 1993). DNA barcoding technique has a definite advantage in these circumstances.

Quality of the commodity is the first requirement to sustain and boost the trade besides ensuring public health. In the present study, we could detect the presence of chilli as low as 1g in 200 g of black pepper powder. The technology developed in the study can be utilized by approved authorities/agencies for adulteration detection/authentication of spice powders. DNA barcoding can be used for routine screening of batches, allowing the immediate rejection of suspected samples or even in case of disputes arising from the quality of a lot.

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