Sequence characterized amplified region markers: A reliable tool for adulterant detection in turmeric powder

K. Dhanya, S. Syamkumar, S. Siju, B. Sasikumar *

Crop Improvement and Biotechnology Division, Indian Institute of Spices Research, Calicut, Kerala, 673 012, India

A R T I C L E   I N F O

A B S T R A C T

Turmeric powder (*Curcuma longa* L.), an important medicinal spice product traded internationally, is subjected to adulteration by design or default with powders of related curcumin containing wild species like *Curcuma zedoaria* and *Curcuma malabarica* leading to toxicity and poor quality of the produce. The present study aims at development of specific, sensitive and reproducible Sequence Characterized Amplified Region (SCAR) markers to detect these adulterants in traded turmeric powder. Two putative RAPD markers, 'Cur 01' and 'Cur 02', generated by random primers OPA 01 and OPE 18 were identified as *C. zedoaria*/*C. malabarica* specific by comparative RAPD analysis of genuine turmeric and market samples of turmeric powder, *C. zedoaria* and *C. malabarica*. These specific RAPD markers were cloned and sequenced. Two pairs of SCAR primers were designed from the RAPD markers 'Cur 01' and 'Cur 02', respectively. Six market samples of turmeric powder and four simulated standards besides the genuine samples were analyzed using the specific SCAR markers. Both the SCAR markers detected the presence of *C. zedoaria*/*C. malabarica* adulteration in four market samples and all the simulated standards prepared in different concentrations. The two SCAR markers developed in the study would be potentially useful for the regulatory agencies to detect *C. zedoaria*/*C. malabarica* adulteration in traded turmeric powder. The analytical strategy being very simple could be used for large scale screening of turmeric powder samples intended for export and domestic uses.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

Turmeric (*Curcuma longa* L. syn. *Curcuma domestica*, family Zingiberaceae) is an important spice as well as medicinal plant. It is generally used in its ground form, turmeric powder, prepared from the processed rhizomes (Bambirra, Junqueira, & Gloria, 2002). The major use of turmeric worldwide is for domestic culinary purpose. Besides its use as a spice, turmeric is now gaining importance globally as a mighty cure to combat a variety of ailments as the rhizome is credited with molecules having antiinflammatory, hypocholestremic, choleratic, antimicrobial, antirheumatic, antifibrotic, antivenomous, antiviral, antidiabetic, antihepatotoxic, anticancerous properties and insect repellent activity (Sasikumar, 2005).

Turmeric is a spice probably most subjected to adulteration since it is frequently traded in ground form (Singhal, Kulkarni, & Rege, 1997). Turmeric powder is adulterated with rhizomes of cheaply available *Curcuma* species especially with those containing the coloring pigment curcumin (Purseglove, Brown, Green, & Robin, 1981) such as *Curcuma zedoaria* (Christm.) Rosc. or 'yellow shoti' syn. *Curcuma xanthorrhiza* Roxb. 'Manjakua' and *Curcuma malabarica* Vel. et al. (Malingre, 1975;

Sasikumar, Syamkumar, Remya, & John Zachariah, 2005; Zwaving & Bos, 1992). *C. zedoaria* and *C. malabarica* are found all over India and other turmeric producing countries, mainly as wild growth and to a limited extent under cultivation. The yield levels of *C. zedoaria* and *C. malabarica* are also high compared to *C. longa* (turmeric) besides being free from any major pests. Though poor in color, (curcumin about 1–2%) the easy availability coupled with very low price would attract turmeric powder manufacturers to use *C. zedoaria*/*C. malabarica* as an adulterant of *C. longa*, irrespective of the spice/medicinal value or its toxicity. *C. zedoaria*/*C. malabarica* are reported as toxic (Lakshmi, Padmaja, & Remani, 2011; Latif, Moris, Miah, Hewitt, & Ford, 1979) and are recently suggested to be synonyms (Skornickova, Sída, & Marhold, 2010). Their contamination in turmeric powder may lead to public health hazard. The conventional analytical methods like microscopy, spectrophotometry, thin layer chromatography etc. are not helpful in identifying these adulterants, in turmeric powder. Nucleic acid analysis represents a good alternative to these conventional methods and is very useful for detecting the plant based adulterants in traded plant materials (Lum & Hirsch, 2006). Although a few reports based on molecular techniques are available for the identification of turmeric in planta (*C. longa*) from other related species (Cao, Sasaki, Fushimi, & Komatsu, 2001; Komatsu & Cao, 2003; Minami et al., 2009; Sasaki, Fushimi, & Komatsu, 2004) no reports are available on adulterant detection in marketed turmeric powder except the report of Sasikumar et al. (2005) using RAPD markers. Here, we report a more reliable and

---

sensitive PCR based method for detecting *C. zedoaria/C. malabarica* adulteration in traded turmeric powder.

2. Materials and methods

2.1. Sample materials and DNA isolation

The six popular branded market samples of turmeric powder procured from the local market at Calicut, Kerala, India were used in the study. Genuine turmeric (*C. longa* L.) and the wild *Curcuma* spp. viz., *C. zedoaria* (Christm.) Rosc. and *C. malabarica* Vel. et al. rhizomes used in the study were obtained from the germplasm collection maintained at the Indian Institute of Spices Research, Experimental Farm at Peruvannamuzhi, Calicut, Kerala, India. Model blends of turmeric and the adulterants viz, *C. zedoaria* and *C. malabarica* prepared in the proportions 99:1, 95:5, 90:10 and 80:20 (weight basis) were used as analytical standards. The popular turmeric cultivars/varieties viz., 'Alleppey', 'Amalapuram', 'Prathiba' and 'Sudarshana' collected from the Indian Institute of Spices Research Experimental Farm also served as analytical standards (reference). Powders were prepared from the boiled and dried rhizomes of the samples using Cyclotec 1093 sample mill. Genomic DNA was extracted from all the samples as per the protocol of Remya, Syamkumar, and Sasikumar (2004). Briefly, one gram of dried powder was mixed with 8 ml of extraction buffer (3% CTAB; 100 mM Tris; 20 mM EDTA; 2 M NaCl; 2% PVP) and incubated overnight with shaking. This was followed by a phenol: chloroform:isoamyl alcohol (25:24:1) and a chloroform:isoamyl alcohol (24:1) extraction step. The aqueous phase was saved and mixed with equal volume of 100% ethanol for DNA precipitation. The pellet was dried, dissolved in nuclease free water and was treated with RNase. This was further purified by several steps of phenol: chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (25:24:1) extractions until a clear aqueous phase was visible. DNA was precipitated from the saved aqueous phase using chilled 100% ethanol. The pellet was washed with 70% ethanol, dried and dissolved in nuclease free water.

2.2. RAPD-PCR

PCR amplification of the DNA was carried out in a 25 µl reaction volume containing 35 ng of DNA, 1× assay buffer (Bangalore Genei, India), 0.2 mM of dNTPs, 2 mM $MgCl_2$, 10 pmol random decamer primer and 1 U of *Taq* DNA polymerase enzyme (Bangalore Genei, India). Amplifications were performed using a thermal cycler (Eppendorf, Master Cycler Gradient S) under the following conditions: initial denaturation at 94 °C for 3 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 37 °C for 1 min, elongation at 72 °C for 1 min, and a final extension at 72 °C for 15 min. PCR products were resolved on a 1.5% [w/v] agarose gel (containing 0.5 µg ml^{-1} of ethidium bromide) electrophoresis in 1× TAE buffer along with 1 Kb DNA ladder (Biogene, USA.) as size marker. The gel was documented using a gel documentation system (Kodak MI, Gel Logic system 200).

2.3. Screening strategy and identification of RAPD amplicons

One hundred random decamer primers procured from Operon Technologies Inc., Alameda, CA, USA were screened for identifying the adulterant specific markers. The primers which gave consistent amplification pattern for genuine turmeric and the adulterants viz., *C. zedoaria* and *C. malabarica* separately were selected for subsequent amplification. Genomic DNA from six market samples of turmeric powder along with genuine turmeric and *C. zedoaria* and *C. malabarica* were amplified and resolved in the same agarose gel. Adulterant specific bands were scored on the basis of their presence in *C. zedoaria* and *C. malabarica* and their absence in genuine turmeric powder.

2.4. Cloning and sequencing of RAPD amplicons

The adulterant specific RAPD bands ('Cur 01' and 'Cur 02') produced in the market sample (sample II), genuine *C. zedoaria* and *C. malabarica* samples were excised from 1.5% agarose gel and purified using Perfectprep Gel Cleanup Kit (Eppendorf, Germany). These DNA fragments were ligated into pTZ57R/T vector system (Fermentas, USA) and transformed into competent *Escherichia coli* strain DH5α using InsTA cloning kit (Fermentas, USA) following the supplier's instructions. Recombinants were identified by blue-white screening on LB plates supplemented with X-gal, IPTG and ampicillin. Further screening was done by colony PCR, in a reaction mix containing 1× assay buffer, 0.2 mM of dNTPs, 1.5 mM $MgCl_2$, 5 pmol of each M13 forward and M13 reverse primers (IDT, USA), 1 µl of lysate and 1 U of *Taq* DNA polymerase enzyme in 25 µl total reaction volume. Thermal cycling conditions for amplification were: 94 °C for 3 min; 30 cycles at 94 °C for 1 min, 48 °C for 1 min and 72 °C for 1 min; and a final extension at 72 °C for 5 min. The amplified products were resolved on a 1.5% agarose gel. Positive transformants were identified based on the insert size and plasmid DNA was isolated from these selected clones using Fast plasmid Mini Prep kit (Eppendorf, Germany). The size of the DNA insert in the plasmids was also confirmed by restriction digestion using *Eco* RI and *Hind* III. The recombinant plasmids were sequenced at the DNA sequencing service unit of Bioseve Biotechnologies, India, using vector specific M13 universal primers.

2.5. Analysis of sequence data and SCAR primer designing

Nucleotide sequence of RAPD markers ('Cur 01' and 'Cur 02') produced in the genuine *C. zedoaria* and *C. malabarica* samples and the market sample II were compared by multiple sequence alignment using Clustal X (Thomson, Gibson, Plewniak, Jeanmougin, & Higgins, 1997) and percent identity was determined using BioEdit Sequence Alignment Editor (Hall, 1999). Nucleotide sequences were submitted to NCBI's GenBank Nucleotide Sequence Database and sequence homologies were determined by performing a nucleotide–nucleotide BLAST (blastn) search (www.ncbi.nlm.nih.gov/BLAST) within the database.

Based on the sequences of the cloned RAPD markers, 'Cur 01' (GenBank accession no. JF927292) and 'Cur 02' (GenBank accession no. JF927293), two pairs of SCAR oligonucleotide primers C1 (C1-F, C1-R) and C2 (C2-F, C2-R) were designed using the program Primer 3 (Rozen & Skaletsky, 2000) (Table 1).

2.6. Sequence specific amplification

The PCR assays for SCAR amplification were carried out in a final reaction volume of 25 µl: 1×*Taq* buffer, 2.5 pmol of each primer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 20 ng genomic DNA, and 1 U of *Taq* DNA polymerase (Sigma Aldrich, USA) with an initial denaturation for 4 min at 94 °C followed by 30 cycles of 30 s at 94 °C, 30 s at the appropriate Ta (Table 1) and 45 s at 72 °C, completed by a final extension at 72 °C for 5 min. Genomic DNA isolated from the genuine turmeric (*C. longa* L.), turmeric varieties/cultivars viz. 'Alleppey', 'Amalapuram', 'Prathiba' and 'Sudarshana', six market samples of turmeric powder, genuine samples of *C. zedoaria* and *C. malabarica* along with the model blends were analyzed using the designed SCAR primers.

3. Results

3.1. DNA isolation

High molecular weight DNA was isolated from all the samples. The yield of genomic DNA isolated from the different samples of turmeric

Table 1
C. zedoaria/C. malabarica specific SCAR primers derived from sequenced RAPD amplicons.

RAPD primer	RAPD marker	SCAR primer	Number of bases	Sequence (5′-3′)	G + C content (%)	Ta (°C)	Expected product (bp)
OPA01	'Cur 01'	C1F	18	CGACGCCTACTGTTCCTC	61.0	60	465
		C1R	20	CCAACGAAGACTTTGATAGG	45.0		
OPE18	'Cur 02'	C2F	18	GCAGAACACGATTTGAAG	44.4	62	367
		C2R	19	GCAGAGGCATTTACAACAC	47.4		

powder and their adulterants, *C. zedoaria* and *C. malabarica* ranged from 48.0 µg g^{-1} to 62.2 µg g^{-1} of dried tissue. An absorbance (A_{260}/A_{280}) ratio of 1.7–1.8 indicated insignificant levels of contaminating proteins and polysaccharides in the DNA.

3.2. RAPD analysis and identification of adulterant specific amplicons

Out of the one hundred RAPD primers screened for amplification in genuine turmeric powder (*C. longa* L.), *C. zedoaria* and *C. malabarica* separately, 35 primers gave amplification in all the three samples. These selected primers (OPA 01, OPA 03, OPA 04, OPA 07, OPA 08, OPA 10, OPA 11, OPA 12, OPA 17, OPA 18, OPA 19, OPB 04, OPB 10, OPB 15, OPB 17, OPC 10, OPC 20, OPD 03, OPD 05, OPD 07, OPD 11, OPD 13, OPD 16, OPD 18, OPD 20, OPE 02, OPE 05, OPE 06, OPE 07, OPE 11, OPE 15, OPE 16, OPE 18, OPE 19, OPJ 18) were used for subsequent amplification. The samples *C. zedoaria* and *C. malabarica* produced similar RAPD profiles with all the primers studied and hence the adulterant specific RAPD markers identified were common to both the samples. Four primers (OPA 01, OPA 12, OPE 11 and OPE 18) generated unique banding patterns which could easily distinguish the *C. zedoaria/C. malabarica* specific bands in the market samples. The primers OPA 01 (5′-CAGGCCCTTC-3′) and OPA 12 (5′-TCGGCGATAG-3′) amplified a product of ~600 bp and ~1100 bp, respectively in four market samples (sample II, sample III, sample IV and sample VI). Primer OPE 18 (5′-GGACTGCAGA-3′) produced a band of ~380 bp in three market samples (sample II, sample III and sample IV). Primer OPE 11 (5′-GAGTCTCAGG-3′) produced adulterant specific bands of ~1400 bp in market sample II, sample IV and sample VI. The putative RAPD amplicons from OPA 01 (~600 bp) (Fig. 1) and OPE 18 (~380 bp) (Fig. 2) named as 'Cur 01' and 'Cur 02', respectively were selected for *C. zedoaria/C. malabarica* specific SCAR marker development, considering their high degree of resolution.

3.3. Cloning and sequence analysis of adulterant specific RAPD amplicons

The putative RAPD markers produced in market sample II, *C. zedoaria* and *C. malabarica* were cloned, separately. Amplification with M13 forward and reverse primer yielded products of ~785 bp

and ~565 bp in all the positive clones of 'Cur 01' and 'Cur 02', respectively. These clones were selected for plasmid DNA isolation. The same size fragments corresponding to the RAPD markers were recovered from *Hind* III and *Eco* RI double digested plasmid DNA. The recombinants were sequenced. The first ten nucleotides at both ends of the sequences obtained matched completely with their corresponding RAPD primers OPA 01 and OPE 18 (Fig. 3). For both the RAPD markers 'Cur 01' and 'Cur 02', the sequence alignment data revealed 100% similarity between sequences obtained from *C. zedoaria*, *C. malabarica* and the market sample II. Nucleotide sequence of 'Cur 01' consisted of 593 bp with 40.30% G + C content (A = 174; C = 151; G = 88; T = 180). The length of the 'Cur 02' obtained was 377 bp with 36.87% G + C content (A = 111; C = 62; G = 77; T = 127). Blast results revealed that both the DNA sequences shared only partial homology with known plant nucleotide sequence.

3.4. SCAR markers for detection of adulteration in turmeric powder

The utility of the two *C. zedoaria/C. malabarica* specific SCAR primer pairs, C1 and C2, developed in the study was tested in simulated samples of turmeric powder and the adulterants made in different concentrations. Both the SCAR primer pairs, C1 and C2, produced single, distinct and brightly resolved bands of 465 bp and 367 bp, respectively in *C. zedoaria* and *C. malabarica* and in all the model blends (Figs. 4 and 5). The two SCAR primers amplified adulterant specific marker in four out of the six market samples screened for adulteration. The SCAR primer pair C1 amplified 465 bp bands in the market sample II, sample III, sample IV and sample VI (Fig. 6). SCAR primer pair C2 also amplified 367 bp bands in the same market samples (Fig. 7). The specific bands were also amplified in *C. zedoaria* and *C. malabarica* samples and were totally lacking in the pure turmeric (including the genuine cultivars/varieties viz., 'Alleppey', 'Amalapuram', 'Prathiba' and 'Sudarshana') powders studied indicating the sensitivity and specificity of the markers. Though the RAPD primer OPE 18 produced marker 'Cur 02' in only three market samples (sample II, sample III and sample IV), the SCAR primer derived from 'Cur 02' could detect adulteration in four market samples. viz., sample II, market sample III, market sample IV and
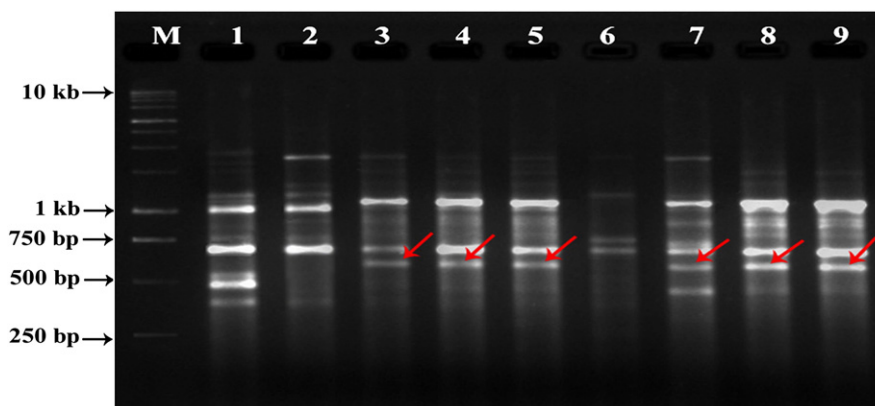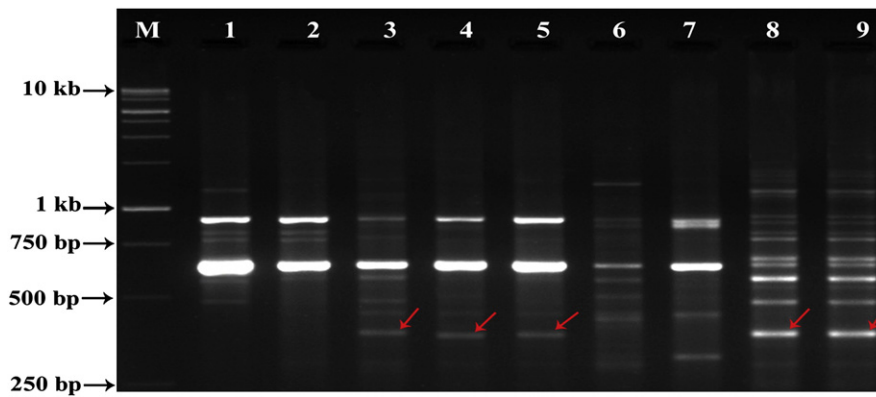

Fig. 1. RAPD profile of genuine turmeric powder, market samples of turmeric powder, *C. zedoaria* and *C. malabarica* amplified using primer OPA 01. Arrows indicate 'Cur 01' bands. M — 1 Kb DNA ladder (Biogene, USA), Lane 1 — genuine turmeric, Lane 2 — market sample I, Lane 3 — market sample II, Lane 4 — market sample III, Lane 5 — market sample IV, Lane 6 — market sample V, Lane 7 — market sample VI, Lane 8 — *C. zedoaria*, Lane 9 — *C. malabarica*.

Fig. 2. RAPD profile of genuine turmeric powder, market samples of turmeric powder, *C. zedoaria* and *C. malabarica* amplified using primer OPE 18. Arrows indicate 'Cur 02' bands. M — 1 Kb DNA ladder (Biogene, USA), Lane 1 — genuine turmeric, Lane 2 — market sample I, Lane 3 — market sample II, Lane 4 — market sample III, Lane 5 — market sample IV, Lane 6 — market sample V, Lane 7 — market sample VI, Lane 8 — *C. zedoaria*, Lane 9 — *C. malabarica*.

market sample VI again attesting the sensitivity and specificity of SCAR primers compared to RAPDs. Hence, these two SCAR primer pairs proved to be *C. zedoaria*/*C. malabarica* specific markers and can extensively be used for adulterant detection in traded turmeric powder.

4. Discussion

For determining the plant based adulterants in turmeric powder, the quality requirements set by PFA (Prevention of Food Adulteration Act, 1957, Govt. of India) such as total curcumin content and ash value are found to be insufficient. Sasikumar et al. (2005) have demonstrated that even adulterated market samples contained acceptable curcumin levels. Chemical profiling of the essential oils (Karig, 1975; Sen, Sen Gupta, & Ghosh Dastidar, 1974; Zwaving & Bos, 1992) for differentiating adulterant *Curcuma* species is also subjective as the adulterant species closely resemble the genuine material and many extrinsic factors such as methods of cultivation, harvesting, drying and storing may affect the ultimate chemical profile of turmeric.

a)

```
  1  CAGGCCCTTCTTTCCTGCATTACAAGTTGGCACAAAAATAAAATATAACAAGAAAGTAAA  60

 61  ATAGTTTAACAGTTATTTTGGTCTTGACTTTAGATTTTTAGTAACATCCTGGGTCAGACC  120

121  GACGCCTACTGTTCCTCTTACCGGGAACGCGCCCTTACCCACTCCACTCGGGAGAATTTA  180

181  CCTGTTGCCAACCAAGTCCTTCAAACTTGTTGGACTTCTACTTAGCCTGATCCTCGACCC  240

241  TCAGAATTTCTACTGCTAGACATCTGGTCCACCTAACCTACCTAGACTTCCACTGCTAGA  300

301  CATCTGGTTCACCTGACCTGCCTAATCTTCCACCTGGTGCACTTGATCTGCCAAGTCTTT  360

361  GTTCAAAGCAACATATACTAAAAGAAACATATATCAGAAATCCAACATTTGATCAAAGTG  420

421  ATTCTCATTTCAATATTATTGTAAAATAGATGTTTATACAAAGAGCAAGGCTTATAGTAT  480

481  ACATCTCAATAATCTCCCACTTATACTAAAAGACCATGCTGCTATAATTGCTGCCATACA  540

541  TCTGATTCTCATCTCCTTCACATGCCTATCAAAGTCTTCGTTGGAAGGGCCTG         593
```

b)

```
  1  GGACTGCAGAACACGATTTGAAGTGAATAGGAAAAGACAACTACATGCTTGTCTTAGCCG  60

 61  ATTTAGAACCTGTAATTGAATGCTGGGACACTACAGCCCCAGACTTTTAGGCGGTTGATT  120

121  TAGAGGTTGAAGGTGTCCAATGTACAAATCTGCAACTTCAGAATATTTTTTCTGATTTTG  180

181  TAGTTGCTAGATGATAAATAAGCAAGTCTGGCTATAGGAAGACCTGGGAATGTAATCTTT  240

241  GTGGCGATGAATGCCCATAATATTATTATCTACCATTATTTTGACGTGTCATTTGTTTAT  300

301  AATTTTCTCTAACATCTTGAGCATTTGCCATTATTAAACATAGAATGATTTCAGTGTTGT  360

361  AAATGCCTCTGCAGTCC                                              377
```

Fig. 3. Nucleotide sequences of RAPD amplicons 'Cur 01' [a] and 'Cur 02' [b] cloned from the market sample II.
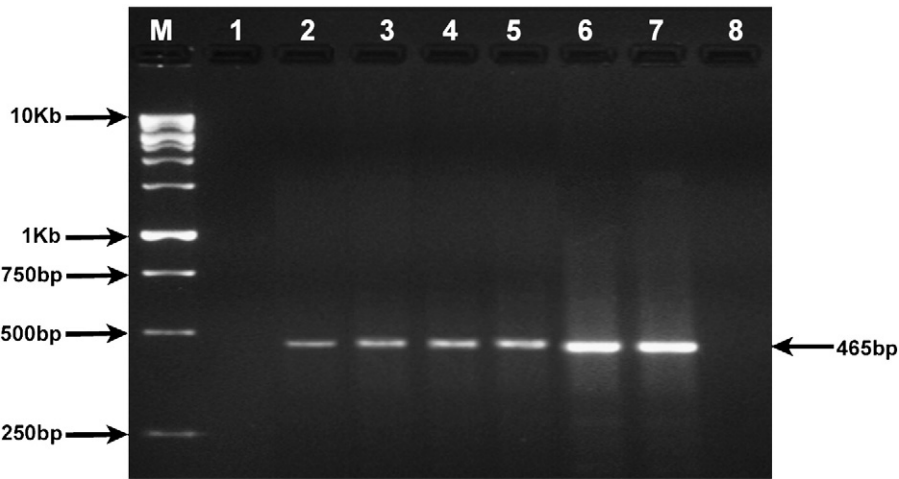
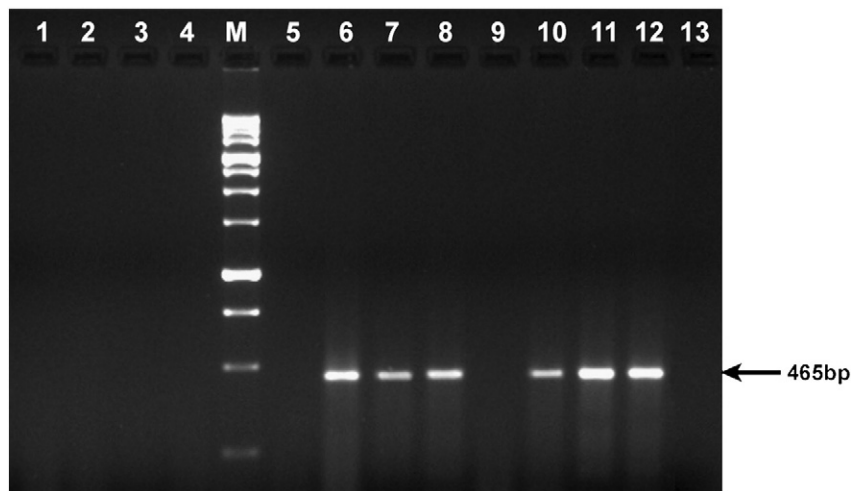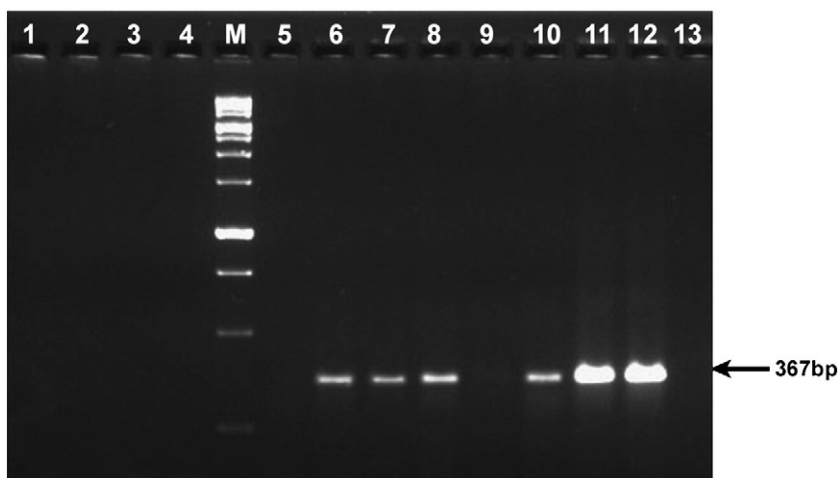**Fig. 4.** PCR amplification of *C. zedoaria*/*C. malabarica* specific SCAR primer pair C1 (C1 F, C1 R) in model blends of turmeric and *C. zedoaria*/*C. malabarica* made in different concentrations. M-1 Kb DNA ladder (Biogene, USA), Lane 1 — genuine turmeric, Lane 2 — simulated sample [turmeric: *C. zedoaria*/*C. malabarica* (99:1)], Lane 3 — simulated sample [turmeric: *C. zedoaria*/*C. malabarica* (95:5)], Lane 4 — simulated sample [turmeric: *C. zedoaria*/*C. malabarica* (90:10)], Lane 5 — simulated sample [turmeric: *C. zedoaria*/*C. malabarica* (80:20)], Lane 6 — *C. zedoaria*, Lane 7 — *C. malabarica* and Lane 8 — negative control (reaction mix without DNA).
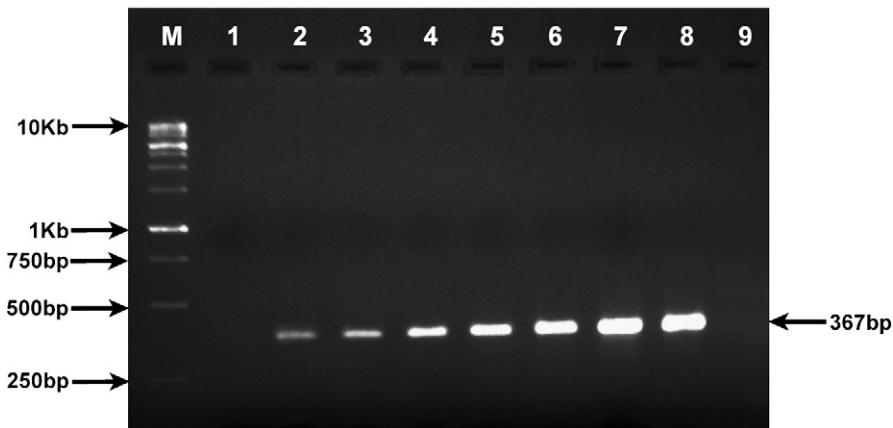
Fig. 5. PCR amplification of *C. zedoaria*/*C. malabarica* specific SCAR primer pair C2 (C2 F, C2 R) in model blends of turmeric and *C. zedoaria*/*C. malabarica* made in different concentrations. M-1 Kb DNA ladder (Biogene, USA), Lane 1 — genuine turmeric, Lane 2 — simulated sample [turmeric: *C. zedoaria*/*C. malabarica* (99:1)], Lane 3 — simulated sample [turmeric: *C. zedoaria*/*C. malabarica* (95:5)], Lane 4 — simulated sample [turmeric: *C. zedoaria*/*C. malabarica* (90:10)], Lane 5 — simulated sample [turmeric: *C. zedoaria*/*C. malabarica* (80:20)], Lane 6 — *C. zedoaria*, Lane 7 — *C. malabarica* and Lane 8 — negative control (reaction mix without DNA).

Fig. 6. PCR amplification of *C. zedoaria*/*C. malabarica* specific SCAR primer pair C1 (C1 F, C1 R) in pure turmeric, market samples of turmeric powder and *C. zedoaria* and *C. malabarica*. Lane 1–'Alleppey', Lane 2–'Amalapuram', Lane 3–'Prathiba', Lane 4–'Sudarshana', Lane 5—market sample I, Lane 6—market sample II, Lane 7—market sample III, Lane 8—market sample IV, Lane 9—market sample V, Lane 10—market sample VI, Lane 11—*C. zedoaria*, Lane 12—*C. malabarica*, Lane 13—negative control (reaction mix without DNA) and M-1 Kb DNA ladder (Biogene, USA).

Fig. 7. PCR amplification of *C. zedoaria/C. malabarica* specific SCAR primer pair C2 (C2 F, C2 R) in pure turmeric, market samples of turmeric powder and *C. zedoaria* and *C. malabarica*. Lane 1—'Alleppey', Lane 2—'Amalapuram', Lane 3—'Prathiba', Lane 4—'Sudarshana', Lane 5—market sample I, Lane 6—market sample II, Lane 7—market sample III, Lane 8—market sample IV, Lane 9—market sample V, Lane 10—market sample VI, Lane 11—*C. zedoaria*, Lane 12—*C. malabarica*, Lane 13—negative control (reaction mix without DNA) and M-1 Kb DNA ladder (Biogene, USA).

Though many workers have demonstrated the efficacy of molecular markers for identifying turmeric adulteration in planta (single nucleotide polymorphism developed by Cao et al. (2001), Komatsu and Cao (2003), Sasaki et al. (2004) and Minami et al. (2009)), the suitability of these methods to detect adulteration in traded turmeric powder is yet to be demonstrated.

Though the RAPD markers analysis could reveal a high degree of polymorphism (Williams, Kubelik, Livak, Rafalski, & Tingey, 1990) their lack of reproducibility and lower annealing temperature may reduce the specificity of the reaction (Macpherson, Eckstein, Scoles, & Gajadhar, 1993). Conversion of RAPD marker to SCAR markers is advantageous as SCAR primers amplify the characterized regions from genomic DNA under stringent conditions, which makes these markers more specific and dependable as compared to RAPD markers (Paran & Michelmore, 1993). The two sets of SCAR primers C1 and C2 developed from RAPD markers in the present study gave specific amplification of adulterant DNA and were found useful for determining adulteration in turmeric powder. RAPD derived SCAR markers are reported to be successfully utilized in the differentiation of plant materials such as *Panax quinquefolius*, *Sinocalycanthus chinensis*, *Artemisia princeps*, *Artemisia argyi*, *Zingiber officinale*, *Lycium barbarum*, *Phyllanthus sps.*, etc. from their adulterants (Chavan, Warude, Joshi, & Patwardhan, 2008; Jain, Shasany, Singh, Khanuja, & Kumar, 2008; Lee et al., 2006; Qian et al., 2006; Sze et al., 2008; Wang, Ha, Ngan, But, & Shaw, 2001). The present study takes into account the detection of adulterants not only in the laboratory artificial mixtures (model blends) but also in the dried and powdered market samples. This demonstrates the applicability of the developed SCAR markers to samples in which DNA might be subject to alterations due to treatment and storage conditions. The applications of SCAR markers for adulterant detection in market samples have also been demonstrated in our previous studies on spices such as black pepper and chili (Dhanya, Syamkumar, Siju, & Sasikumar, 2011; Dhanya, Syamkumar, & Sasikumar, 2009) as well as in Meditteranean oregano by Marieschi, Torelli, Bianchi, and Bruni (2011, 2010); Marieschi, Torelli, Poli, Bianchi, and Bruni (2010).

The PCR method described in the present study is quick and reliable to the already reported molecular methods for detecting adulteration in turmeric powder. The SCAR markers C1 and C2 could successfully detect *C. zedoaria/C. malabarica* adulteration in four out of six market samples used in the study. In addition to this, the efficiency of the SCAR markers developed for detecting adulteration even at lower concentrations (10 g adulterant kg^{-1} of turmeric powder) will substantiate the applicability of the marker as a qualitative diagnostic tool for detection of plant based adulterants in turmeric powder. This work is relevant in the context of the Sanitary and Phytosanitary issues of the WTO agreement as turmeric powder traded globally as a spice and medicine. The technology developed in this study can be utilized to distinguish the adulterant of *C. zedoaria/C. malabarica* in turmeric powders thereby ensuring the quality of the products.

References

Bambirra, M. L. A., Junqueira, R. G., & Gloria, M. B. A. (2002). Influence of post harvest processing conditions of yield and quality of ground turmeric (*Curcuma longa* L.). *Brazilian Archives of Biology and Technology*, *45*, 423–429.

Cao, H., Sasaki, Y., Fushimi, H., & Komatsu, K. (2001). Molecular analysis of medicinally used Chinese and Japanese *Curcuma* based on 18S rRNA gene and trnK gene sequences. *Biological and Pharmaceutical Bulletin*, *24*, 1389–1394.

Chavan, P., Warude, D., Joshi, K., & Patwardhan, B. (2008). Development of SCAR (sequence-characterized amplified region) markers as a complementary tool for identification of ginger (*Zingiber officinale* Roscoe) from crude drugs and multi-component formulations. *Biotechnology and Applied Biochemistry*, *50*, 61–69.

Dhanya, K., Syamkumar, S., & Sasikumar, B. (2009). Development and application of SCAR marker for the detection of papaya seed adulteration in traded black pepper powder. *Food Biotechnology*, *23*, 97–106.

Dhanya, K., Syamkumar, S., Siju, S., & Sasikumar, B. (2011). SCAR Markers for adulterant detection in ground chilli. *British Food Journal*, *113*, 656–668.

Hall, T. A. (1999). BioEdit: A user friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symposium Series*, *4*, 95–98.

Jain, N., Shasany, A. K., Singh, S., Khanuja, S. P. S., & Kumar, S. (2008). SCAR markers for correct identification of *Phyllanthus amarus*, *P. fraternus*, *P. debilis* and *P. urinaria* used in scientific investigations and dry leaf bulk herb trade. *Planta Medica*, *74*, 1–6.

Karig, F. (1975). Schnelle Kennzeichnung von Curcuma-rhizomen mit dem TAS-vertahren. *Deutsche Apotheker-Zeitung*, *115*, 325–328.

Komatsu, K., & Cao, H. (2003). Molecular identification of six medicinal *Curcuma* plants produced in Sichuan: Evidence from plastid trnK gene sequences. *Yao Xue Xue Bao*, *38*, 871–875.

Lakshmi, S., Padmaja, G., & Remani, P. (2011). Antitumour effects of Isocurcumenol isolated from *Curcuma zedoaria* rhizomes on human and murine cancer cells. *International Journal of Medicinal Chemistry*, doi:10.1155/2011/253962.

Latif, M. A., Moris, T. R., Miah, A. M., Hewitt, D., & Ford, J. E. (1979). Toxicity of 'shoti' (Indian arrowroot: *Curcuma zedoaria*) for rats and chicks. *British Journal of Nutrition*, *41*, 57–63.

Lee, M. Y., Doh, E. J., Park, C. H., Kim, Y. H., Kim, E. S., Ko, B. S., et al. (2006). Development of SCAR marker for discrimination of *Artemisia princeps* and *A. argyi* from other *Artemisia* herbs. *Biological and Pharmaceutical Bulletin*, *29*, 629.

Lum, M. R., & Hirsch, A. M. (2006). Molecular methods for the authentication of botanicals and detection of potential contaminants and adulterants. *Acta Horticulturae, 720*, 59–72.

Macpherson, J. M., Eckstein, P. E., Scoles, G. J., & Gajadhar, A. A. (1993). Variability of the random amplified polymorphic DNA assay among thermal cyclers and effects of primer and DNA concentration. *Molecular and Cellular Probes, 7*, 293–299.

Malingre, T. M. (1975). *Curcuma xanthorrhiza* Roxb., temoelawak,als plant met galdrijvende werking. *Pharmaceutics Weekblad, 110*, 601–610.

Marieschi, M., Torelli, A., Bianchi, A., & Bruni, R. (2011). Development of a SCAR marker for the identification of *Olea europaea* L.: A newly detected adulterant in commercial Mediterranean oregano. *Food Chemistry, 126*, 705–709.

Marieschi, M., Torelli, A., Poli, F., Bianchi, A., & Bruni, R. (2010). Quality control of commercial Mediterranean oregano: Development of SCAR markers for the detection of the adulterants *Cistus incanus* L., *Rubus caesius* L. and *Rhus coriaria* L.. *Food Control, 21*, 998–1003.

Minami, M., Nishio, K., Ajioka, Y., Kyushima, H., Shigeki, K., Kinjo, K., et al. (2009). Identification of *Curcuma* plants and curcumin content level by DNA polymorphisms in the trnS-trnfM intergenic spacer in chloroplast DNA. *Journal of Natural Medicines, 63*, 75–79.

Paran, I., & Michelmore, R. W. (1993). Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theoretical and Applied Genetics, 85*, 985–993.

Purseglove, J. W., Brown, E. G., Green, C. L., & Robin, S. R. J. (1981). Turmeric. *Spices, Vol. II*. (pp. 532–580)New York: Longman Publishing Group.

Qian, Y. E., Ying-xiong, Q., Yan-qi, Q., Jian-xin, C., Shu-zhen, Y., Ming-shui, Z., et al. (2006). Species-specific SCAR markers for authentication of *Sinocalycnthus chinensis*. *Journal of Zhejiang University Science, 7*, 868–872.

Remya, R., Syamkumar, S., & Sasikumar, B. (2004). Isolation and amplification of DNA from turmeric powder. *British Food Journal, 106*, 673–678.

Rozen, S., & Skaletsky, H. J. (2000). Primer3 on WWW for general users and for biologist programmers. In S. Krawetz, & S. Misener (Eds.), *Bioinformatics methods and protocols: Methods in molecular biology* (pp. 365–386). New Jersy: Humana Press.

Sasaki, Y., Fushimi, H., & Komatsu, K. (2004). Application of single-nucleotide polymorphism analysis of the *trn*K gene to the identification of *Curcuma* plants. *Biological and Pharmaceutical Bulletin, 27*, 144–146.

Sasikumar, B. (2005). Genetic resources of *Curcuma*: Diversity, characterisation and utilization. *Plant genetic resources—Characterisation and utilisation, 3*, 230–251.

Sasikumar, B., Syamkumar, S., Remya, R., & John Zachariah, T. (2005). PCR based detection of adulteration in the market samples of turmeric powder. *Food Biotechnology, 18*, 299–306.

Sen, A. R., Sen Gupta, P., & Ghosh Dastidar, N. (1974). Detection of *Curcuma zedoaria* and *C. aromatica* in *C. longa* (turmeric) by thin layer chromatography. *Analyst, 99*, 153–155.

Singhal, R. S., Kulkarni, P. R., & Rege, D. V. (1997). Handbook of indices of food quality and authenticity. Cambridge: Woodhead Publishing Limited.

Skornickova, J. L., Sída, O., & Marhold, K. (2010). Back to types! Towards stability of names in Indian *Curcuma* L. (Zingiberaceae). *Taxon, 59*, 269–282.

Sze, S. C., Song, J. X., Wong, R. N., Feng, Y. B., Ng, T. B., Tong, Y., et al. (2008). Application of SCAR (sequence characterized amplified region) analysis to authenticate *Lycium barbarum* (wolfberry) and its adulterants. *Biotechnology and Applied Biochemistry, 51*, 15–21.

Thomson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research, 24*, 4876–4882.

Wang, J., Ha, W. Y., Ngan, F. N., But, P. P. H., & Shaw, P. C. (2001). Application of sequence characterized amplified region (SCAR) analysis to authenticate *Panax* species and their adulterants. *Planta Medica, 67*, 781–783.

Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research, 18*, 6531–6535.

Zwaving, J. H., & Bos, R. (1992). Analysis of the essential oils of five *Curcuma* species. *Flavour and Fragrance Journal, 7*, 19–22.