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Development of Sequence Characterized Amplified Region (SCAR) Marker for the Authentication of *Bacopa monnieri* (L.) Wettst

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Research Article

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ABSTRACT

Aims: To develop Sequence Characterized Amplified Region (SCAR) marker for identification of *Bacopa monnieri* (L.) Wettst.
Study design: Molecular biology tools for authentic identification of *Bacopa monnieri*.
Methodology: RAPD-based SCAR marker was developed to identify *Bacopa monnieri* from its adulterant candidates namely *Centella asiatica*, *Eclipta alba* and *Malva rotundifolia*. 50 random primers were used for initial screening of different accessions of *Bacopa monnieri, Eclipta alba and Malva rotundifolia.* A putative 589 bp marker specific to *Bacopa monnieri* was identified using RAPD technique. This RAPD-amplicon was then sequenced and cloned. Based on the information of cloned sequences a pair of SCAR primers was designed. SCAR primers were then used for authentication of DNA samples of *Bacopa monnieri* and its adulterants. Market samples of *Bacopa monnieri* and *Centella asiatica* collected under the name of Brahmi was put to test with these primers.
Results: Out of 50 random primers, only 14 primers were able to amplify the above plants. A 589 bp polymorphic band obtained with OPAA-3 primer which was specific to *Bacopa monnieri* accessions and not found in other adulterant candidates was selected. This band was eluted, cloned and further sequenced. A pair of SCAR primers (Bac F & Bac R) between 406 bp of 589 bp sequence of RAPD amplicon was designed. A single, bright, distinct band was obtained in *Bacopa monnieri* and not in the adulterants. Further validation was also done in the market samples.
Conclusion: In essence, the study was to develop a RAPD-based SCAR marker for authentication of *Bacopa monnieri*. The SCAR marker was found to be useful for

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preventing the adulteration of other plants in Brahmi and also for screening of crude drug samples intended for export and domestic uses.

Keywords: Bacopa monnieri (L.) Wettst; brahmi; adulteration; molecular authentication; sequence characterized amplification region (SCAR).

1. INTRODUCTION

Bacopa monnieri (L.) Wettst, 'Brahmi', belonging to the family Schrophulariaceae, is a small prostrate herb that grows wild in marshy and damp places near water logs. It is used in traditional medicine to treat various nervous disorders (Mathew et al., 2009). This plant also possesses antiepileptic, antipyretic, analgesic, anti-inflammatory, epilepsy, insanity and anticancer activities (Elangovan et al., 1995; Tripathi et al., 1996; Vohora et al., 1997). It is used for cognitive impairment, thus used as cure of Alzheimer's disease (Dhansekaran et al., 2007; Stough et al., 2008). *Bacopa monnieri* was placed second in the priority list of most important medicinal plant in a sector study of Export–Import bank of India, evaluated on the basis of their medicinal importance, commercial value and potential for further research and development. The medicinal properties of *Bacopa* have been mainly attributed by the presence of different types of saponins such as bacosides A, B, C and D. These are the 'memory chemicals' responsible for cognitive effect and are basically triterpinoids (Rastogi et al., 1994; Singh et al., 1997). *Centella asiatica*, *Eclipta alba* and *Malva rotundifolia* are the common adulterants sold in the name of Brahmi. *Eclipta alba* and *Malva rotundifolia* are basically added to increase its concentration. Since, these drugs are marketed in the form of dried leaves and crude powders, the problem of adulteration is quite common (Zhang et al., 2009). All the above plants belong to different families, *Bacopa monnieri* (Schrophulariaceae), *Centella asiatica* (Apiaceae), *Eclipta alba* (Asteraceae) and *Malva rotundifolia* (Malvaceae) and thus have different constituents. Since the quality and therapeutic effect of herbal medicines are closely related to the authentic identification of species, it is necessary to find a way to distinguish genuine herbal medicine.

Precise identification of crude drugs is essential for the standardization of clinical prescriptions and research of herbal medicines. Subjective methods based on the morphological features such as shape, color, texture, and odor were used for the discrimination of herbal medicines in traditional system. However, it is often difficult to accurately identify medicinal plants from wild populations, or to differentiate species within the same genus based on this subjective evaluation. Furthermore, the use of chromatographic techniques and marker compounds to standardize herbal medicines is also limited because of variable chemical complexity, which is affected by growth, storage conditions, harvest times, and variable sources (Joshi et al., 2004; Zhang et al., 2007). Genetic tools that use hybridization, polymerase chain reaction (PCR), and sequencing techniques provide more objective and reliable methods for identification of herbal medicines (Zhang et al., 2007; Shcher and Carles 2008; Collard et al., 2009). Randomly Amplified Polymorphic DNA (RAPD) analysis has become one of the most effective methods for estimating genetic diversity in plant populations or cultivars because it can reveal high levels of polymorphism. RAPD also has many advantages, such as its high speed, low cost, and requirement of minute amounts of plant material (Williams et al., 1990; Penner et al., 1993). RAPD analysis has been applied in herbal medicine to discriminate between species in various genera (Shcher and Carles, 2008). However, it is less reproducible than other

methods (Hosokawa et al., 2000; Shcher and Carles, 2008). It has other disadvantages as well such as homoplasy, non-homology, nested priming, hetero-duplex formation, collision, non-independence, artefactual segregation which lowers it reliability (Bussel et al., 2005). Paran and Michelmore (1993) used RAPD analysis to develop Sequence Characterized Amplified Region (SCAR) markers, a more accurate and reliable technique to avoid the above problems of RAPD. This technique can be used to develop markers that authenticate herbal medicines by using specific PCR primers derived from RAPD or AFLP fragments. These specific primers result in amplification of products from given samples, and can be used to generate unique amplification products from closely-related samples (Wang et al., 2001; Lee et al., 2006).

In this study, we have developed reliable RAPD-based SCAR marker for the identification of *Bacopa monnieri*. This marker is useful to distinguish *Bacopa monnieri* from *Centella asiatica* and other candidate species namely *Eclipta alba* and *Malva rotundifolia*.

2. MATERIALS AND METHODS

2.1 Plant Materials

Authentic saplings of *Bacopa monnieri* were procured from different part of India, viz., central (Delhi), southern (Banglore and Chennai) and northern (Lucknow and Guwahati). Plant samples of *Centella asiatica, Malva rotundifolia* and *Eclipta alba* were also collected from Delhi and Noida region (Table 1a, b). The saplings were grown in the Herbal Garden of Hamdard University, New Delhi. Fresh leaf samples were collected from live plants, frozen in liquid nitrogen and stored at –80°C. Commercially marketed crude drug samples of *Bacopa monnieri* and *Centella asiatica* were also collected from Delhi market and identification was done on the basis of packed information (Table 2).

Table 1a. Plant materials of *Bacopa monnieri* collected from different regions

Plant name	Sample code	Source	Morphological features	Date of collection
Bacopa monnieri	B1	Delhi	branches *se*, stem *lg*, leaves *g, obl*, corolla *eq*, *v*	2008-10-26
	B2	Banglore	branches *e*, stem *pg*, leaves *g*, *obl*, corolla *ue*, *v*	2008-10-30
	B3	Chennai	branches *e*, stem *lg*, leaves *g*, *obl*, corolla *ue*, *pv*	2009-01-14
	B4	Lucknow	branches *se*, stem *pg*, leaves *pg*, *ob*, corolla *ue*, *pv*	2009-03-12
	B5	Guwahati	branches *se*, stem *pg*, leaves *g*, *ob*, corolla *eq*, *pv*	2009-07-07

Abbreviations used for descriptors are: e=erect, se=suberect, g=green, lg =light green, pg=pale green, ob=obovate, obl=oblanceolate, ue=unequal, eq=equal, v=violet, pv=purple violet. Plant materials were verified by taxonomist, Dr. HB Singh (NISCAIR)

Table 1b. Adulterant Plants collected from different regions

Plant name	Sample code	Source	Morphological features	Date of collection
Centella asiatica	C1	Delhi	stem *sl*, stolon *pr*, leaves *ren*, *g*, *ov*, corolla *p*	2008-11-18
	C2	Lucknow	stem *sl*, stolon *spr*, leaves *ren*, *pg*, *orb*, corolla *p*	2008-12-22
	C3	Noida	stem *sl*, stolon *pr*, leaves *ren*, *g*, *ov*, corolla *p*	2009-02-11
	C4	Banglore	stem *sl*, stolon *spr*, leaves *ren*, *pg*, *ov*, corolla *pp*	2009-04-25
Eclipta alba	E1	Delhi	stem *e*, *r*, leaves *ser*, *g*, flower *w*	2010-08-09
	E2	Noida	stem *e*, *r* ,leaves *ser*, *pg*, flower *w*	2010-08-24
Malva rotundifolia	M1	Delhi	stem *sl*, leaves *pal*, flower *w*	2010-09-10
	M2	Noida	stem *sl*, leaves *pal*, flower *w*	2010-09-26

Abbreviations used for descriptors: sl= slender, e=erect, pr=prostrate, spr=semiprostrate, r=round, ren=renniform, ser=serrate, pal=plamate, ov=ovate, orb=orbicular, g=green, pg=pale green, w=white, p=pink, pp=purple

Table 2. Crude drug samples of 'Brahmi' collected from market, New Delhi

No.	Trade name	Market Samples	Products form	Scientific name	Date of collection
1	BRAHMI	S1	Tablets	*Bacopa monnieri*	2009-05-22
2	BRAHMI	S2	Leaf powder (fine)	*Bacopa monnieri*	2009-07-08
3	BRAHMI	S3	Leaf powder (coarse)	*Centella asiatica*	2009-02-11
4	BRAHMI	S4	Leaf powder (coarse)	*Bacopa monnieri*	2010-01-05
5	BRAHMI	S5	Leaf powder (fine)	*Centella asiatica*	2010-02-24
6	BRAHMI	S6	Leaf powder (coarse)	*Bacopa monnieri*	2010-03-08
7	BRAHMI	S7	Leaf powder (fine)	*Bacopa monnieri*	2010-01-01
8	BRAHMI	S8	Leaf powder (fine)	*Centella asiatica*	2010-04-12

2.2 Isolation of Genomic DNA

Genomic DNA of frozen leaf samples was isolated by modified cetyl trimethyl ammonium bromide (CTAB) extraction method of Doyle and Doyle (1990). 1 g of leaf sample along with polyvinylpyrrolidone was grinded in liquid nitrogen to fine powder. 5 ml of 1% CTAB (100 mM Tris-HCl buffer pH 8.0, 1.4 M NaCl, 20 mM, EDTA, 1% mercaptoethanol) buffer was added to the homogenate, and centrifuged at 3200 × g for about 5 min. The aqueous phase was collected and added 2 vol. 2% CTAB (100 mM Tris-HCl buffer, 1.4 M NaCl, 20 mM EDTA) to it. This mixture was incubated at 60°C for about 45 min with intermittent shaking. The suspension was then cooled to room temperature and equal volume of chloroform and isoamyl alcohol (24:1) was added. The mixture was then centrifuged at 13,000 × g for 10 min. The aqueous phase was taken, and to it was added 0.6 volume of cold isopropanol and 1/30 volume of sodium acetate (3 M, pH 5.2) and incubated at –20°C for 1 h. The sample

was centrifuged at 13,000 × g for 10 min to obtain DNA pellet. The pellet was washed with 80% ethanol twice, air dried and dissolved in TE buffer (10 mM Tris buffer, pH 8.0, 1 mM Na_2EDTA). The isolated DNA was treated with RNase A (10µg/ml) at 37℃ for 30 min. DNA concentration and purity were determined by using Nano drop spectrophotometer (Thermo scientific, USA). The quality of the DNA was also determined using agarose gel electrophoresis stained with ethidium bromide.

2.3 PCR Amplification

The PCR amplification was performed according to the method developed by McClelland et al. (1995). PCR reactions were carried out in 25 µl reaction tubes, using 50 random 10-mer primers of Operon technologies Inc., USA) and Banglore Genei (India) (Table 3). PCR mixtures contained PCR buffer (Promega; 20 mM Tris–HCl (pH8.4), 50 mM KCl), 1.5 mM $MgCl_2$, 300 µM of dNTPs, 0.5 U of Taq polymerase (Promega), and 25 pmol primers. Amplification was performed in a thermal cycler (MasterCycler, Eppendorf, USA). Each reaction tube contained 30 ng DNA template. A standard PCR cycle was used: an initial denaturation step at 95℃ for 2 min, followed by 45 cycles of 95℃ for 1 min, 50℃ for 1 min, and 72℃ for 2 min; the final extension was held for 5 min. PCR products were resolved on 1.2% agarose gel in 1×TAE buffer along with standard 100-bp ladder. The agarose gel containing 0.5 µg/ml ethidium bromide was visualized under UV light on a transilluminator (UVitech, USA), and photograph was taken on gel documentation system (UVitech, USA).

2.4 Cloning and Sequencing of Specific RAPD Fragment

The 589-bp polymorphic band was excised from gel and eluted using a Gel Extraction Kit (Qiagen, Germany). The eluted DNA was cloned into pGEM®-T easy vector (Promega, USA) following the manufacturer's instruction. The ligated plasmid was introduced into *Escherichia coli* strain $DH_{5\alpha}$, following the protocols for preparing competent cells and transformation using the calcium chloride method Saad et al. (2006). White colonies were picked from LB-X-gal plates and grown overnight in LB medium containing ampicilin. The plasmid DNA was isolated from the bacterial culture using plasmid isolation kit (Qiagen, Germany). The inserted fragment was sequenced at the Center for Genomic Application, New Delhi, India with SP6 and T7 primer. Nucleotide sequence of 589 bp RAPD amplicon, specific for all the five accessions of *Bacopa monnieri,* was used for designing primers for development of SCAR marker.

2.5 Analysis of Sequence Data

The DNA sequence was submitted to Gene Bank. Homology searches were performed within GenBank's non-redundant database using the BLAST 2.2.8 algorithm at http://www.ncbi.nlm.nih.gov/BLAST of the National Center for Biotechnology Information (NCBI), with the programme BLASTX.

2.6 SCAR Primer Designing and Validation

A pair of SCAR primers (Bac F & Bac R) between 406 bp of 589 bp sequence of RAPD amplicon was designed. The melting temperature, GC contents and secondary structures of each primer were analysed. The designed SCAR primers (Bac F & Bac R) were 23 bases and 25 bases, respectively (Table 4). PCR conditions for the amplification of genomic DNA of *Bacopa* by SCAR primers were standardized. Annealing temperature (60℃) was

standardized by using gradient PCR, G-storm (UK). Genomic DNA of plant samples were amplified in a thermal cycler (MasterCycler, Eppendorf, USA) with the reaction conditions. SCAR reaction used: an initial denaturation step at 95℃ for 2 min, followed by 40 cycles of 94℃ for 20 sec, 60℃ for 40 sec, and 72℃ for 30 sec; the final extension was held for 5 min. The amplified SCAR product was resolved on 1.5% agarose gel in 1×TAE buffer along with standard 100-bp ladder. The agarose gel containing 0.5 µg/ml ethidium bromide was visualized under UV light on a transilliminator (UVitech, USA), and photograph was taken on gel documentation system (UVitech, USA).

Genomic DNA of *Centella asiatica, Eclipta alba* and *Malva rotundifolia* was isolated and amplified using SCAR primers in a thermal cycler (MasterCycler, Eppendorf, USA) following the same conditions as for *Bacopa monnieri*. Commercial crude drug samples of Brahmi were collected from the market place, New Delhi. DNA was isolated from the above samples using the above isolation method. The gene pool of the samples was then put to test with the above SCAR primers for validation.

Table 3. Nucleotide sequences of selected primers with the number of amplified products and fragment size range (bp)

Primer Code	Primer sequence (5'–3')	No. of amplified products	Fragment size (bp)
OPAA-1	AGACGGCTCC	35	190-868
OPAA-2	GAGACCAGAC	32	222-733
OPAA-3	TTAGCGCCCC	33	215-910
OPAA-4	AGGACTGCTC	40	123-817
OPAA-7	CTACGCTCAC	47	160-964
OPAA-9	AGATGGGCAG	36	291-890
OPAA-10	TGGTCGGGTG	31	113-750
OPAA-14	AACGGGCCAA	24	329-686
OPAA-15	ACGGAAGCCC	35	113-790
BG26	AAGCCTCGTC	43	154-927
BG27	TGCGTGCTTG	48	102-845
BG28	GACGGATCAG	34	175-840
BG29	CACACTCCAG	41	104-840
BG30	TGAGTGGGTG	36	145-836

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 *Bacopa monnieri* and their adulterants, ranged from 50.0 µg g/l to 65.2 µg g/l of fresh 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 Specific Amplicon

RAPD method was performed in search of DNA polymorphism, which can be used for generating informative SCAR marker. 50 RAPD 10-mer primers were screened for amplification of all the accessions of the genuine plant (*Bacopa monnieri*) and its adulterants. Selected primers (OPA 01, OPA 02, OPA 04, OPA 07, OPA 09, OPA 11, OPA 12, OPA 13, OPA 14, OPA 15, Bg 26, Bg27, Bg28, Bg29, and Bg30) produced good quality, reproducible fingerprint patterns and showed a high level of consistency. A total of 515 DNA fragments of 102-910 bp were obtained (Table 3). Confirmation of results was done using three different samples of each accession. Experiment was repeated three times to confirm reproducibility of RAPD pattern under the same conditions of composition of reaction volume, amplification profile and thermal cycler. The putative RAPD amplicon (589 bp) from OPAA-3 was selected for marker development, considering high degree of resolution (Fig. 1).

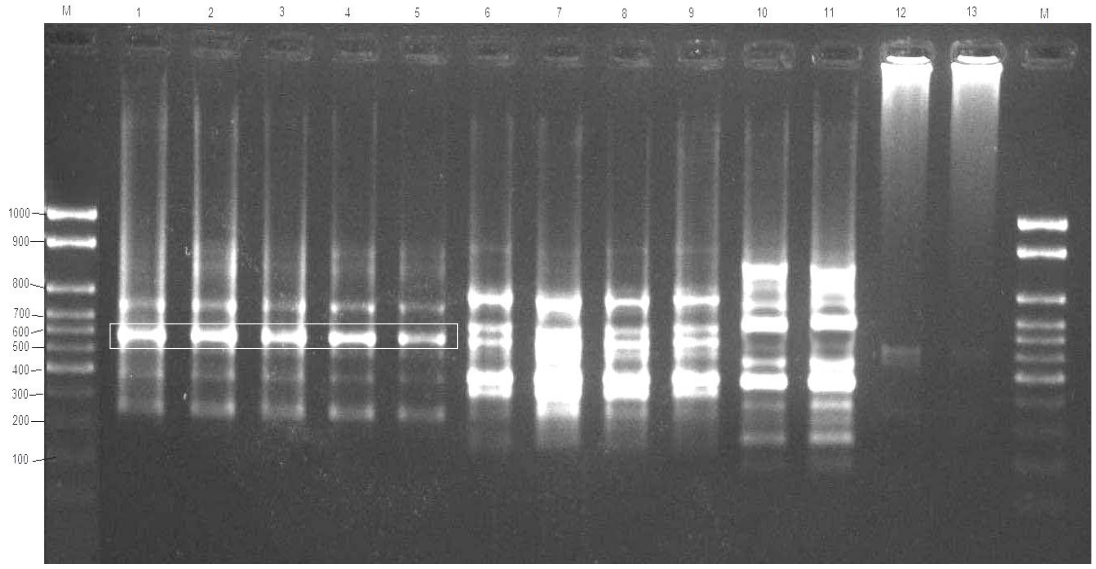

Fig. 1 RAPD pattern with OPAA-3 primer

M: DNA marker; L1−L5: samples of Bacopa monnieri; L6−L9: samples of Centella asiatica; L10−L11: samples of Eclipta alba; L12–L13: samples of Malva rotundifolia.
Collection localities are shown in Table 1.

3.3 Cloning and Sequence Data Analysis

A distinct and bright band of (589 bp), which is specific to all five accessions of *Bacopa monnieri* and which was absent in its adulterants was selected from the RAPD profile. This band was eluted, cloned and sequenced. Restriction digestion analysis using restriction enzymes *EcoR1* revealed a band of ~590 bp on 1.2% agarose gel confirmed presence of insert in the vector. The recombinant was sequenced using primers SP6 and T7. The DNA sequence was submitted to the gene bank (Accession no: JQ429286). The length of Bac 01 marker sequence obtained was 589 with 43.1% G+C content (A=160; T=174; G=111; C=143). BLAST results revealed that the sequence did not have any homology with any known nucleotide sequences (Fig. 2).

3.4 Amplification using SCAR Primers

Based on sequences of this RAPD amplicon, a pair of SCAR oligonucleotide primers (Bac F and Bac R), which could amplify 406 bp of genomic DNA of *B. monnieri* was designed. Bac F (23-mer) was designed as the forward primer and Bac R (25-mer) as reverse primer (Table 4). The sequences were custom-synthesized from IDT, New Delhi (India). A pair of SCAR primers was used for PCR amplifications of genomic DNA from *B. monnieri*, *Centella asiatica*, *Eclipta alba* and *Malva rotundifolia* under same amplification conditions. A single, distinct and brightly resolved band of 406 bp was obtained with DNA isolated from *B. monnieri* only, and no DNA fragment was obtained from the genomic DNA of *Centella asiatica*, *Eclipta alba* and *Malva rotundifolia* (Fig. 3, 4). This established sensitivity and specificity of SCAR marker towards *Bacopa monnieri*. These results further confirm that SCAR marker can be used as a useful tool for the identification of *Bacopa monnieri*, fresh as well as crude drug samples.

1 5' – TGGGCGCACC ATAGCTTGCC TTTCCTCAAA ATTTCTTGGA ACTTTAAAAA

51 ATAAACGAGG CTCCAAGTAA ATGTCCAACT CTCCCAATGA AATCGCCCAT

BacF

101 TTCACGAGTC TTCCCCATGC CTCTGGCTGC TGCTTGACTG ATATAAGTGC

151 CTGCTCGGTT AAGACATTGC TCAAAAGTGA CTGAAAGTAA AGTTTGACCT

201 TCCTAAAGGT CAACATGAGT GCTAGGGCAA ACTTCCCAAT TAATTCTATC

251 TTTTCTCCGG TCCCTGCCTA ATTTTGCTGG TGTAATAGAT AAGTTTATGC

301 TGTTTCGCTT AATCTTGACG AACACCAAAC TTATAGCCTC NTCTGAAACC

351 GCAAACTATA AATAGACCAT CTCTCCTGGT GCGGGCTTGG CAAGTGAAGG

401 AAGGATGGCT AAATATTTCT TGAGTTCCTC CAAAGCAAGG CTACATTCAT

451 GGTCGCACTC ATAGTTTTTA ACCTTCCTTA AAATTTTGAG GAAAGGCAAG

BacR

501 CTCTTGTCCG CCCATCTAGC AATGAATCTA CTAAGGGCCA TTACTCTTCC

551 TACCAACCGC TAGATGTCTT TTATAGATTG GGGGGCCAC – 3'

Fig. 2. Nucleotide Sequence of the RAPD amplicon

Table 4. Sequence of SCAR primers

SCAR primers	No. of base	Sequences (5'-3')	G+C content (%)	Annealing temperature (ºC)
BacF	23	5'-CATGCCTCTGGCTGCTGCTTGAC-3'	44	60
BacR	25	5'-CATTGCTAGATGGGCGGACAAGAGC-3'	41	60

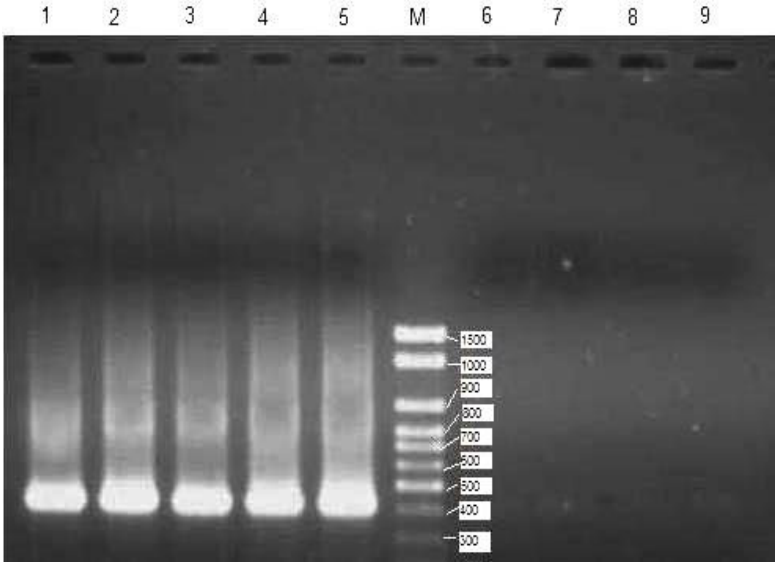

Fig. 3. PCR Amplification of *Bacopa monnieri* (lane 1-5) and *Centella asiatica* (lane 6-9) using SCAR primers (BacF and BacR). Lane M = DNA marker

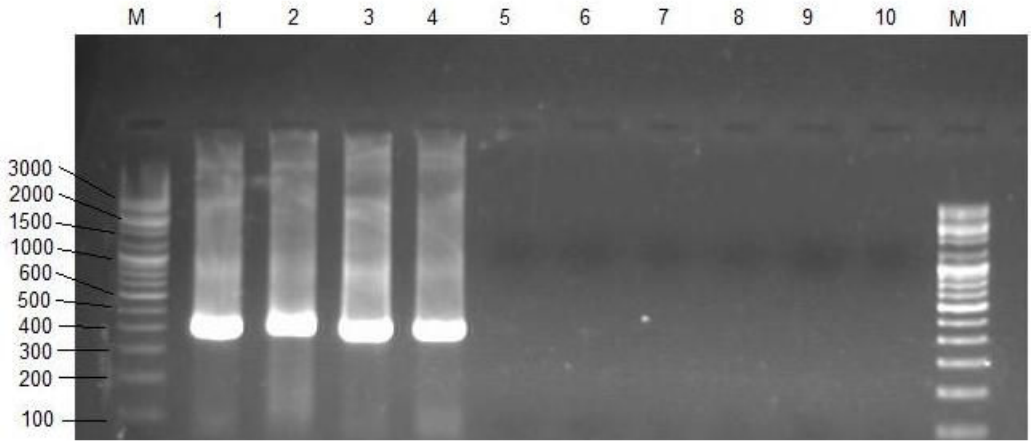

Fig. 4. PCR amplification of *Bacopa monnieri*, *Centella asiatica*, *Eclipta alba* and *Malva rotundifolia* with SCAR primers (BacF and BacR)

M: DNA marker; L1−L4: samples of Bacopa monnieri; L5−L6: samples of Centella asiatica; L7−L8: samples of Eclipta alba; L9−L10: samples of Malva rotundifolia.

3.5 Application of SCAR Primers

To test the applicability of the SCAR marker for molecular authentication of *Bacopa monnieri*, the corresponding SCAR primers were used to amplify DNA from commercial samples. Some commercial crude drug samples (S1 to S8) were purchased from market, New Delhi and were tested with these SCAR primers. The samples which contained *Bacopa monnieri* (S1, S2, S4, S6, and S7) gave positive results with it and the others (S3, S5 and

S8) gave negative results (Fig. 5) further adding to the sensitivity and specificity of the SCAR primers. These results support the commercial applicability of the marker as a useful tool for molecular authentication of *Bacopa monnieri* from fresh as well as dried samples. Even in multicomponent drug samples also it is able to identify *Bacopa monnieri*. These SCAR primers can thus be extensively be used for adulterant detection in traded brahmi samples intended for the export and Import. In the commercial samples the DNA usually appears to be fragmented, therefore shorter bands were selected so that amplification could be easy.

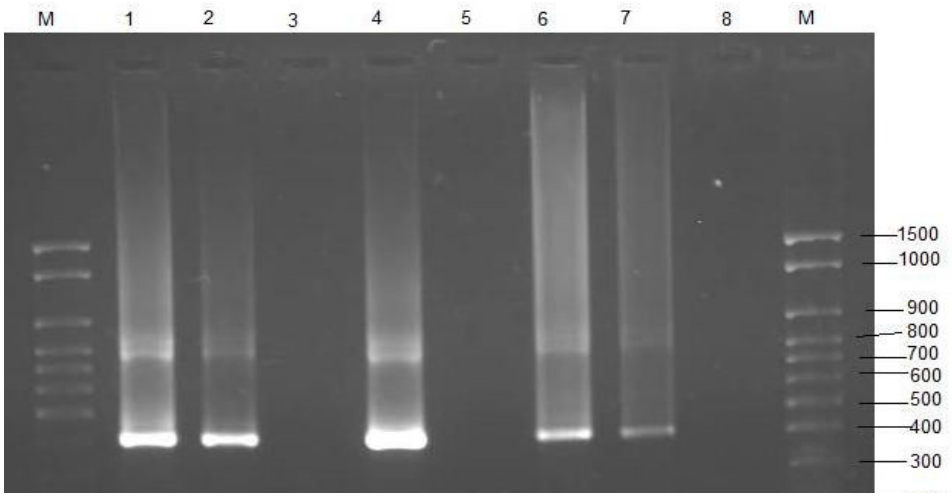

Fig. 5. Amplification of commercial crude drug samples of Brahmi with SCAR primer
M: DNA marker; L1, L2, L4, L6, L7: market samples of Bacopa monnieri; L3, L5, L8: samples of Centella asiatica. Crude drug samples were purchased from Market, New Delhi. Identification was done on the basis of packed information as given in Table 2.

4. DISCUSSION

Herbal medicine has been enjoying renaissance among the customers throughout the world. Use of indigenous drugs from plant origin forms a major part of complementary and alternative medicine/traditional medicine. Adulteration of market samples remains a major problem in domestic and export markets due to confusing nomenclature and lack of botanical identification of traded raw drugs (Patwardhan et al., 2005; Saad et al., 2006). Drug identification is very important to eliminate fraudulent behaviour by unscrupulous individuals. The RAPD technique has been widely adopted for the characterization of the DNA of plants and other organisms (Williams et al., 1990). As the random primers are short (decamers) and the stringency of the reaction is low, the amplified patterns are sensitive to the purity of the DNA preparation, annealing temperature and the concentration of the reagents during the PCR. To circumvent these problems, specific RAPD fragments are sequenced and converted into more reliable SCAR markers. SCAR marker has several advantages over RAPD marker. Annealing conditions are more stringent in SCAR. Contrary to RAPD, SCAR primers can amplify only one locus. Thus, the interpretation of the result is far more straightforward as authentication can be based on the presence and absence of a specific DNA fragment in a given species. Also, the use of longer oligonucleotide primers for SCAR guarantees robust and reliable results. Because of these merits, SCAR has been helpful in differentiating many of the herbal medicines from their close relatives, substituents and adulterants including *Panax* species (Wang et al., 2001), *Astragalus species* (Liu et al.,

2008), *Ehinacea species* (Adinolfi et al., 2007), *Phyllanthus species* (Theerakalpisut et al., 2008), *Pueraria species* (Devaiah et al., 2008), Ginger *species* (Chavan et al., 2008) *Ipomoea species* (Devaiah et al., 2010) and *Cynanchum species* (Moon et al., 2010). SCAR markers have been recently used to detect adulteration in commercially important food products and spices such as turmeric and ground chilli (Dhanya et al., 2011a, 2011b).

In the present study, we have developed a RAPD-based SCAR marker for the identification of *B. monnieri*. *Bacopa monnieri* named as Brahmi is commonly used in ayurvedic herbal drugs. It is a nootropic drug and largely used for its co-gnitive functions (Rajani, 2008). It is an important ingredient of traditional medicine as well as modern medicine such as Brahmighrita, *Brahmi rasayana*, Mentat, Memory plus and BacoMind (Allan et al., 2007). Mentat has been very successful in children with behavioural problems. Thus the demand value increases. To fulfill these demands they have been sometimes substituted/adulterated with other species. Most common substituent is the *Centella asiatica*. There is so much confusion in the herbal market that both the plants are used in place of the other. Both the plants belong to different families but named as 'Brahmi'. Some of the companies use *Bacopa monnieri* in the crude drug formulation and the other use *Centella asiatica*. First of all the DNA was isolated from the different accessions of *Bacopa monnieri* and its adulterants as well. Among them, OPAA-3 primer gave very bright and clear bands both with authentic and adulterant one. A RAPD amplicon of 589 bp was taken and further cloned and sequenced. Based on information of the cloned sequence of marker, Primers (Bac F and Bac R) were designed. The SCAR primers generated a single distinctive band of 406 bp in the all the accessions of *B. monnieri*. The in-house designed SCAR primers, Bac F and Bac R were used to check the other candidate species (*Centella asiatica*, *Eclipta alba* and *Malva rotundifolia*), which are sold in the name of 'Brahmi'. The results showed no amplification of adulterated/substituted species, sold in the name of Brahmi.

In essence, this study was to develop a reliable marker for the identification of genuine 'Brahmi'. This SCAR marker has been used to discriminate between 'Brahmi' and its adulterant/substitute species. The SCAR marker is very successful in this aspect as this method is very easy, rapid and reproducible method. Besides this, it acts as a complementary tool to assist the conventional method. This marker can also be used for high throughput screening of genuine plant sample. Moreover such efficient, precise and sensitive technique will be helpful in quality check of crude drugs marketed in India as well as exported from India.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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