Hindawi Publishing Corporation
Molecular Biology International
Volume 2016, Article ID 1482796, 10 pages
http://dx.doi.org/10.1155/2016/1482796

Research Article

SCAR Marker for Identification and Discrimination of *Commiphora wightii* and *C. myrrha*

Pramod Kumar Sairkar,[1] Anjana Sharma,[2] and N. P. Shukla[1,3]

[1] *Centre of Excellence in Biotechnology, M. P. Council of Science & Technology, Bhopal, Madhya Pradesh, India*
[2] *Bacteriology Laboratory, Department of Post Graduate Studies & Research in Biological Science, Rani Durgawati University, Jabalpur, Madhya Pradesh, India*
[3] *Madhya Pradesh Pollution Control Board, Bhopal, Madhya Pradesh, India*

Correspondence should be addressed to Pramod Kumar Sairkar; sairkar_pramod04@yahoo.com

Received 31 October 2015; Revised 28 January 2016; Accepted 1 February 2016

Academic Editor: Sharad S. Singhal

Copyright © 2016 Pramod Kumar Sairkar et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Commercially important *Commiphora* species are drought-tolerant plants and they are leafless for most of the year. Therefore, it is necessary to develop some molecular marker for the identification. Intended for that, in the present study, species-specific, sequence-characterized amplified regions (SCAR) markers were developed for proficient and precise identification of closely related species *Commiphora wightii* and *C. myrrha*, which may ensure the quality, safety, and efficacy of medicines made from these plants through adulterous mixing of these plants. Two species-specific RAPD amplicons were selected, gel-purified, cloned, and sequenced after screening of 20 RAPD primers. The sequence of 979 and 590 nucleotides (Genebank accession numbers K90051 and K90052) was used for development of 4 SCAR markers, namely, Sc1P, Sc1Pm, Sc2P, and Sc2Pm. Out of them, the Sc1Pm was specific for *C. wightii*, while Sc2P discriminated both the *Commiphora* species. These markers are first reported and will be useful for rapid identification of closely related *Commiphora wightii* and *C. myrrha* species.

1. Introduction

Commiphora spp. of the family *Burseraceae* is being used as a medicinal plant since ancient times and now rated as an endangered plant species [1]. They are found in the arid to semiarid regions of the world, including the deserts of India, Pakistan, Africa, and Saudi Arabia, while in India, it is found in Rajasthan, Madhya Pradesh, Gujarat, Tamilnadu, Orissa, and Karnataka. About 185 species of *Commiphora* were found worldwide, out of them *C. wightii* (synonym *C. mukul*), *C. agallocha*, *C. stocksiana*, *C. berryi*, and *C. myrrha* were found in India [2, 3]. In earlier studies about the flora of India, the "Guggul" plant was known as *Commiphora mukul* (Hook ex Stocks) Engl. or *Balsamodendron mukul* (Hook ex Stocks). Finally, it was named as *C. wightii* (Arn.) by Bhandari in 1964.

C. wightii was well-documented medicinal plant since 3000 years ago [4], having exciting biological activities like being anti-inflammatory, antimicrobial, hepatoprotective, muscle relaxing, antiarthritic, hypolipidemic, hypocholesterolemic, antiobesity, antioxidant, antimalarial, antimycobacterial, antischistosomal, larvicidal, and mollucidal [2, 3, 5–19].

C. wightii contains a bitter gum known as Guggul (Myrrh) in stems and leaves. The yellowish gum oozes upon making an incision and solidifies in the hot environment to a hard brownish resin. Guggul is medicinally important and is used in the treatment of hypercholesterolemia and cardiovascular diseases [9, 20]; it is also shown to have anticancerous activity [21]. The extract of gum Guggul, as gugulipid, guggulipid, or guglipid, is reported as a folk remedy in the Unani and Ayurvedic system of medicine. Two trans-isomers of Guggulsterone, namely, Guggulsterones E and Z, were reported in gum Guggul as important active steroid which are used as cholesterol-lowering agents. The pharmacological properties associated with gum Guggul include anti-inflammatory, antibacterial, anticoagulant, antirheumatic, COX inhibitory,

Table 1: Sequence of SCAR markers designed using 1 kb amplicon.

Name of SCAR	Name of fragments	Sequence (5′-3′)	Total length	Temp	Size (bp)
Sc1P	Sc1P (F)	CTGTGAGGCATTTGTATATTTAA	23 bases	60°C	631
	Sc1P (R)	CTTGTGGTCTTTCAGTCAATAG	22 bases	62°C	
Sc1Pm	Sc1P (F)	CTGTGAGGCATTTGTATATTTAA	23 bases	60°C	910
	Sc1Pm (R)	CTTGAGAACGAAATCTAACAAG	22 bases	60°C	

and hypolipidemic activities that are mostly due to the presence of these steroids [22, 23]. In 1986, Guggul lipids were granted approval in India for marketing as a lipid-lowering drug [24]. Several products of standardized formulations of *C. wightii* were already in human use as cholesterol-lowering agents [22, 25].

Commiphora species have been called "taxonomically difficult," because of being drought-tolerant plants and they are leafless for most of the year [26]. There is resemblance of gum Guggul with gum resin of other species within and outside of the genus, which make high risk of adulteration in commercial samples either deliberately to get more profit or accidentally. Therefore, it is important to validate the *C. wightii* plants and their gum Guggul in commercial samples due to its various pharmacological significances [27]. Many types of markers, namely, morphological, biochemical, and DNA based molecular markers, are commonly used in the identification of species [28]. Molecular markers were used in the identification of species and individual, their origin, and difference at the molecular level in between them [29]. During the last few decades, the use of molecular markers, revealing polymorphism at the DNA level, has been playing an increasing part in plant biotechnology and their genetic studies. These DNA based markers are differentiated into two types: first is non-PCR based RFLP and second is PCR based markers (RAPD, AFLP, SSR, SNP, etc.) [30]. RAPD is a PCR-based technology, based on enzymatic amplification of target or random DNA segments with arbitrary primers. The main advantage of RAPDs is that they are quick and easy to assay, had no sequence data required for primer construction, randomly distributed throughout the genome, and had a dominant nature [31]. However, RAPD marker is not suitable for the species identification, because of their low reproducibility and dominant nature [32]. A RAPD marker can be converted into a codominant and reproducible marker, that is, Sequence-Characterized Amplified Region (SCAR), which may be applicable for authentication of species.

Looking upon these problems, it is necessary to develop some molecular marker for the identification of *C. wightii*. In the present study, an attempt has been made for the development of SCAR markers for *C. wightii*.

2. Materials and Methods

Total 28 accessions of two different species of *Commiphora*, that is, *C. wightii* (17) and *C. myrrha* (11), were collected from Bhopal, Obaidullaganj (Madhya Pradesh), Akola (Maharashtra), Anand (Gujarat), and Jaipur (Rajasthan), and conserved at MPCST Human Herbal Health Care Garden, Bhopal.

2.1. Selection of RAPD Primers and Amplicons. Genomic DNA was isolated from fresh young stem *C. wightii* and *C. myrrha* using the method of Sairkar et al. (unpublished). The yield of DNA was measured using a NanoDrop UV-Spectrophotometer (ND-1000). Genomic DNA was amplified by the 20 primers (Table 1). A cocktail of 40 μL reaction volumes was made with 20 μL, 2x red dye PCR mix (Merck), 1 μL primer (10 pM), and 1 μL template DNA (25 ng/μL) and amplification was performed on the gradient automatic thermal cycler (Eppendorf) following Sairkar et al. [33]. The PCR products were separated electrophoretically on 1.5% agarose gel at 5–10 volts/cm of the gel and visualized by ethidium bromide. The specific amplicon, which discriminates between *C. wightii* and *C. myrrha, was* selected and processed for the development of SCAR marker.

2.2. Cloning of Selected RAPD Amplicon. The selected amplicons were eluted using Medox-Easy Spin Column Cleanup Minipreps kit and ligated with the TA cloning vector (pGEM5Z, Promega). The ligated TA vector was transformed into competent cells of *E. coli* (DH5α), which was prepared using single step ultracompetent cell preparation kit (Medox). The first selection of recombinant clones was based on developed blue and white colonies on LB (Luria Burtani) agar plates containing 0.5 mg/mL ampicillin, 24 μg/mL IPTG, and 30 μg/mL X-gal. The plasmid of white and blue colonies was isolated through Medox-Easy ultrapure spin column plasmid DNA minipreps kit. Three selection steps, that is, clone retardation, restriction digestion, and amplification of plasmid, were adopted to identify positive insert within the plasmid. In retardation step, plasmids were separated electrophoretically to observe the presence of insert within plasmid, while in restriction digestion, plasmids were digested with PvuII enzyme for insert release. In the final step, the plasmids were amplified through the PCR reaction using 50 μL that consist of 25 μL 2x red dye PCR mix (Merck), 1 μL each of forward and reverse M-13 primers (10 pM each), 1 μL of plasmid DNA (25 ng/μL) with a PCR profile of 94°C for 12 minutes, 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C and 45 seconds at 72°C, and final extension on 72°C at 10 minutes using the gradient automatic thermal cycler (Eppendorf).

2.3. Designing and Screening of SCAR Marker. Plasmid having desired amplicon was sequenced by Aristogene Pvt. Ltd., Bangalore, India, using M13 reverse and forward sequencing primers and consensus sequence of amplicons was developed. The homology search of consensus sequences was performed by the NCBI BLAST tool. The primer pairs were designed for these sequences by using PRIMER 3 software [34] and

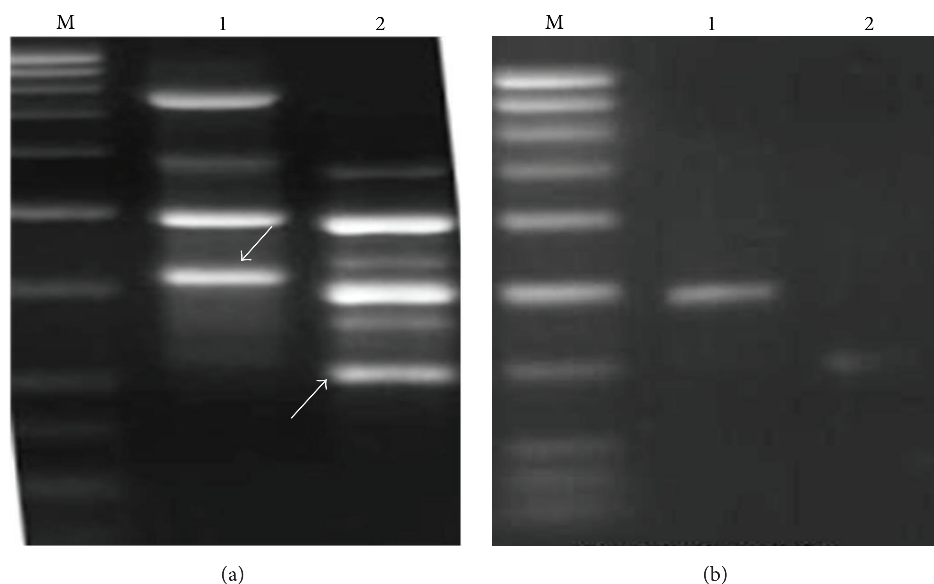

Figure 1: Selection and elution of desired amplicon. (a) PCR product of sample *Commiphora wightii* on low melting agarose gel. Lanes 1 and 2 amplified by primers OPD-02 and OPD-08. (b) Eluted desired amplicon run on agarose gel. Lanes 1 and 2, fragment sizes 1 kb and 0.6 kb, respectively.

used as a candidate for SCAR primer. Four accessions of each species of *C. wightii* and *C. myrrha* were amplified through these primer pairs (synthesized by Aristogene) with a cocktail of 40 μL containing 20 μL of 2x red dye PCR mix (Merck), 1 μL of each of the SCAR primer pair (10 pM each), and 1 μL of template DNA (25 ng/μL). Amplification was performed on the gradient automatic thermal cycler (Eppendorf) with PCR conditions: 94°C for 5 minutes, 30 cycles of 30 seconds at 94°C, 30 seconds at 58°C and 1 minute at 72°C, and final extension on 72°C at 10 minutes. Among the all designed primer pairs, suitable primer pair was selected which discriminate the both species of *Commiphora* and further screened in all the accessions for validation of SCAR marker.

3. Result

3.1. Identification of RAPD Primer and Amplicon. Out of 20 RAPD primers, 1 kb amplicon of OPD-02 and 0.6 kb amplicon of OPD-08 discriminate both *Commiphora* species as it was present only in *C. wightii* accessions (Figure 1(a)). Due to specificity of these amplicons, they were cloned, sequenced, and used for SCAR marker development. These bands were elected from agarose gels and gel electrophoresis revealed that they were appropriate for cloning (Figure 1(b)).

3.2. Cloning and Selection of Positive Clone. White colony of competent cells (*E. coli*) having T vector with 1 kb and 0.6 kb insert was undertaken for plasmid isolation and three selection criteria were performed for the conformation of positive clone. The screening for retardation checking reveals that 17 positive plasmids for 1 kb insert and 5 positive plasmids for 0.6 kb may have proper insert (Figure 2). These positive

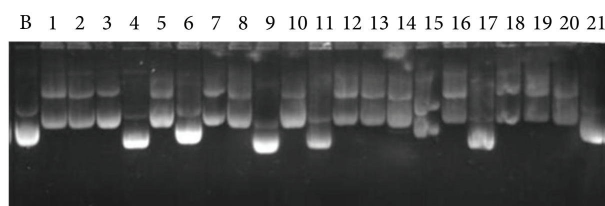

Figure 2: Retardation checking by plasmid run on agarose gel; Lanes 1 to 21: positive cloned plasmids 1 to 21, B: negative cloned plasmid isolated from blue colony.

plasmids were digested with the restriction endonuclease (PvuII) for insert release. A total of 6 positive plasmids of 1 kb insert and 4 positive plasmids of 0.6 kb insert release their respective insert fragment (Figure 3). In the third stage of selection, 4 positive plasmids for 1 kb insert 3 positive plasmids for 0.6 kb insert were finalized for sequencing after amplify with M-13 primer (Figure 4).

3.3. Sequencing and In Silico Application. The clones were sequenced and 979 bp and 590 bp consensus sequences were formed for 1 kb insert 0.6 kb insert, respectively (Figures 5 and 6). The BLAST search was performed for the obtained sequences and no significant homologous sequence was found in the NCBI database. This DNA sequences were deposited in the NCBI gene bank database with accession numbers K90051 and K90052. Two candidate SCAR primer pairs for each DNA sequences were designed, that is, primers Sc1P and Sc1Pm from 979 bp sequences and primer Sc2P and Sc1Pm from 590 bp sequences (Tables 1 and 2). These primers were deposited in the NCBI Prob database with accession number Pr031905450 to Pr031905453.

Table 2: Sequence of SCAR markers designed from 0.6 kb amplicon.

Name of SCAR	Name of fragments	Sequence (5′-3′)	Total length	Temp.	Size (bp)
Sc2P	Sc2P (F)	GTACCCAATGTAGTAATATTCC	22 bases	60°C	491
	Sc2P (R)	TAGTTAGTTTGATGACCATCACA	23 bases	62°C	
Sc2Pm	Sc2P (F)	GTACCCAATGTAGTAATATTCC	22 bases	60°C	570
	Sc2Pm (R)	GTGTGCCCCATTCAACCAAT	20 bases	60°C	

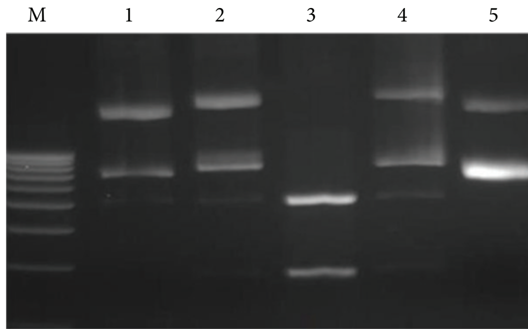

Figure 3: Insert release, digested plasmids by restriction endonuclease PvuII run on agarose gel, plasmid of Lanes 1, 2, 4, and 5 realised 1 kb fragment after digestion.

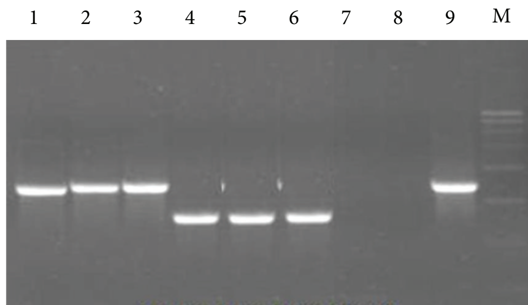

Figure 4: Amplification of plasmid by vector primers (M-13). Lane 1, 2, 3 and 9 produced 1 kb fragment while lanes 4, 5, and 6 produced 0.6 kb fragment.

```
CGTTTCCTTCTTATTAGGGGCGAATTGGGCCCGACGTCGCATGCTCCCGGCCG
CCATGGTTGGACCCAACCCGACCCCTGTGAGGCATTTGTATATTTAAAATAA
ACAACTGTTAAGTAGTTAAGTTAACTAACATTCAACGAAATTAAACCAAAA
CATAGACAAAGTATGATTAGGAGCAATCAGAGAATAAAGAGAGAAAAAGA
AAATAGTACGGAGTAAGGAAGTGTAAATATATATTGTGATTAAATAGTTACT
TTTCTTCGTACATATGATTCATCTCCAGACGTGATTTCATTTGGAATGTATCTA
ATTCGATCTTAATTAAAATTCCATCACTTGCAATGCAGCCGCCGTTAATATAA
TTAACAATATTCGATCCTAGCATGATCGGCATCCTACAGTAAATTAATACAT
AAAAATAGGGTGAAATAGCGACCACTTGGAAATTAATAGTGACGTAATCAT
ACTTTTTTCTTAGACGGGTTAAATTAATATATTAGGTAAGTTATGGTGGTACA
AGAAAATTTTATAAAGATAAAATATTTATAACTAAGGGGACATAATAAATTT
TACTCCTCAAAATGTATCTTTTATACAAAAATAAATATTAAAAATATGGTTA
ATGAACTAATCATAATAAAAGTTAGTCACATCTCTACACCAAGAATATCAAA
TTCATATAAAATGCCTATTGACTGAAAGACCACAAGGTATAATCCTATATTA
AATCTTCTAATATTTAGAATAATAGGTTACCATAAGTTCATATCTAATACATA
AACACACAAATAGAGTTATACAATACTAATAATTTAACATCAATATAATAAT
ATTTGTTTCAACAACATAATTTCATCATCAATTGATAGAAACCATAAGAAAA
TGTGGCTTAAATTAATTTTGATGTCCTAGTAATGGGTATACTAGAATACACAA
ATATGAGAATAAATAAAGGTGTGTGATCTTGTTAGATTTCGTTCTCAAGGGG
GCCGGCAGCCACTTCTTTTTTCAAATGTTCGCAACAATTGGTTGGGTCCATCC
ATGGCCGCGGGATATCACTAGTGCGGCCGCCTGCAGGTCGACCATATGGGA
GAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTTCACCTAAAT
TGCCTAATCCC
```

Figure 5: Consensus sequence (979 bp) of 1 kb fragment (Genebank ID K90051). Red highlights: vector sequence. Green highlight: SCAR primer region. Blue highlight: second reverse SCAR primer. Pink highlight: RAPD primer region.

```
GGCATTCCTTCTATAGGGCGATTGGGCCCGACGTCGCATGCTCCCGGCCGCC
ATGGTTGTGTGCCCCAATGCCAACTTTCTAGAATTTTGGTACCCAATGTAGTA
ATATTCCACCCCTGGTGTGGACGATATTTTGATAAACTCCCATGTCATTTTAA
TTTGTTAATTGGATTTTATTATGATTATTTTTATGCTACTATTAATTTTATTGAT
GGTAATTAAGGATTAAAAGATAAATTTGTCTATTTCAATAGTGATTGATAAA
GTTGATAATTAGGTTGTTTTGTTTAAAGGGATAAAAGTATATGATTAAGGATT
TAAATATAGATAAGGACATGTACCTCATAGCTAATTTTGATAGTCATAAATTT
TCATAGTCACCGGAGTGTTTGGATGGTGAAAGAAATTCCTTACTTCCTCTATT
AAGAGTTAATGGTTTCTTTTGAGTTAGTAGATGAACTTATTAGTGTTTATTGC
ATGGGACCAAAACTTAAAATTTGACAAGATAAAAAGCATAAGCATGTATGT
CTAGAATTGGAGACGTCATCAAAAACTAAATGTGATGGTCATCAAACTAACT
ACTATCTCCCTCCATATGTGATGAATGGGATAAAGAAAAAGATTTTAAATTG
GTTGAATGGGGCACACATCCATGGCCGCGGGATATCACTAGTGCGGCCGCCT
GCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGGATGCATAGCTTGA
GTATTCTATAGTGTCACCCTAAATAGCTTGACGTAATCATGGTCATAGCTGTT
TCCTGTGTGAAATGTTATCCGCTCACAATTCCACACACATACGAGCCGGGAT
GCATAAAGTGTAAAGCCTGTGGTGCCCTAATGAGTGAGCTAACTCACAATTA
ATTGCGTGGCGCTCACTGACCGGCTTTCCAGTCGGGAATCCTGTCGTGCCAG
CCTGCATTTATGAATCGGCCAACGCGCGGGGGAAAGGCGTTTGCGTATTGGG
CGCCTTACCGCTTCCTCCGTCCACATGAC
```

Figure 6: Consensus sequence (590 bp) of 0.6 kb fragment (Genebank ID K90052). Red highlights: vector sequence. Green highlight: SCAR primer region. Blue highlight: second reverse SCAR primer. Pink highlight: RAPD primer region.

3.4. Development of SCAR Marker. The candidate SCAR primer pairs were screened with three accessions of each of *C. wightii* and *C. myrrha* which revealed that the primer Sc1Pm is highly specific for *C. wightii* and amplified 910 bp amplicon, while primer Sc1P had a similar banding pattern in all the samples. Primer Sc2P discriminated both the *Commiphora* species as it gave 491 bp amplicon for *C. wightii*, and 1200 bp for *C. myrrha*, while primer Sc2Pm gives 491 and 570 bp amplicon for *C. wightii* and *C. myrrha*, respectively (Figure 7).

Based on the above results, primers Sc1Pm and Sc2P were authenticated through amplification of eight accession of each species, that is, *C. wightii* and *C. myrrha* (Figure 8). The similar results were observed during this screening as they were discriminated both the species of *Commiphora*.

4. Discussion

Identification of plants at the species level traditionally is a feverish job and needs special care during identification. This

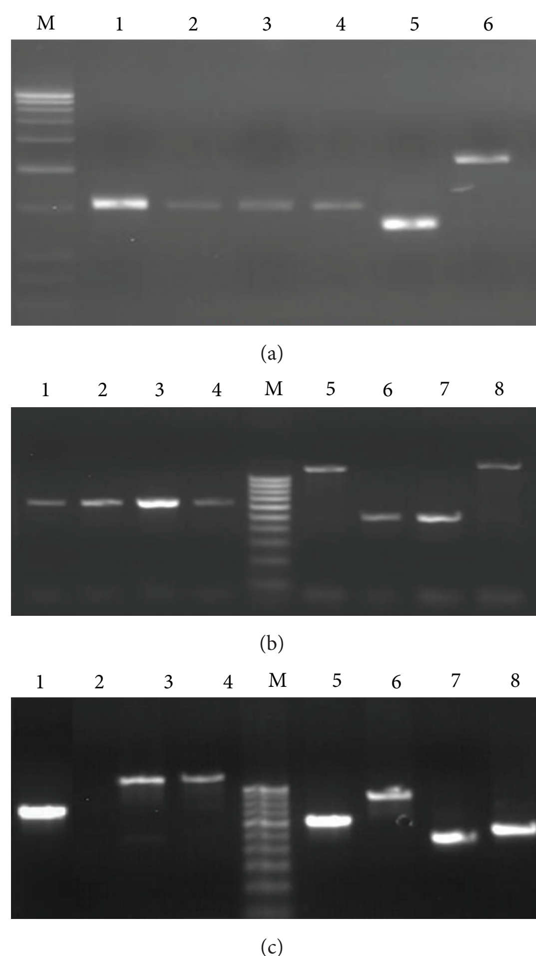

Figure 7: Screening of SCAR primers. (a) Lanes 1 to 2: *C. myrrha* and Lanes 3 to 4: *C. wightii* amplified by primers Sc1P; Lanes 5 to 6: *C. myrrha* and *C. wightii*, amplified by primers Sc2P. (b) Lanes 1 to 2, *C. myrrha* and Lanes 3 to 4, *C. wightii* amplified by primers Sc1P; Lanes 5 to 6, *C. myrrha* and Lanes 7 to 8, *C. wightii*, amplified by primers Sc2P. (c) Lanes 1 to 4, *C. myrrha* amplified by primers Sc1P, Sc1Pm, Sc2P, and Sc2Pm; Lanes 5 to 8, *C. wightii* amplified by primers Sc1P, Sc1Pm, Sc2P, and Sc2Pm.

work usually needs to be a specially trained expert, after that many human errors were observed. To overcome this problem, in the early 1990s many specific molecular identification technologies were popular which are more reliable [35, 36]. Nowadays molecular taxonomists are engaged in preparation of the nucleotide sequence of a short DNA fragment for all living species on earth, which is called DNA barcodes [37–39].

Molecular markers allow the detection of specific DNA sequence differences between tests of individuals of an organism [40]. DNA markers are unlimited in number and are not affected by environmental factors and developmental stages of the plant [41]. The discovery of PCR technology changed the entire molecular biology and a single random oligonucleotide primer (10-bp long) was discovered in 1990 as a universal marker technology called RAPDs [42]. The main advantages of RAPD markers are the following: they are universal and cost-effective and for application of these markers they did not need any genetic information of the target organism and they can map almost completed genomic DNA of the target organism [43]. However, each method analyses different aspects of DNA sequence variation and different regions of the genome. RAPD and AFLP markers appear to frequently target repetitive regions of the genome.

The presence of polysaccharides, polyphenols, and other secondary metabolites in the leaves of *Commiphora* species creates complications in the DNA process. Haque et al. 2008 [44] and Samantaray et al. [45] used various methods and described the process for DNA isolation. Their isolated DNA showed good PCR amplification; therefore, it can further be used in molecular downstream applications. Molecular variations among accessions collected from different localities of Rajasthan and Gujarat were described by Suthar et al. [46]. Intraspecific variation in *Commiphora wightii* populations was described by Haque et al. [47] using Internal Transcribed Spacer (ITS1-5.8S-ITS2) Sequences while Harish et al. [48] studied genetic variations on accessions collected from Indian Thar Desert using RAPD and ISSR markers. Molecular variations among different biotypes of *Commiphora wightii* were done by Vyas and Joshi in 2015 [49] using RAPD markers. Genetic variability among the *C. wightii* germplasm collected from Rajasthan and Haryana was studied by Kulhari et al. [50]. Samantaray et al. [51] used sixty different random decamer primers and identified three primers which produced specific fragment in the female plant of *C. wightii* but failed to do so from the male plant DNAs. Their finding was helpful for the breeding practice of *C. wightii* and our SCAR markers may be useful for identification of *C. wightii* at species level.

The developed SCAR markers by us were used for identification of *C. wightii* and discrimination among *C. wightii* and *C. myrrha*. SCAR markers maybe are developed using sequence of RAPD fragments which are characterized by many advantages, including their specificity, low cost, ease, fast use, reproducibility, abundance, and being polymorphic in nature targeting specific regions of the genomes [52–54] employed with success in plant and animal species identification [30, 55–58].

In this study, RAPD amplicons were selected for cloning, sequencing, and final development of SCAR markers. Specific characters of RAPD markers entice researchers and usually SCAR markers have been developed from RAPD amplicons [58–61]. Amplicon of other fingerprinting methods like AFLP [62–64] and ISSR (Inter Simple Sequence Repeat) [52] was also used to develop SCAR markers.

Developed Sc1Pm marker in this study produced a 910 bp amplicon with *C. wightii*, while in other samples no amplification was observed. These results revealed that this SCAR marker might be used in identification and authentication of *C. wightii*. Many reports are available in which SCAR markers have been used for authentication of medicinal plant species like *Panax ginseng* [65], bent-grass [66], Bamboo [67], *Piper longum* [68], *Artemisia princeps* and *A. argyi* [69], *Phyllanthus emblica* [70], strawberry [71] *Jatropha curcas* [72, 73], *Ganoderma lucidum* [74], *Pueraria tuberosa* [75], *Dendrobium candidum* [76], *Sinapis arvensis* [77], *Cornus officinalis* [78], and *Scrophularia ningpoensis* [79]. Three

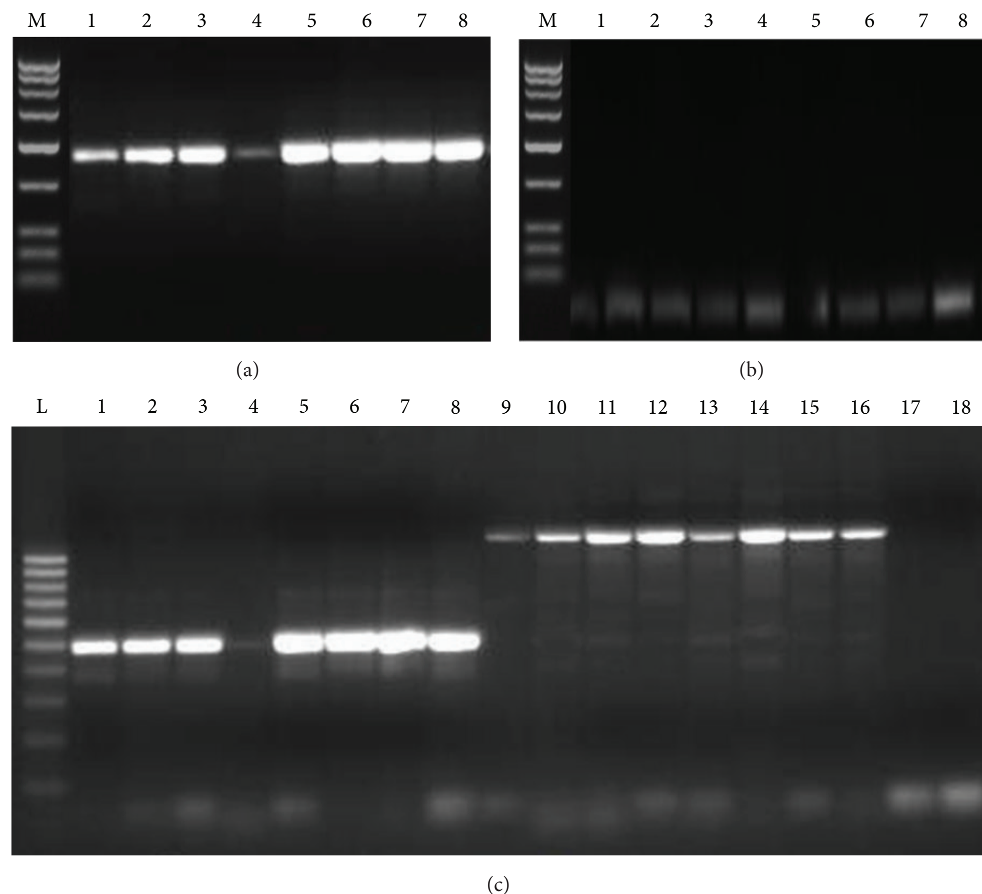

Figure 8: Screening of SCAR primers. (a) Lanes 1 to 8, accessions of *C. wightii* amplified by primers Sc1Pm. (b) Lanes 1 to 8, accessions of *C. myrrha* amplified by primers Sc1Pm. (c) Lanes 1 to 8, accessions of *C. wightii* and Lanes 9 to 16, accessions of *C. myrrha* amplified by primers Sc2P. M = low range ruler (3000, 2500, 2000, 1500, 1000, 600, 300, 200, and 100 bp). L = 100 bp ladder (1000, 900, 800, 700, 600, 500, 400, 300, 200, and 100 bp).

SCAR markers of *Phyllanthus* species were developed from three specific RAPD sequences that can identify and differentiate the morphologically similar *Phyllanthus* species [80].

SCAR markers have been also developed for breeding programs of crops like Rice [81], *Citrus tristeza* [82], *Brassica napus* L. [83], Grapevine [84], Wheat [85], Buckwheat [86], Grape [87], Barley [88], *Atractylodes japonica* and *A. macrocephala* [89], *Diplocarpon rosae* [90], *Puccinia coronata* [91], *Puccinia striiformis* [92], *Thinopyrum elongatum* [93], *Liriope* and *Ophiopogon* [94], *Medicago sativa* [95], *Triticum turgidum* [96], and *Miscanthus sacchariflorus* [97].

Our marker Sc2P produced a prominent amplicon of 491 bp in the *C. wightii*, and 1.2 kb in the *C. myrrha* while other plant samples did not show amplification. The result revealed that this SCAR primer might be used for the discrimination among *C. wightii* and *C. myrrha*. Only few reports are present with a single primer discrimination among two closely related species.

Conflict of Interests

The authors declare that there is no conflict of interests.

Authors' Contribution

Dr. P. K. Sairkar performed wet and dry laboratory work under the supervision of Dr. N. P. Shukla and Professor Anjana Sharma. Professor A. Sharma was cosupervisor of this work and all microbiology related work was performed under her supervision, while Dr. N. P. Shukla was supervisor of this work and probes were developed under his supervision.

Acknowledgments

The authors wish to thank Professor P. K. Verma, Director General, MPCST, and Dr. R. K. Singh, Resource Scientist and Group Head, Advance Research Instrumentation Facility, MPCST, for providing laboratory facilities.

References

[1] IUCN, "IUCN Red List of ThreatenedSpecies," Version 2012.2, 2012, http://www.iucnredlist.org/.

[2] K. R. Kirtikar and B. D. Basu, *Indian Medicinal Plants*, vol. 1, Bishen Singh Mahendra Pal Singh, Dehra Dun, India, 1935.

[3] D. Hocking, *Trees for Drylands*, Oxford and IBH Publishing, New Delhi, India, 1993.

[4] A. Joshi, *Ayurvedic Patrikaritaka Has (History of Ayurvedic Publications)*, Mimeo. Mohan Ayurvedic Pharmacy, Jodhpur, India, 1980.

[5] G. N. Chaturvedi and R. H. Singh, "Experimental studies on anti-arthritic effect of certain indigenous drugs," *Indian Journal of Medical Research*, vol. 53, no. 1, pp. 71–80, 1965.

[6] G. V. Satyavati, C. Dwarkanath, and S. N. Ttripathi, "Experimental studies on the hypocholesterolemic effect of *Commiphora mukul* Engl. (Guggul)," *Indian Journal of Medical Research*, vol. 57, no. 10, pp. 1950–1962, 1969.

[7] S. Kumar and V. Shankar, "Medicinal plants of Indian desert: *Commiphora wightii* (Arnott) Bhand," *Journal of Arid Environments*, vol. 5, pp. 1–11, 1982.

[8] G. V. Satyavati, "Use of plant drugs in Indian traditional systems of medicine and their relevance to primary health care," in *Economic and Medicinal Plant Research*, H. Wagner and N. R. Farnsworth, Eds., pp. 39–56, Academic Press, London, UK, 1990.

[9] R. B. Singh, M. A. Niaz, and S. Ghosh, "Hypolipidemic and antioxidant effects of *Commiphora mukul* as an adjunct to dietary therapy in patients with hypercholesterolemia," *Cardiovascular Drugs and Therapy*, vol. 8, no. 4, pp. 659–664, 1994.

[10] A. D. Bhatt, D. G. Dalal, S. J. Shah et al., "Conceptual and methodologic challenges of assessing the short-term efficacy of Guggulu in obesity: data emergent from a naturalistic clinical trial," *Journal of Postgraduate Medicine*, vol. 41, no. 1, pp. 5–7, 1995.

[11] A. F. Allam, M. H. El-Sayed, and S. S. Kahlil, "Laboratory assessment of molluscidal activity of *Commiphora molmol* (myrrha) on *Biomphalaria alexandrina, Bulinus truncates* and *Lymnea cailliaudi*," *Journal of the Egyptian Society of Parasitology*, vol. 31, pp. 683–690, 2001.

[12] E. S. H. El Ashry, N. Rashed, O. M. Salama, and A. Saleh, "Components, therapeutic value and uses of myrrh," *Pharmazie*, vol. 58, no. 3, pp. 163–168, 2003.

[13] A. M. A. Massoud, F. H. El Ebiary, and N. F. Abd-El-Salam, "Effect of myrrh extract on the liver of normal and bilharzially infected mice—an ultrastructural study," *Journal of the Egyptian Society of Parasitology*, vol. 34, no. 1, pp. 1–21, 2004.

[14] X. Wang, J. Greilberger, G. Ledinski, G. Kager, B. Paigen, and G. Jürgens, "The hypolipidemic natural product *Commiphora mukul* and its component guggulsterone inhibit oxidative modification of LDL," *Atherosclerosis*, vol. 172, no. 2, pp. 239–246, 2004.

[15] F. A. Abbas, S. M. Al-Massarany, S. Khan, T. A. Al-Howiriny, J. S. Mossa, and E. A. Abourashed, "Phytochemical and biological studies on Saudi *Commiphora opobalsamum* L.," *Natural Product Research*, vol. 21, no. 5, pp. 383–391, 2007.

[16] T. Shen, W. Wan, H. Yuan et al., "Secondary metabolites from *Commiphora opobalsamum* and their antiproliferative effect on human prostate cancer cells," *Phytochemistry*, vol. 68, no. 9, pp. 1331–1337, 2007.

[17] A. Sharma, V. K. Patel, S. Rawat, P. Ramteke, and R. Verma, "Identification of the antibacterial component of some indian medicinal plants against *Klebsiella pneumoniae*," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 2, no. 3, pp. 123–127, 2010.

[18] C. Goyal, M. Ahuja, and S. K. Sharma, "Preparation and evaluation of anti-inflammatory activity of gugulipid-loaded proniosomal gel," *Acta Poloniae Pharmaceutica—Drug Research*, vol. 68, no. 1, pp. 147–150, 2011.

[19] T. Al-Howiriny, M. Al-Sohaibani, M. Al-Said, M. Al-Yahya, K. El-Tahir, and S. Rafatullah, "Effect of *Commiphora opobalsamum* (L.) Engl. (Balessan) on experimental gastric ulcers and secretion in rats," *Journal of Ethnopharmacology*, vol. 98, no. 3, pp. 287–294, 2005.

[20] R. Deng, D. Yang, A. Radke, J. Yang, and B. Yan, "The hypolipidemic agent guggulsterone regulates the expression of human bile salt export pump: dominance of transactivation over farsenoid X receptor-mediated antagonism," *Journal of Pharmacology and Experimental Therapeutics*, vol. 320, no. 3, pp. 1153–1162, 2007.

[21] D. Xiao and S. V. Singh, "z-Guggulsterone, a constituent of Ayurvedic medicinal plant *Commiphora mukul*, inhibits angiogenesis *in vitro* and *in vivo*," *Molecular Cancer Therapeutics*, vol. 7, no. 1, pp. 171–180, 2008.

[22] N. Zhu, M. M. Rafi, R. S. DiPaola et al., "Bioactive constituents from gum guggul (*Commiphora wightii*)," *Phytochemistry*, vol. 56, no. 7, pp. 723–727, 2001.

[23] M. R. Meselhy, "Inhibition of LPS-induced NO production by the oleogum resin of *Commiphora wightii* and its constituents," *Phytochemistry*, vol. 62, no. 2, pp. 213–218, 2003.

[24] *Indian Pharmacopeia 2007*, Indian Pharmacopoeia (IP), Government of India, Ministry of Health and Family Welfare, 2007.

[25] P. O. Szapary, M. L. Wolfe, L. T. Bloedon et al., "Guggulipid for the treatment of hypercholesterolemia: a randomized controlled trial," *Journal of the American Medical Association*, vol. 290, no. 6, pp. 765–772, 2003.

[26] F. N. Gachathi, "Recent advances on classification and status of the main gum-resin producing species in the family *Burseraceae*," in *Proceedings of the Regional Conference for Africa on Conservation, Management, and Utilization of Plant Gums, Resins and Essential Oils*, Nairobi, Kenya, October 1997.

[27] H. A. Ahmed, E. T. MacLeod, G. Hide, S. C. Welburn, and K. Picozzi, "The best practice for preparation of samples from FTA®cards for diagnosis of blood borne infections using African trypanosomes as a model system," *Parasites and Vectors*, vol. 4, article 68, 2011.

[28] D. Tautz, P. Arctander, A. Minelli, R. H. Thomas, and A. P. Vogler, "A plea for DNA taxonomy," *Trends in Ecology and Evolution*, vol. 18, no. 2, pp. 70–74, 2003.

[29] N. Techen, S. L. Crockett, I. A. Khan, and B. E. Sheffler, "Authentication of medicinal plants using molecular biology techniques to compliment conventional methods," *Current Medicinal Chemistry*, vol. 11, no. 11, pp. 1391–1401, 2004.

[30] P. Kumar, V. K. Gupta, A. K. Misra, D. R. Modi, and B. K. Pandey, "Potential of molecular markers in plant biotechnology," *Plant Omics Journal*, vol. 2, no. 4, pp. 141–162, 2009.

[31] J. G. K. Williams, M. K. Hanafey, J. A. Rafalski, and S. V. Tingey, "Genetic analysis using random amplified polymorphic DNA markers," in *Methods in Enzymology*, vol. 218, pp. 704–740, Academic Press, New York, NY, USA, 1993.

[32] S. H. Ganiea, P. Upadhyaya, S. Dasa, and M. P. Sharmab, "Authentication of medicinal plants by DNA markers," *Plant Gene*, vol. 4, pp. 83–99, 2015.

[33] P. Sairkar, N. Vijay, N. Silawat et al., "Inter-species association of *Ocimum* genus as revealed through random amplified polymorphic DNA fingerprinting," *Science Secure Journal of Biotechnology*, vol. 1, no. 1, pp. 1–8, 2012.

[34] S. Rozen and H. Skaletsky, "Primer3 on the WWW for general users and for biologist programmers," *Methods in Molecular Biology*, vol. 132, pp. 365–386, 2000.

[35] K.-S. Cheung, H.-S. Kwan, P. P.-H. But, and P.-C. Shaw, "Pharmacognostical identification of American and Oriental ginseng roots by genomic fingerprinting using Arbitrarily Primed Polymerase Chain Reaction (AP-PCR)," *Journal of Ethnopharmacology*, vol. 42, no. 1, pp. 67–69, 1994.

[36] H. Mizukami, K. Ohbayashi, Y. Kitamutra, and T. Ikenaga, "Restriction fragment length polymorphisms (RFLPs) of medicinal plants and crude drugs. I. RFLP probes allow clear identification of *Duboisia* interspecific hybrid genotypes in both fresh and dried tissues," *Biological and Pharmaceutical Bulletin*, vol. 16, no. 4, pp. 388–390, 1993.

[37] P. D. N. Hebert, A. Cywinska, S. L. Ball, and J. R. deWaard, "Biological identification through DNA barcodes," *Proceedings of the Royal Society of London. Series B, Biological Sciences*, vol. 270, pp. 313–321, 2003.

[38] V. Savolainen, R. S. Cowan, A. P. Vogler, G. K. Roderick, and R. Lane, "Towards writing the encyclopaedia of life: an introduction to DNA barcoding," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 360, no. 1462, pp. 1805–1811, 2005.

[39] S. Ratnasingham and P. D. N. Hebert, "BOLD: the barcode of life data system (http://www.barcodinglife.org)," *Molecular Ecology Notes*, vol. 7, no. 3, pp. 355–364, 2007.

[40] P. Langridge and K. Chalmers, "The principle: identification and application of molecular markers," in *Molecular Marker Systems in Plant Breeding and Crop Improvement*, H. Lorz and G. Wenzel, Eds., vol. 49 of *Biotechnology in Agriculture and Forestry*, pp. 129–149, Springer, Berlin, Germany, 2004.

[41] P. Winter and G. Kahl, "Molecular marker technologies for plant improvement," *World Journal of Microbiology & Biotechnology*, vol. 11, no. 4, pp. 438–448, 1995.

[42] J. G. K. Williams, A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey, "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers," *Nucleic Acids Research*, vol. 18, no. 22, pp. 6531–6535, 1990.

[43] S. V. Tingey and J. P. del Tufo, "Genetic analysis with random amplified polymorphic DNA markers," *Plant Physiology*, vol. 101, no. 2, pp. 349–352, 1993.

[44] I. Haque, R. Bandopadhyay, and K. Mukhopadhyay, "An optimised protocol for fast genomic DNA isolation from high secondary metabolites and gum containing plants," *Asian Journal of Plant Sciences*, vol. 7, no. 3, pp. 304–308, 2008.

[45] S. Samantaray, K. P. Hidayath, and S. Maiti, "An isolation protocol of genomic DNA from *Commiphora wightii* (Arnott.) Bhandari: an endangered medicinal plant," *International Journal of Integrative Biology*, vol. 6, no. 3, pp. 127–131, 2009.

[46] S. Suthar, S. Thul, A. K. Kukreja, and K. G. Ramawat, "RAPD markers reveal polymorphism in *Commiphora wightii*, an endangered medicinal tree," *Journal of Cell and Tissue Research*, vol. 8, no. 2, pp. 1477–1480, 2008.

[47] I. Haque, R. Bandopadhyay, and K. Mukhopadhyay, "Intraspecific variation in *Commiphora wightii* populations based on internal transcribed spacer (ITS1-5.8S-ITS2) sequences of rDNA," *Diversity*, vol. 1, no. 2, pp. 89–101, 2009.

[48] Harish, A. K. Gupta, M. Phulwaria, M. K. Rai, and N. S. Shekhawat, "Conservation genetics of endangered medicinal plant *Commiphora wightii* in Indian Thar Desert," *Gene*, vol. 535, no. 2, pp. 266–272, 2014.

[49] P. Vyas and R. Joshi, "Assessment of molecular variations among different biotypes of *Commiphora wightii* (Arnott.) Bhandari, using RAPD markers," *International Journal of Innovative Science, Engineering & Technology*, vol. 2, no. 6, pp. 328–338, 2015.

[50] A. Kulhari, R. Singh, A. Chaudhury, A. K. Dhawan, and R. K. Kalia, "Assessment of genetic variability through ISSR and RAPD markers in *Commiphora wightii* (Arn.) Bhandari," *Acta Physiologiae Plantarum*, vol. 37, article 113, 2015.

[51] S. Samantaray, K. A. Geetha, K. P. Hidayath, and S. Maiti, "Identification of RAPD markers linked to sex determination in guggal [*Commiphora wightii* (Arnott.)] Bhandari," *Plant Biotechnology Reports*, vol. 4, no. 1, pp. 95–99, 2010.

[52] E. Zietkiewicz, A. Rafalski, and D. Labuda, "Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification," *Genomics*, vol. 20, no. 2, pp. 176–183, 1994.

[53] B. Bornet and M. Branchard, "Nonanchored Inter Simple Sequence Repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting," *Plant Molecular Biology Reporter*, vol. 19, no. 3, pp. 209–215, 2001.

[54] B. Bornet, C. Muller, F. Paulus, and M. Branchard, "Highly informative nature of Inter Simple Sequence Repeat (ISSR) sequences amplified using tri- and tetra-nucleotide primers from DNA of cauliflower (*Brassica oleracea* var. *botrytis* L.)," *Genome*, vol. 45, no. 5, pp. 890–896, 2002.

[55] J.-G. Parent and D. Pagé, "Identification of raspberry cultivars by sequence characterized amplified region DNA analysis," *HortScience*, vol. 33, no. 1, pp. 140–142, 1998.

[56] L. Mariniello, M. G. Sommella, A. Sorrentino, M. Forlani, and R. Porta, "Identification of *Prunus armeniaca* cultivars by RAPD and SCAR markers," *Biotechnology Letters*, vol. 24, no. 10, pp. 749–755, 2002.

[57] F. C. F. Yau, K. L. Wong, P. C. Shaw, P. P. H. But, and J. Wang, "Authentication of snakes used in Chinese medicine by sequence characterized amplified region (SCAR)," *Biodiversity and Conservation*, vol. 11, no. 9, pp. 1653–1662, 2002.

[58] R. Bautista, R. Crespillo, F. M. Cánovas, and M. G. Claros, "Identification of olive-tree cultivars with SCAR markers," *Euphytica*, vol. 129, no. 1, pp. 33–41, 2003.

[59] A. S. Parasnis, V. S. Gupta, S. A. Tamhankar, and P. K. Ranjekar, "A highly reliable sex diagnostic PCR assay for mass screening of papaya seedlings," *Molecular Breeding*, vol. 6, no. 3, pp. 337–344, 2000.

[60] O. V. Koveza, Z. G. Kokaeva, S. A. Gostimsky, T. V. Petrova, and E. S. Osipova, "Creation of a SCAR marker in pea *Pisum sativum* L. using RAPD analysis," *Russian Journal of Genetics*, vol. 37, no. 4, pp. 464–466, 2001.

[61] M. S. Arnedo-Andrés, R. Gil-Ortega, M. Luis-Arteaga, and J. I. Hormaza, "Development of RAPD and SCAR markers linked to the Pvr4 locus for resistance to PVY in pepper (*Capsicum annuum* L.)," *Theoretical and Applied Genetics*, vol. 105, no. 6-7, pp. 1067–1074, 2002.

[62] M. S. Negi, M. Devic, M. Delseny, and M. Lakshmikumaran, "Identification of AFLP fragments linked to seed coat colour in *Brassica juncea* and conversion to a SCAR marker for rapid selection," *Theoretical and Applied Genetics*, vol. 101, no. 1-2, pp. 146–152, 2000.

[63] M. Xu and S. S. Korban, "AFLP-derived SCARs facilitate construction of a 1.1 Mb sequence-ready map of a region that spans the Vf locus in the apple genome," *Plant Molecular Biology*, vol. 50, no. 4-5, pp. 803–818, 2002.

[64] H. Schmidt, M. Ehrmann, R. F. Vogel, M. H. Taniwaki, and L. Niessen, "Molecular typing of *Aspergillus ochraceus* and construction of species specific SCAR-primers based on AFLP," *Systematic and Applied Microbiology*, vol. 26, no. 1, pp. 138–146, 2003.

[65] J. Wang, W.-Y. Ha, F.-N. Ngan, P. P.-H. But, and P.-C. Shaw, "Application of sequence characterized amplified region (SCAR) analysis to authenticate *Panax* species and their adulterants," *Planta Medica*, vol. 67, no. 8, pp. 781–783, 2001.

[66] E. A. Scheef, M. D. Casler, and G. Jung, "Development of species-specific SCAR markers in bentgrass," *Crop Science*, vol. 43, no. 1, pp. 345–349, 2003.

[67] M. Das, S. Bhattacharya, and A. Pal, "Generation and characterization of SCARs by cloning and sequencing of RAPD products: a strategy for species-specific marker development in bamboo," *Annals of Botany*, vol. 95, no. 5, pp. 835–841, 2005.

[68] P. Manoj, N. S. Banerjee, and P. Ravichandran, "Development of sex-associated SCAR markers in *Piper longum* L," *Plant Genetic Resources Newsletter*, vol. 141, pp. 44–50, 2005.

[69] M. Y. Lee, E. J. Doh, C. H. Park et al., "Development of SCAR marker for discrimination of *Artemisia princeps* and *A. argyi* from other Artemisia herbs," *Biological and Pharmaceutical Bulletin*, vol. 29, no. 4, pp. 629–633, 2006.

[70] W. Dnyaneshwar, C. Preeti, J. Kalpana, and P. Bhushan, "Development and application of RAPD-SCAR marker for identification of *Phyllanthus emblica* Linn," *Biological and Pharmaceutical Bulletin*, vol. 29, no. 11, pp. 2313–2316, 2006.

[71] R. Rugienius, T. Siksnianas, V. Stanys, D. Gelvonauskiene, and V. Bendokas, "Use of RAPD and SCAR markers for identification of strawberry genotypes carrying red stele (*Phytophtora fragariae*) resistance gene *Rpf1*," *Agronomy Research*, vol. 4, pp. 335–339, 2006.

[72] S. D. Basha and M. Sujatha, "Inter and intra-population variability of *Jatropha curcas* (L.) characterized by RAPD and ISSR markers and development of population-specific SCAR markers," *Euphytica*, vol. 156, no. 3, pp. 375–386, 2007.

[73] S. D. Basha, G. Francis, H. P. S. Makkar, K. Becker, and M. Sujatha, "A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries," *Plant Science*, vol. 176, no. 6, pp. 812–823, 2009.

[74] H. Su, L. Wang, Y. Ge, E. Feng, J. Sun, and L. Liu, "Development of strain-specific SCAR markers for authentication of *Ganoderma lucidum*," *World Journal of Microbiology and Biotechnology*, vol. 24, no. 7, pp. 1223–1226, 2008.

[75] K. M. Devaiah and P. Venkatasubramanian, "Development of SCAR marker for authentication of *Pueraria tuberosa* (Roxb. ex. Willd.) DC," *Current Science*, vol. 94, no. 10, pp. 1306–1309, 2008.

[76] B. Jin, F.-S. Jiang, J. Yu, Z.-S. Ding, S.-H. Chen, and G.-Y. Lv, "Study on sequence characterized amplified region (SCAR) markers in *Dendrobium candidum*," *Zhong Yao Cai*, vol. 33, no. 3, pp. 343–346, 2010.

[77] A. A. Pankin and E. E. Khavkin, "Genome-specific scar markers help solve taxonomy issues: a case study with *Sinapis arvensis* (Brassiceae, Brassicaceae)," *American Journal of Botany*, vol. 98, no. 3, pp. e54–e57, 2011.

[78] S. Chen, X. Lu, and L. Wang, "Study on sequence characterized amplified region (SCAR) markers of *Cornus officinalis*," *Zhongguo Zhong Yao Za Zhi*, vol. 36, no. 9, pp. 1145–1149, 2011.

[79] C. Chen, L.-N. Duan, X.-L. Zhou, B.-L. Chen, and C.-X. Fu, "Molecular authentication of geo-authentic *Scrophularia ningpoensis*," *Journal of Zhejiang University: Science B*, vol. 12, no. 5, pp. 393–398, 2011.

[80] P. Theerakulpisut, N. Kanawapee, D. Maensiri, S. Bunnag, and P. Chantaranothai, "Development of species-specific SCAR markers for identification of three medicinal species of *Phyllanthus*," *Journal of Systematics and Evolution*, vol. 46, no. 4, pp. 614–621, 2008.

[81] N. I. Naqvi and B. B. Chattoo, "Development of a sequence characterized amplified region (SCAR) based indirect selection method for a dominant blast-resistance gene in rice," *Genome*, vol. 39, no. 1, pp. 26–30, 1996.

[82] Z. Deng, S. Huang, S. Xiao, and F. G. Gmitter Jr., "Development and characterization of SCAR markers linked to the *Citrus tristeza* virus resistance gene from *Poncirus trifoliata*," *Genome*, vol. 40, no. 5, pp. 697–704, 1997.

[83] P. Barret, R. Delourme, N. Foisset, and M. Renard, "Development of a SCAR (sequence characterised amplified region) marker for molecular tagging of the dwarf BREIZH (*Bzh*) gene in *Brassica napus* L.," *Theoretical and Applied Genetics*, vol. 97, no. 5, pp. 828–833, 1998.

[84] F. Lahogue, P. This, and A. Bouquet, "Identification of a codominant SCAR marker linked to the seedlessness character in grapevine," *Theoretical and Applied Genetics*, vol. 97, no. 5-6, pp. 950–959, 1998.

[85] P. Hernández, A. Martín, and G. Dorado, "Development of SCARs by direct sequencing of RAPD products: a practical tool for the introgression and marker-assisted selection of wheat," *Molecular Breeding*, vol. 5, no. 3, pp. 245–253, 1999.

[86] J. Aii, M. Nagano, S. H. Woo, C. Campbell, and T. Adachi, "Development of the SCAR markers linked to the Sh gene in buckwheat," *Fagopyrum*, vol. 16, pp. 19–22, 1999.

[87] A.-F. Adam-Blondon, F. Lahogue-Esnault, A. Bouquet, J. M. Boursiquot, and P. This, "Usefulness of two SCAR markers for marker-assisted selection of seedless grapevine cultivars," *Vitis*, vol. 40, no. 3, pp. 147–155, 2001.

[88] G. S. Ardiel, T. S. Grewal, P. Deberdt, B. G. Rossnagel, and G. J. Scoles, "Inheritance of resistance to covered smut in barley and development of a tightly linked SCAR marker," *Theoretical and Applied Genetics*, vol. 104, no. 2, pp. 457–464, 2002.

[89] M. K. Huh and K. H. Bang, "Identification of *Atractylodes japonica* and *A. macrocephala* by RAPD analysis and SCAR markers," *Silvae Genetica*, vol. 55, no. 3, pp. 101–105, 2006.

[90] V. M. Whitaker, J. M. Bradeen, T. Debener, A. Biber, and S. C. Hokanson, "Rdr3, a novel locus conferring black spot disease resistance in tetraploid rose: genetic analysis, LRR profiling, and SCAR marker development," *Theoretical and Applied Genetics*, vol. 120, no. 3, pp. 573–585, 2010.

[91] C. A. McCartney, R. G. Stonehouse, B. G. Rossnagel et al., "Mapping of the oat crown rust resistance gene *Pc91*," *Theoretical and Applied Genetics*, vol. 122, no. 2, pp. 317–325, 2011.

[92] L. Hu, G. Li, H. Zhan, C. Liu, and Z. Yang, "New St-chromosome-specific molecular markers for identifying wheat—*Thinopyrum intermedium* derivative lines," *Journal of Genetics*, vol. 91, no. 2, pp. e69–e74, 2011.

[93] G. H. Xu, W. Y. Su, Y. J. Shu, W. W. Cong, L. Wu, and C. H. Guo, "RAPD and ISSR-assisted identification and development of three new SCAR markers specific for the *Thinopyrum elongatum* E (*Poaceae*) genome," *Genetics and Molecular Research*, vol. 11, no. 2, pp. 1741–1751, 2012.

[94] G. Li and Y.-J. Park, "SCAR markers for discriminating species of two genera of medicinal plants, *Liriope* and *Ophiopogon*,"

Genetics and Molecular Research, vol. 11, no. 3, pp. 2987–2996, 2012.

[95] Y. Wang, B. Bi, Q. H. Yuan, X. L. Li, and J. M. Gao, "Association of AFLP and SCAR markers with common leafspot resistance in autotetraploid alfalfa (*Medicago sativa*)," *Genetics and Molecular Research*, vol. 11, no. 1, pp. 606–616, 2012.

[96] Z. Liu, J. Zhu, Y. Cui et al., "Identification and comparative mapping of a powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. dicoccoides) on chromosome 2BS," *Theoretical and Applied Genetics*, vol. 124, no. 6, pp. 1041–1049, 2012.

[97] J. K. Kim, G. H. An, S.-H. Ahn et al., "Development of SCAR marker for simultaneous identification of *Miscanthus sacchariflorus*, *M. sinensis* and *M. x giganteus*," *Bioprocess and Biosystems Engineering*, vol. 35, no. 1-2, pp. 55–59, 2012.