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SCAR Markers for Authentication of Herbal Drugs

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ABSTRACT

Correct identification and quality assurance is indispensable to ensure reproducible medicinal quality of herbal drugs. Authentication is especially useful in case of those medicinal herbs that are frequently substituted or adulterated with other species or varieties, morphologically and phytochemically indistinguishable. Morphological as well as biochemical markers used in authentication of herbal drugs have many limitations due to the impact of environmental conditions. Molecular markers therefore, are an important tool in quality assurance and preservation of germplasm of medicinal plant species in the plant kingdom. Randomly Amplified Polymorphic DNA (RAPD) is an easy and simple molecular marker, but lack of reproducibility makes it a lesser reliable authentication method for herbal drugs. Besides RAPD, other popular PCR and non-PCR based markers like AFLP, ISSR, SSR and RFLP are also used for authentication. However, these also have disadvantages like use of radioactive isotopes, costly and absolute requirement of sequence information and hence it is a better option to improve the reproducibility of RAPD by converting RAPD amplicons into Sequence Characterized Amplified Region (SCAR) markers. SCAR markers are easy, reliable and reproducible thus, have an advantage over RAPD markers for authentication of medicinal herbs used in the preparation of traditional medicines. These markers however, have been developed for only a few medicinal herbs. This review is an attempt to evaluate critically the role of SCAR markers in authentication of medicinal herbs used in traditional formulations.

Keywords: adulteration, identification, medicinal herbs, molecular markers, quality assurance, RAPD

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INTRODUCTION

Traditional herbal and herbo-mineral drugs have been used since the dawn of civilization to maintain and alleviate human sufferings from diseases. According to an estimate of the World Health Organization (WHO; http://www.who.int/research/en), about 80% of the world population still uses herbs and other traditional medicines for their primary health care needs. From the very beginning, herb authentication has presented a great challenge for people using them for medical purposes. The authentication of medicinal plants is a critical issue for the protection of consumers. Usage of wrong herb may be ineffective or it may worsen the condition and may even cause death. Ideally, authentication should be done from the harvesting of the plant material to the final product. Herbal drugs are normally processed parts of various plants, such as roots, stems, leaves, flowers, fruits, seeds, etc. Due to popularity of herbal drugs globally, their adulteration/substitution aspects are gaining importance at the commercial level. Pharmaceutical companies are procuring materials from traders, who are getting these materials from untrained persons from rural and/or forest areas. This has given rise to wide-spread adulteration/substitution (Meherotra and Rawat 2000), leading to poor quality of herbal formulations. Misidentification of herbs can be non-intentional (processed plant parts are inherently difficult to distinguish) or intentional (profit-driven merchants sometimes substitute expensive herbs with less-expensive look-alike ones).

Traditionally, people authenticate herbs by their appearance, smell and/or taste and some of these methods are still skillful. More sophisticatedly some herbs were authenticated by inspection under microscopes, where the shape and content of various plant cells are examined and analyzed. These methods, based on organoleptic markers or anatomical characters, are sometime imprecise. By and large analytical chromatography, such as thin-layer chromatography, high-performance liquid chromatography, or liquid chromatography has been used for herb authentication. However, during two decades, molecular markers have rapidly complemented the classical strategies. Molecular markers are generally referred to as biochemical constituents, including primary and secondary metabolites in plants and macromolecules, viz. proteins and deoxyribonucleic acids (DNA).

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Mini-Review

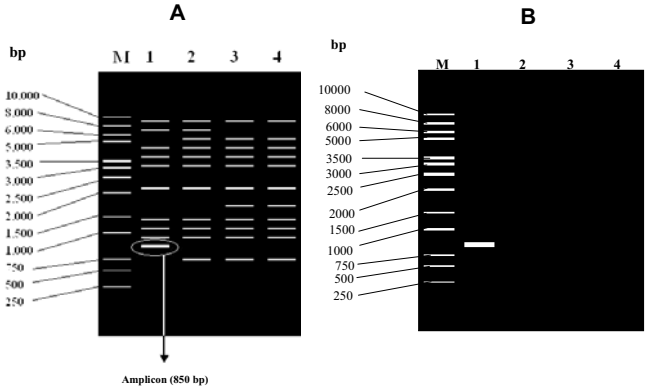

Fig. 1 Diagrammatic representation of SCAR marker development for species 1 (medicinal herb) for its identification and authentication. Species 1 is substituted in market samples with other closely related species (species 2, species 3, species 4). (**A**): Lane M = 1 kb DNA ladder; Lanes 1, 2, 3 and 4 are RAPD fingerprints of species (1, 2, 3 and 4), resolved by electrophoresis on agarose gel, visualized by ethidium bromide staining. (**B**) Lane M = 1 kb DNA ladder; Lanes 1, 2, 3 and 4 are amplified products of species 1, species 2, species 3, species 4, amplified with SCAR primers specifically developed for medicinal herb based on unique RAPD band, resolved by electrophoresis on agarose gel and visualized by ethidium bromide staining. Amplicon of size 850 bp is only obtained in species 1 (medicinal herb) by SCAR primers.

Secondary metabolites as markers have been extensively used in quality control and standardization of herbal drugs, but these also suffer with few limitations. Focus is therefore, on markers based on genetic composition and hence is unique, stable, and ubiquitous to the plants. These DNA markers are not affected by age, physiological condition as well as environmental factors (Chan 2003). Different types of DNA based markers viz., RAPD, RFLP (Restriction Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat) etc., are employed for species discrimination of plants involving taxonomy, physiology, embryology and genetic engineering, etc. (e.g. Joshi *et al*. 2004). Each marker has its own advantages and disadvantages, but none is universally ideal. The choice of technique is therefore, often a compromise that depends upon the nature of research pursued, the genetic resolution needed, financial constraints and the technical expertise available. In this review we have attempted to summarize the information available about SCAR markers and their use in authentication of medicinal herbs.

Sequence Characterized Amplified Regions (SCAR)

A "SCAR", or Sequence Characterized Amplified Region, is an example of a polymorphic region of known sequence. Sequence Characterized Amplified Regions, initially developed for downy mildew resistance genes in lettuce (Paran and Michelmore 1993) are codominant, monolocus, and PCR-based markers that require the use of two specific primers (**Fig. 1**). These markers are based on the sequencing of RAPD or other PCR based markers and hence results with these markers are more reliable with designed specific primers as compared to other DNA based molecular markers (**Table 1**). Specific SCAR sequence primers for amplification may be located at any suitable position within or flanking the SCAR that will identify the polymorphism in a population.

This PCR-based assay is fast, reliable, and easy to conduct in any laboratory. It can be carried out in very short period using unknown genomic DNA from any developmental stage and body part (Kethidi *et al*. 2003). The SCAR amplification profile remains the same in all systems tested. Consequently, SCAR markers, once developed, offer a practical method for screening numerous samples accurately at one time thus, adding to the cost efficiency of the experiment (Kasai *et al*. 2000). SCARs allow for rapid marker development, even though they are not highly polymorphic. SCAR can be used as an allele-specific associated primers (ASAPs) assay to detect the product (Gu *et al*. 1995). Template DNA is amplified using ASAPs which, at stringent annealing temperatures, generate only a single DNA fragment in those individuals possessing the appropriate allele, thus eliminating the need for electrophoresis to resolve the amplifications and increasing the speed of the analysis. The authentication results are reliable, less sensitive to changes in the reaction conditions, reproducible and are not affected by the physiccal forms and age of the sample materials (Hernandez *et al*. 1999). SCAR markers are not affected by the presence of introns that could eliminate the priming sites.

SCAR markers act as both dominant as well as codominant markers. In *Asparagus*, SCAR markers were scored as a dominant marker. Amplification of locus M occurred in both parents at 60°C annealing temperature, but when the annealing temperature was increased to 67°C, only one band was amplified in males and none in females (Jiang and Sink 1997). However, digesting the SCAR fragments produced at the 60°C annealing temperature with four endonucleases (*Hae*III, *Mbo*I, *Rsa*I, *Alu*I) failed to produce a small fragment length polymorphism. This result differs from that of Paran and Michelmore (1993), where codominant SCAR markers were obtained after digesting the monomorphic bands with restriction enzymes.

SCAR markers have been developed for authentication in some medicinal plants, which are easily adulterated viz., *Artemisia* (Lee *et al*. 2006), *Phyllanthus* (Dnyaneshwar *et*

Table 1 Comparison of DNA-based molecular markers.

Parameter	DNA-based molecular marker				
	AFLP	RAPD	SSR	RFLP	SCAR
Quantity of information generated	High and specific	High and nonspecific	High and specific	Low and specific	Low and specific
Replicability	High	Variable	High	High	High
Resolution of genetic differences	High	Moderate	High	High	Low
Ease of use and development	Moderate	Easy	Difficult	Difficult	Easy
Development time	Short	Short	Long	Long	Short
Use of radioactivity	Yes/No	No	Yes/No	Yes	No
Principle	DNA amplification	DNA amplification	DNA amplification	Restriction digestion	DNA amplification
Recurring cost	High	Low	High	High	Low
Reliability	High	Low	High	High	High
Nature of inheritance	Dominant	Dominant	Dominant/Codominant	Codominant	Dominant/Codominant
Single/multiple loci	Multiple	Multiple	Multiple/single	Single	Single
Parts of genome surveyed	Whole genome	Whole genome	Whole genome	Generally low copy region	Part of genome
Skill required	High	Low	High	High	Low
DNA quality required	High	Moderate to high	High	High	Moderate to high
PCR-based	Yes	Yes	Yes	No	Yes

al. 2006), *Panex* (Wang *et al*. 2001), *Atractylodes* (Huh and Bang 2006) and *Echinacea* (Adinolfi *et al*. 2007). To illustrate, some *Artemisia* herbs are used for medicinal purposes. *A. princeps* and *A. argyi* are used as important medicinal material in traditional Korean medicine while *A. capillaris* and *A. iwayomogi* are used for other purposes and are used in place of *A. princeps* and *A. argyi* as these are very difficult to discriminate. Similarly there is pool of material that can be used as adulterant for crude and processed *P. emblica* fruits. The adulterant may be phyllogenetically close or distinct (*e.g.* dried fruit pieces of pumpkin) from *P. emblica* (Dyneshwar *et al.* 2006). The Chinese plant, "Packchul", (*Atractylodes japonica* or *A. macrocephala*), is a very important Chinese medicinal herb plant. The active components of *A. japonica* and *A. macrocephala* are sesquiterpenoids such as atractylon, atractylenolide III and 3β-acetoxyatractylon (volatile oils), but the content levels are different between these two species (Sakamoto *et al.* 1996). Despite being considered less valuable than Korean *A. japonica* (Korean Packchul trades at a price 10 times higher than the Chinese source) with respect to its components and effects, *A. macrocephala* is imported into Korea in huge amounts. These two species can be identified by leaf morphology, flower color and size, and rhizome shape (Bang *et al*. 2003) but it is impossible to distinguish between the two species when the rhizomes are sliced. Therefore, in herbal markets Chinese Packchul is illegally sold either without the correct label or by mixing it with Korean Packchul. In a similar case, Hong Kong is a major entry port for roots of *Panax ginseng* (ginseng) and *P. quinquefolius* (American ginseng). In the market, *P. quinquefolius* from North America is 5 to 10 times more expensive than *P. ginseng* and substitution of the former by the latter is found from time to time (Wang *et al*. 2001).

Development of SCAR markers from other markers

The ease with which a targeted product can be converted into a simple, locus-specific marker varies according to the technique used to produce the original multilocus profile. SCAR primers are oligonucleotides (20-25 bp) that are used to amplify cloned RAPD amplicons (Tartarini *et al*. 1999; Brisse *et al*. 2000; Cao *et al*. 2001) (**Fig. 2**). The designed SCAR primer pair used to amplify genomic DNA from different species of a genus, including targeted species, results in a single, distinct and bright band in the desired sample. SCAR primers deduced from internal sequences are less polymorphic than those including initial RAPD primer sequences, suggesting that the polymorphism is only present in the decamer sequences derived from the RAPD primer sequence (Parasnis *et al*. 2000). The length and GC content of the primer affects the specificity of SCAR marker (Vanichanon *et al*. 2000). Both specificity and the temperature and time of annealing are partly dependent on primer length and its GC content. In general, oligonucleotides between 18 and 24 bases are extremely sequence specific. Primer length is also proportional to annealing efficiency: in general, the longer the primer, the more inefficient the annealing. Ideally the primer should have a near random mix of nucleotides, a 50% GC content and be about 20 bases long.

Both RAPD and SCAR analysis of a 63-plant mapping population of *Asparagus* indicated that polymorphism in the sex expression locus M of *Asparagus* with the two methods corresponded to the same locus (Vos *et al*. 1995). However, SCAR markers are sometimes advantageous over RAPD markers because they detect only a single locus and their amplification is less sensitive to reaction conditions.

Besides RAPD markers, SCAR markers can be developed from more reproducible markers like AFLP (Vos *et al*. 1995), SSR (Litt and Luty 1989; Tautz 1989; Weber and May 1989) and ISSR (Zeitkeinicz *et al*. 1994) (**Fig. 3**). The development of SCAR markers from these markers however, is very costly, difficult, time consuming and may require whole genome sequence information.

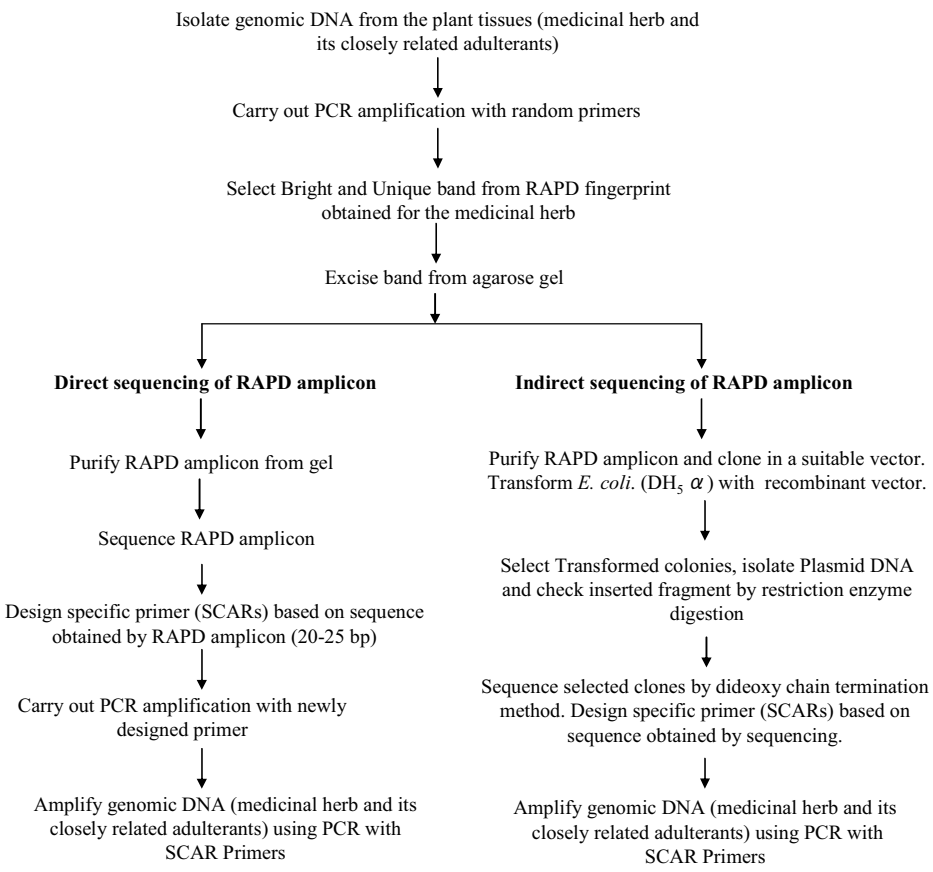

Fig. 2 Schematic representation of SCAR marker development from RAPD fingerprints of a medicinal herb, for its discrimination from closely related adulterants.

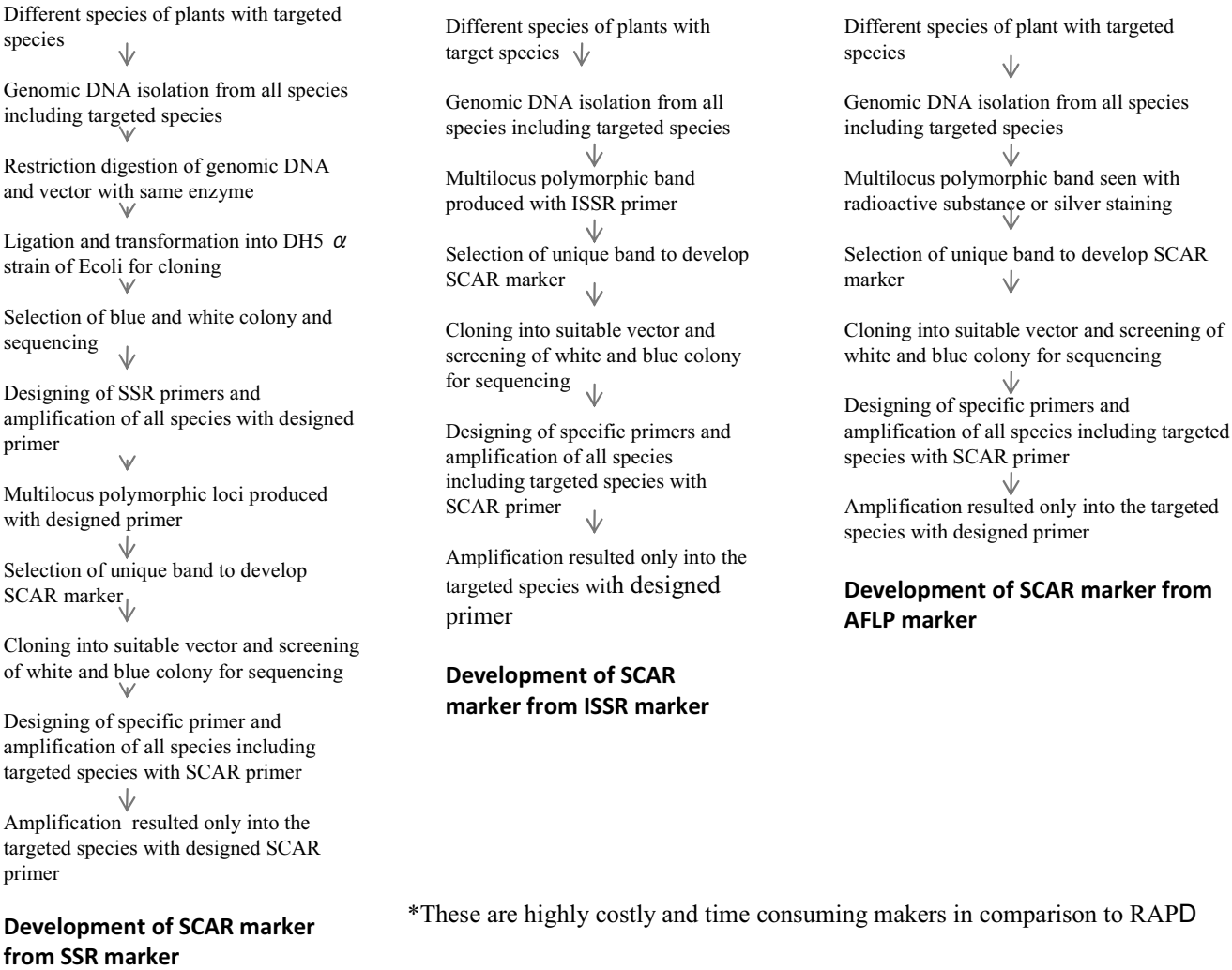

Fig. 3 Development of SCAR markers from AFLP, ISSR and SSR (reproducible) markers*.

Detection efficiency of SCAR markers

SCAR markers are robust and highly efficient. A nanogram or less of DNA sample is sufficient. This method is more specific than other DNA fingerprinting methods using arbitrarily chosen primers. These specific primers generate a sequence-characterized amplified region, which can be particularly useful because they detect only a single locus, their amplification is less sensitive to reaction conditions, and they can potentially be converted into allele-specific markers. To convert a selected unique RAPD, AFLP, ISSR or SSR band to a SCAR marker, each unique band is isolated from agarose gel and the isolated DNA separated by electrophoresis, nucleotide sequence determined using the automatic DNA sequencer, similarities of DNA sequence data analyzed using BLAST and species-specific SCAR primers synthesized.

A small amount (100 mg) of leaf powder of *Echinacea* species and *Phyllanthus emblica* fruit powders (Amlacurna and Triphalacurna) was sufficient to develop a SCAR maker (Dnyaneshwar *et al*. 2006; Adinolfi *et al*. 2007). The SCAR primers used to amplify DNA resulted in a sharp and reproducible band (343 bp) from both the commercial samples of *P. emblica* (Amlachurn and Triphalachurn formulations) (Dnyaneshwar *et al*. 2006).

Authentication of medicinal herbs using SCAR markers

Correct genotype identification of the medicinal herb remains an important concern for the protection of both public health and pharmaceutical industry. Chemo profiling and morphological evaluation are routinely used for identification of medicinal herbs. Chemical complexity and lack of therapeutic markers are some of the limitations associated with the chemical approach while subjective bias in morphological evaluation limits its use. Morphological characteristics are often unreliable or inconclusive, mainly due to the large influence exerted by environmental factors, so that morphological criteria cannot be used as suitable markers to verify the identity of each variety/ species. Many factors may affect the ultimate chemical profile of any herb. Intrinsic factors such as genetics and extrinsic factors such as cultivation, harvesting, drying and storage conditions are a few examples. DNA fingerprint remains the same irrespective of the plant part used, while the phytochemical content may vary with the plant part used, physiology and environment (Joshi *et al.* 2004). DNA fingerprinting ensures presence of the correct genotype but does not reveal the contents of the active principle or chemical constituents. Hence DNA analysis and pharmacognostic techniques for chemoprofiling have to be used hand in hand rather than in isolation.

*Artemisi*a species like *A. princeps*, *A. argyi*, *A. capillaris*, and *A. iwayomogi* are important ingredients in traditional Asian medicinal formulations. Since the dried leaves of these plants are used as medicinal material, which are morphologically indistinguishable from each other, a specific marker is required to efficiently discriminate different species. Two primers Fb and R7, devised to amplify 254 bp fragment of genomic DNA extracted from dried samples of *A. princeps*, and *A. argyi* were developed (Lee *et al*. 2006). This reproducible SCAR marker (254 bp) efficiently discriminates *A. princeps* and *A. argyi* from other *Artemisia* herbs, particularly from *A. capillaries* and *A. iwayomogi* (**Table 2**).

Table 2 Plant parts used and conditions required for developing SCAR markers in some important medicinal plants.

Parameter	***Phyllanthus amarus* (Theerakulpisut *et al.* 2008)**
Part used	Whole plant
Age of plant	Post flowering stage
SCAR primers	*P. amarus*: F(5′-AAACGAGTCCTCCCGGTA-3′); R(5′-CGCAGGGAAGGTGAAGGA-3′)
	P. debilis: F(5′- AACGCCCAATATGCTCGA-3′); R(5′-GTAACGCCCAAAGCCTCA-3′)
	P. urinaria: F(5′- TGTCACTCCTCACCGTCA-3′); R(5′-CATCGGTTCCAGCCACCA-3′)
Amplification conditions*	*Phyllanthus amarus* 64°C; *P. debilis* 62°C; *P. urinaria* 64°C
Size of SCAR marker	*P. amarus* 408 bp; *P. debilis* 549 bp; *P. urinaria* 321 bp
	***Phyllanthus emblica* (Dnyaneshwar *et al.* 2006)**
Part used	Fruit
Age of plant	Post flowering stage
SCAR primers	F(5′-CAGATCTCGTGTAAAAAGCGTTG-3′); R(5′-TGCAGTGAATTCCAAGTGTTTC-3′)
Amplification conditions*	55°C
Size of SCAR marker	343 bp
	***Panex quinquiefolius* (Wang *et al.* 2001)**
Part used	Root
Age of plant	Post flowering stage
SCAR primers	F1(5′-CAAGTCAACTGCAGGGGTTAAGAA-3′); R1(5′-GAGAGAGAAAGATAGTTCAAATAAAAG-3′)
	F2(5′-TTCGCCACCCGGAGCAGCATTGAGATCCGC-3′); R2(5′-GTTCCATCACATAGTTATTGTGGCGA-3′)
Amplification conditions*	SCAR F1 and R1: 56°C; SCAR F2 and R2: 60°C
Size of SCAR marker	420 bp
	***Echinacea purpurea* (Adinolfi *et al.* 2007)**
Part used	Leaves
Age of plant	Pre flowering stage
SCAR primers	F(5′-CGAAAATGGTAAATAAAAGAAT-3′); R(5′-ACTCCCTTGAATACTATA-3′)
Amplification conditions*	51.4°C
Size of SCAR marker	330 bp
	***Atractylodes japonica*, *A. macrocephala* (Huh and Bang 2006)**
Part used	Rhizome
Age of plant	Post flowering stage
SCAR primers	*A. japonica*: F(5'-GGAAGGAATCGAGAAGGCTAACGC-3'); R(5'-AATGGCCGCCATGGTTGAAG-3')
	A. macrocephala: F(5'-CCGTCAATAAACCAAACATCACTG-3'); R(5'-TCCTTGATGCCTACCTCCTGTTAG-3')
Amplification conditions*	39°C
Size of SCAR marker	*Aj* 1117 bp; *Am* 1325 bp
	***Artemisia* (*A. princeps* and *A. argyii*) (Lee *et al.* 2006)**
Part used	Leaves
Age of plant	Pre flowering stage
SCAR primers	F(5′-CATCAACCATGGCTTATCCT-3′); R(5′-GCGAACCTCCCCATTCCA-3′)
Amplification conditions*	Not available
Size of SCAR marker	254 bp

* annealing temperature

Adinolfi *et al*. (2007) developed a SCAR marker to differentiate *Echinacea purpurea* from *E. angustifolia* and *E. pallida*. These different species, due to their difficult identification, are commonly confused and probably used indifferently for the same therapeutic purposes. A species-specific SCAR marker of size 330 bp was developed for *Echinacea purpurea* from the 750 bp RAPD amplicon to distinguish it from related species (*E. angustifolia* and *E. pallida*) (Adinolfi *et al*. 2007).

Panex ginseng and *P. quinquefolius* are very similar in morphology but have diverse physiological activities based on the different combinations and the quantity of various ginsenosides found in each species (Kwan 1995). Wang *et al*. (2001) converted a 420 bp RAPD fragment of *P. quinquefolius* (Ginseng) to a SCAR and used it successfully to authenticate six *Panex* species by the presence of unique amplified band while two common adulterants viz. *M. jalapa* and *P. acinosa* by absence of this unique band. Moreover sequencing and alignment of the SCAR sequences from *P. ginseng* and *P. quinquefolius* indicates the presence of a 25 bp insertion in *P. quinquefolius*.

Phyllanthus emblica L. (Indian gooseberry) fruit has applications in healthcare, food and cosmetic industry. Dyneshwar *et al*. (2006) developed a SCAR marker (343 bp) for correct genotype identification of *P. emblica* from a species-specific amplicon developed by comparative analysis of RAPD profiles of different cultivars of *Phyllanthus*. The marker was further used for authentication of commercial samples of *P. emblica* fruit powders (Amlachurna) as well as Triphalachurna, a multi-component formulation which contains fruit powders of *P. emblica*, *Terminalia chebula* Rotz. and *Terminalia belerica* Roxb.

SCAR markers were also used to provide a simple, cheap, and reliable procedure to identify 22 geographically related olive-tree cultivars. The markers were designed by cloning of prominant RAPD bands obtained in PCR performed on bulk DNA to retain the genetic variability of each cultivar. Clones were partially or totally sequenced and new primers derived from these sequences were used to obtain Sequence Characterized Amplified Region (SCAR) markers (Bautista *et al*. 2003).

SCAR markers for gender discrimination

These markers are very helpful to authenticate plants for gender in certain herbs. There is an unequal distribution of active ingredients in different sexes thus mixing of two affects the efficacy of herbal drug formulations. The SCAR markers, being specific to the genetic composition, are of great help in differentiating the two in a mixture. Further its use for precise and rapid authentication of gender at an early stage would facilitate the cultivation of a particular gender of medicinal herbs. The pistillate plants of *E. ulmoides* are economically more useful than the staminate plants. RAPD was used to screen markers of sex determination in this species. A RAPD marker of size 569 bp linked to sex determination in *E. ulmoides* was developed which was present in all pistillate but not in staminate plants and confirmed by Southern blotting (Xu *et al*. 2005). The MSDE (Marker Linked to Sex Determination in *E. ulmoides*) was

sequenced and specific primers were synthesized to generate a 569 bp pistillate-specific SCAR marker. Thus, SCAR markers helped to authenticate *E. ulmoides* plants for gender even before they reached reproductive maturity, resulting in considerable saving of time and economic resources.

In papaya the different proteinases present in the latex obtained from green unripe fruits have a broad spectrum of activity and are therefore widely used in the food and pharmaceutical industries (Van Droogenbroeck *et al.* 2002). Urasaki *et al*. (2002) developed a 225 bp SCAR marker from a RAPD amplicon namely PSDM (Papaya Sex Determination Marker) (450 bp) for *Papaya* sex determination. The DNA sequence of PSDM exhibited no significant similarity to previously reported sequence markers. Southern hybridization, using PSDM as a probe, showed that PSDM exists in the male and hermaphrodite genomes, but not in the female genome suggesting that PSDM is located on the chromosome region that is specific to the male and the hermaphrodite. The application of the SCARs for precise and rapid authentication of sex in papaya at the seedling stage facilitates the cultivation and breeding of the male and hermaphrodite papaya plants. A similar study conducted to determine the sex types of Colombian cultivars of dioecious papaya genotypes. There are no morphological differences at the chromosome level; therefore the identification of sex types by chromosomal dimorphism is not possible. A RAPD marker of 900 bp was found in male plants, but not in females or hermaphrodites. From this RAPD marker a sequence characterized amplified region (SCAR) was developed and it was possible to amplify fragments from the genomes of male and hermaphrodite plants, but not the female ones (Chaves-Bedoya and Nunez 2007). *Cannabis* species are dioecious in nature. The RAPD and SCAR markers proved to be extremely effective in the discrimination of male plants of *Cannabis sativa*. A SCAR marker was developed from a reproducible RAPD amplicon $OPA8_{400}$ for screening male plants in hemp cultivars (Mandolino *et al*. 1999). It was linked to a male-determining chromosome segment of *C. sativa*; as a consequence, this marker could be used for early determination of sex in hemp, well before the plants reach the reproductive maturity.

FUTURE PROSPECTS

Along with authentication of species identity, prediction of the concentration of active phytochemicals may be required for quality control in the use of plant materials for pharmaceutical purposes. Development of SCAR markers that can correlate DNA fingerprinting data with quantity of selected phytochemical markers associated with that specific medicinal herb would have extensive applications in quality control of raw materials. These designed markers would act as a qualitative/quantitative diagnostic tool for identification of medicinal herbs from harvesting to finished product. Plant breeders, can further use SCAR marker developed for a specific trait, for medicinal crop improvement and conservation. Research is going on similar lines in our laboratory at the Centre for Transgenic Plant Development, Jamia Hamdard, New Delhi, as well as for the authentication of herbal drugs used in traditional Indian system of medicines.

CONCLUSION

The authentication of herbal drugs and discrimination of adulterants from authentic medicinal herbs are essential for both pharmaceutical company as well as public health. There are many molecular markers available in the literature for the identification of herbal drugs. Limitations of chemical and morphological approaches for authentication have generated a need for newer methods in quality control of herbal formulations. SCAR, a PCR-based marker, represents single, genetically defined loci identified by amplification of genomic DNA with a pair of specific oligonucleotide primers. These may contain high-copy number, dispersed genomic sequences within the amplified region. Thus, they are valuable in large-scale and locus-specific applications such as marker-assisted screening and map-based gene cloning. A SCAR generated from polymorphic regions that differ in size between species permit sample authentication based on SCAR size shifts. Different DNA-based markers viz. AFLP, SSR, ISSR and RAPD can be used to generate these markers. However, high reliability of SCAR markers could lead to the displacement of RAPD and other DNA-based markers which are costly, time consuming and tedious. Also high detection sensitivity and avoidable electrophoresis make it an economical molecular tool. Thus, so far SCAR marker seems to be the best suitable technology for authentication of traditional medicinal herbs.

Molecular characterization by SCAR markers allows effective and reliable authentication and discrimination of herbs from their adulterants. The plant species, which are morphologically similar, can be differentiated using these molecular markers. Once a SCAR marker has been developed for a particular species, it can be used to detect the same in homogeneous and heterogeneous formulations used in traditional system of medicines. The medicinal efficacy of male, female and hermaphrodite plants of a species may vary. These markers are a successful tool to distinguish gender before reaching reproductive maturity thus, saving time and cost.

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