Original Article

Significance of molecular markers in pharmacognosy: A modern tool for authentication of herbal drugs

Abstract

Quality evaluation of herbal preparation is an elementary requirement of industry and other association dealing with Ayurvedic and herbal products. The growing use of botanical products now a days is forcing to assess these agents and to develop standards of quality and produce. An attempt has been made through this article to highlight the use of molecular markers for botanicals with special reference to Indian herbal medicine. As the desire for herbal-based products becomes ingrained in our society but standardization of botanicals offers many obstacles like the controversial identity of various plants, deliberated adulteration of plant material, ensuring quality is much more than discovery, specification, and process control. It also includes awareness of every aspect of a manufacturing process from research to shipping. Extensive research on DNA-based molecular markers is in progress for its great utility in the herbal drug analysis and widely used for the authentification of plant species of medicinal importance. DNA markers are reliable for information as the genetic composition is unique for each species and is not affected by age, physiological conditions, as well as environmental factors. DNA markers offer several advantages over conventional phenotypic markers, as they provide data that can be analyzed objectively.

Key words:
Botanicals, genetic markers, herbal drugs, quality control

Introduction

In the last two decades, DNA marker technologies have been revolutionized the plant pathogen genomic analysis and have been extensively employed in many fields of molecular plant pathology. Molecular markers offer also the possibility of faster and accurate identification and early detection of plant pathogen.[1,2] DNA-based molecular markers have utility in the fields such as taxonomy, physiology, embryology, and genetics DNA-based techniques have been widely used for authentication of plant species of medicinal importance.[3]

Molecular Marker

DNA sequence with a known location on a chromosome and associated with a particular gene or trait is known as a genetic marker. It is a variation, which may arise due to mutation or alteration in the genomic loci. A short DNA sequence may be a genetic marker, for example, a sequence surrounding a single base-pair change (single nucleotide polymorphism), or like mini satellites - A long one [Figure 1].[4]

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Desirable properties of ideal DNA markers

- Easily available
- Assay is easy and rapid
- Highly polymorphic and reproducible
- Co-dominant inheritance and recurrent occurrence in genome
- Selectively neutral to environmental conditions or management practices
- Data exchange between different laboratories should be easy
- High genomic abundance.

It is really difficult to get a molecular marker of above criteria. Depending on the type of study undertaken, a marker system can be recognized that would fulfill the above characteristics.[5]

Basic Molecular Marker Techniques

Basic marker techniques can be classified into three categories:

1. Polymerase chain reaction (PCR)-based techniques
2. Non-PCR-based techniques or hybridization based techniques
3. Microsatellite-based marker techniques [Figure 2].

Polymerase chain reaction-based techniques

In the case of PCR-based markers, the primers of known sequence and length are used to amplify genomic and cDNA sequences which are visualized by gel electrophoresis technique. The invention of PCR which is a very versatile and extremely sensitive technique,[6] contributes to use a thermostable DNA polymerase and lead to the development of various molecular marker techniques.[7]

Randomly amplified polymorphic DNA markers

A single species of primer anneals to the genomic DNA at two different sites on complimentary strands of the DNA template. After PCR amplification, a discrete DNA product is obtained if these priming sites are within the amplification range of each other. The introduction of this system produces amplification of several discrete loci.[8]

DNA amplification fingerprinting

In this technique, a single arbitrary primer of only five bases is used to amplify the DNA by PCR. For this marker assay gives simple banding patterns, much-optimized reaction conditions are required and are useful for DNA fingerprinting. Such banding patterns are analyzed by polyacrylamide gel electrophoresis.[9] Arbitrary primed PCR (AP-PCR).

DNA amplification patterns are obtained using single primer of 10–50 bases long in PCR and annealing is carried out under nonstringent conditions.[10]

Sequence characterized amplified regions

Sequence characterized amplified regions (SCARs) are similar to sequence tagged sites (STS) markers but in comparison to random amplified polymorphic DNA (RAPD) they are more reproducible. Although SCARS are mostly dominant markers, also behave as co-dominant markers by digesting them with tetra cutting restriction enzymes. Sex identification of papaya has been carried out using SCAR marker.[11]

Cleaved amplification polymorphic sequence

PCR primers for this process can be synthesized based on sequence information in databank and the electrophoretic patterns are obtained using restriction enzyme digestion of the PCR products.[12,13]

Randomly amplified microsatellite polymorphism

The methodology of these PCR based markers is that first the genomic DNA is amplified using the arbitrary (RAPD) primers. The amplified products thus obtained are then separated electrophoretically and the dried gel is hybridized with microsatellite oligonucleotide probes. Many advantages of oligonucleotide fingerprinting,[14]

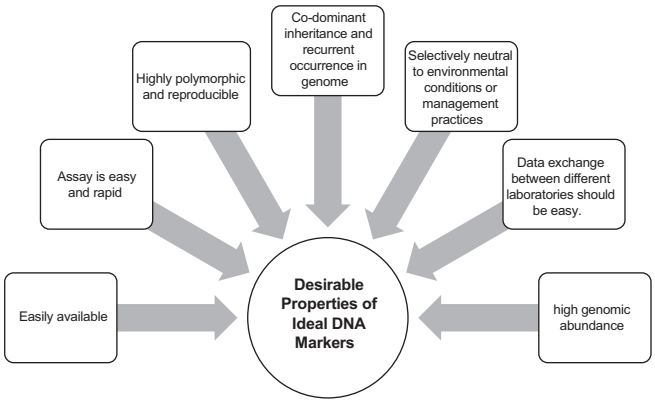


Figure 1: Desirable properties of ideal DNA markers

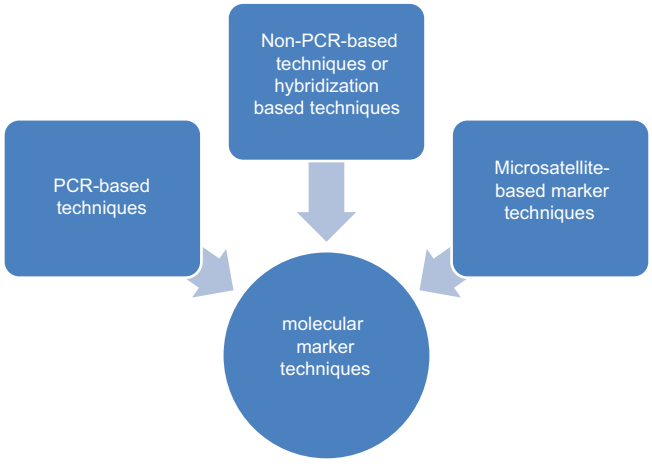


Figure 2: Basic molecular marker techniques

RAPD[15] and microsatellite primed-PCR are thus combined in randomly amplified microsatellite polymorphism.[16,17] Advantages include speed of the assay, high sensitivity, high level of variability detected and no requirement of prior DNA sequence information.[18]

Amplified Fragment Length Polymorphism

Amplified fragment length polymorphism (AFLP) was developed for detection of genomic restriction fragments by PCR amplification, thus fingerprinting patterns are obtained. AFLP is an ingenious combination of restriction fragment length polymorphism (RFLP) and PCR.[19] In the detection of polymorphism between closely related genotypes AFLP is extremely useful.[20,21]

Nonpolymerase Chain Reaction-based Techniques

In the hybridization-based markers or non-PCR based marker, the DNA profiles are visualized by hybridizing the restriction enzyme digested DNA to a labeled probe which is a DNA fragment of known/unknown sequence.

Restriction fragment length polymorphism

The RFLP analysis consists of, restriction endonuclease digested genomic DNA is resolved by gel electrophoresis and then blotted on to a nitrocellulase membrane.[22] Specific banding patterns are then visualized by hybridization with a labeled probe. RFLP are very reliable markers on linkage analysis and breeding and are co-dominant in nature. To constructs a genetic map RFLPs were used for the 1st time to construct a genetic map.[23]

Detailed below are the modifications of the RFLP marker system.

Sequence tagged sites

In this, RFLP probes specifically linked to a desired trait can be converted into PCR based STS oligonucleotide primers based on nucleotide sequence of the probe giving a polymorphic band pattern. This is extremely useful for studying the relationship among several species at a specific locus.[24]

Allele specific associated primers

Specific allele is sequenced and on the base of this sequence, specific primers are designed for DNA template amplification. From this, a single fragment at stringent annealing temperature condition is obtained, and there are used to tag given plant and the gene of interest.[25]

Expressed sequence tag markers

These are introduced for obtaining partial sequencing of random cDNA clones. They are also useful in genome sequencing and mapping programs.[26]

Single strand conformation polymorphism

This is a powerful and popularly used technique for detection of point mutations. It can identify heterozygosity of DNA fragments of the same molecular weight.[27]

Microsatellites and Minisatellites

In virtually all eukaryotic species, 30–90% of the genome is constituted of repetitive DNA, and its nature is highly polymorphic. Microsatellites and minisatellites is one major form of repetitive DNA.[28] With a monomer repeat length of about 11–60 bp, microsatellites are short tandem repeats or simple sequence repeats of 1–6 bp length, repeated several times. Thus, micro and minisatellites form an ideal marker system which simultaneously create complex banding system and detect multiple DNA loci simultaneously. These dominant fingerprinting markers, exhibit high level heterozygosity, and are of Mendelian inheritance.[29]

Detailed below are minisatellite and microsatellite sequence based markers.

Sequence tagged microsatellites sites

Using specific primers designed from sequence 22 data of a specific loci, DNA polymorphism is detected. Primers complementary to the flanking regions of the simple sequence repeat loci yield high polymorphism. For clear banding pattern Di-, tri- and tetra-nucleotide microsatellites are more popular for sequence tagged microsatellites sites analysis.[30] For diversity analysis, dinucleotides which are generally abundant in the genome have been used.[31]

Directed amplification of minisatellite-region DNA

In this case, minisatellites are used as primers for DNA amplification. It is introduced for the 1st time and is found to be useful for species differentiation and cultivar identification.[32,33]

Inter simple sequence repeat markers

This technique was reported for amplifying genomic DNA at the 3'end. They are mostly dominant markers. Number of primers can be synthesized for various combinations of di-, tri-, and tetra and penta-nucleotides.[34]

Advances in Molecular Marker Techniques

Molecular marker techniques have made advances through incorporation of modification in the methodology leads to evolution of several basic techniques.

Organelle microsatellites

Chloroplast DNA and mitochondrial DNA are considered to study the genetic structure and phylogenetic relationships in plants, organelle and genome. Compared

to nuclear alleles, chloroplast and mitochondrial genomes, due to their uniparental mode of transmission, exhibit different patterns of genetic differentiation.[35] Thus, for a widespread understanding of plant population delineation and evolution, three interrelated genomes must be considered, namely nuclear microsatellites, chloroplast and mitochondrial microsatellites have also been developed. Chloroplast microsatellites consisting of relatively short and several mononucleotide sequences are ubiquitous and polymorphic components of chloroplast DNA.[36] Chloroplast genome-based markers uncover genetic discontinuities and distinctiveness among or between taxa with slight morphological differentiation, which nuclear DNA markers cannot reveal sometime.[37]

Sequence-related amplified polymorphism

Sequence-related amplified polymorphism (SRAP) is based on two-primer amplification, mainly 17–21 nucleotides in length. For polymorphism detection SCRAP uses pairs of primers with AT- or GC-rich cores to amplify intragenic fragments. The amplification of open reading frames is the aim of SCRAP technique, SRAP combines simplicity, reliability, moderate throughput ratio, and facile sequencing of selected bands.[38]

Target region amplification polymorphism

The Target region amplification polymorphism technique is a PCR-based convenient technique, which utilizes expressed sequence tag (EST) database information and bioinformatics to generate around targeted candidate gene sequences, polymorphic markers. The fixed primer is designed from the targeted EST sequence in the database; the second primer is an arbitrary primer with either an AT- or GC-rich core to anneal with an intron or exon.[39]

Transposable elements-based molecular markers

The mobile genetic elements which are capable of changing their location in the genome are known as transposons and were discovered in maize almost 60 years ago. Transposable elements, consists of two broad classes and each with its own characteristics properties.[40] Retroelements, such as retrotransposons, short interspersed nuclear elements, and long interspersed nuclear elements, it is the element-encoded mRNA, and not the element itself, that forms the transposition intermediate. The original copy remains intact at the donor site each transposition event creates a new copy of transposons. In contrast, Class II consists of DNA transposons, which change their location in the genome by a "cut and paste" mechanism.[41]

Retrotransposon-based molecular markers

Retrotransposons are the major class of repetitive DNA comprising 40–60% of the entire genome in plants with large genomes.[42] Retrotransposons can be divided into three categories based on structural organization and amino acid similarities among their encoded reverse transcriptases. Long terminal direct repeats (LTRs) flank two of these categories and they encode proteins similar to the retroviruses. These LTR-retrotransposons are known as gypsy-like and copia-like retrotransposons. The LINE1-like or non-LTR retrotransposons are third class of retrotransposons and they lack terminal repeats and encode proteins with significantly less similarity to those of the retroviruses. The replicating process of retrotransposons is by successive transcription, reverse transcription, and insertion of the new cDNA copies back into the genome, copia-like[43,44] and gypsy-like retrotransposons[45] are present throughout the plant kingdom.

Inter-retrotransposon amplified polymorphism and retrotransposon-microsatellite amplified polymorphism

Based on the position of given LTRs within the genome inter-retrotransposon amplified polymorphism (IRAP) and REMP are two amplification based marker methods which have been developed. Proximity of two LTRs using outward-facing primers annealing to LTR target sequences generate the IRAP markers. In retrotransposon-microsatellite amplified polymorphism, amplification between LTRs proximal to simple sequence repeats such as constitutive microsatellites produces markers.[45]

Sequence-specific amplification polymorphism

The technique was first used to investigate the location of BARE-1 retrotransposons in the barley genome.[46] In principle, it is a simple modification of the standard AFLP protocol.[47]

Retrotransposon-based insertion polymorphism

Using the PDR1 retrotransposon in the pea, this technique was developed this technique requires the sequence information of the 50 and 30 regions flanking the transposons.[48]

RNA-based Molecular Markers

Biological responses and developmental programming are regulated by the precise control of genetic expression. Obtaining in depth information about these processes necessitates the study of differential patterns of gene expression. PCR-based marker techniques, such as, cDNA, AFLP, and RNA fingerprinting by arbitrarily primed Polymerase chain reaction (RAP-PCR) are used for differential RNA study selective amplification of cDNAs. Replicated tests show that cDNA-single strand conformation polymorphism reliably separates duplicated transcripts with 99% sequence identity.[49]

RNA fingerprinting by arbitrarily primed polymerase chain reaction

Arbitrarily primer at low stringency for first and second strand cDNA synthesis followed by PCR amplification of cDNA population involves fingerprinting of RNA populations. The method requires nanograms of total RNA and is unaffected by low levels of genomic DNA contamination.[50]

cDNA-amplified fragment length polymorphism

A novel RNA fingerprinting technique to display differentially expressed genes is cDNA-AFLP technique.[51] The methodology includes digestion of cDNAs by two restriction enzymes followed by ligation of oligonucleotide adapters and PCR amplification using primers complementary to the adapter sequences with additional selective nucleotides at the 30 end.[52] The cDNA-AFLP technique is a more stringent and reproducible than RAP-PCR.[53]

DNA Barcoding

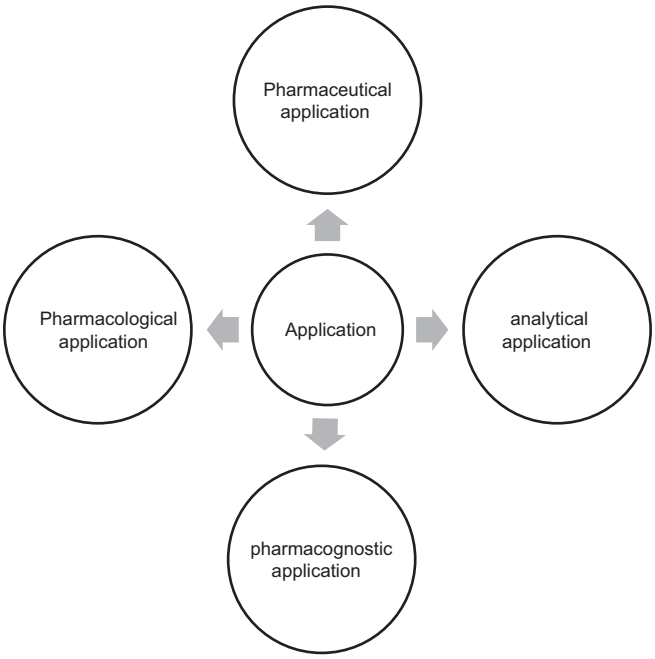
Apart from other technique, it is a dominant method for species identification and discovery since other methods have various limitations and cannot be used in a large scale or in an efficient manner. DNA barcoding uses a short genetic marker in an organism's DNA to identify it as belonging to a particular species. It distinguishes from molecular phylogeny in that the main aim is not to determine classification but to identify an unknown sample in terms of a known classification.[54] Although barcodes are sometimes used in an effort to identify unknown species or assess whether species should be combined or separated, such usage, if possible at all, pushes the limits of what are barcodes capabilities.[55]

Comparison of various aspects of widely used molecular marker techniques [Table 1].

Applications are Prospect of Pharmaceutical Sciences

Pharmacognostic application

Pharmacognosy generally related to quality-control issues using routine organoleptic parameters of crude drugs. DNA-based techniques have been widely used for authentication of plant species of medicinal importance. Since certain rare and expensive medicinal plant species are often adulterated or substituted by morphologically similar, easily available, or less expensive species. For example, *Swertia chirata* is frequently adulterated or substituted by the cheaper *Andrographis paniculata*, therefore, additional methods of identification at the species level have been sought and genome-based methods have been developed for the identification of medicinal plants starting in the early 1990's.[56-59] This work was greatly facilitated by the



invention of the PCR and the introduction of a heat-stable DNA polymerase from the thermophilic bacterium. At present, a practical and powerful tool, i.e. DNA barcodes, is developed for identifying medicinal plants and their adulterants in trade and for ensuring safety in their use.[60] (CBOL Plant Working Group, 2009). Among the PCR-based molecular techniques, RAPD is convenient in performance and does not require any information about the DNA sequence to be amplified.[61] Due to its procedural simplicity, the use of RAPD as molecular markers for taxonomic and systematic analyses of plants.[62] As well as in plant breeding and the study of genetic relationships, has considerably increased.[63] Recently, RAPD has been used for the estimation of genetic diversity in various endangered plant species.[64-68]

This technique remains important in plant genome research with its applications in pharmacognostic identification and analysis.

Pharmacological application

Genetic markers can be used to study the relationship between an inherited disease and its genetic cause (e.g., a particular mutation of a gene that results in a defective protein). Genetic markers have to be easily identifiable, associated with a specific locus, and highly polymorphic, because homozygotes do not provide any information. The methods used to study the genome or phylogenetics is RFLP, AFLP, RAPD, SSR. They can be used to create genetic maps of whatever organism is being studied. The presence of different alleles due to a distorted segregation at the genetic markers is indicative of the difference between selected and nonselected livestock. Endosomal sorting

Table 1: Examples of some medicinal plants and different marker used for various studies and their results

Plants name (family) (reference)	Markers used	Purpose and results
Achillea species (Asteraceae)[70]	AFLP	Nine AFLP primer combinations were used, which produced 301 polymorphic bands. In most species, a high level of genetic variation was detected among the genotypes
A. calamus (Araceae)[71]	RAPD	To study genetic relatedness among accessions. 40 random primers used, 6 primers generated polymorphism
A. mongolicus (Leguminosae)[72]	ISSR	39 out of 99 clear reproducible bands produced while in *Acrobeloides nanus*, 29 out of 112 were polymorphic
A. formosanus (Orchidaceae)[73]	AFLP, ISSR	To assess the genetic variation the total of6 out of 81 primers screened, generating a total of 26 markers, were able to differentiate the 4 lines in the inter single sequence repeat (ISSR) analysis. On the other hand, 17 sets of AFLP primers were chosen to detect the somatic variation among the excised shoots derived from the same individual
A. indica, A. Juss (Meliaceae)[74]	AFLP, SAMPL	AFLP and SAMPL were employed to measure the genetic variation the average genetic similarity values based on Jaccard's coefficient were 0.80 and 0.68, respectively
A. tetracantha (Salvadoraceae)[75]	RAPD	A total of 29 bands were amplified among 6 accessions using 5 primers and the polymorphic bands were 27
B. monnieri (Scrophulariaceae)[76]	RAPD	Among the 40 random primers tested, 29 primers generated one or more polymorphic bands. The number of polymorphic bands generated was primer dependent, ranging from 2 to 8 maximum of 8
Boesenbergia spp. (Zingiberaceae)[77]	RAPD	19 accessions of Zingiberaceae belonging to 11 species of *Boesenbergia*, six species of Kaempferia, and two species of *Scaphochlamys*. 53 amplified bands were observed
C. roseus (Apocynaceae)[78]	RAPD, ISSR	The RAPD primers resulted in the amplification of 56 bands, among which 46 (82%) bands were polymorphic Four ISSR primers amplified 31 loci of which 17 were polymorphic and 14 are monomorphic
C. smyrnioides (Apiaceae)[79]	ISSR	100 primers screened, 18 produced highly reproducible ISSR bands. Using these primers, 196 discernible DNA fragments were generated with 166 (84.7%) being polymorphic, indicating considerable genetic variation at the species level. In contrast, there were relatively low levels of polymorphism at the population level with the PPB ranging from 29.6 to 36.2%
C. morifolium (Asteraceae)[80]	ISSR, SRAP	182 ISSR marker - fragments, as amplified by 22 primers (the PPB: 81.87%), and 243 SRAP marker - fragments as generated by 26 primer pairs (PPB: 75.72%)
C. lanatus (Cucurbitaceae)[81]	ISSR	21 ISSR primers screened, 11 gave bands varying from discrete to large
C. speciosus Koen ex.Retz. (Zingiberaceae)[82]	RAPD	12 decamer random primers was used Four primers showed appreciable molecular polymorphism at amplicon levels
C. pepo (Cucurbitaceae)[83]	ISSR, AFLP, SSR	14 AFLP primers yielded 448 bands of which 280 were polymorphic. Of the 147 ISSR bands scored 108 were polymorphic and SSR scored 20 SSR amplification products
C. longa L. (Zingiberaceae)[84]	RAPD	25 RAPD primers; no significant variation was observed in RAPD profiles of mother plants
C. reflexa (Convolvulaceae)[85]	RAPD	32 decamer oligonucleotide primers were used to amplify the genomic DNA isolated from the dried stems as well as seeds of both the species. Out of the eleven gave faint and non-reproducible, while seven gave species-specific reproducible unique bands
D. officinale (orchidaceous)[86]	RAPD, ISSR	104 reproducible bands were generated using twelve ISSR primers of which 97 were polymorphic, 150 bands produced by RAPD of which 14 were polymorphic
D. obscura (Scrophulariaceae)[87]	RAPD	Seven primers, selected for DNA amplification of D. obscura which showed a high degree of polymorphism among genotypes from different regions or within the same collection area
E. purpurea (Asteraceae)[88]	AFLP	A total of 40 regenerants and 5 donors, using eight primer pairs. The results indicated that a total of 3805 scorable fragments were observed, of which 301 (9.40%) were polymorphic
E. woodii (Zamiaceae)[89]	RAPD, ISSR	RAPD showed 134 bands of which 110 were polymorphic, while ISSR showed 110 bands of which 86 were polymorphic
E. ventricosum (Musaceae)[90]	RAPD	126 oligonucleotide primers initially screened, 12 were chosen that together generated 97 reproducible polymorphic bands Genetic variation within collection sites was relatively high, with values for the Shannon-Weaver diversity index ranging from 0.44 to 0.55
F. carica L. (Moraceae)[91]	ISSR	A total of 33 alleles were detected. A high level of genetic diversity was identified inter cultivars. The clustering grouped the studied cultivars into four clusters with no correlation to geographical origins
F. vesca (Rosaceae)[92]	ISSR	The 23 selected ISSR primers combinations generated 345 amplicons
G. elata Bl. (Orchidaceae)[93]	RAPD, cloning, sequencing and bioinformatics analyses	The distribution of the five DNA sequences varied greatly among the populations. DNA sequences 1 and 5 were found in all the populations studied and determined to be specific DNA molecular markers that differentiate *Gastrodia* from other species
G. Kurroo (Gentianaceae)[94]	RAPD	20 RAPD primers, 5 displayed the same banding profile within all the micropropagated plants and donor mother plant

Contd...

Table 1: Contd...

Plants name (family) (reference)	Markers used	Purpose and results
H. ringens (Lamiaceae)[95]	RAPD, ISSR	17 RAPD primers produced 126 bands of which, 89 were polymorphic (PPB 70.63%), while ISSR amplified 53 bands of which 24 were polymorphic (PPB 45%)
I. younghusbandii (Bignoniaceae)[96]	AFLP	Seven AFLP primer pairs produced 332 reliable bands, of which 185 were polymorphic (55.7%). The number of bands per primer combinations ranged from 38 to 59
I. mauritiana Jacq (Convolvulaceae)[97]	RAPD, SCAR	Identification DNA-based markers have been developed to distinguish *I. mauritiana* from the other Vidari candidates
L. szemaois (Lauraceae)[98]	AFLP, ISSR	Three AFLP primers produced 203 of which 164 were polymorphic and ten ISSR primers produced 77 bands of which 67 were polymorphic
L. angustifolius (Fabaceae)[99]	AFLP, ISSR	3 ISSR primers produced a total of 25 bands of which 6 were monomorphic. 2 AFLP primers produced 82 bands with 50 were monomorphic
M. officinalis (Magnoliaceae)[100]	ISSR	Twelve primer combinations produced a total of 137 unambiguous bands of which 114 (83.2%) were polymorphic
M. chamomilla (Compositae)[101]	RAPD	29 reliable primers that were used, 369 bands were detected and from which 314 (85.44%) bands were polymorphic
M. charantia L. (Cucurbitaceae)[102]	RAPD	29 primers are used which generated a total of 208 reproducible bands, of which 76 (36.50%) were found polymorphic
N. insignis (Asteraceae)[103]	ISSR	11 primers produced 103 reliable ISSR bands, of which 67 were polymorphic
O. europea (Oleaceae)[104]	RAPD, ITS-1, ISSR	Phylogenetic relationship in the *O. europea* complex and phylogeography of 24 populations of *O. europea* were assessed by using nuclear ribosomal ITS-1, RAPD and ISSR
P. bracteatum (Papaveraceae)[105]	AFLP	The two primer combinations generated 254 polymorphic markers
P. emblica L. (Euphorbiaceae)[106]	RAPD	Four primers collectively produced 33 polymorphic bands in these varieties
P. lentiscus L. (Anacardiaceae)[107]	RAPD	For characterization of the genetic variability of Mediterranean *P. lentiscus* genotypes by RAPD. dendrogram based on RAPD analysis gave two main clusters according to their geographical origins
P. corylifolia L. (Fabaceae)[108]	AFLP	To know the variable lengths and independent nature of Ri T-DNA integrations into their genomes using five combinations of *Eco*RI and *Mse*I primers with three selective nucleotides
Rattan genotypes[109]	RAPD	12 primers gave reproducible amplification profiles and 104 polymorphic bands. A considerable degree of polymorphism (98.1%) was detected among the genotypes
R. glutinosa (Scrophulariaceae)[110]	RAPD, ISSR	RAPD primers and ISSR primers amplified average 16.00 and 19.08 bands respectively and the PPBs was 89.58% and 94.32% respectively
R. tanguticum (Polygonaceae)[111]	ISSR	Thirteen selected primers produced 329 discernible bands, with 326 (92.94%) being polymorphic, indicating high genetic diversity at the species level
R. alsia (Crassulaceae)[112]	ISSR	100 primers were screened, 13 produced 140 loci of which 112 (PPB 80%) were polymorphic
R. chrysanthemifolia (Crassulaceae)[113]	ISSR	100 primers were screened, 13 produced highly polymorphic DNA fragments. Using these primers, 116 discernible DNA fragments were generated of which 104 (89.7%) were polymorphic
R. rosea (Crassulaceae)[114]	AFLP	AFLP analysis of 97 *R. rosea* clones using five primer combinations gave a total of 109 polymorphic bands
S. muticum (Fucales, Phaeophyta) (Sargassaceae)[115]	RAPD, ISSR	24 RAPD primers amplified 164 loci of which 124 were polymorphic and 19 ISSR primers amplified 122 loci
Stachys species (Labiatae)[116]	RAPD	Two primers namely OPD6 and OPD14, revealed a DNA polymorphism of the two callus types
Warburgia (Canellaceae)[117]	AFLP	Four AFLP primer pairs (*Eco*RI/*Mse*I) generated a total of 185 amplification products
W. somnifera (*Solanaceae*)[118]	AFLP	Among 64 primers 7 yielded optimum polymorphism. A total of 913 polymorphic peaks were generated using these primers

A. calamus – Acorus calamus; A. mongolicus – Ammopiptanthus mongolicus; A. formosanus – Anoectochilus formosanus; A. indica – Azadirachta indica; A. tetracantha – Azima tetracantha; B. monnieri – Bacopa monnieri; C. roseus – Catharanthus roseus; C. smyrnioides – Changium smyrnioides; C. morifolium – Chrysanthemum morifolium; C. lanatus – Citrullus lanatus; C. speciosus – Costus speciosus; C. pepo – Cucurbita pepo; C. longa – Curcuma longa; C. reflexa – Cuscuta reflexa; D. officinale – Dendrobium officinale; D. obscura – Digitalis obscura; E. purpurea – Echinacea purpurea; E. woodii – Encephalartos woodii; E. ventricosum – Ensete ventricosum; F. carica – Ficus carica; F. vesca – Fragaria vesca; G. elata – Gastrodia elata; G. Kurroo – Gentiana Kurroo; H. ringens – Hesperozygis ringens; I. younghusbandii – Incarvillea younghusbandii; I. mauritiana – Ipomoea mauritiana; L. szemaois – Litsea szemaois; L. angustifolius – Lupinus angustifolius; M. officinalis – Magnolia officinalis; M. chamomilla – Matricaria chamomilla; M. charantia – Momordica charantia; N. insignis – Nouelia insignis; O. europea – Olea europea; P. Bracteatum – Papaver Bracteatum; P. emblica – Phyllanthus emblica; P. lentiscus – Pistacia lentiscus; Psoralea corylifolia – Psoralea corylifolia; R. glutinosa – Rehmannia glutinosa; R. tanguticum – Rheum tanguticum; R. alsia – Rhodiola alsia; Rhodiola chrysanthemifolia; R. chrysanthemifolia – Rhodiola chrysanthemifolia; R. rosea – Rhodiola rosea; S. muticum – Sargassum muticum; W. somnifera – Withania somnifera; PPB – Percentage of polymorphic bands; ITS-1 – Internal transcribed spacer-1; RFLP – Restriction fragment length polymorphism; RAPD – Random amplified polymorphic DNA; ISSR – Inter-simple sequence repeats; AFLP – Amplified fragment length polymorphism

Table 2: Comparison of various aspects of widely used molecular marker techniques

Features	RAPD	RFLP	AFLP	ISSR	DNA barcoding
Level of skills required	Low	High	Medium	Low-medium	Medium
Reproducibility	Unreliable	High	High	Good	Good
Cost per analysis	Low	High	Moderate	Low	Moderate
Automation	Yes	Yes	Yes	Yes	Yes
Accuracy	Good	Very high	High	Good	Good
Prior sequence knowledge	No	Yes	No	No	No
Major application	Gene tagging	Physical mapping	Gene tagging	Genetic diversity	Identification marks
Quantity of DNA required	Low	High	Medium	Low	Low

RFLP – Restriction fragment length polymorphism; RAPD – Random amplified polymorphic DNA; ISSR – Inter-simple sequence repeats; AFLP – Amplified fragment length polymorphism

receptors, useful as molecular markers to define plant endosomal compartments are, for example, the vacuolar sorting receptor (VSR) BP-80 from pea (Pisum sativum) and its Arabidopsis homolog AtELP1, which are known to predominantly associate with prevacuolar compartments/ multivesicular bodies.[68]

Pharmaceutical application

Molecular markers in pharmaceutics relate to drugs and drug development, for example, in silico drug profiling of the human kinome based on a molecular marker for cross-reactivity.[69] Molecular markers focus on molecular mechanistic approaches to the development of bio-available drugs as well as concentrates on the integration of applications of the chemical and biological sciences to advance the development of new drugs and delivery systems.

Other application in plant genome

Examples of some medicinal plants and different marker used for various studies [Table 2].

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Nil.

Conflicts of interest

There are no conflicts of interest.

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