Research in Plant Biology, 2(2): 01-12, 2012 ISSN : 2231-5101
www.resplantbiol.com

Review Article

Molecular markers in characterization of medicinal plants: An overview

Tharachand C, Immanuel Selvaraj C* and Mythili MN

Division of Plant Biotechnology, School of Biosciences and Technology,
VIT University, Vellore 632014, India
*Corresponding author email : immmer@gmail.com

The demand for herbal medicines is increasing day-to-day because of their wide biological activity, higher safety margin than synthetic drugs. Hence, it is important to characterize the medicinal herbs. For the characterization of medicinal plants it is necessary to develop the sensitive and effective technology. Molecular markers can be employed for characterization, since the progress made in genetic markers has been exciting. DNA-based molecular markers have acted as very useful tools in various fields like taxonomy, physiology, embryology, plant breeding, ecology, genetic engineering etc. DNA based markers have their applications in fingerprinting genotypes, determining the seed purity and in phylogenetic analysis by which the conservation of the plant can be made easy. The innovation of Polymerase Chain Reaction (PCR) made the development of DNA based markers easier. Extensive research on molecular markers is in progress in many research institutes all over the world. DNA-based molecular markers have a great utility in the drug analysis and widely used for the characterization of medicinally important plant species. This review is an attempt to evaluate critically the role of different DNA based markers in the characterization of medicinal plants.

A *genetic marker* is "a gene or DNA sequence with a known location on a chromosome and associated with a particular gene or a character". It can be described as a variation, which may arise due to mutation or alteration of nucleotide in the genomic loci that can be observed (Srivatsava *et al*., 2009). DNA-based molecular marker helps in improvement of medicinal plant species. DNA markers are more reliable because the genetic information is unique for each species and is independent of age, physiological conditions and environmental factors (Kalpana *et al*., 2004). The molecular marker technique efficiency is based on the amount of polymorphism it can detect in the given accessions (Leela *et al*., 2009). Restriction fragment length polymorphism (RFLPs) were one of the earliest molecular marker technique used for detecting variations in DNA sequence by restriction digestion of DNA with restriction enzymes. Later-on many methods were developed. A more recent way of detecting individual variations in DNA nucleotides directly is through Single Nucleotide Polymorphism (SNPs). All these methods have expanded the availability of molecular markers which facilitated the creation of genetic maps.

Description of Molecular Markers:

- RFLP (Restriction fragment length polymorphism)
- AFLP (Amplified fragment length polymorphism)

- RAPD (Random amplification of polymorphic DNA)
- VNTR (Variable number tandem repeat)
- SSRs (Simple sequence repeats)
- SNP (Single nucleotide polymorphism)
- STR (Short Tandem Repeats)

Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism. These are hybridization-based methods, polymerase chain reaction (PCR) based methods and sequencing-based methods.

Based on the development of these techniques in the last three decades, classified into three classes (Gupta *et al*., 2001):

a) The first generation molecular markers, including RFLPs, RAPDs and their modifications;
b) The second generation molecular markers, including SSRs, AFLPs and their modified forms,
c) The third generation molecular markers including ESTs and SNPs.

A ***Restriction Fragment Length Polymorphism***, or RFLP, (commonly pronounced "rif lip"), is a technique that exploits variations in homologous DNA sequences. It refers to difference between samples of homologous DNA molecules that come from differing locations of restriction enzyme sites (Gnavi *et al*., 2010). Even though DNA characterization can be done very thoroughly by DNA sequencing techniques, RFLP analysis was developed first and was cheap enough to see wide application in localization of genetic disease genes, determination of risk for a disease, genetic fingerprinting, and paternity testing (Srivatsava and Nidhi, 2009). Jana and Eva (1997) performed the RFLP analysis of the genomic DNA of *Hypericum perforatum* L. They stated that the occurrence of the identical RFLP patterns in some R_0 somaclones and seed-derived plants and their progenies were related to the apomictic mode of reproduction which is assumed to be prevalent in *H. perforatum.* The differences in RFLP patterns of progenies when compared with the maternal plants (1 out of 10 progenies of one control plant and 1 out of 8 progenies of one somaclone) indicated that some progenies have originated through sexual reproduction.

The phylogenetic relationship among the congeneric species (Anthemideae, Asteraceae) in Egypt has studied using RFLP markers along with RAPD and ISSR markers. In this case twenty six RFLP bands were detected after *Eco*RI and *Bam*HI digestion. The average of similarity co-efficient was 0.0 to 0.5. The band products of restricted DNA of *Achillea fragrantissima, Artemisia arborescens, Artemisia Judaica, Matricaria aurea, Glebionis coronaria, Cotula barbata* and *Cotula cinerea* were 6, 4, 5, 4, 3, 2 and 2 bands respectively, while *Achillea santolina, Anacyclus monanthos* and *Matricaria recutita* showed no bands. Based on this study the species were classified as *Matricaria recutita* as out group; *Achillea fragrantissima* and *Artemisia arborescens* were closely related among the group of seven species with less genetic distance (Abd EI-Twab and Zahran, 2010). Using phenotypic evaluation and Southern blot experiments the genomic instability of tobacco plants derived from protoplast was studied. DNA polymorphism (RFLP) was displayed by three regenerates upon Southern blot hybridization and thus confirmed that in such plants alterations in the genome structure could be found and that genotypes of protoplast derived plants frequently differ from the parental genotype (Vyskot *et al*., 1991)

The taxonomy and phylogeny of Indian *Citrus* is revisited using PCR-RFLP with the trnD-trnT and rbcL-ORF regions as well as sequence data analysis of the trnL-trnF intergenic spacer region of

cpDNA by Satya Narayan *et al* (2009). The study was based on 50 accessions of *Citrus* genotypes collected from wild, semi-wild and domesticated stocks. PCR-RFLP analysis resulted in a well resolved phylogenetic tree with branches supported by moderate to high bootstrap values, while the trnL-trnF sequence-based trees showed only moderate to low bootstrap support for the internal tree branches, indicating uncertain origin of some *Citrus* genotypes. However, PCR-RFLP and trnL-trnF data supported the recognition of *Citrus maxima*, *C. medica*, and *C. reticulata* as the basal species of edible citrus.

Amplified fragment length polymorphism (AFLP) is a technique based on the selective PCR amplification of restriction fragments from a total digest of genomic DNA (Dorothea *et al*, 1996). The technique involves three steps:

1. Digestion of total cellular DNA with one or more restriction enzymes and ligation of restriction half-site specific adaptors to all restriction fragments.
2. Selective amplification of some of these fragments with two PCR primers that have corresponding adaptor and restriction site specific sequences.
3. Electrophoretic separation of amplicons on a gel matrix, followed by visualization of the band pattern.

PCR amplification of restriction fragments is achieved by using the adapter and restriction site sequence as target sites for primer annealing. The selective amplification is achieved by the use of primers that extend into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. Using this method, sets of restriction fragments may be visualized by PCR without knowledge of nucleotide sequence. The method allows the specific co-amplification of high numbers of restriction fragments. The number of fragments that can be analyzed simultaneously, however, is dependent on the resolution of the detection system. Typically 50-100 restriction fragments are amplified and detected on denaturing polyacrylamide gels (Vos *et al*., 1991)

AFLP represents dominant marker system among the different marker systems available at present and also provides multi-locus and genome wide marker profiles. These features make the AFLP technology more suitable for molecular characterization and DNA fingerprinting of any germplasm collection (Leela *et al*., 2009). AFLP not only has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques, but it also has the capability to amplify between 50 and 100 fragments at one time. In addition, no prior sequence information is needed for amplification. As a result, AFLP has become extremely beneficial in the study of taxa including bacteria, fungi, and plants, where much is still unknown about the genomic makeup of various organisms (Hao *et al*., 2010). AFLPs can be used to measure recombination rates which can lead to a genetic map with the distance between AFLP loci measured in CentiMorgans (Terashima *et al*., 2002).

AFLP markers have been successfully employed for the development of a high-density linkage map of ryegrass (*Lolium perenne* L.). The application of AFLP markers to genome mapping, in *Lolium* as a prelude to quantitative trait locus (QTL) identification for diverse agronomic traits in ryegrass and for marker-assisted plant breeding was carried out (Bert *et al*., 1999). The usefulness of molecular markers of DNA polymorphism, based on AFLP analysis, to unravel the disputed attributions of *Ocimum* genus has been conducted by Labra *et al*. (2004). The *Ocimum* genus includes more than 150 species. They concluded that the combined analysis of morphological traits, volatile oil

composition and molecular markers represents the optimal approach to verify taxonomy and to correlate it with agronomic traits. In this case the genetic distance was computed by using the Nei and Li's index. This index is more appropriate for AFLP data than others, such as Jaccard's similarity index or the simple band matching distance indexes (Labra *et al*., 2004). Baydar *et al* (2004) investigated the genetic relationships among *Rosa damascena* plants grown in Turkey by using microsatellite and AFLP markers. Twenty three AFLP and nine microsatellite primer pairs were used for this experiment. No polymorphism was detected among the plants, as the marker patterns obtained from different plants are identical. The conclusion from these data is that all *R. damascena* plants studied are derived from the same original genotype by vegetative propagation.

Rapid Amplification of Polymorphic DNA (RAPD) is a type of PCR reaction, but the segments of DNA that are amplified are random (Srivatsava *et al*., 2009). RAPD markers are amplification products of anonymous DNA sequences as primers to amplify nanogram (ng) amounts of total genomic DNA under low annealing temperature. RAPD does not require any specific knowledge of the DNA sequence of the target organism: the identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers sequence (Bardakci, 2001). It is used to analyze the genetic diversity of an individual by using random primers (Arif *et al*., 2010). Nearly all RAPD markers are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). Co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely (Salim khan *et al*., 2010).

The RAPD (Random Amplified Polymorphic DNA) technique was employed for authentication of *Glycyrrhiza glabra* L. from its adulterant *Abrus precatorius* L. by Salim khan *et al* (2009). Fifty two decamer oligonucleotide primers were screened in the RAPD analysis for identification of genuine and adulterant samples. Out of fifty two primers, sixteen primers gave species specific reproducible unique amplicons. The obtained unique amplicons in PCR amplification clearly distinguished genuine as well as adulterant samples having similar morphology and thus, RAPD helping to serve as a complementary tool for quality control. (Salim khan *et al*., 2009).

In a case study by Mehrnia *et al* (2009) the fresh leaves of 20 individual plants belonging to Iranian *Astragalus microcephalus* were collected and analyzed for RAPD markers. A total of 218 bands from eight out of thirty decamer primers were selected. The results showed that RAPDs can be used to study the systematic relationship among closely related species and sub-specific taxa (Mehrnia *et al*., 2005). Similar type of work has been done on *Podophyllum hexandrum* collected from high altitude regions of North Western Himalayas. Random Amplified Polymorphic DNA (RAPD) analysis revealed a high degree of genetic diversity among the 12 collected accessions, attributed to their geographical and climatic conditions. The study demonstrated that RAPD markers are very useful tools to compare the genetic relationship and pattern of variation among such prioritized and endangered medicinal plants (Sultan *et al*., 2010).

Variable Number Tandem Repeats **(VNTR)** A family of genetic loci found in eukaryotes consisting of short (15–100 bp) sequences of DNA repeated in tandem arrays. The alleles for any particular locus all have the same sequence but differ as to how many times the sequence is repeated. VNTR loci contribute to

repetitive DNA and have proved valuable in DNA fingerprinting, especially in forensic science. VNTRs were an important source of RFLP markers used in linkage analysis (mapping) of genomes. Now that many genomes have been sequenced, VNTRs have become essential to forensic crime investigations, via DNA fingerprinting and the CODIS database. The VNTR sequences can be released intact from a DNA sample using restriction endonuclease enzymes and identified using gene probes with Southern blotting . Alternatively, the VNTR alleles can be amplified by the polymerase chain reaction, separated by gel electrophoresis, and the resultant patterns compared without the need for special gene probes. Since each VNTR locus typically has many different alleles, the likelihood of two individuals having identical sets of alleles for even a few such loci is very remote. Hence, a DNA sample can be ascribed to a particular person with a high degree of certainty. The number of repeats can be gauged by dividing the entire molecular weight of the repeated sequence.

VNTRs are similar to Short Tandem Repeats, (STRs) the difference being that in a VNTR, the repeated sequence is no longer about 10-100 base pairs long (Rogstad, 1993). Satsuki reported the VNTR loci found in the mitochondrial DNAs (mt DNAs) of sugar beet and its close relatives. This raises the possibility that the plant mitochondrial genomes may also contain minisatellite like VNTRs. They found four unrelated tandem repeat loci (TR1, TR2, TR3 and TR4) in the mitochondrial genomes of beets with TR1 locus embedded within a three membered family of recombining repeat sequences (the rrn26- repeat) (Satsuki *et al*., 2000).

Microsatellites**, or **Simple Sequence Repeats (SSRs), are stretches of 1 to 6 nucleotide units repeated in tandem and randomly spread in eukaryotic genomes. SSRs are very polymorphic due to the high mutation rate affecting the number of repeat units. It is believed that when DNA is being replicated, errors occur in the process and extra sets of these repeated sequences are added to the strand. Over time, these repeated sequences vary in length between one cultivar and another. Such length-polymorphisms can be easily detected on high resolution gels (for example, sequencing gels), by running PCR amplified fragments obtained using a unique pair of primers flanking the repeat. Using PCR to detect DNA polymorphisms offers improved sensitivity and speed compared with standard blotting and hybridization techniques (Weber and May, 1989).

They are used as molecular markers which have wide-ranging applications in the field of genetics, including kinship and population studies. SSRs have several advantages over other molecular markers. For example, (i) microsatellites allow the identification of many alleles at a single locus, (ii) they are evenly distributed all over the genome, (iii) they are co-dominant, (iv) little DNA is required and (v) the analysis can be semi-automated and performed without the need of radioactivity. With the above mentioned features microsatellites have become the genetic markers of choice in Mammalian and many plant systems (Weber and May, 1989).

The genetic characterization of *Rhodiola rosea* L. using gene specific SSR molecular markers was performed. The phylogenetic relationships of 30 Rhodiola rosea collected from three different valleys were analyzed using 10 PCR markers (5 gene specific SSRs and 5 CAPS). The gene specific SSR analysis yielded 12 polymorphic loci, with an average of 1.8 polymorphic bands per primer. The genetic differentiation observed was considerable low, indicating a high level of

gene flow, which had a strong influence on the genetic structure. The results indicate considerable gene flow among *R. rosea* population that might be a result of seed dispersal rather than cross-pollination (Soni *et al*., 2010).

Guilford *et al*., (1997) reported that screening of an apple genomic library with (GA) 15 and (GT) 15 probes demonstrated that these repeats are abundant, occurring about every 120 and 190 kb, respectively. As few as three microsatellite markers were sufficient to differentiate between all 21 cultivars.

Table 1: Different types of markers used in the study of medicinal plants

Plant	Marker type	No. of markers	Total no. of bands	Poly-morphic fragments	Poly-morphic percentage	Average bands/ primer	Ref.
Jatropha curcas	AFLP	7 pairs	770	680	88	110	Leela *et al.* 2009
Achillea sps	AFLP	9 pairs	313	301	96.1	33.4	Rahim malek *et al.* 2009
Incarvillea young husbandii	AFLP	7 pairs	332	185	55.7	20	Zhu *et al.* 2009
Rosa damascena	AFLP	23 pairs	966	-	-	42	Baydar *et al.* 2004
Huperzia serrata	AFLP	7 pairs	615	532	86.5	38	Huang and He, 2010
Gardenia jasminoides	AFLP	11 pairs	244	165	67.6	15	Han *et al.*, 2007
Ocimum spp.	AFLP	8 pairs	253	150	59.5	31	Moghaddam *et al.*, 2011
Calathea Spp.	AFLP	6 pairs	733	497	67.8	41.1	Chao *et al.*, 2005
Oroxylum indicum	RAPD	40	387	188	49.6	9.6	Jayaram & Prasad 2008
Zeyheria montana	RAPD	9	105	65	61.9	11.6	Bertoni *et al.* 2007
Anisodus tanguticus	RAPD	12	92	76	82.6	7.67	Zheng *et al.* 2008
Swertia przewalskii	RAPD	13	166	156	94	12	Zhang *et al.* 2007
Astragalus sps	RAPD	6	98	98	100	16.3	Adiguzel *et al.* 2006
Podophylum hexandrum	RAPD	3	116	109	93.9	36.3	Sultan *et al.* 2010
Astragalus microcephalus	RAPD	8	218	218	100	27.25	Mehrnia *et al.* 2005
Pongamia pinnata	RAPD	18	210	22	10.4	1.2	Kesari & Rangan, 2011
Jatropha curcas	RAPD	23	90	53	55.8	3.9	Dhakshnamoorti *et al.*, 2011
Ocimum basilicum	RFLP	9	163	83	50.9	9.22	Labra *et al.* 2004
Rhodiola rosea	SSR	5 pairs	12	12	100	1.8	Soni *et al.* 2010
Lycium chinense	SSR	18 pairs	108	91.4	84.7	6	Zhao *et al.*, 2010
Punica granatum	SSR	12 pairs	34	12	35	3	Hasnaoui *et al.*, 2012
Liriope	SSR	67	22	8	32.8	4	Li *et al.*, 2011
Androsace tapeis	ISSR	9	60	50	83	6.6	Geng *et al.* 2009
Rhodiola chrysanthemifolia	ISSR	13	116	104	89.7	8	Xia *et al.* 2009
Rheum tanguticum	ISSR	13	329	326	92.9	25	Hu *et al.*, 2010
Chrysanthemum spp.	ISSR	22	182	148	81.8	6.7	Shao *et al.*, 2010
Magnolia officinalis	ISSR	12	137	114	83.2	9	Yu *et al.*, 2010
Heritiera littoralis	ISSR	11	117	89	76	8	Jian *et al.*, 2010

Single-nucleotide polymorphisms or **SNPs** (pronounced "*snips*") are DNA sequence variations that occur when a single nucleotide (A, T, G and C) in the genome sequence is altered. SNPs constitute the most abundant of molecular markers. The high density and mutational stability of SNPs make them particularly useful DNA markers for population genetics and for mapping candidate genes for disease resistances and abiotic stress tolerance (Alves *et al*., 2008).

For example a SNP might change the DNA sequence AAGGCTAA to ATGGCTAA. For a variation to be considered a SNP, it must occur in at least 1% of the population (Coles *et al*., 2005). SNPs can occur in both coding and non-coding regions of the genome. SNPs within a coding sequence do not necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code. SNPs that are not in protein-coding regions may still affect gene splicing, transcription factor binding, or the sequence of non-coding RNA (Rafalski *et al*., 2002). Several approaches can be used for discovery of new SNPs and about a dozen different methods are now available for SNP genotyping. Some of these methods are also suitable for automation and high throughput approaches. These methods, in principle, make a distinction between a perfect match and a mismatch (at the SNP site) between a probe of known sequence and the target DNA containing the SNP site. The target DNA in most of these methods is a PCR product, except in some cases like 'invasive cleavage assay', and 'Reduced Representation Shotgun (RRS)' devised and used recently (Gupta *et al*., 2001).

Coles *et al*. (2005) used an expressed sequenced tag library in quinoa (*Chinopodium qiuna* willd.) for the discovery of Single nucleotide polymorphism. The SNP markers identified in this work are of particular value in ongoing efforts to characterize gene expression and regulation associated with seed development, while the SNPs identified have immediate application for genetic mapping experiments, germplasm development, and the investigation of evolutionary relationships within the genus *Chenopodium.*

Short Tandem Repeats (STRs) are DNA regions with short repeat units (usually 2-6 base pairs in length). The repeated DNA sequences of a genome come in various sizes and are classified according to the length of the core repeat units, the number of contiguous repeat units, and/or the overall length of the repeat region (Gilmore *et al*., 2003). STRs are found surrounding the chromosomal centromere (the structural center of the chromosomes). STRs have proven to have several benefits that make them especially suitable for identification (Jin *et al*., 2003). The smaller size of STR alleles makes STR markers better candidates for use in forensic applications, in which degraded DNA is common (Gilmore *et al*., 2003). PCR amplification of degraded DNA samples can be better accomplished with smaller target product sizes. By examining enough STR loci and counting how many repeats of a specific STR sequence there are at a given locus, it is possible to create a unique genetic profile of an individual (Nevin *et al*., 2005). Length variations in simple sequence tandem repeats are being given increased attention in plant genetics. Some short tandem repeats (STRs) from a few plant species, mainly those at the dinucleotide level, have been demonstrated to show polymorphisms and Mendelian inheritance (Wang *et al*., 1994).

Authentication of medicinal plants:

DNA based techniques have been widely used for authentication of important medicinal plant species. Molecular biology offers an assortment of techniques that can be very useful for authentication of medicinal

plants. This is especially useful in case of those that are frequently substituted or adulterated with other species or varieties that are morphologically and/or phytochemically indistinguishable (Ngan *et al*., 1999). Some researchers verified the capacity of the AFLP approach to analyze genetic distances among *Ocimum basilicum* L. varieties and to verify the occurrence of any correlation among genetic distance, essential oil profile and morphological description (Labra *et al*., 2004). The RAPD technique has also been used for determining the components of three herbs (*Astragalus membanaceus* (Fisch.) Bge, *Ledebouriella seseloides* Wolff and *Atractylodes macrocephala* Koidz). The components in the herbal formulation have been detected using a single RAPD primer (Adiguzel *et al*., 2006).

Three RAPD primers have been identified that could successfully discriminate between three species of *Podophyllum*, from North Western Himalayas (Sultan *et al*., 2010). In another study, three random primers were used to reveal the genetic variability of *Astragalus* medicine materials sold in Taiwan market (Adiguzel *et al*., 2006). SSCP analysis was also conducted on PCR products from the ITS-1 region of rDNA in order to differentiate the two *Astragalus* species (Mehrnia *et al*., 2005). Primers have been designed for hybridization with the hyper variable ends of microsatellite loci that could reveal DNA-polymorphism among five *Eucalyptus* species (De Melis *et al*., 1999). RAPD analysis has been used for authentication of *Glycyrrhiza glabra* L. from its *Abrus precatorius* L. Fifty two decamer oligonucleotide primers were screened in the RAPD analysis for identification of genuine and adulterant samples. The obtained unique amplicons in PCR amplification clearly distinguished genuine as well as adulterant samples having similar morphology and thus, RAPD helping to serve as a complementary tool for quality control (Salim Khan *et al*., 2009).

A DNA microarray for detecting processed medicinal *Dendrobium* species (*Herba dendrobii*) was constructed by incorporating the ITS1-5.8s- ITS2 sequences of *Dendrobium* species on a glass slide. The established microarray could detect the presence of *D. nobile* in a Chinese medicinal formulation containing nine herbal components (Hao *et al*., 2010). Molecular authentication of the medicinal plant *Angelica sinensis* was carried out based on internal transcribed spacer of nrDNA. The internal transcribed spacer (ITS) regions of *A. sinensis* and seven other Angelica species used as adulterants were sequenced. A pair of specific primers was designed from the polymorphic ITS regions to distinguish *A. sinensis* from the adulterants and regional substitutes. The result showed that there were four samples adulterating *A. sinensis* with regional substitutes. This indicated that *A. sinensis* could be accurately distinguished from the adulterants and regional substitutes. Therefore, the method of molecular authentication based on the ITS sequences may be contributed to raw material production and quality control of *A. sinensis* (Feng *et al*., 2010).

Conclusion:

It can be concluded that markers differ in their ability to differentiate among individuals, in the mechanism of detecting polymorphism, in genome coverage, and in the ease of application. Therefore, they could complement one another depending on technical availability. Molecular technology provides an independent approach for the characterization of medicinal plant materials. It will be more fruitful if a concentrated effort is made to integrate the existing molecular fingerprinting data and to co-ordinate the projects of molecular characterization of medicinal plants.

References:

Abd EI-Twab M.H., and Zahran F.A. 2010. RAPD, ISSR and RFLP analysis of phylogenetic Relationships among Congeneric Species (Anthemideae, Asteraceae) in Egpyt. International Journal of Botany. 6(1): 1-10.

Adiguzel A., Agar G., Baris O., Medine G., Fikrettin S. and Meryem S. 2006. RAPD and FAME analyses of Astragalus species growing in eastern Anatolia region of Turkey. Biochemical Systematics and Ecology. 34: 424-432.

Alves D.M.T., Pereira R.W., Leal-Bertioli S.C.M., Moretzsohn M.C., Guimaraes P.M. and Bertioli D.J. 2008. Development and use of single nucleotide polymorphism markers for candidate resistance genes in wild peanuts (*Arachis* spp). Genetics and Molecular Research. 7(3): 631-642.

Arif I.A., Bakir M.A., Khan H.A., Alfarhan A.H., Al Homaidan A.A., Bahkali A.H., Alsadoon M. and Shobrak M. 2010. Application of RAPD for molecular characterization of plant species of medicinal value from an arid environment. Genetics and Molecular research. 9(4): 2191-2198.

Bayder N.G., Bayder H. and Debener, T. 2004. Analysis of genetic relationships among *Rosa damascena* plants grown in Turkey by using AFLP and microsatellite markers. Journal of Biotechnology. 111: 263-269.

Bert P.F., Charmet G., Sourdille P., Hayward M.D. and Balfourier F. 1999. A high-density molecular map for ryegrass (*Lolium perenne*) using AFLP markers. Theoretical and Applied Genetics. 99: 445-452.

Bertoni B.C., Filho S.A., Martins E.R., Filhi C.F.D., Franca S.C., Pereira A.M.S., Telles H.P.C. and Filho J.A.D. 2007. Genetic variability in natural populations of *Zeyheria Montana* Mart. From the Brazilian cerrado. Scientia Agricola. 64(4): 409-415.

Chao T. C, Devanand P.S. and Chen J. 2005. AFLP analysis of genetic relationships among*Calathea* species and cultivars. Plant Science. 168: 1459-1469.

Coles N.D., Coleman C.E., Christensen S.A., Jellen E.N., Stevens M.R., Bonifacio A., Rojas-Beltran J.A., Fairbanks D.J. and Maughan P.J. 2005. Development and use of an expressed sequenced tag library in quinoa (*Chenopodium quinoa* Willd.) for the discovery of single nucleotide polymorphisms. Plant Science. 168: 439-447.

Dhakshanamoorthy D, Radhakrishnan S and Chidambaram A.L.A. 2011. Induced mutagenesis in *Jatropha curcas* L. using gamma rays and detection of DNA polymorphism through RAPD marker. C. R. Biologies. 334: 24–30.

Dorothea M., Richard M., Brian G.L., Vos P. and Richard P.O. 1996. The use of AFLP finger printing for the detection of genetic variation in Fungi. Mycological Research. 100(9): 1107-1111.

Feng T., Shuang L. and Xing-jin H. 2010. Molecular authentication of the traditional Chinese medicinal plant *Angelica sinensis* based on internal transcribed spacer of nrDNA. Electronic Journal of Biotechnology. 13(1): 1-10.

Fevzi B. 2001. Random Amplified Polymorphic DNA (RAPD) Markers. Turkish Journal of Biology. 25: 185-196.

Geng Y., Tang S., Tashi T., Song Z., Zhang G., Zeng L., Zhao J., Wang L., Shi J., Chen J. and Zhong Y. 2009. Fine and land scape-Scale spatial genetic structure of cushion rock jasmine, *Androsace tapete* (Primolaceae), across southern Qinghai-Tibetan plateau. Genetica. 135: 419-427.

Gilmore S., Rod P. and James R. 2003. Short tandem repeat (STR) DNA markers are hypervariable and informative in Cannabis sativa: implications for forensic investigations Forensic Science International. 131. 65-74.

Gnavi G, Cinzia M.B., Maranna Usai and Massimo E. Maffei. 2010. Comparative characterization of *Santolina insularis* chemotypes by essential oil composition, 5s-rRNA-NTS sequencing and *Eco*RV. Phytochemistry. 71: 930-936.

Guilford P., Prakash S., Zhu J.M., Rikkerink E., Gardiner S., Bassett H. and Forster R. 1997. Microsatellites in *Malus X domestica* (apple): abundance, polymorphism and cultivar identification Theoretical and Applied Genetics. 96: 1069-1076.

Gupta P.K., Roy J.K. and Prasad M. 2001. Single nucleotide polymorphisms: A new paradigm for molecular marker technology and DNA polymorphism detection with emphasis on their use in plants. Current Science. 80(4): 524-535.

Han J, Zhang W, Cao H, Chen S and Wang Y. 2007. Genetic diversity and biogeography of the traditional Chinese medicine, *Gardenia jasminoides*, based on AFLP markers. Biochemical Systematics and Ecology. 35: 138-145.

Hao D.C., Shi-lin C., Pei-gen X. and Yong P. 2010. Authentication of Medicinal Plants by DNA-based Markers and Genomics. Chinese Herbal Medicines. 2(4): 250-261.

Hasnaoui N, Anna B, Sebastini F, Mars M, Zhang D and Vendramin G. 2012. Molecular genetic diversity of *Punica granatum* L. (pomegranate) as revealed by microsatellite DNA markers (SSR). Gene 493: 105–112.

Haung J and He C. 2010. Population structure and genetic diversity of *Huperzia serrata* (Huperziaceae) based on amplified fragment length polymorphism (AFLP) markers. Biochemical Systematics and Ecology. 38: 1137-1147.

Hu Y, Wang L, Xie X, Yang J, Li Y and Zhang, H. 2010. Genetic diversity of wild populations of *Rheum tanguticum* endemic to China as revealed by ISSR analysis. Biochemical Systematics and Ecology 38: 264–274.

Huang C, Liu G, Bai C, Wang W, Zhou S and Yu D. 2010. Estimation of genetic variation in *Cynodon dactylon* accessions using the ISSR technique. Biochemical Systematics and Ecology. 38: 993-999.

Huang Y, Li F and Chen K. 2010. Analysis of diversity and relationships among Chinese orchid cultivars using EST-SSR markers. Biochemical Systematics and Ecology. 38: 93-102.

James L.W. and Paula E.M. 1989. Abundant Class of Human DNA Polymorphisms Which Can Be Typed Using the Polymerase Chain Reaction. The American Journal of Human Genetics. 44: 388-396.

Jana H. and Eva C. 1997. RFLP analysis of *Hypericum perforatum* L. somaclones and their progenies. Euphytica. 95: 229-235.

Jayaram K. and Prasad M.N.V. 2008. Genetic diversity in *Oroxylum indicum* L. Vent. (Bignoniaceae), a vulnerable medicinal plant by random amplified polymorphic DNA marker. African journal of Biotechnology. 7(3): 254-262.

Jian S. G, Tang T, Zhong Y and Shi S.H. 2010. Conservation genetics of *Heritiera littoralis* (Sterculiaceae), a threatened mangrove in China, based on AFLP and ISSR markers. Biochemical Systematics and Ecology 38: 924–930.

Kalpana J., Warude P.C. and Bhushan P. 2004. Molecular markers in herbal drug technology. Current Science. 87(2): 159-165.

Kesari V and Rangan L.2011. Genetic diversity analysis by RAPD markers in candidate plus trees of *Pongamia pinnata,* a promising source of bioenergy. Biomass and Bioenergy. 35: 3123-3128.

Labra M., Mariangela M., Bernardetta L., Fabrizio G., Mauro M. and Francesco S. 2004. Morphological characterization, essential oil composition and DNA

genotyping of *Ocimum basilicum* L. cultivars. Plant Science. 167: 725-731.

Leela T., Suhas P.W., Seetha K., Naresh B., Thakur K.S., David A.H., Prathibha Devi and Rajeev K.V. 2009. AFLP-based molecular characterization of an elite germplasm collection of *Jatropha Curcas* L., a biofuel plant. Plant Science. 176: 505-513.

Li G, Ra W H, Park J, Kwon S, Lee J, Park C and Park Y. 2011. Developing EST-SSR markers to study molecular diversity in *Liriope* and *Ophiopogon*. Biochemical Systematics and Ecology. 39: 241-252.

Lu Z, Wang Z, Peng Y, Korpelainen H and Li C. 2005. Genetic diversity of *populus cathayana* Rehd populations in southwestern china revealed by ISSR markers. Plant Science. 170: 407-412.

Mehrnia M., Shahin Z. and Ashrafoldin S.S. 2005. Intra- and inter-specific relationships within the *Astragalus microcephalus* complex (Fabaceae) using RAPD. Biochemical Systematics and Ecology. 33: 149-158.

Moghaddam M, Reza O and Rezi M.N. 2011. Evaluation of genetic diversity among Iranian accessions of *Ocimum* spp. using AFLP markers. Biochemical Systematics and Ecology. 39: 619-626.

Nevin D.C., Wilson-Wilde L., Robertson J., Jennelle K. and Chris L. 2005. Short tandem repeat (STR) genotyping of keratinised hair Part 2. An optimised genomic DNA extraction procedure reveals donor dependence of STR profiles. *Forensic* Science International. 153: 247-259.

Ngan F., Shaw P., But P. and Wang J. 1999. Molecular authentication of *Panax* species Phytochemistry. 50: 787-791.

Qingsheng J., Dan W., Giovanni M.C., Robert J.H. and Russell F.R. 2003. A single nucleotide polymorphism (SNP) marker linked to the fragrance gene in rice (*Oryza sativa* L.) Plant Science. 165: 359-364.

Rahimmalek M., Badraldin E., Sayed T., Ahmad A. and Nematolah E. 2009. Assessment of genetic diversity among and within *Achillea* species using Amplified Fragment Length Polymorphism (AFLP). Biochemical Systematics and Ecology. 37: 354-361.

Salim Khan, Khanda J.M., Md Tayaab and Malik Z.A. 2009. RAPD profile for authentication of Medicinal plant *Glycyrrhiza glabra* L. Internet Journal of Food safety. 11: 24-28.

SalimKhan, Khanda J.M. and Malik Z.A. 2010. Development of RAPD markers for authentication of medicinal plant *Cuscuta reflexa.* Eurasian Journal of Biosciences. 4: 1-7.

Satsuki N., Tomohiko K. and Tetsuo M. 2000. Variable number of tandem repeat loci in the mitochondrial genomes of beets. Current genetics. 37: 3-38.

Satya Narayan J., Susheel Kumar and Narayanan K. Nair. 2009. Molecular phylogeny in Indian *Citrus* L. (Rutaceae) inferred through PCR-RFLP and trnL-trnF sequence data of chloroplast DNA. Scientia Horticulturae. 119(4): 403-416.

Shao Q.S, Guo Q.S, Deng Y.M, and Guo H.P. 2010. A comparative analysis of genetic diversity in medicinal *Chrysanthemum morifolium* based on morphology, ISSR and SRAP markers. Biochemical Systematics and Ecology 38: 1160–1169.

Sharma A., Ajay G., Namdeo. and Mahadik K.R. 2008. Molecular Markers: New Prospects in Plant Genome Analysis. Pharmacognosy Reviews. 2(3): 23-24.

Soni K., Shruti R., Ankit Gupta, Karma Y., Saurabh Pandit., Pradeep K.N. and Harvinder Singh. 2010. Genetic Characterisation of *Rhodiola rosea* Using Gene Specific SSR and CAPS Molecular Markers. Genetic Engineering & Biotechnology Journal. 11: 1-10.

Srivatsava S. and Nidhi M. 2009. Genetic Markers – A Cutting Edge Technology in

Herbal Drug Research. Journal of Chemical and Pharmaceutical Research. 1(1): 1-18.

Steven H. Rogstad. 1993. Surveying Plant Genomes for Variable Number of Tandem Repeat Loci. Methods in Enzymology. 224: 278-294.

Sultan P., Shawl A.S., Rehman S., Fayaz Ahmed S. and Ramteke P.W. 2010. Molecular characterization and marker based chemotaxonomic studies of *Podophyllum hexandrum* Royle. Fitoterapia. 81: 243-247.

Terashima K., Teruyuki M., Eiji H. and Yukitaka F.N. 2002. A Genetic Linkage Map of *Lentinula edodes* (Shiitake) based on AFLP markers. Mycological Research. 106(8): 911-917.

Vos P., Hogers R., Bleeker M., Reijans M., Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M. and Zabeau M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research. 23(21): 4407-4414.

Vyskot B., Fajkus J., Kuglik P., Blazena K. and Viera K. 1991. Genome modifications in protoplast-derived tobacco plants: Phenotypic evaluation and RFLP Analysis. Biologia Plantarum. 33(6): 455-460.

Wang Z., Weber J.L., Zhong G. and Tanksley S.D. 1994. Survey of plant short tandem DNA repeats. Theoretical and Applied Genetics. 88: 1-6.

Wu Y, Guo Q, He J, Lin Y, Luo L and Liu G. 2010. Genetic diversity analysis among and within populations of *Pogostemon cablin* from China with ISSR and SRAP markers. Biochemical Systematics and Ecology. 38: 63-72.

Xia T., Shilong C., Shengyun C., Zhang D., Zhang D, Gao Q. and Ge X. 2007. ISSR analysis of genetic diversity of the Qinghai-Tibet plateau endemic *Rhodiola chrysanthemifolia.* Biochemical systematics and Ecology.35: 209-214.

Xie W, Zhang X, Cai H, Liu W and Peng Y. 2010. Genetic diversity analysis and transferability of cereal EST-SSR markers to orchardgrass (*Dactylis glomerata* L.) Biochemical Systematics and Ecology. 38: 740-749.

Yongzhu, Yupeng G., Tashi T., Nian L., Qingbiao W. and Yang Z. 2009. High Genetic differentiation and low genetic diversity in *InCarvillea young husbandii,* an endemic plant of Qing ha-Tibetan plateau, revealed by AFLP markers. Biochemical Systematics and Ecology. 37: 589-596.

Yu H.H, Yang Z. L, Sun B and Liu R. 2011. Genetic diversity and relationship of endangered plant *Magnolia officinalis* (Magnoliaceae) assessed with ISSR polymorphisms. Biochemical Systematics and Ecology. 39: 71-78.

Zhang D., Shilong C., Shengun C., Zhang D., Gao Q. 2007. Patterns of genetic variation in *Swertia przewalskii*, an endangered endemic species of the Qinghai-Tibet plateau. Biochemical Genetics. 45: 33-50.

Zhao W.G, Chung J W,Cho Y, Rha W H, Lee G A, Ma K H, Han S H, Bang K H, Park C B, Kim S M and Park Y. 2010. Molecular genetic diversity and population structure in *Lycium* accessions using SSR markers. C. R. Biologies. 333: 793-800.

Zheng W., Wang L., Meng L. and Liu J. 2008. Genetic variation in the endangered *Anisodus tanguticus* (Solanaceae), an alpine perennial endemic to the Qinghai-Tibetan Platueae. Genetica. 132: 123-129.