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Application of ISSR marker in pharmacognosy: current update

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Abstract:
ISSR (Inter Simple Sequence Repeat) is one of the popular techniques of DNA fingerprinting because of several reasons. In many fields, ISSR markers have proved their utility. There are many applications of ISSR in various aspects of medicinal plants. ISSR based markers have utility in the fields like genetics, taxonomy, physiology, embryology etc. and recently the ISSR based markers have found wide applicability in pharmacognostic characterization of medicinal plants. As use of herbal medicines is increasing, there is urgent need of newer technologies and its proper application. In recent years, pharmacognosy has witnessed advent of such new technologies. This review provides detail list of plants, which are studied by ISSR marker and discuss some of the important application in medicinal plant research.
Key words: ISSR, molecular marker, genotyping, genetic diversity, authentication.
Abbreviations: PCR**-** Polymerase Chain Reaction, ISSR- Inter Simple Sequence Repeat**,** RAPD**-** Random Amplification Polymorphic DNA, AFLP-Amplified Fragment Length Polymorphism, SSR- Simple Sequence Repeats, SAMPL-Selective Amplification of Microsatellite Polymorphic Loci.

INTRODUCTION

After discovery in eighties, PCR has become prime importance of any molecular biology laboratory. At same time, application of PCR in various areas related to plants has increased substantially. ISSR (Inter Simple Sequence Repeat) is one of the popular techniques, which become popular because of several reasons. Till date ISSR is widely used for study of plants. Molecular markers such as Random Amplification of Polymorphic DNA (RAPD), Inter-Simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) have been successfully used to assess the genetic diversity in cultivars of many plant species. In this review, we have focused on application of ISSR markers. ISSR markers overcome the shortcomings of the low reproducibility of RAPD; the high cost of AFLP, the complexity of SSR and represent a fast and a cost-efficient technique. The differences that distinguish one plant from another are sequence in the deoxyribonucleic acid (DNA).

In biology and medicine, a molecular marker (biomarker) can be a substance native to the organism whose detection indicates a particular disease state for example; the presence of an antibody may indicate an infection. A molecular marker or genetic marker is a fragment of DNA sequence that is associated to a part of the genome. Molecular markers are used in molecular biology and biotechnology experiments where they use to identify a particular sequence of DNA. As the DNA sequences are very highly specific, they can be identified with the help of the known molecular markers, which can find out a particular sequence of DNA from a group of unknown.

A genetic marker can be defined in one of the following ways; (a) a chromosomal landmark or allele that allows for tracing of a specific region of DNA; (b) a specific piece of DNA with known position on the genome or (c) gene whose phenotype expression is usually easily discerned, used to identify an individual or a cell that carries it, or as a probe to mark a nucleus, chromosome or locus. Since the markers and the genes that mark are close together on the same chromosome, they tend to stay linked as each generation of plants is produced.

TYPES OF MARKER USED

Markers can be broadly classified as follows:

CLASS OF MARKER	LEVEL OF ANALYSIS
Morphological	Phenotype
Biochemical	Gene product
Molecular	DNA profiling

TYPES OF DNA MARKERS

Generally accepted classification of markers is as follows (1,2):

1. Hybridization based markers

Restriction Fragment Length Polymorphism (RFLP)
Variable Number Tandem Repeat
Probes for Micro and Minisatellite
Random Genomic Clone
cDNA Clone

2. PCR based markers

Inter Simple Sequence Repeat
Random Amplification Polymorphic DNA
Amplified Fragment Length Polymorphism
DNA Amplification Fingerprinting
Arbitrary Primed PCR

3. Sequence based marker

Simple Sequence Repeats (SSR)
Sequence Characterized Amplified Region (SCAR)
Cleaved Amplified Polymorphic Sequence (CAPS)
Single Nucleotide Polymorphism (SNP)

ISSR AS A MOLECULAR MARKER

The first studies employing ISSR markers were published in 1994. The initial studies focused on cultivated species and demonstrated the hypervariable nature of ISSR markers. To test the utility of the method in natural populations reexamined a known hybrid complex of four species of *Penstemon* for which three other molecular data sets were available. Their results clearly demonstrated the utility of ISSR markers for addressing questions of hybridization and diploid hybrid speciation. ISSR techniques are nearly identical to RAPD techniques except that ISSR primer sequences are non random designed from microsatellite regions and the annealing temperatures used are higher than those used for RAPD markers, clearly overcoming the limitations of RAPD. Based on the published, unpublished and in-progress studies that have been conducted using ISSR markers it is clear that, ISSR markers have great potential for studies of natural populations.

The basic premise of ISSRs is that primer-annealing sites are distributed evenly throughout the genome such that the primer will anneal to two sites orientated on opposing DNA strands. If these are within an appropriate distance of one another, the region between the two primers will be amplified through PCR. The region would not be amplified if there were divergence at the primer binding sites, if a binding site was lost or if structural rearrangements of the chromosomes had occurred. ISSRs are now being applied to natural populations to address issues such as hybridization. These studies have demonstrated the utility of the technique in a wide range of applications and plant families (Asteraceae, Brassicaceae, Hippocastanaceae, Orchidaceae, Poaceae, Scrophulariaceae, Violaceae).

Compared with other molecular marker such as RAPD, AFLP and SSR, the ISSR marker has its specific advantages, 1) No prior sequence information required 2) Simple and quick operation 3) Amenable to laboratory level 4) High stability 5) Abundance of genomic information 6) Use of radioactivity is not required 7) Show high polymorphism. ISSR marker accesses variation in the numerous microsatellite regions dispersed throughout the various genomes (particularly the nuclear genome) and circumvents the challenge of characterizing individual loci that other molecular approaches require. Microsatellite are very short (usually 10-20 base-pair) stretches of DNA that are "hypervariable", expressed as different variants within populations and among different species. They are characterized by mono-, di- or trinucleotide repeats e.g. AA… or AG… CAG… that have 4-10 repeat units side-by-side. In ISSRs, we specifically target the di- and trinucleotide repeats types of microsatellite because; these are characteristic of the nuclear genome.

The ISSR primers we use to generate the variation in a given DNA sample include one of these highly variable microsatellite sequences and an arbitrary pair of bases at the 3' (rear) end. One samples for variation among DNA samples in small PCR (polymerase chain reaction) reactions using one primer at a time. Where the primer successfully locates two microsatellite regions within an amplifiable distance away on the DNA strands of the DNA sample (the "template DNA") the PCR reaction will generate a band of a particular size (=molecular weight) for that "locus" representing the intervening stretch of DNA between the microsatellites. Usually several too many such "paired" microsatellite areas exist in a particular DNA sample, so one gets that many bands generated in the reaction for that sample. Some of the interesting applications of ISSR are summarized as follows.

APPLICATION OF ISSR MARKERS

Every herbal formulation must be standardized as per WHO guidelines. The objective of WHO guidelines is to define basic criteria for the evaluation of quality, safety and efficacy of drugs herbal medicines. India is one of the world's twelve leading biodiversity centers with the presence of over 45,000 different plant species, out of this about 15,000- 20,000 plants have good medicinal properties of which only about 7,000-7,500 are being used by traditional practitioners. The ISSR based markers have utility in the fields like genetics, taxonomy, physiology, embryology etc. Recently the ISSR based markers have wide applicability in pharmacognostic characterization of medicinal plants.

GENETIC DIVERSITY

Genetic diversity refers to any variation in the nucleotides, genes, chromosomes or whole genomes of the plant. A great degree of genetic diversity exists in non cultivated medicinal plants across the geographical scale, which can be assessed by ISSR marker. Assessment of genetic diversity is one of the main applications of ISSR marker and may be used for medicinal plants related research.

Inter simple sequence repeat (ISSR) markers were used to analyze genetic diversity of *Swertia chirayita* genotypes collected from the temperate Himalayas of India. Allied species of *Swertia chirayita* were used in the study as outliers. Nineteen primers generated a total of 315 ISSR bands, revealing 98.7 % polymorphism among the genotypes assayed. The results revealed a high genetic diversity within the genotypes studied

(3). Fourteen ISSR primers were screened and optimized for detecting the genetic diversity in wild populations of *Glycyrrhiza uralensis* Fisch. By using these primers, 249 polymorphic bands out of a total of 270 (92.2 %) were generated from 70 individuals of 4 wild *G. uralensis* populations (4). The comparison of genetic diversity in *Humulus lupulus* was done using RAPD, STS, ISSR and AFLP molecular methods. Thirty-five RAPD generated 42.3% of polymorphism. Ten STS primer combinations generated 71% of polymorphism. Seven ISSR microsatellite polymorphic primers and seven primer combinations generated 32.6% of polymorphism. A total of 56 AFLP primer combinations generated a 57.6% of polymorphism. All molecular methods accurately distinguished all tested varieties, except for Osvald's clones, which were only distinguished by AFLP (5). Genetic diversity and geographic differentiation of disjunct *Psychotria ipecacuanha* (Rubiaceae) was done by using ISSR markers. Of the 193 bands produced by ISSR primers, 188 (97.4%) were polymorphic at the species level (6). Genetic diversity and hybrid performance of ash gourd *Benincasa hispida* inbred lines based on RAPD and ISSR markers. 42 primers tested four produced monomorphic bands. Thirty-eight primers, which were found to produce intensely stained, polymorphic and reproducible bands. Five ISSR were generated 26 markers bands of which 11 were polymorphic (7). Genetic diversity of cashew germplasm was done using RAPD and ISSR markers, in which 10 selected RAPD primers generated 60 bands of which 51 were polymorphic and with 10 selected ISSR primers 67 bands generated of which 58 were polymorphic showing 86.6% PPB (8). AFLP, selective amplification of microsatellite polymorphic loci (SAMPL), ISSR and RAPD markers were used for the detection of genetic polymorphism in *Tribulas terrestris* (Zygophyllaceae) medicinal herb from samples collected from various geographical regions of India. Six assays each of AFLP and SAMPL markers and 21 each of ISSR and RAPD markers were utilized. AFLP yielded 500 scorable amplified products, of which 82.9% were polymorphic. SAMPL primers amplified 488 bands, of which 462 being polymorphic (94.7%). The ISSR primers amplified 239 bands of which 73.6% showed polymorphism. RAPD assays produced 276 bands, of which 163 were polymorphic (PPB 59%) (9). In another study characterization of surf clam *Mactra veneriformis* was done using ISSR-PCR markers. In which 20 primers showed 240 loci of which 235 were polymorphic (PPB 97.9%) over coming limitation of allozyme and RAPD technique (10). ISSR marker also been used to estimate genetic diversity within and among 10 populations of *Rhodiola chrysanthemifolia* (Crassulaceae). 13 primers screened among 100 showing 116 discernible DNA fragments were generated of which 104(PPB 89.7%) were polymorphic (11). Genetic diversity in Indian bitter gourd (*Momordica charantia* L.) was done using RAPD and ISSR markers. Examining 38 M. charantia accessions with 29 RAPD primers, of which 76 were polymorphic, produced a total of 208 amplicons. While, fifteen ISSR primers produced on average 125 bands in the accessions examined, of which 94 (74.7%) were polymorphic (12). The genetic diversity *Vanilla planifolia* (Orchidaceae) was done by using ISSR primers. A total of 185 reproducible bands were produced by using 20(out of 30) RAPD primers. Out of these, 154 (83.24%) were found to be polymorphic.10 (out of11) ISSR primers were amplified 108 markers bands were generated of which 93 were polymorphic (86.11%) (13).

AUTHENTIFICATION OF PHARMAGNOSTIC PLANTS

ISSR markers have been to authenticate various medicinal important plants. Because various medicinal plants have been adulterated intentionally or unintentionally. Correct botanical identification is possible by the use of ISSR marker, so that better quality herbal drugs can be used. This can be used for detection of adulteration thereby helping quality control. There are many examples of use of ISSR markers in pharmacognosy.

Authentification of most popular mushroom *Flammulina velutipes* was done using strain specific sequence characterized amplified region (SCAR) developed from markers. 8 primers selected from 20 amplified 104 clear and stable bands, of which 81 were polymorphic (14). Development of SSR markers in eucalyptus spp from amplified ISSR in the identification of clones of *Eucalyptous* spp. was previously done by RAPD. The result was not repeatable between laboratories. So, microsatellites markers were developed to fingerprinting of *Eucalyptous*. The primers thus developed were able to amplify the corresponding microsatellite loci from five different spp. namely *E. grandis*, *E. nitens, E. globulus, E. camaldulenssis* and *E. urophylla.* DNA profiling of disputed chilli samples (*Capsicum annum*) was done using ISSR-PCR and FISSR-PCR marker. A total number of 17 ISSR anchored primers produced a total of 212 and 288 bands were resolved by seven di- and eight tri-nucleotide primers respectively. Five out of nine dinucleotide primers and four out of eight trinucleotide primers could unambiguously differentiate all the four disputed Chilli samples. The FISSR-PCR assay revealed a total number of 566 bands using three tri- and one di-nucleotide primers. These four primers could reliably distinguish all the four disputed samples unambiguously (15).

IDENTIFICATION OF PLANT

ISSR markers are proving very useful for correct botanical identification. They can clearly distinguish intra and inter species variation. There are several studies in which these markers are used for species or cultivar identification.

Identification of Mediterranean Diplodus spp. and *Dentex dentex* (Sparidae) was done by means of ISSR markers. The 8 primers used amplified a total of 97 fragments, 95 of which species that were assigned by genotype to a particular species (97.9%) were polymorphic (16). RAPD, ISSR and SRAP (sequence-related amplified polymorphism), were employed for identification and genetic diversity analysis of 35 elite late-bolting radish cultivars. Detected by 35 RAPD primers, 22 ISSR primers and 17 SRAP primer combinations, the proportions of polymorphic bands were 85.44%, 85.2% and 85.41% respectively and the mean genetic similarity coefficients between pairs of genotypes were 0.781, 0.787 and 0.764 respectively. Each of the three molecular marker systems can identify all the cultivars. The level of

polymorphism in tomato (*Lycopersicon esculentum*) was studied using ISSR-PCR. Five tomato species: *Lycopersicon esculentum, Lycopersicon pennellii, Lycopersicon cheesmanii, Lycopersicon humboldtii, Lycopersicon hirsutum* and two *Lycopersicon esculentum* substitution lines IL 6-3 and WSL 6 were analyzed. ISSR-PCR was performed with fourteen primers. Nine of these fourteen primers were individually able to distinguish all tomato species (17). ISSR analysis was performed in eight cultivars of eggplant (*Solanum melongena*) and 12 accessions in eight related Solanum species to evaluate the applicability of this analysis for assessing the phylogenetic relationships and identifying cultivars. A total of 552 polymorphic amplified bands were obtained from 34 of the 100 primers tested and the percentage of polymorphisms was 99.1 % (18).

GERMPLASM AUTHENTIFICATION

Over the last decades, the significance of studies on clonal plants has been widely appreciated and great progress has been made in researching the morphology, physiology, ecology and evolution of clonal plants. To date, much of the interest in clonal plants has focused on how their pattern of development influences the way they grow, how they capture resources and how they respond to environmental variation in space and time. Although allozyme analysis has long been used to identify clones and to study population genetics of clonal plants, it usually underestimates genetic polymorphism and has a limited ability to distinguish genetic individuals. In recent years, a number of PCR-based DNA markers such as RAPD, SSR and ISSR have been widely used to investigate clonal diversity and population genetic structure because they overcome the limitations of allozyme markers.

Clonal fidelity of micropropagated Gerbera (*Gerbera jamesonii*) plants was done by using ISSR markers. Out of 15 ISSR primers, 12 primers showed monomorphic banding pattern within in vitro raised clones and the mother plant. Whereas, polymorphic bands (one in each primer) were detected with three ISSR primers (19). Tung tree (*Vernicia fordii*) is an important woody oil-rich plant in the world. In order to determine the genetic diversity, germplasm resource and breeding method on Tung tree, ISSR was used to investigate the cultivars in China. Among the total 110 bands amplified with 12 primers, 90 were polymorphic. Both UPGMA cluster and PCA showed clear genetic relationship among the 64 Tung cultivars (20). Genetic diversity analysis of 20 exotic germplasm lines and 20 commercial varieties of the two cultivated species (*Corchorus olitorius* and *C. capsularis*) and two wild relatives of jute (*C. aestuans* and *C. trilocularis*) was carried out using sequence tagged microsatellite site (STMS), ISSR and RAPD markers. The four ISSR and 22 RAPD primers employed in the study revealed 98.44% and 100% polymorphism respectively across all the species, while the level of polymorphism was significantly low within a species (21). In coming years, there can be many applications to this field.

GENOTYPING OF PLANT

Non-random distribution of genetic variation usually refers to the genetic structure of a population. Geographic distance is generally regarded as an important influence on both genetic structure and gene flow because; distance confines the movements of gametes, propagules and individuals that change the spatial distributions of genes. The relation of genetic variation and distance scales has been studied for populations of many organisms, including some species of seaweed. A number of DNA markers such as RAPD, AFLP, SSR and ISSR have been used in studies of the genetic structure of populations. Apart from this these markers can be useful for correlation of plant genotype and chemotype, which may prove very useful for checking quality of medicinal plants. Twelve individual genotypes selected from Junisperus populations, varieties and species were analyzed using ITS sequences, RAPDs, ISSRs and leaf volatile terpenoids. These four data sets illustrated that, these data sets can be used at different organizational levels: specific, inter-specific and intraspecific (22). ISSR analysis was used to investigate genetic variations of 184 haploid and diploid samples from nine North Atlantic *Chondrus crispus* population. Twenty-two of 50 primers were selected and 163 loci were scored for genetic diversity analysis. Genetic diversity varied among populations, percentage of polymorphic bands (PPB) ranged from 27.0 to 55.8 % (23). A genetic linkage map of cucumber (*Cucumis sativus* L.) consisting of 116 SRAPs, 33 RAPDs, 11 SSRs, 9 SCARs, 3 ISSRs, and 1 STS, was constructed using 130 F2 progeny derived from a narrow cross between line S94 (Northern China open-field type) and line S06 (greenhouse European type) (24). To assess the genetic integrity of the nuclear, mitochondrial and chloroplast genomes among the hardened regenerants of coffea arabica, the use of DNA markers (RFLP, RAPD, ISSR) for sampling various regions of the genome. A total of 480 genetic loci based on the data obtained from a total of 16 nuclear, mitochondrial and chloroplast gene probes in combination with nine restriction enzyme digests, 38 RAPD and 17 SSR primers were scored in 27 somatic embryo-derived plants and the single control. Among these, 44 loci were observed to be polymorphic. A relatively low level of polymorphism (4.36%) was found in the nuclear genome, while polymorphism in the mitochondrial genome (41%) was much higher (25).

We have summarized details of plants, which are studied by ISSR technology and we hope this list would be useful for further research in the field.

Table 1: Detail list of plant and ISSR marker used for various studies.

Plant (Family)	Marker Used	Result	Reference
Heptacodium miconioides (Caprifoliaceae)	ISSR	Genetic diversity and differenciation among 9 population of *Heptacodium miconioides* were done using	(26)

		ISSR showing high genetic diversity at species level and low at population level.	
Citrus Germplasm	ISSR	ISSR markers were used to study phylogenetic relationship among 33 citrus genotypes using 6 primers showing 234 scorable fragments of which 209 were polymorphic.	(27)
Ceratopteris pteridoides (Perkeriaceae)	ISSR	Genetic diversity is revealed by 65-ISSR primer on 6 randomly selected individuals of which 13 primer shows clear 125 bands of which 56 bands were polymorphic among 72 individuals.	(28)
Sinojackia dolichocarpa (Styraceae)	ISSR	ISSR markers were used to investigate genetic diversity within four natural populations of *S. dolichocarpa*. Leaf samples were collected from 84 individuals. Thirteen ISSR primers selected from 80 give rise to 137 discernible DNA bands, of which 100 were polymorphic.	(29)
Populus cathayana	ISSR	Genetic diversity of *Populus cathayana* was done using eight ISSR primer showed 158 reproducible bands, of which 156 were polymorphic.	(30)
Psammochloa villosa (Posacaae)	ISSR	Genetic variation and clonal diversity of seven *P. villosa* populations was done using ISSR marker. Of the 84 primers screened, 12 produced 173 discernible bands, of which 122 being polymorphic.	(31)
Apterosperma oblata (Theaceae)	ISSR	ISSR markers were used to assess genetic variation and relationship. Twenty one primers generated 168 loci, of which 130 were polymorphic.	(32)
Houttuynia thunb	RAPD, ISSR	Polymorphism of *Houttuynia* germplasm was done using 34 RAPD and 22 ISSR primer showed 199 and 352 bands respectively. RAPD showed 92.9% and ISSR 92.3% polymorphic bands.	(33)
Isoetes hypsophila (Isoetaceae)	ISSR	Genetic diversity of 56 individuals of *I. Hypsophila* was done using twelve ISSR primers selected from sixty-five primers showed 119 bands, of which 82% polymorphic.	(34)
Zinnia elegans	RAPD, ISSR	Genetic diversity of *Z. elegans* was studied using 12 RAPD and 9 ISSR marker showed 147 and 128 polymorphic bands respectively.	(35)
Leersia hexandra (Poaceae)	ISSR	Genetic diversity of *L. hexandra* was studied using 12 ISSR primer showed 175 loci of which 165 were polymorphic.	(36)
Eichhornia crassipes (Pontederiaceae)	RAPD, ISSR	RAPD and ISSR were used to analyze genetic structure of six populations of invasive plant *E. crassipes* using 25 RAPD and 18 ISSR primers. 172 RAPD and 145 ISSR bands were produced but no polymorphic band showing low genetic diversity in *E. crassipes*.	(37)
Caldesia grandis (Alismataceae)	RAPD, ISSR	Although RAPD is more informative than ISSR in case of *C. grandis*. Both RAPD and ISSR detect clonal diversity.	(38)
Phaseolus vulgaris	ISSR, AFLP	78 common bean genotypes were screened for ISSR and AFLP marker. 13 ISSR primers showed 150 bands, of which 50 were polymorphic. While 3 AFLP primers showed 164 bands, of which 54 were polymorphic.	(39)
Astyanax fasciatus (Teleostei, Characidae)	RAPD and ISSR	10 RAPD and 4 ISSR chosened for fingerprinting. The amplification resulted in 35 RAPD loci being 88.14% polymorphic. The ISSR amplification resulted in 35 loci being 91.43 polymorphic.	(40)
Polylepsis australis (Rosaceae)	RAPD, ISSR	RAPD and ISSR were showed diminished gene flow due to recent fragmentation of *polylepis australis*.	(41)

Genus *Sonneratia* (Sonneratiaceae)	ISSR	ISSR marker was used to establish relationship among six *Sonneratia* species. A total 11 primers selected out of 100 showed 480 bands among which 481 polymorphic	(42)
Vicia faba	ISSR	The ISSR markers were used to describe the genetic diversity of greek faba bean. 4 primers used out of 11 showed 192 loci of which 190 were polymorphic.	(43)
Elymus sibiricus (Poaceae, Tritiaceae)	ISSR	ISSR marker was used to assess the genetic diversity and population structure in eight populations of *Elymus sibiricus*. Of the 100 primers screened 13 produced 193 reproducible bands of which 149 were polymorphic.	(44)
Pelargonium reniforme (Geraniaceae)	ISSR	ISSR markers support the recognition of two floral forms of *Pelargonium reniforme*. Of the 9 primers evaluated, 5 were produced 76 bands showed 100% polymorphic bands.	(45)
Gynostemma pentaphyllum (Cucurbitaceae)	ISSR	The genetic diversity of *G. pentaphyllum* was examined using ISSR marker. 14 primers amplified 194 loci, showed 1.03 to 25.26% polymorphic bands at population level, while this value rose to 96.36 at species level.	(46)
Hordeum vulgare (Barley)	ISSR, SSR	Genetic diversity of 90 barley samples was carried using ISSR and SSR. Results showed that Tibetan wild close relatives of barley had higher genetic diversity than those from middle east.	(47)
Encephalartos woodii	RAPD, ISSR	Genetic diversity of *Encephalartos* was studied using RAPD and ISSR marker. RAPD showed 134 bands of which 110 were polymorphic, while ISSR showed 110 bands of which 86 were polymorphic.	(48)
Jatropha curcas (Euphorbiaceae)	ISSR-PCR	Genetic diversity among eight *Jatropha* species was analyzed using ISSR-PCR. 9 ISSR primers generated 64 bands of which 61 were polymorphic.	(49)
Sargassum muticum (Fucales, Phaeophyta)	RAPD, ISSR	RAPD and ISSR markers were used to assess genetic structure of four population of *S. muticum* and one outgroup of *S. fusiforme*. selected 24 RAPD primers amplified 164 loci of which 124 were polymorphic and 19 ISSR primers amplified 122 loci.	(50)
Simmondsia chinensis (Simmondsiaceae)	ISSR	Inter-simple sequence repeat (ISSR) marker for the early detection of male and female plants in *Simmondsia chinensis*. Of the 42 primers analyzed with a bulk sample of pooled male DNA and a bulk sample of pooled female DNA only one primer, UBC-807 produced a unique 1,200 base-pair fragment in the male DNA.	(51)
Olea europea	RAPD, ITS-1, ISSR	Phylogenetic relationship in the *Olea europea* complex and phylogeography of 24 populations of *O. europea* were assessed by using nuclear ribosomal internal transcribed spacer-1(ITS-1), RAPD and ISSR.	(52)
Penstemon (Scrophulariaceae)	ISSR	ISSR marker used in assessing hybridization in natural population of *Penstemon*.	(53)
Genus eleusine	RFLP, RAPD, ISSR	Assessment of genome origin and genetic diversity in genus *Eleusine* was done. Comparison of RFLP, RAPD and ISSR markers in terms of quantity and quality of data indicate that ISSR were promising for analysis of plant genetic diversity.	(54)
Prunus dulcis	RAPD, ISSR	RAPD and ISSR markers were used to analyze the genetic diversity of Portuguese *Prunus dulcis*. Of the tested 6 (out of 60) RAPD and 5(out of 18) ISSR primers were selected for their reproducibility and	(55)

		polymorphism.	
Primula obconica	ISSR	Genetic diversity in *Primula obconica* was done using ISSR markers. ISSR markers produced 249 polymorphic bands and identified 60 genotypes.	(56)
*Vigna mungo (*Blackgram*)*	RAPD, ISSR	Genetic diversity in blackgram genotypes was done using RAPD and ISSR markers. 25 random primers yielded 104 fragments of which 44 were polymorphic and 16 ISSR primers produced 101 bands of which 55 were polymorphic.	(57)
Litsea szemaois (Lauraceae)	AFLP, ISSR	Genetic diversity in *Litsea szemaois* was using AFLP and ISSR primers. Three AFLP primers produced 203 of which 164 were polymorphic and ten ISSR primers produced 77 bands of which 67 were polymorphic.	(58)
Hippophae rhamnoides	ISSR	Genetic diversity of N2 fixing backthorn (*Hippophae rhamnoides*) was done using eight ISSR markers showed 207 polymorphic bands.	(59)
Ficus carica (fig)	RAPD, ISSR, SSR	Nineteen fig varieties were fingerprinted using 13 ISSR, 19 RAPD and 13 SSR primers. All primers produced 258 loci, with the highest number of loci (119) generated by RAPD.	(60)
Euryodendron excelsum (Ternstroemiaceae)	ISSR	ISSR markers were used to access genetic variation and relationship among 14 individuals. Of 225 loci generated by 21 primers, 147 loci were polymorphic.	(61)
Platanus acerifolia (Plantanaceae)	ISSR	ISSR markers were used to access genetic stability of long-term micropropagated plantlets. 16 ISSR primers out of 38 were produced 103 bands of these 17 were polymorphic.	(62)
Dendrobium officinale	RAPD, ISSR	RAPD and ISSR markers revealed genetic diversity in *Dendrobium officinale*. 104 reproducible bands were generated using twelve ISSR primers of which 97 were polymorphic, 150 bands produced by RAPD of which 14 were polymorphic.	(63)
Oxalis tuberosa (oca)	ISSR	ISSR markers were used determine relationship between morphological and molecular systems. 44-accession representing the morphotypes were analysed by 9 ISSR primers produced 69 reproducible bands of which 32 were polymorphic.	(64)
Leucadendron (Proteaceae)	ISSR	ISSR were applied to determine the genetic variation and to discriminate *Leucodendron* cultivars. 25 ISSR primers out of 64 produced 584 bands of which 97% were polymorphic.	(65)
Emmenopterys henryi (Rubiaceae)	ISSR	ISSR markers were used to determine genetic variation and genetic differentiation of nine populations of *Emmenopterys henryi*. 12 primers yielded 157 bands of which 88 were polymorphic at species level and 20.20% bands polymorphic at population level.	(66)
Cucurbita pepo (Cucurbitaceae)	ISSR, AFLP, SSR	Assessment of genetic relationship in *Cucurbita* was done using ISSR, AFLP and 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.	(67)
Vitis vinifera	RAPD, ISSR	Characterization of genetic variation between *Vitis venefera* was done using RAPD and ISSR markers. 11 ISSR primers showed 110 bands of which 40 were polymorphic, RAPD also distinguish *Vitis vinifera.*	(68)
Nelumbo nucifera	ISSR	ISSR markers showed high clonal diversity in plulation	(69)

(Nelumbonaceae)		of *Nelumbo nucifera.*	
Plutella xylostella (Plutellidae)	ISSR-PCR	ISSR-PCR was use to discriminate and genetic structure analysis of *Plutella xylostella* population native different geographical areas.	
Pinus tabulaeformis	RAPD, ISSR	A total of 125 reproducible bands were obtained from 140 individuals of *P. tabulaeformis* using 15 RAPD primers of which 99 were polymorphic. While 5 ISSR primers produced 35 loci of which 28 were polymorphic (PPB 80.0%).	(70)
Ceriops tagal (Rhizophoraceae)	ISSR	The 12 ISSR primers produced 69 bands among 144 individuals. Of the 69 bands only six were polymorphic.	(71)
Lumnitzera littorea (Combretaceae)	ISSR	Across all 82 *L. littorea* individuals, 12 ISSR primers produced 221 reproducible bands, of which 178 were polymorphic (PPB 75.57 and Shannon's index 0.365%)	(72)
Cymbidium goeringii (Orchidaceae)	ISSR	11 ISSR primers produced 127 reproducible bands with 112 were polymorphic. (PPB at species level 88.2% and 63.06% at population level)	(73)
Lamiophlomis rotate (Lamiaceae)	RAPD, ISSR	Genetic diversity and population structure of *L. rotate* was done using RAPD and ISSR. ISSR amplification of all individuals with 18 selected primers gave a total of 214 bands, of which 207 were polymorphic. Also, out of 233 bands produced by RAPD markers, 217 were polymorphic.	(74)
Scrophularia grayana (Scrophulariaceae)	ISSR	Intraspecific morphological and genetic differentiation in *S. grayana* was done using 26 morphological characteristics and ISSR markers respectively. ISSR markers produced 112 loci indicating genetically distinct.	(75)
Lupinus angustifolicus	AFLP, ISSR	*L. angustifolicus* germplasm characterization was done using morphological and molecular markers. 3 ISSR primers produced a total of 25 bands of which 6 were monomorphic. 2 AFLP primers produced 82 bands with 50 were monomorphic.	(76)
Hesperozygis ringens (Lamiaceae)	RAPD, ISSR	The intra- and inter population genetic variability of *H.ringens* was done using RAPD and 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%).	(77)
Ranunculus nipponicus (Ranunculaceae)	ISSR-PCR	The genetic diversity and structure within and eleven extant *R. nipponicus* population were assessed by ISSR-PCR. Nine primers generated 53 bands of which 59 were polymorphic.	(78)
Mikania micrantha and Cuscuta campestris (Asteraceae and convolvulaceae resp.)	ISSR	Clonal structure and diversity of *M. micrantha* and its plant parasite C. campestris revealed by ISSR markers. 12 ISSR primers in *M. micrantha* and *C. campestris* produced a total of 123 and 136 bands respectively. 30 bands were polymorphic in *M. micrantha* while 3 bands were polymorphic in *C. campestris* among.	(79)
Rhodiola alsia (Crassulaceae)	ISSR	Genetic variation among and within population of *R. alsia* was investigated using ISSR markers. Of the 100 primers screened, 13 produced 140 loci of which 112 (PPB 80%) were polymorphic.	(80)
Fragaria vesca (Rosaceae)	ISSR	Development of SCAR markers from ISSR profiles. The 23 selected ISSR primers combinations generated 345 amplicons.	(81)
Northofagus species (Northofagaceae)	RAPD, ISSR	RAPD and ISSR markers were used to characterize and discriminate three Chilean *northofagus species*. 6 RAPD primers produced 42 bands, while 6 ISSR primers	(82)

		produced 63 bands.	
Ammopiptanthus mongolicus (Leguminosae)	ISSR	Genetic diversity and geographic differentiation in *Ammopiptanthus* was done using ISSR markers in *A. mongolicus*, 39 out of 99 clear reproducible bands produced while in *A. nanus*, 29 out of 112 were polymorphic.	(83)
Nouelia insignis (Asteraceae)	ISSR	Genetic diversity of *N. insignis* was examined using ISSR markers. 11 primers produced 103 reliable ISSR bands, of which 67 were polymorphic.	(84)
Lupinus spp.	AFLP, RAPD, ISSR	Twenty-two primers were used in RAPD analysis producing 352 bands, of which only seven were monomorphic. Twelve primer combinations were used for AFLP, resulting in 1340 bands, of which only 12 were monomorphic. While Thirteen ISSR primers were analyzed resulting in 370 different bands, among which only four were monomorphic	(85)
Chrysosplenium iowense (Saxifragaceae)	ISSR	ISSR markers were used to study genetic diversity in *Chrysosplenium iowense*. Four ISSR primers produced 1195 scorable loci of which 1165 (97.5%) were polymorphic	(86)
Oryza meteriana	ISSR	13 ISSR markers were used to anlyze genetic diversity of *Oryza meteriana* . A total of 168 bands were amplified, of which 135 polymorphic bands were discovered and the percentage of polymorphic bands (PPB) was 80.36%	
Citrullus lanatus (Cucurbitaceae)	ISSR	Use of molecular approach using inter-simple sequence repeat (ISSR) markers on three African edible-seeded cucurbits (*Citrullus lanatus* L. Matsumura and Nakai, *Cucumeropsis mannii* L. Naudin and *Cucumis melo* var. *agrestis* L. Naudin).Of the 21 ISSR primers screened, 11 gave bands varying from discrete to large.	(87)
L. perenne (poaceae)	ISSR	In this study ISSR molecular markers used to identify their sequences and to expand the existing *L. perenne* genetic map of the VrnA mapping population.	(88)
Fragaria · ananassa (Strawberry)	RAPD, ISSR	RAPD and ISSR were utilized for determination of genetic relationship of 24 Strawberry cultivars used in breeding program. Polymorphism of investigated genotypes was observed in reactions with 23 out of 48 tested RAPD primes and 41 from 90 tested ISSR primers.	(89)
Platanus acerifolia (Platanaceae)	ISSR	ISSR markers were used to assess the genetic stability of long-term micropropagated plantlets of London plane tree (*Platanus acerifolia*). Out of 38 ISSR primers screened, 16 primers were found to produce clear reproducible bands resulting in a total of 103 distinct bands of which 86 were monomorphic across all 20 of the plants tested and 17 showed polymorphisms	(90)
Section strobus	ISSR	Genetic relationship of 12 species of *Section strobus* was analyzed with ISSR markers. 117 loci were detected with 12 ISSR. Primers. Percentage of polymorphic bands (PPB) varied from 5.93% to 19.92%.	(91)
Rehmannia glutinosa	RAPD, ISSR	RAPD primers and ISSR primers amplified average 16.00 and 19.08 bands respectively and the percentage of polymorphic bands was 89.58% and 94.32% respectively. ISSR marker can detect higher genetic diversity of *R. glutinosa* germplasms than RAPD marker.	(92)
Cistanche species	ISSR	Ninety-four inter-simple sequences repeat (ISSR) primers were used for polymerase chain reaction (PCR) amplification and of which eight primers were found to	(93)

		be sufficient to distinguish different *Cistanche* species.	
Spanish trichinella	ISSR	Genetic variability of the *Spanish trichinella* isolates by ISSR-PCR. ISSR-PCR analysis of the isolates identified as *T. britovi,* showed two different banding profiles compatible with the European *T. britovi* isolate pattern (ISS2) and the autochthonous Spanish *T. britovi* isolate (ISS11).	(94)
Hemarthria compressa	ISSR	Genetic variance of twelve *Hemarthria compressa* populations and one *Hemarthria japonica* population from China were analyzed using inter simple sequence repeat (ISSR). Twelve primers amplified a total of 165 genomic DNA fragments across a total of 148 individuals of which 156 were polymorphic (94.55%).	(95)
Dysosma pleiantha	ISSR	ISSR markers were used to check genetic variation and genetic structure of *Dysosma pleiantha*. The extent of clonality, together with the clonal and sexual reproductive strategies varied among sites and the populations under harsh ecological conditions tended to have large clones with relatively low clonal diversity caused by vegetative reproduction.	(96)

CONCLUSION

As the herbal drugs are on high, need for better standardized plant/ formulations is increasing. ISSR markers are playing pivotal role in this area. Use of these markers will contribute significantly in quality control of herbal formulations. At the same time one cannot underestimate the importance of ISSR marker in botany. In plant breeding, phylogeny, chemotaxonomy and other fields ISSR markers are also finding many applications. One can expect more application ISSR marker for study of different aspects of pharmacognosy in coming days. While dealing with medicinal plants, one encounters many issues like authentication and correct botanical identification, wide range of diversity within and among plants, intentional and unintentional adulteration. At the same time, there is pressing need to correlate genotype and chemotype, also correlation between Ayurvedic and traditional identification with taxonomic identification. We have undertaken research to establish relation between genotype and chemotype of selected medicinal plants. ISSR technology is easy to perform and certainly tackle the above-mentioned issues. This will really help to foster medicinal plant use and research in pharmacognosy.

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