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Microsatellite Markers- A New Practice of DNA Based Markers in Molecular Genetics

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ABSTRACT

Microsatellites are randomly interspersed within eukaryotic genomes. These are also known as SSRs (Simple sequence repeats), STRs (Short tandem repeats), STMs (Sequence tagged microsatellites), and VNTRs (Variable number of tandem repeats), are short 1-8 bp long monomer sequences, which randomly repeated in the DNA sequences. Conservation of the flanking sequence of each microsatellite locus allows the design of primers for PCR amplification. Then amplified products are separated by electrophoresis (either on high resolution agarose or acrylamide gels) to detect the polymorphism in repeat length. These markers don't require the radioactivity for detection and these are extremely robust as well as easily exchanged between the laboratories. Multiplex reactions of SSRs can easily be run to speed up the assay, when the products have non-overlapping size ranges. Microsatellites have emerged as the marker of choice for plant genetic resources and molecular genetic applications due to their abundant and uniform distribution throughout the genome, highly variable nature with regard to repeat number, show co-dominant inheritance, ease of transferability and reproducibility, and found highly efficient in the DNA fingerprinting analysis, as well as can also be utilized in the pedigree analysis of different plant species. This marker system has been proven beneficial for crop improvement and for breeding applications in many species. These are also found the useful tools to detect the polymorphisms in low level of intraspecific diversity. Consequently, in the present review we have described the various characteristic features and properties of these highly polymorphic microsatellite markers, which found beneficial in several molecular genetic and breeding applications.

Key words: Microsatellite markers; Molecular genetics; Amplification; PCR; SSRs

Abbreviations Used: SSRs: Simple sequence repeats; STRs: Short tandem repeats; VNTRs: Variable number of tandem repeats; PCR: Polymerase chain reaction; bp: Base pairs

INTRODUCTION

Molecular markers are biochemical constituents (e.g. secondary metabolites in plants) and macromolecules, viz. proteins and deoxyribonucleic acids (DNA) that plays a very important role in taxonomy, physiology, embryology, plant breeding, ecology, genetic fingerprinting, genetic engineering etc. Analysis of secondary metabolites is restricted to those plants that produce a suitable range of metabolites, which can be easily analyzed and easily distinguish between the varieties. Genetic polymorphism is defined as the instantaneous episodes of a trait in the same population with two or more genotypes (1).

The term Microsatellite was first coined by Lit and Lutty (2). These are the stretches of DNA, consisting of tandemly repeating mono-, di-, tri,-, tetra- and penta-nucleotide units, that are arranged throughout the genomes of most eukaryotic species (3). These are also known as the Simple sequence repeat (SSR); Short tandem repeat (STR). The existence of dinucleotide repeats- poly (C-A), poly (G-T) (i.e. an alternating sequence of cytosine and adenine, with on the opposite strand of the DNA molecule, alternating guanine and thymine) was first documented almost 15 years ago by Hamada and colleagues (4). Subsequent studies by Tautz and Renz (5) have confirmed both the abundance and ubiquity of microsatellites in eukaryotes.

Microsatellites are the 1-8 bp long monomer sequences that are repeated several times. These loci contain tandem repeats that vary in the number of repeat units between genotypes and are referred to as variable number of tandem repeats (VNTRs) or hypervariable regions. In respect of this Minisatellites are tandem repeats with a monomer repeat of length about 11-60 bp. Thus, Microsatellites and minisatellites form an ideal molecular marker system in the plant molecular genetics. These are the useful tools by the amplification of multiple DNA loci, and creating the complex banding pattern. These markers provide the co-dominant genetic fingerprinting in the plant/animal genome analysis (1).

Evaluation of Microsatellites

The era of DNA based molecular genetics had been raised by the development of the restriction fragment length polymorphism (RFLP) technique, which provides the basis of genetic linkage map (6). RFLP-based linkage maps have since been developed in a number of plant and animal species. The polymorphism based on the RFLP is often the result of the absence or presence of an endonuclease restriction site at the specific gene locus (7). These are co-dominant and reliable

markers in linkage analysis, breeding and can be easily determined in homozygous or heterozygous state of an individual. However, the utility of RFLPs has been restricted due to the requirement of the large amount of DNA for the restriction digestion and for the Southern blotting technique. As well as these markers are found expensive, hazardous and time consuming.

Further utilized markers in molecular genetics is the RAPD (random amplified polymorphic DNA) and ISSR (inter simple sequence repeats). RAPD technique was first developed by the Williams *et al*., (8) and provides the simple, polymorphic fingerprints using single arbitrary primers, whereas, ISSR technique requires the microsatellite regions for the amplification reactions. In this technique amplification of the inter-DNA region between the two opposite microsatellites takes place. Both the above techniques are found simple, precise and require the small amount of DNA for the amplification reactions. However, RAPD and ISSR techniques are found dominant in nature and cannot study the allelic variation i.e. heterozygosity of the particular gene locus.

Recent studies using molecular techniques, is mainly based on the microsatellite markers. These, microsatellites or simple sequence repeats (SSRs) represents an ideal class of molecular genetic marker in plant genome analysis, which would be able to disclose multiple alleles and have even distribution throughout the genome and relatively easy to score (9). Microsatellites are found tandem repeats of one to eight base pair long (2, 10) and found suitable as genetic markers for population genetic diversity studies (11).

Attributes of microsatellite markers

The uniqueness and value of microsatellites arises from the following various characteristic features, which are as follows (3):

Detection of multiallelic variation
Hypervariable (i.e. have a high information content)
Co-dominant transmission
Ease of detection by PCR
Relative abundance with uniform genome coverage
Requirement of a small amount of DNA as a starting material
Each locus defined by primer sequence: facilitates exchange
Represent sequence-tagged sites
Act as a universal genetic marker for genetic reagent mapping

Microsatellite amplifications

Amplification of microsatellites takes place by using the developed PCR primer pairs that, found in the conserved flanking regions of the specific SSR locus. These primer pairs allow the amplification of the entire microsatellite locus. Resulting PCR products would vary in size according to the number of repeated DNA units in the microsatellite allele(s) present and can be utilized to detect the high levels of length polymorphism exist as a result of variation in the number of such short tandem repeat units. These PCR amplified products are generally separated on a standard sequencing gel (mainly the PAGE gel) and visualized via autoradiography/or under the U.V. transilluminator.

Utilization of microsatellite markers - Microsatellite markers can be utilized as different other forms to find out the polymorphisms, some other molecular markers, which commonly derived from the microsatellites are as follows:-

Sequence-tagged microsatellite markers (STMS)

In this type of molecular marker studies, development of primers takes place from the sequence data of a specific gene locus. Primers opposite to the flanking region of the microsatellites amplified the specific locus and the variation in the length of the repeating units is found base to detect the polymorphism in the different individuals.

Expressed sequence tagged microsatellite markers (EST-SSRs)

Microsatellite primers of the EST-SSRs are mainly designed to amplify the coding region of a specific gene locus and these will be utilized to detect the polymorphism at the level of gene expression region.

Sequence characterized amplified regions (SCAR) markers

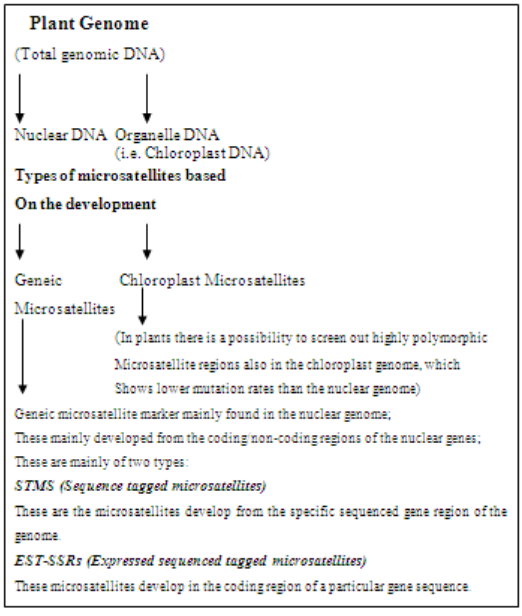
This technique was first introduced by Michelmore and Martin (1991) in which, amplified RAPD regions are first sequenced and then utilized to develop the microsatellite primer pairs for the amplification of a specific gene locus. These primer pairs are found longer in comparison to the RAPD primer pairs mainly 22-24 nucleotide bases long and utilized for specific amplification of a particular gene locus.

Inter simple sequence repeats (ISSR)

In this technique primer pairs are found to the complementary region of the microsatellites. These microsatellites are found the flanking regions of the inter-DNA sequences and the developed primer pairs amplified this inter DNA region by the attachment of the two opposite microsatellites.

Types of simple sequence repeat (microsatellites)

Microsatellites can be classified in to the different forms based on their development, isolation and characterization. Different types of microsatellite markers and their characteristics are as follows:



Exclusivity of Geneic and Organelle Microsatellites

Geneic microsatellites

Locus- specific primers flanking EST- or geneic SSRs designed to amplify the microsatellite loci present in the genes.

Generation of geneic SSR markers is relatively easy and inexpensive.

Geneic SSRs are found the by-product of the sequence data from the genes or ESTs that are publicly available.

EST-SSRs i.e. geneic SSRs are found in the expressed region of the genome and found available easily.

Hypervariable in nature; to detect the polymorphism in the coding region of the genes within and among the different individual genomes.

Due to the public availability of large quantities of gene sequence data, geneic SSRs specifically EST-SSRs can be easily identified, developed and used in a variety of studies, to found out the intra and inter specific variation in a number of plant species (12).

It shows the highly polymorphism and inherited in the Mendelian co-dominant manner (11).

Organelle microsatellites (Chloroplast microsatellites)

These microsatellites developed from the sequenced chloroplast genome of the plant species. These are commonly refereed as the chloroplast microsatellites (cpSSRs).

Polymorphism in cp-SSRs genome is found out in its conserved and variable regions.

These markers prove highly polymorphism in nature between the individuals of a population and among the populations.

These can be easily shared between laboratories, thus providing a common tool for collaborative research by acting as universal genetic markers (13).

Chloroplast microsatellites are uni-parentally inherited might increase the knowledge about the biology of these organisms as well as becoming useful for studying cytoplasmic diversity, cyto-nuclear interactions and monitoring gene flow (11).

These chloroplast microsatellites (cp SSRs) have proved to be of considerable value in numerous studies of plant population structure, diversity, differentiation and paternity analysis (14).

These are also proved to be useful in the plant molecular genetics, evolution and ecology (15).

Inheritance of cpSSRs is also found in the Mendelian co-dominant manner (11).

Expansion of Microsatellites in Genome

There are two methods for the development and isolation of microsatellites i.e.

Standard method

Automated development method

Standard method: Development of microsatellites (SSRs) involves the following steps from this procedure (3):

The creation of a small insert genomic library

Library screening by hybridization

DNA sequencing of positive clones

Primer design and locus-specific PCR analysis

Characterization of polymorphism among different genotypes.

There are various methods available for the creation of genomic library (Table-1). Then the genomic library was hybridized with di-, tri-, tetra-etc enriched oligonucleotides. The positive clones were detected and sequenced. Flanking regions of these sequences were used to develop the microsatellite primer pairs and these primer pairs finally amplified the SSR specific gene locus to find out the polymorphism among the various individuals/accessions in different plant species.

Table-1: Microsatellite enrichment methods

Method	Description	Requirement	References
DNA affinity chromatography	Fragments generated by sonication or endonuclease digestion of genomic DNA are denatured, captured by hybridization to poly (U-G)-Sepharose or nylon bound poly (G-T), eluted and cloned. Options exist to amplify the polymerase chain reaction (PCR) using ligated adaptors.	Genomic DNA	(3)
Duplex-hybridization based formation	Fragments generated by sonication or endonuclease digestion are denatured and hybridized to biotinylated oligonucleotides with a repeat motif complementary to the target sequence. The duplex is captured on streptavidin beads. Fragments may be cloned or products may be selectively amplified by PCR, using primers against added to the starting material by ligation or degenerate PCR.	Genomic or cloned DNA	(3)
Triplex formation	Triple helix formation using duplex DNA (genomic fragments or cloned DNA inserts) and selective oligonucleotides. Potential for RecA protein enhancement, and direct or biotin-mediated capture.	Genomic or cloned DNA	(3)

Oligo-primed second strand synthesis	Single-stranded template generated from cloned DNA in dut ung strain of *Escherichia coli*; second strand synthesis primed using selective repeat oligonucleotides; and transformation into standard *E. coli* for recloning of selected duplexes.	Cloned DNA	(3)
Development of SSR using amplified ISSR region.	Randomly amplified the ISSR region using previously described primer pairs. Then purified the PCR products and cloned. Finally approximately 500-1000 bp of each insert was sequenced for development of the SSR specific primer pairs.	Genomic DNA	(16, 17)

Automated development method:

Initially the identification and development of microsatellites has been done by the publicly available whole genome sequences, gene sequences using the BLASTN and FASTA tools. Subsequently by the development of the several computers based software's or other electronic tools development of microsatellites becomes very easy. Now a day's microsatellites can be directly developed using publicly available DNA sequence/databases from the different modules or programs (Table-2).

Applications of microsatellites

Microsatellites have a large number of applications ranging from identification of a gene locus to improvement of crop/plant varieties. These are useful for marker assisted selection and get extremely popular markers for phylogenetic analysis adding new dimensions to the evolutionary theories. Development of microsatellite markers using genome and their analysis becomes much more popularize in today's molecular genetics.

***Informativeness of microsatellites for genetic analysis*:** Individualization of plant germplasm resources has become important in the present day scenario for their proper management and utilization, as well as for IPR protection, which can be achieved by DNA typing techniques involving use of highly polymorphic markers like SSRs (24). Developed primer pairs for different microsatellite loci in different plant species, can be used to investigate the level of polymorphism. This information can be utilized to detect the genetic variation among the different accessions/individuals within same species (intra-specific), between the species (inter-specific), Intergeneric and among the various different species by the cross amplification technique. These are found useful markers for detecting the allelic diversity among species or accessions as well.

***Genetic mapping and labeling of genes*:** One of the major potential utilities of DNA markers is their use as robust genomic landmarks on the linkage groups that can subsequently be tagged to the gene(s) controlling important traits of interest. This requires generation of reasonably dense linkage maps populated with large number of revisitable DNA markers for which, the SSRs remain the most desired ones (24). Linkage analysis indicates that the developed SSR marker for a particular plant species is dispersed throughout its genome. These markers found suitable for mapping of the total genes with respect to their location and allelic diversity information in a particular genome.

***Phylogenetic studies*:** Previously the phylogenetic studies and relation among the closely related taxa, species were found based on morphological, geographical and taxonomical variations. The techniques of today's molecular genetics i.e. DNA based markers, provide a reliable tool to identify the closely related species and genetic variations among them. Genomic SSRs, specifically EST-SSR markers are found a better choice for application in cross-species phylogenetic studies and also as a valuable tool for plant breeding and for germplasm collection, conservation.

***Transferability and reproducibility*:** The SSR markers have found a great capability of the cross amplifications among the closely related taxa, species and these amplification reactions are found highly reproducible among the different laboratory experimentations.

***Sequence tagged microsatellies (locus- specific markers)*:** STMP provides a cost-efficient and rapid approach for developing the SSR markers. The quantitative relationship between the abundance of individual tags and SSR copy number in the genome provides a tool to help select low copy or unique SSR sequences for primer design, thereby increasing the proportion of locus-specific markers (25). So, these locus-specific STMP markers are provides a valuable tool to detect the genetic variation with respect to a particular trait controlled by the specific gene(s).

***Chloroplast microsatellies markers*:** Chloroplast microsatellies (cpSSRs) are the most polymorphic marker systems in current use because of their high mutation rates and single base pair variation, thus providing a linear relationship between genetic distance based on allelic length differences and time of divergence (24). Sequence variation at coding and non-coding chloroplast loci is the most widely used tool to assess phylogenetic relationships among plant taxa, based on their low rate of sequence evolution and the almost absent recombination in the chloroplast genome (26, 27, 28). Because of this sequence conservation, many studies have used chloroplast DNA sequences for the study of genetic diversity, plant systematic and in breeding applications (29).

Preceding of microsatellites

A lot of work had been previously done by the several researchers in the field of molecular genetics with respect to the development of microsatellite marker and their characterization, identification of polymorphism in various plant species. There are some previously work done and their references are as follows (Table-3).

Table-2: Different computer based programs for microsatellite (SSR) marker development

Software or program	Properties	Link
JSTRING (Java search for tandem repeats in genomes)	JSTRING is a Java program for searching Tandem Repeats (TR) in a DNA sequence.	http://bioinf.dms.med.uniroma1.it/JSTRING/JSTRING.html
MREPS	The following paper describes mreps 2.5 as well as some case examples of its application to genomic studies. Please cite this paper when referring to mreps.	(18, 19) http://bioinfo.lifl.fr/mreps/
PHOBOS	Phobos is a highly accurate and fast search tool for: DNA-microsatellites, DNA-minisatellites and DNA-satellites Developed by: **Dr. Christoph Mayer**	http://www.ruhr-uni-bochum.de/spezzoo/cm/cm_phobos.htm
Poly	Poly is a program for the quantitative analysis of simple sequence repeats (SSRs) in DNA, such as poly (dA).poly (dT) (A tract of adenines with a tract of thymines on the complementary strand).	http://www.bioinformatics.org/poly/wiki/ **programme can download from these sites:** poly-0.0.7.tar.gz poly-0.1.1.tar.gz
SciRoKo 3.4	SciRoKo can be utilized for whole genome microsatellite search and investigation. Given by: **Robert Kofler; Christian Schlotterer; Tamas Lelley**	Available at: http://www.kofler.or.at/bioinformatics/SciRoKo/index.html Download the SciRoKo 3.4 form the following website: www.kofler.or.at/Bioinformatics/
STAR	It mainly searches the approximate tandem repeats (ATR) of a given motif in the prescribed DNA sequences. This software is given by: **Delgrange O. and Rivals E.**	http://www.atgc-montpellier.fr/star/
Tandem SWAN	It is an algorithm for searching of degenerate tandem repeats without insertions and deletions. It is based on calculation of the repeat statistical significance and identifies the length of the repeated unit and the number of repetitions.	http://favorov.imb.ac.ru/swan/
TROLL (Tandem Repeat Occurrence Locator)	The Tandem Repeat Occurrence Locator is a light weight tool for SSR finding and development, which is, given by: **Adalberto Castelo**	http://finder.sourceforge.net/
Tandem repeats finder	Tandem Repeats Finder is a program to locate and display tandem repeats in the DNA	http://tandem.bu.edu/trf/trf.html (20)

	sequences.	
MISA (MIcroSAtellite identification tool)	It identifies the microsatellites in the given FASTA genome sequence.	http://pgrc.ipk-gatersleben.de/misa/misa.html
MREPATT (**M**ultiple **Re**peated **PATT**erns search tool)	It mainly searches the exact occurrences of the SSR patterns like *p, pp, and ppp*… through a set of DNA sequences.	http://alggen.lsi.upc.edu/recerca/search/mrepatt/
SSR Finder (simple sequence repeat finder)	It search as FASTA sequence file and find simple sequence repeats (SSRs; i.e. AAAA..., ATATATAT..., etc.), identify their location and format the file so that it was easy to design primers for SSR motifs.	http://minerva.ufpel.edu.br/~lmaia.faem/ (21)
Build SSR	------	(22)
SSRIT (Simple Sequence Repeat identification tool)	-------	(23)
Sputnik	It also uses for the direct development of microsatellite markers from the whole genome sequence, EST regions or the other gene sequences. Developed by: **C. Abajian**	http://hornbill.cspp.latrobe.edu.au/cgi-binpub/ssrprimer/indexssr.pl; http://abajian.net/sputnik/index.html

Table-3: Preceding of microsatellite (SSR) marker development, characterization and detection of polymorphism in various plant species.

Title	Plant species used	Outcome of work done	Reference
Development and characterization of microsatellite markers in *Cucumis*.	*Melon* (*Cucumis melo* L. and *Cucumis sativus* L.)	Developed set of SSR markers proven beneficial for both genetic and breeding applications. Detection of highly polymorphism among the *melon* genotypes. Phylogenetic analysis of *melons* genotypes.	(30)
Development of microsatellite markers for genome analysis in *Brassica*.	*Brassica napus* and microsatellite distribution in other Brassicaceae species.	Developed microsatellite markers were found, strongly polymorphic among the species. Found suitable as STS markers in genetic analyses. Highly efficient markers for genetic diversity and heterosis analysis in *B. napus* species.	(31)
Isolation and characterization of novel microsatellite markers (104 SSR markers) in *Arachis hypogaea*	*Arachis hypogaea*	Isolated 104 SSR markers showed a good level of PIC value in cultivated *Arachis* germplasm. These markers found useful for genome analysis, linkage mapping, diversity studies and phylogenetic relationships in cultivated groundnut as well as in other related *Arachis* species.	(32)
Development and characterization of	*Taro* (*Colocasia esculenta*)	Found out the polymorphism in low variability showing, vegetatively	(33)

polymorphic microsatellite markers in *taro*.		propagated root crop (i.e. *taro*). Isolated microsatellites were found successful revealing of heterozygosity in *taro* genotypes collected form the Pacific island region.	
Development of new genomic microsatellite markers from r*obusta coffee*.	*Coffee* (*Coffea canephora* Pierre ex A. Froehner)	~92% developed markers showed the transferability across related species/genera of *coffee*. The characterization and validation of new developed SSR markers found useful for genetic diversity analysis in *coffee* germplasm, individualization/bar-coding for germplasm protection, linkage mapping, and taxonomic studies and as a conserved orthologous sets across secondary genepool of *coffee*.	(24)
Characteristics and potential utilization of microsatellites in *Brassica rapa* genome for comparative genomics.	*Brassica rapa*	The number of repeats and the polymorphism information content was higher in dinucleotide microsatellites in comparison to the trinucleotide repeats. The developed SSR primer pairs were found capable for the cross amplification in other cruciferous species and found reliable and effective means for comparative genomics in *Brassica*.	(34)
Discovery, inheritance and variability of microsatellites in *Cassava* (*Manihot esculenta* Crantz).	*Cassava* (*Manihot esculenta* Crantz)	Heterozygosity was detected to found out the usefulness of microsatellites.	(35)
Development of representative, highly informative genotyping set of SSRs for *barley*.	*Cultivated Barley*	Develops the primer pair from the whole genome sequence of *barley* for polymorphism detection.	(36)
Genomic analysis of cultivated *barley* using STMS, PCR based RFLP and SSR markers.	*Cultivated Barley* (*Hordeum vulgare*)	Three types of molecular markers have been compared for their utility in evaluating of genetic diversity among cultivars of *Hordeum vulgare*. Levels of polymorphism and differentiation between groups of cultivars revealed quite high by the SSR markers in comparison to others.	(37)
Analysis of genetic diversity among *European cultivated spelt* by microsatellites.	*Spelt*	Determination of genetic basis of modern *spelt* cultivars in terms of intra-group variability and inter-group distances, by using microsatellite markers developed from common *wheat*. These microsatellites are found the powerful tools for phylogenetic reconstruction in *spelt*.	(38)
Polymorphism of PCR based markers using exons, introns, promoter regions and SSRs in *maize* and introns and repeat sequences in *oat*.	*Maize* (*Zea mays*) *Oat* (*Avena sativa*)	In *maize* 67% of promoter markers, 58% of intron markers, and 13% of exon markers exhibited amplification product polymorphism. Polymorphism was found high in SSR markers followed by intron, exon and promoter markers respectively. In *Avena* genotypes, 64% of SSR markers	(39)

		and 58% of intron markers revealed polymorphism.	
Development and analysis of novel microsatellite markers in *lentils*.	*Lentil* (*Lens culinaris subsp. culinaris*)	Fourteen new microsatellite markers were developed for *lentil* genetic diversity analysis.	(40)
Transferability and polymorphism detection using EST-SSR markers in *barley*.	*Barley* (*Hordeum chilense*)	82 *barley* EST-derived SSR primer pairs tested for transferability. Out of these EST-SSRs, 21 (26%) showed polymorphism among *H. chilense* lines. Identified polymorphic markers were used to test the transferability and polymorphism in other Poaceae family species.	(41)
Evaluation of SSR, ESTs and EST-SSRs as molecular markers in *cotton*.	*Cotton* (*Gossypium barbade*nse and *G. hirsutum*)	21 SSR, 10 EST-SSR and 7-EST primer pairs were used for genetic diversity estimation in 14 *cotton* genotypes including 13 *G. barbadense* genotypes and only one genotype of *G. hirsutum*. Analysis of 14 *cotton* genotypes showed amplification of 119 alleles among, which 79 (66.4%) were found polymorphic.	(42)
Genome-wide identification of microsatellites in *white clover* (*Trifolium repens* L.)	*White clover* (*Trifolium repens* L.)	Primers were designed using the FIASCO procedure and phpSSRMiner program. A total of 191 SSR primers were developed from the two libraries tested for polymorphism in individual clones. Out of total SSR primer pair developed 92% produced amplicons and 66% of them were found polymorphic.	(43)
Development, characterization and utilization of microsatellite markers in *pigeonpea*.	*Pigeonpea* [*Cajanus cajan* (L.) Millsp.]	Primers were designed for 39 microsatellite loci, 20 of which, amplified through PCR and given the products of the expected size. Nineteen of the primer pairs were found polymorphic amongst 15 cultivated and 9 wild *pigeonpea* accessions. These markers provide the evidence for cross-species transferability within the genus *Cajanus*.	(44)
Development of microsatellite markers from an enriched genomic library for genetic analysis of *melon* (*Cucumis melo* L.)	*Melon* (*Cucumis melo* L.)	144 new primer pairs were developed from Tsp-AG/TC genomic library. A sample of the microsatellite markers tested proved efficient for genetic analysis of *melon*, including genetic distance estimates and identity tests. Linkage analysis indicated that the markers developed are dispersed throughout the genome and should be very useful for genetic analysis of *melon*.	(45)
Successful amplification of *Rice* chloroplast microsatellites in Century-old grass samples.	Century old samples of 2 grasses i.e. *Anthoxanthum ordoratum* and *Festuca rubra*	Reported the successful amplification of microsatellite markers for the chloroplast genome from century-old samples of 2 grasses using *rice* chloroplast microsatellite markers.	(46)
Population genetic diversity	*Red pine*	Chloroplast microsatellite markers	(47)

analysis in *red pine*, *Pinus resinosa* Ait. using chloroplast microsatellites.	(*Pinus resinosa* Ait.)	(cpSSRs) DNA were used to study population genetic structure in *red pine* (*Pinus resinosa* Ait.). Using nine cpSSRs loci, a total of 23 chloroplast haplotypes and 25 cpSSRs alleles were found among 159 individuals surveyed in seven widely separated populations. The total genetic diversity was found low.	
Nuclear- and chloroplast-microsatellite variation in A-genome species of *rice*.	A-genome species of *Rice* (*Oryza sativa* and *O. glaberrima* and accessions of wild A-genome species)	Simple sequence length polymorphism analysis was carried out to reveal microsatellite variation and to clarify the phylogenetic relationships among A-genome species of *rice*. Total 29 cultivars and 30 accessions of wild A-genome species were used as a template for PCR to detect 24 nuclear and 10 chloroplast microsatellite loci. Microsatellite amplified clearly in all 59 accessions, with an average of 18.4 alleles per locus.	(48)
Chloroplast microsatellite polymorphisms in *Vitis* species.	*Vitis* species	Three chloroplast microsatellite loci were found to be polymorphic in samples of *Vitis vinifera, Vitis rupestris, Vitis berlandieri* and *Vitis riparia* out of a total 10 consensus primer pairs tested. Chloroplast microsatellite polymorphisms were used to demonstrate the maternal inheritance of chloroplast in *V. vinifera*.	(49)
Development and Mapping of 2240 new SSR markers for *rice* (*Oryza sativa* L.)	*Rice* (*Oryza sativa* L.)	A total of 2414 new di-, tri-, and tetra-nucleotide non-redundant SSR primer pairs, representing 2240 unique marker loci, have been developed and experimentally validated for *rice* (*Oryza sativa* L.). The largest proportion of SSRs in this data set correspond to poly(GA) motifs (36%), followed by poly(AT) (15%) and poly(CCG) (8%) motifs. AT-rich microsatellites had the longest average repeat tracts, while GC-rich motifs were the shortest.	(50)
Development of microsatellite markers by ISSR-suppression-PCR method in *Brassica rapa*	*Brassica rapa*	ISSR-suppression-PCR method was found able for the easy development of the microsatellite markers from the *B. rapa* genome. The major advantage of this method is that library construction and screening can avoided. A total of 55 primer pairs were developed out of which, 51 primer pairs were amplified. Out of total 55 primer pairs, 43 were	(16)

		found perfect type, 3 imperfect and 5 compound types of microsatellite markers.	
Characterization and analysis of microsatellite loci in a mangrove species, *Avicennia marina* (Forsk.) Vierh. (Avicenniaceae)	*Avicennia marina*	Sixteen microsatellite sequences were selected for primer design, and 6 primers were selected to investigate the polymorphism among 15 individuals of *A. marina* from three natural populations of Australia. On average 7 alleles per locus were detected and all microsatellite loci showed high level of genetic diversity (heterozygosity)	(51)
Characteristics of single- and multi-copy microsatellites from *Pinus radiata*.	*Pinus radiata*	Dinucleotide Microsatellites were isolated from *Pinus radiate* using both a standard genomic library and libraries enriched for Microsatellites. Locus-specific primers were designed to amplify 43 unique microsatellies. Thirty two of these loci had found amplifiable in PCR, 11 of which, were found polymorphic in a screen of 19 *P. radiata* individuals.	(52)

CONCLUSION

The selection of the desirable molecular marker is mainly depends on the many issues like, information of genome sequences of a particular organism/individual, costs for marker development, availability of the technology/software, ease of documentation etc. Microsatellite markers are found highly informative, easy to develop by the accessibility of the sequenced genome. These provide an efficient, accurate tool to detect the genetic variation in any organism/individual from, which the DNA can be extracted. These are found abundant in nature, high degree of informativeness, assay via the polymerase chain reaction and the ease of exchange from laboratory to laboratory via the publication of primer sequences. The informativeness of a polymorphic marker depends upon the number of alleles and their relative population frequencies. The greater the number of alleles at a given locus, the more informative will be the marker. This underlies the virtue of microsatellites in linkage analysis and gives measures to the extent to which microsatellites are much more informative than the dimorphic systems such as RFLPs. Above all features makes the microsatellite (SSR) markers a valuable tool in numerous molecular genetic studies/applications.

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