Euphytica (2011) 177:309–334
DOI 10.1007/s10681-010-0286-9

REVIEW

Microsatellite markers: an overview of the recent progress in plants

Rajwant K. Kalia · Manoj K. Rai · Sanjay Kalia · Rohtas Singh · A. K. Dhawan

Received: 25 March 2010 / Accepted: 22 October 2010 / Published online: 9 November 2010

Abstract In recent years, molecular markers have been utilized for a variety of applications including examination of genetic relationships between individuals, mapping of useful genes, construction of linkage maps, marker assisted selections and backcrosses, population genetics and phylogenetic studies. Among the available molecular markers, microsatellites or simple sequence repeats (SSRs) which are tandem repeats of one to six nucleotide long DNA motifs, have gained considerable importance in plant genetics and breeding owing to many desirable genetic attributes including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage including organellar genomes, chromosome specific location and amenability to automation and high throughput genotyping. High degree of allelic variation revealed by microsatellite markers results from variation in number of repeat-motifs at a locus caused by replication slippage and/or unequal crossing-over during meiosis. In spite of limited understanding of the functions of the SSR motifs within the plant genes, SSRs are being widely utilized in plant genome analysis. Microsatellites can be developed directly from genomic DNA libraries or from libraries enriched for specific microsatellites. Alternatively, microsatellites can also be found by searching public databases such as GenBank and EMBL or through cross-species transferability. At present, EST databases are an important source of candidate genes, as these can generate markers directly associated with a trait of interest and may be transferable in close relative genera. A large number of SSR based techniques have been developed and a quantum of literature has accumulated regarding the applicability of SSRs in plant genetics and genomics. In this review we discuss the recent developments (last 4–5 years) made in plant genetics using SSR markers.

Keywords Cross-species transferability · Expressed sequence tags · Molecular markers · Population genetics · Transcription slippage

R. K. Kalia (✉) · M. K. Rai · R. Singh
Plant Molecular Biology Division, Centre for Plant Biotechnology, CCS HAU New Campus, Hisar 125004, Haryana, India
e-mail: rajwantkalia@yahoo.com; rajwant.kalia@ucr.edu

S. Kalia
NRC on Plant Biotechnology, Pusa Campus, New Delhi 110012, India

A. K. Dhawan
Centre for Plant Biotechnology, CCS HAU New Campus, Hisar 125004, Haryana, India

Present Address:
R. K. Kalia
Botany and Plant Sciences, University of California, 2150 Batchelor Hall, Riverside, CA 92521, USA

Introduction

The importance of genetic variations in facilitating plant breeding and/or conservation strategies has long been recognized (Sehgal and Raina 2008). Molecular markers are useful tools for assaying genetic variation and provide an efficient means to link phenotypic and genotypic variation (Varshney et al. 2005a). In recent years, the progress made in the development of DNA based marker systems has advanced our understanding of genetic resources. These molecular markers are classified as: (i) hybridization based markers i.e. restriction fragment length polymorphisms (RFLPs), (ii) PCR-based markers i.e. random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats (ISSRs) and microsatellites or simple sequence repeats (SSRs), and (iii) sequence based markers i.e. single nucleotide polymorphisms (SNPs) (Varshney et al. 2007; Sehgal and Raina 2008). Majority of these molecular markers have been developed either from genomic DNA library (e.g. RFLPs or SSRs) or from random PCR amplification of genomic DNA (e.g. RAPDs) or both (e.g. AFLPs) (Varshney et al. 2007). Availability of an array of molecular marker techiques and their modifications led to comparative studies among them in many crops including soyabean, wheat and barley (Powell et al. 1996; Russell et al. 1997; Bohn et al. 1999). Among all these, SSR markers have gained considerable importance in plant genetics and breeding owing to many desirable attributes including hypervariability, multiallelic nature, codominant inheritance, reproducibility, relative abundance, extensive genome coverage (including organellar genomes), chromosome specific location, amenability to automation and high throughput genotyping (Parida et al. 2009). In contrast, RAPD assays are not sufficiently reproducible whereas RFLPs are not readily adaptable to high throughput sampling. AFLP is complicated as individual bands are often composed of multiple fragments mainly in large genome templates (Varshney et al. 2007). The general features of DNA markers are presented in Table 1.

Microsatellites (Litt and Luty 1989), variously known as Short tandem repeats (STRs, Edwards et al. 1991), Simple sequence repeats (SSRs, Jacob et al. 1991) or Simple sequence length polymorphism (SSLP, Tautz 1989) are tandem repeated motifs of 1–6 bp which have a frequent occurrence in all prokaryotic and eukaryotic genomes analyzed to date (Zane et al. 2002). The existence of microsatellites was demonstrated by Hamada et al. (1982) in various eukaryotes ranging from yeasts to vertebrates. Subsequent studies by Delseny et al. (1983) and Tautz and Renz (1984) confirmed the abundance of microsatellites in plants and in many other eukaryotes. Plants are rich in AT repeats, whereas in animals AC repeat is the most common. This appears to be the general feature distinguishing plant and animal genomes (Powell et al. 1996). SSRs are present in both coding and noncoding regions and are distributed throughout the nuclear genome. These can also be found in the chloroplastic (Provan et al. 2001; Chung et al. 2006) and mitochondrial (Soranzo et al. 1999; Rajendrakumar et al. 2007) genomes. SSRs are characterized by a low degree of repetition per locus (5–100), random dispersed distribution of about (10^4–10^5) per genome (Tautz 1993) and high degree of length polymorphism (Zane et al. 2002). The high length polymorphism is due to different number of repeats in the microsatellite regions, therefore they can be easily and reproducibly detected by polymerase chain reaction (PCR). These markers are amenable to high throughput genotyping

Table 1 Important features of different types of molecular markers

Features	Molecular markers			
	EST–SSRs	SSRs	RFLPs	RAPDs/AFLPs/ISSRs
Need for sequence data	Essential	Essential	Not required	Not required
Level of polymorphism	Low	High	Low	Low-moderate
Dominance	Co-dominant	Co-dominant	Co-dominant	Dominant
Interspecific transferability	High	Low-moderate	Moderate-high	Low-moderate
Utility in Marker assisted selection	High	High	Moderate	Low-moderate
Cost and labour involved in generation	Low	High	High	Low-moderate

and have proven to be an extremely valuable tool for paternity analysis, construction of high density genome maps, mapping of useful genes, marker-assisted selection, and for establishing genetic and evolutionary relationships (Parida et al. 2009). A large number of microsatellite markers are now available for completely sequenced plant genomes such as rice (http://rgp.dna.affrc.go.jp/IRGSP/, IRGSP 2005) and *Arabidopsis thaliana* (http://www.arabidopsis.org/, Arabidopsis Genome Initiative 2000).

Microsatellites have been variously classified depending upon their size, type of repeat unit and its location in the genome. Depending upon the number of nucleotides per repeat unit, SSR's have been classified as mono-, di-, tri-, tetra-, penta- or hexanucleotides (Table 2). Depending upon the arrangement of nucleotides in the repeat motifs, Weber (1990) used the terms perfect, imperfect and compound microsatellites for classification while Wang et al. (2009a) classified microsatellites as simple perfect, simple imperfect, compound perfect or compound imperfect. Perfect repeats are tandem arrays of a single repeat motif, while in imperfect repeats, perfect repeats are interrupted by non-repeat motifs at some locations. In compound microsatellites, two basic repeat motifs are present together in various configurations. Jarne and Lagoda (1996) coined the terms pure and interrupted for perfect or imperfect repeats, respectively as shown in Table 2.

Table 2 Classification of microsatellites

(A) Based on the number of nucleotides per repeat
Mononucleotide (A)n
Dinucleotide (CA)n
Trinucleotide (CGT)n
Tetranucleotide (CAGA)n
Pentanucleotide (AAATT)n
Hexanucleotide (CTTTAA)n (n = number of variables)
(B) Based on the arrangement of nucleotides in the repeat motifs (Weber 1990; Jarne and Lagoda 1996; Wang et al. 2009a)
Pure or perfect or simple perfect (CA)n
Simple imperfect (AAC)n ACT (AAC)n + 1
Compound or simple compound (CA)n (GA)n
Interrupted or imperfect or compound imperfect (CCA)n TT (CGA)n + 1
(C) Based on location of SSRs in the genome
Nuclear (nuSSRs)
Chloroplastic (cpSSRs)
Mitochondrial (mtSSRs)

Most of the genomic SSRs are nuclear SSRs, however, microsatellites are also distributed in mitochondria and chloroplasts. Based on their location in the genome, microsatellites can be classified as nuclear (nuSSR), mitochondrial (mtSSR) or chloroplastic SSRs (cpSSR). Weising and Gardner (1999) discovered (cpSSR) in *Nicotiana*, while mtSSR were discovered by Soranzo et al. (1999) in *Pinus* species. In recent years, cpSSRs have been extensively used to study genomic variations in plants and gene flow in natural populations (Provan et al. 2001) due to their uniparental inheritance. In addition, a comparison of the patterns of variability detected with biparentally (nuSSR) and uinparentally (organellar i.e. cpSSR and mtSSR) transmitted markers can also provide complimentary information for population and evolutionary biologists (Powell et al. 1996).

Microsatellite evolution: mutational mechanism of SSR variation

The SSR evolution i.e. any change in SSR resulting in increase or decrease in repeat number, is associated with its mutation rate. Microsatellite genesis is an evolutionarily dynamic process and has proven to be exceedingly complex (Ellegren 2004; Pearson et al. 2005). Possible explanations for microsatellite genesis include single-stranded DNA slippage, double-stranded DNA recombination (unequal crossing over and gene conversion), mismatch/double strand break repair, and retrotransposition. During DNA replication, slipping of DNA polymerase III on the DNA template strand at the repeat region can cause the newly created DNA strand to expand or contract in the repeat region if the mismatches are not repaired (Wang et al. 2009a). Slip-strand mispairing errors are corrected by mismatch repair (MMR), thus SSR stability depends upon a balance between the DNA slippage and effectiveness of MMR system. When MMR gene mutates or become defective, SSR instability increases. Li et al. (2002) reviewed the relationship between SSR instability due to defective MMR and human cancer. Furthermore, DNA damage caused by external stress such as UV-irradiation, γ-irradiation and oxidative stress etc. can induce slippage mutations and increase mutation rates in

SSRs (Jackson et al. 1998; Chang et al. 2002; Li et al. 2002; Trifonov 2003). A balance between slippage and point mutations is also important for equilibrium distribution of SSRs (Kruglyak et al. 1998; Ellegren 2002). Replication slippage favors growth, whereas point mutation breaks down a long repeat array into two or more shorter ones. Thus, changes in relative frequencies of slippage and point mutations might have direct effect on distribution of SSRs in a genome (Li et al. 2002), and a higher relative rate of slippage giving rise to longer SSRs (Kruglyak et al. 1998). The interaction of slippage and recombination could also affect SSR stability (Li et al. 2002). Retrotransposons are repetitive DNA fragments, which are inserted into chromosomes after they had been reverse-transcribed from any RNA molecule. Microsatellite generation was found to be accompanied by retrotransposition events by analysis of a portion-sequenced human and rice genome DNA (Nadir et al. 1996; Temnykh et al. 2001). Parida et al. (2009) reported that 23.1% of the microsatellite containing sugarcane genomic sequences had significant sequence homology to retrotransposons and suggested the potential use of retrotransposon associated SSR-motif sequences as a source to develop polymorphic class of genetic markers. Abundant distribution of *Copia* and *Gypsy* types LTR retrotransposons was reported in sugarcane (36%, Rossi et al. 2001), maize (32%, Bennetzen 2000), and rice (13%, IRGSP 2005) genomes. The insertion and accumulation of retrotransposons between the genes possibly have played a major role in plant genome expansion (Bennetzen 2000). In wild emmer wheat, interaction between mean repeat length and SSR locus distance from the centromere was reported to have a strong effect on the number of alleles and variation in repeat size at SSR loci (Li et al. 2000). It was suggested that this effect might reflect the possible influence of replication slippage during recombination-dependent DNA repair.

Distribution of SSRs within genes and their effects

Numerous lines of evidence have demonstrated that SSRs constitute a large fraction of non-coding DNA. However, recently many reports have verified that a large number of SSRs are located in transcribed regions of the genomes (Fig. 1), including protein coding genes and expressed sequence tags (ESTs), although, repeat number of SSRs in these regions are comparatively low (Morgante et al. 2002; Li et al. 2004). For instance, in cereals (maize, wheat, barley, sorghum and rice), only 1.5–7.5% SSRs are located in

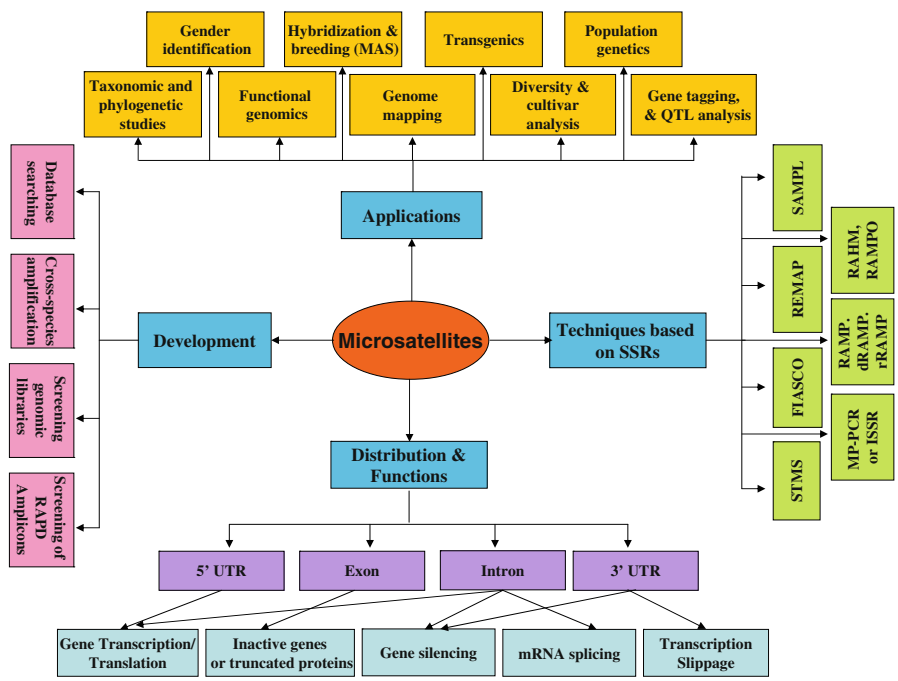

Fig. 1 Microsatellites—a summary of development, distribution, functions and applications

ESTs (Kantety et al. 2002; Thiel et al. 2003). The dinucleotide repeats are most common in many species, but are much less frequent in coding region than in non-coding regions (Li et al. 2002; Wang et al. 1994). In many species, exons have more triplet SSRs than other repeats (Morgante et al. 2002; Li et al. 2004). Such dominance of triplets over other repeats in coding regions may be explained on the basis of the suppression of non-trimeric SSRs in coding regions possibly due to change in reading frame with increase or decrease in number of repeat units. In plants, the most frequent triplet is AAG (Li et al. 2004), although, in cereals, most common triplet is CCG (Cordeiro et al. 2001; Varshney et al. 2002; Thiel et al. 2003). The abundance of CCG repeat is a specific feature of monocots and it may be due to their increased GC content (Morgante et al. 2002) or may be related to high frequencies of certain amino acids. The general frequency of microsatellites is inversely related to the genome size in plants, but the percentage of repetitive DNA appeared to remain constant in coding regions (Morgante et al. 2002) with dicots having more mononucleotide repeats and monocots having more trinucleotide repeats (Lawson and Zhang 2006).

The location of SSR in the genome determines its functional role (Fig. 1). These have the potential to affect all aspects of genetic functions including gene regulation, development and evolution. A microsatellite located in a coding region can affect the activation of a gene and therefore, the expression of a protein. If located in a noncoding or genic region, e.g., the 5′-untranslated regions (UTRs) or introns, the microsatellite may impact gene regulation or gene transcription (Lawson and Zhang 2006). According to available large scale database in wide range of organisms, UTRs (5′-UTR, 3′-UTR and introns) have more SSRs than the coding regions. Majority of intronic SSRs are monomeric and/or dimers in different taxonomic groups (Li et al. 2004). The 5′-UTRs contained more triplets than the 3′-UTRs in *Arabidopsis* (Morgante et al. 2002) and barley (Thiel et al. 2003). In contrast to 5′-UTR, monomers are more common in 3′-UTRs in *Arabidopsis* (Morgante et al. 2002). Presence of SSRs in the 5′-UTRs are required for expression of some genes and variations in SSRs could regulate expression of these genes by affecting transcription and translation. The SSR expansions in 3′-UTRs cause transcription slippage and produce expanded mRNA, which can disrupt splicing and other cellular functions. Intronic SSRs can also regulate gene transcription and translation. In addition, it can also behave as a co-regulator for gene expression alongwith SSRs in the 5′-UTR. SSR expansion in introns and 3′-UTRs can lead to gene silencing or a loss of function. All these effects caused by SSR expansion or contraction within gene can ultimately lead to phenotypic changes (Li et al. 2004); these unusual phenotypic variations cause several diseases in humans. There are more than 40 neurological, neurodegenerative, and neuromuscular disorders associated with human diseases, which are ultimately caused by tandem repeat instability (Orr and Zoghbi 2007). As a diagnostic technique, microsatellite markers have been developed for identifying these neurological, neurodegenerative, and neuromuscular disorders. SSR expansion and/or contraction in exon can lead to a gain or loss of gene function via frame shift mutation or expanded mRNA toxicity (Li et al. 2004). However, the function of genes that contain SSRs and the role of these SSR motifs in plant genes are less studied and poorly understood (Varshney et al. 2005a). Fujimori et al. (2003) suggested that microsatellites located at high frequency in the 5′ flanking regions of plant genes can potentially act as factors in regulating gene expression. In rice, amylose content was correlated with variation in the number of GA or CT repeats in the 5′ UTR of the waxy gene (Bao et al. 2002). In another example, it was found that (CCG)n in the 5′ UTR of ribosomal protein genes of maize was involved in the regulation of fertilization (Dresselhaus et al. 1999).

Development of microsatellite markers

In spite of wide applicability of SSR markers in plant genetics, their development remains a major bottleneck in majority of the species especially the minor crops. This is due to the fact that these need to be isolated de novo from most of the species being examined for the first time. In addition, relatively low frequency of microsatellites in plant genomes compared to animal genome causes technical problems for their large scale isolation (Powell et al. 1996). Microsatellites are usually found in both coding and non-coding regions, however, nucleotide substitution rate is high in non-coding regions. Traditionally, microsatellite loci were isolated from partial genomic libraries of the species of interest by screening several

thousands of clones through colony hybridization with repeat containing probes. This method although relatively simple, especially for microsatellite rich genomes, can turn out to be extremely inefficient for species with low microsatellite frequencies (Zane et al. 2002). Conventional genomic library construction and subsequent screening is cumbersome, tedious and cost intensive process which requires high level of expertise. However, once developed, the running cost of these markers is low enough. AT dinucleotide, which are the most abundant type of SSR in plants, are difficult to isolate from libraries because they are palindromic (Powell et al. 1996). Therefore, several alternative strategies have been devised in order to reduce the time invested in microsatellite isolation and to significantly increase yield of microsatellite loci (Fig. 1). These methods involve identification of SSR sequences in RAPD amplicons, screening of available sequenced EST databases and transferability of markers from related species.

Development of microsatellites through enriched small insert genomic library construction

Two methods have been developed for microsatellite loci isolation from genomic libraries: (i) selective hybridization (Karagyozov et al. 1993; Armour et al. 1994; Kandpal et al. 1994; Hamilton et al. 1999) and (ii) primer extension enrichment (Ostrander et al. 1992; Paetkau 1999). The isolation of microsatellite loci by different methods has been reviewed in detail by Zane et al. (2002). The selective hybridization strategy based microsatellite enrichment technique is a relatively simple, robust, reproducible and cost-effective approach for isolating large number of microsatellites from diverse plant species with higher efficiency. In the first step of selective hybridization approach, fragments generated by sonication or endonuclease digestion of genomic DNA are ligated to a known sequence, a vector or an adaptor. Following the fragmentation-ligation step, DNA is denatured and hybridized with the repeat containing probes. The probes can be bound to a nylon membrane (Karagyozov et al. 1993; Armour et al. 1994) or biotinylated and captured on streptavidin coated beads (Kandpal et al. 1994; Kijas et al. 1994). After the hybridization step and several washes with buffer to remove nonspecific binding, the probe bound DNA is eluted and recovered by PCR amplification. Finally, the recovered fragments are cloned and screened for microsatellites by southern blotting, PCR or direct sequencing (Zane et al. 2002).

Enrichment rates range from 10 to 22% in many crop plants, particularly in the graminaceous species, namely sugarcane (Cordeiro et al. 1999; Parida et al. 2009), rice (Chen et al. 1997), wheat (Pestsova et al. 2000), maize (Sharopova et al. 2002), and sorghum (Bhattramakki et al. 2000). Hindrances are usually observed during the construction of enriched genomic library (Ramsay et al. 2000; Bhattramakki et al. 2000). Cordeiro et al. (1999) reported that only 27% of the total microsatellite containing genomic clones identified from the sugarcane enriched library had primer designing potential. In several other studies the primer designing potential of enriched genomic clones containing microsatellites varied from 51% in wheat (Pestsova et al. 2000) to 66% in sorghum (Bhattramakki et al. 2000) and 90% in sugarcane (Parida et al. 2009). Failure to design primers for all the SSR motifs is due to different factors such as failure of sequencing past the microsatellite motifs and/or location of repeat-motif tract too near to the clone insertion sites, thus offering little or no flanking regions for designing primers (Parida et al. 2009). In spite of these hinderances, this method is widely applicable and is being used in the isolation of microsatellites in a number of plants (Table 3).

Two protocols were proposed to produce genomic libraries that were highly enriched for specific microsatellite repeats using a primer extension reaction (Ostrander et al. 1992; Paetkau 1999). Both methods rely on the construction of a primary genomic library, in which fragmented genomic DNA is inserted into a phagemid or a phage vector in order to obtain a single strand DNA (ssDNA) library. ssDNA is then used as a template for a primer extension reaction, primed with repeat-specific oligonucleotides, which generates a double stranded product only from vectors containing the desired repeat. During the primary library production step, for practical reasons, only a limited portion of the investigated genome is cloned, therefore, the population of inserts undergoes a severe bottleneck that results in loss of rare repeat motifs. Moreover, it is unclear whether the primer-extension approach is effective for tri- and tetranucleotide repeat motifs also. The Ostrander protocol has not been tested for tri- or tetranucleotide repeat enrichment, whereas the Paetkau protocol produced 0–25%

Table 3 Some recent reports on the development of SSRs through genomic library construction

Plant species	Description	References
Adiantum reniforme	Developed and characterized fourteen polymorphic microsatellite loci using the FIASCO protocol	Kang et al. (2006)
Amaranthus hypochondriacus, *A. cruentus*, and *A. caudatus*	Production and characterization of microsatellite markers from three microsatellite-enriched libraries	Mallory et al. (2008)
Arachis hypogaea	Constructed a microsatellite-enriched library from the genotype TMV2	Cuc et al. (2008)
Aucoumea klaineana	Nineteen microsatellite loci were isolated using enrichment protocol	Born et al. (2006)
Ficus montana and *Ficus septica*	Microsatellite markers were developed using genomic libraries enriched for di-, tri- and tetranucleotide repeats	Zavodna et al. (2005)
Hevea brasiliensis	Identified 67 microsatellites in genomic libraries of two clones using enrichment method	Roy et al. (2004)
Hippophae rhamnoides	Developed 26 microsatellite loci using biotin capture method of which 9 were polymorphic among different species	Wang et al. (2008a)
Incarvillea mairei	Developed 13 microsatellite markers using a modified biotin-streptavidin capture method	Ai et al. (2009)
Mangifera indica	Fifteen microsatellite loci were isolated using an enrichment protocol	Schnell et al. (2005)
Michelia coriacea	Developed 12 microsatellite loci using modified biotin–streptavidin capture method	Zhao et al. (2009)
Pinus resinosa	Isolated and characterized 13 nuclear microsatellite loci by screening a partial genomic library	Boys et al. (2005)
Prunus armeniaca	EST–SSRs were developed from cDNA libraries using an enrichment procedure for GA and CA repeats	Decroocq et al. (2003)
Psidium guajava	Constructed (GA)n and (GT)n microsatellite-enriched library and characterized 23 nuclear simple sequence repeat (SSR) loci	Risterucci et al. (2005)
Saccharum sp.	Enriched 6,318 clones from genomic libraries of two hybrid sugarcane cultivars with 18 different SSR repeat-motifs to generate 4.16 Mb high-quality sequences	Parida et al. (2009)
Setaria italica	Developed microsatellite markers from two genomic libraries enriched for (GA)n and (CA)n	Jia et al. (2009)
Solanum melongena	Constructed simple sequence repeat (SSR)-enriched genomic libraries and sequenced more than 14,000 clones	Nunome et al. (2009)
Sonneratia caseolaris	Microsatellite loci from an enriched (AG)n library were isolated using the FIASCO protocol with minor modifications	Chen et al. (2008)
Vitis vinifera	EST–SSRs were developed from cDNA libraries using an enrichment procedure for GA and CA repeats	Decroocq et al. (2003)

positive clones when using a tetranucleotide repeat primer in the extension step. These protocols involve a rather large number of steps, which might explain their limited application (Zane et al. 2002).

Development of microsatellites through screening of RAPD amplicons

To avoid library construction and screening, some authors proposed modifications of the RAPD approach for the amplification of unknown microsatellites, by either using repeat-anchored random primers (Wu et al. 1994) or using RAPD primers and subsequent southern hybridization of polymerase chain reaction (PCR) bands with microsatellite probes (Cifarelli et al. 1995; Richardson et al. 1995). Although not useful for single-locus analyses as no information on microsatellite flanking regions is obtained, these methods inspired alternative strategies for the identification of single microsatellite loci.

Based on the observed abundance of repeat regions in RAPD amplicons, isolation of microsatellite regions was achieved simply by means of southern hybridization of RAPD profiles with repeat containing probes, followed by the selective cloning of positive bands (Ender et al. 1996), or through the cloning of all the RAPD products and screening of arrayed clones (Lunt et al. 1999). Other non-library PCR-based strategies rely on the use of repeat-anchored primers to isolate and then sequence one (Fisher et al. 1996) or both regions (Lench et al. 1996; Cooper et al. 1997) flanking microsatellite repeats. All these methods provide, if successful, a quick alternative to laborious and time-consuming library screening, but their use has not been that frequent (Zane et al. 2002).

Development of microsatellites from EST sequences (genic or EST–SSRs)

Expressed sequence tags (ESTs), obtained by partial random sequencing of cDNA libraries, are 300–500 nucleotide long single read mRNA sequences from many of the genes expressed in a sample from an organism and they represent a snapshot of gene expression in a specific organ or tissue at a specific developmental stage. A wealth of sequence data of ESTs has been generated as a result of sequencing projects for gene discovery from several plant species, giving scientists the flexibility to access many full-length cDNA clones and characterized genes. These sequences are usually available in online databases in public domain, and can be downloaded and scanned for identification of SSRs. These identified SSRs are usually referred to as EST–SSRs or genic microsatellites. In this approach, SSR identification software tools are used to screen the sequence data of ESTs (Varshney et al. 2007). Several search modules or programmes like MISA (MIcroSAtellite), SSRfinder, Sputnik, SSRIT (SSR Identification Tool), SSRSEARCH, TRF (Tandem Repeat Finder) etc. have been developed for recognition of SSR patterns in the sequence files. The generation of genic SSR markers is relatively easy and inexpensive because they are the byproducts of the sequence data from genes or ESTs that are publicly available (Varshney et al. 2005a). Recently, Tang et al. (2008) developed PolySSR, a new pipeline to identify polymorphic SSRs which includes PCR-primer designing for the putatively polymorphic SSR markers taking into account the Single Nucleotide Polymorphisms (SNPs) in the flanking regions thereby improving the success rate of the potential markers. They identified a large number of polymorphic SSRs using publicly available EST sequences of potato, tomato, rice, *Arabidopsis*, *Brassica* and chicken.

EST–SSRs offer advantages over genomic SSRs because they detect variation in the expressed portion of the genome, so that gene tagging should give ''perfect'' marker-trait associations and once developed, these markers, unlike genomic SSRs, may be used across a number of related species (Gupta et al. 2003). Microsatellites have been isolated for a number of plant species using this strategy (Table 4). Greater DNA sequence conservation in transcribed regions, however, leads to lower polymorphism in genic SSRs making them less efficient compared to genomic SSRs for distinguishing the closely related genotypes. Therefore, genomic SSRs are superior over genic SSRs for fingerprinting or varietal identification studies. In addition, the development of genic SSRs is restricted to only those species for which there are sufficient sequence data available (Varshney et al. 2005a). A major drawback of the EST-derived microsatellites is the sequence redundancy that yields multiple sets of markers at the same locus (Parida et al. 2006). To circumvent the problem of redundancy in EST databases, a non-redundant unigene EST data set (random EST sequences assembled into unique gene sequences called unigenes) should be used. The main advantage of unigene-based microsatellite markers (UGMS) is the accurate reflection of density of SSRs in the transcribed regions of the genome. Furthermore, with the availability of large unigene databases, it is possible to systematically search for microsatellites in the unigenes (Parida et al. 2006).

Development of microsatellite through interspecific or intergeneric transferability

Comparative genetics has revealed that gene content and order are highly conserved among closely related species. Sequence data obtained from several crop plants indicate sufficient homology existing between genomes of two or more closely related genera/species. Thus, primer pairs designed on the basis of the sequences obtained from one species could be

Table 4 Some recent reports on the development of SSRs through EST database mining

Plant species	Description	References
Arachis hypogea	881 SSRs were identified from 780 unique ESTs	Liang et al. (2009)
Carthamus tinctorius	Assembled 40,874 reads into 19,395 unigenes, of which 4,416 (22.8%) contained at least one SSR	Chapman et al. (2009)
Cicer arietinum	*MISA* script used for microsatellite identification	Varshney et al. (2009)
Cicer arietinum	Identified 246 SSRs from 2,131 EST sequences	Choudhary et al. (2009)
Citrus sp.	Fourty one EST–SSR markers were produced and were available for citrus genetic studies	Luro et al. (2008)
Coffea arabica and *C. canephora*	Used MISA for microsatellite identification	Aggarwal et al. (2007)
Eleusine coracana ssp. *coracana*	Identified microsatellites using "SSR Primer" an integrated program of SPUTNIK	Arya et al. (2009)
Eucalyptus spp.	Used *MISA* for identification of microsatellites sequence from *Eucalyptus* genome sequencing Project Consortium	Rabello et al. (2005)
Glycine max	Microsatellite identified from soybean non-redundant ESTs in public databases using the FindPatterns module from the GCG software package	Hisano et al. (2007)
Lolium perenne	Identified 955 SSR containing ESTs	Studer et al. (2008)
Lycopersicon esculentum	Developed nearly 700 SSR markers using BAC-end and cDNA sequences	Ohyama et al. (2009)
Nelumbo nucifera	Detected 71 unique SSRs from 125 ESTs	Pan et al. (2010)
Phyllostachys pubescens	Found 3, 241 SSR loci	Tang et al. (2010)
Pinus taeda	Identified 14 species-transferable microsatellite markers from ESTs	Liewlaksaneeyanawin et al. (2004)
Rubus idaeus	Developed 25 polymorphic EST–SSRs	Woodhead et al. (2003)
Solanum tuberosum	Sequence information obtained from Potato Gene Index database using the BLAST search algorithm	Feingold et al. (2005)
Sorghum bicolor	Sequence information obtained from SAMI (Sorghum Assembled Genomic Island)	Li et al. (2009)

used to detect SSRs in related species and even in other genera of the same family. The ability to effectively transfer SSR markers across the taxa, which is commonly known as "transferability", has been successfully demonstrated in many species (Ellis and Burke 2007; Varshney et al. 2007). Both genic and genomic SSR markers can be transferred across species, however, genic SSR markers are expected to have a high transfer rate due to conservation of transcribed regions among related species. Results of many studies have clearly indicated that EST–SSRs can be transferred not only to species within a genus, but in some instances even to multiple genera within a family (Ellis and Burke 2007). The transfer rate will correspond to the phylogenetic distances and extent of sequence conservation between the species under study. Cross transferability of EST–SSRs has been demonstrated in many crops (Table 5). Transfer of EST–SSRs from finger millet (*Eleusine coracana*) to pearl millet (*Pennisetum glaucum*) with an average transfer rate of 64.7% was reported recently by Arya et al. (2009). Parida et al. (2009) tested the cross-transferability potential of sugarcane genomic microsatellites (SEGMS) in other Saccharum species and five cereal species. Transferability rates of 93.2 and 25% of the SEGMS markers designed from hybrid sugarcane cultivar were obtained in other members of Saccharum complex and cereals, respectively.

The extent of cross-transferability of a marker system determines its suitability in comparative genome mapping and phylogenetics. This method of microsatellite detection is especially useful in minor crops where neither sequence information nor the genetic maps are available. Most of the diversity studies and phylogenetic relationships which were primarily based on morphological markers in minor crops can now be assessed more robustly using

Table 5 Some recent reports on interspecific or intergeneric transferability of EST–SSRs

Plant species (source taxon)	Plant species (recipient taxon)	References
Arachis hypogea	17 wild *Arachis* species	Gimenes et al. (2007)
Athyrium distentifolium	3 *Athyrium* spp.	Woodhead et al. (2003)
Bambusa oldhamii	25 species of Bambusoideae	Sharma et al. (2009)
Capsicum annuum	*C. baccatum, C. chacoense, C. chinense, C. frutescens and C. pubescens*	Ince et al. (2010)
Carthamus tinctorius	Eight other pant species of family *Asteraceae*	Chapman et al. (2009)
Castanea sativa and *C. crenata*	*C. mollissima* Blume, *C. seguinii* Dode, and *C. henryi* (Skan.) Rehder & Wilson	Wang et al. (2008b)
Cereals (barley, maize, rice, sorghum, wheat)	*Lolium* sp. (ryegrass)	Sim et al. (2009)
Cereals (wheat, rice, sorghum, maize)	Minor grasses (*Eleusine coracana*; *Paspalum vaginatum*; and *Cynodon dactylon)*	Wang et al. (2005)
Citrus clementina	Other *Citrus* species	Luro et al. (2008)
Coffea sp.	*Psilanthus* spp.	Bhat et al. (2005)
Cucurbita sp.	*C. pepo, C. moschata,C. ecuadorensis*	Gong et al. (2008)
Curcuma longa	13 related species of *C. longa*	Siju et al. (2010)
Eleusine coracana	*Pennisetum glaucum*	Arya et al. (2009)
Festuca arundinacea	Wheat, rice	Saha et al. (2004)
Festuca arundinacea	Six grass species	Saha et al. (2006)
Ficus insipida, *F. racemosa* and *F. rubiginosa*	*F. citrifolia and F. eximia*	Nazareno et al. (2009)
Gossypium arboreum	25 diploid accessions of *Gossypium* species	Guo et al. (2006)
Helianthus annuus	*Helianthus angustifolius, H. verticillatus*	Pashley et al. (2006)
Hevea brasiliensis	12 *H.brasiliensis* varieties, 4 related *Hevea* species and three intergeneric (*Ricinus communis*, *Mannihot utilissima* and *Phyllanthus emblica*) species	Feng et al. (2009)
Hordeum vulgare	*H. bulbosum*, *Triticum aestivum*	Thiel et al. (2003)
	Secale cereale, Oryza sativa	Varshney et al. (2005b)
	H. chilense	Castillo et al. (2008)
Legumes (*Medicago*, soyabean, cowpea and groundnut)	24 different legume genera and species	Wang et al. (2004)
Lolium perenne	*Festuca arundinacea*	Asp et al. (2007)
Medicago truncatula	Three leguminous and three non-leguminous species	Gupta and Prasad (2009)
Medicago truncatula	*Abelmoschus esculentus*	Sawadogo et al. (2009)
Malus × *domestica*	Members of Rosaceae family, representing three genera and 14 species	Gasic et al. (2009)
Olea europaea L.	Other *Olea* species	Rallo et al. (2003)
Oryza sativa, Saccharum sp.	Bamboos	Sharma et al. (2008)
Pinus taeda	*P. contorta* ssp. *latifolia*, *P. ponderosa*, and *P. sylvestris*	Liewlaksaneeyanawin et al. (2004)
Pinus taeda and *P. pinaster*	Six other pine species	Chagne et al. (2004)
Prunus armeniaca	Other Rosaceae members	Decroocq et al. (2003)
Solanum melongena	Other Solanaceae crops (potato, tomato and pepper)	Stagel et al. (2008)
Triticum aestivum	*Hordeum vulgare*, *Oryza sativa* and *Zea mays*	Tang et al. (2006)
Vitis vinifera	Other Vitaceae members	Decroocq et al. (2003)

transferable SSR markers. Comparative mapping of transferable SSRs from related species will help to get a genetic map in minor species and better enrichment of chromosomal regions with fewer markers. Also, SSR marker from a gene with known function can be used for homologous gene identification and cloning in related species if the same is transferable to the related species.

Fingerprinting techniques based on microsatellites

A number of hybridization-based and PCR-based techniques have been developed to exploit the hypervariable nature of microsatellites for applications in plant genomics. In hybridization based technique, labelled synthetic oligonucleotide probes, complementary to microsatellite sequences are used for in-gel hybridization of restriction digested and electrophoretically separated DNA fragments on agarose gels. The ploymorphism detected by this method actually represents polymorphism due to variation in the length of restriction fragments carrying the microsatellites, even when there may be no difference in the length of the microsatellites that are carried by these fragments. The fragments that hybridize with synthetic oligonucleotides range from a few hundred base pairs to as many as 8–10 kb. This technique produced high levels of polymorphism between related genotypes and has proved useful for paternity testing, genotype identification and population genetics (Weising et al. 1995). However, the disadvantages like requirement of large amounts of DNA, tedious and time consuming protocols, non-amenablity to automation and labor inputs associated with this approach limits its use.

A large number of PCR-based methods have also been developed to study SSR based polymorphism in different plant species. DNA polymorphisms can be detected at individual loci by PCR either using locus specific primers flanking the microsatellites (Tautz 1989; Weber and May 1989) or by using random synthetic oligonucleotide primers complementary to the repeat motifs (Meyer et al. 1993; Zietkiewitcz et al. 1994; Wu et al. 1994).

The sequences flanking the microsatellite motifs in the genome are conserved within the particular species and often across the species within a genus and even across related genera (Gupta and Varshney 2000). Therefore, these flanking sequences are used for designing locus-specific primers to amplify individual microsatellite loci and are known as sequence tagged microsatellites sites (STMS) markers (Beckmann and Soller 1990). These markers show polymorphism due to variation in lengths of the microsatellites at individual microsatellite loci. STMS markers are locus specific co-dominant markers used for a variety of plant breeding purposes (Nybom 2004). The differences between alleles are resolved by high-resolution agarose or PAGE gels in combination with ethidium bromide, silver staining or radio-labeling of the primers. Automated detection of microsatellite alleles is also possible using fluorescent primers in combination with semi-automated or automated DNA sequencer. The STMS markers have been extensively used for high throughput genotyping and genome mapping in plants. Development of STMS markers requires identification of microsatellites from the genome, cloning and sequencing, making it cost and labor intensive initially. However, once the locus specific primers become available, its subsequent use becomes cost effective.

Limitations with development of STMS markers led to the development of several other approaches which were PCR based but did not required sequence information to detect polymorphism. Among these, microsatellite-primed PCR (MP-PCR; Meyer et al. 1993) or Inter Simple Sequence Repeat (ISSR; Zietkiewitcz et al. 1994) or Single Primer Amplification Reaction (SPAR, Gupta et al. 1994) is a method wherein arbitary synthetic olegonucleotides, representing a specific microsatellite motif is used as PCR primer. This technique targets the region between two inversely oriented microsatellites within an amplifiable distance. The primers based on tri-, tetra- and pentanucleotide repeat motifs have been found useful since primers representing dinucleotide repeat motifs produce smears attributed to the abundance of dinucleotide repeats in the genome. In anchored ISSR, the di- or trinucleotide repeats are anchored through 1–4-nucleotide repeats at either 5′- or 3′-end. This technique targets the amplification of a subset of regions that are amplified by MP-PCR and therefore, lead to improvement in resolution and reproducibility, a problem that limits the use of MP-PCR.

Random amplified microsatellite polymorphisms (RAMPs; Wu et al. 1994), is another approach in which amplification is performed using a 5′-anchored ISSR primer and a RAPD primer. The amplified products resolve length polymorphism that may be present either at the SSR target site itself or at the associated sequence between the binding sites of the two primers. The RAPD primer binding sites serve as an arbitrary endpoint for SSR-based amplification product. The amplified products may also be digested with a restriction enzyme and are called as digested RAMPs (dRAMPs, Becker and Huen 1995). Reverse random amplified microsatellite polymorphism (rRAMP, Min et al. 2008), allows the detection of nucleotide variation in the 3′ region flanking an SSR using normalized anchored and random primer combinations. The reproducibility and frequency of polymorphic loci in rRAMP is vigorously enhanced by translocation of the 5′ anchor of repeat sequences to the 3′ end position and selective use of moderate arbitrary primers. In Random amplified microsatellite polymorphisms (RAMPO, Richardson et al. 1995) the amplicons are generated using an SSR and a RAPD primer as in RAMP followed by southern blotting and hybrdization with labelled probes. In another approach called Random amplified hybridization microsatellites (RAHM, Cifarelli et al. 1995) the amplicons generated through RAPD are hybridized with SSR-motif containing probes after southern blotting.

Selective amplification of microsatellite polymorphic loci (SAMPL), combines the advantages of both STMS and AFLP (Vos et al. 1995). The AFLP comprises three steps: (1) restriction digestion of genomic DNA with a frequent and a rare cutter, (2) ligation of adaptors of known sequence at both 5′- and 3′-ends of each fragment, and (3) selective amplification of digested adaptor ligated genomic DNA fragments with a primer complementary to adaptor sequence with 1–3 selective nucleotide at its 3′-end. The amplification may involve two steps, the first involving pre-amplification with a selective nucleotide and the second involving 1–3 selective nucleotides at 3′-end of one or both primers. In SAMPL, one AFLP primer with three selected nucleotides is used in combination with a SAMPL primer that is complementary to a microsatellite sequence, during the second amplification step. A polymorphic band on SAMPL fingerprinting gel can also be converted into a conventional, single locus SSR marker by cloning and sequencing the flanking regions. This method though complex, allows researchers to detect microsatellite polymorphism without prior sequence information.

Fast isolation by AFLP of sequences containing repeats (FIASCO, Zane et al. 2002) protocol also relies on the extremely efficient digestion-ligation reaction of the AFLP. DNA is simultaneously digested with *Mse*I and ligated to *Mse*I AFLP adaptor. The digestion-ligation mixture is diluted and directly amplified with AFLP adaptor-specific primers. In this protocol, the amplification is performed by mixing primers carrying all four possible selective bases (*Mse*I-N), thus allowing amplification of all fragments flanked by *Mse*I sites, providing only that they have an appropriate size for PCR. Amplified DNA is then hybridized with a biotinylated probe and hybridized fragments are selectively captured by streptavidin coated beads. The DNA separated from the beads-probe complex is reprecipitated which provides the best candidates for producing a highly enriched microsatellite library.

In Retrotransposon Microsatellite Amplified Polymorphism (REMAP, Kalendar et al. 1999) an anchored microsatellite primer is used in combination with a primer based on LTR of a retrotransposon. The primers are designed using di- and tri-nucleotide microsatellite motifs with addition of a single selective nucleotide at 3′-end.

Applications of microsatellites

Microsatellites have become a marker of choice for an array of applications in plants due to hypervariable nature and extensive genome coverage. These are being used to assess genetic variation at molecular level in a germplasm collection for making appropriate choice of parents for crosses (i.e. hybrid breeding), mapping and tagging of genes or QTLs (quantitative trait loci) for agronomic and disease resistance traits, genome mapping, MAS of promising lines and marker assisted backcrossing (MAB) during breeding programs, gender identification, studying the population structure and taxonomic and phylogenetic relationships (Fig. 1).

Diversity analysis, measurement of genetic similarity or differences among plant species, is important information in crop conservation and varietal

development (Romero et al. 2009). Furthermore, the information is also useful for characterization of accessions in plant germplasm collections and taxonomic studies. In recent years, microsatellite markers have proved to be a powerful tool for estimation of genetic diversity (variation in nucleotide sequence, gene structure, chromosomes and whole genomes) and phylogenetic relationships of species based on sequence conservation due to its high efficiency, codominant nature, reproducibility and high degree of polymorphism. Microsatellites are useful in cultivar identification and are also advantageous in pedigree analysis as these represent a single locus. The multiallelism of these markers facilitates comparative allelic variability detection reliably across a wide range of germplasm (Joshi et al. 1999). Another important application of microsatellites is in the determination of hybridity, wherein the codominant nature of microsatellites play a key role and allows the allelic contribution of each parent to be detected in sexual and somatic hybrids (Powell et al. 1996). Table 6 summarizes some recent reports wherein SSRs have been used for various applications in plants.

A small group of flowering plants are sexually dimorphic where the female plants are commercially valued for production of fruits (papaya, kiwi fruit, dates, seabuckthorn, etc.) and seeds (pistachio, nutmeg, black pepper, jojoba, etc.). However, sex of most of the dioecious plants is not revealed morphologically and the male and female plants cannot be distinguished at seedling stage. This problem is exacerbated in species where the sex of an individual is revealed only after flowering which may take few months (papaya, *Coccinia*) to several years (date palm, nutmeg and jojoba). Sex linked microsatellite markers have been described in several species like hemp (Rode et al. 2005), wild strawberry (Spigler et al. 2008), hop (Jakse et al. 2008), *Carica papaya* (Parasnis et al. 1999) and *Actinidia chinensis* (Fraser et al. 2009). Parasnis et al. (1999) used a microsatellite probe $(GATA)_4$ as a diagnostic marker in papaya and demonstrated the sex-specific DNA variation at any stage of the plant development. Recently, Fraser et al. (2009) constructed gene-rich female, male and consensus linkage maps of the diploid species *A. chinensis* using 644 microsatellite markers. They created genetic linkage maps defining the 29 linkage groups of the haploid genome, revealed the position and extent of the sex-determining locus and also identified, through sex-linked markers, putative X and Y chromosomes.

The development of organelle specific markers (i.e. cpSSR and mtSSR) had a great impact on the determination of structure and variation within a natural population as well as phylogenetic relationships. The uniparental mode of inheritance, conserved gene order and lack of heteroplasmy and recombination of organelle genomes makes them an attractive tool for evolutionary studies mainly patterns of migration, population histories and levels of differentiation (Provan et al. 2001). However, ESTs are also being used for such analysis because in such studies, one actually looks at the evolution of functional genes (Joshi et al. 1999). Evaluation of genetic diversity and phylogenetic relationships has resulted in identification of some misclassified accessions that were reclassified. Genetic diversity assessment and phylogenetic relationship construction will provide important information for choosing parental lines for breeding programs, classification of plant germplasm accessions, and further curation and acquisition of new plant germplasm accessions (Wang et al. 2009a).

Microsatellite markers have been efficiently employed in determination of specific genomic regions that are responsible for the expression of important physiological and agronomic traits. Furthermore, microsatellite markers can also be used in analyzing quantitative trait loci (QTLs) which can lead to the identification of candidate genes for the trait of interest that are particularly vital for a breeding program like yield, disease resistance, stress tolerance, seed and fruit quality etc. (Neeraja et al. 2007; Romero et al. 2009). However, in contrast to microsatellite markers developed from genomic library, EST–SSRs can contribute to direct allele selection because they have known or putative functions and may be associated with targeted trait (Varshney et al. 2005a). Association mapping which refers to significant association of a molecular marker with a phenotypic trait is especially useful for implementing marker-assisted selection for quantitative traits in plant breeding programs (Breseghello and Sorrells 2006b). QTL mapping usually uses a population from a bi-parental cross, while association mapping uses a collection of individuals often with varying ancestry. In recent years, genetic maps have been prepared in several plant species including rice, wheat, barley, cotton, ryegrass, white clover,

Table 6 Some recent reports on application of microsatellites in plants

Plant species	Application	Reference
Genome mapping		
Brassica rapa	Constructed an SSR-based linkage map through comparative genomics with *Arabidopsis thaliana* and mapped QTLs for clubroot resistance through synteny analysis	Suwabe et al. (2006)
Cereals	Interspecific transferability and comparative mapping of barley EST–SSR markers in wheat, rye and rice	Varshney et al. (2005b)
Festuca arundinacea	Developed an SSR- and AFLP-based genetic linkage map	Saha et al. (2005)
Glycine max	Microsatellite discovery from BAC end sequences and genetic mapping to anchor the soybean physical and genetic maps	Shoemaker et al. (2008)
Glycine max	Developed BAC-end sequence-based microsatellite markers and placed them in the physical and genetic maps of soybean	Shultz et al. (2007)
Glycine max	Association mapping of iron deficiency chlorosis loci	Wang et al. (2008c)
Pinus	Cross-species transferability and mapping of genomic and cDNA SSRs in other pines	Chagne et al. (2004)
Quercus	Comparative mapping between *Quercus* and *Castanea*	Barreneche et al. (2004)
Solanum melongena	Gene-based microsatellite development for mapping and phylogeny studies	Stagel et al. (2008)
Solanum tuberosum	Used a probe derived from the tomato Verticillium resistance gene (Ve1)and identified homologous sequences (StVe1) in potato using linkage disequilibrium mapping	Simko et al. (2004)
Spinacia oleracea	Constructed a genetic map of Spinach in a classical back cross population using 101 AFLP and 9 microsatellite markers	Khattak et al. (2006)
Triticum aestivum	Association mapping of kernel size and milling quality in wheat cultivars	Breseghello & Sorrells (2006a)
Triticum aestivum	Microsatellite mapping of adult-plant leaf rust resistance gene Lr22a in wheat	Hiebert et al. (2007)
Triticum aestivum	Enhanced the resolution of an existing linkage map and identified putative functional polymorphic gene loci in hexaploid wheat	Yu et al. (2004a)
Triticum aestivum and Oryza sativa	Existing genetic linkage maps were enhanced using orthologous loci amplified with 58 EST–SSR markers obtained from both wheat and rice ESTs	Yu et al. (2004b)
Vitis vinifera	Developed a reference integrated map from three crosses, based on 283 SSR and 501 SNP-based markers	Vezzulli et al. (2008)
QTL tagging, marker assisted selection (MAS) and marker assisted backcrossing (MAB)		
Glycine max	Evaluated linkage drag for seed yield by using NILs with introgressed quantitative trait loci conditioning resistance to corn earworm and soybean looper	Warrington et al. (2008)
Hippophae L.	Identified ISSR markers associated with dried shrink disease	Ruan et al. (2009)
Hordeum vulgare	Developed a SSR marker, QLB1, tightly linked to Rym4/Rym5 locus conferring resistance to the barley yellow mosaic virus which can be used efficiently in marker-assisted selection	Tyrka et al. (2008)

Table 6 continued

Plant species	Application	Reference
Oryza sativa	A marker-assisted backcross approach for developing submergence tolerant rice cultivars	Neeraja et al. (2007)
Oryza sativa	SSR mapping of brown planthopper resistance gene Bph9 in Kaharamana, an Indica rice	Su et al. (2006)
Pisum sativum	Developed microsatellite markers for powdery mildew resistance	Ek et al. (2005)
Triticum aestivum	Characterization of phytoene synthase 1 gene (*Psy1*) located on chromosome 7A and development of a functional marker	He et al. (2008)
Zea mays	Identified QTL for resistance to maize streak virus disease in maize genotypes used in hybrid development	Lagat et al. (2008)
Hybrid testing		
Arachis hypogea	Used SSR markers to differentiate between selfs and hybrids	Gomez et al. (2008)
Brassica oleracea var. *botrytis*	Purity testing of F_1 hybrid seeds	Astarini et al. (2008)
Capsicum annuum	Hybrid identification and genetic purity testing in pepper	Juhász et al. (2006)
Carthamus tinctorius	Assessment of hybrid seed purity	Naresh et al. (2009)
Juglans nigra × *Juglans regia*	Identification of hybridogenic plants and parentage analysis	Pollegioni et al. (2009)
Lycopersicon esculentum	Seed genetic purity of two commercial hybrid cultivars	Liu et al. (2007)
Oryza sativa	Used SSRs for fingerprinting of rice hybrids and their parental lines and used them in genetic purity assessment of hybrid rice	Tamilkumar et al. (2009)
Oryza sativa	Fingerprinting and genetic purity determination of hybrid seeds	Hashemi et al. (2009)
Zea mays	Compared two methods for choosing parental components in early-generation hybrid testing and indicated that selecting parental components based on molecular marker data is advantageous for the performance of the hybrids	Chuanchai et al. (2010)
Gender determination		
Actinidia chinensis	Constructed the gene-rich female, male and consensus linkage maps of the diploid species using 644 microsatellite markers. Created genetic linkage maps defining the 29 linkage groups of the haploid genome, and revealed the position and extent of the sex-determining locus. Also identified, through sex-linked markers, putative X and Y chromosomes	Fraser et al. (2009)
Cannabis sp.	Identified sex-linked SSR markers	Rode et al. (2005)
Fragaria virginiana	Phenotypic and genetic mapping results supported a model of gender determination with at least two linked loci (or gene regions) with major effects	Spigler et al. (2008)
Humulus lupulus	Identified a trinucleotide microsatellite repeat tightly linked to male sex	Jakse et al. (2008)
Pseudocalliergon trifarium (moss)	Identified Sex-Specific Marker and designed a primer pair to allow the amplification of a 159-bp portion of the female-specific DNA region	Korpelainen et al. (2008)
Spinacia oleracea	Identified a small chromosomal region co-segregating with sex determination in the species with a distance of 1.9 cM to a microsatellite marker termed SO4	Khattak et al. (2006)

Table 6 continued

Plant species	Application	Reference
Genetic diversity, cultivar identification, population genetics, taxonomic and phylogenetic relationships		
Allium cepa	Analysis of genetic variation in a cultivated onion germplasm	McCallum et al. (2008)
Arachis hypogaea	Genetic diversity of cultivated and wild-type peanuts evaluated with M13-tailed SSR markers and sequencing	Barkley et al. (2007)
Arachis hypogaea	Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut	Cuc et al. (2008)
Asparagus	Genetic diversity evaluation among thirty-five cultivars and cultivar identification	Caruso et al. (2008)
Bamboos	Assessment of genetic diversity and phylogenetic relationships of the temperate bamboo collection using transferred EST–SSR markers	Barkley et al. (2005)
Castanea sp.	Characterization in diverse Chestnut cultivars	Inoue et al. (2009)
Citrus sp.	Assessed genetic diversity and population structure in a *Citrus* germplasm collection	Barkley et al. (2006)
Citrus sp.	Studied the effectiveness of transferable EST–SSRs for genetic mapping	Luro et al. (2008)
Crotalaria	Genetic diversity of germplasm assessed through phylogenetic analysis of EST–SSR markers	Wang et al. (2006b)
Cucumis sp.	Molecular phylogeny of *Cucumis* species as revealed by consensus chloroplast SSR marker length and sequence variation	Chung et al. (2006)
Ficus carica	Characterized fig germplasm in Morocco maintained in an *ex situ* collection	Achtak et al. (2009)
Fragaria × *ananassa*	Analysis of genetic diversity and population structure among 92 selected cultivars	Gil-Ariza et al. (2009)
Glycine max	Compared genetic diversity between Chinese and Japanese soybeans using nuclear SSRs	Guan et al. (2010)
Lablab purpureus	Evaluated genetic diversity of the USDA germplasm collection	Wang et al. (2007)
Lespedeza	EST–SSR markers derived from *Medicago*, cowpea and soybean were used to assess the genetic diversity of the USDA *Lespedeza* germplasm collection and clarify its phylogenetic relationship with the genus *Kummerowia*	Wang et al. (2009b)
Olea europaea	Genetic relationships within and between wild and cultivated olives were examined and clarified in an isolated and restricted area of the Mediterranean island of Sardinia	Erre et al. (2010)
Olea europaea	Identification of cultivars diffused in Southern-Italy	Alba et al. (2009)
Oryza sativa	Genetic analysis and genotyping	Singh et al. (2010)
Oryza sp.	Phylogenetic analysis of *Oryza* species, based on mtSSR and cpSSR and their flanking nucleotide sequences	Nishikawa et al. (2005)
Paspalum vaginatum	Characterized seashore paspalum germplasm by transferred SSRs from wheat, maize and sorghum	Wang et al. (2006a)
Pisum sativum	Assessment the genetic relations in *Pisum*	Nasiri et al. (2009)
Prunus persica	Analysis of genetic diversity in primary core collection	Li et al. (2008)
Prunus amygdalus	Genetic diversity analysis of 93 almond genotypes, including 63 Spanish cultivars from different growing regions	Martí et al. (2009)

Table 6 continued

Plant species	Application	Reference
Prunus avium	Assessment of genetic relationships among 126 accessions	Lacis et al. (2009)
Rubus idaeus	Reexamined the genetic diversity and population differentiation	Graham et al. (2009)
Solanum lycopersicum	Genetic variation in 216 tomato cultivars, hybrids, and elite breeding lines	Chen et al. (2009)
Solanum tuberosum	SSR genotyping of landraces supported a major reevaluation of their gene pool structure and classification	Spooner et al. (2007)
Sorghum bicolor	Studied cpSSR variation in 185 Chinese sorghum landraces and 70 cultivated sorghum accessions	Li et al. (2010)
Sorghum bicolor	Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm	Ali et al. (2008)
Spinacia oleracea	Genic microsatellite markers for discrimination of spinach cultivars	Khattak et al. (2007)
Triticeae	Investigated genetic similarity between 150 accessions, representing 14 diploid and polyploid species of the Triticeae tribe using 73 common wheat EST–SSRs	Zhang et al. (2006)

raspberry, potato, sorghum, etc. Once mapped, microsatellite markers could be employed in tagging several individual traits that are particularly important for a breeding program. Association mapping using SSR markers has been successfully conducted in many important crop species such as potato, maize, wheat, and soybean (Wang et al. 2009a). Association between a microsatellite marker and QTL for resistance to *Verticillium dahliae* was identified in potato cultivars which led to cloning of QTL for resistance to *V. dahliae* (Simko et al. 2004). The correlation between SSR markers and wheat kernel size was detected by association mapping using elite germplasm (Breseghello and Sorrells 2006a).

Furthermore, a large number of monogenic and polygenic loci for various traits could be identified and exploited for marker-assisted selection (Joshi et al. 1999). Marker assisted selection can help breeders bypass the traditional phenotype based selections in the field thus expediting the breeding programs. The rice variety Swarna could be efficiently converted to a submergence tolerant variety in three backcross generations, involving a time of two to three years using marker assisted backcrossing (Neeraja et al. 2007). Molecular variation within defined genes underlying specific functions provide candidate gene-based markers which show very close association with the trait of interest and thus should enable to design superior genotypes. For instance, Srinivas et al. (2009) recently explored microsatellite loci in a total of 9,892 subtracted drought stress ESTs of sorghum available in the NCBI dbEST database and suggested that it may be applicable in QTL analysis for drought stress. In another study, Varshney et al. (2009) generated a total of 20,162 drought- and salinity-responsive ESTs from ten different root tissue cDNA libraries of chickpea. Generated set of ESTs serves as a resource of high quality transcripts for gene discovery and development of functional markers associated with abiotic stress tolerance. Transgenic approaches can also be combined with marker-assisted selection for developing insect and disease-resistant cultivars.

Genome mapping is another field where microsatellites are being extensively used. Genome mapping consists of genetic mapping, comparative mapping, physical mapping, and association mapping. Genetic mapping with microsatellite markers in plants were first reported in tropical trees and then reported in soybean, rice, etc. So far there are over 80 genetic maps constructed involving the use of SSR markers from many plant species. Comparative mapping has been successfully conducted in many plant species, including the *Solanaceae* family, grasses, crucifers, legumes and other species (Wang et al. 2009a). Comparative genomics of *Arabidopsis* relatives has great potential to improve our understanding of molecular function and evolutionary processes. Recent studies of phylogenetic relationships within Brassicaceae provide an important framework for

comparative genomics research. Comparative linkage mapping and chromosome painting in the close relatives of *Arabidopsis* inferred an ancestral karyotype of these species. In addition, comparative mapping to *Brassica* identified genomic blocks that have been maintained since the divergence of the *Arabidopsis* and *Brassica* lineages (Schranz et al. 2007). Microsatellite markers have been used for comparative mapping between *Quercus robur* (L.) and *Castanea sativa* (Mill.) (Barreneche et al. 2004). EST–SSR markers were used in comparative mapping in wheat, barley, rye and rice. The conservative chromosome regions between wheat and rice and the presence of orthologues of barley EST–SSRs in different species have been confirmed and identified (Yu et al. 2004b; Varshney et al. 2005b; Stein et al. 2007). SSR markers have also been used to construct whole genome physical maps of model crop species. SSR markers were used for anchoring and comparing the frames of soybean genetic and physical maps (Shultz et al. 2007; Shoemaker et al. 2008). A physical map of a ~2 Mb BAC contig in the region around 80 cM of *Arabidopsis thaliana* chromosome 2 was constructed using SSR markers and BAC end-sequences (Wang et al. 1997).

Advent of new technologies has not affected the use of microsatellites due to their cost effectiveness and use in large scale genotyping. Microsatellite based markers are reliable and easy to use tools for fingerprinting applications including varietal and cultivar discrimination, marker assisted breeding, map based cloning and studies related to gene flow. Future of microsatellite based markers is promising in light of existence of diverse primary and secondary gene pools in many species of plants. Characterization of these gene pools is an immense task, and microsatellite based markers are the only cheap, reproducible and efficient methodology which will be able to account for these characterization studies.

Concluding remarks

Ever since their development, microsatellites markers are constantly being isolated and characterized in a wide range of plants including cereals, legumes, vegetables, forest trees, fruit plants, conifers and other economically important plant species. Microsatellite markers are involved not only in genetic diversity studies, population genetics and evolutionary studies, but are also being used in fundamental research like genome analysis, gene mapping, marker-assisted selection etc. SSR based association mapping holds a great promise for exploiting genetic diversity, characterizing accumulated phenotypic variation, and associating markers with traits in plant germplasm. However, there are several potential drawbacks including the presence of stutter bands, null alleles and heterologous amplicons. Special caution has to be taken if some of the drawbacks are encountered in research (Wang et al. 2009a). The microsatellite markers associated with clear phenotypes can definitely be used in plant breeding programs by MAS to expedite the breeding process. Microsatellite markers can facilitate comparative mapping and help to identify 'linkage blocks', major gene syntenies, chromosome rearrangements, and microsyntenies among species (Wang et al. 2009a). However, the need to isolate them de novo remains a major bottleneck in application of microsatellites as marker in species being examined for the first time. This problem has been partially circumvented by exploiting publicly available genome resources which has been found to be inexpensive and significantly more transferable across taxonomic boundaries than are traditional SSRs (Ellis and Burke 2007). In recent years, EST database is generated continuously for a variety of crops and the trend is towards cross-referencing genes and genomes using sequence and map-based tools (Varshney et al. 2007). Development of transferable SSR markers from sequence information being generated from major crops will provide the initial platform for evaluation, characterization and genetic map development in minor crops. Unigene derived microsatellite markers overcome the problem of redundancy in EST database and have the advantage of assaying variation in the transcribed regions of the genome with unique identity and positions (Parida et al. 2006). A vast amount of genetic diversity exists in plant germplasm and more microsatellite markers will become available as genomic information accumulates. Steady progress and advancement in microsatellites markers will make it more attractive for molecular breeding and plant genetics and ultimately help in major crop improvement.

References

Achtak H, Oukabli A, Ater M, Santoni S, Kjellberg F, Khadari B (2009) Microsatellite markers as reliable tools for fig cultivar identification. J Am Soc Hort Sci 134:624–631

Aggarwal RK, Hendre PS, Varshney RK, Bhat PR, Krishnakumar V, Singh L (2007) Identification, characterization and utilization of EST-derived genic microsatellite markers for genome analyses of coffee and related species. Theor Appl Genet 114:359–372

Ai HL, Wang H, Li DZ, Yang JB (2009) Isolation and characterization of 13 microsatellite loci from *Incarvillea mairei* (Bignoniaceae), an endemic species to the Himalaya–Hengduan mountains region. Conser Genet 10:1613–1615

Alba V, Montemurro C, Antonella WS, Pasqualone AB (2009) SSR-based identification key of cultivars of *Olea europaea* L. diffused in Southern-Italy. Sci Hort 123:1–16

Ali ML, Rajewski JF, Baenziger PS, Gill KS, Eskridge KM, Dweikat I (2008) Assessment of genetic diversity and relationship among a collection of US sweet sorghum germplasm by SSR markers. Mol Breed 21:497–509

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Armour JA, Neumann R, Gobert S, Jeffreys AJ (1994) Isolation of human simple repeat loci by hybridization selection. Hum Mol Genet 3:599–605

Arya L, Verma M, Gupta VK, Karihaloo JL (2009) Development of EST–SSRs in finger millet (*Eleusine coracana* ssp *coracana*) and their transferability to pearl millet (*Pennisetum glaucum*). J Plant Biochem Biotechnol 18:97–100

Asp T, Frei UK, Didion T, Nielsen KK, Lübberstedt T (2007) Frequency, type, and distribution of EST–SSRs from three genotypes of *Lolium perenne*, and their conservation across orthologous sequences of *Festuca arundinacea, Brachypodium distachyon*, and *Oryza sativa*. BMC Plant Biol 7:36

Astarini IA, Plummer JA, Lancaster RA, Yan G (2008) Identification of 'Sib' plants in hybrid cauliflowers using microsatellite markers. Euphytica 164:309–316

Bao JS, Corke H, Sun M (2002) Microsatellites in starch-synthesizing genes in relation to starch physicochemical properties in waxy rice (*Oryza sativa* L.). Theor Appl Genet 105:898–905

Barkley NA, Newman ML, Wang ML, Hotchkiss MW, Pederson GA (2005) Assessment of the genetic diversity and phylogenetic relationships of a temperate bamboo collection by using transferred EST–SSR markers. Genome 48:731–737

Barkley NA, Roose ML, Krueger RR, Federici CT (2006) Assessing geneticdiversity and population structure in a *Citrus* germplasm collection utilizing simple sequence repeat markers (SSRs). Theor Appl Genet 112:1519–1531

Barkley NA, Dean RE, Pittman RN, Wang ML, Holbrook CC, Pederson GA (2007) Genetic diversity of cultivated and wild-type peanuts evaluated with M13-tailed SSR markers and sequencing. Genet Res 89:93–106

Barreneche T, Casasoli M, Russell K, Akkak A, Meddour H, Plomion C, Villani F, Kremer A (2004) Comparative mapping between *Quercus* and *Castanea* using simple-sequence repeats (SSRs). Theor Appl Genet 108:558–566

Becker J, Huen M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol 27:835–845

Beckmann JS, Soller M (1990) Towards a unified approach to genetic mapping of eukaryotes based on sequence tagged microsatellite sites. Nat Biotechnol 8:930–932

Bennetzen JL (2000) Transposable element contributions to plant genome evolution. Plant Mol Biol 42:251–269

Bhat PR, Kumar KV, Hendre PS, Kumar RP, Varshney RK, Aggarwal RK (2005) Identification and characterization of expressed sequence tags-derived simple sequence repeats, markers from robusta coffee variety 'C × R' (an interspecific hybrid of *Coffea canephora* × *Coffea congensis*). Mol Ecol Notes 5:80–83

Bhattramakki D, Dong J, Chhabra K, Hart GE (2000) An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43:988–1002

Bohn M, Utz HF, Melchinger AE (1999) Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. Crop Sci 39:228–237

Born C, Vignes H, Muloko N, Wickings EJ, Hossaert-Mckey M, Chevallier MH (2006) Isolation and characterization of polymorphic microsatellite loci from *Aucoumea klaineana* Pierre (Burseraceae), a tropical rainforest tree of Central Africa. Mol Ecol Notes 6:1054–1056

Boys J, Cherry M, Dayanandan S (2005) Microsatellite analysis reveals genetically distinct populations of red pine (*Pinus resinosa*, Pinaceae). Am J Bot 92:833–841

Breseghello F, Sorrells ME (2006a) Association mapping of kernel size and milling quality in wheat (*Triticum aestivum* L.) cultivars. Genetics 172:1165–1177

Breseghello F, Sorrells ME (2006b) Association analysis as a strategy for improvement of quantitative traits in plants. Crop Sci 46:1323–1330

Caruso M, Federici CT, Roose ML (2008) EST–SSR markers for asparagus genetic diversity evaluation and cultivar identification. Mol Breed 21:195–204

Castillo A, Budak H, Varshney RK, Dorado G, Graner A, Hernandez P (2008) Transferability and polymorphism of barley EST–SSR markers used for phylogenetic analysis in *Hordeum chilense*. BMC Plant Biol 8:97

Chagne D, Chaumeil P, Ramboer A, Collada C, Guevara A, Cervera MT, Vendramin GG, Garcia V, Frigerio JM, Echt C, Richardson T, Plomion C (2004) Cross-species transferability and mapping of genomic and cDNASSRs in pines. Theor Appl Genet 109:1204–1214

Chang CL, Marra G, Chauhan DP, Ha HT, Chang DK, Ricciardiello L, Randolph A, Carethers JM, Boland CR (2002) Oxidative stress inactivates the human DNA mismatch repair system. Am J Physiol Cell Physiol 283: 148–154

Chapman MA, Hvala J, Strever J, Matvienko M, Kozik A, Michelmore RW, Tang S, Knapp SJ, Burke JM (2009) Development, polymorphism and cross-taxon utility of EST–SSR markers from saflower (*Carthamus tinctorius* L.). Theor Appl Genet 120:85–91

Chen X, Temnykh S, Xu Y, Cho YG, McCouch SR (1997) Development of a microsatellite framework map providing

genome-wide coverage in rice (*Oryza sativa* L.). Theor Appl Genet 95:553–567

Chen T, Zhou R, Ge XJ, Shi S (2008) Development and characterization of microsatellite markers for a mangrove tree species *Sonneratia caseolaris* (L.) Engler (Lythraceae *sensu lato*). Conserv Genet 9:957–959

Chen J, Wang H, Shen H, Chai M, Li J, Qi M, Yang W (2009) Genetic variation in tomato populations from four breeding programs revealed by single nucleotide polymorphism and simple sequence repeat markers. Sci Hort 122:6–16

Choudhary S, Sethy NK, Shokeen B, Bhatia S (2009) Development of chickpea EST–SSR markers and analysis of allelic variation across related species. Theor Appl Genet 118:591–608

Chuanchai P, Xuelin T, Silapapun A, Suthipong P, Wei L, Messmer R (2010) Early hybrid testing in tropical maize: are molecular markers useful for selecting the parental component? Kasetsart J Nat Sci 44:70–78

Chung AM, Staub JE, Chen JF (2006) Molecular phylogeny of *Cucumis* species as revealed by consensus chloroplast SSR marker length and sequence variation. Genome 49: 219–229

Cifarelli RA, Gallitelli M, Cellini F (1995) Random amplified hybridization microsatellites (RAHM): isolation of a new class of microsatellite-containing DNA clones. Nucleic Acids Res 23:3802–3803

Cooper S, Bull CM, Gardner MG (1997) Characterization of microsatellite loci from the socially monogamous lizard *Tiliqua rugosa* using a PCR-based isolation technique. Mol Ecol 6:793–795

Cordeiro GM, Maguire TL, Edwards KJ, Henry RJ (1999) Optimization of a microsatellite enrichment technique in *Saccharum* spp. Plant Mol Biol Rep 17:225–229

Cordeiro GM, Casu R, McIntyre CL, Manners JM, Henry RJ (2001) Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs across transferable to *Erianthus* and *Sorghum*. Plant Sci 160:1115–1123

Cuc LM, Mace ES, Crouch JH, Quang VD, Long TD, Varshney RK (2008) Isolation and characterization of novel microsatellite markers and their application for diversity assessment in cultivated groundnut (*Arachis hypogaea*). BMC Plant Biol 8:55

Decroocq V, Fave MG, Hagen L, Bordenave L, Decroocq S (2003) Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor Appl Genet 106:912–922

Delseny M, Laroche M, Penon P (1983) Detection of sequences with Z-DNA forming potential in higher plants. Biochem Biophys Res Commun 116:113–120

Dresselhaus T, Cordts S, Heuer S, Sauter M, Lorz H, Kranz E (1999) Novel ribosomal genes from maize are differentially expressed in the zygotic and somatic cell cycles. Mol Gen Genet 261:416–427

Edwards A, Civitello A, Hammond HA, Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am J Hum Genet 49:746–756

Ek M, Eklund M, Von Post R, Dayteg C, Henriksson T, Weibull P, Ceplitis A, Isaac P, Tuvesson S (2005) Microsatellite markers for powdery mildew resistance in pea (*Pisum sativum* L.). Hereditas 142:86–91

Ellegren H (2002) Microsatellite evolution: a battle between replication slippage and point mutation. Trends Genet 18:70

Ellegren H (2004) Microsatellites: simple sequences with complex evolution. Nat Rev Genet 5:435–445

Ellis JR, Burke JM (2007) EST–SSRs as a resource for population genetic analyses. Heredity 99:125–132

Ender A, Schwenk K, Stdler T, Streit B, Schierwater B (1996) RAPD identification of microsatellites in *Daphnia*. Mol Ecol 5:437–441

Erre P, Chessa I, Muñoz-Diez C, Belaj A, Rallo L, Trujillo I (2010) Genetic diversity and relationships between wild and cultivated olives (*Olea europaea* L.) in Sardinia as assessed by SSR markers. Genet Resour Crop Evol 57:41–54

Feingold S, Lloyd J, Norero N, Bonierbale M, Lorenzen J (2005) Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.). Theor Appl Genet 111:456–466

Feng SP, Li WG, Huang HS, Wang JY, Wu YT (2009) Development, characterization and cross-species/genera transferability of EST–SSR markers for rubber tree (*Hevea brasiliensis*). Mol Breed 23:85–97

Fisher PJ, Gardner RC, Richardson TE (1996) Single locus microsatellites isolated using 5¢-anchored PCR. Nucleic Acids Res 24:4369–4371

Fraser LG, Tsang GK, Datson PM, De Silva HN, Harvey CF, Gill GP, Crowhurst RN, McNeilage MA (2009) A generich linkage map in the dioecious species *Actinidia chinensis* (kiwifruit) reveals putative X/Y sex-determining chromosomes. BMC Genomics 10:102

Fujimori S, Washio T, Higo K, Ohtomo Y, Murakami K, Kenichi M, Kawai J, Carninci P, Hayashizaki Y, Kikuchi S, Tomita M (2003) A novel feature of microsatellites in plants: a distribution gradient along the direction of transcription. FEBS Lett 554:17–22

Gasic K, Han Y, Kertbundit S, Shulaev V, Iezzoni AF, Stover EW, Richard L, Michael B, Wisniewski E, Korban SS (2009) Characteristics and transferability of new apple EST-derived SSRs to other Rosaceae species. Mol Breed 23:397–411

Gil-Ariza DJ, Amaya I, López-Aranda JM, Sánchez-Sevilla JF, Botella MÁ, Valpuesta V (2009) Impact of plant breeding on the genetic diversity of cultivated strawberry as revealed by expressed sequence tag-derived simple sequence repeat markers. J Am Soc Hort Sci 134:337–347

Gimenes MA, Hoshino AA, Barbosa AV, Palmieri DA, Lopes CR (2007) Characterization and transferability of microsatellite markers of the cultivated peanut (*Arachis hypogaea*). BMC Plant Biol 7:9

Gomez SM, Denwar NN, Ramasubramanian T, Simpson CE, Burow G, Burke JJ, Puppala N, Burow MD (2008) Identification of peanut hybrids using microsatellite markers and horizontal polyacrylamide gel electrophoresis. Peanut Sci 35:123–129

Gong L, Stift G, Kofler R, Pachner M, Lelley T (2008) Microsatellites for the genus *Cucurbita* and an SSR-based genetic linkage map of *Cucurbita pepo* L. Theor Appl Genet 117:37–48

Graham J, Woodhead M, Smith K, Russell J, Marshall B, Ramsay G, Squire G (2009) New insight into wild red

raspberry populations using simple sequence repeat markers. J Am Soc Hort Sci 134:109–119
Guan R, Chang R, Li Y, Wang L, Liu Z, Qiu L (2010) Genetic diversity comparison between Chinese and Japanese soybeans (*Glycine max* (L.) Merr.) revealed by nuclear SSRs. Genet Resour Crop Evol 57:229–242
Guo W, Wang W, Zhou B, Zhang T (2006) Cross-species transferability of *G. arboreum*-derived EST–SSRs in the diploid species of *Gossypium*. Theor Appl Genet 112:1573–1581
Gupta S, Prasad M (2009) Development and characterization of genic SSR markers in *Medicago truncatula* and their transferability in leguminous and non-leguminous species. Genome 52:761–771
Gupta PK, Varshney RK (2000) The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. Euphytica 113: 163–165
Gupta M, Chyi YS, Romero-Severson J, Owen JL (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats. Theor Appl Genet 89:998–1006
Gupta PK, Rustgi S, Sharma S, Singh R, Kumar N, Balyan HS (2003) Transferable EST–SSR markers for the study of polymorphism and genetic diversity in bread wheat. Mol Genet Genomics 270:315–323
Hamada HM, Petrino MG, Kakunaga T (1982) A novel repeated element with Z-DNAforming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc Natl Acad Sci USA 79:6465–6469
Hamilton MB, Pincus EL, Di-Fiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA libraries enriched for microsatellites. Biotechniques 27:500–507
Hashemi SH, Mirmohammadi-Maibody SAM, Nematzadeh GA, Arzani A (2009) Identification of rice hybrids using microsatellite and RAPD markers. Afr J Biol 8: 2094–2101
He XY, Zhang YL, He ZH, Wu YP, Xiao YG, Ma CX, Xia XC (2008) Characterization of phytoene synthase 1 gene (*Psy1*) located on common wheat chromosome 7A and development of a functional marker. Theor Appl Genet 116:213–221
Hiebert CW, Thomas JB, Somers DJ, McCallum BD, Fox SL (2007) Microsatellite mapping of adult-plant leaf rust resistance gene Lr22a in wheat. Theor Appl Genet 115: 877–884
Hisano H, Sato S, Isobe S, Sasamoto S, Wada T, Matsuno A, Fujishiro T, Yamada M, Nakayama S, Nakamura Y, Watanabe S, Harada K, Tabata S (2007) Characterization of the soybean genome using EST-derived microsatellite markers. DNA Res 14:271–281
Ince AG, Karaca M, Onus AN (2010) Polymorphic microsatellite markers transferable across capsicum species. Plant Mol Biol Rep 28:285–291
Inoue E, Ning L, Hara H, Ruan S, Anzai H (2009) Development of simple sequence repeat markers in chinese chestnut and their characterization in diverse chestnut cultivars. J Am Soc Hort Sci 134:610–617
IRGSP (2005) The map based sequence of rice genome. Nature 436:793–800
Jackson AL, Chen R, Loeb LA (1998) Induction of microsatellite instability by oxidative DNA damage. Proc Natl Acad Sci USA 95:12468–12473
Jacob HJ, Lindpaintnesr K, Kusumir EL, Bunkery K, Mao IP, Gantenv D, Dzau J, Lander ES (1991) Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. Cell 67:213–224
Jakse J, Stajner N, Kozjak P, Cerenak A, Javornik B (2008) Trinucleotide microsatellite repeat is tightly linked to male sex in hop (*Humulus lupulus* L.). Mol Breed 21:139–148
Jarne P, Lagoda PJL (1996) Microsatellites, from molecules to populations and back. Trends Ecol Evol 11:424–429
Jia X, Zhang Z, Liu Y, Zhang C, Shi Y, Song Y, Wang T, Li Y (2009) Development and genetic mapping of SSR markers in foxtail millet [*Setaria italica* (L.) P. Beauv.]. Theor Appl Genet 118:821–829
Joshi SP, Ranjekar PK, Gupta VS (1999) Molecular markers in plant genome analysis. Curr Sci 77:230–240
Juhász AG, Stágel A, Ács S, Zatykó L (2006) Microsatellite markers and automated fragment analysis techniques for efficient and precise hybrid identification and genetic purity testing in pepper (*Capsicum annuum* L.). Acta Agr Hungarica 54:141–146
Kalendar R, Grob T, Regina M, Suoniemi A, Schulman A (1999) IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques. Theor Appl Genet 98:704–711
Kandpal RP, Kandpal G, Weissman SM (1994) Construction of libraries enriched for sequence repeats and jumping clones, and hybridization selection for region-specific markers. Proc Natl Acad Sci USA 91:88–92
Kang M, Pan L, Yao X, Huang H (2006) Development and characterization of polymorphic microsatellite loci in endangered fern *Adiantum reniforme* var. *sinense*. Conserv Genet 7:807–810
Kantety RV, Rota ML, Matthews DE, Sorrells ME (2002) Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. Plant Mol Biol 48:501–510
Karagyozov L, Kalcheva ID, Chapman VM (1993) Construction of random small-insert genomic libraries highly enriched for simple sequence repeats. Nucleic Acids Res 21:3911–3912
Khattak JZK, Torp AM, Andersen SB (2006) A genetic linkage map of *Spinacia oleracea* and localization of a sex determination locus. Euphytica 148:311–318
Khattak JZK, Christiansen JL, Torp AM, Andersen SB (2007) Genic microsatellite markers for discrimination of spinach cultivars. Plant Breed 126:454–456
Kijas JM, Fowler JC, Garbett CA, Thomas MR (1994) Enrichment of microsatellites from the citrus genome using biotinylated oligonucleotide sequences bound to streptavidin-coated magnetic particles. Biotechniques 16: 656–662
Korpelainen H, Bisang I, Hedena LS, Kolehmainen J (2008) The first sex-specific molecular marker discovered in the moss *Pseudocalliergon trifarium*. J Hered 99:581–587
Kruglyak S, Durrett RT, Schug MD, Aquadro CF (1998) Equilibrium distributions of microsatellite repeat length resulting from a balance between slippage events and point mutation. Proc Natl Acad Sci USA 95:10774–10778

Lacis G, Rashal I, Ruisa S, Trajkovski V, Iezzoni AF (2009) Assessment of genetic diversity of Latvian and Swedish sweet cherry (*Prunus avium* L.) genetic resources collections by using SSR (microsatellite) markers. Sci Hort 121:451–457

Lagat M, Danson J, Kimani M, Kuria A (2008) A quantitative trait loci for resistance to maize streak virus disease in maize genotypes used in hybrid development. Afr J Biotechnol 7:2573–2577

Lawson MJ, Zhang L (2006) Distinct patterns of SSR distribution in the *Arabidopsis thaliana* and rice genomes. Genome Biol 7:R14

Lench NJ, Norris A, Bailey A, Booth A, Markham AF (1996) Vectorette PCR isolation of microsatellites repeat sequences using anchored dinucleotide repeat primers. Nucleic Acids Res 24:2190–2191

Li YC, Fahima T, Korol AB, Peng J, Roder MS, Kirzhner V, Beiles A, Nevo E (2000) Microsatellite diversity correlated with ecological-edaphic and genetic factors in three microsites of wild emmer wheat in North Israel. Mol Biol Evol 17:851–862

Li YC, Korol AB, Fahima T, Beiles A, Nevo E (2002) Microsatellites: genomic distribution, putative functions and mutational mechanisms: a review. Mol Ecol 11:2453–2465

Li YC, Korol AB, Fahima T, Nevo E (2004) Microsatellites within genes: structure, function, and evolution. Mol Biol Evol 21:991–1007

Li TH, Li YX, Li ZC, Zhang HL, Qi YW, Wang T (2008) Simple sequence repeat analysis of genetic diversity in primary core collection of peach (*Prunus persica*). J Integr Plant Biol 50:102–110

Li M, Yuyama N, Luo L, Hirata M, Cai H (2009) *In silico* mapping of 1758 new SSR markers developed from public genomic sequences for sorghum. Mol Breed 24:41–47

Li R, Han Z, Xincheng Z, Yanan G, Fengxia Y, Guoan S, Jiancheng W, Chunqing Z (2010) Genetic diversity in Chinese sorghum landraces revealed by chloroplast simple sequence repeats. Genet Resour Crop Evol 57:1–15

Liang X, Chen X, Hong Y, Liu H, Zhou G, Li S, Guo B (2009) Utility of EST-derived SSR in cultivated peanut (*Arachis hypogaea* L.) and *Arachis* wild species. BMC Plant Biol 9:35

Liewlaksaneeyanawin C, Ritland CE, El-Kassaby YA, Ritland K (2004) Single-copy, species-transferable microsatellite markers developed from loblolly pine ESTs. Theor Appl Genet 109:361–369

Litt M, Luty JA (1989) A hypervariable microsatellite revealed by in vitro amplification of a dinucleotide repeat within the cardiac muscle actin gene. Am J Hum Genet 44: 397–401

Liu LW, Wang Y, Gong YQ, Zhao TM, Liu G, Li XY, Yu FM (2007) Assessment of genetic purity of tomato (*Lycopersicon esculentum* L.) hybrid using molecular markers. Sci Hort 115:7–12

Lunt DH, Hutchinson WF, Carvalho GR (1999) An efficient method for PCR-based identification of microsatellite arrays (PIMA). Mol Ecol 8:893–894

Luro FL, Costantino G, Terol J, Argout X, Allario T, Wincker P, Talon M, Ollitrault P, Morillon R (2008) Transferability of the EST–SSRs developed on Nules clementine (*Citrus clementina* Hort ex Tan) to other *Citrus* species and their effectiveness for genetic mapping. BMC Genomics 9:287

Mallory MA, Hall RV, McNabb AR, Pratt DB, Jellen EN, Maughan PJ (2008) Development and characterization of microsatellite markers for the grain amaranths. Crop Sci 48:1098–1106

Martí ÀF, Alonso JM, Espiau MT, Rubio-Cabetas MJ, Company RS (2009) Genetic diversity in Spanish and foreign almond germplasm assessed by molecular characterization with simple sequence repeats. J Am Soc Hort Sci 134:535–542

McCallum J, Thomson S, Pither-Joyce M, Kenel F (2008) Genetic diversity analysis and single-nucleotide polymorphism marker development in cultivated bulb onion based on expressed sequence tag-simple sequence repeat markers. J Am Soc Hort Sci 133:810–818

Meyer W, Michell TG, Freedman EZ, Vilgalys R (1993) Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. J Clin Biol 31:2274–2280

Min WK, Han JH, Kang WH, Lee HR, Kim BD (2008) Reverse random amplified microsatellite polymorphism reveals enhanced polymorphisms in the 3′ end of simple sequence repeats in the pepper genome. Mol Cells 26:250–257

Morgante M, Hanafey M, Powell W (2002) Microsatellites are preferentially associated with nonrepetitive DNA in plant genomes. Nat Genet 30:194–200

Nadir E, Margalit H, Gallily T, Ben-Sasson SA (1996) Microsatellite spreading in the human genome: evolutionary mechanisms and structural implications. Proc Natl Acad Sci USA 93:6470–6475

Naresh V, Yamini KN, Rajendrakumar P, Kumar VD (2009) EST–SSR marker-based assay for the genetic purity assessment of safflower hybrids. Euphytica 170:347–353

Nasiri J, Haghnazari A, Saba J (2009) Genetic diversity among varieties and wild species accessions of pea (*Pisum sativum* L.) based on SSR markers. Afr J Biotechnol 8: 3405–3417

Nazareno AG, Pereira RAS, Feres JM, Mestriner MA, Alzate-Marin AL (2009) Transferability and characterization of microsatellite markers in two Neotropical *Ficus* species. Genet Mol Biol 32:568–571

Neeraja CN, Maghirang-Rodriguez R, Pamplona A, Heuer S, Collard BC, Septiningsih EM, Vergara G, Sanchez D, Xu K, Ismail AM, Mackill DJ (2007) A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. Theor Appl Genet 115:767–776

Nishikawa T, Vaughan DA, Kadowaki K (2005) Phylogenetic analysis of *Oryza* species, based on simple sequence repeats and their flanking nucleotide sequences from the mitochondrial and chloroplast genomes. Theor Appl Genet 110:696–705

Nunome T, Negoro S, Kono I, Kanamori H, Miyatake K, Yamaguchi H, Ohyama A, Fukuoka H (2009) Development of SSR markers derived from SSR-enriched genomic library of eggplant (*Solanum melongena* L.). Theor Appl Genet 119:1143–1153

Nybom H (2004) Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol 13:1143–1155

Ohyama A, Asamizu E, Negoro S, Miyatake K, Yamaguchi H, Tabata S, Fukuoka H (2009) Characterization of tomato SSR markers developed using BAC-end and cDNA sequences from genome databases. Mol Breed 23:685–691

Orr HT, Zoghbi HY (2007) Trinucleotide repeat disorders. Annu Rev Neurosci 30:575–621

Ostrander EA, Jong PM, Rine J, Duyk G (1992) Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. Proc Natl Acad Sci USA 89:3419–3423

Paetkau D (1999) Microsatellites obtained using strand extension: an enrichment protocol. Biotechniques 26:690–697

Pan L, Xia Q, Quan Z, Liu H, Ke W, Ding Y (2010) Development of novel EST-SSRs from sacred lotus (*Nelumbo nucifera* gaertn) and their utilization for the genetic diversity analysis of *N. nucifera*. J Hered 101:71–82

Parasnis AS, Ramakrishna W, Chowdari KV, Gupta VS, Ranjekar PK (1999) Microsatellite (GATA)n reveals sex-specific differences in papaya. Theor Appl Genet 99: 1047–1052

Parida SK, Kumar ARK, Dalal V, Singh NK, Mohapatra T (2006) Unigene derived microsatellite markers for the cereal genomes. Theor Appl Genet 112:808–817

Parida SK, Kalia SK, Sunita K, Dalal V, Hemaprabha G, Selvi A, Pandit A, Singh A, Gaikwad K, Sharma TR, Srivastava PS, Singh NK, Mohapatra T (2009) Informative genomic microsatellite markers for efficient genotyping applications in sugarcane. Theor Appl Genet 118:327–338

Pashley CH, Ellis JR, McCauley DE, Burke JM (2006) EST databases as a source for molecular markers: lessons from *Helianthus*. J Hered 97:381–388

Pearson CE, Edamura NK, Cleary JD (2005) Repeat instability: mechanisms of dynamic mutations. Nat Rev Genet 6: 729–742

Pestsova E, Ganal MW, Roder MS (2000) Isolation and mapping of microsatellite markers specific for the D genome of bread wheat. Genome 43:689–697

Pollegioni P, Woeste K, Mugnozza GS, Malvolti ME (2009) Retrospective identification of hybridogenic walnut plants by SSR fingerprinting and parentage analysis. Mol Breed 24:321–335

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1:215–222

Provan J, Powell W, Hollingsworth PM (2001) Chloroplast microsatellites: new tools for studies in plant ecology and evolution. Trends Ecol Evol 16:142–147

Rabello E, Souza AN, Saito D, Tsai SM (2005) *In silico* characterization of microsatellites in *Eucalyptus* spp.: abundance, length variation and transposon associations. Genet Mol Biol 28:582–588

Rajendrakumar P, Biswal AK, Balachandran SM, Srinivasarao K, Sundaram RM (2007) Simple sequence repeats in organellar genomes of rice: frequency and distribution in genic and intergenic regions. Bioinformatics 23:1–4

Rallo P, Tenzer I, Gessler C, Baldoni L, Dorado G, Martin A (2003) Transferability of olive microsatellite loci across the genus *Olea*. Theor Appl Genet 107:940–946

Ramsay L, Macaulay M, degli Ivanissevichb S, MacLean K, Cardle L, Fuller J, Edwards KJ, Tuvesson S, Morgante M, Massari A, Maestri E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R (2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

Richardson T, Cato S, Ramser J, Kahl G, Weising K (1995) Hybridization of microsatellites to RAPD: a new source of polymorphic markers. Nucleic Acids Res 23:3798–3799

Risterucci AM, Duval MF, Rohde W, Billotte N (2005) Isolation and characterization of microsatellite loci from *Psidium guajava* L. Mol Ecol Notes 5:745–748

Rode J, In-Chol K, Saal B, Flachowsky H, Kriese U, Weber WE (2005) Sex-linked SSR markers in hemp. Plant Breed 124:167–170

Romero G, Adeva C, Battad Z (2009) Genetic fingerprinting: advancing the frontiers of crop biology research. Philipp Sci Lett 2:8–13

Rossi M, Araujo PG, Sluys MV (2001) Survey of transposable elements in sugarcane expressed sequence tags (ESTs). Genet Mol Biol 24:147–154

Roy CB, Nazeer MA, Saha T (2004) Identification of simple sequence repeats in rubber (*Hevea brasiliensis*). Curr Sci 87:807–811

Ruan CJ, Li H, Mopper S (2009) Characterization and identification of ISSR markers associated with resistance to dried-shrink disease in sea buckthorn. Mol Breed 24: 255–268

Russell JR, Fuller JD, Macaulay M, Hatz BG, Jahoor A, Powell W, Waugh R (1997) Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor Appl Genet 95: 714–722

Saha MC, Mian MAR, Eujayl I, Zwonitzer JC, Wang L, May GD (2004) Tall fescue EST–SSR markers with transferability across several grass species. Theor Appl Genet 109:783–791

Saha MC, Mian MAR, Zwonitzer JC, Chekhovskiy K, Hopkins AA (2005) An SSR- and AFLP-based genetic linkage map of tall fescue (*Festuca arundinacea* Schreb.). Theor Appl Genet 110:323–336

Saha MC, Cooper JD, Mian MAR, Chekhovskiy K (2006) Tall Fescue genomic SSR markers: development and transferability across multiple grass species. Theor Appl Genet 113:1449–1458

Sawadogo M, Ouedraogo JT, Balma D, Ouedraogo M, Gowda BS, Botanga C, Timko MP (2009) The use of cross species SSR primers to study genetic diversity of okra from Burkina Faso. Afr J Biotechol 8:2476–2482

Schnell RJ, Olano CT, Quintanilla WE, Meerow AW (2005) Isolation and characterization of 15 microsatellite loci from mango (*Mangifera indica* L.) and cross-species amplification in closely related taxa. Mol Ecol Notes 5:625–627

Schranz ME, Song BH, Windsor AJ, Mitchell-Olds T (2007) Comparative genomics in the *Brassicaceae*: a family-wide perspective. Curr Opin Plant Biol 10:168–175

Sehgal D, Raina SN (2008) DNA markers and germplasm resource diagnostics: new perspectives in crop improvement and conservation strategies. In: Arya ID, Arya S (eds) Utilization of biotechnology in plant sciences. Microsoft Printech (I) Pvt. Ltd, Dehradun, pp 39–54

Sharma RK, Gupta P, Sharma V, Sood A, Mohapatra T, Ahuja PS (2008) Evaluation of rice and sugarcane SSR markers for phylogenetic and genetic diversity analyses in bamboo. Genome 51:91–103

Sharma V, Bhardwaj P, Kumar R, Sharma RK, Sood A, Ahuja PS (2009) Identification and cross-species amplification of EST derived SSR markers in different bamboo species. Conserv Genet 10:721–724

Sharopova N, McMullen MD, Schultz L, Schroeder S, Sanchez-Villeda H, Davis G, Coe EH (2002) Development and mapping of SSR markers for maize. Plant Mol Biol 48:463–481

Shoemaker RC, Grant D, Olson T, Warren WC, Wing R, Yu Y, Kim H, Cregan P, Joseph B, Futrell-Griggs M, Nelson W, Davito J, Walker J, Wallis J, Kremitski C, Scheer D, Clifton SW, Graves T, Nguyen H, Wu X, Luo M, Dvorak J, Nelson R, Cannon S, Tomkins J, Schmutz J, Stacey G, Jackson S (2008) Microsatellite discovery from BAC end sequences and genetic mapping to anchor the soybean physical and genetic maps. Genome 51:294–302

Shultz JL, Kazi S, Bashir R, Afzal JA, Lightfoot DA (2007) The development of BAC-end sequence-based microsatellite markers and placement in the physical and genetic maps of soybean. Theor Appl Genet 114:1081–1090

Siju S, Dhanya K, Syamkumar S, Sasikumar B, Sheeja TE, Bhat AI, Parthasarathy VA (2010) Development, characterization and cross species amplification of polymorphic microsatellite markers from expressed sequence tags of Turmeric (*Curcuma longa* L.). Mol Biotechnol 44: 140–147

Sim SC, Yu JK, Jo YK, Sorrells ME, Jung G (2009) Transferability of cereal EST–SSR markers to ryegrass. Genome 52:431–437

Simko I, Costanzo S, Haynes KG, Christ BJ, Jones RW (2004) Linkage disequilibrium mapping of a *Verticillium dahliae* resistance quantitative trait locus in tetraploid potato (*Solanum tuberosum*) through a candidate gene approach. Theor Appl Genet 108:217–224

Singh H, Deshmukh RK, Singh A, Singh AK, Gaikwad K, Sharma TR, Mohapatra T, Singh NK (2010) Highly variable SSR markers suitable for rice genotyping using agarose gels. Mol Breed 25:359–364

Soranzo N, Provan J, Powell W (1999) An example of microsatellite length variation in the mitochondrial genome of conifers. Genome 42:158–161

Spigler RB, Lewers KS, Main DS, Ashman TL (2008) Genetic mapping of sex determination in a wild strawberry, *Fragaria virginiana*, reveals earliest form of sex chromosome. Heredity 101:507–517

Spooner DM, Nunez J, Trujillo G, Mdel RH, Guzman F, Ghislain M (2007) Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. Proc Nat Acad Sci USA 104:19398–19403

Srinivas G, Satish K, Madhusudhana R, Seetharama N (2009) Exploration and mapping of microsatellite markers from subtracted drought stress ESTs in *Sorghum bicolor* (L.) Moench. Theor Appl Genet 118:703–717

Stagel A, Portis E, Toppino L, Rotino GL, Lanteri S (2008) Gene-based microsatellite development for mapping and phylogeny studies in eggplant. BMC Genomics 9:357

Stein N, Prasad M, Scholz U, Thiel T, Zhang H, Wolf M, Kota R, Varshney RK, Perovic D, Grosse I, Graner A (2007) A 1,000-loci transcript map of the barley genome: new anchoring points for integrative grass genomics. Theor Appl Genet 114:823–839

Studer B, Asp T, Frei U, Hentrup S, Meally H, Guillard A, Barth S, Muylle H, Roldán-Ruiz I, Barre P, Koning-Boucoiran C, Stunnenberg GU, Dolstra O, Skøt L, Skøt KP, Turner LB, Humphreys MO, Kölliker R, Roulund N, Nielsen KK, Lübberstedt T (2008) Expressed sequence tag-derived microsatellite markers of perennial ryegrass (*Lolium perenne* L.). Mol Breed 21:533–548

Su CC, Zhai HQ, Wang CM, Sun LH, Wan JM (2006) SSR mapping of brown planthopper resistance gene Bph9 in Kaharamana, an Indica rice (*Oryza sativa* L.). Yi Chuan Xue Bao 33:262–268

Suwabe K, Tsukazaki H, Iketani H, Hatakeyama K, Kondo M, Fujimura M, Nunome T, Fukuoka H, Hirai M, Matsumoto S (2006) Simple sequence repeat-based comparative genomics between *Brassica rapa* and *Arabidopsis thaliana*: the genetic origin of clubroot resistance. Genetics 173:309–319

Tamilkumar P, Jerlin R, Senthil N, Ganesan KN, Jeevan RJ, Raveendran M (2009) Fingerprinting of rice hybrids and their parental lines using microsatellite markers and their tilization in genetic purity assessment of hybrid rice. Res J Seed Sci 2:40–47

Tang J, Gao L, Cao Y, Jia J (2006) Homologous analysis of SSR-ESTs and transferability of wheat SSR-EST markers across barley, rice and maize. Euphytica 151:87–93

Tang J, Baldwin SJ, Jacobs JME, van der Linden CG, Voorrips RE, Leunissen JAM, Van EH, Vosman B (2008) Large-scale identification of polymorphic microsatellites using an *in silico* approach. BMC Bioinformatics 9:374

Tang DQ, Lu JJ, Fang W, Zhang S, Zhou MB (2010) Development, characterization and utilization of GenBank microsatellite markers in *Phyllostachys pubescens* and related species. Mol Breed 25:299–311

Tautz D (1989) Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acid Res 17:6463–6471

Tautz D (1993) Notes on the definition and nomenclature of tandemly repetitive DNA sequences. In: Pena SDJ, Chakraborty R, Epplen JT, Jeffreys AJ (eds) DNA fingerprinting: state of the science. Birkhaiiser Verlag, Basel, pp 21–28

Tautz D, Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 12:4127–4138

Temnykh S, DeClerck G, Lukashova A, Lipovich L, Cartinhour S, McCouch S (2001) Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetic marker potential. Genome Res 11:1441–1452

Thiel T, Michalek W, Varshney RK, Graner A (2003) Exploiting EST databases for the development of cDNA derived microsatellite markers in barley (*Hordeum vulgare* L.). Theor Appl Genet 106:411–422

Trifonov EN (2003) Tuning function of tandemly repeating sequences: a molecular device for fast adaptation. In: Wasser SP (ed) Evolutionary theory and processes: nodern horizons, papers in honor of Eviatar Nevo. Kluwer, Amsterdam, pp 1–24

Tyrka M, Perovic D, Wardynska A, Ordon F (2008) A new diagnostic SSR marker for selection of the Rym4/Rym5 locus in barley breeding. J Appl Genet 49:127–134

Varshney RK, Thiel T, Stein N, Langridge P, Graner A (2002) *In silico* analysis on frequency and distribution of microsatellites in ESTs of some cereal species. Cell Mol Biol Lett 7:537–546

Varshney RK, Graner A, Sorrells ME (2005a) Genic microsatellite markers in plants: features and applications. Trends Biotechnol 23:48–55

Varshney RK, Sigmund R, Borner A, Korzun V, Stein N, Sorrells ME, Langridge P, Graner A (2005b) Interspecific transferability and comparative mapping of barley EST–SSR markers in wheat rye and rice. Plant Sci 168:195–202

Varshney RK, Thudi M, Aggarwal R, Börner A (2007) Genic molecular markers in plants: development and applications. In: Varshney RK, Tuberosa R (eds) Genomics-assisted crop improvement: genomics approaches and platforms, vol 1. Springer, Dordrecht, pp 13–29

Varshney RK, Hiremath PJ, Lekha P, Kashiwagi J, Balaji J, Deokar AA, Vadez V, Xiao Y, Srinivasan R, Gaur PM, Siddique KHM, Town CD, Hoisington DA (2009) Comprehensive resource of drought- and salinity responsive ESTs for gene discovery and marker development in chickpea (*Cicer arietinum* L.). BMC Genomics 10:523

Vezzulli S, Troggio M, Coppola G, Jermakow A, Cartwright D, Zharkikh A, Stefanini M, Grando MS, Viola R, Adam-Blondon AF, Thomas M, This P, Velasco R (2008) A reference integrated map for cultivated grapevine (*Vitis vinifera* L.) from three crosses, based on 283 SSR and 501 SNP-based markers. Theor Appl Genet 117:499–511

Vos P, Hogers R, Bleeker M, Reijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabean M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wang Z, Weber JL, Zhang G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88:1–6

Wang ML, Huang L, Bongard-Pierce DK, Belmonte S, Zachgo EA, Morris JW, Dolan M, Goodman HM (1997) Construction of an approximately 2 Mb contig in the region around 80 cM of *Arabidopsis thaliana* chromosome 2. Plant J 12:711–730

Wang ML, Gillaspie AG, Newman ML, Dean RE, Pittman RN, Morris JB, Pederson GA (2004) Transfer of simple sequence repeat (SSR) markers across the legume family for germplasm characterization and evaluation. Plant Genet Resour 2:107–119

Wang ML, Barkley NA, Yu JK, Dean RE, Newman ML, Sorrells ME, Pederson GA (2005) Transfer of simple sequence repeat (SSR) markers from major cereal crops to minor grass species for germplasm characterization and evaluation. Plant Genet Resour 3:45–57

Wang ML, Chen ZB, Barkley NA, Newman ML, Kim W, Raymer P, Pederson GA (2006a) Characterization of seashore paspalum (*Paspalum vaginatum* Swartz) germplasm by transferred SSRs from wheat, maize and sorghum. Genet Resour Crop Evol 53:779–791

Wang ML, Mosjidis JA, Morris JB, Dean RE, Jenkins TM, Pederson GA (2006b) Genetic diversity of *Crotalaria* germplasm assessed through phylogenetic analysis of EST–SSR markers. Genome 49:707–715

Wang ML, Morris JB, Barkley NA, Dean RE, Jenkins TM, Pederson GA (2007) Evaluation of genetic diversity of the USDA *Lablab purpureus* germplasm collection using simple sequence repeat markers. J Hort Sci Biotechnol 82:571–578

Wang A, Zhang Q, Wan D, Yang Y, Liu J (2008a) Nine microsatellite DNA primers for *Hippophae rhamnoides* ssp. *sinensis* (Elaeagnaceae). Conserv Genet 9:969–971

Wang Y, Kang M, Huang H (2008b) Microsatellite loci transferability in chestnut. J Am Soc Hort Sci 133:692–700

Wang JPM, Lee R, Goos RJ, Helms T (2008c) Association mapping of iron deficiency chlorosis loci in soybean (*Glycine max* L. Merr.) advanced breeding lines. Theor Appl Genet 116:777–787

Wang ML, Barkley NA, Jenkins TM (2009a) Microsatellite markers in plants and insects. Part I. Applications of biotechnology. Genes Genomes Genomics 3:54–67

Wang ML, Mosjidis JA, Morris JB, Chen ZB, Barkley NA, Pederson GA (2009b) Evaluation of *Lespedeza* germplasm genetic diversity and its phylogenetic relationship with the genus *Kummerowia*. Conserv Genet 10:79–85

Warrington CV, Zhu S, Parrott WA, All JN, Boerma HR (2008) Seed yield of near-isogenic soybean lines with introgressed quantitative trait loci conditioning resistance to corn earworm (Lepidoptera: Noctuidae) and soybean looper (Lepidoptera: Noctuidae) from PI 229358. J Eco Entomol 101:1471–1477

Weber JL (1990) Informativeness of human $(dC\text{-}dA)_n \cdot (dG\text{-}dT)_n$ polymorphisms. Genomics 7:524–530

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Weising K, Gardner RC (1999) A set of conserved PCR primers for the analysis of simple sequence repeat polymorphisms in chloroplast genomes of dicotyledonous angiosperms. Genome 42:9–19

Weising K, Nybom H, Wolff K, Meyer W (1995) DNA fingerprinting in plant and fungi. CRC Press, Boca Raton

Woodhead M, Russell J, Squirrell J, Hollingsworth PM, Cardle L, Ramsay L, Gibby M, Powell W (2003) Development of EST–SSRs from the alpine lady-fern, *Athyrium distentifolium*. Mol Ecol Notes 3:287–290

Wu K, Jones R, Dannaeberger L, Scolnik PA (1994) Detection of microsatellite polymorphisms without cloning. Nucleic Acids Res 22:3257–3258

Yu JK, Dake TM, Singh S, Benscher D, Li W, Gill B, Sorrells ME (2004a) Development and mapping of EST-derived simple sequence repeat markers for hexaploid wheat. Genome 47:805–818

Yu JK, La Rota M, Kantety RV, Sorrells ME (2004b) EST derived SSR markers for comparative mapping in wheat and rice. Mol Genet Genomics 271:742–751

Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16

Zavodna M, Arens P, Van Dijk PJ, Vosman B (2005) Development and characterization of microsatellite markers for two dioecious *Ficus* species. Mol Ecol Notes 5:355–357

Zhang LY, Ravel C, Bernard M, Balfourier F, Leroy P, Feuillet C, Sourdille P (2006) Transferable bread wheat EST–SSRs can be useful for phylogenetic studies among the Triticeae species. Theor Appl Genet 113:407–418

Zhao XF, Sun WB, Yang JB, Meng J (2009) Isolation and characterization of 12 microsatellite loci for *Michelia coriacea* (Magnoliaceae), a critically endangered endemic to Southeast Yunnan China. Conserv Genet 10:1583–1585

Zietkiewitcz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20:176–183