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Microsatellite Markers in Plants and Insects. Part I: Applications of Biotechnology

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ABSTRACT

Biotechnology is integral to the application of robust, high through-put detection of species-specific and species or genus-transferred microsatellites, or simple sequence repeat (SSR) markers. These short, tandemly repeated stretches of DNA of variable motifs and lengths are relatively evenly distributed throughout eukaryotic nuclear, chloroplasts, and mitochondrial genomes. Microsatellites are inherited as Mendelian co-dominant markers that provide insights into non-Mendelian inheritance such as microsatellite evolution, replication, repair, recombination, and mutation. These characteristics have made microsatellites the genetic marker of choice for most technologically-driven applications in plant and insect genetic studies such as mapping, marker-assisted selection (MAS), and genetic diversity studies. MAS and linkage mapping analyses has greatly assisted breeding programs through the discovery and isolation of many important agronomic genes that underlie respective phenotypes. Linkage maps and genome sequences have provided comparative genomic insights in plants and insects regarding microsatellite distribution, occurrence, and adaptive phenotypic evolution. Furthermore, genomic synteny and SSR sequence conservation have not only provided maximum annotated information for model plants and insects, but have demonstrated cross-species/genera transferability, which is indicative of long evolutionary history. It is the aim of this paper, therefore, to review biotechnology platforms and applications that have made SSR markers so useful as well as to discuss the impact of SSR transferability across species and/or genera.

Keywords: simple sequence repeat, biotechnology, detection platform, specific application, transferability

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INTRODUCTION

Microsatellites or simple sequence repeat (SSR) loci, which have been referred to in the literature as variable number of tandem repeats (VNTRs) and simple sequence length polymorphisms (SSLPs), are found throughout the nuclear genomes of most eukaryotes and to a lesser extent in prokaryotes (Jarne and Lagoda 1996; Vaughan and Lloyd 2003). They have also been identified in plant chloroplasts (Cato and Richardson 1996; Chung *et al.* 2006) as well as in plant and animal mitochondrial genomes (Hoelzel *et al.* 1994; Soranzo *et al.* 1999; van Oppen *et al.* 2000; Rajendrakumar *et al.* 2007). Microsatellites range from one to six nucleotides in length (van Oppen *et al.* 2000) and are classified as mono-, di-, tri-, tetra-, penta- and hexanucleotide repeats. They are tandemly repeated (usually 5-20 times) in the genome with a minimum repeat length of 12 base-pairs (Goodfellow 1992; Vaughan and Lloyd 2003; Ellegren 2004). Microsatellites are further classified as simple perfect, simple imperfect, compound perfect or compound imperfect (Roy *et al.* 2004). Simple-perfect repeats are tandem arrays of a single repeat sequence (e.g., $[AGG]_n$); whereas, simple imperfect arrays consist of one or more repeat units of different lengths (e.g., $[AAC]_n[ACT][AAC]_{n+1}$). Compound-perfect arrays are composed of two or more different repeat motifs of the same length (e.g., $[AGG]_n[AATC]_n$) and compound-imperfect motifs are interrupted by one or more repeats of different length (e.g., $[GGAT]_n[ACT][GTAA]_{n+1}$).

The location of the microsatellite in the genome deter-

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mines its functional role (Lawson and Zhang 2006). Thus, microsatellites have the potential to affect all aspects of genetic function including gene regulation, development, and evolution (Kashi and King 2006; Lawson and Zhang 2006). For this reason microsatellites have been described as "mutator alleles" (King and Kashi 2007a, 2007b). 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). Comparative studies in insects, with some exceptions (Thoren *et al.* 1995; Toth *et al.* 2000) have suggested that microsatellite length and frequency correlate with genome size (Hancock 1996). In plants, the general frequency of microsatellites was not only shown to be inversely related to genome size, 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).

Microsatellites were first identified in humans in 1981 by sequence analysis of alleles at the β globin locus (Miesfeld *et al.* 1981; Spritz 1981) and subsequently found to be naturally occurring and ubiquitous in prokaryotic and eukaryotic genomes (Tautz and Renz 1984; Jeffreys *et al.* 1985; Tautz 1989; Thoren *et al.* 1995; Toth *et al.* 2000). Microsatellite genesis is an evolutionarily dynamic process and has proven to be exceedingly complex (Ellegren 2004; Pearson *et al.* 2005). Trying to understand the process and mechanism may help us to analyze the data and explain the results obtained from microsatellites. Possible explanations for microsatellite genesis include single-stranded DNA slippage, double-stranded DNA recombination, mismatch/ double strand break repair, and retrotransposition (simplified to retroposition). 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. DNA slippage has been confirmed *in vitro* by endonuclease digestion, mutation analysis, and synthesis of simple sequence repeat DNA without using polymerase chain reaction (PCR), and amplification and sequence of DNA containing SSRs using PCR (Streisinger and Owen 1985; Schlotterer and Tautz 1992; Murray *et al.* 1993). Recombination by unequal crossover or gene conversion (crossover and gene conversion are often, but not always associated) in the region containing SSRs may also lead to expansion or contraction of the repeat length (Richard and Paques 2000). However, in genotyping of 362 dinucleotide microsatellite markers on 630 human individuals from 53 pedigrees, there was no significant correlation between microsatellite variation and recombination rate observed (Huang *et al.* 2002). During genome duplication or maintenance, failure of DNA repair to correct the alternation in the repeat region may result in changing the number of tandem repeats. Repair of double strand breaks (DSB) in yeast and human led to frequent expansions and contractions of the repeat length (Liang *et al.* 1998; Paques *et al.* 1998). Retroposons are repetitive DNA fragments, which are inserted into chromosomes after they had been reverse-transcribed from any RNA molecule. Microsatellite generation had been found to be accompanied by retroposition events by analysis of a portion-sequenced human and rice genome DNA (Nadir *et al.* 1996; Temnykh *et al.* 2001). However, there was no clear correlation observed between a high density of transposable elements and a high density of microsatellites on the chromosome 2 of *Arabidopsis thaliana* (Lin *et al.* 1999; Schlotterer 2000). Among these possible explanations, replication slippage may be a major mechanism for microsatellite genesis and evolution, but other mechanisms may also play certain roles and remain to be further investigated (Schlotterer 2000).

Microsatellites, with a mutation rate ranging from 10^{-6} to 10^{-2} (Schlotterer 2000), are highly polymorphic in comparison with other marker systems, which make them an excellent choice for studying insect-plant interactions (Kim and Sappington 2005; Verbaarschot *et al.* 2007; Kim *et al.* 2008b). They are PCR-driven, codominant, abundant (representing ~3% of the human genome DNA), relatively evenly distributed throughout the euchromatic part of genomes (Ellegren 2004; Schlotterer 2004), economic, robust, and reproducible (Weber and May 1989; Schuelke 2000). They are also potentially transferable across species (Varshney *et al.* 2005b; Barbara *et al.* 2007). These features make microsatellites powerful genetic markers for genome (genetic, physical, comparative and association) mapping, genetic diversity, marker-assisted diagnosis / selection, population and evolutionary studies in eukaryotic species including mammals, plants, and insects (Weber 1990; Queller *et al.* 1993; Rafalski and Tingey 1993; Weissenbach 1993; Goldstein and Schlotterer 1999; Tamiya *et al.* 2005; Varshney *et al.* 2005a; Behura 2006). In this article, we review applications of biotechnology on plants and insects in three areas: biotechnology of detection platforms, specific applications, and transferability of microsatellites.

BIOTECHNOLOGY OF DETECTION PLATFORMS

Microsatellites are targeted by designing specific primers that flank either side of the repeat element [i.e. $(TA)_n$]. These primers or, oligonucleotides, are generally 18-24 bp long and target highly conserved regions in the genome. One can easily target and amplify a specific microsatellite locus using PCR technology. Then the difference in repeat length among individuals within a population can be assessed by separating their respective PCR products using electrophoresis. Moreover, these markers can be used to track the inheritance of alleles from progenitor to progeny. In a fairly short period of time, one can generate inter- or intraspecific population genetic data using several microsatellite markers. Generally, as the number of markers increase the chance of gaining interspecific or intraspecific variability also increases. Of course, the separation of PCR products requires a suitable detection system to view and ultimately score the resulting marker data (**Fig. 1A-D**).

There are several detection systems available ranging in overall cost from a few thousand dollars to a few hundred thousand dollars (**Fig. 1A-D**). Before the 1990s and the advent and the general widespread availability of automated sequencers, many laboratories employed polyacrylamide gel electrophoresis followed by gel imaging (i.e. radioactive isotopes, silver staining, or ethidium bromide staining) for detection of the microsatellite alleles. Agarose gels have also been utilized to separate microsatellite alleles (**Fig. 1D**). These systems are very inexpensive to employ for research; however, their main disadvantage is the loss of resolution between alleles that differ by small increments of the microsatellite repeat element in comparison to the high resolving power of polyacrylamide gels.

Many laboratories now have access to at least one type of automated system (capillary or gel based), which have been used extensively to generate microsatellite data in plants and insects (Gyllenstrand *et al.* 2002; Exeler *et al.* 2008; Kim *et al.* 2008a; Pol *et al.* 2008). Slab gel sequencers include the ABI 377 (now discontinued) and the LI-COR 4200, or 4300 (**Fig. 1B, 1C**). The capillary based automated systems are represented by either Beckman CEQ 8000 (**Fig. 1A**), or ABI 310, 3100, 3130, and 3730. These automated sequencers are all based on a fluorophore that is incorporated into the PCR products, which allows the detection of the microsatellite alleles. A diode laser(s), which emits a specified wavelength(s) of light, is absorbed by the fluorophore. A detector then captures the light emitted from the fluorophore and digitally records the light excitation in the form of a band on a gel or a chromatogram. The choice of detection system generally employed by a researcher is dependent on the availability, overall cost of using the equipment, and the ease of use.

Once the detection equipment is acquired, the most

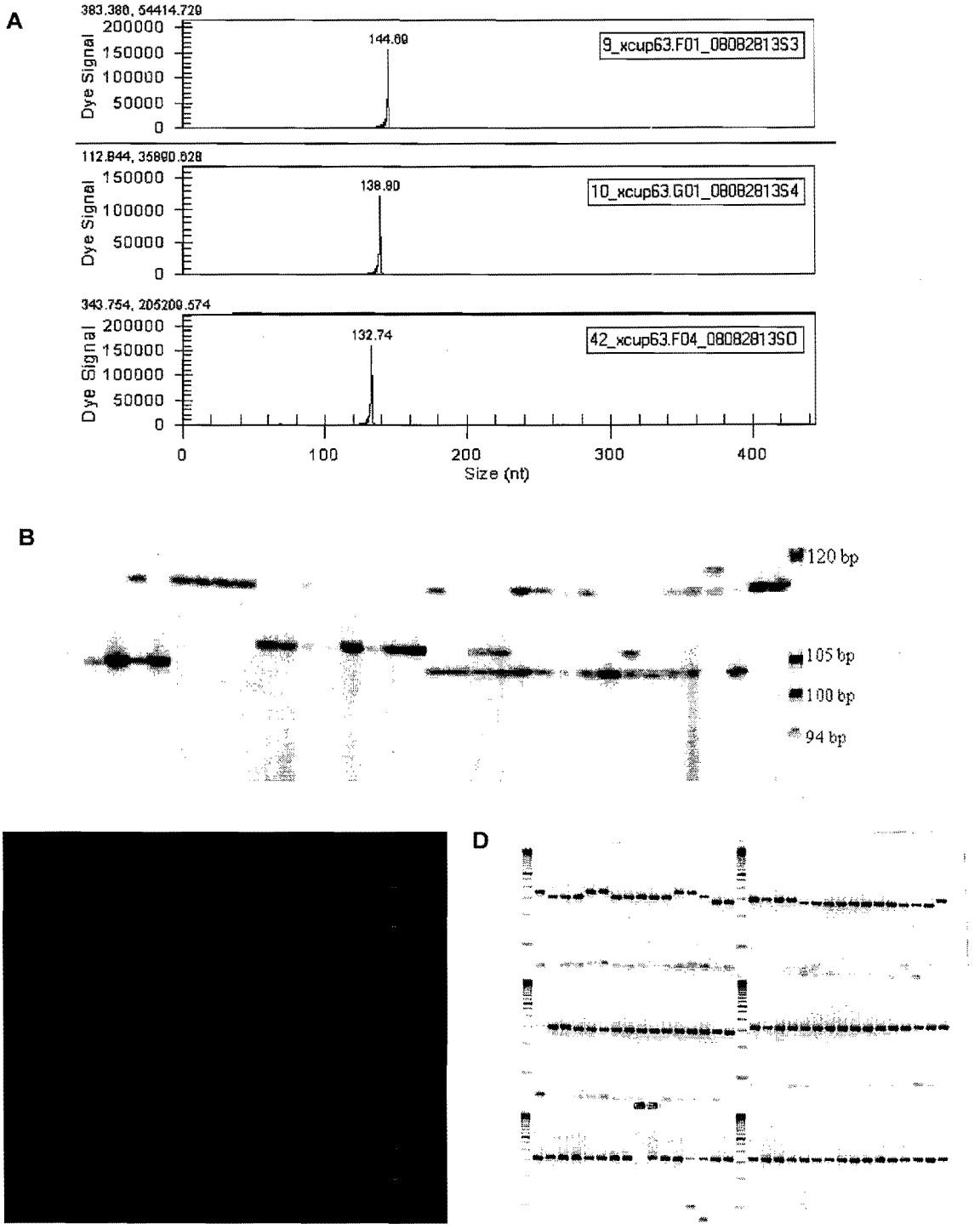

Fig. 1 Separation of microsatellite alleles employing different detection platforms. (**A**) Three accessions distinguished by the same microsatellite marker labeled with same fluorophore by separation on a Beckman CEQ 8000 capillary system. (**B**) Microsatellite alleles labeled with a 700 nm fluorophore separated on a polyacrylamide gel connected to a LI-COR 4200. (**C**) Separation of alleles on an ABI 377 using a microsatellite marker labeled with FAM. (**D**) Allele separation on a 3% agarose gel followed by staining with ethidium bromide and exposure to UV light for visualization.

noteworthy costs of microsatellite experiments are derived from the operational supplies for an automated sequencer and the fluorescent dye chemistries required. If an automated sequencer is not available then the operational cost of polyacrylamide gel electrophoresis followed by DNA staining is fairly minimal in comparison to the automated systems. The necessary supplies required for operating slab gel systems include polyacrylamide gel solution (containing urea, acrylamide, TBE buffer, and water), tetramethylethylenediamine (TEMED), ammonium persulfate (APS), TBE buffer, glass plates, combs, and spacers. On the other hand, main consumables for capillary systems such as the Beckman CEQ 8000 are sample plates, buffer and buffer plates, gel cartridges, and capillary arrays. Fluorophores for allele detection and fluorescently labeled size standards for allele size determination also add to the cost. Unlike the ABI or Beckman systems, the LI-COR system does not require that standards be mixed into each sample. Instead LI-COR standards are loaded into each gel every five or so lanes in order to provide for efficient scoring by LI-COR's SAGA software.

A researcher has two options in regards to ordering primers for any of the above technologies. First, a primer set can be ordered which consists of a single forward primer

Table 1 Platform comparison.

Platform	Fluorophore dyes	Size standard	Multi-plexing	Type	Auto loading	Software	Automatic polyploid scoring
ABI 377	4	Mixed into each sample	Yes	Slab Gel	No	GeneScan, Genotyper	Yes
LI-COR 4300	2	Load standard into a few wells spaced over gel	Yes	Slab Gel	No	SAGA	No
Beckman CEQ 8000	4	Mixed into each sample	Yes	Capillary	Yes	Genetic Analysis Fragments	Yes

labeled with a fluorophore dye attached to the 5′ end and an unlabelled reverse primer. Second, a M13 strategy can be chosen, which can significantly reduce the overall cost (Schuelke 2000). It allows one to order one labeled primer that universally works with each microsatellite primer set. Each PCR reaction requires two unlabelled primers (forward and reverse) and a universal fluorescently labeled M13 primer. The two unlabeled primers consist of a specific SSR targeting forward primer with a 5′ M13 tail (CGTTG TAAAACGACGGCCAGT) and a specific SSR targeting reverse primer. The M13 labeled primer and reverse primer are in excess to the forward primer which is limited. This setup allows the forward M13-tailed primer and reverse primer to initiate the reaction and when the limited primer is depleted, the labeled primer takes the place of the limited forward primer in the remaining PCR cycles. The average cost of a single labeled primer ranges from ~$70-$100; therefore, buying one universal labeled primer for an entire experiment including 30-40 microsatellite markers is much more affordable than purchasing each primer set with a fluorescent label. The M13 labeled tail primer worked well in peanut (Barkley *et al.* 2007) but, not all primer pairs will work after adding a common tail. The primer pairs need to be tested thoroughly before applying it to many DNA samples.

Once the alleles are amplified either by M13 tailing, a single labeled primer, or multiplexed (multiple microsatellites amplified in a single well), they must be separated and scored with appropriate software. Our laboratory has experience operating the LI-COR 4300, ABI 377, and Beckman CEQ 8000 and has employed each one of these machines for microsatellite research. Opinions, therefore, presented here are based on experience with these three machines (**Table 1**). Data scoring can be difficult on all platforms and generally takes a long time to complete. Even though these machines are "automated", all data needs to be carefully evaluated by a knowledgeable scientist and corrected for any software-related scoring inconsistencies. The main problem is that the software programs generally identify too many extraneous bands or peaks. These bands must either be filtered out by the software program and/or manually removed. Another common problem is having the standard mislabeled or not identified, which results in incorrect sample allele size determination. Another potential problem that is only associated with slab gel systems such as the ABI 377 and the LI-COR is that bands of the same size may be scored as different sized fragments due to gel smiling. This occurs when the bands migrate more slowly at the ends of the gel than in the middle and thus the gel images mimic a smile. Lastly, some software programs such as SAGA (LI-COR) are not developed to be able to handle scoring data generated from polyploids. Therefore, when working with polyploid plants, a researcher must choose a different platform that can handle more than two alleles per sample or score the gels by hand.

The overall throughput and cost of each automated platform varies and some of this variability is dependent on the capabilities of the particular researcher using the equipment. For example, LI-COR has 96 well combs available so that an entire plate of samples can be loaded into a single polyacrylamide gel and ultimately be separated in 45 min to 1.5 h dependent on the size of the alleles. Some scientists are very adept, efficient, and precise at loading samples into the very small, narrow wells in these combs. Others, however, do not have the patience for this tedious work. They prefer to either load wider well combs such as 48 well combs or to use a capillary system and not load a gel at all. But, if one reduces the number of samples loaded into a gel then the overall throughput decreases. Capillary systems are advantageous in avoiding gel pouring and loading problems such as spilling samples over into neighboring lanes when loading samples to be separated. But, a 96-well plate on a Beckman CEQ 8000 takes approximately 12 h to complete, which limits the number of samples one can process per day. Multiplexing techniques, however, using the Beckman CEQ 8000 and all ABI models have a slight advantage over the LI-COR because they are capable of detecting four separate flourophores simultaneously. Therefore, they can separate and detect three microsatellite markers simultaneously plus the size standard. LI-COR automated sequencers can only process two microsatellite markers at a time since its detectors are capable of detecting two (700 nm and 800 nm) fluorescent dyes concurrently. The overall cost of detecting microsatellites via automated sequencers is dependent on the platform employed and throughput capabilities previously discussed. In our laboratory, for example, when we compared the overall operating costs of our three platforms (ABI 377, LI-COR 4300, and Beckman CEQ 8000) separating a single marker locus at a time, the ABI 377 and LI-COR 4300 slab gel systems were three to four times less expensive than the Beckman capillary system. Other laboratories may achieve different results. We are unaware of the overall cost of separating microsatellite markers on an ABI capillary systems such as the 3730, and thus, do not have any measure of cost comparison to our Beckman capillary system.

APPLICATIONS OF MICROSATELLITES IN PLANTS AND INSECTS

Microsatellites as DNA markers have been widely used in many living organisms including *E. coli* (Gur-Arie *et al.* 2000; Schlotterer 2000), humans (Beckmann and Soller 1990), mice (Love *et al.* 1990), cows and sheep (Moore *et al.* 1991), plants (Condit and Hubbell 1991), and insects (Hughes and Queller 1993; Goldstein and Clark 1995; de Rosas *et al.* 2008; Exeler *et al.* 2008; Kim *et al.* 2008a; Pol *et al.* 2008) for different kinds of basic genetics research. 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 disorders. In plants and insects, microsatellite markers have been developed for cultivar and insect identification, marker-assisted selection (MAS), quantitative trait loci (QTL) analysis, exploring insect resistance, linkage maps (Bohn *et al.* 2001; Willcox *et al.* 2002; Su *et al.* 2006; Lagat *et al.* 2008), and population and evolutionary genetics studies (Lawson and Zhang 2006). In this section, we will focus on the applications of microsatellites in plants and insects.

In comparison to humans and other vertebrates, plants and insects have less ethical restrictions in regards to collecting samples, increasing population sizes, and controlling mating systems for multiplication. Therefore, there are

some advantages for applications of microsatellites in these systems. Insects play dual roles in ecosystems. Some insects act as beneficial organisms because they can protect and help pollination, produce honey, silk, and other useful compounds. Some insects are pests because they transmit deadly diseases, damage crops, trees, and other natural resources. Using microsatellite markers to understand the evolution process and diversification of insects may help us protect useful insects and control pests in the future. There are many applications of microsatellites in plants and insects, and these applications can be summarized into four categories: 1. genome mapping, 2. cultivar identification and marker-assisted selection, 3. genetic diversity and phylogenetic relationships, and 4. population and evolutionary studies.

Genome mapping

Genome mapping consists of genetic mapping, comparative mapping, physical mapping, and association mapping. Since the purpose of QTL mapping is to identify quantitative trait loci, for convenience it will be covered within the association mapping section. According to the volume of published mapping references from microsatellite markers, the order of magnitude of genome mapping studies from microsatellite markers is genetic mapping, comparative mapping, physical mapping, and association mapping, respectively (see **Fig. 2**).

(1) Genetic mapping: Genetic mapping with microsatellite markers in plants were first reported in tropical trees (Condit and Hubbell 1991), and then reported in soybean (Akkaya *et al.* 1992) and rice (Wu and Tanksley 1993; Zhao and Kochert 1993). Amplification of microsatellites by PCR plus their codominant nature, made this marker system more efficient than other marker systems such as RAPDs and RFLPs (Morgante and Olivieri 1993). In combination with other marker systems, SSR markers were quickly applied for genetic mapping in different plant species (including trees, major and minor crops, fruits and vegetables, ornamentals, and turf grass). So far there are over 80 genetic maps constructed involving the use of SSR markers from many plant species. Since the density of SSR markers in the genome does not always saturate all the chromosomes, SSR-based genetic maps may only narrow down a particular locus of interest or identify a marker or markers closely linked to a trait of interest within a large chromosome region(s). The high density genetic maps generally include numerous types of different molecular markers. In the last decade, sequences from mitochondria DNA (mtDNA) were used as DNA markers for phylogenetic, population genetic and evolutionary studies of insects. In comparison with markers from mtDNA, microsatellite markers are much easier to use because they are highly abundant and polymorphic. Therefore, microsatellites have become popular DNA markers in entomology. In insects, three main methods (single marker analysis, multiple regressions, and marker regression) are used for QTL mapping. A microsatellite-based linkage map of the honeybee (*Apis mellifera* L.) has been constructed (Solignac *et al.* 2004). Two QTL loci for affecting DDT resistance have been mapped in the malaria vector *Anopheles gambiae* using microsatellite markers (Ranson *et al.* 2000).

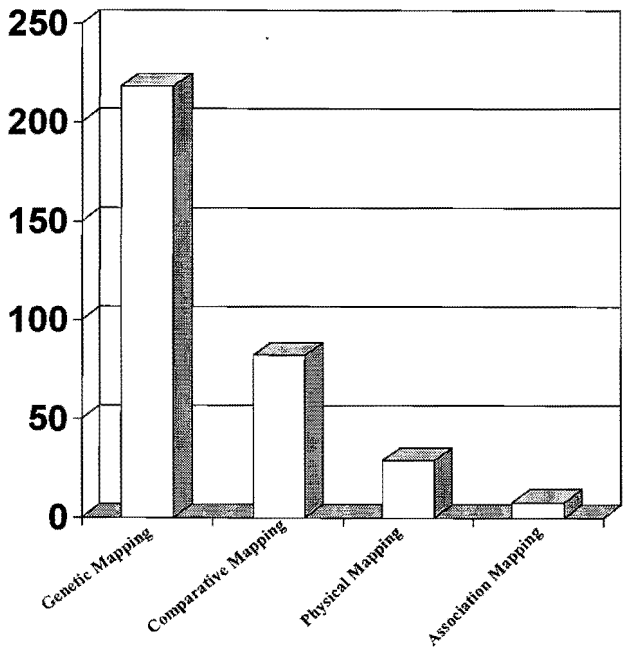

Fig. 2 The number of published references in which plant genome mapping using microsatellites as DNA markers are the focus of the research. The data were collected from NCBI HomePage.

(2) Comparative mapping: Comparative mapping is the alignment of chromosomes or chromosomal fragments of related species based on genetic mapping of common DNA markers and can trace the history of chromosome rearrangements during the evolution of plants, animals, and insects. Comparative analysis of cereal genomes (wheat, rice, maize, sorghum, millet, and sugarcane) has revealed similar genomic building blocks (Moore 1995). Comparative mapping has been successfully conducted in many plant species, including the *Solanaceae* family (Tanksley *et al.* 1988, 1992), grasses (Moore 1995; Gale and Devos 1998), crucifers (Lagercrantz and Lydiate 1996; Lagercrantz *et al.* 1996; Schranz *et al.* 2007), legumes (Zhu *et al.* 2005) and other species. Microsatellite markers have been used for comparative mapping between *Quercus robur* (L.) and *Castanea sativa* (Mill.). Homologies between seven linkage groups were determined and orthologous loci were identified between *Q. robur* and *C. sativa* (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). SSR-based comparative mapping had been conducted between *A. thaliana* and *B. rapa*. Small genomic fragments of *A. thaliana* were scattered throughout an entire *B. rapa* linkage map. A synteny region between *B. rapa* and *A. thaliana* was also identified by comparative mapping (Suwabe *et al.* 2006). Microsatellite markers can facilitate comparative mapping and comparative mapping definitely helps to identify 'linkage blocks', major gene syntenies, chromosome rearrangements, and microsyntenies among species. Major and/or micro-syntenies will further help to develop DNA markers for specific chromosomal regions for marker-assisted selection and even for cross-species homologous cloning.

(3) Physical mapping: SSR markers can be used as anchor markers for joining large pieces of overlapped DNA fragments such as bacterial artificial clones (BACs). Physical maps will give us a real physical distance between markers or genes in bp (base pair) or kbp. SSR markers have been used to construct a whole genome physical map of model crop species. The clones in the tiling-path can be used as DNA templates for whole genome sequencing (such as *Arabidopsis*). SSR markers were also used for anchoring and comparing the frames of soybean genetic and physical maps (Shultz *et al.* 2007; Shoemaker *et al.* 2008), or used for construction of physical maps in specific chromosome regions. 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). Physical maps are useful for helping assemble genome DNA sequences and for positional cloning.

(4) Association mapping: Association mapping has been used interchangeably with linkage disequilibrium (LD) in the literature, but there are some differences between them. Association mapping refers to significant association of a molecular marker with a phenotypic trait; whereas, LD referrers to a non-random association between two markers or two genes (QTLs) or a gene (QTL) and a marker (Gupta *et al.* 2005). Association mapping is especially useful for implementing marker-assisted selection for quantitative traits in plant breeding programs (Breseghello and Sorrells 2006b). The principles of association mapping are not fundamentally different from genetic mapping (genetic linkage analysis) and they are all based on examining genetic recombination. However, genetic mapping or QTL mapping usually uses a population from a bi-parental cross, while association mapping uses a collection of individuals often with varying ancestry. Because the individuals from a population of limited relationships could have gone through many generations of recombination, in comparison with a bi-parental cross, association mapping has four advantages: higher mapping resolution; greater number of alleles, a broader reference population, and less research time in establishing an association (Hastbacka *et al.* 1992; Yu and Buckler 2006). The purpose of association mapping is to detect the correlation between genotypes and phenotypes in a collection of individuals by linkage disequilibrium (LD), which is the nonrandom association between alleles from different loci. Association mapping employed by SSR markers has been successfully conducted in many important crop species such as potato, maize, wheat, and soybean. The identified association between a microsatellite marker and QTL for resistance to *Verticillium dahliae* over a set of 137 tetraploid potato cultivars led to cloning of QTL for resistance to *V. dahliae* (Simko *et al.* 2004). The correlation between SSR markers and wheat kernel size has been detected on three chromosome regions by association mapping using elite germplasm (Breseghello and Sorrells 2006a). The correlation between marker loci and resistance to *Stagnospora nodorum* glume blotch (SNG) was also revealed by association mapping in wheat (Tommasini *et al.* 2007). In soybean, at least two common SSR markers identified in two separate populations were associated with iron deficiency chlorosis by association mapping (Wang *et al.* 2008). A vast amount of genetic diversity exists in plant germplasm and more microsatellite markers will become available as genomic information accumulates. Association mapping holds great promise for exploiting genetic diversity, characterizing accumulated phenotypic variation, and associating markers with traits in plant germplasm. The microsatellite markers associated with clear phenotypes can definitely be used in plant breeding programs by MAS to expedite the breeding process.

Cultivar identification and marker-assisted selection

Since SSR markers are abundant and highly polymorphic, they have been used in the identification of *E. coli* strains (Gur-Arie *et al.* 2000) and yeast strains (Masneuf-Pomarede *et al.* 2007). In soybean, a set of SSR markers has been selected for the identification of soybean cultivars (Song *et al.* 1999). In tomato, a set of 65 SSR markers has been selected for distinguishing 19 diverse tomato cultivars (He *et al.* 2003). A specific set of SSR markers should be developed and selected for each economically-important agricultural species, so that they can be employed to protect the intellectual property rights of plant commercial companies.

SSR marker-assisted selection (MAS) can also greatly enhance the efficiency of plant breeding programs. SSR markers used for selection can be classified into flanking SSR markers (closely linked to the locus for a trait) and targeted gene SSR markers (developed within the targeted gene itself). Obviously, the targeted gene SSR marker is more efficient for selection than the flanking SSR markers, but more efforts will be required for developing gene-targeted SSR markers. SSR marker-assisted selections have been used in many breeding programs. For example, the gene for resistance to the Hessian fly (*H32*), the gene for adult-plant leaf rust resistance and yellow pigment content (*YP*) were successfully selected by flanking SSR markers of Xgwm3 and Xcfd223 (Sardesai *et al.* 2005), GWM296 (Hiebert *et al.* 2007), and Xwmc809 (He *et al.* 2008), respectively, in wheat breeding programs. A major QTL for submergence stress (*Sub1*) was selected by employing flanking SSR markers in a rice backcross breeding program (Neeraja *et al.* 2007). The polymorphic SSR marker QLB1, co-segregated with the locus for resistance to the barley yellow mosaic virus, was used for resistance selection in barley breeding (Tyrka *et al.* 2008). In field pea, when two flanking SSR markers were used to select for powdery mildew resistance, the success rate was 98.4%. These two SSR markers have been successfully used in marker-assisted selection for resistance to powdery mildew in pea breeding (Ek *et al.* 2005). Insect-food crop interactions have created interest in utilizing marker-assisted selection. Host-plant association has been studied in lettuce root aphid, southwestern corn borer (SWCB), rice brown planthopper and other insect herbivore species using microsatellite markers (Willcox *et al.* 2002; Miller *et al.* 2005; Warrington *et al.* 2008). Just as strategies have been studied for host-plant resistance in maize and wheat (Smith *et al.* 1989; Thome *et al.* 1992), interest in strategies for global-sustainable rice production have grown proportional to the global population growth and climate change. There are three specific challenges listed to increasing rice production in rice-producing areas: insect outbreaks and diseases, fertilizer application, and a global water shortage. Thus, identification of insect and disease-resistant genes or functional markers has become a priority. Transgenic approaches could be combined with marker-assisted selection for the purpose of developing insect and disease-resistant rice cultivars. Microsatellite markers have also been used to study the association between host race of *Acyrthosiphon pisum* and their symbiants (Simon *et al.* 2003). As more whole genome sequence projects are completed, more gene-targeted SSR markers should be available for marker-assisted selection.

Genetic diversity and phylogenetic relationships

Genetic diversity refers to any variation in nucleotides, genes, chromosomes, or whole genomes of organisms. Genetic diversity can be assessed at different levels within a species or among species. Phylogenetic relationships reflect the relatedness of a group of species based on a calculated genetic distance (sequence conservation or diversification) in their evolutionary history. SSR markers often are a powerful system for revealing interspecific or intraspecific phylogenetic relationships. For example, the genetic diversity and phylogenetic relationships from germplasm collections such as a temperate bamboo collection (Barkley *et al.* 2005), a citrus variety collection (Barkley *et al.* 2006) and a cultivated and wild peanut collection (Barkley *et al.* 2007; Cuc *et al.* 2008) have been assessed by SSR or transferable SSR markers. The genetic diversity from a primary core collection of peach has been assessed by SSR markers (Li *et al.* 2008). Recently, SSR markers have been developed in eggplant (*Solanum melongena* L.) and the genetic diversity of 38 cultivated eggplant varieties and phylogenetic relationships of its related species have been assessed (Stagel *et al.* 2008). Organelle SSR markers were also developed in rice mitochondrial and chloroplast genomes (Nishikawa *et al.* 2005). SSR markers derived from the chloroplast genome were more polymorphic than the ones derived from mitochondrial genomes. These organelle SSR markers have been used for assessing genetic diversity and phylogenetic relationships of related species within the genus *Oryza*. 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 pro-

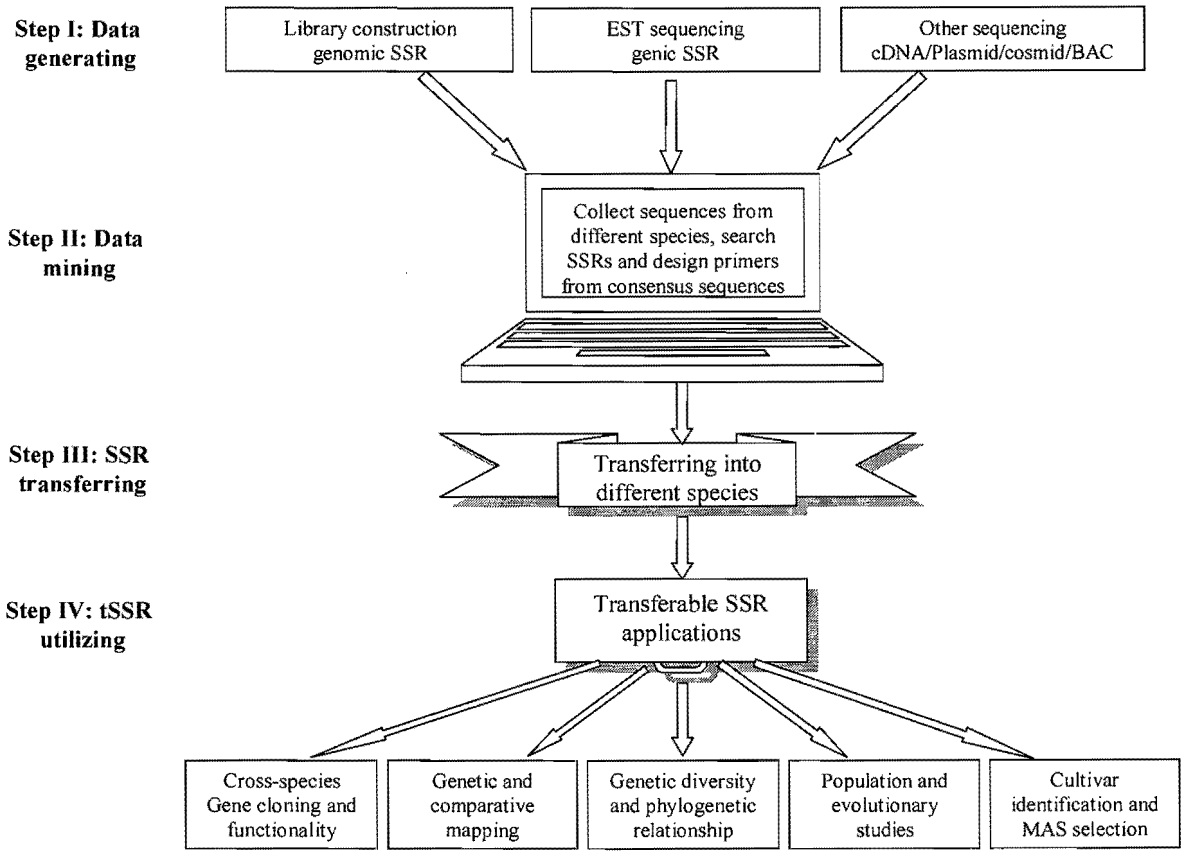

Fig. 3 A flow chart linking the steps involved from marker development to the application of microsatellites.

vide important information for choosing parental lines for breeding programs, classification of plant germplasm accessions, and further curation and acquisition of new plant germplasm accessions.

Population and evolutionary studies

In comparison to animals, plants can not migrate large distances, except in cases of plant introduction and domestication by humans, seed dispersal, and pollen flow, all of which can affect the genetic structure of natural populations. Studies of plant evolution were traditionally based on taxonomic and phenotypic data (such as morphology and karyotype). Researchers can now integrate data collected from molecular assays such as microsatellites, karyotyping, and phenotyping to provide a more robust analysis of their species of interest. Microsatellite markers can be used to determine the population structure within and among natural populations and/or identify the potential progenitors. Chloroplast SSR markers have been used to survey the variation among barley populations (including wild *Hordeum* species, wild progenitors of cultivated barley, barley landraces, and barley cultivars). A decrease in cytoplasmic diversity was observed between wild progenitors and barley cultivars as well as between barley landraces and barley cultivars (Provan *et al.* 1999). Since morphology is a poor character to define most species of cultivated potato, 50 SSR markers have been recently employed to genotype 750 potato accessions (including 742 potato landraces and 8 wild species). The genotyping results confirmed the reclassification of the cultivated potato into four species: *S. tuberosum*, *S. ajanhuiri*, *S. juzepczukii*, and *S. curtilobum* (Spooner *et al.* 2007). As more genomic sequence information becomes available, more SSR markers will be used for genotyping different species, and evolutionary lineages between species or genera will be better understood. For example, tomato (*Lycopersicon esculentum*) has been renamed *Solanum lycopersicon* (Spooner *et al.* 1993; Olmstead *et al.* 1999) and 'Dicots' are being considered as 'Eudicots' (Slotis and Slotis 2003). Since SSR markers are highly polymorphic, abundant genome-wide, and easy to use, they have become the marker of choice for population genetics and evolutionary studies in plants and insects.

TRANSFERABILITY OF MICROSATELLITES

There are three different approaches to obtain microsatellite markers (**Fig. 3**). **(1)** ***Generating*****:** DNA libraries can be constructed and clones can be screened with various probes containing different repeat sequences. The clones that hybridize with a specific repeat element probe [such as $(CA)_n$] contain microsatellites, and thus should be sequenced. Primers can be designed from collected sequence data encompassing repeats to develop SSR targeting markers. Many free web based programs such as Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) exist to input the sequence data and assist in designing optimal primers to target the repeat element. **(2)** ***Mining*****:** There are many DNA sequences available from different research projects. Numerous plant, animal, and insect species are included in the existing DNA databases such as Genbank, EMBL, and DDBJ. These sequences can be downloaded and aligned. One can use the aligned sequences to identify tandem repeats from which primers can be designed for microsatellite marker development. Also, the database can be searched for specific strings of repeat elements in a species of interest. If the sequence data is available in the species or genera of interest, then primers can be designed to target the particular mined microsatellite. **(3)** ***Transferring*****:** There are many SSR markers available from related species in the databases and published literature. These markers can be searched and tested in the species of interest for direct use. Development of transferable SSR markers from data mining has been demonstrated as an efficient approach for

Table 2 Applications of transferable SSR markers in plants.

Classification	Species related	Number of references
Genome characterization	Soybean, red clover, wheat, barley, rye, rice, sorghum, white spruce, peach, *Prunus*, oat, coffee, mint	14
Genetic mapping and comparative mapping	*Triticum* and related species, rice, fragaria, citrus, cucurbita, sorghum, sugar beet, apple, pearl millet, pine, medow fescue, wheat, rice, barley, quercus, *Castanea*	19
Genetic diversity and phylogenetic analysis	*Medicago*, soybean, *Lespedeza*, coffee, sugarcane, bamboo, lablab, wheat, barley, oat, maize, sorghum, seashore paspalum, peanut, clover, guar, *Crotalaria*, tall fescue, forage grass, *Cicer*, almond tree, bamboo, *Crotalaria*, soybean, cowpea	20
Transferability	Cereals, coffee, mint, peanut, pine, *Miscanthus*, pepper, cotton, *Medicago*, pulse, bermudagrass, tall fescue, apple, pear, olive, apricot, grape, peach, sweet cherry, tea, sugarcane, pine, tree, *Caryocar brasiliense*	31
Data mining for SSR	Wheat, rice, maize, sorghum barley, *Coffea*	5
Review		3

many species: cereals (Kantety *et al.* 2002); pine tree (Chagne *et al.* 2004); *Medicago* (Eujayl *et al.* 2004) and coffee (Poncet *et al.* 2006). In the last decade, transferable SSR markers have been used for numerous applications in many plant and insect species (**Table 2**). Of the three microsatellite generating approaches discussed, the first approach is very costly and time-consuming, the second approach is cost-effective, but does not always necessarily produce microsatellites for a species of interest due to a lack of genomic information, and the third approach costs nearly nothing and also is time-saving. Clearly, if SSR marker information is available in a related species, the transfer approach is advantageous. In this section, we will mainly cover development of transferable SSR markers, specific applications of transferable SSR markers, and limitations of SSR marker transferability.

Development of transferable SSR markers

Microsatellites first have to be generated from a species of interest and then their usefulness as a polymorphic genetic marker has to be demonstrated. Some economically important species have enough interest so that the financial resources for marker development are available; whereas, many minor species do not have this luxury. Researchers, however, can test whether the SSR markers developed in other species will work in a related species of their interest. This intention may be the major driving force for development of transferable SSR markers. This situation is especially true for germplasm characterization and evaluation. Transferable SSR marker development can be divided into the following three steps: collection of SSR markers from related species, estimation of the transfer rate of SSR markers, and identification of the polymorphisms (or polymorphism level) of transferable SSR markers.

***Collection of SSR markers*:** Effective SSR markers are available for model or major species (such as *Arabidopsis*, *Medicago*, rice, soybean, and *Drosophila*). SSR markers should be collected from closely related species because these species would be expected to share more conserved sequence sites. If the sequence is conserved between the model/major crop and minor species of interest, the primers will have a higher probability of amplifying DNA fragments in the species of interest. For example, SSR markers derived from *Arabidopsis thaliana* have been used to amplify DNA fragments in *Arabidopsis lyrata*, and SSR markers derived from *Triticum aestivum* have been used to amplify DNA fragments in *Triticum durum*. Based on the origin of the sequences from which the SSR markers were designed, they can be classified into genic SSR markers (from the transcribed gene regions) and genomic SSR markers (from the regions between genes). Both genic and genomic SSR markers should be employed depending on the intended application. Since the transcribed regions should theoretically be conserved among highly related species, primers from genic SSR markers should have a high transfer rate in the species of interest.

***Transfer rate of SSR markers*:** The transfer rate is defined as the percentage of markers which amplify DNA fragments in the species of interest among the total number of SSR markers screened (Wang *et al.* 2004). SSR markers developed in one species might be transferred to several species within the same genus, and even to different genera within the same family. Peakall *et al.* (1998) reported that some SSR markers developed in soybean (*Glycine max* L.) can be successfully amplified in *Glycine*, *Kennedia*, *Vigna* (Phaseoleae), *Vica* (Vicieae), *Trifolium* (Trifolieade) and *Lupinius* (Genisteae) within Papilionoideae, and in *Albizia* within the Mimosoideae (Peakall *et al.* 1998). A set of 42 SSR markers developed in *Arabidopsis thaliana* can be amplified in species of *Arabidopsis halleri*, *Arabidopsis lyrata*, as well as in one more distantly related crucifer, *Arabis drummonidii* (Clauss *et al.* 2002). Some markers developed in olive cultivars (*Olive europaea* L.) were tested in other species within the *Olea* genus and thirteen out of the fifteen tested species amplified corresponding microsatellite loci (Rallo *et al.* 2003). In legumes, several genera (*Medicago*, *Glycine*, *Arachis*, *Trifolium*, *Vigna*, *Cyamopsis*, and *Lablab*) were used for cross-genus amplification (Wang *et al.* 2004). The degree of conservation for SSR-transfer analysis across the legume family was highly variable (8.3% rate of success from peanut to cowpea and 55.0% success from cowpea to lablab). Peanut belongs to Aeschynomenoid, but both cowpea and lablab belong to the same taxonomic lineage, Phaseoloids, which may explain why the microsatellite markers shuttled from cowpea to lablab transferred at such a high rate. Transfer rate of SSR markers in this case was clearly related to phylogenetic distance. In pine trees, SSR markers were developed in *Pinus taeda* and *Pinus pinaster*. Fifty-three primer sets from the pine SSR markers were tested in six other pine species (Chagne *et al.* 2004). The transfer rate of SSR markers in *Pinus* also highly corresponded with phylogenetic distance between species, ranging from 64.6% in *P. canariensis* to 94.2% in *P. radiata*. The transferability of SSR markers were also reported in many other crops such as cereals (Varshney *et al.* 2005b; Wang *et al.* 2005), cotton (Guo *et al.* 2006), peanut (Gimenes *et al.* 2007) and *Citrus* (Luro *et al.* 2008). Overall, the literature cited in plants, demonstrates that the transfer rate of SSR markers is largely dependent on: (i) the phylogenetic distance and sequence conservation of species tested; (ii) the genome size of species tested; (iii) the genome evolution and stability of species tested; and (iv) the number of primers tested and stringency of PCR conditions tested (especially annealing temperature, cycle number, and ion concentration) and the amount of DNA used for PCR. Cross-species or cross-genus amplification to some extent takes advantage of mis-priming, which is similar to amplified fragment length polymorphism (AFLP) where at some base pairs, choice of priming depends on the sequence content of the DNA template (Vos *et al.* 1995).

Recently, transferability of SSR markers was also reported in insects (Weng *et al.* 2007). Among six aphid species investigated, the cross-species transferability of EST-SSR markers was dependent on phylogenetic distance between the species from which the SSR markers were developed

and the species of interest. The cross-species transfer rate in insects seems to be related to generation time, mixed or out-cross breeding systems, and genome size [see review (Barbara *et al.* 2007)]. When comparing genomic SSR markers and genic SSR markers, it was found that the average transfer rate in both plants and insects was much higher from genic SSRs than from genomic SSRs (Chagne *et al.* 2004; Wang *et al.* 2005; Weng *et al.* 2007). The difference in the average transfer rates between genic and genomic microsatellites further implies that the transcribed regions are more conserved than non-transcribed regions across species in both plant and insect genomes.

Polymorphism level of transferable SSR markers: The content of an amplicon from transferable SSR markers could be characterized by three regions: the priming region, flanking region and core microsatellite region. The polymorphism could be either in the priming, flanking or core regions. For transferable SSR marker development, researchers want to get not only a high rate of transfer, but also a high level of polymorphism. There have been contradictory reports in the current literature in regards to comparing the levels of polymorphism from transferable genomic SSR markers and genic SSR markers. Several papers reported that the level of polymorphism was higher from genomic SSR markers than from genic SSR markers (Cho *et al.* 2000; Eujayl *et al.* 2001; Gupta *et al.* 2003). However, some recent studies reported that the level of polymorphism was higher from genic SSR markers than genomic SSR markers (Liewlaksaneeyanawin *et al.* 2004; Varshney *et al.* 2005a). In the grass family, four major species (wheat, rice, maize, and sorghum) and four minor species (durum, finger millet, bermudagrass and seashore paspalum) were estimated for the level of polymorphism using 210 SSR markers (Wang *et al.* 2005) (as an example, see **Fig. 4**). The level of polymorphism detected was significantly higher among species (67%) than within species (34%) and was related to the degree of out-crossing for each species. The level of polymorphism detected within species was 57% from self-incompatible species, 39% from out-crossing species, and 20% from self-pollinated species. To address whether there was a significant difference between transferable genic SSR markers and genomic SSR markers in revealing the level of polymorphism, 30 sorghum genomic SSR markers and 30 sorghum genic markers were selected and tested. Sorghum genomic SSR markers revealed a significantly higher level of polymorphism (69%) than sorghum genic SSR markers (33%) within sorghum. However, when these 60 SSRs were used as transferable markers across species, there was no significant difference in revealing the level of polymorphism between genomic SSR markers and genic SSR markers. The level of polymorphism detected from transferred sorghum genomic SSR markers was 74% among species and 36% within species, while the level of polymorphism detected from sorghum genic SSR markers was 70% among species and 37% within species (differences between types of SSR markers was not statistically significant). The level of polymorphism revealed by transferrable SSR markers probably depends on the phylogenetic distance among species and the genetic distance within the species, but not the types of transferable SSR markers. This may partly explain why there were contradictory published reports. The level of polymorphism detected may not be related to the transfer rate of SSR markers. In other words, high transfer rate markers were not necessarily revealing a high level of polymorphism. In terms of the degree of out-crossing of the species, SSR markers may not be easily transferred to out-crossing species because their sequence divergence may occur rapidly compared with other species. However, once the SSR markers are transferred to the out-crossing species, the level of polymorphism detected should be high within the species.

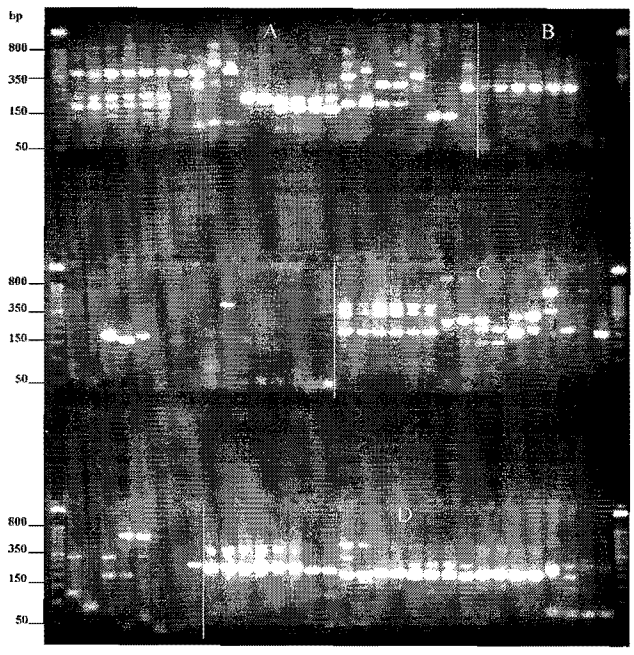

Fig. 4 Amplicons generated by PCR and separated by electrophoresis. Each well contains either 15 µl of molecular marker (150 ng) or 12.5 µl of PCR products. PCR products were amplified with sorghum primers: genomic Xtxp168 (**A**) and Xtxp21 (**B**), EST-derived CNL164 (**C**) and CNL170 (**D**). The first lane of each row was the molecular marker, 50 base pair (bp) ladder followed by W1, W2, D1, D2, D3, D4, R1, R2, M1, M2, S1, S2, F1, F2, F3, F4, C1, C2, C3, C4, P1, P2, P3 and P4 in each panel. The initials W, D, R, M, S, F, C, and P represents wheat, durum, rice, maize, sorghum, finger millet, cynodon, and seashore paspalum, respectively.

Specific applications of transferable SSR markers

The application of transferable SSR markers across species and genera has been reported in many crops such as soybean (Peakall *et al.* 1998), rice (Zhao and Kochert 1993), wheat (Roder *et al.* 1995), sorghum and maize (Brown *et al.* 1996; Cordeiro *et al.* 2001), barley (Thiel *et al.* 2003), sugarcane (Selvi *et al.* 2003), *Crotalaria* (Wang *et al.* 2006b); Oat (Fu *et al.* 2007), *Lablab* (Wang *et al.* 2007), *Lespedeza* (Wang *et al.* 2008), bamboo (Barkley *et al.* 2005), seashore paspalum (Wang *et al.* 2006a) and trees such as peach (Cipriani *et al.* 1999), pine (Karhu *et al.* 2000; Chagne *et al.* 2004), olive (Rallo *et al.* 2003), sweet cherry (Wunsch and Hormaza 2002), and apricot (Decroocq *et al.* 2003). In general, the applications of transferable SSR markers are mainly for minor species, especially for assessing diversity in germplasm studies. The applications of transferable SSR markers can be summarized into the following four areas: genetic and comparative mapping, assessment of genetic diversity and phylogenetic analysis, marker-assisted selection and cultivar identification, and homologous gene identification and cloning.

Genetic and comparative mapping: Since there are not many DNA markers available in minor species, it is hard to pursue genetic mapping. Even in major crops, some chromosomal regions can not be well saturated or covered with DNA markers for certain studies. Transferable SSR markers from related species help to get a genetic map in minor species and better enrich the chromosomal regions with a few markers in major species. For example, transferable SSR markers from barley have been placed on rye and wheat genetic maps (Varshney *et al.* 2005b). Furthermore, SSR markers from related major crops can be utilized for comparative mapping. Transferable genomic and genic SSR markers are highly suitable for comparative mapping. For example, transferable genic SSR markers developed by data mining have been successfully used for comparative mapping among wheat, barley, rye, and rice (Yu *et al.* 2004a, 2004b; Varshney *et al.* 2005b). Comparative mapping among genomes can highlight macro- and micro-synteny

regions among species. These highlights may provide insights on genome evolution or define a small chromosomal region for cross-species gene cloning.

***Assessment of genetic diversity and phylogenetic relationships*:** Due to a lack of DNA markers, especially for minor species, the genetic diversity and phylogenetic relationships for these species were traditionally either assessed with morphological markers (Pengelly and Maass 2001) or organelle (mitochondria or chloroplast) DNA sequences (Nemoto *et al.* 1994). As many SSR markers can be transferred from major crops to minor crop species, the genetic diversity and phylogenetic relationships of many minor species have been assessed by transferable SSR markers (**Table 2**). Assessment of genetic diversity and phylogenetic relationships may be one of the major applications of transferable SSR markers.

***Markers-assisted selection and cultivar identification*:** SSR marker-assisted selection and cultivar identification have been used in plant breeding programs and cultivar commercialization. Transferable SSR markers can be employed for the same purpose in the species of interest, especially for known-function transferable genic SSR markers. These markers could also be redesigned so that they are specific for the minor species of interest by amplifying PCR fragments using microsatellites from a major species, isolating the products, sequencing the PCR product, and redesigning the marker/primers to be specific for the species of interest based on the collected sequence data.

***Homologous gene identification and cloning*:** Homologous genes exist among related species and their functions may also be conserved especially among highly related individuals. If a known-function gene contains SSRs and the SSRs can be transferred to related species, the homologous gene may be cloned using transferable SSR markers in the species of interest. Degenerate oligonucleotide sequences (DOS) and PCR have been utilized to successfully clone cDNAs for specific proteins. DOS primers were also used to amplify unknown members of gene families and homologous genes in different species (Sommer and Tautz 1989; Wilks *et al.* 1989; Cooper and Baptist 1991). To our knowledge, there are no genes which have been cloned across species using transferable SSRs in plants and insects. If these techniques are working in mammals, they should also work in plant and insect species. Research in this area is worth pursuing in the future.

Limitations to transferable SSR markers

Since the amplicons are produced by transferable SSR markers, there are some potential limitations which can cause problems in the application of transferable SSR markers. Highly polymorphic microsatellite markers are widely used for different applications. There are several potential drawbacks including the presence of *stutter bands* (or peaks), *null alleles*, and *heterologous amplicons*. Special caution has to be taken if some of the drawbacks are encountered in research. *Stutter bands* (or peaks): During DNA amplification of the SSR markers, DNA slippage (discussed in Introduction) could occur due to encountering repeat sequences. This can be a problem in transferrable SSRs or when using an SSR marker in the species from which it was originally designed/developed. When PCR products are separated on agarose gels (or acrylamide gels) extra bands (or peaks) could be observed. These extra bands are called stutter bands. Stutter bands are artifacts and are usually shorter and have weaker signal intensity than the actual microsatellite alleles. Due to this technical limitation, when the microsatellite bands or peaks are scored these extra bands have to be eliminated. Improperly scored bands or peaks will affect the data analysis and final results. *Null alleles*: A microsatellite null allele is defined as any allele at a microsatellite locus that consistently fails to amplify due to primer template mismatch or elimination of the gene product. This can occur in normal SSR applications and also in transferable SSR applications. The primary sources of microsatellite null alleles are (a) poor primer annealing due to nucleotide sequence divergence (point mutation or indels) in one or both primers, (b) differential amplification of size-variant alleles (Wattier *et al.* 1998), and (c) fragment rearrangements such as insertions or inversions. These microsatellite null alleles might lead to high frequencies of false parentage exclusions (Dakin and Avise 2004). *Heterologous amplicons*: Here homologous or heterologous amplicons are determined by whether they are located in the same homologous chromosomal region of the original amplicon. Mis-priming or multiple priming may produce either homologous or heterologous amplicons. The content of amplicons can be determined by homologous hybridization (Whitton *et al.* 1997) or direct sequencing. Since the sequence content of homologous or heterologous amplicons could be very different (some of them may not contain simple sequence repeat at all or are located in unrelated chromosomal regions), caution should be taken in their application (for example, comparative mapping). Homologous and heterologous amplicons and homoplasy will be specifically discussed in Part III of this review. Some substantial errors from microsatellite null alleles and heterologous amplicons may be overcome experimentally by redesigning primers, altering PCR programs and conditions, or using a higher quality DNA template.

SUMMARY

Microsatellites or SSRs, as a marker system, have been intensively used for various applications in many different species. As more species are being sequenced, more DNA markers will be developed from single nucleotide polymorphisms (SNPs). Since SNP markers have good genome coverage, they will replace SSR markers for some applications. However, from recent reports in humans (de Bakker *et al.* 2006) and plants (Vezzulli *et al.* 2008), SNP markers can only be transferred to different mapping populations within the same species, but not across species. This will limit the applications of SNP markers on related minor species. In contrast, due to multiple alleles, cost-effectiveness, and transferability, SSR markers will continue to play an important role in different genetic studies in many minor plant and insect species in the future.

DISCLAIMER

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture and the University of Georgia.

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