

Received 12 January 2004

Accepted 9 March 2004

## CHARACTERISTICS AND A COMPARISON OF THREE CLASSES OF MICROSATELLITE-BASED MARKERS AND THEIR APPLICATION IN PLANTS

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**Abstract:** Microsatellites (SSR – simple sequence repeats, STR – short tandem repeats, SSLP – simple sequence length polymorphism, VNTR – variable number of tandem repeats) are the class of repetitive DNA sequences present in all living organisms. Particular characteristics of microsatellites, such as their presence in the genomes of all living organisms, high level of allelic variation, co-dominant mode of inheritance and potential for automated analysis make them an excellent tool for a number of approaches like genotyping, mapping and positional cloning of genes. The three most popular types of markers containing microsatellite sequences that are presently used are: (1) SSR (simple sequence repeats), generated by amplifying in a PCR reaction with the use of primers complementary to flanking regions; (2) ISSR (inter-simple sequence repeats), based on the amplification of regions between inversely oriented closely spaced microsatellites; and (3) SAMPL (selective amplification of microsatellite polymorphic loci), which utilises AFLP (amplified fragment-length polymorphism) methodology, with one exception – for the second amplification, one of the starters is complementary to the microsatellite sequence. The usefulness of the three above-mentioned markers for numerous purposes has been well documented for plants.

**Key Words:** Microsatellite, Marker, Repetitive DNA, SSR, ISSR, SAMPL

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Abbreviations used: SSR – simple sequence repeats; ISSR – inter-simple sequence repeats; SAMPL – selective amplification of microsatellite polymorphic loci; STR – short tandem repeats; SSLP – simple sequence length polymorphism; VNTR – variable number of tandem repeats; EST – expressed sequence tags; RAPD – random amplified polymorphic DNA; RFLP – restriction fragment length polymorphisms; MAS – marker-assisted selection; NIL – nearly isogenic lines; BSA – bulked segregant analysis; QTL – quantitative trait loci; AFLP – amplified fragment-length polymorphism.

## INTRODUCTION

Microsatellites are the class of repetitive DNA sequences present in all organisms, both Eukaryotes [1] and Prokaryotes [2]. They consist of tandemly arranged repeats of several nucleotides, usually 2-6, in tracts up to  $10^2$  bp, that are distributed through the whole genome (though their distribution varies by species and chromosome) and are flanked by highly conserved sequences [3]. Several terms are employed for microsatellites: SSR – simple sequence repeats; STR; SSLP; and VNTR. Chambers and McAvoy [3] suggest using the term VNTR for sequences with core repeats in the range of 7-10 nucleotides, not only from a formal point of view but rather because of the different mutational processes involved in their evolution.

Although microsatellites are present in both non-coding and coding regions, their frequency is higher in transcribed regions, especially in UTRs [1, 4, 5]. The microsatellites in coding and non-coding regions may also differ in their composition. For seven eukaryotic clades, Metzgar *et al.* [6] found that tri- and hexanucleotide repeats were present in both coding and noncoding sequences, but that other repeat types were much less frequent in coding regions than in noncoding regions. These findings suggest that the differences between coding and noncoding microsatellite frequencies arise from specific selection against frameshift mutations in coding regions, resulting from length changes in nontriplet repeats. Morgante *et al.* [1] underlined that the frequency of microsatellites is inversely related to genome size, but remains constant in nonrepetitive DNA. Microsatellites arise in regions consisting of short (up to 8) runs of repetitive nucleotides. Two probable mechanisms generate their variability (DNA replication slippage followed by a high efficiency of the mismatch repair system [3, 7, 8], which is connected with an extraordinary mutation rate ( $10^{-6}$ - $10^{-2}$  per generation).

Microsatellites can be arranged in a simple way, e.g. they consist of several repeats of two or more nucleotides –  $(N_1N_2...N_x)_n$  – or they may have a more complicated structure: two or more adjoining repeats of motifs, e.g.  $(CA)_n(GT)_n$ , or  $(dC-dA)_n(dG-dT)_n$ . Microsatellites called "imperfect" or "compound" (as opposed to "perfect" or "pure") have spacers between motifs repeated several times, e.g.  $(GA)_n(N)_n(CT)_n$ . For more details concerning this nomenclature, see Chambers and MacAvoy [3]. The most frequent microsatellites in plants are composed of dinucleotide motifs, usually  $(AT)_n$  and  $(GT)_n$ , while  $(AC)_n$  repeats are common in animals [1, 5, 9, 10]. TAT repeats prevail among trinucleotides. Some types of microsatellites seem to be specific or much more present for a certain group of plants, e.g. CCG/CGG repeats are much more abundant in rice than in other cereals or dicotyledonous plants [9, 11].

Years ago, repetitive DNA was termed "junk" DNA because it was thought to lack any function. Today, although the role of microsatellites in a plant's DNA is still unknown, it has become an important tool for researchers. Particular characteristics of microsatellites, such as their presence in genomes of all living

organisms, high level of allelic variation, co-dominant way of inheritance and potential for automated analysis make them excellent molecular markers for a number of approaches, like genotyping, mapping or positional cloning of genes [12]. They are especially attractive in the case of species which show a low level of genetic variation, inbred populations and recently derived or geographically close populations where the differentiation may be difficult to discern by the use of other approaches. As the flanking sequences are usually highly conserved, microsatellite primers developed for one species frequently amplify loci in related species [10]. In recent years, the popularity of SSR-based markers has increased considerably [13].

## MICROSATELLITE-BASED MOLECULAR MARKERS

### SSR markers

SSRs are actually considered the most efficient markers, but their use is still limited because of the long and laborious steps to develop them. There are two general strategies to access these regions and create SSR markers: (1) searching for sequences containing microsatellites in the available data bases; or (2) constructing and screening the genomic (or other) library with probes complementary to microsatellite sequences. Exceptionally, some strategies without library construction have been developed.

#### *1. The development of SSR's through data base searches*

This strategy of developing SSR markers is based on searching for sequences containing microsatellites deposited in the data bases (EMBL, GenBank). This method is cost-effective, simple and relatively quick; however, it does show some limitations. It should be underlined that when exploring data from expressed sequences, a considerable amount of potential polymorphism can be lost, as microsatellites are broadly present in the non-coding regions of genomes (although, as cited previously, their frequency is higher in transcribed regions). Additionally, this strategy is limited to plants with high economical or scientific interest which are well represented in the databases. Consequently, their usefulness may be restricted. In rice, Cho *et al.* [14] showed that microsatellites derived from genomic libraries detected a higher level of polymorphism than those derived from ESTs contained in the GenBank database (83.8% vs. 54.0%). The other measures of genetic variability, like the number of alleles per locus, polymorphism information content, and allele size ranges, were higher in the case of the genomic library- than in that of the EST-derived microsatellites. Conversely, in rye, Hackauf and Wehling [15] identified much more effective SSR loci when exploring EST data bases than Saal and Wricke [16] who searched the genomic library. The authors examined more than 8000 rye cDNA sequences from anthers, cold-stressed leaves, and aluminium-stressed and unstressed roots. A total of 157 sequences out of 528 SSRs comprising di-, tri- and tetra-nucleotide motifs turned out to be useful for primer design. One-

hundred EST-derived loci displayed a length polymorphism among 15 rye accessions.

## 2. The development of SSR markers through library construction strategy

### 2.1. Non-enriched libraries

The first strategy is usually used for newly analysed species. The following steps are common for generating SSR markers from a library: isolation of DNA, digestion with the appropriate restriction enzymes, separation by electrophoresis and selection of fragments between 300 and 1000 bp, ligation to the vector, hybridisation with probes composed of several repeats, sequencing of positive clones and design of primers complementary to both flanking regions. Although such an approach has been applied in many cases [10, 16 - 20], a number of disadvantages seems to be common for research starting from library construction, especially in species with large genomes. The most often-admitted problems are: the low effectiveness and specificity of hybridisation as well as the presence of one-side flanks in sequenced fragments. In rye, Saal and Wricke [16] sequenced seventy-four (40.7%) out of 182 positive clones, and the primer pairs were designed for 57 (31.3%) of them. Only 27 primer pairs resulted in specific SSR markers, of which, 20 were mapped. From this calculation comes the final efficiency of about 10%. The sequencing of 1739 positive clones in wheat (511 for GT and 1228 for GA motifs) resulted in obtaining 70 primer pairs, among them only 25 (less than 2%) gave amplified fragments with the expected length [10]. Zane *et al.* [13] informed in their review that the average percentage of positive clones isolated from plants in the traditional way between 1999 and 2001 was as high as 2.3. In order to increase the amount of successful sequencing, positive clones can be pre-screened for insert length, repeat position and orientation by the use of an anchor PCR technique described by Rafalski *et al.* [21]. In this technique, a set of PCR reactions with a combination of four primers (two vector and two degenerated primers complementary to the repeat) is carried out. Clones containing microsatellites positioned either too close or too far from the cloning site are not amplified.

### 2.2. Enriched libraries

Several procedures have been developed to increase the representation of SSRs in screened libraries. Recently, the attractiveness of “enriched protocols” has increased notably, especially in plants [13]. The most popular method of enriched library construction is selective hybridization of DNA fragments using streptavidin-coated magnetic beads or nylon membranes.

The procedure of the construction of enriched libraries using streptavidin-coated magnetic beads or nylon membranes comprises the following steps:

- DNA digestion, ligation of the resulting fragments to double-stranded adaptors;
- their hybridization to biotinylated microsatellite probes, followed by binding to streptavidin-coated magnetic beads;
- the elution of the DNA fragments from the beads, and PCR amplification with primers complementary to the adaptor sequence;

- cloning of the amplified products into the vector;
- transformation of *Escherichia coli*;
- and finally sequencing of the positive clones.

Such an enrichment method has been successfully applied to plants by several authors [22 - 25] with minor modifications, such as additional screenings for the presence of SSRs or the use of  $\lambda$  phagemids instead of *E. coli*. The efficiencies obtained in all cases were higher than in the traditional method and ranged from 55% [25] up to 100% of the clones containing microsatellites and being suitable for primer designation [23]. The 100% efficiency reported by the last cited authors was achieved thanks to the composition of the ligated adaptors (the adaptors provided the primer bind site for subsequent PCR steps and sites to ease cloning of the fragments into the vectors; they were also compatible with the restriction sites in the vector's multiple cloning site).

In spite of the sufficient level of progress in the efficiency of positive clone isolation, the procedure employing magnetic beads allows enrichment in a single or, in the best case, several SSR motifs. This problem can be solved by using Nylon membranes with many bound microsatellite oligonucleotides, as proposed by Edwards *et al.* [26]. Except for the hybridization step, the general idea was here the same as described previously. With this method, authors obtained 50-70% clones containing microsatellites (depending on the species).

### 2.3. Vectorette PCR isolation of microsatellite-containing clones

Vectorette PCR is a method that enables the amplification of specific DNA fragments in situations where the sequence of only one primer is known. This procedure applied to microsatellite isolation relies on PCR amplification using a vectorette-specific primer (forward primer) in combination with an anchored dinucleotide repeat primer (reverse primer), which generate flanking sequences of a given SSR. The vectorette library can be made from plasmid, cosmid and YAC clones [27].

### *3. Other strategies without library construction*

All the methods described above include the step of genomic library construction, which prolongs the procedure by up to one month. To avoid this problem, several procedures without library construction have been proposed. The first group of protocols is based on the fact that RAPD fragments contain SSRs more frequently than random genomic clones. This procedure starts with a random PCR amplification (either with RAPD starters or microsatellite-anchored random primers) followed either by Southern hybridization of PCR products with SSR probes and selective cloning of positive bands, or by cloning and screening all the products [27 - 29]. An interesting "nonlibrary" protocol based on the same idea was proposed by Zane *et al.* [13]. In this protocol, called FIASCO (Fast Isolation by AFLP of Sequences Containing repeats), products derived in a fast and efficient digestion-ligation reaction of AFLP were hybridized with biotinylated probes, followed by selective capturing of microsatellites with streptavidin-coated beads.

The usefulness of SSR markers for numerous purposes has been well documented for plants; among such purposes, the construction of molecular maps has a dominant position [10, 16, 20, 30 – 34]. Expressed sequence tag-derived microsatellite loci were detected and mapped in many species, such as barley [30], alfalfa [35], maize [36] and rice [37]. The SSRs are abundant, ubiquitous and hypervariable in nature; this attracted the attention of breeders who could utilize them for MAS, a modern tool in breeding. Masojć [38] listed four major strategies for finding a molecular marker tightly linked to a target gene of agronomic importance. The first approach uses NILs which are differentiated only by the allelic sets in the gene of interest and in the adjacent chromosomal region. The second one involves BSA. The third one comprises the identification of QTLs, and the last strategy involves computer databases. In the literature, there are several examples of applying SSRs for these purposes. Recently, by means of the BSA strategy, SSR markers closely linked to genes conferring resistance against sugarcane mosaic virus in maize – *Scmv1* and *Scmv2* [39] – and leaf rust in barley – *Rph5* [40] – were identified. Zhou *et al.* [41] showed that the MAS for the major scab resistance QTL with the SSR markers combined with phenotypic selection was much more effective than selection based only on phenotypic evaluation in an early generation. The authors identified markers linked to the major QTL on chromosome 3BS in the original mapping population; these were closely associated with scab resistance. Another interesting application of SSRs in rice breeding was described by Liu and Wu [42]. The authors showed that it is possible to predict heterosis and hybrid performance by the detection of the chromosomal regions influencing yield. However, the use of SSR markers is still relatively expensive for application on a large scale in breeding programs.

Because of the possibility to detect several alleles at a high frequency, SSRs turned out to be an ideal tool for identifying individuals and for establishing genetic diversity between them. It was well demonstrated in the study by Prasad *et al.* [43], who examined 55 elite wheat genotypes with SSR markers, and found that a set of only 12 primer pairs allowed a maximum of 48 genotypes to be distinguished. In the study published by Ashkenazi *et al.* [17], two SSR markers were sufficient to discriminate between 12 potato cultivars.

SSRs have also been applied in phylogenetic investigations for the construction of evolutionary trees, in, among other species, melon [44] and barley [45]. Yaish and Pérez de la Vega [46] were the first to identify (GA)<sub>n</sub> microsatellite-containing loci linked to a putative MADS-box gene (*PVMADS*) in the common bean. Afterwards, the authors constructed an un-rooted phylogenetic tree of the MADS-box genes of *Arabidopsis* and the common bean, which made it possible to show that the *PVMADS* gene is closely related to the *AGL2* group of *Arabidopsis*, involved in floral morphogenesis.

It was demonstrated that microsatellites in plants could even be up to ten-fold more variable than other markers; thus, they are highly recommended for genetic diversity analysis. Russell *et al.* [47] compared the level of polymorphism in

barley as detected by four types of markers: RFLPs, AFLPs, SSRs and RAPDs. Although all four assays were able to detect the polymorphism between 18 cultivated barley accessions, the similarity index was the lowest in the case of SSRs for both the spring and winter types while the diversity index calculated based on SSR data was similar to that obtained for AFLPs.

The high level of DNA polymorphism of SSRs makes them especially useful for self-pollinated species like wheat [10] or barley [30]. However, they have also been used successfully in open-pollinated plants as rye [16] or maize [20]. In rye, a typical open-pollinated species, Saal and Wricke [16] reported that the expected heterozygosity and allele number generated by SSRs were much higher compared with those by RFLPs (0.62 vs. 0.43 and 5.9 vs. 3.4 respectively).

### **ISSR markers**

Microsatellites are usually more or less proportionally dispersed in the genome. However, regions with a greater abundance of these sequences have been found and are named "SSR hot spots" [48 - 50]. Such regions can serve as a source of ISSR markers.

The ISSR technology is based on the amplification of regions (100-3000 bp) between inversely oriented closely spaced microsatellites [50]. Single primers (16-18 bp) consisting of several simple sequence repeats used for an amplification of these regions can be based on any SRR motif and be 5' or 3' anchored by 2-4 (usually) arbitrary selective nucleotides. However, nonanchored primers have also been used [49]. The resulting PCR products are anonymous SSR loci. ISSRs usually amplify 25 to 50 products in one reaction. The number of bands produced may be negatively correlated with the number of nucleotides in the repeat unit of the motif, as shown by Nagaraju *et al.* [51], who investigated the genetic relationship between Basmati and non-Basmati rice varieties. The major advantage of this method is the fact that it does not require a time-consuming (and expensive) step of genomic (or other) library construction. In spite of the fact that ISSRs are mostly inherited as dominant or rarely as codominant genetic markers (if the length of the intervening space between the microsatellites has changed) and are random-type markers, they are thought to be highly useful for many different purposes. This has been confirmed in numerous studies. They seem to be especially suitable for phylogenetic studies, the evaluation of genetic diversity and cultivar identification [50 - 59]. The simplicity of ISSR markers predetermines them for gene tagging. An excellent example was reported on by Ammiraju *et al.* [60], who tested the association of ISSRs with seed size in wheat. The authors found three markers for low seed weight and four markers for high seed weight, and identified QTL-associated ISSRs on three chromosomes. Other examples of gene tagging by means of ISSRs are the identification of a tight linkage between a marker and nuclear restorer gene in rice [61], a gene controlling *Fusarium* wilt resistance in chickpea [62], dominant allele Ns conferring resistance to Potato

virus S in potato [63], and the *Fgr* major locus modulating the fructose to glucose ratio in mature tomato fruit [64].

ISSR marker also turned out to be highly useful for monitoring somaclonal variation [65 - 67]. Leroy and Leon [66] described the application of the ISSR technique for the detection of differences between the hypocotyl-derived calli and leaves of cauliflower. They found polymorphic bands in callus tissues when using primers (GACA)<sub>4</sub> and (GATA)<sub>4</sub>; one of the sequenced bands showed a high similarity to the gene coding for protein kinase of *Arabidopsis thaliana*, which is involved in the regulation of cell proliferation. The authors suggested the ISSR technique to be a highly useful tool for the investigation of genetic instabilities at early stages of *in vitro* culture.

Another benefit of ISSR markers is the possibility to study SSR abundance and distribution in genomes. The bands produced by an ISSR primer with a given microsatellite repeat should reflect the relative frequency of that motif in a given genome. This approach was reported by Van der Nest *et al.* [68] who used an inter-simple sequence repeat technique for an access of microsatellite-rich regions in *Eucalyptus grandis*. The amplification of the microsatellite-rich regions using typical ISSR arbitrary primers was followed by the cloning and sequencing of the PCR products. This made it possible to design a set of SRR primers amplifying mono-, di-, tri-, hexa- and nona-nucleotide repeats, which were also able to generate the corresponding microsatellite loci from other *Eucalyptus* species (*E. grandis*, *E. nitens*, *E. globulus*, *E. camaldulensis* and *E. urophylla*).

ISSRs are considered to be highly informative. In rice, a higher percentage of polymorphic bands was produced with the ISSR technique than with AFLP [52]. Therefore, the ISSRs were more suitable to discriminate between varieties and showed a lower similarity than AFLP – 55.5% vs. 73.3%. A similar conclusion was drawn by Nagaoka and Ogihara [69], Korbin *et al.* [57] and Galvan *et al.* [70], who respectively observed that ISSRs were more informative than RAPDs in wheat, fruit plants (strawberry, apple and *Ribes* species) and the common bean for the evaluation of genetic diversity.

### **SAMPL markers**

SAMPL, another microsatellite-based marker system, is a modification of the AFLP technique [11, 71]. The same template is used as in the case of conventional AFLP – restriction fragments resulting from the digestion of genomic DNA with two endonucleases, ligated with adaptors and preamplified using primers designed on the basis of the synthetic adaptor plus the restriction site and carrying one selective base. The selective amplification is achieved using one of the standard AFLP primers with a SAMPL primer.

The design of the SAMPL primer used in the original procedure was based only on compound SSR sequences consisting of two different adjacent dinucleotide repeats, i.e. G(TG)<sub>4</sub>(AG)<sub>4</sub>A. Later protocols [72, 73] introduced primers complementary to microsatellites and anchored at the 5' end with a non-

microsatellite sequence. Such primers allow the amplification of any type of repeat structure (not only compound microsatellites) and can be extended to different types of tri-, tetra- and pentanucleotide repeats. 3'-anchored SAMPL primers also proved to be useful in producing clear and reproducible banding profiles, as shown for rye [74].

Because SAMPL analysis allows the amplification of microsatellite regions without any previous information on microsatellite flanking sequences and has a high multiplex ratio, it is considered one of the most efficient of all the molecular marker systems known so far [75].

One of the problems occurring while utilizing multiplex fingerprinting techniques is the high complexity of amplification profiles, especially in the case of plants with a large genome size and a high proportion of repetitive DNA. Several ways of dealing with this problem are reported on in published SAMPL protocols. One of them is a removal of restriction fragments with identical adapters at both ends. It can be achieved via affinity capture using streptavidin-coated magnetic beads – as was done in lettuce [76] – or by ligation of a special type of adapters and amplification using suppression PCR technology [72]. Other strategies of simplifying banding profiles are: the choice of an appropriate restriction enzyme – e.g. methylation sensitive *Pst*I [72, 77], or increasing the number of selective nucleotides on the primers [72].

To date, the SAMPL marker system has been established for only a few plant species, namely carrot [73], rye [74], wheat [75], lettuce [76], conifer [77], chicory [78], neem [79], sweet potato [80] and cowpea [81], where it was successfully utilized for studies involving genetic diversity, genotype identification, gene tagging and linkage mapping.

The results of studies comparing the efficiencies of different marker systems reveal that SAMPLs are superior to AFLPs [79] or both AFLPs and RAPDs [81] in resolving differences between closely related genotypes. In the first of the cited studies, devoted to the assessment of intra-population genetic variation in accessions of the medicinally important tropical tree neem, the average percentage polymorphism was respectively detected as 35 and 69% on utilizing AFLP and SAMPL data, and the average genetic similarity values were 0.80 for AFLP and 0.68 for SAMPL. Tosti and Negri [81] found SAMPL to be the most valuable of the three marker systems tested (SAMPL, AFLP, RAPD) in detecting genetic polymorphism in cowpea landraces, due to its highly effective multiplex ratio and the lower number of combinations required. SAMPL markers were also statistically more efficient than AFLPs in detecting parent-specific single-dose markers in Kentucky bluegrass, 75% vs. 32.4% [82].

As an arbitrary multilocus fingerprinting technique, SAMPL also turned out to be a valuable tool for constructing genetic linkage maps, especially for species for which no or only limited previous DNA sequence information was available, and it was used for this purpose on chicory [78], conifer [7] and lettuce [76].

Tab. 1. The application of microsatellite-based markers for different approaches in chosen plant species.

Type of micro-satellite marker	Plant species	Application	References
SSR	winter rye	- linkage mapping, variability analysis	[16]
	wheat	- linkage mapping	[33]
		- variability analysis	[10]
	potato	- phylogenetic and fingerprinting analyses	[17]
		- linkage mapping	[24]
	rice	- linkage mapping	[5, 73]
		- allelic diversity analysis	[5]
		- analysis of allele variation	[14]
	barley	- linkage mapping, analysis of allele variation	[30]
		- evaluation of genetic diversity	[47]
	sunflower	- linkage mapping	[34]
		- gene tagging	[4]
	maize	- linkage mapping, analysis of allele variation	[20]
olive	- linkage mapping	[31]	
maize	- linkage mapping	[36]	
ISSR	wheat	- gene tagging	[60]
		- evaluation of genetic diversity	[69]
	rice	- gene tagging	[61]
		- fingerprinting	[52]
		- evaluation of genetic diversity	[51]
	potato	- gene tagging	[63]
		- evaluation of genetic diversity	[48]
	tomato	- gene tagging	[64]
	chickpea	- gene tagging	[62]
	cauliflower	- detection of somaclonal variation	[66]
	horseradish	- detection of somaclonal variation	[67]
	strawberry, apple and <i>Ribes</i> species	- evaluation of genetic diversity	[57]
	common bean	- evaluation of genetic diversity	[70]
peanut	- evaluation of genetic diversity, phylogenetic analysis, cultivar identification	[58]	
citrus	- cultivar identification	[54]	
SAMPL	lettuce	- linkage mapping,	[76]
		- evaluation of genetic diversity	
	Norway spruce	- linkage mapping	[77]
	carrot	- linkage mapping	[73]
	Kentucky bluegrass	- linkage mapping	[82]
	chicory	- linkage mapping	[78]
	wheat	- evaluation of genetic diversity, gene tagging	[75]
	cowpea	- evaluation of genetic diversity	[81]
	sweet potato	- evaluation of genetic diversity	[80]
	winter rye	- evaluation of genetic diversity	[74]

Tab. 2. A comparison of the main features of microsatellite-based markers

Feature	Marker type		
	SSR	ISSR	SAMPL
Abundance	high	high	medium/high
Locus specificity	yes	no	no
Nature of polymorphism	variation in repeat length/ number of motifs	base changes (insertions, deletions) variation in SSR repeat length/number of motifs	base changes (insertions, deletions) variation in SSR repeat length/number of motifs
Level of polymorphism	high/very high	high/medium	high
Inheritance mode	codominance	dominance /codominance	codominance /dominance
Reproducibility	high	high/medium	high
Sequence information required	yes	no	no
Technical demands	medium/low (except for library construction and screening)	low/medium	medium
Costs	medium	low	medium
Labor	high (a labor-consuming step of library construction and screening)	low	medium
Time	usually a time-consuming step of library construction and screening is needed	low	medium
Main applications	linkage mapping, studies on genetic diversity, gene tagging	identification of cultivars, phylogenetic studies	studies on genetic diversity, linkage mapping
Main advantages	high level of polymorphisms (up to 26 alleles), co-dominant mode of inheritance, very high reproducibility	multilocus and highly polymorphic pattern production per reaction, technical simplicity, low expenses	amplification of many informative bands per reaction, high reproducibility
Problems	frequently a small number of potential microsatellite loci are identified, polymerase slippage when analysing mono- and di- nucleotide repeats, co- migrating fragments not always are homologous	band profiles cannot be interpreted in terms of loci and alleles, dominance of alleles (frequently), similar-sized fragments may not be homologous	relatively time- consuming and labor-intensive procedure, high complexity of amplification profiles may occur

Roy *et al.* [75] demonstrated the applicability of SAMPL markers for gene tagging. Using bulked segregant analysis, they observed associations between SAMPL bands and grain protein content, pre-harvest sprouting tolerance and grain weight in bread wheat. The authors concluded, however, that the conversion of SAMPLs into user-friendly PCR-based locus-specific primers is necessary before they can be used for marker assisted selection in breeding programs.

### FINAL CONCLUSIONS

In recent years, the popularity of SSR-based markers has increased considerably. The main reasons which make microsatellites an especially attractive tool for a number of applications are:

- (1) their high levels of allelic variation and their co-dominant character, which means that they deliver more information per unit assay than any other marker systems, thus reducing costs;
- (2) microsatellites are assayed using PCR, so only small amounts of tissue are required;
- (3) some microsatellite based markers, especially ISSR, can be assayed more rapidly than other types of molecular markers;
- (4) microsatellite primers developed for one species frequently amplify loci in related species.

A summary of the different applications of microsatellite-based markers in plants is given in Tab. 1. However, each type of microsatellite-based markers shows a set of advantages and disadvantages, such as the mode of inheritance, level of informativity and reproducibility, or procedural complicacy, along with economical aspects like costs and the time required to produce the final result. Therefore, the decision on which type of marker should be taken into consideration depends on the nature of the research undertaken. Tab. 2 presents the main features of the above-characterized microsatellite-based molecular markers.

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