Development of functional molecular markers for crop plants

Rajesh Singh, RK Singh

Received: 14 March 2015; Revised Accepted: 28 Apri 2015

ABSTRACT

Molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell are not confounded by the environment, pleiotropic and epistatic effects. Functional markers (FMs) are a good "translator" of gains from emerging technologies into improved crop cultivars. FMs are derived from polymorphic sites within genes causally involved in phenotypic trait variation. Once genetic effects have been assigned to functional sequence motifs, FMs derived from such motifs are used for fixation of gene alleles in a number of genetic backgrounds without additional calibration. FM development requires(1) functionally characterised genes, (2) allele sequences from such genes, (3) identification of polymorphic, functional motifs affecting plant phenotype within these genes and (4) validation of associations between DNA polymorphisms and trait variation.

Keywords: DNA Markers Functional Markers, Polymorphism, RAPD, RFLP, SSR, SNPs

Introduction

Conventional plant breeding is primarily based on phenotypic selection of superior individuals among segregating progenies resulting from hybridization. Although significant strides have been made in crop improvement through phenotypic selections for agronomically important traits, considerable difficulties are often encountered during this process, primarily due to genotype-environment interactions. Besides, testing procedures may be many times difficult, unreliable or expensive due to the nature of the target traits (e.g. abiotic stresses) or the target environment. With the advent of DNA marker technology, several types of and molecular breeding strategies are now available to plant breeders and geneticists, helping them to overcome many of them the problems faced during conventional breeding. Markers that reveal polymorphisms at the DNA level are known as molecular markers. The last two have witnessed a remarkable activity in the development and use of molecular markers both in animal and plant systems. This activity started with low-throughput restriction fragment length polymorphisms and culminated in recent years with single nucleotide polymorphisms (SNPs), which are abundant and uniformly distributed. There have been several reports of the potential applications of molecular markers to plant improvement (Burr *et al*. 1983; Helentjaris *et al*. 1985; Beckman and Soller 1986). A molecular marker is defined as a particular segment of DNA that is representative of the differences at the genome level. Molecular markers may or may not correlate with phenotypic expression of a trait. Molecular markers offer numerous advantages over conventional phenotype based alternatives as they are stable and detectable in all tissues regardless of growth, differentiation, development, or defense status of the cell are not confounded by the environment, pleiotropic and epistatic effects. An increasing number of monogenic, race-specific genes showing a gene-for-gene interaction have been mapped, and agronomically important genes have been correlated to molecular markers, as demonstrated for potato in Table 1. For wheat, such

Rajesh Singh (✉)
Genetics and Plant Breeding, IAS, BHU Varanasi-221005
e-mail: rsingh6361@gmail.com
RK Singh
Visiting Scientist, Plant Genome Mapping Laboratory, 111 River Bend Road, University of Georgia, Athens, GA (USA)

validated markers are available for resistance genes against powdery mildew (Pm1c, Pm17, Pm24, mlRD30), the yellow dwarf virus, the cyst nematodes (Cre1 and Cre3), and the rusts (Lr9, Lr21, Lr24, Lr38, Lr47; Sr38, Yr5, Yr17) and Fusarium head blight (Mohler and Singrun 2005). Presently, the most powerful application of such identified genes and molecular markers is opened up by MAS. It offers the opportunity of combining different genes for a given patho-system in a single genotype (gene pyramiding). A prerequisite for gene pyramiding is that characters are not allelic. Furthermore, knowledge on the gene distances in genetic or better physical maps is very helpful. Using such information, it was possible to combine three race-specific powdery mildew genes (Pm) in a single line which is now under variety test, hoping that such a pyramided resistance will be rather durable (Fig. 2).

The publication of Botstein et al. (1980) about the construction of genetic maps using restriction fragment length polymorphism (RFLP) was the first reported molecular marker technique in the detection of DNA polymorphism. In RFLP, DNA polymorphism is detected by hybridizing a chemically labelled DNA probe to a Southern blot of DNA digested by restriction end nucleases, resulting in differential DNA fragment profile. This differential profile is generated due to nucleotide substitutions or DNA rearrangements like insertion or deletion or single nucleotide polymorphisms. The RFLP markers are relatively highly polymorphic, codominantly inherited and highly reproducible. Because of their presence throughout the plant genome, high heritability and locus specificity the RFLP markers are considered superior. The method also provides opportunity to simultaneously screen numerous samples. The technique is not very widely used because it is time consuming, involves expensive and radioactive/toxic reagents and requires large quantity of high quality genomic DNA. After the invention of polymerase chain reaction (PCR) technology (Mullis and Faloona 1987), a large number of approaches for generation of molecular markers based on PCR were undertaken. The RAPD technique is based on PCR amplification of genomic DNA. It deduces DNA polymorphisms produced by ‘‘rearrangements or deletions at or between oligonucleotide primer binding sites in the genome’’ using short random oligonucleotide sequences (mostly ten bases long) (Williams et al. 1991). As the approach requires no prior knowledge of the genome that is being analyzed, it can be employed across species using universal primers. The major drawback of the method is that the profiling is dependent on the reaction conditions so may vary within two different laboratories and as several discrete loci in the

Table 1 Some important and mapped DNA markers on the example of potato (Wenzel 2006).

Trait	Gene	Chromosome
Potato virus Y	Ry_{adg}	XI
	Ny_{dw}	IV
Potato virus X	*Rx1; Rx2*	XII, V
	Na	XI
	Nb	V
	Nx	IX
Potato leaf roll virus	*PLRV* QTL	XI
Globodera rostochiensis	*Gro*	III, VII, X, XI
	H1	V
Globodera pallida	*Gpa* QTL	IV;V;IX;XII
	Gpa2	XII
Phytophthora infestans	*RB*	X
	R1	V
	R2	IV
	R3; R6; R7	XI
	R_{blc}	VIII
	QTL	V
Synchytrium endobioticum	*Sen1*	XI
Erwinia carotovora	QTL	
Tuber starch, tuber yield	QTL	I–XII
Cold sweetening	QTL	V, IX
Skin colour	QTL	X
Tuber flesh color	QTL	III

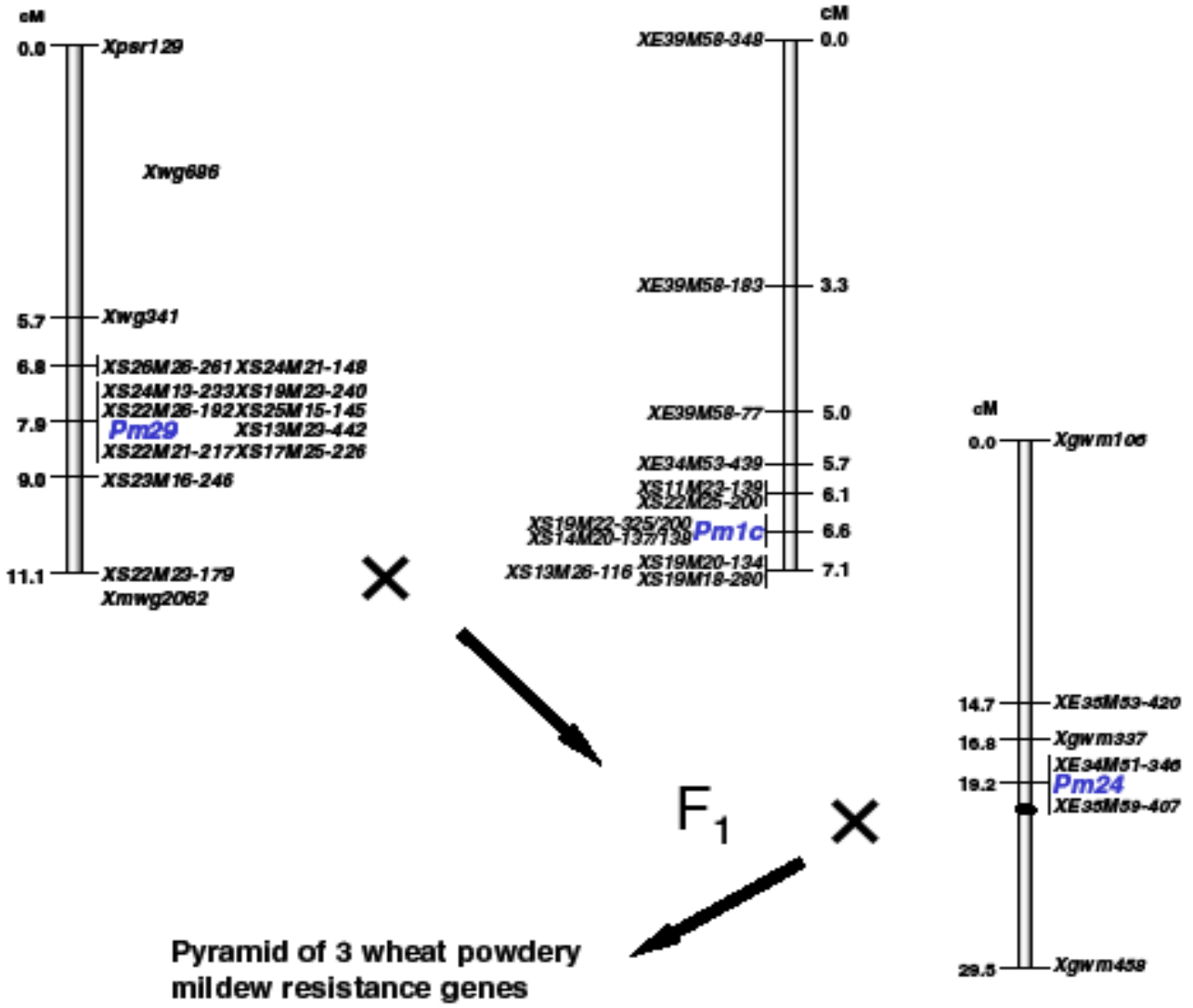
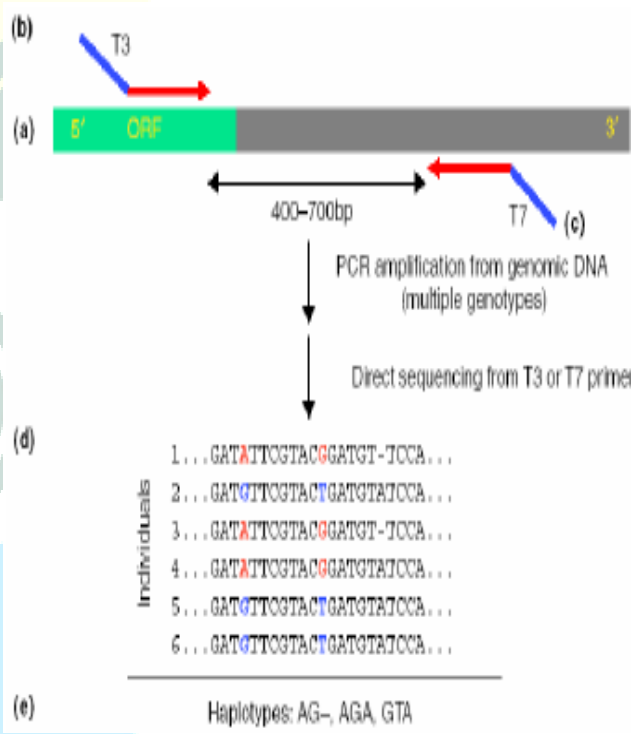


Figure 2 Pyramiding of three powdery mildew genes by marker-assisted selection, resulting in an oligogenic resistance type which should be more durable (Wenzel, G. 2006).

genome are amplified by each primer, profiles are not able to distinguish heterozygous from homozygous individuals (Bardakci 2001). Due to the speed and efficiency of RAPD analysis, high-density genetic mapping in many plant species such as alfalfa (Kiss *et al.* 1993), faba bean (Torress *et al.* 1993) and apple (Hemmat *et al.* 1994) was developed in a relatively short time. The RAPD analysis of NILs (non-isogenic lines) has been successful in identifying markers linked to disease resistance genes in tomato (Lycopersicon sp.) (Martin *et al.* 1991), lettuce (Lactuca sp.) (Paran *et al.* 1991) and common bean (Phaseolus vulgaris) (Adam-Blondon *et al.* 1994). To overcome the limitation of reproducibility associated with RAPD, AFLP technology (Vos *et al.* 1995) was developed. It combines the power of RFLP with the flexibility of PCR-based technology by ligating primerrecognition sequences (adaptors) to the restricted DNA and selective PCR amplification of restriction fragments using a limited set of primers The AFLP technique generates fingerprints of any DNA regardless of its source, and without any prior knowledge of DNA sequence. Most AFLP fragments correspond to unique positions on the genome and hence can be exploited as landmarks in

genetic and physical mapping. The technique can be used to distinguish closely related individuals at the sub-species level (Althoff *et al.* 2007) and can also map genes. Applications for AFLP in plant mapping include establishing linkage groups in crosses, saturating regions with markers for gene landing efforts (Yin *et al.* 1999) and assessing the degree of relatedness or variability among cultivars (Mian *et al.* 2002). Single nucleotide variations in genome sequence of individuals of a population are known as SNPs. They constitute the most abundant molecular markers in the genome and are widely distributed throughout genomes although their occurrence and distribution varies among species. Maize has 1 SNP per 60-120 bp (Ching *et al.* 2002), while humans have an estimated 1 SNP per 1,000 bp (Sachidanandam *et al.* 2001). The SNPs are usually more prevalent in the non-coding regions of the genome. Within the coding regions, an SNP is either non-synonymous and results in an amino acid sequence change (Sunyaev *et al.* 1999), or it is synonymous and does not alter the amino acid sequence. Synonymous changes can modify mRNA splicing, resulting in phenotypic differences (Richard and Beckman 1995). Improvements in sequencing technology and availability of an increasing number of EST sequences have made direct analysis of genetic variation at the DNA sequence level possible (Buetow *et al.* 1999; Soleimani *et al.* 2003). Majority of SNP genotyping assays are based on one or two of the following molecular mechanisms: allele specific hybridization, primer extension, oligonucleotide ligation and invasive cleavage (Sobrino *et al.* 2005). High throughput genotyping methods, including DNA chips, allele-specific PCR and primer extension approaches make single nucleotide polymorphisms (SNPs) especially attractive as genetic markers. They are suitable for automation and are used for a range of purposes, including rapid identification of crop cultivars and construction of ultra high-density genetic maps. However, with the availability of microarrays, SNP platforms have been developed, which allow genotyping of thousands of markers in parallel. Besides SNPs, some other novel marker systems, including single feature polymorphisms, diversity array technology and restriction site-associated DNA markers, have also been developed, where array-based assays have been utilized to provide for the desired ultra-high throughput and low cost. These microarray-based markers are the markers of choice for the future and are already being used for construction of high-density maps, quantitative trait loci (QTL) mapping (including expression QTLs) and genetic diversity analysis with a limited expense in terms of time and money.



Functional Markers

With the advent of high-throughput sequencing technology, abundant information on DNA sequences for the genomes of many plant species has been generated (Goff *et al.* 2002; The Arabidopsis Genome Initiative 2000; Yu et al. 2002). ESTs of many crop species have been generated and thousands of sequences have been annotated as putative functional genes using powerful bioinformatics tools. To gain benefits from plant

genomics, new knowledge must be "translated" into crop varieties with improved characteristics (Thro *et al*. 2004). Functional markers (FMs) are a good "translator" of gains from emerging technologies into improved crop cultivars. FMs are derived from polymorphic sites within genes causally involved in phenotypic trait variation. Once genetic effects have been assigned to functional sequence motifs, FMs derived from such motifs are used for fixation of gene alleles in a number of genetic backgrounds without additional calibration. FM development requires (1) functionally characterised genes, (2) allele sequences from such genes, (3) identification of polymorphic, functional motifs affecting plant phenotype within these genes and (4) validation of associations between DNA polymorphisms and trait variation (Chun *et al*. 2006).

In order to correlate DNA sequence information with particular phenotypes, sequence-specific molecular marker techniques have been designed. Microsatellite or short tandem repeats or simple sequences repeats are monotonous repetitions of very short (one to five) nucleotide motifs, which occur as interspersed repetitive elements in all eukaryotic genomes (Tautz and Renz 1984). Variation in the number of tandemly repeated units is mainly due to strand slippage during DNA replication where the repeats allow matching via excision or addition of repeats (Schlotterer and Tautz 1992). As slippage in replication is more likely than point mutations, microsatellite loci tend to be hypervariable. Microsatellite assays show extensive inter-individual length polymorphisms during PCR analysis of unique loci using discriminatory primers sets. Expressed sequence tag (EST) projects have generated a vast amount of publicly available sequence data from plant species; these data can be mined for simple sequence repeats (SSRs). These SSRs are useful as molecular markers because their development is inexpensive, they represent transcribed genes and a putative function can often be deduced by a homology search. Because they are derived from transcripts, they are useful for assaying the functional diversity in natural populations or germplasm collections. These markers are valuable because of their higher level of transferability to related species, and they can often be used as anchor markers for comparative mapping and evolutionary studies. They have been developed and mapped in several crop species and could prove useful for marker-assisted selection.

Approaches for the development of microsatellites 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.

Data base searching is a cost effective tool for the development of SSR's

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. Additionally, this strategy is limited to plants with high economical or scientific interest which are well represented in the databases. In rice (Cho *et al*. 2000), 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 (2002) identified much more effective SSR loci when exploring EST data bases than Saal and Wricke (1999) 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-, triand tetra-nucleotide motifs turned out to be useful for primer design. One hundred EST-derived loci displayed a length polymorphism among 15 rye accessions.

Cross species amplification leads to the development of SSR markers

Cross species amplification is also a powerful approach to develop microsatellites markers in plants. Database searching is an economic approach for obtaining new microsatellite loci (Brown *et al.* 1996). However, database searching alone is unlikely to provide sufficient markers in plant species for mapping or breeding applications. The application of cross-species transfer of microsatellites was difficult to predict (Brown *et al.* 1996). The taxonomic distance of the species of interest and conservation of the flanking sequence determines whether the correct region is amplified and how much is the variability in the microsatellites. The reaction conditions are often need to be optimized the products sequenced to verify the presence of the microsatellite region. Microsatellites have been transferred between closely related plant species, but there is not much information is available between the genera.

Library construction strategy for development of SSRs

Non-enriched libraries

This strategy is usually used for newly analyzed species. The following steps are involved in 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 of restricted fragments to the vector Hybridisation with probes composed of several repeats Sequencing of positive clones Designing of primers complementary to both flanking regions Although such an approach has been applied in many cases (Roder *et al.* 1995; Saal and Wricke. 1999; Ashkenazi *et al.* 2001; Brown et al. 1996; Panaud *et al.* 1996; Taramino *et al.* 1996) 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 hybridization as well as the presence of one-side flanks in sequenced fragments. In rye, Saal and Wricke (1996) 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 (Roder *et al.* 1995). 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.* 1996. 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.

Enriched libraries are the rich source of SSR markers

Different enrichment methods have been developed to increase the efficiency of microsatellite loci

isolation from genomic DNA libraries. Recently, the attractiveness of "enriched protocols" has increased notably, especially in plants Zane *et al.* (2003). A standard method for the isolation of plant microsatellite loci involves screening colonies/plaque with oligonucleotide probes complementary to microsatellite repeats. Enrichment by primer extention, enrichment by hybridization and enrichment by screening random amplified polymorphic DNA (RAPD) profiles are other approaches for enrichment. Different microsatellite enrichment methods have been given in the table 2.

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 and 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.*
- Sequencing of the positive clones.

Such an enrichment method has been successfully applied to plants by several authors (Fischer *et al.* 1998; Hamilton *et al.* 1999; Milbourne et al. 1998; Prochazka *et al.* 1996) with minor modifications, such as additional screenings for the presence of SSRs or the use of l phagemids instead of *E. coli*. 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.* (1996).

Other strategies without library construction

The construction of genomic library for the development of SSR is time consuming process and it usual takes up to one month. To avoid this problem, several procedures without library construction have been developed. One 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 microsatelliteanchored 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 (Lench *et al.* 1996; Cifarelli *et al.* 1995; Lunt *et al.* 1999). An interesting "nonlibrary" protocol based on the same idea was proposed by Zane et al. (2003). 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 (Roder *et al.* 1995; Saal and Wricke (1996); Taramino *et al.* 1996; Becker *et al.* 1995; De la Rosa. 2003; Hackenberger *et al.* 2003;

Table 2 Summary of microsatellite enrichment method (Adapted from Maguire, T.L. 2001).

Enrichment Method	Level of enrichment	Reference
Enrichment by Primer extension		
Microsatellite oligonucleotide	50-fold compared with un-nriched	Ostander *et al.* (1992)
	24 positive clones sequenced, all contained microsatellites	Paetku (1999)
Degenerate oligonucleotide	15 positive clones sequenced, 13 contained microsatellites	Fisher *et a.l* (1996)
	19 positive clones sequenced, all contained microsatellites	Koblizkova *et al.* (1998)
Enrichment by hybridization		
Streptavidin-coated magnetic beads	48 positive clones sequenced, 29 contained microsatellites	Fisher and Bachmann (1998)
	9 positive clones sequenced, 5 contained Microsatellites 207 positive clones sequenced, 180 contained microsatellites	Prochazka (1996)
	20% positive clones compared with un-enriched with no detectable positive clones	
	12 positive clones sequenced, 8 contained microsatellites	Kijas *et al.* (1994)
	120 positive clones sequenced, all contained microsatellites	Connel *et al.* (1998) Hamilton *et al* (1999)
Nylon membranes	50-70% clones randomly sequenced contained microsatellites	Edwards *et al.* (1996)
Enrichment by screening RAPD profiles	30 positive clones sequenced, 21 contained microsatellites	Ueno *et al.* (1999)
	14 positive clones sequenced, 12 contained microsatellites	Lunt et al (1999)

Roder *et al.* 1998; Tang *et al.* 2002). Expressed sequence tag derived microsatellite loci were detected and mapped in many species, such as barley (De la Rosa *et al.* 2003), alfalfa (Mahalakshmi et al. 2002), maize (Senior *et al.* 1993), and rice (Temnykh *et al.* 2000). 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. Masojc *et al.* 2002 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* (Duble *et al.* 2003), and leaf rust in barley – *Rph*5 (Mammadov *et al.* 2003) were identified. Zhou *et al.* 2003 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 1998. 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.*2000, 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.*2001, 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 (Monforte *et al.* 2003) and barley (Provan *et al.* 1999). Yaish and Perez de la Vega 2003 were the first to identify (GA)n 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. 1997 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 (Roder *et al.* 199)5 or barley (Becker *et al.* 1995). However, they have also been used successfully in open-pollinated plants as rye (Saal and Wricke 1999) or maize (Taramino *et al.* 1996).

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" (Bornet *et al.* 2002a; Bornet *et al.* 2002 b; Zietkiewicz *et al.* 1994). 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 (Zietkiewicz *et al.* 1994). 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 (Bornet *et al.* 2002b) . 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.* (2002), 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 (Zietkiewicz *et al.* 1994; Nagaraju *et al.* 2002; Blair *et al.* 1999; Cavan *et al.* 2000; Fang *et al.* 1997; Gupta *et al.* 1994; Jain *et al.* 1999; Korbin *et al.* 2002; Raina *et al.* 2001; Wolfe 1998). The

simplicity of ISSR markers predetermines them for gene tagging. An excellent example was reported on by Ammiraju *et al.* (2001), 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 (Agaki *et al.* 1996), a gene controlling *Fusarium* wilt resistance in chickpea (Ratnaparkhe *et al.* 1998), dominant allele Ns confering resistance to Potato virus S in potato (Marczewski *et al.* 2002), and the *Fgr* major locus modulating the fructose to glucose ratio in mature tomato fruit (Levin *et al.* 2000). ISSR marker also turned out to be highly useful for monitoring somaclonal variation (Albani *et al.* 1998; Leroy and Leon 2000; Rostiana *et al.* 1999). Leroy and Leon 2000 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)4 and (GATA)4; 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. 2000 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 were produced with the ISSR technique than with AFLP (Blair *et al.* 1999). 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 1997; Korbin *et al.* 2002 and Galvan *et al.* 2003, 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 (Morgante *et al.* 1994; Vos *et al.* 1995). 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)4(AG)4A. Later protocols (Paglia *et al.* 1998; Vivek et al. 1999) 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'-achored SAMPL primers also proved to be useful in producing clear and reproducible banding profiles, as shown for rye (Bolibok *et al.* 2003). Because SAMPL analysis allows the amplification of microsatellite regions

without any previous information on microsatellite flankig sequences and has a high multiplex ratio, it is considered one of the most efficient of all the molecular marker systems known so far (Roy *et al.* 2002). One of the problems occuring 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 (Witsenboer *et al.* 1997) or by ligation of a special type of adapters and amplification using suppression PCR technology (Paglia *et al.* 1998). To date, the SAMPL marker system has been established for only a few plant species, namely carrot (Vivek *et al.* 1999), rye (Bolibok *et al.* 2003), wheat (Roy *et al.* 2002), lettuce (Witsenboer *et al.* 1997), conifer (Paglia et al. 1998), chicory (De Simone *et al.* 1997), neem (Singh *et al.* 2002), sweet potato (Tseng *et al.* 2002) and cowpea (Tosti and Negri. 2002), where it was successfully utilized for studies involving genetic diversity, genotype identification, gene tagging and linkage mapping.. As an arbitary multilocus fingerprintig 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 (De Simone *et al.* 1997), conifer (Paglia *et al.* 1998) and lettuce (Witsenboer *et al.* 1997). A summary of the different applications of microsatellite-based markers in plants is given in Tab. 2. 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. Tab. 3 presents the main features of the above-characterized microsatellite- based molecular markers.

There are two main approaches for the isolation of microsatellite loci from genomic libraries. One method is to screen a large insert genomic library with an end labeled microsatellite oligonucleotide probe. The hybridized clones are purified and divided into subclones. Selected clones are then sequenced and flanking region of microsatellite repeats are used to design PCR primers. Many blot hybridizations requirement and sequencing of large subclones is the drawback of this approach. The alternative is to produce small insert genomic libraries constructed in a plasmid or phage vector. These libraries are suitable for sequencing of entire insert. They can also be highly enriched for the desired microsatellite repeats using enrichment strategies (Edwars *et al.* 1996, Maguire *et al.* 2000).

References

Adam-Blondon AF, Sevignac M, Bannerot H, Dron M (1994) SCAR, RAPD and RFLP markers linked to a dominant gene (Are) conferring resistance to anthracnose in common bean. Theor Appl Genet 88:865-870.

Agaki H, Yokozeki Y, Inagaki A, Nakamura A, Fujimura TA (1996) codominant DNA marker closely linker to rice nuclear restorer gene, *Rf-1*, identified with inter-SSR fingerprinting. Genome 39:1205-1209.

Albani MC, Wilkinson MJ (1998) Inter simple sequence repeat polymerase chain reaction for the detection of somaclonal variation. Plant Breed. 117:573-575.

Althoff DM, Gitzendanner MA, Segraves KA (2007) the utility of amplified fragment length

Table 3 The application of microsatellite-based markers for different approaches in chosen plant species (Rakoczy-Trojanowska and Bolibok. 2004).

Type of microsatellite marker	Plant species	Application	References
SSR	winter rye	linkage mapping, variability analysis	Saal and Wricke. 1999
	wheat	linkage mapping variability analysis	Roder et al.1998 Roder et al. 1995
	potato	phylogenetic and fingerprinting analyses linkage mapping	Ashkenazi et al. 2001 Milbourne et al. 1998
	rice	linkage mapping allelic diversity analysis analysis of allele variation	Panaud et al.1995, Vivek et al. 1999 Panaud et al. 1995 Cho et al. 2000
	barley	linkage mapping, analysis of allele variation	Becker et al. 1995
		evaluation of genetic diversity	Russell et al. 1997
	sunflower	linkage mapping gene tagging	Tang et al. 2002 Hongtrakul et al. 1998
	maize	linkage mapping, analysis of allele variation	Taramino et al. 1996
	olive	linkage mapping	De la Rosa et al. 2003
	maize	linkage mapping	Senior et al. 1993
ISSR	wheat	gene tagging evaluation of genetic diversity	Ammiraju et al. 2001 Nagaoka and Ogihara. 1997
	rice	gene tagging fingerprinting evaluation of genetic diversity	Agaki et al.1996 Blair et al.1999 Nagaraju *et* al. 2002
	potato	gene tagging evaluation of genetic diversity	Marczewski et al. 2002 Bornet et al. 2002a
	tomato	gene tagging	Levin et al. 2000
	chickpea	gene tagging	Ratnaparkhe et al. 1998
	cauliflower	detection of somaclonal variation	Leroy and Leon 2000
	horseradish	Detection of somaclonal variation	Rostiana et al.1999
	strawberry, apple and *Ribes* species	evaluation of genetic diversity	Korbin et al. 2002
	common bean	evaluation of genetic diversity	Galvan et al. 2003
	peanut	evaluation of genetic diversity, phylogenetic analysis, cultivar identification	Raina et al. 2001
	citrus	cultivar identification	Fang et al. 1997
SAMPL	lettuce	linkage mapping, evaluation of genetic diversity	Witsenboer et al. 1997
	Norway spruce	linkage mapping	Paglia et al. 1998
	carrot	linkage mapping	Vivek et al. 1999
	Kentucky bluegrass	linkage mapping	Porceddu et al. 2001
	chicory	linkage mapping	De Simone et al. 1997
	wheat	evaluation of genetic diversity, gene tagging	Roy et al.2002
	cowpea	evaluation of genetic diversity	Tosti and Negri 2002
	sweet potato	evaluation of genetic diversity	Tseng et al. 2002
	winter rye	evaluation of genetic diversity	Bolibok et al. 2003

Table 3 A comparisons of the main features of microsatellite-based markers.

Feature	Marker type		
	SSR	ISSR	SAMPL
Abundance	high	high	medium/high
Locus specifity	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 /dominace
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

polymorphisms in phylogenetics: a comparison of homology within and between genomes. Syst Biol 56:477-484.

Ammiraju JSS, Dholakia BB, Santra DK, Singh H, Lagu MD, Tamhankar SA, Dhaliwal HS, Rao VS, Gupta VS, Ranjekar PK (2001) Identification of inter simple sequece repeat (ISSR) markers associated with seed size in wheat. Theor. Appl. Genet. 102:726-732.

Ashkenazi V, Chani E, Lavi U, Levy D, Hillel J, Veilleux RE (2001) Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. Genome 44:50-62.

Bardakci F (2001) Random amplified polymorphic DNA (RAPD) markers. Turk J Biol 25:185-196

Becker J, Heun M Barley microsatellites: Allele variation and mapping. Plant Mol. Biol. 27:835-845.

Beckman JS, Soller M (1986) Restriction fragment length polymorphisms and genetic improvement of agricultural species. Euphytica 35:111-124.

Blair MW, Panaud O, McCouch SR (1999) Inter-simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (O*ryza sativa* L.). Theor. Appl. Genet. 98:780- 792.

Bolibok H, Rakoczy-Trojanowska M (2003) Evaluating the efficiency of SAMPL marker system in assessing genetic diversity in winter rye (*Secale cereale* L.). 7th Internat. Congress of Plant Mol. Biol. Barcelona, June 23- 28

Bornet B, Goraguer F, Joly G, Branchard M (2002a) Genetic diversity in European and Argentinian cultivated potatoes (*Solanum tuberosum* subsp. *tuberosum*) detected by inter-simple sequence repeats (ISSRs). Genome 45: 481-484.

Bornet B, Muller C, Paulus F, Branchard M (2002b) Highly informative nature of inter simple sequence repeat (ISSR) sequences amplified using triand tetra-nucleotide primers from DNA of cauliflower (*Brassica oleracea* var. *botrytis* L.). Genome 45:890-896.

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314-333.

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzalez-Candelas F, Kresovich S (1996) Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench] Theor. Appl. Genet. 93:90-198.

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzalez-Candelas F, Kresovich S (1996) Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum [*Sorghum bicolor* (L.) Moench]. Theor Appl Genet 93:190-198.

Buetow KH, Edmonson MN, Cassidy AB (1999) Reliable identifi-cation of large numbers of candidate SNPs from public EST data. Nat Genet 21:323-332.

Burr B, Burr FA, Thompson KH, Albertson MC, Stuber CW (1988) Gene mapping with recombinant inbreds in maize. Genetics 118:519-526.

Cavan G, Potier V, Moss SR (2000) Genetic diversity of weeds growing in continuous wheat. Weed Res. 40:301-310.

Ching ADA, Caldwell KS, Jung M, Dolan M, Smith OS, Tingey S, Morgante M, Rafalski A (2002) SNP frequency, haplotype structure and linkage disequilibrium in elite maize inbred lines. BMC Genet 3:19.

Cho YG, Temnykh S, Chen X, Lipovich L, McCouch SR, Ayres N, Cartinhour S (2000) Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.) Theor. Appl. Genet. 100:13-722.

Chun S, Wenzel G, Frei U, Lubberstedt T (2006) Function of genetic material: From genomics to functional markers in maize. Prog Bot 67:53-73.

Cifarelli RA, Gallitelli M, Cellini F (1995) Random amplified hybridization microsatellites (RAHM): isolation of a new class of microsatellitecontaining DNA clones. Nucleic Acid Res. 23:802-3803.

De la Rosa R, Angiolillo A, Guerrero C, Pellegrini M, Rallo L, Besnard G, Berville A, Martin A, Baldoni L (2003) A first linkage map of olive (*Olea europea* L.) cultivars using RAPD, AFLP, RFLP and SSR markers. Theor. Appl. Genet. 106:1273-1282.

De Simone M, Morgante M, Lucchin M, Parrini P, Marocco A (1997) A first linkage map of *Cichorium intybus* L. using a one-way pseudo-testcross and PCR-derived markers. Mol. Breed. 3:415-425.

Duble CM, Quint M, Melchinger AE, Xu ML, Lübberstedt T (2003) Saturation of two chromosome regions conferring resistance to SCMV with SSR and AFLP markers by targeted BSA. Theor. Appl. Genet. 106:485-493.

Edwards KJ, Barker JHA, Daly A, Jones C, Karp A (1996) Microsatellite libraries enriched for several microsatellite sequence in plants.BioTechniques 20:759-760.

Fang DQ, Roose ML (1997) Identification of closely related citrus cultivars with inter-simple sequence repeat markers. Theor. Appl. Genet. 95:408-417.

Fehr WR (ed.) (1987) Principles of cultivar development. Vol. 2. Crop Species. Macmillan Publ. Co., New York.

Fischer D, Bachmann K (1998) Microsatellite enrichment in organisms with large genomes (*Allium cepa* L.). BioTechniques 24:796-802.

Galvan MZ, Bornet B, Balatti PA, Branchard M (2003) Inter simple sequence repeat (ISSR) markers as a tool for the assessment of both genetic diversity and gene pool origin in common bean (*Phaseolus vulgaris* L.). Euphytica 132:297-301.

Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, *et al.* (2002) A draft sequence of the rice genome (Oryza sativa L. ssp. japonica). Science 296:92-100.

Gupta M, Chyi YS, Romero-Severson J, Owen JL (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple sequence repeats. Theor. Appl. Genet. 89:998-1006.

Hackauf B, Wehling P (2002) Identification of microsatellite polymorphisms in an expressed portion of the rye genome. Plant Breed. 121:7-25.

Hackenberger M, Bohn M, Ziegle JS, Joe LK, Hauser JD, Hutton M, Melchinger AE (2002) Variation of DNA fingerprints among accessions within maize inbres lines and implications for identification of essentially derived varietes. I. Genetic and technical sources of variation in SSR data. Mol. Breed. 10:81-191.

Hallauer AR (1990) Methods used in developing maize inbreds. Maydica 35:1-16.

Hamilton MB, Pincus EL, DiFiore A, Fleischer RC (1999) Universal linker and ligation procedures for construction of genomic DNA library enriched for microsatellites. BioTechniques 27:500-507.

Hayden MJ, Sharp PJ (2001) Targeted development of informative microsatellite (SSR) markers. Nucleic Acids Res. 29:e44.

Helentjaris TG, King G, Slocum Siedenstrang MC, Wegman S (1985) Restriction fragment polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol. Biol. 5:109-118.

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Heredity 85:4-11.

Jain A, Apparanda C, Bhalla PL (1999) Evaluation of genetic diversity and genome fingerprinting of Pandorea (Bignoniaceae) by RAPD and inter-SSR PCR. Genome 42:714-719.

Kiss GB, Csanadi G, Kalman K, Kalo P, Okresz L (1993) Construction of a basic linkage map for alfalfa using RFLP, RAPD, isozyme and morphological markers. Mol Gen Genet 238:129-137.

Korbin M, Kuras A, Zurawicz E (2002) Fruit plant germplasm characterisation using molecular markers generated in RAPD and ISSRPCR. Cell. Mol. Biol. Lett. 785-794.

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

Leroy XJ, Leon K (2000) A rapid method for detection of plant genomic instability using unanchored-microsatellite primers. Plant Mol. Biol. Rep. 18:283a-283g.

Levin I, Gilboa N, Yeselson E Shen S, Schaffer AA (2000) Fgr, a major locus that modulates the fructose to glucose ratio in mature tomato fruits. Theor. Appl. Genet. 100:256-262.

Liu XC, Wu JL (1998) SSR heterotic patterns of parents for making and predicting heterosis. Mol. Breed. 4:263-268.

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

Maguire TL, Edwards KJ, Saenger P, Henry R (2000) Characterisation and analysis of microsatellite loci in a mangrove species, Avicennia marina (Forsk.) Vierh. (Avicenniaceae). Theor Appl Genet 101:279-285

Mahalakshmi V, Aparna P, Ramadevi S, Oritz R (2002) Genomic sequence derived simple sequence repeats markers. A case study with *Medicago sativa* spp. Electron. J. Biotechnol. 5:234-242.

Mammadov JA, Zwonitzer JC, Biyashev RM, Griffey CA, Jin Y, Steffenson BJ, Saghai Maroof MA (2003) Molecular mapping of leaf rust resistance gene *Rph*5 in barley. Crop Sci. 43:388-393.

Marczewski W, Hennig J, Gebhardt C (2002) The Potato virus S resistance gene Ns maps to potato chromosome VIII. Theor. Appl. Genet. 105:564-567.

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci 88:2336-2340.

Masojc P (2002) The application of molecular markers in the process of selection. Cell. Mol. Biol. Lett. 7:499-509.

Metzgar D, Bytof J, Wills C (2000) Selection against frameshift mutations limits microsatellite expansion in coding DNA. Genome Res. 10:72-80.

Mian MAR, Hopkins AA, Zwonitzer JC (2002) Determination of genetic diversity in tall fescue with AFLP markers. Crop Sci 42:944-950.

Milbourne D, Meyer RC, Collins AJ, Ramsay LD, Gebhardt C, Waugh R (1998) Isolation, characterization and mapping of simple sequence repeat loci in potato. Mol. Gen. Genet. 259:233-245.

Mohler V, Singrun C (2005) General considerations: marker-assisted selection. In: Lörz H, Wenzel G (eds) Molecular marker systems in plant breeding and crop improvement. Springer, Berlin Heidelberg New York, pp 305-318.

Monforte AJ, Garcia-Mas J, Arius P (2003) Genetic variability in melon based on microsatellite variation. Plant Breed. 122:53-157.

Morgante M, Vogel J (1994) Compound microsatellite primers for the detection of genetic polymorphism. U.S. Patent Appl. 08/326456.

Mullis KB, Faloona F (1987) Specific synthesis of DNA in vitro via polymerase chain reaction. Methods Enzymol 155:350-355.

Nagaoka T, Ogihara Y (1997) Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genet. 94:597-602.

Nagaraju J, Kathirvel M, Kumar R, Siddiq EA, Hasnain SE (2002) Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proc. Natl. Acad. Sci. U.S.A. 99:5836-5841.

Ostrander EA, Jong PM, Rine J, Duyk G (1992) Construction of small-insert genomic DNA libraries highly enriched for microsatellite repeat sequences. Proc Natl Acad Sci U S A. 1992 Apr 15;89(8):3419-23

Paglia G, Morgante M (1998) PCR-based multiplex DNA fingerprinting techniques for the analysis of conifer genomes. Mol. Breed. 4:173- 177.

Panaud O, Chen X, McCouch SR (1995) Frequency of microsatellite sequences in rice (*Oryza sativa* L.). Genome 38:170-1176.

Panaud O, Chen X, McCouch SR (1996) Development of microsatellite markers and characterization of simple sequence length polymorphism

(SSLP) in rice (*Oryza sativa* L.) Mol. Gen. Genet. 252:597-607.

Paran I, Kesseli R, Michelmore R (1991) Identification of restrictionfragment-length-polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near isogenic lines. Genome 34:1021-1027.

Porceddu A, Albertini E, Barcaccia G, Falistocco E, Falcinelli M (2001) Linkage mapping in apomictic and sexual Kentucky bluegrass (*Poa pratensis* L.) genotypes using a two way pseudo-testcross strategy based on AFLP and SAMPL markers. Theor. Appl. Genet. 104:273-280.

Prasad M, Varshney RK, Roy JK, Balyan HS, Gupta PK (2000) The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor. Appl. Genet. 100:584-592.

Prochazka M (1996) Microsatellite hybrid capture technique for simultaneous isolation of various STR markers. Genome Res. 6:646-649.

Provan J, Russel JR, Booth A, Powell W (1999) Polymorphic chloroplast simple sequence repeat primers for systematic and population studies in the genus *Hordeum*. Mol. Ecol. 8:505-511.

Rafalski JA, Tingey SV (1993) Genetic diagnostics in plant breeding:RAPDs, microsatellites and machines. Trends. Genet. 9:275-280.

Rafalski JA, Vogel JM, Morgante M, Powell M, Andre C, Tingey SV (1996) Generating and using DNA markers in plants. In: Nonmammalian genomic analysis. A practical guide (Birren, B. and Lai, E. Eds.) Acad. Press, New York, pp. 75-134.

Raina SN, Rani V, Kojima T, Ogihara Y, Singh KP, Devarumath RM (2001) RAPD and ISSR fingerprints as useful genetic markers for analysis of genetic diversity, varietal identification, and phylogenetic relationships in peanut (*Arachis hypogaea*) cultivars and wild species. Genome 44:763-772.

Rakoczy-Trojanowska M, Bolibok H (2004) Characteristics and a comparison of three classes of Microsatellite-based markers and their application in plants. Cell Mol. Bio. Lettrs. 9:221-238.

Ratnaparkhe MB, Santra DK, Tullu A, Muehlbeur FJ (1998) Inheritance of inter-simple sequence repeat polymorphisms and linkage with a fusarium wilt resistance gene in chickpea. Theor. Appl. Genet. 96:348-353.

Richard I, Beckman JS (1995) How neutral are synonymous codon mutations? Nat Genet 10:259.

Roder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007-2023.

Roder MS, Plaschke J, Konig U, Borner A, Sorrells M, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol. Gen. Genet. 246:327-333.

Rostiana O, Niwa M, Marubashi W (1999) Efficiency of inter-simple sequence repeat PCR for detecting somaclonal variation among leaf-cultureregenerated plants of horseradish. Breed. Sci. 49:245-250.

Roy JK, Balyan HS, Prasad M, Gupta PK (2002) Use of SAMPL for a study of DNA polymorphism, genetic diversity and possible gene tagging in bread wheat. Theor. Appl. Genet. 104:465-472.

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

Saal B, Wricke G (1999) Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome 42:964-972.

Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL (2001) A map of human genome sequence variation containing

1.42 million single nucleotide polymorphisms. Nature 409:928- 933.

Schlotterer C, Tautz D (1992) Slippage synthesis of simple sequence DNA. Nucleic Acids Res 20:2211-2215.

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the target repeats using a CT primer. Genome 36:884-889.

Singh A, Chaudhury A, Srivastava PS, Lakshmikumaran M (2002) Comparison of AFLP and SAMPL markers for assessment of intrapopulation genetic variation in *Azadirachta indica* A. Juss. Plant Sci. 162:17-25.

Sobrino B, Briona M, Carracedoa A (2005) SNPs in forensic genetics: a review on SNP typing methodologies. Forensic Sci Int 154:181-194.

Soleimani VD, Baum BR, Johnson DA (2003) Efficient validation of single nucleotide polymorphisms in plants by allele-specific PCR, with an example from barley. Plant Mol Biol Rep 21:281-288.

Sunyaev S, Hanke J, Aydin A, Wirkner U, Zastrow I, Reich J, Bork P (1999) Prediction of nonsynonymous single nucleotide polymorphisms in human disease-associated genes. J Mol Med 77:754-760.

Tang S, Yu JK, Slabaugh MB, Shintani DK, Knapp SJ (2002) Simple sequence repeat map of the sunflower genome. Theor. Appl. Genet. 105:1124-1136.

Taramino G, Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. Genome 39:277-287.

Tautz D, Renz M (1984). Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 12(10):4127-4138.

Temnykh S, Park WD, Ayers N, Cartinhour S, Hauck N, Lipovich L, Cho YG, Ishii T, McCouch SR (2000) Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa* L.) Theor. Appl. Genet. 100:697-712.

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

Thro AM, Parott W, Udall JA, Beavis WD (2004) The experience of the initiative for future agricultural and food systems. Crop Sci 44:1893.

Torress AM, Weeden NF, Martin A (1993) Linkage among isozyme, RFLP, and RAPD markers. Plant Physiol 101:394-452.

Tosti N, Negri V (2002) Efficiency of three PCR-based markers in assessing genetic variation among cowpea (*Vigna unguiculata* subsp. *unguiculata*) landraces. Genome 45:268-275.

Tseng YT, Lo HF, Hwang SY (2002) Genotyping and assessment of genetic relationships in elite polycross breeding cultivars of sweet potato in Taiwan based on SAMPL polymorphisms. Bot. Bull. Acad. Sin. 43:99-105.

Van der Nest MA, Steenkamp ET, Wigfield BD, Wingfield MJ (2000) Development of simple sequence repeat (SSR) markers in *Eucalyptus* from amplified inter-simple sequence repeats (ISSR). Plant Breed. 119:433-436.

Vivek BS, Simon PW (1999) Linkage relationships among molecular makers and storage root traits of carrot (*Daucus carota* L. ssp. *sativus*) Theor. Appl. Genet. 99:58-64.

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

Wenzel G (2006a) Biotechnology in potato improvement. In: Gopal J, Khurana SMP (eds) Potato production, improvement and post-harvest management. Harworth, New York 109-146.

Wenzel G (2006b) Molecular plant breeding: achievements in green biotechnology and future perspectives. Appl Microbiol Biotechnol 70: 642-.650

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1991) DNA polymorphisms

amplified by arbitrary primers are usefl as genetic markers. Nucleic Acids Res 18:6531-6535.

Witsenboer H, Vogel J, Michelmore RW (1997) Identification, genetic localization and allelic diversity of selectively amplified microsatellite polymorphic loci in lettuce and wild relatives (*Lactuca* spp.). Genome 40:923-936.

Wolfe AD, Xiang QY, Kephart SR (1998) Assessing hybridization in natural populations of *Penstemon* (*Scrophulariaceae*) using hypervariable inter simple sequence repeat markers. Mol. Ecol. 7:1107-1125.

Yaish MWF, Perez de la Vega M (2003) Isolation of (GA)n microsatellite sequences and description of a predicted MADS-box sequence isolated from common bean (*Phaseolus vulgaris* L.). Genet. Mol. Biol. 26:337- 342.

Yin X, Stam P, Dourleijn CJ, MJ (1999) AFLP mapping of quantitative trait loci for yield-determining physiological characters in spring barley. Theor Appl Genet 99:244-253.

Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X *et al*. (2002) A draft sequence of the rice Genome (Oryza sativa L. ssp. indica). Science 296:79-92.

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

Zhou WC, Kolb FL, Bai GH, Domier LL, Boze LK, Smith NJ (2003) Validation of a major QTL for scab resistance with SSR markers and use of marker-assisted selection in wheat. Plant Breed. 122:40-46.

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