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Review

Microsatellite markers: An important fingerprinting tool for characterization of crop plants

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Microsatellites are simple sequence repeats (SSR) of 1-6 nucleotides. They appear to be ubiquitous in higher organisms, both in animal and plant genomes and involving repetitive as well as unique sequences, although the frequency of microsatellites varies between species. They are abundant, dispersed throughout the genome and show higher levels of polymorphism than do other genetic markers. These features coupled with their ease of detection have made them useful markers. Their potential for automation and their inheritance in a co-dominant manner are additional advantages when compared with other types of molecular markers. SSRs are highly polymorphic, genome specific, abundant and co-dominant, and have recently become important genetic markers in cereals including wheat and barley.

Key words: Simple sequence repeat, allelic diversity, polymorphism, co-dominance, microsatellites, genetic diversity.

INTRODUCTION

With the advances in understanding the sequence organization of genomic deoxyribonucleic acid (DNA), a class of repetitive DNA sequences was found to be an integral part of the eukaryotic genome. Genomic simple sequence repeats (SSRs) have been used for a variety of purposes, including gene tagging, physical mapping, genome mapping and estimation of genetic diversity. SSRs/microsatellites are short sequence elements that are arranged in a simple internal repeat structure. SSR or short tandem repeats of DNA sequences (1-6 bp), is present throughout the genome of an individual, both in coding and non-coding regions. Microsatellite loci display co-dominant inheritance, high information content, reproducibility, and even distribution along chromosomes with specificity of locus (Kashi et al., 1997; Roder et al., 1998).

Microsatellite markers could characterize and discriminate all genotypes (Ijaz and Khan, 2009). The frequency of SSR is higher in transcribed regions, especially in the untranslated portions, than in non-transcribed ones (Morgante et al., 2002). SSRs, densely and randomly distributed throughout the whole eukaryotic genome (Tautz, 1989), are tandemly repeated sets of 2 to 8 base pairs, and can vary extensively in the number of repeats, which may be due to slippage of DNA polymerase during replication and unequal crossing over (Schlotterer and Tautz, 1992).

A possible problem, which is associated with the use of simple sequence repeat markers/microsatellite markers, is the occurrence of null alleles (Callen et al., 1993). In comparison with the high polymorphism of genomic SSRs, expressed sequence tag (EST)-SSRs are less powerful in providing information on genetic variations (Eujayl et al., 2002; Thiel et al., 2003).

MICROSATELLITE MARKERS SHOW GREAT ALLELIC DIVERSITY AND POLYMORPHISM

SSR markers are highly mutable loci, present in the eukaryotic genome at several sites, having stretches of tandemly repeated nucleotide motifs that can be as short

Abbreviations: SSR, simple sequence repeats; **DNA,** deoxyribonucleic acid; **EST,** expressed sequence tag; **PCR,** polymerase chain reaction; **PIC,** polymorphism information content; **RFLP,** restriction fragment length polymorphisms.

as 4, 3, 2 or 1 nucleotide. Knowledge of the sequence of these regions are used for designing specific amplifying primers, which then define sequence-tagged microsatellite sites (STMS). SSR markers/microsatellite markers are robust tools that can be exchanged between laboratories, and they are highly polymorphic (Thomas and Scott, 1993). Microsatellites and SSRs are multiallelic and hypervariable, and due to these characteristics, SSR have proven to be highly informative in studies of evolutionary relationships and genetics (Buchanan et al., 1994). Reproducibility of SSR can be efficiently utilized by different research laboratories to generate consistent data (Saghai-Maroof et al., 1994).

SSR markers are those molecular markers that prove to be useful tools for genome mapping, population and parentage analysis, individual identification, phylogenetic studies, cancer diagnostics, etc. The methods for the detection of simple sequence repeats are very quick, easy and feasible for these studies (Lefort et al., 1999). These are polymerase chain reaction (PCR)-based molecular markers and are efficient, reliable, cost-effective and common in rice. Microsatellite markers show a high degree of polymorphism in rice compared to restriction fragment length polymorphisms (RFLPs) and SSR markers are suitable molecular markers for evaluating genetic diversity among closely related rice cultivars (Temnykh et al., 2000). Microsatellite makers are easy to apply once they are developed, as the methodology for their development is complex, difficult and very costly, which are the reasons for restricting their application to important crops such as sugarcane (Scott et al., 2000).

By searching ESTs in sugarcane, 402 SSRs and various dinucleotide and trinucleotide SSRs have been found, and in saccharum species, EST-derived SSRs are useful because they are transferable and polymorphic (da Silva, 2001). Microsatellite markers have greater allelic diversity than other molecular markers (McCouch et al., 2001).

During polyploidization, gene conversion, inter-chromosomal exchange, replication slippages are involved in the evolution of microsatellite/SSR loci (Sayed et al., 2001). Microsatellite markers may be detected in the eukaryotic genome, because interspersed and simple repeats may well overlap regions transcribed by ribonucleic acid (RNA) polymerase, including ESTs in humans, tandem repeat polymorphisms in human genes being more common than generally believed with about 8% of such loci being within the coding sequence and if polymorphic, resulting in a frame shift (Wren et al., 2001).

EST-SSRs belong to that part of the genome that is a transcribed region and may be relatively well conserved, so that any polymorphism that is found and identified using EST-SSRs, may reflect a better relationship between varieties or species. Evaluation of the germplasm with SSRs derived from ESTs enhances or boosts the role of genetic or molecular markers by assaying variations in genes that are transcribed and of known function (Eujayl et al., 2002). For application in marker assisted selection (MAS), microsatellite markers with high polymorphism information content (PIC) values were found to be more useful. The microsatellites derived from genomic sequences were found to be more successful than those SSR markers that were designed from EST (Karakousis et al., 2003). EST-SSR markers have been employed to screen different species of wheat grass, which indicate further chromosome rearrangements or variations in sequences between wheat grass genomes (Mullan et al., 2005), and SSR variations have been identified in 60 durum wheat accessions (Wang et al., 2007).

UTILIZATION OF MICROSATELLITE MARKERS IN BREEDING PROGRAMS

The efficiency of a breeding program is dependent on the diversity of parents that are used in crosses. With the help of SSRs, diversity can be easily found or detected, that exhibits a higher level of polymorphism compared with other isozymes (Trujillo et al., 1995; Belaj, 1998). For microsatellites, heterozygosity values are 7-10 times more than RFLP. SSRs detected heterozygosity among cultivars in rice (McCouch et al., 1988; Wang and Tanksley, 1989; Virk et al., 1995) and in other crops (Kresovich et al., 1994). In plants, SSR markers have proven to be valuable tools for DNA genotyping, population genetics, genetic mapping studies, conservation and the management of genetic resources (Thomas and Scott, 1993; Bowcock et al., 1994; Jarne and Lagoda, 1996; Zane et al., 2002).

Microsatellite/SSR markers are markers of choice due to a high level of polymorphism, as well as higher reliability with the highest polymorphic content (PIC). These characteristics of microsatellites augmented the utilization of these markers as molecular markers in fingerprinting (Weising et al., 1995; Diwan and Cregan, 1997; Ashikawa et al., 1999). These markers have been widely used in soyabean due to their high level of sequence polymorphism, repeatability, co-dominance, rapid and relatively inexpensive use (Maughan et al., 1995; Mudge et al., 1997). Among various SSR loci, there is a significant variation in allelic diversity (Akagi et al., 1997; MCcouch et al., 2001; Ravi et al., 2003). SSR identification in ESTs was reported for a wide range of species (Smulders et al., 1997; Cho et al., 2000; Haung et al., 2000; Lee et al., 2004). The presence of extra microsatellite/SSR motifs demonstrates that, as found in other plant genomes, there are many compound SSRs, suggesting that there may be clustering of SSRs in some genomic regions (Fisher et al., 1996). Those polymorphisms that are based on variation in the number of microsatellites have been successfully applied to plant breeding programs. Primers have been studied that are obtained from the sequence flanking the repeat unit; they are used with genomic DNA in PCR reactions and show SSR

polymorphisms that represent different alleles at that locus (Gupta et al., 1996).

Inter-microsatellite amplification (IMA) markers in the F2 population of peach, for economically important traits, including the four agronomic characters peach/ nectarine, flat/round fruit, acid/non-acid fruit, and pollen sterility, have been studied and mapped to 11 linkage groups. Between pairs of markers, the average density is 4.5 cM (Dirlewanger et al., 1998). The genetic attributes of SSRs or microsatellites as DNA markers, coupled with their presence in most, if not all, sugarcane genotypes make SSR/microsatellites the preferred and reliable method for use in the construction of a reliable framework of the genetic map of sugarcane (Cordeiro et al., 1999). These markers are abundant, genome specific, co-dominant, and cover all 21 wheat chromosomes and have been successfully employed to characterized genetic diversity in seed bank collections of improved wheat germplasm (Borner et al., 2000; Huang et al., 2002) and wild relatives (Hammer, 2000). Morphologically, *Triticum urartu* and *Triticum baeoticum* are very similar. It was possible to distinguish between these two species by using these markers. Therefore, SSR markers are a new powerful tool that supports the determination of critical races in diploid wild wheat species. They also allow the discussion of evolutionary pathways within Triticum (Hammer, 2000). So, for efficient screening of the germplasm by saturating more regions of wheat genome, more polymorphic wheat microsatellites could be used (Ijaz and Khan, 2009).

In sunflower, 980 clones from a genomic DNA library enriched for several dinucleotide and tetranucleotide SSR motifs were identified and then sequenced, of these, 35.5% were unique sequences, 71% of the amplified products displayed polymorphisms among 16 elite inbred lines; primers sets were designed for 87% of the single clones, and 74.5% of the primer sets amplified clean SSRs for genomic DNA (Yu et al., 2000). Resistance to green bug biotype E, and to map the resistance quantitative trait loci (QTLs) by using a microsatellite linkage map, it was studied and found that microsatellite markers are closely linked to the major resistance QTLs (YQ et al., 2007). The presence of locus in OS1E6 locus in various cereal genotypes have been found by analyzing these cereal genotypes. The presence of this locus in various cereal genotypes indicates its conservation across different cereal members (Davierwala et al., 2000). Microsatellite markers or SSR markers linked to the gene Pm 24 in an F2 progeny in wheat by applying bulk segregation analysis (BSA) have been identified (Huang et al., 2000). Three microsatellite markers have been mapped, namely Xgwm 106, Xgwm 337 and Xgwm 458, in the coupling phase to the Pm 24 locus. An allele of microsatellite locus Xgwm 337 was located 2.4 + -1.2 cM from Pm 24.M.

CONCLUSION

In conclusion, microsatellite markers/SSR markers show co-dominant expression and multiallelism, and because of these characteristics, simple sequence repeat markers/microsatellite markers exhibit high PIC values (Ferreira and Grattapaglia, 1998). These markers display high gene diversity scores, and these characteristics make them useful in distinguishing closely related genotypes (Giancola, 1998). Hence, SSRs have been found to be highly polymorphic, genome specific, abundant and co-dominant, and have recently become important genetic markers in cereals including wheat and barley.

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