Menara Perkebunan 2014 82(1), 39-50 **Review**

Molecular markers and their application for DNA fingerprinting and genetic diversity studies in *Coffea* species

Marka molekuler dan penerapannya untuk studi sidik jari DNA dan keragaman genetik pada spesies Coffea

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Abstrak

Strategi klasik yang meliputi perbandingan anatomi, fisiologi dan sitogenetika telah banyak diterapkan untuk mengidentifikasi karakter tertentu serta untuk menentukan keragaman dan hubungan antar dan intra spesies. Namun, saat ini penanda molekuler telah melengkapi strategi sebelumnya dengan sangat cepat. Berbagai jenis penanda molekuler digunakan untuk menilai tingkat polimorfisme DNA. Penanda molekuler ini diklasifikasikan sebagai penanda berbasis hibridisasi dan berbasis Polymerase Chain Reaction (PCR). *Dalam beberapa tahun terakhir, sistem penanda DNA yang berbeda seperti* Restriction Fragment Length Polymorphisms (RFLPs), Random Amplied Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) *yang juga disebut Mikrosatelit,* Single Nucleotide Polymorphims (SNPs) *dan lain-lain telah dikembangkan dan diterapkan pada berbagai spesies tanaman. Penanda molekuler ini dapat digunakan untuk sidik jari DNA dan studi keragaman genetik. Sidik jari berdasarkan DNA telah banyak digunakan dalam ilmu forensik, juga memiliki berbagai aplikasi dalam pemuliaan tanaman. Tulisan ini memberikan* overview *tentang berbagai penanda molekuler dan aplikasinya untuk sidik jari dan kajian keragaman genetik tanaman berdasarkan DNA pada berbagai spesies tanaman, dan secara khusus pada* Coffea *sp.*

[Kata Kunci: Polimorfisme DNA, pemuliaan tanaman, fragmen restriksi, PCR]

Abstract

Conventional strategies including comparative anatomy, physiology and cytogenetics were applied to identify the certain character as well as to determine inter- and intra-species diversity and relationships. However, more recently molecular markers have very rapidly complemented the previous strategies. Various types of molecular markers are used to assess DNA polymorphism. They are classified as hybridization-based markers and polymerase chain reaction (PCR) based markers. In recent years, different DNA marker systems such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplied Polymorphic DNAs (RAPDs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs) which also called as microsatellites, Single Nucleotide Polymorphims (SNPs) and others have been developed and applied to a range of plant species. These molecular markers can be used for DNA fingerprinting and genetic diversity study. DNA fingerprinting has been widely used in forensic science, but is has also a variety of application in plant breeding. This paper provides an overview about various molecular markers and their application for DNA plant fingerprinting and genetic diversity, especially in *Coffea* sp.

[Keywords: DNA polymorphism, plant breeding, restriction fragment, PCR]

Introduction

Molecular markers, also known as a genetic marker, include biochemicals, proteins and mainly DNA. Moreover, molecular markers can be found at specific locations of the genome. They are used to 'flag' the position of a particular gene or the inheritance of a particular characteristic. In a genetic cross, molecular markers will typically stay linked with the characteristics of interest. Thus, individuals can be selected in which the molecular marker is present, since the marker indicates the presence of the desired characteristic. Deeply, DNA markers are specific fragments of DNA that can be identified within the whole genome. It is nowadays acceptable that DNA markers represent the most significant advance in breeding for the last few decades and currently constitute the most important application of molecular biology to plant breeding (Jiang, 2013; Grover & Sharma, 2014).

Various types of genetic markers are used to assess DNA polymorphism. These genetic markers are classified into morphological-based, biochemical and molecular markers (Kumar *et al*., 2009). Notably for molecular markers, they are classified as hybridization-based markers and polymerase chain reaction (PCR) based markers (Jiang, 2013; Semagn *et al*., 2006). In the first one, DNA profiles are visualized by hybridizing a given labelled DNA probe to genomic DNA digested by various restriction enzymes. PCR-based markers involve *in vitro* amplification of DNA sequences induced with specifically or arbitrarily chosen oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme.

The amplified fragments are separated by electro-phoresis and band patterns are characterized by different methods such as staining and autoradiography (Schlotterer, 2004). PCR is a versatile technique invented during the mid- 1980s. Since

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thermostable DNA polymerase was introduced in 1988, the use of PCR has tremendously increased in research and clinical laboratories. The primer sequences are chosen to allow base-specific binding to the template. This technique is deemed to be extremely sensitive and rapid. Its application has open up a multitude of new possibilities in the field of molecular biology. DNA fingerprinting and genetic diversity studies are the two most used applications of molecular markers in plant biology (Hassanpour *et al.*, 2013; Liao *et al.*, 2012; Geleta *et al.*, 2012; Acosta-Quezada *et al.*, 2012; Todd *et al.*, 2011)

In this paper, firstly, various molecular markers were described in order to review the wide-range uses of each marker in diverse plant species, including the advantages and disadvantages. In comparison, added information about biochemical marker was also reviewed. Secondly, the review adressed about the utilization of these markers in *Coffea* sp. in comparison with other plant species to develop state of the art about genetic research and advances in this species. Thirdly, the applications of molecular markers in DNA fingerprinting and genetic diversity studies were robustly discussed in plant species in comparison with *Coffea* sp. Conclusively, previous and present researches on *Coffea* sp. using molecular markers in Indonesia are trying to establish better understanding on the genetic diversity of this species, on the establishment of core collection and furthermore to be used in the future breeding program.

1. *Biochemical marker*

1.1. *Isozymes*

Isozymes are based on proteins with identical function, but different electrophoresis mobility. Isozymes were first described by Hunter and Markert who defined them as different variants of a given enzyme having identical function (Hunter & Markert, 1957). This definition encompasses (1) enzyme variants that are the products of different genes and thus represent different loci (described as *isozymes*) and (2) enzymes that are the products of different alleles of one single gene (described as *allozymes*). Isozymes are usually the result of gene duplication and from polyploidization. The technique is based on allelic variation existing for many different proteins (Kumar *et al*., 2009). For example, two alleles of malic dehydrogenase would both perform the same enzymatic function, but their electrophoresis mobility may differ. Therefore, the two enzymatic forms would migrate to different locations on a gel.

Whilst isozymes may be almost identical in function, they may differ in other ways. In particular, amino acid substitutions that change the electric charge of the enzyme (such as replacing aspartic acid with glutamic acid are easy to be traced using gel electrophoresis. This principle forms the basis for the use of isozymes as molecular markers (Kumar *et al.*, 2009). The weakness of isozyme markers relies on the expression of analyzed proteins which depend on tissue/organ, development stage, and also environment (Kumar *et al*., 2009). Therefore a reliable comparison between different individuals requires several sampling and analyses for differentiating genetic from environment causes. Due to various drawbacks these protein based markers have now been largely superseded by more informative DNA-based approaches not sensitive to environmental conditions. However, they are still amongst the quickest and cheapest marker systems to develop, and they remain an excellent choice for projects that only need to identify low levels of genetic variation, e.g. quantifying mating systems.

2. *Molecular marker types.*

2.1. *Randomly Amplified Polymorphic DNA (RAPD).*

Randomly Amplified Polymorphic DNA (RAPD) is based on the differential PCR amplify-cation of DNA samples induced by short oligo-nucleotide primers on random locations of the genome (de Lima *et al*., 2011). In this reaction, a single set of primer anneals to genomic DNA at two different sites on complementary strands of DNA template. If these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through PCR amplification. This procedure detects DNA polymorphisms mainly on the binding sequence of the selected primer. The number of amplification products is related to the number and orientation of the complementary sequences to the primer and therefore also to the size of the genome. Each primer induces amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence poly-morphism between individuals (Schlotterer, 2004). Therefore, RAPD technique can be performed in a moderate laboratory for most of its applications. Despite the reproducibility problem, the RAPD method will probably be important as long as other DNA-based techniques remain unavailable in terms of cost, time and labour (Lashermes *et al*., 1996).

The standard RAPD technology utilises short synthetic oligonucleotides (10 bases long) of random sequences as primers to amplify nanogram amounts of total genomic DNA under low annealing temperatures by PCR technique. Amplification products are generally separated on agarose gels and stained with ethidium bromide. Decamer primers are commercially available from various sources. In 1990, Welsh and McClelland developed a new PCR-based genetic assay; whos independently developed a similar methodology using primers about 15 nucleotides long and different amplification and electrophoretic con-ditions from RAPD and called it the arbitrarily primed polymerase chain reaction (AP-PCR) technique

(Welsh & McClelland, 1990). PCR amplification with primers shorter than 10 nucleotides, specifically called as DNA amplification fingerprinting (DAF) has also been used producing more complex DNA fingerprinting profiles (Welsh & McClelland, 1990). Although these approaches are different with respect to the length of the random primers, amplification conditions and visualisation methods, they all differ from the standard PCR condition in which only a single oligonucleotide of random sequence is employed and no prior knowledge of the genome subjected to analysis is required. Unfortunatelly, RAPD are dominant markers, meaning they could not differentiate homozygous A/A from heterozygous A/a which give limitations for fingerprinting and mapping. However, they have been used as markers of important traits to be introgressed in near isogenic lines (Pinheiro *et al*., 2009).

2.2. *Amplified Fragment Length Polymorphism (AFLP).*

Amplified fragment length polymorphism (AFLP) technology is based on the amplification of restriction fragments from the whole genome, and separation of labeled amplified products by denaturation on the polyacrilamide gel electrophoresis. This technique combines the accuracy of digestion by restriction endonucleases with the precision of the polymerase chain reaction (Sabir *et al*., 2014).

The restriction nucleases restrict the DNA at a precise location within this recognized sequence, generating fragments of DNA. The simultaneous application of different restriction enzymes can produce hundreds of fragments of variable length from the DNA sample. After having been submitted to a pre-selective and followed to selective amplify-cation the fragments of interest are amplified by PCR for further visualization by gel electrophoresis.

The observed fragment patterns reveal DNA differences between individuals. Two different individuals processed with the same restriction nuclease enzyme(s) will yield a different DNA pattern/ fingerprint. The presence or absence of bands is referred to as a polymorphism as for RAPD.

Compared to other DNA screening techniques AFLP has several advantages. The amount of required DNA for the analysis is far less (sensitivity), while the information obtained can be more (power). The reproducibility of AFLP is greater than other techniques, increasing the confidence of the results from experiment to experiment (Hamon *et al.*, 2005). Compared with RFLP, AFLP is faster, and more cost-effective. It also allows simultaneous identification of a large number of amplification products. However, like RAPD, AFLP is a dominant marker which not allows the differentiation between some heterozygous and homozygous individuals.

2.3. *Restriction Fragment Length Polymorphism (RFLP).*

Restriction Fragment Length Polymorphism (RFLP) is based on the differential hybridization of cloned DNA probes to fragments from DNA digested by restriction enzyme. The marker is specific of a single probe/restriction enzyme combination. They are simply inherited in naturally occurring Mendelian characteristics (Lander & Botstein, 1989).

The first step is to develop a set of DNA probes that can be used to identify the restricted fragments. Random genomic clones are a poor choice as hybridization probes because, plant genomes containing a large percentage of repeated sequences, they will generate a number of hybridization bands that are difficult to analyze. Therefore the two primary sources of probes for RFLP are cDNA clones and PstI-derived genomic clones representing respectively expressed genes and non-methylated ones (Podio *et al*., 2012).

In RFLP analysis, DNA from genotypes to be analyzed is digested with a serie of endonucleases, then resolved by gel electrophoresis and blotted on nylon membrane (Southern, 1975). Specific banding patterns are visualized by hybridization with labelled probes. Some probes show a distinctive hybridization pattern for each genotype. The differences in band pattern for one probe represent a restriction fragment length polymorphism (Southern, 1975). The selection of appropriate source for RFLP probe varies within the considered application. Though genomic library may exhibit greater variability than cDNA libraries, these remain controversial (Garcia *et al*., 2011). This might be due to the detection by cDNA probes of variation not only in coding regions but also in flanking regions and introns.

The RFLPs are codominant markers and could potentially detect all alleles from a given locus which may be highly desirable for detecting recessive traits (Kesawat & Das Kumar, 2009). RFLP analysis is therefore a very powerful tool in genetic, taxonomic, and evolutionary studies of plants. However, this technique is time-consuming and labour-intensive due to blotting and hybridization with radio labelled probes (Kumar *et al*., 2009). Their inability to detect single base change outside the restriction site also limits their power (Schlotterer, 2004).

2.4. *Simple Sequence Repeats (SSRs).*

A major step forward in molecular markers was the discovery that about 30–90% of the genome of virtually all the species is constituted of polymorphic repetitive DNA. These regions contain loci comprising several hundred allelic forms differing in length, sequence or both. They are interspersed in tandem arrays ubiquitously. The repetitive DNA regions are also the location of numerous genomic

mutations. Thus repetitive DNA and mutations together form the basis of a number of markers that are useful for various applications in plant genome analysis. The markers belonging to this class are both hybridization-based and PCR-based.

The term of Simple Sequence Repeats (SSR), also called microsatellites and minisatellites was introduced (Jeffreys *et al.*, 1985). Both are multilocus probes creating complex banding patterns and are usually non-species specific. They essentially belong to the repetitive DNA family. Minisatellites are tandem repeats with a monomer repeat length of about 11–60 bp, while microsatellites consist of 1 to 6 bp long repeated monomer sequence (Jeffreys *et al.*, 1985; Tourmente *et al.*, 1998). These loci contain various numbers of tandem repeat units between genotypes. In the genome, they are occasionally referred as variable region if a single locus contains variable numbers of tandem repeats between individuals. They may also be referred as hyper-variable regions if numerous loci contain tandem repeats within a genome generating high levels of polymorphism between individuals. The number of repeats depends on a sequence specific slippage rate (Spackman *et al.*, 2010).

One of the prominent features of these SSR markers is their co-dominant characteristic allowing to detect and to evaluate the level of heterozygosity. Moreover, they are valuable genetic markers because of their multi-allelic nature, and they are abundant, variable, and easy to genotype. Compared with RFLP, SSR markers are easier to handle, cheaper to use, suitable for automation, and high reproducible (Wang *et al.*, 2014). Therefore SSR could be used for high throughput genotyping. Moreover this markers showed the high transferability among wide species, such as wheat, rye, and triticale (Castillo *et al.*, 2008, Sim *et al.*, 2009). This knowledge upraised the fact that SSR markers can better define genetic diversity across genera between plant species (Kumar *et al.*, 2009; Zhang *et al.*, 2014a; Zhang *et al.*, 2014b).

Kantety and colleagues described a marker system named Inter-Simple Sequence Repeat (ISSR) (Kantety *et al.*, 1995). The ISSR analysis involves PCR amplification of regions between adjacent, inversely oriented microsatellites using single simple sequence repeat (SSR)-containing primers. The technique can be applied for any species that contains a sufficient number and distribution of SSR motifs and has the advantage that genomic sequence data is not required (Muzila *et al.*, 2014). The primers used in ISSR can be based on any di-, tri-, tetra-, or pentanucleotide SSR motifs found at microsatellite loci, giving a wide diversity of possible amplification products (Alikhani *et al.*, 2014).

Subsequently, microsatellites developed from expressed sequence tag (ESTs), popularly known as EST-SSRs, can be deduced from database searches and other in silico approaches (Fang *et al.*, 2014). Furthermore, EST-SSR markers are expected to show high inter-specific transferability as they belong to conserved regions of the genome. Due to the recent increasing interest for functional genomic, large dataset of ESTs have been developed, and with bio-informatics tools it is now possible to identify and develop EST-SSR markers at a large scale in a time and cost-effective manner (Varshney *et al.*, 2005). Because of the above advantages and accessibility of large EST databases, increasing SSR markers are now being identified and used for a variety of applications in a number of species like, cereals (Varshney *et al.*, 2005), coffee (Aggarwal *et al.*, 2007), and durum wheat (Wang *et al.*, 2007).

In genetic diversity study in *Coffea*, previous study showed that 110 arabica SSR markers have shown a good transferability on six African species and the amplification ranged from 72.7 to 86.4% (Poncet *et al.*, 2004). Furthermore a set of 40 polymorphic SSRs developed from *C. canephora* genomic library were tested in accessions of *C. heterocalyx* and *C. pseudozanguebariae* belonging to different African geographical clades. 38 of them (95%) were amplified in *C. heterocalyx* and *C. pseudozanguebariae*, indicating a very high transferability across the genus *Coffea* (Poncet *et al.*, 2007). Subsequently, transferability of 44 robusta derived SSR markers was also tested on 13 related *Coffea* and two *Psilanthus* species. Overall, an average transferability of 92% was observed (Hendre *et al.*, 2008). Other research groups reported the high transferability of 60 SSR markers developed from robusta and arabica coffee into 15 coffee species (Cubry *et al.*, 2008). These markers allowed detecting a large part of the diversity available within wild species for breeding application. These features make SSR desirable markers for genome mapping, crop improvement breeding, or population genetic studies and possibly quality control of varieties.

2.5. *Single Nucleotide Polymorphisms (SNPs).*

The most recently developed molecular marker is Single Nucleotide Polymorphisms (SNP). They are based on DNA sequence variations in a single nucleotide. They occur at a frequency of about one SNP per 1000 nucleotides in genomic DNA (Mmeka *et al.*, 2013). They can be used to detect alleles responsible for a trait of interest. Thus various large scale discovery projects are currently aimed at identifying SNPs from a broad range of organisms, including crop plants. The discovery of SNPs and insertions/deletions has been simplified by recent developments in sequencing technology. SNP discovery in many crops, such as corn and soybean, is relatively straightforward because of their high level of intraspecific nucleotide diversity and the availability in public database of many genes and EST sequences. For these species, direct readout of SNP haplotypes is possible. Haplotype-based analysis is more informative than individual SNPs and therefore

has more power in genetic association with phenotypes. For many crops the elite germplasm has been subjected to various evolutionary and breeding bottlenecks increasing the amount of linkage disequilibrium (LD) and facilitating the association of candidate SNP to phenotypes. Whole-genome scans may help identifying genome regions associated with interesting phenotypes if sufficient LD is present (Rafalski, 2002).

The abundance, ubiquity and interspersed nature of SNPs give them a huge potential in genetic mapping, marker-assisted breeding development, gene cloning and functional genomics. High automation in SNPs development has become incredibly convenient by various automatized systems after transformation in CAPS (cleaved amplified polymorphism sequence), dCAPS (derived-CAPS), or allele-specific PCR markers (Liu & Zhang, 2006). This feature has made them usable for highthroughput genotyping.

2.6. *Sequence-Related Amplified Polymorphism (SRAP)*

Sequence-related amplified polymorphism (SRAP) is a novel, PCR-based molecular marker technique developed by Li and Quiros (Li and Quiros 2001). This marker system aims at the amplification of open reading frames (ORFs) using two primers (forward and reverse) for amplification and produces a number of co-dominant markers per amplification. It has been found immensely useful in several crops. The SRAP marker system is a new, simple and efficient marker system that can be adapted for a variety of purposes such as linkage map construction (Yeboah *et al*., 2007), genomic and cDNA fingerprinting, gene tagging (Li & Quiros, 2001), genetic diversity analysis (Li *et al*., 2009) and map-based cloning (Zhang *et al*., 2010).

Merits of SRAP over other marker systems rely on (1) its simplicity and reliability, (2) reasonable throughput rate, (3) most importantly targets ORFs in genome, (4) numerous co-dominant and clear high-intensity, (5) bands rarely overlapping, (6) not crop-specific, (7) easy isolation of bands for sequencing, (8) cost-effective, (9) as any of the forward primers can be, combined with any of the reverse primers, many primer combinations are possible, hence reduces the cost of PCR, (10) multi-loci and multi-allelic features, which makes it potentially more efficient for genetic diversity analysis, gene mapping and fingerprinting genotypes (Robarts & Wolfe, 2014).

Limitations of SRAP markers have not yet been described, as these markers are relatively new and their use is still in its early stages. Similar to other markers scored as dominant, SRAP amplicons cannot yield heterozygosity descriptors such as Hardy–Weinberg equilibrium. Conclusions made as to taxonomic placement of examined individuals, especially in cultivated taxa, may not be appropriate, as selective pressures (anthropogenic or otherwise) may have directly affected the patterns of diversity elucidated by SRAP markers and not reflect evolutionarily relevant systematic relationships (Guo *et al*., 2012).

As PCR-based markers, SRAP markers have many advantages, including the requirement for a relatively small amount of template genomic DNA, and good levels of polymorphism in many plant species. In particular, these types of DNA markers can be detected without any prior knowledge of the genome sequence (Robarts & Wolfe, 2014). Due to this fact, the SRAP technique is unique in the sense that it can allow mapping of gene expression markers or gene expression quantitative loci (QTL). In future years, SRAP will emerge as a highly productive technique in crops where the genome sequence is not available for marker-assisted breeding and related applications.

The existence of various molecular techniques and differences in their principles and methodologies require careful consideration in choosing one or more of such marker types. The comparison of each marker was provided in Table 1.

3. *Molecular markers and DNA fingerprinting studies*

DNA fingerprinting has been widely used in forensic science, but it has also a variety of application in plant and animal breeding. It uses the techniques based on PCR to reveal the specific DNA profile of a given organism. A DNA fingerprint is independent of the environment, and is consistent throughout different parts and developmental stages of the organism. Similarity of DNA fingerprints depends on genetic closeness of tested individuals. DNA fingerprinting can distinguish plants from different families, genera, species, cultivars (cultivated variety), and even sibling plants (Malik *et al*., 2014). Over the years, scientists have developed many DNA fingerprinting techniques with variation in complexity, setting-up and operation costs, reliability and throughput. The terminology of "DNA fingerprinting" was introduced for the first time in 1985 to describe bar-code-like DNA patterns generated by multilocus probes after electrophoresis separation of genomic DNA fragments (Jeffreys *et al*., 1985). The obtained patterns make up a unique feature of the analysed individual and are currently considered to be the ultimate tool for biological individualization.

The requirement of World Trade Organization on member states to provide protection for new plant varieties has given some protection opportunities to new plant varieties in many countries. With the possibility of getting specific DNA profile for a single plant, DNA fingerprint gives new possibilities to satisfy criteria for granting protection. These criteria have to include distinctiveness, uniformity, and stability. For administrators of plant property rights, DNA fingerprinting can help select most suitable

Table 1. Recapitulation of several characteristics of molecular marker discussed in this paper. The data are collected based on comparison of several review papers (Robarts & Wolfe, 2014; Jiang, 2013; Schlotterer, 2004).

Tabel 1. Rekapitulasi beberapa karakteristik marka molekuler yang didiskusikan dalam paper ini. Data dikumpulkan dari beberapa publikasi (Robarts & Wolfe, 2014; Jiang, 2013; Schlotterer, 2004).

Characteristics *Karakteristik*	RAPD	AFLP	RFLP	SSR	ISSR	SRAP
Genomic abundance	High	High	High	High	Medium-High	High
Polymorphism level	Medium	Medium	Medium	High	Medium	High
Locus specificity	No	No	Yes	Yes	No	Yes
Co-dominance of alleles	No	No/Yes	Yes	Yes	No	Yes
Reproducibility	Low	Medium	High	High	Medium	High
Labor-intensity	Low	Medium	High	Low	Low	Low
Technical demands	Low	Medium	High	Low-Medium	Low-Medium	Medium
Operational costs	Low	Medium	High	Low	Low-Medium	Low
Development costs	Low-Medium	Low	Medium-High	High	Low	Low
Required DNA quantity	Low	Medium	High	Low	Low	Low
Amenability to automation	Yes	Yes	No	Yes	Yes	Yes

reference varieties for morphological comparison and save cost. It is also most effective in enforcing protection by proving infringement of property rights.

For industrial processed products, DNA fingerprinting has been applied in several crops, such as in herbal drugs. It requires intact genomic DNA fragments from plant samples after processing. Adulterants can be distinguished even in processed samples, enabling the authentication of the drug. Many studies have reported the genotyping of several medicinal plants, and have made available their DNA fingerprints. The brief account of various DNA-based technologies that are useful in genotyping and quick identification of botanicals with suitable examples for herbal drug technology was reviewed (Joshi *et al*., 2004). However these results should be taken with a grain of salt as the plants are often sourced from a variety of locations through the world (Ha *et al*., 2002). Most recently, the variety of cocoa beans purchased from Ecuador was routinely traced by molecular markers at Nestle R&D Center Tours. By this procedure, they could detect the proportion of fine flavouring cocoa versus common varieties.

In the coffe industrial, the coffee market is regularly developing finished products based on a single variety. Some of them are well recognized by the consumer for example Maragogype, Blue Mountain or Bourbon. The distinction of coffee varieties at the green coffee stage is almost impossible using physical or chemical analytical tools. A DNA method was developed to allow the identification of varieties through the value chain, from the field to the finished product. The method is applied on routine basis to guarantee the purity and authenticity of raw material used by Nespresso. The quality control test was recently applied and fine-tuned using green bean batches from farms in Southern Brazil, which grow red and yellow bourbon varieties. This Arabica blend is at the origin for the sensory specificity of "Dulsão do Brazil" capsule. By using a set of eight micro-satellite markers (SSRs) selected for their ability to discriminate the Bourbon origins (Morel *et al*., 2012).

For planting material industrial, an authentication of *Panax ginseng* and *P. quinquefolius* can be described by using AFLP (Ha *et al*., 2002). Recently, analysis of somaclonal variation of somatic embryogenesis by using this marker in Peach Palm was succesfully carried out (Steinmacher *et al*., 2007). Moreover, assessment of genetic stability of clonal materials and determining of genetic stability throughout the process of somatic embryogenesis can also use the SSR markers. By this technique Lopes and colleagues concluded that the simple somatic embryogenesis protocol described has potential for the commercial propagation of cork oak because it results in a low percentage of mutations (Lopes *et al*., 2006).

Other than usual use, DNA fingerprinting can be contribute in plant breeding to identify genetic off-types within breeding population, to differentiate accessions, cultivars, and species that might be difficult to characterize due to similar visible characteristics, but also to validate transformation events after genetic engineering (Priyono *et al*., 2005) and assesment of genetic variability within and among arabica coffee progenies and cultivars (Silveira *et al*., 2003). Moreover, the combination of AFLP and SSR marker could be used to study the origin of cultivated *C. arabica* varieties (Anthony *et al*., 2002). By using ISSR, the parents of six interspecific coffee hybrids were well determined (Ruas *et al*., 2003). Genetic fingerprinting of the *Listada de Gandía* eggplant landrace using genomic SSRs and EST-SSRs was also reported (Muñoz-Falcón *et al*., 2011). Several international germplasm collection centres are using DNA fingerprinting to focus their limited resources on maintaining and propagating the core individuals from their collections, including in cocoa collections at Nestle R&D Center Tours.

4. *Molecular markers and genetic diversity studies*

Information on genetic diversity and relationships within and among crop species and their wild relatives is essential for the efficient utilization of plant genetic resource collections (Shirasawa *et al*., 2014). Moreover, the availability of this genetic diversity is a pre-requisite for any breeding of the crop improvement programs. Efforts have also been made to predict the off spring from a cross by the measurement of genetic distance between the parents, since it can be used as an estimation of expected genetic variance in different sets of segregating progenies derived from different crosses (Hendre & Aggarwal, 2007).

Previously, high level of genetic diversity and relationships among either species within genus or cultivar within species was detected by using isozymes for several crops, such as *Hevea brasiliensis* (Lidah *et al*., 2006). Moreover, DNA markers have been used for genetic diversity study in plants. For coffee, the RFLP markers were used to study the polymorphism level (Priyono *et al*., 1999), and to estimate the heterozygosity level and segregating pattern in *C. canephora* (Priyono *et al*., 2000).

RAPD marker has also been applied for studying genetic diversity of various species. They have been used for assessment of diversity in highland bananas from the National Banana Germplasm Collection (Nsabimana & Van Staden, 2007), study of genetic polymorphism among camelina germplasm (Vollmann *et al*., 2005), and analysis of variation among *Hordeum spontaneum* accessions (Karsai *et al*. 2004). Recently, this marker was used for assessment of clonal fidelity of the regenerated plants of *Capparis decidua* (Tyagi *et al.*, 2010). For coffee, this marker was also used for studying of genetic diversity between cultivated and wild accession of *C. arabica* (Lashermes *et al*., 1996), and to study of genetic polymorphism among *C. arabica* cultivar (Sera *et al*., 2003).

Moreover, assessment of genetic diversity through AFLP markers was reported for various crops, such as *Guizotia abyssinica* (L.f.) Cass (Geleta *et al*., 2008) and tetraploid switchgrass (Todd *et al*., 2011). Moreover these markers were also used to study the genetic variation and relationships of pedigree-known oat, wheat, and barley cultivars (Fu *et al*., 2006) and in accessions from the different cultivar groups and origins in the tree tomato (Acosta-Quezada *et al*., 2012). For coffee, AFLP marker was succesfully used for assessment of genetic diversity for *C. arabica* cultivars. Using 10 AFLP markers, 28 genotypes were independently distinguished and did not cluster according to collection region (Dessalegn *et al*., 2008).

Recently, studies of crop genetic diversity were carried out by using SSR markers, for example the genetic diversity has been assessed with SSRs for closely related wheat cultivars (Wang *et al*., 2007). This marker has also been reported to study the genetic diversity of Chinese spring soybean germplasm (Wang *et al*., 2008), genetic characterization and species relationships among selected Asiatic *Vigna* Savi (Vir *et al*., 2010), assessment of genetic diversity and relationships of upland rice accessions (Tang *et al*., 2010), genetic polymorphism in cassava (Montero-Rojas *et al*., 2011), genetic structure and relationships within and between cultivated and wild sorghum (*Sorghum bicolor* (L.) Moench) (Mutegi *et al*., 2011), and genetic structure and diversity analysis in *Vitis vinifera* (Doulati-Baneh *et al*., 2013). Futhermore, Inter-simple sequence repeats (ISSR) markers has been used for evaluation of genetic diversity in Brazilian cultivated *Jatropha curcas* L. accessions (Grativol *et al*., 2011), estimation of genetic diversity in some Iranian wild *Prunus* subgenus *Cerasus* accessions (Shahi-Gharahlar *et al*., 2011) and estimation of genetic diversity in some Iranian cornelian cherries (*Cornus mas* L.) accessions (Hassanpour *et al*., 2013).

Moreover, SSR markers have been developed in *Coffea* and some of them have been evaluated among *Coffea* species. SSR markers used in coffee species have shown an average polymorphism information content (PIC) of 0.6, 0.3 and 0.22 for diploid species, wild tetraploids species and cultivated tetraploid species, respectively (Moncada & McCouch, 2004). Futhermore, ISSR has been used genetic diversity for *C. arabica* (Ruas *et al*., 2003). Deeply, the genetic diversity of the Indonesian *C. canephora* collection was studied using 19 microsatellite (SSR) markers for 1886 accessions. However, due to possible gene introgressions of other *Coffea* species and also presence of duplicates, only 1382 accessions were finally kept for the analysis. Genetic structure study identified three genetic groups in the collection. Robusta accessions controls included in this study have allowed the identification of two of these three groups as SG1 and SG2. The third genetic group identified appears to be unique with a low level of heterozygosity. According to Indonesian Coffee and Cacao Research Institute archives, this new group was originated from Republic Democratic Congo and closer to the location of group SG2. Principal Component Analysis clearly differentiates this group among the two others (Sumirat *et al*., 2012). Moreover, SNPs have also been applied for genetic diversity in some crops such as *Zea mays*, *Glycine max* (Rafalski, 2002), and *Oryza sativa* (Liu & Zhang, 2006).

Using only one type of marker to quantify genetic diversity generates results that have been questioned in terms of reliability, when compared to the combined use of different markers. Some study of genetic diversity using different types of nuclear genome markers were reported for some crops. The RFLP and RAPD markers have been used simultaneously for analysing the genetic diversity of *Coffea* acessions (Paillard *et al*., 1993).

On the contrary, the study of dispersal of durum wheat landraces across the Mediterranean basin has been carried out using the combination of AFLP and SSR marker (Moragues *et al*., 2007).Their results support two dispersal patterns of durum wheat in the Mediterranean basin, one through its North side and a second one through its South side. For coffee, these marker combinations were applied to reveal polymorphism profile in a *Coffea* interspecific backcross progeny (Hamon *et al*., 2005).

To compare the efficiency of the use of single versus multiple markers, Leal and colleagues quantified genetic diversity among 10 S7 inbred popcorn lines using both RAPD and SSR markers (Leal *et al*., 2010). When comparing the groups formed using SSR and RAPD markers, there were similarities in the combinations of genotypes from the same genealogy. Correlation between genetic distances obtained through RAPD and SSR markers was relatively high (0.5453), indicating that both techniques are efficient for evaluating genetic diversity. In case of popcorn, the use of RAPD has generated more polymorphisms than SSR markers (Leal *et al*., 2010). Another example was reported for study of genetic diversity analysis of elite pearl millet inbred lines. They showed that the cluster and principal component analysis of the combined dataset from RAPD and SSR markers indicated moderate genetic divergence among the elite pearl millet germplasm, besides unraveling the genetic relationships among the male sterile lines and the restorers (Chandra-Shekara *et al*., 2007).

The using of combination RAPD and ISSR markers was used for genetic study of some crops, for example on Greek *Aegilops* species (Thomas & Bebeli ,2010). In conclusion, their results showed that there is genetic diversity in the Greek *Aegilops* species studied, and clustering based on genetic similarities was in agreement with botanical classifications. Futhermore, the evaluation of genetic divergence among accessions of elephant grass has been reported (de Lima *et al*., 2011). In this report, they showed the correlation of 0.76 between the genetic distances achieved by the RAPD and ISSR markers, which is highly significant by the Mantel test. Based on UPGMA grouping, considering the point of sudden change, five and six groups were formed for the data from the RAPD and ISSR markers, respectively. Both markers provided partially concordant groups, indicating that these techniques can provide consistent information and consequently could be used in studies of genetic diversity among accessions.

Finaly, sequence-related amplified polymorphism (SRAP) markers were used for genetic study. This type of markers was used in combination with RAPD to study the molecular variation of sugarcane smut, *Sporisorium scitamineum* in Mainland China (Que *et al*., 2012). Results of RAPD, SRAP, and RAPD-SRAP combined analysis showed that, whereas the molecular variation of *S. scitamineum* was associated with geographic origin, there was no evidence of co-evolution between sugarcane and the pathogen. Complementary result from these combinations of markers also did not provide any information about race differentiation of *S. scitamineum*. This suggests that the mixture of spores from sori collected from different areas should be used in artificial inoculations for resistance breeding and selection (Que *et al*., 2012). Moreover, SRAP markers were also used in combination with ISSR to study the genetic diversity analysis of *Prunella vulgaris* in China (Liao *et al*., 2012). The main result proposed that all the 26 *P. vulgaris* populations possessed high levels of genetic diversity. ISSR markers showed a slightly higher proportion of polymorphic loci (PPL = 89.19%) than did the SRAP markers (PPL = 87.93%). The results also showed that the 26 populations could be clustered into three groups by cluster analysis, which matched the geographic distribution of this species. The data indicated that ISSR and SRAP are both reliable and effective tools for analyzing genetic diversity in *P. vulgaris* (Liao *et al*., 2012). In specific case of Arabica coffee, SRAP markers were recently utilized to discriminate the profile of parents and hybrids (Mishra *et al*., 2011). As generally known, the phenotypic as well as genetic variability has been found low because of the narrow genetic basis and self fertile of *Coffea arabica*. Due to this high similarity in phenotypic appearance, selection of parental lines for inter-varietal hybridization and hybrids was difficult (Mishra *et al*., 2011).

Conclusion and Prospective

Due to advances in molecular biology techniques, large numbers of highly informative DNA markers have been developed for the fingerprinting and identification of genetic polymorphism. This technique has been developed for plant breeding in many laboratories. Specifically in coffee, previous and on going researches has been conducted using these molecular markers. According this aspect, in previous collaboration with Nestle R&D Centre Tours, Indonesian Coffee and Cocoa Research Institute (ICCRI) has selected the Robusta coffee parental clones based on RFLP probes. Present collaboration, by the same research institute, the evaluation of the genetic diversity of more than two thousand coffee accestion by using microsatelite marker is ongoing research. This present result will be used to construct the Coffee Core Collection. This review is expected to assist breeders in order to elaborate the best strategy to manage the genetic diversity according to the molecular and phenotypic data. To conclude, it will also be used to guide the breeding program of *C. canephora* in Indonesia.

.References

Acosta-Quezada P, S Vilanova, J Martínez-Laborde & J Prohens (2012). Genetic diversity and relationships in accessions from different cultivar groups and origins in the tree tomato (*Solanum betaceum* Cav.). *Euphytica* 187, 87-97.

Aggarwal R, P Hendre, R Varshney, P Bhat, V Krishnakumar & L Singh (2007). Identification, characterization and utilization of EST-derived genic microsatellite markers for genome analyses of coffee and related species. *Theor Appl Gen,* 114**,** 359-372.

Alikhani L, MS Rahmani, N Shabanian, H Badakhshan & A Khadivi-Khub (2014). Genetic variability and structure of *Quercus brantii* assessed by ISSR, IRAP and SCoT markers. *Gene* 552**,** 176-183.

Anthony F, M Combes, C Astorga, B Bertrand, G Graziosi & P Lashermes (2002). The origin of cultivated *Coffea arabica* L. varieties revealed by AFLP and SSR markers. *Theor Appl Genet* 104**,** 894-900.

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

Chandra-Shekara AC, BM Prasanna, SR Bhat & BB Singh (2007). Genetic diversity analysis of elite pearl millet inbred lines using RAPD and SSR markers. *J Plant Biochem Biotechnol* 16**,** 23-28.

Cubry P, P Musoli, H Legnaté, D Pot, F de Bellis, V Poncet, F Anthony, M Dufour & T Leroy (2008). Diversity in coffee assessed with SSR markers: structure of the genus *Coffea* and perspectives for breeding. *Genome,* 51**,** 50-63.

de Lima RSN, RF Daher, LSA Gonçalves, DA Rossi, AT do Amaral Júnior, MG Pereira & FJS Lédo (2011). RAPD and ISSR markers in the evaluation of genetic divergence among accessions of elephant grass. *Genet. Mol Res* 10, 1304-1313.

Dessalegn Y, L Herselman & MT Labuschagne (2008) AFLP analysis among Ethiopian Arabica coffee genotypes. *African J Biotechnol* 7 (18), 3193-3199.

Doulati-Baneh H, SA Mohammadi & M Labra (2013). Genetic structure and diversity analysis in *Vitis vinifera* L. cultivars from Iran using SSR markers. *Scientia Horticult* 160, 29-36.

Fang P, S Niu, H Yuan, Z Li, Y Zhang, L Yuan & W Li (2014). Development and characterization of 25 EST-SSR markers in *Pinus sylvestris* var. mongolica (Pinaceae). *Appl in Plant Sci* 2(1), apps.1300057.

Fu YB, WG Legge, JM Clarke & KW Richards (2006). Genetic variation and relationships of pedigree-known oat, wheat, and barley cultivars releaved by bulking and single-plant sampling. *Genet Res and Crop Evolution,* 53, 1153-1164.

Garcia D, N Carels, DM Koop, LA de Sousa, SJ d Andrade Junior, V Pujade-Renaud, CR Reis Mattos & JC de Mattos Cascardo (2011). EST profiling of resistant and susceptible *Hevea* infected by *Microcyclus ulei*. *Physiol Mol Plant Pathol* 76, 126-136.

Geleta M, T Bryngelsson, E Bekele & K Dagne (2008). Assessment of genetic diversity of *Guizotia abyssinica* (L.f.) Cass. (Asteraceae) from Ethiopia using amplified fragment length polymorphism. *Plant Genet Res* 6, 41-51.

Geleta M, I Herrera, A Monzón & T Bryngelsson (2012). Genetic Diversity of Arabica Coffee (*Coffea arabica* L.) in Nicaragua as estimated by simple sequence repeat markers. *The Sci World J,* 2012**,** 11. 802607.

Grativol C, C da Fonseca Lira-Medeiros, A Hemerly & P Ferreira (2011). High efficiency and reliability of inter-simple sequence repeats (ISSR) markers for evaluation of genetic diversity in Brazilian cultivated *Jatropha curcas* L. accessions. *Mol Biol Rep* 38, 4245-4256.

Grover A & PC Sharma (2014). Development and use of molecular markers: past and present. *Critical Rev in Biotechnol*, 1-13.

Guo D, J Zhang, C Liu, G Zhang, M Li & Q Zhang (2012). Genetic variability and relationships between and within grape cultivated varieties and wild species based on SRAP markers. *Tree Genet & Genom* 8, 789-800.

Ha WY, PC Shaw, J Liu, FCF Yau & J Wang (2002). Authentication of *Panax ginseng* and *Panax quinquefolius* using amplified fragment length polymorphism (AFLP) and directed amplification of minisatellite region DNA (DAMD). *J Agricult Food Chem* 50, 1871-1875.

Hamon P, I Coulibaly & B Revol (2005). AFLP and SSR polymorphism in a *Coffea* interspecific backcross progeny ((*Coffea heterocalyx* x *C. canephora*) x *C. canephora*). *In*: *Proc 20th Internat Conf on Coffee Sci,* 11-15 October 2004, Bangalore, India. p. 699-701.

Hassanpour H, Y Hamidoghli & H Samizadeh (2013). Estimation of genetic diversity in some Iranian cornelian cherries (*Cornus mas* L.) accessions using ISSR markers. *Biochem Systems Ecol* 48, 257-262.

Hendre P, R Phanindranath, V Annapurna, A Lalremruata & R Aggarwal (2008). Development of new genomic microsatellite markers from robusta coffee (*Coffea canephora* Pierre ex A. Froehner) showing broad cross-species transferability and utility in genetic studies. *BMC Plant Biol* 8, 51.

Hendre PS & RK Aggarwal (2007). DNA markers: development and application for genetic improvement of Coffee. *In*: R. Varshney & R. Tuberosa (eds) *Genomics-Assisted Crop Improvement.* Netherlands. Springer, p. 399-434.

Hunter RL & CL Markert (1957). Histochemical demonstration of enzymes separated by zone electro-phoresis in starch gels. *Sci* 125, 1294-1295.

Jeffreys AJ, V Wilson & SL Thein (1985). Hypervariable `minisatellite' regions in human DNA. *Nature* 314, 67-73.

Jiang GL (2013). *Molecular Markers and Marker-Assisted Breeding in Plants*. New York.

Joshi K, P Chavan, D Warude & B Patwardhan (2004). Molecular markers in herbal drug technology. *Curr Sci* 87, 159-165.

Kantety RV, X Zeng, JL Bennetzen & BE Zehr (1995). Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbred lines using inter-simple sequence repeat (ISSR) amplification. *Mol Breed* 1, 365-373.

Karsai I, PM Hayes, J Kling, IA Matus, K Mészáros, L Láng, Z Bedő & K Sato (2004). Genetic variation in component traits of heading date in *Hordeum vulgare* subsp. spontaneum accessions characterized in controlled environments. *Crop Sci* 44, 1622-1632.

Kesawat M & B Das Kumar (2009). Molecular markers: It's application in crop improvement. *J Crop Sci Biotechnol* 12, 169-181.

Kumar P, VK Gupta, AK Misra, DR Modi & BK Pandey (2009). Potential of molecular markers in plant biotechnology. *Plant Omics* 2, 141-162.

Lander ES & D Botstein (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genet* 121, 185-199.

Lashermes P, P Trouslot, F Anthony, MC Combes & A Charrier (1996) Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. *Euphytica* 87, 59-64.

Leal AA, CA Mangolin, AT do Amaral Júnior, LS A Gonçalves, CA Scapim, AS Mott, IBO Eloi, V Cordovés & MFP da Silva (2010). Efficiency of RAPD versus SSR markers for determining genetic diversity among popcorn lines. *Genet Mol Res* 9, 9-18.

Li G & CF Quiros (2001). Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. *Theor Appl Genet* 103, 455-461.

Li Y, X Fan, T Shi, Q Zhang & Z Zhang (2009) SRAP marker reveals genetic diversity in tartary buckwheat in China. *Frontiers of Agricul in China,* 3**,** 383-387.

Liao L, Q Guo, ZY. Wang, L Liu & ZB Zhu (2012). Genetic diversity analysis of *Prunella vulgaris* in China using ISSR and SRAP markers. *Biochem System and Ecol* 45, 209-217.

Lidah YL, ASP N'Guetta, M Fanjavola, A Clément-Demange & GM Rodier (2006). Etude par les isozymzs de la pollination libre de génotypes d'hévéas sauvages en verger à graines. *Biotechnol Agr Soc et Environ* 10, 209-216.

Liu CG & GQ Zhang (2006). Single nucleotide polymorphism and its application. *Yi Chuan,* 28, 737-744.

Lopes T, G Pinto, J Loureiro, A Costa & C Santos (2006). Determination of genetic stability in long-term somatic embryogenic cultures and derived plantlets of cork oak using microsatellite markers. *Tree Physiol* 26, 1145-1152.

Malik W, J Ashraf, MZ Iqbal, A Ali Khan, A Qayyum, M Ali Abid, E Noor, M Qadir Ahmad & G Hasan Abbasi (2014) Molecular markers and cotton genetic improvement: current status and future prospects. *The Sci World J* 2014, 15.

Mishra MK, N Suresh, AM Bhat, N Suryaprakash, SS Kumar, A Kumar & Jayarama (2011). Genetic molecular analysis of *Coffea arabica* (Rubiaceae) hybrids using SRAP markers. *Rev de Biol Trop* 59, 607-617.

Mmeka EC, AI Adesoye, BI Vroh & KI Ubaoji (2013). Single nucleotide polymorphism (SNP) markers discovery within *Musa* spp (plantain landraces, AAB genome) for use in beta carotene (Provitamin A) trait mapping. *American J Biol and Life Sci* 1, 11-19.

Moncada P & S McCouch (2004). Simple sequence repeat diversity in diploid and tetraploid *Coffea* species. *Genome,* 47, 501-509.

Montero-Rojas M, AM Correa & D Siritunga (2011). Molecular differentiation and diversity of cassava (*Manihot esculenta*) taken from 162 locations across Puerto Rico and assessed with micro-satellite markers. *AoB Plants,* 2011.

Moragues M, M Moralejo, M Sorrells & C Royo (2007). Dispersal of durum wheat [*Triticum turgidum* L. ssp. turgidum convar. durum (Desf.) MacKey] landraces across the Mediterranean basin assessed by AFLPs and microsatellites. *Genet Res Crop Evolution,* 54, 1133-1144.

Morel E, L Bellanger, F Lefebvre-Pautigny, C Lambot & D Crouzillat (2012). DNA trace-ability for variety purity in Nespresso Product. *In*: *Proc 24th Int Conf on Coffee Sci Costa Rica*, p. 1446-1450.

Muñoz-Falcón JE, S Vilanova, M Plazas & J Prohens (2011) Diversity, relationships, and genetic finger-printing of the Listada de Gandía eggplant landrace using genomic SSRs and EST-SSRs. *Sci Horticult* 129, 238-246.

Mutegi E, F Sagnard, K Semagn, M Deu, M Muraya, B Kanyenji, S de Villiers, D Kiambi, L Herselman & M Labuschagne (2011). Genetic structure and relationships within and between cultivated and wild sorghum (*Sorghum bicolor* (L.) Moench) in Kenya as revealed by microsatellite markers. *Theor Appl Genet* 122, 989-1004.

Muzila M, G Werlemark, R Ortiz, J Sehic, M Fatih, M Setshogo, W Mpoloka & H Nybom (2014). Assessment of diversity in *Harpagophytum* with RAPD and ISSR markers provides evidence of introgression. *Hereditas,* 151, 91-101.

Nsabimana A & J Van Staden (2007). Assessment of genetic diversity of highland bananas from the national banana germplasm collection at Rubona, Rwanda using RAPD markers. *Sci Horticult,* 113, 293-299.

Paillard M, N Duchateau & V Petiard (1993). Diversite genetique de quelques groupes de cafeiers: utilisation des outils moleculaires, RFLP et RAPD. *In: Proc 15th Int Conf on Coffee Sci* Montpellier.

Pinheiro PV, JC d Faria, EOP e L Nogueira & FJL Aragão (2009). Transgene inheritances and genetic similarities of near isogenic lines of genetically modified common beans. *Pesquisa Agropecuária Brasileira,* 44, 1168-1176.

Podio M, MP Rodríguez, S Felitti, J Stein, EJ Martínez, LA Siena, CL Quarin, SC Pessino & JPA Ortiz (2012). Sequence characterization, in silico mapping and cytosine methylation analysis of markers linked to

apospory in *Paspalum notatum*. *Genet Mol Biol* 35, 827-837.

Poncet V, M Dufour, P Hamon, S Hamon, A de Kochko & T Leroy (2007). Development of genomic microsatellite markers in *Coffea cane-phora* and their transferability to other coffee species. *Genome,* 50, 1156-1161.

Poncet V, P Hamon, J Minier, C Carasco, S Hamon & M Noirot (2004). SSR cross-amplification and variation within coffee trees (*Coffea* spp.). *Genome,* 47, 1071-1081.

Priyono, A Budiani, S Mawardi & Siswanto (2005) Confirmation of transgenic Robusta coffee (*Coffea canephora*) transformed by chitinase-encoding gene and its propagation through somatic embryogenesis. *Pelita Perkebunan,* 21, 73-89.

Priyono, AM Henry, A Deshayes, B Purwadi & S Mawardi (1999). The polymorphism level of *Coffea canephora* in several clones couples, restriction enzymes, and probes sources. *Pelita Perkebunan,* 15, 152-161.

Priyono, AM Henry, A Deshayes, B Purwadi & S Mawardi (2000). Heterozygosity and segre-gating pattern estimation of *Coffea canephora* using RFLP technique. *Pelita Perkebunan,* 16, 1-10.

Que Y, L Xu, J Lin, R Chen & MP Grisham (2012). Molecular variation of *Sporisorium scitamineum* in mainland China revealed by RAPD and SRAP markers. *Plant Dis* 96, 1519-1525.

Rafalski A (2002) Applications of single nucleotide poly-morphisms in crop genetics. *Curr Opinion in Plant Biol* 5, 94-100.

Robarts DWH & AD Wolfe (2014). Sequence-related amplified polymorphism (SRAP) markers: A potential resource for studies in plant molecular biology. *Appl in Plant Sci* 2, 1400017.

Ruas PM, CF Ruas, L Rampim, VP Carvalho, EA Ruas & T Sera (2003). Genetic relationship in *Coffea* species and parentage determination of interspecific hybrids using ISSR (Inter- Simple Sequence Repeat) markers. *Genet Mol Biol* 26, 319-327.

Sabir JSM, S Abo-Aba, S Bafeel, TA Zari, S Edris, AM Shokry, A Atef, NO Gadalla, AM Ramadan, MA Al-Kordy, FM El-Domyati, RK Jansen & A Bahieldin (2014). Characterization of ten date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia using AFLP and ISSR markers. *Comptes Rendus Biol* 337, 6-18.

Schlotterer C (2004). The evolution of molecular markers - just a matter of fashion? *Nat Rev Genet* 5, 63-69.

Semagn K, A Bjornstad & MN Ndjiondjop (2006). An overview of molecular marker methods for plants. *African J Biotechnol* 5, 2540-2568.

Sera T, PM Ruas, CdF Ruas, LEC Diniz, VdP Carvalho, L Rampim, EA Ruas & SRd Silveira (2003). Genetic polymorphism among 14 elite *Coffea arabica* L. cultivars using RAPD markers associated with restriction digestion. *Genet Mol Biol* 26, 59-64.

Shahi-Gharahlar A, Z Zamani, R Fatahi & N Bouzari (2011). Estimation of genetic diversity in some Iranian wild *Prunus* subgenus *Cerasus* accessions using inter-simple sequence repeat (ISSR) markers. *Biochem System Ecol* 39, 826-833.

Shirasawa K, S Isobe, S Tabata & H Hirakawa (2014). Kazusa Marker DataBase: a database for genomics, genetics, and molecular breeding in plants. *Breed Sci* 64, 264-271.

Silveira SR, PM Ruas, CdF Ruas, T Sera, VdP Carvalho & ASG Coelho (2003). Assessment of genetic variability within and among coffee progenies and cultivars using RAPD markers. *Genet Mol Biol* 26, 329-336.

Sim SC, JK Yu, Yk Jo, ME Sorrells & G Jung (2009). Transferability of cereal EST-SSR markers to ryegrass. *Genome* 52, 431-437.

Southern EM (1975). Detection of specific sequences among DNA fragments separated by gel electro-phoresis. *J Mol Biol* 98, 503-517.

Spackman ME, FC Ogbonnaya & JS Brown (2010). Hypervariable RAPD, ISSR and SSR markers generate robust taxonomic groups among *Puccinia striiformis* formae speciales of importance to Australian agriculture. *Australian Plant Pathol* 39, 226-233.

Steinmacher DA, NG Krohn, ACM Dantas, VM Stefenon, CR Clement & MP Guerra (2007). Somatic embryogenesis in peach palm using the thin cell layer technique: induction, morpho-histological aspects and AFLP Analysis of somaclonal variation. *Ann Bot* 100, 699-709.

Sumirat U, L Bellanger, V Lanthoene, S Mawardi, D Nugroho, Priyono, T Wahyudi, P Broun, C Lambot & D Crouzillat (2012) Genetic diversity assesment in Indonesian *Coffea canephora* collection using SSR markers. *In*: *Proc 24th Int Conf on Coffee Sci* Costa Rica, 1354-1358.

Tang S, Y Zhang, L Zeng, L Luo, Y Zhong & Y Geng (2010). Assessment of genetic diversity and relation-ships of upland rice accessions from southwest China using microsatellite markers. *J Plant Biosystems* 144 (1), 85-92.

Thomas KG & PJ Bebeli (2010). Genetic diversity of Greek Aegilops species using different types of nuclear genome markers. *Mol Phylogenet Evolution* 56, 951-961.

Todd J, YQ Wu, Z Wang & T Samuels (2011). Genetic diversity in tetraploid switchgrass revealed by AFLP marker polymorphisms. *Genet Mol Res* 10, 2976-2986.

Tourmente S, A Lazreg, J Lafleuriel, S Tutois, C Cuvillier, MC Espagnol & G Picard (1998). Identification of new minisatellites loci in *Arabidopsis thaliana*. *J Experiment Bot* 49, 21-25.

Tyagi P, S Khanduja & SL Kothari (2010). In vitro culture of *Capparis decidua* and assessment of clonal fidelity of the regenerated plants. *Biol Planta* 54, 126-130.

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

Vir R, T Jehan, KV Bhat & S Lakhanpaul (2010). Genetic characterization and species relationships among selected Asiatic Vigna Savi. *Genet Res and Crop Evolution* 57, 1091-1107.

Vollmann J, H Grausgruber, G Stift, V Dryzhyruk & T Lelley (2005). Genetic diversity in camelina germplasm as revealed by seed quality charac-teristics and RAPD polymorphism. *Plant Breed* 124, 446-453.

Wang HY, YM Wei, ZH Yan & YL Zheng (2007). EST-SSR DNA polymorphism in durum wheat (*Triticum durum* L.) collections. *J Appl Genet* 48, 35-42.

Wang LX, RX Guan, YH Li, FY Lin, WJ Luan, W Li, YS Ma, ZX Liu, RZ Chang & LJ Qiu (2008). Genetic diversity of Chinese spring soybean germplasm revealed by SSR markers. *Plant Breed* 127, 56-61.

Wang S, Y Liu, L Ma, H Liu, Y Tang, L Wu, Z Wang, Y Li, R Wu & X Pang (2014). Isolation and characterization of microsatellite markers and analysis of genetic diversity in Chinese Jujube (*Ziziphus jujuba* Mill.). *PLoS ONE* 9, e99842.

Welsh J & M McClelland (1990). Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res* 18, 7213-7218.

Yeboah MA, C Xuehao, CR Feng, G Liang & M Gu (2007). A genetic linkage map of cucumber (*Cucumis sativus* L) combining SRAP and ISSR markers. *African J Biotechnol* 6, 2784-2791.

Zhang P, X Liu, H Tong, Y Lu & J Li (2014a) Association mapping for important agronomic traits in core collection of rice (*Oryza sativa* L.) with SSR markers. *PLoS ONE,* 9**,** e111508.

Zhang W, D Tian, X Huang, Y Xu, H Mo, Y Liu, J Meng & D Zhang (2014b). Characterization of flower-bud transcriptome and development of genic SSR markers in Asian Lotus (*Nelumbo nucifera* Gaertn.). *PLoS ONE,* 9, e112223.

Zhang W, H He, Y Guan, H Du, L Yuan, Z Li, D Yao, J Pan & R Cai (2010). Identification and mapping of molecular markers linked to the tuberculate fruit gene in the cucumber (*Cucumis sativus* L.). *Theor Appl Genet* 120, 645-654.