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Review Article

Review on Coffee (*Coffea arabica* L.) Genetic Diversity Studies Using Molecular Markers

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Abstract: Coffee (*Coffea arabica* L.) is one of the most consumed beverages in the world and is the second largest traded commodity after petroleum. It greatly contributes to the economy of many developing countries in South and Central America, Asia, and Africa. Currently, many countries are producing and supplying coffee to the world market and there is a high competition among producers to meet the demand both in quantity and quality. Hence, it is vital to improve the current productivity as well as the quality of coffee for each producing country. In this regard, variability study is a pre-requisite for improvement of any crop and knowledge of the genetic variability among genotypes is important for the transfer of useful genes and to maximize the use of available germplasm resources. Hence, genetic diversity analysis in coffee (*Coffea arabica* L.) has paramount importance in continuously developing coffee varieties through hybridization and selection. Morphological markers allow the assessment of genetic variability based on individual phenotypic differences yet there are limitations associated with these markers. These limitations led to the development of molecular markers. Molecular marker techniques are based on naturally occurring polymorphisms in DNA sequences. Studies, which have been conducted to assess the level of genetic diversity of *Coffee arabica* L. using different DNA markers (RAPD, AFLP, ISSR, and SSR), demonstrated that all DNA marker techniques could be applied for measuring the degree of variability within, and between wild and cultivated coffee populations. Despite all the four DNA markers are efficient in coffee genetic diversity analysis, all have their own disadvantages. However, the previous studies showed that the polymorphism observed in coffee Arabica is generally low as compared to other diploid coffee species and this is probably the result of a narrow genetic basis of the cultivated coffee (*Coffea arabica* L.) as well as the mating system of the crop. Hence, it is recommended to use the observed variability for the coffee breeding program to improve the yield and quality of coffee.

Keywords: Coffea Arabica, Molecular Markers, Polymorphisim, Simple Sequence Repeat Markers, Variability

1. Introduction

Coffea arabica L. is an amphidiploids species (2n=4x= 44) [1] native to the highlands of Southwest Ethiopia (Sylvain, 1955). It is the only polyploidy coffee species and is self-fertile at approximately 90% [2] while other species are generally self-incompatible.

Knowledge of genetic diversity within and among genotypes of any crop is fundamental for estimating the potential of genetic gain in a breeding program and for effective conservation of available genetic resources [3]. It may also be important for selecting promising parental lines in hybrid variety development [4]. Currently, different methods such as morphological and molecular markers are available for the estimation of genetic diversity within and among genotypes. Morphological markers while still useful in plant breeding have some limitations because they are influenced by the environment and limited in number. Besides, they require lengthy and expensive evaluation during the whole growth stage, especially in perennial plants like coffee [5-6].

Nevertheless, advances in biochemistry and molecular biology during the past few decades have helped to overcome these constraints by providing breeders with many powerful

molecular markers. DNA marker is a small region of DNA sequence showing polymorphism (base deletion, insertion, and substitution) between different individuals. Various molecular approaches have been devised in many plants using DNA based markers for the detection of genetic diversity. These markers are generally independent of environmental factors and are more numerous than phenotypic characters providing clear information of underlying variation in the genome of an organism. The DNA- based marker systems are generally classified as hybridization-based (non-PCR) and PCR-based markers [7-9].

Hybridization-based /Non-PCR based / genetic markers: DNA-based marker systems may be described as hybridization-based makers when the DNA profiles are visualized by hybridizing fragments to labeled probes. The restriction fragment length polymorphisms (RFLPs) marker technology is the first generation of DNA markers and one of the best for plant genome mapping. These variations are co-dominantly inherited. The markers became possible as a result of the discovery of restriction endonucleases, bacterial enzymes with characteristic restriction, or recognition sites (cleave DNA only at sections where a unique and recognizable nucleotide sequence occurs, e g., AATT) [10]. In Arabica coffee, RFLP markers were employed to identify the origin of the *C. arabica* genome. By comparing the RFLP patterns of wild diploid species with those of *C. arabica*, [1] concluded that the *C. arabica* genome was formed by hybridization between *C. eugenioides* and *C. canephora*, or ecotypes related to these diploid species.

PCR-based DNA markers: With the development of the polymerase chain reaction (PCR), many PCR-based DNA molecular techniques have been, and still are being developed for plant genome analysis. These techniques include RAPD (random amplified polymorphic DNA) [11], AFLP (amplified fragment length polymorphism) [12]; ISSR (inter-simple sequence repeat) [13], and SSR (simple sequence repeat) or microsatellite [14]. A series of molecular marker types are available for plant genotyping, but no single technique is generally applicable to the wide range of questions in plant genome analysis; each available technique exhibits both assets and drawbacks, thus, a decision is needed on which marker system should be used for which research aim. The marker types differ in information content, number of scorable polymorphisms per reaction, and degree of automation. In addition, the choice of method often depends on the genetic resolution needed as well as on technological and financial constraints [15]. Although genetic diversity analysis using molecular marker techniques is reliable and was done on several plant species; such types of studies are rare on Arabica coffee. Besides, the available information on the limited research done on Arabica coffee is not compiled comprehensively. Therefore, the present review was aimed at compiling coffee genetic diversity studies conducted using RAPD, AFLP, ISSR, and SSR markers.

2. Some Genetic Diversity Studies of Arabica Coffee (*Coffea arabica* L.) Using Molecular Markers

The results of genetic diversity studies conducted on coffee Arabica using RAPD, AFLP, ISSR, and SSR markers are discussed in the following section.

2.1. Randomly-Amplified Polymorphic DNA Marker (RAPD)

It is the first PCR-based molecular marker [16]. The randomly-amplified polymorphic DNA marker (RAPD) detects nucleotide sequence polymorphism in DNA by using a single primer of arbitrary nucleotide sequence (Oligonucleotide primer, mostly ten bases long) [16]. In the reaction, a single species of primer anneals to the genomic DNA at two different sites on complementary strands of DNA template. The disadvantage is its dominant nature, relatively short primers and lack of reputability. RAPD markers have a very high genomic abundance, a fast and efficient method. RAPDs are also commercially available and no sequence data is required for prime construction [17]. RAPD markers were used to study the genetic diversity among wild *Coffea arabica* L. accessions [18-20] and among cultivated Arabica coffee accessions [21-25]. Moreover, RAPD plus restriction digestion was also employed in detecting the variability of cultivated Arabica coffee [26-28].

The genetic diversity of 88 spontaneous and sub-spontaneous accessions, six cultivars of C. arabica collected from different parts of Ethiopia and two accessions derived from the populations of Typica and Bourbon were studied using RAPD-markers and the the result showed that Typica and Bourbon-derived accessions were differentiated from Ethiopian materials. However, they did not observe within-accession polymorphism and confirmed the low level of polymorphism in C. Arabica [18]. Similarly, low to moderate polymorphism was also reported by the researchers who investigated the genetic diversity among 16 forest coffee populations of four important coffee-growing zones of Ethiopia (Wollega, Jimma, Illu-Abobora and Bale) using RAPD markers [19].

Apart from the forest coffee, RAPD markers were also used to study the variability among the cultivated varieties. In this regard, investigators reported the existence of a narrow genetic base in commercial cultivars of C. Arabica and they have also confirmed that the RAPD markers were effective in detecting the variability in Arabica coffee as well as in classifying the accessions but were not able to distinguish the cultivars belonging to the same group, either Bourbon or Typica [21]. Similarly, researchers reported that RAPD markers were able to make distinctions among eight coffee Arabica commercial cultivars and/or lines [23]. The contradictory results might have been attributed to the variation in the materials used and also the number of RAPD markers used for the studies. The low genetic diversity of Arabica coffee was further confirmed by the study conducted

on coffee Arabica inbreed lines widely grown in Brazil and on commercial coffee varieties, advanced selections, and museum collections in Kenya using RAPD markers [24-25].

However, despite most of the above studies showing low variability, higher polymorphism were reported in Tanzanian cultivated Arabica coffee accessions and in the Colombian replica of FAO Ethiopian wild and semi-wild coffee germplasm collections [20-21]. The inconsistencies observed in the degree of polymorphism of RAPD markers in the above studies may be attributed to the variations in the number of genotypes, number of primers, and fragment scoring methods used for the studies.

Furthermore, the capacity of the RAPD marker in detecting variability among the coffee accessions can further be enhanced through the use of a combination technique. For example, in genetic diversity studies conducted in Tanzania and in Brazil, it was proved that the combination of the techniques was very efficient for the estimation of genetic relationship among *C. arabica* genotypes and the ability to estimate viability among coffee accessions was greater for RAPD plus restriction digestion than for RAPD alone [26-28].

Therefore, from the above studies, it can be concluded that RAPD markers appear to be very effective in revealing the genetic variation in *C. arabica*. Hence, testing the polymorphism level of RAPD markers under uniform sample size, primers, and fragment scoring methods is essential. Besides, RAPD associated with a prior digestion of genomic DNA with restriction digestion is vital.

2.2. Amplified Fragment Length Polymorphic DNA (AFLP) Analysis

Amplified fragment length polymorphism (AFLP) technology was developed by the Dutch company, Keygene due to reproducibility limitations associated with RAPD [12]. It is essentially an intermediate between RFLPs and PCR. AFLP refers to molecular markers obtained by selective PCR amplification of restriction fragments [29]. The major disadvantage of AFLP markers is that these are dominant markers [17]. However, AFLP's strength lies in their high genomic abundance, considerable reproducibility, generation of many informative bands per reaction, wide range of applications, and no sequence data for primer construction are required [12].

In Arabica coffee, AFLP has been used to study to genetic diversity within and among cultivars. In this regard, the results of the genetic diversity of 26 coffee Arabica accessions representing four groups revealed that the AFLP markers allowed the grouping of the Typica-and Bourbon-derived accessions by their genetic origin, confirming the existence of two genetic bases of the cultivated material but the results revealed low genetic variation within groups [30]. Moreover, sixty-one Coffea accessions composed of 58 coffee Arabica accessions, and three diploid species were analyzed with six AFLP primer combinations and the result revealed small differences between and within Arabica cultivars [31]. Furthermore, the low generic variation of Arabica coffee was confirmed with the studies conducted using AFLP markers [24, 32-33].

Hence, despite low genetic variation observed in Arabica coffee similar to other DNA markers, AFLP markers proved to be effective for characterizing the two genetic bases (Typica and Bourbon) and also for detecting the variability within coffee Arabica accessions.

2.3. Inter Simple Sequence Repeat Marker (ISSR)

The Inter-SSR (ISSR) also called MP-PCR (microsatellite-primed PCR) or ISA (Inter-SSR Amplification) or RAMP (Randomly Amplified Microsatellite Amplification) marker system was first developed in 1994 [13]. The ISSR technique is a PCR-based method, which involves the amplification of DNA segments present at an amplifiable distance between two identical microsatellite repeat regions oriented in opposite directions [34]. Inter-SSRs are semi-arbitrary markers amplified by PCR in the presence of one or two primers complementary to a target microsatellite. Similar to RAPD markers, ISSR markers do not require genome sequence information but provide multi-loci amplification [35]. The primers are not proprietary and can be synthesized by anyone. The technique is simple, and quick, and the use of radioactivity is not essential. ISSR markers usually show high polymorphism [36]. However, reproducibility, dominant inheritance, and homology of co-migrating amplification products are the main limitations of ISSRs.

The ISSR markers have also been applied to characterize coffee germplasm in Ethiopia [5, 8, 37-38]. From the study conducted to see the genetic variation of forest coffee trees (*Coffea arabica* L.) on a total of 160 individuals representing 16 populations from four regions of Ethiopia, it was reported that most of the variability was observed between populations than within populations [5]. However, the results genetic diversity studies conducted using ISSR markers on wild coffee of Berhane Kontir and Yayu forest and Harenna Forest of Bale Mountain showed that the higher proportion of genetic diversity was observed within the population rather than between populations [8, 37].

The higher proportion of the within-population genetic variation observed in the previous studies [8] and [37] in contrast to [5] is probably attributed to the high outcrossing rate of wild Arabica coffee populations in the study areas due to the abundance heavy pollinators which increases the variability within the population. The earlier report of higher outcrossing /self-fertility of only 40 to 60% in wild coffees planted at Jimma [39] and the recent study report of 76% of multi-locos outcrossing in wild Arabica coffee [40] confirmed the higher outcrossing rate in wild coffee Arabica. The differences in the number of samples and ISSR primers in each study and differences in coffee management intensity might have contributed to the observed variation between the studies.

Apart from forest coffee, the diversity of cultivated coffee landraces was also confirmed using ISSR markers. In this regard, a high genetic variability was observed within 87 *Coffea arabica* landraces collected from Southern Ethiopia

and this study showed the presence of higher diversity within and among populations of cultivated coffee in the south region [38]. Therefore, the above studies have shown that the ISSR markers are efficient in estimating the genetic diversity of both wild and cultivated coffee Arabica accessions.

2.4. Simple Sequence Repeat (SSR) Markers

SSR markers are ideal genetic markers for detecting differences between and within species of genes of all eukaryotes [41]. It consists of tandemly repeated 2-7 base pair units arranged in repeats of mono-, di-, tri-, tetra, and penta-nucleotides (A, T, AT, GA, AGG, AAAG, etc) with different lengths of repeat motifs. These repeats are widely distributed throughout the plants and animal genomes that display high levels of genetic variation based on differences in the number of tandemly repeating units of a locus. The variation in the number of tandemly repeated units results in highly polymorphic banding patterns [41] which are detected by PCR, using locus-specific flanking region primers where they are known. Some of the prominent features of these markers are that they are dominant fingerprinting markers and co-dominant sequence- tagged microsatellites (STMS) markers [9].

Diversity Among Coffee Species, Between Wild and Cultivated Varieties, and Within Arabica Coffee Genotypes

Microsatellites were applied to compare the diversity among C. arabica, C. canephora and related coffee species [42-46] to investigate polymorphisms among wild and cultivated C. arabica accessions [47-48] and to study the diversity within and between forest coffee populations and Arabica coffee landraces grown in Ethiopia [5-6, 49].

2.4.1. Diversity Among Coffee Species

Thirteen coffee species including *C. arabica and C. canephora* were characterized using 11 SSR markers. These microsatellite primers, however, found little variation in *C. arabica* Similarly, nine SSR markers were developed by the researchers to identify polymorphism in C. arabica, C. canephora, and 17 species of Coffea and the related genera Pilanthus [43]. The results of diversity analysis by these markers also revealed very low polymorphism across the 45 arabica genotypes. Moreover, the genetic diversity of 15 coffee species with particular emphasis on four major species (*C. arabica, C. canephora, C. congensis, and C. liberica*) was studied using 64 SSR markers and the results revealed that *Coffea arabica* showed the lowest diversity whereas *Coffea canephora* appears to be the most diverse [44]. Furthermore, the genetic diversity of coffee representing different genetic origins, Arabica, Robusta, Híbrido de Timor, triploids, and racmosa were compared using SSR markers and result revealed that the highest percentage of polymorphism was observed within the genetic groups of Robusta (89.2%) while the lowest was within Arabica: The lowest genetic diversity within Arabica species was further confirmed and low genetic variation was observed within *C. arabica* genotypes as compared to the diploid species. Besides, the results showed a high genetic resemblance between *C. arabica* and *C. eugenioides* as compared to *C. Canephora*, thus supporting the previous research which confirmed *C. eugeniodes* as the maternal parent of *C. arabica* [45].

The low genetic variability of Arabica coffee as compared to the diploid species is probably the result of a narrow genetic basis of the cultivated coffee, as very few accessions from Yemen and Ethiopia were introduced and are the basis of all breeding programs. Also, *C. arabica* is an autogamos.

2.4.2. Diversity Between Wild and Cultivated Coffee Arabica Varieties

Apart from comparing the diversity of different coffee species, SSR markers have also been used to compare the diversity of sub-spontaneous and cultivated Arabica coffee. Differnt investigators reported that the SSR markers revealed higher polymorphism within the sub- spontaneous-derived accessions than within cultivated accessions [5, 30, 47]. They have identified a total of 28 alleles out of these alleles only eight (28.5%) were identified within each Typica and Bourbon derived accessions but all (100%) the identified alleles in this particular study were observed in the sub-spontaneous-derived accessions. In addition, the results of study on the genetic diversity of 30 coffee accessions (seven diploid accessions and 23 wild and cultivated tetraploid accessions of *Coffea arabica*) using 34 SSR markers revealed more allelic diversity among the five diploid species than among the 23 tetraploid genotypes [47]. According to the authors, fifty-five percent of the alleles found in the wild tetraploids were not shared with cultivated *C. arabica* genotypes, supporting the idea that the wild tetraploid ancestors from Ethiopia could be used as a source of novel genetic variation to expand the gene pool of elite *C. arabica* germplasm. from the study conducted on coffee samples collected from different forest coffee populations of Ethiopia using SSR markers, moderate genetic diversity exists within and among a few forest coffee populations [5]. Moreover, high polymorphism has been repored in spontaneous and sub-spontaneous derived accession of Ethiopia in other similar diversity analyses conducted using SSR markers [48]

2.4.3. Diversity Within Arabica Coffee Genotypes

Despite the low genetic variation of Arabica coffee genotypes has been reported by several researchers who studied genetic diversity on samples collected from coffee Arabica accessions grown out of the center of diversity, Ethiopia, microsatellites were able to show moderate to high genetic diversity among Yemen and Brazil accessions [48]; among coffee accession grown in Nicaragua [50], among coffee samples collected from coffee ccessions grown in Ethiopia [49, 6].

The results of diversity study conducted on 28 Arabica coffee genotypes collected from northwestern and southwestern Ethiopia using six simple sequence repeat (SSR) primers revealed the presence of considerable variability among some of the genotypes, with relatively higher genetic diversity among coffee genotypes from Southwestern Ethiopia as compared to those from Northwestern Ethiopia [49]. Moreover, the high genetic diversity among coffee

Arabica accession grown in Ethiopia and the potential of SSR markers for coffee genetic variability studies further confirmed and the diversity study conducted on a total of 132 coffee Arabica genotypes (most of them grown in Ethiopia) using 32 SSR markers revealed a high genetic variability reserve with a lot of specificity in Ethiopian Arabica coffees [6]. Similalrly, high level of genetic diversity in Ethiopian commercial arabica coffee varieties reported on the study conducted in 40 released coffee cultivars using 14 SSR markers [51]. The authors reported high average number of polymorphic alleles (7.5) and polymorphic information content (PIC = 80%) per locus. The genetic similarity among varieties using the Jaccard's similarity coefficient ranged from 0.14 to 0.78, with a mean of 0.38. Similarly, the diversity of 64 promising Arabcia coffee genotypes originating from different coffee-growing areas of Ethiopia using 14 SSR and the result revealed the presence of a high level of genetic diversity and wide genetic dissimilarity within and among elite breeding lines from the same and different geographical origins [52].

However, the low genetic diversity reported for coffee Arabica accessions grown out of Ethiopia is because of the coffee accessions were derived from a few coffee trees established from a few seeds introduced to different coffee-growing countries from Ethiopia through Yemem. Therefore, from the above three studies, it can be concluded that there is sufficient variability among coffee genotypes grown in Ethiopia to use for future coffee breeding programs. In general, the potential application of SSR markers in differentiating different coffee species, studying the variability between wild and cultivated Arabica accessions, and to study the diversity within Arabica coffee genotypes have been confirmed.

3. Summary and Conclusion

Diversity based on phenotypic and morphological characters, usually varies with environments, and evaluation of traits requires growing the plants to full physiological maturity before identification, but now the rapid development of biotechnology allows easy analysis of a large number of loci distributed throughout the genome of the plants.

The results of many experiments conducted using molecular markers such as RAPD, AFLP, ISSR, and SSR in coffee revealed that most of these markers can differentiate among different coffee species, between wild and cultivated coffee varieties, and can also successfully be used to estimate the genetic diversity within coffee Arabica to use such genetic information in breeding program. Moreover, all the studies confirmed that the genetic variation within Arabica coffee is low as compared to the diploid species due to its autogamous nature and narrow genetic base of coffee Arabica grown out of the center of diversity. However, the variability in Arabica coffee is sufficient among coffee genotypes grown in the center of diversity, Ethiopia to use for future coffee breeding programs.

The precision of assessing cultivar diversity study largely depends on sample size and the number of polymorphic markers. However, the number of markers, the number of genotypes, and band scoring methods were not consistent across the studies. Therefore, to avoid inconsistencies and to deliver comprehensive information for a specific DNA markers, using a large number of genotypes uniformly, and the number of markers as well as the techniques of band scoring methods should also be similar.

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