Analysis of genetic diversity of Arabica coffee [Coffea arabica L.] in Solok Regency by SRAP molecular markers

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Abstract. Coffee is one of the most widely grown plantation crops in Indonesia. There are two types of coffee plants that are most widely cultivated, namely Robusta and Arabica which are distributed in several provinces including West Sumatra. Solok Regency is one of the coffee production centers in West Sumatra. Information regarding the genetic diversity of Arabica coffee that scattered in several locations is still not available. Due to this fact, the genetic diversity of coffee can be identified by DNA analysis. The DNA-based molecular marker that can be applied as a genetic marker of plants is SRAP. This study aimed to analyze the genetic diversity of coffee by the SRAP marker system. Fifteen samples consisted of five varieties of Andungsari, Sigararutang, Kartika, Gayo, Sinar Harapan were collected from three locations namely Air Dingin, Subarang Danau, Simpang Tanjung Nan IV and amplified using 8 selected primary combinations. Diversity analysis was performed by POPGENE and NTSYS. The results showed that the average value of genetic diversity [H] between samples ranged from 0.2812-0.3638, while Shannon's Information index [I] ranged from 0.4330-0.5346. The average number of polymorphic loci ranged from 19 to 23 with the percentage of polymorphic loci are ranging from 82.61% to 100%. Cluster analysis shows that there are three main clusters [I, II, III] where cluster I can be separated into four nodes, cluster II into three nodes, cluster III into five nodes. The SRAP molecular marker is effectively used to analyze molecular genetic diversity in the Arabica coffee population.

1. Introduction

Coffee is a plant that is widely planted in Indonesia. This has led to Indonesia being dubbed "Home of world finest coffee" because it contributes to being one of the largest coffee exporting countries in the world. There are two types of coffee plants that are most widely cultivated in Indonesia, namely Robusta coffee and Arabica coffee. Both types of coffee are distributed in several provinces in Indonesia, including West Sumatra. Solok Regency is one of the Arabica coffee production centers in West Sumatra with an average production area in 2015 of 11,054 ha [1].

Genetic diversity is one of the factors that play a role in breeding programs. Lacking information related to the genetic diversity of Arabica coffee which is cultivated in Solok district, causes the assembling of superior new varieties to be less effective. The availability of information related to genetic diversity is very important and is needed to design breeding programs and maintain genetic diversity in a specific gene pool [2]. Genetic diversity can be analyzed using biochemical,
morphological, and molecular-based markers. The molecular-based marker system is a more effective and efficient technology for distinguishing between species and between cultivars that are closely related compared to the other two markers [3].

One of the most widely used molecular-based marker systems lately is the Sequence-related Amplified Polymorphism [SRAP] developed by Li and Quiros [4]. Some of the advantages of using SRAP compared to other marker systems are multi-locus and multi-allele. Those characteristics make the SRAP marker system to be more efficient for genetic diversity analysis, gene mapping and fingerprint genotyping. Furthermore, it is also believed to be more cost-effective, easy and reliable, using forward and reverse primers so that the primer combinations could be more alternatives [5]. SRAP has been widely used to identify the genetic diversity of several types of plants such as Arabica coffee plants from Yemen [2], hybrid robusta coffee [6] elephant grass [7], cereal crops [8], rice [9], bananas [10], some medicinal plants [11], [12]. The aim of this study was to analyze the genetic diversity of coffee in the Solok Regency using the SRAP technique application.

2. Materials and methods

2.1. Plant materials

The plant material used in this study were the leaves of a coffee plant that consisted of 5 varieties, namely Andungsari, Sigararutang, Kartika, Gayo, and Sinar Harapan. The samples were collected from three locations namely Air Dingin, Subarang Danau, Simpang Tanjung Nan IV. Analysis of genetic diversity of coffee plants using SRAP molecular markers consisting of stages of DNA isolation, DNA quality, and quantity testing by electrophoresis and nanodrop, primers selection, amplification using PCR [Polymerase Chain Reaction] technique.

2.2. DNA isolation and primer selection

DNA isolation was carried out using the protocol provided by GeneJET Plant Genomic DNA Purification Mini Kit with a slight modification. The primers selection was done through BSA [Bulked Segregant Analysis] approach using SRAP primers consisting of 16 combinations. Primers were chosen based on their ability and capability to produce DNA fragments in all accessions, clear, and high polymorphism. DNA amplification was done using 8 selected primer combinations namely A [EM1-Me1], C [EM1-Me3], E [EM2-Me1], J [EM3-Me2], K [EM3-Me3], L [EM3-Me4], N [EM4-Me2] and O [EM4-Me3].

2.3. PCR amplification and fragment scoring

The SRAP protocol was based on [4] with modifications to the composition for the PCR reaction and the last elongation time. The composition of the PCR cocktail as much as 50 μl consisted of 2 μl DNA templates, 1.5 μl reverse primers, 1.5 μl forward primers, 20 μl free nuclease water and J 25 μl KOD DNA polymerase [Toyobo-Japan]. Amplification was run on a thermocycler [Biometra-Germany] with the following program: pre-denaturation of 94°C for 5 minutes, then the first cycle of 5 cycles consisted of a denaturation phase of 94°C for 1 minute, annealing at 35°C for 1 minute, then followed by the second cycle of 35 cycles consisting of a denaturation phase at a temperature of 94°C for 1 minute, annealing at a temperature of 50°C for 1 minute and elongation phase at 72°C for 1 minute. The PCR reaction was terminated in the elongation phase at 72°C for 8 minutes and 4°C as the holding temperature. Amplification products were checked using 0.7% agarose gel electrophoresis run at 100 volts for 40 minutes. Visualization of the results of electrophoresis using a UV light on the gel documentation system [Biometra-Germany]. Data obtained from the gel documentation was used for scoring. If the fragment was available, then it was scored “1” and if there was no fragment, then it was scored “0”. Data obtained were analyzed using POPGENE 3.2. and NTSYS ver 2.02.
3. Results and discussion

3.1. Primer selection
For a quick selection of primers, the BSA [Bulked Segregant Analysis] approach was applied. Figure 1 shows the results of a selection of 16 primer combinations based on the BSA approach. It shows that some fragments are visible and but some fragments are unclear. Based on this result 8 primer combinations were selected determined on the quality of the amplified fragments and polymorphic fragments produced in each primer. The 8 primers combinations are A, C, E, J, K, L, N, and O with a total of 70 fragments. Primer C produced the most fragments [11], while 7 other primers produced only 8 to 10 fragments.

![Figure 1. Results of amplification of 16 primer combinations using the BSA approach in Arabica coffee population](image)

3.2. Genetic diversity of Arabica coffee
Based on Table 1, it is identified that the highest value of Ne is found in the population L1.1 [1.6484] and the lowest value is found in population L3.2 [1.4574]. Genetic variation can be seen from the H value among populations. The H values for 15 coffee populations ranged from 0.2812-0.3638. The highest genetic diversity value is shown by the population L1.3 [0.3638] while the lowest genetic diversity value is shown by population L3.2 [0.2812]. The percentage of polymorphic loci ranged from 82.61% to 100%.

| Population | Na  | Ne  | H     | I     | NPL | PLP      |
|------------|-----|-----|-------|-------|-----|----------|
| L1.1       | 1.9565 | 1.6485 | 0.3633 | 0.5327 | 22  | 95.65 %  |
| L1.2       | 1.9130 | 1.6315 | 0.3529 | 0.5161 | 21  | 91.30 %  |
| L1.3       | 1.9565 | 1.6425 | 0.3638 | 0.5346 | 22  | 95.65 %  |
| L1.4       | 1.9130 | 1.6189 | 0.3499 | 0.5137 | 21  | 91.30 %  |
| L1.5       | 1.9565 | 1.5517 | 0.3265 | 0.4917 | 22  | 95.65 %  |
| L2.1       | 1.9130 | 1.6311 | 0.3552 | 0.5203 | 21  | 91.30 %  |
| L2.2       | 2.0000 | 1.5774 | 0.3420 | 0.5152 | 23  | 100.00 % |
| L2.3       | 2.0000 | 1.5440 | 0.3266 | 0.4965 | 23  | 100.00 % |
| L2.4       | 2.0000 | 1.6159 | 0.3582 | 0.5342 | 23  | 100.00 % |
| L2.5       | 2.0000 | 1.5606 | 0.3334 | 0.5048 | 23  | 100.00 % |
| L3.1       | 1.9565 | 1.4822 | 0.2966 | 0.4559 | 22  | 95.65 %  |
| L3.2       | 1.9130 | 1.4574 | 0.2812 | 0.4330 | 21  | 91.30 %  |
| L3.3       | 1.8261 | 1.5454 | 0.3638 | 0.5346 | 22  | 95.65 %  |
| L3.4       | 2.0000 | 1.6051 | 0.3465 | 0.5177 | 23  | 100.00 % |
| L3.5       | 2.0000 | 1.5619 | 0.3375 | 0.5105 | 23  | 100.00 % |
| Average    | 1.9536 | 1.5783 | 0.3362 | 0.5021 | 21.933 | 95%      |
Notes:  
* L1 = Air Dingin  
* L2 = Subarang Danau  
* L3 = Simpang Tanjung Nan IV  
* Na = Observed number of alleles  
* Ne = Effective number of alleles  
* H = Gene diversity  
* I = Shannon's Information index

The ability of 8 SRAP primer combinations to produce a high level of polymorphism with a range of 82.61% to 100%, proved that the SRAP marker is very effective to be used to identify the genetic diversity. The effectiveness of the SRAP marker in identifying genetic diversity is also shown by the results published by Mishra et al [13] for the identification and analysis of hybrid Arabica coffee populations. The SRAP marker was also successfully used in the assessment of the genetic differentiation of coffee genotypes obtained from various valleys in Yafea City, Yemen [2]. Based on those results, the SRAP marker is regarded very effective and has the potential to be used in gene mapping, genotype fingerprinting, and genetic diversity analysis because this molecular marker system has multi-allelic and multi loci [8].

The SRAP marker also successfully demonstrated the level of diversity among 15 coffee populations taken from three different locations. In Figure 2 it can be seen that the similarity coefficient between populations ranges from 0.725 - 0.92. The highest similarity value [0.92] was found between Sigararutang genotypes collected from the over the side of Solok Lake, followed by the Sigararutang genotype collected from the Air Dingin location and then Kartika genotype collected from Simpang Tanjung Nan IV. The lowest similarity value [0.725] was shown by the Andungsari genotype originated from Air Dingin and Sinar Harapan located in Simpang Tanjung. Nan IV.

![Figure 2. Genetic similarity coefficient among 15 Arabica coffee populations collected from three different locations](image)

The genetic similarity coefficient among coffee genotypes collected from the three locations ranging from 0.725 to 0.92, showing that coffee has a wide genetic diversity. This can happen because in one field farmers usually cultivate several different varieties. Such cultivation systems can cause natural crossing between coffee varieties planted close together. According to [14], such conditions could increase opportunity in triggering of gene flow among species and even between cultivars. Gimase et al [15] stated that the presence of external gene introgression will result in new genetic diversity within the same cultivar, and such conditions are the basis for genetic variation in Arabica coffee populations.
Based on Figure 2, it is known that the cluster I group generally consists of populations originating from Air Dingin namely Andungsari, Gayo, Sigarratung. These results indicate that SRAP molecular markers succeeded in grouping plants based on the same cultivation location. Similar results were obtained at the Tempuyung plant where the SRAP marker was able to group several Tempuyung accessions from the same location into one cluster [16]. A similar geographical location will usually correlate with high genetic similarity [17].

However, in Figure 2 it can also be seen that several genotypes of Arabica coffee grown in the same location do not cluster in one cluster [cluster II and III]. Some factors that might influence are 1. Propagation materials used by farmers, especially those from seeds, are taken from different populations. This can happen because most farmers still plant several varieties on the same land; 2. Cross-pollination naturally occurs [18] Coffee varieties planted in adjacent locations, and 3. Heterozygosity residues from the original parents which are still existing [19].

4. Conclusion
The SRAP marker is effective to identify the genetic diversity of Arabica coffee populations, showing a high level of polymorphism ranged from 82.61% to 100%. The Arabica coffee in Solok Regency has a wide genetic diversity ranging from 0.725 to 0.92.

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