Journal of Tropical Crop Science Vol. 11 No. 3, October 2024
www.j-tropical-crops.com

Received 14/12/2023; Revised 24/04/2024; Accepted 15/05/2024
https://doi.org/10.29244/jtcs.11.03.207-216

RESEARCH ARTICLE

Genetic Diversity of Yellow and Red Berries Arabica Coffee Populations Grown in a Mix Populations in Garut, West Java, Indonesia, Based on SSR Markers

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Abstract

Farmers in Garut, West Java, grow mixed varieties of Arabica coffee (*Coffea arabica* L.). Subsequently, they use harvested beans as seeds. Intercrossing among varieties may result in hybrid progenies and harvesting hybrid progenies as seed results in genetic variations. This research aims to evaluate the genetic diversity of Arabica coffee grown in a mixed population. Ninety-one Arabica coffees comprised 37 Arabica cv. “Ahernt GRT KN” (yellow-), 45 “Sigararutang” (red-), and nine “S795” (red berries) were sampled. Twenty SSR primer pairs were validated using 15 samples representing three varieties; six were polymorphic and used to genotype 91 Arabica accessions. Genetic data were analyzed using PowerMaker 3.25 and Darwin version 6 software. The results showed that the six SSR loci generated from 2 – 3 alleles, with an average of 2.17 alleles per locus. Genetic analysis of Arabica coffee from Garut, West Java, generated SSR markers with an average PIC of 0,33 across loci and varieties. The PIC within Arabica coffee cv. “Ahernt GRT KN” and “Sigararutang” were low, and within “S795” was moderate. Those PICs indicate the presence of more genetic diversity within “S795” than the other two cultivars. The Ho across Arabica coffee cv. “Sigararutang” and “S795” were lower than the He values, confirming their self-pollination nature. However, the Ho values of Arabica coffee cv. “Ahernt GRT KN” was larger than the others, indicating the presence of residual heterozygosity and a low percentage of recent outcrossing. The low Ho values of “Sigararutang” suggest that Arabica coffee is homozygous. Arabica coffee cv. “S795” also showed a low Ho value, but its moderate He value indicates the presence of more genetic diversity than the other cultivars.

Keywords: Cluster Analysis, *Coffea arabica* L., He and Ho values, outcrossing rate, simple sequence repeats

Introduction

Arabica coffee (*Coffea arabica* L.) holds significant economic value and is cultivated in numerous countries, including Indonesia. It is esteemed for its flavor, making it more costly than Robusta, Liberica, and other coffee varieties (Dias and Benassi, 2015). The price of Arabica coffee varies depending on its quality, with specialty flavors and superior bean quality commanding higher prices than regular Arabica coffee. Specialty Arabica coffee, on average, fetches 4.5 times the price of non-specialty Arabica coffee (Traore et al., 2018). Factors influencing Arabica coffee’s specialty flavor include the coffee variety, altitude, location of the farm, and processing method (Traore et al., 2018). Planting specialty Arabica coffee varieties can yield greater profits for farmers, as the income from specialty coffee is higher per harvest than regular Arabica coffee.

Garut Regency is among the regions in Indonesia known for Arabica coffee production. Arabica farmers in Garut cultivate several varieties with specialty flavors, including “Ahernt GRT KN.” “Sigararutang.” “S795.” and “Buhun.” among others (Randriani et

Received 14/12/2023; Revised 24/04/2024; Accepted 15/05/2024
https://doi.org/10.29244/jtcs.11.03.207-216

al., 2018). These farmers typically use beans from mature trees for planting, a practice observed not only in Garut but also in various coffee-growing regions worldwide (Campuzano-Duque and Blair, 2022; Megersa, 2022; Passos et al., 2020; Scalabrin et al., 2020). Arabica coffee is considered self-pollinating (Gómez et al., 2023), leading farmers to believe that the offspring will resemble the parent. However, farmers often collect seeds from random trees, leading to genetic diversity within the variety due to mutations, segregation, or gene flow from a low frequency of crossbreeding among Arabica coffee varieties. In Garut, farmers commonly plant multiple Arabica coffee varieties on the same land, potentially leading to outcrossing between varieties (Dani et al., 2016; Izzah et al., 2015).

Genetic diversity analysis is essential to assess genetic variation within varieties, which can influence genetic identity within specific cultivars. Moreover, it helps trace potential gene flow among Arabica coffee varieties, i.e., among the yellow and the red berries Arabica coffee varieties. Molecular markers, particularly simple sequence repeats (SSR) markers, are commonly used for genetic diversity analysis due to their stability and effectiveness in measuring genetic variability (Oliveira et al., 2022). This research aims to describe genetic diversity within Arabica coffee varieties and trace gene flow among the varieties.

Material and Methods

Research Locations and Genetic Material

The sampling location was Margamulya village, Cikajang sub-district, Garut Regency, West Java, Indonesia (GPS coordinates 7° 21' 29.6" S and 107° 44' 45.5" E). Laboratory analysis was conducted at the Plant Molecular Biology Laboratory (PMB lab), Department of Agronomy and Horticulture, Faculty of Agriculture, IPB University. The plant materials used were 91 samples of Arabica coffee, consisting of 37 samples of Arabica coffee cv. "Ahernt GRT KN," 45 genotypes of Arabica coffee cv. Sigararutang, and nine genotypes of Arabica coffee cv. "S795." The geo-locations of each Arabica coffee sample were not recorded.

DNA Isolation

Leaf samples (200 mg) from each genotype and seedling were isolated using the modified CTAB method (Larekeng et al., 2015). The quality and quantity of the isolated DNA were only estimated by horizontal electrophoresis using 1% agarose in 1x SB (sodium borate) buffer at a voltage of 100 volts for 30 minutes and comparing it with the known concentration of 1 kb DNA size marker.

SSR Primer Selection

A total of 20 SSR primers (Baltazar and Fabella, 2020; Dani et al., 2016; Izzah et al., 2015) were screened for polymorphism using 15 samples, comprising six samples of Arabica coffee cv. "Ahernt GRT KN," seven of "Sigararutang," and two "S795," respectively. The sequences of the primers used are presented in Table 1. The examples of the eliminated and selected SSR primers after primer selection analysis are presented in Figure 1.

PCR Amplification

The selected polymorphic primers were used for the PCR amplification of 91 Arabica coffee samples, and the PCR amplification was performed using a Biorad T100™ Thermal Cycler machine. The Mytaq HS Mix PCR kit (Bioline) with a total reaction of 7 ul was used. The PCR reaction consisted of 3 ul of PCR mix, 0.15 ul forward and reverse primers (10 uM), and 3.7 ul of DNA working solution (DNA diluted 30-50 times, depending on the estimated concentration). The PCR program for DNA amplification was as follows: the first stage was pre-denaturation at 95 ^{o}C for 5 minutes, followed by the second stage of denaturation at 95 ^{o}C for 30 seconds, annealing at 55 ^{o}C for 30 seconds, and extension at 72 ^{o}C for 30 seconds. The second stage was repeated for 35 cycles. The third stage is the final extension at 72 ^{o}C for 10 minutes (Izzah et al., 2015; Larekeng et al., 2015). The presence of amplicons was confirmed using 1% agarose gel in 1x SB (Sodium Borate) buffer at 100 volts for 6 minutes. A 50 bp ladder was added to the leftmost well to confirm the amplicon sizes. Electrophoresis was carried out using the Mupid-EXu Submarine Electrophoresis System.

Polyacrylamide Gel Electrophoresis (PAGE)

The confirmed amplicons were further analyzed on 6% polyacrylamide gel electrophoresis (PAGE) using 1x SB buffer (Larekeng et al., 2015). The pre-run process was carried out at 100 volts for 60 minutes. The run process was carried out for 120 minutes at 60 volts. PAGE results were stained with the silver staining using the Creste et al. (2001) method. The silver staining results were air-dried overnight and scanned using a printer scanner. The scan results were then used to score amplified alleles manually.

Data Analysis

Summary of population statistical analysis (number

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of alleles per locus [Na], major allele frequency, expected heterozygosity/genetic diversity [He], observed heterozygosity/individual heterozygosity [Ho], and Polymorphism Information Content [PIC]) was carried out using PowerMaker 3.25 software. Cluster analysis used the Sattah and Tversky method (Tree construction – Scores menu in Darwin Software (Perrier and Jacquemoud-Collet, 2019) because it produces better clustering results than UPGMA (Saitou, 2013) and is as good as the neighbor-joining method but the clustering results are easy to interpret.

Result and Discussion

SSR Primer Selection

The selected primers should generate polymorphic amplicons (SSR alleles), and their size differences are easy to score. Overlapping alleles that are close in size are difficult to score, and the sample genetic makeups are challenging to determine whether they are homozygous or heterozygous. Such SSR primers are discarded since they are useless for tracing outcrossing occurrences. Out of the 20 primers evaluated (Baltazar and Fabella, 2020; Dani et al., 2016; Izzah et al., 2015), Missio et al. (2009), Table 1), six primers are polymorphic and can be used for further analysis (M24, Ca052, M32, CaM16, M101, Ca087). The examples of amplicons generated using discarded SSR primers (M25) and selected ones (M101) are presented in Figures 1A and 1B.

Summary Statistics of SSR Markers Across Arabica Varieties

Population genetic parameters calculated based on 91 samples of three Arabica coffees cultivars, genotyped using six SSR primer pairs were presented in Table 2. Genotyping across 91 samples, genotypes using six SSR marker loci showed major allele frequency (MAF) values ranging from 0.50 - 0.93 (Table 2), and CaM16 SSR locus has the highest MAF (0.93) while M101 was the lowest (0.50). A pair of SSR primers (Ca087) generated three SSR alleles, while the others generated two. The average number of alleles per locus was 2.17 (Table 2). The number of alleles per locus observed in this experiment did not differ from the previous allele number found in Arabica coffee, as reported by Al-Murish et al. (2013). In their study, an average of 2.31 alleles per locus were obtained. Our findings were also similar to those reported by Moncada and McCouch (2004). Their report found an average of 1.9 alleles per SSR locus in the cultivated Arabica coffee and 2.53 alleles per locus in the wild Arabica coffee. The low number of alleles per locus in Arabica coffee significantly differed from those in Robusta coffee. The average allele number per locus in Robusta coffee ranges from 3.1 - 7.8 (Kiwuka et al., 2021; Musoli et al., 2009). Such data indicated a lower genetic diversity within Arabica coffee than in Robusta.

The expected heterozygosity (He) values represent the genetic diversity in a population (Harris and DeGiorgio, 2017), while the observed heterozygosity (Ho) values represent heterozygosity at the individual level. Across 91 samples, the He values in this study ranged from 0.12 - 0.50, with an average of 0.43 (Table 2). In contrast, the Ho values range from 0.00 - 0.19, with an average value of only 0.06 (Table 2). Those low Ho values indicate that the tested Arabica coffees were genetically homozygous and showed a low outcrossing level. Since gene flow should increase heterozygosity (Bell et al., 2019). The higher He values than the Ho was also reported by Josia et al. (2021) when testing several pure lines of corn. In contrast, hybrid corn populations had both high He and Ho values. The low observed heterozygosity in Arabica coffee was also reported by Scalabrin et al. (2020) based on sequencing data of the Arabica coffee genome.

Polymorphism information content (PIC) values across 91 Arabica coffee samples for six SSR loci range from 0.12 - 0.37, with an average PIC per locus of 0.33 (Table 2). The Ca087 SSR locus showed the highest PIC (0.44), while the CaM16 showed the lowest PIC (0.12, Table 2). The PIC value in this study ranged from 0.12 to 0.44, with an average of 0.33. Five primers had moderate values (PIC 0.25-0.50), and one primer had a low PIC (PIC <0.25). This result showed that the primer could detect moderate levels of genetic diversity. Previous studies in Arabica coffee have reported that the heterozygosity values depended on the evaluated primers. Some primers resulted in 100% heterozygosity, while others resulted in 0% heterozygosity (Benti et al., 2021; Dani et al., 2016; Izzah et al., 2015). Since Arabica coffee is an allotetraploid comprising A and B genomes, multi-allele amplification from the A and B genomes of the current Arabica coffee may occur, resulting in a heterozygous score (Scalabrin et al., 2020; Teressa et al., 2010). In reality, what appears to be heterozygous are amplifications of two homozygous loci from A and B genomes having different alleles, i.e., AA and BB. This study confirms that Arabica coffee appears to have a low level of heterozygosity.

Summary Statistics of SSR Markers within Arabica Varieties

Within the Arabica coffee varieties, the He, Ho, and PIC of Arabica coffee CV are “Ahernt GRT KN,”

Received 14/12/2023; Revised 24/04/2024; Accepted 15/05/2024
https://doi.org/10.29244/jtcs.11.03.207-216

Table 1. Characteristics of 20 SSR primers selected in 15 Arabica coffee genotypes.

No.	Primer's ID*	Primer sequences	Tm (ºC)
1	M2a	F : 5'-AGTGGTAAAAGCCGTTGGTG-3' R : 5'-GCGGTTGTTGGTGAGTTGAA-3'	51.8 51.8
2	M24	F : 5'-GGCTCGAGATATCTGTTTAG-3' R : 5'-TTTAATGGGCATAGGGTCC-3'	49.7 51.9
3	M25	F : 5'-CCCTCCCTGCCAGAAGAAGC-3' R : 5'-AACCACCGTCCTTTTCCTCG-3'	57.9 53.8
4	M20	F : 5'-CTTGTTTGAGTCTGTCGCTG-3' R : 5'-TTTCCCTCCCAATGTCTGTA-3'	51.8 49.7
5	Ca052	F : 5'-GATGGAAACCCAGAAAGTTG-3' R : 5'-TAGAAGGGCTTTGACTGGAC-3'	51.5 54.2
6	Ca018	F : 5'-GTCTCGTTTCACGCTCTCTC-3' R : 5'-ATTTTTGGCACGGTATGTTC-3'	53.8 47.7
7	M29	F : 5'-GACCATTACATTTCACACAC-3' R : 5'-GCATTTTGTTGCACACTGTA-3'	47.7 47.7
8	M32	F : 5'-AACTCTCCATTCCCGCATTC-3' R : 5'-CTGGGTTTTCTGTGTTCTCG-3'	55.1 53.6
9	M42	F : 5'-ATCCGTCATAATCCAGCGTC-3' R : 5'-AGGCCAGGAAGCATGAAAGG-3'	51.8 53.8
10	M47	F : 5'-TGATGGACAGGAGTTGATGG-3' R : 5'-TGCCAATCTACCTACCCCTT-3'	51.8 51.8
11	M324	F : 5'-GCCCTTCCTTTCTTCATTTC-3' R : 5'-TGGGTGTTCCTCTTTCTCTG-3'	49.7 51.8
12	M329	F : 5'-ACTCAGACAAACCCTTCAAC-3' R : 5'-GATGTTTTGCATCTATTTGG-3'	49.7 45.6
13	CaM16	F : 5'-AAGGCAGCTGAAGCGGGACAAA-3' R : 5'-TGGGGAGAGCTGCAGTTGGAGG-3'	61.9 63.5
14	DCM06	F : 5'-GTAGTCGGTGGGCTTGTGTT-3' R : 5'-AACGCGGACTAATTGAGGAA-3'	53.8 49.7
15	R278	F : 5'-TGTAGATTTGAAACCCAATC-3' R : 5'-AAGTCTCGACAAGTTTTGAC-3'	45.6 47.7
16	M101	F : 5'-TATGTCTCTAACTTTCCTATTTT-3' R : 5'-AGAGACTACATTTACACAGAAGA-3'	47.0 50.8
17	Ca021	F : 5'-GCTGAGAGTTTTGAGGGAAA-3' R : 5'-CCGACGTAGTTGATGATTGA-3'	49.7 49.7
18	Ca087	F : 5'-TCACTCTCGCAGACACACTAC-3' R : 5'-GCAGAGATGATCACAAGTCC-3'	56.2 53.0
19	CaM03	F : 5'-CGCGCTTGCTCCCTCTGTCTCT-3' R : 5'-TGGGGGAGGGGCGGTGTT-3'	60.4 57.2
20	Sat227	F : 5'-TGCTTGGTATCCTCACATTCA-3' R : 5'-ATCCAATGGAGTGTGTTGCT-3'	50.5 49.7

Note: Tm = Melting temperature. * Primer sources: Baltazar and Fabella (2020), Dani et al. (2016); Izzah et al. (2015), Missio et al. (2009)

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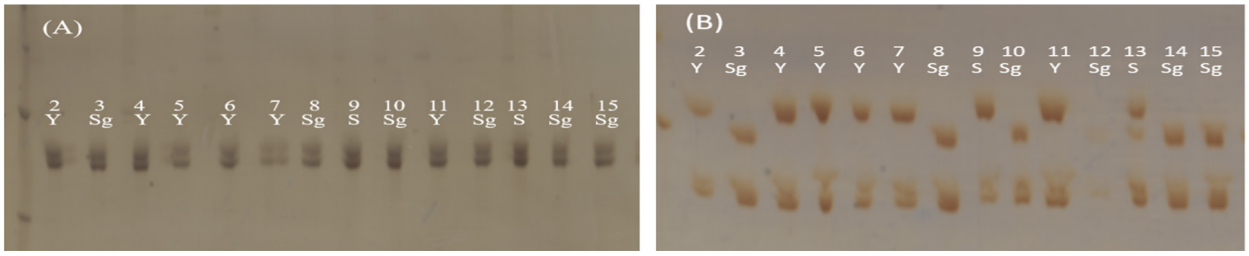

Figure 1. The sample of eliminated and selected SSR primers after primer selection analysis. (A) An example of a generated monomorphic PCR amplicon using M25 SSR primers, and the primers are excluded from further analysis. (B) An example of polymorphic SSR amplicons generated using M101 SSR primers and the primers are included for further study.

Table 2. Calculated population genetic parameters based on 91 samples of three Arabica coffees cultivars. The Arabica coffee samples were genotyped using six SSR primer pairs.

Primer ID	Mayor allele frequency	Alleles numbers (Na)	Expected heterozygosity (He)	Observed Heterozygosity (Ho)	Polymorphism Information Content (PIC)
M24	0.65	2.00	0.46	0.19	0.35
Ca052	0.67	2.00	0.44	0.18	0.34
M32	0.52	2.00	0.50	0.01	0.37
CaM16	0.93	2.00	0.12	0.00	0.12
M101	0.50	2.00	0.50	0.01	0.38
Ca087	0.53	3.00	0.54	0.00	0.44
Mean	0.63	2.17	0.43	0.06	0.33

Table 3. Expected heterozygosity (He) and observed heterozygosity (Ho) within Arabica coffee cv. “Ahern GRT KN,” “Sigararutang,” and “S795.” The three Arabica coffee varieties were genotyped using six SSR primer pairs.

Primer’s ID	“Ahernt GRT KN” (n=39)			“Sigararutang” (n=47)			“S795” (n=9)		
	He	Ho	PIC	He	Ho	PIC	He	Ho	PIC
M24	0.50	0.47	0.37	0.43	0.00	0.34	0.20	0.00	0.18
Ca052	0.50	0.43	0.37	0.40	0.02	0.31	0.20	0.00	0.18
M32	0.05	0.00	0.00	0.00	0.00	0.00	0.28	0.11	0.24
CaM16	0.00	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.35
M101	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.11	0.10
Ca087	0.00	0.00	0.00	0.00	0.00	0.00	0.57	0.00	0.49
Means	0.18	0.15	0.12	0.14	0.00	0.11	0.30	0.04	0.25

Notes: n = number of samples

ranging from 0 - 0.50, 0 - 0.47, and 0 - 0.37 (Table 3). Arabica coffee cv. “Ahernt GRT KN” had a moderate He value (i.e. 0.50) in two primers (M24 and Ca052, Table 3). On the other hand, the average He and Ho values of Arabica coffee cv. “Ahernt GRT KN” was low (i.e., 0.18 and 0.15, Table 3), indicating the low genetic diversity within Arabica coffee cv. “Ahernt GRT KN.” Such low He and Ho values occur because the other four SSR loci evaluated generate a monomorphic amplicon from Arabica coffee “Ahernt GRT KN,” the He value was zero. The presence of heterozygosity in two SSR loci and homozygosity in the other four indicated that the population of Arabica coffee cv. “Ahernt GRT KN” still has residual heterozygosity.

The He, Ho, and PIC of Arabica coffee cv. “Sigararutang” ranged from 0 - 0.43, 0 - 0.02, and 0 - 0.34 (Table 3). The He values of Arabica coffee

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cv. "Sigararutang" was also moderate (i.e. 0.43 and 0.40) in the M24 and Ca052 SSR loci. Like "Ahernt GRT KN," the average He and Ho values of Arabica coffee cv. "Sigararutang" was also low (i.e., 0.14 and 0.00, Table 3), indicating genetic uniformity of Arabica coffee cv. Sigaratutang samples. Low He and Ho values also occur because the other four SSR loci evaluated did not generate amplicons from Arabica coffee cv. "Sigararutang" and the He value was zero. Out of the 45 samples of Arabica coffee cv. "Sigararutang", only one sample had a heterozygous allele in one SSR locus (i.e., Ca052).

Meanwhile, the He, Ho, and PIC of Arabica coffee cv. "S795" ranged from 0.10 - 0.57, 0 - 0.11, and 0.10 - 0.49 (Table 3). The He values of Arabica coffee cv. "S795" was moderate (i.e., 0.43 and 0.40) in the CaM16 and Ca087 SSR loci and low (i.e., 0.10 - 0.28) in the other four SSR loci (Table 3). Moreover, the He and PIC values in the "S795" were contributed by six SSR loci. Such data indicated that Arabica coffee cv. "S795" was relatively more diverse than Arabica coffee cv. "Ahernt GRT KN" and Sigararutang. The average He value of Arabica coffee cv. "S795" was also moderate (i.e., 0.30), and the Ho value was low (i.e., 0.04), also indicating genetic uniformity of Arabica coffee cv. "S795" samples. The low Ho value occurs because four SSR loci evaluated have zero Ho, and only two loci (M32 and M101) have 0.11 Ho values. Only two samples of Arabica coffee cv. "S795" had heterozygous alleles, one sample in primer M32 and the other in primer M101.

Phylogenetic Analysis Among Samples of Arabica Coffee Cultivars

The phylogenetic analysis of 91 samples from three Arabica coffee cultivars (Arabica coffee cv. "Ahernt GRT KN," "Sigararutang," and "S795") revealed three major clusters, as depicted in Figure 2. Cluster 1 primarily consisted of Arabica coffee cv. "Sigararutang" (Figure 2). Cluster 2 comprised eight samples of Arabica coffee cv. "S795" and one 'Ahernt GRT KN,' while cluster 3 included 36 samples of Arabica coffee cv. "Ahernt GRT KN" and one Arabica coffee cv. "S795" (Figure 2).

Further analysis of Arabica coffee cv. "Sigararutang" revealed four distinct clades. Clades "Sigararutang" type 1 and type 4 consisted of numerous individual samples, with 11 and 32 samples, respectively (Table 4). Conversely, "Sigararutang" types 2 and 3 each had only one and two individual members, respectively (Table 4). The genetic distance among Arabica coffee

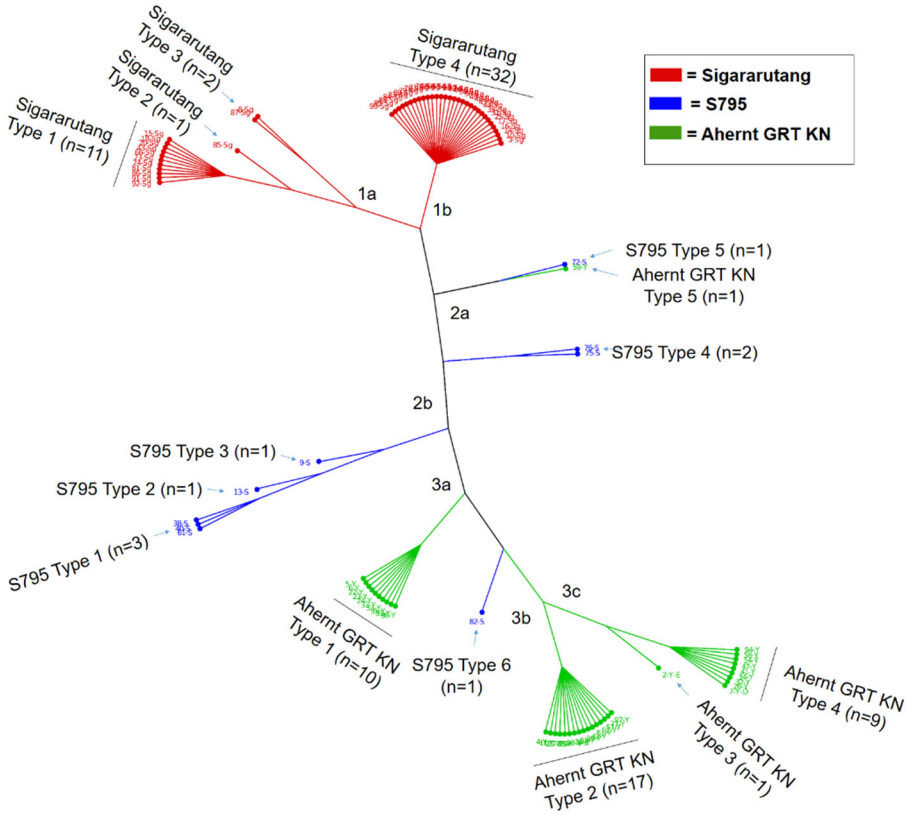

Figure 2. Results of cluster analysis of 91 Arabica coffee samples, constructed using six polymorphic SSR markers. The clustering only presents the topology of all samples by setting all edge lengths to 1. The calculated genetic distance among individuals is presented in Table 4.

Received 14/12/2023; Revised 24/04/2024; Accepted 15/05/2024
https://doi.org/10.29244/jtcs.11.03.207-216

cv. "Sigararutang" types ranged from 0.18 to 0.30 (Table 5).

The contributing alleles separating Arabica coffee cv. "Sigararutang" into four different clades are the allelic configuration of the M24 and Ca052 SSR loci since the other three loci had the same allele configuration (i.e., 1/1 for M32 and M101, 0/0 for CaM16 and

Table 4. The presence of genotypic variations based on the allelic patterns of the three polymorphic SSR marker loci (Ca05, CaM1, and Ca08) and for each Arabica coffee "Sigararutang," "S795," and "Ahernt GRT KN." Samples belonging to different types represent differences in their allelic constitutions.

Arabica coffee cultivars and types	The SSR locus genotypes based on the allele composition					
	M24	Ca052	M32	CaM16	M101	Ca087
"Sigararutang" type 1 (n=11)	1/1	2/2	1/1	0/0	1/1	0/0
"Sigararutang" type 2 (n=1)	99/99	1/2	1/1	1/1	1/1	0/0
"Sigararutang" type 3 (n=2)	1/1	1/1	1/1	0/0	1/1	0/0
"Sigararutang" type 4 (n=32)	2/2	1/1	1/1	0/0	1/1	0/0
"S795" type 1 (n=3)	2/2	1/1	2/2	1/1	2/2	1/1
"S795" type 2 (n=1)	2/2	1/1	2/2	1/1	1/2	1/1
"S795" type 3 (n=1)	2/2	1/1	2/2	1/1	2/2	0/0
"S795" type 4 (n=2)	2/2	1/1	2/2	0/0	2/2	0/0
"S795" type 5 (n=1)	2/2	1/1	1/1	1/1	2/2	2/2
"S795" type 6 (n=1)	1/1	2/2	1/2	0/0	2/2	1/1
"Ahernt GRT KN" type 1 (n=10)	2/2	1/1	2/2	0/0	2/2	2/2
"Ahernt GRT KN" type 2 (n=16)	1/2	1/2	2/2	0/0	2/2	2/2
"Ahernt GRT KN" type 3 (n=1)	1/2	2/2	2/2	0/0	2/2	2/2
"Ahernt GRT KN" type 4 (n=9)	1/1	2/2	2/2	0/0	2/2	2/2
"Ahernt GRT KN" type 5 (n=1)	99/99	1/1	1/1	0/0	2/2	2/2

Note: 1/1 = homozygote "1-1," 1/2 = heterozygote "1-2," 2/2= homozygote "2-2," 99/99 = missing allele.
*n = number of samples belonging to a specific type.

Table 5. Matric of genetic distances among Arabica coffee cultivars and types within cultivars.

Types within cvs	Sg T1	Sg T2	Sg T3	Sg T4	S T1	S T2	S T3	S T4	S T5	S T6	Y T1	Y T2	Y T3	Y T4
Sg T2	0.3													
Sg T3	0.2	0.2												
Sg T4	0.3	0.3	0.2											
S T1	0.8	0.8	0.7	0.6										
S T2	0.9	0.9	0.7	0.6	0.1									
S T3	0.8	0.8	0.6	0.6	0.2	0.2								
S T4	0.7	0.7	0.6	0.5	0.4	0.5	0.4							
S T5	0.8	0.7	0.6	0.5	0.4	0.4	0.3	0.4						
S T6	0.9	0.8	0.7	0.6	0.7	0.7	0.6	0.6	0.6					
Y T1	0.7	0.6	0.5	0.4	0.4	0.4	0.3	0.2	0.3	0.5				
Y T2	0.7	0.7	0.6	0.5	0.6	0.6	0.5	0.4	0.5	0.3	0.4			
Y T3	0.8	0.8	0.7	0.6	0.6	0.7	0.6	0.5	0.6	0.4	0.5	0.1		
Y T4	0.8	0.8	0.7	0.6	0.7	0.7	0.6	0.5	0.6	0.4	0.5	0.1	0.1	
Y T5	0.6	0.6	0.5	0.4	0.5	0.5	0.4	0.3	0.4	0.5	0.3	0.4	0.4	0.3

Note: Sg = Sigararutang, S = "S795," Y = Ahernt GRT KN, T1-Tn = types within each Arabica coffee cultivar based on their SSR allele profiles. See Table 4 for detailed allele profiles for each type.

Received 14/12/2023; Revised 24/04/2024; Accepted 15/05/2024
https://doi.org/10.29244/jtcs.11.03.207-216

Ca087 SSR loci, respectively). Allelic configurations of the M24 and Ca052 SSR loci for clade 1 of Arabica coffee cv. "Sigararutang" are 1/1 and 2/2, while those for clade 4 are 2/2 and 1/1. Allelic configurations of the M24 and Ca052 SSR loci for clade 2 of Arabica coffee cv. "Sigararutang" are 99/99 (missing data) and 1/2, while those for clade 3 are 1/1 and 1/1.

The M24 and Ca052 SSR loci are reported as two linked loci residing in the Arabica coffee genome's same chromosome (chromosome 6). The position of the two SSR loci are in sequence 37,272,573 (M24 locus) and 49,779,001 (Ca052) of the Arabica coffee genomes, separated by 12.5 megabase pairs (Mbps) (Scalabrin et al., 2020). Based on our data, the allelic configuration of M24 and Ca052 loci were either 1/1 or 2/2, as in Arabica coffee cv. "Sigararutang" type 1 or 2/2 and 1/1 as in type 4. Therefore, the predicted allelic configurations for the missing alleles in the M24 locus of "Sigararutang" type 2 are 1/2. The "Sigararutang" type 2 sample was probably a hybrid between type 1 and type 4 parents. Moreover, "Sigararutang" type 3 samples showed M24 and Ca052 loci allelic configuration of 1/1 and 1/1, which indicates that the two members of "Sigararutang" type 3 are the product of chromosome crossing over between M24 and Ca052 loci.

Based on the clustering analysis results, nine samples of Arabica coffee cv. "S795" is divided into six clades (i.e. "S795" type 1 - 6). Each "S795" type comprised 1, 2, or 3 individual samples (Table 4). The contributing alleles separating Arabica coffee cv. "S795" is divided into six clades, the six SSR loci allelic configurations. Allelic configurations of the M24 and Ca052 SSR loci for clade 1 - 5 of Arabica coffee cv. "S795" are 2/2 and 1/1, while those for clade 6 are 1/1 and 2/2 (Table 4). The "S795" type 6 sample showed a heterozygous allele configuration in the M32 locus, and it may have represented a hybrid progeny. The high genetic diversity among "S795" samples may have come from intercrossing "S795" with other cultivars or because of the intra-varietal variability. Arabica coffee cv. "S795" resulted from the selection of hybrid progenies between Arabica coffee cv. Kent and S288 (Waller et al., 2007). Meanwhile, Arabica coffee cv. S288 originated from hybridization between *C. liberica* and *C. arabica* (Nayani et al., 2017). Such hybridization origins may make Arabica coffee cv. "S795" heterozygous and heterogeneous populations showing intra-varietal genetic diversity. Subsequently, the offspring of "S795" may segregate and form homozygous and heterogeneous populations with high genetic diversity through self-pollination.

Based on the clustering analysis, Arabica coffee cv. "Ahernt GRT KN" samples are categorized into five distinct clades: "Ahernt GRT KN" type 1, 2, 3, 4, and 5. Among these, types 1, 2, and 4 comprise 10, 16, and 9 individual samples, respectively, while types 3 and 5 each contain one sample. Similar to "Sigararutang," the allelic configurations of the M24 and Ca052 SSR loci significantly differentiate the clades within Arabica coffee cv. Ahern GRT KN. Notably, type 2 samples exhibit a heterozygous allelic configuration (1/2) for these loci, while types 1 and 4 display configurations of 2/2 and 1/1, respectively.

The genetic distance among "Sigararutang" types 1-4 ranges from 0.2 to 0.3, indicating relatively low diversity within this cultivar. Similarly, the genetic distance among Arabica coffee cv. "S795" types 1-6 range from 0.1 to 0.7, reflecting higher diversity than Sigararutang. For Arabica coffee cv. "Ahernt GRT KN," the genetic distance among types 1-5 ranges from 0.1 to 0.5. The diversity within "Sigararutang" and "Ahern GRT KN" is primarily attributed to M24 and Ca052 SSR loci variations. Conversely, higher diversity was observed in Arabica coffee cv. "S795" is due to contributions from six SSR loci.

There is a need to reselect the genetic identity of "S795" and distribute the selected varieties to Arabica coffee farmers, given the high genetic diversity among samples of Arabica coffee cv "S795," This ensures the maintenance of desired traits and genetic purity within the cultivar.

Conclusions

Twenty SSR primer pairs were tested for their ability to generate SSR markers using 15 samples of Arabica coffee, resulting in six primers showing allele polymorphism. Genetic analysis of three cultivars of Arabica coffee from Garut, West Java, using SSR markers, revealed a moderate average Polymorphic Information Content (PIC) value of 0.33 across loci and varieties. However, the PIC value within Arabica coffee cv. "Ahernt GRT KN" and "Sigararutang" were low, whereas within "S795," it was moderate. Such data suggest a higher genetic diversity within "S795." Further analysis indicated the observed heterozygosity (Ho) across Arabica coffee cv. "Sigararutang" and "S795" were much lower than the expected heterozygosity (He), indicating the predominantly self-pollinating nature of these cultivars. Conversely, the Ho values of Arabica coffee cv. "Ahernt GRT KN" were larger, suggesting residual heterozygosity and a low percentage of recent outcrossing. Consequently, using harvested seeds from the mixed plantation of Arabica coffee cv "Ahernt GRT KN" could result in heterogeneous progenies. On the other hand, the low Ho values of "Sigararutang"

Received 14/12/2023; Revised 24/04/2024; Accepted 15/05/2024
https://doi.org/10.29244/jtcs.11.03.207-216

suggest it is more homozygous compared to "Ahernt GRT KN," and seeds harvested from "Sigararutang" should be more homogeneous. Although Arabica coffee cv. "S795" also exhibited a low Ho value; the moderate He value within "S795" indicates high genetic diversity. Therefore, intra-specific selection of "S795" is necessary before utilizing seeds harvested from mixed gardens. Cluster analysis based on genotyping data revealed four types of Arabica coffee cv. "Sigararutang," five types of "Ahernt GRT KN," and six types of "S795" samples.

Acknowledgment

The authors thank the Ministry of Finance of the Republic of Indonesia through the Education Fund Management Institute (LPDP) for financing the primary author's MSc. degree program and partially providing research funds.

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