Hindawi
Scientifica
Volume 2023, Article ID 6676038, 12 pages
https://doi.org/10.1155/2023/6676038

Research Article

Estimating the Genetic Diversity of Oranges *Citrus* spp. in South Sulawesi, Indonesia, Using RAPD Markers

Mustika Tuwo,[1,2] **Tutik Kuswinanti**,[3] **Andi Nasruddin**,[3] **and Elis Tambaru**[2]

[1]*Doctoral Program of Agricultural Science, Graduate School Universitas Hasanuddin, Makassar 90245, Indonesia*
[2]*Department of Biology, Faculty of Mathematics and Natural Science, Universitas Hasanuddin, Makassar 90245, Indonesia*
[3]*Department of Plant Pest and Disease, Faculty of Agriculture, Universitas Hasanuddin, Makassar 90245, Indonesia*

Correspondence should be addressed to Mustika Tuwo; mustikatuwo@gmail.com

Received 1 May 2023; Revised 14 October 2023; Accepted 28 October 2023; Published 8 November 2023

Academic Editor: Carsten Wrenger

Oranges hold significant economic importance, being cultivated extensively worldwide and having a large global market. Indonesia, ranked eighth globally as a producer of oranges, is one of the countries with high genetic diversity of oranges. This diversity is distributed across various regions of Indonesia, including South Sulawesi. Despite the advancements in DNA-based molecular marker techniques for assessing genetic diversity, information on orange diversity in South Sulawesi is currently unavailable and under-researched. In this study, random amplified polymorphic DNA (RAPD) markers were utilized to analyze the genetic diversity of oranges in five production centers in South Sulawesi. Leaf samples of 13 orange varieties were collected from the five production centers: Pangkep, Sidrap, Bantaeng, North Luwu, and Selayar in South Sulawesi, Indonesia. Genomic DNA extraction from the orange leaves followed the protocol of the DNA Mini Kit Geneaid. DNA amplification was carried out using the RAPD method with 14 primers: OPE-04, OPH-04, OPH-15, OPN-14, OPN-16, OPR-08, OPR-20, OPW-06, OPW-09, OPX-07, OPX-11, OPX-17, UBC-18, and UBC-51. The RAPD primers yielded 109 amplified fragments ranging in size from 200 to 2000 base pairs (bp), and all RAPD primers showed 100% polymorphism. The genetic diversity value (He) of oranges in South Sulawesi was moderate (0.236). Cluster analysis based on a similarity coefficient of 77% divided the 175 orange genotypes into five groups. The most closely related genotypes were SB6 and SB7, exhibiting 100% similarity, followed by genotypes JS8 and JS9 and JS13 and JS17, with genetic similarities exceeding 99% for each pair. Genotypes P9 and SI5 displayed the highest genetic distance, with a similarity coefficient of 57%. The dendrogram diagram can serve as a basis for selecting desired plant traits in the improvement of plant characteristics through both conventional breeding and genetic engineering activities.

1. Introduction

Oranges are considered the most important fruit commodity worldwide, both in fresh and processed forms [1, 2]. According to data from the Food and Agriculture Organization (FAO) of the United Nations, global orange production exceeded 75 million tons in 2019 [3]. This is in line with data from the Central Statistics Agency (BPS) of Indonesia, which reported that orange (tangerine/mandarin) production in Indonesia reached 2.72 million tons in 2022, representing a 13.2% increase compared to the previous year's production of 2.4 million tons [4]. These figures position Indonesia as the eighth largest orange producer in the world, with Brazil, India, and China as the top three orange-producing countries [5]. Indonesia has high genetic diversity in *Citrus* fruits, with production centers relatively dispersed throughout the country, including South Sulawesi. In South Sulawesi, the production centers for oranges are in the districts of Selayar, known for its tangerines, and Pangkep, known for its pomelos, which have been established as long-standing production areas. In addition to these two districts, there are three other districts that have emerged as new production areas: North Luwu for siam oranges, Bantaeng for batu oranges, and Sidrap for lime and kaffir lime. The increase in global orange production is proportional to the high demand for oranges worldwide, indicating that oranges are a favored fruit among the population. This is supported by the nutritional content of

oranges, which is beneficial for health. Oranges are a source of energy and carbohydrates (sucrose, glucose, and fructose), providing good dietary fiber that helps prevent gastrointestinal diseases. They are also rich in vitamin C and antioxidants [6]. Oranges are a source of phytochemicals, including phenols, carotenoids, phytoestrogens, and sulfides, which have potential antioxidant properties and health benefits for the human body [7].

The abundance of orange varieties and cultivars makes it difficult for researchers to differentiate them, necessitating the use of numerical taxonomy for grouping [8]. Diversity represents a valuable resource in the national orange germplasm. However, if this diversity does not reflect genetic diversity, it can lead to confusion in *Citrus* breeding activities, considering that the seed sources used by farmers in national *Citrus* centers are interrelated. Genotypic variation becomes important as genetic information that can be identified and analyzed through molecular marker applications. Despite advancements in DNA-based molecular marker techniques for studying genetic diversity, information regarding orange diversity in South Sulawesi is currently unavailable and under-researched. To address the lack of genetic diversity data for oranges in South Sulawesi, this study utilized the random amplified polymorphic DNA (RAPD) molecular marker. RAPD technology remains relevant and can be used to assess genetic diversity among cultivars originating from the same ancestors [8]. RAPD is the first and simplest PCR-based molecular marker developed for assessing genetic diversity among plant species [9], genetic diversity within populations [10, 11], selection of cultivars with genetic tolerance to salt [12], genetic conservation programs [13], and analysis of ecological aspects [14]. This study aims to analyze the genetic diversity of orange plants in five production centers in South Sulawesi using the RAPD molecular marker. The results of this study are expected to support more accurate characterization of oranges, which can serve as a basis for further research, plant breeding, and development of oranges, particularly in South Sulawesi, Indonesia.

2. Material and Methods

2.1. Plant Materials and DNA Isolation. Leaf samples of *Citrus* plants were collected from five *Citrus* cultivation centers located in the South Sulawesi Province at different elevations. Sampling was conducted from July 2021 to February 2022. Ten young leaves were collected from each plant, and leaves were taken from 10 plants for each orange variety. Detailed information of the leaf samples collected from the five locations is provided in Table 1 and Figure 1.

The extraction of DNA genomes from young *Citrus* leaves was performed following the DNA Mini Kit Geneaid protocol. The DNA quantity was measured using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific) with the Invitrogen QubitTM dsDNA BR Assay Kit, 100 assay (2–1000 ng). The quality of the DNA was assessed using a 1% (w/v) agarose gel containing ethidium bromide (0.5 μg/ml) at 100 V for 90 minutes.

2.2. PCR Amplification and Electrophoresis. The RAPD amplification reactions were analyzed using 14 primers to generate reproducible bands (Table 2). The PCR reaction mixture (13.5 μl) consisted of 3 μl genomic DNA, 3 μl ddH2O, 1.25 μl of each RAPD primer, and 6.25 μl KAPA2G Fast ReadyMix. The PCR process was performed using a SensiQuest PCR machine. The PCR amplification steps included an initial denaturation at 95°C for 30 seconds, followed by 35 cycles of annealing (adjusted to the primer temperature) for 50 seconds, extension at 72°C for 1 minute, and a final postextension at 72°C for 5 minutes. The amplified products were analyzed on a 1% (w/v) agarose gel containing ethidium bromide (0.5 μg/ml) at 100 V for 90 minutes, alongside a 50 bp DNA ladder, and documented using a Gel DOC UV-transilluminator. All PCR results were tested for reproducibility repeated three times.

2.3. Data Analysis. The DNA band profiles obtained from RAPD analysis were scored based on the presence or absence of amplification bands observed on the agarose gel, taking into consideration clear and reproducible DNA bands selected for analysis. A score of 1 was assigned to bands that appeared, while a score of 0 was given to bands that did not appear for each primer. The presence or absence of bands was manually observed through the electropherogram. The calculated data included the percentage of polymorphism, heterozygosity value, and polymorphic information content (PIC). The percentage of polymorphism was calculated as the percentage of polymorphic loci out of the total loci obtained per primer.

The heterozygosity value was calculated using the following formula [15, 16]:

$$\mathrm{He} = 2^{*} p^{*} q, \tag{1}$$

where for binary diploid data and assuming Hardy–Weinberg equilibrium, $q = (1\text{-Band Freq.})\,\hat{}\,0.5$ and $p = 1 - q$ [15].

The value of polymorphic information content (PIC) was calculated using the following formula [17]:

$$\mathrm{PIC} = 2\,\mathrm{fi}\,(1 - \mathrm{fi}). \tag{2}$$

Annotation: fi = frequency of allele.

A similarity matrix of the binary data was used for cluster analysis using the UPGMA (unweighted pair group method with arithmetic averages) and SAHN (sequential agglomerative hierarchical and nested) algorithms to obtain a dendrogram using NTSYS-pc version 2.10e software [18, 19]. Principal coordinate analysis (PCoA) was performed based on random amplified polymorphic DNA (RAPD) data to further understand the similarity among cultivars using the PCoA package in NTSYS-pc 2.1 [20].

3. Results

3.1. RAPD Analysis. A total of 14 primers, selected based on previous studies [21], were used (listed in Table 3).

Table 1: *Citrus* spp. cultivar collected from South Sulawesi, Indonesia.

Sample site	Varieties of oranges	Geographical coordinates	Altitude (m asl.)
Ma'rang (Pangkep Regency)	Red pomelos, white pomelos, and sweet pomelos	Lat S-4o42′ ″long E 119°34″	32
Pitu Riase (Sidrap Regency)	Lime and kaffir lime	Lat S-3.84° long E 119.81°	205
Bisappu (Bantaeng Regency)	Batu orange	Lat S-5o32′ ″long E 119°51″	265
Malangke Barat (North Luwu)	Sweet santang, siam orange, and dekopon	Lat S-2o50′ ″long E 120°19″	17
Bontomatene and Bontona Saluk (Selayar Regency)	Seeded selayar, selayar-selayar, JC-selayar, and JC	Lat S-6°8′1 ″long E 120°27″	268.5

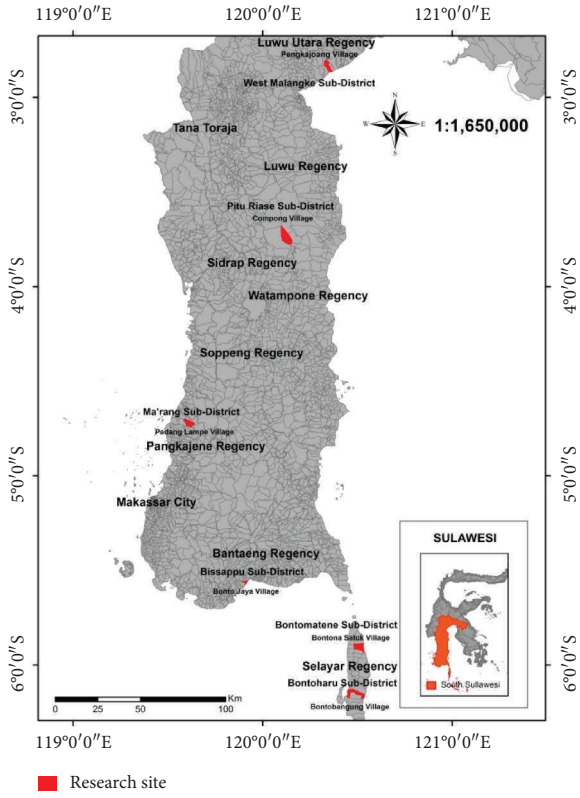

Figure 1: Sampling locations of *Citrus* leaf specimens in South Sulawesi, Indonesia.

Table 2: List of RAPD primers used in this study.

Primer	Primer sequences (5′-3′)	Tm (°C)
OPE-04	GTG ACA TGC C	33.2
OPH-04	GGA AGT CGC C	37.5
OPH-15	AAT GGC GCA G	37.1
OPN-14	TCG TGC GGG T	43.2
OPN-16	AAG CGA CCT G	35.1
OPR-08	CCA TTC CCC A	33.2
OPR-20	TCG GCA CGC A	44.5
OPW-06	AGG CCC GAT G	39.3
OPW-09	GTG ACC GAG T	33.9
OPX-07	GAG CGA GGC T	39.5
OPX-11	GGA GCC TCA G	35.4
OPX-17	GAC ACG GAC C	36.8
UBC-18	GGG CCG TTT A	35.0
UBC-51	CTA CCC GTG C	36.9

Primer screening was conducted to determine the appropriate annealing temperature and select polymorphic primers. This was done by amplifying PCR reactions using different primers and DNA samples under the same conditions [22]. A total of 109 amplified fragments were obtained using the 14 primers, and all fragments generated were found to be polymorphic. Each primer yielded an average of 7.79 amplified fragments, with a minimum of 4 fragments produced by primer OPX-17 and a maximum of 12 fragments with primer OPH-15 (Figure 2). The size of the amplified products ranged from 200 to 2000 bp. The polymorphic information content (PIC) values of the primers ranged from 0.143 for primer OPX-11 to 0.388 for primer OPH-04, with an average value of 0.253. The dendrogram was obtained from the UPGMA analysis of the binary RAPD data, resulting in five clusters.

3.2. Genetic Diversity. Genetic diversity can be defined as the variation within and between species in terms of genetic composition. Populations with high genetic diversity are more likely to exhibit enhanced adaptation [23]. Genetic diversity can be assessed based on the values of heterozygosity. Heterozygosity is a parameter used to measure the level of genetic diversity within a population. The average value of heterozygosity (He) is 0.236 (Table 4). The highest heterozygosity value was observed in type red pomelos (M), which is 0.299, while the lowest was observed in type JC-selayar (JS), which is 0.167. The values of He among the *Citrus* cultivar populations varied considerably, ranging from 0.167 to 0.299. The average heterozygosity value for the *Citrus* population is 0.236.

Cluster analysis results of 175 *Citrus* genotypes using 14 primers can be seen in Figure 3. At a similarity level of 0.69, all analyzed *Citrus* genotypes can be separated into 2 main clusters. Cluster 1 can be further divided into subclusters with different genetic distances. Based on the genetic distance at a coefficient of genetic similarity of 0.77, 5 clusters were identified, each having distinct genetic relationships. Cluster 1 consists of 54 genotypes (SB, SS, JS, B, P, JSI, SI, SM, JC, and M), cluster 2 consists of 40 genotypes (SB, SS, JS, D, SI, MSI, SI, and SM), cluster 3 consists of 41 genotypes (JC, SS, B, SM, JS, and NN), cluster 4 consists of 30 genotypes (M, P, and G), and cluster 5 consists of 10 genotypes (N) (Table 5).

Principal coordinate analysis (PCoA) is an analysis used to determine the proximity of individuals based on the similarity of their characteristics through dimensionality reduction. Figure 4 shows the results of the principal coordinate analysis derived from the binary RAPD data. The PCoA analysis grouped the *Citrus* genotypes based on their types, including red (M), white (P), and sweet (G) pomelo cultivars, as well as the lime (N) cultivar. This indicates that each *Citrus* cultivar is distinct from the others. The pomelo group exhibits higher diversity compared to other cultivars as evidenced by the scattered distribution of points within the group compared to the tendency of other cultivar groups to cluster together.

4. Discussion

The application of molecular markers is an appropriate strategy for analyzing the genetic diversity of *Citrus* species and cultivars. Molecular markers such as RAPD have been widely used in germplasm characterization, genetic diversity studies, systematic analysis, and phylogenetic analysis [24]. RAPD has proven to be quite efficient in detecting genetic variations [25]. For the purpose of identifying genetic diversity, the choice of primers is crucial in distinguishing between species varieties or cultivars [26]. Amplification of

Table 3: Details of RAPD primers and obtained polymorphisms.

Primer	Primer sequences (5′-3′)	Ta (°C)	Fragment size (bp)	Number of band	Number of fragment polymorphic	% fragment polymorphic	PIC
OPE-04	GTG ACA TGC C	30.4	400–1300 bp	5	5	100	0.378
OPH-04	GGA AGT CGC C	40.3	250–1600 bp	6	6	100	0.388
OPH-15	AAT GGC GCA G	35.4	200–1200 bp	12	12	100	0.196
OPN-14	TCG TGC GGG T	43.8	200–1800 bp	7	7	100	0.258
OPN-16	AAG CGA CCT G	34.5	250–1100 bp	9	9	100	0.291
OPR-08	CCA TTC CCC A	33.8	300–2000 bp	8	8	100	0.192
OPR-20	TCG GCA CGC A	45.1	250–1200 bp	10	10	100	0.178
OPW-06	AGG CCC GAT G	37.6	250–1400 bp	9	9	100	0.274
OPW-09	GTG ACC GAG T	37.6	200–700 bp	7	7	100	0.220
OPX-07	GAG CGA GGC T	41.2	200–1600 bp	8	8	100	0.288
OPX-11	GGA GCC TCA G	36.0	350–1000 bp	8	8	100	0.143
OPX-17	GAC ACG GAC C	36.2	350–1000 bp	4	4	100	0.350
UBC-18	GGG CCG TTT A	32.3	350–1000 bp	5	5	100	0.198
UBC-51	CTA CCC GTG C	41.3	200–1500 bp	11	11	100	0.188
Total				109	109		3.542
Mean						100	0.253

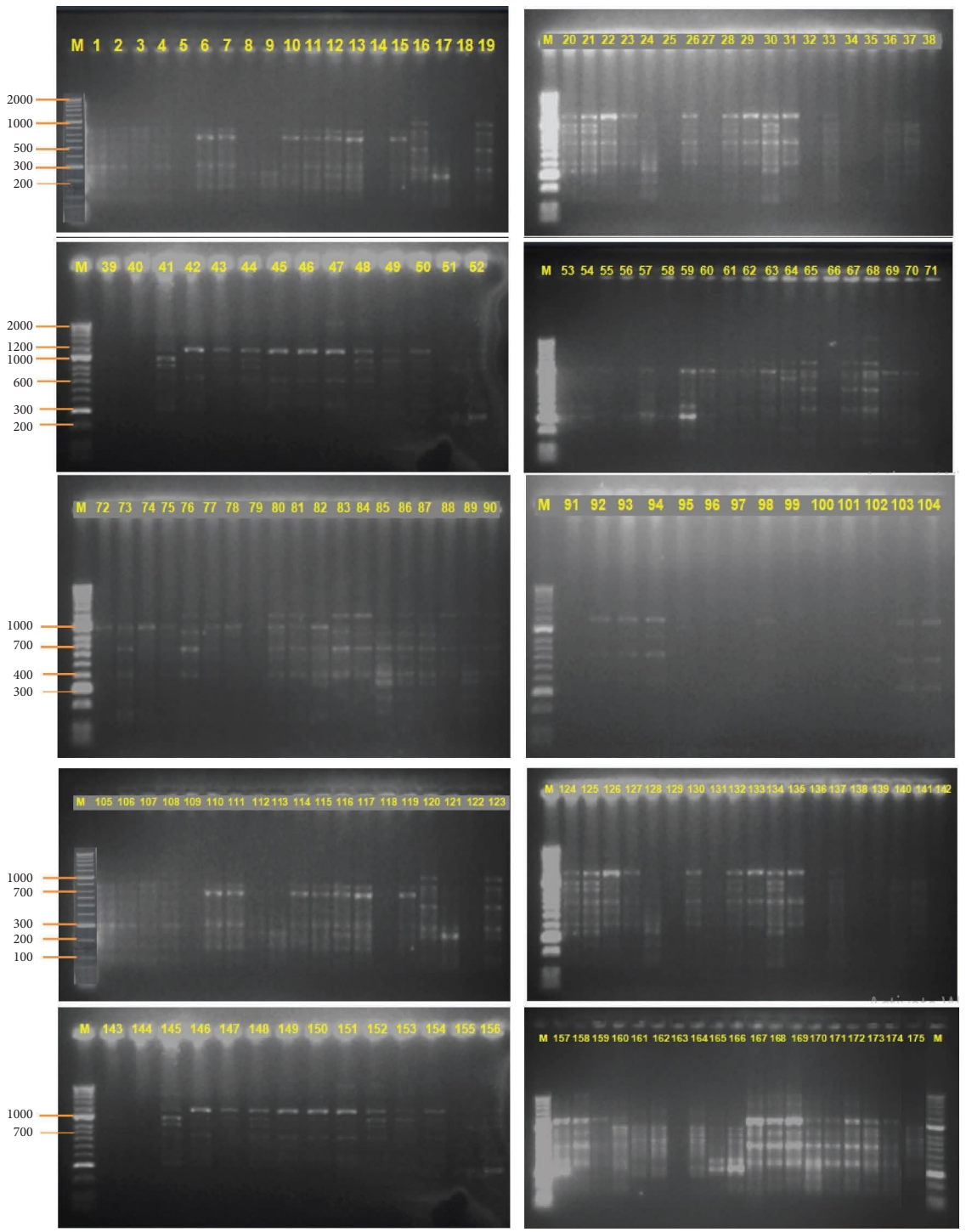

Figure 2: RAPD profile using primer OPA-15. M = marker, 1–10 = SB (seeded selayar), 11–20 = SS (selayar-selayar), 21–30 = JS (JC-selayar), 31–40 = M (red pomelo), 41–50 = P (white pomelo), 51–60 = G (sweet pomelo), 61–70 = JC (japanche citroen), 71–90 = B (batu orange), 91–110 = SI (siam orange), 111–130 = N (lime), 131–140 = NN (kaffir lime), 141–160 = SM (sweet santang), and 161–175 = D (dekopon orange).

the total genomic DNA of 175 *Citrus* genotypes was performed using 14 primers (Figure 2). Each primer produced a different number of DNA fragments. The appearing fragments exhibited variations in base size and fragment intensity. Differences in fragment intensity are influenced by the distribution of primer binding sites on the genome, as well as the purity and concentration of the genomic DNA in the reaction. The number of fragments generated by each primer depends on the distribution of homologous sites in the genome [25]. The presence of differences in DNA fragment patterns (in terms of quantity and size) reflects the existence of a highly complex plant genome [27].

Table 4: Heterozygosity values.

No.	Varieties of oranges	Heterozygosity (He)
1	Seeded selayar (SB)	0.204
2	Selayar-selayar (SS)	0.202
3	JC-selayar (JS)	0.167
4	Red pomelo (M)	0.299
5	White pomelo (P)	0.268
6	Sweet pomelo (G)	0.290
7	JC orange (JC)	0.211
8	Sweet santang (SM)	0.195
9	Batu orange (B)	0.233
10	Siam orange (SI)	0.229
11	Lime (N)	0.268
12	Kaffir lime (NN)	0.289
13	Dekopon orange (D)	0.212
	Average	0.236

Despite the newly developed genetic markers, the use of RAPD as a genetic marker remains a preferred choice for rapid estimation of genetic diversity status. The key feature of the RAPD technique is its high polymorphism detection capability [28], which is consistent with the findings of this study, where each primer produced a varying number of DNA fragments, up to 12 fragments. The percentage of polymorphic bands for all RAPD primers was 100%, indicating that the utilized RAPD markers had a high level of polymorphism. This is in line with previous primer selection results [21], where these primers were identified as generating polymorphic band patterns for *Citrus* varieties. This indicates that the used RAPD markers possess a high level of polymorphism (>50%). RAPD profiles reveal that each primer can yield prominent bands that can serve as RAPD markers to detect differences among the 13 varieties. Polymorphic bands can depict the genomic state of the plant, with a greater number of polymorphic bands indicating higher genetic diversity [29].

The success of a primer in amplifying template DNA is determined by the presence of nucleotide sequence homology between the primer and the template sequence. Other factors that also influence amplification include the quantity and quality of DNA, the concentration of MgCl2, Taq DNA polymerase enzyme, and annealing temperature [30]. The quality of RAPD markers is evaluated through the polymorphic information content (PIC) value. RAPD primers yielded PIC values ranging from 0.143 to 0.388, indicating that all the primers used in this study are suitable for the genetic characterization of *Citrus*. According to Botstein et al. [31], PIC classification is highly informative if $PIC > 0.5$, moderately informative if $0.5 > PIC > 0.25$, and weakly informative if $PIC < 0.25$. RAPD markers can be recommended for use in *Citrus* breeding programs. To date, RAPD is still widely used to assess genetic diversity in various plant species [8, 10, 32–36]. In *Citrus* plants, RAPD markers have been used for cultivar identification, mapping, genetic diversity assessment, and other breeding programs [37]. The application of RAPD has been successful in characterizing sweet orange varieties, enabling the differentiation and distinction of each variety from one another [28]. The utilization of RAPD has proven effective in analyzing phylogenetic relationships and genetic diversity among *Citrus* varieties [38].

DNA markers commonly used to reveal genetic diversity and relationships are RAPD markers, which are one of the many techniques used in molecular biology research. RAPD is considered a simple DNA marker because it does not require prior information from DNA sequence data [39], it is simple in preparation [40], it is fast and easy to analyze, it can be distributed throughout the genome [41], and it can be performed at any stage of plant development [42]. Additionally, RAPD does not require highly pure DNA, meaning it is tolerant to varying levels of DNA purity [43]. RAPD markers are effective and reliable molecular markers for assessing genetic variation accurately [44]. RAPD generates a higher number of genetic loci compared to phenotypic and biochemical markers [45]. One drawback of RAPD markers is their low reproducibility [46]. However, this can be minimized by optimizing PCR conditions, testing the reproducibility of selected primers by repeating PCR amplification three times under the same amplification conditions [47], choosing suitable primers [48], and ensuring optimal extraction methods [49]. Reproducibility in RAPD refers to the extent to which the results of RAPD analysis can be consistently reproduced when performed by different laboratories or individuals. In this study, efforts have been made to minimize factors affecting reproducibility, such as the quality of DNA obtained, which averaged between 39.93 and 85.20 ng/μg. This range is considered more than sufficient for RAPD analysis, where the required DNA concentration is typically 10–100 ng/μg [50]. The PCR technique, including the PCR reaction conditions (temperature, time, and cycle number), the choice of primers, and the electrophoresis conditions, has been standardized, and researchers followed the same protocol during repetitions. Internal reproducibility was also conducted within this study, with experiments repeated three times in the same laboratory, and external reproducibility involved collaboration with three different laboratories: the Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Hasanuddin University; the Laboratory of Research and Development in Sciences, Faculty of Mathematics and Natural Sciences, Hasanuddin University; and the Microbiology Laboratory, Hasanuddin University.

One of the parameters used to assess genetic diversity is genetic variation or heterozygosity (He) [51]. The highest genetic diversity is found in the red pomelo (M) population with a He value of 0.299. The lowest genetic diversity is observed in the JC-selayar population (JS) with a He value of 0.167. This is likely due to the fact that the JS population originates from the same parent. Low genetic diversity is estimated to have a negative impact on species survival and is a major concern for conservation efforts [52]. The average He value for all tested genotypes is 0.236. Dominant markers like RAPD can only produce two alleles at each locus. Therefore, the maximum He value is 0.5 [24]. Based on the analysis of He values, the genetic diversity of *Citrus* in South Sulawesi is considered moderate. According to the criteria, He values greater than 0.30 indicate high diversity, values between 0.20 and 0.30 indicate moderate diversity, and

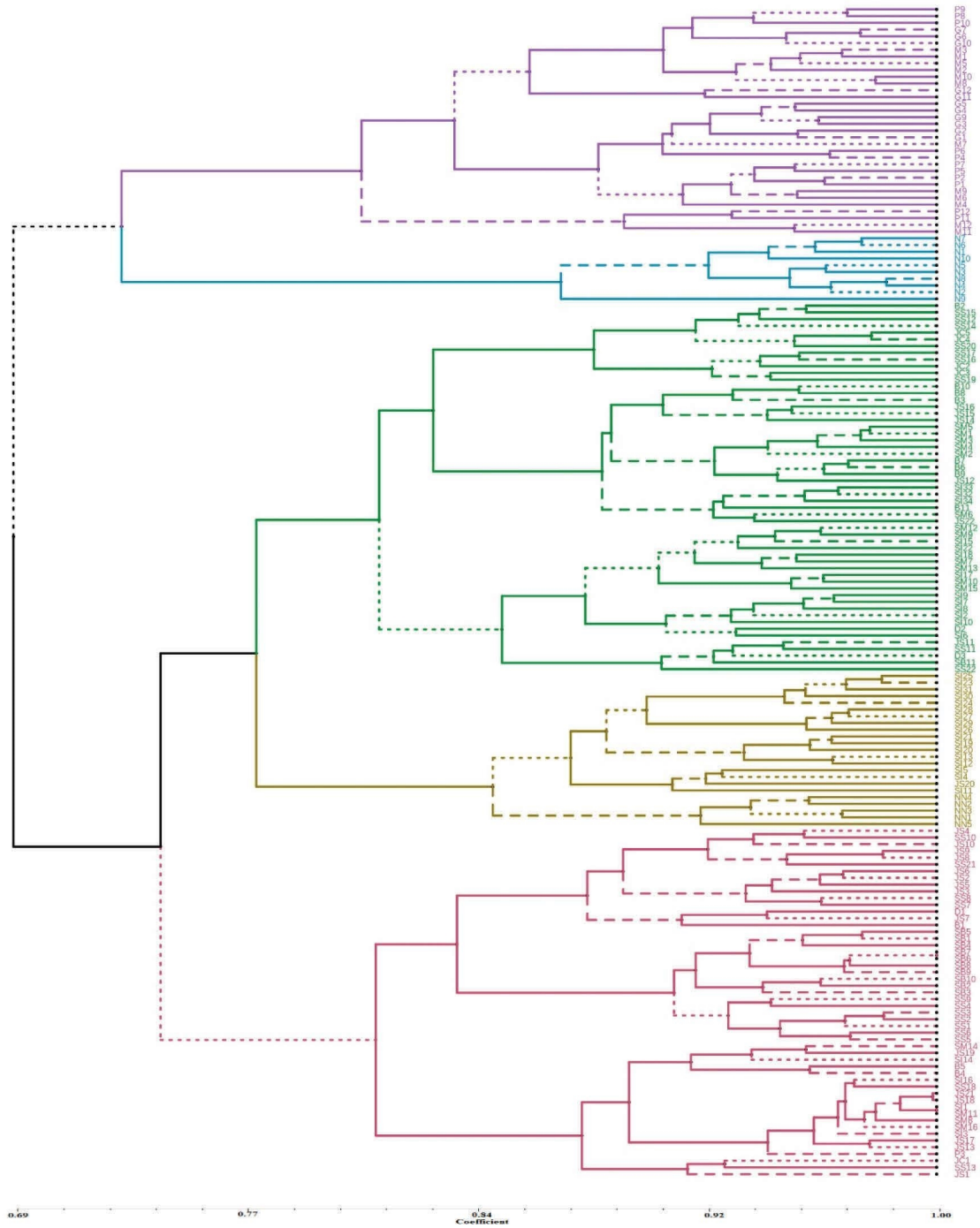

Figure 3: Dendrogram generated from UPGMA cluster analysis of 175 *Citrus* genotype samples.

values less than 0.20 indicate low diversity [53]. Populations with high genetic diversity have the ability to withstand diseases and extreme climatic changes, allowing them to persist over multiple generations [23]. The high genetic diversity in the red pomelo (M) population is likely influenced by the larger population size compared to other locations [49]. The high diversity may also be attributed to cross-pollination facilitated by pollinator agents, which play a significant role in successful fertilization. In such conditions, the likelihood of inbreeding is reduced. Cross-pollination can lead to genetic material mixing among different parent trees [54, 55].

Populations with high genetic diversity are highly valuable as they provide a diverse gene pool for genetic conservation and plant breeding programs [11]. According to the previous studies [56], populations with high genetic diversity can be attributed to several factors: (i) the population already had high genetic diversity since its formation, (ii) the population has been minimally disturbed by human activities, preserving its condition, and (iii) random

Table 5: Grouping of 175 *Citrus* cultivar genotypes at a genetic similarity coefficient of 70%.

Cluster	Genotype
I	SB1, SB5, SB6, SB7, SB8, SB9, SB4, SB2, SB3, SB10, SS1, SS2, SS3, SS7, SS8, SS10, SS9, SS5, SS6, JS2, JS5, JS6, JS3, JS8, JS9, JS10, JS4, JS7, SS4, SS21, B1, JS1, P3, SS18, JSI3, JSI17, JSI18, JSI21, SI1, SM11, SM8, SI3, SM16, SI16, JSI9, SM14, JC1, B4, B5, SS13, M11, M12, P11, and P12
II	SB11, SS11, JS11, D1, D2, D3, SI2, SI4, SI5, SI10, SI6, SI7, SI8, SI9, SI12, SI13, SI18, SI19, MSI9, SI20, SI21, SI14, SI17, SI15, SM9, SM10, SM15, SM12, SM13, SM7, SI22, SI23, SI24, SI25, SI26, SI27, SI29, SI28, SI30, and SI31
III	JC2, SS16, SS17, SS19, JC3, JC4, JC5, SS12, SS20, SS14, SS15, SS22, B2, SM2, B6, B7, B9, SM1, SM3, SM5, SM4, JS12, B3, B10, B8, B11, JS22, SI32, SI33, SI34, SM6, SI11, JS14, JS15, JS16, JS20, NN1, NN2, NN3, NN4, and NN5
IV	M1, M3, M2, M5, M6, M7, M9, M8, M10, M4, P1, P2, P4, P6, P7, P5, P8, P9, P10, G1, G2, G10, G3, G9, G6, G7, G4, G5, G11, and G12
V	N1, N7, N3, N6, N2, N5, N4, N8, N9, and N10

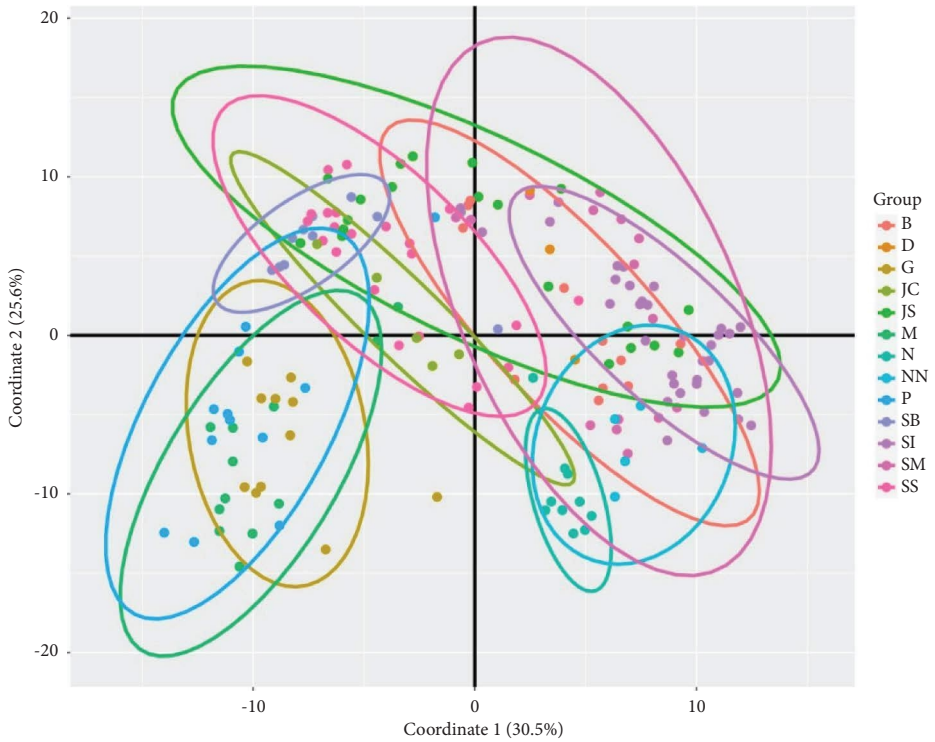

Figure 4: Results of principal coordinates analysis (PCoA) on 175 *Citrus* plant genotypes.

mating between individuals leading to genetic recombination and increased genetic diversity within the population. Conversely, low genetic diversity in a population indicates that it is threatened, fragmented, and damaged by human activities. The genetic diversity of a plant serves as the foundation for plant breeders to identify germplasm for trait improvement, viability analysis, rootstock purity, and enhancing fruit production quality and quantity [57]. Understanding phylogenetic relationships and genetic variability plays a crucial role in determining relatedness, characterizing germplasm, and establishing *Citrus* breeding programs [58]. The analysis of relatedness aims to cluster plant populations based on shared characteristics to determine their distant or close relationships [59]. To determine the genetic relationships among the 13 *Citrus* varieties, scoring data were used to calculate a similarity matrix, which was subsequently used in cluster analysis to generate a dendrogram.

The dendrogram (Figure 3) shows the separation of *Citrus* varieties into several clusters, with some clusters based on their populations. Some populations are also randomly grouped as their distribution patterns are not influenced by geographic location. This is evident in the dendrogram where the populations from Selayar Regency are grouped with varieties from other regencies such as Sidrap, Bantaeng, and North Luwu. Based on the genetic distance calculated using Nei's coefficient [60], with a similarity coefficient of 0.77, the 175 *Citrus* genotypes are

divided into 5 distinct groups with separate genetic relationships. The clustering results show that several genotypes belonging to the pomelo varieties, namely, red pomelo (M), white pomelo (P), and sweet pomelo (G), are grouped together. The PCoA analysis also confirms that the pomelo varieties M, P, and G form separate clusters, as shown in Figure 4. PCoA can be used for further confirmation of genetic diversity. The same pattern is observed for the kaffir lime genotype (N) which forms a separate cluster. However, not all genotypes with the same parentage are grouped together randomly, such as seeded selayar (SB), selayar-selayar (SS), JC-selayar (JS), Japanshe citron (JC), Siam (SI), sweet santang (SM), dekopon (D), batu (B), and kaffir lime (NN). This is likely due to the high heterozygosity of *Citrus* plants, resulting in different characteristics among genotypes derived from the same parent combination. The dendrogram reveals 5 distinct main clusters. The first cluster consists of 54 genotypes, the second cluster consists of 40 genotypes, the third cluster consists of 41 genotypes, the fourth cluster consists of 30 genotypes, and the fifth cluster consists of 10 genotypes. There is some mixing of varieties collected from three regions (Selayar, North Luwu, and Bantaeng), as seen in clusters I, II, and III. This is likely due to *Citrus* breeders using desired plant material and grafting or propagating it onto different plants or selling it to different locations. Clusters IV and V consist only of varieties collected from Pangkep and Sidrap.

The relationship among the tested genotypes ranges from 0.69 to 1, indicating that the 13 varieties exhibit varying degrees of genetic relatedness, from close to distant. All genotypes can be differentiated among the different varieties. High genetic distances indicate relatively distant relationships between varieties, and while small genetic distances indicate close genetic relatedness. Genetic distance is used to detect relationships among populations and between species. Based on the RAPD marker analysis, the *Citrus* genotypes SB6 and SB7 exhibit the closest genetic relationship, with a similarity coefficient of 100%. This is followed by genotypes JS8 and JS9, as well as JS13 and JS17, with genetic similarity values exceeding 99%. The high genetic similarity between SB6 and SB7 suggests that they are likely the same genotype. Both genotypes belong to the Keprok *Citrus* type originating from Selayar. Similarly, genotypes JS13 and JS17 have a genetic similarity value of >99% and both belong to *Citrus* varieties obtained through grafting the JC rootstock with the Selayar Keprok scion. The genotypes P9 and SI5 exhibit the furthest genetic relationship, with a similarity coefficient of 57%. These two genotypes belong to different types. SI5 is a Siam *Citrus* variety from North Luwu characterized by its greenish-yellow and shiny fruit skin, as well as a smooth fruit surface texture. On the other hand, P9 is a white pomelo from Pangkep, characterized by its large fruit size with an average diameter of 15–22 cm, and in some cases even larger than 30 cm. The fruit has a relatively thick skin measuring 2.1–3.73 cm and a strong adhesion to the flesh. Increasing genetic distance between genotypes leads to a higher heterosis effect. However, to produce desirable recombinants, agronomic characteristics should also be considered. One factor influencing genetic variation in nature is the mating system in plants [61]. This mechanism depends on flower structure, mutations, migration, and mating systems [50, 62, 63]. Genetic variation is a key factor in the conservation of biodiversity [64], as the loss of genetic variation can hinder a species' ability to respond to natural selection [65]. The observed genetic variation among samples taken from different regions with varying ecological conditions and elevations may be attributed to differences in seed sources or the influence of mutations and natural crossbreeding [66].

5. Conclusion

The genetic diversity value (He) of *Citrus* in South Sulawesi is moderate (0.236). Genetic diversity plays an important role in improving plant traits through plant breeding. Cluster analysis based on a similarity coefficient of 77% divided the 175 *Citrus* genotypes into 5 groups. The most closely related genotypes are SB6 and SB7 with a similarity coefficient of 100%, followed by JS8 and JS9, as well as JS13 and JS17, with genetic similarity values exceeding 99% for each pair. Genotypes P9 and SI5 exhibit the furthest genetic relationship, with a similarity value of 57%. The dendrogram diagram can serve as a basis for selecting desired plant traits in improving plant characteristics through both conventional breeding and genetic engineering activities.

Data Availability

The data used to support this study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors would like to express their gratitude to the Biotechnology and Tree Breeding Laboratory, Faculty of Forestry, Hasanuddin University; the Laboratory of Research and Development in Sciences, Faculty of Mathematics and Natural Sciences, Hasanuddin University; and the Microbiology Laboratory, Hasanuddin University Teaching Hospital for their support and facilities. We also thank Devi Bunga Pagalla, S. Si., M. Sc., Farham, SP, and Nurindah Rezky, S. Si for their assistance in sample collection, and Nurul Afiah Abd. Majid, S. Si. for her support in data processing.

References

[1] P. Zhang and Z. Zhou, "Postharvest ethephon degreening improves fruit color, flavor quality and increases antioxidant capacity in 'Eureka' lemon (*Citrus* limon (L.) Burm. f.)," *Scientia Horticulturae*, vol. 248, pp. 70–80, 2019.

[2] N. Kusumawati, A. B. Santoso, A. Wijiastuti et al., "Extraction, optimization, and dyeing standardization using fresh orange *Citrus* peel on cotton fabrics," *International Journal on Advanced Science, Engineering and Information Technology*, vol. 10, no. 3, pp. 1278–1283, 2020.

[3] Portal Informasi Indonesia, "Melestarikan Manisnya Jeruk Siompu," 2023, https://bit.ly/41NWdK5.

[4] Data Indonesia, "Produksi Jeruk Siam di Indonesia Sebanyak 2.7 Juta Ton pada," 2021, https://bit.ly/3AoPBFN.

[5] Katadata Media Network, "10 Negara Penghasil Jeruk, Indonesia Masuk Daftar," 2023, https://bit.ly/44aGvu7.

[6] A. Saeid and M. Ahmed, "*Citrus* fruits: nutritive value and value-added products," in *Citrus - Research, Development and Biotechnology*, IntechOpen, London, UK, 2021.

[7] W. F. Abobatta, "Nutritional benefits of *Citrus* fruits," *American Journal of Biomedical Science and Research*, pp. 303–306, 2019.

[8] S. S. Arsih, N. Fitrianto, E. Azizah, W. Herawati, and Rochmatino, "Anatomical profile and genetic variability of sweet potato (Ipomoea batatas) cultivars in Banyumas, Central Java, based on RAPD Markers," *Biodiversitas*, vol. 21, no. 4, pp. 1755–1766, 2020.

[9] A. A. Dar, R. Mahajan, S. Sharma, R. Mahajan, and S. Sharma, "Molecular markers for characterization and conservation of plant genetic resources," *The Indian Journal of Agricultural Sciences*, vol. 89, no. 11, pp. 1755–1763, 2019.

[10] M. Hamouda, "Molecular analysis of genetic diversity in population of Silybum marianum (L.) Gaertn in Egypt," *Journal of Genetic Engineering and Biotechnology*, vol. 17, no. 12, pp. 1–9, 2019.

[11] P. Zulfahmi and A. Y. Edi Purwanto dan, "Genetic diversity and population structure of Eurycoma apiculata in Eastern Sumatra, Indonesia," *Biodiversitas*, vol. 22, no. 10, pp. 4431–4439, 2021.

[12] S. R. Mazumder, H. Hoque, B. Sinha et al., "Genetic variability analysis of partially salt tolerant local and inbred rice (Oryza sativa L.) through molecular markers," *Heliyon*, vol. 6, no. 8, pp. e04333–7, 2020.

[13] G. Elyasi Zarringhabaie, A. Javanmard, and O. Pirahary, "Random amplified polymorphic markers as indicator for genetic conservation program in Iranian pheasant (*Phasianus colchicus*)," *The Scientific World Journal*, vol. 2012, no. 640381, pp. 1–5, 2012.

[14] F. Indriani, U. J. Siregar, D. D. matra, and I. Z. Siregar, "Ecological aspects and genetic diversity of shorea balangeran in two forest types of muara kendawangan nature reserve, west kalimantan, Indonesia," *Biodiversitas*, vol. 20, no. 2, pp. 482–488, 2019.

[15] M. Nei, "Estimation of average heterozygosity and genetic distance from a small number of individuals," *Genetics*, vol. 89, no. 3, pp. 583–590, 1978.

[16] L. Wallace, *Methods Available for the Analysis of Data from Dominant Molecular Markers*, Department of Biology, University of South Dakota, 2003.

[17] Z. H. Guo, K. X. Fu, X. Q. Zhang et al., "Molecular insights into the genetic diversity of Hemarthria compressa germplasm collections native to Southwest China," *Molecules*, vol. 19, no. 12, pp. 21541–21559, 2014.

[18] F. J. Rohlf, *NTSYSpc: Numerical Taxonomy and Multivariant Analysis System*, Exeter Publication, New York, NY, USA, 1998.

[19] M. Pangkey, N. N. Wahibah, and N. Sofiyanti, "Polimorfisme peroksidase ramin (gonystylus bancanus (miq.) kurz) di Hutan Pt. Diamond raya timber provinsi riau," *JOM FMIPA*, vol. 1, no. 2, pp. 340–350, 2014.

[20] K. S. Ibrahim, G. Gurusubramanian, R. P. Zothansanga Yadav et al., *Bioinformatics-A Student's Companion*, Springer Science+Bussiness Media, Singapore, 2017.

[21] M. Tuwo, T. Kuswinanti, A. Nasruddin, and E. Tambaru, "RAPD primer screening as a preliminary study to analyze the genetic diversity of *Citrus* spp. in South Sulawesi, Indonesia," *IOP Conference Series: Earth and Environmental Science*, vol. 886, pp. 1–7, 2021.

[22] S. H. Larekeng, M. Restu, A. Arif, Y. F. Cahyaningsih, and J. Mukti, "A genetic approach to study mating system on jabon merah (Anthocephalus macrophyllus Roxb.) from three different provenances in South Sulawesi," *IOP Conference Series: Earth and Environmental Science*, vol. 235, 2019.

[23] L. Siti Halimah, R. Paelongan, Y. Fitri, C. Nurhidayatullah, and M. Restu, "Primer screening and genetic diversity analysis of jabon putih (Anthocephalus cadamba (Roxb) Miq.) based on Random Amplified Polymorphic DNA (RAPD) Markers," *International Journal of Current Research and Review*, vol. 12, no. 24, pp. 173–178, 2020.

[24] K. Weising, H. Nybom, K. Wolf, and G. Kahl, *DNA Fingerprinting in Plants Principle, Methods and Application*, Taylor and Francis, Boca raton, Florida, 2005.

[25] J. G. Williams, A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey, "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers," *Nucleic Acids Research*, vol. 18, no. 22, pp. 6531–6535, 1990.

[26] Y. S. Poerba and F. Ahmad, "Genetic variability among 18 cultivars of cooking bananas and plantains by RAPD and ISSR makers," *Biodiversitas*, vol. 11, no. 3, pp. 118–123, 2010.

[27] D. Grattapaglia, J. Chaparro, P. Wilcox et al., "Mapping in woody plants with RAPD markers: application to breeding in forestry and horticulture. Application of RAPD Technology to Plant Breeding," in *Proceedings of the Joint Plant Breeding Symposia Series CSSA/ASHS/AGA*, Minneapolis, November 1992.

[28] A. S. A. Al-Janabi, S. J. A. Al-Awadi, and I. H. M. Al-Zaidi, "Genetic diversity assessment of some *Citrus* species cultivated in Iraq based on RAPD molecular marker," *Al-Kufa University Journal for Biology*, pp. 23–31, 2016.

[29] S. B. Primrose and R. M. Twyman, "Principles of gene manipulation and genomics," *American Journal of Human Genetics*, Blacwell, Carlton North, Australia, 2006.

[30] I. M. S. Sembiring, L. A. p Putri, and H. Setiado, "The Application of five RAPD primers for genetic diversity analysis of North Sumatera's Andaliman (Zanthoxylum acanthopodium DC)," *Jurnal Agroteknologi*, vol. 4, no. 1, pp. 1748–1755, 2015.

[31] D. Botstein, R. L. White, M. Skolnick, R. W. L. Davis, M. Skolnick, and R. W. Davis, "Construction of a genetic linkage map in man using restriction fragment length polymorphisms," *American journal of human genetics*, vol. 32, no. 3, pp. 314–331, 1980.

[32] S. R. Mazumder, H. Hoque, B. Sinha, W. R. Chowdhury, M. N. Hasan, and S. H. Prodhan, "Genetic variability analysis of partially salt tolerant local and inbred rice (Oryza sativa L.) through molecular markers," *Heliyon*, vol. 6, no. 8, pp. e04333–7, 2020.

[33] A. Rehman, S. Saeed, and A. Ahmed, "Genetic diversity and population structure of Seriphidium sub-genus of Artemisia from different terrains of Balochistan, Pakistan," *Biodiversitas*, vol. 22, no. 5, pp. 2658–2664, 2021.

[34] S. Ramadiana, D. Hapsoro, R. Evizal, K. Setiawan, A. Karyanto, and Y. Yusnita, "Genetic diversity among 24 clones of robusta coffee in Lampung based on RAPD markers," *Biodiversitas*, vol. 22, no. 6, pp. 3122–3129, 2021.

[35] I. W. Pangestika, A. Susilowati, and E. Purwanto, "Genetic diversty of Coffea canephora Pierre ex A. Froehner in

Temanggung District, Indonesia based on molecular marker RAPD," *Biodiversitas*, vol. 22, no. 11, pp. 4775–4783, 2021.

[36] I. G. A. S. Wangiyana, A. N. Supriadi, Sunarpi, and I. G. A. A. H. Triandini, "Diversity of Gyrinops versteegii from several agarwood plantation on Lombok Island (Indonesia) as raw material of Gyrinops tea," *Biodiversitas*, vol. 23, no. 1, pp. 178–186, 2022.

[37] M. Bidisha, D. Reya, and R. Saha, "Application of dna based molecular marker for the assessment of genetic transformation in *Citrus* sinensis," *International Journal of Science and Research*, vol. 6, pp. 2319–7064, 2013.

[38] K. Shahzadi, S. Nasz, and S. Ilyas, "Genetic diversity of *Citrus* germplasm in Pakistan based on random amplified polymorphic DNA (RAPD) markers," *The Journal of Animal and Plant Sciences*, vol. 26, no. 4, pp. 1094–1100, 2016.

[39] S. Hisada, T. Akihama, T. Endo, T. Moriguchi, and M. Omura, "Expresssed sequence tags of *Citrus* fruit during rapid cell development phase," *Journal of the American Society for Horticultural Science*, vol. 122, no. 6, pp. 808–812, 1997.

[40] B. Abbas, M. H. Bintoro, M. Sudarsono Surahman, and H. Ehara, "Genetic relationship of sago palm (metroxylon sagu rottb.) in Indonesia based on RAPD markers," *Biodiversitas*, vol. 10, no. 4, pp. 168–174, 2009.

[41] M. Sarwat, S. Das, and P. S. Srivastava, "Analysis of genetic diversity through AFLP, SAMPL, ISSR and RAPD markers in Tribulus terrestris, a medicinal herb," *Plant Cell Reports*, vol. 27, no. 3, pp. 519–528, 2008.

[42] M. Fernandez R, V. A. Sofia, and B. L. Claudio, "RAPD and freezing resistance in Eucalyptus globulus," *Electronic Journal of Biotechnology*, vol. 9, no. 3, pp. 303–309, 2006.

[43] T. K. Prana and N. S. Hartati, "Identifikasi sidik jari DNA talas Colocasia esculenta L. Schott Indonesia dengan teknik RAPD (Random Amplified Polimorphic DNA): skrining primer dan optimalisasi kondisi PCR," *Jurnal Natur Indonesia*, vol. 5, no. 2, pp. 107–112, 2003.

[44] L. Bidyaleima, R. Kishor, and G. J. Sharma, "Chromosome numbers, RAPD and ISSR profiles of six Zingiber species found in Manipur, India," *Biodiversitas*, vol. 20, no. 5, pp. 1389–1397, 2019.

[45] Y. Yonemoto, A. K. Chowdury, H. Kato, and M. M. Macha, "Cultivar identification and their genetic relationships in Dimocarpus longan subspecies based on RAPD markers," *Scientific Horticulture*, vol. 109, pp. 147–152, 2006.

[46] C. J. Jones, K. J. Edwards, S. Castagiole et al., "A Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories," *Molecular Breeding*, vol. 3, no. 5, pp. 382–390, 1997.

[47] Y. S. Poerba and E. A. Widjaya, "Prime screening for dyera costulata (miq) hook.F random amplified polymorphic DNA analyses," *Biodiversitas*, vol. 10, no. 1, pp. 12–18, 2009.

[48] A. Aminah, A. Supriyanto Suryani, and I. Z. Siregar, "Genetic diversity of Pongamia pinnata (Millettia pinnata, aka. malapari) populations in Java Island, Indonesia," *Biodiversitas*, vol. 18, no. 2, pp. 677–681, 2017.

[49] W. Arianto, E. A. M. Zuhud, A. Hikmat, T. sunarminto, and Z. S. Iskandar, "Genetic diversity of Amorphophallus titanium in Bengkulu, Indonesia based on RAPD markers," *Biodiversitas*, vol. 19, no. 5, pp. 1783–1790, 2018.

[50] J. Sambrook and D. W. Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, New York, NY, USA, 2001.

[51] R. Finkeldey, *Pengantar Genetika Hutan Tropis. Bogor: Fakultas Kehutanan, IPB. Terjemahan Dari: An Introduction to Tropical Forest Genetics*, Springer, Berlin, Germany, 2005.

[52] E. Milot, H. Weimerskirch, P. Duchesne, L. Bernatchez, P. Duchesne, and L. Bernatchez, "Surviving with low genetic diversity: the case of albatrosses," *Proceedings of the Royal Society B: Biological Sciences*, vol. 274, no. 1611, pp. 779–787, 2007.

[53] M. Na'iem, "Genetic variation of shorea leprosula miq," in *Three Population in Indonesia: Implication for Ex Situ Conservation*, Buletin Kehutanan, Yogyakarta, Indonesia, 2001.

[54] J. L. Hamrick and M. J. W. Godt, "Isozyme and the analysis of genetic structure in plant population," in *Isozyme in Plant Biology. Oregon: Dioscoridos Press*, D. E. Soltis and P. S. Soltis, Eds., Springer, Dordrecht, 1989.

[55] J. Kinho, M. Na'iem, and S. dan Indrioko, "Study on genetic diversity of Diospyros rumphii bakh in North Sulawesi based on isoenzym markers," *Jurnal Pemuliaan Tanaman Hutan*, vol. 10, no. 2, pp. 95–108, 2016.

[56] A. Azman, K.-K.-S. Ng, C.-H. Ng et al., "Low genetic diversity indicating the threatened status of *Rhizophora apiculata* (Rhizophoraceae) in Malaysia: declined evolution meets habitat destruction," *Scientific Reports*, Article ID 19112, 2020.

[57] X. Li, R. Xie, Z. Lu, and Z. Zhou, "The origin of cultivated *Citrus* as inferred from internal transcribed spacer and chloroplast DNA sequence and amplified fragment length polymorphism fingerprints," *Journal of the American Society for Horticultural Science*, vol. 135, pp. 341–350, 2010.

[58] R. Herrero, M. J. Asíns, E. A. Carbonell, L. Navarro, E. A. Carbonell, and L. Navarro, "Genetic Diversity in the orange subfamily Aurantioideae. I. Intraspecies and intragenus genetic variability," *Theoretical and Applied Genetics*, vol. 92, no. 5, pp. 599–609, 1996.

[59] U. D. L. Azizah, F. Yulianti, A. L. Adiredjo, and D. Sitawati, "Genetic relationship analysis of strawberry (Fragaria sp.) based on morphology character and Random Amplified Polymorphic DNA (RAPD)," *Plantropica Journal of Agricultural Science*, vol. 4, no. 1, pp. 77–85, 2019.

[60] M. Nei, "Genetic Genetic Distance between Populationsistance between population," *The American Naturalist*, vol. 106, no. 949, pp. 283–292, 1972.

[61] M. Sedgley and A. R. Griffin, *Sexual Reproduction of Tree Crops*, Academic Press incorporated Harocourt Brace Jovanovich Publishers, Harocourt Brace Jovanovich Publishers, San Diego, CA, USA, 1898.

[62] A. H. Hamid, N. A. Ab-Shukor, and A. L. Senin, "Morphometric and genetic variation of six seed sources of Azadirachta excelsa (Jack) Jacobs," *Journal of Biological Sciences*, vol. 8, pp. 702–712, 2008.

[63] Y. Iskandar, Z. Siregar, N. Wijayanto, I. G. K. Tapa Darma, and D. Syamsuwida, "Genetic variation of Melia azedarach in community forests of West Java assessed by RAPD," *Biodiversitas*, vol. 12, no. 2, pp. 64–69, 2011.

[64] B. Thomas, S. Macdonald, M. Hicks, D. Asams, and R. Hodgetts, "Effects of reforestation methods on genetic diversity of lodgepole pine: an assessment using microsatellite and randomly amplified polymorphic DNA markers," *Theoretical and Applied Genetics*, vol. 98, pp. 793–801, 1999.

[65] J. Gul, S. Saeed, A. Ahmed et al., "Genetic diversity and morphological variation of Pinus gerardiana along the environmental gradient from Zhob, Balochistan, Pakistan," *Nusantara Bioscience*, vol. 13, no. 1, pp. 121–128, 2021.

[66] M. C. Vidal, A. Setiawan, and Y. Wahyu, "RAPD based genetic diversity, agronomic characters, and nutrition content of Timor Leste kidney bean (Phaseolus vulgaris) genotypes," *Biodiversitas*, vol. 20, no. 9, pp. 2612–2619, 2019.