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Application of RAPD Molecular Technique to Study the Genetic Variations of Citrus in South Sulawesi, Indonesia

Mustika Tuwo [a,b], Tutik Kuswinanti [c,*], Andi Nasruddin [c], Elis Tambaru [b]

[a] *Doctoral Program of Agricultural Science, Graduate School, Hasanuddin University, Makassar 90245, Indonesia*
[b] *Department of Biology, Faculty of Mathematics and Natural Science, Hasanuddin University, Makassar 90245, Indonesia*
[c] *Department of Plant Pest and Disease, Faculty of Agriculture, Hasanuddin University, Makassar 90245, Indonesia*
*Corresponding author: *koeswinanti@yahoo.com*

***Abstract*— Indonesia is a tropical country with superior local citrus species and varieties. Citrus fruits were very useful for cultivation, especially to meet the nutritional needs of the wider community. A province in Indonesia, namely South Sulawesi is one of the centers for citrus development. A preliminary study showed various cultivated citrus in South Sulawesi namely *Mandarin Orange* cultivar *Selayar*, *Mandarin Orange* cultivar *Batu*, *JC* lime, *Pummelo Pangkep*, *tangerine*, *Santang Madu*, *Dekopon*, lime, and *Kaffir* lime. This study aims to evaluate genetic diversity at several citrus plantation centers in South Sulawesi using the RAPD technique. The analysis was carried out using five primers. In this study, RAPD primers could be used to characterize the genetic diversity and similarity of thirteen citrus cultivars in South Sulawesi. One informative RAPD primer based on its PIC value was OPC-09. The results of the genetic similarity analysis are presented in the form of a dendrogram. The first cluster consisted of *Mandarin Orange* cultivar *Selayar* (*Seeded Selayar, Selayar-Selayar, JC-Selayar*), *JC* lime, *Mandarin Orange* cultivar *Batu*, *Santang Madu*, and *Pummelo Pangkep* (cultivar *Pangkep Merah*, *Pangkep Putih*, *Pangkep Golla-golla*). The second cluster consisted of *Mandarin Orange* cultivar Selayar (*selayar-selayar, JC-selayar*), *Santang Madu*, tangerine, *Mandarin Orange* cultivar *Batu*, *Dekopon*, lime, and *Kaffir* lime. The clusters with the most distant genetic relationship are cluster A with cluster B, with a genetic similarity of 62%. Meanwhile, clusters with the closest genetic relationship are clusters I and II, with 79% genetic similarity.**

***Keywords*— Citrus; South Sulawesi; genetic relationship; genetic diversity; RAPD.**

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I. INTRODUCTION

Indonesia is a tropical country with the second highest level of biodiversity in the world [1] and consists of 16,671 islands that name has been verified as of 2018 [2]. One of Indonesia's leading horticultural commodities is fruit, and Indonesia has local superior citrus species and varieties that are spread throughout the archipelago. Citrus is one of the world's major fruit crops [3], grown in many regions [4], which has high economic value [5]. Citrus fruits belong to the family Rutaceae and subfamily Aurantioideae and can be grown in tropical and sub-tropical climates [6].

Citrus fruits were very useful for cultivation, especially to meet the nutritional needs of the wider community so that it is in line with a healthy lifestyle (by getting back to nature) and so that the consumption of citrus fruits increases along with the population that continues to increase from year to year [7], [8]. It is a source of vitamin C, minerals, phenolic compounds, flavonoids, folic acid, potassium, and pectin, and good sources of antioxidants [7]–[9].

There are differences in citrus in several aspects, such as fruit morphology, quality, embryo, inflorescence, the direction of growth, and adaptability [10], [11]. The diversity of oranges is indicated by the high number of taxonomic units [12]. However, currently in general, the world's citrus classification is still based on the classification system according to Swingle and Tanaka that in the genus Citrus there are sixteen species [13]. Citrus varieties planted in Indonesia are tangerines (75%), mandarin oranges (24%), and other varieties (1%), including grapefruit, orange, lemon, and lime [14]. Citrus fruits that are developed in almost every province in Indonesia are tangerine, mandarin orange, pummelo, sweet orange, lime, lemon, and kaffir lime [15]. The taxonomy of the genus citrus is complex mainly because of sexual compatibility between species and genera [16]. The results of the exploration of citrus species show that Indonesia, including Sulawesi, is rich in diversity of citrus.

South Sulawesi is one of the centers of citrus fruit development. Citrus fruits cultivated in South Sulawesi are mandarin orange (Selayar, Bantaeng), JC lime or mandarin lime (Selayar), orange cultivar Batu (Bantaeng), tangerine (North Luwu), lime and kaffir limes (Sidrap), pummelo (Pangkep), orange cultivar Santang Madu (Bantaeng, North Luwu), and dekopon orange (North Luwu).

Characterization of the various types of citrus fruits is needed as one of the first steps to guarantee the characteristics of citrus fruit varieties. Information on plant diversity is needed in the determination of kinship relationships, breeding programs, and taxonomy [17], [18], [19]. The more available this information is, the easier it is to determine the genetic position or relationship among varieties that can be used as the basis for plant selection.

Diversity can be studied using morphological, physiological, anatomical, palynological, cytological, biochemical, embryological, and molecular characteristics [20], [21]. Morphological characters are most often used in identification because they are easy to observe. However, morphological characters tend to be unstable because they are influenced by the environment [22]. Morphological characters are still not sufficient to determine a rank in the taxonomic level clearly; thus, it is necessary to complement other methods to evaluate genetic relationships [22]–[24].

Rapid technological developments encourage many molecular diversity studies to be carried out. Molecular markers such as RAPD, RFLP, AFLP, ISSR, and SSR have been used to research germplasm characterization, genetic diversity, and systematic and phylogenetic analysis [25], [26]. Random Amplified Polymorphism DNA (RAPD) has the advantage that with a simple procedure, relatively inexpensive price, and a small amount of DNA for analysis, it can produce highly polymorphic DNA representative of the entire genome [27]–[29]. RAPD has been widely applied to citrus plants, among others, for studying the genetic variations of citrus germplasm [5], [30]. In this study, an evaluation of genetic diversity at several citrus plantation centers in South Sulawesi was conducted using the RAPD technique.

II. Materials and Method

A. Sampling

This study was conducted from April to September 2021. Citrus leaf samples were collected from 13 cultivars (Table 1) with the condition that the plants were biologically healthy and growing in citrus growing regions in Pangkep Regency, Sidrap Regency, Bantaeng Regency, North Luwu Regency, North Luwu Regency, and Selayar Islands Regency. Sampling was done by taking 5 young leaves from each of 10 citrus plant cultivars using the purposive random sampling method.

B. DNA Isolation and PCR

DNA isolation was conducted based on the procedure of Geneaid. The DNA quality was checked by electrophoresis. The amplification process was conducted using KAPA2G Fast ReadyMix (KAPA Biosystems). The analysis process was done at the Laboratory of Biotechnology and Tree Breeding, Faculty of Forestry, Hasanuddin University.

DNA amplification employed RAPD markers (Table 2) with 10.5 µl of PCR reaction composition mix (KAPA Mix 6.25 µl; primer 1.25 µl; ddH_2O 3 µl; DNA template 3 µl). The steps of PCR refer to Tuwo et al. [31]. The qualitative test was performed using 1% agarose gel electrophoresis with TAE 1X for 60 minutes at 120 volts and imaging on Gel DOC UV-transilluminator.

TABLE I
CITRUS CULTIVARS AND THEIR REGIONS OF ORIGIN

No.	Cultivars	Sample Code	Origin
1.	Seeded selayar *Citrus reticulata*	S	Bontomatene, Selayar
2.	Selayar-selayar *Citrus reticulata*	SS	Bontomatene, Selayar. Bisappu, Bantaeng
3.	JC-selayar *Citrus reticulata*	JS	Bontomatene, Selayar
4.	JC (Japansche Citroen) *Citrus limonia*	JC	Bontomatene, Selayar
5.	Pangkep merah *Citrus maxima*	M	Padang lampe, Pangkep
6.	Pangkep putih *Citrus maxima*	P	Padang lampe, Pangkep
7.	Pangkep golla-golla *Citrus maxima*	G	Padang lampe, Pangkep
8.	Mandarin orange cv. Batu *Citrus reticulata*	B	Bisappu, Bantaeng
9.	Santang madu *Citrus reticulata*	SM, BM	Malangke Barat, Luwu Utara
10.	Tangerine *Citrus nobilis*	JSi, MSI	Malangke Barat, Luwu Utara. Bisappu, Bantaeng
11.	Lime *Citrus auratifolia*	N	Pitu Riase, Sidrap
12.	Kaffir lime *Citrus hystrix*	NN	Pitu Riase, Sidrap
13.	Dekopon *Citrus reticulata* Shiranui	D	Malangke Barat, Luwu Utara

C. DNA Isolation and PCR

The PCR results were converted into binary data. The profiles of DNA bands from the RAPD analysis were scored based on the presence or absence of amplification results. A score of 1 indicates the DNA band that appears, and a score of 0 is for the DNA band that does not appear in each primer. The binary data were then converted into a similarity matrix based on the SM (Simple Matching) coefficient. The similarity value is used for grouping analysis using the SAHN (Sequential Agglomerative Hierarchical Nested Cluster Analysis) function with the UPGMA (Unweighted Pair Group Methods with Arithmetic Average) in the NTSYSpc 2.10e program [32], [33]. The heterozygosity value was calculated using the following formula [33], [34].

$$qi = \left(\frac{individuals\ that\ do\ have\ strand}{number\ of\ individuals\ observed}\right)^{1/2} \quad (1)$$

$$pi = 1 - qi \quad (2)$$

$$He = 1 - pi^2 - qi^2 \quad (3)$$

Annotation:
qi = frequency of null allele
pi = frequency of dominant allele

The value of polymorphic information content (PIC) was calculated using the following formula [33], [35]:

$$PIC = 2fi\ (1 - fi) \quad (4)$$

Annotation:
Fi = frequency of allele

There are 3 group of the PIC value, namely highly = > 0.5; moderate = 0.25 > 0.5; and slightly informative = < 0.25 [36], [37].

TABLE II
RAPD PRIMERS SEQUENCE USED IN THIS STUDY

No.	Primer	Primer Sequences 5'-3'	Tm (°C)	Ta (°C)	No. of bands	No. polymorphic bands	% Polymorphism	Amplicon size range (bp)	PIC
1.	OPA-05	AGG GGT CTT G	32.6	35.4	4	4	100	400-1100	0.22
2.	OPA-09	GGG TAA CGC C	37.4	35.6	7	7	100	200-1100	0.33
3.	OPA-17	TCG GCG ATA G	35.7	40.2	7	7	100	100-1100	0.25
4.	OPC-09	GAC CGC TTG T	36.2	35.6	3	3	100	300-1000	0.45
5.	OPC-17	CTC ACC GTC C	37.4	40.2	2	2	100	300-500	0.35

III. Result and Discussion

A. RAPD Analysis

The RAPD molecular technique was applied to characterize and determine genetic diversity and genetic similarity (phylogenetic) in 12 citrus cultivars in South Sulawesi. RAPD is widely used on whole genomic DNA and random primers to assess genetic diversity among plants [38]. DNA isolates were obtained through the extraction process from 174 samples of orange leaves using Kit (Geneaid). The DNA isolates were then amplified using five primers (Table 1).

The banding pattern of the PCR results was then analyzed through the electrophoresis process. DNA amplification of citrus fruits resulted in a total of 23 bands in 174 samples, where all of these bands are polymorphic bands so that the polymorphic bands produced are 100%, meaning the DNA bands formed are not monomorphic bands (bands that are present in all samples). Thus, this study showed that the 13 citrus cultivars tested had high genetic diversity. As stated by Lizawati et al. [38], the presence of high polymorphic bands means the genetic diversity of the analyzed species is high.

Each primer produced a different pattern of DNA bands with an amplicon range of 100-1100 bp. The amplicon range of the OPA-05 primer is 400-1100 bp, the OPA-09 primer has an amplicon range of 200-1100, the OPA-17 primer has a range of 100-1100 bp, OPC-09 has a range of 300-1000 bp, and the OPC-17 primer has a range of 300-500 bp (Table 2). A DNA band that is present or absent among species is called a polymorphic band, while a band is called a monomorphic band if it appears in all analyzed species [38]. Polymorphism results from changes in nucleotide bases that alter the amplification region's primary binding, insertion, or deletion site [39].

The difference in polymorphism is caused by the difference in the amount of genetic variation that exists between different accessions [38]. Polymorphic information generated by DNA markers is needed in plant breeding programs to improve plant quality [40]. One of the most important features of the RAPD molecular technique is the ability to detect high levels of polymorphism, and this feature has been fulfilled in this study. However, some samples of citrus fruits did not produce bands on certain primers. This is probably due to the absence of homologous primary sequences in the genome. The number of DNA amplification bands depends on the attachment of the homolog to the DNA template [33]. Other possible causes are technical errors, amplification processes, and inappropriate temperatures of certain primers for certain samples [38]. Also influenced by several factors, including PCR conditions, quality/quantity of DNA, and concentration of PCR components [41]. The detection of RAPD-based polymorphisms is based on the variation of the annealing primer site in the PCR process. Further analysis regarding primers and samples of certain citrus cultivars needs to be done [38].

The PIC value is information to detect primers that are capable of producing polymorphic bands in a population [38], [42]. The high level of genetic diversity is influenced by the level of polymorphism of genetic markers used. Thus, the genetic markers that will be used need to be considered carefully. The value of polymorphic information content (PIC) is standardized for evaluating genetic markers based on DNA bands of PCR amplification results.

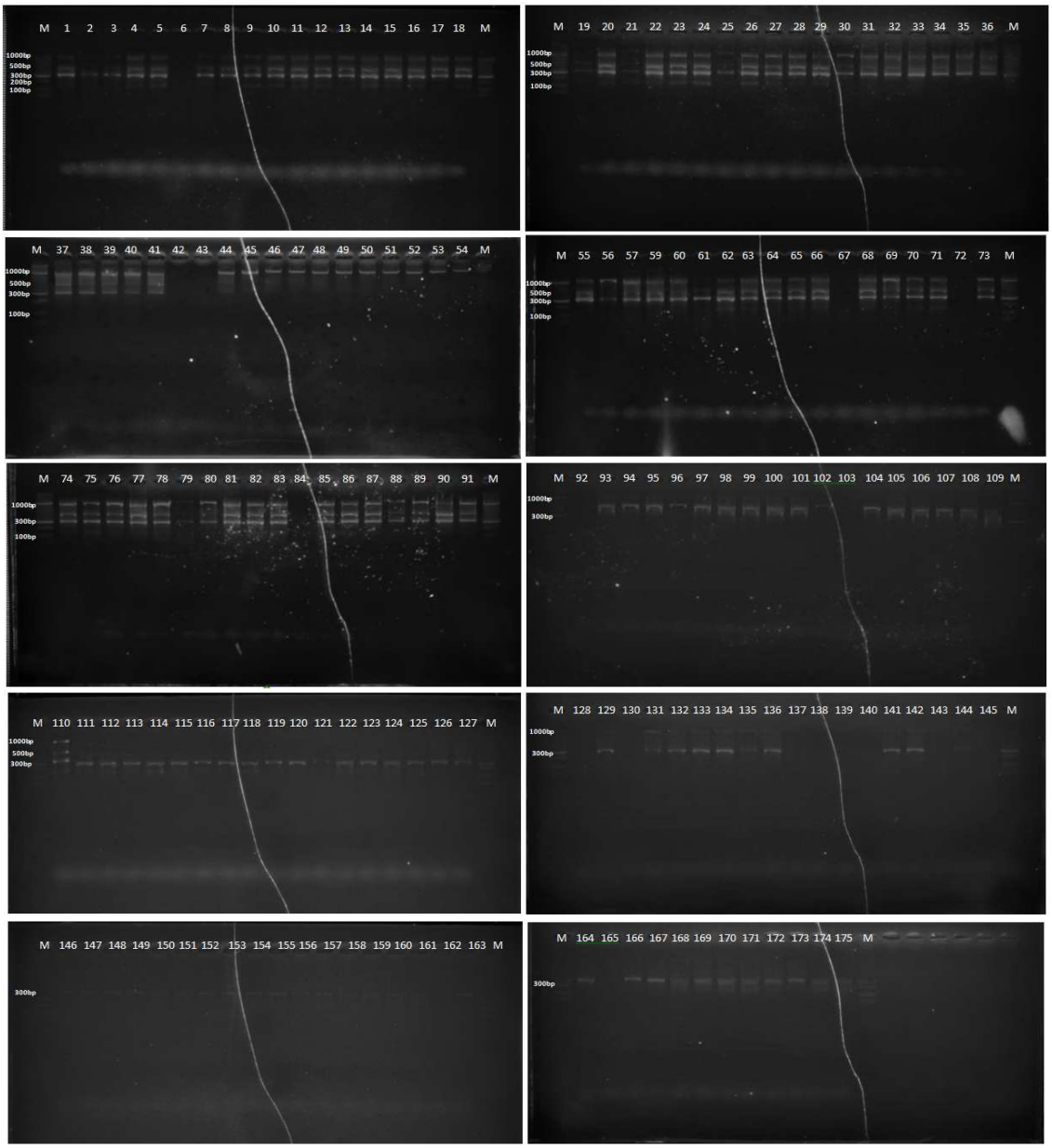

Fig. 1 The resulting RAPD profiles for 175 citrus cultivars on OPC-09 primers. M= 50 bp marker; lanes 1-18 represent the S-SS-coded samples, lanes 19-36 represent the SS-JS-M-coded samples, lanes 37-54 represent the M-P-G-coded samples, lanes 55-73 represent the G-B-coded samples, lanes 74 -91 represents the B-JS-coded sample, lanes 92-109 represents the JS-JSI-coded sample, lanes 110-127 represents the JSI-N-NN coded sample, lanes 128-145 represents the MSI-SM-coded samples, lanes 146-163 represents the SM-coded samples, lanes 164-17 5 represent the SM-coded samples

The maximum PIC value for the RAPD marker is 0.5. The PIC values are used to consider which primer is the best in the RAPD marker and reflect the diversity and allele frequency among the samples. The higher the PIC value, the better the primer is to be used in analyzing genetic variation [43]. Based on the calculation of the PIC value, each primer had a different value. The highest PIC value was discovered in the OPC-09 primer, which is 0.45, and the lowest PIC value was discovered in the OPA-05 primer. PIC value is divided into three classes: PIC > 0.5 = highly informative; 0.25 > PIC > 0.5 = moderately informative, and PIC < 0.25 = slightly informative [36]. The PIC values of 0PA-09, OPC-09, and OPC-17 primers were categorized as moderately informative, and those of OPA-05 and OPA-17 primers were categorized as slightly informative. PIC values below 0.25 are not recommended in genetic studies [43]. The PIC value of each primer can be seen in Table 2.

B. Cluster Analysis

The RAPD molecular technique using DNA as a template showed a pattern of bands that vary in size and number. The total number of DNA bands is used for cluster analysis, where the banding pattern obtained in each species is a score based on the presence or absence of each DNA band that appears. Each banding pattern of DNA amplification products is an informative profile or character to display the construction of genetic diversity and genetic relationships (similarity) between samples. DNA analysis with RAPD marker OPC-09 is shown in Figure 1. Heterozygosity is one of the parameters that is used to measure the level of genetic diversity in a population based on allele frequency at each locus [44].

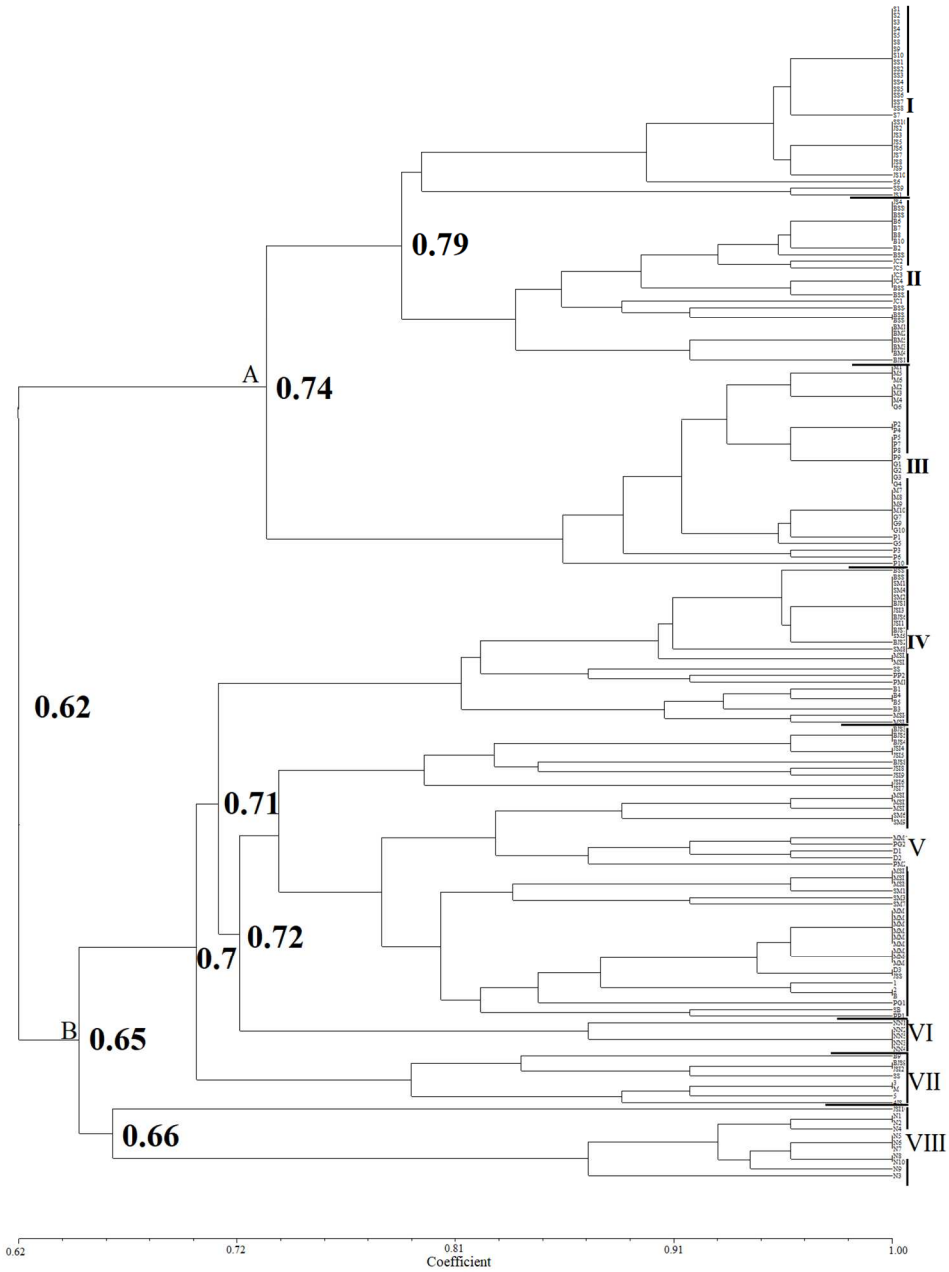

Fig. 2 Dendrogram showing the 174 groups of citrus fruits

Heterozygosity (He) is a fundamental measure of genetic diversity in a population that explains the proportion of heterozygous genotypes under Hardy-Weinberg equilibrium [45]. High heterozygosity in a population means that the genetic variability in the population is high, whereas low heterozygosity means that the genetic variability is also low [46].

Heterozygosity is one of the most important resources in breeding programs because it is associated with genetic variability [45]. Dominant markers such as RAPD can only produce two alleles at each locus [44], and therefore, the maximum heterozygosity value obtained is 0.5. The value of genetic diversity (He) 0.2349 ≤ is categorized as high [47]. The heterozygosity value is obtained from manual DNA visualization scoring results and tabulated into the heterozygosity (He) formula. Each band that appears on the gel is a specific allele [44]. The allele is then translated into binary data, which is assigned a value based on the presence or absence of an allele.

TABLE III
HETEROZYGOSITY VALUES

No.	Cultivar	Sample code	Heterozygosity
1.	Seeded Selayar	S	0.33
2.	Selayar-selayar	SS	0.38
3.	JC-selayar	JS	0.39
4.	JC	JC	0.36
5.	Pangkep merah	M	0.31
6.	Pangkep putih	P	0.28
7.	Pangkep golla-golla	G	0.31
8.	Batu	B	0.24
9.	Santang madu	SM	0.16
10.	Dekopon	JSI	0.22
11.	Tangerine	MSI	0.20
12.	Lime	N	0.27
13.	Lime kaffir	NN	0.26
Average			**0.29**

A value of 1 will be assigned if there is an allele, and a value of 0 will be assigned if there is no allele. The He value of each citrus population is quite diverse, ranging from 0.16-0.39. The average He value of the citrus population in South Sulawesi is 0.29. Based on the results of DNA analysis in this study, it can be said that the genetic diversity of the citrus population is high.

The dendrogram that was obtained based on the RAPD banding pattern of the tested citrus cultivars is presented in Figure 2 below. The data above shows that at the level of similarity of 62%, two main clusters, namely cluster A and cluster B, were obtained. Cluster A consisted of sub-clusters I, II, and III. Sub-cluster I consisted of seeded mandarin orange cultivar Selayar, mandarin orange cultivar Selayar-Selayar, and mandarin orange cultivar JC-Selayar. Sub-cluster II consisted of JC lime, mandarin orange cultivar batu, and orange cultivar santang madu while sub-cluster III consisted of pummelo cultivar Pangkep merah, pummelo cultivar Pangkep putih, and pummelo cultivar Pangkep golla-golla cultivars.

Meanwhile, cluster B consisted of sub-clusters IV, V, VI, VII and VIII with the number of individuals in each cluster varying. Cluster IV consisted of orange cultivar santang madu and tangerine, cluster V consisted of dekopon orange and tangerine, cluster VI consisted of kaffir lime, cluster VII consisted of tangerine, and cluster VIII consisted of lime. The clusters that have the most distant genetic relationship are cluster A and cluster B with a similarity of 62%. Meanwhile, clusters with the closest genetic relationship are clusters I and II with 79% similarity.

TABLE IV
CLUSTERS AND CODES OF CITRUS FRUIT SAMPLES

No.	Sub-cluster	Sample code
1	I	S1, S2, S3, S4, S5, S8, S9, S10, SS1, SS2, SS3, SS4, SS5, SS6, SS7, SS8, S7, SS10, JS2, JS3, JS5, JS6, JS7, JS8, JS9, JS10, S6, SS9, JS1
2	II	JS4, BSS9, BSS10, B6, B7, B8, B10, B2, BSS8, JC2, JC5, JC3, JC4, BSS1, BSS3, JC1, BSS4, BSS5, BSS6, BM1, BM2, BM5, BM3, BM4, BJS1
3	III	M1, M5, M6, M2, M3, M4, G6, P2, P4, P5, P7, P8, P9, G1, G2, G3, G4, M7, M8, M9, M10, G7, G9, G10, P1, G5, P3, P6, P10
4	IV	BSS2, BSS7, SM10, SM4, SM2, BJS10, JSI3, BJS6, JSI1, BJS7, SM5, BJS2, SM8, MSI3, MSI5, SS, PP2, PM1, B1, B4, B5, B3, MSI4, MSI6
5	V	BJS3, BJS5, BJS4, JSI4, JSI5, BJS8, JSI9, JSI6, JSI7, MSI1, MSI2, MSI7, SM6, SM9, MM9, PG2, D1, D2, PM2, MSI8, MSI10, MSI9, SM1, SM3, SM7, MM1, MM10, MM7, MM5, MM8, MM2, MM3, MM4, MM6, D3, JSS1, SS, SB, PG1, SB, PP1
6	VI	NN1, NN2, NN5, NN3, NN4
7	VII	B9, BJS9, JSI2, SS3, MM5, JSI4, JS6, M7, N8, O
8	VIII	JSI10, N1, N2, N4, N5, N6, N7, N8, N10, N9, N3

The data above shows that at the level of similarity of 62%, 2 main clusters, namely cluster A and cluster B, were obtained. Cluster A consisted of sub-clusters I, II, and III. Sub-cluster I consisted of seeded mandarin orange cultivar Selayar, Mandarin orange cultivar Selayar-Selayar, and mandarin orange cultivar JC-Selayar. Sub-cluster II consisted of JC lime, mandarin orange cultivar batu, and orange cultivar santang madu while sub-cluster III consisted of pummelo cultivar Pangkep merah, Pangkep putih, and Pangkep golla-golla cultivars. Meanwhile, cluster B consisted of sub-clusters IV, V, VI, VII and VIII with the number of individuals in each cluster varying. Cluster IV consisted of orange cultivar santang madu and tangerine, cluster V consisted of dekopon orange and tangerine, cluster VI consisted of kaffir lime, cluster VII consisted of tangerine, and cluster VIII consisted of lime. The clusters that have the most distant genetic relationship are cluster A and cluster B with a similarity of 62%. Meanwhile, clusters with the closest genetic relationship are clusters I and II with 79% similarity.

IV. CONCLUSION

By applying the RAPD molecular technique to 12 citrus fruit cultivars in South Sulawesi, it was found that the diversity of citrus fruits in South Sulawesi is high, making it possible for plant breeding activities to be conducted. Five primers (OPA-05, OPA-09, OPA-17, OPC-09, and OPC-17) that were used succeeded in producing polymorphic bands and were suitable to be used as markers in detecting genetic diversity of citrus fruits where OPC-09 primer was the most effective one. A total of 12 citrus cultivars tested were grouped into 2 main clusters with a genetic distance of 62%. It is necessary to do further analysis using larger amount of primers to complete the genetic information of citrus fruits in South Sulawesi.

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REFERENCES

[1] Maskun, H. Assidiq, N. H. Al Mukarramah, and S. N. Bachril, "Threats to the sustainability of biodiversity in Indonesia by the utilization of forest areas for national strategic projects: A normative review," in *IOP Conf. Series: Earth and Environmental Science*, UK, 2021, pp. 1-9.

[2] United Nations Group of Experts on Geographical Names, *Country report of the Republic of Indonesia*. Geospatial Information Agency of Indonesian, 2019, Accessed on: Jan. 24, 2022. [Online] Available: https://unstats.un.org/unsd/geoinfo/UNGEGN/docs/1st-session/GEGN.2_2019_CRP.132_Country_Report_Indonesia_2019.pdf.

[3] J. P. T. Kumar, A. Thirugnavel, D. Y. Upadhyay, S. A. Kamde, P. R. Jalamkar, and A. A. Murkute, "Genetic diversity and population structure of sweet orange [*Citrus sinensis* (L.) Osbeck] germplasm of India revealed by SSR and InDel Markers, " *bioRxiv.,* 2022.

[4] Y. G. D. Anggraheni, E. S. Mulayaningsih, and D. Priadi, "Genetic variability of Citrus spp. in Cibinong germplasm garden using random amplified polymorphic DNA," in *IOP Conf. Series: Earth and Environmental Science.,* UK, 2021, pp. 1-9.

[5] A. Tolangara, A. D. Corebima, A. Mas'ud, and Sundari, "Genetic diversity of Lemon(*Citrus* spp.) from Ternate island (Indonesia) based on morphological and molecular characterization,' *Biodiversitas*., vol. 21, no. 5, pp. 1908-1913, May 2020.

[6] C. Liu, H. Liu, J. Hurst, M. P. Timko, and C. Zhou, "Recent advances on citrus yellow vein clearing virus in citrus," *Horticultural Plant Journal*., vol. 6, no. 4, pp. 216-222, July 2020.
[7] G. D. Vita, M. Borrello, R. Vecchio, G. Gulisano, and M. D'Amico, "Purchasing drivers of fresh citrus fruits in urban Italy: is it all about taste?", *Nutrients.,* vol. 12, pp. 1-10, April 2020.
[8] R. Visvanathan and G. Williamson, "Effect of citrus fruit and juice consumption on risk of developing type 2 diabetes: evidence on polyphenols from epidemiological and intervention studies," *Trends in Food Science & Technology*., vol. 115, pp. 133-146, Sept. 2021.
[9] I. Jevtic, D. I. Udovicic, M. Ljubica, and A. Matic, "The Possibility of satisfying vitamin C daily needs by consuming fresh orange and grapefruit," *Journal of Hygienic Engineering and Design,* pp. 52-57, 2019.
[10] P. Inglese and G. Sortino, "Citrus history, taxonomy, breeding, and fruit quality", in Research Paper - University of Oxford. Oxford University Press USA: Oxford Research Encyclopedia of Environmental Science, 2019, pp. 1-22. [Online] Available: https://www.researchgate.net/publication/331399212_Citrus_History_Taxonomy_Breeding_and_Fruit_Quality_Citrus_History_Taxonomy_Breeding_and_Fruit_Quality_Summary_and_Keywords.
[11] M. Kim, S. H. Kim, H. B. Kim, Y. C. Park, and K. J Song, "Some factors affecting the efficiency of hybrid embryo rescue in the 'Shiranuhi' mandarin," *Horticultural Science and Technology.,* vol. 38, pp. 271-281, Jan. 2020.
[12] J. Singh, V. Sharma, K. Pandey, S. Ahmed, M. Kaur and G. S. Sidhu, "Horticultural classification of citrus cultivars", Intechopen, 2021, pp. 1-24. [Online] Available: https://www.researchgate.net/publication/331399212_Citrus_History_Taxonomy_Breeding_and_Fruit_Quality_Citrus_History_Taxonomy_Breeding_and_Fruit_Quality_Summary_and_Keywords.
[13] V. Zech-Matterne and G. Fiorentino, "Archaeology and history of citrus fruit in the mediterranean: acclimatization, diversifications, uses. Publications du Centre Jean Bérard: Naples. 2018.
[14] H. Zainuri and K. Yamaura, "New farmers' incentives under the new tangerine farming support: the case of Tuban, Indonesia," *International Journal of Fruit Science*., vol. 21, pp. 392-399, Mar. 2021.
[15] Balitjestro, *Types of Oranges Growing in Indonesia*. 2020. Accessed on: Jan. 26, 2022. [Online] Available: http://balitjestro.litbang.pertanian.go.id/jenis-jeruk-yang-berkembang-di-indonesia/.
[16] M. Addi, A. Elbouzidi, M. Abid, D. Tungmunnithum, A. Elamrani and C. Hano, "An overview of bioactive flavonoids from citrus fruits," *An Overview of Bioactive Flavonoids from Citrus Fruits.,* vol. 12, pp. 1-15, Dec. 2021.
[17] R. Chen, K. Takamura, K. Sugiyama, D. Kami, K. Shimomura, and Y. Yoshioka, "Genetic diversity assessment of *Cucurbita* genetic resources in Japan by nuclear and organelle DNA markers," *The Horticulture Journal.,* vol. 90, pp. 382-392, July 2021.
[18] R. Herawati, Alnopri, Masdar, U. K. Joko, and B. G. Murcitro, "Assessment of genetic diversity in various yield traits to determinea high yielding new type of upland rice," *International Journal on Advanced Science Engineering Information Technology.,* vol. 11, pp. 828-833, Apr. 2021.
[19] A. F Yusuf and B. S. Daryono, "Studies of genetic and morphological characteristics of indonesian melon (*Cucumis melo* L. 'Hikapel') germplasm," *International Journal on Advanced Science Engineering Information Technology.,* vol. 11, pp. 2023-2030, Oct. 2021.
[20] F. Akohoue, E. G. Achigan-Dako, C. Sneller, A. V. Deynze, and J. Sibiya, "Genetic diversity, SNP-trait associations and genomic selection accuracy in a west african collection of Kersting's groundnut [*Macrotyloma geocarpum* (Harms) Marechal & Baudet]," *Plos One.,* vol. 15, pp. 1-24, Jun. 2020.
[21] M. A. Sihalohoa, D. S. Hanafiab, E. Juliantic, and M. Basyuni, "Morphological characters of local origin durian (*Durio zibethinus* Murr.) fruits and seeds from Central Tapanuli Regency, North Sumatra, Indonesia," *International Journal on Advanced Science Engineering Information Technology.,* vol. 11, pp. 213-222, Feb. 2021.
[22] Kristamtini, S. Widyayanti and H. Purwaningsih, "Genetic diversity of local red rice cultivars collections of Yogyakarta AIAT, Indonesia based on morphological character," in *IOP Conf. Series: Earth and Environmental Science*, UK, 2020, pp. 1-6.
[23] F. Yulianti, A. L. Adiredjo, L. Soetopo, and S. Ashari, "Morphology and genetic characteristics of potential citrus rootstock in Indonesia," *Biodiversitas*., vol. 21, pp. 5514-5520, Nov. 2020.
[24] F. P. Sabdanawaty, Purnomo, and B. S. Daryono,"Species diversity and phenetic relationship among accessions of api-api (*Avicennia* spp.) in Java based on morphological characters and ISSR markers," *Biodiversitas*., vol.22, pp. 193-198, Jan. 2021.
[25] G. R. Aristya, R. Kasiamdari, M. F. Arif, F. Amalia, and R. D. Pawestri, "Comparison of molecular markers ISSR, SSR and RFLP to study the genetic variation of strawberry cultivars," *American Journal of Agricultural and Biological Science.,* vol. 14, pp. 61-68, Mar. 2019.
[26] S. Amiteye, "Basic concepts and methodologies of DNA marker systems in plant molecular breeding," *Heliyon.,* vol. 7, pp. 1-20, Sep. 2021.
[27] M. R. Pahlevi, S. Indriyani, R. Mastuti, and E. L. Arumingtyas, "Genetic variation based on RAPD profiling and production loss of cayenne pepper due to periodic flooding," *International Journal on Advanced Science Engineering Information Technology.,* vol. 11, pp. 834-842, Apr. 2021.
[28] M. Cehula, J. Mlcek, T. Juríková, J. Žiarovská, O. Paulen, L. Dokoupil, A. Adámková, and R. Babosova, "Assessment of genetic diversity of edible honeysuckle monitored through RAPD in relative to bioactive substances," *Agronomy.,* vol. 10, pp. 1-11, Jun. 2020.
[29] D. Yuan, H. Qin, D. Chen, and J. Chen, "Genetic diversity analysis of Chinese Leishmania isolates and development of L. donovani complex-specific markers by RAPD," *BMC Infectious Diseases*., vol. 21, pp. 1-10, May 2021.
[30] I. B. K. Mahardika, I. N. Rai, M. S. Mahendra, and R. Dwiyani, "Genetic diversity and fruit qualityof several pomelo "Jeruk Bali"(*Citrus grandis* L. Osbeck) cultivars in Bali," *International Journal of Bioscience and Biotechnology*., vol. 5, no. 1, pp. 43-59. Sept. 2017.
[31] 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," in *IOP Conf. Series: Earth and Environtmental Science,* UK, 2021, pp. 1-7.
[32] R. S. Kasiamdari, G. R. Aristya, H. Prabowo, C. Ariesta, and M. F. Arif, "Genetic diversity of Indonesian's sugarcane varieties (*Saccharum officinarum*) based on RAPD molecular markers," *Biodiversitas*., vol. 20, pp. 3005-3010, Oct. 2019.
[33] Uslan and M. Pharmawat, "Genetic diversity of *Sterculia quadrifida* in Kupang, Indonesia based on RAPD (Random Amplified Polymorphic DNA) markers," *Biodiversitas*., vol. 21, pp. 3407-3414, Jul. 2020.
[34] L. Wallace, "Methods available for the analysis of data from dominant molecular markers. Department of Biology," University of South Dakota, 2003.
[35] Z. H. Guo, K. X. Fu, X. Q. Zhang, S. Q. Bai, Y. Fan, Y. Peng, L. K. Huang, Y. H. Yan, W. Liu, and X. Ma, "Molecular insights into the genetic diversity of *Hemarthria compressa* germplasm collections native to Southwest China," *Molecules.,* vol. 19, no. 12, pp. 41-59, Dec. 2014.
[36] D. Botstein, R. L. White, M. Skolnick, and R. W. Davis, "Construction of a genetic linkage map in man using restriction fragment length polymorphisms," *Am. J. Hum. Genet.,* vol. 32, pp. 314–331, May 1980.
[37] H. Sadiyah, S. Ashari, B. Waluyo, and A. Soegianto, "Genetic diversity and relationship of husk tomato (*Physalis* spp.) from East Java Province revealed by SSR markers," *Biodiversitas*., vol. 22, pp. 184-192, Jan. 2021.
[38] Lizawati, S Nusifera, Neliyati, Y Alia and Antony, "RAPD-PCR primer selection to analyze genetic diversity of Cinnamon plan," in *IOP Conf. Series: Earth and Environmental Science*, UK, 2021, pp. 1-7.
[39] J. G. Williams, A. R. Kubelik, K. J. Livak, K. J. Rafalski, and S. V. Tingey, "DNA polymorphisms amplified by arbitrary primers are useful as genetic markers," *Nucleic Acid Research.*, vol. 18, no. 22, pp. 6531-6535, Nov. 1990.
[40] Annisa, R. Hafzari, T. Setiawati, B. Irawan, and J. Kusmoro, "Evaluation of RAPD markers for molecular identification of five bamboo genera from Indonesia," *Folia Forestalia Polonica.,* vol. 61, no. 4, pp. 255-266, Dec. 2019.
[41] J. Normala, V. T. Okomada, A. A. Mohd, A. A. Nur, A. B. Abol-Munafi, and S. Md. Sheriff. "Genetic variation between triploid and diploid *Clarias gariepinus* (Burchell, 1822) using RAPD markers," *Veterinary Sciences*., vol. 8, no. 75, pp. 1-12, May 2021.
[42] S. R. Dalimunthe, L. A. M. Siregar, L. A. P. Putri, T. Chairunnisa, and A. Hairmansis, "Polymorphism levels of some SSR markers (Simple Sequence Repeat) for parental line identification on low temperature

tolerance," in *IOP Conf. Series: Earth and Environmental Science*, UK, 2020, pp. 1-9, 2020.

[43] Serrote, C. M. Lemos, Reiniger, L. Silveira, Rejane, Silva, K. Buuron, Rabaiolli, S. M. Santos, Stefanel, and C. Moro, "Determining the polymorphism information content of a molecular marker," *Journal pre-proofs.,* Elsevier. 2019.

[44] A. S. Shaibu, H. Ibrahim, Z. L. Miko, I. B. Mohammed, S. G. Mohammed, H. L. Yusuf, A. Y. Kamara, L. O. Omoigui, and B. Karikari, "Assessment of the genetic structure and diversity of soybean (*Glycine max* L.) germplasm using diversity array technology and single nucleotide polymorphism markers," *Plants.,* vol.11, pp. 1-13, D2022.

[45] Z. Luo, J. Brock, J. M. Dyer, T. Kutchan, D. Schachtman, M. Augustin, Y. Ge, N. Fahlgren, and H. Abdel-Haleem, "Genetic diversity and population structure of a *Camelina sativa* spring panel," *Frontiers in Plant Science*, vol. 10, no. 184, pp. 1-12, Feb. 2019.

[46] Q. B. Zhao, H. Sun, Z. Zhang, Z. Xu, B. S. Olasege, P. P. Ma, X. Z. Zhang, Q. S. Wang, and Y. C. Pan, "Exploring the structure of haplotype blocks and genetic diversity in chinese indigenous pig populations for conservation purpose," *Evolutionary Bioinformatics.,* vol. 15, pp. 1–8, Jan. 2019.

[47] Siregar, U. Juniarti, and R. D. Olivia, "Genetic Diversity of Sengon (*Paraserianthes falcataria* (L)) in Java Community Forest based on RAPD Marker," *Journal of Tropical Silviculture*., vol. 3, pp. 1-7, Feb. 2012.