

Genetic Diversity of Three Types of *Curcuma* Using Molecular Sequence-Related Amplified Polymorphism Markers

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Received February 3th, 2025; Accepted March 9th, 2025

ABSTRACT

The genus *Curcuma* comprises numerous species with high medicinal value, many exhibiting morphological similarities that complicate taxonomic identification and hinder breeding efforts. This study aimed to elucidate the genetic diversity and relationships among three closely related species, *Curcuma longa* (turmeric), *Curcuma purpurascens* (temu tis), and *Curcuma mangga* using Sequence-Related Amplified Polymorphism (SRAP) markers. Genetic material was extracted from rhizome samples and amplified using five SRAP primer combinations, producing 56 loci with an average polymorphism percentage of 69.64%. The result showed a high genetic similarity (82%) between *C. longa* and *C. purpurascens*, whereas *C. mangga* exhibited a more distant relationship (55% similarity), indicating a narrower genetic base among the former two species. Additionally, total flavonoid content and antioxidant activity, with turmeric showing the highest flavonoid concentration (25.95% w/w), and *C. mangga* exhibiting the strongest antioxidant activity (42.12% wb). These findings highlight the effectiveness of SRAP markers in differentiating morphologically similar species and provide valuable genetic and phytochemical insights to support the conservation, breeding, and pharmacological development of *Curcuma* species. Integrating molecular and biochemical data underscores the potential for selecting superior varieties for both agricultural and medicinal applications.

Keywords: *Curcuma* spp.; Genetic diversity; SRAP markers; Flavonoid content; Antioxidant activity

Cite this as (CSE Style): Setyawati A, Samanhudi, Bachtiar ZA. 2025. Genetic diversity of three types of *Curcuma* using molecular sequence-related amplified polymorphism markers. Agrotechnology Res J. 9(1):15–21. <https://dx.doi.org/10.20961/agrotechresj.v9i1.84303>.

INTRODUCTION

The *Curcuma* genus has a long history as a traditional medicine. Over 70 *Curcuma* species are found globally, with significant cultivation occurring in Asian countries, Australia, and West Africa. Numerous studies focused on phytochemical, pharmacological, and molecular aspects have been conducted across various *Curcuma* species worldwide (Ayati et al. 2019; Ewon and Bhagya 2019). Many species within this genus exhibit significant therapeutic potential, enabling the treatment of various health conditions such as stomach ulcers, spleen and liver enlargement, liver disorders, skin ailments, chest pain, cough, diabetes, rheumatism, and for blood detoxification (Mishra et al. 2018; Rahaman et al. 2021).

A total of 3 species from the *Curcuma* genus were used in this study, namely turmeric (*Curcuma longa*) as a comparison, then temu tis (*Curcuma purpurascens*), and mangga temu (*Curcuma mangga*). Turmeric, temu tis, and temu mangga are medicinal plants of the temu tribe (Zingiberaceae). The Zingiberaceae tribe is a type

of temu plant with a characteristic rhizome. The Zingiberaceae family, in general, is better known as a group of ginger plants that have characteristics, including herbaceous stature with rhizomes that contain volatile oil with an aromatic smell (Auliani et al. 2014).

In addition to having the same family, the three *Curcumas* have similarities in morphology and content. The turmeric rhizome is cylindrical, the surface color is yellowish and segmented, the flesh color is yellow-orange (Setiawan et al. 2018). Turmeric is a plant rich in curcuminoid compounds with antioxidant properties (Ferreira et al. 2013). Then, the morphology of the temu tis rhizome is similar to turmeric, but the cross-section of the temu tis rhizome is slightly larger, and the color is paler than that of turmeric (Hong et al. 2014). While the rhizome of the temu mangga is cylindrical, the color of the flesh is yellowish-white (Setiawan et al. 2018). Fresh mangga rhizomes, when cut, produce a mangga-like aroma (Raihana et al. 2011). Jalip et al. (2014) states that *Curcuma purpurascens* has the highest antioxidant compared to *Curcuma mangga*, *Curcuma heyneana*, *Curcuma aeruginosa*, and *Curcuma phaeocaulis*. It can be concluded that the temu tis and the temu tis also have antioxidant activity. Still, the temu tis has a higher antioxidant activity than the temu tis. Based on the

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antioxidant content, the three *Curcumas* have similarities, especially morphologically.

However, the species used in this study have not been widely cultivated and are rarely studied for genetic diversity and kinship patterns. Turmeric, Temu Tis, and Temu mangga look very similar. Therefore, it is necessary to analyze genetic diversity to find information on these plants' kinship relationships and genetic variations. Analysis of plant genetic diversity was carried out using molecular markers. One of the molecular markers that can be used is Sequence Related Amplified Polymorphism (SRAP). According to Subositi and Mujahid (2013), Sequence Related Amplified Polymorphism (SRAP) is a molecular marker that was found later than other molecular markers such as Restriction Fragment Length Polymorphism (RFLP), Amplified Fragment Length Polymorphism (AFLP), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), and Inner Simple Sequence Repeat (ISSR). SRAP combines the ease of the RAPD technique and the high-accuracy results of AFLP and can detect polymorphisms in coding sequences commonly found in cultivar genomes with relatively low mutation rates. The SRAP marker not only amplifies the interval between the gene and the non-coding flanking region but is also closely associated with the actual gene, which will generate a fingerprint of the coding sequence and allow easy isolation of the band for sequencing (Li et al. 2014). The molecular marker SRAP is more reproducible, stable, and very simple in terms of operation compared to other molecular marker techniques (Oluoch et al. 2018).

This study uses a molecular marker, Sequence-Related Amplified Polymorphism (SRAP), to obtain information on genetic diversity and kinship between turmeric, temu tis, and mangga plants. Thus, this genetic information is expected to assist in the breeding and preservation of *Curcuma longa*, *Curcuma purpurascens*, and *Curcuma mangga* varieties.

MATERIALS AND METHODS

This research was conducted from April 2021 until November 2021. The analysis was conducted at the Laboratory of Genetics and Plant Breeding, Faculty of Agriculture, Gadjah Mada University, Yogyakarta. The tools used in this study included a micropipette (Eppendorf Research Plus), water bath (Advantec TBS221AA), vortex (Barnstead Thermolyne Maxi Mix II), spectrophotometer (GeneQuant 1300), centrifuge (Eppendorf mini spin plus), PCR (BIO-RAD T100 Thermal Cycler), UV Transilluminator (Trans WD Transilluminator), scale (ACIS AD-300i), refrigerator -20 (Panasonic Econavi Inverter), and electrophoresis tank (VWR electrophoresis 700-0777). The materials used were three samples of rhizome, namely turmeric (Surakarta), Temu tis (Wonogiri), and Temu mangga (Garut). Materials used for molecular analysis include PCR mix (GoTaq Green Master Mix Promega), NFW

(Nuclease Free Water Promega), Ladder (SMOBIO AccuBandTM 100 bp+3K DNA ladder II), DNA stain (FloroSafe DNA Stain 1st Base), Agarose (GeneDirex), and TBE (Tris Boric EDTA)

DNA amplification

The research stages began with sample preparation and 2% CTAB extraction buffer, DNA isolation, PCR, and DNA electrophoresis. The variables observed were the concentration and purity of DNA, as well as parameters of genetic diversity in a population, which included the percentage of polymorphic loci (PLP), the number of observed alleles (Na), the number of effective alleles (Ne), and genetic variation (He). Data analysis was conducted by scoring the bands appearing at each locus in the three species. If a band appears, it is given a score of 1; if not, it is given a score of 0. Scoring is done manually, using a 100bp ladder (smobio) to compare the size of the DNA band that appears. The dendrogram was visualized using NTSYSpc-2.02 software with the Dice similarity index. To create the cluster, use the Unweighted Pair Group Method With Arithmetic Averages (UPGMA) method of the similarity index.

Flavonoid level test

Standard raw curve creation. Quercetin standard weighed as much as 10.0 mg. Then 0.3 ml of 5% sodium nitrite was added (settled for 5 minutes). Then 0.6 ml of 10% aluminum nitrate was added (left for 5 minutes), and 2 ml of 1 M sodium hydroxide was added. The volume was adjusted until it reached 10 ml through dilution according to the concentration standard curve. Then the absorbance was read at 510 nm.

Determination of total flavonoid equivalent quercetin UV-vis spectrophotometric method. Samples weighed as much as ± 50 mg. Then 0.3 ml of 5% sodium nitrite was added (settled for 5 minutes). Then 0.6 ml of 10% aluminum nitrate was added (left for 5 minutes), and 2 ml of 1 M sodium hydroxide was added. The volume was adjusted to reach a volume of 10 ml with a 5-fold dilution. The absorbance was read at 510 nm.

RESULTS AND DISCUSSIONS

DNA concentration and purity

The results of DNA isolation in Table 1 show that the highest DNA concentration value was obtained by turmeric at 400 ng.μL⁻¹. Meanwhile, the value of DNA purity with absorbance 260/280 ranged from 1,000-4,000, and absorbance 260/230 ranged from 0.375-0.571. According to (Ningsih et al. 2018), pure DNA has an absorbance ratio of 260/280 nm ranging from 1.8 to 2.0. If the DNA purity has a value lower than 1.8, then the DNA sample is contaminated by protein, while a DNA purity value higher than 2.0 means that RNA contaminates the DNA sample. At the absorbance ratio 260/230 nm, pure DNA ranged from 2 to 2.2. If the value is lower than 2, the DNA is contaminated with carbohydrates, organic matter, or other chemicals.

Table 1. Concentration and purity of DNA 3 types of *Curcuma*

Sample	DNA concentration (ng.µL ⁻¹)	260/280	260/230
Temu Mangga (TM)	300	1,000	0.375
Temu Tis (TT)	200	1,000	0.500
Turmeric (K)	400	4,000	0.571

It can be concluded that the purity of the DNA obtained from the three samples was not very good because it was contaminated. However, contamination can be reduced through DNA dilution. When the DNA is diluted, the impurities will be reduced. The contaminated DNA here does not interfere with PCR activity because there is no smear when performing PCR. A smear is a DNA band in the form of an elongated stain on the electrophoresis gel, which is most likely caused by polysaccharides that are also extracted when DNA isolation is carried out (Rahayu et al. 2015).

DNA amplification of three types of *Curcumas*

Analysis of genetic diversity using the molecular marker technique SRAP (Sequence Related Amplified Polymorphism). Table 2 is the primary code that will be used to analyze genetic diversity. There are five combinations of SRAP primers used in Table 2. Five SRAP primer combinations that will be used in the genetic diversity analysis of 3 types of *Curcuma* are the Me1-Em1 primer combination, the Me2-Em2 primer, the Me3-Em3 primer, the Me2-Em1 primer, and the Me2-Em3 primer. SRAP molecular markers have two primers: forward primers and reverse primers. Forward primary with code Me, and reverse primary with code Em, as seen in Table 2.

Table 2. Primary SRAP genetic diversity analysis of 3 rhizome samples

Code Primary	Primary Sequence (5'-3')
Me1	TGAGTCCAAACCGGATA
Me2	TGAGTCCAAACCGGAGC
Me3	TGAGTCCAAACCGGAAT
Em1	GACTGCGTACGAATTAAT

Em2	GACTGCGTACGAATTTGC
Em3	GACTGCGTACGAATTGAC

An electropherogram is a basic representation of the composition of DNA samples (Cowell et al. 2015). In Figure 1, it can be seen that the DNA Electropherogram of 3 types of *Curcuma* using five combinations of SRAP primers produces various kinds of bands. Polymorphic bands and monomorphic bands appear at certain sizes. The data in

Table 3 can be generated from the Electropherogram image above.

The results of DNA amplification in

Table 3 indicate that the amplification using five combinations of SRAP primers in the analysis of genetic diversity resulted in 56 loci. The primer combination that produced the most loci was Me2-Em1 with 18 loci. While the lowest loci that appeared as many as six were found in the primer combination. Me2-Em2. The molecular weight of the DNA bands produced from the combination of Me1-Em1 primers ranged from <100 to 700 bp. The combination of Me2-Em2 primers produces DNA bands with a molecular weight of 200-900 bp. The combination of Me3-Em3 primers produces DNA bands with a molecular weight of <100-1490 bp. The combination of Me2-Em1 primers produces DNA bands with a molecular weight of 100-2100 bp. Meanwhile, the Me2-Em3 primer combination produces DNA bands with a molecular weight of 190-2000 bp.

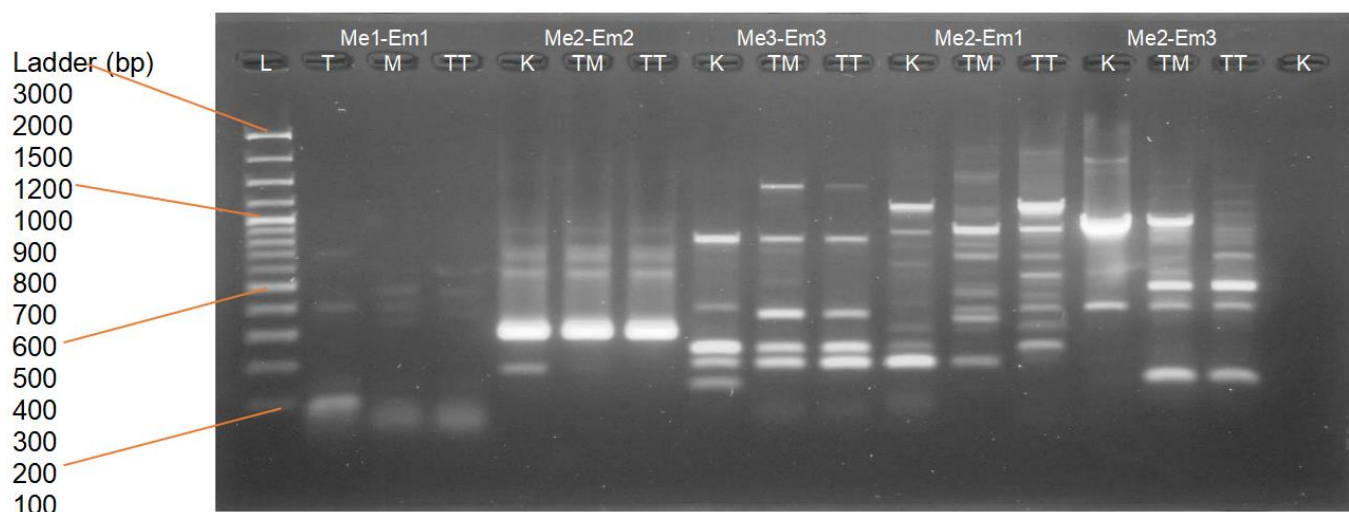


Figure 1. Electropherogram of 3 types of *Curcuma* DNA using five combinations of SRAP primers

Table 3. The total polymorphic loci were amplified using 5 SRAP primers, and the percentage of polymorphic bands in the three types of *Curcuma*

Primary Combination	Molecular weight (bp)	Locus	Polymorphic Locus	Monomorphic Locus	Polymorphic Percentage (%)
Me1- Em1	<100-700	8	8	0	100
Me2-Em2	200-900	6	1	5	16.66
Me3-Em3	<100-1490	12	9	3	75.00
Me2-Em1	100-2100	18	11	7	61.11
Me2-Em3	190-2000	12	10	2	83.33
Total		56	39	17	
Average					69.64

The results of DNA amplification using five combinations of SRAP primers showed that the DNA bands had molecular weights in the range <100-2100. From the polymorphism data in

Table 3, it can be concluded that, of the five primers used, only one primer produced 100% polymorphic locus patterns, namely the Me1-Em1 primer combination. At the same time, the lowest polymorphism is 11.76% in the primary combination Me2-Em2. The average polymorphism in 5 SRAP primer combinations was 69.64%. According [Kawengian et al. \(2016\)](#) the number of polymorphic DNA bands in the analysis of genetic diversity greatly determines a population's diversity level. The more polymorphic DNA bands will better describe the state of the plant genome and will minimize bias because certain parts of the genome are not represented.

Parameters of genetic diversity and genetic kinship

According to [Ellegren and Galtier \(2016\)](#), genetic diversity is considered a reflection of the balance

between the emergence and disappearance of genetic variants (alleles). Analysis of genetic diversity can be seen through the parameters shown in [Table 4](#). The genetic diversity of the three types of *Curcuma* resulted in 39 polymorphic loci, and the percentage of polymorphic loci was 69.64%. [Table 4](#) produces an average Shannon information index value of 0.045. Then the average allele observed (N_a) was 1.696. The average effective allele (N_e) was 1.486.

The higher the N_e value, the greater the number of heterozygous individuals ([Pasaribu 2017](#)). The expected heterozygosity value (H_e) has an average of 0.276. According [Istiqomah et al. \(2016\)](#), the value of heterozygosity is one of the parameters that can measure the level of genetic diversity in a population, the higher the value of H_e (Heterozygosity), the higher the level of genetic diversity. In previous studies, he classified heterozygosity (H_e), which has a value of more than 0.20, as high for common plant families, especially rare plants ([Irsyad et al. 2020](#)).

Data analysis was conducted by scoring the bands appearing at each locus in the three species. If a band appears, it is given a score of 1; if not, it is given a score

of 0. Scoring is done manually, using a 100bp ladder (smobio) to compare the size of the DNA band that appears. The dendrogram was visualized using NTSYSpc-2.02 software with the Dice similarity index. The Unweighted Pair Group Method With Arithmetic Averages (UPGMA) method is used from the similarity index to create the cluster.

Analysis of the genetic diversity of 3 types of *Curcuma* using five combinations of SRAP primers can be seen in Figure 2, with a similarity index of 55-82% or a diversity value of 18-45%. This shows a narrow genetic diversity between the three types of *Curcuma*. The result is that temu mangga has a similarity index of 55% to that of Temu tis and Turmeric. At the same time, temu tis with turmeric has a very high similarity index of 82%. This shows that temu tis and turmeric have a very close genetic relationship. The higher the similarity index value, the closer the kinship between the samples tested. If the similarity index is >70%, it indicates that the varieties being compared have close similarities, resulting in lower genetic variation. This is due to the high similarities and similarities of characters in these varieties, because the higher the character similarities between varieties, the lower the level of diversity (Mustofa et al. 2014).

Total flavonoid level test

Analysis of total flavonoid content using the UV-vis spectrophotometry method. The analysis aimed to determine the total flavonoid content in turmeric, temu

tis, and temu mangga. According to Aminah et al. (2017), the UV-vis spectrophotometry method was used to analyze total flavonoids because flavonoids have a conjugated aromatic system so that they can show strong absorption bands in the ultraviolet and visible light spectrum regions. The standard curve results can be seen in Figure 3, and the results of the total flavonoid analysis are in Table 5.

The total flavonoid content was determined based on the calibration curve $y = 0.00318777x - 0.00255132$ with $r^2 = 0.99921$, and the R value was 0.9996. The equation generated from the quercetin calibration curve was used to compare the concentration of total flavonoid compounds in turmeric, mangga, and turmeric. In Figure 3, it can be concluded that the concentration (mg.L^{-1}) is directly proportional to the absorbance. The higher the concentration used, the higher the absorbance. In Table 5, it can be concluded that turmeric has the highest total flavonoid content, which is 25.95% w/w. Then the lowest flavonoid content was in temu tis, amounting to 1.76% w/w. Flavonoid compounds are secondary metabolites found in plants. Flavonoid compounds are included in the group of phenolic compounds, which are known to have antioxidant abilities (Arrisujaya et al. 2019). This can be proven by the results of antioxidant tests on temu tis, temu mangga, and turmeric, which have been carried out by the Food and Nutrition Laboratory, Faculty of Agriculture, Sebelas Maret University, Surakarta. The results of the antioxidant tests for Temu Tis, Temu Mangga, and Turmeric can be seen in Table 6.

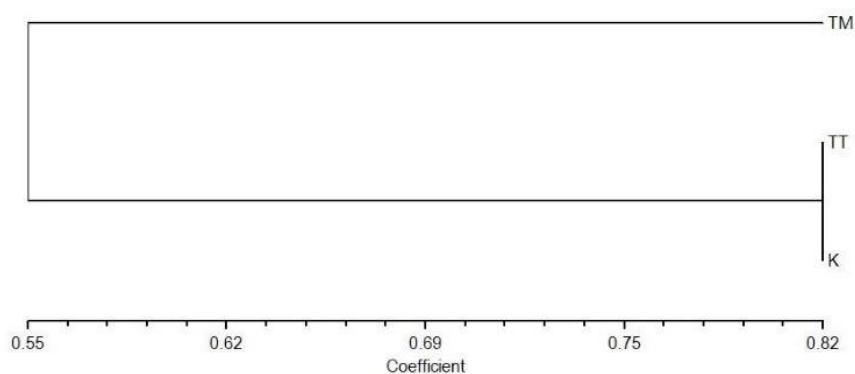


Figure 2. The *Curcuma* 3 type dendrogram uses the molecular marker SRAP

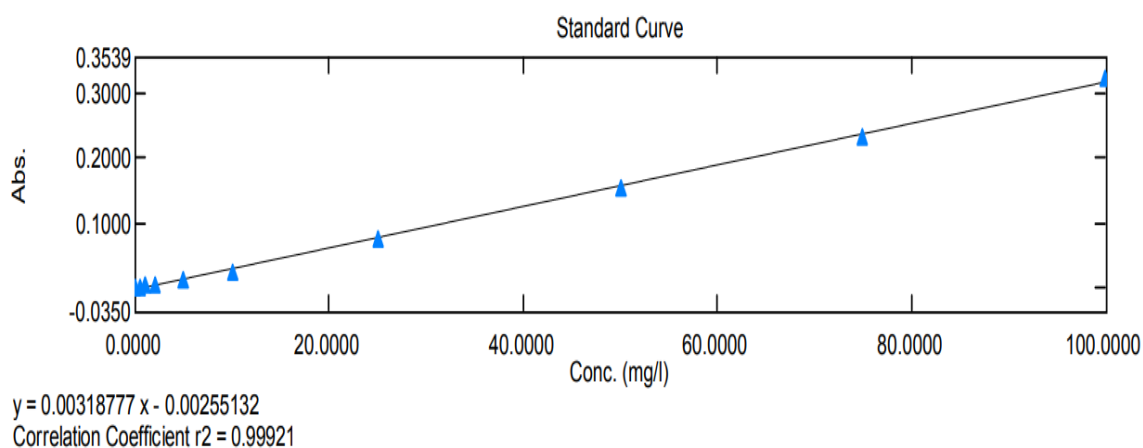


Figure 3. Quercetin standard curve

Table 4. Parameters of genetic diversity using SRAP molecular markers on 3 types of *Curcuma*

JLP	PLP		N	Na	Ne	I	He
34	69.64%	mean	3,000	1,696	1,486	0.045	0.276
		SE	0.000	0.062	0.052	0.038	0.027

Remarks: number of polymorphic loci, PLP= percentage of polymorphic loci, N= number of samples, Na = number of alleles observed, Ne = number of effective alleles, I = Shannon information index, He = expected heterozygosity

Table 5. Test results of total flavonoid levels in temu tis, temu mangga and turmeric

Sample	Total Flavonoids	Unit	Method
Temu Tis Powder	1.76	% w/w	UV-vis spectrophotometry
Temu Mangga Powder	13.96	% w/w	UV-vis spectrophotometry
Turmeric Powder	25.95	% w/w	UV-vis spectrophotometry

Table 6. Results of antioxidant test analysis on temu tis, temu mangga, and turmeric conducted

Sample Code	Kinds of Analysis	Analysis Method	Analysis results (% wb)	
Temu Tis	Antioxidant	Spectrophotometry	18.73	18.52
			18.30	
Turmeric	Antioxidant	Spectrophotometry	40.93	40,20
			39.46	
Temu Mangga	Antioxidant	Spectrophotometry	41.83	41.98
			42.12	

Table 6 shows that temu tis, mangga, and turmeric contain antioxidant compounds. Temu tis contains an antioxidant of 18.52% wb, then temu mangga contains an antioxidant of 41.98% wb, and turmeric contains an antioxidant of 40.20% wb. The highest antioxidant activity was held by temu mangga, while the lowest was by temu tis. So, it was concluded that temu tis, mangga, and turmeric contain flavonoids and antioxidants. Antioxidants are very easily oxidized, so free radicals will oxidize antioxidants and protect other molecules in cells from damage due to oxidation by free radicals or reactive oxygen (Werdhasari 2014).

CONCLUSIONS AND SUGGESTIONS

The conclusions from the genetic diversity study of three *Curcuma* species using the molecular marker Sequence Related Amplified Polymorphism are as follows: analysis of genetic diversity of three *Curcuma* species with SRAP markers showed a close relationship, the molecular marker SRAP was able to reveal the relationship between closely related cultivars, three types of *Curcuma* contain high levels of flavonoids and antioxidants. Additionally, significant differences in flavonoid and antioxidant content suggest potential for targeted breeding based on both genetic and phytochemical traits.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Faculty of Agriculture, Universitas Sebelas Maret, facilities provided in the laboratory so that this research can be done successfully.

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