Molecular characters of melon (*Cucumis melo* L. 'Kinaya') using inter simple sequence repeat

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ABSTRACT. Cultivar 'Kinaya' is a melon produced from breeding between 'Sonya' and 'Kinanti'. This study on phenotypic and molecular characters is carried out to support 'Kinaya' as a superior cultivar. Our study aimed to analyze the genetic variation of the melon 'Kinaya Bulat Kuning' and 'Kinaya Kuning Lonjong' and its parental 'Sonya' and 'Kinanti'. The research was conducted in the Greenhouse of Mutihan, D.I.Yogyakarta, and the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada. Quantitative characters were determined by morphometric comparisons, fruit weights, and the number of seeds. Qualitative characters included fruit color, fruit skin color, aroma, texture, and taste. The molecular characterization method was inter simple sequence repeat (ISSR) and included DNA isolation, spectrophotometry, amplification of DNA target using PCR, and visualization of DNA target. Molecular characters were analyzed using spectrophotometry and visualization of DNA bands by electrophoresis using the MVSP 3.1 program. PCR used four random primers such as UBC 807, 809, 810, and 812, which obtained 11 polymorphic and 12 monomorphic DNA bands with a polymorphism rate of 47.8%. It is known that the cultivar 'Kinaya' has a similarity of 72% with its inductees 'Sonya' and 'Kinanti'.

Keywords: cultivar 'Kinanti' and 'Sonya'; dendrogram construction; genetic variation; phenotypic characters; polymorphism percentage

INTRODUCTION

Melon (*Cucumis melo* L.) is a horticulture plant that belongs to the Cucurbitaceae family that grows widely in Asia and Middle East countries and is consumed in large quantities as a traditional diet (Paris *et al*., 2012; Shin *et al*., 2019; Gómez-García *et al*., 2020). Melons are thus a good source of vitamins, minerals, and fiber for the body. Melons contain calories, sodium and low in fat, source of potassium (K), vitamin C, vitamin B6, and vitamin A (Poverenov *et al*., 2014; Sultana & Rahman, 2014). The cucurbitacin-β, lithium, iron, and zinc found in melons can help prevent cancer and boost the immune system (Ittiyavirah *et al*., 2013; Raji & Orelaja, 2014; Sangamithra & Ragavi, 2020). Melons have been extensively developed and bred in Indonesia in order to generate superior cultivars and eliminate the need for imported melon cultivars.

'Kinaya' is one of the new melon cultivars developed by the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada. Research on genetic traits supports 'Kinaya' as a superior melon cultivar. 'Kinaya' is the third derivative of the 'Kinanti' and 'Sonya', which was cultivated by the Laboratory of Genetic and Breeding, Faculty of Biology, Universitas Gadjah Mada. 'Sonya' has phenotypic characters round fruit shape, having a net on the skin, green fruit skin, while the characteristics of 'Kinanti' are round fruit shape, have no net on the skin, and yellow fruit skin.

Fruit production is one of the challenges in melon cultivation, which varies considerably in shape, and the color is different from other melons, generally appearing when planting seeds from previous plantings (Ajuru & Okoli, 2013; Pitrat, 2016; Flores-León *et al*., 2021). Variations in melon plants consist of phenotype and genetic variations. Phenotype variations can be observed from morphological characters that appear qualitatively and quantitatively, while genetic variations can be seen through DNA analysis (Liu *et al*., 2013; Granier & Vile, 2014; Nadeem *et al*., 2017).

Inter simple sequence repeat (ISSR) relies on simple sequence repetitions in primers to avoid the need for sequence information in primer synthesis (Son *et al*., 2012; Nanda *et al*., 2013). ISSR analysis employs investigations involving genetic identity, parentage, genetic distance authentication, clone and hybrid varieties identification, and taxonomy research of closely related species because of the multi-locus fingerprinting profiles acquired (Devarumath *et al*., 2012; Dangi *et al*., 2014; Tahseen *et al*., 2018). Using ISSR, we have successfully analyzed the genetic variation of *C. melo* L. 'Melonia' in the previous studies (Daryono *et al*., 2019). Similar studies include Iran melon (Raghami *et al*., 2013), Indian melon (Singh *et al*., 2015), Azerbaijan melon (Guliyev *et al*., 2018) were successfully evaluated the genetic diversity among melon accessions. To the best of our knowledge, this research is the first to estimate the polymorphism of multi-loci spectra for cultivar *C. melo* L. 'Kinaya' used four valuable primers.

The present study aimed to verify genetic variations and similarity index based on ISSR molecular markers. This study will help devise future hybrid breeding and conservation approaches.

MATERIALS AND METHODS

The research was conducted in the greenhouse of Mutihan, Madurejo, Sleman, D.I.Yogyakarta, and Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada from November 2019 to August 2020.

DNA extraction. DNA was isolated from leaf and root samples using the Cytiva Nucleon™ PhytoPure™ Genomic DNA Extraction Kits (Cytiva RPN8511). Leaf and root samples were cut and weighed 0.3 g in a cold state. The leaves and roots were crushed using a mortar and gradually added 500 µl of PhytoPure I. PhytoPure I was used as a buffer solution to prevent enzymes in cells from damaging the DNA structure. The buffer solution used contains SDS, which separates protein from DNA. RNA contaminants were removed using RNAse. Samples were put into a 1.5 ml tube, and 200 µl of PhytoPure II was

added and shaken to be homogeneous. Samples were incubated in a water bath at 65°C for 20 min and continued by transferring samples to the freezer at 4°C. Incubation at 65°C aims to optimize the extraction process. Cooling the samples at a low temperature aims to stop the enzymatic process to not interfere with the extraction process. After the samples cooled in the freezer, cold chloroform was added. The extraction process is followed by adding PhytoPure™ Resin. Samples were centrifuged at 1300 rpm for 10 min. Isopropanol was added into the supernatant, then centrifuged. DNA pellet was washed with 70% ethanol, then dried with ethanol, added to a cold 1xTE buffer, and stored in the freezer to prevent DNA from being degraded (Aristya *et al*., 2019; Daryono *et al*., 2019).

Spectrophotometry (quantitative test). DNA concentration and purity were quantitatively measured using Spectrophotometers NanoDrop at 260 nm and 280 nm.

DNA amplification. DNA isolation results were applied using Polymerase Chain Reaction (PCR) (Bio-Rad Thermal Cycle) method with four primers: UBC 807, UBC 809, UBC 810, and UBC 812. The PCR reaction volume used was 25 µl, which consists of 12.5 µl master mix PCR (PCR Kit, Bioline), 2 µl primer, 2 µl DNA template, and 8.5 µl ddH$_2$O sterile. PCR amplification reaction includes steps: initial denaturation at 95°C for 3 min, denaturation at 95°C for 15 s, annealing at 46.2°C-51°C for 30 s, and extension at 72°C for 45 s. The cycle was repeated 35 times (Daryono *et al*., 2019).

Visualization of PCR. DNA was electrophoresed on the agarose gel 2% for 55 min and visualized using Gel Doc. The study used a DNA ladder of 100-1500 bp (VC 100bp DNA Ladder).

Data analysis. Data obtained from morphological characters were analyzed using tables and ANOVA Levane's test analysis. Genetic characters were analyzed by showing DNA purity. Visualization of DNA bands resulting from electrophoresis and dendrogram reconstruction using the Multi-Variate Statistical Package (MVSP) ver. 3.1 (Daryono *et al*., 2020). This analysis was used to

determine phenetic relationships based on a molecular basis. The DNA bands that emerged from the DNA amplification results in each primer were analyzed using binary data to assess the level of similarity and kinship.

RESULTS AND DISCUSSION

The observation results at the greenhouse showed a variety of phenotypic characters of six samples. The phenotypic characters were then divided into qualitative and quantitative characters.

Spectrophotometry (quantitative test). Measurement of DNA concentration for six samples at A260/A280 was showed in Table 1.

The purity of melon DNA ranged from 1.7-2.0 (Table 1), and DNA depth ranged from 1000-3000 ng/µl. The isolated DNA produced is pure and free from contamination of proteins, RNA, phenols, or other compounds that can interfere with PCR process. The highest concentration was found in SNY 1, which was 3134.07 ng/µl, and the lowest was found in BK 2, which was 1326.9 ng/µl. Referring to the protocol used, the minimum DNA concentration used for the PCR process is

around 200 ng/µl so that all samples can be used for the PCR process.

Table 1. DNA quantitative test results using a spectrophotometer.

No	Samples	Code	Conc. (ng/µl)	Purity (λ260/ λ280)
1	'Kinaya Kuning Lonjong' 1	KL1	2387.35	1.835
2	'Kinaya Kuning Lonjong' 2	KL2	2706.42	1.848
3	'Kinaya Bulat Kuning' 1	BK 1	1457.5	1.929
4	'Kinaya Bulat Kuning' 2	BK 2	1326.9	1.749
3	'Kinanti' 1	KNT1	2645.42	1.746
4	'Kinanti' 2	KNT2	2478.57	1.819
5	'Sonya' 1	SNY1	3134.07	2.018
6	'Sonya' 2	SNY 2	2435.15	1.777

DNA amplification. DNA amplification was carried out using the PCR-ISSR method. The objective was to determine the genetic variation of melon cultivars 'Kinaya', 'Kinanti', and 'Sonya'.
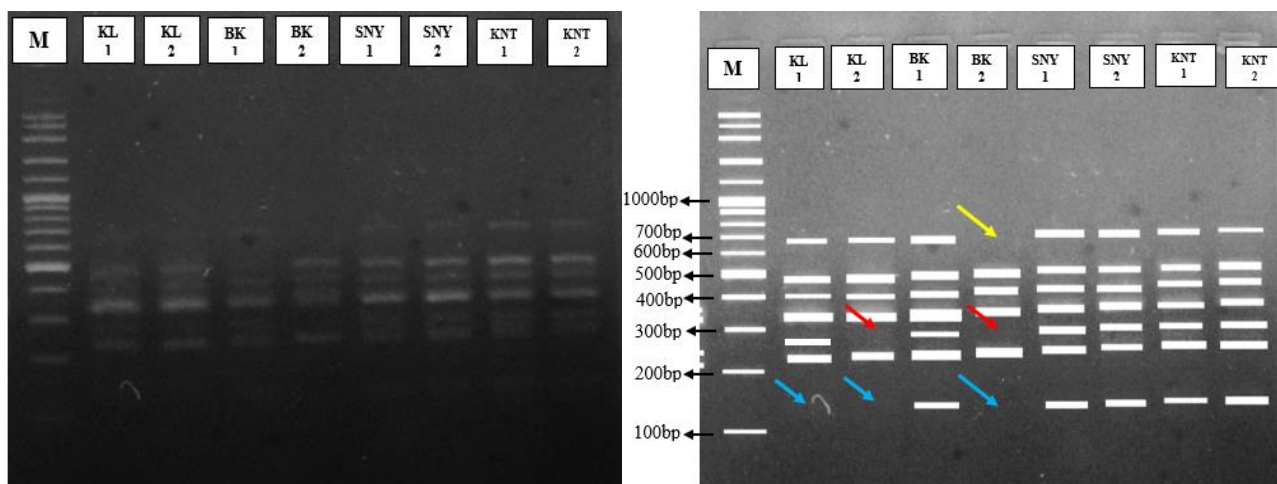

Fig. 1. Electrophoresis results and reconstruction of cultivars 'Kinaya', 'Sonya' and 'Kinanti' using the primer UBC 809 (M= Marker; KL= 'Kinaya Kuning Lojong'; BK= 'Kinaya Bulat Kuning'; SNY= 'Sonya'; KNT= 'Kinanti').

Based on PCR-ISSR visualization using primers UBC 809 (Fig. 1), we obtained seven DNA bands ranged from 150-700 bp. The seven DNA bands consist of three polymorphic and four monomorphic DNA bands. DNA bands measuring ±700 bp (yellow arrows) were found

in all samples except BK 2. DNA bands with a size of ±270 bp (red arrows) were found in samples KL 1, BK 1 3, SNY 1, SNY2, KNT 1, and KNT 2. DNA bands with a size of ±150 bp (blue arrows) were found in samples BK 1, SNY 1, SNY2, KNT 1, and KNT 2.
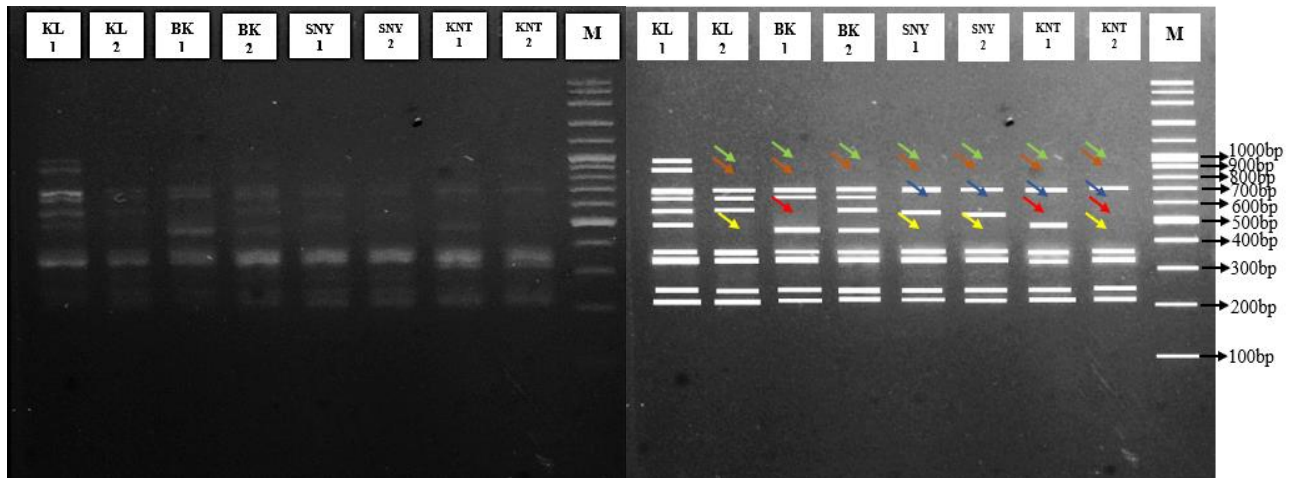
Fig. 2. Electrophoresis results and reconstruction of cultivars 'Kinaya', 'Sonya' and 'Kinanti' using the primer UBC 807 (M= Marker; KL= 'Kinaya Kuning Lojong'; BK= 'Kinaya Bulat Kuning'; SNY= 'Sonya'; KNT= 'Kinanti').

Primer UBC 807 (Fig. 2) showed ten DNA bands ranged from 200-1000 bp, consisting of five polymorphic and five monomorphic DNA bands. About ±490 bp (yellow arrows) were only found in samples KL 1, BK 1, BK 2, and KNT 1. DNA bands with ± 550 bp (red arrows) were found in samples KL 1, KL 2, BK 1, SNY 1, and SNY 2. DNA bands with ±690 bp were found in samples KL1, KL 2, BK 1, and BK 2, while DNA bands with ± 900 bp and ± 1000 bp were only found in KL 1 samples.
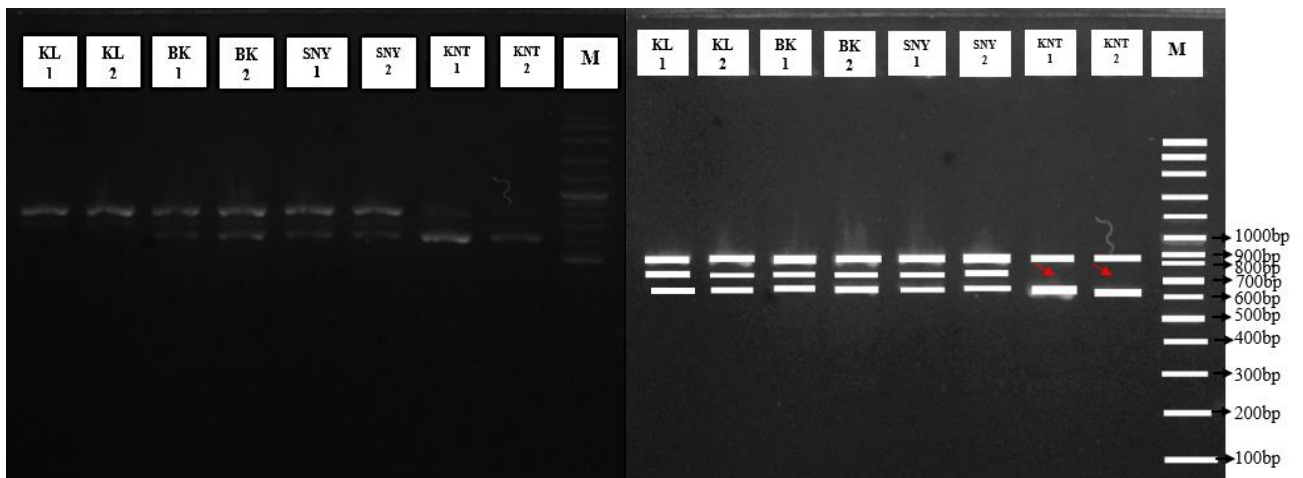

Fig. 3. Electrophoresis results and reconstruction of cultivars 'Kinaya', 'Sonya' and 'Kinanti' using the primer UBC 810 (M= Marker; KL= 'Kinaya Kuning Lojong'; BK= 'Kinaya Bulat Kuning'; SNY= 'Sonya'; KNT= 'Kinanti').

A total of three DNA bands measuring 600-900 bp were visualized on PCR-ISSR using primer UBC 810 (Fig. 3). The three DNA bands consist of one polymorphic and two monomorphic DNA bands. DNA bands with ±600 bp and ±900 bp were found in all samples. DNA bands with a length of ± 700 bp were only found in samples KL 1, KL2, BK 1, BK 2, SNY 1, and SNY2.
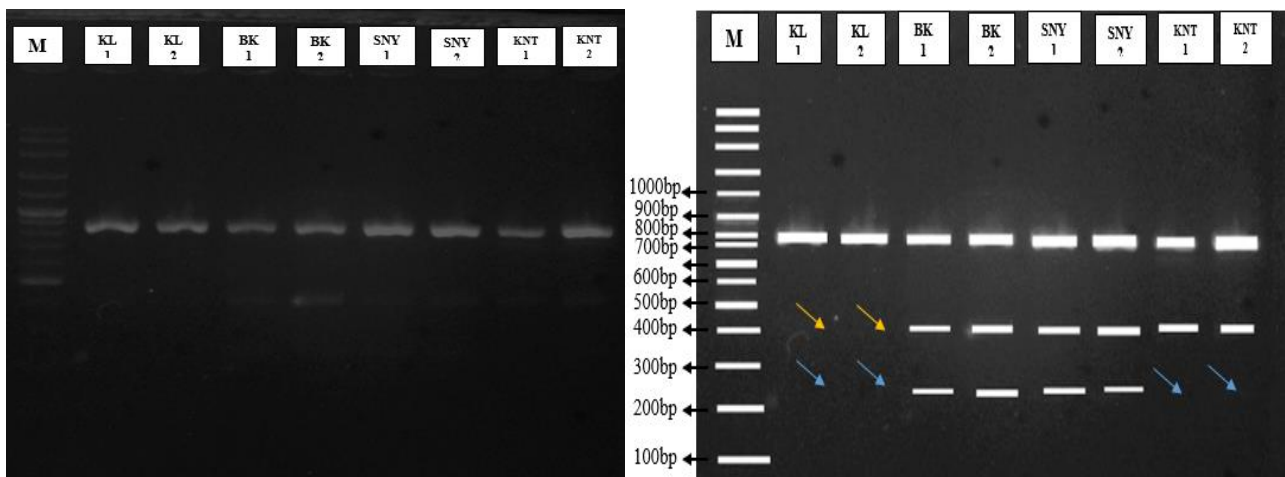
Fig. 4. Electrophoresis results and reconstruction of cultivars 'Kinaya', 'Sonya' and 'Kinanti' using the primer UBC 812 (M= Marker; KL= 'Kinaya Kuning Lojong'; BK= 'Kinaya Bulat Kuning'; SNY= 'Sonya'; KNT= 'Kinanti').

We experienced three DNA bands ranged from ±200-800 bp using primer UBC 812 (Fig 4) according to PCR-ISSR visualization, consisting of two polymorphic and one monomorphic character. DNA bands ±800 bp were found in all samples. DNA bands ± 400 bp were found in samples BK 1, BK 2, SNY 1, SNY 2, KNT 1, and KNT 2, while DNA bands about ± 250 bp were found in samples BK1, BK 2, SNY 1, and SNY 2. Due to the fact that each primer's annealing temperature is optimized, primer ISSR can detect considerable polymorphism (Wang, 2011; Gebrehiwet *et al.*, 2019). Each primer generates an extra DNA band, which resulting a distinct base size and intensity (Ng & Tan, 2015; Rohela *et al.*, 2019). Differences in the strength of DNA bands are influenced by the distribution of primer slinging sites throughout the genome, the purity of the genome, and the concentration of the genome in reactions (Ahmed *et al.*, 2014; Healey *et al.*, 2014; Shahid *et al.*, 2014; Boyd *et al.,* 2019). The variation in DNA band patterns is due to the quantity and size of the bands, which accurately describe the highly complex genomes of plants (Pellicer *et al.*, 2018; Lucas *et al.*, 2019; Ragupathy *et al.*, 2019).

Table 2. Percentage of polymorphic DNA bands using four specific primers.

No	Primer	Number of DNA bands	Number of DNA Monomorphic bands	Number of DNA polymorphic bands	DNA polymorphic (%)
1	UBC 807	10	5	5	50%
2	UBC 809	7	4	3	42.8%
3	UBC 810	3	2	1	33.3%
4	UBC 812	3	1	2	66.7%
Total		23	12	11	47.8%

According to Table 2, the percentage of polymorphism DNA bands generated by four primers varies. The UBC 812 primer had the highest polymorphism level at 66.7%, while the UBC 810 primer had the lowest percentage.
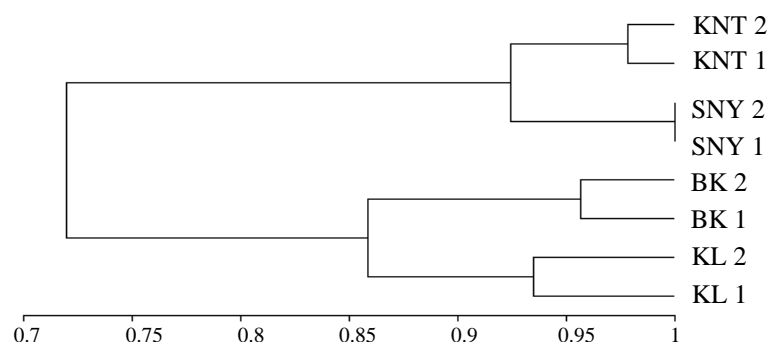
Fig. 5. Dendrogram of the phenetic kinship of melon based on ISSR with UPGMA method (Jaccard's Coefficient) (KL= 'Kinaya Kuning Lojong'; BK= 'Kinaya Bulat Kuning'; SNY= 'Sonya'; KNT= 'Kinanti').

Cultivars 'Kinaya Bulat Kuning' and 'Kinaya Kuning Lonjong' are clustered together, indicating that all samples are closely related (Fig. 5). These four melon samples are combined into a cultivar 'Kinaya', and differ only in fruit morphologies. 'Kinaya Bulat Kuning' and 'Kinaya Kuning Lonjong' are the outcome of segregating crossed elders 'Sonya' and 'Kinanti' that could not yield stable phenotypes. The stability and diversity of phenotypic features are owing to a large number of qualitative characteristics being controlled by a single gene inherited from elders.

'Kinaya' is the third derivative of crossed ♀ 'Kinanti' and ♂ 'Sonya' cultivated by the Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada. The first derivative of the cross is named KSL, and the second derivative is called KSL 4. KSL 4 has oblong fruit phenotypes, a net, and yellow fruit skin. KSL 4 seeds are replanted and produce 'Kinaya' as the third derivative. 'Kinaya' has two forms of fruit that are oblong and round. 'Kinaya' also has a yellow fruit skin color that is derivative of 'Kinanti', and the fruit's skin is shrouded in net according to its elder 'Sonya'. With similar properties and phenotypes, the cultivars 'Kinaya Kuning Lonjong' and 'Kinaya Bulat Kuning' are in one cluster. The cultivars 'Sonya' and 'Kinanti' exhibit a 72% similarity with the cluster 'Kinaya'. Both cultivars are parental of the cultivar 'Kinaya' which have a high similarity rate of $\geq 70\%$. The higher the similarity index indicates the close phenetic kinship between tested cultivars. Since samples tested have a similarity index $\geq 70\%$,

all cultivars used in this study are one species. The dendrogram construction using the simple matching coefficient (SSM) and Jaccard coefficients (SJ) obtained relatively the same results, consisting of two clusters with the same members of each group.

The choice of molecular markers used in our research can influence the construction results in determining the genetic variation of melons. Primers such as the ISSR can amplify melon sequences in the genome with varying numbers. In line with Akash *et al*. (2019) that investigated 17 *C. melo* var. *flexuosus* using 14 ISSR molecular markers and showed 43% polymorphic rate. Mohamed *et al*. (2020) also studied 12 *C. melo* var. *flexuosus* uses six ISSR primers that produce varying and reproducible polymorphic levels. This molecular marker can be used to demonstrate that melon cultivars are related despite growing in a distinct environments. The evaluation of diversity and kinship using molecular markers is stable and is unaffected by environmental or tissue impacts. It is observable at an early stage of development and exhibits genetic variances. The information across the cultivars demonstrates the technique's utility for detecting genetic variation, which may aid in future genetic improvement projects for this fruit crop.

CONCLUSION

The genetic variation analysis of the cultivars 'Kinaya', 'Sonya', and 'Kinanti' using four ISSR primers UBC 807, UBC 809, UBC 810, and UBC 812, revealed 23 DNA bands, 11 polymorphic and 12 monomorphic. The polymorphism rate was 47.8%. Jaccard

coefficient analysis showed 72% similarity of cultivar 'Kinaya' with its parental 'Sonya' and 'Kinanti'.

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