RESEARCH ARTICLE

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GENETIC DIVERSITY OF *Cucumis* and *Mukia* (Cucurbitaceae) BASED ON ISSR MARKERS

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SUMMARY

The genera *Cucumis* L. and *Mukia* Arn. are closely related based on nuclear and chloroplast DNA. However, morphologically, *Cucumis* and *Mukia* have different characteristics. Therefore, the aims of this study were to determine genetic diversity by using inter simple sequence repeat (ISSR) markers and the fingerprints of *Cucumis* and *Mukia*. Samples were collected from Java, Madura, Kalimantan, Maluku, Ambon, and Papua. *Cucumis* (53) and *Mukia* (42) accessions were analyzed. Data were coded in the form of binary data and arranged in matrix form by using simple matching coefficients and similarity coefficients, and a dendrogram was constructed by using the unweighted pair group method with arithmetic mean method. The amplification of *Cucumis* and *Mukia* DNA by using 20 ISSR primers produced 246 bands and 245 polymorphic bands. The highest diversity was observed in *Mukia javanica* (Miq.) C. Jeffrey (0.101) and the lowest in *Mukia maderaspatana* L. (0.037). Principal component analysis grouped *Cucumis* and *Mukia* accessions into four groups: group I united all accessions of *M. javanica*, group II united those of *M. maderaspatana*, group III united those of *Cucumis melo* L., and group IV united those of *C. sativus* L. Five specific bands on primer H3, H7, H11, H1, H8, H6, H14 were found for *Cucumis*; three specific bands of species *C. melo* accession Golden melons, Bhalungkak, and Orange fruit melons; and two specific bands on *C. sativus* accession of Dayak cucumber. *M. maderaspatana* accessions from Kalimantan had specific bands for primers H6, H11, and H14. The results of the analysis indicated that ISSR markers can be used to distinguish *Cucumis* and *Mukia* into separated genera.

Keywords: *Cucumis*, ISSR, Malesia, Microsatellite, *Mukia*

Key findings: Genetic diversity was determined to verify *Cucumis* cultivars and *Mukia* accessions based on molecular markers. The ISSR markers can be used to

determine the genetic diversity of the genera *Cucumis* and *Mukia* and determine the fingerprints of *Cucumis* cultivars.

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INTRODUCTION

The genera *Cucumis* and *Mukia*, which are members of Cucurbitaceae, are phylogenetically closely related (Renner *et al.*, 2007). Previous research based on nuclear and chloroplast DNA grouped *Mukia* within the genus *Cucumis* (Schaefer, 2007). However, the two genera are morphologically distinct, i.e., flower size, fruit size, ripe fruit color, seed shape, seed surface, and seed edge (de Wilde and Duyfjes, 2010). Recent studies by Pratami *et al.* (2019) based on the morphological characteristics of seeds supported the opinion of de Wilde and Duyfjes (2010), who kept *Mukia* apart from *Cucumis*. The significant discrepancy between their distinct morphological characteristics and their similarity based on molecular data has caused the taxonomic status of *Cucumis* and *Mukia* to be debated.

Cucumis has long been cultivated throughout the world. As a consequence, morphologically, the Cucumis cultivars have many variations including leaf shape, leaf venation, leaf tips, leaf edges, female flowers, fruit stalk color, fruit spines, and fruit skin texture. *Cucumis* cultivars also vary in anatomy and isozyme characteristics. As a result, three cultivars of *C. sativus*, i.e., timun wuku, timun saloyo, and timun jepang, can be grouped itself and separated from six of *C. melo* cultivars i.e., krai randu, timun suri, krai kapasan, bhalungkak, blewah and melon (Rahayu and Hartana, 2002). However, the cultivars in the species cannot be separated by morphological, anatomical, and isozyme approaches. Therefore, in this study, molecular markers are applied to verify *Cucumis* cultivars and *Mukia* accessions.

Cucumis is a plant genus that has high economic value and has been widely cultivated. Widening of *Cucumis* genetic diversity is much needed for further crop improvement both in terms of quality and quantity. One way to expand genetic diversity is by crossing with *Cucumis* close relatives which have certain advantages such as *Mukia* that has a high resistance to pests and diseases. As one source of germplasms for the development of *Cucumis*, knowledge genetic diversity knowledge of *Mukia* is very critical to investigate. The genetic diversity of *Mukia* and *Cucumis* can be performed using molecular markers.

Several molecular markers have been used for genetic diversity analysis (Nadeem *et al.*, 2018), such as restriction fragment length polymorphism (Azrai, 2005), random amplified polymorphic DNA (RAPD) (Welsh and McClelland 1990; Behera *et al.*, 2008; Erdinc *et al.* 2013; Abdel-Ghani and Mahadeen, 2014), inter simple sequence repeat (ISSR) (Zietkiewicz *et al,.* 1994; Irshad and Idrees 2014; Ng and Tan, 2015), simple sequence repeat (SSR) (Tautz 1989) and amplified fragment length polymorphism (AFLP) (Yashiro *et al.*,

2005; Akash *et al.*, 2013; Shamasbi *et al.*, 2014) markers. ISSR markers are a repetitive, effective, simple, and fast marker technology that combines the utility of RAPD, SSR, and AFLP markers. These markers are inexpensive, and the amount of DNA required is relatively small. Besides that, ISSR markers have greater stability in repetition and show the amount of variability (Arefrad *et al.*, 2015; Akash *et al.*, 2019). These markers are capable of distinguishing between the closely related individuals of *Cucumis* and other plants (Reddy *et al.,* 2002; Pharmawati, 2009; Javan *et al.*, 2012; Tonk *et al.*, 2014; Singh *et al.*, 2016; Akash *et al.*, 2019).

The analysis of *Cucurbita pepo* and *Cucumis melo* by using ISSR markers produced higher polymorphism compared with RAPD or AFLP markers (Paris *et al*. 2003; Sensoy *et al*.*,* 2007). These results confirm that ISSR markers not only show polymorphy within a species but also are efficient for distinguishing between individuals and useful for the analysis of any genetic relationship and in breeding programs of *Cucurbitaceae* (Arefrad *et al.*, 2015; Guliyev *et al.*, 2018). The purpose of this present study was to determine the genetic diversity of the genera *Cucumis* and *Mukia* by using ISSR markers and the fingerprints of cultivars in *Cucumis*.

MATERIALS AND METHODS

Materials

The plant material used consisted of 53 *Cucumis* accessions representing two species (*C. melo* and *Cucumis sativus*) and 42 *Mukia* accessions representing two species (*Mukia javanica* and *Mukia maderaspatana*). *C. melo* was composed of three groups that are commonly known in Indonesia, i.e., the Reticulatus, Inodorus, and Cantaloupensis groups (Lim, 2011). The Reticulatus group was represented by amanta melons, roxy melons, and orange fruit melons; the inodorus group consisted of krai, bhalungkak, golden melon, golden tiger melon, cranshaw melon, timun suri, papua small melon, papua large melon, maluku melon, agrestis melon, ambon melon, and black melon; and the cantalupensis group was represented by blewah and timun mas. *C. sativus* consisted of kenya dayak cucumber, punan dayak cucumber, benlebat cucumber, taruna cucumber, baby cucumber, and timun jepang. *M. javanica* and *M. maderaspatana* were each composed of three populations (Table 1).

Methods

Sampling

Cucumis and *Mukia* sampling was done in several regions of Indonesia, namely, West Java (Bogor, Lembang, and Mekarsari), Central Java (Demak, Brebes, and Purwokerto), East Java (Probolinggo, Surabaya, Banyuwangi, Baluran, and Jombang), Madura (Bangkalan, Sampang, and Sumenep), Kalimantan (Samarinda and Berau), Maluku (Ambon), and Papua (Sentani) (Figure 1). Samples were taken from several healthy leaves from each accession and then placed in a plastic bag containing silica gel.

Investigation of *Cucumis* and *Mukia* genetic diversity

Molecular analysis was performed in three stages, i.e., DNA isolation, DNA

Table 1. Plant material.

Accessions	Local Name	Taxon	Status	Origin
CM1	Krai	*C. melo*	Local cultivars	Central Java
CM2,3,4,11,15,25	Timun suri	*C. melo*	Local cultivars	Java
CM5,35	Golden melon	*C. melo*	Cultivated	Mekarsari
CM6-7	Blewah	*C. melo*	Local cultivars	East Java
CM8-10,17	Bhalungkak	*C. melo*	Local cultivars	East Java & Madura
CM12	Papua small melons	*C. melo*	Local cultivars	Papua
CM13	Papua large melons	*C. melo*	Local cultivars	Papua
CM14	Timun mas	*C. melo*	Local cultivars	East Java
CM16, 36	Golden tiger melon	*C. melo*	Cultivated	Seed packaging
CM18	Cranshaw melon	*C. melo*	Cultivated	Seed packaging
CM19	Orange fruit melon	*C. melo*	Cultivated	Seed packaging
CM20	Melon	*C. melo*	Cultivated	Seed packaging
CM21, 37	Amanta melon	*C. melo*	Cultivated	Seed packaging
CM22, 38	Roxy melon	*C. melo*	Cultivated	Seed packaging
CM23	Timun suri	*C. melo*	Local cultivars	Java
CM24	Melon	*C. melo*	Cultivated	Seed packaging
CM26	Melon	*C. melo*	Cultivated	Seed packaging
CM27-29	Maluku melon	*C. melo*	Naturalized	Maluku
CM30, 39	Black melon	*C. melo*	Cultivated	Seed packaging
CM31, 40	Agrestis melon	*C. melo*	Naturalized	Nusa Tenggara Barat
CM32, 41	Ambon melon	*C. melo*	Naturalized	Ambon
CM 33-34	Orange fruit melon	*C. melo*	Cultivated	Purwokerto
CS1,7	Kenya Dayak cucumber	*C. sativus*	Local cultivars	East Kalimantan
CS2,8	Punan Dayak cucumber	*C. sativus*	Local cultivars	East Kalimantan
CS3,9	Benlebat cucumber	*C. sativus*	Cultivated	Seed packaging
CS4,10	Taruna cucumber	*C. sativus*	Cultivated	Seed packaging
CS5,11	Baby cucumber	*C. sativus*	Cultivated	Seed packaging
CS6,12	Timun jepang	*C. sativus*	Cultivated	Seed packaging
MJ1-20	Bebontengan	*M. javanica*	Naturalized	Sukamaju
MJ21-28	Bebontengan	*M. javanica*	Naturalized	Cibeber
MJ29-32	Bebontengan	*M. javanica*	Naturalized	Pabangbon
MM1-6	Bebontengan	*M. maderaspatana*	Naturalized	Madura
MM7-8	Bebontengan	*M. maderaspatana*	Naturalized	Kalimantan
MM9-10	Bebontengan	*M. maderaspatana*	Naturalized	East Java

amplification via polymerase chain reaction (PCR) technique, and amplicon visualization. The molecular analysis of *Cucumis* and *Mukia* was performed by using ISSR markers. The isolation of DNA from leaves was performed by using the cetyl trimethyl ammonium bromide method (Doyle and Doyle, 1987). DNA amplification was performed by using 20 ISSR primers (Table 2) and GoTaq Green Master Mix (Promega, United States). The PCR process was conducted with a total volume of 10 µl consisting of 5 µl of GoTaq Green, 2 µl of primers (0.5 µM), 1 µl of a purified DNA template (250 ng), and 2 µl of ddH2O. Amplification was performed by using a 35-cycle PCR machine (SelectCycler II, Bioproduct, USA) and pre-denaturation for 5 min at 94 °C. Each cycle consisted of denaturation at 94 °C for 1 min, annealing at 44 °C –55 °C for 30 s, elongation at 72 °C for 1 min, and final extension at 72 °C for 5 min. The DNA fragments, including standard 1 kb DNA ladder (Thermoscientific, USA), were separated on 1.5% agarose gel for 60 min at 100 V. Then, the gel was

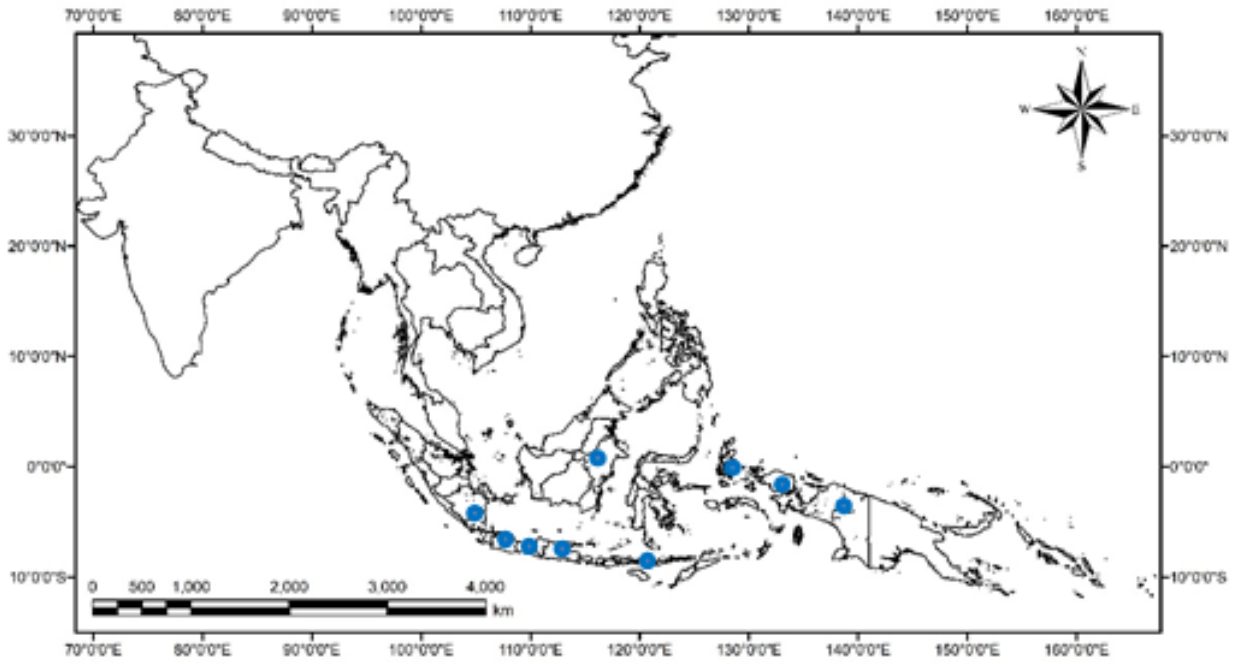

Figure 1. Map of sampling sites of *Cucumis* and *Mukia.*

Table 2. Polymorphic bands of 20 ISSR primers in *Cucumis* and *Mukia.*

Primers	Sequence (5′----3′)	Annealing temperature (°C)	Number of visible bands	Number of polymorphic bands	Polymorphism (%)
G 10	$(AG)_8C$	48.1	15	15	100
M 14	$(AC)_8G$	48.9	13	13	100
P 1	$(GA)_9AT$	48.9	9	9	100
P 6	$(AGG)_5$	53.9	10	10	100
P 9	$(AG)_8T$	51.7	11	11	100
H 1	$(GGGGT)_3$	51.7	13	13	100
H 2	$(GA)_9T$	52.9	10	10	100
H 3	$T(GA)_9$	45.4	18	18	100
H 5	$(CT)_8T$	45.4	6	6	100
H 6	$(GT)_8T$	44.4	9	9	100
H 7	$(AG)_8T$	46.6	16	15	93.7
H 8	$(AG)_8C$	49.6	17	17	100
H 9	$(AC)_8T$	55.0	12	12	100
H 10	$(AG)_8TC$	46.6	16	16	100
H 11	$(AG)_8YA$	49.7	18	18	100
H 12	$(GA)_8YC$	48.9	7	7	100
H 13	$(CA)_8RC$	53.9	13	13	100
H 14	$(AC)_8YA$	45.9	8	8	100
H 15	$(AC)_8YG$	54.9	15	15	100
M 11	$(TG)_8RT$	48.9	10	10	100
Total			246	245	
Average			12.3	12.2	95%

Table 3. Genetic diversity parameters of *Cucumis* and *Mukia.*

Species	N	Na	Ne	I	*h*	%P
C. melo	41	1.667	1.296	0.303	0.189	83.33%
C. sativus	12	1.923	1.395	0.403	0.254	92.31%
M. javanica	32	1.918	1.197	0.248	0.142	93.15%
M. maderaspatana	10	1.878	1.263	0.303	0.180	87.80%

N=Number of Samples; Na=Number of Alleles; Ne=Number of Effective Alleles; I=Shannon Index; h=Genetic Diversity (Polymorphic Information Content, PIC); % P=Percentage of Polymorphic Loci.

visualized, and the amplified band pattern was documented by using a UV–transilluminator instrument (WiseDoc, Daihan Ltd, South Korea).

Data analysis

ISSR data were analyzed on the basis of the presence (score 1) or absence (score 0) of visible DNA bands. Molecular data were arranged into a binary matrix and then further incorporated by using simple matching (SM) similarity coefficients. A dendrogram of *Cucumis* and *Mukia* was constructed by generating the unweighted pair group method with arithmetic mean (UPGMA) method. All data were analyzed by using NTSYS-PC program version 2.11a (Rohlf, 2000). For generating the dendrogram of cultivation status of *Cucumis*, we only incorporated accession CM1-CM32 and CS1-CS6. By contrast, we removed the accession of CM33-CM41 and CS7-CS12 to avoid the redundancy of the monomorphic band in the dendrogram.

Genetic diversity was analyzed by using GenAlex 6.5 (Peakall and Smouse, 2012). A high Na value in the GenAlex program means that more individuals are homozygous (Maleki *et al.*, 2017). In accordance with GenAlex analysis, we also analyzed genetic diversity on the basis of analysis of molecular variation (AMOVA) and the Shannon index. According to Silva *et al.* (2015), the Shannon index may vary from 0 to 1, and lower genetic diversity is indicated by values closer to zero.

RESULTS

The PCR analysis using 20 ISSR primers generated 6–18 bands per primer. A total of 246 bands were produced, among which 245 bands were polymorphic, and the average number of bands was 12.3 bands per primer. The primers that produced the most polymorphic bands were $(GA)_9T$ and $(AG)_8YA$ with a total of 18 bands. Conversely, the lowest number of polymorphic bands (6 bands) was produced by primers $(CT)_8T$ (Table 2).

Analysis of genetic diversity

The number of alleles (Na) for all accessions of *Cucumis* and *Mukia* or the combination was higher than the effective allele number (Ne). The Na values were 1,667–1,923, whereas the Ne values were 1,197–1,395 (Table 3).

The Shannon diversity information index values (I) and the genetic diversity index (h) for *C. sativus* were the highest (0.403 and 0.254) and those for *M. javanica* were the lowest (0.248 and 0.142) (Table 3). These results indicated that the genetic diversity of *Cucumis* and *Mukia* is relatively low.

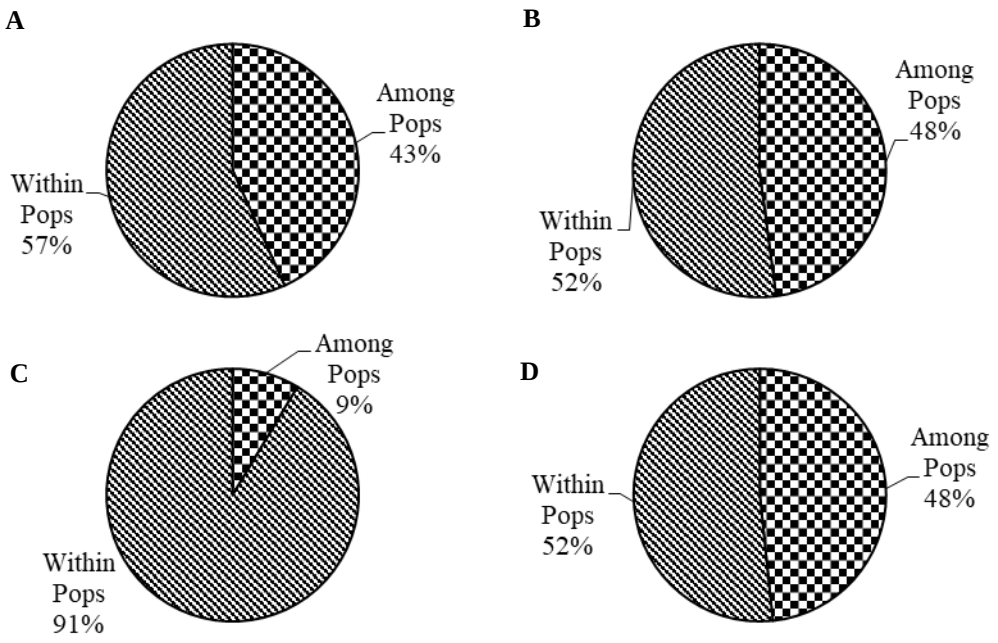

Figure 2. Percentage of genetic variation within and among populations (pops) of *C. melo* (A), *C. sativus* (B), *M. javanica* (C), and *M. maderaspatana* (D) based on ISSR markers.

The genetic diversity analysis of *Cucumis* and *Mukia* based on AMOVA showed that both genera had higher genetic variation within a population (57% [*C. melo*], 52% [*C. sativus*], 91% [*M. javanica*], and 52% [*M. maderaspatana*]) than among populations (43% [*C. melo*], 48% [*C. sativus*], 9% [*M. javanica*], and 48% [*M. maderaspatana*]) (Figure 2). A previous study on *Cucumis sativus* reported similar results (Panyanitikoon *et al.*, 2018).

Genetic relationships analysis of *Cucumis* and *Mukia*

The molecular data analysis of 53 *Cucumis* accessions and 42 *Mukia* accessions based on ISSR markers divided all accessions into two groups, Group A and B, at a similarity coefficient of 70% in the dendrogram (Figure 3). Group A contained all *C. melo* accessions, and group B consisted of *C. sativus* and *Mukia* accessions. At 71% similarity, the two species of *Mukia* were separated from *Cucumis*. Group C united all *C. sativus* accessions with a similarity coefficient of 74%, and the dayak cucumber was separate from the other cucumbers with a similarity coefficient of 84%. benlebat cucumber, taruna cucumber, and baby cucumber were clustered together, whereas Timun Jepang, which is characterized by dark green fruit skin and harder flesh, was separated from other *C. sativus* cultivars at a similarity coefficient of 90%. Local cultivars (kenya dayak and punan dayak cucumber) from Kalimantan were separated from other *C. sativus* cultivars at a similarity

coefficient of 79%. *Mukia* (group D) was divided into two groups in accordance with species. *Mukia javanica* was separated from *M. maderaspatana* at a similarity coefficient of 83%.

Cucumis melo was divided into three groups in accordance with their cultivation status: (1) naturalized, (2) local cultivars, and (3) cultivated (Figure 4). All groups were classified in accordance with their status although they vary in fruit morphology. Principal component analysis (PCA) showed a similar pattern of grouping (according to species level), which divided the materials into four groups, i.e., *M. javanica*, *M. maderaspatana*, *C. melo*, and *C. sativus* (Figure 5).

Cluster analysis through PCA divided the 53 *Cucumis* and 42 *Mukia* accessions into four groups. Group I contained *M. javanica* (accessions from Sukamaju, Cibeber, and Pebangbon) and group II contained *M. maderaspatana* (accessions from Bangkalan, Baluran, and Kalimantan). Group III contained *C. melo* (accessions from Demak, Brebes, Purwokerto, Mekarsari, Probolinggo, Bogor, Papua, Jombang, Semarang, Nusa Tenggara Barat, Maluku, Ambon, and Sampang), and group IV contained *C. sativus* (accessions from Samarinda, Berau, Purwakarta, Jember, and Yogyakarta) (Figure 5).

Specific bands

A total of five specific bands were found for *Cucumis*; these bands consisted of three specific bands for *C. melo* and two specific bands for *C. sativus* (Table 4). In *C. melo*, the 750 bp of H1 primers was a specific band for golden melons, the 250 bp of H3 primers was a specific band for bhalungkak, and the 250 bp of H7 primers was a specific band for orange fruit melons. By contrast, in *C. sativus*, the specific band for dayak cucumber was the 500 bp of H8 primers and 500 bp of H11 (Table 4, Figure 6). In *Mukia*, the accession of *M. maderaspatana* from Kalimantan had specific bands at 500 bp of H6, 400 bp of H11, and 750 bp of H14 (Table 4). These results indicated that some specific bands, as a genetic profile, can be used to identify cultivars of *C. sativus* and species of *Mukia*. Similar to the results of this study, specific bands can be used as markers to identify the species or infraspecies of many plants, i.e., *Citrus* (Agisimanto *et al.*, 2007).

DISCUSSION

The low value of genetic diversity of *Cucumis* or *Mukia* is influenced by environmental factors and the number of accessions (Innark *et al.*, 2013; Innark *et al.*, 2014). Environmental factors, including temperature, light intensity, altitude, and humidity, reduce the variation in *Cucumis* and *Mukia* populations. All alleles of *Cucumis* and *Mukia* have varying frequencies. This result is similar to that found by Riupassa *et al.* (2015). The number of accessions of *C. sativus* and *M. maderaspatana* was lower than that of *C. melo* and *M. javanica*, which had more than 30 accessions (41 and 32, respectively). In Iran, the lowest Shannon information index, expected heterozygosity, and percentage of polymorphic loci are shown by 'Fars2' and 'Markazi1' accessions (*C. melo*). The Shanon index and expected heterozygosity of Iranian flexuosus obtained by using

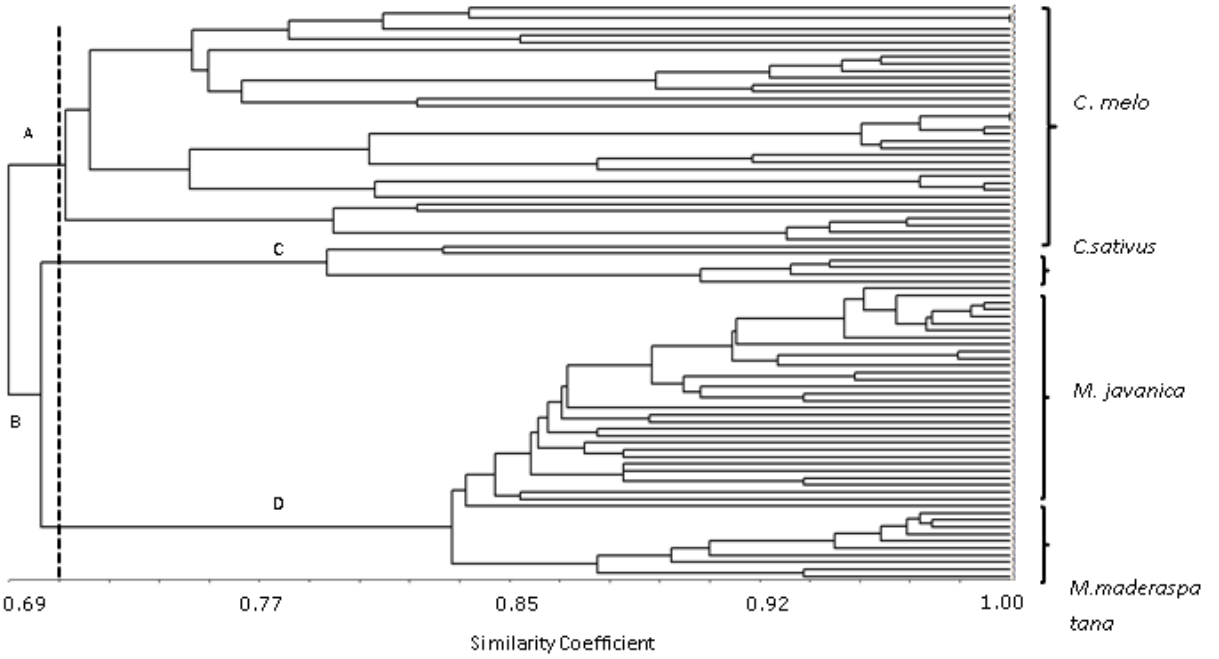

Figure 3. Dendrogram of *Cucumis* and *Mukia* based on ISSR markers using UPGMA method.

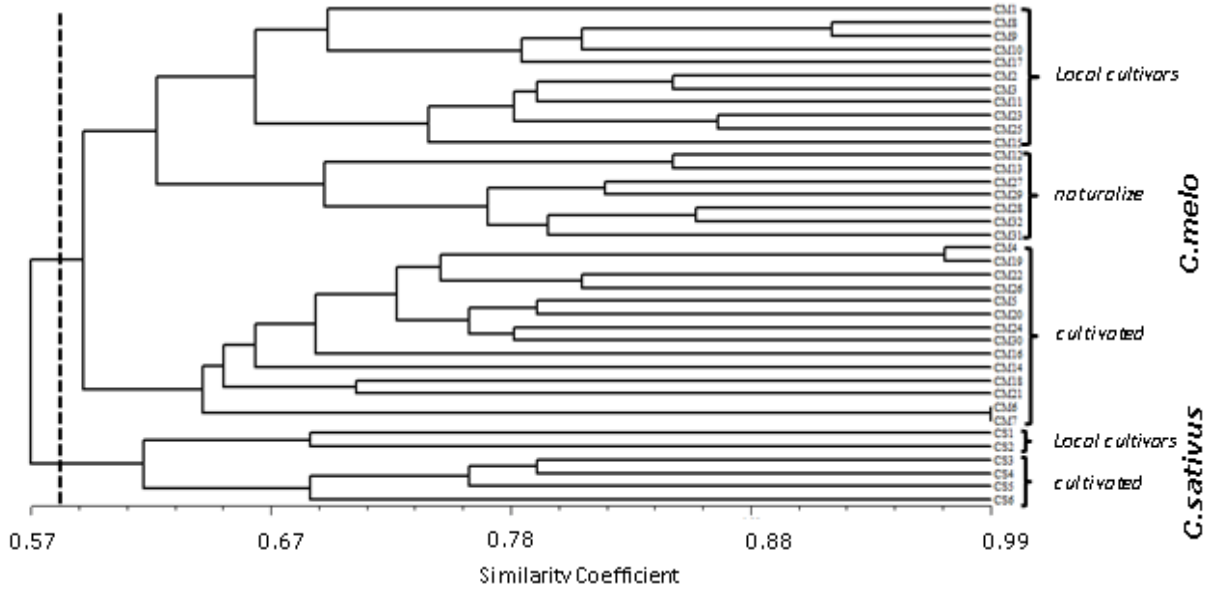

Figure 4. Dendrogram of *Cucumis* based on ISSR markers using UPGMA method.
CM1 = Krai, CM2 = Timun suri 1, CM3 = Timun suri 2, CM4 = Melon, CM5 = Golden Melon, CM6 = Blewah, CM7 = Blewah, CM8 = Bhalungkak 1, CM9 = Bhalungkak 2, CM10 = Bhalungkak 3, CM11 = Timun suri 3, CM12 = Papua small melons, CM13 = Papua large melons, CM14 = Timun mas, CM15 = Timun suri 4, CM16 = Golden tiger melon, CM17 = Bhalungkak 4, CM18 = Cranshaw melon, CM19 = Orange fruit melon, CM20 = Melon 1, CM21 = Amanta melon, CM22 = Roxy melon, CM23 = Timun suri 5, CM24 = Melon 2, CM25 = Timun suri 6, CM26 = Melon 3, CM27 = Maluku melon 1, CM28 = Maluku melon 2, CM29 = Maluku melon 3, CM30 = Black melon, CM31 = Agrestis melon, CM32 = Ambon melon, CS1 = Kenya Dayak cucumber, CS2 = Punan Dayak cucumber, CS3 = Benlebat cucumber, CS4 = Taruna cucumber, CS5 = Baby cucumber, CS6 = Jepang cucumber. The monomorphic bands of the accessions CM33-CM41 and CS7-CS12 was removed of this analyze.

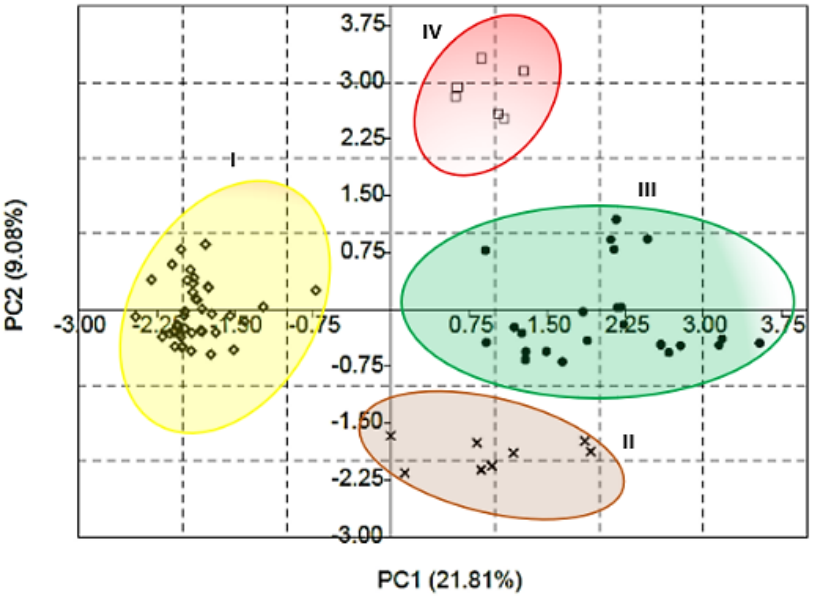

Figure 5. Principal components analysis of *Cucumis* and *Mukia* accessions. ◇ (*M. javanica*): Accession from Sukamaju, Cibeber, and Pebangbon. x (*M. maderaspatana*): Accession from Bangkalan, Baluran, and Kalimantan. • (*C. melo*): Accessions from Demak, Brebes, Purwokerto, Mekarsari, Probolinggo, Bogor, Papua, Jombang, Semarang, Nusa Tenggara Barat, Maluku, Ambon, and Sampang. □ (*C. sativus*): Accessions from Samarinda, Berau, Purwakarta, Jember, and Yogyakarta. PC1: Principal Component Value 1. PC2: Principal Component Value 2.

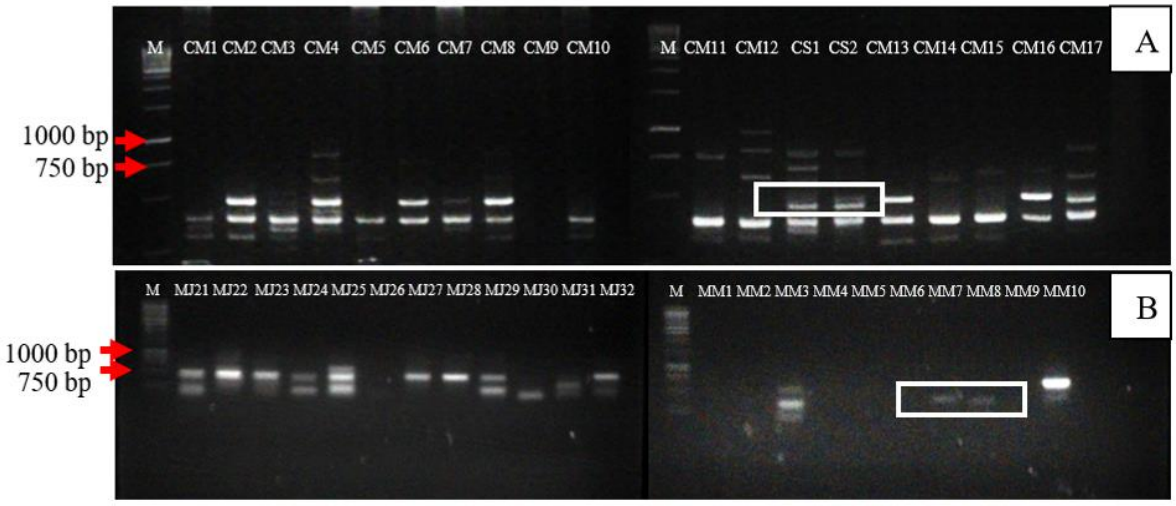

Figure 6. Gel electrophoresis of ISSR markers of *Cucumis* and *Mukia* using primer H11. (A) the specific band of the Dayak cucumber cultivars (*C. sativus*) with a size of 500 bp and (B) the specific band of Bebontengan (*M. maderaspatana*) from Kalimantan with a size of 400 bp.

Table 4. Specific bands for *Cucumis* and *Mukia* identification.

Accessions/ Local Names (Species)	Primer Name {Sequence}$^{\text{Specific Bands}}$
Golden melon (*C. melo*)	H1 $\{(GGGGT)_3\}^{750}$
Bhalungkak (*C. melo*)	H3 $\{T(GA)_9\}^{250}$
Orange fruit melon (*C. melo*)	H7 $\{(AG)_8T\}^{250}$
Dayak cucumber cultivars (*C. sativus*)	H8 $\{(AG)_8C\}^{500}$, H11$\{(AG)_8YA\}^{500}$
Bebontengan (*M. maderaspatana*) from Kalimantan	H6 $\{(GT)_8T\}^{500}$, H11$\{(AG)_8YA\}^{400}$, H14$\{(AC)_8YA\}^{750}$

RAPD markers are 0.201 and 0.25, respectively (Soltani *et al.*, 2010). Low genetic diversity results with a value of I = 0.121 were also reported by Maleki *et al.* (2017) for 27 accessions of Iranian melons.

The low value of genetic diversity among populations is because *Cucumis* and *Mukia* have unisexual flowers and consequently cross-pollinate. This is also seen in durian tengkurak (*Durio tanjungpurensis* Navia) (Riupassa *et al.*, 2015) and in rye (*Secale cereale* L.) (Persson and Bothmer, 2000). Self-pollinating plants have a higher genetic diversity variation between populations (Wright *et al.*, 2013). A genotype that undergoes self-pollination and fertilization has an increased homozygous proportion but a decreased proportion of heterozygotes (Mangoendidjojo, 2003).

Our results indicate that the variation in genus *Mukia* is smaller than in *Cucumis*. Nakata *et al.* (2005) classified *C. melo* cultivars from Japan into three horticultural groups: Group Cantalupensis, Group Inodorus, and Group Conomon. In *C. sativus*, 16 selected cucumber cultivars were classified into four groups on the basis of several characteristics, namely, fruit flavor (i.e., aroma and taste), and dry matter. *C. melo* has higher morphological diversity than *C. sativus*, which has caused more complexity in evaluating the phylogenetic analysis (Zhang, 2012).

In this study, various morphological characteristics of *C. melo* w, viz. flower size, fruit shape, fruit size, fruit skin surface, fruit flesh thickness, fruit storage capability, and seed size, were found in the three groups. This study is in line with the study of Rahayu and Hartana (2002) who grouped local cucumber (timun wuku and timun saloyo) into *C. sativus* but included 'krai randu,' 'timun suri,' 'krai kapasan,' 'bhalungkak,' 'blewah,' and cantaloupe in *C. melo*. By contrast, Heyne (1950) did not distinguish among 'blewah' (Javanese), 'bhalungkak' (Maduranese), 'krai' (Javanese), and 'bonteng suri' (Sundanese), whereas Ochse and Bakhuinzen van den Brink (1931) included 'krai' and 'bonteng suri' in *C. sativus*. Besides, they stated that 'bhalungkak' is 'krai'. The results of our study indicated that *C. melo* developed from naturalized varieties into cultivars. Thus, we found that differences among the germplasm of melons, local cultivars of melons, and commercial cultivars play a role in modern melon cultivar development. All accessions of *C. melo* that were investigated in this study were grouped on the basis of their cultivation status, i.e., naturalized types, local cultivars, and cultivated types. The naturalized type of *C. melo* has a closer relationship with local cultivars at a similarity coefficient of 75% than with cultivated and patented cultivars at 73%. This result

provides useful information on new developments in *C. melo* from naturalized types to cultivars.

This study revealed that the genetic variation in *C. melo* was significantly higher than that in *Mukia maderaspatana* likely because the locations of the *C. melo* samples were from a wider area and they comprised three cultivation statuses, namely, naturalized types, local cultivars, and cultivated plants, whereas *Mukia* is only found as naturalized type. Similar to this study, wild cucumbers, especially genus *Mukia*, are only found as naturalized type (Singh *et al.*, 2016). Specific band sizes in the genus *Cucumis infinity* and flexuosus genotypes are relatively higher than others based on ISSR markers (Singh *et al.*, 2016; Akash *et al.* 2019). *Cucumis* is found in many locations in Indonesia with a different status, e.g., naturalized type, local cultivars, and cultivated. *Mukia* is found only in several locations as a naturalized type. In our study, the location of sampling and the cultivated status of the plants affected the diversity of *Cucumis* and *Mukia*. Wild and cultivated plants found in more locations and with diverse statuses will have higher diversity than those that are found only in a few locations and with naturalized types because they have not experienced considerable human intervention. However, diversity is more variable between the genera *Cucumis* and *Mukia*. The high genetic distance between *Cucumis* and *Mukia* showed that the two genera are best regarded as distinct (Table 5).

Greek and Cypriot traditional cultigens were grouped within subspecies melo and showed to be different from flexuosus accessions (Tzitzikas *et al.*, 2009). By contrast, analysis with RAPD markers showed that Greek flexuosus landraces and Greek inodorus landraces are distinctly different (Staub *et al.*, 2004). In our study, the naturalized group consisted of maluku melons 1,2,3, papua small melons, papua large melons, ambon melons, and agrestis melons. The plants in this group are not cultivated and not used by local people, but are usually eaten by animals. The group of local cultivars (semi cultivated) included krai, bhalungkak, and timun suri, which are found in Central Java, East Java, and Madura. The third group of cultivated melons consisted of timun mas and blewah which are traded in farm shops.

Genetic distance shows the similarity of genetic characteristics between two populations, and higher genetic distance shows a lower level of similarity of genetic diversity (Kuwi *et al.*, 2018). As shown in Table 5, *Cucumis* and *Mukia* have a relatively large genetic distance. This study

Table 5. Genetic distance between *Cucumis* and *Mukia* populations.

Populations	Cm	Cs	Mj	Mm
Cm	0.00			
Cs	0.25	0.00		
Mj	0.47	0.38	0.00	
Mm	0.49	0.43	0.30	0.00

Legends: Cm=*C. melo*; Cs=*C. sativus;* Mj=*M. javanica*; Mm=*M. maderaspatana*

confirmed that the significant differences in the morphological characteristics of the two genera (Pratami *et al.*, 2019) are manifestations of genetically diverse alleles between the two.

The genetic distance between species within the genera *Cucumis* and *Mukia* is relatively low (0.25 and 0.30, respectively). The genetic distance of *C. melo* and *C. sativus* is also relatively low (0.25). The low genetic distance between the two *Cucumis* species occurred because their alleles show a high degree of genetic similarity based on the ISSR band pattern. Accessions belonging to the genus *Cucumis* tend to group and have a low genetic diversity value, indicating that accessions of *C. melo* and *C. sativus* belong to one separate group. Low genetic distance among populations could be caused by intraspecific hybridization during evolutionary periods such that alleles are similar. The similarity of these alleles results in a relatively low polymorphism, and the genetic distance among populations will be low (Cidade *et al.*, 2013; for a grass species). The same result was found for *Mukia* species, wherein *M. javanica* has a low genetic distance from *M. maderaspatana*. The low genetic distance of both *Mukia* species confirms that *Mukia* is separate and different from *Cucumis*.

The specific bands that we have found can assist local plant breeders in developing the purity of relatively fixed alleles and determine the limits of grouping the same cultivars of *Cucumis* and *Mukia* accessions. Specific gel bands can be further purified and then sequenced to obtain specific allele sequences and specific single nucleotide polymorphisms (SNPs) markers. Recently, SNP markers combined with PCR techniques have been shown to be alternative markers that are cheap, reproducible, repetitive, and valid for signifying and specifically identifying a cultivar, species, or other cultivated plants (Ganal *et al.*, 2012).

Genetic diversity in *Cucurbitaceae* has been extensively studied, especially at the genus level. A previous study reported that a combined analysis of the chloroplast sequences of *C. sativus* and *C. melo* resulted in a lower genetic distance than morphological characters (Renner *et al.*, 2007). In contrast, Zhang *et al.,* (2012) reported a relatively high genetic diversity between *C. sativus* and *C. melo* according to molecular and morphological characteristics. Moreover, although large morphological diversity was reported among *C. melo* and *C. sativus* accessions, the genetic diversity identified by using molecular characterization is significantly larger in *C. melo* than in *C. sativus* (Zhang *et al.*, 2012). The phylogenetic analysis of *Cucurbitaceae* by using the chloroplast DNA sequence of two regions (Spacer and Intron) also showed high diversity between *C. melo* and *C. sativus* (Kocyan *et al.*, 2007).

CONCLUSION

The genetic diversity of *Cucumis* and *Mukia* based on ISSR markers was similar to that based on morphological data. Shannon's information index value and genetic diversity index revealed that the genetic diversity of *Cucumis* and *Mukia* was low. *Cucumis melo* had the highest genetic diversity. Genetic variation within a population was higher than that among

populations in all studied species. As many as five specific bands were found for *Cucumis*, three for *C. melo* and two for *C. sativus,* and three specific bands were found for *M. maderaspatana* from Kalimantan. This study supported the view that *Cucumis* and *Mukia* are two distinct genera.

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