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Genetic variability of Indonesian eggplant (*Solanum melongena*) based on ISSR markers

UNI B. HUSNUDIN[1,♥], BUDI S. DARYONO[2,♥♥], PURNOMO[3,♥♥♥]

[1]Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Sleman 55281, Yogyakarta, Indonesia. Tel./fax.: +62-274-580839, ♥email: uni.baroroh@gmail.com

[2]Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Sleman 55281, Yogyakarta, Indonesia. Tel./fax.: +62-274-580839, ♥email: bs_daryono@mail.ugm.ac.id

[3]Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sekip Utara, Sleman 55281, Yogyakarta, Indonesia. Tel./fax.: +62-274-580839, ♥email: purnomods@ugm.ac.id

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Abstract. *Husnudin UB, Daryono BS, Purnomo. 2019. Genetic variability of Indonesian Eggplant (*Solanum melongena*) based on ISSR markers. Biodiversitas 20: 3049-3055.* Eggplant (*Solanum melongena* L.) is one of important vegetable in Indonesia and this country has a variety of eggplant germplasm, whereas molecular data of Indonesian eggplant is limited. This study describes an analysis of genetic variation based on ISSR markers to determine the diversity and phenetic relationship of Indonesian eggplants. 23 samples were collected from the Indonesian Center for Agricultural Biotechnology Research and Development (ICABIOGRAD) and some commercial cultivars. Data were analyzed by ISSR analysis using UBC 809, UBC 880, UBC 888, UBC 892 and UBC 895 primers. Similarity index was counted by Jaccard Coefficient formula based on molecular scoring. Cluster analysis was conducted by Unweighted Pair Group Methods using Arithmetic averages (UPGMA) method to create a dendrogram with Multivariate Statistical Program (MVSP) v.3.1 software. The results showed that ISSR markers were effective in the estimation of the genetic variability of eggplant accessions characterized by different level of polymorphism. Five ISSR primers generated 40 polymorphic bands (64.5% of the total). The dendrogram divided 23 eggplant accessions into 2 main clusters and one accession ("terong jawa"/K18) located outside from the main cluster. Results suggested that all accessions were grouped randomly into some clusters not in accordance with the locations of sample collection.

Keywords: Genetic, Indonesia, phenetic relationship, *Solanum melongena*, variability

INTRODUCTION

Eggplant (*Solanum melongena* L.) is one of the important vegetables in tropical and subtropical regions across the world. This species is member of the large and species-rich genus *Solanum* which it has approximately 1400 species that occur on all continents where eggplant included in subgenus *Leptostemonum* Bitter (Knapp et al. 2013) and *S. melongena* is included in section *Melongena* Dunal (Lester and Daunay 2003). Most researchers agree that the origin of section *Melongena* was African or East Africa (Daunay and Janick 2007; Knapp et al. 2013) followed by radiation into Asia (Levin et al. 2006). Vavilov (1951) proposed an Indo-Burma origin and it is generally accepted that eggplant was domesticated in tropical Asia. The greatest diversity of landraces and cultivars is found in Asia (India, China, and southeast Asia), with secondary centers in the Middle East and around the Mediterranean (Knapp et al. 2013).

Eggplant fruit is consumed widely as vegetables and some vegetative parts (e.g. peduncles, roots, stalks, and leaves) are used for traditional medicine (Daunay and Janick 2007). Eggplants have an important nutritional value such as protein, carbohydrate, fiber, vitamins, some minerals like potassium, calcium, sodium, and iron (USDA 2018) and high antioxidant capacity mainly due to chlorogenic acid and the anthocyanin pigments (Raigón et al. 2008). Anthocyanin pigments are found abundantly in the pigmented peel of eggplant fruit (Azuma et al. 2008).

Indonesia is one of the top 10 eggplant-producing countries in the world after China, India, Egypt, Turkey, and Iran with eggplant production in 2017 reached 0.53 thousand tons (FAOSTAT 2017). The cultivated eggplant in Indonesia can be found in some areas by their local names (Kurniawan et al. 2010). Collections of eggplant in this country resulting more than 200 accessions eggplant (ICABIOGRAD 2018) and 78 cultivars have been registered in the information system of plant varieties protection of the Indonesian Ministry of Agriculture (PPVT 2017). The diversity is available in among cultivars of eggplant (Frary and Doganlar 2013). Several characterization studies in eggplant with standardized morphological and agronomic descriptors have revealed that are suited for providing a useful characterization for eggplant breeders (Prohens et al. 2005; Kumar et al. 2008; Muñoz-Falcón et al. 2009; Begum et al. 2013; Kaushik et al. 2016).

The development of biotechnology techniques such as the molecular marker methods in the last few decades has proved valuable in crop breeding, especially in studies on genetic variation, diversity and gene mapping (Kumar et al. 2009; Reddy et al. 2002). This condition may raise

breeder's concern, since genetic diversity provides the genetic base for crop enhancement of environmental adaptation, yield or disease resistance (Ali et al. 2011). The most appropriate genetic marker has depended on the specific application, the presumed level of polymorphism, the presence of sufficient technical facilities and financial limitations. The earlier studies in eggplant using molecular markers are restriction fragment length polymorphism (RFLP) markers (Isshiki et al. 1998; Isshiki et al. 2003), amplified fragment length polymorphism (AFLP) markers (Mace et al. 1999; Nunome et al. 2001; Furini and Wunder 2004), random amplified polymorphic DNA (RAPD) markers (Karihaloo et al. 1995; Sifau et al. 2014; Asad et al. 2015), and simple sequence repeat (SSR) markers (Tümbİlen et al. 2009; Sunseri et al. 2010; Caguiat and Hautea 2014). These methods have major limitations, there is low reproducibility of RAPD, high cost of AFLP/RFLP and the need to know the flanking sequences to develop species-specific primers for SSR polymorphism (Reddy et al. 2002; Kumar et al. 2009). Inter simple sequence repeat (ISSR) is a marker that overcomes most of these limitations, because the technique combines most of the benefits of AFLP, RFLP and microsatellite analysis with the universality of RAPD (Zietkiewicz et al. 1994; Reddy et al. 2002). ISSR markers have been successfully used in eggplant for genetic diversity of Southern Chinese long-eggplant cultivars (Weihai et al. 2008), analysis of variation and identifying cultivars of eggplant and related species (Isshiki et al. 2008), molecular diversity analysis of Chinese-cultivated eggplant (Ali et al. 2011), and molecular identification of eggplant (Mahmoud and El-Mansy 2012).

Genetic variation analysis of four Indonesian eggplants using RAPD markers has been reported by Susilo and Setyaningsih (2018). Based on these references, we know that molecular data of Indonesian eggplant is limited. This study describes an analysis of genetic variation based on ISSR markers to determine the diversity and phenetic relationship of Indonesian eggplants.

MATERIALS AND METHODS

Plant materials

A total of 21 eggplant accessions and 2 commercial cultivars were used in this study (Figure 1). Eggplant accessions were collected from the Indonesian Center for Agricultural Biotechnology Research and Development (ICABIOGRAD), Bogor and grown in the greenhouse. Sample was obtained from different regions in Indonesia. Observations have been conducted from December 2017 to March 2018. The accession number, local name, and origin of samples are listed in Table 1.

DNA extraction, PCR amplification, and gel electrophoresis

Total DNA was isolated from fresh young leaves using GeneAid Plant Genomic DNA Mini Kit (GeneAid Biotech Ltd. Taiwan). The purity of DNA was determined by the ratio of spectrophotometer reading at 260 nm and 280 nm using 1.0% (w/v) of DNA samples. PCR reaction was carried out in a volume of 25 µl containing 1 µl DNA, 12,5 µl Bioline My Taq HS Red Mix, 11 µl of ddH_2O and 1 µl primer ISSR. Five primers of UBC primer set (University of British Columbia) were used for ISSR analysis based on Isshiki et al. (2008) are listed in Table 2.

PCR reaction was performed in Bio-Rad thermocycler using the following PCR profile for each primer: one cycle pre-denaturizing for 3 minutes at 94ºC, followed by 45 cycles of denaturizing for 30 seconds at 94ºC, annealing for 45 seconds at recommended ISSR primer temperature (Table 2), elongation for 2 minutes at 72ºC and post-elongation for 5 minutes at 72ºC. A total 5 µl of PCR product (ISSR) were separated by electrophoresis on 2% agar gel in 1.0 x TBE buffer containing 3 µl FloroSafe DNA stain and running at 50 v for 40 minutes using electroporator machine MUPID-exU. 4 µl DNA ladder (BenchTop 100bp DNA ladder Promega) was loaded to estimate the sizes of ISSR markers in base pairs. DNA bands were visualized by Gel Documentation.

Scoring and analysis

Each ISSR marker fragment was scored as present (1) or absent (0). The binary data matrix of ISSR compiled by the 5 primers of 23 eggplant accessions. Similarity coefficients were calculated with Jaccard Coefficient (Sokal and Sneath 1963). Based on similarity index data, cluster method algorithm Unweighted Pair-Group Method Using Arithmetic Average (UPGMA) was used to construct dendrogram using Multivariate Statistical Program (MVSP) software version 3.1 pc (Kovach 2007).

Table 1. List of eggplant accessions from Indonesia used in this study

A.N.	Local name of accession	Code	Origin of accession
9	Terong gelatik	K14	Pandeglang, Banten, West Java
32	Terong butuh	K17	Serang, Banten, West Java
49	Terong ungu	K116	Bogor, West Java
68	Terong gelatik; terong lalap	K32	Ciamis, West Java
94	Terong gelatik	K20	Gunungkidul, Yogyakarta
95	Terong	K62	Gunungkidul, Yogyakarta
156	Terong gelatik kecil	K13	Cilacap, Central Java
181	Terong telunjuk	K88	Deli Serdang, North Sumatra
199	Terong kecap	K90	Deli Serdang, North Sumatra
217	Terong hijau	K178	Tanah Karo, North Sumatra
240	Terong manggis	K110	Padang Pariaman, West Sumatra
257	Terong talang	K69	Ogan Ilir, South Sumatra
267	Terong apel	K50	Muaraenim, South Sumatra
271	Terong lalap rebus	K83	Ogan Komering Ulu, South Sumatra
288	Terong kercil	K135	Muaraenim, South Sumatra
606	Terong gading	K118	Kubu Raya, West Kalimantan
615	Terong pinang	K57	Singkawang, West Kalimantan
637	Terong asam	K51	Bengkayang, West Kalimantan
759	Faimatak	K82	Belu, West Nusa Tenggara
801	Poki-poki	K93	Minahasa, North Sulawesi
150	Terong jawa	K18	Cilacap, Central Java
-	Terong ungu	TU	Commercial cultivar
-	Kania F1	KF1	Commercial cultivar

Note: A.N.= accession number

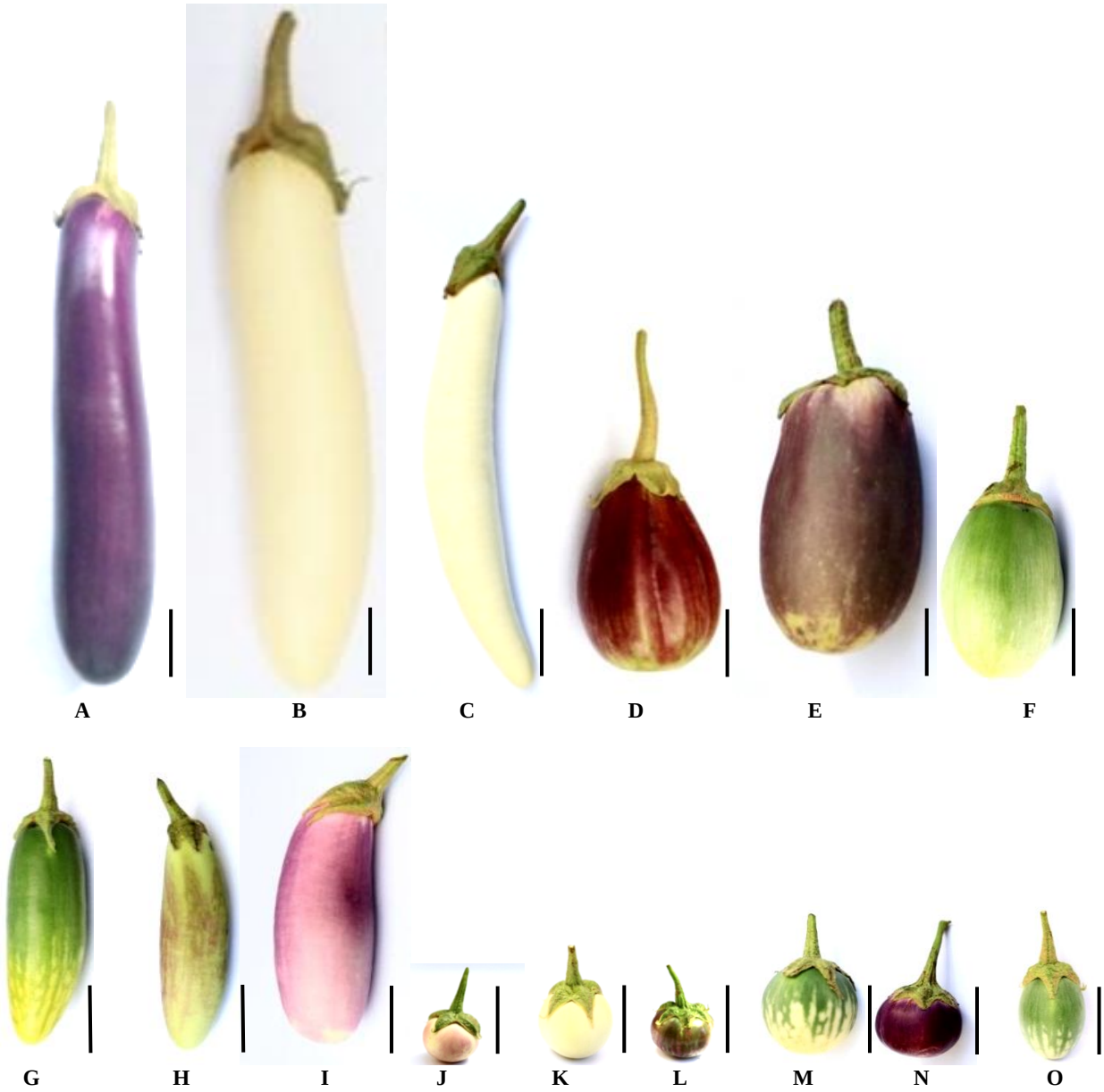

Figure 1. Samples of *S. melongena* collected in this study with different shapes and colours. A. Terong Ungu (TU); B. Kania F_1 (KF1); C. Terong Gading (K118); D. Poki-poki (K93); E.Terong Telunjuk (K88); F. Terong Ungu (K116); G. Terong Butuh (K17); H. Terong Jawa (K18); I. Terong Pinang (K57); J. Terong Gelatik Kecil (K13); K. Terong Apel (K50); L. Terong Gelatik (K14); M. Faimatak (K82); N. Terong Manggis (K110); O. Terong Kecap (K90). Bar = 3 cm

Table 2. ISSR primers and their nucleotides base sequence used in this study

Primer	Sequence 5'-3'	Annealing temp. (°C)
UBC 809	AGAGAGAGAGAGAGAGG	56.5
UBC 880	GGAGAGGAGAGGAGA	55.1
UBC 888	BDBCACACACACACACA	58
UBC 892	TAGATCTGATATCTGAATTCCC	49.8
UBC 895	AGAGTTGGTAGCTCTTGATC	58

Note: B = (C, G, T); D = (A, G, T)

RESULTS AND DISCUSSION

Analysis of polymorphism by ISSR

The ISSR profile produced by primers for 23 accessions is shown in Figure 2. The reproducible polymorphic bands generated by the 5 primers were detected in the 300 to 3,000 bp range, with a total number of 62 amplified bands. Amplified polymorphic and percentage of polymorphic bands produced by each primer are shown in Table 3. The total bands produced 62 bands with 40 (64.5%) are polymorphic bands and 22 (35.6%) are monomorphic bands. The UBC 880, 892, 895 primers produce a higher percentage of polymorphisms than UBC 809, 888 primers which produce lower polymorphisms.

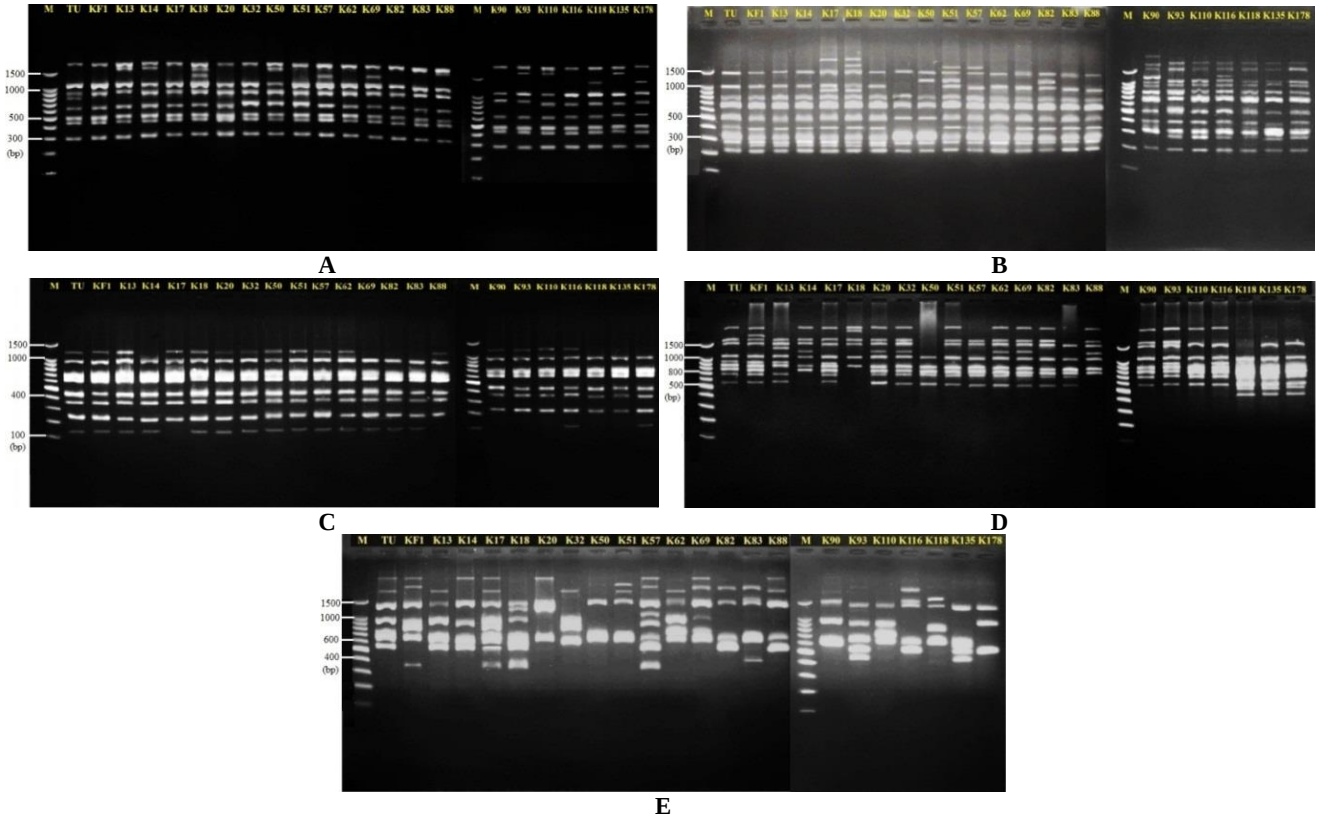

Figure 2. ISSR marker profiles of eggplants: (A) UBC 809 primer, (B) UBC 880 primer,(C) UBC 888 primer, (D) UBC 892 primer and (E) UBC 895 primer

PCR results with UBC 809 primers show a DNA band that is only owned by TU at the size of 970 bp, so the DNA band becomes specific band markers of "terong ungu" (TU). The UBC 892 Primer produced a DNA band with 2317 bp length become a specific band of "terong jawa" (K18) accessions, a band with 2007 bp length was a specific band of "Kania F1" (KF1) and a band with 1421 bp length was a specific band of "terong gelatik kecil" (K13).

Genetic variability of eggplants

Based on the similarity matrix between accessions (Table 4), with UPGMA cluster analysis method and MVSP 3.1 software, 23 eggplant accessions formed a dendrogram (Figure 3). Dendrogram showed that 23 eggplant cultivars form 2 main cluster and one accession located outside from the main cluster with Jaccard's similarity coefficient ranging from 0.661-0.881. Accession "terong jawa" (K18) from Java (Cilacap, Central Java) is located outside from main cluster at 0.661 similarity coefficient.

Dendrogram shows that cluster A consisting of 3 eggplant accessions there are "terong hijau" (K178), "terong" (K135) and "terong gading" (K118) at 0.683 similarity coefficient with 2 accessions were collected from Sumatra (K178, K135) and K118 was from Kalimantan. Most of the accessions were grouped in Cluster B. This cluster is consists of 4 sub-clusters, there are sub-cluster I consists of "terong gelatik" (K32) at 0.692 similarity coefficient, sub-cluster II consists of "terong kecap" (K90) at 0.697 similarity coefficient, and sub-cluster III consists of "terong apel" (K50) at 0.701 similarity coefficient. Sub-cluster IV formed by 16 other eggplant accessions (Figure 3) above 0.725 similarity coefficient.

Discussion

Several types of molecular markers have been used to study the relationships between the cultivated *S. melongena* and related species. The earlier markers used in the study of eggplant genetic diversity were allozyme (Isshiki et al. 1994) and RAPDs (Karihaloo et al. 1995). ISSR markers have also been used for assessment of genetic diversity relationships in eggplant and related species (Isshiki et al. 2008; Weihai et al. 2008). Based on the results of this study, ISSR primers are effective to show polymorphism in Indonesian eggplant accessions with 64.5% polymorphic band and show genetic diversity of eggplant. This result shows lower level of polymorphism than some previous studies by Mahmoud and El-Mansy (2012) that reported 66.2% polymorphism of 10 eggplant genotypes, Weihai et al. (2008) show the percentage of ISSR polymorphisms from Southern Chinese long-eggplant cultivars was 71%, and Isshiki et al. (2008) reported the percentage polymorphisms of 8 Japanese eggplant and 12 related

Solanum species using 34 ISSR primers was 99.1%. High levels of polymorphism showed that ISSR markers are suitable for genetic diversity studies. But low level of molecular polymorphisms are shown in some study, Isshiki et al. (1994) observed Japanese cultivars of eggplant using allozyme markers show low-level polymorphisms between them, Ali et al. (2011) obtained 13.5% (landraces), 20.7% (hybrid cultivar) and 15.3% (across) level of polymorphism in Chinese cultivated eggplant revealed by ISSR primers. Nunome et al. (2003) assumed that this low level of the polymorphisms was caused by the narrow genetic background of the cultivated eggplant and very small gene pool existed from which the cultivated forms arose (Karihaloo et al. 1995).

A dendrogram constructed by UPGMA cluster analysis from the ISSR data showing genetic relationship among accessions with similarity coefficient ranging from 0.661-0.881 (Figure 3). Sifau et al. (2014) indicating a fairly wide and diverse genetic base with high-level polymorphism and similarity coefficient ranging from 0.74 to 0.94 of Nigerian eggplant using RAPD markers. Marsolais et al. (1993) suggested that range of similarity coefficient 0.50 using RAPD may be implied the occurrence of interspecific hybrid, while range between 0.61-0.99 could suggest genetic similarity at the species level in Lilac. Different results are shown in dendrogram analysis based on the AFLP data revealed that Turkish eggplants have moderate molecular genetic diversity with genetic similarity ranging between 0.30 and 0.95 (Tümbİlen et al. 2009). Moderate genetic diversity was also observed in the *S. melongena* accessions studied by Mace et al. (1999) and Furini and Wunder (2004) using AFLP data. Behera et al. (2006) found broader diversity in 92 South Asian *S. melongena* accessions (genetic similarity between 0.37 and 0.90) using microsatellite markers.

Eggplant accessions divided into 2 main clusters (A and B) with one accession is located outside from the main cluster based on the dendrogram. Accession "terong jawa" (K18) from Java (Cilacap, Central Java) is located outside from the main cluster at 0.661 similarity coefficient. "Terong jawa" was separated from the main cluster by the presence of a band at 2317 bp in UBC 892 primer which is only owned by this accession, it was seemed to be most distinct and the DNA contains the greatest number of novel alleles (Kusumadewi 2011). This accession has violet skin color, obovate fruit shape, and the number of fruit per plant less than the other accessions with large fruit size.

Dendrogram shows that eggplant clustering was high level of similarity among them. Cluster A consisting of 3 eggplant accessions with morphological differences and there were collected from Sumatra and Kalimantan. Most of the accessions, including 2 commercial eggplants "terong ungu" (TU) and "Kania F1" (KF1) were grouped in cluster B with morphological differences. This cluster is consists of accessions from varied regions, there are from Java, Sumatra, Kalimantan, Sulawesi, and West Nusa Tenggara (NTB). The grouping of that commercial eggplant into main cluster may be implied genetic similarity between them caused by commercial cultivars resulted from breeding process that sharing the same genetic base (Aguilera et al. 2011). This result is different from the earlier study, Isshiki et al. (2008) found that 8 Japanese eggplant cultivars were categorized into the single group from the related *Solanum* with ISSR markers with combining the ISSR marker bands obtained by a few of the 34 primers, that was enough for identification of the eight cultivars. Caguiat and Hautea (2014) also analyzed genetic variation of eggplant using SSR markers, they found that eggplant formed 3 groups namely wild eggplant groups, cultivar groups, and landraces groups.

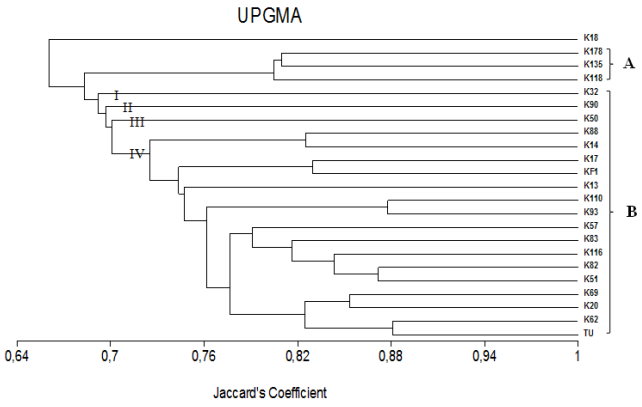

Figure 3. Dendrogram illustrating the variability and relationship among accessions of *S. melongena* based on ISSR analysis. The name of each accession is listed in Table 1.

Table 3. Polymorphic band markers from 23 eggplant accessions with 5 primers ISSR

Primer	Sequence	Σ ISSR fragment	Σ polymorphic fragment	Σ monomorphic fragment	Percentage of polymorphic bands (%)
UBC 809	AGAGAGAGAGAGAGAGG	10	3	7	43%
UBC 880	GGAGAGGAGAGGAGA	14	8	6	57%
UBC 888	BDBCACACACACACACA	8	2	6	25%
UBC 892	TAGATCTGATATCTGAATTCCC	15	13	2	87%
UBC 895	AGAGTTGGTAGCTCTTGATC	15	14	1	93%

Note: Y = (C, T); R = (A, G); H = (A, C, T); B = (C, G, T); V = (A, C, G); D = (A, G, T)

Table 4. Similarity matrix between eggplant accessions based on ISSR analysis

	TU	KF1	K13	K14	K17	K18	K20	K32	K50	K51	K57	K62	K69	K82	K83	K88	K90	K93	K110	K116	K118	K135	K178
TU	1																						
KF1	0.804	1																					
K13	0.818	0.761	1																				
K14	0.733	0.717	0.727	1																			
K17	0.771	0.83	0.729	0.688	1																		
K18	0.647	0.667	0.708	0.739	0.706	1																	
K20	0.795	0.818	0.75	0.786	0.745	0.688	1																
K32	0.711	0.733	0.705	0.738	0.739	0.612	0.762	1															
K50	0.674	0.659	0.707	0.619	0.596	0.574	0.725	0.675	1														
K51	0.795	0.778	0.75	0.667	0.708	0.653	0.81	0.721	0.769	1													
K57	0.761	0.783	0.717	0.674	0.68	0.694	0.773	0.617	0.69	0.814	1												
K62	0.881	0.778	0.791	0.744	0.745	0.653	0.854	0.805	0.725	0.854	0.733	1											
K69	0.795	0.818	0.711	0.786	0.708	0.653	0.854	0.721	0.683	0.81	0.814	0.854	1										
K82	0.767	0.75	0.721	0.714	0.646	0.625	0.78	0.651	0.737	0.872	0.786	0.78	0.825	1									
K83	0.727	0.75	0.721	0.636	0.681	0.592	0.78	0.651	0.784	0.825	0.786	0.78	0.78	0.795	1								
K88	0.791	0.696	0.744	0.825	0.702	0.681	0.805	0.674	0.675	0.762	0.727	0.805	0.762	0.732	0.69	1							
K90	0.66	0.646	0.617	0.574	0.688	0.633	0.705	0.622	0.659	0.786	0.711	0.705	0.667	0.756	0.714	0.659	1						
K93	0.8	0.745	0.795	0.711	0.787	0.729	0.733	0.652	0.69	0.773	0.739	0.773	0.733	0.744	0.744	0.767	0.75	1					
K110	0.773	0.717	0.727	0.682	0.723	0.702	0.744	0.659	0.744	0.786	0.711	0.829	0.744	0.714	0.756	0.738	0.762	0.878	1				
K116	0.761	0.745	0.717	0.638	0.68	0.66	0.733	0.617	0.732	0.857	0.778	0.733	0.733	0.829	0.829	0.689	0.791	0.818	0.791	1			
K118	0.638	0.625	0.63	0.553	0.667	0.58	0.644	0.6	0.675	0.721	0.689	0.682	0.682	0.651	0.69	0.636	0.659	0.689	0.738	0.689	1		
K135	0.646	0.6	0.674	0.667	0.673	0.653	0.652	0.574	0.683	0.689	0.696	0.652	0.652	0.698	0.738	0.682	0.667	0.814	0.786	0.733	0.805	1	
K178	0.717	0.667	0.711	0.705	0.64	0.688	0.689	0.644	0.643	0.727	0.773	0.727	0.767	0.738	0.698	0.644	0.667	0.773	0.744	0.733	0.805	0.81	1

The pattern of eggplant grouping not related to the morphological characters (fruit color, shape, and sizes) and this is an indication of some form of genetic relatedness among them despite differences in morphological features. Genetic variability can be caused by environment or internal factors such as mutation (Sari et al. 2018). Based on dendrogram (Figure 3) we knew that also clustering wasn't in accordance with the collection area of eggplant, it can be seen in cluster B that accession from Sulawesi (K93) and NTB (K82) joined with accessions from Java, Sumatra, and Kalimantan. Some similar result ware also found in Mao et al. (2006) were analyzing genetic diversity of Southern Chinese long-eggplant cultivars using ISSR markers the result is 57 eggplant cultivars divided into 6 groups that had no connection with regions of cultivars origin, Sifau et al. (2014) analyzing genetic variation using the RAPD marker also states that grouping is not related to the origin area of eggplant accession and study by Sari et al. (2018) using RAPD markers show that edible canna (*Canna indica* L.) in Indonesia divided into cultivar group based on morphological characters and was not influenced by geographical origin. Caguiat and Hautea (2014) also analyzed genetic variation of eggplant using SSR markers, they found that eggplant formed 3 groups that not related to the origin area of eggplant namely wild eggplant groups, cultivar groups, and landraces groups.

This is the report of using ISSR markers in detecting genetic variability of Indonesian eggplant accessions. Cluster analysis dendrogram based on the present ISSR markers distinguished eggplant into 2 main clusters and one accession ("terong jawa"/K18) located outside from the main cluster. All of eggplant accessions were grouped randomly into some clusters not in accordance with the locations of sample collection.

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