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Characterization of Carambola (*Averrhoa carambola*) Plant Collection of Cibinong Plant Germplasm Garden Based on Phenotypic and Genetic Characters

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Abstract

Indonesia as a rich biodiversity country has many superior fruit plant germplasms such as sweet star fruit or carambola (*Averrhoa carambola*). Some varieties of carambola which collected at the Germplasm Garden of Research Center for Biotechnology-LIPI have been used for parent trees of fruit plant production. Therefore, they have to be characterized both phenotypically and genetically. The objective of the study was to analyze the relationship between eight varieties of carambola i.e. Malaysia, Penang, Rawasari, Bangkok, Sembiring, Dewabaru, Demak and Dewimurni at the germplasm garden based on phenotypic and genetic characters. Phenotypic characters were observed directly in the field, whereas genetic characters were observed with RAPD markers using 10 primers. Phylogenetic analysis was done using NT-SYS software showed that there were three clusters of carambola varieties. Meanwhile, Malaysia and Penang varieties have closed relationships (96%) compared with the other varieties. The result of the study would be dedicated to updating and completing the existing fruit plant collection database of Plants Germplasm Garden.

How to Cite

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INTRODUCTION

Germplasm Garden of Cibinong is one of *in situ* conservation site of fruit plants belongs to Research Center for Biotechnology-LIPI. Currently, there are nine superior and local varieties of sweet star fruit (carambola) from all over provinces of Indonesia and abroad were collected in the garden. The majority of the fruit plant collection in the garden have been registered by the Institute for Seed Control and Certification of West Java, as a source of scions for seedling production. Local varieties which have poor fruit quality but have good roots structure was retained in this garden for rootstocks.

Carambola is tropical to the subtropical evergreen tree with 6-10 m in height. The species is thought to be indigenous to South Asia. This plant has been cultivated in South East Asia and Malaysia for several hundred years (Small, 2012). In Java Island, this plant grows wild in many places at an altitude of 500 m above the sea level. Depok region in West Java is a carambola fruit production center in West Java (Pansila *et.al.*, 2011).

Plant genetic resources play an important role in generating new high yielding crop varieties. Morphological, biochemical and molecular procedures have been exploited for evaluating these resources as crop germplasm. Diversity in germplasm is important for any breeding program since it directly affects the potential for genetic gain through selection (Kotal *et al.*, 2010). Kuswandi *e.t al.* (2014) reported that morphology and molecular markers can be used to analysis relatedness and genetic variability.

Randomly amplified polymorphic DNA (RAPD) is one of the methods to identify polymorphism that can be used to elicit information on divergence, variation, diversity analysis, phylogeny, quantitative traits, marker-assisted selection etc. (Nagaral *et.al.*, 2009). Goraniya *et. al.* (2013) reported that RAPD markers can be used to analyze the interrelationship and genetic polymorphism between *Manilkara hexandra* Roxb and *Averrhoa carambola* Yulita (2011) reported that RAPD markers potential to characterize genetic variation and relatedness of two new species of star fruit. The RAPD markers also can solve the taxonomy problems such as complexity in star fruit species. Gajera *et. al.* (2014) used RAPD to study genetic diversity and relationship among mango. The information of the extent of genetic variability is useful in the choice of parents for breeding hybrids.

The objective of the study was to assess the genetic variability and relationship between eight carambola varieties at the germplasm garden in order to update and complete the existing database of fruit plant collection of Plant Germplasm Garden.

METHODS

Plant source

Plant, flower and fruit samples of carambola were obtained from 20-year-old plant collection of Germplasm Garden of Research Center for Biotechnology-LIPI at Cibinong, West Java. Eight varieties of carambola i.e. Dewimurni, Rawasari, Sembiring, Malaysia, Demak, Penang, Dewabaru and Bangkok were used in this study. Carambola variety of Demak, Dewabaru and Dewimurni were released as superior fruit plant varieties by the Ministry of Agriculture (Prastowo *et al.*, 2006).

Phenotypic characters

Morphological observation of vegetative and generative parts of the carambola plant was done daily from March to October 2015. Visual observation techniques were done directly in the field using a digital SLR camera (Canon EOS 600D) and a measuring tool (digital caliper) (Nankai).

Genetic character

The 10 RAPD primers used in the study for genetic analysis were S97 (ACGACCGACA), S103 (AGACGTCCAC), S122 (GAGGATCCCT), S123 (CCTGATCACC), S126 (GGGAATTCGG), S197 (TGGGGACCAC), S199 (GAGTCAGCAG), S5 (TGCGCCCTTC), S29 (GGGTAACGCC) and S33 (CAGCACCCAC). The PCR reaction was performed with a total volume of 12.5 µl contained 1 µl of genomic DNA, 2.5 µl 5x PCR buffer (Kappa, Genetika Science), 0.25µl dNTP (10mM), 1µl of primer (10 µM) and 0.25 u of Taq polymerase (Kappa 2G Fast DNA Polymerase 5 u/µl). Amplified was carried out using a PCR machine 2720 Thermal Cycle Applied Biosystem which was set for initial denaturation step of 3 minutes at 94ºC followed by 40 cycles of 1 min at 94ºC (denaturation), 1 min at 36ºC (annealing) and 1 min at 72ºC (extension) and followed by one cycle of 10 minutes at 72ºC (final extension), then held at 4ºC.

PCR products were loaded on 2 % agarose gel in 0.5 × Tris Borate EDTA (TBE) buffers which added 2.5 µl Sybr safe / 100 ml agarose. Electrophoresis was carried out for 2 h at 50 Volt. The gel was examined under ultraviolet transillu-

minator and photographed using Gel Documentation System (Biodoc Analyser). The presence of a particular band was scored as 1 and absence as 0. Data were analyzed using the NTSYS program to calculate similarity values and then the similarity matrix was converted into dendrogram using unweighted pair group method with the arithmetic average (UPGMA).

RESULTS AND DISCUSSION

Phenotypic characters

The result of the observation of morphological diversity of eight varieties of carambola collection of Germplasm Garden of Research Center for Biotechnology-LIPI is shown in Table 1.

Morphological characterization is the easiest activity to be done because it is simple, inexpensive and useful to determine the relatedness between accessions (Nasution & Hadiati, 2014). Table 1 showed that all varieties observed have the same leaf and flower characters, with the exception of corolla color of Dewimurni and Rawasari varieties (reddish violet), Sembiring (whitish light violet) and the rest varieties are whitish violets. Detailed phenotypic characters of the eight carambola varieties as follows:

Dewimurni variety has a convex leaf surface; thin, opposite, round shaped leaves extending to the side; the leaf base rounded at the top of leaves and asymmetric on the edge, pointed leaf tips. Flower is round, small, arranged in a cluster of dark red color, purplish red flower, yellow stamens.

Rawasari variety has a concave leaf surface; leaves are stiff, opposite, round shaped and widened downwards, pointed leaf base on the top leaf and asymmetric on the edge. Sepal color is red when the buds, blend between pink and white when in bloom. Young fruit is light green and yellowish white when ripe.

Sembiring variety has a concave leaf surface; more rigid, alternate, round shaped leaves, pointed leaf base on the top leaf and asymmetric on the edge. The flower sepal is larger, open and separated, different when fruit was juvenile.

Malaysia variety has a concave leaf surface; rather thin, opposite, round shaped leaves, pointed leaf base on the top leaf and asymmetric on the edge. This variety has smaller white and purple flowers. Flower stalk has many branches.

Demak variety has a concave leaf surface, thin, opposite, round shaped leaves, pointed leaf base on the top leaf and asymmetric on the edge.

Penang variety has a convex leaf surface, thin, opposite, round shaped leaves, pointed

Table 1. Variation in the leaf and flower of eight carambola varieties at the Germplasm Garden of Cibinong

Variety	Leaf color		Leaflets composition	Leaf tip	Leaf shape	Flower color			
	Young	Mature				Corolla	Petal	Pistil	Stamen
Dewimurni	Yellowish red	Dark green	Opposite	Acute	Ovate	Reddish violet	Red, White at the edge	Yellowish white	Yellowish
Rawasari	Yellowish red	Dark green	Opposite	Acute	Ovate	Reddish violet	Red, White at the edge	Yellowish white	Yellowish
Sembiring	Yellowish red	Dark green	Opposite	Acute	Ovate	Whitish light violet	Red, White at the edge	Yellowish white	Yellowish
Malaysia	Yellowish red	Dark green	Opposite	Acute	Ovate	Whitish violet	Red, White at the edge	Yellowish white	Yellowish
Demak	Yellowish red	Dark green	Opposite	Acute	Ovate	Whitish violet	Red, White at the edge	Yellowish white	Yellowish
Penang	Yellowish red	Dark green	Opposite	Acute	Ovate	Whitish violet	Red, White at the edge	Yellowish white	Yellowish
Dewabaru	Yellowish red	Dark green	Opposite	Acute	Ovate	Whitish violet	Red, White at the edge	Yellowish white	Yellowish

leaf base on the top leaf and asymmetric on the edge, The flower has 5 stamens. The sepal clearly look like a tube. Sepal color is red when the buds, blend between pink and white when in bloom, is opened when the bud and closed when in.

Dewabaru variety has a concave leaf surface; more rigid, alternate, round shaped leaves. The flowers are compound, delicate flower stalk, has a maximum of 3 flowers each stalk. The flower has 5 sepals and petals which are located alternately.

Bangkok variety has a convex leaf surface; opposite, round shaped, pointed leaf base on the top leaf and asymmetric on the edge. The flower has a dense stalk, each flower has 5 petals which are attached to each other, the color of flower petals are purple with pink edges. The flower has 5 sepals, alternate with petals, long and short stamens, and tubular pistils. Morphology of leaves and flowers of eight carambola varieties is presented in Figure 1.

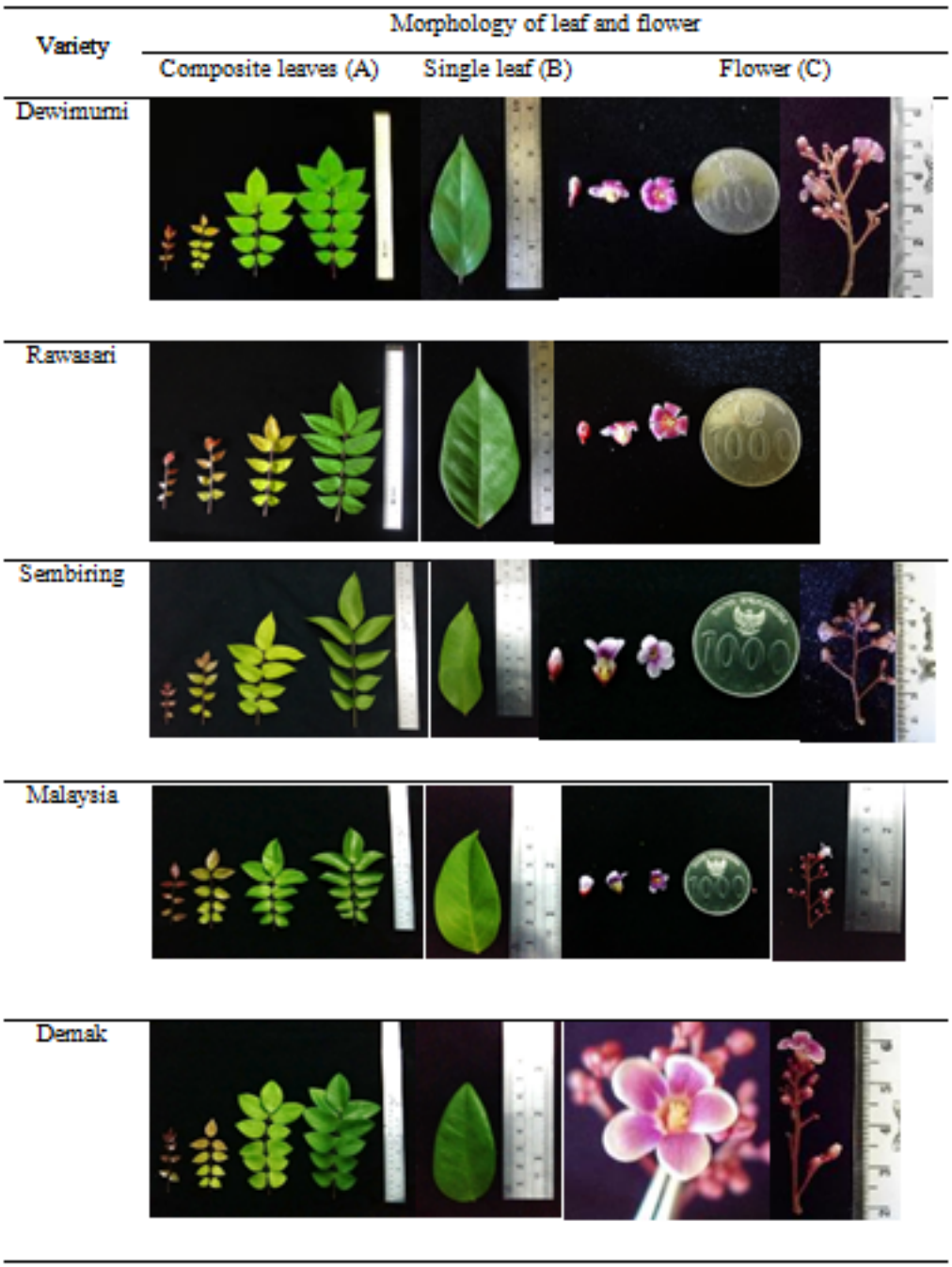


Figure 1. Morphology of leaves and flower of eight varieties of carambola at the Germplasm Garden of Cibinong A. Composite leaves, B.Single leaf, and C. Single flower,

A previous study on the relationship between nine carambola varieties conducted by Priadi and Cahyani (2011) based on phenotypic characters showed that the Malaysia and Penang variety has closely related with 80.2% similarity (Figure 2). It was suggested that both varieties come from the same region.

Genetic characters

DNA analysis was conducted to eight varieties of carambola to estimate genetic diversity and relatedness among carambola varieties. The 10 RAPD primers were used in this study showed 3 monomorphic primers and 7 polymorphic primers. The 10 RAPD primers generated a total of 59 RAPD bands (Table 3). The number of amplification products or bands per primer varied from 3 to 9, with a mean of 5.9. In the present study, 7 polymorphic RAPD markers showed 38.0 % polymorphism among the varieties (18 polymorphic bands).

The UPGMA dendrogram based on RAPD marker data clearly discriminated among varieties (Figure 4). The cluster analysis clearly distinguished the 8 carambola varieties. The dendrogram is divided into three clusters. Group 1 included 5 varieties namely Malaysia, Penang, Rawasari, Bangkok, and Sembiring. The result of group 1 showed that Malaysia and Penang have closely related with maximum similarity 96%. It is because of the same geographical origin between Malaysia and Penang. Group 2 con-

Table 2. Results of leaf and flower measurement of eight varieties of carambola at the Germplasm Garden

Variety	Leaf		Leaflet			Flower				
	Length	Wide	Length	Wide	Total	Length	Wide	No. of petal	No. of corolla	Pistil length
	(cm)	(cm)	(cm)	(cm)		(mm)	(mm)			(mm)
Dewimurni	23.6	15	7.4	4	5	6.3	3.5	5	5	2.7
Rawasari	23.3	15.3	7.8	3.3	5	6.1	3.7	5	5	2.5
Sembiring	25.8	17.2	8.8	3.4	4	4.8	2.8	5	5	3.0
Malaysia	21.7	15.9	7.7	3.8	5	4.6	3.2	5	5	2.9
Demak	20.6	13.7	6.8	3.3	5	4.6	3.7	5	5	2.7
Penang	23.3	15.6	7.8	3.2	5	5.0	3.3	5	5	2.9
Dewabaru	29.5	19.2	9.4	3.9	5	5.1	3.1	5	5	3.0
Bangkok	24.5	16.9	8.4	4.5	5	3.7	2.8	5	5	2.2
Average	24.0	16.1	8.0	3.7	4.9	5.0	3.2	5	5	2.7
Variance	7.4	2.8	0.7	0.2	0.1	0.7	0.1	0	0	0.1
Stand. Dev.	2.7	1.7	0.8	0.5	0.4	0.8	0.4	0	0	0.3

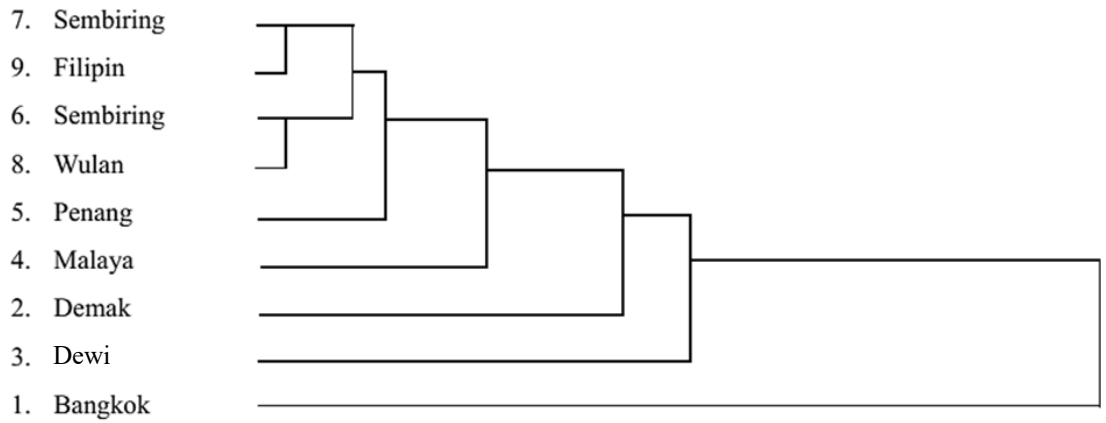
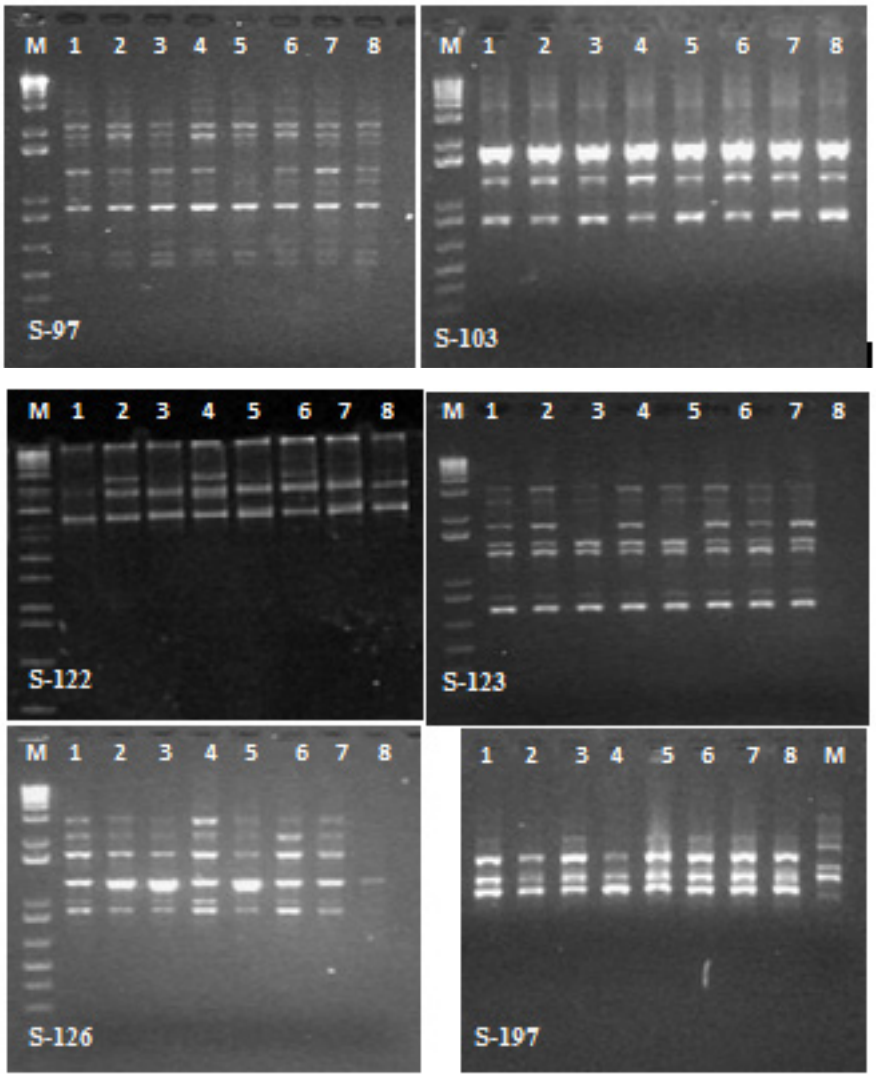


Figure 2. Dendogram for nine carambola varieties based on the morphology of leaves, flower and fruit (Priadi & Cahyani, 2011).

Table 3. Primers used for amplification and their polymorphism percentage

Primer	Sequences	Monomorphic bands	Polymorphic bands	Total no. of bands	Percentage of polymorphism (%)
S97	ACGACCGACA	6	2	8	25.0
S103	AGACGTCCAC	3	0	3	0
S122	GAGGATCCCT	3	1	4	25.0
S123	CCTGATCACC	6	1	7	14.3
S126	GGGAATTCGG	1	5	6	83.3
S197	TGGGGACCAC	3	1	4	25.0
S199	GAGTCAGCAG	4	5	9	55.6
S5	TGCGCCCTTC	4	0	4	0
S29	GGGTAACGCC	6	0	6	0
S33	CAGCACCCAC	5	3	8	37.5
Total		41	18	59	
Average				5.9	38.0



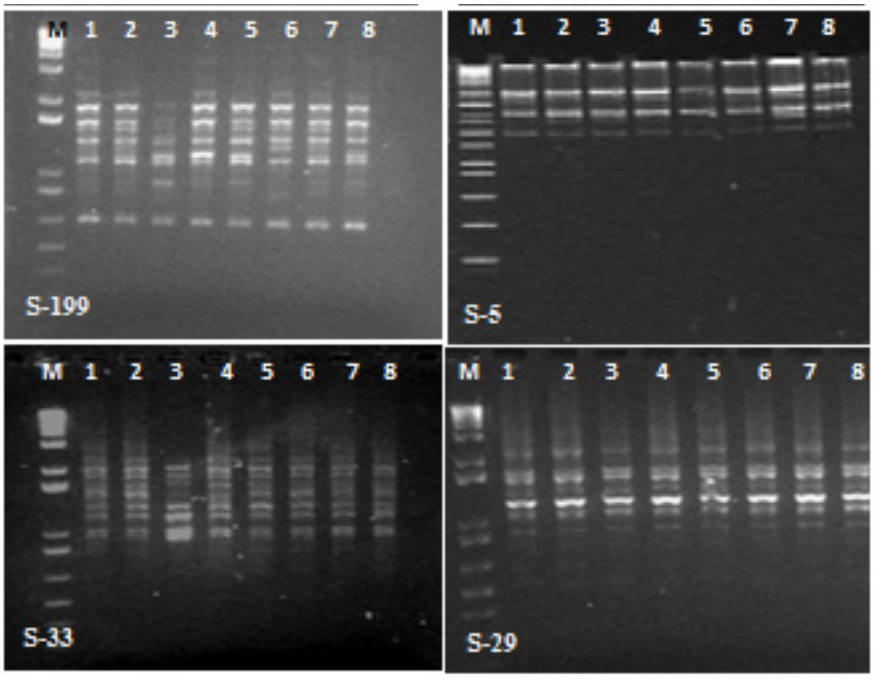


Figure 3. Amplification profile of eight varieties of carambola using 10 RAPD primers. 1. Malaysia, 2. Penang, 3.Dewabaru, 4. Bangkok, 5. Demak, 6. Sembiring, 7. Rawasari, 8. Dewimurni

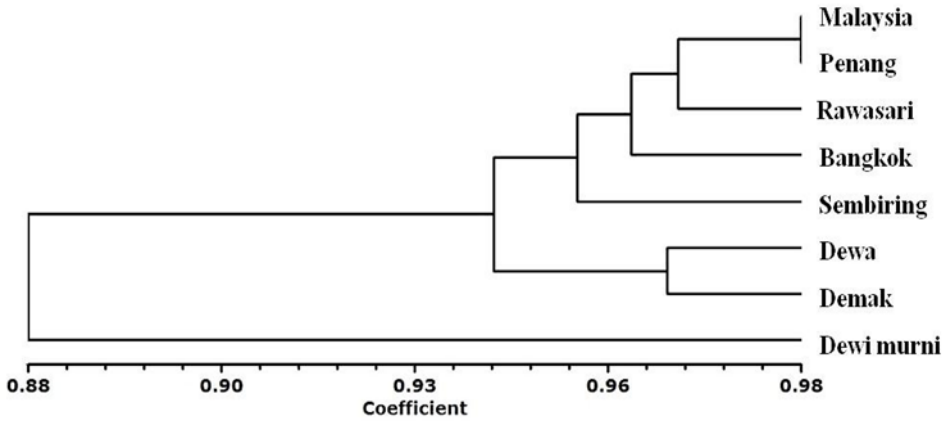


Figure 4. Dendogram for eight carambola varieties constructed for RAPD analysis using UPGMA

sisted of Dewabaru and Demak. Group 3 consisted of only one variety (Dewimurni) and is the most genetically diverse variety than others.

RAPD analysis would be very useful in breeding for verification of lines/cultivars. RAPD analysis has been successfully used for genetic diversity analysis of many plants such as maize (Carvalho *et al*., 2004), *Jatropha curcas* (Dhakshanamoorthy *et al*., 2015) and sugarcane (Kawar *et al.,* 2009).

CONCLUSION

Genetic characterization of eight carambola varieties using RAPD markers resulted in three relationship cluster. A variety of Malaysia and Penang has closely related (similarity 96%) at the same cluster with a variety of Rawasari, Bangkok, and Sembiring. In the previous study, the same result was also obtained by phenotypic characterization with the similarity of 80.2 %.

Phenotypic analysis can be used for characterization of carambola plant collection, alt-

hough it has to be confirmed by genetic analysis in order to obtain a more accurate result.

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