Pak. J. Biotechnol. Vol. 13 (2) 87- 94 (2016)
www.pjbt.org
ISSN Print: 1812-1837
ISSN Online: 2312-7791

GENETIC VARIABILITY AND RELATIONSHIP AMONG DURIAN CULTIVARS (*Durio zibethinus* Murr) IN THEKAMPAR, INDONESIA ASSESSED BY RAPD MARKERS

Rosmaina[1], Joko Warino[2], Suhaida[3], Zulfahmi[1]*

[1]Department of Agrotechnology, Faculty of Agriculture and Animal Science, Islamic State University of Sultan SyarifKasim(UIN SUSKA) Riau, Panam Campus, Pekanbaru 28293, Riau, Indonesia. [2]Alumni of Department of Agrotechnology, Faculty of Agriculture and Animal Science, Islamic State University of Sultan SyarifKasim(UIN SUSKA) Riau.[3]Department of Agriculture and Food Crops, District of KepulauanMeranti, Indonesia. E-mail*: zulfahmi@uin-suska.ac.id

Article Received 15.2.2016; Revised 25.3.2016; Accepted 7.4.2016

ABSTRACT

Durian (*Durio zibethinus* Murr) is an important tropical fruit tree economically and ecologically. This research aims to observe the genetic variability and relationship among durian cultivars based on RAPD(Random Amplified Polymorphic DNA) markers. The results of this study obtained a total of 49DNA fragments of which 34 fragments (69.39%) were polymorphic. Overall size of the PCR amplified product bands ranged from 200 bp (OPO-11) to 1800 bp (OPO-05). Six primers (OPT-09, OPO-05 OPY-16, OPY-15, OPD-08, and OPY-14) can distinguish among five durian cultivars e.g. Bakul, Ome Kampar, Tembaga, Sijantung and Keong Mas. The genetic distance among durian cultivars ranged from 0.276-0.477. Based on UPGMA dendrogram, seven durian cultivars were divided into four groups. The first cluster consisted of Sijantung cultivar, second cluster consisted of Bakul cultivar, third cluster consisted of Keong Mas and Tembaga cultivars, and fourth cluster consisted of Empu Kunyit, Ome Kampar, and Montong cultivars. The results of this study will helpthe breeders toformulate the breeding strategy for the durian improvementin future.

Key words*: Genetic variability, Durio zibethinus* Murr*, Kampar, RAPD markers.*

INTRODUCTION

Durian (*Durio zibethinus* Murr) is a fruit tree species belonging to Bombacaceace family (Brown, 1997, Yumoto, 2000, Bumrungsri *et al*., 2009). It is one of popular seasonal fruit in South east Asia, especially in Thailand, Indonesia, Malaysia and Philippines. Durian is widely distributed and cultivated in Indonesia. Nowadays, Indonesia has more than 103 of durian cultivars and each cultivar is different morphologically in its characteristics such as its leaf, seed shape, fruit shape, aroma and flavor of fruit (Nafsi, 2007) but information concerning the genetic variation and relationship among cultivated durian varieties in Indonesia are limited. The durian is an open pollinated species, so that naturally there is always cross pollination among cultivars (Lim andLuders, 1998). In addition, they are pollinated by some pollinators, for example, spider hunter, bird, honey bees and stingless bees (Yumoto, 2000). Durian is an important tropical fruit crop economically. Not only durian fruits but also durian timber has high economic value. The durian fruits have antioxidant properties (Arancibia-Avila *et al*., 2008) and contained numerious nutrients such as carbohydrate, protein, fat and other nutrients (Brown, 1997), whereas the hard wood of this species is suitable for heavy construction, flooring, to make poles, furniture, window panels and doors.

Kampar district is one of the regions in Riau province-Indonesia that have many durian cultivars but seven cultivars of them, namely Tembaga, Ome Kampar, Bakul, Empu Kunyit, Sijantung, Keong Mas and Montong cultivars produce the high quality fruits compared to others. Three cultivars (Tembaga, Sijantung and Ome Kampar) have been released by Ministry of Agriculture of Indonesia as superior national variety. They have been cultivated many years and are propagated by grafting on to seedlings of randomly selected rootstocks. During past decade, the naming of durian cultivars in Kampar referred to some morphological traits, particularly in the shape fruit, color fruit and other morphological characters. The using of these characters are often arising confusion and doubted in determine of durian cultivars because performances or phenotypes of plants are often in consistent since they are influenced by environment factors and plants age (Hariyati *et al.,* 2013). Another problem is that most of durian cultivars are certified based on morphological traits such as fruit characteristics that are not

continuously present. Thus, improvement in the method of cultivar identification must become a priority for durian breeders in order to verify identity and assist in confirming proprietary rights. Besides, the morphological traits have limitation to differentiate genetically similar individuals since they do not cover the entire genome, and require to extensive observation in the mature plants (Moreno and Trujillo, 2005; Vanijajiva, 2011, Hariyati *et al.,* 2013).

Knowledge of the genetic diversity and genetic relationship among durian cultivars are an important to beutilized in the breeding programmme. These in formations are used by breeder to plan hybridization and development of superior cultivars more quickly and commercially in future. Moreover, avaibility of genetic diversity is interesting for crop breeder since a representative reserve of alleles and genotype may become useful for responding to future changes (Moreno and Trujillo, 2005). Until now, information on genetic variation and genetic relationship between durian cultivated in Kamparusing molecular techniques are not available. Therefore, this study is necessary to be implemented as a basic consideration to formulate the breeding strategies of durian in Kampar district in future.The molecular approach for identification and to understand the relationship between plants seem to be more effective than morphological markers because it allows direct access to the hereditary material, independent from season, no limit of plant sage and no effect of environment (Williams *et al*., 1990, Paterson *et al*., 1991).

RAPD markers have been known as one of the most commonly used techniques for estimating the genetic diversity of plants, cultivar identification, hybrid purity, genome maping, and genetic structure of population (Spooner *et al.,* 2005, Weising *et al.,* 2005,Muchugi *et al.,* 2008, Kumar and Gurusubramanian, 2011) due to their advantages compared to other DNA molecular techniques such as its simplicity, fast, cost effective, short primers of arbitrary sequences, require small amounts of target DNA as well as no need of prior sequence information on the target species and radioactivity free procedure (Welsh and McClelland, 1990, Williams *et al.,* 1990, Spooner *et al.,*2005,Weising *et al.,* 2005, Muchugi *et al.,* 2008). On the other hand, RAPD has some limitations, such as dominant mode of inheritance and poor reproducibility. However this weakness can be resolved by optimizing the reaction conditions (Weising *et al.,* 2005,Muchugi *et al.,* 2008). The RAPD markers have been applied to genetic analysis and cultivar identification in the several fruit trees such as *Durio zibethinus* (Ruwaida *et al.*, 2009, Vanijajiva, 2011, Hariyati *et al.,* 2013), duku (Hanum *et al.,* 2011), Citrus (Baig *et al*., 2009, Leng *et al*., 2012), Pear (Lisek and Rozpara, 2010) and Mango steen (Mansyah *et al.*, 2007). The objective of this study was to assess the genetic variability and relationship of durian cultivars in Kampar District using RAPD markers.

MATERIALS AND METHODS

Samples Collection and DNA Extraction: Young leafs of seven durian cultivars (Empu Kunyit, Keong Mas, Ome Kampar, Montong, Tembaga, Bakul and Sijantung) were collected from nursery in Tambang subdistrict, Kampar-Indonesia. The number of sampled for each cultivar collected was one individual. Leafs sampled were entered into plastic bags that containing silica gel with rasio leafs and silica gel was 1:5 (w/w) for reducing of leaf moisture content and for preventing of samples from fungi attack, and then samples were carried to laboratory and were stored in freezer at temperature –20^{o}C until DNA extraction was carried out. Total genomic DNA was isolated using the Cetyl-Trimethyl Ammonium Bromide (CTAB) method as described by Doyle and Doyle (1990). Good quality DNA samples from isolation were diluted and stored in freezer at -20^{o}C until RAPD analysis was performed.

Primer Screening, Amplification and DNA Electrophoresis: Twenty random primers were initially screened to determine the suitability of each primer for this study. Out of twenty primers, twelve primers showed success DNA amplifycation, and then ten primers were selected since they yielded strong, intense and unambiguous bands. These primers were used for further analysis. DNA amplification was carried out in CFX 96TM Real Time DNA engine Thermal cycler (BioRad). The amplification was program med as follows: initial denaturation for 5 minutes at 95^{o}C, followed by 39 cycles of denaturation for 1 minute at 95^{o}C, annealing for 1 minute at 37^{o}C, extension for 1 minute at 72^{o}C, and final extension for 10 minutes at 72^{o}C. Each PCR reaction comprising 13 µl, contained 2.0 µl DNA templates(5-10 ng), 1.5µlprimers (5 pmol/ µl), 2.0µl water free RNase, and 7.5 µl Hot Star Taq Master Mix (Qiagen). PCR products were separated in a 1.0% (w/v) agarose gel in 1X TAE buffer at 100 volts for about 30-45 minutes, and then gels were stained in 1.0% (v/v) ethidium bromide solution. The banding patterns of gel

were observed under UV light apparatus and photographed using Gel Doc system (BioRad). A 100 bp DNA ladder (Amresco) was included in the gels as a size reference or molecular weight standard. Analysis of banding patterns was performed using an Image Lab version 2.0.1 (BioRad) Software.

Data Analysis: Amplification products were scored as '1' for presence and '0' for absence of each band in individual lanes. The scores were entered as a binary matrix for analysis. The genetic diversity was estimated in two ways: (i) percentage of polymorphic loci (PPL), that was calculated by dividing the number of polymerphic bands at the cultivar by the total number of bands surveyed, (ii)genetic distance (do) and similarity among individuals (S), they were calculated using the formula Nei and Li (1978). All parameters were calculated using the software of POPGEN version 1.31 (Yeh *et al*., 1999). UPG MA (Un-weighted Pair-Group Method Arithmatic) dendogram analysis based on Nei and Li genetic distance (1978) was cons-tructed using NTSYS version 2.00 (Rohlf 1998) soft wares.

RESULTS AND DISCUSSION

Genetic variability: The results of RAPD amplification with ten primers selected are displayed in Table 1. Total fragment scorable was 49 and maximum numbers of bands were observed in OPY-15 primer (seven fragments) and minimum numbers of fragments are generated by OPT-7 primer (two fragments). Each primer produced the number of fragment are different because it has nucleotide sequence different or interaction between primer and DNA template and cultivar analyzed. The number of fragment polymorphic was 34 fragments (69%). The low polymorphism was found in OPT-07 primer and high polymerphism was observed in OPO-05, OPT-09 and OPY-16 primers, respectively (Table-1). Polymorphism is a form of genetic variation in the DNA chain. According to Weising *et al*., (2005) Polymorphism is generated by several types of events, namely (1) insertion of a large piece of DNA between the primer binding sites may exceed the capacity of PCR, resulting no fragment is detected; (2) insertion or deletion of a small fragment of DNA will lead to a change in size of the amplified fragment; (3) the deletion of one of the two primer annealing sites results in either the loss of a fragment or an increase in size; and (4) a nucleotide substitution within one or both primer target sites may affect the annealing process, which can lead to a presence versus absence polymorphismor to a change in fragment size.

Table-1: List of RAPD primers selected, their sequence, size range of fragments, total number of bands amplified, the number of polymorphic bands among seven of durian cultivars

Primer name	Sequence of the primer	Size ranges (bp)	Total number of bands amplified	Number of polymorphic bands	Proportion of polymorphic bands (%)
OPO-05	5’CCCAGTCACT’3	400 - 1000	6	6	100%
OPO-06	5’CCACGGGAAG’3	400-1800	5	3	60%
OPO-11	5’GACAGGAGGT’3	200-1250	6	2	33%
OPT-07	5’GGCAGGCTGT’3	450-880	2	0	0%
OPT-09	5’CACCCCTGAG’3	400-950	3	3	100%
OPD-08	5’GTGCCCCATG’3	230-750	6	4	60%
OPY-14	5’GGTCGATCTG’3	320-1270	5	3	60%
OPY-15	5'AGTCGCCCTT'3	350-1300	7	5	71%
OPY-16	5'GGGCCAATGT'3	300-950	5	5	100%
OPY-20	5'AGCCGTGGAA'3	280-600	4	3	75%
Total	-	-	49	34	69.39%

The banding patterns of different durian cultivars using six primers are shown in Figure 1 to 3. The fragment sizes generated were ranged from 200 to 1800 bp. Strong and weak bands were produced in the RAPD reactions. Weak bands result from low homology between the primer and the pairing site on the DNA strand (Thormann *et al*., 1994). The weak bands were, therefore, disregarded to increase analysis precision and reproducibility of the banding pattern in this study was confirmed by two replicated reactions indenpently with the same primer and performed at different times. Genetic variation is an important for plants to adapt and evolve through environmental changes so that they can thrive and be stable for a long time. In addition, high genetic variability can be further exploited by breeder for crop improvement. Genetic variability of durian in this study was high categorized (69%),and it was higher than other long lived perennial plant species (48.1%) that is reported by Hamrick and Godt (1996).

Ruwaida *et al*., (2009) selected six primers after screening; those generated a total of 90 scoreable bands of which 74 fragments (81.88%) were polymorphic. Hariyati *et al*., (2013) selected five primers after screening; those generated a total of 79 scoreable bands of which 59 fragments (81.88%) were polymorphic while Vanijajiva (2011) found only 37.77% of polymorphic fragments in 14 of durian cultivars in Thailands. A diverse level of polymorphism between previous studies and present study could be explained by differences in the primers used, criteria for selecting markers and the number and origin of the cultivar tested.

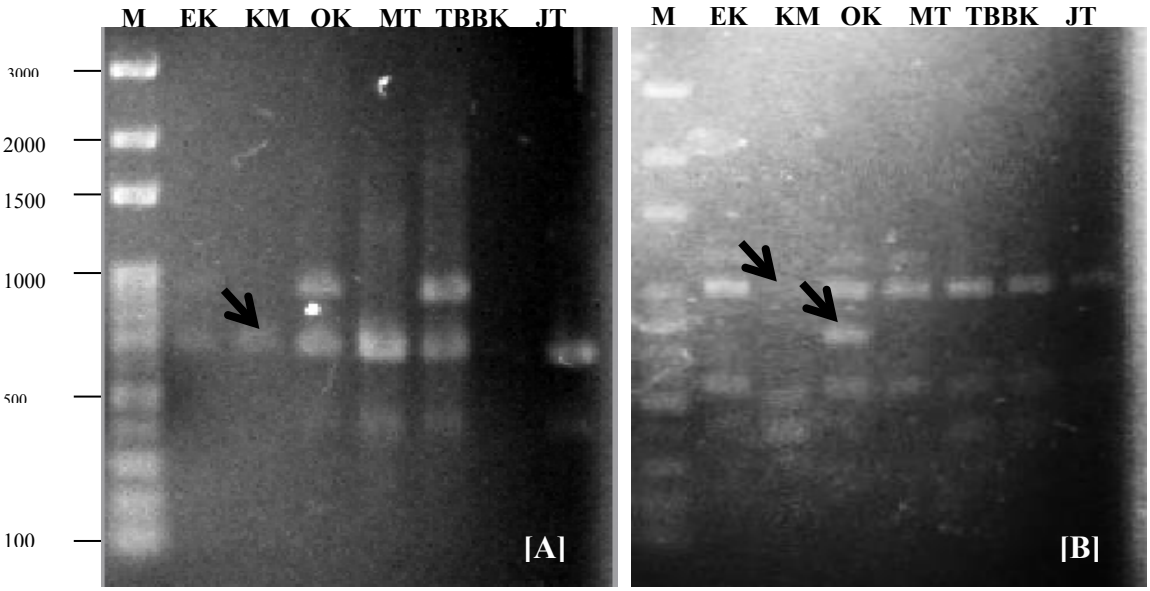

Figure- 1: RAPD amplification Products of OPT-09 [A]; OPO-05 [B] primers; EK [EmpuKunyit]; KM [Keong Mas];OK [Ome Kampar]; JT [Sijantung]; MT [Montong]; TB [Tembaga]; BK [Bakul] cultivars.

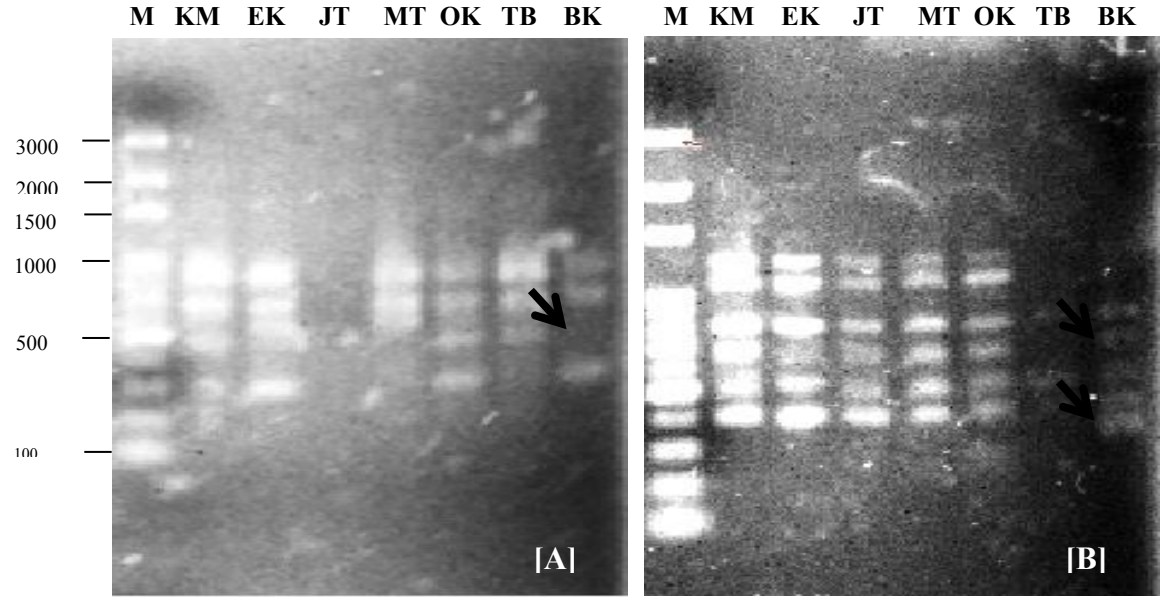

Figure -2: RAPD amplification Products of OPY-16 [A]; and OPY-15 [B] primers. KM [Keong Mas]; EK [EmpuKunyit]; JT [Sijantung]; MT [Montong]; OK [Ome Kampar]; TB [Tembaga]; BK [Bakul] cultivars.

Identification of durian cultivars: Out of ten primers selected for analysis, six primers can distinguish the five cultivars (Bakul, Ome Kampar, Tembaga, Sijantung and Keong Mas) from seven cultivars analyzed in this study. The Bakul cultivar can be identified with primer OPT-09 which no band generated when is amplified. Using Primer OPT-09, all cultivars except Bakul showed a common band of 700 bp, and this band acts as markers for KeongMas

cultivar because other bands shared by other cultivars are absent in Keong Mas cultivar (Figure 1A). The Ome Kampar cultivar can be identified specifically with a band size of 750 bpin primer OPO-05 (Figure 1B) and Keong Mas cultivar can be also identified by this primer with absent of band size of 1000 bp. The Sijantung cultivar was not yielded band when is amplified with primer OPY-16, and this acts as markers for Sijantung cultivar. Bakul cultivar can be identified by primer OPY-16 with absent of band size of 500 bp (Figure 2A).PrimerOPY-15 can distinguish between Tembaga and Bakul cultivars, Tembaga cultivar showed no band size of 700 bp while Bakul cultivar produced specifically a band size of 350 bp (Figure 2B). Bakul cultivar can be also identified specifically with a band size of 600 bp and 700 bp in OPD-08 primer (Figure 3A) and OPY-14 primer (Figure 3B), respectively. The primer OPD-08 is also useful in identifying Tembaga cultivar with absent band size of 500 bp (Figure 3A). After analyzing the data generated by six primers, it was concluded that some primers gave unique bands in a specific durian cultivar as shown in Table 2. Information on the band specific can be utilized by breeder and farmer. Breeder used this information as a basic to select the parental for improvement of durian, protection of cultivars or clones and detection of seedling purity. While for farmer, this information could be used to verify the originality of seedling that will be cultivated. Durian is long live species, therefore, seedlings that cultivated do not mistake because it will disadvantage economically and times.

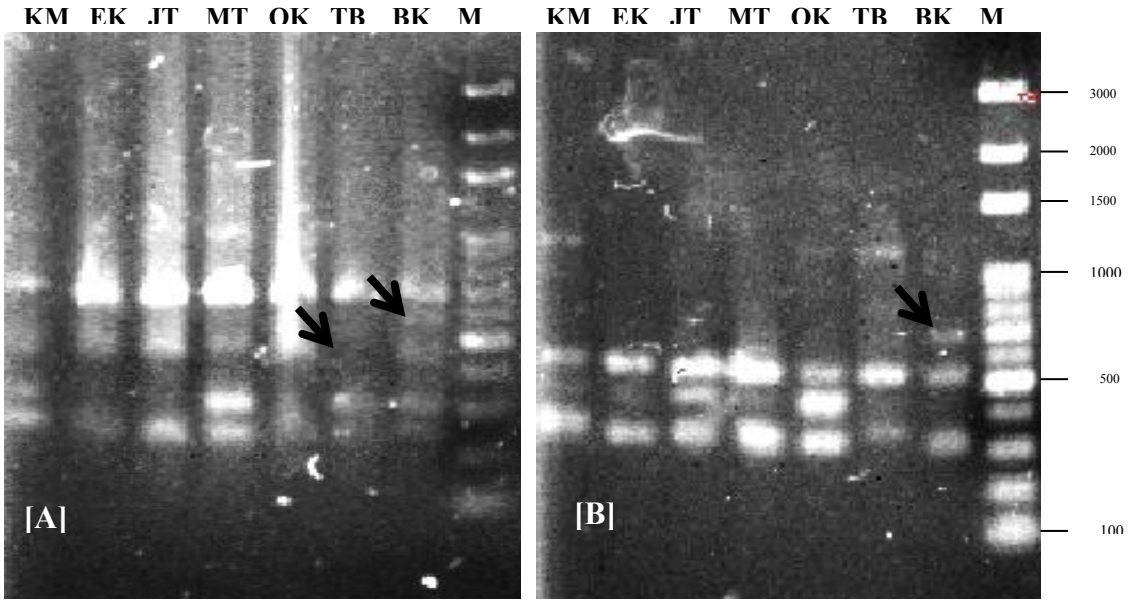

Figure -3: RAPD amplification Products of OPD-08 [A] and OPY-14 [B] primers. KM [KeongMas]; EK [EmpuKunyit]; JT [Sijantung]; MT [Montong]; OK [Ome Kampar]; TB [Tembaga]; BK [Bakul] cultivars.

Table -2: Unique markers for identifying of durian cultivar and criteria used

Cultivars	Unique markers	Identification Criteria
Tembaga	OPD-08 (500)	Absence
Tembaga	OPY-15 (700)	Absence
Bakul	OPT-09	Not amplified
Bakul	OPD-08 (600)	Present
Bakul	OPY-14 (700)	Present
Bakul	OPY-15 (350)	Present
Bakul	OPY-16 (500)	Absence
Sijantung	OPY-16	Not Amplified
Ome Kampar	OPO-05 (750)	Present
Keong Mas	OPO-05 (1000)	Absence
Keong Mas	OPT-09 (900)	Present

Genetic Relationship among Durian Cultivars: Genetic distance indicated the genetic relationship among durian cultivars where the genetic distance values ranged from 0-1 (Weising *et al*., 2005). The genetic distance values among durian

cultivars ranged from 0.1306 to 0.4906 (Table-3). The minimum of genetic distance value was observed between Empu Kunyit and Ome Kampar cultivars (0.1306) while the maximum of genetic distance value was observed between Tembaga and Sijantung cultivars (0.4906). The different genetic distance values among cultivars reflected from wide variation among durian cultivars. High genetic variability between varieties indicated that this species was rich genetic material. This study indicated that Tembaga and Sijantung cultivars showed the highest genetic variation while the lowest genetic variation was observed between Empu Kunyit and Ome Kampar cultivars.

Table-3: Nei's Genetic identity (above diagonal) and genetic distance (below diagonal) values between seven durian cultivars

Cultivars	EmpuKunyit	Keong Mas	Ome Kampar	Montong	Tembaga	Bakul	Sijantung
Empu Kunyit	****	0.7551	0.8776	0.8367	0.7143	0.6939	0.6939
Keong Mas	0.2809	****	0.7143	0.7551	0.7551	0.6531	0.6939
Ome Kampar	0.1306	0.3365	****	0.7959	0.6735	0.6939	0.6939
Montong	0.1782	0.2809	0.2283	****	0.6735	0.6939	0.7347
Tembaga	0.3365	0.2809	0.3953	0.3953	****	0.6939	0.6122
Bakul	0.3655	0.4261	0.3655	0.3655	0.3655	****	0.6735
Sijantung	0.3655	0.3655	0.3655	0.3083	0.4906	0.3953	****

UPGMA dendogram based on Nei's genetic distance (1972) displayed that seven durian cultivars were divided into four groups (Figure 4). The first cluster consisted of durian Sijantung, second cluster consisted of durian Bakul, third cluster consisted of Keong Mas and Tembaga, and fourth cluster consisted of durian Empu Kunyit, Ome Kampar and Montong. Information on genetic relationship among durian cultivars can be used as a basic consideration to formulate the breeding program of durian in future, particularly in selected parental for out crossing. Julisaniah (2008) explained that close genetic relationship of parental will increase the successful of pollination while the distant genetic relationship among parental tend to fail the fertilization.

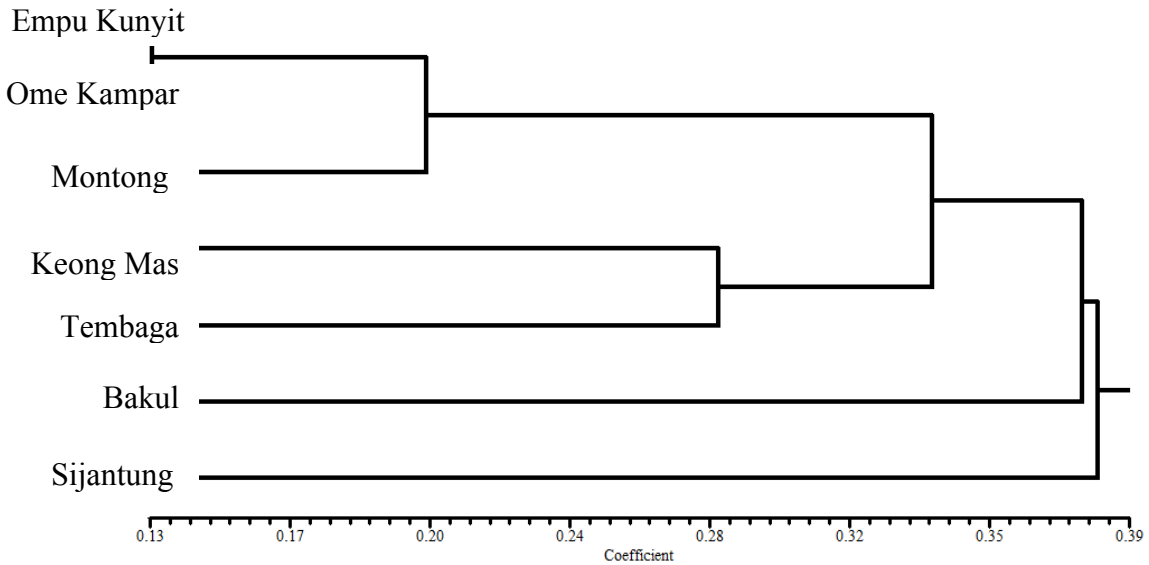

Figure-4: UPGMA Dendogram of seven cultivars of durian based on genetic distance (Nei, 1978)

The polymorphism detected among durian cultivars can be used in breeding works to maximize the use of genetic resources for improvement of durian cultivar. From this study, it was revealed that the highest genetic identity (0.8776) exists between Empu Kunyit and Ome Kampar cultivars. On the other hand, the lowest genetic identity (0.6122) was observed between Tembaga and Sijantung cultivars (Table 3). These finding indicated that Empu Kunyitvs and Ome Kampar cultivars and Tembagavs Sijantung cultivars could be used in the crop breeding program for development of new durian variety in future. Dendogram was also showed that the Tembaga

and Sijantung cultivars were most distantly related to each other and hence, we recommended that these two cultivars should be used in hybridization program to create maxi-mum genetic diversity for improvement of durian in Kampar.

In conclusion, RAPD markers provide a fast and efficient tool for genetic diversity assessment among the durian cultivars. This was a prelim-nary study, and a more detailed molecular study such as SSR and AFLP markers could help to discriminate other cultivars that have been cultivated in Kampar, Indonesia.

ACKNOWLEDGEMENTS: Authors thank to the Directorate of Islamic Higher Education of Ministry of Religious Affair of Indonesia for supporting financial to this study.

REFERENCES

Arancibia-Avila, P., F.Toledo, Y.S.Park, S.T. Jung, S.G. Kang, B.G. Heo, S.H. Lee, M. Sajewicz, T. Kowalska and S. Gorinstein, Antioxidant properties of durian fruit as influenced by ripening. LWT - Food SciTechnol. 41: 2118-2125 (2008). doi:10. 1016/j.lwt.2007.12.001

Baig, M.N.R., S. Grewal, S. Dhillon, Molecular characterization and genetic diversity analy-sis of citrus cultivars by RAPD markers. Turk J. Agric. For. 33: 375-384 (2009) doi:10.3906 /tar-0804-27

Brown, M.J., *Durio* - A Bibliographic Review in R.K. Arora, V. R. Rao and A.N. Rao, Eds. IPGRI office for South Asia, New Delhi. (1997)

Bumrungsri, S., E.Sripaoraya, T. Chongsiri, K. Sridith and P.A. Racey, The pollination ecology of durian (*Duriozibethinus*, Bomba-caceae) in Southern Thailand. J. Trop. Ecol. 25: 85-92 (2009)

Doyle, J.J. and J.L. Doyle, Isolasi of Plant DNA From Fresh Tissue. *Focus* 12: 13-15 (1990).

Hamrick, J.L. and M.J.W.Godt. Effects of life history trait on genetic diversityin plant species. Phil. Trans. R. Soc. Lond. B. 351: 1291-1298 (1996).

Hariyati, T., J.Kusnadi and E.L.Arumingtyas, Genetic diversity of hybrid durian resulted from cross breeding between *Duriokutejensis* and *Duriozibethinus* based on random amplified polymorphic DNAs (RAPDs). Am. J. Mol. Biol. 3: 153-157 (2013).

Hanum,L., R.S.Kasiamdari, Santosa and Rugayah, Genetic Relatedness among Duku, Kokosan, and Pisitan in Indonesia Based on Random Amplified Polymorphic DNA Markers. I. J. Biotech. 17(2): 121-131 (2012).

Kumar, N.S. and G. Gurusubramanian, Random amplified polymorphic DNA (RAPD) markers and its applications. Sci. Vis. 11(3): 116-124 (2011).

Mansyah, E., A.Baihaki, R.Setiamihardja, J.S. Darsa and Sobir, Analysis of genetic variability of mangosteen (*Garcinia mango stana* L.) in Java and West Sumatra using RAPD technique. Zuriat. 14(1): 35-44 (2003)

Muchugi, A., C.Kadu, R.Kindt, H.Kipruto, S. Lemurt, K.Olale, P.Nyadoi, I.Dawson and R.Jamnadass, *Molecular Markers for Tro-pical Trees, a Practical Guide to Principles and Procedures*. ICRAF Technical Manual No. 9.I. Dawson and R. Jamnadass (eds.) Nairobi: World Agroforestry Centre (2008)

Moreno, I. and I. Trujillo, Genetic characteri-zation and relatedness among cherry cultivars in a germ plasm bank by random amplified polymorphic DNA analysis. Agric. Conspec. Sci. 70(4): 105-111 (2005).

Leng, X.P., H.R.Li, G.Y.Zhong, C.N. Song, X. Sun and J.G. Fang, Employment of a new strategy for identification of loose-skin mandarin (Citrus reticulata Blanco) cultivars using RAPD markers. Rom Biotech Lett. 17(2): 7073-7083(2012).

Lisek, A. and E.Rozpara, Identification of Pear cultivars with RAPD and ISSR markers. J. Fruit Ornam. Plant Res. 18(2): 17-22 (2010).

Lim,T.K. and L.Luders, Durian flowering, polli-nation and incompatibility studies. Ann. Appl. Biol. 132: 151-165 (1998).

Nei, M. and W.H. Li, Mathematical modes for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA.76: 5269–5273(1978)

Nafsi, N.I., Diversity analysis of durian (*Duriozi-bethinus* Murr.) varieties using microsatellite markers [Thesis]. School of Bioscience and Biotechnology, Bandung Institute of Technology, Bandung, Indonesia (2007).

Paterson, A.H., S.D.Tanksley and M.E.Sorreis, DNA markers in plant improvement. Adv. Agron. 46: 39–90 (1991).

Rohlf, F.J., NTSYS-pc version 2.0. Numerical Taxonomi and Multivariate Analisis System. *Exeter Software, Setauket*. New York(1998).

Ruwaida,I.P., Supriyadi and Parjanto,Variability analysis of Sukun durian plant (*Duriozi-bethinus*) based on RAPD marker. Nusantara Bioscience 1: 84-91(2009)

Spooner, D., R. Van-Treuren and M.C. De-Vicente, Molecular Markers for Gene Bank Management. IPGRI Technical Bulletin No. 10. International Plant Genetic Resources Institute, Rome, Italy (2005).

Thormann, C.E., M.E. Ferreira, L.E.A. Camargo, J.G. Tivang and T.C. Osborn, Comparison of RFLP and RAPD markers for estimating genetic relationships with in and among Cruciferous species. Theor. Appl. Genet. 88: 973-980 (1994).

Vanijajiva, O., Genetic variability among Durian (*Duriozibethinus* Murr.) cultivars in the Nonthaburi province, Thailand detected by RAPD analysis. Journal of Agricultural Technology 7(4):1107-1116 (2011).

Weising, K., H. Nybom, K. Wolff and G. Kahl, DNA Fingerprinting in Plants: Principles, Methods, and Applications. Second Edition. Taylor and Francis Group, Boca Raton (2005).

Welsh, J. and Mc-Clelland, Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res. 18: 7213-7218(1990).

Williams, J.G.K., A.R.Kubelik, K.J.Livak, J.A. Rafalski and S.V.Tingey, DNA polymorphisms ampli fied by arbitrary primer are useful as genetic markers. Nucleic Acids Res. 18(22): 6531-6535 (1990).

Yeh, F.C., R. Yang and T. Boyle, Popgen Version 1.31. Microsoft window based for population genetic analysis. Department renewable resources, University of Alberta, Edmonton, Alberta, Canada (1999).

Yumoto, T., Bird Pollination of three durio species (Bombacaceae) in a tropical rain forest in Serawak, Malaysia. Am. J. Bot. 87(8): 1181–1188 (2000).