ACCEPTED MANUSCRIPT

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) PROFILES OF SEVERAL ISOLATES OF *Ganoderma* spp. FROM BANYUMAS, CENTRAL JAVA, INDONESIA

Ratnaningtyas NI, Susanto AH, Yulia A

DOI: 10.11598/btb.2019.26.2.884

To appear in : BIOTROPIA Vol. 26 No. 2 August 2019 Issue

Received date : 07 August 2017
Accepted date : 06 November 2017

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) PROFILES OF SEVERAL ISOLATES OF *Ganoderma* spp. FROM BANYUMAS, CENTRAL JAVA, INDONESIA

Nuniek Ina Ratnaningtyas*, Agus Hery Susanto and Ana Yulia
Faculty of Biology, Jenderal Soedirman University, Purwokerto 53122, Indonesia
*Corresponding author's e-mail: nuniek.ratnaningtyas@unsoed.ac.id

Running title: RAPD profiles of *Ganoderma* spp.

ABSTRACT

A study on the RAPD profiles of several isolates of *Ganoderma* spp. from Banyumas Regency, Central Java, Indonesia and their genetic inter-relationship based on RAPD markers was conducted using a survey method, where random sampling technique was applied. Collected *Ganoderma* spp. fruiting bodies were first morphologically characterized, after which RAPD analysis utilizing four random primers, i.e. OPC-1, OPC-2, OPC-4 and OPC-5 was carried out. The results revealed that the four primers used generated polymorphic bands toward 10 samples with polymorphism level of 100%, showing absolutely high genetic diversity. The level of genetic similarity ranged between 0.48 and 0.82, indicating moderate similarities among samples. A constructed dendogram indicated that the isolates of *Ganoderma* spp. were grouped into three clusters at a coefficient of 0.63, but neither according to geographical locations nor growth substrates.

Keywords: Banyumas, *Ganoderma* spp. isolate, RAPD

INTRODUCTION

Ganoderma spp. are soil-borne fungi that can be both favourable and unfavourable to humans. These fungi have long been used as an ingredient for several herbal medicines for human health (Boh B. 2013), but on the other hands they can act as parasites, causing root and stem rot in many annual tropical plant species (Mercière *et al.* 2015). The genus *Ganoderma* has a world-wide distribution, growing on numerous perennial, coniferous and palmaceous hosts (Flood *et al*. 2000). The very wide distribution of *Ganoderma* spp. are including in Banyumas Regency, Central Java, Indonesia (Ratnaningtyas & Samiyarsih 2012), so that their diversity in the region should be sufficiently high. It is well known that information on species abundance and diversity are critical for the sustainable use of natural communities in a given ecosystem (Nuryanto & Susanto 2010). Moreover, a better understanding on the relationships among genera and species is needed for genetic improvement (Mathius *et al.* 2009). In the other hand, *Ganoderma* is also a notoriously variable and difficult fungus to characterize and this has led to much past confusion in disease aetiology and epidemiology (Flood *et al.* 2000).

According to Flood *et al.* (2000), the number of known *Ganoderma* species can be estimated at about 60-80 laccate and 10-30 non-laccate species, and it is likely that new taxa are yet to be

discovered in poorly studied tropical region. The use of traditional taxonomic method has been inconclusive for establishing a stable classification of the group, and these methods are useless for characterzation of intragroup of *Ganoderma spp.* grouping. However, an accurate identification system and a phylogenetically based classification of *Ganoderma* taxa together with the development of genetic markers for intragroup of its grouping would have practical implications in epidemiology studies, the wood industry, and pharmacology. For instance, it would have help in monitoring fungal propagation within and between fields and in bioprospecting for new genes and new metabolites and would provide useful information for genetic engineering or breeding of commercial strains (Hseu *et al*. 1996).

A previous study of *Ganoderma* exploration during rainy season for about two months with purposive sampling found 43 fruit bodies were found in the area, ranging from lowlands to moderate altitudes, which generated absolutely high diversity in macromorphology and micromorphology characterization. However, characterizations based on macromorphological and micromorphological characters were insufficient to identifiy the fungi to species level, grouping, and fungicidal kinship of *Ganoderma* spp. (Ratnaningtyas & Samiyarsih 2012). Based on this fact, a molecular analysis is needed to be done, among others, using RAPD as a marker. The RAPD procedure is relatively inexpensive, requires only a small quantity of DNA samples (0.5 – 50 ng), does not involve radioisotope usage, and fast and easy to perform. However, this technique has some limitations, i.e uncertain reproducibility, possibility of producing same-sized fragments of different sequences, and revealing only dominant markers (Demeke & Adams 1994). This study aims to determine the RAPD profiles of several isolates of *Ganoderma* spp. found in the Banyumas Regency, Central Java, Indonesia and establish the genetic relationship among these isolates.

MATERIALS AND METHODS

Sampling and characterization of *Ganoderma* spp.

Sampling and characterization of *Ganoderma* spp. were done following Ratnaningtyas & Samiyarsih (2012) and Steyaert (1972). A total of 10 *Ganoderma* samples were randomly collected from four different locations around Banyumas Regency, Central Java, Indonesia. Young and fresh fruiting bodies were taken from the substrates, plastic-wrapped, and subsequently stored in a plastic box at room temperature. Before collected from their natural habitat, the fruiting bodies were photographed and the coordinates of the locations and the substrate were recorded. The macromorphological characterizations of the fruiting bodies were carried out based on the growth substrate (dead rot or live tree), color, edge, edge color, thickness, and the diameter of the length and width of the dorsal, and ventral surface of pileus, colour of edge of pileus, the body of the fruit (tube length, cutis length, number of pores/mm^2, and concentric zone), and stalks (color, length,

thickness) – all of which were observed directly from fresh samples. Micromorphological characterizations were performed on *Ganoderma*. isolates colony appearance.

Isolation of *Ganoderma* spp. isolates

Isolation of *Ganoderma* isolates was done following Ratnaningtyas & Samiyarsih (2012). The surface of the fruiting bodies were washed with clean water and dried with tissue papers. Subsequently, the fruiting bodies were cut into 0.5 cm × 0.5 cm and then re-washed with sterile distilled water for approximately 15 – 30 seconds, and dried using sterile filter papers placed on petri dishes. The tissues of the fruiting body were then inoculated using a Potato Dextrose Agar (PDA) medium and incubated at room temperature (25°C). After mycelia growth was observed from the tissues, the young hyphae was taken and inoculated on a PDA medium. After considerable growth was observed, the pure culture was obtained by transferring it to a new PDA medium for rejuvenation, and subsequently to a slant PDA medium for collection.

Extraction of genomic DNA

Extraction of *Ganoderma* spp. genomic DNA was done based on the methods described by Orozco-Castillo *et al.* (1994) with modifications. The modification included frozen storage temperature of samples in lower temperature (-80°C instead of -60°C), sample weight (0.3 g mycelia instead of 0.1 g mycelia), precipitation solution (absolute ethanol instead of isopropanol), duration of precipitation process (12 hours instead of 30 minutes).

Measurement of DNA quantity and purity

DNA integrity was checked qualitatively using 1% agarose gel electrophoresis with 1x TBE buffer solution at 90 volt and 500 mA current strength for approximately 75 minutes. DNA quantity was calculated using NanoDrop™ 8000 Spectrophotometer. DNA concentration was measured at 260 nm wavelength, while DNA purity was assessed at a ratio of 260 to 280 nm (Sambrook *et al.* 1989).

***Ganoderma* spp. DNA fragment amplification using RAPD method**

The methods to run RAPD was based on Williams *et al.* (1990). DNA samples of *Ganoderma* spp. were prepared in a concentration of 100 ng/µL. RAPD reactions consisted of 1 µL DNA samples, 1 µL primers, 4 µL KAPA Master Mixes, and 3 µL Nuclease Free Water. Four different random oligonucleotide primers, i.e. OPC-1 (5'-TTCGAGCCAG-3'), OPC-2 (5'-GTGAGGCGTC-3'), OPC-4 (5'-CCGCATCTAC-3'), and OPC-5 (5'-GATGACCGCC-3') were used in this study, since the primers produced polymorphic bands when applied to *Ganoderma*

samples (Palupi 2010). The PCR cycles were as follows: pre-denaturation at 94°C for 3 minutes 30 seconds, followed by 40 cycles of 1 minute denaturation at 94°C, 1 minute annealing at 34°C, 1 minute extension at 72°C, continued with a final extension at 72°C for 5 minutes. The amplification products were then separated using 1.4% agarose gel electrophoresis and visualized using UV transilluminator.

Data analysis of RAPD band profiles was descriptive. The RAPD band profile for each primer was analyzed independently based on the presence or absence of bands at each locus in all the samples according to a binary scoring system. A score of 1 was assigned if a band was present and 0 if there was no band, regardless its intensity. Cluster analysis was performed and a dendogram was constructed using the Unweighted Pair-Group with Aritmetic Mean (UPGMA) method (Sokal & Michener 1958) based on the Numerical Taxonomy and Multivariate System (NTSYS) software for pc version 2.02i (Rohlf 1997), then the confidence level of dendogram was performed using WinBoot software with replication of 2000 bootstrap analysis (Yap & Nelson 1996).

RESULTS AND DISCUSSION

Morphological characterizations of *Ganoderma* spp.

Ten samples of *Ganoderma* fruiting bodies were obtained randomly from four different locations at Banyumas Regency, Central Java, Indonesia, i.e. four samples from North Purwokerto sub-district, four samples from Patikraja sub-district, one sample from Baturraden sub-district, and one sample from East Purwokerto sub-district. This is considerably less in number than previous exploration obtained by Ratnaningtyas & Samiyarsih (2012) in the same sampling period of time and duration, i.e. 43 fruiting bodies. According to Ratnaningtyas and Samiyarsih (2012), the findings of wild *Ganoderma* spp explorations are influenced by environmental factors, including weather and altitude. *Ganoderma* spp. tend to be difficult to find during the dry season, particularly in the lowlands due to low rainfall, low humidity and high temperature. Alexopoulus *et al.* (1996) and Pacioni (1981) stated that the growth of the fruiting bodies and spread of Basidiomycetes are highly influenced by environmental factors, such as humidity, altitude, and rainfall. Fungus spores can be optimally distributed through air or substrate moreover if each type of fungus has only a certain range of tolerated temperature. The hyphae are able to grow and develop well on moist soil. Humidity factors also greatly affect the ability of Basiodiomycetes class to form the fruiting body.

Each sample was obtained from distinct substrates, i.e. from dead *Swietenia macrophylla* stems, *Dypsis lutescen* roots, *Tamarindus indica* trees, *Delonix regia* trees and *Albizia chinensis* trees. The morphological characterization of the samples were done both macromorphologically and micromorphologically. The macromorphological characteristics are shown in Figure 1,

demonstrating variation in color, shape, and size of the fruiting bodies, while the micromorphological characteristics are shown in Table 1, including the examination of cultural characteristics of *Ganoderma*. Also in the table are shown the detail macromorphological and micromorphological characteristics.

Figure 1. Macromorphological diversity of several *Ganoderma* spp. fruiting bodies in Banyumas Regency (1: Gano-1, 2: Gano-2, 3: Gano-3, 4: Gano-4, 5: Gano-5, 6: Gano-6, 7: Gano-7, 8: Gano-8, 9: Gano-9, and 10: Gano-10).

Table 1. Morphological characterizations of *Ganoderma* spp. from Banyumas Regency

Sample Code and Origin	Coordinate and Growth Substrate	Hood	Stalk	Tube and Fruiting Body	Pore/ mm^2 and Concentric Zone	Colony Appearance
Gano-1, North Purwokerto Sub-regency	7º24'33.7''S 109º15'14.8"E, Dead *Swietenia macrophylla* Trunk	DDHL 5 cm, DDHW 3.5 cm, DVHL 5 cm, DVHW 3 cm, white edge colour	present, whitish to brownish, 2.8 cm thick, 2.5 cm long	0.7 cm long, fruiting body has 2.30 cm thick, pale grey pileus colour	11 pores, cutis 0.2 cm long, not forming concentric zone	Surface is white with whitish to yellow reverse colors
Gano-2, North Purwokerto Sub-regency	7º24'34.5''S 109º15'15.2"E, *Dypsis lutescen* Root	DDHL 6 cm, DDHW 6 cm, DVHL 7.7 cm, DVHW 6 cm, white edge colour	present, brownish to blackish, 2 cm thick, 2.8 cm long	0.95 cm long, fruiting body has 0.59 cm thick, dark brown pileus colour	14 pores, cutis 0.1 cm long, forming concentric zone	Surface is white with brown reverse colors
Gano-3, North Purwokerto Sub-regency	7º24'33.7''S 109º15'14.5"E, Dead *Swietenia macrophylla* Trunk	DDHL 4.3 cm, DDHW 9.5 cm, DVHL 4.3 cm, DVHW 9.5 cm, dark brown edge colour	Absent	0.2 cm long, fruiting body has 1.07 cm thick, dark brown pileus colour	9 pores, cutis 0.2 cm long, forming concentric zone	Surface is white with brown reverse colors
Gano-4, Patikraja Sub-regency	7º28'01.1''S 109º13'06.2"E, *Tamarindus indica* Tree	DDHL 9.8 cm, DDHW 8.5 cm, DVHL 9 cm, DVHW 7.5 cm, yellowish	absent	0.1 cm long, fruiting body has 1.41 cm thick, brownish red pileus colour	4 pores, cutis 0.2 cm long, not forming concentric zone	Surface is white with whitish to yellow reverse color

		white edge colour				
Gano-5, Patikraja Sub-regency	7º28'01.1''S 109º13'06.2"E, *Tamarindus indica* Tree	DDHL 12.5 cm, DDHW 9.5 cm, DVHL 13.5 cm, DVHW 8.5 cm, reddish brown edge colour	Absent	0.4 cm long, fruiting body has 2.17 cm thick, reddish brown pileus colour	4 pores, cutis 0.2 cm long, not forming concentric zone	Surface is white with whitish to yellow reverse colors
Gano-6, Patikraja Sub-regency	7º28'01.1''S 109º13'06.2"E, *Tamarindus indica* Tree	DDHL 10.5 cm, DDHW 9 cm, DVHL 10.5 cm, DVHW 9 cm, blackish brown edge colour	Absent	1.1 cm long, fruiting body has 2.14 cm thick, blackish brown pileus colour	4 pores, cutis 0.1 cm long, not forming concentric zone	Surface is white with whitish to yellow reverse colors
Gano-7, Patikraja Sub-regency	7º28'01.1''S 109º13'06.2"E, *Tamarindus indica* Tree	DDHL 9.5 cm, DDHW 7.3 cm, DVHL 9.2 cm, DVHW 7, brownish red edge colour	present, brownish to blackish, 4 cm thick, 2.8 cm long	1.1 cm long, fruiting body has 0.59 cm thick, brownish red pileus colour	5 pores, cutis 0.5 cm long, forming concentric zone	Surface is white with whitish to yellow reverse colors
Gano-8, North Purwokerto Sub-regency	7º23'29.8''S 109º14'43.1"E, *Delonix regia* Tree	DDHL 5.5 cm, DDHW 5.2 cm, DVHL 5.4 cm, DVHW 5.7 cm, blackish brown edge colour	present, brownish to blackish, 1.5 cm thick, 2.9 cm long	0.5 cm long, fruiting body has 1.25 cm thick, blackish brown pileus colour	5 pores, cutis 0.5 cm long, forming concentric zone	Surface is white with whitish to yellow reverse colors
Gano-9, Baturraden Sub-regency	7º20'39.5''S 109º14'02.7"E, *Albizia chinensis* Tree	DDHL 7.9 cm, DDHW 7 cm, DVHL 8.5 cm, DVHW 6.5 cm, white edge colour	present, brownish to blackish, 0.5 cm thick, 3.7 cm long	1.19 cm long, fruiting body has 0.59 cm thick, pale brown pileus colour	3 pores, cutis 0.2 cm long, not forming concentric zone	Surface is white with brown reverse colors
Gano-10, East Purwokerto Sub-regency	7º24'15.1''S 109º13'54.1"E, *Tamarindus indica* Tree	DDHL 11 cm, DDHW 13.4 cm, DVHL 7 cm, DVHW 13 cm, yellowish white edge colour	present, reddish brown, 2.1 cm thick, 8.6 cm long	1.11 cm long, fruiting body has 0.59 cm thick, brownish red pileus colour	7 pores, cutis 0.1 cm long, forming concentric zone	Surface is white with whitish to yellow reverse colors

Notes: DDHL = Diameter of Dorsal Hood Length
DDHW = Diameter of Dorsal Hood Width
DVHL = Diameter of Ventral Hood Length
DVHW = Diameter of Ventral Hood Width

Ganoderma species occur throughout the world in both temperate and tropical regions (Flood *et al*. 2000). *Ganodermataceae* have attracted the attention of mycologists for many years. They have been considered as either plant pathogens (Adaskaveg *et al*. 1993), or useful medicinal herbs (Mizuno *et al*. 1995). Because of these fundamentally different view points among collectors, the taxonomy of these fungi is very subjective and confusing (Flood *et al*. 2000). Contributions to

178 the morphology and taxonomy of the Ganodermataceae have been made by many mycologists,
179 including Steayert (1972), Furtado (1981), Corner (1983) and Zhao (1989). However, the great
180 variability in macroscopic and microscopic characters of the basidiocarps has resulted in a large
181 number of synonyms and in a confusing taxonomy, especially in the genus *Ganoderma* (Gilbertson
182 & Ryvarden 1986). Adaskaveg & Gilbertson (1988) also stated that the basidiocarps of *Ganoderma*
183 species have a very similar appearance, causing confusion in identification among species.

184 Although morphological characterization of *Ganoderma* spp. were carried out in this study,
185 the data can not sufficiently provide identification of those 10 isolates into level of species even
186 tentatively. Similarly, several previous studies revealed such a difficulty, e.g. Seo & Kirk (2000),
187 found very similar pileus colour, zonation and pattern of stipe attachment among *G. lucidum*
188 (ATCC 64251 and ASI 7004), *G. oregonense* (ATCC 64487), *G. resinaceum* and *G. oerstedii*
189 (ATCC 52411). Meanwhile, cultural characteristics of *Ganoderma* species have been studied and
190 employed to determine taxonomic arrangement (Adaskaveg & Gilbertson 1986, 1989), but these
191 attempts caused more confusion as they were often quite different from classical identifications
192 based on morphological features. For example, Nobles (1948, 1958) described the differences in the
193 cultural characteristics of *G. lucidum*, *G. tsugae*, and *G. oregonense*. Later, the isolates previously
194 listed as *G. lucidum* were changed by Nobles (1965) to *G. sessile*. Vegetative incompatibility
195 between intraspecific dikaryotic pairings of *Ganoderma lucidum* and *Ganoderma tsugae* also found
196 by Adaskaveg & Gilbertson (1987). This corresponds to Flood *et al*. (2000) stating that other fungi
197 have been shown to have different mating systems within the same genus.

198 In addition to limitation of morphological-based identification, tentative identification of
199 *Ganoderma* spp. can not also be performed based on their hosts and/or types of growth substrates.
200 For instance, Steayert (1967) reported that *G. boniense* and *G. zonatum* were found mostly on
201 palms, *G. miniatotinctum* and *G. tornatum* were found only on palm, while *G. cupreum* and *G.*
202 *xylonoides* were found on both palms and woody dicots. Meanwhile, Semangun (1988) stated that
203 although all scientists agree that stem bark on oil palm is caused by the genus *Ganoderma*, but until
204 now there are still differences of opinions about the fungus species in question. Various species
205 have been mentioned, e.g. *G. lucidum* (Leyss.) Karst., *G. laccatum* Kalchbr., *G. tropicum*, and *G.*
206 *cochlear*. Turner (1981) noted that other species of Ganoderma can cause stem rot, e.g. *G.*
207 *boninense* Pat., *G. chalceum*, *G. colossus*, *G. fornicatum*, *G. miniatocinctum*, *G. pediforme,* and *G.*
208 *tornatum*. As for the cause of red rot disease, *G. pseudoferreum* is a species that can infect various
209 host including rubber, tea, and quinine. Tea-protective trees such as *Albizzia falcata* and gliricide
210 (*Gliricidia sepium*) are also susceptible to this disease. Semangun (1988) reported that *G. lucidum*
211 or better known by its common name, Lingzhi, which has been used as medicinal herb since many
212 years ago, can also be found as a pathogen to roots of pepper plants and coconut trees in West

Kalimantan, causing the death of the plants. Flood *et al*. (2000) reviewed a previous study finding that *G. lucidum* infected coconut, causing basal stem rot disease.

Given the difficulties of taxonomic identification of *Ganoderma* collections using traditional methods, the ease and reducing costs of PCR amplification and direct sequencing techniques, as well as the rapid expansion of molecular databases for a broad array of fungi, might become the much easier way to identify *Ganoderma* and other problematic fungal strain. One of the approaches is by means of RAPD technique (Flood *et al*. 2000). This can be used to differentiate between isolates of *G. lucidum* having identical sequences by another marker, i.e. ITS (Hseu *et al*. 1996).

RAPD analysis

The results of the RAPD amplifications, along with their interpretations, are shown in Figure 2. Individual band on the gel represents an amplified locus of each isolate.

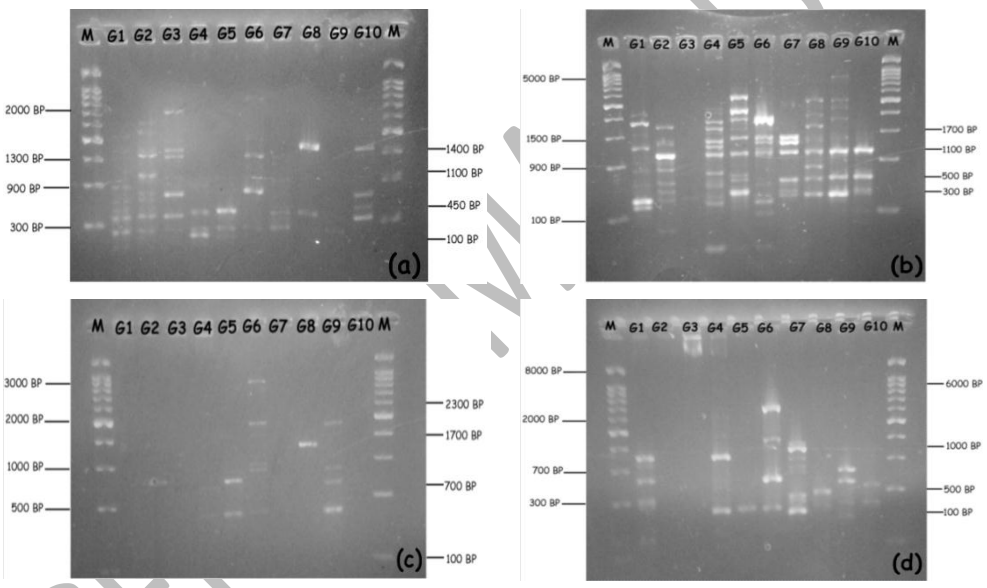

Figure 2. RAPD Amplification products of several isolates of *Ganoderma* spp. from Banyumas Regency using (a) OPC-1 (b) OPC-2 (c) OPC-4 (d) OPC-5 (M = Marker, G1 = Gano-1, G2 = Gano-2, G3 = Gano-3, G4 = Gano-4, G5 = Gano-5, G6 = Gano-6, G7 = Gano-7, G8 = Gano-8, G9 = Gano-9, G10 = Gano-10)

RAPD analysis of the 10 isolates of *Ganoderma* spp. from Banyumas Regency revealed that all primers used generated unique pattern. In other words, it can also be shown that the genetic variation among those isolates are very high. Four random primers used were capable of detecting 43 bands or polymophic loci. The average number of polymorphic bands generated was 10 bands per primer. The highest polymorphism was produced by OPC-2 primer, while the lowest one was produced by OPC-4 primer (Table 2). Previous studies investigating genetic variation among 13

isolates of *Ganoderma* spp. from Papua New Guinea using OPA-18 and OPA-20 also found high variabilities among the isolates (Flood *et al*., 2000).

The difference in the number of amplified RAPD markers was due to the ability of each primer in recognizing genomic DNA sequences. According to Gusmiaty *et al.* (2012), the differences in DNA fragment total counts and sizes were due to the primer's attachment site distribution on the nucleotide base sequence within the genome. The distance between these amplified sites would produce DNA fragments in various sizes. Table 2 demonstrates that overall, the sizes of the resulting amplified DNA bands ranged from 100 to 5,000 bp. A previous study on Russian *G. lucidum* Complex isolates obtained RAPD band sizes of 500 – 3,000 bp (Postnova & Skolotneva 2010). Meanwhile Brazilian and Chinese *G. lucidum* isolates produced RAPD band sizes from 100 to 10,000 bp (Rolim *et al.* 2011), and *Ganoderma* spp. isolates produced 100 – 2,500 bp RAPD band sizes (Mei *et al.* 2014). The differences in the results were due to the difference in the primers used and the length of the DNA sites of primer attachments in the samples (Innis & Gelfand 1990).

Table 2. Polymorphism of *Ganoderma* spp. DNA from Banyumas Regency based on RAPD markers

Primers	**Sequences (5' - 3')**	**Length of Loci (bp)**	**Number of Poly-morphic bands**	**Number of Mono-morphic bands**	**Total**	**G+C Content**
OPC-1	5'-TTCGAGCCAG-3'	100-2,000	9	0	9	60%
OPC-2	5'-GTGAGGCGTC-3'	100-5,000	19	0	19	70%
OPC-4	5'-CCGCATCTAC-3'	100-3,000	5	0	5	60%
OPC-5	5'- GATGACCGCC-3'	100-2,000	10	0	10	70%
		Total	43	0	43	
			100%	0%		

The results obtained also show that certain *Ganoderma* spp. DNAs were not amplified by some primers but were amplified by some others. This could be observed in the Gano-9 sample, which does not show any RAPD band when amplified using OPC-1 primer, but shows bands when subjected to other primers. According to Azizah (2009), the absence of RAPD band might be due to sequence incompatibility between primer and target, as well as the efficiency and optimization of the PCR process.

Previous RAPD studies on several *Ganoderma* species showed different level of polymorphisms. Rolim *et al.* (2011) detected genetic diversity with 63% polymorphism in the Brazilian and Chinese *G. lucidum* isolates. Postnova and Skolotneva (2010) reported 85% polymorphism in the *G. lucidum* Complex isolates from Russia, and Palupi (2012) obtained 100%

polymorphism in the *Ganoderma* spp. isolates associated with plantation crops (cocoa) and its protective crops (*Albizia chinensis*, mahogany, and *Leucaena leucocephala*) from various regions in Indonesia. These differences in polymorphism illustrated the complexity of the genome samples observed (Nurhidayati, 2016). The level of polymorphism produced could be used as an indicator to genetic diversity and is very useful in detecting genetic inter-relationships among samples (Azizah, 2009).

Cluster analysis of several *Ganoderma* spp. isolates from Banyumas regency

Similarity coefficient matrix or genetic distance among *Ganoderma* spp. isolates from Banyumas Regency based on the RAPD profile is presented in Table 3. The similarity coefficients show genetic distance values ranging from 0.4871 to 0.8205. The longest genetic distance is shown between Gano-4 and Gano-9, Gano-5 and Gano-6, Gano-6 and Gano-8 with similarity coefficient value of 0.4871, while the shortest genetic distance is shown between Gano-7 and Gano-10 with a similarity coefficient value of 0.8205.

Table 3. Similarity coefficient matrix of several *Ganoderma* spp. isolates from Banyumas Regency based on RAPD markers

	G-1	G-2	G-3	G-4	G-5	G-6	G-7	G-8	G-9	G-10[a]
G-1	1.0000									
G-2	0.6923	1.0000								
G-3	0.6923	0.6410	1.0000							
G-4	0.7692	0.7179	0.6153	1.0000						
G-5	0.6410	0.5897	0.5897	0.6666	1.0000					
G-6	0.5897	0.5384	0.6410	0.5641	0.4871	1.0000				
G-7	0.6666	0.7692	0.7179	0.6923	0.7692	0.6153	1.0000			
G-8	0.6410	0.7435	0.6923	0.6153	0.6923	0.4871	0.7692	1.0000		
G-9	0.5641	0.5128	0.5641	0.4871	0.6666	0.5641	0.6410	0.6666	1.0000	
G-10	0.7435	0.7435	0.7948	0.6153	0.6923	0.5897	0.8205	0.7948	0.6666	1.0000

[a]G-1: Gano-1, G-2: Gano-2, G-3: Gano-3, G-4: Gano-4, G-5: Gano-5, G-6: Gano-6, G-7: Gano-7, G-8: Gano-8, G-9: Gano-9, G-10: Gano-10

A lower genetic similarity coefficient value signifies a distant relationship, whereas a higher coefficient signifies a closer relationship. The distance of genetic relationship between individuals affects the level of genetic differences. A dendogram arranged based on the genetic distance values among the isolates of *Ganoderma* spp. from Banyumas Regency can be seen in Figure 3. As such, this dendogram describes the genetic relationship among the 10 samples of *Ganoderma* spp. from the region.

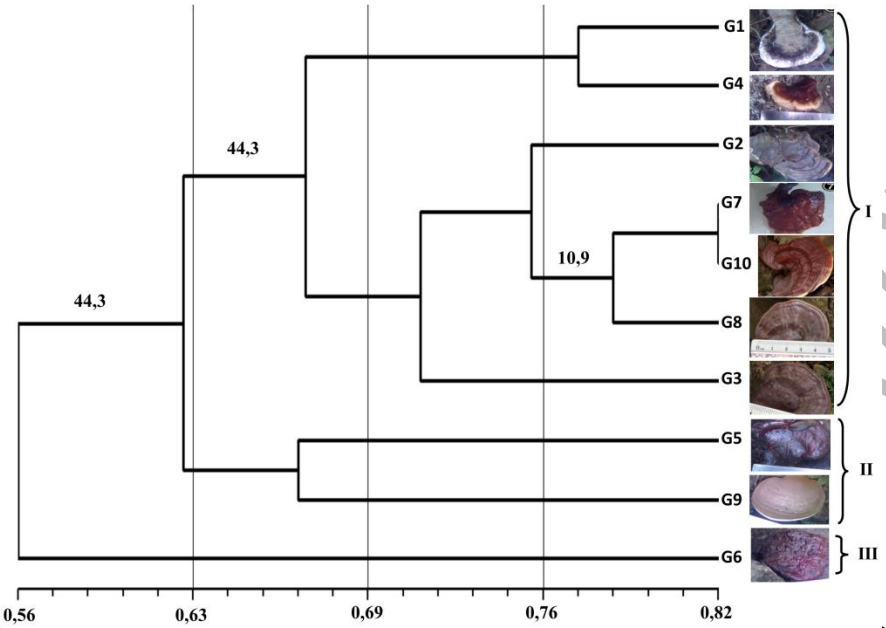

Figure 3. Dendogram of *Ganoaerma* spp. isolates from Banyumas Regency based on RAPD markers

According to the dendogram, the lowest genetic relationship among the samples was 0.56, which was shown between Gano-6 and the other nine isolates. The genetic relationship level between two tested genotypes ranged from 0.48 to 0.82 (Table 2). This signifies that the 10 genotypes possess close to distant genetic relationship. At a coefficient of 0.63 there would be three clusters, i.e. Clusters I, II, and III, but they were not grouped either according to geographical regions or growth substrates. This was in contrast with a previous study of ITS phylogeny summarized by Moncalvo *et al*. (2000), where *Ganoderma* taxa repeatedly showed similar patterns of geographic distribution, between and/or within cdlades; e.g. disjunction between temperate and tropical taxa and connection between the more tropical regions of the southern hemisphere (nothern Australia and Papua New Guinea) and South-East Asia.

Then, at a coefficient of 69%, Cluster I will be splitted into two sub-clusters separating Gano-1 (North Purwokerto, *Swietenia macrophylla* tree trunk) and Gano-4 (Patikraja; *Tamarindus indica* tree) in the first sub-cluster from Gano-2 (North Purwokerto, *Dypsis lutescen* root), Gano-3 (North Purwokerto, *Swietenia macrophylla* tree trunk), Gano-7 (Patikraja; *Tamarindus indica* tree), *Gano*-8 (North Purwokerto, *Delonix regia* tree), and Gano-10 (East Purwokerto, *Tamarindus indica* tree) in the second sub-cluster. Cluster II consisted of two isolates, i.e. Gano-5 (East Purwokerto, *Tamarindus indica* tree) and Gano-9 (Baturraden, *Albizia chinensis* tree). On the other hands,

Cluster III consisted of only one isolate, i.e. Gano-6 (Patikraja, *Tamarindus indica* tree), which could be considered as the outgroup. Clusters I and II could be considered as Major Cluster I, while Cluster III could be seen as Major Cluster II. The closest relationship was shown between Gano-7 and Gano-10 with a similarity coefficient of 0.82 and a low confidence level (<50%), while the furthest relationship was shown between Gano-4 and Gano-9, Gano-5 and Gano-6, Gano-6 and Gano-8 with a similarity coefficient of 0.42 and a low confidence level (<50%).

CONCLUSIONS

It can be concluded that the four RAPD primers used resulted in polymorphism level of 100% among the ten isolates of *Ganoderma* spp. from Banyumas Regency. The level of genetic similarity indicates moderate similarities among the isolates, while at a similarity coefficient of 0.63, they were grouped into three clusters, but neither by geographical regions nor growth substrates.

ACKNOWLEDGEMENT

Part of this work was conducted at the Department of Fisheries and Animal Husbandry of the Banyumas Regency. Therefor, we are grateful to Mrs. Alice Yuniaty and Mr. Agus Nuryanto for the laboratory facilities and their valuable support.

REFERENCES

Adaskaveg JE & RL Gilbertson. 1986. Cultural studies and genetics of sexuality of *G. lucidum* and *G. tsugae* in relation to the taxonomy of the *G. lucidum* Complex. Mycologia 78(5):694-705.

Adaskaveg JE & RL Gilbertson. 1987. Vegetative incompatibility between intraspecific dikaryotic pairings of *Ganoderma lucidum* and *Ganoderma tsugae.* Mycologia 79(4):603-613.

Adaskaveg JE & RL Gilbertson. 1988. Basidiospores, pylocystida, and other basidiocarp characters in several species of the *Ganoderma lucidum* complex. Mycologia 80:493-507.

Adaskaveg JE & RL Gilbertson. 1989. Cultural studies of four North American species in the *Ganoderma lucidum* complex with comparisons to *Ganoderma lucidum* and *Ganoderma tsugae.* Mycological research 92:182-191.

Adaskaveg JE, Miller RW, Gilbertson RL. 1993. Wood decay, lignicolous fungi, and decline of peach trees in South California. Plant disease 77:707-711.

Alexopoulus CJ, Blackwell M, Mims CW. 1996. Introductory micology 4th edition. New York (UK): John Wiley & Sons Inc.

Azizah A. 2009. Perbandingan pola pita amplifikasi DNA daun, bunga, dan buah kelapa sawit normal dan abnormal. [Thesis], Bogor (ID): Fakultas Matematika Dan Ilmu Pengetahuan Alam Institut Pertanian Bogor.

Boh B. 2013. *Ganoderma lucidum*: a potential for biotechnological production of anti-cancer and immunomodulatory drugs. Recent patent anti-cancer drug discovery 8(3):255-287.

Demeke T, Adams RP. 1994. The use of PCR-RAPD analysis in plant taxonomy and evolution. In:: Griffin HG, Griffin AM, editors. PCR technology current innovations. London (UK): CRC Press. p. 179-191.

Flood J, PD Bridge, M. Holderness. 2000. *Ganoderma* diseases of perennial crops. Oxon, UK:: CABI Publishing.

Gilbertson RL, Ryvarden L. 1986. North Americal polypores. Part I. Oslo, Norway: Fungiflora.

Gusmiaty, Restu M, Pongtuluran. 2012. Seleksi primer untuk analisis keragaman genetik jenis bitti (*Vitex coffassus*). Jurnal Perennial 8(1):25-29.

Hseu RS, His-Hua W, Huei-Fang W, Jean-Marc M. 1996. Differentiation and grouping of isolates of the *Ganoderma lucidum* Complex by random ampified polymorphic DNA-PCR compared with grouping on the basis of internal transcribed spacer sequences. Applied and environmental microbiology, 62(4):1354-1363.

Innis MA, Gelfand DH. 1990. Optimization of PCRs. In: MA Innis, DH Gelfand, JJ Sninsky, TJ White, editors. PCR Protocols: a guide to methods and applications. San Diego (USA): Academic Press. p. 1-12.

Mathius NT, Rahmawati D, Anidah. 2009. Genetic variations among *Aquarilia* species and *Gyrinops versteegii* using amplified lenght polymorphism markers. BIOTROPIA 16(2):88-95.

Mei Z, Luquan Y, Md Asaduzzaman Khan, Manman Y, Chunli W, Weichan Y, Xiaoning P, Mousumi T, Hao Zhang, Xiaotao Li, Juniang F. 2014. Genotyping of *Ganoderma* species by improved random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) analysis. Biochemical systematics and ecology 56:40-48.

Mercière M, Laybats A, Carasco-Lacombe C, Tan, JS, Klopp C, Durand-Gasselin T, Alwee SSRS, Camus-Kulandaivelu1 L, Breton F. 2015. Identification and development of new polymorphic microsatellite markers using genome assembly for *Ganoderma boninense*, causal agent of oil palm basal stem rot disease. Mycological progress 14(103):1-11.

Moncalvo JM, Lutzoni FM, Rehner SA, Johnson J, Vylgalis R. 2000. Phylogenetic relationships of agaric fungi based on nuclear large subunit ribosomal DNA sequences. Systematic biology 49 (2):278-350.

Mizuno T, Wang GY, Zhang J, Kawagishi H, Nishitoba T, Li JX. 1995. Reishi, *Ganoderma lucidum* and *Ganoderma tsugae*: bioactive substances and medicinal effects. Food reviews international 11:151-166

Nobles MK. 1948. Studies in forest pathology. VI. Identification of cultures of wood-rooting fungi. Canadian journal of research 26:281-431.

Nobles MK. 1958. Cultural character as aguide to the taxonomy and phylogeny of the Plyporaceae. Canadian journal of botany 36:883-926.

Nobles MK. 1965. Identification of cultures of wood-inhabiting Hymenomycetes. Canadian journal of botany 43:1097-1139.

Nurhidayati RD. 2016. Keragaman genetik kultivar duku (*Lansium parasiticum* (Obseck) K.C. Sahni & Bennet) di Purbalingga berdasarkan penanda RAPD. [Thesis]. Purwokerto (ID): Fakultas Biologi Universitas Jenderal Soedirman.

Nuryanto A, Susanto AH. 2010. Genetic variability of *Polymesoda erosa* population in the Segara Anakan Cilacap. BIOTROPIA 17(1): 22-30.

Orozco-Castillo C, Chalmers KJ, Waugh R, Powell W. 1994. Detection of genetic diversity and selective gene in coffee using RAPD markers. Theoretical and applied genetics 87:934-940.

Pacioni G. 1981. Guide To Mushrooms. New York (USA): Simon & Schuster's, Inc. In: Proborini MW. 2012. Eksplorasi dan identifikasi jenis-jenis jamur kelas Basidiomycetes di kawasan Bukit Jimbaran Bali. Jurnal biologi 16(2):45-47.

Palupi DLN. 2010. Analisis keragaman genetik *Ganoderma* spp. menggunakan penanda molekuler Random Amplified Polymorphic DNA (RAPD). [Thesis]. Bogor (ID): Departemen Biokimia Fakultas Matematika dan Ilmu Pengetahuan Alam Institut Pertanian Bogor.

Postnova EL, Skolotneva ES. 2010. *Ganoderma lucidum* Complex: some individual groups of strains. Microbiology 79(2):270-276.

Ratnaningtyas NI, Samiyarsih S. 2012. Karakterisasi *Ganoderma* spp. di Kabupaten Banyumas dan uji peran basidiospora dalam siklus penyakit busuk batang. Biosfera 29(1):36 – 41.

Rohlf FJ. 1997. NTSYS-pc version. 2.02i numerical taxonomy and multivariate analysis system. New-York (USA): Applied Biostatistics Inc., Exeter Software.

Rolim LDN, Cavalcante MADQ, Urben AF, Buso GSC. 2011. Use of RAPD molecular markers on differentiation of Brazilian and Chinese *Ganoderma lucidum* strains. Brazilian archives of biology and technology 54(2):273-281.

Sambrook J, Fritsch EF, Maniatis T. 1989. Molecular cloning: a laboratory manual 2nd ed. New-York (USA): Cold Spring Harbor Laboratory Press.

Semangun, Haryono. 1988. Penyakit-penyakit tanaman perkebunan di Indonesia. Yogyakarta (ID): Gadjah Mada University Press.

Sokal R, Michener C. 1958. A statistical method for evaluating systematic relationships. University of Kansas science bulletin 38: 1409–1438.

Steyaert RL. 1972. Species of *Ganoderma* and related genera mainly of the Bogor and Leiden herbaria. Rijksherbarium 7(1):55-118.

Turner PD. 1981. Oil palm disease and disorders. United Kingdom: Oxford University Press.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. 1990. DNA polymorphism amplified by arbritary primers are useful as genetic markers. Nucleic acid research 18(22): 6531-6535.

Yap IV, Nelson RJ. 1996. Winboot: A program performing bootstrap analysis of binary data to determine the confidence limits of UPGMA based dendrograms. IRRI Paper Series 14: 1–20.

Yuwono T. 2006. Teori dan aplikasi polymerase chain reaction: panduan eksperimen PCR untuk memecahkan masalah biologi terkini. Yogyakarta (ID): Penerbit ANDI.