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

Ratnaningtyas NI, Susanto AH, Yulia A

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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 characterization 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 identify 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/mm², 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. Alexopoulos *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 Basidiomycetes class to form the fruiting body.

Each sample was obtained from distinct substrates, i.e. from dead *Swietenia macrophylla* stems, *Dypsis lutescens* 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>
<thead>
<tr>
<th>Sample Code and Origin</th>
<th>Coordinate and Growth Substrate</th>
<th>Hood</th>
<th>Stalk</th>
<th>Tube and Fruiting Body</th>
<th>Pore/mm² and Concentric Zone</th>
<th>Colony Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gano-1, North Purwokerto Sub-regency</td>
<td>7°24′33.7″S 109°15′14.8″E, Dead <em>Swietenia macrophylla</em> Trunk</td>
<td>DDHL 5 cm, DDHW 3.5 cm, DVHL 5 cm, DVHW 3 cm, white edge colour</td>
<td>present, whitish to brownish, 2.8 cm thick, 2.5 cm long</td>
<td>0.7 cm long, fruiting body has 2.30 cm thick, pale grey pileus colour</td>
<td>11 pores, cutis 0.2 cm long, not forming concentric zone</td>
<td>Surface is white with whitish to yellow reverse colors</td>
</tr>
<tr>
<td>Gano-2, North Purwokerto Sub-regency</td>
<td>7°24′34.5″S 109°15′15.2″E, <em>Dypsis lutescens</em> Root</td>
<td>DDHL 6 cm, DDHW 6 cm, DVHL 7.7 cm, DVHW 6 cm, white edge colour</td>
<td>present, brownish to blackish, 2 cm thick, 2.8 cm long</td>
<td>0.95 cm long, fruiting body has 0.59 cm thick, dark brown pileus colour</td>
<td>14 pores, cutis 0.1 cm long, forming concentric zone</td>
<td>Surface is white with brown reverse colors</td>
</tr>
<tr>
<td>Gano-3, North Purwokerto Sub-regency</td>
<td>7°24′33.7″S 109°15′14.5″E, Dead <em>Swietenia macrophylla</em> Trunk</td>
<td>DDHL 4.3 cm, DDHW 9.5 cm, DVHL 4.3 cm, DVHW 9.5 cm, dark brown edge colour</td>
<td>Absent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gano-4, Patikraja Sub-regency</td>
<td>7°28′01.1″S 109°13′06.2″E, <em>Tamarindus indica</em> Tree</td>
<td>DDHL 9.8 cm, DDHW 8.5 cm, DVHL 9.5 cm, DVHW 7.5 cm, yellowish</td>
<td>absent</td>
<td>0.1 cm long, fruiting body has 1.41 cm thick, brownish red pileus colour</td>
<td>4 pores, cutis 0.2 cm long, not forming concentric zone</td>
<td>Surface is white with whitish to yellow reverse color</td>
</tr>
<tr>
<td>Sub-regency</td>
<td>Location</td>
<td>Taxonomy</td>
<td>Dorsal Hood Width</td>
<td>Dorsal Hood Length</td>
<td>Dorsal Hood Colour</td>
<td>Dorsal Hood Edge Colour</td>
</tr>
<tr>
<td>-------------</td>
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<td>------------------------</td>
</tr>
<tr>
<td>Gano-5, Patikraja Sub-regency</td>
<td>7°28'01.1''S, 109°13'06.2''E, <em>Ganoderma</em></td>
<td><em>Ganoderma</em></td>
<td>1.1 cm long, blackish brown pileus colour</td>
<td>5 pores, cutis 0.5 cm long, forming concentric zone</td>
<td>blackish brown pileus colour</td>
<td>Surface is white with whitish to yellow reverse colours</td>
</tr>
<tr>
<td>Gano-6, Patikraja Sub-regency</td>
<td>7°28'01.1''S, 109°13'06.2''E, <em>Ganoderma</em></td>
<td><em>Ganoderma</em></td>
<td>1.1 cm long, blackish brown pileus colour</td>
<td>5 pores, cutis 0.5 cm long, forming concentric zone</td>
<td>blackish brown pileus colour</td>
<td>Surface is white with whitish to yellow reverse colours</td>
</tr>
<tr>
<td>Gano-7, Patikraja Sub-regency</td>
<td>7°23'29.8''S, 109°14'43.1''E, <em>Tamarinus indica</em></td>
<td><em>Tamarinus indica</em></td>
<td>0.5 cm long, blackish brown pileus colour</td>
<td>5 pores, cutis 0.5 cm long, forming concentric zone</td>
<td>blackish brown pileus colour</td>
<td>Surface is white with whitish to yellow reverse colours</td>
</tr>
<tr>
<td>Gano-8, North Purwokerto Sub-regency</td>
<td>7°20'39.5''S, 109°14'02.7''E, <em>Albizia chinensis</em></td>
<td><em>Albizia chinensis</em></td>
<td>1.19 cm long, pale brown pileus colour</td>
<td>3 pores, cutis 0.2 cm long, not forming concentric zone</td>
<td>pale brown pileus colour</td>
<td>Surface is white with brown reverse colours</td>
</tr>
<tr>
<td>Gano-9, Baturraden Sub-regency</td>
<td>7°24'15.1''S, 109°13'54.1''E, <em>Ganoderma</em></td>
<td><em>Ganoderma</em></td>
<td>1.11 cm long, red edge colour</td>
<td>7 pores, cutis 0.1 cm long, forming concentric zone</td>
<td>red pileus colour</td>
<td>Surface is white with whitish to yellow reverse colours</td>
</tr>
</tbody>
</table>

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 viewpoints among collectors, the taxonomy of these fungi is very subjective and confusing (Flood et al. 2000). Contributions to
the morphology and taxonomy of the Ganodermataceae have been made by many mycologists, including Steayert (1972), Furtado (1981), Corner (1983) and Zhao (1989). However, the great variability in macroscopic and microscopic characters of the basidiocarps has resulted in a large number of synonyms and in a confusing taxonomy, especially in the genus Ganoderma (Gilbertson & Ryvarden 1986). Adaskaveg & Gilbertson (1988) also stated that the basidiocarps of Ganoderma species have a very similar appearance, causing confusion in identification among species.

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

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

**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 polymorphic 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

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequences (5' - 3')</th>
<th>Length of Loci (bp)</th>
<th>Number of Polymorphic bands</th>
<th>Number of Monomorphic bands</th>
<th>Total</th>
<th>G+C Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPC-1</td>
<td>5'-TTCGAGCCAG-3'</td>
<td>100-2,000</td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>60%</td>
</tr>
<tr>
<td>OPC-2</td>
<td>5'-GTGAGGGCTC-3'</td>
<td>100-5,000</td>
<td>19</td>
<td>0</td>
<td>19</td>
<td>70%</td>
</tr>
<tr>
<td>OPC-4</td>
<td>5'-CCTGACATCTAC-3'</td>
<td>100-3,000</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>60%</td>
</tr>
<tr>
<td>OPC-5</td>
<td>5'-GATGACGCC-3'</td>
<td>100-2,000</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>70%</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>43</td>
<td>0</td>
<td>43</td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>G-1</th>
<th>G-2</th>
<th>G-3</th>
<th>G-4</th>
<th>G-5</th>
<th>G-6</th>
<th>G-7</th>
<th>G-8</th>
<th>G-9</th>
<th>G-10a</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-1</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-2</td>
<td>0.6923</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-3</td>
<td>0.6923</td>
<td>0.6410</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>G-4</td>
<td>0.7692</td>
<td>0.7179</td>
<td>0.6153</td>
<td>1.0000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G-5</td>
<td>0.6410</td>
<td>0.5897</td>
<td>0.5897</td>
<td>0.6666</td>
<td>1.0000</td>
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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.
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 clades; e.g. disjunction between temperate and tropical taxa and connection between the more tropical regions of the southern hemisphere (northern Australia and Papua New Guinea) and South-East Asia.

Then, at a coefficient of 69%, Cluster I will be split 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).
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**

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**REFERENCES**


