

ARBUSCULAR MYCORRHIZAL FUNGI ASSOCIATED WITH *BISBUL* (*Diospyros blancoi*)

DWI RETNO NINGSIH¹, KARTINI KRAMADIBRATA²,
and AGUSTIN WYDIA GUNAWAN^{1,3*}

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Institut Pertanian Bogor,
Darmaga Campus, Bogor 16680, Indonesia

²Herbarium Bogoriense, Botany Division, Research Center for Biology - LIPI,
Jalan Raya Jakarta-Bogor Km 46, Cibinong 16199, Indonesia

³Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia,
Jalan Jendral Sudirman 51, Jakarta 12930, Indonesia

Received 15 September 2013/Accepted 10 December 2013

ABSTRACT

Bisbul (*Diospyros blancoi*) is a kind of edible fruit and could be beneficial as inhibitor for skin ageing process. Majority of root plants have symbiotic associations with arbuscular mycorrhizal fungi (AMF); however, AMF in *bisbul* has never been reported. The objective of this study was to observe AMF colonies and to identify AMF spores in *bisbul* tree rhizospheres and in pot-cultured *Pueraria javanica*. Roots and soil samples from *bisbul* rhizospheres were collected from three locations in Bogor area. Roots were stained using trypan blue 0.05%. Soil samples were air dried, then parts of them were used for spore isolation and the rest were used for pot cultures. Spores were isolated by wet sieving and decanting method and then continued with centrifugation. The results showed that the structures of AMF colonies in *bisbul* roots were arbuscules, vesicles, coiled hyphae, and internal hyphae. Isolated AMF spores were identified as *Acaulospora longula*, *A. scrobiculata*, *A. tuberculata*, *Claroideoglossum geosporum*, *Funnelformis etunicatum*, *Gigaspora candida*, *G. ramisporophora*, *Glomus albidum*, *G. glomerulatum*, and *Scutellospora calospora*. This is the first report of mycorrhizal infection in the root of *bisbul* and AMF association with *bisbul* root.

Key words: AMFungi, symbiosis, *Diospyros blancoi*

INTRODUCTION

At present, researches on the diversity of arbuscular mycorrhizal fungi (AMF), which have mutualistic symbiotic associations with fruit trees, are starting to gain attention. One of the fruit plant considered to have economic value is *bisbul*. *Bisbul* (*Diospyros blancoi*) is a kind of red fruit indigenous to the Philippines and was

* Corresponding author : agustinwydiagunawan@yahoo.com

introduced to Bogor Botanical Garden in 1881. The wood from *bisbul* trees can be used as material for the roof and handicraft. The fruit contains vitamins beneficial to make the skin smooth, maintain healthy eyes, and prevent constipation (Coronel 1992).

Symbioses of AMF with fruit plants in Indonesia have been reported in salak (*Salacca edulis*) (Retnaningsih 1998); persimmon (*Diospyros kaki*), mango (*Mangifera indica*), papaya (*Carica papaya*), and soursop (*Annona muricata*) (Septyarini 1999); durian (*Durio zibethinus*) (Chairani *et al.* 2002); rambutan (*Nephelium lappaceum*) (Muliawan *et al.* 2002); mangosteen (*Garcinia mangostana*) (Lucia 2005); banana (*Musa paradisiaca*) and tomato (*Solanum lycopersicum*) (Duaja & Jasminarni 2008); and jambu-jambuan (*Syzygium* sp.) (Setiadi & Setiawan 2011). However, there has been no report on AMF in *bisbul* tree yet. Therefore, the first step to examine the symbiosis of AMF in *bisbul* roots is to observe their AMF colonies and to identify AMF spores in *bisbul* rhizospheres as well as in pot cultures with *Pueraria javanica* as the host.

MATERIAL AND METHODS

Sampling of Roots and Soil

The samples of roots and soil were collected from three locations in Bogor, i.e. IPB Darmaga campus, Cibinong, and Ciampea. Three *bisbul* trees were selected from every location, and sampling was replicated two times for every tree. For each replication \pm 500 g sample was taken from each position, which was 100 cm from the tree and at a depth of 15-20 cm. The samples were then mixed to make one composite sample. Root samples were immediately cleaned and immersed in 70% alcohol solution, while soil samples were air-dried for spore isolation and pot cultures.

AMF Colonies in *Bisbul* Roots

AMF colonies were observed using trypan blue stain (Phillips & Hayman 1970) with a modification in cell clearing with 10% KOH for 120 min. The structures of AMF colonies were observed in 20 randomly picked root cuts. The roots that showed the structures of arbuscules, vesicles, coiled hyphae, and internal hyphae indicated the occurrence of symbiosis between the roots and AMF. These mycorrhizal roots were examined to calculate the percentage of AM in *bisbul* roots using the following formula:

$$\frac{\text{Number of fields-of-view containing mycorrhizae}}{\text{Total observed fields-of-view}} \times 100\%$$

Pot Cultures

Pot cultures with *P. javanica* as the host were prepared with planting media as follow: 50 g sterile zeolite, 100 g soil sample from *bisbul* rhizosphere, and then 50 g sterile zeolite as the cover layer. Three pot cultures were prepared from each rhizosphere soil sample of every *bisbul* tree, so there were 18 pots. Then, the plants

were maintained for three months in order to produce AMF spores. Each pot was watered daily with sterile water. Fertilizer containing 5% P was used; the amount of water for each plant was 100 mL. Fertilizing was done when the plants were two weeks old, and then repeated every week until they were three months old. After three months, the plants were left to dry for three weeks.

Spore Isolation and Identification.

AMF spores were isolated from 100 g rhizosphere soil sample for every *bisbul* tree, so the total amount of soil sample for every location was 300 g. Likewise for the pot cultures, 100 g was isolated from every pot culture so there was 300 g soil sample for each *bisbul* tree, or 900 g for each location. AMF spores were isolated employing wet-sieving and decanting method and centrifugation (Walker *et al.* 1982). Collected spores were preserved by mounting them on slides in *polyvinyl alcohol lacto glyserol* as media. Identification was conducted based on morphology, while the nomenclature followed Schüßler & Walker (2010).

RESULTS AND DISCUSSION

AMF Colonies in *Bisbul* Roots

Bisbul roots are pigmented, so it took up to 120 min to clear the cells using 10% KOH in order to observe the internal structures of arbuscular mycorrhizae clearly. The structures of the observed *bisbul* roots were found to be arbuscules, vesicles, coiled hyphae, and internal hyphae (Fig. 1) which indicated that the *bisbul* roots had symbiotic associations with AMF. The average of colonization was 41% in *bisbul* roots collected from IPB Darmaga Campus, 49% from Cibinong, and 64% from Ciampea.

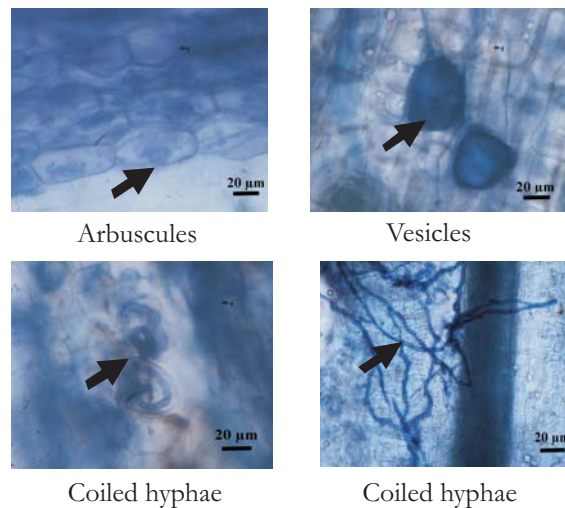


Figure 1. Arbuscular mycorrhizal structures (arrow) in *bisbul* roots.

The occurrence of the structures proved that *bisbul* roots had symbiotic association with AMF, and that AMF spores could be found in the soil of *bisbul* rhizospheres. Its close relative, *Diospyros kaki* (Septyarini 1999), *D. nigrocartex* (Zhao *et al.* 2001), *D. scabrida* (Hawley and Dames 2004), and *D. pendula* (Delvian 2010) were also reported to have symbiotic association with AMF. The arbuscular mycorrhizae observed in *bisbul* roots were classified as arum-paris type.

AMF Spores

Different number of spores were isolated from the soil of *bisbul* rhizospheres collected from three locations in Bogor (Table 1). AMF spores collected from *bisbul* rhizosphere soil in IPB Darmaga campus had the highest quantity as well as diversity, 17 isolated spores consisted of 7 AMF species: *Acaulospora longula*, *A. scrobiculata*, *A. tuberculata*, *Gigaspora ramisporophora*, *Funneliformis etunicatum*, *Claroideoglossum geosporum*, and *Glomus glomerulatum*. Spores in *bisbul* rhizospheres from Cibinong were *C. geosporum*, *G. glomerulatum*, and 2 other species different from those found in Darmaga, they were *Glomus albidum* and *Scutellospora calospora*. AMF spores found in *bisbul* rhizospheres in Ciampea were *Acaulospora longula*, *A. scrobiculata*, and *C. geosporum*.

Table 1. Total spores of arbuscular mycorrhizal fungi (AMF) obtained from *bisbul* rhizospheres and pot cultures with *Pueraria javanica* as the host

AMF	Total AMF spores					
	Darmaga ^a		Cibinong ^a		Ciampea ^a	
	BR ^b	PC ^c	BR ^b	PC ^c	BR ^b	PC ^c
<i>Acaulospora longula</i>	1	13	0	16	4	16
<i>Acaulospora scrobiculata</i>	4	8	0	16	1	7
<i>Acaulospora tuberculata</i>	3	2	0	3	0	4
<i>Claroideoglossum geosporum</i>	3	85	2	34	1	48
<i>Funneliformis etunicatum</i>	2	3	0	2	0	9
<i>Glomus albidum</i>	0	7	3	17	0	24
<i>Glomus glomerulatum</i>	2	4	1	6	0	9
<i>Scutellospora calospora</i>	0	2	1	6	0	2
Total	15	124	7	105	6	123

Notes:

BR: *bisbul* rhizospheres; PC: pot culture

^a soil pH: Darmaga (5.8-6.0), Cibinong (5.8-6.0), and Ciampea (6.2-6.6)

^b AMF spores collected per 300 g soil of BR,

^c AMF spores collected from 300 g PC media, which was an average of 3 pot culture replications of each *bisbul* rhizosphere soil in 1 location.

When the soil from *bisbul* rhizospheres was used in pot cultures with *P. javanica* as the host, the AMF species observed in *bisbul* rhizospheres were also found in their pot cultures. This study produced a high quantity of AMF spores, so it was possible to conduct the identification in order to confirm the identity of the AMF obtained directly from *bisbul* rhizospheres, and also to trap AMF propagules *Gigaspora candida* which spores could not be isolated directly from the soil of *bisbul* rhizospheres.

1. *Acaulospora longula* Spain & N.C. Schenck

The spores were globose to subglobose, yellow to brown in color, measured 90-132 × 93-150 µm. Spore wall was yellow to brown and without ornamentation. Total wall thickness was < 6-9 µm. *Acaulospora longula* was successfully obtained from Darmaga (pH 5.7-5.9) and Ciampea (pH 6.2-6.6). Chairani *et al.* (2002) reported that this species was found in durian in Bogor with pH 4.2-6.3 and Fahriny (2013) in *Areca* rhizospheres at pH 5.9. It seemed that this species could survive in a wide range of acidic pH. *A. longula* had similarities in color and form compared with those first described by Schenck *et al.* (1984). The spore size in this study was bigger compared to that reported by Schenck *et al.* (1984), (60-)70-90(-110) µm, and Fahriny (2013), 126-14 × 96-117 µm. In this study, the sporiferous saccule of *A. longula* was not found. Schenck *et al.* (1984) reported that sporiferous saccule was found at the terminal hyphae and after the spores matured, the sporiferous saccule would be left empty.

Specimens examined: DRN 6, DRN 18, DRN 19, DRN 20, DRN 25, DRN 26, DRN 36, DRN 38, DRN 39, DRN 47, DRN 50, DRN 51, DRN 52, DRN 68, DRN 70, DRN 76, DRN 77, DRN 80, DRN 82, DRN 97, DRN 98, DRN 103, DRN 105, DRN 109, DRN 111, DRN 112, DRN 113, DRN 114, DRN 118, DRN 119, DRN 120, DRN 121, DRN 122, DRN 123, DRN 127, DRN 132, DRN 136, DRN 139, DRN 144, DRN 146, DRN 160, DRN 171, DRN 173, DRN 175, DRN 176, DRN 178, DRN 179, DRN 182, DRN 184, DRN 187, DRN 191, DRN 195, DRN 193, DRN 194, DRN 196 and DRN 199.

2. *Acaulospora scrobiculata* Trappe

The spores were globose to subglobose, yellow in color, and measured 90-115 × 93-112 µm. Spore surface had ornamentation of evenly spaced, linear elliptical crescent-like forms. Spore wall was hyaline to yellow. Total wall thickness was 6-9 µm. *Acaulospora scrobiculata* was obtained in Darmaga soil at pH 5.7-5.9 and Ciampea (pH 6.2-6.6). This species had been reported by Chairani *et al.* (2002) from durian rhizospheres in Bogor at pH 4.2-6.5 and Fahriny (2013) from *Areca* rhizospheres at pH 6.7. *A. scrobiculata* had the same color, form, and ornamentation as those described by Trappe (1977). This spore size was smaller compared with that reported by Trappe (1977), 100-240 × 100-220 µm; Widiastuti and Kramadibrata (1992) 100-200 × 100-200 µm; Septyarini (1999) 105.6-211.2 × 96-220.8 µm; as well as Haerida and Kramadibrata (2002) 108.9-144 × 108.9-144 µm; Kramadibrata (2009) 90-(130)-250 × 100-(120)-250 µm. However, it was bigger compared to that reported by Lucia (2005), i.e. 82-125 × 67-120 µm.

Specimens examined: DRN 3, DRN 10, DRN 20, DRN 29, DRN 30, DRN 33, DRN 34, DRN 35, DRN 37, DRN 41, DRN 43, DRN 44, DRN 45, DRN 56, DRN 65, DRN 72, DRN 85, DRN 99, DRN 111, DRN 118, DRN 119, DRN 124, DRN 125, DRN 127, DRN 130, DRN 131, DRN 132, DRN 136, DRN 137, DRN 140, DRN 142, DRN 143, DRN 144, DRN 146, DRN 148, DRN 149, DRN 150, DRN 152, DRN 156, DRN 157, DRN 158, DRN 159, DRN 167, DRN 176, DRN 178, DRN 181 and DRN 182.

3. *Acaulospora tuberculata* Janos & Trappe.

The spores were globose to subglobose, yellow to brown in color, measured 114-272 × 111-272 µm. Spore surface had ornamentation of smooth beads, dense, and uniform. Spore wall was yellow to brown. Total wall thickness was 9-12 µm. *Acaulospora tuberculata* was successfully obtained from Darmaga (pH 5.7-5.9). Chairani *et al.* (2002) reported the presence of this species in durian in Bogor with pH range of 4.2-6.5 and it had the same form, color, and ornamentation as those described by Janos and Trappe (1982). The spore size was relatively smaller compared to that reported by Janos and Trappe (1982), i.e. 255-327 × 255-340 µm. Likewise, *A. tuberculata* spores reported by Septyarini (1999), Chairani *et al.* (2002), Lucia (2005) and Kramadibrata (2009) were smaller than that of Janos and Trappe (1982).

Specimens examined: DRN 2, DRN 44, DRN 55, DRN 70, DRN 80, DRN 101, DRN 103, DRN 122, DRN 124, DRN 126, DRN 145, DRN 150, DRN 155, DRN 177, DRN 180, DRN 186, DRN 190, DRN 192, DRN 193 and DRN 199.

4. *Claroideoglossum etunicatum* (W.N. Becker & Gerd.) C. Walker & A. Schüßler

The spores were globose to subglobose, yellow to brown in color, and measured 81-108 × 81-111 µm. Spore wall was yellow to brown. Total wall thickness was 3-9 µm. *Claroideoglossum etunicatum* (syn./formerly: *Glomus etunicatum*) was observed in Darmaga at pH range of 5.7-5.9. Chairani *et al.* (2002) was successful in obtaining this species at pH range of 4.2-5.0. This species had the same color and form as those described by Becker dan Gerdemann (1977). The spore size in this study was relatively the same or bigger compared to that reported by Becker dan Gerdemann (1977) which was 68-144(-162) µm, Lucia (2005) 47-107 × 43-99 µm, Kramadibrata *et al.* (2007) 48-77(-156) × 48-77(-156) µm and Kramadibrata (2009) 150 × 150 µm.

Specimens examined: DRN 8, DRN 10, DRN 15, DRN 46, DRN 56, DRN 77, DRN 86, DRN 87, DRN 97, DRN 103, DRN 107, DRN 121, DRN 123, DRN 153, DRN 159, DRN 169, DRN 170, DRN 175, DRN 176, DRN 178, DRN 179, DRN 181, DRN 184, DRN 187, DRN 190, DRN 192, DRN 193, DRN 194 and DRN 197.

5. *Funnelformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler

The spores were globose to ellipsoid, brown to red in color, and measured 75-158 × 72-132 µm. Spore wall was brown. Total wall thickness was 6-15 µm. *Funnelformis geosporum* was found in all study area, at pH range of 5.7-6.6. These spores had the same color and form as those described by Walker (1982). The spore size in this study was smaller than what was reported by Walker (1982), which measured 110-190 µm (syn./formerly *Glomus geosporum*). However, the spore size was relatively the same or bigger than Lucia (2005) which measured 99-124 × 92-107 µm and smaller than Kramadibrata (2009) which measured 100 × 280 µm.

Specimens examined: DRN 4, DRN 9, DRN 11, DRN 12, DRN 17, DRN 22, DRN 23, DRN 24, DRN 25, DRN 26, DRN 27, DRN 28, DRN 29, DRN 30, DRN 31, DRN 32, DRN 33, DRN 34, DRN 35, DRN 36, DRN 37, DRN 38, DRN 39, DRN 40, DRN 42, DRN 43, DRN 47, DRN 48, DRN 49, DRN 52, DRN 53, DRN 54, DRN 55, DRN 56, DRN 57, DRN 59, DRN 60, DRN 61, DRN 62, DRN 63, DRN 64, DRN 66, DRN 67, DRN 69, DRN 71, DRN 72, DRN 73, DRN 74, DRN 75, DRN 76, DRN 79, DRN 81, DRN 82, DRN 83, DRN 86, DRN 87, DRN 88,

DRN 89, DRN 90, DRN 91, DRN 92, DRN 93, DRN 94, DRN 95, DRN 96, DRN 97, DRN 100, DRN 101, DRN 103, DRN 104, DRN 106, DRN 107, DRN 108, DRN 109, DRN 110, DRN 113, DRN 114, DRN 115, DRN 116, DRN 122, DRN 123, DRN 125, DRN 128, DRN 130, DRN 131, DRN 132, DRN 133, DRN 136, DRN 137, DRN 138, DRN 139, DRN 140, DRN 141, DRN 149, DRN 150, DRN 151, DRN 152, DRN 153, DRN 155, DRN 157, DRN 159, DRN 161, DRN 162, DRN 165, DRN 166, DRN 168, DRN 169, DRN 172, DRN 174, DRN 175, DRN 174, DRN 178, DRN 179, DRN 180, DRN 183, DRN 184, DRN 187, DRN 188, DRN 190, DRN 191, DRN 192, DRN 193, DRN 194, DRN 195, DRN 196, DRN 197, DRN 198, DRN 199 and DRN 200.

6. *Gigaspora candida* Bhattacharjee, Murkeji, J.P.Tewari & Skoropad

The spores were globose to subglobose, greenish white in color, and 90-225 × 90-228 µm in size. Spore wall was white. Total wall thickness 3-9 µm. Bulbous suspensor was white to yellow and 27-42 × 21-39 µm in size. *Gigaspora candida* had the same color and form as those described by Bhattacharjee and Mukerji (1982). The obtained spores were 90-225 × 90-228 µm in size, this is smaller compared to that described by Bhattacharjee and Mukerji (1982), i.e. 200-300 µm. The suspensor was also smaller in size, compared to that reported by Bhattacharjee and Mukerji (1982), i.e. 30-50 µm.

Specimens examined: DRN 150, DRN 155, DRN 170 and DRN 171.

7. *Gigaspora ramisporophora* Spain, Sieverd. & N.C. Schenck

The spores were globose to subglobose, brown to reddish brown in color, and 150-372 × 165-372 µm in size. Spore wall was brown. Total wall thickness 6-9 µm. Bulbous suspensor was yellow to brown and 27-36 × 21-39 µm in size. Only 2 spores of *Gigaspora ramisporophora* were found in Darmaga at soil pH 5.7-5.9. These spores had the same color and form as those described by Spain *et al.* (1989). The spore size was 150-372 × 165-372 µm, still in the same range as that described by Spain *et al.* (1989), i.e. (143-)150-400 × 200-450(-501) µm, Septyarini (1999) 230-576 × 288-595 µm, and Lucia (2005) 182-317 × 172-317 µm. The obtained suspensor relatively smaller than that reported by Spain *et al.* (1989) sized (32-)40-60(72) × (50-) 60-83 µm.

Specimens examined: DRN 1, DRN 2, DRN 126, DRN 128, DRN 132, DRN 133, DRN 136, DRN 138, DRN 139, DRN 144, DRN 148, DRN 149, DRN 170, DRN 181 and DRN 185.

8. *Glomus albidum* C. Walker & L.H. Rhodes

The spores were globose to subglobose, hyaline to yellowish white in color, and 69-120 × 66-135 µm in size. Spore wall was hyaline to yellow. Total wall thickness was < 3-9 µm. *Glomus albidum* was found in Cibinong soil with pH range of 5.9-6.0. Fahriny (2013) reported that this species associated with *Areca* at pH 6.9. *G. albidum* had the same color and form as those described by Walker and Rhodes (1981). In this study, the spore size was smaller compared to that reported by Walker and Rhodes (1981) which measured (85-) 95-168(-198) × (85-)95-168(-177) µm and Fahriny (2013) which measured 48-144 × 66-171 µm and Kramadibrata (2009) which measured 85-160 × 85-160 µm.

Specimens examined: DRN 13, DRN 14, DRN 29, DRN 41, DRN 43, DRN 44, DRN 45, DRN 58, DRN 61, DRN 62, DRN 84, DRN 91, DRN 99, DRN 101, DRN 111, DRN 117, DRN 118, DRN 119, DRN 121, DRN 123, DRN 125, DRN 126, DRN 127, DRN 128, DRN 129, DRN 135, DRN 136, DRN 137, DRN 141, DRN 143, DRN 144, DRN 145, DRN 146, DRN 148, DRN 150, DRN 152, DRN 153, DRN 154, DRN 155, DRN 156, DRN 158, DRN 159, DRN 160, DRN 161, DRN 163, DRN 164, DRN 166, DRN 168, DRN 169, DRN 171, DRN 175, DRN 176, DRN 177, DRN 179, DRN 190, DRN 192, DRN 193, DRN 196, DRN 197 and DRN 199.

9. *Glomus glomerulatum* Sieverd

The spores were globose to subglobose, yellow to brown in color, and $70-117 \times 70-123 \mu\text{m}$ in size. Spore wall was yellow to brown. Total wall thickness was 6-9 μm . *Glomus glomerulatum* was successfully obtained from Darmaga (pH 5.7-5.9) and Cibinong (pH 5.9-6.0). The specimens had the same color and form as those described by Sieverding (1987). The size of these spores was bigger compared to that reported by Sieverding (1987) which measured 40-70 μm , while in this study the spores were $70-117 \times 70-123 \mu\text{m}$ in size.

Specimens examined: DRN 7, DRN 9, DRN 46, 52, DRN 61, DRN 73, DRN 77, DRN 97, DRN 98, DRN 99, DRN 103, DRN 105, DRN 107, DRN 114, DRN 117, DRN 118, DRN 121, DRN 122, DRN 123, DRN 136, DRN 139, DRN 140, DRN 163, DRN 164, DRN 170, DRN 175, DRN 176, DRN 177, DRN 179, DRN 180, DRN 181, DRN 186, DRN 190, DRN 194 and DRN 196.

10. *Scutellospora calospora* (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders

The spore was globose to subglobose, greenish yellow to brown in color, and $135-234 \times 141-210 \mu\text{m}$ in size. Spore wall was hyaline to yellow. Total wall thickness was 6-18 μm . Bulbous suspensor was hyaline to brown and $27-45 \times 24-39 \mu\text{m}$ in size. The quantity of AMF spores *S. calospora* was the lowest, only 1 spore was found from Cibinong at pH (5.9-6.0). It had the same color and form as those described by Koske and Walker (1986). The spore size was same range as that described by Koske and Walker (1986), which was $114-285(-511) \times 110-412(-511) \mu\text{m}$, but this spore was bigger than that reported by Kramadibrata *et al.* (2007), which measured $86-134 \times 86-134 \mu\text{m}$, Kramadibrata (2009) $150 \times 150 \mu\text{m}$ (globose) and $100-160 \times 165-250 \mu\text{m}$ (oblong). The suspensor was $27-45 \times 24-39 \mu\text{m}$ in size, relatively smaller than what was reported by Koske and Walker (1986), which measured 33-48 μm .

Specimens examined: DRN 16, DRN 83, DRN 84, DRN 85, DRN 119, DRN 126, DRN 133, DRN 134, DRN 138, DRN 139, DRN 144, DRN 146, DRN 159, DRN 164, DRN 185 and DRN 192.

Ten species of AMF spores were found in the rhizosphere of *bisbul* in Bogor, also eleven species of AMF were found in persimmon rhizosphere in Cibinong (Septyarini 1999), but only 3 same species of AMF were recorded from both plants i.e. *A. scrobiculata*, *A. tuberculata*, and *G. ramisporophora*.

The quantity and diversity of AMF species obtained from every location were different from each other. It could be influenced by different locations and soil types. IPB Darmaga campus and Cibinong soil belong to ultisol (dominated by clay), while in

Ciampea is alfisol (dominated by fine clay) (Soil Survey Staff 2010). Widiastuti and Kramadibarata (1992) suspected that the soil dominated by clay fraction was suitable for the development and growth of *Glomus* spp. spores, while Koske (1987) reported that the spores from genera *Gigaspora* and *Scutellospora* were found in large quantity in sandy soil. AMF species diversity can also be influenced by the soil pH which was different in each location (Moreira-Souza *et al.* 2003).

Funneliformis geosporum was the dominant genus in *bisbul* rhizospheres and pot cultures. Carvalho *et al.* (2001) reported that this species (formerly *G. geosporum*) was the dominant fungi in saline soil. Furthermore, Gai *et al.* (2006) also reported that the spread of *Glomus* (including syn. *Claroideoglomus* and *Funneliformis*) was wider in tropical and subtropical regions compared to other genera.

CONCLUSIONS

This study is the first report on *bisbul* mycorrhiza and it showed that AMF was associated with *bisbul* trees in the form of arum-paris type. The spores found in *bisbul* tree rhizospheres were identified as *Acaulospora longula*, *A. scrobiculata*, *A. tuberculata*, *Claroideoglomus geosporum*, *Funneliformis etunicatum*, *Gigaspora candida*, *G. ramisporophora*, *Glomus albidum*, *G. glomerulatum*, and *Scutellospora calospora*.

REFERENCES

- Becker WN, Gederhmann JW. 1997. *Glomus etunicatus* sp. nov. *Mycotaxon*. 6:29-32.
- Bhattacharjee M, Mukerji KG. 1982. Structure and hyperparasitism of a new species of *Gigaspora*. *Trans Br mycol Soc*. 78(1):184-188.
- Carvalho LM, Cacador I, Loucao MAM. 2001. Temporal and spatial variation of arbuscular mycorrhizas in salt marsh plants of the Tagus estuary (Portugal). *Mycorrhiza*. 11:303-309. doi:10.1007/s00572-001-0137-6.
- Chairani, Gunawan AW, Kramadibrata K. 2002. Mikoriza durian di Bogor dan sekitarnya. *J Mikrobiol Indones*. 7(2):44-46.
- Coronel RE. 1992. Edible fruits and nuts. In *Plant Resources of South-East Asia 2*. Edited by Verheij EWM, Coronel RE. Bogor (ID): Prosea Foundation. p 151-152.
- Delvian. 2010. Keberadaan cendawan mikoriza arbuskula di hutan pantai berdasarkan gradien salinitas. *J Ilmu Dasar*. 11(2):133-142.
- Duaja MD, Jasminarni. 2008. Isolasi dan karakterisasi cendawan mikoriza arbuskular di rhizosfer beberapa jenis tanaman di kebun percobaan Fakultas Pertanian, Universitas Jambi. *J Agron*. 12(2):34-38.
- Fahriny RA. 2013. Ragam Cendawan Mikoriza Arbuskula pada *Areca* di Kebun Raya Bogor. [skripsi]. Bogor (ID): Institut Pertanian Bogor.
- Gai JP, Christie P, Feng G, Li XL. 2006. Twenty years of research on community composition and species distribution of arbuscular mycorrhizal fungi in China: a review. *Mycorrhiza*. 16:229-239. doi:10.1007/s00572-005-0023-8.
- Haerida I, Kramadibrata K. 2002. Identifikasi jamur mikoriza arbuskula pada rizosfer tanaman jagung manis di Jawa. *Floribunda*. 2(2):33-37.
- Hawley GL, Dames JF. 2004. Mycorrhizal status of indigenous tree species in forest biome of the Eastern Cape, South Africa. *South Africa J Sci*. 100(11):633-637.

- Janos DP, Trappe JM. 1982. Two new *Acaulospora* species of tropical America. *Mycotaxon*. 15:515-522.
- Koske RE, Walker C. 1986. Species of *Scutellospora* (*Endogonaceae*) with smooth-walled spores from maritime sand dunes: two new species and redescription of the spores of *Scutellospora pellucida* and *Scutellospora calospora*. *Mycotaxon*. 27:219-235.
- Koske RE. 1987. Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. *Mycologia*. 79(1):55-68.
- Kramadibrata K. 2009. *Glomeromycota* recovered from cacao soil. *Reinwardtia* 12(5): 357-371.
- Kramadibrata K, Prastyo H, Gunawan AW. 2007. Jamur arbuskula pada bambu di Jawa. *Berita Biol*. 8(6):531-536.
- Lucia Y. 2005. Cendawan mikoriza arbuskula di bawah tegakan tanaman manggis dan peranannya dalam pertumbuhan bibit manggis (*Garcinia mangostana* L.). [thesis]. Bogor (ID): Institut Pertanian Bogor.
- Moreir-Souza M, Trufem SFB, Gomes-da-Costa SM, Cardoso EJB. 2003. Arbuscular mycorrhizal fungi associated with *Araucaria angustifolia* (Bert.) O. Ktze. *Mycorrhiza*. 13:211-215. doi:10.1007/s00572-003-0221-1.
- Muliawan J, Gunawan AW, Kramadibrata K. 2002. Mikoriza rambutan di Bogor dan sekitarnya. *J Mikrobiol Indones*. 7(1):24-25.
- Phillips JM, Hayman DS. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br mycol Soc*. 55(1):158-161.
- Retnaningsih E. 1998. Biodiversitas cendawan mikoriza arbuskula pada rizosfer salak. [skripsi]. Bogor (ID): Institut Pertanian Bogor.
- Schenck NC, Spain JL, Sieverding E, Howeler RH. 1984. Several new and unreported vesicular-arbuscular mycorrhizal fungi (*Endogonaceae*) from Colombia. *Mycologia*. 76(4):685-699.
- Schüßler A, Walker C. 2010. *The Glomeromycota: a species list with new families*. Edinburgh (UK): Royal Botanic Garden Edinburgh.
- Septyarini. 1999. Cendawan mikoriza arbuskula di kebun plasma nutfah Puslitbang Bioteknologi-LIPI, Cibinong. [skripsi]. Bogor (ID): Institut Pertanian Bogor.
- Setiadi Y, Setiawan A. 2011. Studi status fungi mikoriza arbuskula di areal rehabilitasi pasca penambangan nikel (Studi kasus PT INCO Tbk, Sorowako, Sulawesi Selatan). *J Silvikul Trop*. 3(1):88-95.
- Sieverding E. 1987. A VA-mycorrhizal fungus, *Glomus glomerulatum* sp. nov., with two hyphal attachments and spores formed only in sporocarps. *Mycotaxon*. 29:73-79.
- Soil Survey Staff. 2010. *Keys to Soil Taxonomy*. 11th ed. Washington (US): US Department of Agriculture Natural Resource Conservation Service :85-573.
- Spain JL, Sieverding E, Schenck NC. 1989. *Gigaspora ramisporophora*: a new species with novel sporophores from Brazil. *Mycotaxon*. 34(2):667-677.
- Trappe JM. 1977. Three new Endogonaceae: *Glomus constrictus*, *Sclerocystis clavisporea*, and *Acaulospora scrobiculata*. *Mycotaxon*. 6(2):359-366.
- Walker C, Mize CW, McNabb Jr HS. 1982. Population of endogonaceous fungi at two locations in Central Iowa. *Can J Bot*. 60(12):2518-2529. doi:10.1139/b82-305.
- Walker C, Rhodes LH. 1981. *Glomus albidus*: a new species in the *Endogonaceae*. *Mycotaxon*. 12(2):509-514.
- Walker C. 1982. Species in the *Endogonaceae*: a new species (*Glomus occultum*) and a new combination (*Glomus geosporum*). *Mycotaxon*. 15:49-61.
- Widiastuti H, Kramadibrata K. 1992. Jamur mikoriza beresikula arbuskula di beberapa tanah masam di Jawa Barat. *Menara Perkebunan*. 60(1):9-19.
- Zhao ZW, Xia YM, Qin XZ, Li XW, Cheng LZ, Sha T, Wang GH. 2001. Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi in the tropical rain forest of Xishuangbanna, southwest China. *Mycorrhiza*. 11:159-162.