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Indigenous Vesicular Arbuscular Mycorrhizal (VAM) Fungi in Cashew nut (*Anacardium occidentale* L.) Plantation of North East-Bali Island - Indonesia

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Abstract

A study on indigenous VA-mycorrhizal fungi associate with “cashew nut” plants (*anacardium occidentale* L.) was carried out at arid areas of North-East of Bali Indonesia from April 2011 up to February, 2012 representing dry and rainy seasons consecutively.

Soil samples were randomly collected from rhizosphere of either seedling or adult cashew-nut plants with 2 months interval. Spores of myccorhizal fungi were extracted from the soil by wet-sieving and decanting method and morfologically identified.

This study found 5 genera consisiting of 13 species i.e. *Acaulospora* (4 species), *Gigaspora (2 species), Scutellospora* (1 species)*, Entrophospora* (1 species) and *Glomus* (5 species). The most frequent genera found was *Glomus* and the least was *Entrophospora*. The number of spore varied temporally and showed opposite trend to the monthly total of rain fall. The average of spore number was 52 to 91 per 250 g soil sample.

Keywords: Arbuscular mycorrhizae, *A. occidentale* L. Arid area, Bali Island

1. Introduction

North-East region of Bali island is one of the three dry areas in Bali. The region is characterised by sandy soil structure thought it has been washed away since it erupted at 1960s, lack of water and extreme temperature during dry season. All those lead to low productivity farming due to limitation of soil water (Antara, 2004 ; Aditya,2011). The local people relies onto the limited farming during rainy season and cashew nut plantation that grow and adapted to such condition. Currently The cashew farming, however, is facing a serious problem caused by infection of *Rhidigosporus* sp (“white root fungi”) and *Phytopthora sp* (Proborini, unpublished).

In accordance with the potential of VAM fungi as biofertiliser for plant suggested by many studies and organic farming program promoted by the Government of Bali province, VAM fungi has been applied and developed for plant farming. The premises were that Vesicular Arbuscular Mycorrhizal (VAM) fungi are abundant in almost all natural terrestrial communities and form obligate symbiotic associations with over than 80% of vascular plants (Smith *et al*, 2010). VAM- fungi takes roles in maintaining ecosystem processes by promoting plant fitness through a range of mechanisms (Brundrett *et al*, 2008); protecting plant host from soil pathogens (Smith, 2000) and improving soil structure, enhancing water and nutrient uptake (Smith *et al*. 2010); increase the efficiency of fertilizer use and plant growth (Douds *et al*. 2010).

Regarding those above, some efforts have been undertaken to raise up farming productivity in Bali by applying michorizal fungi on nut and corn farming in field as a pilot project (Sukasta and Proborini , unpublished data). The result was not optimum yet which might be due to either less adapted mycorrizhal spore or ineffective spore dose. Similarly goverment regional technical assistance unit of Bali province is undergoing a research to diminish the impact of “ white root disease “. These efforts applied commercial mycorrhizal fungi explored from other region, instead of indigenous mycorhizae. The indigenous michorizal spore, however, has not been developed yet. The other part of this study showed that local plants at North-East Bali were highly associate with VAM fungi indicated by hyphal infection in plant root. Therefore it is urgent to explore the diversity of indigenous micorizha in Cashew plantation rizosphere.

2. **Material and Methods**

2.1 Soil collection

The study were carried out on cashew farming (*A. Ocidentale* L) at Village of Sukadana Suburb of Kubu, Regency of Karangasem North-East Bali Indonesia , one of three dry regions in Bali. The study was carried out from April 2011 up to February 2012, representing dry and seasons consecutively, with two month interval sampling period. Soil sample of each sampling period was randomly taken from five sampling points over the area of cashew farm. Soil sample (*ca*. 2kg) of each sampling point was collected from either seedling or cashew plant rhizosphere (± 20 – 30 cm in depth). The cashew plant roots were also included as sample.

2.2. Isolation of VAM spores

VAM Spore was isolated from the soil sample by wet sieving and decanting method described by Gerdemann and Nicolson (1963). Sub-sampled soil (250 gr) from each sample was suspended in 1 L of water and stirred. The supernatant was sift through a series of sieve mesh size i.e.500, 125, 90 and 45 microns consecutively. s retained on the last three sieve mesh size were collect and placed in beaker glass containing water. Such process was replicated four times for every sub-sample soil. The collected spores were then centrifuged by sucrose density gradient method to remove soil attach to spore.

2.3. Spore identification

The VAM fungal spores mounted in polyvinil alcohol-lactic acid glycerol (PVLG) and Melzer reagent were identified based on its morphological characters (i.e. shape, colour, diameter, ornament, hifa).

The described spores characters were then compared to the descriptions of VAM species by Walker (1983); Schenck and Perez (1990); Morton and Benny (1990); Kramadibrata, (2008); Schuβler et al.,(2001) and the online reference culture database published at INVAM (http://invam.caf.wvu.edu). The identified fungal spore was then species-base seperated and counted its number.

3. **Results and Discussion**

3.1. Type of VAM spores

The total number of spores wet-sieved from the 30 soil samples was 2205 VAM spores or sporocarp. In accordance with the described spore characters, the VAM fungi found were five genera consisting of 13 species i.e. *Glomus* (5 species). *Acaulospora* (4 species), *Gigaspora (2 species), Scutellospora* (1 species) and *Entrophospora* (1 species) (Figure 1). The morphological description of the fungal spores found were described beneath.

1. ***Glomus etunicatum* Becker &Gerdemann**

Single spore, globose -sub globose, orange-red brown diameter 91-149 x 108-170 μm. Cell wall thickening extend up to 16 μm without ornaments.

2. ***Glomus mosseae* (Nicol.&Gerd) Gerd & Trape**

Circular spore; yellow- brownish color, diameter 105-194.50 μm. Cell wall 5.43-7.77 μm thickness. Smooth cell walls without ornaments.

3. ***Glomus aggregatum* Schenck &Smith**

Spores globose; yellow color; diameter 51.06-(76.59) -115 μm. hifa 1.11 - (1,51)-3.33 μm thickness. Smooth cell wall without ornaments

4. ***Glomus intraradices*** **Schenck &Smith**

Spore globose- sub globose, sometime irregular with many elliptical shapes, color pale cream-yellow brown diameter 40.5-(98.5) -115 (119) μm

5. ***Glomus rubiformis*** **Gerdemann & Trappe**

Globose to subglobose sporocarps; yellow-brownish color; 140-280 x 180-330 μm ; sporocarps with radially arranged chlamydospores. Chlamydospores are arranged on a thick-walled central plexal cell , color yellow-yellowish brown.

6. ***Acaulospora foveata Trappe & Janos***

Spores single without mother spores; globose-elipsoid; brown-reddish color; diameter 124-
246 x 148-256 μm; three layer of cell walls; outer layer of wall, brown-redish color, 12-13μm thickness; inner layer transparent, yellowish color, 3 -7 μm thickness.

7. ***Acaulospora tuberculata Janos &Trappe***

Spores single without mother spores; globose-elipsoid. yellow – brownish color; diameter 93-300 x 93-300 μm. Two layers of cell wall,outer layer yellowish, 4-6μm thickness, with smooth spina; second layer, yellowish (transparent), 3-5 μm thickness.

8. ***Acaulospora cf. undulata*** **Sieverding**

Spores single without mother spores, globose-elipsoid. diameter 73-102 x 73-102 μm, white – creamish color. Does not react with melzer's reagent; Two layers of cell wall, outer layer yelowish, 4-6 μm thickness; inner layer yellowish (transparent) 1-2 μm thickness

9. ***Acaulospora Scrobiculata*** **Trappe**

Sporocarps unknown, form singly in soil, spores globose to subglobose; diameter 92-156 x 98-168μm; light-brownish color of mature spore. Two layers of cell wall, hyaline to light greenish, 2-4 μm thickness, outer layer ornamented with profuse minute

10. ***Gigaspora albida*** **Schenck &Smith**

Spore shape globosa-subglobosa, cream-yellowish color, diameter 260-(265)-270 μm. smooth surface of outer wall with subtending hyphae. Spore does not react to Melzer's reagent.

11. ***Gigaspora margarita*** **Becker & hall**

Single spore in soil; Globose; white-yellowish color, does not flourescens, spore diameter 240-300 μm; cell wall structure smooth 14 to 21 μm thickness does not react to Melzer's reagent.

12. ***Scutellospora cf. heterogama*** **(Nicol. & Gerd.) Gerd. & Trappe**

Single spore; subglobose- globosa; color dark orange to brown-red in Melzer's reagent the spores will turn to almost black; diameter 200-320 x 200-320 μm. Outer wall ornamented

13. ***Entrosphospora infraquens*** **Hall**

Single spore, globose-sub globose, color orange-redbrown; diameter 220-300 x 220-300 μm ; two layers of cell wall; outer layer hyaline to light greenish color, 3-7 μm thickness, outer wall ornamented with pith.

All VAM fungi species found in this study belong to the phylum of Glomeromycota (Schüβler *et al*., 2001). Among the Glomeromycotan fungi, the Gigasporaceae (genera Scutellospora and Gigaspora) are distinguished by the formation of the spores on a "bulbous suspensor" (Oehl *et al*., 2008). All species of Gigasporaceae is characterized by producing spores without ornamentation and having germ tubes that growth directly from the cell wall (Gerdemen and Trappe, 1974 ; Morton and Benny, 1990).

The Glomineae (genera *Acaulospora* and *Glomus*) have single asexual stages and often in aggregate forms in soil or plant roots or sporocarp (Morton and Benny, 1990). Meanwhile, morphological character of another Glomineae (genera *Entrophospora*) was intra-hyphal spore formation within the neck of a soporiferous saccule. The spores of *E infraquens* formed of subtending hyphae has larger sacccules than other species of *Entrosphospora* (Oehl *et al*., 2008).

This finding of study is the first result reporting the diversity of indigenous VAM fungi in sandy soil at the North- East Bali even might be in Bali. The previous research of Kramadibarta (2008) was only short study of cacao plantations soil at Jembrana Bali and found three genera, i.e. *Acaulospora, Gigaspora* and *Glomus*.

The finding of this study (i.e 13 species) also indicates that the indigenous VAM fungi species at North-East of Bali is high diverse. Compare to study of Kramadibrata (2008) that found 19 VAM species from 11 sites of cacao plantation in Java and Bali and the study of Widyastuti (2004) at sawit plantation found 10 species.

3.2. Number of spores

The number of spore collected was 2205 spores over the sampling period and the average number of spore was vary from 52 up to 91 spores per 250 gr. The number of spore showed temporal variation over the year (Figure 2), the spore number increased from April to June and relatively steady at the period June up to October. The spore number decreased at the period from October 2011 up to February 2012. Such temporal variation of total number of VAM spore indicated opposite correlation with the monthly total rain fall (Figure 2). Spores number was high over dry season (Agust to October 2011) and was tend to decreased at rainy sesaons (Des 2011 to Feb 2012).

This finding provide another evidence that VAM fungi is influenced by soil water content (Delvian, 2006a). The mycorrhizal fungi tend to sporulate during the dry season and will germinate to hyphal shape during the rainy periods (Smith and Read, 1997; Hartoyo *et al*., 2011). During rainy periods the VAM spore germinate to hyphal-shape and the hyphae expand through soil to absorb nutrient and they tend to infect plant root as mutual symbioses (Delvian, 2006b; Alizadeh, 2011). The formation of spore seems as a mechanism of VAM fungi adaptation and self protection to extreme environment e.g. droughtness and temperature as reviewed by Smith and Read (1997).

The range of total spore number observed was 261 up to 456 (totally 2205 spores). The average of spore number was rangely from 52 up to 91per 250 gr soil. The dynamic of species based spore number was showed at (Figure 3). The figure indicated that spores number of Glomus, Acaulospora and Gigaspora were higher than Scutelospora and *Enthrophospora eff. Infrequens* along period of this study. The figure also showed that the highest spore number was of *Glomus* and the least was of *Scutelospora* and *Enthrophospora*. This finding fit to the study of Morton (1988) that *Glomus* species is common species in the soil all over the world and make up for more than 75% of total isolates, followed by *Acaulospora* and *Gigaspora*. *Glomus* is also predominantly distributed genus in India (Khare *et al*.,2011) and Pakistan (Burni *et al*.,2011). Such of G*lomus* dominance may be attributed to adaptability of the genera to soil alkalinity (neutral and alkaline) (Mosse, 1991) and to high soil temperatures (Shi *et al*., 2007). During rainy season, *Glomus* germinates and forms hyphae faster than *Gigaspora, Scutelospora* or *Acaulospora*, it can be addressed to that *Glomus* has smaller size spore (Chalimah *et al*. 2007 ; Hartoyo *et al*. 2011). This finding indicates the *Glomus* is potential to be developed as bio-fertilizer for cashew plantation in North East of Bali.

Interestingly, this study also found *Entrophospora eff. Infraquens* only at June and October. This species is rarely found in the natural field (Karmadibrata-pers com) because less germinate in the nature otherwise in propagation, the species , therefore, is recognized as “infrequent species” (Oehl, *et al*. 2008).

4. **Conclusion**

The finding of this study showed highly diversity species of VAM fungi in sandy soil at North-East of Bali Island of Indonesia. The genera *Glomus* was mostly found. The spore number of VAM fungi showed temporal dynamic in relation to the seasons (rainy and dry).

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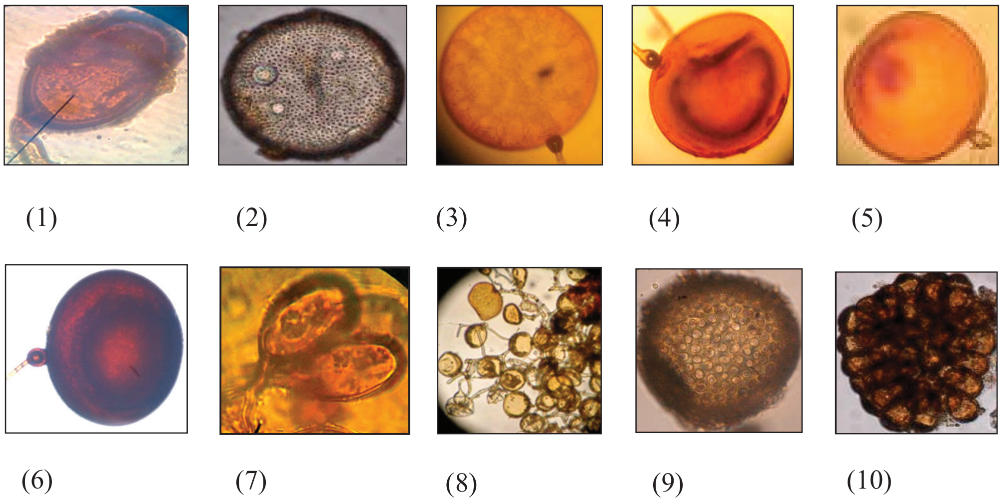

Figure 1: Morphological character of AMF spore under light microscopes (400 X magnification);
(1. *Acaulospora foveata*; 2 *Acaulospora. scrobiculata;* 3. *Gigaspora margarita,* 4.*Gigaspora. albida,* 5. *Glomus moseae,* 6.*Glomus. etunicatum;* 7.*Glomus.aggregatum;* 8. *Glomus intraradices* , 9. *Enterophospora* 10. *Glomus. rubiformis*)

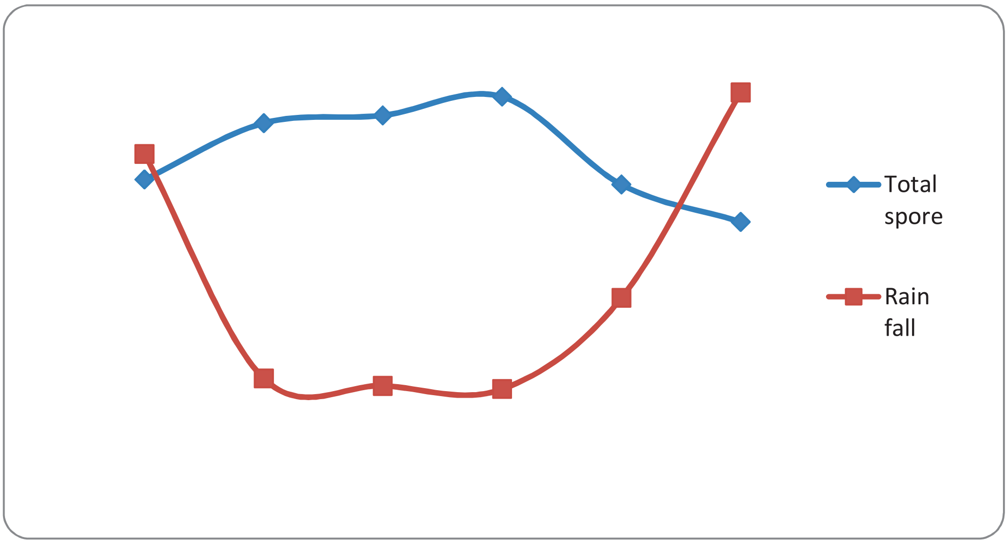

Figure 2. The VAM spores number of Cashew nut rhizosphere in a year

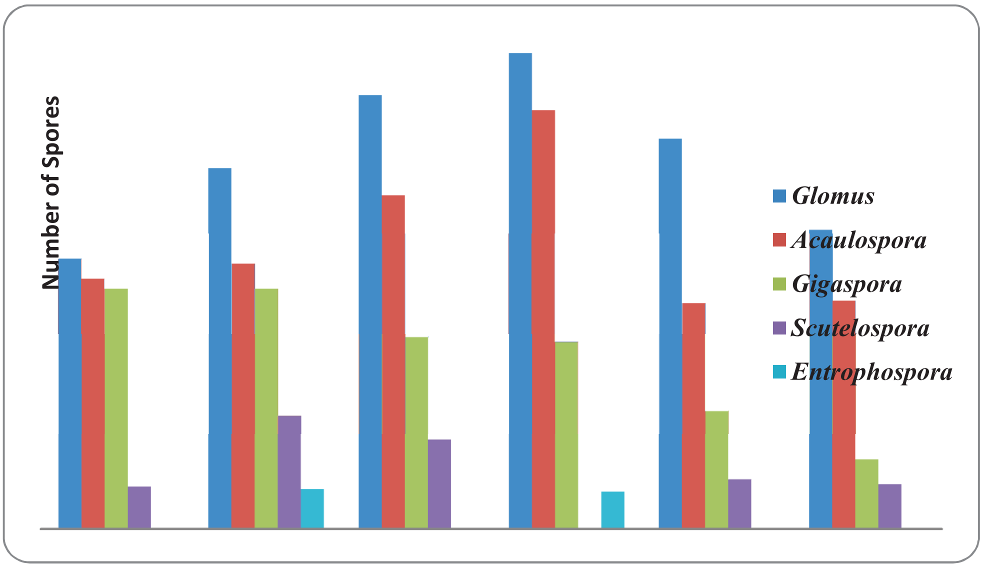

Figure 3. Number of VAM-spores (*Glomus, Acaulospora, Gigaspora, Scutelospora* and *Entrospora*) in cashew nut rhizosphere