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Spatial and Seasonal Distribution of Endomycorrhiza from Cashew Nut in Bali

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Abstract

Plants growing in poor soil areas are commonly associated with endomycorrhizalfungi as a mutualism symbiotic. The fungus takes a role on enhancement water from the soil and uptake soil mineral particularly Phosphorus, Magnesium and Potassium. Poor soil conditions at Bali Island are spatially located at Gerokgak-Buleleng Suburb (North-west of Bali) and Sukadana-Karangasem Suburb (North-East of Bali). One of the economical plants growing well at the regions is cashew nut plant. However, less study on the endomycorrizhal fungi associated with cashew nut plat has been undertaken. The objective of this study is to observe the spatial and temporal variation of VAM presence at the cashew nut planting area, consisting of number of spores and percentage of infection (colonization) at Cashew nut plant root. The number of spores was counted by wet sieving and decanting methods and the root infection were observed by means of Grid-line Intersect method. The result showed that the cashew nut plant at both regions live symbiotic mutually with VAM. The VAM spore density in cashew nut rizhospher shows either temporal or spatial variation. The spore density in Rizhospher tend to increase over dry season (June up to December) and decrease over raining season (April up to June and December up to February). The spore density is considerably higher in rizhosphere at Sukadana region than those at Gerokgak region. Such spatial variation seems likely related to the soil properties. The VAM colonization was found as vesicle, urbuscle, internal-external hyphae and spore forms. The VAM colonization shows similar temporal variation at both areas, it is considerably related to the temporal variation of rain fall level. Percentage of VAM colonization over raining season (April up to June and December up to February) is higher than over dry season (June up to December). Such VAM colonization, nevertheless, does not show spatial variation.

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Poor soil

Introduction

Endomycorrhiza is an order of Fungi that lives in close association with higher plants, providing them with additional nutrients such as Phosphorous and Nitrogen (Smith *et al.*, 2010). Especially the Arbuscular Mycorrhizal Fungi (AMF or VAM) are classical symbiotic mutualists between soil fungi and the absorbing organs of the vascular plants, in the benefit of both sides through the reciprocal exchange of minerals and organic resources (Johnson *et al.*, 1997, Smith and Read 1997, Brundrett *et al.*, 2008). Because of these benefits, over 70% of the vascular plants (Angiosperms and Gymnosperms) are living associated with mycorrhizal fungi (Deacon, 1997). Over 13 species and 4 Families of mycorrhiza have been discovered to date (Proborini *et al.*, 2013).AMF take a significant role in some aspects of plant survival in arid regions, promoting plant growth and increasing plant productivity (Delvian, 2008; Proborini, 2011). Consequently, they play an important role in maintaining the ecosystem processes by promoting plant fitness through a range of mechanisms (Brundrett *et al.*,2008), e.g. protecting the host plants from soil pathogens (Smith, 2000) and improving texture and structure of soil, enhancing water and nutrient uptake (Smith *et al.*, 2010). On the other hand, the species distribution of mycorrhiza depends on the associated plants as well as the soil composition, moisture, and number of nutrient in the soil (Smith, 2000). Bonfirm *et al.*, (2016) provideevidence the diversity of Arbuscular Mycorrhizal Fungi in a Brazilian Atlantic Forest. Similarly Renuka (2012) reported a seasonal dependency of mycorrhiza in tropical regions.

Cashew nut (*A. occidentale* L) is one of the important crops in arid regions, reaching a productivity of42 ton / ha (Witjaksono *et al*., 2002). This leads to an income of the farmers up to 543.000 US$ in a year (Biro Pusat Statistik, 2002). Beside cashew nuts, only few crops can be cultivated on these soils, leading very often to monoculture. In Indonesia, the cashew nut plant has been planted through the government for reforestation program especially in Bali, Sulawesi, Central of Java in Indonesia, because it is and well adapted to dry areas and growing well in these regions. Now-days, the cashew nut plant is one of the main sources of income for the local communities.

The cashew nut has been introduced to Bali in 1976 (Antara, 2004), and especially promoted during 1990 through the Governmental of Bali Province program. The arid regions in Bali Island are mostly in the Northern Bali region i.e. Western of Buleleng Suburb (i.e. Gerokgak region) and Western of Karangasem Suburb (i.e. Sukadana region). These areas are dominated by plant communities adapted to the dry regions, such as Cassava and Cashew nut Plants (Antara, 2004; Daryana, 2010; Proborini, 2011).

Balinese cashew nuts have been demonstrated to mutualize with 13 species of mycorriza by Proborini *et al.*, (2013). It is most likely that the good performance of Cashew nut plants in Bali depend on this symbiosis. So far, no studies on the special and regional distribution of the different mycorriza species in Bali have been carried out. This study analyses the occurrence of mycorriza communities associated with cashew nut plants over two different areas in the arid region of Bali, during the dry and rainy season.

Materials and Methods

Soil and plant root sampling

Soil and plant sample were taken from western of Karangasem Suburb (i.e. Sukadana region and Western of Buleleng (i.e. Gerokgak region). The regions are known as cashew nut plantation area. Sampling of soil and Cashew nut plant root were 2 monthly intervals from cashew nut plantation area from April of 2011 up to February 2012. Soil sampling method was adapted from Widiastuti (2004). The soil was taken from rizhospere soil at surrounded of cashew plant root ± 20 – 30 cm depth. Two composite sample bags (@Ca 2 kg) per site were collected from five sampling sites randomly chosen (totally 20 bags per sampling period).

Sample of root was *tertiary root* of young cashew nut plant (plant height *ca* 40 cm). Five individual plant of cashew nut were sampled from each sampling region (totally 10 individual of cashew nut plant per sampling period).

Percentage of plant root colonization

Percentage of plant root colonization was calculated by means of the method of Kormanik and Mc.Graw (1982) modified (Proborini, 1998). Plant roots sample were processed i.e. clearing, staining and destaining consecutively. The observation of VAM colonization in root cortex and endomycorrhizal hifa was carried out by means of dissecting-set and binocular microscopes. To count the percentage of mycorrrhizal colonization by using formula is as below:

$$\%\ \text{root colonization} = \frac{\sum \text{Vertical cross} + \text{horizontal cross hypha}}{\textit{Total root length examined}} \times 100\%$$

Isolation and spore density

The spore isolation from rizhosphere soil was carried out by the means of the method of Brundrett *et al.*, (2008) i.e. wet decantation method. Soil sample i.e. 250 gram soaked in 1 L of water (ratio of water: soil is 1:4) and steered. The supernatant was decanted in five consecutive sieves (AnalysensiebEckhardt 5657 Haan W. Germany) with top down mesh size 500 µm, 300 µm, 200 µm, 63 µmdan 45 µm consecutively. The spores found were observed under dissecting microscope. The total number of spore was counted and the spore was fixed in a glass bottle filled containing sterilized water.

Physical and chemical properties of soil

Soil was collected from cashew nut plantation area at either Sukadanaregion-Karangasem Suburb or Gerokgak region-Buleleng Suburb. Five bags of rizhosphere soil (@ 2 kg) were composite per sampling period. Soil sample was 2 monthly interval sampled over exploration study. The physico-chemical of soil properties were analysed at the Laboratory of Soil, Faculty of Agriculture University of Udayana and Technical Service of Analytical Laboratory University of Udayana.

Rain fall Frequency and rain water level

The data of rain fall frequency and the rain water level was obtained from BMKG Bali Regional. The data collected was for one year period i.e. April 2011 up to April 2012.

Results and Discussion

Rain fall frequency and rain water level

A data of rain fall frequency and rain water level over one year period (January 2011 up to February 2012) at both regions is presented at Figure 1 and 2. Though raining season seems longer at Sukadana region than at Gerokgak region, the data showed clearly similar temporal dynamic at both regions. Raining season occurs at April up to June and December up to February; Dry season is at June up to December.

Physico-chemical soil properties

The status of spatial and temporal of physico-chemical soil properties is presented at Table 1 and 2. The data showed clearly that, except K level, soil water level, C, N and P are higher at Gerokgak region than those at Sukadana region. These data depicts the soil status of Gerokgak region is overall better than those of Sukadana region. The soil water level at both regions fix to either the dynamic of rain fall frequency or rain water level. The soil water level of Sukadana region is lower than those of Gerokgak region, it likely related to the soil texture. Sandy soil textures tend to have lesser capacity to retain water than clay soil texture, because sandy soil has more pores than clay soil. High soil pores could also lead to high soil nutrients wash (C, N and P) at sandy soil compare to clay soil texture. Though some of the physico-chemical soil properties show difference spatially, overall Sukadana and Gerokgak regions could be classified as arid regions, leading to the conclusion that *A. occidentalle* plant at the regions is highly rely on to mutual symbiosis with VAM.

Endomycorrhizal spores density

A total of 120 sample bags were examined. Direct microscopic counting showed massive number of VAM spores at the regions over sampling period is presented at Figure 3. The spore density showed convincingly variation either spatially or temporally (Fig. 3).

Spatially, the spore density in the rizhospere of cashew nut plant (*A. occidentale* L) sampled from Sukadana region showed consistently more abundant than those sampled from Gerokgak region. Take account to the soil physico-chemical properties, such spore density variation confirm to the spatial variation of the soil properties i.e. texture, nutrient and water level (Table 1 and 2). Soil Water level is likely to be the most determinant factor affecting such spatial variation of spore density, water stimulates spore to germinate. There-fore spore density tends to decrease during rainy season because the spore will germinate to form hypha (Smith, 2000, Delvian, 2006 b). This study found that level of Phosphorous (P) and N level seem positive linear to the spore density. This is in-contrast to Khumar *et al.,* (2016) who showed negative linear of phosphorous availability and spore density. While, the finding on N level - spore density correlation agrees to the study of Gobharani *et al.,* (2012) and Sawant and Bhale (2016) who showed the VAM spore density supported by higher available N in soil. However, this finding does not fit to the study of Khumar *et al.*, (2016) who revealed that N soil content do not have any influence on spore density. The spore density is found high at Sukadana region having high pores soil texture i.e. sandy clay. This is in contrast to the study Okiobe *et al.*, (2015) who showed Clay-sand mixture (2: 1) to have more spore density. Nevertheless, soil pore is likely to exert positive role on VAM spore formation.

Temporally, the dynamic of spore density at both regions are clearly fixed to the temporal dynamic of rain fall frequency and water level. The figure showed opposite trend between spore density and the rain fall frequency and water level, the spore number tend to increase when rain fall frequency and water level decrease (June up to December) and *vice versa* (April up to June and

December up to February). Spore is resulted by hypha of Mycorizhal fungi and spore formation is one of the mechanisms of self-defense when environmental condition does not support the development of hypha. This finding fit to Smith (2000) and Delvian (2006 b), the existence of endomycorrhiza spore in the nature is season related, the spore number tends to decrease during rainy season because the spore will germinate to form hypha and the hypha will grow to find out soil or supply water over dry season.

Fig.1 Graphic of rain fall frequency and average of water level at Sukadana region

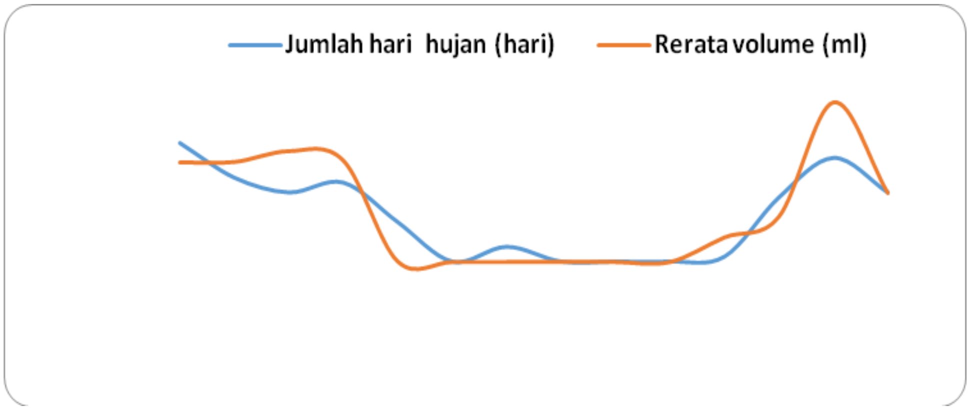

Fig.2 Graphic of rain fall frequency and average of water level at Gerogak region

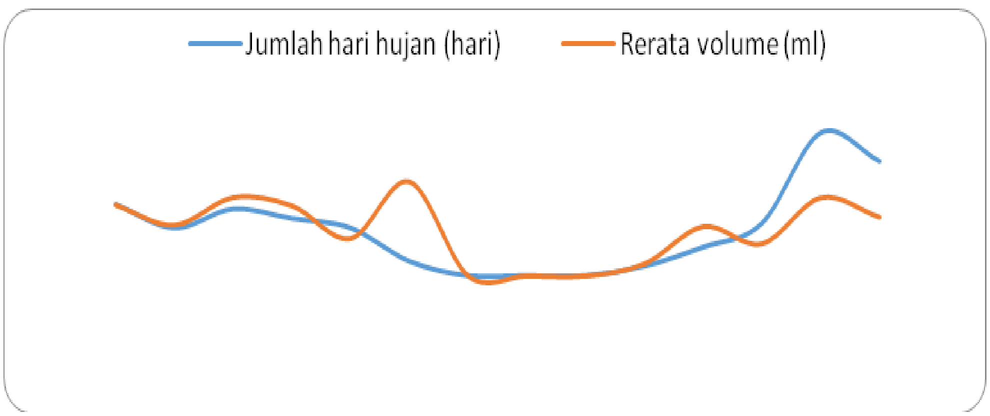

Fig.3 Temporal and spatial of spore density in rizhosphere of Cashew plants

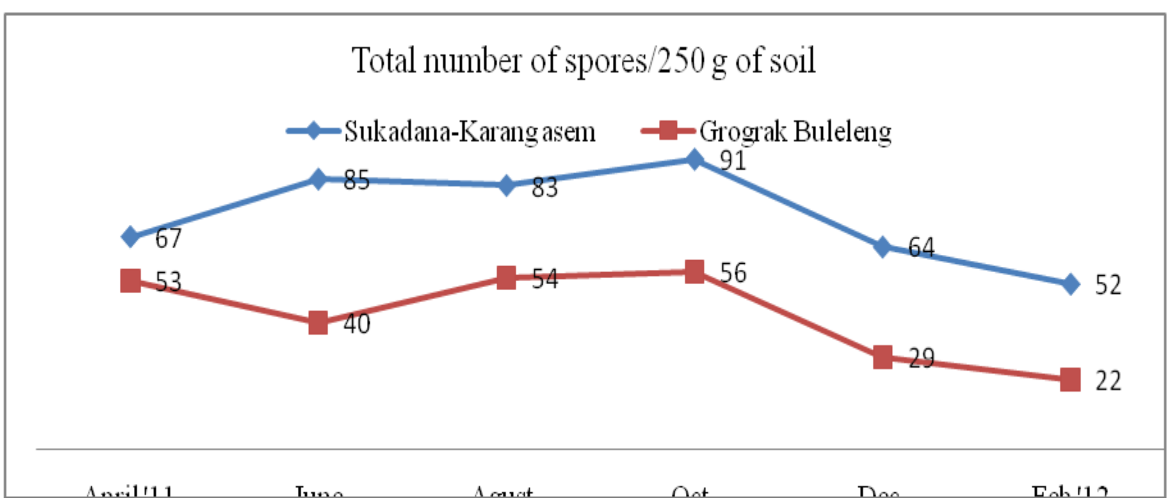

Fig.4 Spatial and temporal variation of average percentage of endomycorrhiza

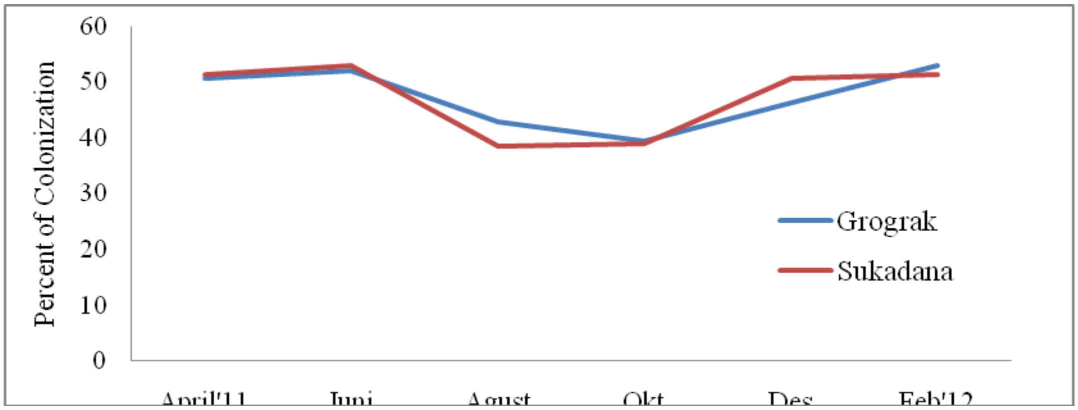

Fig.5 AMF colonization in the root (Binoculer microscope 100x) (Proborini, 2013)

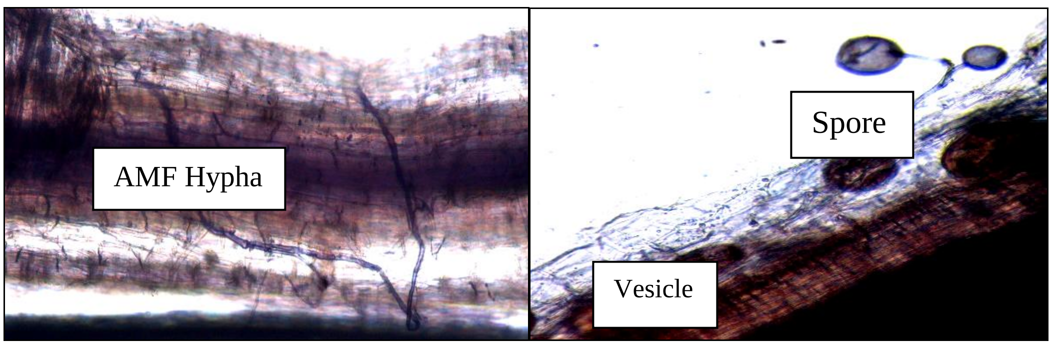

Table.1 the analysis of nutrients on the soil from Gerogak and Sukadana region

	Grograk-Buleleng					Sukadana-Karangasem				
	pH	C-organic (%)	Total N (%)	K-available (ppm)	P-available (ppm)	pH	C-organic (%)	Total N (%)	K-available (ppm)	P-available (ppm)
April'11	6,1(SA)	0,79 (VL)	0,05(VL)	79,65 (VH)	53,54(VL)	6,8(N)	1,28(L)	0,12(L)	61,60(VH)	662,07(VH)
June	6,8 (N)	0,78(VL)	0,06(VL)	102,25(VH)	87,44(L)	6,8(N)	1,26(L)	0,18(L)	48,85(VH)	665,22(VH)
August	6,7(N)	0,79(VL)	0,05(VR)	64,10(VH)	51,34(VL)	6,9(N)	0,70(VL)	0,16(L)	69,04(VH)	774,11(VH)
Oct	6,9(N)	0,78(VL)	0,13(L)	111,51(VH)	54,24(VL)	6,5(N)	0,42(VL)	0,15(L)	68,10(VH)	525,26(VH)
Dec	6,5(N)	0,69(VL)	0,08(VL)	98,67(VH)	56,67(VL)	6,2(SA)	0,52(VL)	0,13(L)	66,67(VH)	520,21(VH)
Feb'12	6,3(SA)	0,66(VL)	0,07(VL)	92,46(VH)	54,46(VL)	6,7(N)	1,25(L)	0,10(L)	63,42(VH)	512,34(VH)

Table.2 The analysis of water content and soil texture from Sukadana and Gerogak region

	Sukadana-Karangasem					Grograk-Buleleng				
	Water content		Soil Texture			Water content		Soil Texture		
	KU (%)	KL (%)	Sand (%)	Dust (%)	Clay (%)	KU (%)	KL (%)	Sand (%)	Dust (%)	Clay (%)
April'11	1,03	11,98	88,82	8,32	2,86	9,41	23,69	35,87	46,74	17,40
			Sand					clay		
June	0,62	13,55	85,46	10,90	3,64	7,92	30,69	30,68	50,37	18,95
			Sandy-clay					clay-sandy		
August	0,81	12,07	86,22	10,66	3,12	8,98	31,24	28,29	56,64	15,07
			Sandy-clay					clay-sandy		
Oct	0,45	11,78	84,16	13,50	2,34	8,30	29,16	37,94	45,44	16,62
			Sandy-clay					clay		
Dec	0,86	12,86	85,36	13,31	2,33	9,23	24,87	35,87	46,74	17,40
			Sandy-clay					clay		
Feb'12	0,94	12,22	85,92	10,71	3,27	8,76	23,76	34,87	47,74	17,40
			Sandy-clay					clay		

Percentage of root colonization

A total of 120 individual samples examined showed clearly the colonization of VAM in the root of cashew nut plant (Figure 5). The average percentage of endomycorrhizal colonization in the samples from both sampling regions is presented at Figure 4. The root colonization showed convincingly temporal dynamic but not spatially (Fig. 4).

This study, based on spatial observation of vesicle, arbuscule and mycelia in *A. occidentalle* root**,** the root colonization at both regions varries from 38.38 % up to 52.94. This finding confirms the high level symbiosis between VAM and *A. occidentalle at the regions.* The finding of this study also showed that despite difference in soil properties in the regions studied, no significant difference in root colonisation.High root colonisation at Gerokgak region can be addressed to the low of Phosphorous availability. One adaptation of plant to low Phosphorous availability is symbiosis with VAM, the fungi re-mineralizes Phosphorous and delivers Phosphate (P) and other minerals in exchange for carbohydrate (Smith *et al.*, 2010). Though Phosphat (P) availability is very high at Skadana region that could supress the VAM colonisation, it is likely that the low level of N overruled Phosphat supressive (Carbonnel and Gutjahr, 2014).

The result of temporal dynamic of root colonisation showed similar trend at Sukadana and Gerokgak regions, the trend fits to the dynamic of rail fall frequency and rain water level. The root colonisation tend to high over raining season(April up to June 20111 and December up to February 2012) and decrease gradually over dry season (june up to December 2011).

This dynamic of root colonisation is likely related to again soil water level, water stimulate VAM spore to germinate and form hypha that immediately colonize plant root (Chalimah *et al.*, 2007). The result also agrees with Delvian (2006b) dan Smith *et al.,* (2010), hypha colonization is season related. Such colonization facilitate endomycorrhyza gets benefit from the host plant i.e. glucose (Hapsoh, 2008). While the host plant gets some benefits from endomycorrhiza colonization i.e. the endomycorrhiza takes a role on re-mineralization of Phosphorous and providing Phosphate (P) in soil (Smith *et al.*, 2010), on water absorption and mineral uptake (Renuka *et al.*, 2012) and on producing plant growth hormone-like similar to auxin, cytokine and Gyberelin (Imas *et al.*, 1989).

Recall all explanation above, it can be concluded that *A. acidentalle* at Sukadana and Gerokgak regions have mutual symbiosis with VAM fungi. Nevertheless, in accordance with soil properties difference, the VAM seems to take role on water supply and soil mineral delivery (i.e. N) at Sukadana region and to take a role on soil minerals delivery (i.e. N and P) at Gerokgak region.

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