INTERNATIONAL JOURNAL OF BIOSCIENCES AND BIOTECHNOLOGY • Vol. 5 No. 2 • April 2018
ISSN: 2303-3371
https://doi.org/10.24843/IJBB.2018.v05.i02.p09

SPORE PROPAGATION OF INDIGENOUS ENDOMYCHORIZA FROM SEVERAL ROOTING AREAS OF SNAKE FRUIT ON DIFFERENT SOIL WATER CONTENT

I Nyoman Rai[1]*, I Ketut Suada[1], M. Praborini[2], and I Wayan Wiraatmaja[1]

[1]Departement of Agroecotechnology, Faculty of Agriculture, Udayana University
[2]Departement of Biology, Faculty of Mathematics and Natural Sciences, Udayana University
**Corresponding author: rainyoman@unud.ac.id*

ABSTRACT

Cultivation of organic snake fruit in Bali which is done on dry land with the irrigation depends on rainfall and the fertilization generally only uses uncertain amount of fallen leaves, it reduces the productivity, quality, and continuity of fruit production over time. In rhizosphere of snake fruit there are various types of indigenous endomycorrhiza that potentially can be developed as biofertilizer, but as a biofertilizer, the low number of spores population for inoculum becomes a limiting factor in using indigenous endomycorrhiza. The effort that can be done are to propagate the spores by giving water stress treatment. This study aimed to obtain the best rhizosphere location of snake fruit as the source of spores and the level of soil water content to multiply the spores. The research was conducted in the green House, Farm Station, Agriculture Faculty, Udayana University at Jalan Pulau Moyo, Denpasar, from October 2017 to January 2018. The spore propagation used nested experiment with Randomized Block Design patterns. The treatments were rhizosphere location as the source of indigenous endomycorrhizas spore consists of three levels (Bebandem District Karangasem Regency, Payangan District Gianyar Regency, and Pupuan District Tabanan Regency) and soil water content consists of three levels (100%, 70%, and 40% field capacity). The results of the research showed that the source of indigenous endomychorrizae from snake fruit rhizosphere in Payangan District gave the highest number of spores found and the highest percentage of the spore increase after propagation. While in the soil water content treatments, the highest number of spores found and the percentage of the spore increase after propagation were obtained at soil water content of 40% field capacity. The percentage of root infections from different sources of indigenous endomychorrhizae and different levels of soil water content is same i.e 100%.

Keywords: Organic snake fruit, moisture content, spores, indigenous endomychorriza, biofertilizers.

INTRODUCTION

Organic snake fruit cultivation in Bali has done on dry land with irrigation depends on rainfall, whereas fertilization generally only uses uncertain amount of fallen leaves and unplanned (Sukewijaya et al., 2009). The impacts of this way of cultivation caused the N, P, and K leaf nutrient content of snake fruit were very low. It's indicated by the C-organic content, N, P, and K soils status are very low (Rai *et al*. 2010). If this condition ignored, the productivity, quality, and continuity of organic snake fruit production in Bali will be decreasing over time.

The development of mycorrhiza as biofertilizer is expected can be solved the problem that faced by the farmers in cultivating of organic snake fruits.

Mycorrhiza is a fungus that lives based on the mutualism symbiotic between funguses (myces) with root (rhiza) of the plants. Mycorrhiza infects plant roots but does not harmful to the plant, but mutual reciprocity. The host plant obtains nutrients and water from mycorrhiza while the mycorrhiza obtains carbohydrates or foodstuffs from host plant (Menge, 1985; Finlay, 2008; Zasvari et al., 2012; Sarah and Ibrar, 2016).

Based on the way of infection, mycorrhiza is divided into two groups, there are ectomycorrhiza and endomycorrhiza. Ectomycorrhiza infects the surface of the plant roots especially between root tip cells that covered by soil. As a result of the infection, white hyphae tissue formed in the hairs of the roots, known as Hartig nets. The infection causes root changes morphologically, root shortening, swelling, branching of the dichotomes, and form pigments. The host plants for ectomycorrhiza are generally forestry crops (Smith and Read, 2008; Finlay, 2008; Brundrett, 2009). While the endomycorrhiza infects inner part of root tip cells. The hyphae from outside the roots enter the cells and fill the spaces between cells, forming arbuscules, i.e the hyphae tissue that penetrates the sidelines of the cells through plasmalemma. Besides, the inner cells of hyphae will form vesicles, i.e a small bubble in the form of granules in lipid-containing cytoplasm developed to be vegetative reproductive apparatus. Vesicles and arbuscules on endomycorrhiza expand the space caused by nutrient uptake by the roots. Endomycorrhizal infections do not cause root changes morphologically but alter the appearance of cells and root tissue. The most host plants for endomycorrizha are important agricultural commodities, such as food crops (beans, rice, corn, etc.), horticultural crops (fruits, vegetables, and ornamental plants), and industrial plants (cocoa, rubber, coffee, cashew, etc.) (Schenck and Perez, 1990; Aguilar and Barea, 1997; Hempel *et al*., 2007; Smith and Read, 2008; Brundrett, 2009). Based on the natural characteristic of endomycorrhizal which most of its host is the agricultural crop, the development of biofertilizer from mycorrhiza is directed from this fungus (Wang and Yong Shi, 2008; Sadhana, 2014)

Rai *et al*. (2015) obtained that snake fruit cv. Gula Pasir fertilized with endomycorrhiza biofertilizer (purchased from IPB, Bogor) not only increase fruit production per tree but also produce off-season fruits. Endomycorrhiza biofertilizer which had applied increases the soil fertility, improves the process of photosynthesis that effected on the increase of the total sugar, reducing sugar, leaf sucrose content, and significantly increase the N, P, K, and Mg leaf tissue content. A similar result was obtained by Juliadewi *et al*. (2014) that snake

fruit cv. Gula Pasir fertilized with endomycorrhiza biofertilizer with the dose of 75 g/tree increase fruits number and fruit weight per tree and produce off-season fruits. It was related to increasing of the percentage of the fruit-set (flower to be fruit) on the plants that received endomycorrhiza biofertilizer compared to control, i.e 77.22% in the "*Gadu*" season and 85.98% in the "*Sela*" season, and it was positively correlated with longer and more extent of infected roots by endomycorrhiza so that their ability to absorb water and nutrients greater.

Currently, various brands of endomycorrhiza biofertilizer packaging in the form of powders, granules, or pellets ready for application have been available. However, according to Brundrett (2009) and Jha and Kumar (2011), indigenous endomycorrhiza or local species (taken from local site) are much more adaptive and effective because hyphae/mycelium and/or fungal spores are more adaptable, better and more optimal in colonizing the in the root system of the host plants. The potentiality is also much better because of the diversity of the indigenous endomycorrhizas, also called Vesicular Arbuscular Mycorrhizal (MVA) fungus in nature is very large (Hempel *et al*., 2007; Wang and Yong Shi, 2008; Avio *et al*., 2009; Baslam *et al*., 2011; Proborini, 2013; Suamba *et al*., 2014). Rai *et al*. (2017) have successfully identified two types of indigenous endomycorrhizas in soil rhizosphere (rooting areas) of snake fruit plants, i.e *Glomus* and *Acaulospora*. The root infection percentages of both types on the snake fruit are very high, i.e 96.67% but the average population of the spore in the soil was very low, those are only 20 spores/100 g of soil. Thus, to obtain sufficient quantities of spores as inoculums in making biofertilizers it is necessary to find the method/technique of propagation.

The abundance and activities of the mycorrhiza in the soil rhizosphere layer is largely determined by the environmental factors, especially degree of drought (Schubler *et al*., 2001; Moreira *et al*., 2007; Hernadi, 2012; Sadhana, 2014). Fertile soil with enough water content decreases the infection rate and effectiveness of mycorrhiza, otherwise drought increase degree of root infection and effectiveness of N, P and K nutrient uptake by mycorrhiza (Allen and Boosalis, 1983; Tahat and Sijam, 2012; Kavitha and Nelson, 2013; Mo *et al*., 2016). Manurung *et al*. (2015) showed, soil water content on 80% and 60% field capacity causing dry weight of rubber seeds inoculated by endomycorrhiza genus *Acaulospora* was significantly higher than that of on 100% field capacity, due to on 80% and 60% field capacity increase of colonization/root infection and nutrient uptake of N and P. Similar results was expressed by Quiroga *et al*. (2017) that the

rate of colonization of mycorrhiza increase significantly in dry conditions and this caused corn crops to be more resistant to drought. Leal *et al*. (2016) reported that the number of endomycorrhiza spores of genus *Glomus* and *Acaulospora* in Eucalyptus camaldulensis suffer drought increase more than 300-fold compared to controls. According to Mathimaran *et al*. (2017), moderate drought caused colonization of arbuscular mycorrhizae increase sharply with increase root extending ability up to 80%, but at severe dryness growth rate of plant and ability of colonization decrease significantly.

Based on the above description, to obtain sufficient quantity of spores as a source of inoculant in making biofertilizer for snake fruit plant is needed to conduct research concerning isolation of indigenous endomycorrhiza from rooting area of snake fruit and do multiplication trial on different levels soil water content to find out the most effectiveness method to propagate spore of indigenous endomycorrhiza.

MATERIALS AND METHODS

The research was conducted at Green House, Farm Station, Faculty of Agriculture, Udayana University at Jalan Pulau Moyo, Denpasar, from October 2017 to January 2018.

This propagation trial of indigenous endomycorrhiza used nested experiment with Randomized Block Design patterns. The treatments were rooting area location as the source of spore of indigenous endomycorrhiza (RAL) and soil water content (W). The rooting area location as the source of spore of indigenous endomycorrhiza consists of three levels, namely rooting area of snake fruit in Bebandem District, Karangasem Regency (RAL_B), Payangan District, Gianyar Regency (RAL_Y), and Pupuan District, Tabanan Regency (RAL_P). Meanwhile, soil water content consists of three levels, i.e 100%, (W_1), 70% (W_2), and 40% field capacity (W_3). Calculation of the field capacity soil water content is done by watering the experimental pot containing media with excess water (watering volume is recorded) then left for one day. Dripping water from excess watering is accommodated in the container, then field capacity soil water content calculated by the volume of watering minus the volume of water drip.

Materials used include corn seeds "Ketan variety" as the host plant, aquades, 60% glucose, 10% KOH, 3% H_2O_2, 1% HCl, lactoglycerol, and trypan blue. Required tools include scissors, stereo microscope, compound microscope, centrifuge machine, centrifuge tube, ose needle, petri dish, autoclave, object glass, glass cover, and a set of sieve spores with hole diameter 1 mm, 500 μm, 212 μm, 106 μm and 53 μm.

Spore isolation, counting the number of spores, and root infection were done at Laboratory of Genetic Resources and Molecular Biology, Udayana University. The soil samples from rhizosphere rooting area which will be isolated were taken from around stem of snake fruit tree (30 cm distance from the base of the stem and 0-30 cm depth from the soil surface), at 3 sites of the source of indigenous endomycorrihza treatment. Spore isolation was done using wet screening technique followed by centrifugation technique according to Brunndret *et al*. (2009). The soil sample of 100 g was dissolved in 1,000-1,200 ml of water and stirred evenly, then filtered in a set of filtration with diameter holes size from small to large i.e 1 mm, 500 μm, 212 μm, 106 μm, and 53 μm, respectively. The dissolution and filtration were repeated 2-3 times, and then the rest of the soil was poured on the top filter. The top filter was then sprayed with tap water to facilitate the filtered material to pass. After filtering process on the top screen was completed, then proceed to the second filter, third, and so on. The remaining soil in 500 μm, 212 μm, 106 μm and 53 μm sieves were transferred to centrifuge tubes, then aquades were added 25-40 mL and centrifuged at 2,000 rpm for 5 min. The supernatant of the centrifuge process was thrown away and 60% glucose was added. The centrifuge tube was sealed and then centrifuged at 2000 rpm for 1 minute. After 1 min, the sugar-containing supernatant in each sieve was rinsed with water on the sieve with a diameter of 53 μm and the result placed in a petri dish then the spores are isolated. The isolated spores were placed on the carrier, mixture of zeolite and quartz sand, which has been sterilized.

The implementation of indigenous endomycorrhiza propagation experiment was conducted according to Brundrett *et al*. (2009) method, but modified using mixed media consisting of zeolite, quartz sand, and soil, which had previously been sterilized by heating at 121 °C in the autoclave for 30 minutes. The order of placement of propagation media (zeolite, quartz sand, and soil) in plastic pots was at the bottom layer filled 250 g composite of zeolite and quartz sand which has been given 20 spores per pot; at the middle layer put 500 g of sterilized soil only; and at the top layer put 50 g composite zeolite and quartz sand as the cover. After that, plastic pots were planted with corn seeds as host plants. From planting until the age of 2 weeks the plants were watered up to field capacity, after that irrigated according to soil water content treatments. At the age of 6 weeks from planting, shoot of plants was cut (topping) a half part of the height, aimed to stimulate indigenous endomycorrhiza form much more spores. By toppings, host plants and indigenous endomycorrhiza will run into stress, then the host plant died and

indigenous endomycorrhiza tried to defend itself where its hyphae shrunk and form spores. One week since topping, the died host plants were harvested and the number of spores on media be counted.

The observed variables were the number of spores, percentage increase number of spores, root infection or percentage of root colonization of host plants, and growth of host plant includes the number of leaves, plant height, stem diameter, dry-oven shoot weight, dry-oven root weight and dry-oven total weight of plants per pot.

Counting number of spore result of propagation was done by observing the isolates under the microscope, while the percentage increase number of spores was calculated by the number of spores reduced by the number of initial spores (20 pieces per pot) divided by the number of initial spores.

Calculating percentage of root colonization was done using slide method according to Giovannetti and Mosse (Nurhandayani et al., 2013) by the formula: percentage of the infected root is the number of infected roots divided by the total of all roots observed multiplied by 100%. Levels of root infection are classified into 5 classes, i.e very low (root infection 0-5%), low (root infection 6-25%), moderate (root infection 26-50%), high (root infection 51- 75%), and very high (root infection 76-100%). Observation of root infection was done by root staining with trypan blue. Root staining was proceded by root washing until clean and then roots at one point were cut 2-5 cm length. After that, 20 pieces of 2-5 cm root length were put into the test tube; added 10% KOH until all part of roots were submerged, heated at 250 °C for 10 minutes in the microwave and then stored for ± 12 hours at room temperature. After 12 hours KOH was removed by washing the roots with tap water (washing were done 3 times) then added 3% H_2O_2 and stored for 12 hours at room temperature. After 12 hours H_2O_2 was removed by washing the roots with tap water (washing were done 3 times), then added 1% HCl and stored for 12 hours at room temperature. After 12 hours HCL was thrown away, added trypan blue, heated at 250 °C for 5 minutes in the microwave and then stored for 12 hours at room temperature. Then, trypan blue was removed, added lactoglycerol and heated at 250 °C for 5 minutes on the microwave for 12 hours at room temperature. Finally, the roots were taken with tweezers, placed in a row and observed under the microscope to calculate the presence or absence of infection and saw the mycorrhizal structure (vesicles, arbuscules, and hyphae)

Plant height and stem diameter were measured shortly before the host plant were

topped, dry-oven shoot weight was the sum of topping shoot and the remaining part of the shoot after topping at harvest, dry-oven root weight was measured after harvesting, while dry-oven total weight was sum of the dry-oven shoot weight and dry-oven root weight.

The collected data was analyzed using Analysis of Variance (Anova). If there was a significant difference between treatments then tested further with Least Significance Different (LSD) Test.

RESULTS AND DISCUSSION

The result of variance analysis showed that rooting area location as the source of spore of indigenous endomycorrhiza (RAL) had significant different effect on number of spores and percentages increase the number of spore after propagation, while on root infection and growth of host plants were non significant different effect. The level of soil water content in each source of indigenous endomycorrhiza location was very significant or significant different effect on almost all of the observed variables, except for the root infection was not significant (Table 1).

The most number of spores after multiplication was obtained on RAL_Y i.e 223.84 units and significantly different than that of from RAL_B and RAL_P with the number of spores 82.33 units and 121.62 units, respectively (Table 2). Thus, with the number of initial spores given on the medium was same i.e 20 spores per pot, its mean that the highest increase number of spore after propagation was obtained on RAL_Y 1,019.22%, whereas on RAL_B and RAL_P was 311.67% and 508.08%, respectively. These data indicated that in the manufacturing of indigenous endomycorrhiza biofertilizer, spore propagation source should be taken from rooting area of snake fruit from Payangan District (RAL_Y).

Table 1. The significance of the effect of rooting area location as the source of spore of indigenous endomycorrhiza and soil water content treatment to the observed variables

No.	Variables	RAL	W		
			RAL_B	RAL_Y	RAL_P
1.	Number of spore after propagation (unit)	**	**	*	*
2.	Percentage increase number of spore after propagation (%)	**	*	*	*
3.	Percentage of root infection (%)	ns	ns	ns	ns
4.	Number of leaf of host plant (unit)	ns	*	**	**
5.	Height of host plant (cm)	ns	**	*	*
6.	Stem diameter of the host plant (cm)	ns	*	*	*
7.	Dry-oven shoot weight of host plant (g)	ns	**	**	**
8.	Dry-oven root weight of host plant (g)	ns	**	**	**
9.	Dry-oven total weight of host plant (g)	ns	**	**	**

Description: RAL = rooting area location as the source of spore of indigenous endomycorrhiza, RAL_B = Bebandem, RAL_Y = Payangan, RAL_P = Pupuan, ** = very significant different on F Test 1%, * = significant different on F Test 5%, ns = non significant different.

Table 2. The number of spores, percentage increase number of spores and root infections after propagation due to influence by rooting area location as the source of spore of indigenous endomycorrhiza and soil water content treatment.

Treatments	Number of spores after propagation (unit)	Percentage increase number of spore after propagation (%)	Percentage root infection (%)
RAL			
RAL_B	82.33 b	311.65 b	100 a
RAL_Y	223.84 a	1.019.20 a	100 a
RAL_P	121.62 b	508.08 b	100 a
LSD 5%	49.60	448.00	ns
RAL_B			
100% FC (W_1)	47.33 b	136.65 b	100 a
70% FC (W_2)	73.33 ab	266.65 ab	100 a
40% FC (W_3)	126.33 a	531.65 a	100 a
RAL_Y			
100% FC (W_1)	193.73 b	868.65 b	100 a
70% FC (W_2)	222.33 ab	1.011.65 ab	100 a
40% FC (W_3)	235.47 a	1.077.35 a	100 a
RAL_P			
100% FC (W_1)	76.60 b	283.00 b	100 a
70% FC (W_2)	80.60 ab	303.00 ab	100 a
40% FC (W_3)	108.80 a	444.00 a	100 a
LSD 5%	29.15	159.75	ns

Description: - In the same column, the numbers followed by the same letter for the treatment of auxin type and concentration of each type of auxin showed no significant effect on the level of 5% LSD.
- FC = Field Capacity

Although number of spores and percentages increase of number of spores after multiplication were significantly different among RAL, but to growth of host plant (maize) the effect of RAL was not significantly different, indicated by leaf number, plant height, stem diameter, dry-oven shoot weight, dry-oven root weight, and dry-oven total weight (Table 3). It was probably related to root infection among RAL was not significantly different namely 100% at all RAL. Root infections 100% indicated that isolated indigenous endomycorrhiza from all location rooting area of snake fruit was able to interact well with maize, and the infected root increase the development of hair roots which leads to high nutrient uptake and overall growth of host plant was not significantly different among source of indigenous endomycorrhiza sites. Beltrano *et al*. (2013) and Sarah and Ibrar (2016) suggested that the density of spores and colonization of host roots is largely determined by the compatibility of mycorrhiza with host plants, environmental factors, and interaction between mycorrhiza

and chemical compounds produced by host plants.

Table 3. The number of leaves, plant height, stem diameter, dry-oven shoot, dry-oven root and dry-oven total of host plant due to influence by rooting area location as the source of spore of indigenous endomycorrhiza and soil water content treatment.

Treatments	Number of leaves (unit)	Plant height (cm)	Stem diameter (cm)	Dry-oven shoot weight (g)	Dry-oven root weight (g)	Dry-oven total weight (g)
RAL						
RAL_B	10.00 a	16.63 a	0.70 a	3.47 a	0.51 a	3.98 a
RAL_Y	8.70 a	11.28 a	0.59 a	4.59 a	0.55 a	5.14 a
RAL_P	10.58 a	13.45 a	0.63 a	3.55 a	0.41 a	3.96 a
LSD 5%	ns	ns	ns	ns	ns	ns
RAL_B						
100% FC (W_1)	10.33 a	22.79 a	0.72 a	3.19 a	0.70 a	3.89 a
70% FC (W_2)	9.94 ab	17.19 b	0.73 a	2.67 a	0.65 a	3.22 a
40% FC (W_3)	9.72 b	9.91 c	0.64 b	1.55 b	0.30 b	1.85 c
RAL_Y						
100% FC (W_1)	9.22 a	12.96 a	0.72 a	5.77 a	0.71 a	6.48 a
70% FC (W_2)	8.78 b	10.79 ab	0.54 b	4.23 b	0.58 ab	4.81 b
40% FC (W_3)	8.11 c	10.10 b	0.52 b	3.78 b	0.36 b	4.14 b
RAL_P						
100% FC (W_1)	12.00 a	14.67 a	0.69 a	4.56 a	0.62 a	5.18 a
70% FC (W_2)	11.11 b	14.22 ab	0.67 ab	3.17 b	0.46 ab	3.62 b
40% FC (W_3)	11.11 b	12.27 b	0.61 b	2.69 b	0.21 b	2.90 b
LSD 5%	0.54	2.19	0.07	0.79	0.32	0.77

Description: - In the same column, the numbers followed by the same letter for the treatment of auxin type and concentration of each type of auxin showed no significant effect on the level of 5% LSD.
- FC = Field Capacity

In the treatment of soil water content, the lower soil water content from field capacity the higher number of spore and percentage increase number of spores was obtained after propagation. it occurred at all RAL. Table 2 showed that the highest number of spores and percentage increase number of spores after propagation at RAL_B were obtained at soil water content of 40% FC (126.33 units and 531.65%) and the lowest was obtained at 100% FC (47.33 units and 136.65%). At RAL_Y the highest number of spores and percentage increase number of spores after propagation were also obtained at soil water content of 40% FC (235.47 units and 1,077.35%) and the lowest was obtained at 100% FC. Similarly, at RAL_P the highest number of spores and percentage increase number of spores after propagation were also obtained at soil water content of 40% FC (108.80 units and 444.00%) and the lowest at 100% FC (76.60 units and 283.00%). The number of spores and percentage increase number of spores between soil water content

of 100% FC and 70% FC were not significantly different, either at RAL_B or RAL_Y and RAL_P. These data indicated that soil water content greatly affects the ability of indigenous endomychorrhizae to multiply. The more decrease of soil water content from 100% FC the more increase number of spores was obtained. The most increased number of spores at soil water content of 40% FC compared to 100% FC namely reached 1,077.35% was obtained at RAL_Y while that of at RAL_B and RAL_P was subsequently 531.65% and 444.00%. The results of this study were in accordance with the result of research by Schubler *et al*. (2001) and Sadhana (2014) that the abundance and activity of the mycorrhiza in the soil rhizosphere is greatly determined by the degree of drought. Quiroga *et al*. (2017) also got that drought increases the number of spores in roots of maize and drought tolerant cultivars was higher in colonization than drought-sensitive cultivars. Similarly, Leal *et al*. (2016) reported that the number of endomychorhiza spores of the genus *Glomus* and *Acaulospora* in *Eucalyptus camaldulensis* suffer drought increased more than 300-fold compared to controls. Based on the results of this study, to obtain enough number of spores to make biofertilizer, soil should be taken from rooting area of snake fruit in Payangan (RAL_Y, then multiplication by giving soil water content of 40% FC (W_3).

Table 3 showed that the best growth of maize crops was obtained at 100% FC soil water content, while the worst at 40% FC, either at RAL_B or at RAL_Y and RAL_P. At RAL_B, the lowest dry-oven total weight, dry-oven root weight, and dry-oven shoot weight per pot were obtained at 40% FC i.e 1.85 g, 0.30 g, and 1.55 g, respectively, and significantly different from those of at 100% FC i.e 3.89 g, 0.70 g, and 3.19 g, respectively. Similarly, at RAL_Y and RAL_P the lowest dry-oven total weight, dry-oven root weight, and dry-oven shoot weight per pot were obtained at 40% FC, subsequently 4.14 g, 0.36 g, and 3.78 g at RAL_Y, and subsequently 2.90 g, 0.21 g and 2.69 g at RAL_P. Those values at 40% FC were significantly different to those of at 100% FC but not significantly different to those of at 70%. The decreasing of dry-oven total weight of corn on 70% and 40% FC related to decreasing the number of leaves at the treatment, either at RAL_B or at RAL_Y and RAL_P. The declining of leaf number caused photosynthesis process goes poorly and it caused the growth of plants disturbed so the host plant height, stem diameter, and accumulation of dry weight at soil water content of 70% and 40% FC decreased. This was different compared to Manurung *et al*. (2015) that soil water content of 80 and 60% field capacity causes the dry weight of rubber seeds inoculated by endomychorrizae genus

Acaulospora significantly higher than that of in 100% field capacity.

When viewed the relationship between the number of spore increase after propagation with host plant growth, data of this study showed that decrease of soil water content from 100% FC to 70% FC and 40% FC caused increase the number of spores but decline the growth of host plant. Whereas, according to Mathimaran *et al*. (2017) moderate drought causes colonization of arbuscular mycorrhizaa increased sharply with an increase in root extension up to 80% but in severe dryness plant growth and the ability of colonization of mycorrhizal also decreased significantly. The high increase number of spore at 40% FC soil water content compared to 100% FC were 531.65%, 1,077.35%, and 444,00% subsequently in RAL_B, RAL_Y and RAL_P indicated that adaptation capability of indigenous endomychorrizae which taken from rooting area to drought was very high. The high adaptability on multiplied the number of spores has not been matched by its ability to prevent the decrease of host plant growth predicted due to the research being conducted in small pots so that high amounts of colonization and high number of mycorrhizae can not able to expand for getting water or nutrient because the rhizosphere zone is restricted just in pots.

ACKNOWLEDGMENTS

The author would like to express his profuse thanks to the Ministry of Research, Technology and Higher Education of Republic of Indonesia which has funded this research through the Project of National Competitive Grant 2017.

REFERENCES

Aguilar, C., & Barea, J. M. (1997). Applying Mycorrhiza Biotechnology to Horticulture: Significance and Potentials. *Scientia Horticulturae*, 68 (1), 1-24.

Allen, M. F., & Boosalis, M. G. (1983). Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *New Phytologyst*, 93, 67-76. Doi: 10.1111/ j.1469-8137.1983.tb02693.x.

Avio, L., Cristani, C., Strani, P., & Giovannetti, M. (2009). Genetic and Phenotypic Diversity of Geographically Different Isolates of Glomus Mosseae. *Canadian Journal of Microbiology*, 55, 242–253.

Baslam, M., Garmendia, I., & Goicoechea, N. (2011). Arbuscular Mycorrhizal Fungi (AMF) Improved Growth and Nutritional Quality of Greenhouse-Grown Lettuce. *Journal of Agricultural and Food Chemistry,* 59(10), 5504-5515.

Beltrano, J., Ruscitti1, M., Arango, M. C., & Ronco, M. (2013). Effects of Arbuscular Mycorrhiza Inoculation on Plant Growth, Biological and Physiological Parameters and Mineral Nutrition in Pepper Grown under Different Salinity and P Levels. *Journal of Soil Science and Plant Nutrition,* 13(1), 123-141.

Brundrett, M. (2009). Mycorrhizal Associations and other Means of Nutrition of Vascular Plants: Understanding the Global Diversity of Host Plants by Resolving Conflicting Information and Developing Reliable

Means of Diagnosis. *Plant and Soil*, 320, 37–77.

Finlay, R. D. (2008). Ecological Aspects of Mycorrhizal Symbiosis: with Special Emphasis on the Functional Diversity of Interactions Involving the Extraradical Mycelium. *Journal of Experimental Botany*, 5(5), 1115-1126.

Hempel, S., Renker, G., & Buscot, F. (2007). Differences in the Species Composition of Arbuscular Mycorrhizal Fungi in Spore, Root and Soil Communities in a Grassland Ecosystem. *Environmental Microbiology*, 9, 1930–1938.

Hernadi, P., Sasvari, Z., Albrechtova, J., Vosatka, M., & Posta, K. (2012). Arbuscular Mycorrhizal Inoculants Increase Yield of Spice Pepper and Affects Indigenous Fungal Community in the Field. *Hort Science,* 47(5), 603-606.

Jha, S., & Kumar, K. N. (2011). Potential of Mycorrhizal Fungi in Ecosystem: A Review. *International Journal of Research in Botany*, 1(1), 1-7.

Juliadewi, K. A. C., Rai, I. N., & Kartini, N. L. (2014). Application Dose of Endomikoriza Fungus to Increase Production of Snake Fruit cv. Gula Pasir. *Jurnal Plumula,* 4(3), 19-25.

Kavitha, T., R., & Nelson, (2013). Diversity of Arbuskular Mycorrhizal Fungi (AMF) in the Rhizophere of *Helianthus annuus L. American-Eurasian Journal Agriculture & Environment Science,* 13(7), 982-987.

Leal, P. L., Varon-Lopez., Prado., M. I. G. O., dos Santos, J.V, Soares., C.R.F.S. Siqueira., J. O., & Moreira. F. A. S. (2016). Enrichment of arbuscular mycorrhizal fungi in a contaminated soil after rehabilitation. *Brazilian Journal of Microbiology*, 47, 853–862. Doi: org/10.1016/j.bjm.2016.06.001.

Manurung, Y. C., A. Asmarlaili., Hanafiah., P., & Marbun. (2015). Pengaruh Berbagai Kadar Air Tanah Pada Efektifitas Mikoriza Arbuskular Terhadap Pertumbuhan dan Serapan Hara Bibit Karet (*Hevea brassiliensis* Muell. Arg.) di Rumah Kasa. *Jurnal Online Agroekoteknologi*, 3(2), 465-475.

Mathimaran, N., M. P. Sharma, M. B. Raju., D. J., & Bagyaraj. (2017). Mycosphere Essay 17 Arbuscular mycorrhizal symbiosis and drought tolerance in crop plants. *Mycosphere,* 8(3), 361–376. Doi:10.5943/mycosphere/8/3/2.

Menge, J. A. (1985). Effect of Soil Fumigants and Fungicides on Vesicular Arbuscular Fungi. *Phytopathology*, 72, 1125-1132.

Mo, Y. Y., Wang, R., Yang, J., Zheng, C., Liu, H., Li, J., Ma, Y., Zhang, C., Wei, X., & Zhang. (2016). Regulation of Plant Growth, Photosynthesis, Antioxidation and Osmosis by an Arbuscular Mycorrhizal Fungus in Watermelon Seedlings under Well-Watered and Drought Conditions. *Frontier Plant Science*, 7, 644. Doi: 10.3389/fpls.2016.00644

Moreira, S. M., Dilmar, S., & M. Tsai. (2007). Biodiversity and Distribution of Arbuscular Mycorrhizal Fungi in *Araucaria angustifolia* Forest. *Journal agriculture,* 64 (2), 393-399.

Nurhandayani, R. R., Linda, S., & Khotimah. (2013). Inventarisasi Jamur Mikoriza Vesikular Arbuskular dari Rhizosfer Tanah Gambut Tanaman Nanas (*Ananas comosus* L. Merr). *J. Protobiont* 2 (3), 146-151.

Proborini, M. W., M. Sudana, W., & Suarna, P. Ristiati. (2013). Indigenus Vesicular Arbuscular Mycorrhizal (VAM) Fungi in Cashew Nut (*Anacardium occidentale* L.) Plantation of North East Bali Island- Indonesia. *Journal of Biology, Agriculture and Healthcare,* 3(3), 114 -121.

Quiroga, G. G. Erice., R. Aroca, F., Chaumont, J.M., & Ruiz-Lozano. (2017). Enhanced Drought Stress Tolerance by the Arbuscular Mycorrhizal Symbiosis in a Drought-Sensitive Maize Cultivar Is Related to a

Broader and Differential Regulation of Host Plant Aquaporins than in a Drought-Tolerant Cultivar. *Frontier Plant Science*, 8, 1056. Doi: 10.3389/fpls.2017.01056.

Rai, I. N., Semarajaya., C. G. A., Wiraatmaja., I. W. (2010). A Study on the Flowering Phenophysiology of Gula Pasir Snake Fruit to Prevent Failure of Fruit-set. *Journal of Horticulture*, 20(3), 216-222.

Rai, I. N., Wiraatmaja., I W. Semarajaya, C. G. A., Alit Astiari, N. K. N., & Astawa., G. (2015). Application of Mycorhiza Arbuscular Dosage and Organic Fertilizer to Produce Off-season Fruit of Salak Gula Pasir (*Salacca zalacca* var. Gulapasir). *Article Presented on International Conference of Bioscience and Biotechnology*. Udayana University, September 18-19th, 2015.

Rai, I. N., Suada, I. K., Proborini., M. W., & Wiraatmaja., I. W. (2017). Exploration of Mychoriza on Snake Fruit Plantation as Biological Fertilizer. Papper presented on Workshop on Development and Implementation of Innovative Agricultural Technologies to Increase Sustainability of Agro-ecological Systems. *Moscow State University, Russia, 21-23 Agust 2017*.

Sarah, S., & Ibrar. M. (2016). Effects of arbuscular mycorrhizal fungi on spores density and root colonization of four hybrids of sunflower (*Helianthus annuus* L.) at different rock phosphate levels. *Sarhad Journal of Agriculture,* 32(4), 258-266. http://dx.doi.org/10.17582/journal.sja/2016.32.4.258.266.

Sadhana, B. (2014). Arbuscular Mycorrhizal Fungi (AMF) as a Biofertilizer: a Review. *International Journal of Current Microbiology Applied Science* , 3(4), 384-400.

Sasvari, Z., Magurno1. F., Galanics. D., Nhu Hang., T. T. T. T., Hong Ha, N. D., Luyen, L. M., Huong, K., & Posta. (2012). Isolation and Identification of Arbuscular Mycorrhizal Fungi from Agricultural Fields of Vietnam. *American Journal of Plant Sciences*, 3, 1796-1801.

Schenck, N. C., & Y. Perez. (1990). Manual for the Identification of V. A. Mycorrhizal (VAM) Fungi. *University of Florida Press, Florida, USA*. 241p.

Schubler, A. D., Schwarzott, C., & Walker. (2001). A New Fungal Phylum, the Glomeromycota: Phylogeny and Evolution. *Tropical Ecology*. 44(2): 207-215.

Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis. Third edition: Academic Press. *Elsevier Ltd. New York, London, Burlington, San Diego*.768p.

Suamba, I. W., Wirawan, I. G. P., & Adiartayasa, W. (2014). Isolasi dan Identifikasi Fungi Mikoriza Arbuskular (FMA) secara Mikroskopis pada Rizosfer Tanaman Jeruk (*Citrus* sp.) di Desa Kerta, Kecamatan Payangan, Kabupaten Gianyar. *E-Jurnal Agroekoteknologi Tropika*, 3(4), 201-208. http://ojs.unud.ac.id/index.php/JAT.

Sukewijaya, I. M., Rai., I. N. M., & Mahendra., S. (2009). Development of Salak Bali as an Organic Fruit. *Asian Journal of Food and Agro-Industry*. Special Issue:37-43.

Tahat, M. M., & Sijam, K. (2012). Mycorrhizal Fungi and Abiotic Environmental Conditions Relationship. *Research Journal of Enveronmental Sciences*, 25, 431-440. http://dx.doi.org/10.3923./rjes.2012.

Wang, F. Y., & Z. Yong Shi. (2008). Biodiversity of Arbuscular Mycorrhizal Fungi in China: a Review. *Advances in Environmental Biology,* 2(1), 31-39.