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THE EFFECT OF ROOT STIMULANT ON ECTOMYCORRHIZAL COLONIZATION OF GNETUM SEEDLINGS (*Gnetum gnemon L.*)

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ABSTRACT

Ectomycorrhizal fungi usually infect the young lateral roots. Root stimulant can induce the growth of new lateral roots after application of root pruning. This research is aimed at determining the exact amount of root stimulant concentration that suffices the condition of increased root colonization of gnetum seedling by mycorrhizal fungi. The research were tested in a greenhouse in a completely randomized design for 8 months. Four levels of root stimulant concentation was used, i.e. 0.00%, 1.00%, 1.25% and 1.67%. All treatments were treated with root pruning and ectomycorrhizal fungi (*Scleroderma* spp.) inoculation. Growth parameters observed were the number of new roots, the percentage of ectomycorrhizal colonization, diameter, height, shoot wet biomass, shoot dry biomass, root wet biomass, and root dry biomass. The results showed that all the roots of *Gnetum* seedlings infected by *Scleroderma* spp. Root stimulant had significant effects on increasing the growth of new roots. Root stimulant with concentration 1.67% gave the best growth response on all parameters except root dry biomass. The number of new roots was 35.50, the percentage of ectomycorrhizal colonization 58.47%, diameter 3.57 mm, height 48.21 cm, shoot wet biomass 8.57 g, shoot dry biomass 3.56 g, and root wet biomass 5.81 g.

Key words: *Gnetum gnemon*, mycorrhizal colonization, plant growth, root stimulant, *Scleroderma*

INTRODUCTION

Melinjo (as known in Indonesia) (*Gnetum gnemon L.*) is able to live and grow in a land that possesses few nutrient and water supply (Cadiz and Florido 2011) due to its capability to mutually associate with the ectomycorrhiza fungi of *Scleroderma* genus, in which results in two types of ectomycorrhiza morphotypes, the yellow one and the white one. The type of yellow morphotype is recognized as more widely spread and identified as *Scleroderma sinnamariense* while the white type of morphotype is identified as *Scleroderma* sp. (Bechem and Alexander 2012).

Ectomycorrhiza is a form of mutualistic symbiosis between ectomycorrhiza-forming fungi and the root of high-level plants. The symbiosis, since it is mutualism, gives profit to both sides, the plants receives nutrient from the fungi while the fungi receives the result of plant's photosynthesis in form of carbon compound (Smith and Read 2008). In addition, ectomycorrhiza also gives usefulness to the plants, such as helps the plants to absorb the nutrient of the soil (Allen *et al*. 2003; Dehlin *et al*. 2004; Lilleskov *et al*. 2002; Baghel *et al*. 2009). A mycorrhizal seedling is able to easily adapt to its current environment, or its new environment due to its nature which establishes its recognition as one of significant factor in the developing of new forest area (Valdes *et al*. 2009).

The effort of the uplift of mychorrhizal seedlings production is done by combining root pruning and artificial inoculation which are conducted when the plants are still in young age (Wulandari and Supriyanto 2013). The use of organic root stimulant towards gnetum seedling in this research serves as the means to raise the colonization of ectomycorrhizal seedlings since the newly lateral-root is still easy to infect and thus increases its growth (Setiadi 2009). In support to the above statement, Hariangbanga (2009) also states that organic root stimulant contains several elements that made it possible to affect in such a way, they are humic acid, amino acid, mineral and catalyst. Thus, it is expected that combining root pruning and ectomycorrhiza's fungi inoculation to organic root stimulant is able to increase the root colonization of gnetum seedling by mycorrhiza fungi.

Thus, this research is aimed at determining the exact amount of root stimulant concentration that suffices the condition of increased root colonization of gnetum seedling by mycorrhizal fungi, in which the the gnetum seedling has already received mycorrhizal fungi inoculation and root pruning.

METHODS

The preparation of the materials

Planting media

This research requires certain planting media which includes soil, organic fertilizer, cocopeat, and chaffed charcoal. Then, before all the media being mixed, initial sterilization is necessary. The process of sterilization is

conducted by autoclaving the media in 121°C and 1 atm of pressure for one hour. The initial stage of the preparation is by mixing soil, cocopeat, and organic fertilizer with 4:2:4 ratios. Then, chaffed charcoal is added to the mixed media by the ratio of 9:1, and ends up by putting the completely mixed media to a polybag.

The gnetum seedlings

The gnetum seedlings that not yet infected by any mycorrhiza and measured approximately 30-40 cm of height is required for this research. In order to deprive attached soil on the root, it must be washed on flowing water before it made into use. Then, the lateral root of the seed is reduced approximately 30% to stimulate the growth of new root and the leaf is also reduced approximately 50% to decrease the transpiration.

The liquid of organic root stimulant

In this research, the researcher acquires the liquid of organic root stimulant from CV Akar Langitbumi. The liquid of organic root stimulant is made in four level of concentrations, i.e. 0.00%, 1.00%, 1.25%, and 1.67% which is based on the previous research. On the concentration of 0.00%, no organic root stimulant is needed to add since it serves as the control. Moreover, 1.00% concentration is formulated by dissolving 10 mL of organic root stimulant to 1 L of water (1:100), 1.25% is formulated by dissolving 12.5 mL of organic root stimulant to 1 L of water (1:80), and 1.67% concentration is formulated by dissolving 16.7 mL of organic root stimulant to 1 L of water (1:60).

Inoculation of ectomycorrhiza fungi

This research uses soil inoculum that is taken from planting media of ectomycorrhizal gnetum seedlings and at the rate of 5g/seed. The types of inoculation in use are *Sclerodema sinnamariense* and *Sclerodema* sp.

The conduct of the research

First**,** root-pruned gnetum seedlings are marinated for 24 hours in a glass of liquid of organic root stimulant. Simultaneously, the researcher makes planting holes at the planting media which already packed in polybags. Then, the researcher puts 5g/seed of inoculated ectomycorrhyza and, consequently, the completely marinated gnetum seedlings into the planting media in the polybags. Last, the planting holes are being covered again with planting media. The preservation of the gnetum seedlings are constituted of two activities: (1) watering the seed in the morning once per two or three days, depending on the dampness of the soil, and (2) weeding the seeds if any occurrences of weeds are found in the polybags.

Observation and data collection

The conducts of observation and data collection are based on the previous research conducted by Wulandari and Supriyanto (2013) which includes: the height of the seeds, the diameter of the rods, biomass, and root observation.

The height of the seedlings (cm)

The measurement is conducted by using ruler as the main and only tool. The seedling is measured from the neck of the root (the line between rod and root above the surface) until the tip of the seedling, and the measurement is conducted once every two weeks.

The diameter of the rod (mm)

The measurement of the diameter of the rod is conducted using caliper. The seeds are measured 1--2 cm above the neck of the root, in which it has been marked with wooden sticks to avoid any mistakes or miscalculation. The measurement is conducted once per six weeks.

The biomass of root and tip (g)

The measurement of the biomass is conducted by calculating the wet mass and dry mass of root and tip after reaching 33rd week. The calculation of the wet mass is conducted by separating the plant from the planting media and washing it in order to remove any dirt and soil from the plant. The completely clean plant is then sliced in order to gain an independent part of the plant (shoot and root). Then, the wet biomass is acquired by summing the mass of shoot and root after pondered by scale. Moreover, the calculation of dry biomass is conducted by continuing the process of the wet biomass. The shoot and the root are dried in the oven for five days in 70 °C of temperature and also pondered by scale to acquire the dry biomass of the plant. The sum of shoot and root's mass also serves as the total of the biomass.

Root observation

The observation is conducted by separating the plant off the planting media and scrutinizing it with a magnifying glass, in which also conducted in the 8rd month. The observation is conducted in order to gain any knowledge concerning the percentage of ectomycorrhizal colonization, after-pruning root growth, and the sum of infected seedlings. The root growth is determined by summing the branched root due to root pruning and calculating the amount of branches formed. The percentage of ectomycorrhizal colonization and infected seedlings is acquired by the following formula:

$$\%\ \text{colonization} = \frac{\sum \text{ectomycorrhizal lateral root}}{\sum \text{total of lateral roots}} \times 100\%$$

$$\%\ \text{ectomycorrhizal seedlings} = \frac{\text{the sum of ectomycorrhizal seedlings}}{\text{the sum of observed seedlings}} \times 100\%$$

Design of the experiment and data analysis

The experiment is conducted using completely randomized design with only one factor involved, the organic root stimulant (0.00%, 1.00%, 1.25%, and 1.67%). Each conduct of the experiment is consisted of four trials, in which every trial consists of five units of gnetum seedling. The data which are collected from the observation and calculation are analyzed using variance

analysis. In case of any significant variation occurred, extended experiment is conducted using Duncan's multiple range test.

RESULTS AND DISCUSSION

Results

The observation of gnetum seedling includes the development of the root and the the shoot of the gnetum seedlings. The result of gnetum seedling's growth variance analysis which is given certain level of organic root stimulant can be seen at Table 1.

The organic root stimulant 1% gives no significant impact to the growth of seeds' shoot and root compared to the control. Meanwhile, gnetum seedlings given organic root stimulant 1.25% show more significant impact than the previous one since the growth of height and root's wet biomass are recognized as better than control. Yet, the best result of the experiment comes from gnetum seedlings given organic root stimulant 1.67%. It can be recognized by the growth it gives to the variables: the sum of branched roots, the sum of new branches, diameter, height, shoot's wet biomass, root's wet biomass, and shoot's dry biomass, compared to the other concentration levels. However, it has no significant impact on root's dry biomass of gnetum seedlings.

The growth of the root and ectomycorrhizal colonization

The organic root stimulant 1.67% stimulates the occurrences of new lateral roots compared to roots given no organic root stimulant which suffers highly insignificant growth of branched roots and occurrences of new lateral roots (Table 1); whereas the sum of branched roots increases from one to three roots, and each branch forms two to four new roots at roots given the 1.67% rate. Thus, consequently, the more branches formed by the roots results in smaller root's diameter.

In this research, all seeds are inoculated and its roots are pruned by 30%. Table 2 shows that all gnetum seedlings in this research are colonized by ectomycorrhiza. Gnetum seedlings given organic root stimulant 1.67% possess highest level of ectomycorrhizal colonization among the others treatment.

The numbers in the same column, which precedes the same letter, have no significant impact on 5% of test level; tn: insignificant, *: significant at 5% of test level.

According to research, gnetum seedlings which are colonized by *Scleroderma sinannmarense* ectomycorrhiza fungi have certain distinctive feature compared to those colonized with others of the genus *Scleroderma*. Its mantle and hyphae are yellow colored which eases the differentiation with others ectomyrrhiza fungi's hyphae, in which white colored. In addition, its another characteristic is having monopodial branching.

Discussion

Gnetum seedlings which are given organic root stimulant 1% fail to provide significant impact compared to the control seeds (without root stimulant). Furthermore, seedlings which are given the stimulant 1.25% show impact on two variables while seedlings which are given the stimulant 1.67% show impact on seven to eight observed variables. According to the preceded research, giving more than 1.67% level of stimulant is considered as too high and may cause harm to the gnetum seedlings which is indicated by dried leaf of the plant started from its tip, or, in other words, look like recently burned. Parnata (2010) states that plants which are indicated given excessive fertilizer may be poisoned and show its condition by the appearance of dried stem and leaf. Thus, giving more than 1.67% of organic root stimulant to gnetum seedlings is highly not recommended.

Table 1 The growth of gnetum seedling which is given organic root stimulant treatment in the 8th month of observation

Variables	Test of F	Concentration of root stimulant (%)			
		0.00	1.00	1.25	1.67
Root growth					
Sum of branching roots	*	3.32^{b}	3.31^{b}	3.00^{b}	4.12^{a}
Sum of new branches	*	2.19^{b}	2.57^{b}	2.62^{b}	3.75^{a}
Shoot growth					
Diameter (mm)	*	2.57^{b}	2.59^{b}	2.68^{b}	3.57^{a}
Height (cm)	*	38.52^{b}	38.37^{b}	41.67^{ab}	48.21^{a}
Shoot wet biomass (g plant^{-1})	*	7.15^{b}	7.24^{b}	7.69^{b}	8.57^{a}
Shoot dry biomass (g plant^{-1})	*	2.57^{b}	2.51^{b}	2.52^{b}	3.56^{a}
Root wet biomass (g plant^{-1})	*	3.90^{b}	4.39^{b}	5.04^{ab}	5.81^{a}
Root dry biomass (g plant^{-1})	ns	1.19^{a}	1.13^{a}	1.20^{a}	1.38^{a}

The numbers in the same column, which precedes the same letter, have no significant impact on 5% of test level; ns: not significant, *: significant at 5% of test level.

Table 2 The level of ectomycorrhizal colonization on gnetum seedlings given organic root stimulant treatment

Variables	Concentration of organic root stimulant (%)			
	0	1.00	1.25	1.67
Ectomycorrhizal seedlings (%)	100.00[a]	100.00[a]	100.00[a]	100.00[a]
Ectomycorrhizal colonization (%)	25.71[b]	18.67[b]	32.88[b]	58.47[a]

Giving organic root stimulant 1.67% to gnetum seedlings gives the best result in all observed variables except the variable of root dry biomass. To be more detailed, there are several increased dimension of gnetum seedlings which are given the stimulant, 25.16% for the height, 38.91% for the diameter, and 31.38% for the biomass. The increased dimensions are caused by stimulant's capability to stimulate the forming of root branching and new lateral root. According to Godbold and Brunner (2007), the architecture of root system is a result from several processes such as branching, extending, gravitropic response, and secondary growth. Root plays important roles in absorbing the water and nutrient from the soil, transferring the carbon to the soil, and keeping the physical stability of the soil. The ability of the root to absorb is varying, depending on the pigmentation of the root, architecture of the root, and the structure of the branching. Moreover, pigmented roots are anatomically divided into two classes, (1) posses thick tannins zone and absorbing ability (the roots are pigmented to primary structure), and (2) posses fully developed secondary periderm and xylem which have no ability to absorb. Thus, the presence of periderm influences the function of root absorbability.

The lateral roots of wooden forest plants are usually attached by ectomycorrhizal fungi, so then they are covered by the hyphae of ectomycorrhizal fungi which results on the broadened area of absorb. Gnetum is a kind of plant that is able to associate with ectomycorrhizal fungi. Smith and Read (2008) state that roots that are attached by ectomycorrhizal fungi are showing distinctive characteristics, it is usually covered by mantle that envelops the surface of lateral roots, *Hartig-net*, and extraradicular hyphae. The volume of fungi occurred on the rhizosphere of a plant cannot be precisely determined, similar to the significance of the hyphae (in term of quantities) which serves to absorb water and nutrient from the soil (Cermak *et al*. 2013).

The ectomycorrhizal colonization is formed at lateral root, and can be further developed, at gnetum seedlings, using root pruning technique (Wulandari *et al*. 2013). Root pruning and ectomycorrhizal inoculation give significant impact to ectomycorrhizal colonization, the percentage of colonized plants, the sum of branched roots, and the sum of branches of the gnetum seedlings during three to four months after the seedlings inoculated (Wulandari and Supriyanto 2013). Those things are the result of root pruning that stimulates the growth and development of branched roots and thus made possible to be attached by ectomyzorrhizal fungi. Comas *et al*. (2012) state that morphological variations of roots are highly related to planting strategy which is carefully chosen to ensure its growth at different kind of environment. The development of roots' length and the wide of the surface are necessary in order to improve its effectiveness in absorbing soil nutrition.

In this research, the conduct of root pruning is combined with giving the organic root stimulant to inoculated roots in which results in better root colonization by ectomycorrhizal fungi (the sum of branched roots, the sum of new branches, diameter, height, shoot's wet biomass, root's wet biomass, and shoot's dry mass) compared to those of root pruning only. Moreover, it is caused by the colonization of ectomycorrhiza to the roots that stimulate its capability in absorbing soil nutrient. Setiadi (2009) states that the improved P element within the plant could result in hastening the cell division particularly in the development of plant's meristem system, and thus further affect the growth of the height and diameter of gnetum seedlings.

Organic root stimulant 1.67% gives best result since the level of ectomycorrhizal colonization is increased to 58.47%. The stimulant affects the development of branched roots to a degree of one to three and the sum of new branches, counting from two to four. The development of the roots can be accelerated by the use of root pruning activity and giving organic root stimulant to the plant. In addition, giving organic root stimulant stimulates more roots branching occurred because its use stimulates the growth of lateral roots and thus increases the nutrient absorbing and the growth of gnetum seedlings. Organic root stimulant also stimulates the growth of the lateral roots colonized by ectomycorrhiza since it stimulates the growth of gnetum seedlings' roots. In addition, organic root stimulant also contains amino acid which is needed for the growth of ectomycorrhizal fungi (Hariangbanga 2009).

CONSLUSION

The gnetum seedlings which are given organic root stimulant 1.67% shows best result in the growth of gnetum seedlings. The sum of branched roots, the sum of new branches, diameter, height, shoot's wet biomass, root's wet biomass, and shoot's dry biomass have significant advantage of growth compared to others treatments.

SUGGESTION

Since the organic root stimulant 1.67% is giving significant impact to gnetum seedlings, its application to other plants is worth to analyze. Thus, it is also suggested to conduct further analysis of organic root stimulant at the rate of 1.67% and 2.50% to find out the better improvement of its application.

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