EFFECTIVENESS OF INDIGENOUS ENDOMYCORRHIZAL BIOFERTILIZER PROTOTYPE ON ORGANIC SALAK LEAVES AND FRUITS IN BALI

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ABSTRACT

Organically cultivated salak (*Salacca zalacca*) on dry land has limited production in Bali. Typically, fertilization is carried out using leaf litter or other organic fertilizers because soil fertility is low for salak plantations. The present study analyzed the effectiveness of an indigenous endomycorrhizal biofertilizer on the nutrient and total carbohydrate content of salak leaves and fruits. The study used a randomized block design with nine replicates. The treatment consisted of three levels, i.e., 1. fertilization with leaf litter only, as is practiced by the farmers and as control (C); 2. fertilization with indigenous endomycorrhizae biofertilizer prototype (P); and 3. combined fertilization with leaf litter and indigenous endomycorrhizae biofertilizer prototype (PM). Spores of indigenous endomycorrhizae from salak plantations were used for making a biofertilizer prototype. The results showed that P and CP treatments provided beneficial results such as: 1. significantly increased the chlorophyll, relative water content of leaves, as well as the number and weight of fruits per tree; 2. improved fruit quality by increasing sweetness and weight per fruit; and 3. had a positive effect on water uptake and nutrient absorption as indicated by high N and P of leaf tissue and high carbohydrate content of leaves.

Keywords: biofertilizer, endomycorrhizae, organic, prototype, *Salacca zalacca*

INTRODUCTION

Bali Province is a major tourist destination in Indonesia. Increasing the number of domestic and international tourists leads to an increased demand for fruits, including organic salak fruits. Salak cultivation in Bali has been carried out organically since the 1500s and is hugely beneficial to the farmers; however, the quantity, quality, and continuity of organic salak fruit produced is low (Rai *et al.* 2014).

This low productivity can be attributed to the nature-dependency of the farmers, who have not yet implemented adequate agricultural practices. Typically, fertilization only uses leaf litter, while irrigation relies only on rainfall. Thus, the fertilization of organic salak renders low soil fertility thereby decreasing the plant productivity over time (Rai *et al.* 2014). Rai *et al.* (2010) found that the nutrient content of N, P, and K in the leaf tissue of organic salak in Bali was low; also, the soil fertility was low as indicated by the levels of C-organic and the content of N, P, and K in the soils. In order to maintain the soil fertility and environmental sustainability for high organic salak productivity, the cultivation should be carried out with indigenous endomycorrhizae biofertilizer based on the fact that the diversity of indigenous endomycorrhizae fungal species in nature is very large (Baslam *et al.* 2011; Proborini 2013; Suamba *et al.* 2014; Sarah & Ibrar 2016; INVAM 2017).

Kim *et al.* (2017) established a close correlation between soil, plants, and endomycorrhizae. Indigenous endomycorrhizae isolated from plants in certain locations, will be more effective if applied directly to the target plant concerned where it is taken as compared

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to the endomycorrhizae brought from outside (non-indigenous endomycorrhizae). The arbuscular mycorrhizal symbiosis between plants and fungi exerts a positive impact on improving the soil structure, expanding the water absorption areas, increasing the plant tolerance to biotic and abiotic stresses, increasing the nutrient uptake, and increasing the plant growth and yield (Nikhat 2014; Olagunju *et al.* 2014; Soka & Ritchie 2016). In addition, endomychorrizae increases plant tolerance to various biotic and abiotic stresses, including alkalinity and metal toxicity (Abbasi *et al.* 2015; Bitterlich *et al.* 2018). The endomycorrhizae fungus which is also called arbuscular mycorrhizae is beneficial to the host plants as it plays a role in increasing nutrient absorption through the endomycorrhizal structures that form large root surface areas so that the plant roots can absorb the nutrients (Baslam *et al.* 2011; Sasvari 2012; Brundrett & Tedersoo 2018). Endomycorrhizae plays a role in increasing plant resistance to root pathogen attack through antibiotics produced during symbiosis with plant roots that weaken and also kill the pathogenic bacteria, viruses, and fungi (Brundrett 2017; Sasvari, 2012).

Furthermore, the diversity and activity of indigenous endomycorrhizae in plant roots are mainly determined by the type of the host plant (Ningsih *et al.* 2013; Sadhana 2014), environmental growth factors, such as climate and soil moisture/drought (Hernadi 2012; Quiroga *et al.* 2017; Mathimaran *et al.* 2017; Kehri *et al.* 2018), and the level of soil fertility (Tahat & Sijam 2012; Kavitha & Nelson 2013; Mo *et al.* 2016). Rai *et al.* (2019a) explored and identified morphologically and genetically the indigenous endomycorrhizae from salak root areas in three regencies in Bali: Karangasem, Gianyar, and Tabanan. In our study, two genera of indigenous endomycorrhizae in salak were identified i.e., *Glomus* and *Entrophospora.* The genus *Glomus* consisted of three species (*Glomus cubens, Glomus custos,* and *Glomus indicum*), while genus *Entrophospora* consisted of only one species (*Entrophospora*_sp_*SH197095.06FU*). After propagation, the spores of these species were formulated into prototypes of biological fertilizers/biofertilizer with carrier media zeolite, quartz sand, sea sand, and volcanic sand. The most effective method to be applied in salak seedling is a prototype composed of a mixture of 100 spores from *Glomus* species in 500 g volcanic sand carrier media. This phenomenon was shown by the optimal growth of salak seedling and the maximum (100%) colonization of endomycorrhizae at the roots (Rai *et al,* 2019b). Positive test results on salak seedling growth need to be followed up by evaluating the efficiency of the prototype of indigenous endomycorrhizae biofertilizer on organic salak plantations in the field. Thus, the present study aimed to determine the effect of prototype indigenous endomycorrhizae biofertilizers on organic salak production and its effect on the nutrient and carbohydrate content of the leaves.

MATERIALS AND METHODS

The study was conducted from February to December 2019 in organic salak farms owned by farmers in Sibetan Village, Bebandem District, Karangasem Regency. The study used 15-year-old salak trees and a randomized block design with nine replicates. Three levels of fertilization were applied as follows: 1. fertilized with leaf litter only, as is practiced by the farmers and as control (C); 2. fertilized with prototype indigenous endomycorrhizae biofertilizer (P); and 3. combination of leaf litter and prototype indigenous endomycorrhizae biofertilizer (CP). Therefore, 27 salak trees with a total plot size of 216 m square were needed.

The spores of indigenous endomycorrhizae were taken from salak roots in Sibetan Village. The isolation of the spores was carried out using the wet filtering technique, followed by centrifugation as described by Brundrett (2017). These spores were propagated according to the method described by Rai *et al.* (2018), using corn as a host plant with water stress treatments. Topping or shoot cutting was carried out at the age of 4 weeks. Watering to field capacity was carried out from the beginning of seed planting to 1 week of age, followed by watering to 50% of field capacity until 4 weeks of age. After topping at the end of the 4th week, watering was stopped so that the plant experienced stress. Then, the plant was dismantled to harvest the spores from the multiplication at the end of the 5th week. Subsequently, the resulting spores were placed on volcanic sand: 100 spores/500 g sand.

Fertilization was conducted by making a hole, 30 cm wide and 20 cm deep, around the tree at a distance of 40 cm from the base of the tree. The salak trees in treatment C were fertilized with 4 kg leaf litter of salak/tree. The leaves were cut into 20-cm-long pieces, placed in a hole, and covered with soil. On the other hand, the trees in the P treatment were fertilized with prototype indigenous endomycorrhizae made from a mixture of 100 spores of three *Glomus* species (*Glomus cubense*, *Glomus Custos,* and *Glomus indicum*) in 500 g volcanic sand carrier media per plant. The prototype of the biofertilizer was evenly spread around the tree and covered with soil. Interestingly, the CP treatment was carried out by spreading a prototype of the biofertilizer made from a mixture of 100 spores of three *Glomus* species in 500 g of volcanic sand carrier media per plant, followed by spreading the salak leaf litter on it evenly around the tree.

The chlorophyll content and the relative water content (RWC) of the leaves, the percentage of fruit set, the number and weight of fruits per tree, weight per fruit, fruit diameter, fruit sweetness level, N and P content of the leaves, and total sugar, reducing sugar (R-sugar) and sucrose content of the leaves, and root colonization by indigenous endomycorrhizae were recorded. The colonization of roots by indigenous endomycorrhizae was observed by the slide method of Giovannetti and Mosse (1980) using the following formula: percentage of colonized roots = the number of colonized roots/the total number of roots observed × 100%. The percentage of fruit set was calculated as follows: the number of flowers developed into fruits/the number of flowers × 100%. The leaf chlorophyll content measured three times by Chlorophyll Meter SPAD-502 in April, June, and August, and the average was calculated. The total sugar was analyzed by anthrone method, R-sugar by Nelson–Somogyi method, and the sucrose content was calculated by subtracting the value of total sugar content from that of R-sugar, and multiplied by 0.95. The RWC was measured from matured leaves. After being cut from the tree, the mature leaves were then immediately wrapped in aluminium foil, stored in an icebox, and transported to the laboratory for further analysis. Thirty pieces of leaf samples (10 pieces from the tip of the leaf, 10 pieces from the middle, and 10 pieces from the bottom), with a diameter of 1 cm, were taken from leaf sheaths using a round punch and weighed (W_1). These leaf samples were immersed in water and irradiated with 40 W fluorescent light at room temperature for 5 h. Then, each piece of leaf sample was carefully dried with paper towel, and weighed (W_2), followed by oven-drying at 70 °C for 24 h before being estimated (W_3). The value of RWC was calculated by the formula: $(W_1\text{-}W_2)/(W_3\text{-}W_2) \times 100\%$. The nutrient content of N and P leaves was analyzed by Kjeldahl method and the P-availability was analyzed by Olsen method. The number and weight of fruits per tree were calculated cumulatively at the end of the study. Moreover, the weight of each fruit was calculated as follows: total weight of fruit per tree/the number of fruits per tree.

These data were analyzed using analysis of variance with SPSS (Statistical Product and Service Solution) software version 26. If the F test showed a significant difference, the least significant difference (LSD) test was performed to differentiate the average value among tretaments.

RESULTS AND DISCUSSION

The results of the analysis of variance (ANOVA) showed that the fertilization treatment significantly affected all the observed variables. The weight and number of fruits per tree (790.80 g and 16.81 fruits, respectively) in the treatment of indigenous endomycorrhizae biofertilizer prototype (100 spores/500 g volcanic sand carrier media) were significantly higher than those fertilized with leaf litter/control (653.81 g and 14.94 fruits, respectively), but were not significantly different from CP (790.14 g and 16.56 fruits, respectively) (Table 1). The quality of salak fruit in the P and CP treatments was significantly improved as compared to that in treatment C, as shown by the increased sweetness of the fruit (Table 2) and the weight per fruit (Table 1). The high number and weight of fruit per tree, as well as the increased fruit quality due to the administration of indigenous endomycorrhizae biofertilizer prototype, elevated the N and P content of the leaves as compared to the control (Table 2); also, the chlorophyll content and RWC of the leaves were increased (Table 1). Visually, the trees treated with C and CP treatments had dark green and fresh leaves as compared to the control.

Table 1 Effect of prototype indigenous endomycorrhizae biofertilizers on the leaf chlorophyll content, RWC of leaves, number and weight of fruits per tree, weight per fruit, and fruit sweetness in salak plants

Treatments	Leaves chlorophyll content (SPAD)	Leaves relative water content (%)	Number of fruit per tree (unit)	Weight of fruit per tree (g)	Weight per fruit (g)	Fruit sweetness (% Brix)
C	68.82 b	55.03 b	14.94 b	653.81 b	44.09 a	12.89 b
P	76.83 a	65.37 a	16.81 a	790.80 a	47.61 a	13.72 a
CP	77.46 a	65.75 a	16.56 a	790.14 a	47.97 a	14.66 a
LSD 5%	0.67	0.98	1.59	96.34	7.27	0.51

Note: Numbers followed by the same letter in the same column did not differ significantly in the LSD 5% level.

Higher production and yield quality in P and CP treatments as compared to control (C) were related to high leaf chlorophyll content (Table 1) and the ability of salak tree to absorb nutrients and water (Table 2). The high P and N content in the leaves resulted from the P and CP treatments was significantly associated with root colonization as compared to the C treatment. Table 2 displayed that root colonization by indigenous endomycorrhizae in the P and CP treatments was 100%, but only 23.14% in treatment C. The high root colonization in P and CP revealed a mutualism symbiosis between indigenous endomycorrhizae and salak trees, which in turn, increased the P and N content and the RWC in the leaves. The endomycorrhizae infects the roots of plants but does not cause injury, which in turn causing mutual reciprocal processes. Host plants obtain nutrients from endomycorrhizae, while endomycorrhizae obtain carbohydrates or food from host plants (Hernadi *et al.* 2012; Zasvari *et al.* 2012).

High nutrient content and RWC in the leaves resulted from the P and CP treatments increased the chlorophyll content, which improved the photosynthesis as indicated by elevated total sugar, R-sugar, and sucrose content in the leaves resulted from the P and CP treatments as compared to that in C (Table 2). The present study showed that indigenous endomycorrhizae fungus isolated from root of salak trees is suitable to be used as a biofertilizer. The administration of indigenous endomycorrhizae resulted in the absorption of nutrients and water through the endomycorrhizae structure that enlarges the surface area of salak root, so that the plant roots can absorb the nutrients and water (Baslam *et al.* 2011; Zasvari 2012; Brundrett & Tedersoo 2018, Bitterlich *et al.* 2018).

Spore density and root colonization of host plants are largely determined by the compatibility of endomycorrhizae with host plants, environmental factors, and interactions between endomycorrhizae and chemical compounds produced by host plants (Beltrano *et al.* 2013; Sarah & Ibrar 2016). Thus, a correlation between indigenous endomycorrhizae biofertilizers prototype and salak trees can be suspected, which increases the yield, the quality of the yield, and the physiological processes of salak trees. The results of this efficiency test are in accordance with those of previous studies wherein endomycorrhizal fungi biofertilizer can increase the growth, production and quality of pineapple yield (Nurhandayani *et al.* 2013), chili (Tanwar *et al.* 2013), teak (Proborini, 2013), tea (Nepaleon *et al.* 2012) and apple (Fediala *et al.* 2018). Endomycorrhizae plays a role in increasing plant resistance to drought or lack of water in the dry season because the root of the plants possess mycelium that can reach water in the wider rhizosphere area of the soil and adsorb water despite limited availability (Sasvari 2012). Furthermore, it is involved in producing various growth regulators such as auxins, cytokinins, and gibberellins and vitamins that can increase the growth of plant organs and roots that do not rapidly age and hence can function effectively in the absorption of nutrients and other solutes (Baslam *et al.* 2011; Tanwar *et al.* 2013). The endomycorrhizae also improve the soil structure because the mycelium on the outside of the roots of plants (covering soil grains) produces polysaccharide gels that increase the stability of soil aggregates (Kruger 2011; Sasvari 2012; Sadhana 2014; Jansa *et al.* 2016; Kim *et al.* 2017).

Table 2 Effect of prototype indigenous endomycorrhizae biofertilizer application on the N and P nutrient, total sugar, R-sugar, and sucrose content of the leaves and root colonization on salak plants

Treatments	N content of leaves (%)	P content of leaves (%)	Total sugar content of leaves (%)	R-sugar content of leaves (%)	Sucrose content of leaves (%)	Root colonization (%)
C	0.50 a	0.23 b	21.35 b	1.96 b	18.91 b	23.14 b
P	0.52 a	0.26 a	23.48 a	3.35 a	21.41 a	100.00 a
CP	0.53 a	0.27 a	24.71 a	3.41 a	21.73 a	100.00 a
LSD 5%	0.03	0.01	0.51	0.22	0.79	3.16

Note: Numbers followed by the same letter in the same column did not differ significantly in the LSD 5% level.

Although C was not given indigenous endomycorrhizae biofertilizer, root colonization occurred albeit with significantly lower intention as compared to that in P and CP treatments. The data showed that indigenous endomycorrhizae were naturally present in salak root area; however, for a positive influence on the production and quality of salak, the population needed to be increased by giving indigenous endomycorrhizae biofertilizer for the optimal production of the plants. The indigenous endomycorrhizal fungi, also known as arbuscular mycorrhizal fungi, in nature is diversified (Baslam *et al.* 2011; Proborini 2013; Suamba *et al.* 2014), with a positive effect on the salak trees (Juliadewi *et al.* 2014). Indigenous endomycorrhizae is naturally associated with plant roots without human intervention and has a high potential for extensive colonization because it recognizes the host plant and has a higher tolerance to environmental conditions, such as high stress (Sadhana 2014; Quiroga *et al.* 2017). Based on these characteristics, it can be speculated that indigenous endomycorrhizae isolated from salak roots has great potential to be developed as biofertilizer because it adapts to host plants to be fertilized (Sasvari 2012; Sadhana 2014; Hohmann & Messmer 2017).

CONCLUSIONS

The current prototype of indigenous endomycorrhizae biofertilizer increased the yield and quality of the fruits in organic salak. Also, the nutrient level, the relative water content of the leaves, and the photosynthesis process were improved.

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