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Response of Rubber Stump Grown on Red Yellow Podzolic Soil Amended with Native Arbuscular Mycorrhizae of West Sumatra Indonesia.

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ABSTRACT

Indonesia has a target to become the world's main producer of rubber *Havea brasilensis* in 2025. To achieve that goal, one of the prioritized programs is a mass-production of prime seedling quality. The purpose of this study was to improve stump growth quality of rubber grown on red yellow podzolic soil (RYPS), which originated from sprout, by using native arbuscular mycorrhizae derived from West Sumatra. This research was designed in a randomized block design: 5 treatments and 5 replications. Stump was cultivated in 10 kg of unsterilized RYPS in a black polyethylene bag. Treatments were as follows: without mycorrhizae (control), 2.5 g inoculums of mycorrhizae, 5.0 g inoculums of mychorrizae, 7.5 g inoculums of mycorrhizae and 10g inoculums of mycorrhizae per plant, per polyethylene bag. The elements observed were plant height; stem diameter; the amount, width and length of leaf and mycorrhizae dependency. DNMRT 95% and one way ANOVA was used to analyze the effect of the treatments. The best treatment to improve stump growth quality which were indicated on plant height, stem diameter and amount of leaf in this study was by the application of 10 g of inoculums, significantly different with other dosages of inoculums treatments. The result was supported by the highest percentage of mycorrhizae colonization (75.13%) and high dependency level in stump's root. The results showed that amended RYPS with arbuscular mycorrhizae enhanced mycorrhizae colonization in stumps roots (high dependency) thus proven in increasing seedling growth quality.

Keywords: *Havea brasiliensis*, main producer, red yellow podzolic soil, stump quality, high dependency.

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INTRODUCTION

Rubber (*Havea brasiliensis* Muell Arg) is one of the most important export commodities of Indonesia. In the last 30 years, the exports have been continually increased from 1.0 million tons in 1985 (4) to 3.1 million tons in 2014 valued at US$4.7 billions. With the assumption that the world's rubber growing production is 2.5% per year and the increase of international trade is 2.6% per year (33), Indonesia plans to be the main rubber production country in the world by developing its plantation area up to 4.5 million ha to reach 3.5 million tons of yield in 2025 (14, 4). This opportunity is further enlarged as the price of rubber will remain stable until 2025 at US$ 2.00 per kg (29).

Currently, Indonesia's rubber plantation is 3.65 million ha, consisting of 80% of public plantation and 20% of state or private plantation. The largest plantation is currently in the island of Sumatra (70%), other plantations can be found in Kalimantan (20%), in Java (5%) and in other islands (5%). However, the vast area of plantation is not balanced by sufficient production of rubber. Indonesia's rubber production is at 900 kg/ha/yr and the quality of rubber produced is not satisfactory, while Malaysia, Vietnam and Thailand produce 1510, 1720 and 1800 kg/ha/yr respectively (11, 13). Some reasons behind this due to rubber tree aging, replanting with low quality of seedling, pests and diseases, poor maintenance of the majority the public plantation and cultivation on low quality of soil such as at red yellow podzolic soil (RYPS). There are 48.3 million ha RYPS in Indonesia spread in Sumatra (the largest), Kalimantan, Sulawesi, Papua and West Java (31, 24). This type of soil can be used for developing rubber plantation program, therefore procurement of qualified seedlings becomes an urgent action.

The advantage of using mycorrhizae to increase seedling quality have previously proven on papaya, banana, citrus, mangosteen and oil palm (22, 27, 23), through its significant role in absorbing minerals/nutrients and sustainable characters (1, 2, 19, 21, 37, 11, 7, 20). According to Suswati *et al* (35), weight of banana cv Kepok increased up to 40% caused by addition of arbuscular myccorhiza P-10. Nasir *et al* (27) reported that the treatment of mycorrhiza was also able to reduce time / day of banana seedlings maintenance in the nursery up to 30%, compared to seedlings without mycorrhiza, thus successfully saving maintenance costs between 20-40%. Myccorhiza also acts as an effective biological control agent for pathogens (32, 33). It is become more important in Indonesia as rubber plantations are being attacked by most destructive pathogen on rubber, namely white root disease caused by *Rigidoporus microporus* (Swartz. fr)van Ov) (`12). With the target to increase rubber plantation to 1 million hectare focusing in RYPS in 2025, and considering that the necessity of 1 ha is 400 plants, then Indonesia will require 400 millions prime qualified rubber seedlings. The potential of native arbuscular mycorrhiza fungi that improve the growth and nutrient uptake of rubber stump is therefore of great interest. The purpose of this study is to find the appropriate application dose of mycorrhiza that can improve the quality of rubber stump.

METHODOLOGY

Stump preparation:

Study was conducted at a farmer property in the District of Bintan, The Province of Riau Island Sumatra Indonesia from February to December 2015. Germinated seeds that were provided for rubber stumps, originated from Rubber Research Institute at Sungai Putih North Sumatra. The qualified germinated seeds were then grown for stumps based on Damanik *et al* (5) and planted in 5 kg of unsterilized RYPS in 25 cm x 50 cm black polyethylene bags for 2 months, before inoculating with native arbuscular mycorrhiza fungi.

Inoculum treatments:

The design used was a randomized block design (RBD) with 5 treatments and 5 replications. The treatments were without mycorrhizae (control), 2.5 g inoculums of mycorrhizae, 5 g inoculums of mycorrhizae, 7.5 g inoculums of mycorrhizae and 10 g inoculums of mycorrhizae. Treatment was conducted by immersing mycorrhizae (based on the treatment's dosage) as deep as 5 cm in the rhizosphere area of germinated seeds which are called now stumps. Registered native arbuscular mycorrhizae of West Sumatra derived from Tropical Fruit Research Institute in Solok West Sumatra Indonesia was used in this study. The inoculum is a mixing of *Glomus* sp and *Acaulospora* sp in granular sand. The amount of rubber stumps in a single unit activity were 1250.

Parameter observation:

For observation in each treatment, stumps were selected at random, and then the chosen stumps became the permanent samples. This number of fixed samples were 15% of plants per treatment. These samples then became replications of the treatment. Analyze the impact of treatments those were plant high, trunk diameter, leave amount and leave width were measured per week. This study used analyses of variance (one way ANOVA) and Duncan's multiple range test (DNMRT) 95% by using SAS 7.0 (Stat Soft 2007). Myccorhiza dependency was analysed based on Habite and Majunath (7). Roots of stumps were cleaned and washed with water and cut 1 cm length, soaked in 10% KOH for 24 hours, washed with aquades and re-soaked in 2% HCl for 3 minutes. The 1 cm roots then were stained for 24 hours. Staining composition were 400 ml gliserin + 400 ml lactate acid + 0.1 g trypan blue. After 24 hours in staining process, it was continued with distaining procedure. Distaining composition similar with staining but without trypan blue. The percentage of mycorrhiza colonization in root tissue was calculated under microscope using the following formula :

$$\text{Colonization} = \frac{\text{The amount of spores on the marked surface}}{\text{The amount of spores on the entire surface}} \times 100\%$$

RESULTS AND DISCUSSION

Pre-activity Soil Analysis Result

Most of the area used for rubber plantation in the District of Bintan is red yellow podzolic soil (RYPS). Soil sample was analyzed at Soil Laboratory of Tropical Fruit Research Institute in Solok, West Sumatra, Indonesia by using Kjeldahl method (9). Results of soil analyze before the study shown that soil had acidic pH, very low level of organic C, low levels of N, K, Mg, Al-dd and H^+, and had medium P_2O_5 and Ca (Tabel 1). In general, the soil analyze indicated the low level of soil fertility (30). One of the alternatives to increase the soil's quality is by introducing mycorrhizae, mainly for the availability of water, N, P and K (1, 20). In this study, native and registered mycorrhizae originated from West Sumatra Indonesia (22) was used.

Table 1. Results of soil analysis of land assessment prior to activity

Description	Content*	Classification**
pH (H2O)	3,69	Low (very acidic)
C organic (%)	1,894	Very low
N (%)	0,15	Low
P2O5 (ppm)	12,3	Medium
K (me/100 g)	0,39	Low
Ca (me/100 g)	7,31	Medium
Mg (me/100 g)	0,62	Low
CEC	12,62	Low
AL-dd (me/100 g)	0,10	Low
H+ (me/100 g)	0,05	Low

*Conducted at Soil Laboratory, Tropical Fruit Research Institute, Solok, West Sumatra. Indonesia.
**Classification by Soil Science Research Center, 1999. Bogor, West Java. Indonesia.

Mycorrhiza colonization and dependency:

` Data obtained in this experiment showed that RYPS of experimental used had a lower level of mycorrhizae content (Table 2). Mycorrhiza colony in the root stumps grown in RYPS without mycorrhiza treatment (control) was due unsterilized soil used in this study. However the level of colonization was still low (16.73%) and far below mycorrhiza colonization in root stumps planted with mycorrhiza treatment. Table 2 shows high sensitivity of rubber stumps, as shown by the high percentage of mycorrhiza colonization in root, even with the lowest level of treatment (2.5 g/stump/polybag). This relationship was also found in the percentage of mycorrhiza dependency of rubber stump against the treatment of 7.5 and 10.5 g inoculums / stump / polybag which approaches the "very high" classification, respectively 75.04 and 75.13%.

Table 2. Mycorrhizae root colonization and dependency in rubber stumps at week 12.

Treatment (g inoculum mycorrhizae/ plant)	Stump	
	Colonization (%)	Dependency
Control (0)	16.73	Low
2.5	68.24	High
5.0	70.38	High
7.5	75.04	High
10.0	75.13	High

This is supported by The Institute of Mycorrhizal Research and Development USDA, which classifies 50-75% colonization as high and 76-100% as very high (7). This finding indicates that native arbuscular mycorrhiza introduced to this RYPS can develop in roots as well as can survive and develop in soil. This is detected from the average percentage of mycorrhiza colonization in stumps roots grown in soil amended with mycorrhiza which ranges from 68.24 to 75.13%, compared to control which only reached 16.73% (Table 2). The percentage of mycorrhiza colonization of the treatment with mycorrhiza was 4.07 to 4.49 times more than treatment without mycorrhiza (control). This result also proves that the ability to infect of arbuscular mycorrhiza used in this study is high. This ability reaches maximum capacity particularly in marginal soils (1, 10, 20) , as RYPS is poor nutrient soil type (Table 1). Soil analyses after mycorrhiza introduction was not conducted due to error of procedure at the time of soil sampling.

Plant Height

There was a high inconsistency of plant height during 11 weeks observation (Tabel 3). However at the end of the observation at week 12, the highest plant (37.02 cm) was recorded at 10 g application of arbuscular mycorrhizae and significantly different with other treatments. This data was supported by the highest of mycorrhizae colonization and dependency in stump roots at the treatment of 10 g inoculums/stump/polybag (Tabel 2). According to Abbot *et al* (1) and Nasaruddin (25), the higher the dose of mycorrhizae, the higher the growth of plants. This result is supported by other studies, which concludes that mycorrhizae produces an intensive tangle of hyphae and high colonization in roots effectively increased the total absorption surface of inoculated plants, thus improved plant access to water and nutrients such as P, Cu and Zn (16, 27, 29, 23).

Table 3. The average height of rubber stumps grown in amended soil with arbuscular mycorrhizae fungi

Treatment (g inoculum mycorrhizae/ plant)	Weeks after inoculation (WAI)					
	2	4	6	8	10	12
Control	24.36 ns	26.88 ab	29.80 b	32.26 ns	33.28 ab	33.30 b
2,5	24.86	27.64 ab	30.46 ab	33.00	34.30 ab	34.48 b
5,0	25.32	28.20 a	31.16 a	32.94	34.62 a	34.76 b
7,5	24.72	27.38 ab	30.24 ab	32.58	33.94 ab	33.96 b
10	24.08	26.56 b	29.56 b	31.80	33.38 b	37.02 a
CV	4.14	3.68	5.75	2.83	2.59	6.42

Numbers located on the same column are not significantly different from the Turkey test 5%.
ns=not significant

Stem Diameter

The treatments of 7.5 and 10.0 g inoculums/plant resulted the best stem diameter (0.50 cm) and significantly different with the result from other treatments. Similar to plant height, significant effect of the treatments on stem diameter appeared at week 12 (Table 4). This result was supported by high mycorrhizae colonization and dependency in stump roots at the treatments of 7.5 and 10.0 g inoculums/plant (Table 2). Differed with this study, Nasaruddin (25) reported that effect on stem diameter of cocoa shown 20 weeks after application. Eventhough both of cocoa and rubber are in the same group of perennial plants, in this study, the effect of mycorrhizae on rubber appeared 8 weeks earlier (week 12). This effect was not only by the dose of inoculum or plant rhizosphere system, but also caused by effectiveness of mycorrhiza species. The effectiveness of the arbuscular mycorrhizae used in this study was high, which was indicated by the percentage of mycorrhizae colonization in roots, thus parallel with increasing dose of the treatments (Table 2). Reports of the difference appearance of myccorhiza effect on difference plant species were also mentioned by Abbot *et al* (1).

Table 4. The average diameter of the assessed stump grown in soil amended with arbuscular mycorrhizal fungi.

	Weeks after inoculation (WAI)					
Treatment (g inoculum mycorrhizae/plant)	**2**	**4**	**6**	**8**	**10**	**12**
Control	0.30 a	0.30 a	0.30 a	0.30 a	0.30 a	0.40 b
2,5	0.30 a	0.30 a	0.30 a	0.30 a	0.30 a	0.40 b
5,0	0.20 b	0.20 b	0.20 b	0.30 a	0.30 a	0.40 b
7,5	0.30 a	0.30 a	0.30 a	0.30 a	0.30 a	0.50 a
10	0.20 b	0.20 b	0.20 b	0.30 a	0.30 a	0.50 a
Cv	0,0	0,0	0,0	4.6	4.6	0

Numbers located on the same column are not significantly different from the Turkey test 5%.

Number of Leaves

As to the observation of plant height (Table 3) and stem diameter (Table 4), the best results on the observation of the number of leaves (17.62) were also obtained from the treatment of 10 g inoculum myccorhiza (Table 5). The result was supported by Nakmee *et al* (24) which accredited arbuscular mycorrhiza for the increase in the number in sorghum treated. According to Nasaruddin (25), an additional one unit dose of mycorrhizae increases the number of leaves of the cocoa plant as much as 0.84 sheet. Mycorrhiza can explore areas within the soil that are unreachable by roots, thus enabling it to absorb the nutrients needed by plants, including micro-elements (27, 17). The volume of soil that can be explored by the roots associated with external hyphe of mycorrhiza range between 5-200 times more and widespread, compared to root exploration without mycorrhiza (1). According to Zachee *et al* (35), mycorrhiza's significant role is found in the formation of nodule on *Arachis hypogea* L which affect vegetative growth parameters (plant height, leaf number, number of branches) and production.

Table 5. The average number of leaves assessed for the improvement of health and quality of the rubber stumps amended with arbuscular mycorrhizal fungi.

Treatment (g inoculums mycorrhiza /plant)	Weeks After Inoculation (WAI)					
	2	**4**	**6**	**8**	**10**	**12**
Control (0 g)	8.14 ns	10.12 ab	13.08 ab	14.94 a	15.32 ab	15.44 b
2,5	7.64	9.90 b	11.72 b	13.90 b	15.14 b	15.36 b
5,0	8.52	10.48 ab	13.06 ab	14.90 ab	16.08 ab	16.46 ab
7,5	7.78	12.50 ab	12.54 ab	14.40 ab	15.72 ab	15.76 b
10	8.56	10.92 a	13.62 a	15.72 a	17.08 a	17.62 a
Cv	10.35	9.46	9.19	8.88	7.97	6.73

Numbers located on the same column are not significantly different from the Turkey test 5%.
ns = not significant

Leaf Width

Not all mycorhhiza arbuscular treatments giving a positive response at the same time by the plant. Nakmee *et al* (24) found that all the observations of vegetative and production parameters on *Sorghum bicolor*

Linn increased, except for plant height. Also no differences were found on seedlings height of *Sorea leprosula* Miq treated with symbionts of *Gigaspora* sp and *Glomus* sp. While Fitriyah (6) did not find a higher increment of rice treated with mycorrhiza 55 days after sowing, whereas other observational components provide a response. It is also common in this study, in which all observation parameters were observed (plant height, leaf number, stem diameter and mycorrhiza colonization) were affected by the dose of treatments, except leaf width (Table 6). According to Hadley (8) there are parts of plants growth are based on genetically control.

Table 6. The average width of leaves of the rubber stumps amended with with arbuscular mycorrhiza fungi.

Treatment (g inoculums mycorrhizae/plant)	Weeks After Inoculation					
	2	4	6	8	10	12
Control (0)	0	2.84 a	4.26 ab	5.53 b	5.75 b	6.42 a
2,5	0	2.98 a	4.50 a	5.74 ab	5.78 b	6.40 a
5,0	0	3.08 a	4.52 a	6.19 a	6.49 ab	6.57 a
7,5	0	3.19 a	4.01 b	5.43 b	6.65 a	6.81 a
10	0	2.90 a	4.18 ab	5.44 b	6.62 ab	6.75 a
Cv	0.0	10.76	8.39	6.81	7.62	12.77

Numbers located on the same column are not significantly different from the Turkey test 5%.

From observations on the treatment of stem height (Table 3), stem diameter (Table 4) and the number of leaves (Table 5), it were found that the treatment of 10.0 g mycorrhiza inoculation / plant give the best results. This was supported by the percentage of mycorrhiza colonization and dependency which is high in this study (Table 2). Even at the dose of 10.0 g, the level of plant dependency on mycorrhiza can be categorized as high.

In this study, the role of multiple symbionts of *Glomus* sp and sp *Acaulospora* in promoting vegetative growth of rubber stumps (height, stem diameter and number of leaves) were evident at week 12, except at the leaf width. Both of these mycorrhizae species are among the most effective inoculants and have been demonstrated to be the pioneer fungus that colonized plant hosts (1, 24, 14). The evidence is the accumulation of plant host colonization from multiple symbionts which result in complementary effect with respect to plant growth (3, 10, 14, 15) as it was also found on rubber stump in this study.

CONCLUSION

The quality growth of rubber stumps planted in red yellow podzolic soil can be improved simultaneously with the application of native arbuscular mycorrhizae from West Sumatra. This study found that the percentage of colonization and mycorrhiza dependency of rubber stump is high. The results of this research presents an enormous opportunity to use red yellow podzolic soil which is amended with arbuscular mycorrhiza, for the development of new rubber plantation in Indonesia.

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