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Utilization of Biopore Infiltration Hole and Cross Drain Technology to Improve Root Geometry and Mycorrhizal Colonization in Skidding Road

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Abstract

Root geometry is important factor in planting in skidding roads. Soil compaction, lack of mycorrhizal population and stunted seedling growth are the common problem in skidding road for reforestation success. Implementation of bio-pore infiltration hole (BIH) and cross drain (CD) technology may improve the skidding road problems. The aim of the study was to examine the use of BIH and CD technology to improve root geometry and mycorrhizal colonization in skidding roads. *Shorea leprosula* and *Shorea parvifolia* seedlings were planted in planting hole of 30 x 30 x 30 cm and surrounded with 4 BIHs. The planting distance was 2.5 x 2.5 m. Each unit experiment was bordered with cross drain to minimize the erosion and run off. The results showed that BIH and CD, contributed significantly to root geometry that is reflected in their shoot root ratio. Root geometry in *S.parvifolia* was better than in *S.leprosula* due to the increment in number and the length of primary and secondary roots in BIHs. The ectomycorrhizal colonization in both species was 45.6 to 62.7 %.

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A massive mantle sheets was formed in root tips in one layer with the thickness 7.2 to 14.8 µm. Hartig's net penetration reached to 10.3 to 14.7 µm. It was formed in between two radially elongated epidermis cells (REEC).

***Keywords*:** bio-pore infiltration hole, cross drain, root geometry, mycorrhizal, skidding road.

1. Introduction

Soil plays an important role for tree growth, due to its water and mineral resources, medium of tree for root growth [1, 2]. [3] stated that soil greatly affects the implementation of silvicultural practices such as species selection, prediction success on planting technology, seedling growth and stand production. One of the soil properties that affect root development is the soil physical properties [4]. Components of soil physical properties that directly related to the ability root development and root volume are texture and structure of soil as well as the bulk density resulted from soil compaction [3]. Various activities of heavy equipment in skidding road causes soil compaction [5]. Soil compaction that occurs in a long period of time may have a negative impact on soil properties and plant growth. Compaction of the soil will alter the soil structure and the soil pores, so that the soil water content in skidding road will also change [4]. The decrease in soil porosity and increased bulk density and low soil water availability can interfere root development.In these conditions, the roots will adjust itself to grow and develop properly and in turn will impacting on root geometry [6].

The results of the study in skidding road in this study shows that the bulk density in skid trails after Bio-pore Infiltration Hole (BIH) and the Cross Drain Technology (CD) are made up of between 1.28 and 1:36 gr/cc^3. But establishment BIHs around plants and CDs has increasedthe soil permeability to 30.03%, increasing the CEC (19.87 meg/100 grams), P available in the BIH increased40%, content of Al^{+3} undetected and increase soil microbial respiration. The existence of BIH and CD reduced runoff and erosion. Thus BIH and CD could improve the soil properties on skidding road.

Root geometry is a root morphological form as a result of the growth made by the apical meristem development located at the tip of the root. Lynch [7] explains that the term of root geometry has been widely used to express the forms or types of root systems which constitute the genetic aspects and is an important aspect in plant productivity. Kramer & Kozlowski [8] reported that the root system of trees has large roots and many roots branches. Variations in root distribution and root status is very important because roots are able to penetrate in different soil structure to form branching system and to absorb water and minerals from the soil as tree growth medium.

Application of soil and water conservation techniques such as the use of BIH and CD is important to support root development. Establishment on BIH and CD are expected to improve the condition soil medium to support plant growth and to improve plant root geometry that will improve both rooting quality and quantity. Besides BIH, seedling inoculation with ectomycorrhizal can also be performed. Ectomycorrhizal fungi inoculation are expected to extend root absorption surface in order to reach difficult areas to be penetrated by root system[9]. Mycorrhizal mycelia are probably much more effective than root hairs in reestablishing early contact with the soil. The fungi that are involved need only a supply of reserve foods from the plant and the proper conditions of moisture and temperature to resume growth; consequently, they do not depend on resumption of full activity by the whole plant. It is significant that most tree species normally enter into mycorrhizal relationships and may require them to survive or thrive after planting [10]. The conditions of both treatments are expected to be able to improve the process of nutrients and water absorption in skidding road soils that have undergone a process of soil compaction.

The objective of the study was to examine the use of BIHs and CDs technology to improve root geometry and mycorrhizal colonization on skidding road.

2. Materials and Methods

The experiment was conducted in the natural forest of PT. Suka Jaya Makmur Forest Concession Holder, West Kalimantan for a year from October 2012 to September 2013. Study area is located at 159 km from Ketapang District. Geographically located between study sites of $110^{o}27$ E - 111^{o} 25 'E and $01^{o}00$'-$01^{o}55$'S with altitude of 700 masl.

The materials used in this experiment were the root of meranti (*S. leprosula* and *S. parvifolia*), age of one year after planting, planted near the bio-pore infiltration holes. Tools used include calipers, rulers, analytical balance, petri dish, digital microscope, solution of FAA (Formaldehyde acetic acid), histological dyes (safranin, Alcian blue and paraffin), counters, markers, preparations, tisue, binocular microscopes and books and stationary.

Seedlings of *S.leprosula* and *S.parvifolia* were planted in skidding road after cross drain constructions. The planting hole was surrounded by four BIHs. The distance between two CD was 20 m. The plot size of each treatment was 4 m x 20 m. The planting distance was 2.5 m x 2.5 m. The experimental plot was repeated in three places of skidding road. Factorial design in completely randomized design was used.

Data collection on root geometry was done by digging and observing the root system of one year old plant of *S.leprosula* and *S.parvifolia* seedlings. Plant roots were classified as horizontal roots (H_{root}) if the angle between the root and the vertical plane is greater than or equal to 45^{o} ($\geq 45^{o}$). If the angle is less than 45^{o} ($<45°$), the roots were classified as vertical root (V_{root}). Horizontal root fraction is the ratio between the surface area of the roots horizontally with a total surface area of the root (horizontal + vertical). Shoot-root ratio were expressed by the ratio between the total cross-sectional area at the root of the stem cross-sectional area or basal area. In addition to the shoot-root ratio, counting the number of primary and secondary roots and root length were also conducted.

Root distribution was observed by calculating shoot-root ratio, which is calculated from the square of stem diameter (d^2) and the sum of the squares of all root diameter ($\Sigma\ dr^2 + H + V_{roots}$) of each individual tree according to the following formula [11]:

$$\text{Shoot-root ratio} = \frac{d^2}{\sum_{1}^{n} d_r^{\,2}}$$

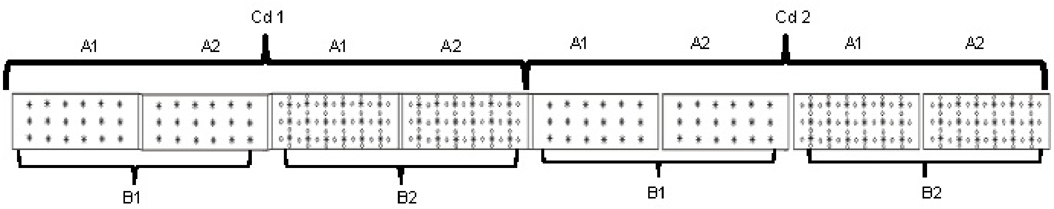
where:

n = Number of all roots (horizontal and vertical)

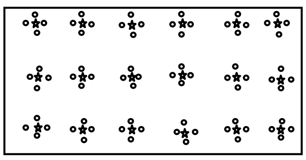
H = Horizontal Root

V = Vertical roots

The percentage of mycorrhizal roots were observed visually and then calculated its of colonization level. Observations mycorrhizal roots was performed using a microscope according to method described by [12].



Remarks:
Cd 1 =No Cross drain
Cd 2 = with Cross drain
A1 = *Shorea leprosula*
A2 = *Shorea parvifolia*
B 1 = No bio-pore infiltration hole
B 2 = with bio-pore infiltration hole



Remarks :

* = planting hole

= Bio-pore infiltration hole

Figure 1. Lay out of experimental plot in study site.

$$\text{The percentage of mycorrhizal colonization} = \frac{\text{Total of infected root length by ectomycorrhizal}}{\text{Total of infected and uninfected root length}} x\ 100\%$$

Mycorrhizal colonization observations were performed with the following phases:

1. Taking primary, secondary and tertiary roots and calculation of the root diameter.
2. Tertiary roots were collected and put into a solution of FAA
3. Tertiary roots were washed and observed then further determination of the number of roots under compound microscope.
4. Root anatomy for observation was made by the method of [13] .This method starts with the root fixation using the FAA for 24 hours, and followed by dehydration process using ethanol at concentration of 40 %, 60 %, 80 % and 95 % . The next process is pre-parafination by using the xylol solution at concentration 25 %, 50 %, 75 % and 100 %, which aims to eliminate ethanol from plant tissue then replaced by paraffin, this process uses 100% ethanol and xylol with multiple stages. Parafination process was done by using mixture of xylol and paraffin with different concentrations and different phases during 24 hours. This process was carried out in anoven with a temperature of 55 °C.
5. Results of parafination were then printed and cut using a rotary microtome with a thickness size of 5-10 µm, and then proceed with the root stainingstage. Root staining techniques performed in 8 stages (among others: fixation in FAA,dehydration, praparafination,parafination, blocking, cutting roots, root staining and root observations). Safranin dye 0.5% and 1% alcian blue was used. Once this was done, the whole process of preparation of the roots can be observed under photonic microscope to observe root anatomy.

Observations were made on the root morphology (% colonization, root system) and root anatomy. Root anatomy observations were made by observing the mantle thickness and Hartig net depth of root histology results.Observations were made on several samples of the colonized roots of *S. leprosula* and *S. Parvifolia*seedlings.

3. Results And Discussion

3.1. Shoot-root ratio

Shoot-root ratio on *S.parvifolia* and *S.leprosula* plants are presented in Table 1. Single treatment with BIH and no BIH increased shoot-root ratio of *S.leprosula* from 37.1 % to 55.6 % while in *S.parvifolia* increased from 106.8 % to 139.6 %. It means the presence of BIH increase the shoot root ratio. It is possible that BIH improved the root system of *S.leprosula* and *S.parvifolia* planted in skidding road. Meanwhile the treatment CD did not affect the shoot root ratio either in *S.leprosula* and *S.parvifolia*. Application of soil and water conservation techniques such as making bio-pore infiltration hole affected plant shoot-root ratio of both *S.leprosula* and *S.*

parvifolia. Bio-pore infiltration hole is instrumental in providing growing space for plant roots. Infiltration bio-pore hole will increase water reserves in the soil and prevent the flow of water in the soil surface [14]. The holes will be filled with air and facilitate the passage of water in the soil [15]. Bio-pore infiltration holes made around the plant will be able to build systems that better root geometry. In addition, bio-pore infiltration hole can also help the development of various types of soil microbes including mycorrhizal fungi that play important role in nutrient and water absorption for seedling growth. In over all, it appears that shoot- root ratio value in *S.parvifolia* was higher than in *S.leprosula.*

Table1.1 Effect of bio-pore infiltration hole (BIH) and cross drain (Cd) on shoot-root ratio in *Shorea leprosula* and *Shorea parvifolia* seedlings

Treatment	*Shorea leprosula* (%)	*Shorea parvifolia* (%)
No Biophore Infiltration Holes	37.1 a	106.8 a
With Biophore Infiltration Holes	55.6 b	139.6 b
No*cross drain*	49.9	134.5 a
With *cross drain*	40.8	110.5 b

Description: values followed by different letters means significantly difference at Duncan test

$p > 0.05$

3.2. Root Length

Inhibition of root elongation is not necessarily correlated with inhibited uptake of mineral nutrients [16]. In compacted soils the contact between roots and soil increases and thereby also the delivery rate for mineral nutrients [17], as indicated, for example, by higher uptake rates per unit root length of nitrate [18] and [19]. The increase in phosphorus uptake per unit root length can occur for various reasons, not only the higher buffer power of soils. It may be, for example, expression of a higher demand placed by the shoot on a smaller root system [20] or brought about by root-induced changes in the rhizospore. An increase in soil bulk density decrease the total root length of maize very considerably but did not alter the percentage of photosynthates allocated to the roots, thus leading to a twofold increase in consumption of photosynthates per unit root length [21].

Despite the various compensatory reactions of root systems in compacted soils, plants usually grow poorly in soils of high bulk density [22]. Insufficient water and nutrient supply might play a role, but often both shoot growth and transpiration are first reduced, regardless of the plant nutrient and water status. In compacted soil shoot growth is also often more depressed than root growth, suggesting root-derived hormonal signals in response to soil compaction [23]. Most likely the root cap is also the sensor of this stress factor. In shoot responses to soil compaction considerable differences occur not only between plant species but also genotypes within species like barley and wheat. There is a tendency, for genotypes with lower growth rates in non-compacted soils to respond less to soil compaction than genotypes with high growth rates [24].

3.3. Root Volume Restriction

Restriction of the rooting volume, for example , by small pot size, has inhibitory effects on shoot growth similar to those of soil compaction. In soil-grown plants inhibited shoot growth by 'root restriction stress' (bonsai effect) might be caused at least in part by limited nutrient and water supply to shoots. However, the same effect occurs in sand cultures percolated with nutrient solutions [25] or in containers with a flow-through hydroponic culture system. Root activity, expressed as oxygen consumption per unit root weight is distinctly lower under root restriction stress, and some increase in ethylene formation occurs, but this is probably a secondary event [26].

Under root-restriction stresses there is a relatively similar decline in root and shoot dry weight with decrease in pot size [25]. A reduction in leaf elongation rates is the most distinct response to root-restriction stress, and this reduction is primarily the result of reduced cell division and not so much of cell size [27]. Thus, inhibited shoot growth under root-restriction stress is most likely a process regulated by root-derived hormonal signals in which nutritional factors or plant water relations may, or may not, play a secondary role. Root-restriction stress has practical implications for pot-grown plants in horticulture, but also deserves more attention in pot experiments.

The effect of soil water content (or of the soil water potential) on root growth is expressed in the form of a typical optimum curve for which chemical and physical factors in the soil are the responsible parameters. In dry soils mechanical impedance and low soil water potential are the dominant stress factors. In saline soils the salt accumulation in general and the root surface in particular further decrease soil water potential and, in combination with ion imbalance in the rhizosphere, impose an additional stress factor root growth and functioning [27].

Interaction treatment between BIH and CD, *S.leprosula* has the longest primary root length (7.62 cm) and significantly different from other treatments (Table 2.). Table 2 also shows that the treatment did not significantly affect root length of secondary roots. In contrast to *S. leprosula*, secondary root length *Shorea parvifolia* plants were not affected by interaction treatment between BIH and CD, (Table 3).

Utilization bio-pore infiltration hole (BIH) and cross drain on skidding road and for reducing the soil run off. are expected to enhance the water absorption and retention capacity of the soil as well as for increased the soil fertility. In addition, the level of soil moisture will provide suitable place for root development. Table 2 showed that thelength of primary roots were not affected by the BIH and CD but those treatments affected the length and number of secondary roots.

In overall, the combination treatments of BIH and CD were not significantly affected by the treatment to root system of *S.parvifolia* (Table 3). Mycorrhizal symbioses play a prominent role in the biology and ecology of forest trees. They involve soil fungi and roots of trees, which together as a symbiosis provide the fungi with carbohydrates and enhance the uptake of water and nutrients for the trees, and also have a major protective role for the roots [29].

Table 2.Effect of interaction treatments between bio-pore infiltration hole (B) and cross drain (Cd) treatment on the primary root length, secondary root length, number of roots primer, and the number of secondary of *Shorea leprosula* seedlings.

Treatment	*Cross-drain*	The length of primary root	The length of secondary root	The number of primary roots	The number of secondary roots
No bio-pore infiltration hole (B1)	No *Cross-drain* (Cd1)	4.53 a	8.63 b	7.52 a	36.78 c
	With *Cross-drain* (Cd2)	5.32 a	7.27 b	7.22 a	49.78 b
With bio-pore infiltration hole (B2)	No *Cross-drain* (Cd1)	5.90 a	10.09 a	5.00 c	47.00 a
	With *Cross-drain* (Cd2)	7.62 a	10.71 a	10.67 b	47.60 a

Description: values followed by different letters means significantly difference at Duncan test a>0.05

Table 3. Effect of interaction treatments between bio-pore infiltration hole (B) and cross drain (Cd) treatment on the primary root length, secondary root length, number of roots primer, and the number of secondary of *Shorea parvifolia* seedlings.

Treatment	*Cross-drain*	The length of primary root	The length of secondary root	The number of primary roots	The number of secondary roots
No bio-pore Infiltration hole (B1)	No *Cross-drain* (Cd1)	5.07 ns	7.87 ns	5.00 ns	31.89 ns
	With *Cross-drain* (Cd2)	4.06 ns	6.32 ns	8.00 ns	44.00 ns
With bio-pore infiltration hole (B2)	No *Cross-drain* (Cd1)	4.38 ns	6.84 ns	9.11 ns	39.67 ns
	With *Cross-drain* (Cd2)	8.50 ns	8.98 ns	10.33 ns	94.00 ns

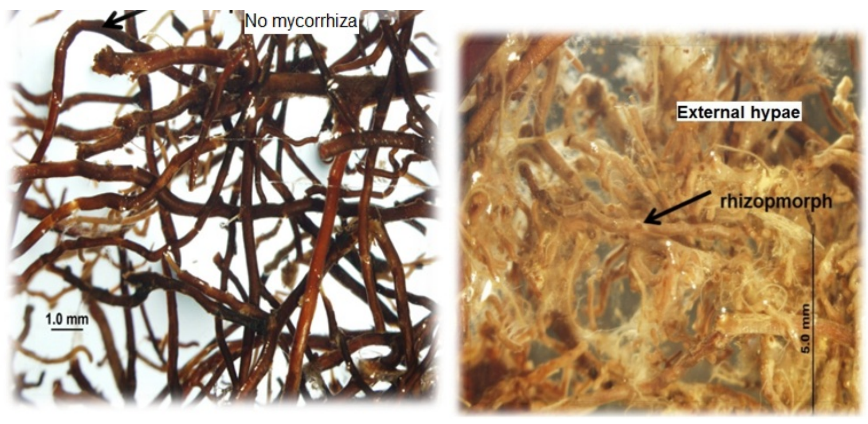
Description: values followed by different letters means significantly difference at Duncan test (a < 0.05), ns = no significant difference at Duncan test $\alpha > 5$ %

The effect of BIH and CD on ectomycorrhizal colonization percentage is presented in Table 4. The effect of BIH on *S.leprosul* and *S.parvifolia* on mycorrhizal colonization percentage was not significantly different. Ectomycorrhizal colonization on roots of *S.leprosula* and *S.parvifolia* was at moderate levels (40% - 60%). It means that Dipterocarpaceae is associated naturally with ectomycorrhizal fungi.[30] stated that the symbiosis ectomycorrhizal fungi in Dipterocarpaceae could happen naturally if the soil contain sufficient probable number of ectomycorrhizal inoculants in the soil. *S.leprosula* and *S.parvifolia* were also associated with Scleroderma sp as reported by [31].It is also possible that treatment had similar effect on mycorrhizal colonization due to the environmental conditions. The availability of sufficient water, moisture and air could support the development of ectomycorrhizal [32]. In overall, bio-pore infiltration hole increased the ectomycorrhizal colonization in *S.leprosula* and *S.parvifolia.* It is also possible that treatment had similar effect on mycorrhizal colonization due to the environmental conditions. In overall, bio-pore infiltration hole increased the ectomycorrhizal colonization in *S.leprosula* and *S.parvifolia.*

Table 4. Effect of bio-pore infiltration hole (BIH) and *Cross drain* (CD) on percentage of colonization in *Shorea leprosula* (A1) and *Shorea parvifolia* (A2) seedlings

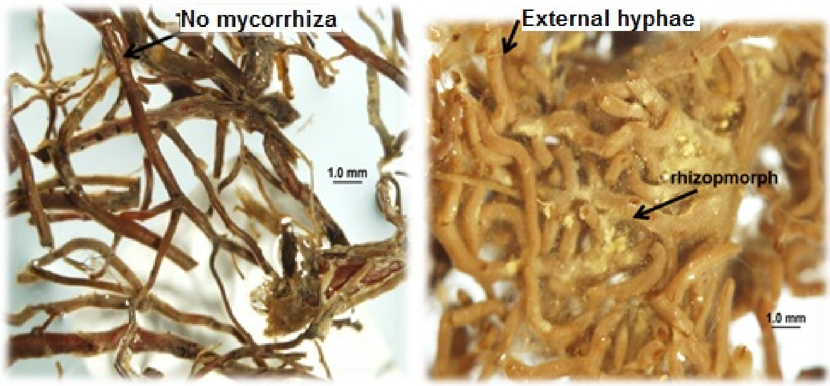
Treatment	*Shorea leprosula* (%)	*Shorea parvifolia* (%)
No Bio-pore infiltration holes	56.1 a	46.8 a
With bio-pore infiltration holes	62.7 a	57.2 a
No *cross drain*	58.6 a	45.6 a
With *cross drain*	60.1 a	58.5 a

Description: values followed by different letters means significantly differences (P < 0.05)



(A) (B)

Figure 1. Non mycorrhizal root (A) and mycorrhizal roots (B) in the root system of *Shorea leprosula*



(A) (B)

Figure 2. Non-mycorrhizal root (A) and mycorrhizal roots(B) in the root system *Shorea parvifolia*

Figure 1 and 2 showed clearly non mycorrhizal and mycorrhizal roots either in *S.leprosula* and *S.parvifolia* seedlings. Mycorrhizal roots are heavily sheeted with external hyphae in white color.

3.4. Ectomycorrhizal structure

Ectomycorrhizal structure at the root of *S. leprosula* and *S.parvifolia*is presented in Tables 5 and 6. Hartig net and mantle in the root system of *S. leprosula* on the were affected by the combination treatments of BIH and CD (14.7 μm and 14.8 μm) (Table 5). The highest root diameter in *S.leprosula* was found in the combination treatment BIH and No CD (197.6 μm) while [30] found colonized root diameter was 0.3 to 0.6 mm covered by hyphae mantle. Its Hartig net develops between root-hair-like out-growth of the epidermal cell in the root system of *Neea off floribunda*. In overall the presence of BIH improved the mycorrhizal colonization in *S.leprosula*.

Table 5. Effect of interaction treatments between BIH and CD on Hartig net and mantel formation, root radian on *S.leprosula*

Treatment		Hartig net (μm)	Mantle (μm)	Root diameter (μm)
No BIH (B1)	No cross drain (Cd1)	12.3 ab	13.4 a	107.6 c
	cross drain (Cd2)	10.4 b	7.3 b	127.4 b
With BIH (B2)	No cross drain (Cd1)	13.7 ab	14.8 a	197.6 a
	cross drain (Cd2)	14.7 a	14.8 a	133.0 b

Description: values followed by different letters means significantly differences (P < 0.05)

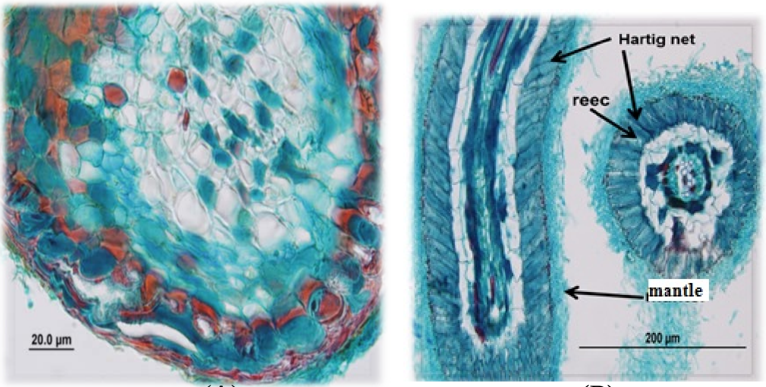
Table 6. Effect of interaction treatment between BIH and CD on Hartig net and mantel formation, root diameter on *S.parvifolia*

	Treatment	Hartig Net (μm)	Mantle (μm)	Root diameter (μm)
No BIH (B1)	Nocross drain (Cd1)	10.9a	13.8 a	138.2a
	cross drain (Cd2)	10.30a	8.2 b	123.6a
With BIH (B2) (B2)	No cross drain (Cd1)	17.3a	7.2 b	167 a
	cross drain (Cd2)	18.3a	14.8 a	172.2a

Description: Values followed by different letters means significantly differences (P < 0.05)

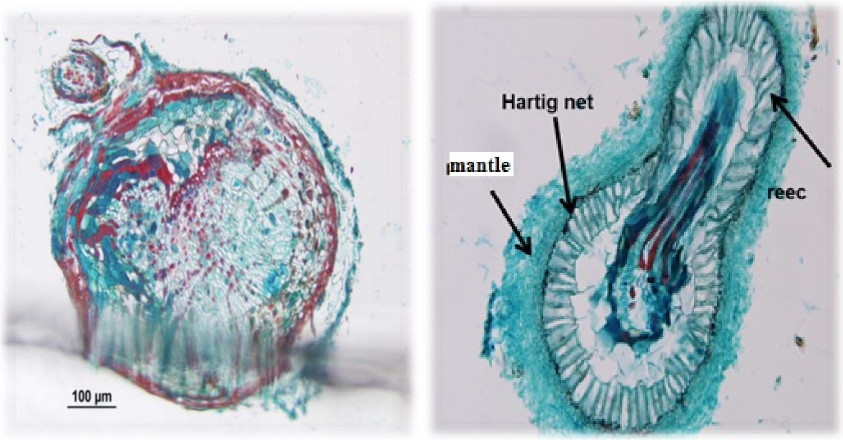
Table 6 shows that BIH and CD did not significantly effected the hartig net and root diameter in *S.parvifolia* but the mantel formation was affected by both treatments. Histology structure of mycorrhizal roots *S.parvifolia* and *S.leprosula* can be seen in Figure3. Ectomycorrhizal root structure in both Dipterocarpaceae species has thicker mantel, Hartig net and elongated radially epidhermal cells are (reec). The mantel thickness in those combination treatments was 14.8 μm. [32] found the thickness of mantle in *Pakaraimaea dipterocarpaceae spp nitida* (Dipterocarpaceae in Venezuela) is 15 – 25 μm wide, with two layers.The existence of radially elongated epidhermal cells (REEC) are characteristic of Dipterocarpaceae roots associated with ectomycorrhizal [34].

Ectomycorrhizal colonization in the root system are mostly in the root tip. It is in accordance with [35] that twenty four tree species were found to be symbiotically associated with ectomycorrhizal fungal species on the basis of root tip colonization.



(A) (B)

Figures 3. Cross-section of nonmycorrhizal root (A) and mycorrhizal root (A) of *Shorea leprosula*



(A) (B)

Figures 4. Cross-section of non mycorrhizal (A) and mycorrhizal roots (B) of *Shorea parvifolia* Description : reec = radially elongated epidermis cells

Supriyanto [36] stated that the existence of mantle and Hartig net tissues on the root system of Dipterocarpaceae species explained the status of the compatibility between the host plant with ectomycorrhizal fungi. Other supported research results were also done by [37] and [38] on *Shorea javanica* seedlings Hartig net is the place for nutrient exchange between fungi and plant as well as for carbohydrate from plant to fungi and water from fungal hyphae to plant [30]. According to [39] autoradiographic analysis of the flow of carbohydrates from root cells into the Hartig net cells and then to mantle cells also in other opposite direction is found also the flow of phosphate to the Hartig net and mantle cell to the root cells.

The mantle is a barrier between the plant roots with soil. Some researcher have been done to look at the function and mantle form [40, 41, 42]. Mantle function is a place for storage of various organic matters, minerals, heavy metal elements that could potentially be toxic to the plants, so the plants will be protected from poisoning and protect the roots from water loss and pathogen attack [30]. The number of mantle layers in generally related to the compatibility level between plant and ectomycorrhizal fungi.

The presence of massive external hyphae in root system of *S.leprosula* and S.*parvifolia* are mostly due to the BIH treatments. With these results BIH and CD is promising silviculture techniques to improve or increase the seedling growth the skidding road (Figure 5).

(A) (B)

Figure 5. Growth of *Shorea leprosula* (A) and *Shorea parvifolia* (B) at skidding road in one year after planting.

4. Conclusion

Implementation bio-pore infiltration hole (BIH) and cross drain (CD) contributed root geometry of seedlings planted in skidding road. In addition to the distribution of the roots, these two techniques create good growing conditions for soil organisms such as ectomycorrhizal. *S. leprosula* and *S.parvifolia* have different root distribution and root symbiosis with ectomycorrhizal fungi , where its colonization level belong to medium level.

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