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Stomata and morphology root of oil palm seeds using organic fertilizer in Ultisol media

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Abstract Organic fertilizer application did not affect stomatal characters, number, stomata density and stomata index. Organic fertilizer treatment changed in tissues and root morphology. The treatments of organic liquid fertilizer of 100 ml.L^{-1} with manure that made root size larger than 50 ml.L^{-1} and 0 ml.L^{-1}. Organic fertilizer treatment increased in the number of transport vessels, size of the transport vessels and cortex which was a sign of Al accumulation in the roots.

Keywords: Acid soil, Organic fertilizer, Root

Introduction

Bengkulu Province is one of the centres of palm oil production in Indonesia. The productivity of palm oil in Bengkulu is the fourth highest in Indonesia, reaching 4,063 tons.ha^{-1} with an enhancement production of 1.06 million tons and an area of 318,352 ha in 2020 (Statistics, 2020). The development of oil palm cultivation continues to increase with the expansion of planting areas. Oil palm cultivations are started from pre-nursery to mature plants that have limitations on planting media or soil conditions. The pre-nursery stage determines oil palm production. Pre-nursery nurseries by nursery companies plant mainly used ultisol as acid soil.

The chemical, physical and biological conditions of acid soil are classified as soil fertility decline. In acid soils, aluminium (Al) is the main factor limiting plant growth because it inhibits root growth and impairs metabolism physiology (Singh *et al*., 2017). Analysis of Al stress plants occurred accumulation of Al in the roots. Al can inhibit plant nutrient absorption like P, K, and Mg (Silva *et al.,* 2010). Al is concentrated in cortical

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root cells, especially in the protoplasm and nucleus (Rorison, 1973). The absence of a shortage of organic matter complicates nutrient deficiencies in acid soils due to poor microbial ecology caused by a lack of metabolic substrates (Michael, 2021). One of the efforts to reduce the level of stress acidity is organic matter amendment. Organic fertilizers not only role factor in increasing the availability of soil nutrients but also can control soil acidity. affecting plant growth. Soil organic matter is the most important indicator of soil productivity, such as an increase in soil C organic which impacts soil properties (Wani *et al.,* 2019). Organic matter is essential in soil components because it acts as a soil buffer against a decreasing pH (Assefa and Tadesse, 2019). Adding organic fertilizers can increase soil pH, organic C, total N, availability of P, exchangeable K, and reduction of Al exchangeable (Ermadani *et al.,* 2019). The effect of acid soil from Al poisoning on oil palm prenursery can be identified through the roots, which activate the cellular response (Song *et al.,* 2022). In addition, identification through the leaf, stomata, is an indicator of the change effect of the environment (Hetherington and Woodward, 2003) as Al affects the growth of oil palm seeds. The main effect of organic fertilizer as a soil ameliorant is to reduce the impact of acid soil on oil palm seedlings. Therefore, the effectiveness of applying organic fertilizer on acid soil in increasing soil fertility is seen through the morphology of the roots and stomata on the leaves of oil palm seedlings. The study aimed to determine the stomata and morphology of the root of palm oil in the pre-nursery phase in acid soil with organic fertilizer application.

Materials and methods

The experiment was conducted from July until October 2021 at the Research Experiment Faculty of Agriculture in Beringin Raya, Bengkulu City, 3°45'14.8"S 102°16'09.5"E, 5 m asl.

Experimental design

The experiment was performed in a Split Split Plot design with three replications and three factors. The main plot was shaded at 50% and 75% shading. The subplot was liquid organic fertilizer (LOF) made from household waste with three levels of B1=0 $ml.L^{-1}$, B2=50 $ml.L^{-1}$ and B3=100 $ml.L^{-1}$. The application of LOF was done five times during the experiment. The subplot was manure kinds with four levels P1=cow manure, P2=chicken manure, P3=goat manure and P4=no manure. The manure was applied 50 g/polybag one week before planting. The total number of experimental units was 72.

The pre-nursery of oil palm using polybag was 15 x 23 cm. Ultisol soil was used as media in the experiment which a pH level of 4.06. The chalk is given two weeks before planting with 5 g.polybag^{-1}. The experiment used seedling from Oil Palm Research Centre Indonesia, Simalungun variety.

Stomata analysis

The leave oil palm as preparation was slashed longitudinally at the leave lower surface. Then place on glass object, drip aquadest and cover by cover glass. Observed the preparation start with 4 x magnification. Measurement of the length and width of stomata obtained magnification 100 x with the formula:

22 oculer = 2 objective
1 oculer = 2 x10 µm/22
= 20/22
1 oculer = 0.9 µm

So, all the values obtained in the measurements multiplied by 0.9 µm.
Stomata density obtained by :

$$Stomata\ density = \frac{the\ number\ of\ stomata}{wide\ field\ of\ view}$$

$$wide\ field\ of\ view = magnification\ \times (length\ \times width)$$

Stomata indeks obtained by :

$$stomata\ indeks = \frac{the\ number\ of\ stomata}{the\ number\ of\ stomata\ \times epidermis\ cell}$$

Histological analysis

The root of the oil palm was slashed as thin as possible and placed on a glass object. The aquadest was dripped and covered by cover glass. Observed the preparation start with 4 x magnification by microscope. Measurement of the root and petioles size obtained magnification 4 x with the formula.

2 oculer = 5 objective
1 oculer = 5 x10 µm/2
= 50/2
1 oculer = 25 µm

So, all the values obtained in the measurements were multiplied by 25 µm.

Results

Stomata oil palm seeds use organic fertilizer in Ultisol media

Based on microscopic observations, the stomata of oil palm seedlings were 21.3-26.7 m long and 6.6-16.8 m wide (Figure 1). The density of stomata occurs at 5.68 to 33.75 mm^2. Stomata size on pre-nursery oil palm leaves showed no difference between organic fertilizer treatments without organic fertilizer, manure and liquid organic fertilizer. However, stomata length was greater in treating 100 $ml.L^{-1}$ liquid organic fertilizer + cow manure.

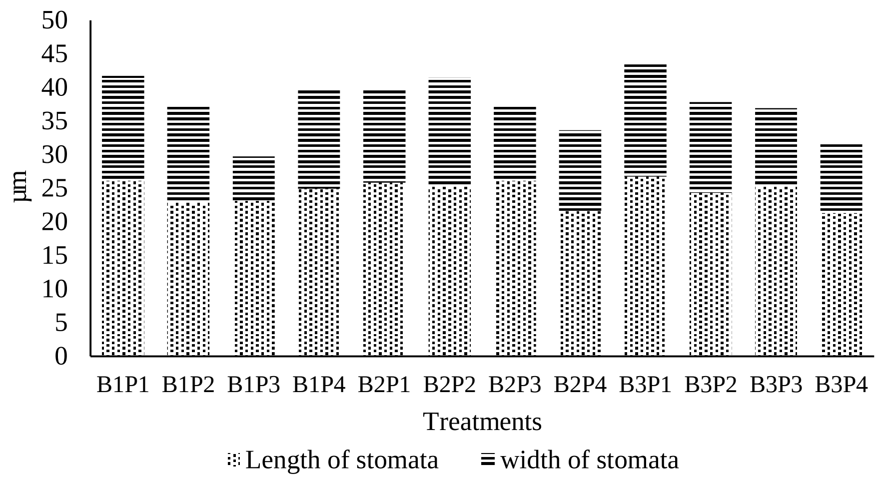

Figure 1. Length and width stomata of LOF and manure application in ultisol soil media

The results of stomatal characters, the number, stomata density and stomata index did not show any effect from the treatment given and without organic fertilizer. The goat manure treatment showed more stomata with an average of 8.3 mm. In contrast, the stomata index was higher than applying 50 $ml.L^{-1}$ liquid organic fertilizer and chicken manure (Table 1). The lowest stomatal density was in the treatment without LOF + goat manure, and the highest was without LOF + cow manure. The addition of organic matter did not have a significant effect on the density of stomata palm oil.

Morphologycal of root oil palm seeds use organic fertilizer in Ultisol media

Generally, the treatments of organic liquid fertilizer 100 $ml.L^{-1}$ with manure made root size larger than 50 $ml.L^{-1}$ and 0 $ml.L^{-1}$. This treatment was adjusted to the size and number of vascular bundles in the roots which are also larger than the others (Table 2). Based on microscopic observations of the root tissue of oil palm seedlings, the cortical layers formed were thin in the treatment without organic fertilizers. In contrast, the cortical network was larger in the treatment with organic fertilizers (Figure 2).

Table 1. The number of stomata, stomata density and index stomata palm oil seeds pre-nursery at organic fertilizer treatments

No	Treatments	The Number of stomata (mm)	Stomatal density (mm^{-2})	Stomatal Indeks (µm)
1	B_1P_1	1,35	33,75	0,075
2	B_1P_2	9,2	28,75	0,067
3	B_1P_3	8,7	5,68	0,10
4	B_1P_4	8,3	23,05	0,093
5	B_2P_1	6,1	16,94	0,053
6	B_2P_2	7,6	19	0,132
7	B_2P_3	8,7	31,07	0,076
8	B_2P_4	3,8	15,20	0,044
9	B_3P_1	8,6	19,54	0,058
10	B_3P_2	7,3	22,81	0,069
11	B_3P_3	8,1	27,93	0,063
12	B_3P_4	7,4	33,63	0,098

Table 2. The root cell analysis palm oil seeds at organic fertilizer treatments

No	Treatments	Root size (mm)	The number of vascular bundles	vascular bundles size (µm)	Standard deviation
1	B_1P_1	2.00	15	112.5	0.70
2	B_1P_2	1.88	9	125	0
3	B_1P_3	1.65	7	112.5	0.70
4	B_1P_4	3.00	16	112.5	0.70
5	B_2P_1	2.45	14	137.5	0.70
6	B_2P_2	1.65	9	112.5	0.70
7	B_2P_3	1.75	14	87.5	0.70
8	B_2P_4	1.98	9	100	0
9	B_3P_1	2.58	18	137.5	0.70
10	B_3P_2	2.85	19	162.5	2.12
11	B_3P_3	2.50	11	212.5	0.70
12	B_3P_4	1.85	13	112.5	0.70

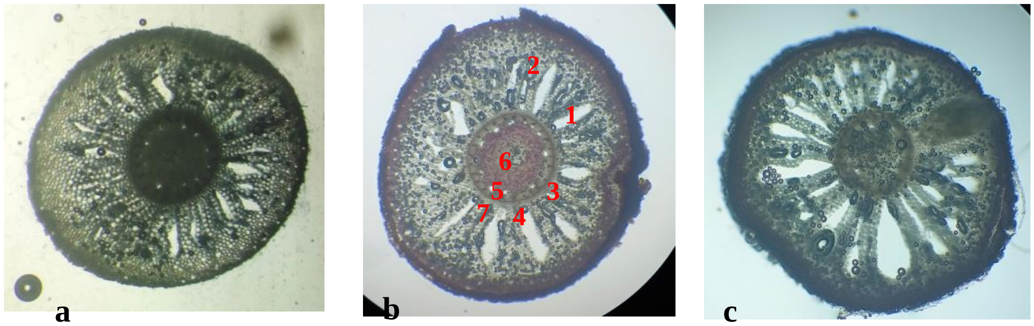

Figure 2. Root tissue oil palm seeds at non and LOF applications, 1. Cortex; 2. Epidemis; 3. Endodermis; 4. Vacular cylinder; 5. Core of parenchyma cells; 6. Phloem; 7. Xylem; a. 50 $ml.L^{-1}$ LOF; b. 100 $ml.L^{-1}$ LOF; c. Non LOF

Visually, the root of oil palm seeds morphology in Figure 3 showed that treatment without LOF (B1) and 50 $ml.L^{-1}$ (B2) had little root hair compared to 100 $ml.L^{-1}$ (B3). Morphology of root without manure (P4) treatment appeared the length of root longer than others. The application of 100 $ml.L^{-1}$ LOF + chicken manure (B3P2) formed a lot of root hairs and quarternary roots.

Discussion

The results of the microscopic analysis showed that the length of stomata palm oil seeds belonged to small stomata from 21.3 to 26.7 µm, and stomatal density occurred from 5.68 to 33.75 mm-2. The range size stomata from about 10-80 µm and densities occur 5 - 1000 $mm-^{2}$ of epidermis that differently on species and environment (Hetherington and Woodward, 2003). The length of stomata correlated positively with stomatal sensitivity that affected leaf water potential (Aasamaa *et al.*, 2001).

The organic fertilizer treatment did not affect stomata size, but the addition of 100 ml.L-1 LOF and cow manure resulted in a larger stomata length. Stomata respond quickly to environmental changes, especially leaf water potential. Stomatal size plays a key role in this control, and there is a negative relationship between stomatal pore length and sensitivity to increased drought. (Hetherington and Woodward, 2003). Al toxicity significantly changes stomatal density, index and shape coefficient (Smirnov *et al.*, 2014). The results of stomatal characters, such as the number of stomata, density and stomata index, did not show any effect from the treatment and without organic fertilizer. Applying organic material in acidic soil conditions can control the condition of leaf water potential that maintained water movement from the soil to the leaf.

However, stomata length was non-significant with organic fertilizer and non-organic fertilizer on ultisol media.

Figure 3. The morphology of roots palm oil seeds at organic fertilizer treatments

Al toxicity in plants can reduce P, K and Mg levels in wheat crops (Silva *et al*., 2010). This caused disturbance in the osmotic balances for cell extension. In addition, endodermal differentiation can be effected by the minimal accumulation of Al in the roots of tolerant wheat plants (Silva *et al*., 2010). The root tissue bulging between the application of organic fertilizer and without application on the size of the cortex were differed. It indicated the accumulation of Al in the cortex. However, the interaction of Al with nutrients depended on the level of plant tolerance. According to Supena *et al*. (2014) stated that the Al content obtained by plants was around 30-90% localized in the root apoplast, and most of the Al accumulates in the cell walls (Supena *et al*., 2014; Iqbal, 2012 and Supena *et al*. 2014).

Based on the morphological appearance of the roots of oil palm seedlings, it showed symptoms of Al toxicity or plant growth disturbance in the roots. The differentroot length in organic fertilizers in each treatment was due to Al-dd in different soils. Changing morphology of oil palm roots under Al stress were changed in primary root length, which decreased by 24.3% at 300 ppm Al stress (Supena *et al.*, 2014). It can be seen in the 0 ml.L-1 LOF treatment with short roots. Al toxicity causes deformation at the root tips and brittleness because the root tips, root cap, meristem and elongation zone are sensitive to Al (Iqbal, 2012). The Simalungun variety is Al-tolerant with relatively unchanged roots up to 300 ppm Al stress (Supena *et al*., 2014). However, in this study, oil palm seedlings of the Simalungun variety obtained symptoms of Al toxicity.

Besides root length, reduced root hair development is the earliest recognized symptom of Al toxicity (Singh *et al*., 2017). A small amount ofroot hairs were seen in the treatment without LOF and manure (P4). While the root hairs that formed the most were in the application of LOF 100 ml.L-1 + chicken manure. Adding chicken manure can improve soil texture, affecting the number of growing root hairs. Organic matter releasing the organic compounds into the soil led more negative charges that can retain the acid cations (Michael, 2021).

The capacity of the soil to absorb organic matter depended on the content of Al oxides. Several factors increased the concentration of dissolved organic matter in solution that increased in pH, decreased in ionic strength of electrolyte solution and the valence of cations in solution, and decreased in the strength of anions in solution (Haynes, 2005). Al exchangeable can be reduced due to the application of organic waste fertilizer because it increased organic C up to 24% (Ermadani *et al.*, 2019). Organic matter can increase soil pH due to the oxidation of organic acid anions present in decomposing residues, ammonification of residue N, specific adsorption of organic molecules produced during decomposition, and reduction reactions induced by anaerobiosis (Haynes and Mokolobate, 2001). However, the nutrients from organic fertilizers are slowly released and depended on the microorganisms presented in the organic fertilizers (Michael, 2021).

Organic fertilizer treatment provided the changes in tissues and root morphology. It increased the number of transport vessels, the size of the transport vessels and the size of the cortex which showed a sign of least Al accumulation in the roots. Morphologically, the chicken manure forms are given quaternary roots that contributed to nutrient absorption.

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