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Assessment of Drought Adaptation in Hevea Brasiliensis PB 260 Clone Seedlings During El Nino Events

Yayuk Purwaningrum[1*], Yenni Asbur[1], Dedi Kusbiantoro[1], Dian Hendrawan[2], Khairunnisyah Nasution[2], Fiqi Alfisar Lubis[3]

[1] Department of Agrotechnology, Universitas Islam Sumatera Utara, 20144 Medan, Indonesia
[2] Department of Agribusiness, Universitas Islam Sumatera Utara, 20144 Medan, Indonesia
[3] Department of Agrotechnology, Universitas Amir Hamzah, 20371 Deli Serdang, Indonesia

[*] Correspondence: Yayuk Purwaningrum (yayuk.purwaningrum@fp.uisu.ac.id)

Received: 01-05-2024 **Revised:** 03-16-2024 **Accepted:** 03-24-2024

Citation: Purwaningrum, Y., Asbur, Y., Kusbiantoro, D., Hendrawan, D., Nasution, K., & Lubis, F. A. (2024). Assessment of drought adaptation in Hevea brasiliensis PB 260 clone seedlings during El Nino events. *Org. Farming*, *10*(1), 1-12. https://doi.org/10.56578/of100101.

Abstract: The atmospheric El Nino phenomenon, characterized by elevated sea surface temperatures in the eastern Pacific Ocean, leads to reduced precipitation and increased temperatures in Indonesia due to diminished influx of moist air. These conditions necessitate the development of drought-resistant rubber (Hevea brasiliensis) seedlings, particularly for regions susceptible to such climatic variations. This study focuses on the PB 260 clone, investigating the efficacy of burnt husk applications in enhancing drought resilience. Employing a non-factorial randomized block design (RBD), three treatments were administered to the seedlings: no burnt husk, burnt husk as mulch, and burnt husk as a planting medium, with each treatment replicated three times and utilizing 30g of burnt husk per polybag. It was observed that the application of burnt husk as mulch significantly promoted root growth compared to the other treatments. This was quantified by measurements showing an increase in root length (98.7m), surface area (45.54m²), and volume (30 mL). These results suggest that the use of burnt husk as mulch might offer a viable strategy for enhancing drought adaptation in Hevea brasiliensis, providing a foundation for earlier tapping maturity through improved root development under drought conditions.

Keywords: El Nino; Hevea brasiliensis; Drought adaptation; Burnt husk mulch

1. Introduction

The impacts of global warming and climate change, such as increased temperatures and droughts, inhibit plant growth (Muhammad et al., 2023). Java, Lampung, Aceh and North Sumatra are among the high-risk drought areas (Rejekiningrum et al., 2022). This is very worrying because these areas are the center of Indonesia's rubber plantations (Muhammad et al., 2023). Climate change significantly impacts rubber plantations by altering the duration of the tapping period and diminishing latex yield. Therefore, it is necessary to pay attention to climate change and create drought-resistant adaptation strategies for rubber plants (Fischer et al., 2024).

Global warming increases the evaporation rate of groundwater and causes a decrease in soil moisture, resulting in a lack of water, which inhibits seedling growth and affects the length of tapping time. According to reports from various regions, rubber production decreased by an average of 30% per year in 2010 due to rainfall that fell throughout the year (Liu et al., 2021). A water deficit has an impact on cell division, elongation and differentiation. In addition, it decreases carbon dioxide (CO_2) fixation, which reduces photosynthetic yields and the accumulation of reactive oxygen species (ROS) compounds, thereby reducing plant production. This condition stimulates the responses of plants through their water deficit, affecting their morphological and physiological changes (Chandra et al., 2021; Lupascu et al., 2023).

One common morphological indication is the effect on root elongation (Cheng et al., 2022). In cotton plants, this strategy is indicated by root elongation and differences in the number of shoots (Abro et al., 2023). An early approach to overcome the problem of drought stress in rubber plants is to select recommended clones, aiming to obtain clones that are relatively more tolerant to drought stress conditions, especially rootstocks in the nursery.

Drought tolerance is a condition in which plants can survive despite drought stress/water deficit (Bhandari et al., 2023).

One of the efforts to overcome the lack of water in the planting medium is to provide organic material (husk charcoal) either as mulch or a media mixture. Burnt husk has the advantage of binding water and nutrients. Therefore, it is expected to have a positive impact on its application. Plant roots can more easily absorb the nutrients for growth and development once bound with the husk (Das et al., 2021). Nutrients contained in burnt husk include nitrogen at 0.32%, phosphate at 0.15%, potassium at 0.31%, calcium at 0.96%, iron at 180 ppm, zinc at 14.10 ppm, manganese at 0.4 ppm and a pH ranging from 8.5 to 9.0 (Sama, 2019).

The addition of rice husk to the soil can improve its physical and chemical properties (Sarigar, 2021; Syarifuddin et al., 2020). Notably, it increases soil pH, augments fertility, and increases nutrient availability. Furthermore, it elevates the activity of microorganisms and humus levels, alongside improvements in soil structure (Sarigar, 2021). Additionally, the husk acts similarly to zeolite, enhancing nutrient retention within the soil, thus preventing nutrient leaching by water while ensuring that nutrients are readily releasable when required by plants (Ariyani et al., 2022).

As a result, more research needs to be done to find out how burnt husk affects the growth of PB 260 clone rubber seedlings so that drought-resistant adaptive rubber seedlings can be made.

2. Materials and Methods

2.1 Site Description

The research was conducted at the Experimental Farm of the Faculty of Agriculture, Islamic University of North Sumatra Medan, Jalan Karya Wisata, located in Medan Johor District, North Sumatra Province. The site, situated at an elevation of 25 meters above sea level, features flat topography and is characterized by inceptisol soil. The geographical coordinates are 03°30'24" N and 98°26'00" E.

2.2 Experimental Design

A non-factorial group randomized design with three replications was employed, where the treatment involved the application of organic matter. Specifically, 30g of burnt husk per polybag was applied across three different treatments, namely, no application, surface application (as mulch), and mixed into the soil (as a planting medium).

2.3 Observation Variables

2.3.1 Root length (m)

Root length was measured at the end of the study. The sample plant was dissected from the polybag and placed on a plastic-coated nail board (Figure 1). Then the sample was washed to remove soil and impurities. Subsequently, the roots were dried and their lengths were calculated.

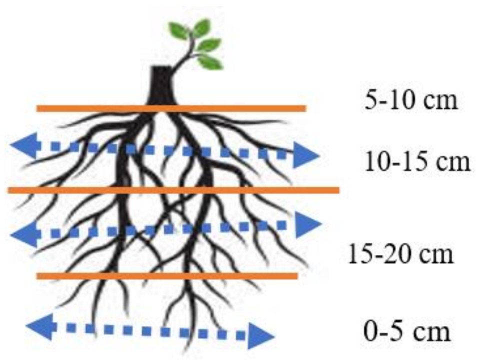

Figure 1. Calculation and measurement of root length (Huang et al., 2023)

The total root length was measured using the line intersection method by segmenting the roots into intervals (0-5 cm, 5-10 cm, etc.) up to the length of the longest root. Within each interval, the width between the furthest roots was measured (Figure 1) and the number of roots, including primary, secondary, tertiary, and quaternary, was counted. The total root length (R) was calculated using the formula:

$$R = \pi NA / 2H$$

where, R is the total root length, N is the number of roots, A is the square area, H is the total length of straight lines, and $\pi = 3.14$.

Then the lengths of the roots at other intervals were measured in the same way. For each interval, the width of the roots and the total number of roots should be measured. Upon reaching the depth of the longest root, the lengths from each depth were aggregated to compute the total root length.

For instance, at the 0-5 cm interval with a root width of 6 cm and 150 roots, the calculation is as follows:

$$R = \pi NA / 2H \rightarrow R = (3.14 \times 150 \times 30) / (2 \times 5) \rightarrow R = 14{,}130 / 10 \rightarrow R = 1413\text{cm}$$

2.3.2 Root surface area (m^2)

The root surface area and diameter were also measured at the end of the study. To obtain the root surface area, the root projection area was measured. Assuming the root is cylindrical, the root projection area is $2RP$, where R is the radius, and P is the root length. The root surface area is the area of the cylindrical skin without caps at both ends of the root, which can be calculated by the circumference multiplied by the root length, i.e., $2\pi RP$.

Additionally, the average root diameter was derived as $2R$ (Huang et al., 2023). Root surface area and root diameter were also measured per root interval, and then summed.

Before calculating the root surface area, the root projection area was calculated using the tube area formula, i.e., $2\pi Rt$, where $\pi = 22/7$ or 3.14, R is the radius, and t is the height. For the polybags used in this study, with dimensions of 12 cm × 17 cm, the radius was determined to be 6 cm and the height 17 cm. Therefore, the root projection area was calculated to be 2 × 3.14 × 6 cm × 17 cm = 640.56 cm^2.

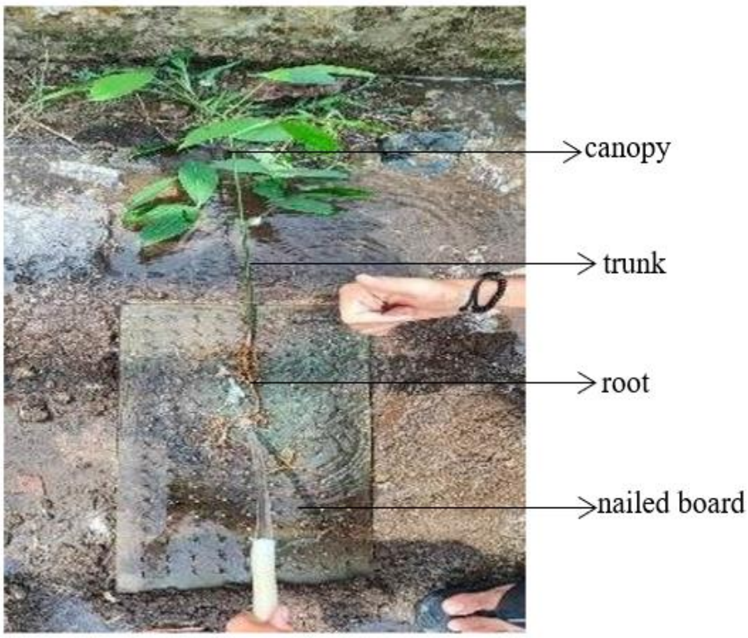

Figure 2. A board with nails
Source: Miftah 2023

Then water was sprayed to separate the soil from the seedling roots (Figure 2). Once cleaned, the roots were arranged on a plastic sheet affixed to one side, ensuring the roots were spaced evenly between the affixed plastic. The roots were then left to dry until they became dry and rigid. Subsequently, the rigid roots were segmented into 10 cm intervals, such as 0-10 cm, 10-20 cm, etc. Each segment was then analyzed to enumerate the roots. In addition, a graph of the root distribution pattern was made, based on the length and surface area of each segment (Huang et al., 2023).

2.3.3 Root volume (ml)

Root volume was measured at the end of the study. The volume was determined by inserting the roots into a Becker glass with 50 mL of water, though this volume was adjusted (100 mL, 150 mL, or more) based on the size of the roots being measured. The increase in water volume consequent to the insertion of the roots was recorded. The volume of the roots was calculated as the difference between the water volume with the roots and the initial water volume (without roots), as depicted in Figure 3.

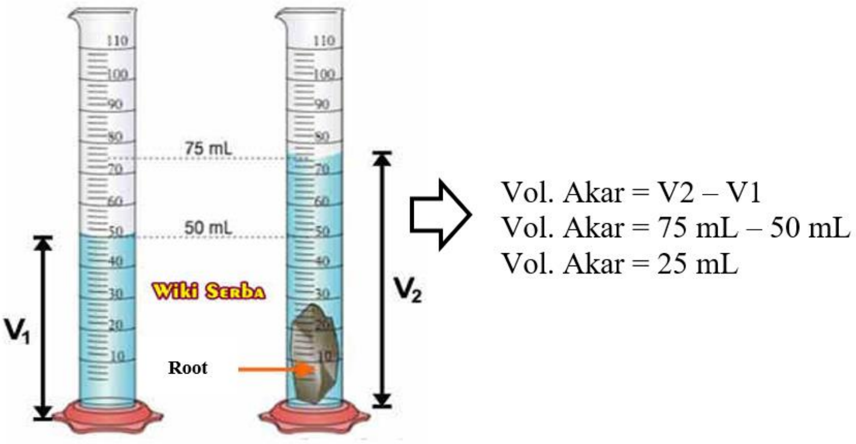

Figure 3. Measurement of root volume
Source: Sigalingging 2021

2.3.4 Head root ratio (NAT)

The root-shoot ratio (NAT) was observed at the end of the research using the following formula:

$$\mathrm{NAT} = \frac{\text{Dry Weight of Roots}}{\text{Dry Weight of Shoots}}\,(g)$$

2.3.5 Stomata density (mm^2)

Leaf samples were collected from fully expanded leaves located on the first and second umbrellas, with four leaves sampled per treatment. Each leaf was cleaned on both the upper and lower surfaces to remove dust and other particulates using tissue, and was then cut transversely. Subsequently, the leaves were sliced using a razor blade to ensure extremely thin sections were obtained, including cuts from the tip, center, and base of the leaf.

Leaf sections were immersed in chlorox (Bayclin) for five minutes to bleach the tissue, after which they were rendered visibly white. The sections were then extracted using tweezers and needles and subsequently rinsed thoroughly in distilled water to remove any residual chlorox. Furthermore, the sections were soaked in 1% safranin for one minute to stain the tissues, facilitating the distinction between stomata and epidermis. After staining, the sections were again washed in distilled water to eliminate excess dye. Finally, the stained sections were placed on microscope slides and a mounting medium was applied using a pipette to preserve the tissue for microscopic analysis.

After staining, the leaf sections were covered with cover glass. The prepared slides were labeled and subsequently examined under a microscope at 400x magnification. Data collection was conducted quantitatively by counting the number of stomata in each stomatal field of view. The density of stomata was calculated using the formula (El-Sharkawy et al., 1985):

$$\text{Stomata Density} = \frac{\text{Number of Stomata}}{\text{Stomata Field of View}}$$

The area of the field of view at 400x magnification was used to measure stomatal density. The field of view, being rectangular, was measured using a camera (Optilab) attached to a microscope and calibrated using the ImageRaster software. The area of the field of view can be calculated as follows:

$$\begin{aligned}
\text{Field of view} &= \text{Length} \times \text{Width} \\
&= 362.26\ \mu m \times 342.37\ \mu m \\
&= 0.36226\ mm \times 0.34237\ mm \\
&= 0.1240269562\ mm^2 \text{ (simplified to } 0.12\ mm^2\text{)}
\end{aligned}$$

2.4 Statistical Analysis

Differences in burnt husk treatment for different observation variables were analyzed using a two-way Analysis of Variance (ANOVA) followed by the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$.

3. Results and Discussion

3.1 Root Growth of PB 260 Clone Rubber Seedlings

The application of burnt husk affects the length, surface area and volume of roots. The application of burnt husk as mulch showed the best growth in the length, surface area and volume of roots compared to the application as a planting medium and the treatment without application (Divyabharathi et al., 2024). Table 1 shows the conditions of PB 260 clone rubber seedlings at 22 Weeks After Planting (WAP).

Table 1. PB 260 clone rubber seedlings at 22 WAP

Treatment	Root Length (m)	Root Surface Area (m^2)	Root Volume (mL)
No burnt husk (A_0)	54.54B	25.06B	25B
Burnt husk mulch (A_1)	98.74A	45.54A	30A
Grilled husk media (A_2)	22.60C	4.87C	2C

Note: Values in the same column and row followed by different notations indicate statistically significant differences ($P < 0.05$) based on the 5% LSD test.

When conditions are wet, the application of burnt husk as a planting medium is excessive or saturated with water. As a result, the disturbance of root respiration prevents the roots from absorbing water and nutrients, leading to a shortage of water and nutrients for root growth. Meanwhile, the treatment without the application of organic material showed that the plants lacked water due to high evaporation without mulch covering the soil surface (Chemura, 2014).

A B C

Figure 4. Root performance of PB 260 clone rubber seedlings (A) treated without burnt husk, (B) application of burnt husk as mulch, and (C) application of burnt husk as a planting medium
Source: Miftah 2023

The growth of the length, surface area and volume of roots is good if water is available to suit the needs of the plant, such as through the application of burnt husk as a planting medium. The roots grow well in the mulch treatment because the planting media have balanced concentrations of oxygen (O_2) and water, leading to faster root penetration and optimal absorption of water and nutrients. Water and nutrients greatly influence the results of photosynthate for root growth (i.e., length, surface area and volume of roots), as shown in Figure 4 and Figure 5.

A B C

Figure 5. Root growth of PB 260 clone rubber seedlings (A) treated without burnt husk, (B) application of burnt husk as mulch, and (C) application of burnt husk as a planting medium
Source: Miftah 2023

The good physical properties of soil have an impact on the development of deeper and wider roots. As a result, plant growth is ultimately supported by better absorption of nutrients and water (Divyabharathi et al., 2024). Figure 5 shows that the treatment using burnt husk as mulch has the best root growth, followed by no application of burnt husk. The treatment using burnt husk as a planting medium has the poorest root growth. Compared with the treatment without applying burnt husk, the treatment using organic material as mulch has better root growth because the burnt husk can retain ground water needed for plant growth (Chemura, 2014).

When using burnt husk as mulch, the soil water content is the highest because the treatment without mulch increases the evaporation rate of the soil, reducing the amount of ground water available and causing a lack of water for the growth and development processes (Varela Milla et al., 2013). Compared with other treatments, the treatment using burnt husk as a planting medium has the poorest root growth, suggesting that the planting medium experiences excess water during the rainy season. With a water holding capacity of 353% (Varela Milla et al., 2013), burnt husk can bind excess water during wet conditions as a planting medium and cause the planting medium to become overwatered. When there is high rainfall, gas exchange in the planting medium experiences obstacles because the planting medium begins to become saturated with water (Samouëlian et al., 2012). This happens because the macropore space, which should be filled with air, is also filled with water, leading to breathing obstacles for the roots.

Limited O_2 dramatically affects the growth, development and existence of plants. O_2 is very important in relation to the respiration of plant roots and soil microorganisms. Plant roots respire to obtain energy and absorb nutrients. Excess water in the soil disrupts nutrient absorption (Topp et al., 1997). This opinion is in accordance with the research findings of Waśkiewicz et al. (2016), which state that the fairly high rainfall during the research has an unfavorable influence on the growth of mustard greens. The planting medium contains a lot of water which can result in inadequate nutrient absorption by plant roots. If burnt husk is added to the soil, it can bind water. Therefore, soil media with added burnt husk can improve the porosity of the media, which is good for root respiration and can maintain soil moisture (Ardian et al., 2022). A medium is considered good if it meets the balance requirements between humidity and aeration.

Maintaining humidity is crucial to prevent the risk of media drying out (Kolb et al., 2017). Apart from inhibited cell activity, the dry root penetration area (low soil moisture) also inhibits root development. Therefore, if newly formed roots cannot penetrate, ultimately the root tip dies (Kolb et al., 2017). Water stress inhibits cell formation and development, resulting in few plant roots with a small size and a relatively narrow distribution area (Wu et al., 2022). In conditions of water shortage, most of the assimilate in the plant body obtained from the source is distributed to the roots so that the roots can grow and meet the plant's need for water. If the length of roots increases, it means that water and nutrients are available (Nugmanov et al., 2023). If soil moisture falls below field capacity, the roots form more branches and elongate more quickly to obtain water for consumption (Asbur, 2006). Therefore, plant roots in soil with a water content below field capacity always branch. Root length describes the plant's ability to obtain water and nutrients in deeper soil layers.

Root length is significantly and positively correlated with root surface area (r = 0.96**), and growth in root length is always followed by an increase in root surface area (Asbur, 2006). Therefore, root surface area can be used as an indicator of the root system's ability to absorb water and nutrients (Seleiman et al., 2021). The greater the root surface area, the bigger the absorption of water and nutrients required to survive drought-stress conditions. The treatment using burnt husk as mulch showed the best growth in the length, surface area and volume of roots compared with the treatment without burnt husk and burnt husk as a planting medium. In the treatment using burnt husk as mulch, the condition of the soil media was balanced between O_2 and water so that root respiration was not disturbed, which resulted in optimal water and nutrient absorption rates (Leogrande & Vitti, 2019).

With sufficient water and nutrients, the rate of photosynthesis produces photosynthate which is used for growth in the length, expansion and volume of roots. There are no obstacles to the growth, development and penetration of roots through soil media because soil porosity is quite good.

If the roots are good, other parts of the plant also grow well because the roots absorb the nutrients needed by the plant (Wu et al., 2016). An environment lacking water affects root volume, hampering root development (Liu et al., 2018). Root growth, including root elongation and widening, is influenced by media factors and environmental factors. The planting media factor closely supports root growth as an organ that absorbs water and nutrients.

3.2 Distribution of Roots

The distribution of root length at different intervals is presented in Table 2 and Figure 6.

As shown in Table 2 and Figure 6, the treatment method of applying burnt husk as mulch shows the highest and most even distribution of lateral roots/root hairs at every interval, with the longest tap/primary root up to a depth of 30 cm. The application of burnt husk as a planting medium shows that root growth is inhibited by low root distribution and a small number of lateral roots. Therefore, in the treatment method of applying burnt husk as mulch, the environmental conditions for root growth are suitable and do not interfere with root penetration. However, when using burnt husk as a planting medium, the water saturation of the planting medium hinders root respiration, resulting in stunted root growth and root death, as evidenced by the very low root distribution.

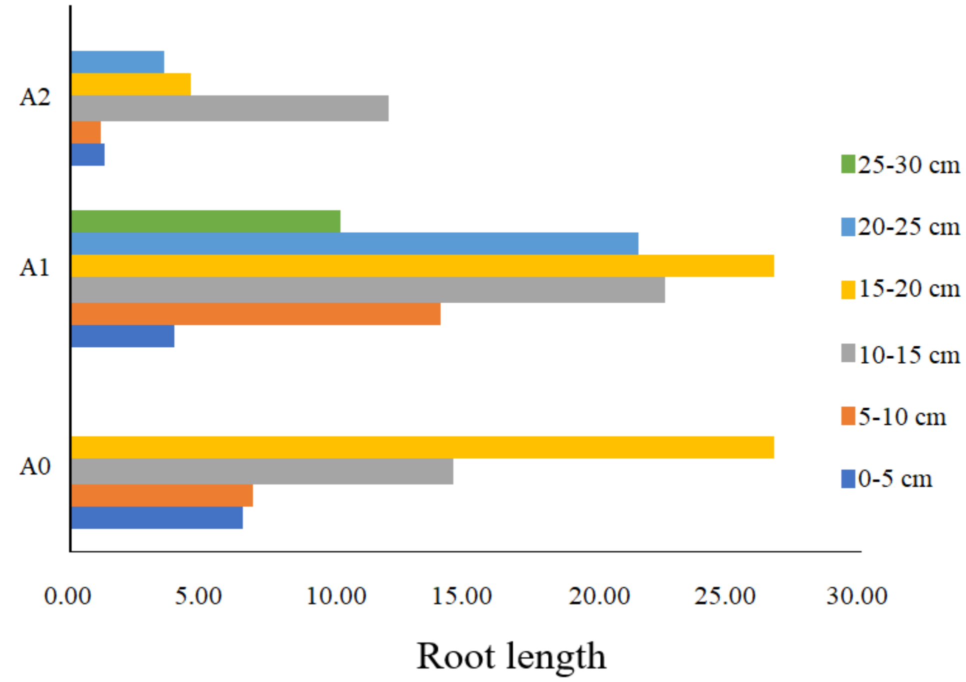

Figure 6. Root distribution based on root length (m)

Table 2. Root distribution based on root surface area (m^2) of PB 260 clone rubber seedlings at 22 WAP

Treatment	Soil Profile Depth (cm)					
	0-5	5-10	10-15	15-20	20-25	25-30
No burnt husk (A_0)	4.52	4.65	6.76	9.14		
Burnt husk mulch (A_1)	3.86	7.26	9.21	10.01	9.00	6.20
Grilled husk media (A_2)	0.57	0.55	1.74	1.07	0.94	

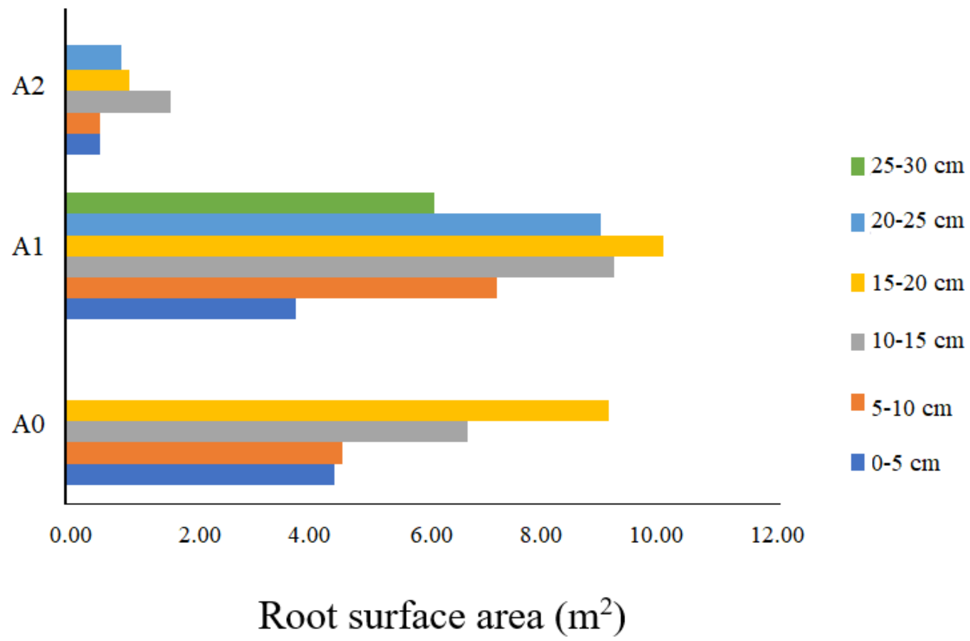

Figure 7. Root distribution based on root surface area (m^2)

Factors that influence root distribution patterns include soil temperature, aeration, water availability and nutrient availability. Root system architecture varies greatly between different plant species, but within a single species, it is flexible and changes depending on soil conditions (Lynch et al., 2022). The pattern of root distribution based on root length indirectly shows the ability of roots to absorb water and nutrients from several soil depths. Plants with evenly distributed roots at various depths in the soil profile tend to absorb more water because the roots penetrate deeper into the soil compared to plants with shallow and unevenly distributed roots (Madhu & Hatfield, 2013).

Plants' ability to survive on dry (marginal) land is also determined by their root architecture, such as rooting depth and the spread of lateral roots along with smaller root hairs (Purwaningrum, 2006). The biomass and size of tap roots increase with age compared to lateral roots (Al-Kayssi, 2023). Table 3 shows the root distribution based on the root surface area (m^2).

Table 3. Average root and shoot dry weights and root-shoot ratio of PB 260 clone rubber seedlings with three treatments in dry conditions (22 WAP)

Treatment	Root Length (m)	Root Surface Area (m^2)	Root Volume (mL)
No burnt husk (A_0)	2.7B	5.0A	0.55B
Burnt husk mulch (A_1)	3.8A	3.4B	1.13A
Grilled husk media (A_2)	0.7C	1.5C	0.45B

Note: Values in the same column and row followed by different notations indicate statistically significant differences (P<0.05) based on the 5% LSD test.

Figure 7 demonstrates a positive correlation between root length and root surface area. This aligns with the research by Asbur (2006), demonstrating a highly significant positive correlation between root length and root surface area (r = 0.96**), where an increase in root length consistently leads to an increase in root surface area.

Roots are plant organs that determine the plant's ability to absorb water and nutrients in the plant medium. Therefore, the root surface area can be used as an indicator of the root system's ability to absorb water and nutrients. Furthermore, it serves as a gauge for the development of the root system (Wang et al., 2021), and the transfer of seedlings with wider roots to the field promotes their optimal growth (Sallam et al., 2024).

3.3 Root and Shoot Dry Weights and the Root-Shoot Ratio

As shown in Table 3, the treatment using the burnt husk as mulch shows root and shoot dry weights with balanced growth. However, the treatment without the burnt husk shows greater shoot growth compared to root growth because the shoot dry weight is greater than the root dry weight. The application of burnt husk as a planting medium shows that the dry weight of the roots and crown is very low. Figure 8 shows the stomata density of PB 260 clone rubber seedlings with three treatments.

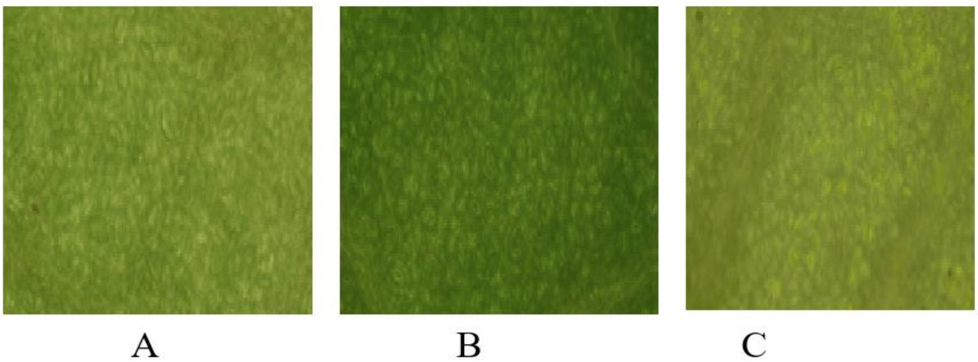

Figure 8. Stomata density of PB 260 clone rubber seedlings with three treatments (A) Without burnt husk (stomata density 13888.14/mm; leaf area 43.79 cm^2); (B) Applying burnt husk as mulch (stomata density 14730.08/mm; leaf area 27.37 cm^2); and (C) Applying burnt husk as a planting medium (12626.08/mm; leaf area 43.23 cm^2)
Source: Miftah 2023

If the planting medium is excessive/saturated with water, the roots cannot absorb the water and nutrients needed by the plant, especially for crown growth. Stunted crown growth also hampers the roots. Likewise, if the planting medium has sufficient water, the growth of the crown and roots is balanced. The dry weights of roots and shoots are presented in Figure 9 and Figure 10.

Figure 9. Dry weight of roots of PB 260 clone rubber seedlings with three treatments: (A) Without burnt husk (A_0); (B) Applying burnt husk as mulch (A_1); and (C) Applying burnt husk as a planting medium (A_2)
Source: Miftah 2023

Figure 10. Dry weight of crowns of PB 260 clone rubber seedlings with treatment: (A) Without burnt husk (A_0); (B) How to apply burnt husks as mulch (A_1); and (C) How to apply burnt husks as a planting medium (A_2)
Source: Miftah 2023

Basically, root and stem growth is very complex, especially in terms of photosynthate mobilization because many factors influence plants. Under limited conditions, plants encourage root growth to obtain more nutrients and water (Liu et al., 2024). The development of the root system influences that of the seed crown, i.e., the growth in height and diameter of the seed. The roots provide the nutrients and water needed by the seedling crown for photosynthetic activities, while the seedling crown provides the results of photosynthesis needed for the growth of roots and other parts (Lamasrin et al., 2023). In general, the addition of burnt husk increases the effective development of the roots of Jabon seedlings tested in sub-soil media (Schurr et al., 2006).

The role of roots in plant growth is as important as the canopy, whose function is to provide carbohydrates through the process of photosynthesis. Therefore, the function of the roots is to provide the nutrients and water needed for plant metabolism (Puhe, 2003). A plant's ability to absorb nutrients can be determined by measuring the porosity and length of its roots, as well as its fresh and dry root weights. Root factors influence a plant's ability to grow. It is known that plant crown growth is correlated with root growth. The quality of annual plant growth begins with the growth conditions from sowing (Ramayana et al., 2024).

Roasted burnt husk provides a better response to the wet and dry weights of the plant because it is more crumbly than other planting media (Yanti et al., 2020). It is thought that the characteristic makes it easier for the roots of the tested Brassica alboglabra seedlings to penetrate the media, thereby expanding the root elongation area and accelerating root development (Fried et al., 2019).

The percentage difference in growth increase indicates that the root dry weight has increased more than the shoot dry weight. This shows that the addition of burnt husk has a greater influence on increasing root development in wasian cempaka seedlings compared to the shoots, which also has a positive effect on crown growth and root dry weight (Nejad et al., 2010).

Water deficits reduce the root-shoot ratio in corn plants. Under drought conditions, biomass allocation to roots usually increases to access water sources (Volpe et al., 2013). Douglas fir and Ponderosa pine seedlings can survive drought conditions by expanding the root system and reducing the shoot-to-root ratio. Meanwhile, at a watering volume of 70-80%, the shoot-root ratio decreases at a watering interval of three days and then increases at a watering interval of seven days (Kasiman et al., 2017). This decrease in the canopy occurs due to efforts to expand the root system for water uptake, while the increase in rooting occurs due to efforts to utilize water uptake for canopy growth (Mackay et al., 2015).

4. Conclusions

The study findings indicate that the application of burnt husk as mulch significantly enhances root growth compared to the absence of husks or their use as a planting medium. Using burnt husk as mulch increased the water holding capacity by up to 353%, thereby maintaining the soil water required for plant growth. Based on these results, it is recommended that burnt husk be utilized as mulch to promote the cultivation of drought-adaptive rubber seedlings.

Data Available

The data used to support the research findings are available from the corresponding author upon request.

Acknowledgement

Gratitude is extended to the Research Institute of the Islamic University of North Sumatra and and the Sungei Putih Research Unit for their provision of laboratory support equipment.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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