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Evaluation of Drought Resistance of Rubber Seedlings of PB 260 and GT 1 Clones through Watering Frequency

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Abstract: Rising temperatures due to global warming increase evaporation and reduce soil water availability, inhibiting the growth of rubber seedlings (*Hevea brasiliensis*), especially roots. This study aims to analyze the growth characteristics of rubber seedlings of PB 260 and GT 1 clones, test the effect of water deficit due to watering frequency on the growth of PB 260 clones, and assess the impact on GT 1 clones. The method used a Separate Plots Design in a factorial Randomized Group Design with two factors: clone (PB 260 and GT 1) and watering frequency (once every 1, 3, 5, and 7 days), repeated three times. The research was conducted in the experimental field of Faculty of Agriculture, Islamic University of North Sumatra, Medan. The results showed the best root growth in watering every 3 days (PB 260) and every 5 days (GT 1). Daily watering causes water saturation, inhibiting root growth. The study contributes to early selection of drought-tolerant clones in nurseries.

Keywords: plant physiology; water stress; seedling adaptation

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1. Introduction

Low rainfall can cause drought conditions, which have a direct impact on plants. When groundwater is limited, the osmotic pressure around the roots increases, making it difficult for plants to absorb water. As a result, the turgor pressure in plant cells decreases, causing the plants to wilt. If the drought continues, plant tissue can be damaged or even die (Pamungkas, 2022).

Water shortages (water deficits) also disrupt plant physiological processes, such as inhibiting cell division, reducing the rate of photosynthesis, and triggering the accumulation of damaging reactive oxygen species (ROS) (Hasanuzzaman et al., 2020; Zhang et al., 2021; Wu et al, 2022). In response, plants adapt by modifying their morphology, for example by lengthening the roots to reach deeper water (Pasaribu et al., 2023). One of the initial solutions to overcome the impact of drought is to select plant clones that are resistant to dry conditions (Cahyo et al., 2022).

Rubber tree (*Hevea brasiliensis*) clone selection should consider agroecosystems and cultivation objectives as they affect latex productivity. Clones are divided into Quick Starter (fast growing, e.g., RRIM 900, PB 260) and Slow Starter (slow growing, e.g., GT 1, BPM 1) (Zhang et al., 2024; Cahyo et al., 2022b). Quick Starter clones are suitable for short growing seasons, while Slow Starter clones tend to be more stable, resistant to environmental stress, and more efficient in long-term nutrient use (Priyadarshan, 2021; Yudistira, et al., 2023).

Quick and Slow Starter clones respond differently to drought. GT1 clones exhibit stronger drought avoidance by markedly reducing stomatal conductance and transpiration under water deficit, whereas PB 260 shows higher stomatal activity and greater drought sensitivity (Cahyo et al., 2022). GT1 also adapts by limiting transpiration and adjusting leaf osmotic potential to maintain water status (Wan et al., 2022). In contrast, PB 260 relies on osmoregulation through solute accumulation to maintain cell turgor, although it remains relatively drought-susceptible compared with GT1 (Xiao et al., 2023).

Research on the frequency of watering in Quick Starter (PB 260) and Slow Starter (GT 1) clones is important to understand physiological responses to drought stress. Limited water availability reduces cell turgor pressure, causing leaves to lose elasticity and disrupting stomatal function.

Consequently, physiological processes such as photosynthesis, transpiration, and respiration do not operate optimally (Liu et al., 2023; Hagpanah et al., 2024). This condition slows down cell division and root system development, which plays a crucial role in nutrient uptake (Cahyo et al., 2022). Each clone exhibits different physiological adaptation strategies; for instance, PB 260 shows a faster initial growth response, whereas GT 1 tends to be slower, and these differences influence their requirements and responses to watering frequency (Pasaribu et al., 2023; Cahyo et al., 2022b).

This test is also important because the identification of drought-tolerant rootstocks is still not optimal. Therefore, a fast and accurate physiological detection method is needed to support the selection of superior clones under water stress conditions (Pasaribu et al., 2023).

2. Materials and Methods

The research was conducted at the Experimental field of the Faculty of Agriculture, Islamic University of North Sumatra, Jln. Karya Wisata, Medan Johor District, Medan City, North Sumatra Province, coordinates 3031'45 "N 98040'52" E with an altitude of ± 25 m above sea level, flat topography with Inceptisol order soil type.

Experimental Design

This study used a Split Plot Design arranged in a Factorial Randomized Block Design (RBD) with two factors and three replications. This design was chosen because it was able to accommodate differences in the level of influence and control over each factor studied. With this approach, it is expected that the observation results will be more accurate and in accordance with the physiological characteristics of the rubber plants being tested.

The first factor as the main plot is the rubber plant clone (C) consisting of two levels, namely C_1 = PB 260 (*Quick Starter*) and C_2 = GT 1 (*Slow Starter*). These two clones were chosen because they have different growth rates and initial adaptation characteristics that can affect the response to drought stress. The placement of clones as the main factor allows for an in-depth analysis of genetic contributions in dealing with water deficits.

The second factor as a subplot is the frequency of watering (F), which consists of four levels, namely: F_1 = watering every day, F_2 = every 3 days, F_3 = every 5 days, and F_4 = every 7 days. This frequency variation aims to simulate multilevel drought stress conditions and evaluate plant physiological responses such as root growth, turgor pressure, and water use efficiency in each clone. Each treatment combination uses a watering amount of 900 mL/polybag, with a polybag size of 12 cm × 17 cm (3 kg). The experiment was carried out on an area of 200 m^2 × 200 m^2, with treatments arranged using a completely randomized block design (RBD) and three replications. This design is expected to provide accurate information regarding the interaction between clones and watering frequency in increasing the resistance of rubber plants to drought stress.

Observational Variable

Total increase in leaf area (cm^2)

Leaf area measurement on rubber seedlings was done by recording the length and width of each leaf. Furthermore, leaf area was calculated using the formula: Area = Length × Width × 0.75, according to the method of (Pasaribu et al., 2023). Observations were made on day 0, day 21, and day 42 after planting. The area of all leaves was averaged, then multiplied by the number of leaves in one plant. These results were used to see the increase in leaf area over time as an indicator of plant growth.

Stomatal density (number/mm^2)

Stomatal density is calculated by dividing the number of stomata observed by the area of the microscope field of view (in mm^2). The formula used is: Stomatal Density = amount of Stomata / Area of Field of View (mm^2). The results of this calculation indicate the number of stomata per unit area, which functions as an important indicator in the process of transpiration and photosynthesis.

The observation steps are carried out by cleaning the leaves first, then making a thin incision on the lower surface of the leaf. The incision is soaked in Bayclin solution for 5 minutes, rinsed using distilled water, then stained with 1% safranin solution for 1 minute. After that, the sample is placed on a glass object with additional glycerin to be observed under a microscope at a magnification of 1000x. The number of stomata is then counted in a certain area of the field of view to determine its density (Scarpeci et al., 2017; Jayakody et al., 2017; Zhu et al., 2018; Stein et al., 2024).

Leaf Relative water content (RWC) (%)

Leaf Relative water content was calculated using the equation of (Hultine and Marshall, 2001; Scarpeci et al., 2017; Stein et al., 2024): $\mathbf{RWC} = \frac{\mathbf{FW-DW}}{\mathbf{TW-DW}} \times \mathbf{100}\%$

RWC = Relative Water Content

FW = Fresh weight (wet weight) of leaves obtained by weighing the fresh leaves on the sample plants

Turgid Weight /TW = Weight gain, obtained by fresh leaves on the sample plants after being weighed then soaked in distilled water (distilled water) for 4-5 hours, after which the leaf surface is dried by rubbing with tissue paper until dry, then weighed.

DW= Measurement of relative water content (RWC) of leaves was carried out on the 42nd day after planting, before watering. The procedure began by weighing the fresh biomass of the plant crown, then the part was soaked in water for 5–6 hours so that the tissue was saturated with water. After soaking, the samples were dried using an oven at 80°C for 24 hours until the weight was stable. The dry weight of the leaves obtained was then used to calculate the RWC value as an indicator of water status in plant tissue.

Root Length (m)

Root length measurements were taken at 63 Days After Planting (DAP) by opening the polybags, removing the soil, and drying the roots. Root length was calculated using the line intersection method by dividing the roots at depth of 0-5 cm, 5-10 cm, etc., then measuring the width and number of roots per depth. Total root length was calculated using the formula: R=NA/2H where R = total root length, N = number of roots, A = area of a rectangle, H = length of straight line, and π=3.14 (Chang et al, 2013; Delory et al., 2017).

Root Volume (mL)

Root volume formula = Volume of water with roots (mL) - Initial water volume (mL) (Pang, 2011). Measurements were taken once at the end of the study by dipping the roots into a beaker glass filled with water (50 mL, 100 mL, or according to the size of the roots), then recording the difference in the increase in water volume.

Root and Shoot Dry Weight (g)

Shoot Dry Weight is measured by weighing the crown of sample plants that have been dried in an oven at 80°C until constant weight (±48 hours) (Poorter et al., 2012).

Shoot Root Ratio (SRR) is calculated by the formula $\mathrm{SRR}=\frac{\textbf{Dry Weight of roots}}{\textbf{Dry Weight of shoots}}(\mathbf{g})$. SRR observations were made once at the end of the study to assess plant biomass allocation.

Groundwater Volume

Groundwater volume is calculated by the formula: $\mathbf{V} = [(\mathbf{W1} - \mathbf{W2}/\boldsymbol{\rho}] \times \mathbf{100}\%$

with V = water volume (%), W_1 = wet soil weight (g), W_2 = oven dried soil weight (g), and ϱ = soil density (g/cm³). Measurements were taken at field capacity (saturated soil dried 48 hours) and age 21 Days After Planting (soil dried 48 hours) (Li et al.,2023).

Statistical Analysis

Data analysis was performed using the SPSS version 25.0 statistical program. Treatments in the exploitation system were analyzed using Two-Way ANOVA, then continued with the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$ to determine differences between treatments on various observation variables.

3. Results (or Results and Discussion)

Combinations of clone types and watering frequency

The combined treatment of clones and watering frequency has not affected the increase in crown growth (leaf surface area) but affects the number and density of stomata, leaf RWC, and groundwater volume. In line with the results of research by Webster et al., (2019) said that there was no interaction between the type of clone and the level of water stress.

Likewise, the effect of various types of rubber clones, in general, has not affected the growth of rubber seedlings. Similarly, the results of research by Robertson et al., (2023) showed that the effect of watering frequency treatment with various types of rubber clones has not affected the growth of rubber seedlings in polybags, because none of the variable observation parameters had an effect.

When viewed from the treatment numbers the combination of C_1F_3 (PB 260 clone with watering frequency once every 5 days), C_2F_3 (GT 1 clone with watering frequency once every 5 days), and C_2F_4 (GT 1 clone with watering frequency once every 7 days) showed lower shoot growth due to lower water volume compared to C_1F_1, C_1F_2, C_2F_1, and C_2F_2, but the growth increment of PB 260 clone was higher compared to GT 1 clone.

This can be explained that the growth of each clone is different, which is influenced by genetic traits, especially the speed of metabolism, and the adaptation/tolerance of clones to water shortage is also different (Table 1). (Hasanuzzaman et al., 2020; Zhang et al., 2021).

Water limitation affects the RWC value of leaves Wang et al., (2023). Yang (2022). stated that the limited water available in the media resulted in the limited amount of water absorbed by plants, so that the RWC of the leaves decreased. Drought stress treatment also causes a decrease in RWC in rubber seedlings. Leaf RWC reflects the status of water in plant tissues and can describe the level of stress that occurs. Leaf RWC is a variable of plant resistance to drought stress (Abo, et al., 2025),

because it describes the water status and turgor pressure of leaf cells, especially when plants experience a decrease in water potential.

The value of relative water content is inversely proportional to leaf water potential, where plants that are treated with drought stress will experience more severe stress due to the amount of water used by plants to maintain osmotic pressure and transpiration is greater, so that the water potential decreases (Al Abdooli et al., 2025).

The dominant effect of drought on plant physiological processes is changes in stomatal conductance. The closing of stomata is triggered by the hormone abscisic acid which is produced in the roots and carried to the leaves as a stomatal informer when drought stress occurs. Stomatal conductance also affects the magnitude of photorespiration (Zhang et al., 2023).

Table 1. Average increase in canopy growth (total leaf area), number of stomata and stomata density, as well as leaf Relative Water Content (RWC) and soil water volume in rubber seedlings from seedlings at 21 and 42 days after sowing in the combination of clone treatment and watering frequency

Treatment	Total Leaf Area (cm^2)		Number of Stomata	Stomata Density (mm^2)	Relative Water Content (RWC) Leaf (%)	Groundwater Volume (%)
	21	42	42	42	42	42
PB 260						
C_1F_1	155.50	57.50	30.00a	12.63a	94.55a	1261.80a
C_1F_2	175.03	105.91	28.00a	11.81ab	91.67ab	1031.50c
C_1F_3	125.32	70.25	25.00ab	10.55ab	90.91b	851.40e
C_1F_4	125.34	37.32	15.00c	6.33d	87.10c	783.20g
GT1						
C_2F_1	142.06	91.94	20.00bc	8.44cd	89.39bc	1250.10b
C_2F_2	161.94	42.69	18.00bc	7.59d	87.65c	953.40d
C_2F_3	134.07	59.06	24.00ab	10.13abc	87.80c	820.50f
C_2F_4	131.88	74.56	20.00bc	8.44cd	87.69c	754.90h

Note: Numbers in the same column followed by different letters significantly different according to LSD test (P = 0.05).

Root Length (m), Root Surface Area (m2) and Root Volume (mL)

Table 2 shows that the clone characteristics clone (C) has an effect on the growth of root length and volume. GT1 clone (C_2) showed longer root growth of 30.16 m compared to PB 260 clone of 26.82 m, but on the contrary showed lower root volume of 12.25 mL while PB 260 clone was 15.00 mL. This can be explained that GT1 clones have deeper rooting characteristics to seek water as one of the adaptations to drought conditions, which spurs the growth of the dominant apical meristem so that the roots are longer, while the growth of the lateral meristem is inhibited and this is consistent with the lower root volume of GT1 clones compared to PB 260 clones. The root volume of PB 260 clone is greater than that of GT1 clone which is influenced by genetic factors, namely high metabolic rate (Zhu et al., 2022).

Some plant traits, especially those related to roots, may reflect the dominant influence of genetic factors. This means that these traits are more determined by the genetic information possessed by the plant than by environmental influences (Li et al., 2023). From a plant physiology perspective, root traits play a major role in responding to drought stress, because roots are the main organs that function to absorb water and nutrients.

In dry conditions, plants with longer, deeper, and wider root systems have a better ability to find water sources in the soil. Therefore, traits such as root length increase, root distribution and depth, root dry weight, root volume, root to crown ratio, and root length to plant height ratio are important indicators in assessing plant resistance to drought (Wang et al., 2023).

Tabel 2. Root growth (length, surface area, root volume) of PB 260 and GT 1 clones treated with watering frequency

Treatment	Clone (C)	
	PB 260 (C_1)	GT 1(C_2)
Watering frequency (F)	**Total Root Length (m)**	
Every day (F_1)	13.64f	33.31c
Once every 3 days (F_2)	49.93a	25.73d
Once every 5 days (F_3)	26.22d	18.87e
Once every 7days (F_4)	17.47e	42.72b
	Root Surface Area (m^2)	
Watering frequency (F)		
Every day (F1)	41.39f	64.68c
Once every 3 days (F2)	79.19b	54.85d
Once every 5 days (F3)	81.16b	48.68e
Once every 7days (F4)	66.25c	103.60a
	Root Volume (mL)	
Watering frequency (F)		
Every day (F1)	10.00b	10.00b
Once every 3 days (F2)	10.00b	10.00b
Once every 5 days (F3)	20.00a	10.00b
Once every 7days (F4)	20.00a	20.00a

Note: Numbers in the same column followed by different letters significantly different according to LSD test (P = 0.05).

Rubber plant clones that have more active root physiological characteristics and develop well under water stress tend to show higher resistance to drought stress. These traits can be used as a reference in the selection of superior clones based on physiological adaptation to extreme environmental conditions.

According to Zhao et al., (2023) an increase in the root system is generally followed by a decrease in crown growth. Plants that prioritize root growth over the crown have a better ability to survive drought conditions. The results of Zheng et al., (2024) showed that root growth increased significantly with decreasing water availability, while shoot growth experienced the opposite.

The results showed that the frequency of watering every 7 days (F_4) was able to stimulate the growth of rubber plant roots more optimally compared to watering every day (F_1). The F_4 treatment produced a root length of 30.09 cm, a root surface area of 84.92 m^2, and a root volume of 20 mL. From a physiological perspective, longer watering intervals encourage plants to adapt to water stress through elongation and expansion of the root system, as an effort to obtain water from deeper soil layers (Rodríguez et al., 2023). This physiological response is known as the avoidance mechanism, which is a plant strategy to avoid drought through changes in root structure (Chen et al., 2022).

On the other hand, the F_1 treatment (watering every day) caused the media to be saturated with water because the watering volume reached 1255.9 mL, exceeding the soil field capacity of only 1224 mL. In this condition, root growth is inhibited because the oxygen content in the media decreases. Physiologically, roots require a balance between water and air in the soil for cellular respiration. An overly wet environment can cause hypoxia, which disrupts root metabolism and reduces the efficiency of water and nutrient absorption (Nishiuchi et al., 2021; Zhang et al., 2023). This causes the plant root system to not develop optimally.

In the F_4 treatment, the volume of water given was only 769.1 mL, far below field capacity. This condition creates a drier planting medium environment, thus triggering a physiological response in the form of root system adjustments. Plants will increase root length, expand lateral root tissue, and increase root volume as an adaptation to drought (Chada and Ofoe, 2023). This mechanism also plays a role in maintaining water pressure in the tissue by increasing the efficiency of water absorption and

reducing water loss (Siaga et al., 2024). Therefore, less frequent watering frequency treatments can actually optimize root development and increase plant resistance to environmental stress.

The low root system in the F_1 treatment is due to saturated soil water, where the soil macro pores are filled with water which results in reduced O_2 which inhibits root respiration to produce energy in absorbing water and also the lack of O_2 causes some roots to rot and die, especially the roots that are on the surface, the lack of water in the seedlings also inhibits crown development, namely the lowest increase in leaf surface area of 128.6 cm^2 because the leaves function to carry out the photosynthesis process and the photosynthate formed is in accordance with the photosynthesized leaf area for root growth (Azhar and Sathornkich, 2022).

Treatment F_4, which is watering every 7 days, makes the planting medium have enough oxygen, but the water content is low. Conditions like this make the roots respond by growing longer and wider to find water in deeper parts of the soil. This can be seen from the increase in root surface area and root volume. Plants divert energy to strengthen the root system so that it can continue to absorb water even though the soil conditions are dry (Fischer et al., 2023). This is one form of drought adaptation strategy, where plants focus more on growing roots than their crowns (leaves and upper stems). Because most of the energy is used for root growth, leaf growth is slower. In treatment F_4, the leaf area only increased to 148.8 cm^2. In contrast to F_2 (watering every 3 days), where plants get enough water and can grow in balance, so the leaf area increases more, namely 168.5 cm^2. In sufficient water conditions, the water pressure in plant cells remains stable, the photosynthesis process runs well, and leaves can grow more optimally (Chada and Ofoe, 2023; Siaga et al., 2024).

The difference in these results shows that plants adjust the distribution of energy based on the amount of water available. If water is low (as in F_4), plants prioritize root growth in order to survive. However, if water is sufficient (as in F_2), plants can grow roots and leaves in a balanced manner. This understanding is important for determining the right watering schedule, especially for perennial plants such as rubber, so that root and crown growth remain balanced and plants are not stressed (Ahmed, et al 2023; Zhang, et al., 2024).

In treatment F_3 (watering 5 days a time), the volume of soil water is 922.5 mL and it is suspected that in this condition the air and water systems are balanced so that the growth of roots and crowns are balanced. Plant needs for water can be met by absorption by the roots. The water content in the soil and the ability of the roots to absorb water greatly affect the amount of water absorbed by the roots (Yan et al., 2022). The availability of soil moisture is very influential on growth; water is a basic requirement for plants and the main constituent of cell protoplasm. In addition, water is the main component in the photosynthesis process and plays a role in transporting it to all parts of the plant through water movement in the plant.

One of the factors that influence the growth of rubber plants is the availability of water. (Huang et al., 2023). Under water shortage conditions, the distribution of assimilate in the plant body obtained from the source will mostly be distributed to the roots, so that the roots can grow and can meet the plant's need for water (Stefaniak et al., 2024).

Pan et al., (2021) state that drought stress limits root growth and development, plus the use of limited planting media will affect the root adaptation space so that the spread of roots to increase water and nutrient absorption is limited. The limited water available in the soil causes the absorption of water by the roots to not be maximized as a result the need to grow is not met and will affect the development of the root system resulting in a low root surface area (Goufo et al., 2022; Ahmed et al., 2023). If this situation continues, the visible consequences are stunted plants, wilting, low production, reduced quality, and so on. Plants lacking water can lead to death, whereas excess water can cause damage to plant roots, due to lack of air in stagnant soil (De, 2022).

According to Bhatla and Lal, (2023) the division and development of plant cells in general is strongly influenced by the presence of water both inside and outside plant cells. Drought will inhibit cell division and development, so that the roots are not able to carry out maximum distribution and expansion. Minimal root distribution affects root size including root surface area. Stressed roots will become smaller in volume due to less water content in the root body (Li et al., 2023), and the higher the root length value, the smaller the root diameter (Huang et al., 2022).

Based on Table 2, the treatment combination of C_1F_4 (PB 260 clone with watering frequency once every 7 days) showed the lowest growth of root length, root surface area, and root volume compared to other treatment combinations. This can be explained by the low volume of soil water, the water absorbed for root growth is also low and root growth is in accordance with the metabolism of the clone.

Shahzad et al., (2024) in their research showed that the decrease in soil moisture content will affect the absorption of nutrients by plant roots which will further affect the metabolism and growth of plants, to get the best growth of rubber seedlings in polybags, the soil moisture content must be maintained at field capacity. Al-Zahrani et al., (2023) stated that plants that are able to maintain the water content in the leaves in water shortage, means that the plant is able to hold stomatal closure in drought conditions so that leaf water preservation occurs.

5. Conclusions

The study showed that the combination of clone type and watering frequency affected the physiological responses of plants, especially root growth and leaf water content (RWC), but did not affect leaf growth. The PB 260 clone produced a larger root volume, while the GT1 clone had longer roots, reflecting differences in genetic response to drought.

Watering frequency once every 7 days (F_4) encourages roots to grow more actively as a form of adaptation to water shortages encountered in the GT1 clone (C_2). However, more plant energy is directed to the roots, so leaf growth is reduced. Conversely, watering every day (F_1) makes the soil saturated with water, inhibits root respiration and reduces water absorption efficiency.

Watering frequency every 5 days (F_3) provides a balance of water and air, so that root and leaf growth is optimal. Root characteristics such as length and volume are important as indicators of drought resistance, because a wide root system helps plants absorb water more effectively.

Therefore, selecting drought-resistant clones and setting the right watering frequency are very important in rubber cultivation, especially in dry land or in facing climate change.

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