Pertanika J. Sci. & Technol. 32 (6): 2807 - 2826 (2024)

SCIENCE & TECHNOLOGY

Journal homepage: http://www.pertanika.upm.edu.my/

Physiological Behavior of Rubber Plants (*Hevea Brasilliensis*) to Different Soil Moisture Conditions

Rohaizad Mislan[1], Zulkefly Sulaiman[1]*, Siti Nor Akmar Abdullah[2], Mohd Razi Ismail[1], Sarker Mohammad Rezaul Karim[1], Mohd Aswad Abdul Wahab[3] and Aizat Shamin Noran[4]

[1]*Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[2]*Institute of Plantation Studies, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[3]*Department of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[4]*Production Development Division, Rubber Research Institute of Malaysia, Malaysian Rubber Board, 47000, Sg. Buloh, Selangor, Malaysia*

ABSTRACT

Drought conditions can severely impact rubber (*Hevea brasiliensis*) plantations, leading to economic loss in Malaysia. The study aimed to assess the impact of varying soil moisture levels on the physiological characteristics of five latex timber clones (LTCs) of rubber, with the goal of identifying the most suitable clone for specific soil moisture conditions. These conditions include (1) field capacity, (2) 75% available water (AW), (3) 50% AW, (4) 25% AW, and (5) wilting point, with the ultimate objective of optimizing cultivation methods and fostering sustainable rubber production in Malaysia. The five clones under investigation include RRIM3001, RRIM2025, RRIM2001, RRIM928, and PB350. Leaf chlorophyll content, stomatal conductance, and net photosynthesis were measured 4 and 8 months after treatment (MAT). The findings indicated significant effects of moisture stress on various physiological attributes, including total chlorophyll content, relative chlorophyll content, stomatal conductance, and net photosynthesis rate. At 4 and 8 MATs, the clones subjected to field capacity exhibited the highest values for these physiological characteristics, followed by those exposed to 75% available water, with the lowest values observed at the wilting

ARTICLE INFO

Article history:
Received: 22 September 2023
Accepted: 01 February 2024
Published: 25 October 2024

DOI: https://doi.org/10.47836/pjst.32.6.21

E-mail addresses:
rohaizadmislan@gmail.com (Rohaizad Mislan)
zulkefly@upm.edu.my (Zulkifly Sulaiman)
snaa@upm.edu.my (Siti Nor Akmar Abdullah)
razi@upm.edu.my (Mohd Razi Ismail)
sarker.mohammad@upm.edu.my (Sarker Mohammad Rezaul Karim)
mohdaswad@upm.edu.my (Mohd Aswad Abdul Wahab)
aizat@lgm.gov.my (Aizat Shamin Noran)
* Corresponding author

ISSN: 0128-7680
e-ISSN: 2231-8526

point. RRIM3001 consistently exhibited the highest total chlorophyll content, stomatal conductance, and net photosynthesis among the clones at both sampling dates. The highest net photosynthesis was observed in the RRIM3001 clone under field capacity conditions. Furthermore, a significant positive correlation was identified between total chlorophyll and relative chlorophyll contents, as well as between net photosynthesis and stomatal conductance. These findings carry practical implications for water management during the initial growth phase of rubber seedlings and for replanting initiatives in rubber plantations.

Keywords: Chlorophyll content, *Hevea brasiliensis*, latex timber clones, net photosynthesis, soil moisture regimes, stomatal conductance, volumetric soil water content

INTRODUCTION

Rubber (*Hevea brasiliensis*) is a significant economic crop in Malaysia, contributing to the country's agricultural sector and foreign exchange earnings. The success of rubber cultivation depends on various factors, including soil moisture, which plays a crucial role in plant growth, development, and productivity. Understanding the physiological adaptations of rubber clones to different soil moisture regimes is essential for optimizing cultivation practices and ensuring sustainable rubber production in Malaysia (Mislan et al., 2020; Mokhatar et al., 2011). Malaysia experiences variable rainfall patterns throughout the year, leading to fluctuating soil moisture conditions. By studying the physiological adaptations of different rubber clones to different soil moisture regimes, we can identify the most suitable moisture conditions for maximizing rubber growth and yield. Drought is a significant environmental stressor that affects rubber cultivation, particularly in regions with water scarcity or seasonal dry spells. By examining the physiological responses of rubber clones to reduced soil moisture, we can identify the mechanisms and traits associated with drought tolerance. This information can be used to develop improved rubber clones that are more resilient to water scarcity, reducing yield losses and ensuring the sustainability of rubber production in drought-prone areas (Cahyo et al., 2022).

Limitations in soil moisture elicit a range of plant responses, from recently elucidated acoustic signals to intricate physiological alterations (Waqas et al., 2023). The physiological changes are interconnected and influence overall plant performance, especially in chlorophyll contents, photosynthetic rate, and stomatal conductance in rubber clones in response to different soil moisture regimes. Investigating these interactions provides insights into the underlying mechanisms governing the physiological adaptations of the rubber clones (Kunjet et al., 2013). Understanding how different moisture regimes affect key physiological processes, such as chlorophyll content, photosynthesis, and stomatal regulation, enables us to comprehensively assess the impact of soil moisture on rubber plant physiology and productivity (Wang, 2014).

Climate change poses significant challenges to agricultural systems worldwide, including rubber cultivation. Alterations in precipitation patterns, temperature regimes, and extreme weather events can profoundly affect soil moisture dynamics. By studying the physiological adaptations of rubber clones under different soil moisture regimes, we can anticipate and prepare for potential future changes in climate (Jacob et al., 2022). With these ends in view, the research was undertaken with the following objectives – to assess the impact of varying soil moisture regimes on the physiological responses of five rubber clones, to identify the best-adapted rubber clones to different soil moisture levels, and to determine the optimal soil moisture conditions for maximizing rubber growth and yield. The research aims to identify the most suitable clones for specific soil moisture regimes, ensuring sustainable and efficient rubber production in Malaysia.

MATERIALS AND METHODS

Location and Treatments

The experiment was conducted under simulated water stress environments in a shed house at Ladang 2, Faculty of Agriculture, Universiti Putra Malaysia ($3^0$20` N, $101^0$420` E, 31 m elevation). Five latex timber clone (LTC) seedlings (budded plant) aged from 4 to 5 months with 4 leaves whorls, e.g., RRIM3001, RRIM2025, RRIM2001, RRIM928 and PB350, were used as plant materials and Munchong soil series (typic hapludox/haplic ferralsol) was used as a planting media in pots. Agronomic practices, including fertilization, weed removal, and pest control, were applied to sustain the seedlings as recommended by the Malaysia Rubber Board (Malaysian Rubber Board, 2009). The soil moisture treatments included are (1) moisture at field capacity (FC), (2) 75% available water (AW), (3) 50% AW, iv) 25% AW, and (4) moisture at wilting point (WP).

Development of Munchong Soil Series Curve

Soil samples were collected from Ladang 10, Taman Pertanian, UPM, using 7.6 cm diameter and 4.0 cm depth double core rings. Water Retention Analysis determined the soil moisture content of the Munchong soil series following Teh and Jamal (2006), and the Munchong soil water retention curve was developed (Figure 1). Collected samples were divided into five equal portions and were saturated with water for 24 hours on porous plates. These samples were placed

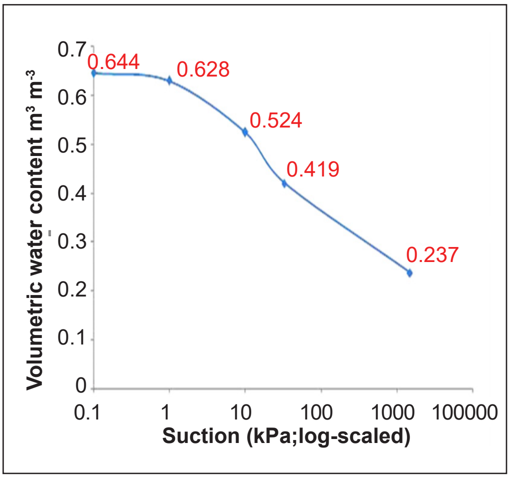


Figure 1. Munchong series soil water retention curve

inside the corresponding pressure chambers of a Pressure Membrane Chamber apparatus and connected to the outflow hub. Pressure heads ranging from 0 kPa to 1500 kPa were adjusted. The air pressure was applied uniformly until equilibrium (4–7 days) was attained, signifying no further water outflow. Afterward, air pressure was released gradually, and the samples were weighed. Oven drying at 105°C for 24 hours yielded the dry weights. Moisture content under various pressures was determined using wet and dry weights.

Bulk density was also assessed using Teh and Jamal's (2006) method (Equation 1).

$$\text{Bulk density} = \frac{\mathrm{D_w\ (gm)}}{\mathrm{R_v\ (cm^3)}} \qquad [1]$$

Where D_w = Dry weight of the soil; and R_v = Ring volume.

The final moisture percentage was calculated using Equations 2 and 3.

$$\%\ \text{Moisture (v/v)} = \%\ \text{moisture (dry basis)} \times \text{bulk density of soil (g cm}^{-3}) \qquad [2]$$

$$\text{Moisture (\%)} = \frac{\mathrm{W_d - D_d\ (g)}}{\mathrm{D_d - D\ (g)}} \times 100 \qquad [3]$$

% Moisture content ($^{w}/_{w}$) = Dry Basis (%)

% Moisture content ($^{v}/_{v}$) = Moisture (%) × B_d (g cm^{-3})

Where W_d = Wet weight of the soil with dish; D_d = Dry weight of the soil with dish; B_d = Bulk density of soil; and D = Dish weight.

The experiment was replicated four times, and average moisture values were plotted on a log scale of volumetric water content (Y-axis) against soil suctions (X-axis) at different pressures (e.g., 0 kPa, 1 kPa, 10 kPa, 33 kPa, and 1500 kPa), as suggested by Teh and Jamal (2006). Moisture at 33 kPa (0.419) represented field capacity (FC), and at 1500 kPa (0.237) indicated a wilting point (WP).

Determination of Other Treatments [% Available Water (AW)]

i) 50% AW = (moisture at FC – moisture WP) × 0.50 + moisture at WP = 0.328 $m^3\ m^{-3}$
ii) 75% AW = (moisture at FC – moisture at WP) × 0.75 + moisture at WP = 0.3735 $m^3\ m^{-3}$
iii) 25% AW = (moisture at FC – moisture at WP) × 0.25 + moisture at WP = 0.2825 $m^3\ m^{-3}$

Estimation of Total Chlorophyll Content

Chlorophyll contents were measured at 4 and 8 MAT according to the method described by Witham et al. (1986). Sample rubber leaves were cut and put in a water bucket to prevent the desiccation of leaves, and then they were brought into the laboratory. The fresh leaf was cut into pieces with scissors and weighed on a digital balance of 200 mg. The fresh weight of the sample was recorded, and samples were then transferred into a plastic vial containing 20 ml of 80% acetone. The vial was kept in the dark for 72 hours. The absorbance was recorded at 645nm and 663nm wavelengths using the light spectrophotometer (UV-3101P, Labomed Inc, USA). Chlorophyll content was calculated and expressed as mg g^{-1} of plant leaf tissue with Equation 4.

$$\text{Total chlorophyll (mg g}^{-1}\text{fresh leaf)} = \frac{20.2\ (A_{645}) - 8.02\ (A_{663})}{1000} \times \frac{V}{W} \qquad [4]$$

Where A_{645} = Absorbance of the solution at 645 nm; A_{663} = Absorbance of the solution at 663 nm; V = Volume of the solution in mL; and W = Weight of fresh sample in gram.

Relative chlorophyll content (leaf greenness) was estimated using the ratio of transmitted red light and NIR (Near-infrared) light emitted by a red and an NIR LED, respectively, through the leaf. Transmitted red light through a leaf is inversely related to the chlorophyll content because chlorophylls absorb red light efficiently (Taiz et al., 2015). Relative chlorophyll content was estimated by Yamamoto et al. (2002) by monitoring the SPAD index using a Chlorophyll meter (SPAD-502, Konica-Minolta Corp, Japan). After calibration of the meter, the sample leaves were placed under the sensor, and the readings were recorded by pressing the sensor gently on the leaf surface. Data were taken from four fully expanded and matured leaves of the clones randomly selected per treatment.

Measurement of Stomatal Conductance and Photosynthetic Rate

The stomatal conductance and photosynthesis rates in rubber plants were measured using a LI-6400 Porometer and a portable open gas exchange system with an Infrared Gas Analyzer. On sunny days, three uppermost fully expanded and matured leaves were selected from different angles for each treatment at 4 and 8 MATs (months after treatments). Stomatal conductance was assessed by the LI-6400 Porometer, which quantifies the rate of vapor diffusion through the stomata, yielding values in $molH_2Om^{-2}s^{-1}$. Simultaneously, the rate of photosynthesis ($\mu molCO_2\ m^{-2}s^{-1}$) was determined using the LI-6400 gas exchange system within a 6400-02B LED light source chamber. These measurements provide valuable insights into the physiological responses of the plants under varying conditions, contributing to a comprehensive understanding of their performance and adaptation mechanisms.

Statistical Analysis

A two-factorial experiment consisting of 25 treatment combinations (5 LTCs × 5 soil moisture regimes) was conducted and was arranged in a randomized complete block design with four replications. The overall effects of soil moisture regimes and LTCs were determined by means of a two-way analysis of variance as per Gomez and Gomez (1984). The correlation between total chlorophyll and relative chlorophyll contents was determined. The data were subjected to statistical analysis using the SPSS Statistical Program. Analysis of variance was done to see the significant difference between the treatments, and the Studentised T-test was used to compare the means at 5% probability ($P \leq 0.05$).

RESULTS AND DISCUSSION

Effects on Chlorophyll Contents

The chlorophyll content of leaf samples from five different latex timber clones (LTCs) was significantly influenced by varying soil moisture regimes, as illustrated in Figures 2 and 3. Among these selected clones, RRIM3001 displayed the highest total chlorophyll content, registering at 3.50 mg g^{-1}, a significant 9.14%, 17.14%, 10.86%, and 8.57% higher compared to RRIM2025, RRIM2001, RRIM928, and PB350, respectively.

The interaction effects of soil moisture regimes on total chlorophyll content were highly significant ($P \leq 0.0001$), revealing distinct responses across LTCs in *H. brasiliensis* seedlings at 4 and 8 months after treatment (MAT) (Table 1). Generally, the highest total chlorophyll content was recorded under field capacity (FC) moisture conditions (4.72 mg g^{-1}), followed by 75% available water (4.01 mg g^{-1}), with the lowest values noted under wilting point (WP) conditions (1.97 mg g^{-1}). Soil moisture stress was observed to decrease the total chlorophyll content, as depicted in Figure 3. A parallel effect of soil moisture regimes was observed in rubber leaves' relative chlorophyll content (SPAD index) (Figures 4 and 5). Clone RRIM3001 displayed the highest leaf relative chlorophyll content (56.91) compared to RRIM2001, RRIM928, and PB350. No significant difference was found between RRIM3001 (56.91) and

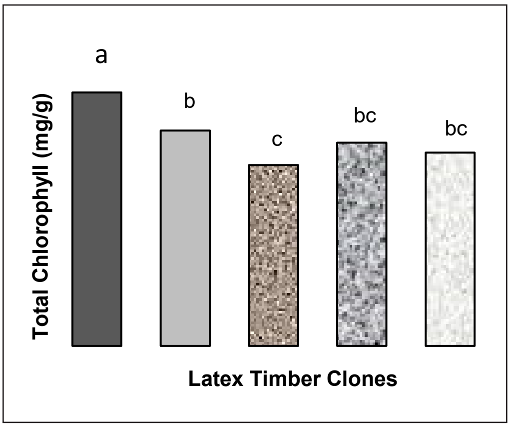


Figure 2. Total chlorophyll content (mg g^{-1}) extracted from leaf in five LTCs of *H. brasiliensis* seedlings
Note. Data are the average of two sampling dates crossing over five soil moisture regimes. At each LTC, the means with the same letters are not significantly different by the LSD at $\alpha = 0.05$. LTC = Latex timber clone

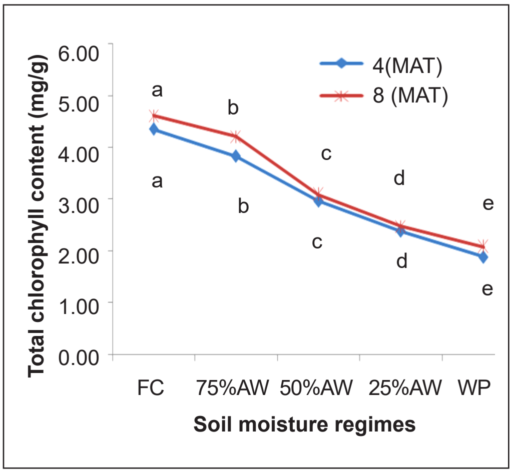


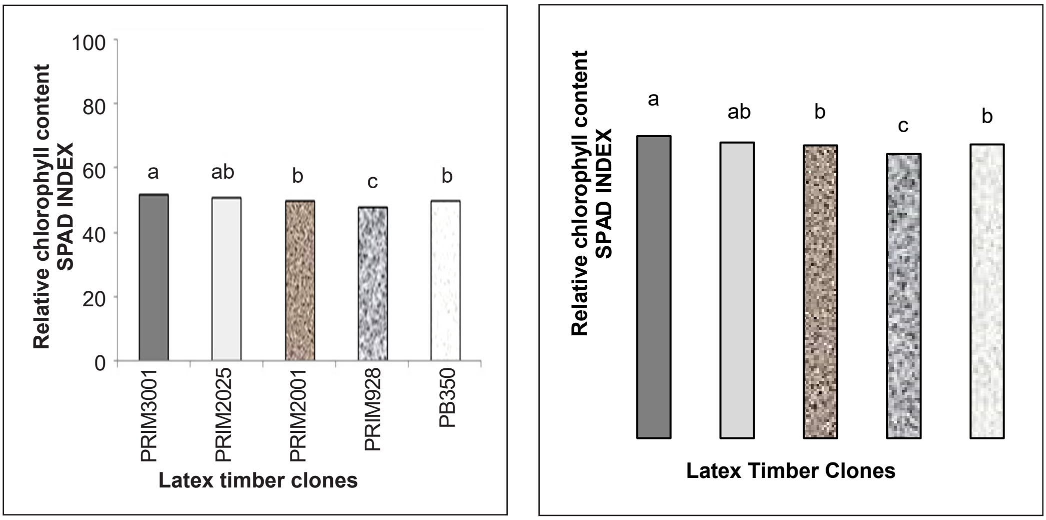
*Figure 3***.** Total chlorophyll content (mg g^{-1}) extracted from the leaf as affected by different soil moisture regimes at 4 (blue) and 8 (red) MATs
Note. Data are the average of five LTCs. At each soil moisture regime, means with the same letters at a particular sampling date are not significantly different by the LSD at $\alpha = 0.05$. MAT = Months after treatment, FC = Field capacity, AW = Available water, WP = Wilting point

Table 1
Total chlorophyll content extracted from leaf as affected by different soil moisture regimes in five LTCs of H. brasiliensis

Clones	Soil moisture regimes	Total chlorophyll content (mg g^{-1}) 4 MATs	Total chlorophyll content (mg g^{-1}) 8 MATs
RRIM 3001	FC	5.19 ±0.35a	5.07 ±0.79a
	75% AW	4.92 ±0.73ab	4.39 ±0.34ac
	50% AW	3.59 ±0.41cg	2.62 ±0.26eh
	25% AW	2.76 ±0.70fj	2.17 ±0.22fh
	WP	2.17 ±0.20hj	2.11 ±0.11gh
RRIM 2025	FC	4.42 ±0.98ac	4.31 ±0.37ac
	75% AW	4.19 ±0.84ad	3.75 ±0.30cd
	50% AW	2.93 ±0.08ei	3.09 ±0.66de
	25% AW	2.46 ±0.31hj	2.82 ±0.21eg
	WP	1.84 ±0.23ij	2.00 ±0.56gh
RRIM 2001	FC	3.83 ±0.63bf	4.47 ±0.30ac
	75% AW	2.97 ±0.36eh	4.04 ±0.23bc
	50% AW	2.64 ±0.22gj	2.85 ±0.32eg
	25% AW	2.08 ±0.15hj	2.16 ±0.16fh
	WP	1.77 ±0.33j	2.16 ±0.19fh
RRIM 928	FC	4.37 ±0.43ac	4.40 ±0.12ac
	75% AW	3.91 ±0.24be	4.28 ±0.16ac
	50% AW	2.53 ±0.15gj	3.00 ±0.24df
	25% AW	2.33 ±0.24hj	2.73 ±0.31eh
	WP	1.80 ±0.21j	1.89 ±0.13h
PB 350	FC	3.92 ±0.80be	4.78 ±0.023ab
	75% AW	3.11 ±0.57dh	4.53 ±0.45ac
	50% AW	3.11 ±0.11dh	3.83 ±0.21cd
	25% AW	2.25 ±0.18hj	2.47 ±0.47eh
	WP	1.81 ±0.22j	2.21 ±0.11eh

Note. Values followed by a common letter within each column are not significantly different by the LSD at α = 0.05. AW = Available water, FC = Field capacity, WP = Wilting point, MAT= months after transplant

RRIM2025 (55.43) concerning relative chlorophyll content at 4 and 8 MAT. The trends observed in relative chlorophyll content mirrored those in total chlorophyll content and were negatively affected by soil moisture stress (Figure 5). Specifically, at 4 MAT, FC moisture conditions exhibited the highest relative chlorophyll content (66.06), outperforming the 75% available water, 50% available water, 25% available water, and WP treatments. Similar patterns were noted at 8 MATs across the five LTCs of *H. brasiliensis* seedlings. These results underscore the significant influence of soil moisture regimes on the total and relative chlorophyll contents of rubber leaves in different LTCs. Moisture conditions at FC were associated with higher chlorophyll contents, while moisture stress conditions resulted in



(a) (b)

Figure 4. Relative chlorophyll content (SPAD index): (a) at 4 MAT; and (b) at 8 MAT in five LTCs of *H. brasiliensis* seedlings

Note. Values are an average of five soil moisture regimes. At each LTC, the means with the same letters are not significantly different by the LSD at α = 0.05. MAT = Months after treatment, LTC = Latex timber clone

lower chlorophyll contents. It aligns with previous research by Wang et al. (2015), which emphasized the correlation between plant water availability and chlorophyll content.

There was a significant interaction between LTCs and moisture stresses in producing relative chlorophyll content at both 4 and 8 MATs (Table 2).

Soil moisture plays a pivotal role in various aspects of plant physiology, particularly chlorophyll production, which is indispensable for photosynthesis. Adequate soil moisture is essential for water uptake by plant roots, and water is a crucial component in chlorophyll synthesis, facilitating multiple biochemical reactions during the process (Wang et al., 2019). It also acts as a medium for transporting

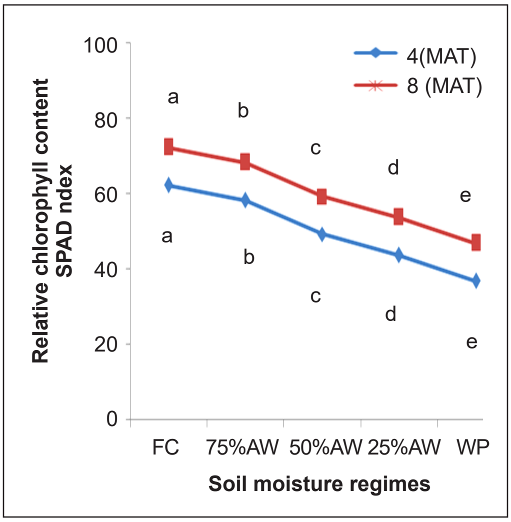


Figure 5. Relative chlorophyll content (SPAD index) at 4 MATs (blue) and 8 MATs (red) under different soil moisture regimes

Note. Values are an average of five LTCs. At each soil moisture regime, means with the same letters are not significantly different by the LSD at α = 0.05. FC = Field capacity, AW = Available water, WP = Wilting point, MAT = Months after treatment nutrients and minerals vital for chlorophyll production, including nitrogen (N), magnesium (Mg), and iron (Fe). These nutrients are essential for chlorophyll molecule formation, with their uptake facilitated by water movement in the soil. Chlorophyll molecules capture light energy during photosynthesis, and optimal soil moisture levels support efficient photosynthesis by maintaining turgor pressure within leaf cells. Turgor pressure, influenced by water availability, ensures proper alignment of chloroplasts within leaf cells for maximum light absorption (Li & Wang, 2021). Soil moisture levels also regulate leaf temperature through transpiration, which leads to cooling. This cooling effect helps maintain an appropriate temperature range for optimal chlorophyll synthesis and function. Additionally, soil moisture availability influences the stomatal aperture. Stomata, small

Table 2

Relative chlorophyll contents as affected by different soil moisture regimes in five LTCs of H. brasiliensis

Clones	Soil Moisture Regimes	Relative chlorophyll content (SPAD index)	
		4 MATs	8 MATs
RRIM3001	FC	64.45 ±0.96a	74.15 ±0.99a
	75% AW	61.20 ±1.10ac	72.20 ±1.10ab
	50% AW	50.12 ±0.25f	60.22 ±0.24f
	25% AW	44.90 ±0.73fh	55.90 ±0.74fh
	WP	37.97 ±0.76jk	47.97 ±0.75jk
RRIM2025	FC	62.40 ±0.94a-b	72.40 ±0.94ab
	75% AW	58.62 ±1.24cd	68.62 ±1.24cd
	50% AW	49.80 ±0.40f	59.80 ±0.40f
	25% AW	44.00 ±0.61hi	54.01 ±0.61hi
	WP	37.32 ±1.13k	47.33 ±1.14k
RRIM2001	FC	61.80 ±2.15ac	71.81 ±2.16ac
	75% AW	57.50 ±1.67de	67.51 ±1.68de
	50% AW	48.55 ±1.34f	58.54 ±1.34f
	25% AW	44.57 ±1.40gh	54.57 ±1.40gh
	WP	36.92 ±1.51k	47.91 ±1.51k
RRIM928	FC	60.50 ±1.10bd	59.50 ±1.09bd
	75% AW	55.07 ±1.02e	55.07 ±1.01e
	50% AW	48.02 ±1.25gj	58.02 ±1.24gj
	25% AW	41.05 ±1.90ij	51.05 ±1.89ij
	WP	35.72 ±1.85k	43.72 ±1.84k
PB350	FC	61.80 ±1.70ac	71.80 ±1.70ac
	75% AW	58.77 ±2.50cd	68.77 ±2.50cd
	50% AW	49.77 ±0.84f	59.77 ±0.84f
	25% AW	43.65 ±1.28hi	53.65±1.28hi
	WP	36.30 ±1.48k	36.30 ±1.48k

Note. Values followed by a common letter within each column are not significantly different by the LSD at α = 0.05. AW = Available water, FC = Field capacity, WP = Wilting point, MAT= months after transplant openings on the leaf surface, are responsible for gas exchange and water vapor release. In conditions of limited soil moisture, plants may close their stomata to conserve water, potentially reducing carbon dioxide availability for chlorophyll production and impacting its synthesis. Furthermore, recent findings by Swoczyna et al. (2022) highlighted the utility of chlorophyll as a potential indicator of stress conditions in plants, shedding light on its role in determining overall plant growth.

Correlation Between Total Chlorophyll Content and Relative Chlorophyll Content

The results indicated that the total chlorophyll content extracted from the leaves was significantly ($P \leq 0.0001$) correlated with the relative chlorophyll content readings obtained from the Minolta SPAD-502 device. The correlation coefficients, representing the strength and direction of the relationship, were reported as 0.74841 at 4 MATs and 0.8159 at 8 MATs.

The strength of the correlation suggests that the relative chlorophyll content readings obtained from the SPAD-502 can serve as a reliable indicator of the total chlorophyll content in rubber leaves (Jiang et al., 2017). These findings have implications for assessing the chlorophyll status of rubber leaves non-destructively and quickly using the SPAD-502 device. By measuring the relative chlorophyll content, which is strongly related to the total chlorophyll content, researchers and rubber growers can gain insights into the rubber leaves' photosynthetic activity and overall health without the need for labor-intensive and time-consuming laboratory extractions.

Effects on Stomatal Conductance

The effect of different soil moisture regimes on the stomatal conductance of five timber latex clones (LTCs) of rubber plants has been investigated. The results indicate that soil moisture regimes significantly influenced the stomatal conductance of the rubber leaves. Among the clones, RRIM2001 exhibited the highest stomatal conductance (0.062 $molH_2Om^{-2}s^{-1}$), followed by PB350 (0.052 $molH_2Om^{-2}s^{-1}$), while RRIM928 displayed the lowest value (0.050 $molH_2Om^{-2}s^{-1}$) (Figure 6). It suggests that the clones responded differently to the soil moisture conditions, with RRIM2001 showing the highest stomatal conductance and RRIM928 exhibiting the lowest. Carr (2012) stated that the water relations of rubber trees are affected by drought stress, and clones differ in their susceptibility to cavitation, which occurs at xylem water potentials in the range of −1.8 to −2.0 MPa. Clone RRII105 is capable of maintaining higher leaf water potentials than other clones because of stomatal closure, supporting its reputation for drought tolerance.

Figure 7 provides insights into the effects of various soil moisture regimes on stomatal conductance. Moisture at field capacity (FC) resulted in the highest stomatal conductance (0.0715 $molH_2Om^{-2}s^{-1}$), followed by 75% available water (AW) (0.0625 $molH_2Om^{-2}s^{-1}$). In contrast, the lowest stomatal conductance was observed under water stress conditions at the wilting point (WP) (0.0358 molH$_2$Om^{-2}s^{-1}). These results indicate that sufficient soil moisture, such as at FC, promotes higher stomatal conductance, while water deficit conditions restrict stomatal opening and reduce conductance. As a result of soil drought, leaf water potential (ΨL) decreases, and stomata close, which limits transpiration and photosynthesis. Manzoni et al. (2013) investigated the optimization of stomatal conductance for maximum carbon gain under dynamic soil moisture. They found that stomatal conductance is affected by changes in soil moisture. Liang et al. (2023) conducted a study on the stomatal responses of terrestrial plants to global change. They found that stomatal conductance is also sensitive to changes in soil moisture regimes.

The interaction effects of LTCs and soil moisture regimes on stomatal conductance were also significant (Table 3). Although all the LTCs showed the highest stomatal conductance under moisture at FC, the LTCs RRIM2001, RRIM2025, and PB350 only showed the second-highest stomatal conductance under soil moisture at 75% AW (Table 3). It is important to note that there were no significant differences between the five clones in terms of stomatal conductance under soil moisture at FC.

Apart from RRIM2001, there were no significant differences between the clones regarding stomatal conductance under WP conditions (Table 3). In conclusion, adequate soil moisture, such as that at field capacity, promotes higher stomatal conductance, facilitating the exchange of gases, including water vapor, between the leaf and the surrounding environment. As represented by the wilting point, water stress conditions lead to reduced

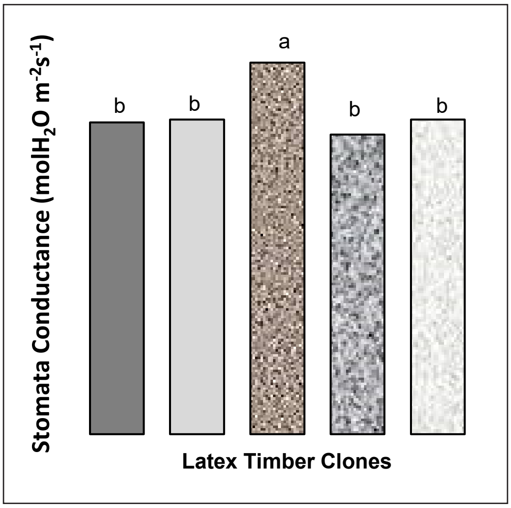


Figure 6. Stomatal conductance (molH$_2$O m^{-2}s^{-1}) in five LTCs of *H. brasiliensis* seedlings
Note. Data are the average of two sampling dates crossing over five soil moisture regimes. At each LTC, means with the same letters are not significantly different by the LSD at α = 0.05. LTC = Latex timber clone

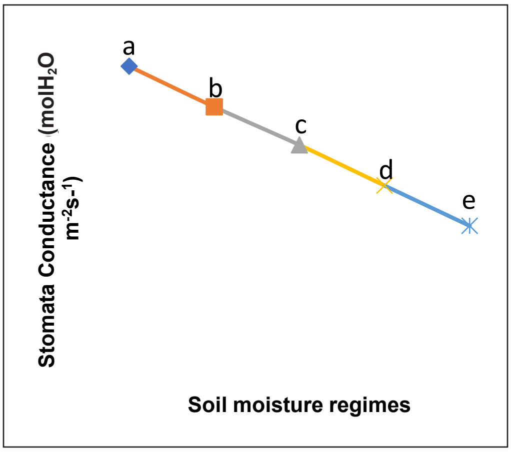


Figure 7. Stomatal conductance (molH$_2$O m^{-2}s^{-1}) as affected by different soil moisture regimes in five LTCs of *H. brasiliensis* seedlings
Note. Data are the average of two sampling dates crossing over five LTCs. At each soil moisture regime, means with the same letters are not significantly different by the LSD at α = 0.05. LTC = Latex timber clone, FC = Field capacity, AW = Available water, WP = Wilting point

Table 3
Stomatal conductance (molH$_2$O m^{-2}s^{-1}) and net photosynthesis rate (µmolCO$_2$ m^{-2}s^{-1}) as affected by different soil moisture regimes in five LTCs of H. brasiliensis

Clones	Soil moisture regimes	Stomatal conductance (molH$_2$O m^{-2}s^{-1})	Net photosynthesis rate (µmolCO$_2$ m^{-2}s^{-1})
RRIM3001	FC	0.0700 ±0.0040a	11.55 ±0.05a
	75% AW	0.0600 ±0.0001c	10.56 ±0.26bc
	50% AW	0.0525 ±0.0025de	7.90 ±0.03f
	25% AW	0.0420 ±0.0025f	7.45 ±0.10g
	WP	0.0350 ±0.005gh	6.18 ±0.29ij
RRIM2025	FC	0.0725 ±0.0025a	10.82 ±0.17b
	75% AW	0.0625 ±0.0025bc	10.67 ±0.11bc
	50% AW	0.0525 ±0.0025de	7.62 ±0.21fg
	25% AW	0.0420 ±0.0025f	7.35 ±0.21g
	WP	0.0320 ±0.0025h	6.03 ±0.20ij
RRIM2001	FC	0.0725 ±0.0025a	10.60 ±0.20bc
	75% AW	0.0675 ±0.0025ab	9.96 ±0.09de
	50% AW	0.0625 ±0.0025bc	7.34 ±0.06g
	25% AW	0.0570 ±0.0047cd	6.68 ±0.04h
	WP	0.0500 ±0.004e	5.21 ±0.04k
RRIM928	FC	0.0700 ±0.0001a	10.70 ±0.15b
	75% AW	0.0600 ±0.0001c	9.61 ±0.04cf
	50% AW	0.0500 ±0.0001e	6.67 ±0.04h
	25% AW	0.0400 ±0.0001fg	5.89 ±0.03j
	WP	0.0300 ±0.0001h	2.31 ±0.09m
PB350	FC	0.0725 ±0.0025a	10.70 ±0.11b
	75% AW	0.0625 ±0.0025bc	10.30 ±0.06cd
	50% AW	0.0525 ±0.0025de	6.34 ±0.01hi
	25% AW	0.0420 ±0.0025f	6.10 ±0.21ij
	WP	0.0320 ±0.0025h	4.69 ±0.04l

Note. Data are the average of two sampling dates. Values followed by a common letter within each column are not significantly different by the LSD at α = 0.05. LTC = Latex timber clone, AW = Available water, FC = Field capacity, WP = Wilting point

stomatal conductance, indicating a restriction in gas exchange and potential water loss prevention by the plants. The variations observed among the different LTCs suggest that each clone possesses a distinct response to soil moisture conditions. Praba et al. (2009) opined that soil moisture stress can affect plants' osmotic adjustment and photosynthetic activity in rice and wheat. Stomata respond to soil moisture content compared to leaf water status, and the plants respond to biochemicals for triggering low water availability. The stomatal conductance was higher in clones with better moisture access, hinting at their potential for better photosynthesis compared to those in drier conditions.

Effects on Net Photosynthesis Rate

The findings indicate that both factors, e.g., LTCs and soil moisture regimes, significantly influenced the net photosynthesis rate. Figure 8 demonstrates the variations in net photosynthesis rate among different LTCs. The rubber clone, RRIM3001, exhibited the highest net photosynthesis rate (8.728 $\mu molCO_2m^{-2}s^{-1}$), which was significantly higher than the other clones (RRIM2025, RRIM2001, PB350, and RRIM928). RRIM928 recorded the lowest net photosynthesis rate of 7.036 $\mu molCO_2m^{-2}s^{-1}$. These results suggest variations in the photosynthetic capacity among the different clones, with RRIM3001 displaying the highest rate and RRIM928 exhibiting the lowest. Sterling et al. (2019) noted variations in photosynthetic rate in different rubber clones. They recorded that the clones FX4098, FDR4575, MDF180, GU198 and FDR5788 represent genotypes with the best photosynthetic performance (greater photosynthetic rates and better ability of the photosynthetic apparatus to capture, use and dissipate light energy). Carr (2012) also noted similar differences in the rubber clones with respect to their net photosynthetic rates.

The highest photosynthesis rate was observed under soil moisture at field capacity (FC) (10.874 $\mu molCO_2m^{-2}s^{-1}$), followed by 75% available water (AW) (10.220 $\mu molCO_2m^{-2}s^{-1}$). At the same time, the lowest value was recorded under water stress conditions (wilting point, WP) (4.884 $\mu molCO_2m^{-2}s^{-1}$) (Figure 9). These findings indicate that optimal soil moisture conditions, such as those at FC, promote higher net photosynthesis rates, while water deficit conditions restrict photosynthetic activity and result in lower rates. Santos et al. (2019) observed that physiological mechanisms responsible for tolerance to and recuperating from drought conditions in four rubber clones include changes in growth patterns and biomass allocation, diminishing stomatal conductance, and CO_2 assimilation rate.

The interaction between the LTCs and soil moisture regimes was significant, indicating that the response of net photosynthesis rate to moisture regimes varied among different clones (Table 3). Specifically, the rubber clone RRIM3001 exhibited the significantly highest net photosynthesis rate (11.55 $\mu molCO_2m^{-2}s^{-1}$ when subjected to soil moisture at FC. However, the clone, RRIM2001 treated with FC and 75% AW, did not significantly differ in net photosynthesis rate. On the other hand, RRIM928 displayed the lowest net photosynthesis rate (2.31 $\mu molCO_2\ m^2s^1$) when subjected to WP conditions. In conclusion, the LTCs and soil moisture regimes significantly affect rubber plants' net photosynthesis rate. Optimal soil moisture conditions, particularly those at field capacity, promote higher photosynthetic rates, while water stress conditions restrict photosynthetic activity.

These findings highlight the importance of proper soil moisture management in rubber plantations to optimize photosynthetic efficiency. Maintaining favorable soil moisture levels, especially around field capacity, ensures adequate water availability and promotes optimal net photosynthesis rates. Considering the specific moisture requirements of different clones, implementing appropriate irrigation strategies and water conservation practices

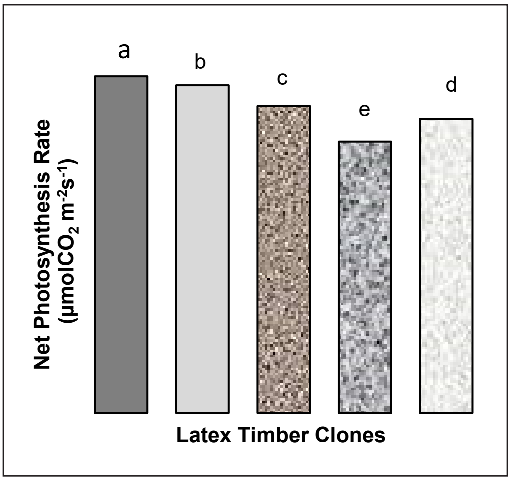


Figure 8. Net photosynthesis rate (µmolCO$_2$ m^{-2}s^{-1}) in five LTCs of *H. brasiliensis* seedlings
Note. Data are the average of two sampling dates crossing over five soil moisture regimes. At each LTC, means with the same letters are not significantly different by the LSD at α = 0.05. LTC = Latex timber clone

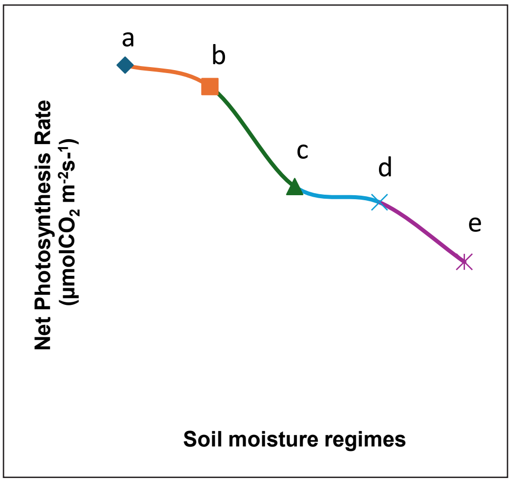


Figure 9. Net photosynthesis rate (µmolCO$_2$ m^{-2}s^{-1}) under different soil moisture regimes
Note. Data are the average of two sampling dates crossing over five LTCs. At each soil moisture regime, means with dissimilar letters are significantly different by the LSD at α = 0.05. LTC = Latex timber clone

can contribute to improved photosynthetic performance and overall plant productivity. Generally, chlorophyll measurements relate, directly or indirectly, to all stages of light-dependent photosynthetic reactions, including photolysis of water, electron transport, pH gradient formation across the thylakoid membrane, and ATP synthesis and thus the general bioenergetic condition of the photosynthetic machinery (Bernat et al., 2012).

It is noted that soil moisture directly affects water availability in rubber plants. Sufficient soil moisture ensures an adequate water supply to the plant roots, allowing for efficient nutrient uptake and transport to the leaves. Water is a crucial component for photosynthesis, as it serves as the medium for various metabolic reactions and as a source of electrons in light-dependent reactions. Therefore, optimal soil moisture levels are essential for maintaining high net photosynthesis rates. Soil moisture conditions also have a significant impact on stomatal conductance, which is related to gas exchange, allowing for the uptake of carbon dioxide (CO_2) needed for photosynthesis and the release of oxygen (O_2) produced during the process. In a study, Sterling et al. (2019) investigated the comprehensive effects of atmosphere and soil drying on the stomatal behavior of different plant types. They found that high vapor pressure deficit and low soil water content limit the stomatal conductance of plants, which in turn affects the net photosynthesis rate. When soil moisture is limited, plants close their stomata partially or completely to reduce water loss through transpiration.

However, stomatal closure also restricts the entry of CO_2, leading to a decline in net photosynthesis rates. Soil moisture influences leaf turgor pressure; the pressure water exerts within the plant cells. Sufficient soil moisture maintains optimal turgor pressure, ensuring that plant cells are adequately hydrated and able to maintain their structural integrity. Leaf cells with optimal turgor pressure are more efficient in capturing and utilizing light energy for photosynthesis. In contrast, water stress conditions can reduce turgor pressure, resulting in cellular damage, decreased photosynthetic capacity, and lower net photosynthesis rates. Figure 10 provides insights into the influences of different LTCs and soil moisture conditions on the relatively healthy appearance of rubber leaves.

Soil moisture affects the rubber plants' availability and uptake of essential nutrients. Adequate soil moisture levels facilitate the movement of nutrients in the soil solution, making them accessible to the plant roots. Nutrients such as nitrogen, phosphorus, and potassium are crucial for various physiological processes, including photosynthesis. When soil moisture is limited, nutrient uptake and transport may be hindered, leading to nutrient deficiencies and reduced photosynthetic activity. The net photosynthesis rate followed a similar pattern exhibited in chlorophyll contents and stomatal conductance, i.e., the clones with more water available showed higher rates than those experiencing moisture stress.

Notably, RRIM3001 stood out among the clones by consistently showing higher chlorophyll content, better stomatal conductance, and higher net photosynthesis rates across various soil moisture levels. Compared to RRIM2025, RRIM2001, RRIM928, and PB350, RRIM3001 demonstrated a more robust and adaptable response to changes in soil moisture. Even under different moisture conditions, RRIM3001 maintained superior physiological performance, indicating its better ability to handle varying water availability compared to the other studied clones.

The study highlighted that sufficient soil moisture is crucial for optimal physiological performance in rubber plants. The differences in how clones responded to varying moisture levels emphasize the potential for selecting or developing clones better suited to specific moisture conditions, which could significantly contribute to sustainable rubber production methods.

The suggestion to maintain soil moisture at field capacity could significantly influence rubber plantation practices by enhancing plant health and productivity. However, achieving and maintaining this optimal moisture level might pose challenges in different environments. Environmental factors like irregular rainfall patterns or high temperatures could make it challenging to sustain field capacity consistently. Implementing irrigation systems might help regulate moisture levels, but this could raise concerns about water availability and management, especially in regions prone to water scarcity. Therefore, balancing the need for optimal moisture with the practicality and sustainability of water usage in diverse environmental conditions would be crucial in implementing this recommendation in rubber plantations.

Figure 10. Effects of different soil moisture conditions on the leaf-appearance of five TLCs of *H. brasiliensis Note*. It is obvious that the healthy appearance and dark green color were observed under the soil moisture at field capacity and at 75% available water, and it decreased with an increase in soil moisture stresses. At the top of each plate, the name of each latex timber clone (black) is given. FC = Field capacity, AW = Available water, WP = Wilting point

Correlation Between Stomatal Conductance and Net Photosynthesis Rate

A significant positive correlation (coefficient of correlation = 0.829) between the net photosynthesis rate and stomatal conductance of rubber leaves under different soil moisture regimes is noticed. As stomatal conductance increases, the net photosynthesis rate increases, indicating a positive relationship between these two variables. These results highlight the importance of stomatal conductance in regulating the photosynthetic activity of rubber leaves and emphasize the role of soil moisture in influencing this relationship.

The study found correlations between different measurements. For example, photosynthesis and stomatal conductance were also lower when chlorophyll levels were lower. It suggests that when plants lack water, multiple aspects of their health and functioning are affected together. Understanding these connections can help future research by guiding scientists to focus on improving multiple factors at once, like finding rubber clones that can handle water stress better while keeping their chlorophyll levels up. In practical terms, this knowledge might help select or develop rubber plants more resilient to water shortages, ensuring better growth and productivity in rubber farms.

Certain cultural practices should be implemented to mitigate the impacts of soil moisture stress on the rubber clones. These practices may include implementing efficient irrigation methods to maintain optimal soil moisture levels, especially during critical growth stages (Somjan & Sadude, 2008). It was found that irrigation caused marked leaf-shedding in some groups of rubber trees, which reduced the production of new leaves by about 10-20% compared to trees that were not irrigated. The study also found that sufficient irrigation eliminated foliar injury and resulted in a high photosynthetic rate. Mulching the soil surface to reduce evaporation and maintain soil moisture is helpful to combat the negative effects of soil moisture stresses (Shen et al., 2019). The study found that mulching with organic materials improved soil moisture retention and reduced soil temperature, improving rubber plant growth and yield. The mulching also reduced weed growth and soil erosion, contributing to the rubber plants' overall health.

The study specifically investigated the effect of rubber wood biochar as a mulching material and found that it significantly increased the height, stem diameter, and biomass of the rubber plants compared to the control treatment without mulch. Applying organic matter or compost to improve soil structure and water-holding capacity will also reduce the impact of soil moisture stresses (Bhadha et al., 2017). The study found that applying organic waste materials, such as compost, improved soil fertility and structure, promoting root growth of the rubber trees. The study also found that the organic amendments increased the soil's water-holding capacity, which contributed to the improved growth of the rubber trees. By implementing these cultural practices, it may be possible to minimize the negative effects of moisture stress on rubber plantations, leading to improved chlorophyll production, stomatal conductance, net photosynthesis rate and overall plant health.

CONCLUSION

The study highlighted that keeping the soil well-watered, especially when growing rubber plants, improves their health and productivity. So, when planting new rubber trees, it is essential to ensure they have enough water for better growth. Also, choosing a rubber clone, like RRIM3001, which can handle different water levels well, could be a good idea for replanting in rubber farms. It means paying attention to how much water these plants need and adjusting irrigation methods accordingly during replanting to get the best results. Maintaining soil moisture at field capacity is important to ensure optimal rubber clone performance. Giving the right amount of water at the right times can help grow healthier and more productive rubber plants in Malaysia.

The discovery that RRIM3001 performed well even when there was less water in the soil is crucial for regions facing water scarcity in rubber production. This finding suggests that RRIM3001 could be a valuable choice for areas with limited water resources. Cultivating this specific clone might help regions with low water availability, as it showed better resilience to soil moisture deficits. The implication is that by selecting and focusing on clones like RRIM3001, rubber cultivation practices in water-scarce areas could become more sustainable, potentially reducing the impact of water shortages on rubber production in Malaysia.

Furthermore, the SPAD meter offers an advantage by providing a quick and non-destructive way to measure relative chlorophyll content in rubber plants. This device allows for on-the-spot assessment without damaging the plants, offering immediate feedback on their health. Its use enables efficient monitoring across a plantation, aiding in identifying areas or specific plants needing attention due to lower chlorophyll levels. This quick assessment facilitates targeted resource allocation, directing efforts like irrigation or nutrient application precisely where needed. Ultimately, the SPAD meter's utilization supports timely interventions, optimizing resource allocation and enhancing overall crop health assessment in rubber plantations.

ACKNOWLEDGEMENTS

The authors thank Universiti Putra Malaysia for providing the facilities to carry out the research. We gratefully acknowledge all the laboratory assistants of Universiti Putra Malaysia, Serdang, Selangor, who graciously contributed to the success of this work.

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