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Response of different avocado microclonal rootstock varieties to etiolation time

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ABSTRACT: The potential impact of uniform, consistent, rapid, easy-to-transport, and disease-free clonal propagation of avocado (*Persea americana* Mill.) is significant for the industry. This research, conducted with a meticulous Split Plot arrangement in a Completely Randomized Design, provides valuable insights into optimizing microcloning techniques for avocado clonal propagation, specifically regarding varietal responses to etiolation time for more efficient and productive avocado cultivation. The study investigated the response of clonal rootstocks to etiolation as a means of microcloning, using different lengths of etiolation time (0, 2, 4 and 6 weeks) as the main plots and three clonal varieties of avocado rootstocks ('Malagkit', 'Evergreen', and 'Hass') as the subplots. Results revealed an interaction effect in leaf chlorophyll content, with 'Hass' rootstocks etiolated for six weeks exhibiting the highest value of 41.59 SPAD. Individually, 'Hass' rootstocks demonstrated superior performance in various parameters, including stem length (9.97 cm), stem diameter (.3880 cm), and leaf number (8.37). The 'Evergreen' clonal rootstocks exhibited the largest leaf area (53.54 cm^2) and the darkest leaf (4.82) and stem (5.00) color. The findings suggest significant variability among the three avocado clonal rootstock varieties in response to etiolation time. Specifically, 'Hass' rootstocks performed optimally when etiolated for four to six weeks.
Keywords: clonal rootstocks; disease-free propagation; microcloning.

Resposta de diferentes variedades de porta-enxertos microclonais de abacateiro ao tempo de etiolamento

RESUMO: O potencial impacto da propagação clonal uniforme, consistente, rápida, fácil de transportar e livre de doenças do abacateiro (*Persea americana* Mill.) é significativo para a indústria. Esta pesquisa fornece informações valiosas para otimizar técnicas de micropropagação para propagação clonal de abacateiros, especificamente as respostas varietais ao tempo de etiolamento para uma cultivação de abacateiros mais eficiente e produtiva. Este estudo, utilizando um arranjo de Parcela Dividida em um Design Completamente Aleatório, investigou a resposta de porta-enxertos clonais ao etiolamento como meio de micropropagação. A pesquisa utilizou diferentes períodos de etiolamento (0, 2, 4, 6 semanas) como parcelas principais e três variedades clonais de porta-enxertos de abacateiro ('Malagkit', 'Evergreen' e 'Hass') como subparcelas. Os resultados revelaram um efeito de interação no teor de clorofila foliar, com os porta-enxertos 'Hass' etiolados por seis semanas apresentando o maior valor de 41.59 SPAD. Individualmente, os porta-enxertos 'Hass' demonstraram desempenho superior em vários parâmetros, incluindo comprimento do caule (9.97 cm), diâmetro do caule (0.3880 cm) e número de folhas (8.37). Os porta-enxertos clonais 'Evergreen' exibiram a maior área foliar (53.54 cm^2) e a cor mais escura de folhas (4.82) e caules (5.00). No geral, os resultados sugerem uma variabilidade significativa entre as três variedades clonais de porta-enxertos de abacateiro em resposta ao tempo de etiolamento. Especificamente, os porta-enxertos 'Hass' apresentaram desempenho ótimo quando etiolados por quatro a seis semanas.
Palavras-chave: porta-enxertos clonais; propagação livre de doenças; microclonagem.

1. INTRODUCTION

Microcloning represents a novel approach to asexual propagation in fruit crops, offering many benefits for avocados (*Persea americana* Mill.). This innovative technique ensures consistency in clonal propagation, rapid multiplication, ease of transportation, mitigation of soil-related problems, and the production of robust, disease-resistant clones (ERNST, 1999; DE VILLIERS; ERNST, 2007; SPANN, 2018).

Building upon methodologies outlined by Frolich; Platt (1972), and Brokaw (1987), the microcloning process typically commences with the establishment of nurse seedlings onto which clonal rootstocks are grafted. Subsequently, the clonal rootstocks undergo a period of etiolation before being rooted and eventually grafted with the preferred microclones.

Previous research undertakings have consistently demonstrated the superior growth of avocado microclones on clonal rootstocks compared to those grafted onto clonal seedlings. Notably, some varieties emerged as highly favored clonal rootstocks for their tolerance and resistance to root rot compared to seedling rootstocks, such as 'Duke 7', 'Thomas,' 'Barr Duke,' 'Toron Canyon,' and 'Merensky II (WITNEY, 2002; MICKELBERT et al., 2012). Furthermore, investigations into the etiolation phenomenon of avocado clonal rootstocks have garnered significant attention within the realm of avocado cultivation (GLEESON et al., 2016; DUMAN et al., 2020). Scholars have explored the application of etiolation over varying durations, spanning from a brief window of 72 to 216 hours (SARKER; GOMASTA, 2024) to more extensive periods of up to four weeks (ALLESBESTE, 2009)

Despite the positive reviews and potential of avocado microcloning (RURAL DELIVERY, 2014; MITTER, 2016; GREEN AFRICA MAGAZINE, 2023), it remains relatively underexplored in the Philippines, where conventional methods of using clonal seedlings to graft commercial scions still predominate. The bulky nature of grafted clonal seedlings and transportation challenges due to soil media further complicate Filipino avocado farmers' adoption of this plant propagation technique.

Given the promising stage of avocado microcloning adoption among Filipino nursery operators, local avocado varieties with potential as clonal rootstocks have yet to be thoroughly studied. Limited literature on avocado microcloning, clonal rootstock etiolation, and promising local avocado varieties, originating from the Philippines underscores the need for further exploration. Several institutions and individuals (THE EAST AFRICAN AGRICULTURAL JOURNAL, 1940; FAO, 1999; CHO et al., 2018; WILLIAMS et al., 2024) have mentioned that avocados are propagated asexually by marcotting, inarching, grafting, and budding. However, there is a shortage of information about microcloning as another asexual propagation technique for this fruit.

In response to these challenges, the researchers delved into assessing the responses of different avocado microclonal varieties to varying etiolation durations, aiming to contribute to advancing microcloning practices in avocado cultivation. Specifically, they determined the best etiolation time that could promote optimum results for the clonal rootstocks, the rootstock variety that had the best response to etiolation time, and the level of interaction between variety and etiolation time on the performance of avocado clonal rootstock.

2. MATERIAL AND METHODS

2.1. Time and Place of the Study

The assessment of nursery performance of different avocado microclonal rootstocks, as influenced by the length of etiolation time, was conducted at the Central Mindanao University College of Agriculture Horticulture Nursery (7.8592º N, 125.0515º E, 311 masl) (WORLDWIDE ELEVATION MAP FINDER, 2024) on November 2023 to February 2024. The climatic data (Figure 1) from DOST-PAGASA (2024) for November 2023 to February 2024 show a gradual decrease in maximum and minimum temperatures. Maximum temperatures dropped from 31.59°C in November to 29.29°C in February, while minimum temperatures decreased from 22.97°C to 21.18°C during the same period. Relative humidity ranged from 66.62% to 74.94%, with the highest level recorded in January 2024. The observed climatic trends underscore the importance of meticulous environmental monitoring and adaptive management practices in avocado culture. By leveraging insights from climatic data, the researchers implemented targeted strategies to optimize avocado clonal rootstock production, mitigate environmental stressors, and enhance overall crop resilience in response to changing climatic conditions.

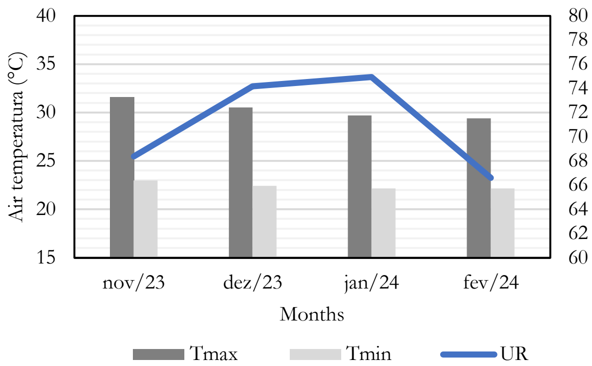

Figure 1. Minimum and maximum air temperatures (°C) and air relative humidity (%) in the Experimental Area. Source: DOST-PAGASA, Central Mindanao University, AgroMet Station, Musuan, Maramag, Bukidnon, Philippines (2024).

Figura 1. Temperaturas mínima e máxima do ar (°C) e umidade relativa do ar 9%) na área experimental. Fonte: DOST-PAGASA, Universidade Central de Mindanao, Estação AgroMet, Musuan, Maramag, Bukidnon, Filipinas (2024).

2.2. Materials and Equipment

The study utilized avocado nurse seedlings of the 'Evergreen' variety alongside clonal rootstocks such as 'Malagkit,' 'Evergreen,' and 'Hass.' These were cultivated in a nursery shed using various substrates, including cocopeat, ordinary garden soil, old/decayed rice hull, and vermicompost. Watering was done via a water sprinkler/hose system, and the seedlings were housed in polyethylene bags measuring 8" x 10". Tools such as bolo, spade, scissors, and cutters were used for maintenance tasks.

For precise data collection and analysis, the researchers employed a range of instruments and software. Measurements were taken using an AMSPEC® ruler, a Want® SPAD meter for assessing chlorophyll levels, a digital Vernier caliper, and the Easy Leaf® App and MunCell® App for digital analysis. Data were processed and analyzed using MS Excel and the Statistical Tool for Agricultural Research (STAR) version 2.0.1 software.

Additionally, the research involved using black Geena Pongee cloth to cover the etiolation boxes, providing a controlled environment for studying plant growth and development. Overall, the comprehensive array of materials, tools, and analytical techniques employed in the study facilitated rigorous experimentation and reliable data interpretation.

2.3. Experimental Design and Treatments

This experiment employed a Split Plot arrangement within a Randomized Complete Block Design (RCBD) framework. The experimental layout consisted of four (4) main plots, each subdivided into three (3) subplots. The entire design was replicated three (3) times to ensure the robustness and reliability of the results.

Randomization of treatments and field layout were meticulously executed using the Statistical Tool for Agricultural Researches (STAR) Version 2.0.1. This software, developed by the reputable International Rice Research

Institute (IRRI), provided a systematic approach to randomization and field layout, thereby minimizing potential biases and enhancing the validity of the experimental findings. By implementing the Split Plot arrangement within the RCBD framework and employing the STAR software for randomization and field layout, the experiment was well-structured to effectively assess the effects of different treatments on the avocado nurse seedlings and clonal rootstocks.

Main plots (etiolation time)

A1 = no etiolation (Control)
A2 = 2 weeks of etiolation after bud burst
A3 = 4 weeks of etiolation after bud burst (Recommendation, [ALLESBESTE, 2009])
A4 = 6 weeks of etiolation after bud burst

Subplots (Varieties of clonal rootstocks)

B1 = 'Malagkit' Variety
B2 = 'Evergreen' Variety
B3 = 'Hass' Variety

2.4. Collecting and Gathering of Planting Materials

'Evergreen' avocado seeds, as well as the 'Malagkit' and 'Evergreen' scions, were procured from local farmers in Barangay Kulaman Valley, Arakan, Cotabato, Philippines. This sourcing strategy ensured the use of locally adapted genetic material often well-suited to the region's environmental conditions. Furthermore, the clonal rootstocks of the 'Hass' variety were obtained from a private avocado grower located in Toril District, Davao City, Philippines. This sourcing decision likely aimed to access high-quality rootstock material from a reputable source.

2.5. Establishment of Nursery Shed

An existing nursery shed belonging to the College of Agriculture at Central Mindanao University served as the facility for conducting this experiment. Minor repairs and modifications were undertaken to adapt the shed to the study's specific requirements and ensure its suitability. Depending on the experiment's needs, these adjustments might have included repairing structural damage, enhancing ventilation, optimizing lighting conditions, or improving drainage systems (Figure 2).

2.6. Pre-germination of 'Evergreen' Avocado as a Nurse Seed

The process began with pre-germination of the 'Evergreen' avocado nurse seeds, a crucial step to ensure successful seedling establishment. For this purpose, germination beds were prepared, with 100% cocopeat chosen as the recommended germination media. Cocopeat, known for its moisture retention and aeration properties, provides an ideal environment for seed germination and early root development.

Once the seeds had germinated, they were transplanted into long, narrow-sleeved polyethylene bags measuring 8 inches by 10 inches. These bags served as individual containers for the seedlings, providing ample space for root growth and ease of handling. The choice of polyethylene bags allows for efficient space utilization and facilitates uniformity in plant management.

The growing media used in the polyethylene bags consisted of a carefully formulated blend to provide optimal nutrients and substrate characteristics for avocado seedling growth. This standard growing media comprised 30% ordinary garden soil (OGS), 30% vermicompost (VC), 30% soil compost, and 10% decomposed rice hull. Each component was selected based on its nutrient content, water retention capacity, and overall suitability for avocado cultivation.

Including vermicompost and soil compost enriches the growing media with organic matter, essential nutrients, and beneficial microorganisms, fostering healthy root development and overall plant vigor. Adding a decomposed rice hull helps improve soil structure, enhance drainage, and promote aeration within the growing medium.

2.7. Growing of Seedling Nurse

The 'Evergreen' stock seedlings underwent careful nurturing until their stems attained the requisite thickness, typically measuring at least 6 mm in diameter, akin to the size of a pencil. This critical threshold indicated that the seedlings had reached an appropriate stage of maturity for grafting. During this growth period, attention was given to optimizing growing conditions to promote healthy stem development and robust root systems, ensuring the success of the subsequent grafting process.

2.8. Grafting of Clonal Rootstocks

Once the 'Evergreen' stock seedlings attained the target size of "pencil size" or 6 mm in diameter, indicating their readiness for grafting, the desired clonal stocks ('Malagkit', 'Evergreen', and 'Hass') were promptly grafted onto them. This grafting process typically occurred approximately eight (8) weeks after the initial planting of 'Evergreen' seeds in polyethylene bags. The grafting procedure aimed to achieve a close and secure union between the scion and the rootstock, facilitating the successful integration of desired traits from the clonal stocks onto the 'Evergreen' rootstock. By timing the grafting operation to coincide with the optimal growth stage of the stock seedlings, the researchers aimed to maximize the chances of grafting success and subsequent healthy growth of the grafted avocado plants.

2.9. Construction of Etiolation Boxes

Etiolation boxes were meticulously crafted to envelop the experimental plants, providing a controlled environment conducive to specific growth conditions. These boxes were constructed using wooden frames, ensuring stability and support, and were carefully covered with black Geena Pongee cloth. The choice of black Geena Pongee cloth served multiple purposes: it effectively blocked out light, induced etiolation by restricting photosynthesis, and helped maintain consistent environmental conditions within the boxes. This setup facilitated the study of plant responses to reduced light exposure, allowing researchers to investigate morphological and physiological changes associated with etiolation.

2.10. Placing the Grafted Seedling in Etiolation Boxes

After the bud break of the clonal rootstocks had initiated, they were carefully transferred into spacious etiolation boxes designed to accommodate the microclones for each treatment. These boxes were adequately ventilated to ensure proper airflow and were sized appropriately to accommodate the experimental setup. Once placed inside the etiolation boxes, the buds were subjected to a controlled environment where they developed in darkness under ambient temperatures. In this study, a specific protocol was followed regarding the duration of etiolation. Two shoots per graft were allowed to develop, and the timing of etiolation was carefully manipulated. Etiolation times were selected as 0, 2, 4, and 6 weeks after bud break, representing different stages of shoot development under reduced light conditions.

Figure 2. Graphical abstract of the methods undertaken for the study. 1. Collecting and gathering planting materials. 2. Establishment of nursery shed. 3. Pre-germination and growing of 'Evergreen' seedling nurse. 4. Grafting of clonal rootstocks. 5. Construction of germination boxes. 6. Etiolation of avocado clonal rootstocks.

Figura 2. Resumo gráfico dos métodos utilizados para o estudo. 1. Coleta de materiais de plantio. 2. Implantação de galpão de berçário. 3. Pré-germinação e cultivo de mudas 'Evergreen'. 4. Enxertia de porta-enxertos clonais. 5. Construção de caixas de germinação. 6. Estiolamento de porta-enxertos clonais de abacateiro.

2.11. Monitoring the daily air temperature and relative humidity

The daily local climatic data on rainfall, humidity, and temperature (minimum and maximum) were obtained from the Department of Science and Technology, Philippine Atmospheric Geophysical, and Astronomical Services Administration (DOST-PAGASA) local station at Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. Moreover, an outdoor thermometer/hygrometer was placed in the nursery area to monitor the immediate environment's daily temperature and relative humidity. This was done at 8:00 a.m. and 2:00 p.m. to take note of the minimum and maximum temperatures and relative humidity for a particular day. Sprinkling the area and the plants with water was done to maintain the humidity above 70 percent.

2.12. Statistical Analysis

Following the data collection, the next step involved analyzing variance (ANOVA) to assess the significance of the observed treatment differences. This statistical analysis was performed using the Statistical Tool for Agricultural

Research (STAR) version 2.0.1 software, which offers advanced capabilities for data analysis in agricultural research settings. By subjecting the collected data to ANOVA, researchers could determine whether the observed variations in the measured parameters were statistically significant.

Subsequently, a post hoc analysis was conducted using Tukey's Honestly Significant Difference (HSD) test for parameters where significant differences were detected based on the calculated F-value from the ANOVA. Tukey's HSD test allows for pairwise comparisons of treatment means, enabling researchers to identify specific differences between treatment groups. This post hoc analysis is essential for gaining deeper insights into the nature and magnitude of differences among treatments, helping elucidate experimental variables' effects on the measured outcomes with greater precision. Overall, the combination of ANOVA and Tukey's HSD test facilitated rigorous statistical analysis and interpretation of the experimental results, enabling researchers to draw meaningful conclusions from the data.

3. RESULTS

Table 1 summarizes the analysis of variance conducted on avocado clonal rootstocks, revealing a significant interaction between etiolation time and rootstock variety, particularly impacting leaf chlorophyll content. Rootstock variety independently influenced stem length, diameter, leaf number, area, and chlorophyll. Etiolation time they significantly affected only the leaf chlorophyll content. Table 2 details the individual effects of etiolation time and rootstock varieties. Further insights into their interaction are presented in Table 3, illuminating key factors influencing avocado clonal rootstock growth.

Table 1. Summary of analysis of variance of the two factors and their interactions on growth parameters of avocado clonal rootstocks.
Tabela 1. Resumo da análise de variância dos dois fatores e suas interações nos parâmetros de crescimento de porta-enxertos clonais de abacateiro.

Factors	Stem Length (cm)	Stem Diameter (cm)	Number of Leaves	Leaf Area (cm^2)	Leaf Chlorophyll (SPAD)
Etiolation time	ns	ns	ns	ns	**
Variety	**	**	*	**	*
Etiolation Time x variety	ns	ns	ns	ns	*

** - highly significant, * - significant, ns – not significant (Tukey's HSD test of significance)

3.1. Etiolated Stem Length (cm)

The findings indicate that no statistically significant interaction effect was observed between etiolation time (main plot) and variety (sub-plot), nor was there an independent effect of etiolation time on the stem length of various avocado microclonal rootstocks. However, a notable independent effect was detected for variety, which significantly influenced stem length (cm) across different avocado clonal rootstocks, with a significance level of 1% according to the Honestly Significant Difference (HSD) test.

Remarkably, the 'Hass' variety exhibited the longest stem length, measuring 9.97 cm, significantly greater than the stem length of the 'Malagkit' variety, recorded at 6.90 cm. Notably, the 'Evergreen' stem length, at 7.78 cm, demonstrated comparability with both 'Hass' and 'Malagkit' varieties. As delineated in Table 2 and illustrated in Figure 3, these findings underscore the varietal influence on stem elongation in avocado clonal rootstocks, with 'Hass' emerging as particularly noteworthy.

3.2. Stem Diameter (cm)

The analysis revealed the absence of a significant interaction effect between etiolation time and variety and an independent effect of etiolation time on the stem diameter of diverse avocado clonal rootstocks. However, a prominent effect was observed concerning varieties (subplot), which exhibited a notably significant impact, reaching a level of significance at 1% based on the Honestly Significant Difference (HSD) test.

Specifically, the varieties 'Hass' and 'Evergreen' demonstrated significantly larger stem diameters, measuring 0.3880 cm and 0.3845 cm, respectively, compared to the 'Malagkit' variety, which recorded a smaller diameter of 0.3268 cm. These findings underscore the substantial influence of varietal characteristics on stem diameter in avocado clonal rootstocks, with 'Hass' and 'Evergreen' varieties emerging as noteworthy contributors to greater stem diameter dimensions.

Table 2. Response of different avocado microclonal rootstock varieties to etiolation time.
Tabela 2. Resposta de diferentes variedades de porta-enxertos microclonais de abacate ao tempo de estiolamento.

Treatments	Stem Length (cm)	Stem Diameter (cm)	Number of Leaves	Leaf Area (cm^2)
Individual mean comparison of etiolation time (weeks)				
0 week	7.01	0.379	9.01	45.60
2 weeks	8.03	0.361	7.68	49.06
4 weeks	8.66	0.375	7.34	48.68
6 weeks	9.18	0.351	6.91	51.33
HSD (0.05*)	2.14^{ns}	0.47^{ns}	4.27^{ns}	1.38^{ns}
Individual mean comparison of clonal stock variety				
'Malagkit'	6.90b	0.3268b	7.02b	42.00b
'Evergreen'	7.78ab	0.3845a	7.82ab	53.54a
'Hass'	9.97a	0.3880a	8.37a	50.46a
HSD (0.01**, 0.05*)	6.65**	6.89**	4.15*	26.86**

3.3. Number of Leaves

The analysis unveiled the absence of a significant interaction effect between etiolation time and variety and an independent effect of etiolation time on the number of leaves among distinct avocado microclonal rootstocks. Remarkably, it was discerned that solely the variety exhibited a notable and statistically significant impact, with a significance level set at 5% based on the Honestly Significant Difference (HSD) test.

Specifically, the 'Hass' variety demonstrated the highest number of leaves, averaging at 8.37, while the 'Malagkit' variety exhibited the lowest count, recording an average of 7.02 leaves. Conversely, the 'Evergreen' variety yielded an intermediate leaf count of 7.82, comparable to the 'Malagkit' and 'Hass' varieties. These findings underscore the pronounced influence of varietal attributes on leaf production in avocado microclonal rootstocks, with 'Hass' variety notably outperforming others in leaf abundance (Figures 3 and 4).

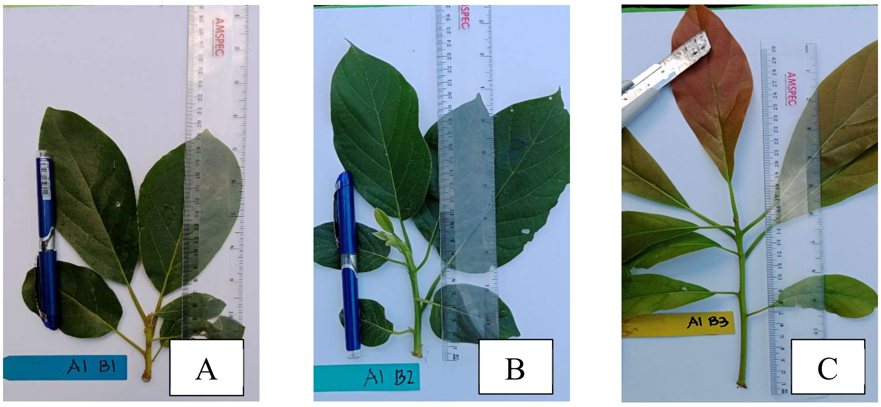

Figure 3. The different varieties of avocado (A – Malagkit; B – Evergreen; C – Hass) clonal rootstocks. Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. 2024.
Figura 3. As diferentes variedades de porta-enxertos clonais de abacateiro (A – Malagkit; B – Evergreen; C – Hass). Universidade Central de Mindanao, Musuan, Maramag, Bukidnon, Filipinas. 2024.

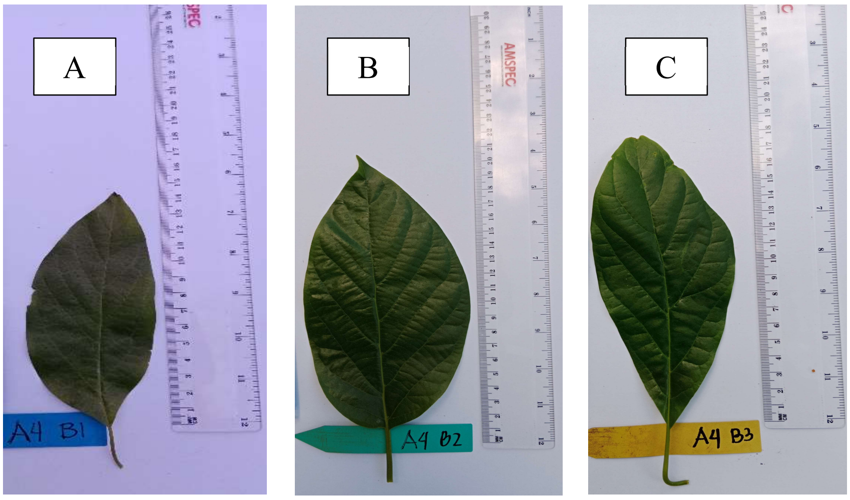

Figure 4. Leaves of different varieties of avocado (A – Malagkit; B – Evergreen; C – Hass) clonal rootstocks etiolated for six (6) weeks. Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. 2024.
Figura 4. Folhas de diferentes variedades de abacateiro (A – Malagkit; B – Evergreen; C – Hass) porta-enxertos clonais estiolados por seis (6) semanas. Universidade Central de Mindanao, Musuan, Maramag, Bukidnon, Filipinas. 2024.

3.4 Leaf Area (cm^2)

The analysis revealed that only the variety (subplot) exerted a statistically significant and independent effect, with a significance level of 5% according to the Honestly Significant Difference (HSD) test, on the leaf area (cm^2) of avocado clonal rootstocks. Notably, 'Evergreen' and 'Hass' varieties exhibited larger leaf areas, measuring 53.54 cm^2 and 50.46 cm^2, respectively. In contrast, the 'Malagkit' variety displayed a smaller leaf area of 42 cm^2, underscoring its comparatively reduced leaf size compared to the varieties above. Conversely, no significant interaction effect between etiolation time and variety, nor an independent effect of etiolation time, was observed on avocado clonal rootstocks. These results indicate that variations in leaf area among avocado varieties are primarily influenced by varietal characteristics rather than temporal factors such as etiolation time. These findings, as depicted in Table 2 and Figure 4, elucidate the differential responses of avocado clonal rootstocks to varietal attributes, particularly regarding leaf area, thereby contributing to a deeper understanding of plant morphology and growth dynamics in avocado cultivation.

3.5 Leaf Chlorophyll Content (SPAD Value)

The results in Table 3 indicated a significant interaction effect (etiolation time x variety) on this parameter. In the comparison of etiolation time at each level of varieties, six weeks and four weeks of etiolating the 'Malagkit' variety resulted in a higher amount of leaf chlorophyll (41.54 and 40.10 SPAD values, respectively) as compared to two weeks (31.02 SPAD value) and control (29.45 SPAD value) of etiolation time. Also, six and four weeks of etiolating the 'Evergreen' variety garnered the highest amount of chlorophyll (38.70 and 38.43 SPAD values). These were higher amounts of chlorophyll than what control (27.08

SPAD value) and two weeks (26.29 SPAD value) of etiolation had produced. Furthermore, six weeks of etiolating 'Hass' variety resulted in 41.59 chlorophyll content (SPAD value). This was the highest compared to the leaf chlorophyll contents produced at four weeks, two weeks, and zero etiolation time (31.65, 31.39, and 25.09 SPAD values, respectively).

In the comparison of varieties at each level of etiolation time, it was revealed that the 'Malagkit' variety obtained the highest chlorophyll content of 29.45 SPAD value for the batch that did not receive any etiolation time. This value was comparable with the amount of chlorophyll produced by 'Evergreen' (27.08 SPAD value) and 'Hass' varieties (25.09 SPAD value). Moreover, when different clonal rootstocks were subjected to a 2-week etiolation period, the 'Hass' variety garnered the highest leaf chlorophyll content of 31.39 SPAD value. This, however, was comparable with the 'Malagkit' (31.02 SPAD value) and the 'Evergreen' (26.29 SPAD value) varieties. Furthermore, when the same avocado varieties were etiolated for four weeks, it was observed that the 'Malagkit' variety contained the highest amount of chlorophyll in its leaves (40.10 SPAD value), which was also comparable with the 'Evergreen' variety (38.43 SPAD value). The two varieties were far superior in their leaf chlorophyll content compared to the 'Hass' variety (31.65 SPAD value). Finally, all three varieties of clonal rootstocks obtained comparable results of leaf chlorophyll content when subjected to six weeks of etiolation. The results showed that the 'Hass' variety had 41.59, followed by the 'Malagkit' variety with 41.54, and the 'Evergreen' variety with 38.70 leaf chlorophyll content (SPAD value).

3.6. Leaf Color

The MunCell Digital Color Chart analysis yielded insightful findings regarding leaf coloration across avocado varieties. Notably, the mean leaf color of 'Evergreen' (4.82) and 'Malagkit' (4.53) avocados appeared darker in comparison to 'Hass' avocado (3.65). Furthermore, a discernible trend was observed in Table 4, indicating that as the etiolation time progressed, there was a gradual darkening in the mean leaf color of avocado leaves: 0 weeks (3.20), 2 weeks (4.53), 4 weeks (4.60), and 6 weeks (5.00).

These observations underscore the dynamic nature of leaf coloration influenced by both varietal characteristics and temporal factors such as etiolation time. The darker hues observed in 'Evergreen' and 'Malagkit' avocados suggest potential varietal differences in pigment composition or density, contributing to variations in leaf color. Additionally, the progressive darkening of leaf color with increasing etiolation time reflects the physiological responses of avocado leaves to reduced light exposure, a phenomenon commonly associated with enhanced chlorophyll accumulation or alterations in pigment synthesis pathways. These findings offer valuable insights into the intricate interplay between genetic factors and environmental stimuli in shaping leaf coloration dynamics in avocado cultivation.

Table 3. Interaction effect of etiolation time and variety on the avocado clonal rootstocks.
Tabela 3. Efeito da interação do tempo de estiolamento e variedade nos porta-enxertos clonais de abacateiro.

Etiolation Time	Variety	Stem Length (cm)	Stem Diameter (cm)	Number of Leaves	Leaf Area (cm^2)	Leaf Chlorophyll (SPAD)
0 week	'Malagkit'	5.92	0.337	8.54	40.35	29.45^{b,A}
	'Evergreen'	7.50	0.438	9.50	51.53	27.08^{b,A}
	'Hass'	7.61	0.363	9.00	44.92	25.09$^{b, A}$
2 weeks	'Malagkit'	6.71	0.319	6.72	40.05	31.02^{b,A}
	'Evergreen'	7.21	0.364	7.67	54.77	26.29^{b,A}
	'Hass'	10.18	0.400	8.66	52.35	31.39^{b,A}
Four weeks	'Malagkit'	6.01	0.334	6.30	40.30	40.10^{a,A}
	'Evergreen'	9.16	0.387	7.52	54.20	38.43^{a,A}
	'Hass'	10.80	0.403	8.20	51.53	31.65^{b,B}
Six weeks	'Malagkit'	8.97	0.318	6.53	47.31	41.54^{a,A}
	'Evergreen'	7.25	0.349	6.60	53.64	38.70^{a,A}
	'Hass'	11.31	0.385	7.61	53.05	41.59^{a,A}
c.v. (a)%		23.25	15.66	17.03	12.35	12.66
c.v. (b)%		25.84	12.37	14.89	8.20	8.51

Note: for leaf chlorophyll, small letters indicate statistically significant differences among etiolation time (main plot) at each level of varieties (subplot), while capital letters indicate statistically significant differences among varieties (subplot) at each level of etiolation time (main plot)

Table 4. Response of different avocado microclonal rootstock varieties to etiolation time (leaf and stem colors).
Tabela 4. Resposta de diferentes variedades de porta-enxertos microclonais de abacate ao tempo de estiolamento (cores das folhas e caules).

B (Variety)	A (Etiolation Time)				Mean
	0 week	2 weeks	4 weeks	6 weeks	
Leaf Color					
'Malagkit'	3.00	5.13	5.00	5.00	4.53
'Evergreen'	4.60	4.73	4.93	5.00	4.82
'Hass'	2.00	3.73	3.87	5.00	3.65
Mean	3.20	4.53	4.60	5.00	
Stem Color					
'Malagkit'	5.00	5.00	5.00	4.67	4.92
'Evergreen'	5.00	5.00	5.00	5.00	5.00
'Hass'	5.00	4.67	5.00	5.00	4.92
Mean	5.00	4.89	5.00	4.89	

Scale	Description
1	5 YR (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12 (Chroma) to 7.5 YR (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12 (Chroma)

2	10 YR (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12 (Chroma) to 2.5 Y (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12 (Chroma)
3	5 Y (Hue); N1-N9 (Value); 1,2,3,4,6,8,10 (Chroma) to 7.5 Y (Hue); N1-N9 (Value); 1,2,3,4,6,8,10 (Chroma)
4	10 Y (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12 (Chroma) to 2.5 GY (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12 (Chroma)
5	5 GY (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12 (Chroma) to 7.5 GY (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12,14 (Chroma)
6	10 GY (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12,14,16,18 (Chroma) to 2.5 G to (Hue); N1-N9 (Value); 1,2,3,4,6,8,10,12 (Chroma)

3.7. Stem Color

Utilizing the digital MunCell Chart for comparison, it was observed that the stem color among different avocado varieties exhibited no significant distinctions. The mean stem color measurements for 'Malagkit,' 'Evergreen,' and 'Hass' varieties were consistent, with values of 4.92, 5.00, and 4.92, respectively. Furthermore, an analysis of stem color variation over different etiolation periods indicated minimal changes, with mean stem color measurements at zero, two, four, and six weeks recorded as 5.00, 4.89, 5.00, and 4.89, respectively.

These findings suggest uniformity in stem coloration across avocado varieties, reflecting inherent genetic traits or developmental patterns contributing to consistent pigmentation. Additionally, the stability of stem color over varying etiolation periods underscores its resilience to temporal environmental influences, highlighting the robustness of this morphological characteristic. This comprehensive assessment of stem color dynamics enhances our understanding of avocado plant morphology and provides valuable insights for cultivar selection and management practices in avocado cultivation.

4. DISCUSSION

The findings regarding etiolated stem length (cm) underscored the superior performance of the Hass variety as a clonal rootstock, exhibiting the longest etiolated stems suitable for microcloning techniques in avocado propagation. However, it is noteworthy that irrespective of etiolation time, the length of avocado stems did not exhibit significant variation among different varieties. Furthermore, the observed range of stem length across various etiolation periods (weeks) spanned from 7.01 to 9.18 centimeters. Notably, specimens exposed to direct sunlight tended to manifest relatively shorter stem lengths than those maintained within etiolation boxes over several weeks. These results align with existing literature, such as those of Ndagire (2021), Arpaia; Smith (2009), and Roe et al. (1999), who have extensively documented the superior performance of Hass avocado scions in nurseries characterized by enhanced bud sprouting, increased height, and overall superior horticultural outcomes. Furthermore, corroborating studies by Tang et al. (2022), Kocurek et al. (2020), and Corre (1983) have elucidated that plants subjected to low light conditions, shading, or etiolation environments often exhibit heightened vertical growth and elongated stems. This convergence of findings underscores the robustness and consistency of the observed trends and contributes to a deeper understanding of avocado cultivation practices and managing varietal traits in horticultural settings.

Based on the comprehensive analysis of stem diameter (cm) data, it can be inferred that irrespective of etiolation time, the 'Hass' and 'Evergreen' varieties consistently exhibited thicker stem diameters than the 'Malagkit' varieties. The range of stem diameter across etiolation periods ranged from 0.351 cm to 0.379 cm. Notably, observations indicated that specimens subjected to etiolation typically displayed thinner stems than those not. This trend underscores the significant influence of environmental factors, such as light exposure intensity, duration, and quality, on stem morphology in avocado crops. Furthermore, existing literature supports these findings, as documented by Tamu (2023), Grant (2021), Shafiq et al. (2021), Chaffey (2014), and Maynard; Bassuk (1996). Their research elucidates that crops exposed to sunlight typically exhibit larger stem diameters, reflecting the positive impact of optimal light conditions on plant growth and development. Conversely, plants subjected to etiolation or grown under shaded or dark conditions tend to manifest thinner stems, highlighting the crucial role of light availability in shaping stem morphology. These findings contribute to a deeper understanding of the intricate interplay between environmental stimuli and plant physiological responses in avocado cultivation, informing strategic approaches for optimizing crop productivity and quality.

Furthermore, the findings suggest that 'Hass' emerged as the most prolific producer of leaves among the three varieties examined. Notably, the range of leaf numbers across etiolation periods spanned from 6.91 to 9.01, with specimens directly exposed to sunlight immediately after bud growth demonstrating the highest leaf counts. In contrast, those subjected to six weeks of etiolation exhibited the lowest leaf numbers. These results underscore the pivotal role of sunlight exposure in leaf production, as documented by Pierson et al. (1990) and Evans (1973), who reported that plants exposed to sunlight typically exhibit greater leaf abundance, whereas those grown under shade or subjected to etiolation tend to display fewer leaves, as observed in studies by Cookson; Granier (2006) and Cookson et al. (2006). Moreover, in alignment with these findings, Liu et al. (1999) noted that Hass avocado plants can produce 9-11 leaves approximately 25 days after the emergence of the first leaf, a range consistent with the leaf production observed in this study. This convergence of findings highlights the robustness and reliability of the observed trends across diverse studies, reaffirming the significant impact of environmental factors on leaf proliferation in avocado cultivation.

Moreover, the findings regarding leaf area (cm^2) imply that irrespective of leaf morphology, the 'Evergreen' and 'Hass' cultivars exhibited greater leaf area, suggesting a potential superiority in foliar biomass accumulation. Additionally, when evaluating leaf area across different etiolation durations, it was observed that the range spanned from 45.60 cm^2 to 51.33 cm^2. Notably, specimens subjected to etiolation demonstrated larger leaf areas than their non-etiolated counterparts. The dimensions of Hass avocado leaves typically fall within the range of 4-10 cm in width x 10-30 cm in length, corresponding to a leaf area of 40 cm^2 – 300 cm^2 (BRITANNICA, 2024; SPECIALTY PRODUCE, 2022). This aligns with similar avocado varieties, characterized by leaf dimensions ranging from 6-30 cm in length and 3.5-19 cm in width (equating to 21-570 cm^2 in leaf area) (ABRAHAM et al., 2018; YOKOHAMA et al., 1991; CHIA et al., 1988).

Furthermore, the present findings corroborate with previous research by Science And Plants For Schools (2022), Gibson et al. (2001), and Lichtenthaler (1985), which emphasized that shaded or etiolated plants tend to exhibit broader and larger leaves compared to those receiving direct sunlight exposure. This convergence of results underscores the influence of environmental factors, such as light availability, on leaf morphology and area in avocado plants.

As indicated by SPAD values, the data about leaf chlorophyll content elucidate a complex interaction between the duration of etiolation and the specific cultivars of clonal rootstocks, yielding significant implications for chlorophyll accumulation. Notably, the 'Malagkit' and 'Evergreen' avocado varieties exhibited optimal chlorophyll levels following 4-6 weeks of etiolation, while the 'Hass' cultivar demonstrated peak chlorophyll content after a six-week etiolation period. These findings align with the assertions put forth by Ernst (1999), who advocated for a minimum etiolation period of four weeks to achieve optimal chlorophyll synthesis in avocado clonal rootstocks. Conversely, conflicting perspectives are evident in the work of Bender et al. (2002), suggesting that the etiolation duration for clonal rootstocks could be abbreviated to a mere 10 days. This discrepancy underscores the need for further investigation into etiolation duration's nuanced effects on avocado cultivars' chlorophyll dynamics, thereby contributing to a more comprehensive understanding of optimal cultivation practices in avocado production.

The findings of the current investigation provide valuable insights into the foliar characteristics of different avocado cultivars. Specifically, the 'Evergreen' and 'Malagkit' varieties are distinguished by their deep, verdant foliage, exhibiting a robust dark green hue. In contrast, the 'Hass' cultivar presents a striking contrast with its light green leaves, often accompanied by subtle reddish undertones. This variation in leaf pigmentation, characterized by red, yellow, or copper-like hues, may be attributed to several environmental factors, including photoinhibition, nocturnal cold exposure, or fluctuations in carbon fixation during vegetative flushing phases, as elucidated by Mandemaker (2007). Such chromatic nuances are frequently observed during the early developmental stages of clonal rootstocks as they undergo vigorous growth, extending both stems and foliage. Conversely, in contrast to the discernible differences in leaf coloration among varieties, the coloration of stems remains largely consistent across all cultivars and etiolation durations. Irrespective of variety or etiolation regimen, the stems consistently exhibit a light green hue, falling within the spectrum delineated by the digital MunCell Chart. This uniformity in stem pigmentation is indicative of the health and vigor of young avocado clonal rootstocks, signifying their ability to efficiently transport essential substances between roots and leaves, as highlighted by Berry et al. (2022) and Valverdi et al. (2022). Such visual cues provide valuable insights into avocado plants' physiological status and practical indicators of their overall health and vigor within agricultural contexts.

5. CONCLUSIONS

Based on the results presented, several conclusions can be drawn regarding the growth parameters of avocado clonal rootstocks under different conditions: The variety of avocado clonal rootstocks significantly influenced various growth parameters, including stem length, stem diameter, number of leaves, leaf area, and leaf chlorophyll content. The 'Hass' variety generally exhibited superior growth characteristics compared to the 'Malagkit' variety, with the 'Evergreen' variety also performing well in certain aspects; etiolation time significantly affected the leaf chlorophyll content but not other parameters studied. Longer etiolation periods generally resulted in increased leaf chlorophyll content, particularly in the 'Malagkit' and 'Evergreen' varieties; and a significant interaction between etiolation time and rootstock variety was found in the leaf chlorophyll content, indicating that the effect of etiolation time on chlorophyll content varied depending on the rootstock variety. These findings suggest that the 'Hass' variety may be the most suitable for use as a clonal rootstock due to its superior growth characteristics. However, further studies are needed to explore these differences' underlying mechanisms and optimize growth conditions for different avocado varieties.

One notable limitation of this study is its focus on avocado clonal rootstocks, which may constrain the generalizability of the results to other plant species. The study primarily assessed growth parameters such as stem length, stem diameter, number of leaves, leaf area, and leaf chlorophyll content while overlooking potential confounding variables such as soil composition, water availability, and temperature, which could significantly impact the growth of avocado clonal rootstocks. Furthermore, the study did not explore the long-term effects of etiolation on avocado clonal rootstocks, thereby limiting the understanding of the sustainability and broader implications of this microcloning technique.

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