

Growth vitamin hormone and shading intensity affect the rhizo-caulogenesis of etiolated avocado microclonal rootstocks

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Abstract. *Esgrina FJO, Tan RJ. 2025. Growth vitamin hormone and shading intensity affect the rhizo-caulogenesis of etiolated avocado microclonal rootstocks. Asian J Agric 9: 160-173.* The propagation of avocado clonal rootstocks is essential for a resilient, productive, and quality commercial cultivation. Optimizing growth conditions, particularly shading intensity and vitamin hormone concentration, plays a critical role in enhancing rhizogenesis, caulogenesis, and other physiological traits. Despite substantial research, the combined effects of these factors remain insufficiently explored. Thus, this study investigated the effects of shading intensity (40, 60, and 80%) and vitamin hormone concentrations (0, 0.1, 0.2, and 0.3 mL/L of water) on key morphological and physiological parameters of etiolated avocado clonal rootstocks, using a split-plot arrangement in randomized complete block design. The results revealed significant interaction effects (1%, Tukeys' HSD) between shading intensity and hormone concentration in all parameters. The highest root number (1.33), root length (0.53 cm), chlorophyll content (61.10 SPAD), fresh weight (25.53 g), dry weight (10.33 g), were observed under 80% shading with 0.2-0.3 mL of vitamin growth hormone/L of water. Moderate shading (60%) combined with 0.1-0.2 mL of vitamin growth hormone promoted stem elongation (25.81 cm), and leaf area (100.99 cm²). These findings underscore the necessity of tailoring propagation protocols to specific environmental and hormonal conditions. The study provides comprehensive insights into optimizing avocado rootstock propagation, contributing to sustainable agricultural practices and supporting the growing global demand for avocados.

Keywords: Avocado clonal rootstocks, rhizogenesis and caulogenesis, rootstock propagation techniques, shading intensity, vitamin hormone concentration

INTRODUCTION

Avocado (*Persea americana* Mill.), known for its exceptional nutritional benefits and economic potential, has become increasingly popular across the globe in recent years (Bhore et al. 2021; Bangar et al. 2022; Sora 2023; Subba et al. 2023). This popularity stems from the fruit's high content of healthy fats, vitamins, and antioxidants, which contribute to its growing demand in both the food industry and health sectors. Despite the widespread recognition of avocado's value, the commercial success of its cultivation heavily relies on the effective management of its propagation, especially the use of clonal rootstocks. These rootstocks play a critical role in improving avocado's resilience to environmental stresses, enhancing fruit quality, and ensuring consistent yield. Clonal rootstocks are integral to ensuring consistent yields and reducing the impact of environmental challenges. Thus, understanding the factors that influence the propagation of these rootstocks is essential for maximizing production efficiency and ensuring the sustainability of the avocado industry.

Rootstock propagation, especially when using clonal methods, is influenced by several important factors, including shading intensity and the use of growth hormones. Shading intensity plays a big role in how well a plant can photosynthesize, which directly affects its growth and overall health. If there's too much or too little shade,

the plant struggles to get the right amount of light for photosynthesis, slowing down growth and weakening its roots. On the other hand, growth hormones like auxins and cytokinins help regulate key processes like root development and cell division, making it easier for the plant to grow strong and healthy. By carefully managing both shading and hormone treatments, growers can optimize the propagation process and produce rootstocks that are more resilient and productive (Blakey et al. 2015; Alon et al. 2022; Lahak et al. 2024). Meanwhile, the application of specific growth hormones regulates processes which are vital for successful propagation (Hiti-Bandaralage et al. 2017; Khawas and Upadhyay 2022; Shindre et al. 2023). Many studies - previous and current - have highlighted how these factors influence critical physiological processes, such as photosynthesis (Yang and Li 2017; Müller and Munné-Bosch 2021; Wang et al. 2021; Elango et al. 2023), root development (Miotto et al. 2021; Yoon et al. 2021; Zhou et al. 2022), and auxin distribution (Iglesias et al. 2018; Yang et al. 2018; Xie et al. 2022), which are essential for robust plant growth.

Although there has been considerable research on the asexual propagation of avocado (Barceló-Muñoz and Pliego-Alfaro 2003; Kasana et al. 2024; Li et al. 2024; Williams et al. 2024), the combined effects of shading intensity and hormone concentration on rootstock development remain poorly understood. Despite progress

in understanding how individual factors like light and hormones affect plant growth, there is still a gap in knowledge regarding how these variables interact to influence the development of avocado rootstocks. Existing studies have largely focused on these variables in isolation, failing to investigate their potential synergistic interactions (Lovatt and Salazar-García 2005; Tinyane et al. 2018; Supriyanto and Yulianto 2022; Esgrina and Tan 2024; Ibtissem et al. 2024). This research gap has resulted in a lack of comprehensive guidelines for optimizing avocado rootstock propagation protocols, which could otherwise enhance efficiency and success rates in commercial production. Therefore, there is a pressing need to explore how these factors interact, as their combined influence could potentially offer new insights into better propagation practices.

To address this knowledge gap, this study explored shading and vitamin hormone interaction effect on the rhizogenesis (root formation) and caulogenesis (stem development) of etiolated avocado clonal rootstocks. By systematically varying shading and hormone treatments, it seeks to identify the most effective combinations that promote root and shoot development.

The potential impact of this research extends beyond theoretical understanding. By advancing propagation techniques for avocado clonal rootstocks, the findings could contribute to sustainable agricultural practices, enhancing the global supply of high-quality avocados. Moreover, by bridging the current knowledge gap regarding the combined effects of shading intensity and hormone application, this study could offer valuable insights to growers and researchers, enabling them to develop more efficient propagation protocols. Ultimately, the study's outcomes would not only improve the economic viability of avocado cultivation but also contribute to its ecological sustainability, ensuring that future avocado production meets the growing global demand.

MATERIALS AND METHODS

Study area

The study on the interaction of shading intensity and growth vitamin hormone on the shoot and root development of etiolated avocado microclonal rootstocks was conducted at the Horticulture Nursery, College of Agriculture, Central Mindanao University (7.8592°N, 125.0515°E, 311 masl) (googlemap.com 2024) on March to October 2024.

The local climatic data (Figure 1) from Department of Science and Technology Philippine Atmospheric, Geophysical, and Astronomical Administration (DOST-PAGASA) (2024), show a general trend of warm temperatures and fluctuating humidity levels. Minimum temperatures range from 21.93°C in July to 23.17°C in May, while maximum temperatures peaked at 34.98°C in May and dropped to 30.84°C in September. Humidity levels are relatively high, particularly in July (75.6%) and September (75.9%), with June, August, and October also showing values above 70%. Overall, the data indicate a

consistent warm climate with higher humidity towards the mid-year months, particularly during the rainy season, with a slight decrease in humidity during the hotter months of April and May (El Niño phenomenon).

Avocado growth thrives within temperature of 20-30°C but the recorded maximum temperatures and lower humidity (<70%) suggest conditions slightly above or below the optimal. Hence, the researchers aligned their propagation practices with these environmental factors. There was, in fact, an El Niño phenomenon during this time. Thus, irrigation and misting system were implemented during dryer months to maintain a humid microclimate in an outdoor nursery setting which would be conducive for the clonal rootstocks. The provision of shading nets also helped maintain moderate temperature in the area and reduced the occurrence of plant stress.

Materials and equipment

In this study, the researchers used a range of materials and tools to support the propagation and study process. They employed 'Hass' avocado clonal rootstock and 'Evergreen' nurse seedlings for propagation, provided net shading to the clonal stocks, and applied Hormex® growth vitamin hormone as an exogenous auxin to promote root and shoot development. In an outdoor nursery setup, the soil mix of cocopeat, garden soil, decayed rice hull, and vermicompost was used for planting. They also utilized water sprinklers, misting system, polyethylene bags (8" x 10"), and various working tools like bolos, spades, scissors, and cutters.

For measurement and data collection, they used rulers to take length, SPAD meter (Want® Brand) to measure the amount of chlorophyll, Vernier caliper for taking the diameter, and mobile applications like Easy Leaf® and MunCell® to assess leaf area and leaf color. Statistical analysis was done using Statistical Tool for Agricultural Research (STAR) software ver. 2.0.1, a program developed by the International Rice Research Institute (IRRI), Black Geena pongee cloth was used to control light exposure during etiolation of clonal rootstocks in the boxes. These materials and tools were crucial for assessing the impact of growth conditions on the development of Hass avocado rootstocks.

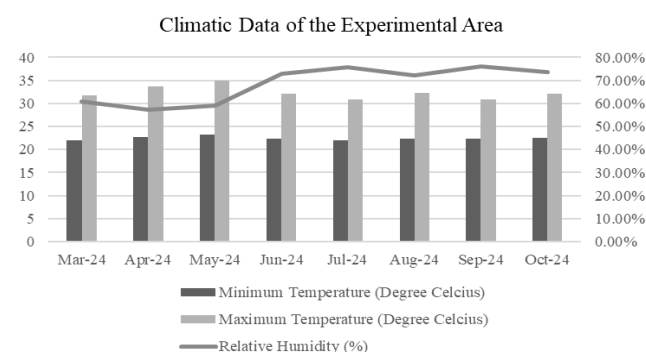


Figure 1. Local climatic data in Central Mindanao University, Bukidnon, Philippines from March to October 2024. Source: DOST-PAGASA, CMU Sub-Station

The researchers utilized an existing outdoor nursery shed as the experimental area, performed necessary repairs and modifications to meet the study's specific requirements and ensure its suitability. They repaired structural damage, improved ventilation, optimized lighting, installed misting systems, and enhanced the drainage system.

'Evergreen' avocado seeds, sourced from local farmers in Barangay Kulaman Valley, Arakan, Cotabato, Philippines, were used as seedling nurses. This approach ensured the use of genetically adapted material that is typically well-suited to the area's environmental conditions. Additionally, clonal rootstocks of the 'Hass' variety were acquired from a private grower in Makilala, Cotabato, Philippines, likely chosen to ensure high-quality rootstock from a trusted supplier.

Experimental design and treatments

The experiment was carried out using a Split Plot arrangement within a Randomized Complete Block Design (RCBD), where the intensity of shading served as the main plot and the concentrations of vitamin hormone were assigned as subplots. Three main plots and four subplots were replicated three times, with each treatment consisting of seven microclones-five of which were used as sample plants and two as buffers. In total, 252 'Evergreen' nurse seedlings and 252 'Hass' clonal rootstocks were used in the study. The researchers used the STAR ver. 2.0.1 to carry out the randomization and field layout of the study.

The treatments applied were as follows: for the main plot, the intensity of net shading included 40% (A1), 60% (A2), and 80% (A3) net shading, while the subplot involved varying concentrations of Hormex growth vitamin hormone: control (B1), 0.5 recommended rate (0.1 mL of vitamin hormone per liter of water, B2), recommended rate (0.2 mL of vitamin hormone per liter of water, B3), and 1.5 recommended rate (0.3 mL of vitamin hormone per liter of water, B4).

Pre-germinating and growing of 'Evergreen' seedling nurse

The activity began with pre-germinating 'Evergreen' avocado seeds in beds of 100% cocopeat (Figure 2.A), chosen for its moisture retention and aeration properties. Once germinated, the seeds were transplanted into individual polyethylene bags (8" × 10") to provide space for root growth and uniform development. The growing medium consisted of 30% garden soil, 30% vermicompost, 30% soil compost, and 10% decomposed rice hull, balancing nutrients, water retention, and drainage. Organic materials enriched the soil, while rice hulls improved aeration. The seedlings were nurtured until their stems reached at least 6 mm in diameter, signaling readiness for grafting, with careful attention to optimal conditions for both stem and root growth.

Grafting of clonal rootstocks

Once the 'Evergreen' stock seedlings reached the desired 6 mm diameter, or "pencil size," they were ready for grafting (Figure 2.B). This occurred about eight weeks after planting the 'Evergreen' seeds in polyethylene bags. The 'Hass' clonal stocks were then grafted onto the

'Evergreen' seedlings, with the aim of establishing a strong connection between the scion and rootstock. By grafting at the optimal growth stage of the stock seedlings, the researchers maximized the chances of successful grafting and healthy plant development.

Etiolating grafted clonal rootstocks

Etiolation boxes were especially designed to create a controlled environment for the experimental plants, using wooden frames for stability and covering them with black Geena pongee cloth (Figure 2.C). This cloth served to block light, inducing etiolation. This setup allowed researchers to observe plant responses to reduced light exposure and monitor the morphological and physiological changes associated with etiolation. After bud break in the clonal rootstocks, the microclones were transferred into the etiolation boxes that provided sufficient ventilation and space for each treatment. The plants were kept in darkness at ambient temperatures, with shoots allowed to grow for 4 weeks post-bud break, representing different stages of shoot development under limited light conditions.

Selecting the most desirable etiolated clonal rootstock

200 to 300 mm (20 to 30 cm) long clonal rootstocks were selected for the rooting experiment (Figure 2.D), as this length is considered optimal for successful rooting. According to the studies by Ernst (1999) and Esgrina and Tan (2024), the ideal length for clonal rootstocks, which allows for better root initiation, the rootstocks were subjected to a period of 2 to 6 weeks of etiolation. By this process, rootstocks were placed in the etiolation boxes covered with Geena pongee cloth to allow low light promote elongation. It helped the clonal rootstocks reach the desired size and characteristics that enhance their ability to form roots once exposed to the different shading intensities and vitamin growth hormone concentrations.

Applying Hormex® growth vitamin hormone

Different concentrations of the growth vitamin hormone were applied to the etiolated 'Hass' clonal stocks through an incision made at the base of the etiolated stem (Figure 2.E). The concentrations tested included 0, 0.1, 0.2, and 0.3 mL/L of water. The vitamin hormone formulation consisted of Thiamine Hydrochloride (Vitamin B1) at 25%, Naphthyl Acetic Acid (NAA) at 24%, and Indole Butyric Acid (IBA) at 13%.

Fixing the rooting devices at the base of the etiolated shoot

Spherical rooting devices, each measuring 5 centimeters in diameter, were filled with sterile 100% cocopeat as the rooting media to ensure a clean and controlled environment for root development (Figure 2.F). To further support the growth of the clonal rootstocks, a pinch of slow-release fertilizer (Osmocote) was placed on the surface of the cocopeat within each rooting device. This fertilizer provided a steady supply of nutrients over time, promoting healthy root and shoot growth while reducing the need for frequent fertilization. The combination of cocopeat and Osmocote helped create an optimal rooting medium that

encouraged the successful establishment of the clonal rootstocks.

Placing the plants in shade

The plants, with their etiolated shoots and leaves protruding from the media-filled micro-containers, were exposed to varying light intensities of 40, 60, and 80% net shading (Figure 2.G). This controlled light exposure triggered photosynthesis, gradually strengthening and hardening off the plants as they adapted to increasing light conditions. As the plants acclimated, both root initiation and shoot elongation occurred simultaneously, with the roots beginning to form and the shoots continuing to grow and elongate. Sixty days after this, data were gathered by the researchers (Figure 2.H).

Monitoring the daily air temperature and relative humidity

The daily local climate data, including humidity and temperature (minimum and maximum), were obtained from the Department of Science and Technology, Philippine Atmospheric Geophysical and Astronomical Services Administration (DOST-PAGASA) station at Central Mindanao University, Musuan, Maramag, Bukidnon, Philippines. Additionally, an outdoor thermometer/ hygrometer was placed in the nursery area to monitor the daily temperature and relative humidity of the immediate environment. Readings were taken at 8:00 a.m. and 2:00 p.m. to record the minimum and maximum temperatures and humidity levels for each day. To mitigate high temperatures and maintain humidity above 70%, the plants were regularly sprinkled, and a misting system was used in the outdoor nursery shed.

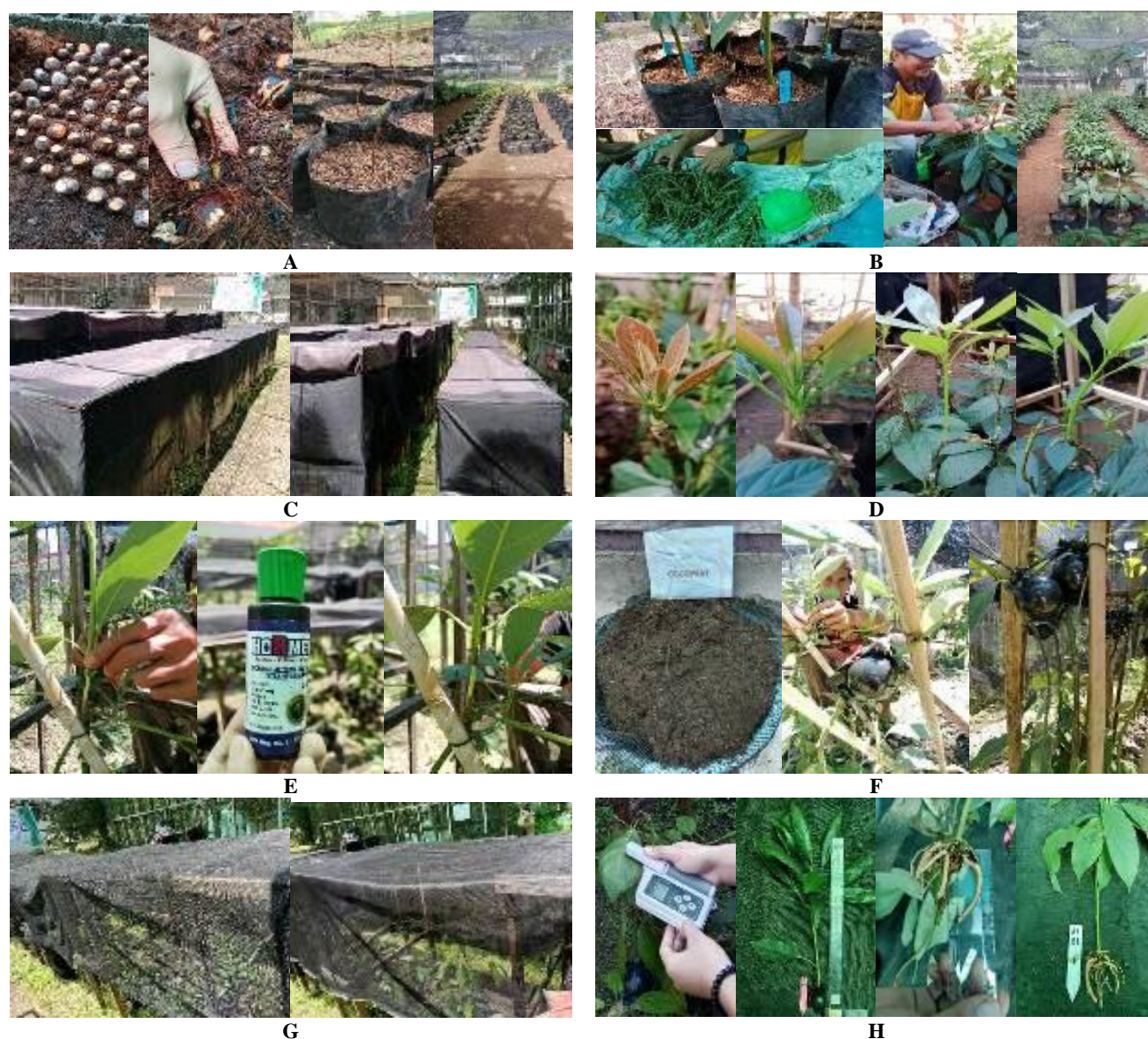


Figure 2. Graphical sketch of the study's activities: A. Pre-germinating and growing of seedling nurse; B. Grafting of clonal rootstocks; C. Etiolating grafted clonal rootstocks; D. Selecting the most desirable etiolated clonal rootstocks; E. Applying growth vitamin hormone; F. Fixing the rooting devices at the base of the etiolated shoots; G. Placing the plants in shade; and H. Gathering of data

Statistical analysis

After data collection (Figure 2.H), an Analysis of Variance (ANOVA) was conducted to assess the significance of the treatment differences. This statistical analysis was performed using the Statistical Tool for Agricultural Research (STAR) version 2.0.1 software, which is specifically designed for advanced data analysis in agricultural research. ANOVA allowed the researchers to determine whether the observed variations in the measured parameters were statistically significant. Following ANOVA, a post hoc analysis was done using Tukey's Honestly Significant Difference (HSD) test for parameters that showed significant differences based on the F-value from the ANOVA. Tukey's HSD test enabled pairwise comparisons of treatment means, allowing researchers to pinpoint specific differences between treatment groups. This step was crucial for understanding the nature and extent of differences among treatments, providing more detailed insights into how experimental variables affected the outcomes. In summary, the use of ANOVA and

Tukey's HSD test provided a thorough statistical framework for analyzing and interpreting the results, helping researchers draw meaningful conclusions from the data.

RESULTS AND DISCUSSION

Table 1 displays the results of the Analysis of Variance (ANOVA) performed on avocado clonal rootstocks, highlighting the impact of shading intensity, hormone concentration, and their interaction on different root and shoot parameters, except for the stem diameter. Shading intensity independently influenced certain parameters, including root number, root length, and stem diameter. On the other hand, the concentration of growth hormone significantly impacted all the parameters listed in the table. Moreover, Table 2 shows that all parameters, except stem diameter, were significantly affected by the interaction effect of shading intensity and vitamin growth hormone.

Table 1. Summary of the analysis of variance for the effects of shading intensity, hormone concentration, and their interaction on root and shoot parameters of avocado clonal rootstocks

Factors	Root number	Root length (cm)	Stem length (cm)	Stem diameter (cm)	Leaf number	Leaf area (cm ²)	Fresh shoot weight (g)	Dry shoot weight (g)	Leaf chlorophyll content (SPAD)
Shading intensity	**	**	**	ns	ns	ns	ns	ns	ns
Hormone concentration	**	**	**	**	**	**	**	**	**
Shading intensity x hormone concentration	**	**	**	ns	**	**	**	**	**

Note: ** = highly significant; ns = not significant (Tukeys' HSD test of significance)

Table 2. Interaction effect of shading intensity and growth hormone concentration on the different parameters of avocado clonal rootstocks

Shading intensity (%)	Growth hormone concentration (mL/L of water)	Root number **	Root length (cm)**	Stem length (cm)**	Stem diameter (cm)ns	Leaf number*	Leaf area (cm ²)**	Fresh weight (g)**	Dry weight (g)**	Leaf chlorophyll content (SPAD)**
40%	Control (0)	0.40b,A	0.90a,A	25.85a,B	0.625	9.07b,AB	85.37b,B	21.47a,AB	8.27a,AB	46.85b,B
	0.1	0.00b,B	0.00b,B	32.07a,A	0.634	10.40a,A	83.59b,B	25.13a,A	10.13a,A	57.04a,A
	0.2	0.00a,B	0.00a,B	18.87b,C	0.623	7.07b,B	110.56a,A	17.27a,B	6.93ab,B	56.18b,A
	0.3	0.00b,B	0.00b,B	20.09a,C	0.626	9.53ab,A	102.78b,A	21.67ab,AB	8.73ab,AB	50.71b,B
60%	Control (0)	0.00c,B	0.00b,B	21.99b,B	0.601	9.53b,B	99.49a,A	20.27a,B	7.60a,AB	53.31a,A
	0.1	0.20 ^a ,A	0.72a,A	25.81b,B	0.678	9.53a,B	100.99a,A	25.47a,A	9.60a,A	56.65a,A
	0.2	0.00a,B	0.00a,B	30.99a,A	0.559	13.40a,A	74.30c,B	19.93a,B	8.93a,AB	47.04c,B
	0.3	0.00b,B	0.00b,B	23.02a,B	0.559	9.13b,B	99.73b,A	19.20b,B	7.47b,B	57.04a,A
80%	Control (0)	1.07a,B	0.887a,A	27.27a,A	0.575	12.80a,A	94.58ab,B	21.87a,A	8.07a,B	49.35ab,B
	0.1	0.00b,C	0.00b,C	27.90b,A	0.665	9.93a,B	75.99b,C	24.20a,A	10.33a,A	59.45a,A
	0.2	0.00a,C	0.00a,C	18.00b,B	0.551	5.13b,C	88.30b,B	12.80b,B	4.87b,C	61.10a,A
	0.3	1.33a,A	0.527a,B	21.53a,B	0.579	11.40a,B	145.12a,A	25.53a,A	10.33a,A	49.91b,B
c.v. (a)%		23.09	8.65	6.42	5.78	10.86	9.00	7.68	17.67	3.95
c.v. (b)%		31.74	13.21	6.98	6.19	10.86	5.28	9.59	10.71	3.81

Note: Lowercase letters represent statistically significant differences among shading intensities (main plot) at each growth hormone concentration level (subplot), while UPPERCASE LETTERS indicate statistically significant differences among growth hormone concentrations (subplot) at each shading intensity level (main plot)

Root number

The number of roots of different clonal rootstocks was measured after a 60-day rooting period, revealing a significant interaction effect (1%, HSD) between shading intensity and hormone concentration (Table 1). The comparison of shading (main plot) at each level of vitamin hormone (subplot) (Table 2) shows that under 80% shade without Hormex hormone, avocado clonal rootstocks produced the highest number of roots, averaging 1.07 roots. This result was significantly greater than the root production under 40% shade (0.40) and 60% shade (0.00) at the same hormone level. At the hormone concentration of 0.1 mL/L, clonal rootstocks under 60% shade yielded the highest root count at 0.20 root, which was greater compared to those exposed to 40% shade (0.00) and 80% shade (0.00). However, all clonal rootstocks subjected to any shading level and treated with 0.2 mL/L of rooting hormone failed to produce any roots. Interestingly, when 0.3 mL/L of rooting hormone were applied, rootstocks under 80% shade achieved the highest number of roots, averaging 1.33 root. This was significantly higher than the root counts under 60% shade (0.00) and 40% shade (0.00).

Moreover, in the detailed comparison of hormone concentration (subplot) at each level of shading intensity (main plot) (Table 2), it was found out that under the control treatment (no rooting hormone), clonal rootstocks placed under 80% shade developed the highest number of roots, with an average of 1.07 roots. This was greater than the root counts under 40% shade (0.40) and 60% shade (0.00). At 0.1 mL of rooting hormone/liter of water, the highest root production was observed under 60% shade (0.20). In contrast, no root was produced under 40% shade or 80% shade. When 0.2 mL of rooting hormone/liter of water was applied, none of the clonal rootstocks developed roots, regardless of shading intensity. Finally, the highest root production, averaging 1.33 roots, was achieved under 80% shade with 0.3 mL of rooting hormone/liter of water. No root was formed under 40 or 60% shade at this hormone level.

Root length (cm)

After a 60-day rooting period (Figure 3) a statistically significant interaction (1%, HSD) was observed in the interaction between shading intensity (main plot) and hormone concentration (subplot) on the root length (cm) of

etiolated avocado clonal rootstocks (Table 1). When comparing shading intensity (main plot) at each level of hormone concentration (subplot) (Table 2), it was found out that among the treatments without hormone application, avocado rootstocks grown under 40% shade exhibited the greatest mean root length at 0.90 cm. This was comparable to the root length under 80% shade (0.887 cm) but significantly longer than the roots under 60% shade (0.00 cm). At the 0.1 mL/L hormone concentration, rootstocks grown under 60% shade achieved the longest mean root length of 0.72 cm. In contrast, no root development was observed under either 40 or 80% shade. When treated with 0.2/L hormone, no root was observed under any shading intensity. Under the highest hormone concentration of 0.3 mL/L, rootstocks exposed to 80% shade produced the longest roots, with a mean length of 0.527 cm. However, rootstocks under both 40 and 60% shade failed to develop roots at this concentration.

The comparison of vitamin hormone concentration at each level of shading intensity on the length (cm) of roots of clonal rootstocks (Table 2) reveals the significant interaction (1%, HSD) between rooting hormone concentrations (subplot) and shading intensities (main plot) on the root length (cm) of avocado clonal rootstocks. The results showed that both environmental factors and hormone applications significantly influenced root elongation, with varying effects at different levels of shade and hormone concentration. Under 40% shade, the control group (without vitamin hormone) produced roots with the longest mean length of 0.90 cm, significantly surpassing all other treatments that received rooting hormone applications (0.1, 0.2, and 0.3 mL/L of water), as none of them produced roots. At 60% shade, only the rootstocks treated with 0.1 mL/L of rooting hormone developed roots, with an average length of 0.72 cm. In comparison, all other treatments, including the control group and those treated with higher hormone concentrations (0.2 and 0.3 mL/L), failed to produce roots. For rootstocks placed under 80% shade, the control group (no hormone) once again exhibited the greatest root length, averaging 0.887 cm. This was significantly longer than the roots produced under the same shade intensity with 0.3 mL/L rooting hormone (0.527 cm). Notably, no root was observed for rootstocks treated with 0.1 or 0.2 mL/L of rooting hormone under this shading condition (Figure 3).



Figure 3. Roots and calluses are formed from avocado clonal rootstocks with the interaction of shade intensity and vitamin growth hormone level

Stem length (cm)

The results show that there was a highly significant interaction (1%, HSD) between the main plots and subplots of the experiment. It was, however, only the subplot (hormone concentration) which caused highly significant individual effect on the stem length; shade intensity (main plot) had no significant effect at all (Table 1). In the absence of hormone application, the rootstocks subjected to 80 and 40% shading exhibited the longest stem lengths, measuring 27.27 and 25.85 cm, respectively. These values were statistically superior to those grown under 60% shading, which reached only 21.99 cm. When the plants were treated with 0.1 mL/L hormone, the 40% shade condition yielded the longest stem length at 32.07 cm. This value was significantly higher than those measured under 60% (21.99 cm) and 80% (27.90 cm) shade. Applying 0.2 mL/L of hormone shifted the optimal shade condition. Under this treatment, plants exposed to 60% shade reached 30.99 cm, statistically outperforming those in 40% (18.87 cm) and 80% (18.00 cm) shade conditions. Interestingly, when the hormone concentration was increased to 0.3 mL/L, the stem lengths across the three shading intensities (60, 80, and 40%) showed no statistically significant differences, with values of 23.02, 21.53, and 20.09 cm, respectively.

The comparison of hormone concentration across different levels of shading intensity (Table 2) revealed distinct patterns in stem length development among avocado clonal rootstocks. At 40% shade, rootstocks treated with 0.1 mL/L hormone achieved the longest stem length of 32.07 cm, significantly outperforming the control group (25.85 cm). On the other hand, rootstocks treated with 0.3 and 0.2 mL/L of hormone exhibited the shortest statistically comparable stem lengths of 20.09 and 18.87 cm, respectively. When the shading intensity was increased to 60%, the trend shifted. Rootstocks treated with 0.2 mL/L of hormone produced the longest stems, measuring 30.99 cm, which was statistically superior to all other treatments. Meanwhile, rootstocks treated with 0.1 mL/L of hormone resulted in a stem length of 25.81 cm, statistically similar to both the 0.3 mL/L (23.02 cm) and the control group (21.99 cm). Under 80% shade, the 0.1 mL/L treatment (27.90 cm) yielded the highest stem length, though it was statistically

comparable to the control group (27.27 cm). Conversely, the 0.3 mL/L treatment (21.53 cm) and 0.2 mL/L treatment (18.00 cm) resulted in the shortest stem lengths (Figure 4).

Stem diameter (cm)

The stem diameter of etiolated avocado clonal rootstocks was significantly influenced (1%, HSD) by rooting hormone concentration (Table 1). The application of 0.1 mL of vitamin hormone per liter of water resulted in the largest average stem diameter of 0.660 cm. This is the most effective concentration for promoting stem thickening. In contrast, rootstocks that received none (0), 0.2, and 0.3 mL of the hormone per liter of water exhibited statistically comparable but smaller diameters of 0.60, 0.588, and 0.577 cm, respectively. Interestingly, the main plot factor-shading intensity-did not produce a statistically significant individual effect on the stem diameter. Additionally, the interaction between shading intensity and rooting hormone concentration did not yield any significant interaction effects (Tables 1 and 2).

Leaf number

The number of leaves (Table 1; Figure 4) of etiolated avocado clonal rootstocks varied across hormone concentrations and shading intensities (1%, HSD), showing distinct patterns in leaf production. Without the application of rooting hormone, clonal rootstocks exposed to 80% shade produced the most leaves (12.80). In contrast, rootstocks under 60% shade (9.53 leaves) and 40% shade (9.07 leaves) yielded lesser and statistically similar values. When treated with 0.1 mL/L of rooting hormone, all shading levels produced statistically comparable leaf counts: 10.40 leaves under 40% shade, 9.93 leaves under 80% shade, and 9.53 leaves under 60% shade. However, rootstocks treated with 0.2 mL/L of hormone showed a different trend, producing the most leaves (13.40) under 60% shade. This was significantly superior to the leaf counts under both 40% shade (7.07 leaves) and 80% shade (5.13 leaves). Interestingly, when 0.3 mL/L of hormone was applied, the rootstocks under 80% shade produced the most leaves (11.40). However, leaf production decreased under 60% shade (9.13 leaves), and the count under 40% shade (9.53 leaves) was statistically similar to the values obtained under 60 and 80% shade.



Figure 4. Clonal avocado rootstocks are rooted using these circular devices. Their stem length (cm) and diameter (cm), and leaf numbers and area (cm²) are collected upon termination time

The comparison of hormone concentrations at each level of shading intensity (Table 2) revealed important trends in the leaf production of etiolated avocado clonal rootstocks. At 40% shade, rootstocks treated with 0.1 mL/L of rooting hormone produced the most leaves (10.40). However, this was statistically comparable to those treated with 0.3 mL/L and the control group with no hormone application, having 9.53 and 9.07 leaves, respectively. The 0.2 mL/L treatment produced the fewest leaves of 7.07. At 60% shade, a shift in the trend was observed. Rootstocks treated with 0.2 mL/L of rooting hormone produced the most leaves (13.40), which was statistically superior to all other treatments. In contrast, rootstocks treated with 0.1 mL/L, 0.3 mL/L, and the control group produced statistically similar leaf counts of 9.53, 9.13, and 9.53, respectively. When rootstocks were subjected to 80% shade, those without any hormone application produced the most leaves (12.80), although this result was comparable to the 0.3 mL/L treatment (11.40 leaves). The 0.1 mL/L treatment resulted in a slightly lower count of 9.93 leaves, while the 0.2 mL/L treatment produced the fewest leaves of 5.13.

Leaf area (cm²)

The leaf area (cm²) (Figure 4) measurements of avocado clonal rootstocks highlight the significant interaction between shading intensity (main plots) and rooting hormone concentration (subplots), as shown in Table 1. This interaction (Table 2) suggested that both factors significantly influenced (1%, HSD) the growth response of the clonal rootstocks. When no rooting hormone was applied, the largest leaf area (99.49 cm²) was observed in rootstocks under 60% shade. Rootstocks under 80% shade followed closely with a statistically similar value of 94.58 cm², while those under 40% shade had the smallest leaf area (87.37 cm²). Upon applying 0.1 mL/L of rooting hormone, the highest value was again recorded under 60% shade (100.99 cm²). It significantly outperformed the rootstocks under 40% (83.59 cm²) and 80% (75.99 cm²) shade. The application of 0.2 mL/L of rooting hormone, however, resulted in a shift in optimal shading conditions. In this case, rootstocks under 40% shade exhibited the largest leaf area (110.56 cm²). This surpassed those under 80% shade (88.30 cm²). The plants under 60% shade showed a significant decline, producing the smallest leaf area (74.30 cm²). When 0.3 mL of rooting hormone was applied, a dramatic increase in leaf area was seen under 80% shade (145.12 cm²), far exceeding the values for 40% (102.78 cm²) and 60% (99.73 cm²) shade, which were statistically similar.

Table 2 compares the significant effects (1%, HSD) of different rooting hormone concentrations across various shading intensities on the leaf area of etiolated avocado clonal rootstocks. The data revealed distinct patterns in how hormone application and shading intensity interacted to influence leaf area growth. Under 40% shade, rootstocks treated with either 0.3 mL or 0.2 mL/L of rooting hormone produced statistically similar leaf areas of 102.78 and 110.56 cm², respectively. These values were significantly higher than those recorded for the untreated rootstocks

(85.37 cm²) and those treated with 0.1 mL/L of vitamin hormone (83.59 cm²). When exposed to 60% shade, rootstocks treated with 0.1 mL/L of rooting hormone, 0.3 mL/L, and the control (no hormone) all achieved comparable leaf areas (100.99, 99.73, and 99.49 cm², respectively). Interestingly, the application of 0.2 mL/L of rooting hormone resulted in a significantly smaller leaf area of 74.30 cm². Finally, for rootstocks under 80% shade, the application of 0.3 mL/L of rooting hormone produced the largest leaf area of 145.12 cm², far exceeding the leaf areas of untreated rootstocks (94.58 cm²) and those treated with 0.2 mL/L of rooting hormone (88.30 cm²). Rootstocks treated with 0.1 mL/L of hormone exhibited the smallest leaf area (75.99 cm²).

Fresh weight (g)

The fresh weight (g) of different clonal rootstocks was measured, with results showing a significant interaction (1%, HSD) between shading intensity (main plot) and rooting hormone concentration (sub plot). Table 2 demonstrates that at lower rooting hormone concentrations (0-0.1 mL/L), the fresh weights of the clonal rootstocks were statistically similar across all shading intensities (40, 60, and 80%). However, the application of 0.2 mL/L of rooting hormone significantly affected fresh weight depending on shading intensity. Rootstocks under 60 and 40% shade produced the heaviest and statistically similar weights of 19.93 and 17.27 g, respectively. Furthermore, 0.3 mL/L of rooting hormone yielded the heaviest fresh rootstock weight of 25.53 g under 80% shade. Interestingly, this result was statistically similar to the fresh weight of rootstocks under 40% shade (21.67 g). In contrast, rootstocks under 60% shade produced the lightest fresh weight (19.20 g) under the same hormone concentration.

When comparing the significant effects (1%, HSD) of rooting hormone concentrations (subplot) across different shading intensities (main plot), the results revealed several notable patterns (Table 2). Under 40% shade, clonal rootstocks treated with 0.1 mL/L of rooting hormone achieved the heaviest fresh weight at 25.13 g. This value was statistically comparable to those treated with 0.3 mL/L of hormone (21.67 g) and even the untreated rootstocks (21.47 g). Interestingly, the lightest fresh weight in this shading condition was observed when 0.2 mL/L of vitamin hormone were applied, producing only 17.27 g. Similarly, under 60% shade, the application of 0.1 mL/L of rooting hormone consistently resulted in the highest fresh weight of 25.47 g. It significantly outperformed the untreated rootstocks (20.27 g) and those treated with both 0.2 mL/L (19.93 g) and 0.3 mL/L (19.20 g) of rooting hormone. Under 80% shade, clonal rootstocks treated with 0.3 mL rooting hormone/L of water produced the heaviest fresh weight at 25.53 g. This was statistically comparable to those treated with 0.1 mL hormone/L of water (24.20 g) and the untreated rootstocks (21.87 g). Similar to the other shading intensities, the application of 0.2 mL rooting hormone/L of water resulted in the lightest fresh weight (12.80 g).

Dry weight (g)

The dry weight (g) of avocado clonal rootstocks, as presented in Table 1, showed notable interactions (1%, HSD) between shading intensity (main plot) and rooting hormone concentration (subplot). Rootstocks that were untreated (control) produced statistically similar values across different shading rate: 8.27 g (40% shade), 7.60 g (60% shade), and 8.07 g (80% shade). The same was true for those treated with 0.1 mL of rooting hormone/L of water, with statistically comparable dry weights of 10.13 g (40% shade), 9.60 g (60% shade), and 10.33 g (80% shade). When 0.2 mL of rooting hormone/L of water was applied, the clonal rootstocks exposed to 60% shade achieved the heaviest dry weight of 8.93 g. In contrast, the rootstocks subjected to 40% shade produced a dry weight of 6.93 g. This was statistically similar to those grown under 80% shade (4.87 g). Interestingly, the highest hormone concentration of 0.3 mL of hormone/L of water led to the heaviest dry weight of 10.33 g for rootstocks under 80% shade. However, this result was statistically comparable to rootstocks under 40% shade, which produced a dry weight of 8.73 g. The lightest dry weight (7.47 g) occurred under 60% shade.

When comparing the significant effects (1%, HSD) of rooting hormone concentrations (subplot) across different shading intensities (main plot), the results in Table 2 reveal several key trends regarding the dry weight of avocado clonal rootstocks. Under 40% shade, the application of 0.1 mL of rooting hormone/L of water resulted in the highest dry weight of 10.13 g. This was statistically comparable to rootstocks treated with 0.3 mL of hormone/L of water (8.73 g) and even to the untreated control group (8.27 g). The use of 0.2 mL of rooting hormone/L of water, however, produced the lightest dry weight (6.93 g) of clonal rootstocks. Under 60% shade, rootstocks treated with 0.1 mL of rooting hormone/L of water again showed the heaviest dry weight of 8.93 g. This value was statistically similar to those treated with 0.2 mL of hormone/L of water (8.93 g) and untreated rootstocks (7.60 g). Interestingly, the lightest dry weight (7.47 g) was observed in rootstocks treated with 0.3 mL of hormone/L of water. For rootstocks under 80% shade, the application of 0.3 and 0.1 mL of rooting hormone/L of water resulted in the highest and statistically similar dry weights (10.33 g each). The untreated rootstocks trailed behind with a dry weight of 8.07 g, while the lightest dry weight (4.87 g) was observed in rootstocks treated with 0.2 mL of rooting hormone/L of water.

Leaf chlorophyll content (SPAD value)

The study measured the leaf chlorophyll content (SPAD value) of different avocado clonal rootstocks, focusing on the interaction between shading intensity and hormone concentration levels (Table 1). The results revealed a highly significant interaction (1%, HSD) between these factors, which influenced the chlorophyll content in avocado clonal rootstocks. When no rooting hormone was applied, the highest chlorophyll content (53.31 SPAD) was observed in clonal rootstocks subjected to 60% shade,

which was statistically comparable to those under 80% shade (49.35 SPAD). However, when 0.1 mL of rooting hormone/L of water was applied, shading intensity had no significant effect. All plants under 40, 60, and 80% shade showed statistically similar SPAD values of 57.04, 56.65, and 59.45, respectively. When 0.2 mL of hormone/L of water was applied, plants subjected to 80% shade had the highest chlorophyll content (61.10 SPAD). This was followed by those under 40% shade (56.18 SPAD). In contrast, plants under 60% shade had the lowest chlorophyll content (47.04 SPAD). With 0.3 mL of hormone/L of water, plants under 60% shade showed the highest chlorophyll content (57.04 SPAD), while those under 40 and 80% shade attained similar but lower SPAD values of 50.71 and 49.91, respectively.

The comparison of subplots (rooting hormone concentrations) at each level of shading intensity revealed variations in leaf chlorophyll content of avocado clonal rootstocks (Table 2). At 40% shading intensity, the highest chlorophyll content was achieved when 0.1 mL of rooting hormone/L of water was applied, resulting in 57.04 SPAD values. This was statistically similar to the 0.2 mL treatment (56.18 SPAD) but significantly higher than both the 0.3 mL treatment (50.71 SPAD) and the untreated clonal stocks (49.91 SPAD). At 60% shading intensity, the 0.3 mL treatment attained the highest chlorophyll content (57.04 SPAD). This, however, was statistically comparable to the 0.1 mL treatment (56.65 SPAD) and the untreated control (53.31 SPAD). Notably, the 0.2 mL treatment had the lowest chlorophyll content (47.04 SPAD). Under 80% shading intensity, the application of 0.2 mL of rooting hormone/L of water resulted in the highest chlorophyll content (61.10 SPAD). It was statistically similar to the 0.1 mL treatment (59.45 SPAD). The 0.3 mL treatment and the control were both lower, with chlorophyll contents of 49.91 SPAD and 49.35 SPAD, respectively.

Leaf color

Using the Munsell Color Application, the leaf color of different rootstocks was measured. Table 3 evaluates the leaf color of etiolated avocado clonal rootstocks under varying concentrations of growth vitamin hormone (Hormex) and different shading intensities. It used a numerical scale (Esgrina and Tan 2024) from 1 to 6 based on the Munsell color system. The control group (no hormone) showed a leaf color score of 4.60 (40% shading), which increased to 5.00 (at both 60 and 80% shading), with an overall mean of 4.87. In contrast, the 0.1 mL of hormone/L of water maintained a consistent score of 5.00 across all shading intensities, resulting in a mean of 5.00. Similarly, the 0.2 mL treatment also consistently scored 5.00 at all shading levels, matching the overall mean of 5.00. For the 0.3 mL treatment, the score was 5.00 (40% shading), dropped to 4.40 (60% shading), and returned to 5.00 (80% shading), yielding a mean of 4.00, indicating variation in leaf color response based on shading intensity. The mean leaf color scores across the shading levels were 4.90 (40% shading), 4.85 (60% shading), and 5.00 (80% shading).

Table 3. Leaf color of etiolated avocado clonal rootstocks as affected by growth vitamin hormone and shading intensity

Subplot (Hormex hormone concentration)	Main plot (shading intensity)			Mean
	40%	60%	80%	
Control (no hormone)	4.60	5.00	5.00	4.87
0.1 mL/L of water	5.00	5.00	5.00	5.00
0.2 mL/L of water	5.00	5.00	5.00	5.00
0.3 mL/L of water	5.00	4.40	5.00	4.00
Mean	4.90	4.85	5.00	

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)

Discussions

The results of this study highlight the significant interaction between shading intensity and hormone concentration in influencing the rhizogenesis and caulogenesis of avocado clonal rootstocks. The effects of shading and growth hormone applications were observed across various morphological and physiological parameters, supporting previous studies on plant growth regulation under different environmental conditions (Brini et al. 2022; Ghorbel et al. 2023).

Optimal hormone concentrations under specific shading levels

Higher shading intensity (80%) in combination with 0.2-0.3 mL/L of hormone resulted in the highest root number, root length, chlorophyll content, fresh weight, and dry weight of avocado seedlings. These findings align with previous studies suggesting that reduced light intensity enhances root initiation by lowering transpiration rates and improving auxin stability (Hossain and Kamaluddin 2005; Pacholczak et al. 2017). This is consistent with findings by Aly et al. (2019), who reported that reduced light intensity positively influences root formation and overall biomass in ginger plants. The results also align with studies on shading effects in strawberries, where Cordoba-Novoa et al. (2022) found that shading reduced plant water deficits, thereby improving vegetative growth. Moderate shading (60%) with 0.1-0.2 mL/L hormone concentration was most effective for stem elongation and callus formation, highlighting the role of intermediate light exposure in

promoting cell expansion and differentiation (Štefančič et al. 2005; Kanmegne et al. 2017; Dev et al. 2018; Jiang et al. 2020; Khandaker et al. 2022; Feng et al. 2023). Conversely, at 40% shading, root formation of avocado clonal stocks was significantly limited, particularly when 0.2 mL/L of hormone was applied. This suggests that excessive light exposure may reduce endogenous auxin accumulation (Lee et al. 2022; Xin et al. 2022), counteracting the exogenous application of hormones (Sun et al. 2023). The balance between endogenous and exogenous hormone levels is crucial for successful rhizogenesis and caulogenesis (Hunt et al. 2011; Muttaleb et al. 2017; Chen et al. 2023; Khan et al. 2024). Similar trends were observed by Dewi et al. (2022), who found that excessive light exposure reduced the efficacy of growth regulators in hybrid corn. This suggests that light-induced auxin degradation may have inhibited root formation, which has also been observed in studies on tomato and cotton seedlings under shading conditions (Echer et al. 2019; Zhang et al. 2020; Liphon and Detpiratmongkol 2020).

Physiological mechanisms underlying the observed interactions

The differential responses observed in avocado rootstocks can be explained by key physiological mechanisms influenced by shading and hormone application:

Auxin and root development

The role of auxin in root initiation is well established, but its effectiveness on avocado root initiation and development depends on light conditions (Pantoja-Guerra et al. 2023; Calatrava et al. 2024). Under high shading (80%), reduced photodegradation of auxin may have contributed to higher root proliferation and elongation (Tanimoto 2005; Zhao et al. 2016; Olatunji et al. 2017). The data support findings that moderate-to-high shade (40-80%) enhances root development in woody species (Arévalo-Gardini et al. 2021; Xue et al. 2023) including avocados. Studies by Hersch et al. (2014) and Brini et al. (2022) indicate that light intensity regulates auxin transport and signaling pathways, thereby affecting shade avoidance responses. The positive effect of shading on auxin stability and root proliferation has also been noted in previous work on shade-tolerant Mediterranean species (García-Pérez et al. 2021; Tivendale and Millar 2022).

Shading and stem growth

Stem elongation of avocado scions was most pronounced under moderate shading (60%) with 0.1-0.2 mL/L hormone, resulted in the longest stem lengths, suggesting that intermediate light exposure optimizes stem elongation (Kaur 2017; Hussain et al. 2019). Shading has been shown to increase internodal elongation by altering auxin transport (Abdel-Mawgoud et al. 1996; Collins and Wein 2000; Zhiyu et al. 2007; Wu et al. 2017a; Mishra et al. 2020; Formisano et al. 2022; Luo et al. 2023). Our findings align with previous reports indicating that moderate shading enhances shoot elongation in various crops (Wu et al. 2017b; Sosnowski et al. 2023). Similar results were

found in chili pepper studies, where moderate shading improved biomass accumulation (Hariyono et al. 2021). The enhanced shoot elongation observed under 60% shading may be attributed to a shift in hormonal regulation, as shading has been shown to increase gibberellin activity while modifying auxin distribution (Khan and Nabi 2023). A study by Mroue et al. (2017) further supports the role of auxin in integrating environmental cues to regulate plant development.

Chlorophyll content and shading

The highest chlorophyll content in the leaves of avocado seedlings was recorded under 80% shading with 0.2 mL/L hormone, supporting related literatures that shading reduces chlorophyll degradation and enhances pigment synthesis (Duan et al. 2018; Chen et al. 2021; Esgrina and Tan 2024). It is also consistent with findings in basil, where increased shading and light regulation enhanced pigment accumulation (Eghbal et al. 2024). Hormone application further stabilized the chlorophyll levels of avocado seedling leaves, likely by improving nitrogen assimilation and delaying senescence (Mazzoni-Putman et al. 2021; Huang et al. 2022; Feng et al. 2023; Mason 2023; Trösch 2023). Studies in kalmegh also indicate that shading improves chlorophyll stability, which aligns with the observed increase in SPAD values in avocado seedlings (Valio 2001; Liphon and Detpiratmongkol 2020). The role of auxins in maintaining chlorophyll content and delaying leaf senescence has been documented in work on tomatoes (Yuan et al. 2018) and other horticultural crops (Guimarães et al. 2020; Khan and Nabi 2023).

The findings of this study are consistent with earlier research demonstrating that shading and hormone treatments interact synergistically (Khajehpour et al. 2014; Chaiwanon et al. 2016; de Wit et al. 2016; Ibukun 2016; Lymperopoulos et al. 2018; Sulaiman et al. 2020) to enhance propagation success. Auxin-based hormones have been shown to enhance root biomass and shoot development, as observed in both avocado and other tree crops. For instance, similar studies on tree seedlings have shown that moderate-to-high shading improves root initiation while excessive hormone concentrations can have inhibitory effects (Oumahmoud et al. 2023; Xue et al. 2023). Moreover, auxin-based hormones have been linked to improvements in both root and shoot biomass, as observed in this study (Takahashi 2013; Pacheco-Villalobos et al. 2016; Zain et al. 2022; Wang et al. 2024). However, deviations from expected outcomes—such as the limited root formation of avocado clonal stocks at 40% shading despite hormone application—suggest that additional environmental factors, such as growing media, temperature and humidity, may influence hormone activity. Similar variations have been noted in experiments where light intensity fluctuations affected tomato seedling growth (Zheng et al. 2023). Future studies should consider incorporating additional physiological assessments, such as hormonal quantification and gene expression analysis, to further elucidate the underlying mechanisms.

This study underscores the importance of tailoring propagation protocols to specific environmental conditions. The interaction between shading intensity and hormone

concentration significantly affects key growth parameters. Specifically, 80% shading with 0.2-0.3 mL/L hormone optimized root formation, leaf area, chlorophyll content, and biomass, while 60% shading with 0.1-0.2 mL/L hormone was most effective for stem elongation. Based on these findings, it is recommended that propagation strategies be adjusted depending on the desired growth outcome. For maximizing overall plant growth, 80% shading with 0.3 mL/L hormone is ideal, while for better shoot elongation and chlorophyll retention, 60% shading with lower hormone concentrations (0.1-0.2 mL/L) is preferable. These insights contribute to refining avocado rootstock propagation techniques, supporting both commercial cultivation and sustainable agricultural practices.

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