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Effect of auxins on the rooting of the avocado rootstock 'Duke 7'

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Abstract: Clonal propagation of avocado rootstocks through etiolated shoot rooting represents a key strategy to enhance genetic uniformity, plant health, and productivity in commercial orchards. However, its success largely depends on the rooting phase, where auxins play a critical role. This study evaluated the effect of auxin-based rooting agents (types and concentrations) on root induction and quality in etiolated shoots of the 'Duke 7' rootstock. Five agents (IAA, NAA, IBA, K-IBA, and IBA + NAA combination) were tested at three concentrations (24.6, 34.4, and 44.2 mM) under a completely randomized factorial design (5 × 3) with three replicates per treatment. Morphological variables included rooting percentage, survival rate, root number/length/diameter, secondary root development, callus formation, and root quality index (RQI). Results revealed significant effects of agent type, concentration, and their interaction. NAA (34.4 mM) was the most effective for root number (55.3) and RQI (154.9 cm), albeit with high callus formation and reduced secondary roots. The IBA + NAA combination (34.4 mM) also showed high RQI (140.4 cm), with greater root length and less negative impact on root architecture. IBA alone achieved 100% rooting with moderate root development, balancing efficacy and physiological tolerance. Overall, intermediate concentrations of NAA and IBA + NAA yielded optimal results. These findings can refine clonal propagation protocols for 'Duke 7', with direct applications in commercial nurseries producing high-performance rootstocks.

1. Introduction

Commercial avocado trees result from the combination of tissues from two distinct plants: A scion that forms the canopy and a rootstock that provides the root system (Gleeson *et al*., 2016). This propagation technique enables cultivars to achieve early productivity while preserving their phenotypic traits, even when grafted onto juvenile plants (Melnyk, 2017).

In Mexico, commercial avocado nursery production primarily relies on seed-propagated rootstocks derived from native trees exhibiting broad yet poorly characterized genetic diversity (Salazar-García *et al*., 2004 a). This genetic variability leads to heterogeneous tree growth (Medina-Urrutia *et al*., 2017) and increased susceptibility to diseases, pests, and abiotic stressors such as drought, salinity, or nutrient imbalances (Salazar-García *et al*., 2004 a). Furthermore, rootstock type has been documented to directly influence key agronomic parameters including yield, tree size and vigor, as well as fruit quality and postharvest life (Barrientos-Priego, 2017).

The most effective strategy to mitigate issues arising from genetic heterogeneity involves using clonal rootstocks (Salazar-García *et al*., 2004 b; Cohen *et al*., 2023). Although avocado trees grafted onto clonal rootstocks are more expensive than those on seedling rootstocks (Cohen *et al*., 2023), certain Mexican production areas affected by *Phytophthora cinnamomi* Rands (Ochoa-Fuentes *et al*., 2007; Sánchez-González *et al.*, 2019), clay soils (Salazar-García *et al*., 2015), alkaline pH, and soil salinity (Medina-Urrutia *et al.*, 2017) would significantly benefit from their implementation.

While clonal rootstocks can standardize production, they present inherent technical limitations, particularly during the rooting phase (Ernst, 1999; Gleeson *et al*., 2016). Current commercial methodologies for clonal propagation of avocado species are primarily derived from the technique established by Frolich and Platt (1972). This approach, albeit with potential minor modifications, involves performing air layering on an etiolated shoot, separating it from the mother plant once a root system develops, and grafting the commercial cultivar during the rooting phase when stem diameter permits (Ernst, 1999; Ernst *et al*., 2013).

The formation of adventitious roots in woody species such as avocado is regulated by the balance of growth regulators, with auxin application being one of the most effective strategies to promote rooting (Zhao *et al*., 2022). Synthetic auxins like indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) serve as the primary regulators of adventitious root formation through complex interactions that modulate metabolic, transport, and signaling processes (Lakehal and Bellini, 2019).

Recent studies demonstrate that auxin type and concentration influence rooting quality through mechanisms such as molecular stability, transport, and metabolite conjugation (Damodaran and Strader, 2019; Gomes and Scortecci, 2021). However, current avocado propagation protocols remain based on classical work (Frolich and Platt, 1972), typically limited to IBA application at 7000 mg L^{-1} (Ernst, 1999), without considering the efficacy of other auxin types or concentrations. Furthermore, few recent studies have explored these aspects (Li *et al*., 2024) for standard rootstocks like 'Duke 7'.

In this context, the objective of this study was to evaluate the effect of different auxin types and concentrations on root induction and rooting quality in etiolated shoots of the 'Duke 7' rootstock. This work aims to establish an efficient vegetative propagation protocol to enhance production of clonal avocado rootstocks in Mexico, thereby improving genetic uniformity and resilience of commercial plantations.

2. Materials and Methods

Experimental site and plant material

This study adopted the propagation method proposed by Hofshi (1996) and Ernst (1999), adapted to the environmental conditions of a greenhouse located in Chapingo, Texcoco, State of Mexico (19.4904322, -98.8734917) at 2264 meters above sea level. The research was conducted during 2022 and 2023.

Nurse plants were produced using West Indian avocado seeds (70±17.4 g) from Veracruz, a size determined optimal for etiolated shoot development during rooting (Castro *et al*., 2021). Seeds were sown on November 1, 2022, in 1000 cm^3 polyethylene bags filled with a 1:1:1 (v/v/v) substrate mixture of peat moss, volcanic rock, and perlite. Plants received light irrigation and preventive applications of fungicide (benomyl 1 g L^{-1}) and insecticide (imidacloprid 1 mL L^{-1}) until grafting.

On February 17, 2023, nurse plants were grafted with mature 'Duke 7' buds at 5 cm above substrate level, retaining only two buds per scion. Graft wounds were sealed with polyvinyl acetate resin to prevent desiccation. During graft union formation, buds were covered with transparent polyethylene bags (5 × 8 cm) which were removed when plants were transferred to the etiolation chamber.

Experimental establishment

Once etiolated shoots reached 25-30 cm in length (Ernst, 1999), treatments were applied. At the base of each shoot, air layering was performed using a cutting blade periodically disinfected with ethanol (70%). A wound of approximately 2 cm in length was caused, to which 100 µL of rooting growth regulator was applied. Each treated shoot was then placed in a 150 cm³ transparent plastic container filled with coconut coir dust.

The treated plants were maintained in a shaded area within the greenhouse and watered periodically to maintain adequate moisture levels in both the air-layering substrate and the nurse plant's growing medium. All rooting formulations were prepared fresh on the day of treatment application and were not stored for subsequent use, ensuring consistent growth regulator activity and concentration for each experimental unit. During the study period, greenhouse conditions exhibited natural variability, with mean temperatures gradually increasing from 16.3 to 23.7°C while relative humidity fluctuated between 54% and 64% (Fig. 1).

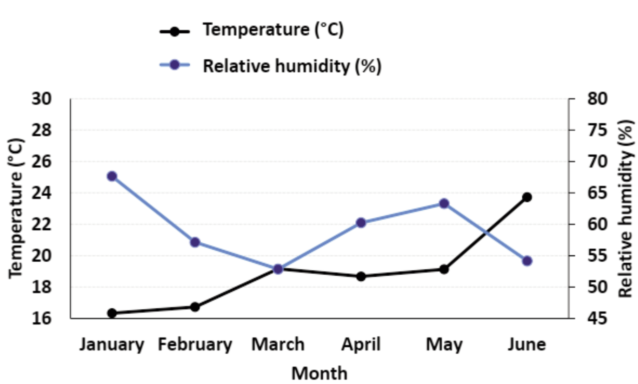

Fig. 1 - Average monthly ambient temperature and relative humidity variation during the experimental period.

Experimental design

The study employed a completely randomized factorial design (5 × 3) with three replications per treatment. Each experimental unit consisted of three air-layered plants. The first factor represented auxin type with five levels, while the second factor comprised three concentrations: 24.6, 34.4, and 44.2 mM (Table 1). This design enabled systematic evaluation of 15 treatment combinations under standardized conditions.

For each treatment, 20 mL of solution was prepared using distinct protocols according to auxin type. The potassium salt of indole-3-butyric acid (K-IBA) and commercial IBA+NAA formulation (Dip'N Grow®) were prepared exclusively with distilled water. For other auxins (IAA, NAA, IBA), a two-step dissolution was employed: initial solubilization in 96% ethanol (5 mL for 24.6 mM, 7 mL for 34.4 mM, or 9 mL for 44.2 mM) followed by volume completion with distilled water. This methodology ensured complete auxin solubility while maintaining precise target concentrations across all experimental treatments.

Evaluated variables

Eighty days after treatment application, the air-layered shoots were carefully separated from nurse plants and the substrate adhered to the roots was removed through water immersion with intermittent manual agitation. Quantitative assessments included: shoot survival (percentage of viable shoots), rooting success (proportion of shoots that emitted at least one root of 0. 5 cm in length or more), root number (mean per rooted shoot), root dimensions (length and diameter of five primary roots measured with Mitutoyo® digital caliper, ±0.01 mm precision), secondary root development (percentage of shoots with secondary roots), callus formation (percentage of shoots showing callus at wound site), and the Root Quality Index (RQI) calculated as the product of mean

Table 1 - Experimental treatments: auxin types and concentrations evaluated in the rooting of 'Duke 7' avocado rootstock etiolated shoots

Treatment Rooting agent (auxin type)	Concentrations (mM)		
Indole-3-acetic acid (IAA, Sigma®)	24.6	34.4	44.2
1-naphthaleneacetic acid (NAA, Sigma®)	24.6	34.4	44.2
Indole-3-butyric acid (IBA, Sigma®)	24.6	34.4	44.2
Potassium salt of indole-3-butyric acid (K-IBA, Sigma®)	24.6	34.4	44.2
Combination of IBA + NAA (Dip'N Grow®)	24.6	34.4	44.2

root number and average length (cm). This comprehensive evaluation protocol enabled systematic comparison of treatment effects on both root initiation and development.

Statistical analysis

For statistical analysis, mean values per experimental unit were used. The data were processed using the GLM procedure in SAS® statistical software (SAS OnDemand for Academics, version 3.1.0; SAS, 2021). Variables expressed as proportions or percentages were subjected to arcsine square root transformation of the original decimal fraction values to meet assumptions of normality and homogeneity of variances.

Following analysis of variance (ANOVA), when significant differences (P<0.05) were detected for treatment effects or their interactions, means were compared using Tukey's test. Data values were back-transformed to their original units for presentation and interpretation.

The statistical model applied was as follows:

$$Y_{ij} = \mu + A_i + C_j + AC_{ij} + \varepsilon_{ij}$$

where: Y_{ij} is the value of the variable evaluated with the i-th rooting agent and the j-th concentration; μ is the overall mean; A_i is the fixed effect of the i-th rooting agent; C_j is the fixed effect of the i-th concentration; AC_{ij} is the fixed effect of the interaction of the i-th rooting agent with the j-th concentration; and ε_{ij} is the experimental error.

3. Results and Discussion

The experimental results demonstrate the significant influence of auxin type and concentration on root development in etiolated shoots of avocado rootstock 'Duke 7'. Our findings reveal distinct morphological responses to different auxin treatments, with particular combinations showing optimal performance in root initiation and development. These outcomes are analyzed through three critical lenses: Their physiological implications for adventitious root formation, practical applications for commercial clonal propagation systems, and comparative relevance to established literature in avocado propagation. The data presentation focuses on treatment efficacy across measured parameters including rooting percentage, root architecture features, and callus formation patterns, providing a comprehensive evaluation of auxin effects on this economically important rootstock cultivar.

Analysis of variance

Analysis of variance revealed significant differences (P<0.05) among treatments for most evaluated variables, confirming the influence of auxin type and concentration on adventitious root development in etiolated avocado shoots. Specifically, the rooting agent type showed highly significant effects on root quality index (P<0.001), root number (P<0.001), root length (P<0.01), and callus formation (P<0.01). Concentration significantly affected only root number (P<0.01), while the Rooting agent × Concentration interaction was significant for survival rate, rooting percentage, root length and diameter, and secondary root presence (P<0.05) (Tables 2 and 3).

These findings demonstrate that both the composition of the rooting agent and its concentration distinctly influence various aspects of the rooting process, and that their interaction can substantially modify the morphological response of etiolated shoots.

Effect of the rooting agent

The rooting agent type exerted a decisive

Table 2 - Mean squares obtained in the analysis of variance to evaluate the effect of five rooting agents and three concentrations on variables related to the rooting of etiolated shoots of avocado rootstock 'Duke 7'

Variation source	DF	SSP	Rooting		PCP
			Percentage	Quality index	
Rooting agent (A)	4	0.135 NS	0.133 NS	89156.6 ***	0.987 **
Concentration (C)	2	0.067 NS	0.046 NS	8474.8 NS	0.488 NS
A × C	8	0.269 *	0.135 *	12153.5 NS	0.183 NS
Experimental error	30	0.253	0.057	40686.9	0.230

DF= Degrees of freedom; SSP= Shoot survival percentage; PCP= Percentage of callus presence; NS= Not significant (P>0.05); * Significant (P<0.05); **Significant (P<0.01); ***Highly significant (P<0.001); ×= Interaction between factors.

Table 3 - Mean squares obtained in the analysis of variance to evaluate the effect of five rooting agents and three concentrations on variables related to the rooting of etiolated shoots of avocado rootstock 'Duke 7'

Variation source	DF	Roots number	Length	Diameter	SRP
Rooting agent (A)	4	2545.16 ***	1.154 **	0.106 NS	2.133 ***
Concentration (C)	2	675.16 **	0.018 NS	0.012 NS	0.020 NS
A × C	8	128.08 NS	0.460 *	0.283 *	0.431 *
Experimental error	30	133.93	0.120	0.123	0.144

DF= Degrees of freedom; SRP= Secondary roots presence; NS= Not significant ($P>0.05$); * Significant ($P<0.05$); ** Significant ($P<0.01$); ***Highly significant ($P<0.001$); ×: Interaction between factors.

influence on multiple root development parameters in etiolated 'Duke 7' shoots. Although all treatments achieved rooting rates exceeding 94% (Table 4), significant variations were observed in three critical aspects: 1) post-treatment survival rates, 2) root system quality (including architecture and developmental patterns), and 3) callus formation intensity at wound sites. These differential responses highlight the importance of precise auxin selection in clonal propagation protocols, where optimal root system architecture must be balanced with minimal callus interference for successful transplant establishment.

Among the evaluated rooting agents, 1-naphthaleneacetic acid (NAA) promoted the highest root number (55.3) and root quality index (154.9 cm) (Table 4), confirming its efficacy as a potent inducer of adventitious root formation. This response likely stems from NAA's enhanced stability in plant tissues, reduced susceptibility to enzymatic degradation, and prolonged persistence at the application site (da Costa *et al*., 2013; Raggi *et al*., 2020). However, this treatment also reduced shoot survival to 98.1% (Table 4), suggesting phytotoxic effects potentially linked to ethanol solvent use, and the heightened sensitivity of etiolated tissues to elevated auxin concentrations (Amri, 2010; Grossmann, 2009; Ludwig-Müller, 2020). These findings underscore the need to balance rooting efficacy with tissue tolerance, particularly for etiolated shoots whose cell walls exhibit modified xyloglucan and pectin composition. Such alterations increase tissue flexibility but also enhance susceptibility to apoplastic pH imbalances when critical auxin thresholds are exceeded (Duman *et al*., 2020; Wang *et al*., 2025).

In contrast, indole-3-acetic acid (IAA) and indole-3-butyric acid potassium salt (K-IBA) exhibited

Table 4 - Average values by type of rooting agent for the variables: percentage of shoot survival, percentage of rooting, rooting quality index, and percentage of callus presence in the rooting of etiolated shoots of the avocado rootstock 'Duke 7'

Rooting agent	SSP (%)	Rooting		PCP (%)
		Percentage	Quality index (cm)	
IAA	100.0 a[z]	94.2 a	40.1 c	68.86 ab
NAA	98.1 b	96.0 a	154.9 a	87.89 a
IBA	100.0 a	100.0 a	100.2 b	13.74 b
K-IBA	100.0 a	94.2 a	34.0 c	68.86 ab
IBA + NAA	100.0 a	100.0 a	98.3 b	83.56 a
CV (%)	5.94	16.44	43.07	50.88
HLSD	1.57	10.20	50.36	37.09
Average	99.93	98.46	85.51	65.33

[z] Average values in the same column followed by different letters indicate statistical differences (Tukey, $P<0.05$). IAA= indole-3-acetic acid; IBA= indole-3-butyric acid; NAA= 1-naphthalene acetic acid; K-IBA= indole-3-butyric acid potassium salt; SSP= Shoot survival percentage; PCP= Percentage of callus presence; CV= Coefficient of variation; HLSD= Honest least significant difference.

maximum survival rates (100%) but produced limited root formation (14.9 and 14.4 roots, respectively; Table 5) and significantly lower root quality indices (Table 4). This reduced efficacy likely stems from IAA's inherent instability, being rapidly degraded by peroxidase enzymes and light exposure (Roussos, 2023; Yun *et al*., 2023). Furthermore, IAA readily forms biologically inactive conjugates with amino acids and sugars, substantially reducing its bioavailability and root-promoting activity (Pincelli-Souza *et al*., 2024).

While potassium indole-3-butyric acid potassium salt (K-IBA) offers greater stability than IAA and eliminates the need for organic solvents in solution preparation (Lesmes-Vesga *et al*., 2021), its efficacy as a rooting inducer appears constrained by distinct physiological transport limitations (Yang *et al*., 2022). In its ionic form, K-IBA demonstrates restricted apoplastic diffusion, significantly impeding passive transport to target cells near the application site in etiolated shoots. Effective mobilization instead requires active transport mechanisms (Roussos, 2023) mediated by specialized carrier proteins, including AUX1/LAX family influx transporters and PIN-FORMED (PIN) and ABCB efflux transporters, which collectively regulate auxin distribution across cellular membranes (Hammes *et al*., 2021). Crucially, K-IBA must undergo conversion to its non-ionic (protonated) form to cross plasma membranes and subsequently trigger adventitious root formation (Pincelli-Souza *et al*., 2024), adding a metabolic conversion step that may delay or limit its biological activity compared to more mobile auxin forms.

In contrast, both IBA and the commercial IBA + NAA combination (Dip'N Grow®) demonstrated an optimal balance between shoot survival and root quality (Table 4), establishing them as viable candidates for clonal avocado propagation protocols. These rooting agents produced consistent, reliable responses particularly valuable for nurseries requiring both high rooting success and preservation of etiolated shoot viability. The observed performance suggests these formulations effectively navigate the critical compromise between root induction efficacy and minimal phytotoxicity, a decisive advantage for commercial scale production of 'Duke 7' rootstock.

The evaluation revealed significantly higher callus formation with NAA (87.9%) and IBA+NAA (68.9%) treatments compared to IBA alone (13.7%) (Table 4), consistent with previous reports of synthetic auxins promoting unorganized tissue proliferation (Zhai and Xu, 2021). While callus formation may initially facilitate root primordia initiation, excessive development can negatively impact rooting success through three primary mechanisms: physical obstruction of emerging roots, disruption of normal root system architecture, and competition for essential metabolic resources that would otherwise support root growth (Chen *et al*., 2020).

The current study revealed that callus development was solely influenced by the type of rooting agent applied, with no consistent correlation observed between callus presence and root quantity. While NAA treatment produced both the highest callus formation (87.89%) and root number (55.29), IBA which generated minimal callus (13.74%) still induced intermediate root formation (34.04) (Tables

Table 5 - Mean values by rooting agent type for the variables number, length, and diameter of roots, and presence of secondary roots in the rooting of etiolated shoots of the avocado rootstock 'Duke 7'

Rooting agent	Roots			SRP (%)
	Number	Length (cm)	Diameter (mm)	
IAA	14.92 c [z]	2.29 b	1.49 a	80.7 a
NAA	55.29 a	2.83 ab	1.59 a	39.8 b
IBA	34.07 b	2.90 a	1.53 a	95.8 a
K-IBA	14.40 c	2.27 b	1.60 a	58.7 a
IBA + NAA	32.11 b	3.03 a	1.77 a	58.7 a
CV (%)	38.37	16.77	22.06	44.17
HLSD	15.82	0.61	0.48	24.59
Average	30.16	2.67	1.59	57.33

[z] Average values in the same column followed by different letters indicate statistical differences (Tukey, P<0.05). IAA= indole-3-acetic acid; IBA= indole-3-butyric acid; NAA= 1-naphthalene acetic acid; K-IBA= indole-3-butyric acid potassium salt; SRP= Secondary roots presence; CV= Coefficient of variation; HLSD= Honest least significant difference.

4 and 5).

Contrasting with findings in woody *Eucalyptus* species where adventitious roots originate from callus tissue (Fett-Neto *et al*., 2001; Zhang *et al*., 2022), our observations demonstrated direct root emergence from stem tissue above the auxin application site, without visible callus involvement (Fig. 2). This response suggests etiolated 'Duke 7' shoots maintain an intrinsic capacity for direct rhizogenesis, a phenomenon previously documented in other avocado rootstocks like 'VC801' (Duman *et al*., 2020).

Morphological analysis revealed significant suppression of secondary root growth following NAA application (Fig. 2), likely due to auxin-induced temporal inhibition of lateral root development in primary root tissues. This phenomenon aligns with observations in *Arabidopsis thaliana* (Biswas *et al*., 2019), where supraoptimal auxin levels negatively affect lateral root formation through disruption of polar auxin transport in pericycle cells, cell cycle arrest in lateral root primordia, and downregulation of lateral root-promoting genes such as ARF7 and ARF19. While NAA effectively stimulates primary root formation in avocado rootstock 'Duke 7', our results indicate that higher concentrations may delay optimal root system development by inhibiting secondary branching. This architectural limitation presents key practical challenges: extended production timelines due to delayed shoot separation from nurse plants, and potential requirement for additional agronomic interventions (e.g., supplemental growth regulator treatments) to promote secondary root growth before transplanting. These findings suggest that while NAA remains a potent rooting agent, commercial nurseries should carefully evaluate the trade-off between rapid root initiation and subsequent root system complexity when selecting auxin formulations for clonal propagation.

The findings of this study offer valuable guidance for optimizing clonal propagation of avocado rootstocks in commercial settings. For nurseries prioritizing root quantity, NAA emerges as the most effective option despite its tendency to reduce secondary root development. IBA presents a balanced alternative, producing intermediate root numbers (34.0) while maintaining excellent shoot survival rates (100%), making it particularly suitable for operations where plant viability is paramount. The commercial IBA + NAA formulation (Dip'N Grow®) provides a practical ready-to-use solution that combines the benefits of both auxins while simplifying nursery workflows. Importantly, the results demonstrate that IAA and K-IBA are unsuitable for large-scale propagation due to their limited root induction capacity (14.9 and 14.4 roots respectively) and inherent biochemical instability. These evidence-based recommendations allow propagation specialists to select auxin treatments

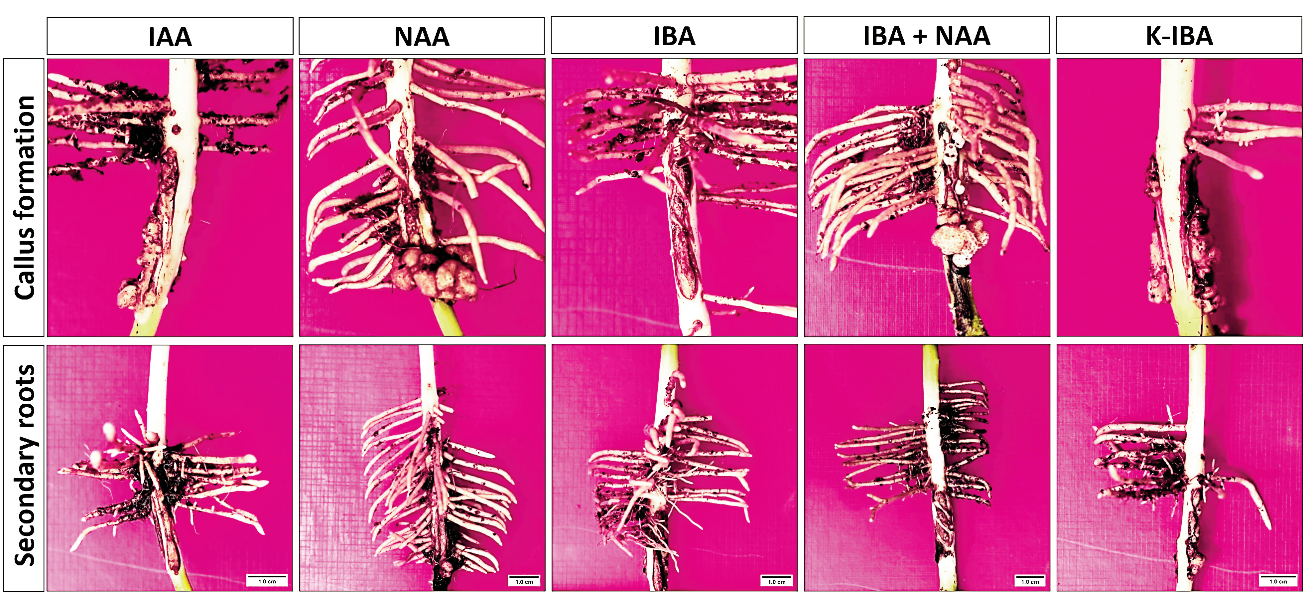

Fig. 2 - Influence of five rooting agents on callus formation and secondary root development in the rooting of etiolated shoots of the avocado rootstock 'Duke 7'. IAA= indole-3-acetic acid; IBA= indole-3-butyric acid; NAA= 1-naphthalene acetic acid; K-IBA= indole-3-butyric acid.

based on their specific production requirements, whether the priority is maximizing root biomass, ensuring transplant success, or streamlining operational efficiency.

These findings represent a significant advancement over traditional IBA-only protocols (Ernst, 1999), which typically employ high concentrations (34.4 mM). The demonstrated benefits of auxin diversification align with recent studies in other woody species like apple (*Malus* spp.) and mulberry (*Morus alba*), where combined auxin treatments have outperformed IBA in promoting adventitious root formation (Sourati *et al*., 2022; Tahir *et al*., 2022; Wang *et al*., 2024).

The research confirms that auxin selection critically impacts not just rooting efficiency but also root system morphology, both determining factors for successful clonal propagation of avocado rootstocks. These morphological qualities ultimately influence field establishment and long-term productivity of grafted trees.

Effect of the concentration

The study revealed significant auxin concentration effects on key root development, particularly root number (Table 6). A clear dose-dependent response was observed, with progressive increases in root formation corresponding to higher auxin concentrations: from 24.6 roots per shoot at 24.6 mM to 37.6 roots at 44.2 mM (Table 6). This pattern aligns with classical auxin response curves reported in the literature (Nissen, 1985), where rooting typically improves with increasing auxin concentrations up to an optimal threshold, beyond which phytotoxic effects suppress root initiation (Nissen, 1985; Sahoo *et al*., 2021).

Historically, avocado clonal propagation has employed varying concentrations of auxins, particularly indole-3-butyric acid (IBA) as the most commonly used rooting agent. Reported applications range from 500 to 10,000 mg L^{-1}, equivalent to approximately 2.5-51.3 mM (Rogel-Castellanos *et al*., 2000; Mindêllo-Neto *et al*., 2006; Li *et al*., 2024). Significant cultivar-specific differences emerge from these protocols: In ‘Duke 7’, IBA application at 2,500 mg L^{-1} achieved 56.6% rooting success (Li *et al*., 2024), while ‘Fuerte’ required five-fold lower concentrations (500 mg L^{-1}) to reach 47.5% efficacy (Mindêllo-Neto *et al*., 2006). Notably, the rootstock ‘Dusa’ demonstrated striking physiological-state dependence, with 82% rooting in etiolated shoots versus less than 10% in non-etiolated tissue at 2,500 mg·L^{-1} (Li *et al*., 2024), highlighting the critical importance of the plant material’s physiological condition in propagation success. These collective findings underscore the dual influence of genetic factors and tissue physiology in determining optimal auxin protocols for different avocado cultivars.

These historical precedents contrast sharply with the findings of the current study, where the tested concentration range (24.6 to 44.2 mM) achieved rooting success rates exceeding 95% while maintaining shoot survival rates in most treatments (Table 3). Furthermore, the results demonstrate that precise concentration adjustments can simultaneously maximize rooting efficiency and improve root system quality without inducing the severe adverse effects (particularly phytotoxicity) typically observed when exceeding optimal NAA concentration (Yan *et al*., 2014). This refined

Table 6 - Mean values by rooting hormone concentration for the variables number, length, and diameter of roots and presence of secondary roots in the rooting of etiolated shoots of the avocado rootstock ‘Duke 7’

Concentration	Roots			SRP (%)
	Number	Length (cm)	Diameter (mm)	
24.6 mM	24.62 b [z]	2.65	1.56	61.50 a
34.4 mM	28.24 ab	2.65	1.60	55.23 a
44.2 mM	37.62 a	2.71	1.62	55.23 a
CV (%)	38.37	16.77	22.06	44.17
HLSD	10.42	0.40	0.32	11.22
Average	30.16	2.67	1.59	57.33

[z] Average values in the same column followed by different letters indicate statistical differences (Tukey, P<0.05). SRP= Secondary roots presence; CV= Coefficient of variation; HLSD= Honest least significant difference.

approach represents a significant improvement over traditional protocols, as it achieves near-universal rooting success while avoiding the compensatory trade-offs between root quantity and plant viability that characterize many existing methods. The study specifically identified 34.4 mM as the most balanced concentration for commercial applications, combining high rooting percentages (98.1%) with excellent root architecture development and minimal callus formation (Tables 6 and 7).

The clonal propagation of ‘Duke 7’ rootstock has presented particular difficulties. Previous studies reported limited success, with only 26% rooting after 180 days when using non-etiolated shoots treated with Dip’N Grow® at 3,000 mg L^{-1} (approximately 15.2 mM equivalent) (Salazar-García *et al*., 2004 b). Alternative approaches using IBA-saturated wood chips (10,000 mg L^{-1}) on etiolated shoots improved rooting to 60% (Escobedo and Escobedo, 2011). In marked contrast, the current study demonstrates that optimized auxin selection and concentration in etiolated shoots can achieve 100% rooting efficiency with superior morphological quality, results that substantially surpass all previously reported values for this challenging rootstock. This breakthrough reflects both the importance of physiological preconditioning (etiolation) and precise auxin formulation in overcoming the historical propagation barriers for ‘Duke 7’.

From a physiological perspective, the enhanced efficacy observed at higher auxin concentrations may stem from increased hormone availability at the application site, promoting activation of key genes involved in cellular differentiation (such as WOX11/12, *ARF*, and *LBD*) that are essential for adventitious root formation (Lakehal and Bellini, 2019; Li *et al*., 2024). However, this concentration-dependent effect was not uniform across all measured parameters. Callus formation, root length and diameter, and secondary root development showed no significant differences between concentrations (Tables 6 and 7), suggesting that structural root system quality may be modulated by additional factors beyond concentration alone, including auxin type, formulation characteristics, and local hormonal interactions (Druege *et al*., 2016; Lakehal and Bellini, 2019). These differential responses highlight the complex regulatory networks governing root organogenesis, where concentration primarily drives root initiation while other factors determine subsequent root architecture development.

Interactive effects of rooting agent type and concentration

The significant interaction between auxin type and concentration across multiple key rooting variables demonstrates that the morphogenic response of etiolated ‘Duke 7’ shoots depends not merely on the auxin type or applied dose in isolation, but rather on their specific combination (Tables 1 and 2). This interaction was particularly pronounced for critical parameters including: survival rate, rooting percentage, root length and diameter, and secondary root presence (Fig. 3). The non-additive effects reveal complex phytohormonal regulation where certain auxin-concentration combinations synergistically enhance rhizogenesis while others exhibit antagonistic relationships, suggesting tissue-specific saturation thresholds for different auxin

Table 7 - Mean values by rooting agent concentration for the variables: Survival percentage, rooting percentage, quality index, and callus presence percentage in the rooting of etiolated shoots of the avocado rootstock ‘Duke 7’

Concentration	SSP (%)	Rooting percentage	Quality Index (cm)	PCP (%)
24.6 mM	100.00 a [z]	98.91 a	71.74 a	75.00 a
34.4 mM	100.00 a	99.33 a	80.56 a	74.66 a
44.2 mM	99.30 a	96.55 a	104.24 a	58.42 a
CV (%)	5.94	16.44	43.07	50.88
HLSD	0.68	4.51	33.15	17.46
Average	99.93	98.46	85.51	65.33

[z] Average values in the same column followed by different letters indicate statistical differences (Tukey, P<0.05). SSP= Shoot survival percentage; PCP= Percentage of callus presence; CV= Coefficient of variation; HLSD= Honest least significant difference.

formulations. These findings necessitate a dual-parameter optimization approach for clonal propagation protocols, as neither factor alone sufficiently predicts rooting performance.

In contrast to other treatments, increasing IAA concentrations showed a positive correlation with rooting percentage, improving from 66.6% at 24.6 mM to 88.8% at 34.4 mM, and reaching 100% at 44.2 mM. For both IBA and the IBA + NAA combination (Dip'N Grow®) maintained consistent 100% rooting across all three tested concentrations, indicating a broad efficacy window for these formulations. NAA exhibited optimal performance at 24.6 mM and 34.4 mM (100% rooting), but efficacy declined to 77.7% at 44.2 mM, likely reflecting phytotoxic effects at higher doses. Notably, K-IBA performed best at the lowest concentration (100% at 24.6 mM), with progressively reduced rooting at higher levels (88.8% at 34.4 mM and 66.6% at 44.2 mM). These results clearly demonstrate the significant impact of the auxin type × concentration interaction on the rhizogenic response of etiolated avocado shoots (Fig. 3).

Previous studies have demonstrated that the transport and physiological activity of auxins can vary significantly depending on their structure and molecular form (Korasick *et al.*, 2013). For example, IBA undergoes conversion to both IAA and IBA conjugates during plant tissue transport, resulting in prolonged, multiphasic rooting promotion (Damodaran and Strader, 2019). In contrast, externally applied IAA tends to remain in its free form during transport, making it more susceptible to enzymatic inactivation and oxidative degradation (Hayashi *et al.*, 2021). This difference may explain why IBA shows higher efficacy at lower concentrations, while IAA requires higher doses to induce comparable rooting responses.

NAA exhibits superior chemical stability compared to other auxins, allowing prolonged activity in plant tissues (da Costa *et al.*, 2017). This stability stems from its synthetic molecular structure and likely involves specialized transporters that facilitate its movement and accumulation at target sites (Yang *et al.*, 2006; Napier, 2021). Studies report that NAA resists rapid degradation or conjugation in plant tissues, enhancing its capacity to induce abundant root formation (Nissen and Sutter, 1990; Gomes and Scortecci, 2021) (Fig. 4). However, at 44.2 mM, NAA application resulted in the highest root numbers but reduced rooting percentage (77.8%) and suppressed secondary root development (Fig. 4). These findings

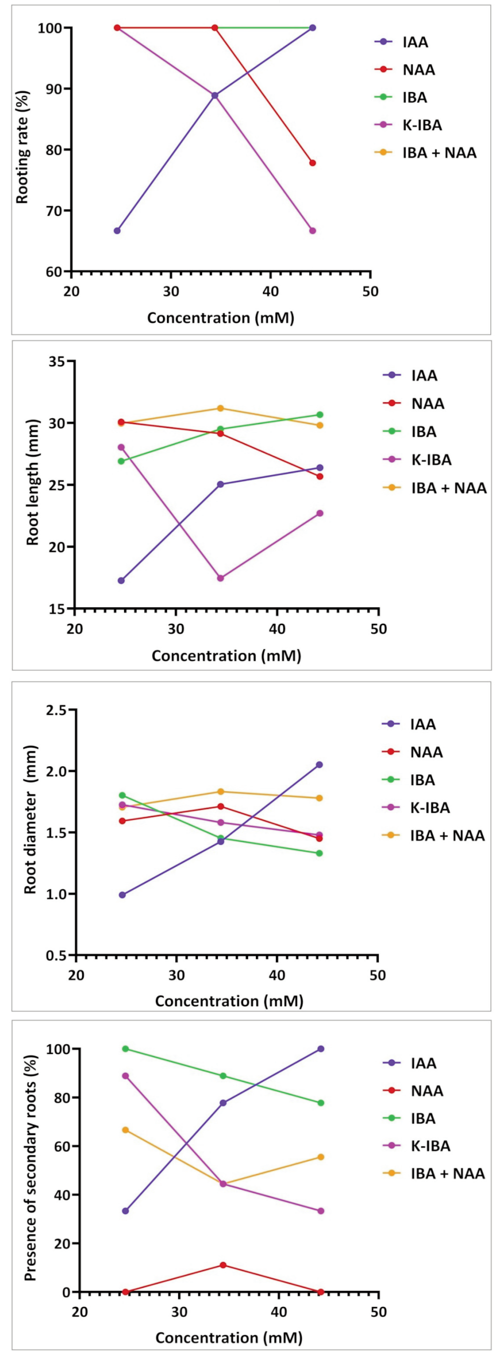

Fig. 3 - Interaction of auxin type and concentration on rooting of etiolated shoots of avocado rootstock 'Duke 7'. IAA: indole-3-acetic acid, NAA= 1-naphthalene acetic acid, IBA= indole-3-butyric acid, K-IBA= indole-3-butyric acid potassium salt, IBA + NAA= indole-3-butyric acid + 1-naphthalene acetic acid (Dip'N Grow®).

suggest that while certain formulations effectively promote primary root formation, they may also cause unintended effects like lateral root inhibition, potentially due to localized hormonal imbalance at the rooting site (Lakehal and Bellini, 2019; Bhalerao *et al.*, 2002).

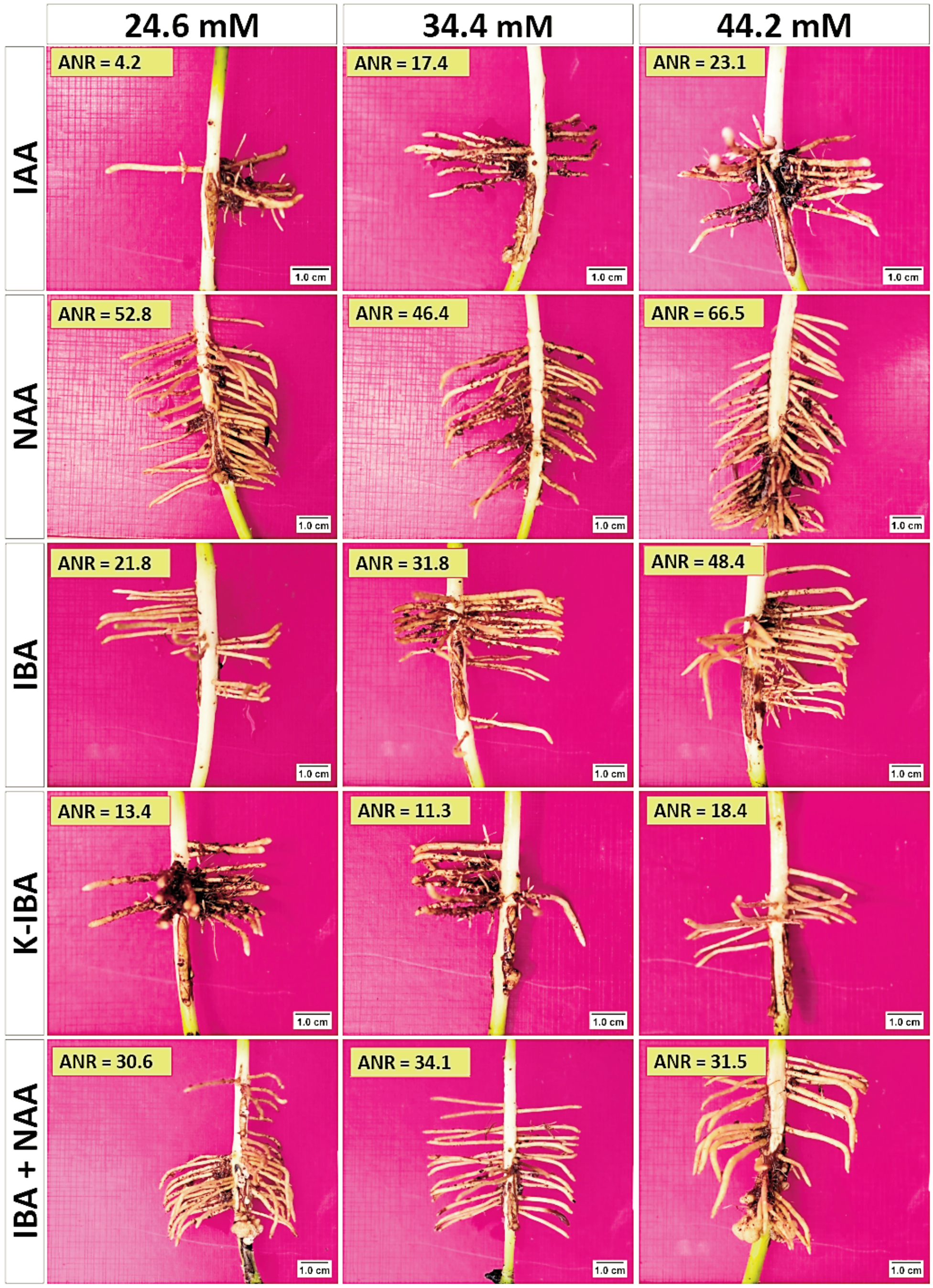

Fig. 4 - Rooting of etiolated shoots of 'Duke 7' rootstock with the application of IAA (indole-3-acetic acid), NAA (1-naphthalene acetic acid), IBA (indole-3-butyric acid), K-IBA (indole-3-butyric acid potassium salt) and IBA + NAA (indole-3-butyric acid + 1-naphthalene acetic acid) at three concentrations. ANR= Average number of roots; Scale bar: 1.0 cm.

The interaction between auxin type and concentration significantly influenced primary root length. The greatest average length (31.19 mm) was achieved with the IBA + NAA combination (Dip'N Grow®) at 34.4 mM, suggesting a synergistic effect favoring root elongation. In contrast, the shortest roots formed with K-IBA at 34.4 mM (17.46 mm) and IAA at 24.6 mM (17.22 mm), indicating reduced efficacy in promoting cellular elongation under these specific conditions. For the remaining treatments, no statistically significant differences were observed, with an average length of 27.83 mm, suggesting a more uniform response across these combinations (Fig. 3).

Root diameter was similarly affected by concentration, particularly in the case of IAA. At 24.6 mM, IAA produced thinner roots (0.97 mm), while at 34.4 mM, it generated thicker roots (2.05 mm), demonstrating a concentration-dependent response in radial root expansion. The other auxins, regardless of concentration, produced roots of intermediate diameter, averaging 1.60 mm with no statistical differences between treatments, indicating more stable growth patterns in terms of root thickness (Fig. 3).

The rooting agent type and concentration interaction exerted a clear effect on secondary root formation. NAA consistently restricted lateral root development across all three tested concentrations (Fig. 4), likely due to its potent growth regulator activity and potential induction of apical dominance or excessive local accumulation in basal tissues (Aloni *et al*., 2006). Recent studies indicate that synthetic auxins like NAA exhibit enhanced stability and tissue persistence, creating strong but localized hormonal signaling that may suppress lateral root initiation through downregulation of Lateral Organ Boundaries Domain (LBD) transcription factors critical for adventitious rooting, and prolonged activation of AUX/IAA repressor proteins that inhibit auxin-dependent gene expression by blocking ARF transcription factors (Lakehal and Bellini, 2019; Jing and Strader, 2019). This dual regulation at the genetic level explains NAA's capacity to simultaneously promote primary root growth while inhibiting secondary root formation.

In contrast, IAA demonstrated a positive and progressive effect on secondary root formation as concentration increased, likely attributable to its lower stability. This auxin undergoes rapid metabolic conversion or conjugation, preventing excessive accumulation while enabling dynamic tissue transport, characteristics that facilitate lateral root differentiation (Casanova-Sáez *et al*., 2021; Zhang *et al*., 2023).

These findings underscore the importance of developing tailored propagation protocols that carefully consider both auxin type and optimal concentration for specific plant materials. For commercial nurseries where consistency and productivity are paramount, pre-mixed formulations such as IBA+NAA (Dip'N Grow®) may serve as practical solutions, though they require precise concentration adjustments to prevent phytotoxic effects in sensitive etiolated tissues. Critical implementation considerations include establishing appropriate concentration thresholds for different auxin combinations, accounting for tissue-specific sensitivity variations, and carefully balancing the trade-offs between root quantity and overall root system quality. The research demonstrates that successful clonal propagation depends on this multifaceted optimization approach rather than relying on standardized auxin applications.

The significant interaction between auxin type and concentration conclusively refutes the concept of a "universal concentration" suitable for all rootstocks or growing conditions. This research instead provides empirical evidence supporting the development of customized propagation protocols through careful refinement of auxin formulations, adjustments based on the physiological state of plant material, and optimization of multiple interdependent parameters.

These insights prove particularly valuable for enhancing clonal propagation of difficult-to-root rootstocks such as 'Duke 7', where well-balanced root system architecture critically determines subsequent field performance. The data-driven methodology established in this study could be effectively adapted to improve propagation protocols for other commercially significant avocado cultivars, potentially revolutionizing nursery production standards through science-based precision agriculture approaches.

4. Conclusions

The results demonstrated that auxin type and concentration significantly and differentially influenced adventitious root induction in etiolated shoots of 'Duke 7' avocado rootstock. These effects were evident in both rooting percentages and the morphological quality of the root system.

NAA promoted the highest root number and quality index, but also induced substantial callus formation and reduced secondary root development, suggesting potential phytotoxicity at elevated concentrations. In contrast, IAA showed a more balanced dose-dependent response and enhanced secondary root growth, while IBA and its commercial formulation with NAA (IBA + NAA) provided stable and reliable performance.

Auxin concentration modulated rooting efficiency, with dose-dependent physiological responses being most pronounced for IAA and K-IBA. The tested concentration range (24.6-44.2 mM) proved effective for rooting (>95%) while maintaining shoot survival in most treatments.

A clear auxin type × concentration interaction was observed, emphasizing the need for genotype (and physiological state) specific optimization of both factors. The responses documented are not universal and must be considered when designing propagation protocols for avocado rootstocks.

The combination of etiolated shoots with properly selected and dosed auxins achieved 100% rooting in certain treatments, surpassing results from traditional protocols. These findings represent significant progress toward standardizing and optimizing clonal propagation of 'Duke 7' for commercial production.

This study provides experimental evidence for developing more efficient, reproducible, and economically viable protocols applicable in commercial nurseries. By improving the availability of clonal avocado rootstocks, these advances will enhance the sustainability and competitiveness of avocado production systems. The optimized protocols specifically address three industry needs: consistent rooting success, superior root system architecture, and scalable production methods. Future research should explore applications to other commercially important rootstock varieties while maintaining the precision agriculture approach demonstrated here.

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