

Article

Beyond Conventional Auxins: Evaluating DCPE and DCP Pulse Applications for Enhanced Rooting in *Lavandula angustifolia* Mill.

Hajer Darouez and Stefaan P. O. Werbrouck * 

Laboratory for Applied In Vitro Plant Biotechnology, Ghent University, 9000 Ghent, Belgium; hajer.darouez@ugent.be

* Correspondence: steffa.werbrouck@ugent.be

Abstract

Efficient adventitious root formation is crucial for *Lavandula angustifolia* Mill. propagation. This study evaluated the effects of continuous and short-duration pulse applications (1 min, 1 h, and 1 day) of the auxin dichlorprop (DCP) and its prodrug dichlorprop-2-ethylhexyl ester (DCPE) at varying concentrations on adventitious rooting and callus formation. DCPE generally proved more effective than DCP in promoting rooting, especially at lower concentrations, with continuous application of 0.1 μ M DCPE yielding the highest number of adventitious roots. Notably, a brief 1 min pulse of 2.5 μ M DCPE induced superior rooting, including high root number and weight, while minimizing callus formation compared to longer exposures. In contrast, 1 h pulse treatments showed a positive correlation between auxin concentration and root number but led to substantial callus development. These findings highlight DCPE's potential as an efficient auxin source for lavender propagation, likely due to its rapid hydrolysis to active DCP within plant tissues, facilitating systemic distribution. The enhanced rooting achieved with short pulse treatments offers significant implications for optimizing commercial propagation for this economically important aromatic plant.

Keywords: adventitious rooting; auxin prodrugs; *L. angustifolia*; auxin pulse treatment; in vitro propagation



Academic Editor: Alfonso Albacete

Received: 31 May 2025

Revised: 2 July 2025

Accepted: 8 July 2025

Published: 10 July 2025

Citation: Darouez, H.; Werbrouck, S.P.O. Beyond Conventional Auxins: Evaluating DCPE and DCP Pulse Applications for Enhanced Rooting in *Lavandula angustifolia* Mill. *Agronomy* **2025**, *15*, 1677. <https://doi.org/10.3390/agronomy15071677>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The perennial aromatic shrub, *Lavandula angustifolia* Mill., commonly known as lavender, holds considerable commercial value. Its essential oil is widely utilized across the perfume, cosmetic, pharmaceutical, and food sectors, further complemented by its medicinal attributes and ornamental appeal [1]. Given the increasing worldwide demand for lavender and its derivatives, optimizing propagation methods is crucial for developing sustainable and cost-effective production systems [1]. While both sexual (seed) and asexual (vegetative) propagation are possible, vegetative methods are favored in commercial settings to guarantee genetic uniformity and the retention of desirable traits [2]. Despite this preference, a key hurdle in achieving successful vegetative propagation remains the consistent and efficient formation of adventitious roots [3].

Auxins, a class of plant growth regulators (PGRs), are crucial for initiating adventitious roots and improving rooting in various plant species [4–6]. Key conventional auxins include indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NAA).

IAA, the most abundant natural auxin, controls root system architecture and development, with exogenous application consistently affecting root growth [7]. IBA also promotes adventitious root development [8,9] and is more stable than IAA, persisting longer in plant tissues [10–12].

Dichlorprop-P-2-ethylhexyl (DCPE, Corasil) belongs to the bipartite auxin prodrugs. The concept of prodrugs, originating and extensively developed within human pharmacology, offers a strategy to overcome inherent limitations in the direct administration of active compounds [13]. This paradigm involves the design of inactive compounds that are subsequently converted into their active forms within a biological system. DCPE is an auxin ester that needs activation within the plant through hydrolysis by esterases. This mechanism distinguishes it from classical auxins, as it exhibits different uptake and transport characteristics [14]. The active form, dichlorprop (DCP), is a versatile auxin-type phenoxy compound used in agriculture and horticulture, where it serves as a selective herbicide. DCPE functions as a selective herbicide but also has commercial applications as a plant growth regulator, increasing fruit size and preventing premature fruit drop in citrus [15,16].

Notably, both DCPE and DCP, at a concentration of 1 μM , induced not only callus but also abundant aerial in vitro root formation in poplar, a phenomenon never seen with traditional auxin treatments [14]. We observed a similar exaggerated auxin effect in *L. angustifolia*. This led us to hypothesize that these potent auxins could be precisely controlled by applying low concentrations in short pulses. Such an approach could refine rooting protocols, not only for lavender, but ultimately contribute to the efficient micropropagation of woody perennials.

2. Materials and Methods

2.1. Propagating In Vitro Shoot Cultures

In vitro propagation of *Lavandula angustifolia* ‘Hidcote’ was conducted using foliated nodal segments (approximately 1.5 cm in length). These explants were cultured in a modified Murashige and Skoog (MSmod3B) medium (Duchefa, Haarlem, The Netherlands) [17], which contained half the standard concentrations of NH_4NO_3 and KNO_3 . The medium was supplemented with 3% sucrose and 0.7% Plant Agar (Duchefa, Haarlem, The Netherlands). Meta-Topolin Riboside (mTR) was added at a concentration of 0.74 mg/L prior to adjusting the pH to 5.8. Sterilization of the medium was achieved by autoclaving for 20 min at 121 °C. Cultures were maintained at 25 ± 1 °C under a 16/8 h light/dark photoperiod. Illumination was provided by a 40 W LED panel (Ledstores Europe, Amsterdam, The Netherlands) emitting warm white fluorescent light (3000 K) at a photosynthetic active radiation (PAR) of $40 \mu\text{mol m}^{-2} \text{s}^{-1}$. Subculturing was executed every six weeks.

2.2. Root Induction Through Continuous Application of DCP and DCPE

Internodal stem segments, measuring 1.5 ± 0.2 cm in length, were excised from existing shoot cultures. These segments were then transferred to 380 mL glass containers containing MSmod3B medium supplemented with one of five concentrations of either DCP or DCPE (0, 0.01, 0.1, 1, or 10 μM) following autoclaving. The experiment employed a completely randomized design with five treatments (each concentration of DCP/DCPE as a separate treatment). Each treatment consisted of three replicate containers, with five in vitro shoots serving as the experimental units within each container. The entire experiment was repeated three times independently. Cultures were maintained under controlled environmental conditions: a temperature of 25 ± 1 °C and a 16/8 h light/dark photoperiod. After a four-week incubation period, plantlets were carefully removed from the culture medium and thoroughly washed with distilled water to remove any remaining

agar. A detailed evaluation was then performed on each of the 45 explants per treatment. This evaluation included manually counting the number of adventitious and lateral roots. Root lengths (average and maximum) and shoot length were measured using a ruler. For fresh weight measurements, roots and callus were immediately separated and weighed using an analytical balance with a precision of 0.0001 g.

2.3. Root Induction Through Pulse Treatment of DCP and DCPE in *L. angustifolia*

Five foliated nodal explants, each measuring approximately 1.5 cm in length, were inoculated into 380 mL glass vessels containing 100 mL of MSmod3B medium. For root induction, microshoots were treated with MS medium supplemented with different concentrations of DCP and DCPE (1.25, 2.5, and 5 μM) for three distinct pulse durations: 1 min, 1 h, and 1 day. To ensure uniform hydration and physiological condition, all explants were freshly excised and immediately placed in hormone-free modified MS medium while preparing the entire batch. Once all explants were prepared, they were simultaneously transferred into the auxin-containing pulse medium for the assigned duration (1 min, 1 h, or 1 day). This approach minimized variation in exposure timing and ensured consistent pre-treatment handling across all replicates. All pulse treatments were performed under ambient laboratory conditions ($25 \pm 1^\circ\text{C}$). Following these pulse treatments, the explants were transferred to hormone-free MSmod3B medium for subsequent development. Each treatment included three containers, each containing five in vitro shoots as experimental units. The cultures were maintained at $25 \pm 1^\circ\text{C}$ with a 16/8 h light/dark cycle. After four weeks, we recorded the number of adventitious roots, lateral roots, average adventitious root length, maximum root length, root fresh weight, callus fresh weight, and shoot length.

2.4. Statistical Analysis

Experiments were conducted in triplicate. Statistical analyses were carried out using IBM SPSS Statistics for Windows, version 29 (IBM Corp, North Castle, NY, USA). The normality of the data was determined by the Kolmogorov–Smirnov test. Given the non-normal distribution, the Kruskal–Wallis test (non-parametric) was applied to assess inter-group differences at $\alpha = 0.05$.

3. Results

3.1. Root Induction Through Continuous Application of Auxin Analogs

Figures 1 and 2 illustrate the dose response of continuous administration of DCP and DCPE on various root parameters in *L. angustifolia* after 28 days of in vitro culture. At 0.01 μM , both DCP and DCPE stimulated the formation of adventitious roots to a greater extent than the control, with DCPE showing a more pronounced effect. This positive influence on adventitious root number was even more evident at 0.1 μM , where DCPE induced the highest number of roots, significantly outperforming DCP at the same concentration. Lateral root formation also responded positively to both compounds at 0.1 μM , showing a substantial increase compared to the control. However, 0.1 μM of both analogs had a minimal impact on lateral root development. The effect on average adventitious root length followed a different pattern. The application of DCP at 0.1 μM resulted in the longest roots, followed by DCPE at 0.01 μM , both leading to significantly longer roots than the control. Root biomass, as indicated by weight, was enhanced by both compounds, with the 0.1 μM DCPE yielding the greatest root weight, significantly surpassing both the control and the higher concentration of DCP. Similarly, shoot length was improved by all treatments compared to the control. However, this enhanced growth was associated with increased callus formation at 0.1 μM for both compounds, with DCPE inducing more callus than DCP.



Figure 1. Effects of DCP and DCPE concentrations on in vitro root induction in lavender after 28 days. (a) Hormone-free control and (b–e) increasing concentrations of DCP (0.01, 0.1, 1, and 10 μ M), and (f–i) increasing concentrations of DCPE (0.01, 0.1, 1, and 10 μ M). Scale bar = 1 cm.

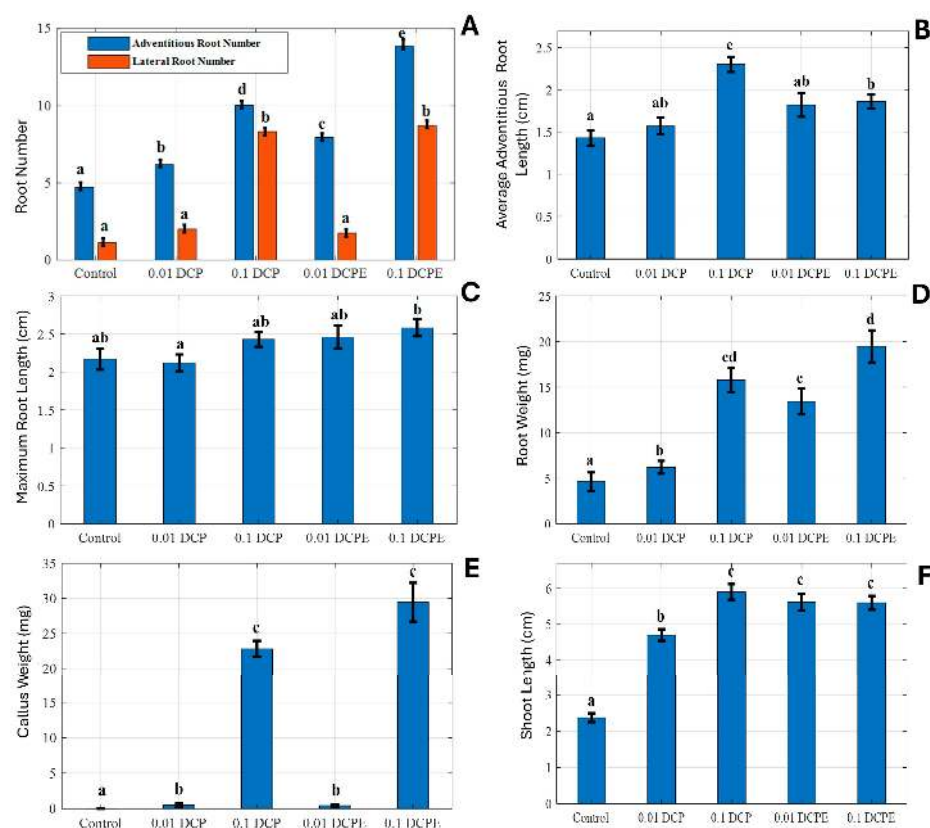


Figure 2. Influence of continuous low concentrations (0.01 and 0.1 μ M) of DCP and DCPE on root, shoot, and callus formation in *L. angustifolia* after 28 days of in vitro culture. (A) Root number; (B) average adventitious root length; (C) maximum root length (cm); (D) root weight (mg); (E) callus weight (mg); (F) shoot length (cm). Bars represent averages $\pm$ SE. Bars sharing the same letter are not significantly different ($\alpha = 0.05$).

At a concentration of 1 μM , both DCP and DCPE induced rooting so extensively that it could not be accurately quantified. As Figure 3 illustrates, aerial root development was observed across the basal, middle, and upper sections of the plants after 15 days. Root induction following DCPE treatment appeared to result in stronger and more widespread rooting compared to DCP, particularly in the basal and middle stem regions, characterized by a high density of root hairs and substantial callus formation. In comparison, we tested the effects of IBA and NAA at 10 μM each (Figure A1 and Table A1) but did not observe similar rooting responses. At 1 μM , both IBA and NAA induced limited root formation, often accompanied by moderate callus development. At 10 μM , both compounds showed signs of toxicity, also resulting in callus formation but without significant root elongation or density. These findings highlight the superior rooting performance of DCP and particularly DCPE under the tested conditions.



Figure 3. Rooting responses of *L. angustifolia* stems and leaves to 1 μM DCP (a–d) and DCPE (e–h) after 15 days. Panels show rooting at different stem positions: (a,e) basal, (b,f) middle, and (c,g) upper; (d,h) rooting on leaves from middle stems. Scale bar = 1 mm.

3.2. Root Induction Through Pulse Treatment of DCP and DCPE in *L. angustifolia*

3.2.1. A 1-Day Pulse Treatment with 1.25 μM DCP and DCPE

Figure 4 shows that exposure to a 1-day pulse at 1.25 μM induced substantial and potentially adverse morphological responses in both treatment groups. Callus formation and aerial root development were observed with both DCP and DCPE. However, DCPE-treated plants (a, b) exhibited a more pronounced response, characterized by more extensive root development and stronger overall morphological changes than those treated with DCP (c, d), which also displayed these features. In fact, the extensive root proliferation observed, particularly in the DCPE treatment, often made accurate quantification of root number and length parameters impractical in these pulsed treatments. This suggests a high bioactivity for both compounds, with DCPE eliciting a more potent effect on plant morphology.

3.2.2. A 1 h Pulse Treatment

Table 1 and Figure 5 illustrate that a 1 h pulse treatment with DCP and DCPE (1.25–5 μM) significantly enhanced root development in lavender, exhibiting distinct concentration-dependent responses. While 5 μM DCPE yielded the highest adventitious root number, DCPE generally outperformed DCP, especially at lower concentrations, where it also promoted greater root biomass. Higher auxin concentrations, while boosting root

initiation, inhibited root elongation. Notably, a substantial callus formation occurred across all 1 h treatments, increasing with concentration, suggesting a potential shift towards callus proliferation at longer exposures. Shoot length was consistently enhanced by all auxin treatments, irrespective of concentration.

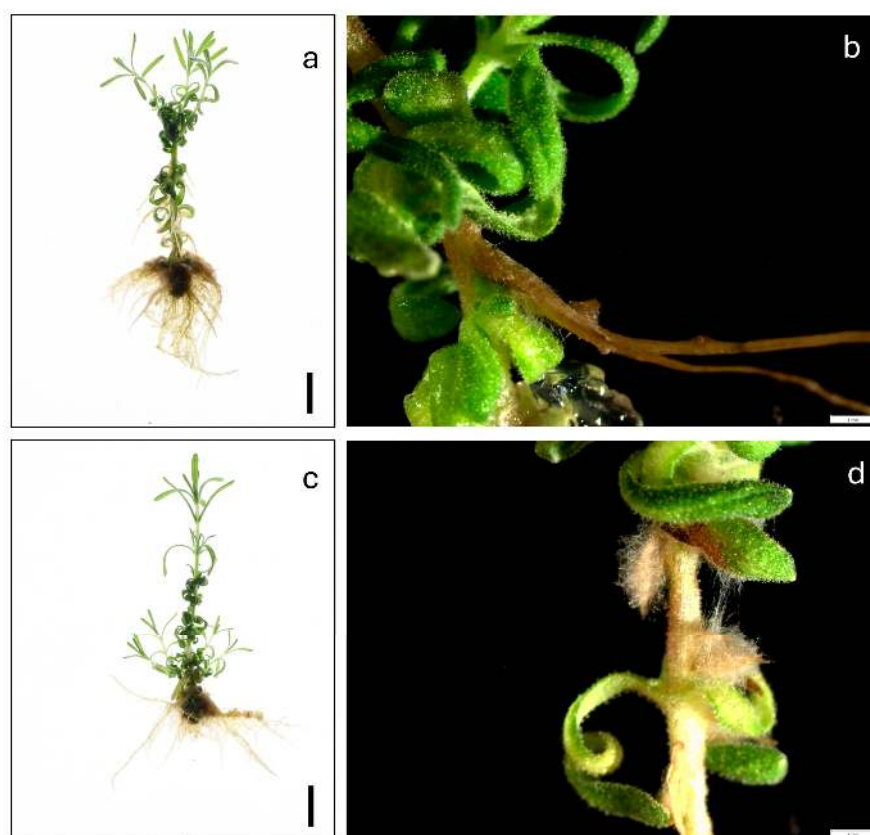


Figure 4. Effect of a 1-day pulse treatment of 1.25 μM DCP and DCPE on *L. angustifolia* rooting. (a) Plant treated with DCPE (scale bar = 1 cm); (b) close-up of middle section treated with DCPE; (c) plant treated with DCP (scale bar = 1 cm); (d) close-up of middle section treated with DCP (scale bar = 1 mm).

Table 1. Effects of a 1 h pulse treatment of DCPE and DCP on adventitious and lateral root development, including root number, average adventitious root length, maximum root length (cm), and root and callus fresh weights (mg) in *L. angustifolia* after 28 days of in vitro culture.

Auxin Prodrug Treatments (μM)	Adventitious Root Number	Lateral Root Number	Average Adventitious Root Length (cm)	Maximum Root Length	Root Weight (mg)	Shoot Length (cm)	Callus Weight (mg)
Control	4.49 ± 0.27^a	0.82 ± 0.17^a	1.07 ± 0.09^a	1.39 ± 0.09^a	3.23 ± 0.5^a	3.93 ± 0.54^a	0 ± 0^a
1.25 DCP	8.42 ± 0.35^b	4.36 ± 0.29^b	2.13 ± 0.12^d	3.46 ± 0.13^e	9.65 ± 1.59^b	5.45 ± 0.22^b	15.92 ± 1.33^b
2.5 DCP	9.44 ± 0.42^b	7.93 ± 0.44^c	1.69 ± 0.10^c	2.49 ± 0.14^d	8.11 ± 1.04^b	5.23 ± 0.27^b	26.52 ± 1.99^c
5 DCP	12.04 ± 0.46^c	3.91 ± 0.34^b	1.25 ± 0.09^{ab}	1.93 ± 0.21^b	12.62 ± 1.52^c	5.02 ± 0.26^b	41.76 ± 2.58^d
1.25 DCPE	11.02 ± 0.35^c	4.04 ± 0.38^b	2.32 ± 0.13^d	3.23 ± 0.16^e	17.4 ± 1.51^d	5.16 ± 0.18^b	23.42 ± 1.49^c
2.5 DCPE	11.91 ± 0.45^c	3.87 ± 0.39^b	1.37 ± 0.07^b	3.31 ± 0.73^d	9.28 ± 1.27^{bc}	4.99 ± 0.2^b	28.71 ± 2.04^c
5 DCPE	13.38 ± 0.76^c	7.64 ± 0.48^c	1.13 ± 0.05^a	2.68 ± 0.63^c	11.2 ± 1.19^{bc}	5.04 ± 0.18^b	45.31 ± 4.43^d

Averages $\pm$ SE followed by the same letter in the same column are not significantly different at $p < 0.05$ according to the Kruskal–Wallis H test.

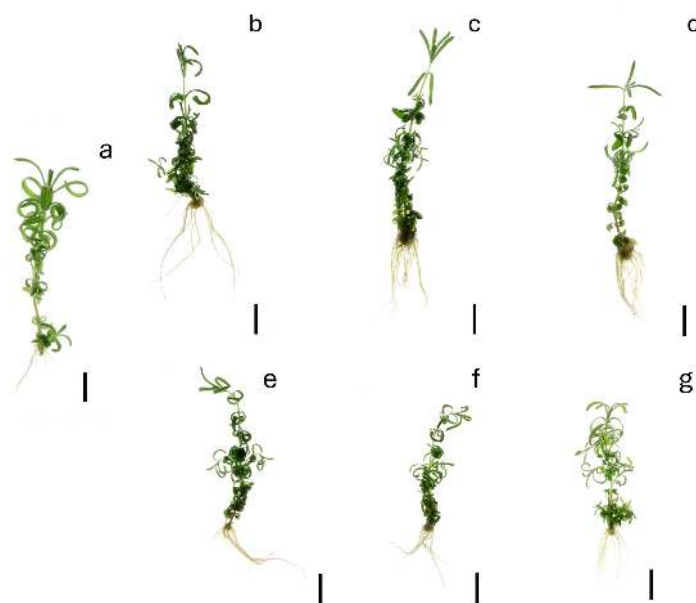


Figure 5. Rooting and development of *L. angustifolia* after a 1 h pulse treatment with DCPE and DCP, observed after 28 days. Treatments included (a) control; (b–d) DCPE at 1.25, 2.5, and 5 μ M; and (e–g) DCP at 1.25, 2.5, and 5 μ M. Scale bar = 1 cm.

3.2.3. A 1 Min Pulse Treatment

As illustrated in Table 2 and Figures 6 and A2, a brief one-minute pulse treatment with DCP and DCPE (1.25–5 μ M) significantly improved root development in lavender. Specifically, 2.5 μ M of DCPE proved exceptionally effective, leading to the highest numbers of adventitious and lateral roots, and superior average and maximum root lengths and root weight, when compared to all other treatments. It is worth noting that the 5 μ M pulse treatments with both DCP and DCPE induced callus formation, a characteristic not observed at lower concentrations, which remained callus-free. Furthermore, one-minute pulses of DCP and DCPE did not negatively impact lateral root length, total root length, or root weight. A decline in these parameters only began to manifest at the 5 μ M concentration.

Table 2. Effects of a 1 min pulse treatment with DCPE and DCP on adventitious and lateral root development, including root number, average adventitious root length, maximum root length (cm), and root and callus fresh weights (mg) in *L. angustifolia* after 28 days of in vitro culture.

Auxin Prodrug (μ M)	Adventitious Root Number	Lateral Root Number	Average Adventitious Root Length (cm)	Maximum Root Length	Root Weight (mg)	Shoot Length (cm)	Callus Weight (mg)
Control	4.49 $\pm$ 0.27 ^a	0.82 $\pm$ 0.17 ^a	1.07 $\pm$ 0.09 ^a	1.39 $\pm$ 0.09 ^a	3.23 $\pm$ 0.5 ^a	3.93 $\pm$ 0.54 ^a	0 $\pm$ 0 ^a
1.25 DCP	6.09 $\pm$ 0.3 ^b	1.02 $\pm$ 0.19 ^a	2.21 $\pm$ 0.13 ^c	2.68 $\pm$ 0.13 ^b	8.64 $\pm$ 1.27 ^b	5.15 $\pm$ 0.22 ^b	0 $\pm$ 0 ^a
2.5 DCP	10.51 $\pm$ 0.38 ^{de}	12.78 $\pm$ 0.85 ^c	3.21 $\pm$ 0.14 ^d	5.1 $\pm$ 0.22 ^c	23.83 $\pm$ 2.56 ^c	5.29 $\pm$ 0.18 ^b	0 $\pm$ 0 ^a
5 DCP	7.2 $\pm$ 0.4 ^c	10.51 $\pm$ 0.62 ^b	2.22 $\pm$ 0.12 ^c	2.7 $\pm$ 0.14 ^b	11.49 $\pm$ 1.67 ^b	4.44 $\pm$ 0.27 ^b	8.79 $\pm$ 1.06 ^c
1.25 DCPE	9.64 $\pm$ 2.04 ^d	1.27 $\pm$ 0.21 ^a	1.77 $\pm$ 0.1 ^b	2.77 $\pm$ 0.11 ^b	11.12 $\pm$ 1.27 ^b	4.88 $\pm$ 0.21 ^b	0 $\pm$ 0 ^a
2.5 DCPE	11.36 $\pm$ 0.58 ^e	17.53 $\pm$ 0.86 ^d	3.47 $\pm$ 0.14 ^d	5.73 $\pm$ 0.23 ^c	30.14 $\pm$ 3.14 ^c	5.4 $\pm$ 0.15 ^b	0 $\pm$ 0 ^a
5 DCPE	8.02 $\pm$ 0.47 ^c	13.78 $\pm$ 0.81 ^c	2.41 $\pm$ 0.14 ^c	5.3 $\pm$ 0.25 ^c	13.26 $\pm$ 2 ^b	5 $\pm$ 0.23 ^b	10.13 $\pm$ 3.5 ^b

Averages $\pm$ SE followed by the same letter in the same column are not significantly different at $p < 0.05$ according to the Kruskal–Wallis H test.

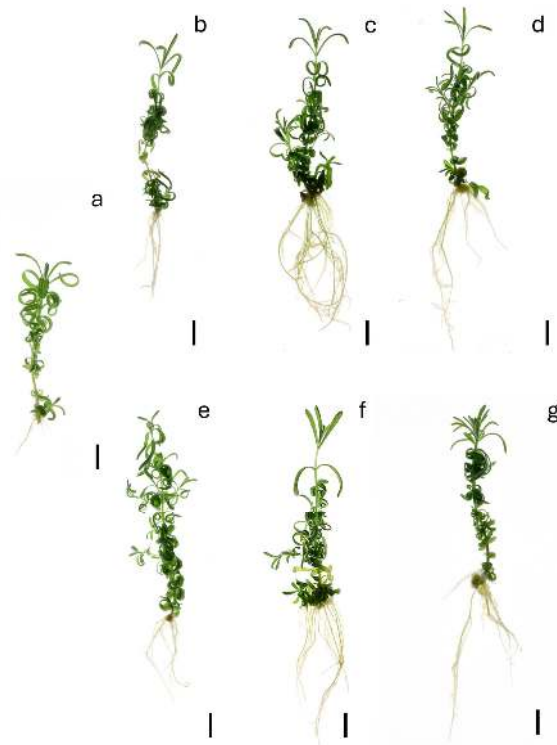


Figure 6. Effect of a 1 min pulse of DCPE and DCP on rooting and development of *L. angustifolia* after 28 days of in vitro culture. Treatments included (a) control; (b–d) DCPE at 1.25, 2.5, and 5 μM ; and (e–g) DCP at 1.25, 2.5, and 5 μM . All pulses were 1 min in duration. Scale bar = 1 cm.

4. Discussion

Upon continuous exposure, DCPE and DCP uniquely induce root initiation systemically across the entire plant. This differs significantly from conventional auxins like indole-3-butylic acid (IBA) and 1-naphthaleneacetic acid (NAA). Although IBA and NAA are known to promote strong root formation in many tree species (e.g., IBA in *Prunus avium* [4,5,18], *Syzygium maire*, *Poirertia latifolia*; NAA in *Phoenix dactylifera* [19], *Pyrus communis* [20]), they lack this distinct whole-plant rooting effect. Given the extensive rooting observed, we proceeded to examine the concentration-dependent responses to DCP and DCPE. While both compounds successfully promoted adventitious root formation, DCPE exhibited greater efficacy at lower concentrations (0.01 and 0.1 μM), indicating a more potent or optimized mode of action. This advantage is likely attributable to the lipophilic nature of its ester structure, which may facilitate improved penetration into plant tissues and a gradual intracellular hydrolysis that releases active DCP in a controlled manner [21]. This mechanism leads to improved systemic availability of the auxin without causing a sudden surge of the free acid [22]. Darouez et al. [14] demonstrated that in *Populus × canadensis*, DCPE rapidly converts to active DCP and exhibits enhanced systemic movement, inducing both adventitious and aerial roots. This distinct metabolic behavior is reflected in the extreme aerial rooting responses observed in *L. angustifolia* in the current study, particularly with continuous exposure to the auxin prodrug. While continuous exposure to auxin, even at 1 μM , which stimulated aerial root (AR) formation, indicates a strong rooting capacity, it might not be ideal for micropropagation due to potential handling challenges and reduced plantlet quality. Increasing the concentration to 10 μM did not further increase the number of basal ARs; instead, the ARs became shorter and gradually surrounded by callus tissue. This observation is consistent with the well-known biphasic action of active auxins, which promote root initiation but can inhibit their elongation [23–25].

In *L. angustifolia*, 1 h and 1-day pulse treatments with both DCP and DCPE significantly promoted root formation but also induced substantial callus development, especially at higher concentrations. This outcome likely stems from the overstimulation of auxin signaling caused by prolonged exposure, even in these transient treatments. The well-documented biphasic effect of auxins reveals that while optimal concentrations and durations facilitate organized organogenesis like adventitious rooting, excessive or prolonged exposure often triggers dedifferentiation and callus formation [26]. Our findings suggest that auxin accumulation during the 1 h and, particularly, the 1-day treatments surpassed the physiological threshold required for targeted root induction, leading to broader, less specific developmental responses. As demonstrated by Dubrovsky et al. [27], localized auxin maxima are crucial for specifying lateral root founder cells. The disruption of these precise spatial signals, whether due to auxin overload or systemic saturation, can impair the cellular patterning necessary for root primordia formation. Furthermore, sustained auxin exposure may saturate auxin receptors or alter tissue polarity, resulting in widespread stimulation of cell division and unorganized growth, as previously described by Benjamins et al. [28]. This principle is broadly supported by studies on plant hormones. For instance, in vitro studies on chestnut and oak shoots demonstrate that while moderate auxin treatments promote adventitious rooting, excessive auxin leads to callus formation and shoot tip necrosis [29]. Justamante et al. [30] demonstrated in vitro that exogenous application of IBA significantly improved the rooting performance of *Prunus* rootstock microcuttings, though the response was strongly genotype-dependent. While moderate IBA treatments enhanced adventitious root formation, prolonged or excessive exposure led to increased callus formation and reduced root length, indicating that a short pulse of IBA is optimal for triggering root initiation. Similarly, Bai et al. [31] found in apple rootstocks that IBA promotes adventitious root initiation, but extended or high-concentration treatments inhibit root elongation, an effect mediated in part by increased ethylene production.

The reduced effectiveness of the 1.25 μM DCPE pulse treatment (1 min duration) compared to the best treatment, 2.5 μM DCPE, can be attributed to an insufficient auxin signal to fully initiate the adventitious root formation process. Auxin acts as a local morphogenetic trigger, and its role in root initiation is highly dose-dependent. Dubrovsky et al. [27] demonstrated that a critical threshold of auxin accumulation is necessary to specify founder cells and initiate lateral root formation. At a low concentration and brief exposure, the amount of DCPE absorbed and subsequently hydrolyzed into active DCP may not have been adequate to activate the necessary auxin-responsive genes and downstream pathways. Furthermore, the absence of callus formation at 1.25 μM suggests that while the treatment was non-phytotoxic, it was also too mild to trigger a full morphogenic response. In contrast, 2.5 μM DCPE likely surpassed this threshold, providing a sufficient and transient auxin stimulus that activated root-specific developmental pathways without causing stress-related effects like excessive callusing. This finding supports the broader understanding that auxin effectiveness depends not only on presence but also on achieving a critical dose–time combination capable of inducing organogenic commitment [15,26]. Recent research highlights that the effectiveness of auxin in inducing organogenesis depends not only on its presence but also on achieving a critical combination of concentration and exposure time. For successful organogenic commitment, plant cells must experience auxin above a threshold level for a sufficient duration, a principle demonstrated both in vitro and in vivo [32,33]. This dose–time relationship is fundamental to practical horticultural techniques, such as the quick-dip method, where cuttings are briefly immersed in concentrated auxin solutions to initiate root formation without causing inhibitory effects associated with prolonged or excessive exposure [34]. Such approaches ensure that auxin stimulates root initiation efficiently, reflecting the synchronized cellular and molecular

processes that govern this development [35]. Our findings provide a mechanistic basis for why horticultural practices like quick dips in auxin solutions are effective: they deliver a critical, temporally controlled auxin pulse that is sufficient to trigger root initiation without the deleterious effects of prolonged exposure [36]. Interestingly, increasing the DCPE concentration to 5 μM for the same 1 min pulse led to a reduction in root number and some callus formation, suggesting a possible threshold beyond which temporary toxicity or receptor saturation may occur. This phenomenon is not new. It is similar to what has been observed with other auxins, such as NAA, where high concentrations are known to inhibit root initiation [26,37]. The success of short DCPE pulses in *L. angustifolia* aligns with similar results using pulse applications of IBA in *Prunus avium* [18], reinforcing that a brief, optimized auxin signal is sufficient for effective rooting across species. The superior rooting achieved by DCPE with this short pulse treatment holds significant promise for commercial propagation, suggesting that effective rooting can be achieved with a brief auxin application, potentially minimizing phytotoxicity and lowering production costs, especially for valuable crops such as lavender [38].

All auxin treatments (both DCP and DCPE, at various concentrations and durations) consistently improved shoot length compared to the control. This effect is attributed to the fundamental roles of auxins in promoting both cell elongation and cell division, which are essential processes for plant growth and development. By stimulating cell expansion and proliferation within shoot tissues, auxins contribute to increased stem elongation and overall plant vigor. For example, research has shown that application of auxins such as indole-3-butyric acid (IBA) leads to significant increases in shoot length and leaf area in various plant species [39,40]. These findings are supported by broader reviews of plant hormone action, which confirm that auxins are critical regulators of shoot development and elongation [26,34,41]. Future studies could investigate the molecular mechanisms underlying DCPE's enhanced efficacy, explore its application in other woody perennials, or optimize large-scale propagation systems based on these findings.

5. Conclusions

This study effectively demonstrates the superior efficacy of the auxin prodrug dichloroprop-2-ethylhexyl ester (DCPE) in fostering adventitious root formation in *Lavandula angustifolia*. A particularly notable finding is that a brief 1 min pulse treatment with 2.5 μM DCPE yielded exceptional rooting, characterized by a high number of roots and significant root weight, while concurrently minimizing undesirable callus development compared to longer exposure times. This outcome underscores the potential of short-duration DCPE applications to precisely regulate auxin availability, thereby facilitating efficient root initiation without the typical drawbacks associated with prolonged exposure to conventional auxins. Future research could investigate the molecular mechanisms underlying the enhanced efficacy of DCPE pulses. This could involve exploring its application in other woody perennials, not only in vitro but also for the rooting of recalcitrant horticultural cuttings in vivo.

Author Contributions: Conceptualization, S.P.O.W. and H.D.; methodology, H.D.; software, H.D.; validation, H.D.; formal analysis, H.D.; investigation, H.D.; resources, S.P.O.W.; data curation, H.D.; writing—original draft preparation, S.P.O.W. and H.D.; writing—review and editing, S.P.O.W. and H.D.; visualization, H.D.; supervision, S.P.O.W.; project administration, S.P.O.W.; funding acquisition, S.P.O.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request.

Acknowledgments: This article is based upon work from COST Action CA21157 “COPYTREE, European Network for Innovative Woody Plant Cloning” supported by COST (European Cooperation in Science and Technology) (www.cost.eu).

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

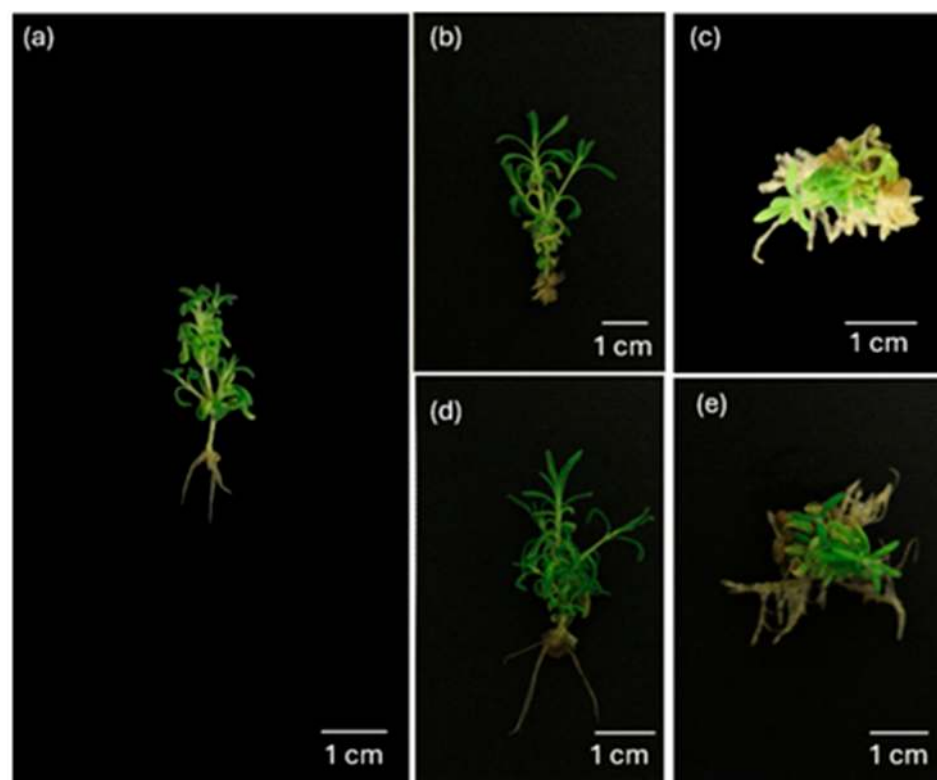


Figure A1. Influence of auxin treatments on rooting and shoot growth in *L. angustifolia* after three weeks of in vitro culture. Treatments include (a) control (hormone-free medium); (b) 1 μ M 1-naphthaleneacetic acid (NAA); (c) 10 μ M NAA; (d) 1 μ M indole-3-butyric acid (IBA); (e) 10 μ M IBA.

Table A1. Effect of auxins on root parameters, shoot length, and callus weight of lavender after three weeks of in vitro culture.

Auxins (μ M)	Adventitious Root Number	Lateral Root Number	Average Adventitious Root Length (cm)	Root Weight (mg)	Shoot Length (cm)	Callus Weight (mg)
no auxin	4.22 $\pm$ 0.27a	0.73 $\pm$ 0.13b	0.82 $\pm$ 0.09b	0.76 $\pm$ 0.07a	0.9 $\pm$ 0.05a	0 $\pm$ 0a
1 μ M NAA	8.13 $\pm$ 0.36b	0 $\pm$ 0a	0.43 $\pm$ 0.02a	3.8 $\pm$ 0.34c	1.17 $\pm$ 0.07b	27.13 $\pm$ 2.93b
10 μ M NAA	4.89 $\pm$ 0.41a	12.04 $\pm$ 1.16c	1.12 $\pm$ 0.09c	76.24 $\pm$ 5e	1.4 $\pm$ 0.04c	155 $\pm$ 10.37d
1 μ M IBA	5.24 $\pm$ 0.34a	0.11 $\pm$ 0.69a	1.16 $\pm$ 0.08c	1.63 $\pm$ 0.28b	1.2 $\pm$ 0.08b	19.99 $\pm$ 1.85b
10 μ M IBA	4.13 $\pm$ 0.31a	10.64 $\pm$ 0.82c	1.6 $\pm$ 0.08d	38.11 $\pm$ 3.3d	1.5 $\pm$ 0.07c	82.67 $\pm$ 6.06c

Averages $\pm$ SE followed by different letters in the same column are significantly different at $p < 0.05$ according to the Kruskal–Wallis test.

Appendix B

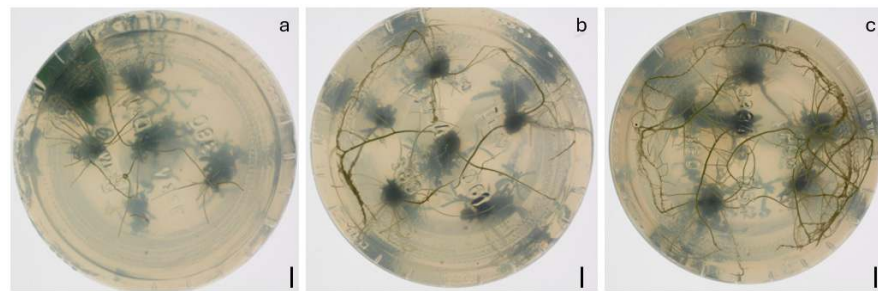


Figure A2. Effect of a 1-min pulse of DCPE and DCP on rooting and development of *L. angustifolia* after 4 weeks of in vitro culture (scale bar = 1 cm). The treatments included (a) control (hormone-free medium); (b) 2.5 µM DCP; (c) 2.5 µM DCPE.

References

- Opanasenko, V.F.; Havryliuk, Y.V.; Shevchenko, T.M. Prospects for the Use of Growth Regulators in Vegetative Propagation Technology of *Lavandula angustifolia*. *Med. Perspect.* **2024**, *29*, 89–95. [\[CrossRef\]](#)
- Babanina, S.S.; Yegorova, N.A.; Stavtseva, I.V.; Abdurashitov, S.F. Genetic stability of lavender (*Lavandula angustifolia* Mill.) plants obtained during long-term clonal micropropagation. *Russ. Agric. Sci.* **2023**, *49*, 132–139. [\[CrossRef\]](#)
- George, E.F.; Debergh, P.C. Micropropagation: Uses and Methods. In *Plant Propagation by Tissue Culture*, 3rd ed.; George, E.F., Hall, M.H., De Klerk, G.J., Eds.; Springer: Dordrecht, The Netherlands, 2008; Volume 1, pp. 29–64. [\[CrossRef\]](#)
- Verdi, R.; Bettoni, J.C.; Werner, S.S.; Boff, M.I.; Boff, P. Effects of the phenological stage, type of cutting and plant growth regulators on the propagation by stem cutting of *Poirertia latifolia* Vogel, a Brazilian native medicinal plant. *Rev. Colomb. Cienc. Hortic.* **2020**, *14*, 267–274. [\[CrossRef\]](#)
- Bettoni, J.C.; van der Walt, K.; Souza, J.A.; McLachlan, A.; Nadarajan, J. Sexual and asexual propagation of *Syzygium maire*, a critically endangered Myrtaceae species of New Zealand. *N. Z. J. Bot.* **2023**, *62*, 35–52. [\[CrossRef\]](#)
- Loconsole, D.; Cristiano, G.; De Lucia, B. Image analysis of adventitious root quality in wild sage and glossy abelia cuttings after application of different indole-3-butyric acid concentrations. *Plants* **2022**, *11*, 290. [\[CrossRef\]](#)
- Ilczuk, A.; Jacygrad, E. The effect of IBA on anatomical changes and antioxidant enzyme activity during the in vitro rooting of smoke tree (*Cotinus coggygia* Scop.). *Sci. Hortic.* **2016**, *210*, 268–276. [\[CrossRef\]](#)
- Guasso, L.; Lattuada, D.S.; de Souza, P.V.D. In vitro root development under different IBA concentrations and culture media. *Comun. Sci.* **2025**, *16*, e4313. [\[CrossRef\]](#)
- Sekhukhune, M.K.; Maila, M.Y. Exogenous IBA stimulatory effects on root formation of *Actinidia deliciosa* rootstock and *Actinidia arguta* male scion stem cuttings. *Front. Sustain. Food Syst.* **2024**, *8*, 1461871. [\[CrossRef\]](#)
- Fattorini, L.; Velocchia, A.; Della Rovere, F.; Angeli, S.D.; Falasca, G.; Altamura, M.M. Indole-3-butyric acid promotes adventitious rooting in *Arabidopsis thaliana* thin cell layers by conversion into indole-3-acetic acid and stimulation of anthranilate synthase activity. *BMC Plant Biol.* **2017**, *17*, 121. [\[CrossRef\]](#)
- Aihebaier, S.; Muhammad, T.; Wei, A.; Mamat, A.; Abuduaini, M.; Pataer, P.; Yigaimu, A.; Yimit, A. Membrane-protected molecularly imprinted polymer for the microextraction of indole-3-butyric acid in mung bean sprouts. *ACS Omega* **2019**, *4*, 16789–16793. [\[CrossRef\]](#)
- da Costa, C.T.; Ruedell, C.M.; Fett-Neto, A.G.; de Almeida, M.R.; Maraschin, J.S.F. When stress and development go hand in hand: Main hormonal controls of adventitious rooting in cuttings. *Front. Plant Sci.* **2013**, *4*, 133. [\[CrossRef\]](#) [\[PubMed\]](#)
- Rautio, J.; Kumpulainen, H.; Heimbach, T.; Oliyai, R.; Oh, D.; Järvinen, T.; Savolainen, J. Prodrugs: Design and Clinical Applications. *Nat. Rev. Drug Discov.* **2008**, *7*, 255–270. [\[CrossRef\]](#) [\[PubMed\]](#)
- Darouez, H.; Werbrouck, S.P. In Vitro Rooting of Poplar: Effects and Metabolism of Dichlorprop Auxin Ester Prodrugs. *Plants* **2025**, *14*, 108. [\[CrossRef\]](#) [\[PubMed\]](#)
- Ljung, K. Auxin Metabolism and Homeostasis During Plant Development. *Development* **2013**, *140*, 943–950. [\[CrossRef\]](#)
- Zhao, Y. Auxin Biosynthesis and Its Role in Plant Development. *Annu. Rev. Plant Biol.* **2010**, *61*, 49–64. [\[CrossRef\]](#)
- Murashige, T.; Skoog, F. A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiol. Plant* **1962**, *15*, 473–497. [\[CrossRef\]](#)
- Quambusch, M.; Schürz, J.; Hanke, M.-V.; Flachowsky, H.; Peil, A. Improved in vitro rooting of *Prunus avium* microshoots using a dark treatment and an auxin pulse. *Sci. Hortic.* **2017**, *220*, 52–56. [\[CrossRef\]](#)

19. Kaewchangwat, N.; Thanayupong, E.; Jarussophon, S.; Niamnont, N.; Yata, T.; Prateepchinda, S.; Unger, O.; Han, B.-H.; Suttisintong, K. Coumarin-caged compounds of 1-naphthaleneacetic acid as light-responsive controlled-release plant root stimulators. *J. Agric. Food Chem.* **2020**, *68*, 6268–6279. [\[CrossRef\]](#)
20. Khan, S.; Bi, T.B. Direct shoot regeneration system for date palm (*Phoenix dactylifera* L.) cv. Dhakki as a means of micropropagation. *Pak. J. Bot.* **2012**, *44*, 1965–1971.
21. Ortiz Maria, F.; Figueiredo Marcos, R.; Nissen Scott, J.; Wersal Ryan, M.; Ratajczyk William, A.; Dayan Franck, E. 2,4-D and 2,4-D butoxyethyl ester behavior in Eurasian and hybrid watermilfoil (*Myriophyllum* spp.). *Pest Manag. Sci.* **2022**, *78*, 626–632. [\[CrossRef\]](#)
22. Stella, V.J.; Nti-Addae, K.W. Prodrug Strategies to Overcome Poor Water Solubility. *Adv. Drug Deliv. Rev.* **2007**, *59*, 677–694. [\[CrossRef\]](#) [\[PubMed\]](#)
23. Christian, M.; Hannah, W.B.; Lüthen, H.; Jones, A.M. Identification of Auxins by a Chemical Genomics Approach. *J. Exp. Bot.* **2008**, *59*, 2757–2767. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Du, M.; Bou Daher, F.; Liu, Y.; Steward, A.; Tillmann, M.; Zhang, X.; Gray, W.M. Biphasic control of cell expansion by auxin coordinates etiolated seedling development. *Sci. Adv.* **2022**, *8*, eabj1570. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Abts, W.; Vandenbussche, B.; De Proft, M.P.; Van de Poel, B. The role of auxin–ethylene crosstalk in orchestrating primary root elongation in sugar beet. *Front. Plant Sci.* **2017**, *8*, 444. [\[CrossRef\]](#)
26. Davies, P.J. (Ed.) *Plant Hormones: Physiology, Biochemistry and Molecular Biology*; Springer Science & Business Media: Dordrecht, The Netherlands, 2013. [\[CrossRef\]](#)
27. Dubrovsky, J.G.; Sauer, M.; Napsucialy-Mendivil, S.; Ivanchenko, M.G.; Friml, J.; Shishkova, S.; Benkova, E. Auxin Acts as a Local Morphogenetic Trigger to Specify Lateral Root Founder Cells. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 8790–8794. [\[CrossRef\]](#)
28. Benjamins, R.; Scheres, B. Auxin: The Looping Star in Plant Development. *Annu. Rev. Plant Biol.* **2008**, *59*, 443–465. [\[CrossRef\]](#)
29. Vielba, J.M.; Vidal, N.; Ricci, A.; Castro, R.; Covelo, P.; San-Jose, M.C.; Sanchez, C. Effects of Auxin and Urea Derivatives on Adventitious Rooting in Chestnut and Oak Microshoots. *Isr. J. Plant Sci.* **2020**, *67*, 52–68. [\[CrossRef\]](#)
30. Justamante, M.S.; Mhimdi, M.; Molina-Pérez, M.; Albacete, A.; Moreno, M.Á.; Mataix, I.; Pérez-Pérez, J.M. Effects of auxin (Indole-3-butyric Acid) on adventitious root formation in peach-based *Prunus* rootstocks. *Plants* **2022**, *11*, 913. [\[CrossRef\]](#)
31. Bai, T.; Dong, Z.; Zheng, X.; Song, S.; Jiao, J.; Wang, M.; Song, C. Auxin and its interaction with ethylene control adventitious root formation and development in apple rootstock. *Front. Plant Sci.* **2020**, *11*, 574881. [\[CrossRef\]](#)
32. Galvan-Ampudia, C.S.; Cerutti, G.; Legrand, J.; Brunoud, G.; Martin-Arevalillo, R.; Azais, R.; Vernoux, T. Temporal integration of auxin information for the regulation of patterning. *eLife* **2020**, *9*, e55832. [\[CrossRef\]](#)
33. Reinhardt, D.; Mandel, T.; Kuhlmeier, C. Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* **2000**, *12*, 507–518. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Woodward, A.W.; Bartel, B. Auxin: Regulation, action, and interaction. *Ann. Bot.* **2005**, *95*, 707–735. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Yalamanchili, K.; Vermeer, J.E.; Scheres, B.; Willemsen, V. Shaping root architecture: Towards understanding the mechanisms involved in lateral root development. *Biol. Direct* **2024**, *19*, 87. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Zhao, Y.; Chen, Y.; Jiang, C.; Lu, M.Z.; Zhang, J. Exogenous Hormones Supplementation Improve Adventitious Root Formation in Woody Plants. *Front. Bioeng. Biotechnol.* **2022**, *10*, 1009531. [\[CrossRef\]](#)
37. Pant, M.; Bhandari, A.; Husen, A. Adventitious Root Formation and Clonal Propagation of Forest-Based Tree Species. In *Environmental, Physiological and Chemical Controls of Adventitious Rooting in Cuttings*; Academic Press: Cambridge, UK, 2023; pp. 471–490. [\[CrossRef\]](#)
38. Crişan, I.; Ona, A.; Vârban, D.; Muntean, L.; Vârban, R.; Stoie, A.; Mihăiescu, T.; Morea, A. Current Trends for Lavender (*Lavandula angustifolia* Mill.) Crops and Products with Emphasis on Essential Oil Quality. *Plants* **2023**, *12*, 357. [\[CrossRef\]](#)
39. Yang, T.; Davies, P.J. Promotion of Stem Elongation by Indole-3-Butyric Acid in Intact Plants of *Pisum sativum* L. *Plant Growth Regul.* **1999**, *27*, 157–160. [\[CrossRef\]](#)
40. Sarropoulou, V.N.; Dimassi, K.N.; Therios, I.N.; Tsabolatidou, E.G. Effect of Indole-3-butyric Acid (IBA) on Growth Parameters and Leaf Anatomy of Olive (*Olea europaea* L.) Rooted Cuttings. *J. Plant Growth Regul.* **2016**, *35*, 612–624.
41. Li, S.-W.; Xue, L.; Xu, S.; Feng, H.; An, L. Integrating the Roles for Cytokinin and Auxin in De Novo Shoot Organogenesis. *Int. J. Mol. Sci.* **2021**, *22*, 8554.

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.