

Physiological maturation and hormonal profiles associated with rooting success of leafy cuttings in *Prunus subhirtella* 'Autumnalis'

Petra Kunc

`petra.kunc@bf.uni-lj.si`

University of Ljubljana

Aljaz Medic

University of Ljubljana

Mariana Cecilia Grohar

University of Ljubljana

Tanja Mrak

Slovenian Forestry Institute

Gregor Osterc

University of Ljubljana

Research Article

Keywords: Topophysis, ornamental cherry, phytohormones, adventitious roots, root morphology

Posted Date: February 12th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8765137/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Plant Biology on March 27th, 2026.
See the published version at <https://doi.org/10.1186/s12870-026-08644-1>.

Abstract

The success of vegetative propagation in woody ornamentals is strongly influenced by the physiological age and positional origin of donor shoots, yet the underlying mechanisms linking topophytic origin, hormonal dynamics, and root system quality remain unclear. This study examined adventitious rooting, root system morphology, and endogenous phytohormone profiles in basal (inner/lower crown) and terminal (outer/upper crown) leafy cuttings of *Prunus subhirtella* 'Autumnalis' from a mature 60-year-old tree. Despite similar rooting success between cutting types (66.7%), basal cuttings produced more extensive root systems, with greater total length, surface area, and numbers of tips and forks, whereas terminal cuttings formed thicker roots and a higher proportion of coarse roots (> 2mm). Free indole-3-acetic acid (IAA) peaked at 4 hours post-severance in both types. However, terminal cuttings exhibited elevated levels of IAA conjugates and oxidative metabolites, as well as transiently higher jasmonic acid immediately after excision indicating that they exhibited stronger stress after wounding compared with basal cuttings. In contrast, the basal cuttings maintained higher indole-3-butyric acid (IBA) and distinct 4-chloro-indole-acetic acid (4-Cl-IAA) dynamics. These results suggest that differences in auxin metabolism and jasmonate dynamics shape root system morphology and quality. Integrating topophytic origin and hormonal profiling provides valuable insights for optimizing clonal propagation and improving root system performance in woody ornamentals.

Introduction

The physiological status of woody plants plays a crucial role in determining the success of vegetative propagation and regeneration. Throughout ontogenetic development, the regenerative potential of plant tissues generally declines—a phenomenon known as physiological maturation. This process progressively reduces the morphogenetic competence of cells, thereby limiting their capacity to form adventitious roots and shoots and constraining the overall regenerative plasticity of mature tissues [1, 2]. Consequently, successful vegetative propagation is often restricted to physiologically juvenile plant material, which maintains a higher ability to respond to auxin stimulation and initiate root primordia [1, 3, 4].

Physiological age is not determined solely by the chronological age of the plant or organ but results from the combined influence of three key factors: cyclophysis, referring to the aging of the entire plant and its parts; topophysis, describing the positional effect of an organ's origin within the plant; and periphysis, representing environmental influences acting upon that organ [4]. Among these, topophysis has received increasing attention as an important determinant of rooting ability, particularly in clonal propagation of woody plants. It is defined as the positional effect of a shoot within the crown on its subsequent morphogenetic, anatomical, and physiological behaviour [5].

Numerous studies have demonstrated that the origin of cuttings within the donor plant significantly affects their rooting capacity. Shoots arising from the basal and inner crown regions generally exhibit greater rooting potential than those collected from apical or outer crown parts [4, 6]. This is because

basal shoots—especially trunk suckers or coppice sprouts—tend to retain more juvenile physiological features compared to apical shoots, which are more mature due to prolonged exposure to differentiation and reduced cell competence [7]. The terminal shoots of trees often undergo more cell divisions, leading to greater developmental age and a lower capacity to form roots [8]. Consequently, cuttings taken from lower crown positions or rejuvenated sprouts usually root more readily.

The influence of topophysis and maturation on rooting success is not only of theoretical significance but also of great practical relevance for nursery production and the clonal propagation of elite genotypes. Understanding the positional gradients of rejuvenation and applying appropriate management techniques—such as hard pruning of stock plants or the use of basal shoots—can substantially improve propagation efficiency and uniformity [4] [9].

Beyond morphological and developmental gradients, differences in rooting competence are increasingly attributed to variations in endogenous hormonal status. Phytohormones play a pivotal role in regulating the physiological and molecular processes involved in adventitious root (AR) formation. Among them, auxins are the primary inductive signals promoting root initiation, while jasmonates are recognized as modulators of wound response, defence signalling, and hormonal crosstalk during AR development [1, 10, 11].

Despite the importance of vegetative propagation in woody ornamentals, studies addressing the combined effects of topophytic origin, rooting quality, and endogenous hormonal status remain scarce, and qualitative differences between rooted basal and terminal cuttings have not yet been comprehensively evaluated. The objective of this study was to compare rooting success and the quality of adventitious root systems in basal and terminal cuttings of *Prunus subhirtella* 'Autumnalis' and to evaluate whether differences in rooting performance are associated with variations in endogenous auxin- and jasmonate-related hormonal profiles in the induction phase of AR formation. Basal cuttings, representing a more juvenile physiological state, were expected to display a hormonal balance more conducive to root induction, characterized by higher levels of active auxins and lower content of auxin conjugates and catabolites, while elevated jasmonate levels were hypothesized to be linked to reduced rooting competence. By integrating topophytic origin, hormonal status, and root system quality, this study provides new insight into the physiological mechanisms governing clonal propagation success in woody ornamental species.

Material and Methods

Plant material

The study was conducted on the ornamental cherry (*Prunus subhirtella* Miq. 'Autumnalis') (Fig. 1a, Fig. 1b, Fig. 1c), a mature tree approximately 60 years old, growing at the experimental field of the Biotechnical Faculty, University of Ljubljana (46°3'4" N; 14°30'18" E), Slovenia. This tree was selected because the topophytic effect—the influence of the positional origin of shoots within the crown on their

physiological and morphogenetic characteristics—becomes particularly pronounced in older woody plants.

Two types of shoot material were collected based on their positional origin within the crown (Fig. 2):

- i. terminal shoots from the outer, upper parts of the canopy, representing physiologically more mature material (hereafter referred to as terminal shoots), and
- ii. basal shoots originating from the inner and lower crown zones, representing physiologically more juvenile material (hereafter referred to as basal shoots).

Experimental design

Cuttings were collected from basal and terminal portions of donor plants of *Prunus subhirtella* 'Autumnalis', representing two physiologically distinct shoot types differing in their topophytic position within the crown. To investigate temporal changes in endogenous hormone content following excision, samples were taken at six time points: immediately after severance (0 min) and after 30 min, 1 h, 2 h, 4 h, and 24 h post-severance. Cuttings were placed in the propagation bed within the fogging system, according to the marked plots—randomly selected (see Supplementary Material, Fig. S1)—and were sampled at specific times.

The experiment followed a completely randomized two-factorial design, with shoot position Basal (B) vs. Terminal (T)) and time after severance 0 min (0), 30 min (30), 1 h (1), 2 h (2), 4 h (4), 24 h (24)) as fixed factors, resulting in 12 treatment combinations (T0–T24 and B0–B24). For each treatment, three biological replicates were prepared, each consisting of seven leafy cuttings (See Supplementary material, Fig. S1). Cuttings belonging to the same replicate were immediately placed into labelled bags (e.g., T0–1, T0–2, T0–3; etc.), frozen in liquid nitrogen, lyophilized, and ground to a fine powder prior to hormone extraction.

In addition to samples used for hormonal profiling, two additional treatment groups, designated T rooting and B rooting, were established to evaluate the rooting performance of terminal and basal cuttings, respectively (See Supplementary material, Fig. S1). Each of these treatments consisted of three replicates of eight cuttings maintained under standard rooting conditions until the end of the growing season (end of October). At that time, the rooting success was assessed based on the percentage of rooted cuttings, percentage of cuttings that formed callus tissue, and a detailed root analysis was also conducted.

Phytohormone extraction and analyses

Lyophilized samples were ground into a fine powder in liquid nitrogen using a mortar and pestle. The samples were stored in vacuum-sealed bags to prevent moisture absorption and potential hormone degradation. Hormone extraction was initiated four days after lyophilization. Hormonal analyses were conducted on three independent biological replicates per treatment, each representing pooled material

from seven cuttings. We analysed a comprehensive set of auxin-related compounds—indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 4-chloro-indole-3-acetic acid (4-Cl-IAA), Indole-3-acetyl-aspartic acid (IAA-Asp), Indole-3-acetyl-glutamic acid (IAA-Glu), Oxindole-3-acetic acid (ox-IAA) and 2-Oxindole-3-acetyl-aspartic acid (ox-IAA-Asp)—as well as jasmonate-related molecules, including jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile).

Extraction and quantification of phytohormones were performed largely as previously described by Kunc et al., [12, 13]. Briefly, 100 mg of powdered plant tissue was extracted with isopropanol, followed by shaking on ice extraction. After centrifugation, the supernatant was collected, evaporated to dryness, reconstituted in methanol, and clarified by a second centrifugation prior to analysis.

Phytohormone analysis was conducted using UHPLC coupled to tandem mass spectrometry operating in selected reaction monitoring (SRM) mode. Identification and quantification were based on external standards, with calibration curves used for concentration determination see Table 1). Final hormone levels were corrected for extraction recovery as described previously (see Supplementary Table S1).

Table 1 SRM transitions for phytohormone quantifications.

Phytohormone	Retention time	Pseudo molecular ions	Fragmentation pattern
	(min)	(<i>m/z</i>)	(relative peak intensity %)
IAA	4.57	174	130 (100)
IBA	6.23	202	158 (100)
4-Cl-IAA	5.33	208	164 (100)
IAA-Asp	3.90	289	132 (100), 174 (90),
IAA-Glu	3.93	303	128 (100), 285 (92), 146 (25), 174 (15)
Ox-IAA	3.60	190	146 (100)
Ox-IAA-Asp	3.30	305	132 (100), 190 (65), 287 (45), 261 (40)
JA	7.40	209	165 (100)
JA-Ile	9.50	322	129 (100)
IAA: Indole-3-acetic acid; IAA-Asp: Indole-3-acety-aspartic acid; IAA-Glu: Indole-3-acetyl-glutamic acid; ox-IAA: Oxindole-3-acetic acid; ox-IAA: Asp 2-Oxindole-3-acetyl-aspartic acid; JA: Jasmonic acid; Ja-Ile: Iso-jasmonoyl-L-isoleucine.			

Growing conditions

The experiment was conducted in a greenhouse at the Biotechnical Faculty, University of Ljubljana (46°3'4" N, 14°30'18" E). Cuttings were maintained under high relative humidity (98–100%) using an automated high-pressure fogging system (Plantog, Fischamend, Austria) to reduce transpiration and prevent desiccation. The propagation substrate consisted of a 1:1 (v/v) mixture of peat and sand supplemented with a controlled-release fertilizer (Osmocote Exact; ICL Group Ltd., Israel).

The fogging system operated daily between 08:00 and 20:00 h in cyclic intervals, with misting duration adjusted according to weather conditions. Natural daylight conditions followed the local photoperiod in Ljubljana from June to November.

Measurement of climate parameters

Substrate temperature, leaf lamina temperature, and air temperature measured 3 cm above the leaf lamina were continuously monitored at hourly intervals throughout the entire experimental period, from 26 June 2025 to 31 October 2025, using T-Soil and Lat-B3 sensors (Ecomatik, Germany). Continuous measurements were conducted to ensure precise characterization of the microclimatic conditions during the rooting experiment.

Rooting assessment and root morphology

The rooting success was assessed based on the percentage of rooted cuttings, percentage of cuttings that formed callus tissue. For root morphology analysis roots were cleaned off propagation substrate under tap water and scanned in water-filled tray on Epson Perfection V850 Pro (Epson, Suwa, Japan) scanner at 400 dpi. Scans were evaluated with WinRhizo Pro 2021 (Regent Instruments Inc., Quebec, Canada) software to obtain total root length, mean root diameter, root surface area, length of roots per each root diameter class (i.e. length of all roots whose diameter fit into selected diameter span), number of root tips and forks. Number of root tips and forks was expressed per length to obtain density. Percentage of root length in each root diameter class was calculated by dividing root length of selected diameter class by total root length. After scanning, the roots were dried and weighed. Specific root length (SRL) was calculated by dividing total length of the root system by its weight.

Chemicals

For the extraction and subsequent analytical procedures, the following reagents were utilized: isopropanol and methanol (Merck KGaA, Darmstadt, Germany), formic acid (Kemika d.d., Zagreb, Croatia), and acetonitrile (Sigma-Aldrich Chemie GmbH, Steinheim, Germany).

The following standards were employed for the identification and quantification of phytohormones: indole-3-acetic acid, jasmonic acid, and iso-jasmonoyl-L-isoleucine (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), indole-3-acetyl-L-aspartic acid (Biosynth Ltd., United Kingdom), Indole-3-acetyl-glutamic acid, oxindole-3-acetic acid, 2-Oxindole-3-acetyl-aspartic acid (CymitQuímica, S.L., Barcelona, Spain)

Statistical analyses

Data were processed using Microsoft Excel 2016 and subjected to statistical analysis using R Commander (Rcmdr package, version 4.1.0). To evaluate significant differences between the means of two independent groups, a Student's t-test was performed. One-way analysis of variance (ANOVA) was employed to assess differences among multiple groups. Where applicable, Duncan's multiple range test was used for post-hoc pairwise comparisons to identify statistically significant differences. Results are expressed as means $\pm$ standard error (SE), and statistical significance was determined at a 95% confidence level (p -value < 0.05).

For multivariate data visualization, phytohormone profiles were visualized using a standardized heatmap generated in R with the *pheatmap* package. Data were pre-processed using *dplyr* and *tidyr*, and mean hormone content were calculated for each Time $\times$ Stock plant combination, with sampling times ordered chronologically and stock plant types defined as basal or terminal. Additional data visualization was performed using RAWGraphs 2.0. For this purpose, data were normalized using Min–Max scaling, transforming all parameters to a range between 0 (minimum) and 1 (maximum) to ensure comparability across variables with different measurement units. A stacked bar chart was generated in RAWGraphs 2.0 to illustrate the percentage of root length within each root diameter class, categorized according to the type of cutting. Percentages were calculated relative to the total root length per treatment.

Results

Phytohormone profile

The heatmap revealed notable differences in hormone levels and their timing across both cutting types (Fig. 3). IAA content increased significantly over time in both basal and terminal cuttings, reaching maximum values at 4 h after severance. In basal cuttings, IAA levels remained low during the first hour but increased significantly at 2 and 4 h, reaching a maximum at 4 h ($79.93 \text{ ng g}^{-1} \text{ DW}$), before declining at 24 h. Terminal cuttings showed a similar temporal pattern, with significant increases at 2 and 4 h ($57.49 \pm 12.15 \text{ ng g}^{-1} \text{ DW}$ and $61.00 \pm 18.28 \text{ ng g}^{-1} \text{ DW}$, respectively) after severance, and sustained elevated levels at 24 h. Terminal cuttings showed very high initial 4-Cl-IAA levels at 0 min ($3562.76 \text{ ng g}^{-1} \text{ DW}$), which declined markedly by 24 h. In contrast, basal cuttings exhibited lower initial 4-Cl-IAA content, followed by a rapid increase within the first 2 h after severance (from 438.74 to $2553.58 \text{ ng g}^{-1} \text{ DW}$). IBA peaked between 30 min and 1 h after severance in both types and declined thereafter. A pronounced increase in IAA-Asp was observed at 4 h, particularly in basal cuttings ($674.29 \text{ ng g}^{-1} \text{ DW}$). IAA-Glu content in basal cuttings began at a moderate level ($78.35 \text{ ng g}^{-1} \text{ DW}$) and steadily declined, reaching its lowest point at 4 h ($25.39 \text{ ng g}^{-1} \text{ DW}$). Terminal cuttings started with significantly higher IAA-Glu levels ($151.99 \text{ ng g}^{-1} \text{ DW}$) than basal cuttings, but this level dropped by more than half within the first 30 minutes ($66.15 \text{ ng g}^{-1} \text{ DW}$). Ox-IAA content was higher in terminal cuttings at early time points, with a transient peak at 30 min ($86.37 \text{ ng g}^{-1} \text{ DW}$), followed by a decline at later time points. Basal cuttings maintained lower and more stable ox-IAA levels. Ox-IAA-Asp content was higher in terminal cuttings during the early time points (30 min and 1 h) and decreased at 2 and 4 h, whereas basal cuttings showed

low early levels and a significant increase at 24 h. Jasmonic acid (JA) levels showed no significant temporal variation in basal cuttings, while terminal cuttings exhibited significantly higher JA content at 0 min (747.98 ng g⁻¹ DW), followed by a rapid decrease by 30 min that persisted through 24 h. JA-Ile content was highest at 0 min in both cutting types and declined rapidly thereafter. In basal cuttings, JA-Ile partially recovered at 24 h, whereas terminal cuttings remained at low levels throughout the remaining time points (see Supplementary Material Table S2, Fig. 3).

Terminal cuttings showed significantly higher content of JA 0 min after severance, IAA-Asp 0 min, 30 min, 1 h, 24 h after severance, IAA-Glu 0 min, 4 h after severance, ox-IAA 0 min, 1 h, 2 h, 24 h after severance, and 4-Cl-IAA 0 min, 24 h after severance. Basal cuttings exhibited significantly higher IBA content at 4 h, 24 h after severance and 4-Cl-IAA 1h after severance. No significant differences were found for free IAA at any time point (Table 2)

Table 2 Statistical significance (*p*-values) of hormonal differences between basal and terminal cuttings.

Phytohormone	0 min	30 min	1 h	2 h	4 h	24 h
IAA	0.440 (ns)	0.141 (ns)	0.449 (ns)	0.916 (ns)	0.527 (ns)	0.135 (ns)
IAA-Asp	0.002 (**)	0.026 (*)	0.002 (**)	0.189 (ns)	0.584 (ns)	0.042 (*)
IAA-Glu	0.004 (**)	0.058 (ns)	0.270 (ns)	0.791 (ns)	0.029 (*)	0.124 (ns)
IBA	0.978 (ns)	0.342 (ns)	0.878 (ns)	0.962 (ns)	0.028 (*)	<0.001 (***)
JA	0.005 (**)	0.218 (ns)	0.671 (ns)	0.901 (ns)	0.102 (ns)	0.414 (ns)
JA-Ile	0.067 (ns)	0.118 (ns)	0.507 (ns)	0.731 (ns)	0.179 (ns)	0.118 (ns)
ox-IAA	0.002 (**)	0.107 (ns)	0.011 (*)	0.012 (*)	0.119 (ns)	0.006 (**)
ox-IAA-Asp	0.056 (ns)	0.009 (**)	0.373 (ns)	0.101 (ns)	0.895 (ns)	0.781 (ns)
4-Cl-IAA	0.004 (**)	0.064 (ns)	0.003 (**)	0.053 (ns)	0.073 (ns)	0.007 (**)
ns: not significant ($p > 0.05$); *: significant at $p \leq 0.05$; **: significant at $p \leq 0.01$; ***: significant at $p \leq 0.001$ (n=3)						

Air, leaf lamina, and rooting substrate temperature dynamics

In June, mean monthly air, leaf, and rooting substrate temperatures were 31.6 °C, 31.7 °C, and 33.9 °C, respectively, with daily minima around 05:00–06:00 and maxima at 15:00–16:00. In July, mean monthly temperatures were 26.9 °C (air), 27.1 °C (leaf), and 29.6 °C (rooting substrate); daily minima occurred early morning (05:00–07:00) and maxima in the afternoon (14:00–17:00). In August, mean monthly temperatures were 26.5 °C (air), 26.7 °C (leaf), and 28.9 °C (rooting substrate), with lowest temperatures at 05:00–07:00 and highest at 15:00–16:00. In September, mean monthly temperatures were 22.1 °C (air and leaf) and 23.7 °C (rooting substrate); minima occurred early morning and maxima in the afternoon (15:00–17:00). In October, mean monthly temperatures dropped to 11.6 °C (air), 12.0 °C (leaf), and 13.5

°C (rooting substrate), with minima at 07:00–08:00 and maxima at 15:00 (see Supplementary Material Fig. S2, S3, S4).

Rooting assessment and root morphology

In terminal and basal cuttings, 66.67% of the cuttings successfully formed roots, while 33.33% either failed to root or rooted but subsequently failed (See Supplementary Material Table S3). All successfully rooted cuttings formed roots without callus formation. Significant differences in root system morphology were observed between the two cutting types. Basal cuttings showed superior performance in most growth-related parameters, whereas terminal cuttings exhibited a higher average diameter, as shown in Fig. 4.

Basal cuttings showed significantly higher values for total root length, surface area, and the number of root tips (Table 3). The number of forks was nearly double in basal cuttings compared to terminal cuttings, a difference that is highly significant ($p = 0.0099$). Interestingly, terminal cuttings produced roots with a significantly larger average diameter (0.67 ± 0.02 mm) than basal cuttings (0.60 ± 0.02 mm). No statistically significant differences ($p > 0.05$) were observed for the number of root tips or forks per length, dry weight, or specific root length (SRL) (Table 3).

Table 3 Morphological root parameters of basal and terminal cuttings

Parameter	Basal cuttings (Mean $\pm$ SE)	Terminal cuttings (Mean $\pm$ SE)	<i>p</i> -value	Significance
Length (cm)	407.13 $\pm$ 46.24	229.35 $\pm$ 27.15	0.0048	**
Surface area (cm ²)	75.79 $\pm$ 9.45	47.95 $\pm$ 5.39	0.0162	*
Average diameter (mm)	0.60 $\pm$ 0.02	0.67 $\pm$ 0.02	0.0124	*
Number of tips	801.00 $\pm$ 97.05	469.50 $\pm$ 62.37	0.0074	**
Number of forks	1208.31 $\pm$ 204.49	577.13 $\pm$ 96.65	0.0099	**
Number of Tips/cm	2.09 $\pm$ 0.18	2.26 $\pm$ 0.31	0.6265	ns
Number of Forks/cm	2.80 $\pm$ 0.13	2.43 $\pm$ 0.14	0.0562	ns
Dry weight (g)	0.32 $\pm$ 0.05	0.21 $\pm$ 0.04	0.1311	ns
SRL (m/g)	15.27 $\pm$ 1.18	12.92 $\pm$ 1.32	0.1666	ns
ns (not significant): $p > 0.05$ * (significant): $p \leq 0.05$ ** (highly significant): $p \leq 0.01$, (n=16)				

Although both cutting types exhibited similar overall distribution patterns of root length in each root diameter class, statistically significant differences were observed in three specific diameter classes

(Table 4). Basal cuttings exhibited a significantly higher percentage of roots in the 0.2–0.3 mm class (26.04%) compared with terminal cuttings (20.78%). In contrast, terminal cuttings showed significantly higher proportions of roots in the 0.7–0.8 mm and 0.8–0.9 mm diameter classes.

Table 4 Percentage of root length in each root diameter class according to the type of cutting

Root diameter class	Basal cuttings (%)	Terminal cuttings (%)	<i>p</i> -value	Significance
0.0<L ≤0.1 mm	1.16	1.40	0.389	ns
0.1<L ≤0.2 mm	14.77	12.64	0.296	ns
0.2<L ≤0.3 mm	26.04	20.78	0.015	*
0.3<L ≤0.4 mm	18.84	17.89	0.444	ns
0.4<L ≤0.5 mm	7.11	7.29	0.731	ns
0.5<L ≤0.6 mm	9.16	10.65	0.182	ns
0.6<L ≤0.7 mm	4.27	5.00	0.146	ns
0.7<L ≤0.8 mm	3.24	4.09	0.0097	**
0.8<L ≤0.9 mm	2.79	4.03	0.0076	**
0.9<L ≤1.0 mm	2.21	2.57	0.256	ns
1.0<L ≤1.5 mm	6.91	8.95	0.0597	ns
1.5<L ≤2.0 mm	2.39	3.21	0.249	ns
2.0<L ≤3.0 mm	0.77	0.97	0.389	ns
3.0<L ≤5.0 mm	0.23	0.36	0.106	ns
>5.0 mm	0.10	0.17	0.129	ns
ns (not significant): $p > 0.05$ * (significant): $p \leq 0.05$ ** (highly significant): $p \leq 0.01$ (n=16)				

This data indicates that both basal and terminal cuttings developed root systems dominated by fine roots (< 2 mm). 59.64 % of the total root length was concentrated within the 0.1–0.4 mm diameter range in basal cuttings, and 51.31 % in terminal cuttings. As root diameter increased, the proportion of total root length consistently declined. Among all diameter classes, the 0.2–0.3 mm interval contributed the greatest proportion to total root length in both basal and terminal cuttings (See Supplementary Material Fig. S5).

Discussion

In our study, we investigated the topophytic effect in cuttings of *Prunus subhirtella* 'Autumnalis' obtained from the basal and terminal parts of a mature tree. Topophysis is the occurrence of physiological

differences within a single tree, caused by variation in physiological age among different plant parts [14]. Although propagules from basal and inner parts typically exhibit higher rooting capacity due to their physiological juvenility [9, 15] we observed no significant difference in overall rooting success. This is likely due to the inherent high rooting ability of this species [12, 13], further evidenced by the complete absence of callus formation, which is often a marker of difficult-to-root material [13, 16]. While rooting success was uniform, significant differences emerged in root system architecture. Basal cuttings developed significantly longer and more extensive root systems (total root length and surface area). These parameters are critical indicators of soil exploration capacity and resource acquisition efficiency [17]. The superior root development observed in basal cuttings may be attributed to the retention of physiological juvenility, which could promote greater biomass allocation to the root system. Interestingly, SRL did not differ between cutting types, suggesting that both types employed a similar economic strategy in terms of biomass investment per unit of root length [18–21]. A higher number of root forks facilitates a more extensive and complex distribution of the root system within the soil profile [22]. Our results showed a significantly higher number of root tips and forks in basal cuttings. However, no significant differences were detected when these parameters were expressed on a relative basis (i.e., number of root tips and forks per length), indicating that the increased branching observed in basal cuttings was primarily a consequence of their greater overall system size rather than differences in branching intensity. Fine roots are commonly defined using a diameter threshold of ≤ 2 mm, although this criterion varies widely among studies and is often not explicitly reported. Alternative thresholds ranging from 0.5 to 5 mm have been applied, highlighting the lack of standardization in fine-root classification [23]. In species-specific approaches, such as in *Ficus carica* L., roots developing in cuttings are further classified into very fine (< 0.5 mm), fine (0.5–1 mm), and coarse (> 1 mm) roots, which is consistent with the focus of our study on roots formed in cuttings [24]. Root diameter is a critical functional trait with ecological and modeling relevance. Species with smaller-diameter roots tend to be less dependent on mycorrhizae and exhibit more efficient phosphorus and water uptake [19, 24, 25]. Substantial investment in fine roots represents an important ecological strategy, particularly in woody plants [24]. Our study revealed both types of cuttings developed root systems predominantly composed of fine roots, which is consistent with the species' reputation as easy to root. Nonetheless, we observed differences in average root diameter, with terminal cuttings exhibiting characteristically thicker roots. In addition, terminal cuttings had a higher proportion of coarse roots, suggesting that root system characteristics vary depending on the origin of the cutting, despite both being derived from the same plant. These differences highlight the influence of cutting origin on root system architecture and potential early-stage performance, even within genetically identical material.

The formation of adventitious roots (AR) is regulated by interactions between environmental and endogenous factors, among which phytohormones—particularly auxins—play a central role [24, 26]. Rooting capacity is often correlated with IAA concentration, and the effect of IAA on root induction is restricted to a short period immediately after propagation begins [9]. In *Vigna radiata*, an IAA peak occurs at the base of cuttings 24 h after severance [27], whereas in *Prunus subhirtella* 'Autumnalis' the peak appears earlier, 4 h after severance [12, 13, 15]. Our findings demonstrate that both cutting types

reached a peak in free IAA concentration at 4 h post-severance, a timing that is consistent with previous studies [12, 13, 15]. While the absolute content of free IAA remained statistically similar between basal and terminal cuttings during first 24 h after severance, the successful initiation of the rooting process in both groups can be attributed to this timely accumulation. Free IAA levels are regulated by *de novo* biosynthesis, reversible conjugation with sugars and amino acids (IAA-Asp, IAA-Glu), and irreversible oxidation to oxIAA [28]. In *Arabidopsis*, oxIAA-Asp and oxIAA-Glu have been identified as endogenous metabolites [28, 29]. Our results indicate that although the primary trigger for rooting—the transient peak in free IAA at 4 h was common to both cutting types, auxin homeostasis was regulated through distinct metabolic pathways. Terminal cuttings exhibited significantly higher levels of conjugated and oxidative auxin metabolites at multiple time points. While the 4 h free IAA peak appears sufficient to initiate adventitious root formation in both types, the increased flux toward conjugated and oxidative forms in terminal cuttings likely constrains the duration and/or intensity of the auxin signal. This refined hormonal regulation suggests that these so-called “inactive” or “storage” forms are not merely metabolic by-products, but active contributors to the modulation of rooting quality and root system architecture. Notably, this pattern contrasts with our previous observations [13] in rejuvenated plant material, which—despite appearing physiologically younger—exhibited higher IAA-Asp levels than mature plants. In addition to IAA, 4-Cl-IAA is a naturally occurring auxin with 10–20 times higher auxin activity than IAA, and halogenated auxins have been shown to positively affect root induction, although their role during the induction phase remains unclear [30]. These opposing trends in 4-Cl-IAA dynamics may reflect differences in cutting quality and physiological readiness for propagation. Given that the specific role of 4-Cl-IAA in adventitious root (AR) formation remains largely unexplored, the observed temporal shift suggests its possible function in the early stages of the rooting process. IBA, originally synthesized but later identified as a natural auxin, is considered the second most important auxin after IAA. While IBA application does not increase the percentage of rooted cuttings, it significantly improves root system quality, as reflected by increased root length, root volume, dry matter accumulation, and fresh and dry root weight [31]. Higher IBA concentrations in the basal cuttings likely contributed to the improved quality of these root parameters observed in this study. In addition to auxins, JA is rapidly synthesized in response to wounding during cutting preparation, with a transient increase at the cutting base that is crucial for AR initiation. JA activity depends on its conversion to the active form JA-Ile, which is essential for JA signaling during adventitious root formation [32]. In our study JA levels were significantly higher in terminal cuttings at 0 min and remained relatively stable thereafter, whereas levels in basal cuttings showed no significant variation over the 24-hour period. This observation is consistent with our previous study [13], in which cuttings derived from physiologically older stock material exhibited higher initial JA content compared to rejuvenated material. Furthermore, the dynamics of JA-Ile align with earlier findings [12]; levels rose immediately following severance (wounding), followed by a rapid decline. In terminal cuttings, this depletion occurred within the first 30 minutes, while in basal cuttings, the decline was slightly delayed, occurring after 1 hour.

Conclusion

This study is the first to investigate detailed root morphology in *Prunus subhirtella* 'Autumnalis' cuttings as influenced by different cutting types. Previous studies addressing topophysis have not included root system quality parameters, such as root morphology, nor have they examined such a broad range of phytohormone profiles involved in adventitious root formation. Our results revealed significant differences in root morphology among cuttings taken from the same tree but differing in physiological age. As expected, basal cuttings, which are physiologically younger, exhibited superior morphological root traits. In addition, differences in hormonal profiles were detected between cutting types, suggesting a relationship between hormone dynamics and root morphology. Although an IAA peak at 4 h after severance—considered critical for AR formation—was observed in both cutting types, notable differences were found in other phytohormones. Future studies should include overwintering and transplanting experiments to assess how these parameters affect plant survival, which was not possible in the present study due to the destructive nature of dry mass measurements. Further research should also address root morphology and hormone profiles in species with lower rooting capacity and expand the investigation of phytohormones, particularly those whose roles in root quality are still poorly understood.

Declarations

Availability of data and materials The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests The authors declare that they have no competing interests.

Acknowledgments Not applicable.

Ethics, Consent to Participate, and Consent to Publish:

Not applicable.

Clinical Trial Registration:

This study is not a clinical trial; therefore, trial registration is not applicable.

Author Contributions Conceptualization, P.K. and G.O.; Data curation, P.K. and G.O.; Formal analysis, P.K. and A.M.; Funding acquisition, G.O.; Investigation, P.K.; Methodology, P.K., M.C.G. and T.M.; Project administration, G.O.; Resources, G.O. Visualization, P.K.; Writing—original draft, P.K.; Writing—review & editing, G.O., A.M., M.C.G. and T.M.

All authors have read and agreed to the published version of the manuscript.

Funding This work is part of the program Horticulture P4-0013-0481 supported by the Slovenian Research and Innovation Agency (ARIS). The contribution of TM was possible through ARIS research core funding P4-0107 Forest biology, ecology and technology.

References

1. da Costa CT, de Almeida MR, Ruedell CM, Schwambach J, Maraschin FS, Fett-Neto AG. When stress and development go hand in hand: Main hormonal controls of adventitious rooting in cuttings. *Frontiers in Plant Science*. 2013;4. <https://doi.org/10.3389/fpls.2013.00133>.
2. Osterc G, Štampar F. Maturation changes auxin profile during the process of adventitious rooting in *Prunus*. *Eur J Hortic Sci*. 2015;80:225–230. <https://doi.org/10.17660/eJHS.2015/80.5.4>.
3. Wilson SB, Davies FT, Geneve RL. *Hartmann and Kester's Principles and Practices of Plant Propagation*. 9th ed.
4. Osterc G, Štefančič M, Štampar F. Juvenile stockplant material enhances root development through higher endogenous auxin level. *Acta Physiol Plant*. 2009;31:899–903. <https://doi.org/10.1007/s11738-009-0303-6>.
5. Hansen J. Influence of cutting position and stem length on rooting of leaf-bud cuttings of *Schefflera arboricola*. 1986.
6. Pijut PM, Woeste KE, Michler CH. Promotion of adventitious root formation of difficult-to-root hardwood tree species. In: *Horticultural Reviews*. Wiley; 2011. p. 213–251. <https://doi.org/10.1002/9780470872376.ch6>.
7. Olesen P. On cyclophysis and topophysis. *Silvae Genet*. 1978;27.
8. Greenwood MS, Hutchison KW. Maturation as a developmental process. In: *Clonal Forestry I*. 1993. p. 14–33.
9. Wendling I, Trueman SJ, Xavier A. Maturation and related aspects in clonal forestry – Part II: Reinvigoration, rejuvenation and juvenility maintenance. *New Forests*. 2014;45:473–486. <https://doi.org/10.1007/s11056-014-9415-y>.
10. Lakehal A, Dob A, Novák O, Bellini C. A DAO1-mediated circuit controls auxin and jasmonate crosstalk robustness during adventitious root initiation in *Arabidopsis*. *Int J Mol Sci*. 2019;20. <https://doi.org/10.3390/ijms20184428>.
11. Druege U. Overcoming physiological bottlenecks of leaf vitality and root development in cuttings: A systemic perspective. *Front Plant Sci*. 2020;11. <https://doi.org/10.3389/fpls.2020.00907>.
12. Kunc P, Medic A, Osterc G. Wound-induced dynamics of selected auxins and jasmonates suggest interhormonal crosstalk during the induction phase of adventitious root formation in *Prunus subhirtella* 'Autumnalis'. *Acta Physiol Plant*. 2026;48. <https://doi.org/10.1007/s11738-025-03877-3>.
13. Kunc P, Medič A, Hudina M, Veberič R, Osterc G. Physiological age of stock plants determines phytohormonal changes in leafy cuttings of *Prunus subhirtella* 'Autumnalis'. *J Plant Growth Regul*. 2024. <https://doi.org/10.1007/s00344-024-11479-5>.
14. Hamann A. Effects of hedging on maturation in loblolly pine: rooting capacity and root formation. 1995.
15. Osterc G, Petkovšek MM, Štampar F. Quantification of IAA metabolites in the early stages of adventitious rooting might be predictive for subsequent differences in rooting response. *J Plant Growth Regul*. 2016;35:534–542. <https://doi.org/10.1007/s00344-015-9559-9>.

16. Kunc P, Medic A, Veberič R, Osterc G. Does the physiological age of stock plant material affect the uptake of indole-3-butyric acid (IBA) in leafy cuttings of *Prunus subhirtella* 'Autumnalis'? *Horticulturae*. 2024;10. <https://doi.org/10.3390/horticulturae10030296>.
17. Pan X, Wang P, Wei X, Zhang J, Xu B, Chen Y, et al. Exploring root system architecture and anatomical variability in alfalfa (*Medicago sativa* L.) seedlings. *BMC Plant Biol*. 2023;23. <https://doi.org/10.1186/s12870-023-04469-4>.
18. Ostonen I, Püttsepp Ü, Biel C, Alberton O, Bakker MR, Lõhmus K, et al. Specific root length as an indicator of environmental change. *Plant Biosyst*. 2007;141:426–442. <https://doi.org/10.1080/11263500701626069>.
19. Eissenstat DM. Costs and benefits of constructing roots of small diameter. *J Plant Nutr*. 1992;15:763–782. <https://doi.org/10.1080/01904169209364361>.
20. Rose L. Pitfalls in root trait calculations: How ignoring diameter heterogeneity can lead to overestimation of functional traits. *Front Plant Sci*. 2017;8. <https://doi.org/10.3389/fpls.2017.00898>.
21. Ryser P. The mysterious root length. *Plant Soil*. 2006;286:1–6. <https://doi.org/10.1007/s11104-006-9096-1>.
22. Cao T, Zhang H, Chen T, Yang C, Wang J, Guo Z, et al. Research on the mechanism of plant root protection for soil slope stability. *PLoS One*. 2023;18. <https://doi.org/10.1371/journal.pone.0293661>.
23. Fantozzi D, Montagnoli A, Trupiano D, Di Martino P, Scippa GS, Agosto G, et al. A systematic review of studies on fine and coarse root traits measurement: towards the enhancement of urban forests monitoring and management. *Frontiers in Forests and Global Change*. 2024;7. <https://doi.org/10.3389/ffgc.2024.1322087>.
24. Mafrica R, Bruno M, Fiozzo V, Caridi R, Sorgonà A. Rooting, growth, and root morphology of the cuttings of *Ficus carica* L. (cv. 'Dottato'): Cutting types and length and growth medium effects. *Plants*. 2025;14. <https://doi.org/10.3390/plants14020160>.
25. Kou X, Han W, Kang J. Responses of root system architecture to water stress at multiple levels: A meta-analysis of trials under controlled conditions. *Front Plant Sci*. 2022;13. <https://doi.org/10.3389/fpls.2022.1085409>.
26. Pacurar DI, Perrone I, Bellini C. Auxin is a central player in the hormone cross-talks that control adventitious rooting. *Physiol Plant*. 2014;151:83–96. <https://doi.org/10.1111/ppl.12171>.
27. Nag S, Saha K, Choudhuri MA. Role of auxin and polyamines in adventitious root formation in relation to changes in compounds involved in rooting. *J Plant Growth Regul*. 2001;20:182–194. <https://doi.org/10.1007/s003440010016>.
28. Hladík P, Petřík I, Žukauskaitė A, Novák O, Pěňčík A. Metabolic profiles of 2-oxindole-3-acetyl-amino acid conjugates differ in various plant species. *Front Plant Sci*. 2023;14. <https://doi.org/10.3389/fpls.2023.1217421>.
29. Yee Tam Y, Epstein E, Normanly J. Characterization of auxin conjugates in *Arabidopsis*. 2000.

30. Pincelli-Souza RP, Tang Q, Miller BM, Cohen JD. Horticultural potential of chemical biology to improve adventitious rooting. *Horticulture Advances*. 2024;2. <https://doi.org/10.1007/s44281-024-00034-7>.
31. Pinto KGD, Albertino SMF, Leite BN, Soares DOP, de Castro FM, da Gama LA, et al. Indole-3-butyric acid improves root system quality in guarana cuttings. *HortScience*. 2020;55:1670–1675. <https://doi.org/10.21273/HORTSCI14984-20>.
32. Li M, Wang F, Li S, Yu G, Wang L, Li Q, et al. Importers drive leaf-to-leaf jasmonic acid transmission in wound-induced systemic immunity. *Mol Plant*. 2020;13:1485–1498. <https://doi.org/10.1016/j.molp.2020.08.017>.

Figures

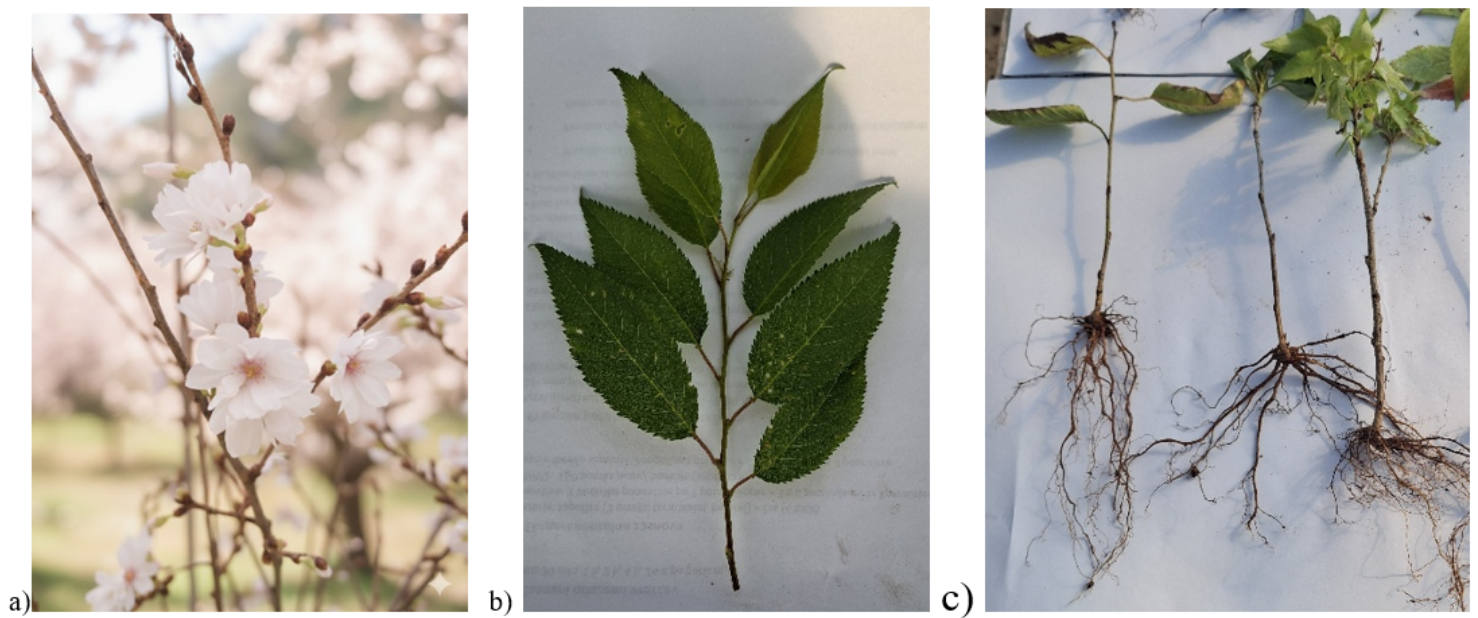


Figure 1

Prunus subhirtella 'Autumnalis' a) flowers b) leafy cutting c) successfully rooted leafy cuttings

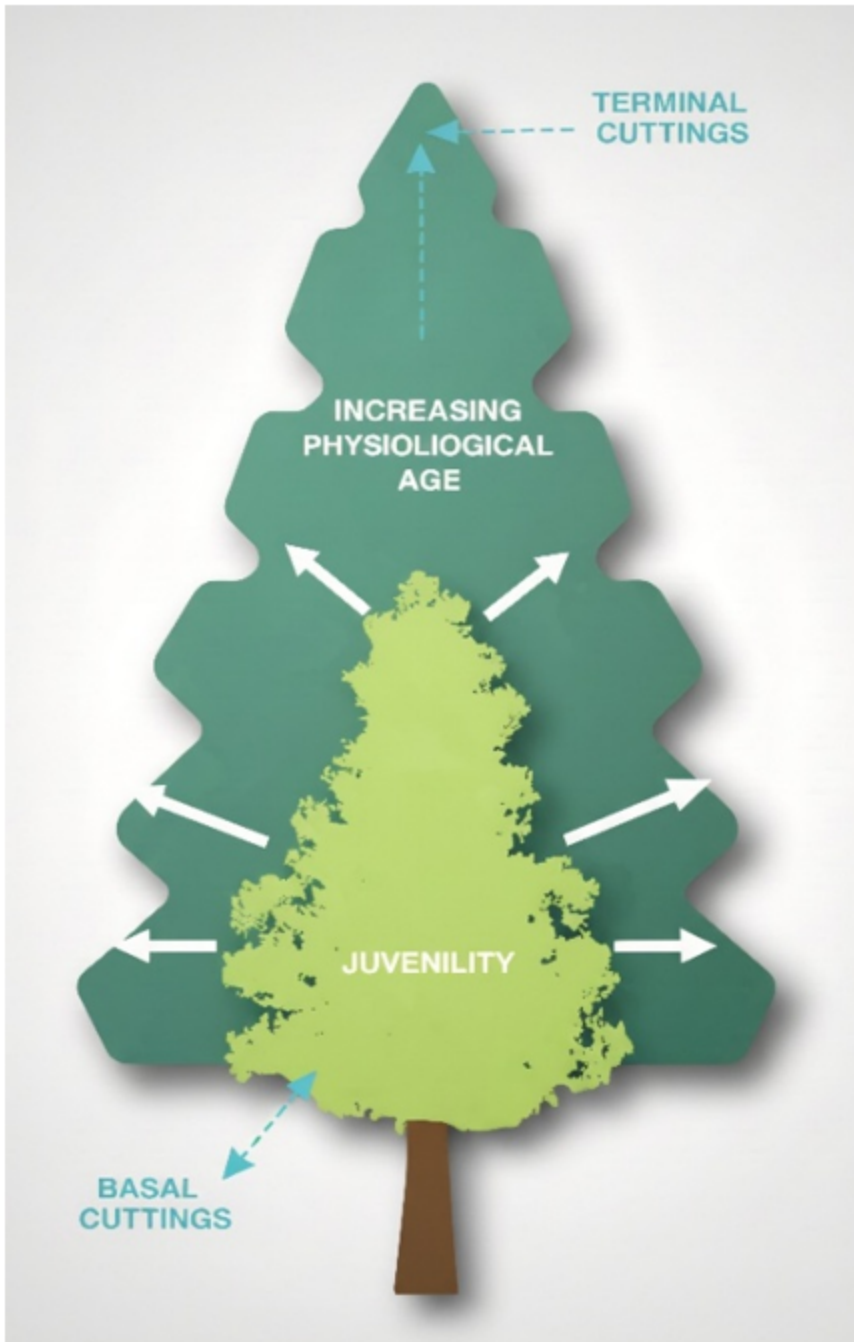


Figure 2

A pronounced topophytic effect, characterized by increasing physiological age from inner and basal regions to outer and terminal regions, with marked sampling positions for basal and terminal cuttings.

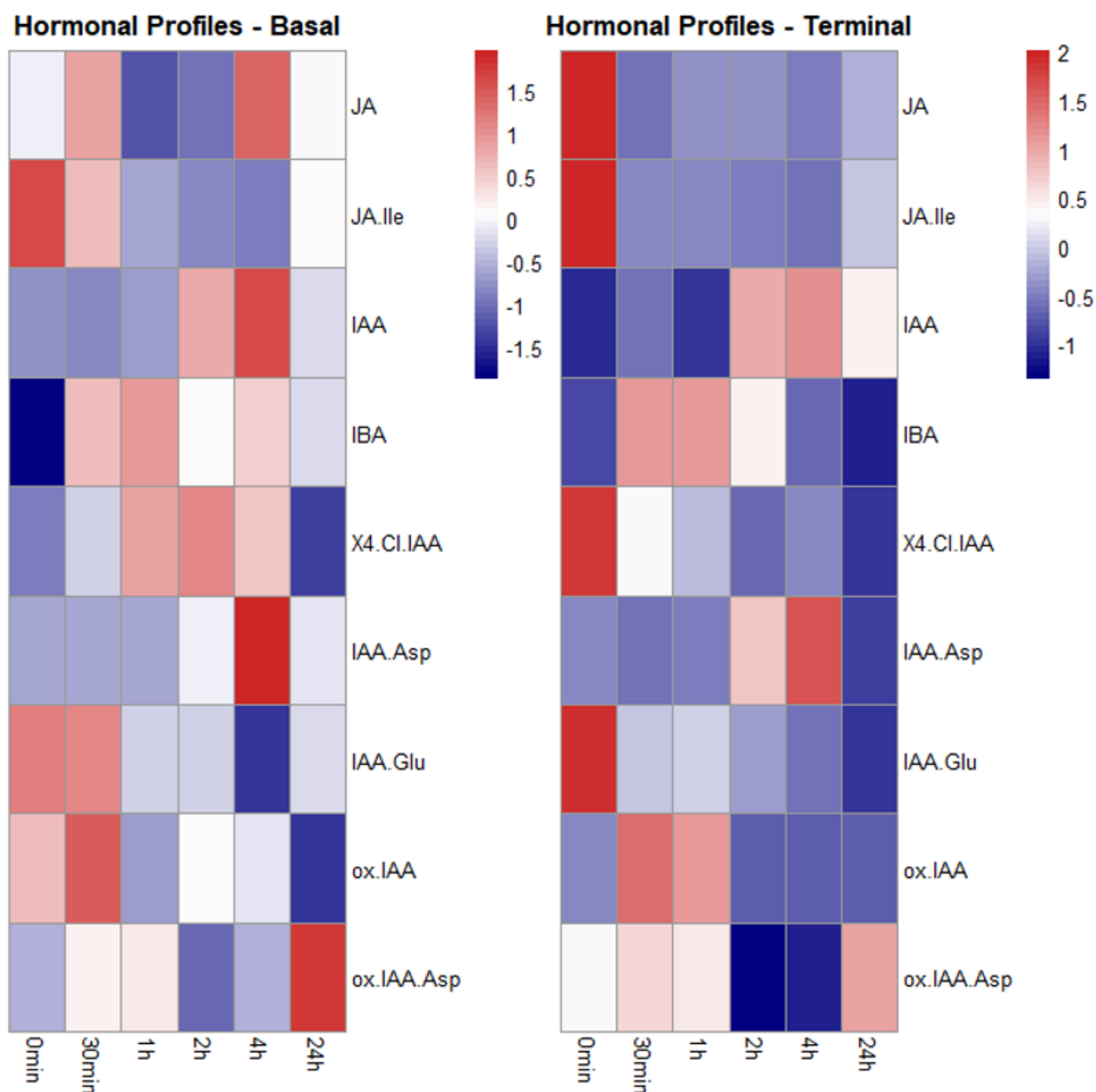


Figure 3

Heatmap representing the Z-score normalized content of individual phytohormones measured at six time points for basal and terminal cuttings. Red indicates higher relative content, while blue indicates lower relative content. (Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA), 4-chloro-indole-3-acetic acid (X4.Cl.IAA), Indole-3-acetyl-aspartic acid (IAA.Asp), Indole-3-acetyl-glutamic acid (IAA.Glu), Oxindole-3-acetic acid (ox-IAA), 2-Oxindole-3-acetyl-aspartic acid (ox.IAA.Asp), Jasmonic acid (JA), Jasmonoyl-isoleucine (JA.Ile))

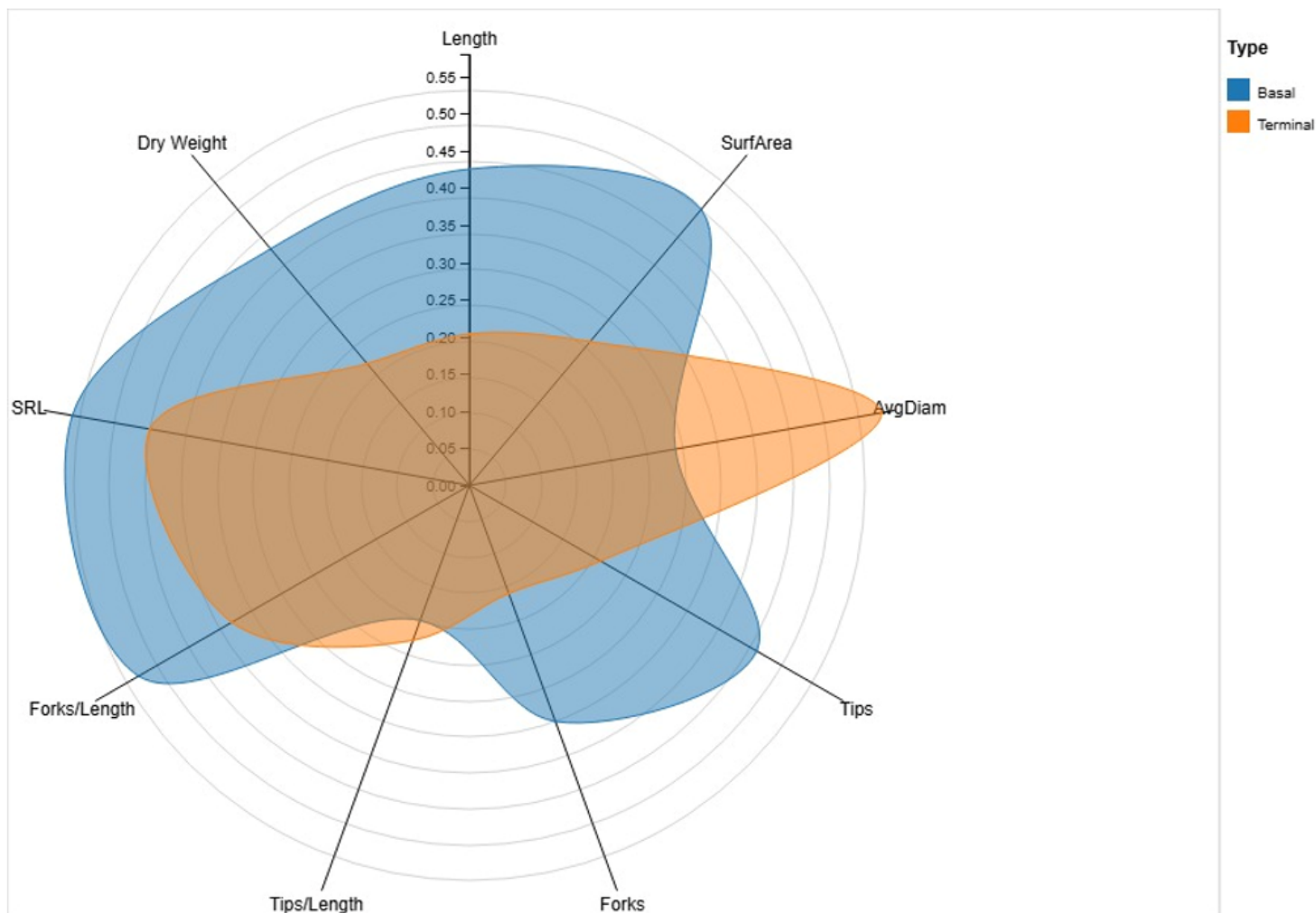


Figure 4

Comparison of normalized morphological root parameters between basal and terminal cuttings.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementarymaterial.docx](#)