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Improved root architecture and seedling performance in pistachio (*Pistacia vera* L.) via radicle-tip excision

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Abstract

Background Root system architecture is a key determinant of plant establishment, particularly in species such as Pistachio (*Pistacia vera* L.), which inherently display limited lateral root formation and low survival rates after transplanting. This study evaluated the impact of radicle-tip excision on root architecture and overall seedling vigor in 'Ohadi' pistachio cultivar. A factorial experiment was conducted using five distinct radicle length categories at the time of excision: L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm), and three cutting sizes of 1 mm (CS1), 3 mm (CS3) and 5 mm (CS5) while untreated seedlings were used as the control group. Following radicle-tip excision, the seedlings were grown in perlite-filled pots and irrigated weekly with half-strength Hoagland's nutrient solution. Growth, vitality, and root architectural traits were assessed at end of the experiment.

Result Remarkably, the L3CS3 treatment involving excision of 3 mm from the radicle tip when its length reached L3 (2.01–3.0 cm) resulted in significant enhancements in key morphological traits, including shoot and root fresh/dry weights, leaf area and plant height. In addition, root system characteristics such as the number of lateral roots (NLR), network depth (NWDP), network volume (NWVL), and convex area (NWCA) showed substantial improvement. Ordinal regression analysis revealed a strong relationship between lateral root traits and overall seedling vigor, confirming the physiological relevance of the root manipulation. Complementary correlation analysis further supported the notion that improved root traits positively influence shoot growth, indicating an integrative effect.

Conclusion These findings underscore the potential of radicle-tip excision as a cost-effective and scalable approach to improve root functionality and transplant performance in pistachio, with broader applicability to other species that exhibit limited lateral root development in nursery production systems.

Keywords Growth parameters, Lateral roots, Radicle-tip excision, Root system, Transplanting, Vitality

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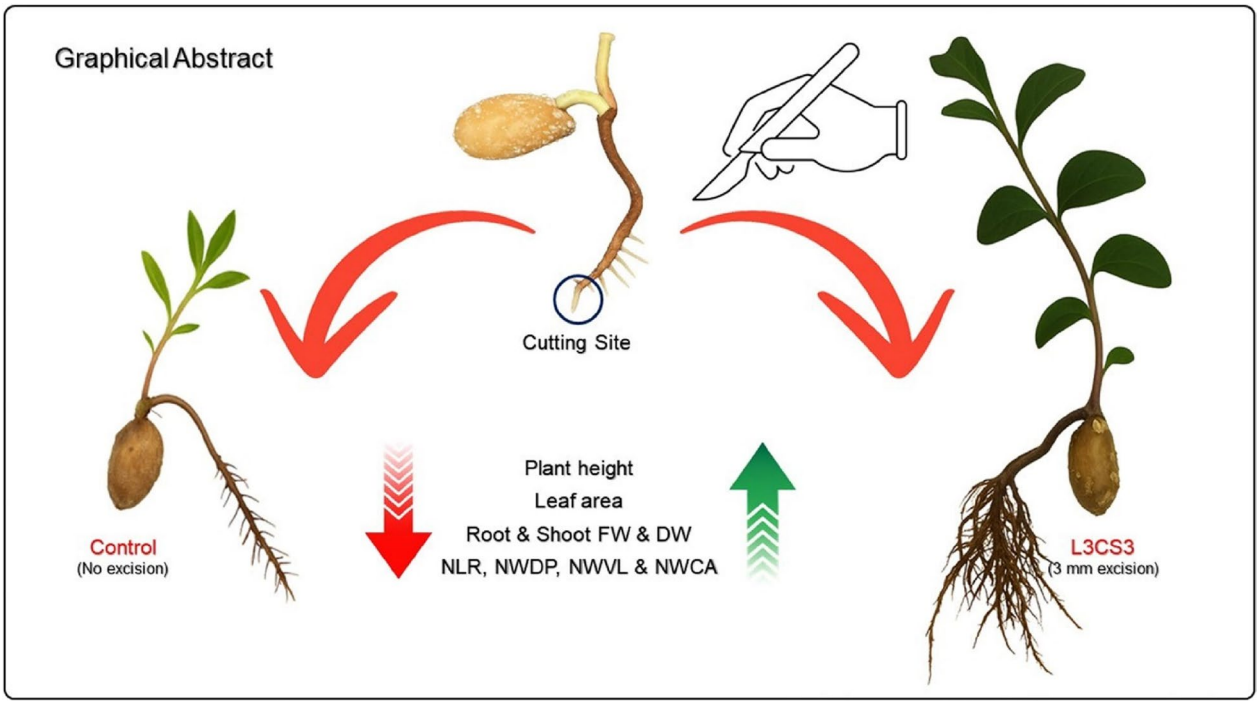
Graphical abstract



Introduction

The genus *Pistacia*, a member of the Anacardiaceae family, comprises several species, among which Pistachio (*Pistacia vera* L.) is cultivated for its commercially valuable nuts [1]. The pistachio plant develops a dominant taproot but exhibits poor lateral root branching, which may compromise its capacity for early establishment and efficient water and nutrient uptake from the soil [2]. The production of rootstocks with strong roots and high establishment potential plays a key role in enhancing the yield of this economically significant crop [3].

Tree roots are typically classified into two categories based on diameter: coarse roots, which exceed 2 mm in thickness, and fine roots, which are less than 2 mm in diameter [4]. Coarse roots, particularly those exhibiting increased suberization, are primarily responsible for transporting water from deeper soil layers. In contrast, fine roots, characterized by a dense network of root hairs, are more effective in absorbing water and nutrients from the upper soil layers [5]. These root types differ in several key attributes, including their longevity, biomass, and lignin content [6].

Root architecture describes the spatial arrangement of a plant's root system within the soil, including its structure, distribution, and branching patterns. This encompasses both primary roots (taproots) and secondary roots (lateral roots). The root system serves as a critical interface between the soil and the aerial parts of the plant, functioning as a sensor for abiotic and biotic stresses [7]. Moreover, it plays a pivotal role in regulating plant growth and development, enabling plants to adapt to edaphic conditions and ensuring their survival across a wide range of ecological conditions [8].

A significant challenge in pistachio cultivation is the poor establishment of plants after transplanting from the nursery to the field [2]. Pistachio is particularly vulnerable to transplant stress, primarily due to damage to the main root tip, which increases the risk of desiccation. As a result, pistachio trees are typically propagated in nurseries or directly from seeds in the field. The production of high-quality seedlings is critical for enhancing post-transplant survival rates and minimizing recovery periods [9]. Pistachio seedlings have an axial root system but often lack sufficient lateral roots, making them vulnerable to transplant shock. A limited root system reduces seedling resilience, leading to lower survival and slower growth in the field [10–12]. To improve post-transplant success, a well-developed and branched root system is essential for enhancing water and nutrient uptake, which ultimately supports better bud growth and overall plant performance [2, 12].

The root systems of seedlings can be manipulated to mitigate transplantation shock by promoting an increase in the number of roots. This enhancement can be achieved through root pruning in the nursery, which encourages the expansion of lateral roots (LRs) [13]. LRs

are essential for increasing stress resilience and boosting overall plant productivity. A significant challenge lies in the intricate interactions between hormones and genes during LRs development. Previous research has shown that every stage of root formation, from the initiation of lateral root primordia to their emergence from the parent root, is governed and regulated by both internal and external signals [14]. The ratio of auxins to cytokinins controls plant growth and, most critically, regulates the initiation of both primary and lateral roots [15].

Active growth of dicot primary roots often signifies apical dominance, a condition in which lateral root development is inhibited [16]. However, the removal of the primary root tip triggers the rapid initiation of lateral root primordia, which are typically absent in intact roots [17]. Thus, upon discovering an effective method to stimulate lateral root formation and emergence, growers have adopted the radicle-tip excision technique, which involves trimming pistachio root tips [2]. Root tip excision has been used in various plant species as a strategy to promote root growth by inducing lateral root formation and enhancing plant establishment. Root system manipulation has also proven effective in improving transplant performance in woody species. For instance, pre-transplant root pruning significantly enhanced lateral root formation and post-transplant shoot growth in species such as *Quercus bicolor* and *Carpinus caroliniana* [18].

To optimize pistachio seedling establishment and emphasize the pivotal role of lateral root development and optimized root architecture, we conducted an innovative study investigating the effects of radicle-tip excision on growth and root system structure. Our research addresses the limited scientific resources in this area, proposing that trimming the root tip shortly after radicle emergence at the cell division zone stimulates root branching, thereby promoting sustained growth following transplantation. The findings provide insights that may contribute to enhancing lateral root development and improving post-transplantation survival rates in pistachio cultivation.

Materials and methods

Plant material

Half-sibling seeds of *Pistacia vera* L. cv. 'Ohadi' were obtained from a commercial pistachio orchard located in Rafsanjan, Kerman Province, Iran, and served as the plant material in this study. The 'Ohadi' cultivar is among the most commonly cultivated pistachio varieties in Iran, known for its moderate vegetative vigor and broadly spreading canopy architecture [19]. It is well-suited to the environmental and soil conditions prevalent in major pistachio-producing areas of the country.

To overcome seed dormancy, stratification was conducted by placing the seeds at 4 °C for a period of 60 days. After cold stratification, the seeds were immersed in water for 12 h and subsequently disinfected by soaking in a 0.01% Captan solution for 20 min [20]. Treated seeds were then sown in plastic trays containing fine-grade (particle size 1–2 mm) perlite and maintained under greenhouse conditions.

Experimental setup

The experiment was designed as a factorial trial based on a completely randomized layout, incorporating two experimental factors. A total of 15 treatment combinations, along with a control group, were included. Each treatment and the control were replicated three times, with 10 seedlings per replicate, resulting in an initial population of 480 plants. The first factor involved the radicle length at the time of excision, categorized into five levels: L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm). The second factor was cutting size from the radicle tip, set at three levels: 1 mm (CS1), 3 mm (CS3) and 5 mm (CS5). In the control group, the radicle tips remained intact (Fig. 1).

Radicle-tip excision was performed 7 to 14 days after sowing, once the radicles had emerged and reached the target length. A scalpel was used to carefully remove the specified portion of the radicle in order to minimize any potential damage to the developing root. The excision length was measured precisely using a digital caliper. Immediately following the excision, seedlings were transferred into 2-liter plastic pots, measuring 20 cm in both diameter and height, containing fine-grade perlite. To maintain uniformity among experimental units, any weak or abnormal seedlings were discarded after transplanting, ensuring that only one healthy seedling remained per pot.

Throughout the experiment, the greenhouse maintained day and night temperatures of approximately 24 °C and 19 °C, respectively, with relative humidity levels ranging from 51% during the day to 75% at night. Beginning one week post-transplantation, the seedlings received weekly applications of Hoagland's solution at half-strength, meaning the macronutrient concentrations were reduced by half [21]. Root traits and growth parameters were evaluated twelve weeks after planting.

Measurements

Root architecture

At the end of the experiment, the roots were gently removed from the pots, and then separated from the crown. Images of the roots were captured using an RGB digital camera and subsequently analyzed with GiaRoots® software [23, 24]. The parameters derived from image processing included the number of lateral roots (NLR),

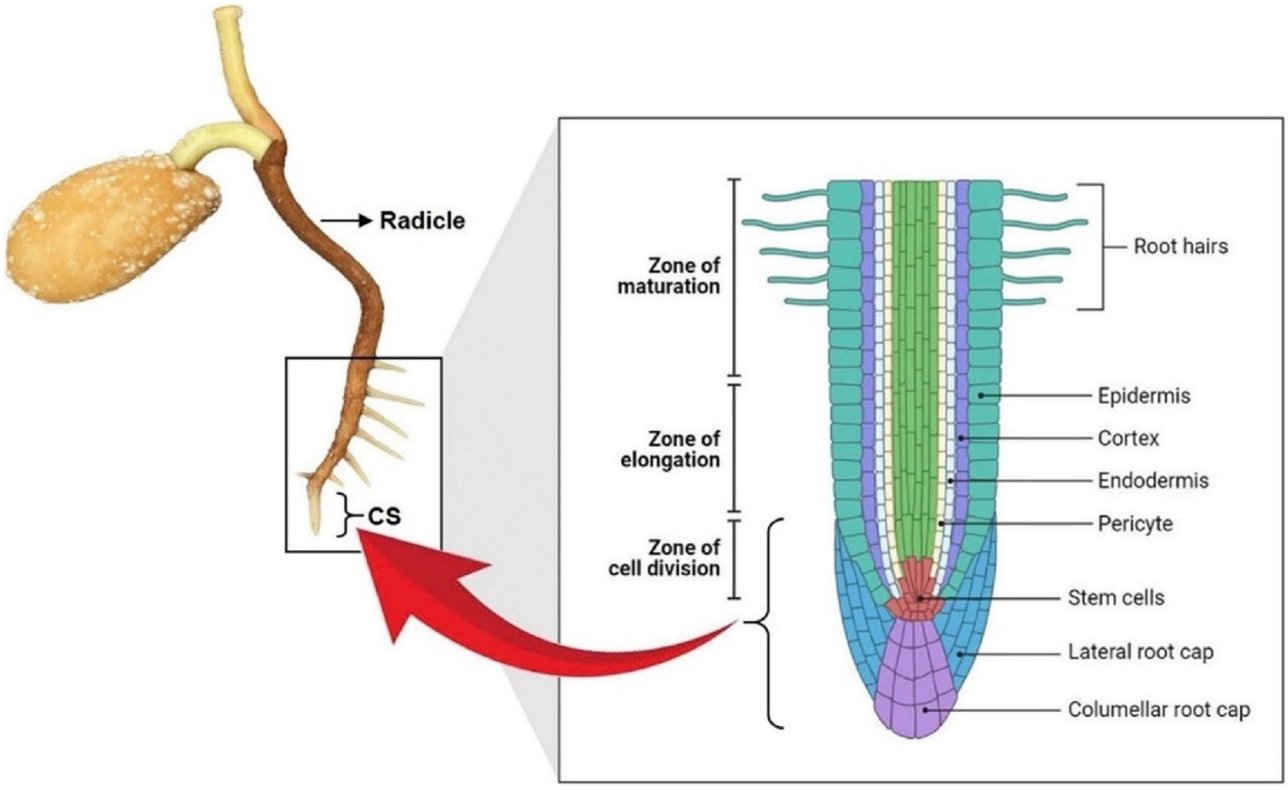


Fig. 1 Anatomical structure of the pistachio radicle illustrating the cutting site (CS). The schematic image is sourced from Shelden and Munns, 2023 [22]

network depth (NWDP: number of pixels in the vertical direction from the upper-most network pixel to the lower-most network pixel), ellipse aspect ratio (ELAX: ratio of the minor to the major axis of best fitting ellipse), network length distribution (NWLD: fraction of network pixels found in the lower 2/3 of the network which is defined based on network depth), network convex area (NWCA: area of the convex hull that encompasses the image) and network volume (NWVL: sum of the local volume at each pixel of the network skeleton, as approximated by a tubular shape whose radius is estimated from the image).

Growth characteristics

The growth characteristics assessed in this experiment included leaf area, plant height, and the fresh weight (FW) and dry weight (DW) of both the shoot and root. To measure leaf area, all leaves from each plant were arranged flat without overlapping, photographed, and analyzed using Digimizer® software [25]. Plant height was measured in centimeters (cm) using a ruler, from the soil surface to the apical meristem. The fresh and dry weights of both roots and shoots were recorded using a digital scale with an accuracy of 0.0001 g. After removal from the soil, plants were separated into shoots and roots, which were then oven-dried at 70 °C for 72 h to determine their dry weight. Measurements were repeated until a constant mass was achieved, ensuring accuracy [26].

Plant vitality

The plant vitality index was evaluated using a visual scoring method based on overall plant appearance, leaf color and turgor. Scores ranged from 1 (indicating the most severely damaged plants with yellowing, wilted or necrotic leaves) to 5 (representing completely green, turgid and healthy plants with no visible stress symptoms) [27].

Statistical analysis

Analysis of variance was conducted in R (v4.3.2) using various packages by function: "tidyverse" and "dplyr" for data manipulation; "car", "MASS", "easyanova", and "report" for statistical modeling; "ggplot2", "ggstatsplot", and "sjPlot" for visualization; and "flextable" for reporting. Means were compared with Duncan's Multiple Range Test ($P < 0.05$). Trait normality was assessed via the Shapiro-Wilk test. Vitality, measured on an ordinal scale, was analyzed using ordinal logistic regression via the *polr* function from "MASS". Odds ratios (OR), 95% CIs, and p-values were calculated relative to the control group. A Pearson correlation network was visualized using the *ggraph* package [28].

Results

Root architecture

An analysis of the root system revealed that the interaction between cut size and radicle length at the time of tip cutting had a significant impact on the number of lateral roots (NLR) ($P < 0.01$). Based on the results, different treatments showed varying responses in terms of NLR, NWDP, and NWCA. Plants from which 3 mm of the radicle tip was removed (CS3) when the radicle length reached L3 (2.01–3.0 cm) produced the highest average number of lateral roots (mean NLR = 32). By increasing seedling root lengths to L4 (3.01–4.0 cm) and L5 (4.01-5.0), lateral root numbers (NLR) decreased. No significant difference was found between L1CS1, L1CS5 and the uncut control. These displayed the lowest NLR (Fig. 2).

The root network convex area

NWCA was lowest in L1CS5 and did not differ significantly from the control, L1CS3, and L1CS5 treatments. The greatest NWCA belonged to the L3CS3, which also had the highest number of lateral roots. NWCA ascended from L1 to L3, but when the length of the radicle reached L4 and L5, and excision was done, NWCA decreased (Fig. 3).

Network depth

The impact of radicle-tip cutting on NWDP showed that the L3CS1 treatment had the highest NWDP, and no significant difference existed between the control and this treatment. The L1CS5 treatment had the lowest NWDP (Fig. 4)

Root network volume

NWVL was significantly influenced by radicle length and cutting size × radicle length interaction ($P < 0.01$). The L3CS3 treatment resulted in the highest NWVL. Radicle lengths of 0.5–1.0 cm were deemed unsuitable for cutting, as they led to a reduction in lateral root numbers. Control plants exhibited similar NWCA and NWVL to those cut at L1 length, with no significant difference between them (Fig. 5).

Network length distribution

Root architecture data indicated that NWLD was significantly affected by radicle length ($P < 0.01$) and size of the cutting site ($P < 0.05$), as shown in the root architecture data. Although control plants showed the highest NWLD, the difference between control and L3CS3 was not statistically significant (Table 1).

Ellipse aspect ratio

ELAX was unaffected by the treatments, with only the interaction of radicle length × cut size proving significant ($P < 0.05$). Control plants exhibited the highest ELAX. There were no significant differences in ELAX between L1 and L5 (Table 1).

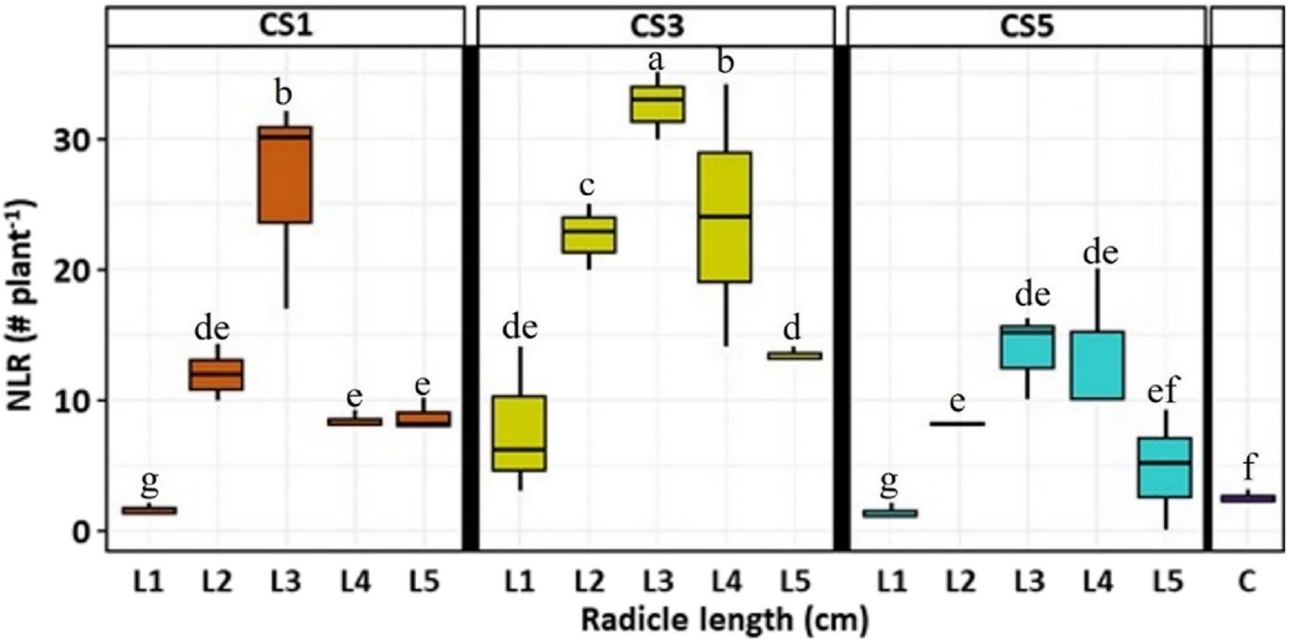


Fig. 2 The number of lateral roots (NLR) (± SD) of pistachio (cv. Ohadi) subjected to radicle-tip excision treatments. L refers to the radicle length during cutting, categorized as L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm). CS represents the cutting site distance from radicle tip including 1 (CS1), 3 (CS3), and 5 mm (CS5). The control (C) indicates no excision. Different letters indicate statistically significant differences between groups ($P < 0.05$)

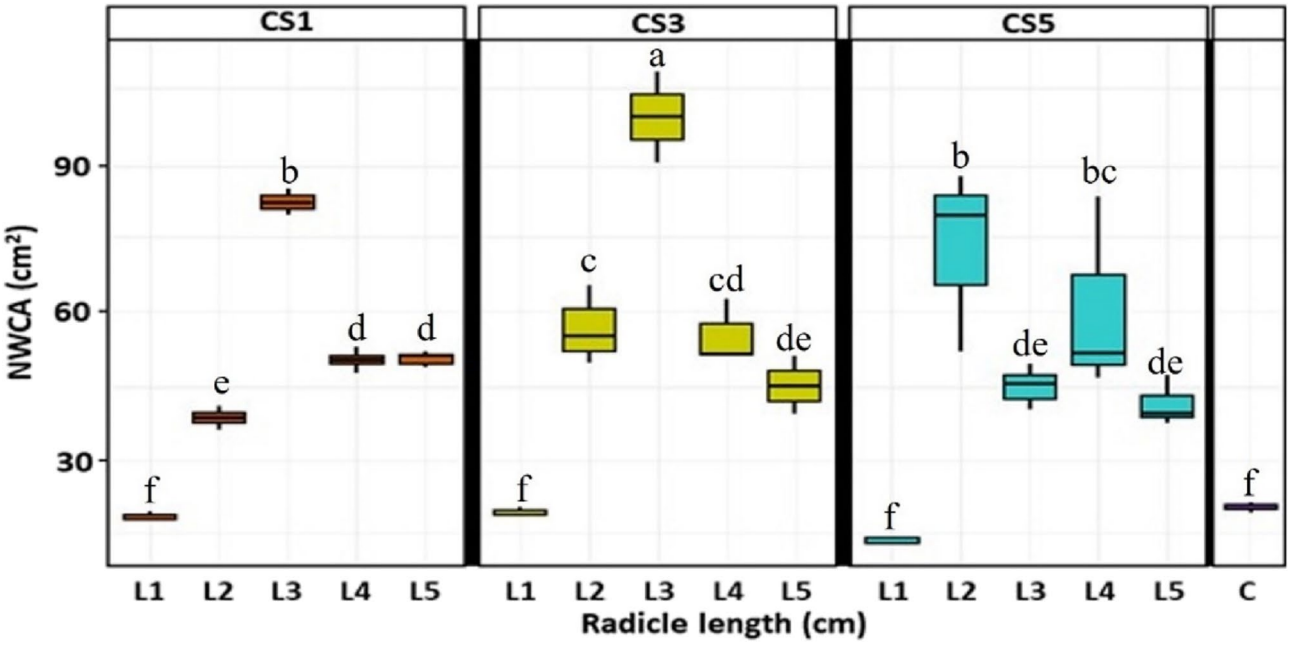


Fig. 3 The root network convex area (NWCA) (± SD) in pistachio (cv. Ohadi) subjected to radicle-tip cutting treatments. L represents the radicle length during cutting, categorized as L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm). CS represents the cutting site distance from the radicle tip, including 1 (CS1), 3 (CS3) and 5 mm (CS5). The control (C) indicates no excision. Different letters indicate statistically significant differences between groups ($P < 0.05$)

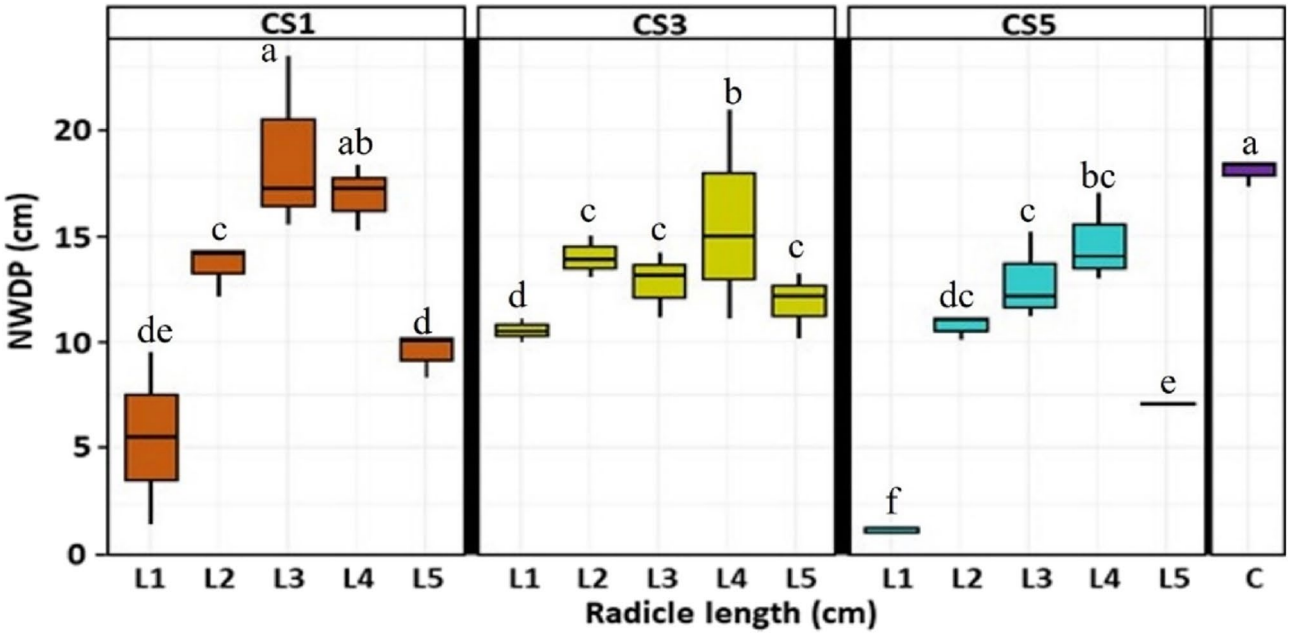


Fig. 4 The root network depth (NWDP) (± SD) in pistachio (cv. Ohadi) subjected to radicle-tip cutting treatments. L denotes the radicle length during cutting, categorized as L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm). CS indicates the cutting site distance from the radicle tip, including 1 (CS1), 3 (CS3), and 5 mm (CS5). The control (C) indicates no excision. Different letters indicate statistically significant differences between groups ($P < 0.05$)

Growth characteristics

The results revealed significant impacts of the studied treatments ($P < 0.01$) on the FW and DW of the shoot and root. The lowest FW and DW for both shoot and root were observed in L5CS5 and L1CS5 treatments. Differences occurred in the height of pistachio seedlings across treatments, with the tallest plants (22.6 cm) achieved through the L3CS3 treatment. Control plants had an

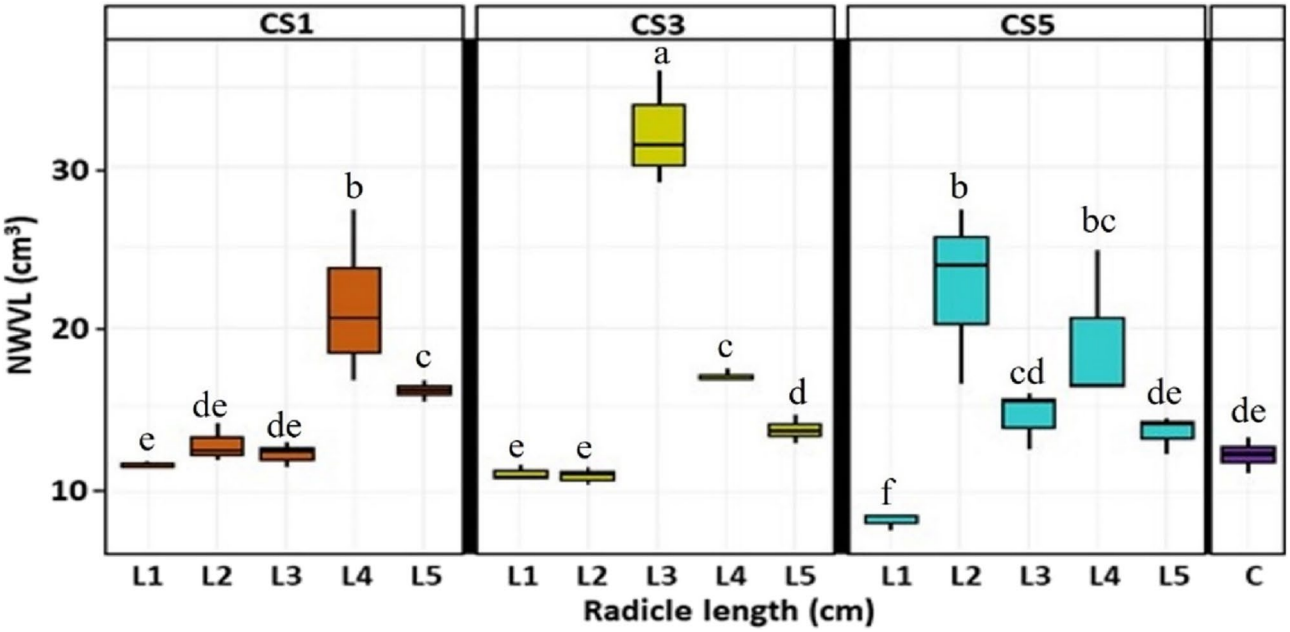


Fig. 5 The root network volume (NWVL) (± SD) of pistachio (cv. Ohadi) under radicle-tip cutting treatments. L denotes the radicle length during excision, categorized L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm). CS indicates the cutting site distance from radicle tip, including 1 (CS1), 3 (CS3), and 5 mm (CS5). The control (C) indicates no excision. Different letters indicate statistically significant differences between groups ($P < 0.05$)

Table 1 Mean values for plant height, leaf area, ellipse axes ratio, and network length distribution in pistachio seedlings under radicle-tip cutting treatments

Treatment		Characteristic			
Radicle length (L)	**Cutting size (CS)**	**Plant height (cm)**	**Leaf area (cm^2)**	**ELAX**	**NWLD**
L1	CS1	7.5 ± 0.51 jk	0.28 ± 0.02 h	0.2 ± 0.01 de	0.13 ± 0.01 g
	CS3	8.83 ± 0.73 j	0.65 ± 0.05 gh	0.13 ± 0.01 e	0.2 ± 0.02 fg
	CS5	6 ± 0.44 k	0.28 ± 0.01 h	0.22 ± 0.05 de	0.13 ± 0.03 g
L2	CS1	16.6 ± 0.82 d-f	3.4 ± 0.13 b	0.54 ± 0.02 b	0.37 ± 0.04 de-g
	CS3	21.3 ± 1.13 ab	2.06 ± 0.23 c-e	0.3 ± 0.01 c-e	0.53 ± 0.03 b-d
	CS5	13.33 ± 1.10 gh	3.11 ± 0.33 b	0.3 ± 0.01 c-e	0.43 ± 0.02 c-f
L3	CS1	18.33 ± 1.56 cd	3.11 ± 0.45 b	0.324 ± 0.02 c-e	0.54 ± 0.01 b-d
	CS3	22.6 ± 2.12 ab	4.71 ± 0.66 a	0.284 ± 0.01 c-e	0.7 ± 0.02 ab
	CS5	14 ± 1.34 f-h	2.46 ± 0.91 b-e	0.33 ± 0.04 b-e	0.65 ± 0.03 bc
L4	CS1	15 ± 0.77 fg	1.55 ± 0.55 e-g	0.37 ± 0.01 b-d	0.53 ± 0.01 b-d
	CS3	20 ± 1.78 bc	1.69 ± 0.78 d-f	0.374 ± 0.02 b-d	0.48 ± 0.02 b-e
	CS5	9.66 ± 0.65 ij	1.49 ± 0.45 e-g	0.394 0.03 b-d	0.45 ± 0.01 b-e
L5	CS1	11.66 ± 0.63 hi	1.98 ± 0.33 de	0.36 ± 0.05 b-d	0.34 ± 0.03 d-g
	CS3	15.5 ± 0.87 e-g	2.65 ± 0.68 b-d	0.304 ± 0.03 c-e	0.26 ± 0.04 e-g
	CS5	5.33 ± 0.12 k	0.86 ± 0.06 h	0.355 ± 0.01 b-d	0.194 ± 0.01 fg
Control	-	9.66 ± 0.21 ij	1.89 ± 0.38 de	0.932 ± 0.02 a	0.92 ± 0.10 a
L		**	**	ns	**
CS		**	**	ns	*
L×CS		**	**	*	*

Values are presented as Mean ± Standard Deviation (SD). Means followed by different lowercase letters within the same column are significantly different at $P < 0.05$ according to Duncan's multiple range test.*, **, ns: Significant differences at 5% and 1% probability levels and non-significantly different, respectively.

ELAX ellipse aspect ratio, *NWLD* network length distribution, *L* the length of the radicle when its tip is being cut including L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm), *CS*the length of cutting site from radicle tip, including 1 (CS1), 3 (CS3) and 5 mm (CS5), *C* control (no excision).

average height of 9.6 cm, surpassing the L1 treatments (L1CS1, L1CS3, L1CS5) and L5CS5 (Table 1). In addition, the L3CS3 treatment demonstrated significantly higher leaf area, FW, and DW for both root and shoot compared to the other treated seedlings and the control. This treatment proved to be the most effective in terms of growth parameters, including the induction of lateral roots and root architecture parameters (Figs. 6 and 7).

Plant vitality

Based on the results of the ordinal logistic regression (Table 2), the treatments with radicle lengths of L2, L3 and L4, as well as the control group, displayed significantly higher vitality compared to L1 and L5. Specifically, the L3CS3 treatment had the highest odds ratio (OR = 1.8, p-value = 0.002), suggesting an 80% increase in the likelihood of higher vitality scores compared to the control. On the other hand, L1 and L5 treatments exhibited lower vitality, with L1CS1 showing a 69% decrease in the odds of higher vitality (OR = 0.31, p-value < 0.001). These results suggest that optimal radicle tip cutting length and site can improve the vitality and growth of pistachio seedlings, with moderate lengths (2.01–3.0 cm) and appropriate cutting sizes (around 3 mm from the tip) being most effective for promoting root and plant health.

The analysis includes the odds ratios (OR), 95% Confidence Intervals (CI), p-values, and the percentage change in odds for each treatment compared to the control group. *, **, ns: Significant differences at 5% and 1% probability levels and non-significantly different, respectively.

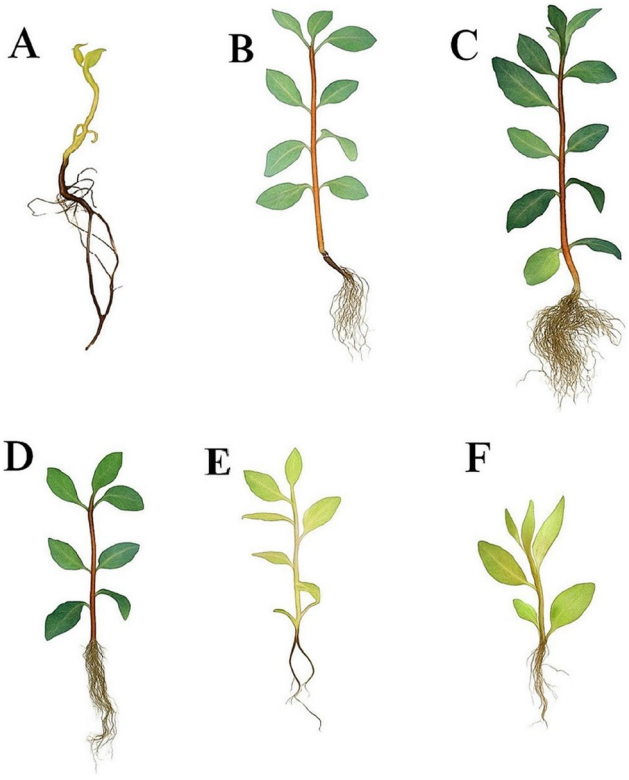


Fig. 6 Representative pistachio seedlings (cv. Ohadi) under 3 mm radicle-tip excision (CS3) treatments. A: L1CS3; B: L2CS3: C: L3CS3, D: L4CS3; E: L5CS3; F; Control. L denotes the radicle length during excision, categorized L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm)

Correlation analysis

Pearson correlation analysis revealed significant relationships between root development and growth characteristics in pistachio seedlings (cv. Ohadi). NLR showed strong positive correlations with both root and shoot biomass, including fresh weight (FW) ($r = 0.85$, $P < 0.01$) and dry weight (DW) ($r = 0.76$, $P < 0.01$), indicating that more lateral roots contribute to increased biomass. NLR was also moderately correlated with plant height ($r = 0.68$, $P < 0.05$), suggesting that taller plants tend to have more lateral roots. Additionally, NLR was strongly correlated with NWCA ($r = 0.91$, $P < 0.01$) and NWVL ($r = 0.87$, $P < 0.01$), highlighting the importance of larger root networks in supporting lateral root growth. NWCA also positively correlated with plant height ($r = 0.75$, $P < 0.01$) and LA ($r = 0.78$, $P < 0.01$), linking root expansion to better shoot growth. NWDP was positively correlated with plant height ($r = 0.72$, $P < 0.01$) and root fresh weight ($r = 0.80$, $P < 0.01$), suggesting deeper roots enhance overall growth, particularly biomass. Vitality, measured through ordinal logistic regression, was strongly associated with NLR ($r = 0.85$, $P < 0.01$), root dry weight ($r = 0.79$, $P < 0.01$), and shoot fresh weight ($r = 0.72$, $P < 0.01$), indicating that better root and biomass development leads to higher plant vitality. ELAX did not show significant correlations with other growth traits, likely due to its lack of variation across treatments. However, NWLD exhibited positive correlations with plant height ($r = 0.60$, $P < 0.05$) and LA ($r = 0.70$, $P < 0.01$), suggesting that a more evenly distributed root length supports taller plants and larger leaves (Fig. 8).

Discussion

Root system architecture (RSA) plays a crucial role in determining plant growth and resilience, particularly under stressful environmental conditions. A well-developed RSA, including an extensive lateral root network, enhances water and nutrient uptake efficiency, increases transplant success, and contributes to greater biomass and product quality. Moreover, manipulating RSA during early stages through root-tip excision can improve seedling uniformity, reduce production costs, and enhance field establishment and performance [27, 29–31]. In pistachio seedlings, which naturally develop a taproot-dominant system, modifying root structure through precise radicle-tip excision at the appropriate stage offers a

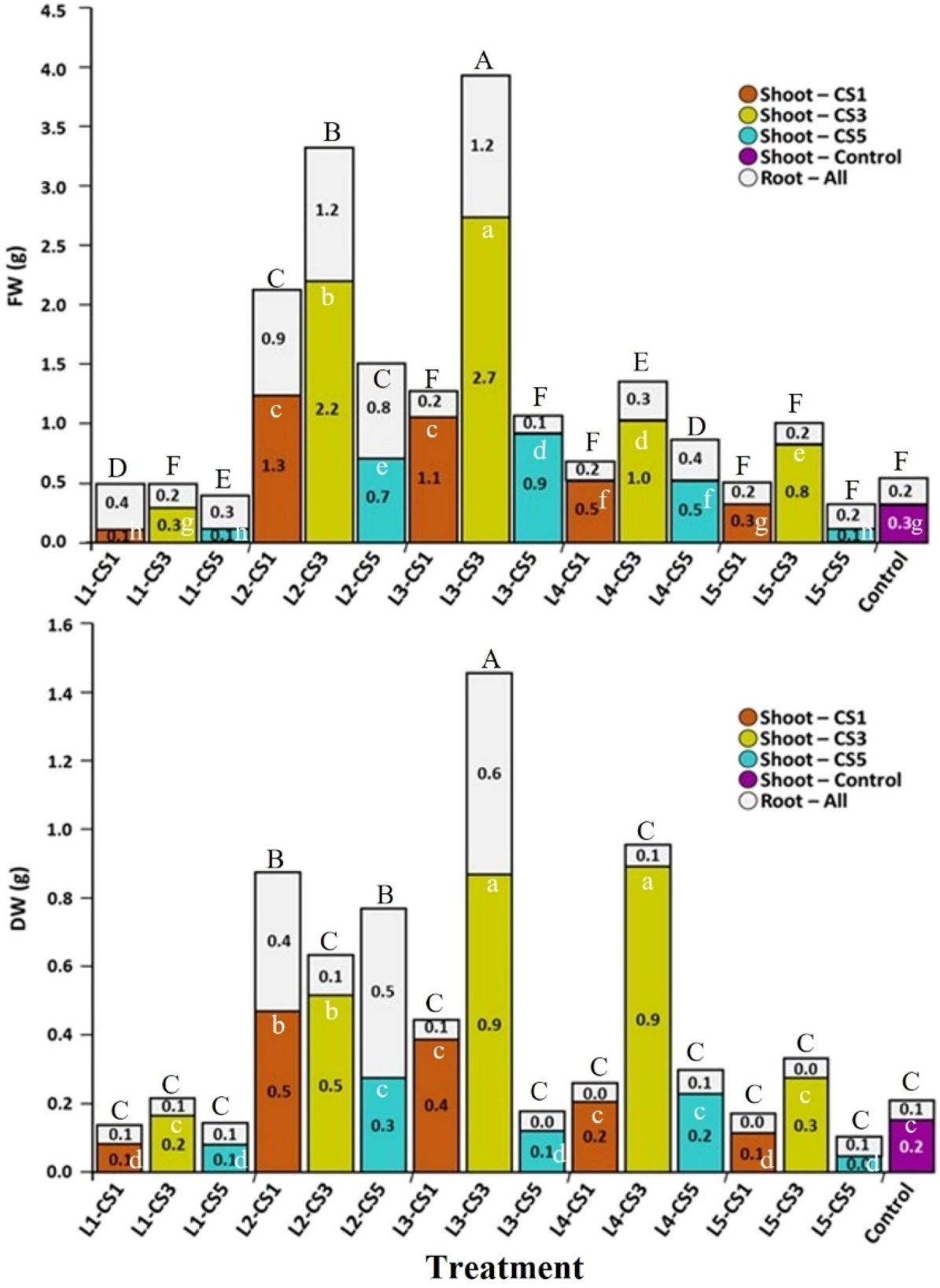


Fig. 7 The fresh (FW) and dry (DW) weight of pistachio seedlings (cv. Ohadi) under radicle-tip cutting treatments. L denotes the radicle length during excision, categorized L1 (0.5–1.0 cm), L2 (1.01–2.0 cm), L3 (2.01–3.0 cm), L4 (3.01–4.0 cm) and L5 (4.01–5.0 cm); CS: the length of cutting site from radicle tip, including 1 (CS1), 3 (CS3) and 5 mm (CS5). C: control (no excision). Each column is divided into shoot (lower part, small letters) and root (upper part, capital letters). Different letters indicate significant differences between treatments at $P < 0.05$

Table 2 Ordinal logistic regression analysis for vitality in pistachio seedlings under radicle-tip cutting treatments

Treatment	OR	95% CI	*P*-value	Change in Odds (%)
Control (Ref)	**1.0**	–	–	–
L1-CS1	0.31	0.18–0.53	$<0.001^{**}$	↓69%
L1-CS3	0.35	0.20–0.60	$<0.001^{**}$	↓65%
L1-CS5	0.4	0.23–0.68	0.001^{**}	↓60%
L2-CS1	1.2	0.75–1.92	0.45^{ns}	↑20%
L2-CS3	1.35	0.85–2.13	0.21^{ns}	↑35%
L2-CS5	1.1	0.70–1.72	0.64^{ns}	↑10%
L3-CS1	1.4	0.88–2.24	0.15^{ns}	↑40%
L3-CS3	1.8	1.30–3.39	0.002^{**}	↑80%
L3-CS5	1.6	1.00–2.56	0.048^{*}	↑60%
L4-CS1	1.1	0.68–1.78	0.69^{ns}	↑10%
L4-CS3	1.45	0.90–2.34	0.12^{ns}	↑45%
L4-CS5	1.25	0.78–2.01	0.34^{ns}	↑25%
L5-CS1	0.5	0.30–0.84	0.008^{**}	↓50%
L5-CS3	0.48	0.28–0.82	0.006^{**}	↓52%
L5-CS5	0.55	0.33–0.90	0.017^{*}	↓45%

practical solution to address limitations in lateral root formation [32]. Such methods are crucial for refining nursery practices, accelerating post-transplantation acclimatization, and ultimately enhancing seedling performance in the field [3].

Given the growing need for sustainable and high-efficiency orchard establishment in arid and semi-arid regions, such as Iran, enhancing root functional traits through simple and low-cost interventions can have a significant agronomic impact [33, 34]. Unlike traditional approaches that often focus solely on shoot growth or fertilization strategies, root-targeted manipulations provide a fundamental shift toward improving whole-plant resilience from the base.

This study seeks to refine radicle-tip excision techniques in pistachio roots to improve seedling establishment in the orchard. By investigating how the timing and precision of cutting influence root system morphology, the research addresses a critical knowledge gap in pistachio nursery management, where root system quality directly affects transplant success and long-term productivity. Ultimately, these findings may inform protocol development not only for pistachio but also for other species with poor lateral root expression, offering broader applications in horticulture and forestry.

Pistachio root systems are often deficient in lateral root development [34], leading to higher transplantation costs and lower survival rates. The effectiveness of lateral root induction is influenced by both environmental and physiological factors, including the balance of phytohormones [15, 17]. Among the effective interventions is pinching or cutting the radicle tip to suppress apical dominance, which promotes lateral root emergence [35].

Our findings demonstrate that radicle-tip excision, particularly when performed at L3 (2.01–3.0 cm) radicle length with a 3 mm excision (treatment L3CS3), significantly enhances RSA. This was evidenced by marked increases in the number of lateral roots (NLR), network volume (NWVL), and network convex area (NWCA). Improved RSA correlated with higher shoot fresh weight (FW), shoot dry weight (SDW), root dry weight (RDW), and leaf area (LA), indicating a substantial impact on overall seedling growth and vigor. The correlation between enhanced RSA and improved above-ground traits highlights the functional benefits of optimal radicle-tip cutting. Specifically, the L3CS3 treatment yielded the highest recorded values for all major growth parameters, demonstrating that precise control over radicle length and cutting depth can significantly enhance seedling establishment metrics.

Anatomically, lateral root formation typically initiates in the differentiation zone, where cells complete elongation and begin to acquire specialized functions. The developmental transition from the apical meristem through the elongation zone to this region is accompanied by a decline in mitotic activity, creating conditions favorable for lateral root primordia initiation in response to external or internal cues [36, 37]. Excision within the meristematic region, such as at 3 mm, likely removes apical dominance and stimulates lateral root formation due to the higher density of undifferentiated, actively dividing cells [2, 17]. Although hormonal levels were not measured in this study, previous research has highlighted the antagonistic roles of auxin (IAA) and cytokinins (CKs) in regulating lateral root development. IAA promotes lateral root initiation, while elevated CK concentrations in the root cap can suppress it [14, 17]. It is plausible that radicle-tip excision disrupts the hormonal gradient at the root apex, thereby favoring lateral root formation in distal zones. This hypothesis aligns with earlier findings in pistachio seedlings and other species [2, 33, 38]. Future studies measuring hormonal dynamics post-excision would help clarify the physiological basis of the observed root responses.

One of the most critical contributors to this improved functionality is the increase in lateral root formation, which expands total root surface area, enhances anchorage and improves soil exploration capacity [8]. Our findings revealed a strong correlation between the number of lateral roots (NLR) and increased network convex area (NWCA), both of which contribute to greater resource absorption potential. Elevated NWCA and network depth (NWDP) serve as indicators of superior spatial root deployment, supporting better nutrient uptake and leading to more vigorous above-ground growth [8, 31].

Interestingly, pre-existing lateral roots formed before radicle-tip excision showed limited elongation and

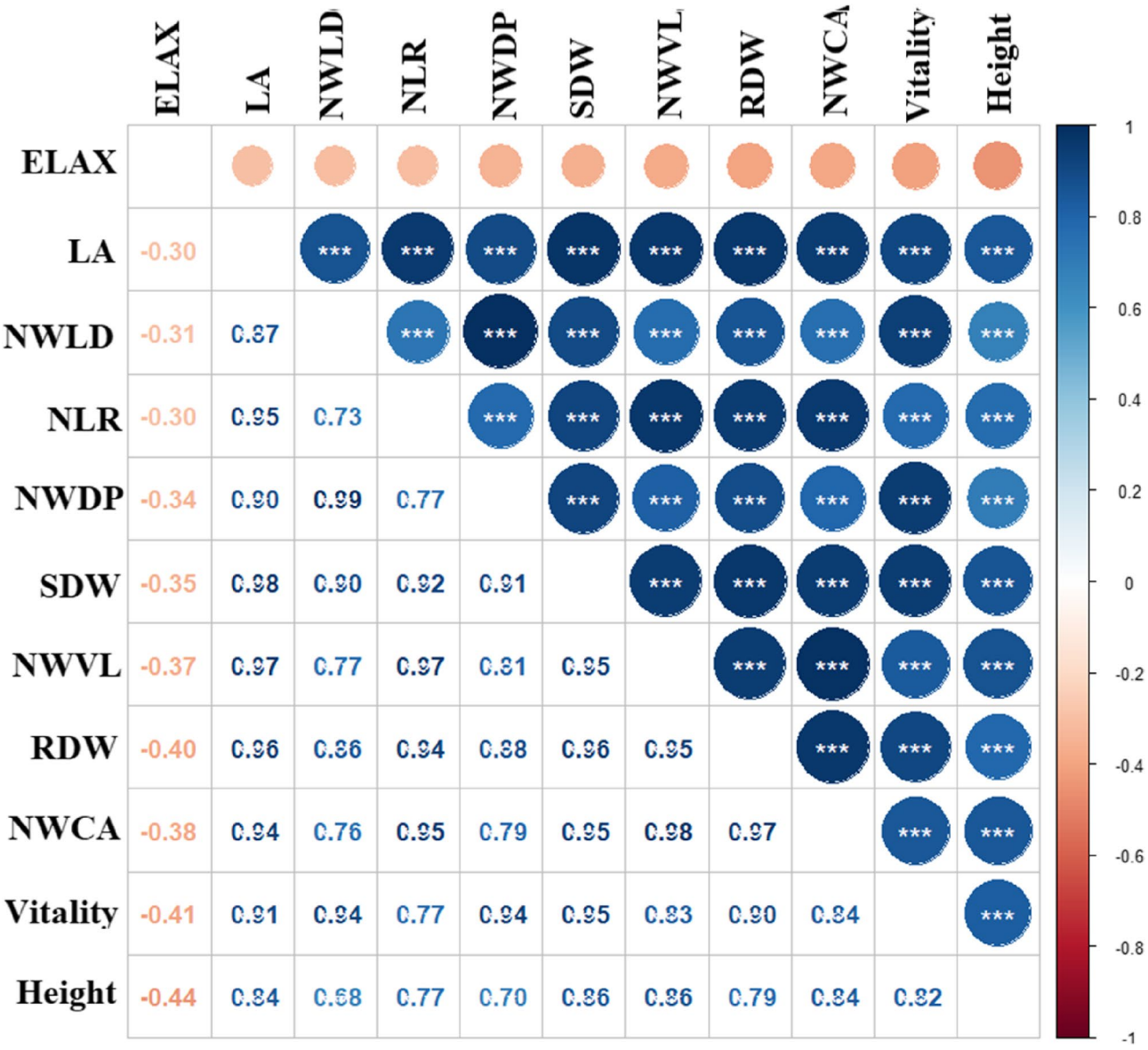


Fig. 8 Correlation coefficient between all traits studied in this research in pistachio seedlings (cv. Ohadi). The color spectrum, bright blue to bright red, represents highly positive to highly negative correlations. Stars in circle indicate the significance of correlations (*$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$). LA: leaf area, SDW: shoot dry weight, RDW: root dry weight, NLR: number of lateral roots, NWDP: network depth, ELAX: ellipse aspect ratio, NWLD: network length distribution, NWCA: network convex area, NWVL: network volume

slower growth rates. However, lateral roots that emerged post-excision exhibited accelerated, unimpeded elongation, often extending close to the length of the taproot itself though without penetrating deeper layers [39, 40]. This observation suggests a potential trade-off between vertical root elongation and lateral expansion following radicle-tip removal [41]. The suppression of apical dominance likely redirects resources and hormonal signaling toward the initiation and elongation of lateral roots. While this architectural shift may limit deep soil penetration, it enhances horizontal soil exploration, which is especially advantageous in shallow, nutrient-rich nursery beds [42, 43]. For species like pistachio, where early root establishment is critical for transplant survival, such a shift may confer more benefits than drawbacks. However, in deep-rooting species or water-scarce environments, this trade-off must be carefully evaluated to balance long-term drought tolerance with early-stage vigor [44]. This phenomenon underscores the developmental advantage conferred by radicle-tip removal, particularly in species prone to weak lateral root expression and poor post-transplant survival [45].

At the cellular level, the developmental competency of pericycle cells is spatially restricted. Only those within

the basal meristem regions preconditioned for division are capable of initiating lateral root formation [46]. The process involves a coordinated sequence of periclinal divisions that result in development of lateral root primordia [47]. As the radicle elongates, the distance from the meristematic zone increases, leading to reduced cell division potential and, consequently, lower lateral root initiation frequency [37].

Our results demonstrate that excision of the radicle at precisely 3 mm of its tip (L3CS3) interrupts this elongation trajectory within the zone of high meristematic activity. This disruption transforms the radicle into a more branched architecture, enabling the rapid emergence of lateral roots before the onset of cellular differentiation. The enhanced branching pattern promotes a denser, more functional root system, which significantly contributes to higher transplant success rates. Additionally, genotypic variability may influence the responsiveness of seedlings to radicle-tip cutting treatments [48].

Genetic factors such as the density of meristematic cells, root growth rates, and hormonal sensitivity could determine the extent of lateral root induction post-excision [49]. Pistachio varieties may differ in their endogenous cytokinin-to-auxin ratios or the developmental competence of their pericycle cells, affecting their regenerative capacity. Therefore, screening multiple genotypes for optimal responses to L3CS3 treatment could provide valuable insights for selective breeding programs aimed at enhancing transplant success and root system quality through both mechanical and genetic strategies [50].

From a practical viewpoint, the proposed 3 mm excision distance provides an effective benchmark for maximizing meristem cell availability and triggering robust lateral root development. The observed improvements in post-transplant field survival strongly endorse this technique as a cost-effective and scalable solution. Furthermore, its potential for mechanization presents promising opportunities for future research, particularly for commercial nurseries seeking to minimize labor demands while enhancing seedling establishment in pistachio orchards and other cropping systems.

Conclusion

This study is the first to determine the optimal timing and excision size for promoting lateral root formation in pistachio seedlings. We demonstrated that radicle-tip excision at 3 mm, when the radicle length reaches 2.01 to 3.0 cm (L3CS3), significantly enhances root branching, shoot and root biomass, and overall plant performance. These improvements are attributed to the disruption of the meristematic zone, which facilitates lateral root initiation, likely through hormonal regulation involving cytokinins. This practical, cost-effective method offers a scalable and reliable strategy to enhance root architecture and post-transplant performance in pistachio, with promising applicability to other species with poor lateral root development. Its simplicity and potential for automation also present valuable opportunities for broader adoption and further research in nursery and field conditions. Further investigations incorporating root activity-related assays, such as root respiration rate, enzyme activities, hormone profiling, or nutrient uptake efficiency, could provide deeper insight into the physiological mechanisms underlying the observed morphological changes and support more refined nursery practices.

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
Conceptualization: M.R.R.; Methodology: M.R.R., S.S., and M.O.; Software: M.O.; Validation: M.R.R., M.M.A. and R.S.M.; Original draft preparation: M.O.; Review and editing: M.O., M.R.R., S.L., M.M.A., R.S.M. and K.V.; Visualization: M.R.R.; Supervision: M.R.R. and S.S.

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Declarations

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Not applicable.

Consent for publication
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Competing interests
The authors declare no competing interests.

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