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Genotype-dependent *In Vitro* Propagation Responses of Newly Registered Turkish Hazelnut Cultivars(§)

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ABSTRACT

Hazelnut (*Corylus avellana* L.) genetic resources are essential for biodiversity conservation and breeding programs. Recently registered cultivars with valuable agronomic traits require efficient clonal propagation systems to ensure the production of disease-free planting material and the establishment of uniform orchards. Although micropropagation offers a promising alternative to conventional propagation methods, hazelnut remains a recalcitrant species due to low regeneration capacity and frequent latent contamination. In this study, four newly registered Turkish hazelnut cultivars (Okay 28, Allahverdi, Çetiner, and Süslü) were evaluated for their *in vitro* propagation responses. Axillary nodal explants collected between May and August 2025 were surface-sterilized and cultured on Nas and Read Medium (NRM) supplemented with 5 mg L^{-1} BA and 0.01 mg L^{-1} IBA. After four weeks, contamination and shoot induction rates were recorded. The highest contamination levels were observed in 'Okay 28' (84%) and 'Çetiner' (85%), whereas 'Süslü' (23%) and 'Allahverdi' (39%) showed the lowest contamination. Correspondingly, 'Süslü' and 'Allahverdi' exhibited the highest shoot induction rates (56% and 44%, respectively). During multiplication, 'Süslü' produced the highest number of shoots per explant (2.3) and the greatest shoot length (4.6 cm). Rooting on NRM supplemented with 2 mg L^{-1} IBA revealed significant genotype-dependent variation. This study represents the first report on the *in vitro* propagation of these newly registered hazelnut cultivars and highlights the importance of genotype-specific optimization for efficient micropropagation and germplasm conservation.

Keywords: *Corylus avellana,* hazelnut, micropropagation

Introduction

Hazelnut (*Corylus avellana* L.) is among the most economically significant nut crops worldwide, with Türkiye serving as the leading producer and exporter, supplying approximately 61% of global hazelnut production (FAOSTAT, 2024). Hazelnut cultivation is the principal livelihood and an essential economic pillar of the Black Sea region. Türkiye's diverse ecological conditions have facilitated the accumulation of rich *Corylus* germplasm, resulting in extensive genetic variability and numerous locally adapted landraces. This genetic wealth forms the backbone of breeding programs aimed at improving yield, kernel quality, and resilience to biotic and abiotic stress factors.

The Hazelnut Research Institute (HRI) in Giresun maintains the national hazelnut germplasm collection, comprising 459 *C. avellana* genotypes originating from different production zones (Şekerli, 2024). These genotypes represent a valuable reservoir for conservation, breeding, and biotechnological studies. However, conventional propagation method by suckers, it is slow, seasonal, and susceptible to pathogen transmission, while also showing considerable variability among propagules (Beyhan et al., 2018). Consequently, there is a growing need for efficient *in vitro* propagation systems that enable the rapid and reliable multiplication of elite cultivars while preserving genetic fidelity.

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Plant tissue culture provides a powerful platform for the clonal propagation, conservation, and improvement of woody species. Although several studies have reported *in vitro* propagation of Turkish cultivars such as 'Tombul', 'Çakıldak', and 'Foşa' (Şekerli, 2024; Elçiçek 2020), no data are available regarding the micropropagation responses of recently registered cultivars developed through national breeding efforts. Currently, 23 hazelnut cultivars are officially registered in Türkiye, each with distinct agronomic properties and adaptation capacities (HRI, 2026). Among these, four recently registered cultivars have not previously been evaluated under *in vitro* conditions. Understanding the morphogenic responses of these newly released cultivars under controlled culture conditions is therefore critical for establishing robust propagation protocols and enhancing the management of hazelnut genetic resources.

In this study, four recently registered Turkish hazelnut cultivars with distinct agronomic traits were evaluated. 'Okay 28', developed through breeding programs at HRI, is noted for its larger nut and kernel size compared with the widely cultivated 'Tombul' (Balık et al., 2015). The 'Allahverdi' cultivar, obtained through selection breeding and registered in 2015, displays a leafing time that is on average 10-15 days later than that of 'Tombul' (Balık et al., 2016). 'Çetiner' registered in 2022, is the earliest leafing cultivar among all examined varieties and exhibits a comparable kernel ratio along with reduced susceptibility to biennial bearing (Bektaş and Çil 2023). The most recently registered cultivar, 'Süslü' (TTSM, 2025), is a red-leaved ornamental hazelnut with distinctive husk coloration, contributing both genetic and aesthetic diversity to Turkish hazelnut germplasm.

The aim of this study was to evaluate the *in vitro* shoot induction and proliferation responses of these four new Turkish hazelnut cultivars cultured on Nas and Read medium (NRM) (Nas and Read, 2004). Genotype-specific differences were assessed to determine optimal conditions for high-quality shoot production. The findings provide a foundation for large-scale propagation of these cultivars and contribute to the long-term conservation and utilization of Türkiye's hazelnut genetic resources.

Materials and Methods

Plant Materials

One-year-old shoots were collected from approximately 10-year-old field-grown mother trees of the hazelnut cultivars 'Okay 28', 'Allahverdi', 'Çetiner', and 'Süslü' maintained in the germplasm collection orchard of the Hazelnut Research Institute (HRI), Giresun, Türkiye. Explant collection was carried out during the first weeks of June, July, and August 2025. During each sampling period, shoots from all four cultivars were collected and used as biological replicates for the study. The mother trees were pruned in March 2025 to promote the development of vigorous current-season shoots suitable for *in vitro* culture. No fungicides, plant growth regulators, or other chemical treatments were applied to the mother trees prior to explant collection.

Surface Sterilization and Culture Initiation

After the leaves had been removed, the shoots were rinsed under running tap water for 1 hour. The shoots were cut into 2-3 cm nodal segments and surface-sterilized under a laminar flow hood by immersion in 70% ethanol for 30 seconds followed by 1% (v/v) sodium hypochlorite (NaOCl). Preliminary sterilization trials were conducted to optimize the exposure time, and a treatment time of 5 min was selected as the optimal condition for all subsequent experiments. The explants were then rinsed three times with sterile distilled water and blotted dry on sterile filter paper.

Following the surface sterilization procedure, the explants were cultured individually in 16 × 100 mm culture tubes containing 5 mL of Nas and Read Medium (NRM; Nas and Read, 2004) supplemented with 5 mg L^{-1} 6-benzyladenine (BA), 0.01 mg L^{-1} indole-3-butyric acid (IBA), 30 g L^{-1} sucrose, and 0.6% (w/v) plant agar (Duchefa). The pH of the medium was adjusted to 5.7 before the addition of agar, after which the medium was autoclaved. Cultures were maintained at 23 ± 2°C under a 16 h photoperiod with a light intensity of 40 µmol m^{-2} s^{-1}. After four weeks of culture, the percentages of contaminated explants and shoot induction were recorded (Figure 1).

Shoot Multiplication

At the end of the initiation stage, the original woody explants were discarded, and only newly developed, contamination-free microshoots approximately 2 cm in length and containing two to three nodes were selected for the multiplication stage. The basal leaves of the selected microshoots were removed prior to transfer to fresh culture medium. The microshoots were then transferred to fresh NRM with the same composition as described above.

Microshoots were cultured in culture jars containing 50 mL of medium, with five microshoots per jar. Three biological replicates were used for each cultivar, and each replicate consisted of four culture jars. The cultures were maintained under the same environmental conditions described for the initiation stage. Microshoots were transferred onto fresh medium of the same composition at four-week intervals without shoot excision or division.

After a total culture period of eight weeks, the number of shoots per explant and the mean shoot length were measured and recorded (Figure 2).

Root Induction

Microshoots longer than 4 cm obtained after two consecutive four-week subcultures in the multiplication stage were transferred to fresh NRM supplemented with 2 mg L^{-1} IBA, 30 g L^{-1} sucrose, and 0.6% (w/v) plant agar for root induction. The pH of the medium was adjusted to 5.7 before the addition of agar and autoclaving. After autoclaving, filter-sterilized IBA (0.22 µm membrane filter, Isolab) was aseptically added to the cooled medium.

Microshoots were cultured in culture jars containing 50 mL of medium, with five microshoots per jar. Three biological replicates were used for each cultivar, and each replicate consisted of four culture jars. Cultures were maintained under the same environmental conditions described above. After six weeks of culture, the rooting percentage, the mean number of roots per microshoot, and the length of the longest root were recorded (Figure 3).

Acclimatization

Rooted plantlets cultured for six weeks on rooting medium were transferred into paper pots containing a substrate mixture of peat:perlite (2:1:1, v/v/v). The paper pots were placed in transparent plastic containers with closed lids to maintain high relative humidity and kept in a growth chamber at 23 ± 1°C, 70% relative humidity, and a 16 h photoperiod. After four weeks, humidity was gradually reduced by progressively opening the container lids, which were completely removed after 30 days. Plantlets were fertilized weekly with Hoagland nutrient solution (Hoagland and Arnon, 1938). Following acclimatization, the plantlets were transferred to a temperature-controlled greenhouse maintained at 25 ± 2°C and equipped with an automated misting system**.** The plantlets were grown in seedling trays under low plastic tunnels to maintain high humidity and promote successful establishment.

Data Collection and Analysis

The experiments were conducted using a completely randomized design. For culture initiation, each explant was cultured individually in a separate culture tube, and each tube was considered an experimental unit. The initiation experiment consisted of three independent experimental runs, conducted during the first weeks of June, July, and August 2025, with 100 explants per cultivar in each run. For the multiplication and rooting experiments, five microshoots were cultured in each jar, and each culture jar was considered an experimental unit. For each cultivar, the experiment consisted of three independent experimental runs, with four jars per run, resulting in a total of 12 jars and 60 microshoots per cultivar. Percentage data, including contamination, shoot induction, and rooting percentages, were subjected to arcsine square-root transformation before analysis of variance. All data were analyzed by analysis of variance (ANOVA) using IBM SPSS Statistics version 18.0. Differences among cultivar means were compared using Tukey's honestly significant difference test at $p < 0.05$. Untransformed percentage means are presented in the tables and figures for ease of interpretation.

Results

Microbial contamination and shoot induction rates varied significantly among the cultivars tested ($p < 0.05$) (Figure 4). The highest contamination levels were observed in 'Okay 28' (84%) and 'Çetiner' (85%), while 'Süslü' (23%) and 'Allahverdi' (39%) exhibited the lowest levels. Consistent with this trend, 'Süslü' and 'Allahverdi' showed the highest shoot induction frequencies, 56% and 44%, respectively. In contrast, 'Okay 28' (7%) and 'Çetiner' (10%) demonstrated the lowest shoot formation rates.

Contamination-free explants were subsequently transferred to multiplication medium. Among the cultivars, 'Süslü' produced the highest mean number of shoots per explant (2.3) and the greatest shoot length (4.6 cm). 'Allahverdi', 'Okay 28', and 'Çetiner' generated 1.31, 1.02, and 0.78 shoots per explant, respectively (Figure 5). Shoot elongation followed a similar pattern: 'Allahverdi' reached an average shoot length of 3.51 cm, followed by 'Okay 28' (2.15 cm), whereas 'Çetiner' exhibited the shortest mean shoot length (2.05 cm) (Figure 5).

Rooting performance demonstrated substantial variation among the cultivars (Figure 6). 'Süslü' achieved the highest rooting success (90%), producing an average of 4.56 roots per micro-cutting. This was followed by 'Allahverdi' with 83% rooting and a mean of 3.51 roots per micro-cutting, and 'Okay 28' with 72% rooting and an average of 2.15 roots. 'Çetiner' exhibited the lowest rooting success (56%), with a mean of 2.05 roots per micro-cutting.

Discussion

Extensive breeding and selection programs in hazelnut have led to the development of several superior cultivars with improved agronomic traits. Rapid, disease-free, and clonal propagation of these cultivars is essential for their widespread adoption and for establishing uniform orchards. In Türkiye,

where propagation is largely carried out through suckers, conventional methods remain insufficient to meet the growing demand for high-quality planting material and are unsuitable for sustainable large-scale production. In contrast, micropropagation enables continuous, controlled, and clonal plant production throughout the year, making it a valuable tool for modern hazelnut cultivation.

In the present study, mature field-grown trees served as the source of explants. Consequently, the observed microbial contamination rates were higher than those reported in studies using juvenile material or plants grown under controlled conditions. This outcome is consistent with previous work demonstrating that the use of potted or greenhouse-grown donor plants significantly reduces both bacterial and fungal contamination during culture initiation (Messeguer and Mele 1983; Yu and Reed 1995; Bacchetta et al., 2008; Şekerli, 2024). Similarly, explants derived from mature trees often show lower establishment success compared with juvenile tissues, as reported in other woody species and in hazelnut (Bassil et al., 1991; Diaz-Sala et al., 1990; Messeguer and Mele, 1987). Studies comparing juvenile and mature nodal explants of *C. avellana* have consistently shown higher culture establishment and regeneration capacity in juvenile plant material, supporting the results of the current study.

Regeneration capacity in hazelnut is well known to be highly genotype-dependent, and previous studies have reported considerable variability among cultivars in both shoot induction and rooting responses (Hand et al., 2014; Akin et al., 2017). The findings of the present study are consistent with these observations. Among the cultivars evaluated, 'Süslü' exhibited the highest shoot and root induction capacity, whereas 'Okay 28' and 'Çetiner' displayed the lowest performance. 'Okay 28', one of the two Turkish cultivars developed through breeding programs (Balık et al., 2015), is primarily recognized for its large nut size but appears to possess limited regenerative capacity *in vitro*. The 'Allahverdi' cultivar, a registered variety developed through selection breeding, is distinguished by leaf emergence occurring approximately two weeks later than that of the reference cultivar 'Tombul' (Balık et al., 2016), and exhibited an intermediate regeneration performance among the four cultivars tested. Although the physiological basis of the superior regeneration performance of 'Süslü' remains unclear, genotypic differences in endogenous biochemical and hormonal profiles may contribute to the observed variation (Lupo et al., 2025). In line with this, other red-leaved hazelnut types, such as *Corylus maxima* Mill., have also been reported to synthesize substantial levels of flavonoids and diarylheptanoids with notable bioactive properties (Riethmüller et al., 2015; Felegyi-Tóth et al., 2022). The red-leaved phenotype and ornamental value of 'Süslü' further underscore its potential as a promising candidate for both horticultural and biotechnological applications. Overall, the genotype-dependent variation observed in this study highlights the necessity of cultivar-specific optimization when developing micropropagation protocols for hazelnut.

Conclusions

This study presents the first report on the *in vitro* propagation responses of the newly registered Turkish hazelnut cultivars 'Okay 28', 'Allahverdi', 'Çetiner', and 'Süslü'. The successful regeneration of contamination-free plant material from these cultivars provides a valuable foundation for future tissue culture and biotechnology research. The ability to obtain clean, clonal stock material is essential for breeding programs, conservation strategies, and large-scale propagation efforts aimed at supporting Türkiye's hazelnut industry. Further optimization studies focusing on medium composition, growth regulators, and rooting conditions will help enhance the efficiency and reliability of propagation protocols for these important cultivars.

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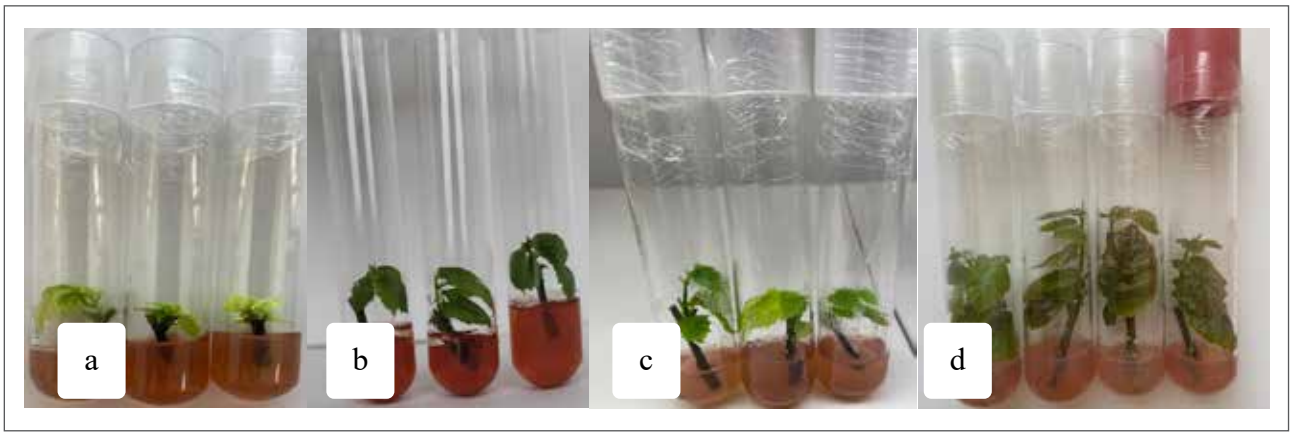

Figure 1. Culture initiation from axillary nodal explants. a) 'Okay 28' b) 'Allahverdi' c) 'Çetiner' d) 'Süslü'.

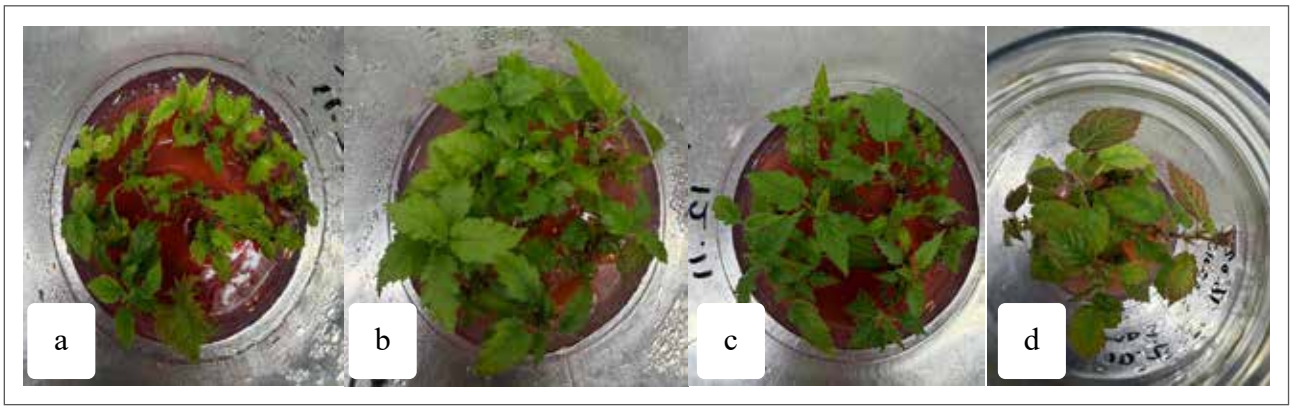

Figure 2. Shoot multiplication of four cultivars. a) 'Okay 28' b) 'Allahverdi' c) 'Çetiner' d) 'Süslü'

Figure 3. *In vitro* rooting of 'Süslü' cultivar.

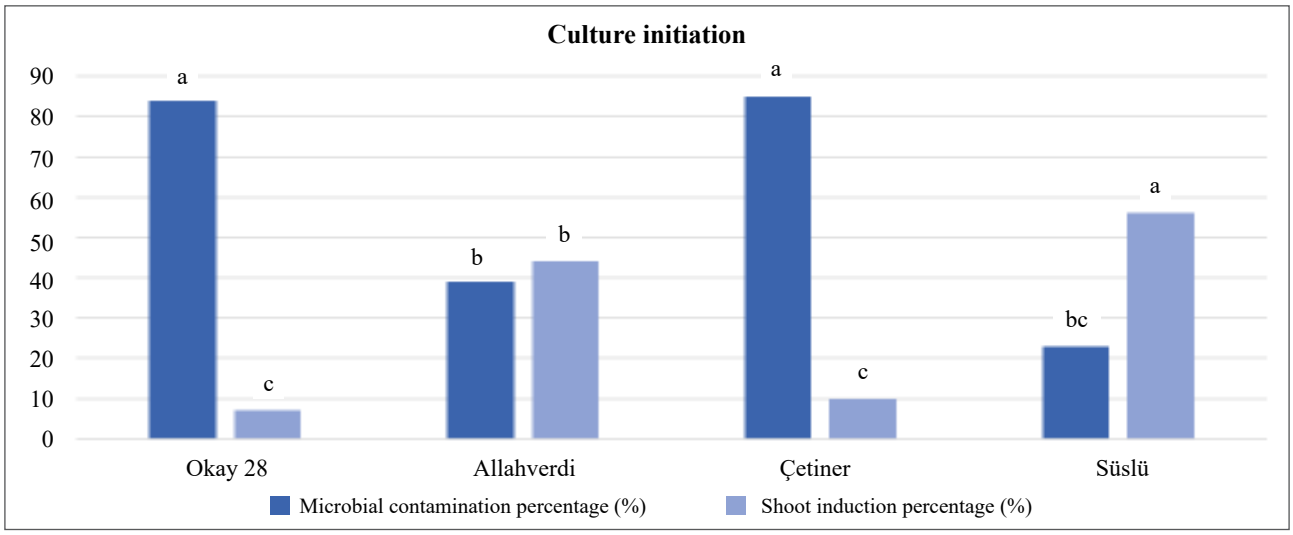

Figure 4. Visual microbial contamination and shoot induction percentage of four cultivars. Different uppercase letters indicate significant differences between genotypes (Tukey's HSD test at $p < 0.05$).

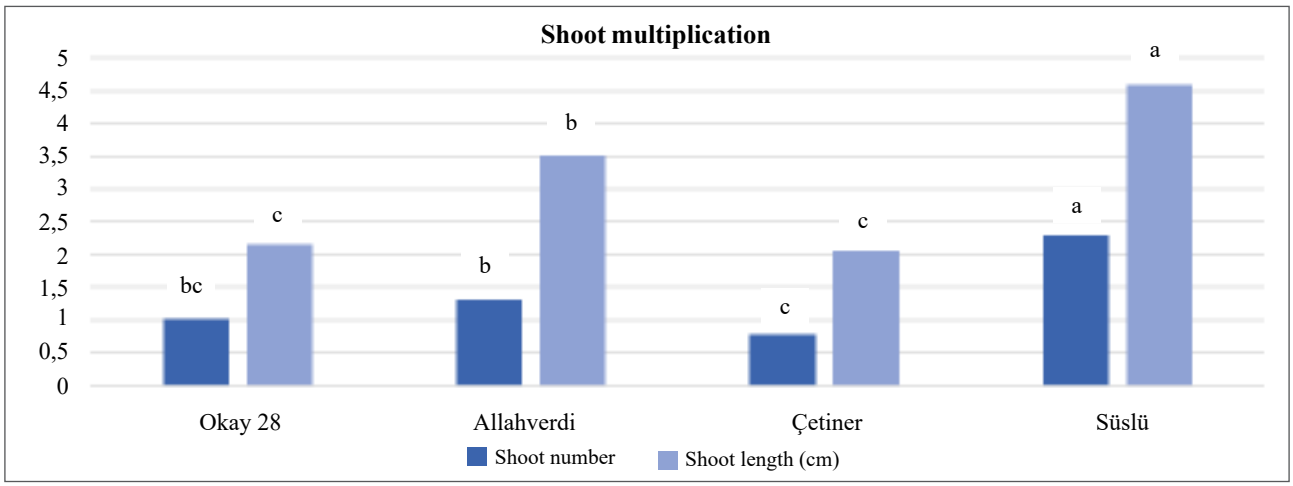

Figure 5. Average shoot number and shoot length of four cultivars in multiplication medium for eight weeks. Different uppercase letters indicate significant differences between genotypes (Tukey's HSD test at $p < 0.05$).

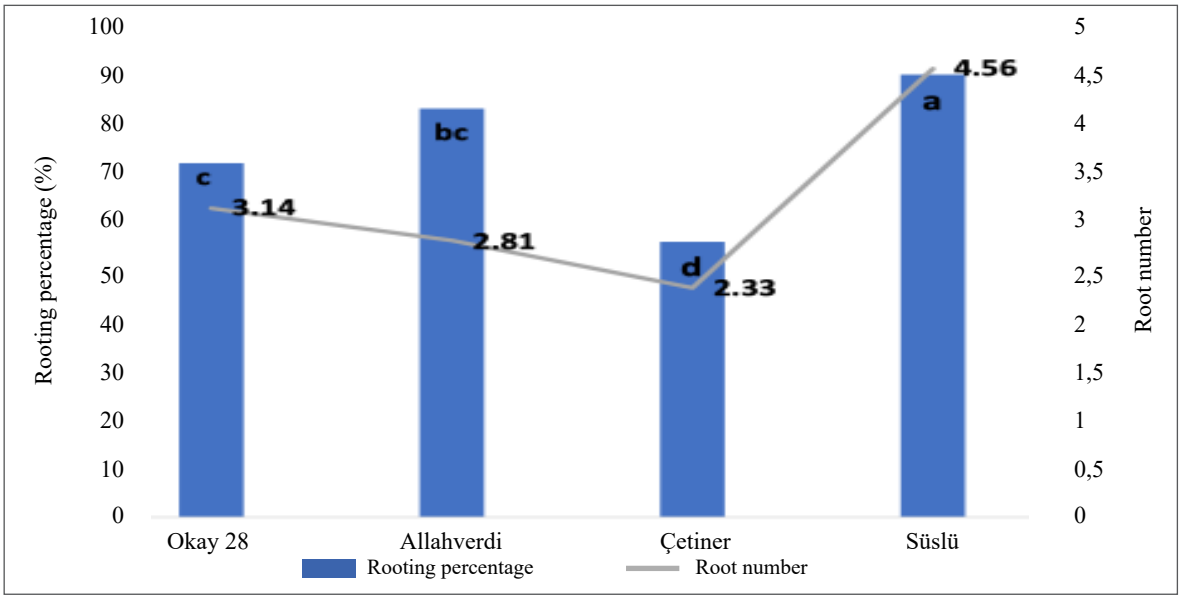

Figure 6. Rooting percentage and average root numbers of four cultivars in rooting medium for six weeks. Different uppercase letters indicate significant differences between genotypes (Tukey's HSD test at $p < 0.05$).

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