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Changes in nutritional components and physiological parameters of *Acer griseum* stem cuttings during rooting

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Abstract: *Acer griseum* is an ornamental tree species with high horticultural value, but its cutting propagation is limited by poor rooting performance. However, the physiological basis for this persistent rooting failure, particularly the temporal dynamics of nutritional status and physiological responses during the cutting period, remains poorly understood. To address this gap, this study evaluated temporal changes in nutritional components and physiological indices during the cutting process under 1-naphthaleneacetic acid (NAA) treatment and a water control. Throughout the experimental period (0–50 days), contents of starch, soluble sugars, and soluble protein continuously decreased, indicating progressive depletion of internal nutrient reserves. Antioxidant enzyme activities generally decreased during the cutting period, whereas malondialdehyde content increased progressively, suggesting enhanced membrane lipid peroxidation. Notably, no adventitious roots were formed in either treatment throughout the entire experimental period. These results indicate that rapid nutrient depletion and unfavourable physiological status may contribute to the low rooting ability of *A. griseum* cuttings, providing useful information for improving vegetative propagation strategies of this species.

Keywords: paperbark maple; propagation; rooting; nutrients; 1-naphthaleneacetic acid; NAA

Acer griseum (Franch.) Pax is a deciduous species of *Acer* (Sapindaceae) with high ornamental, economic, and ecological value. However, because its natural populations are small and fragmented, and habitats have been severely disturbed, this species is threatened with extinction and was listed as endangered by the International Union for Conservation of Nature (IUCN) in 2009. Therefore, developing effective propagation techniques is essential for its conservation and sustainable utilisation. Propagation of *A. griseum* is constrained by strong reproductive limitations. Sexual reproduction is inefficient due to a high proportion of empty seeds and prolonged seed dormancy, which greatly restricts seedling production (Zhang et al. 2012; Guo et al. 2017; Sun et al. 2022; He, Song 2023). Accordingly, vegetative propagation has been considered an alternative strategy to bypass seed-related constraints; however, cutting propagation of *A. griseum* is still limited in practice because of poor adventitious root formation.

Previous studies on *A. griseum* have mainly focused on cultivation techniques (Chen et el. 2010; Sun et al. 2022), seed dormancy mechanisms (Zhang et al. 2012; Guo et al. 2017), resource surveys (Chen 2012; Zhang et al. 2015), molecular

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genetics (Sun et al. 2014; Wang 2016; Ye et al. 2017; Wang et al. 2017; Fu 2022), and leaf colour variation (Fan et al. 2024). In contrast, physiological changes during the cutting process – particularly those associated with internal nutrient status and physiological responses underlying rooting failure – remain insufficiently investigated.

Adventitious root formation is a complex process regulated by internal nutrient reserves and physiological control. Carbohydrates (especially starch and soluble sugars) provide energy and carbon skeletons for cell division and respiratory metabolism, while soluble proteins serve as essential nitrogen sources supporting metabolic activities related to root formation (Li et al. 2020; Xie et al. 2022; Yin 2022; Su et al. 2023). Enzymes involved in reserve mobilisation are also closely related to this process: α-amylase (α-AL) promotes the conversion of stored starch into soluble sugars (Wu et al. 2002; Han, Wei 2006; Li et al. 2018), whereas lipase (LPS) participates in lipid catabolism (Passardi et al. 2005; Cosio, Dunand 2009) and is associated with metabolic regulation and ageing-related processes (Quettier, Eastmond 2009; Barros et al. 2010; Yuan et al. 2018; Sankar, Ponnuraj 2020). Rooting also involves oxidative metabolism (Xu 2020): peroxidase (POD) contributes to rooting by regulating reactive oxygen species and cellular redox balance, and catalase (CAT) maintains hydrogen peroxide homeostasis by catalysing its decomposition and participates in redox regulation and stress responses during rooting (Scott et al. 1999; Yang, Poovaiah 2002; Hong et al. 2005; Queval et al. 2007; Mhamdi et al. 2010). Under stress conditions, membrane lipid peroxidation leads to malondialdehyde (MDA) accumulation, and MDA is widely used as an indicator of membrane damage and oxidative stress in plants (Davey et al. 2005; Yamauchi et al. 2008; Murtaza et al. 2019).

Therefore, this study aimed to investigate temporal changes in nutritional components and physiological indices during the cutting process of *A. griseum*, to elucidate physiological factors associated with persistent rooting failure and to provide a scientific basis for improving vegetative propagation strategies of this species.

MATERIAL AND METHODS

The experiment commenced on May 15, 2022, at a location designated as Northwest A&F University (108°04'E, 34°16'N), situated in a warm temperate monsoon climate region. Experimental materials were obtained from 15-year-old seed-grown *A. griseum* trees located in the Northwest A&F University Botanical Garden. One-year-old branches exhibiting robust growth were selected from the outer canopy. Branches with diameters of 6–8 mm were collected from the middle portion of the shoots and cut into 15 cm long stem cuttings. Each cutting was prepared with a straight cut at the upper end and a 45° angled cut at the basal end. During the experimental period, cuttings were maintained under controlled laboratory conditions with suitable temperature, humidity, shading, and regular watering to ensure favourable rooting conditions.

Prior to the formal experiment, a preliminary screening was conducted to evaluate the propagation performance of *A. griseum* cuttings by testing different substrate mixtures, types and concentrations of growth regulators, soaking durations, seasonal timing, and cutting positions. Although no adventitious roots were formed under any of the tested combinations, differences in cutting survival were observed. Under the same substrate condition, soaking cuttings in 1 000 mg/L 1-naphthaleneacetic acid (NAA) for 1 h resulted in the lowest mortality rate. Based on these observations and to ensure experimental stability and reproducibility, this treatment was selected for subsequent physiological measurements.

The formal experimental design consisted of one treatment group and one control group. Cuttings in the treatment group were soaked in a 1 000 mg/L solution of NAA for 1 h, while cuttings in the control group were soaked in water for the same duration. After treatment, cuttings were inserted into a substrate composed of perlite, vermiculite, and peat soil mixed at a ratio of 1 : 1 : 2 (v/v/v). The day of insertion was designated as day 0. Physiological and nutritional parameters were measured on day 0 and subsequently at 10-day intervals, resulting in a total of six sampling times. Each treatment consisted of three biological replicates, with five cuttings per replicate. At each sampling time, 15 cuttings per treatment were randomly sampled from the propagation container for analysis.

Nutritional components measured in the cuttings included starch, soluble sugars, and soluble proteins. Soluble sugars and starch were determined

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using anthrone colourimetry (Yemm, Willis 1954) and expressed as mg/g. Soluble protein content was determined using a colourimetric method (Bradford 1976) and expressed as mg/mL. Physiological indices measured included MDA, POD, CAT, LPS, and α-AL. MDA content was determined using the thiobarbituric acid (TBA) method (Hodges et al. 1999) and expressed as nmol/g. POD activity was measured spectrophotometrically (Chance, Maehly 1955), whereas CAT activity was determined by monitoring the decomposition of hydrogen peroxide at 240 nm (Aebi 1984); both enzyme activities were expressed as U/g. LPS activity was determined using the copper soap method (Cherry, Crandall 1932) and expressed as U/g. α-AL activity was determined using the dinitrosalicylic acid (DNS) method (Bernfeld 1955) and expressed as mg/min/g.

Statistical analyses were conducted using SPSS 26.0. Differences among sampling times within each treatment were evaluated using analysis of variance (ANOVA). When significant differences were detected, post hoc comparisons were performed. Differences were considered statistically significant at $P < 0.05$.

RESULTS

This section presents the temporal dynamics of nutritional components and physiological indices in *A. griseum* cuttings during the cutting process under NAA treatment and water control conditions, and analyses their variation patterns to characterise physiological responses associated with the cutting process and persistent rooting failure.

Changes in nutrient content during the cutting process of *Acer griseum*

Changes in starch content. During the cutting process of *A. griseum*, starch content was observed to decrease in both the control group (CK) and the NAA-treated group. From day 0 to day 10 post-cutting, the rate of starch decreased in the NAA-treated group exceeded that of the control group (Figure 1), suggesting that NAA treatment hastened the starch consumption in the cuttings. This period is critical as the cuttings require significant energy for growth and development, leading to the ongoing breakdown of starch into soluble sugars.

From day 10 to day 50 post-cutting, the rate of starch decrease in the NAA-treated group began to stabilise; meanwhile, the starch content in the control group followed a pattern of "rapid decline – increase – slow decline." This behaviour suggests that compared to the NAA-treated group, which initially consumed a significant amount of starch, the control group cuttings retained more residual starch, enabling a more prolonged consumption period.

Changes in soluble sugar content. The analysis reveals that the changes in soluble sugar content in *A. griseum* cuttings during the cutting process mirrored the trends observed in starch content for both the CK and the NAA-treated group (Figure 2). From day 0 to day 30 post-cutting, soluble sugar levels in both the CK and NAA-treated groups exhibited a continuous decline. Notably, the rate of decline in the NAA-treated group was significantly higher than in the CK group during the initial 10 days, indicating a substantial difference and suggesting that NAA treatment accelerated the consumption of soluble sugars in the cuttings.

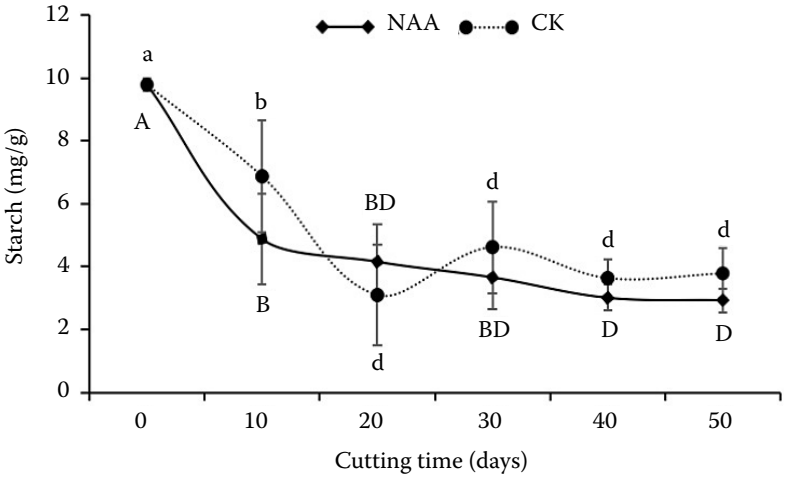

Figure 1. Temporal changes in starch content in *Acer griseum* cuttings under NAA treatment and water control during the cutting process

NAA – 1-naphthaleneacetic acid (NAA); CK – control group; values are means ± SE ($n = 3$); different letters indicate significant differences among sampling times within the same treatment at $P < 0.05$

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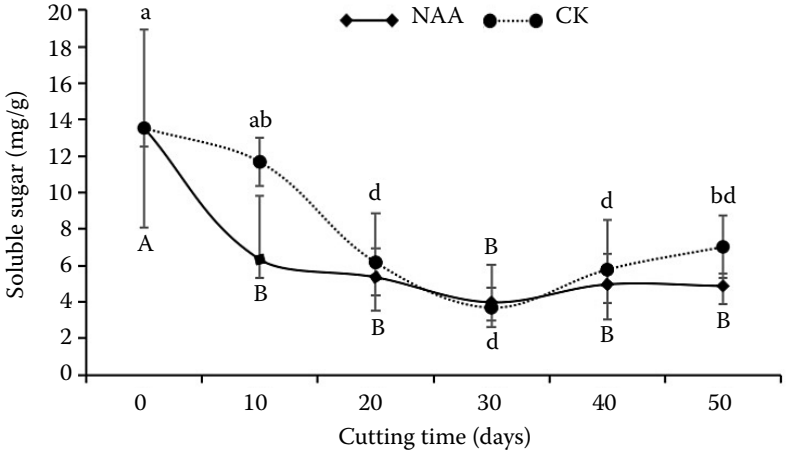

Figure 2. Temporal changes in soluble sugar content in *Acer griseum* cuttings under NAA treatment and water control during the cutting process
NAA – 1-naphthaleneacetic acid (NAA); CK – control group; values are means ± SE (n = 3); different letters indicate significant differences among sampling times within the same treatment at $P < 0.05$

From day 30 to day 50 post-cutting, the soluble sugar content in the NAA-treated group stabilised at a lower level, while the control group showed a slight increase in soluble sugar content. This rise could be attributed to the presence of compounds in *A. griseum* cuttings that may directly or indirectly impede the normal physiological functions of cells, thereby preventing the cuttings from absorbing and utilising the soluble sugars derived from starch hydrolysis, leading to their accumulation. Moreover, due to significant early starch consumption, the NAA-treated group may lack the capacity for further hydrolysis of soluble sugars, which could contribute to the failure to induce rooting in the cuttings.

Changes in soluble protein content. Figure 3 illustrates the changes in soluble protein content within the cuttings of *A. griseum* throughout the cutting process. Both the NAA-treated group and the CK displayed a consistent decline in soluble protein levels. This pattern suggests that the cuttings were continually using up soluble proteins to secure necessary nutrients. However, due to the inability to successfully induce adventitious roots, which would replenish these proteins, the levels of soluble proteins dwindled to a minimum by the end of the cutting period. This decrease mirrors the trends observed in starch and soluble sugar content. After 10 days post-cutting, the rate of soluble protein consumption in the NAA-treated group notably increased. From day 15 to day 45 post-cutting, the soluble protein levels in the NAA-treated group consistently remained below those in the CK group, potentially because the NAA treatment increased the activity of certain proteases in the cuttings, thereby speeding up protein breakdown.

Changes in physiological indices during the cutting process of *Acer griseum*

Changes in MDA content. The experimental data revealed a consistent pattern of change in MDA content during the cutting process of *A. griseum*,

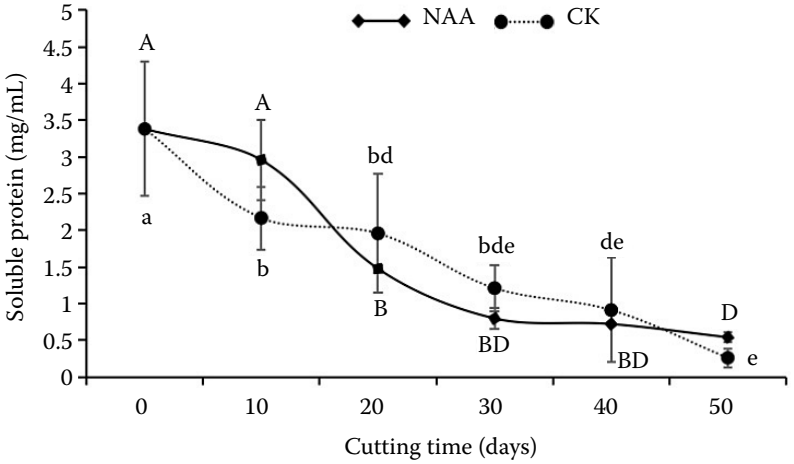

Figure 3. Temporal changes in soluble protein content in *Acer griseum* cuttings under NAA treatment and water control during the cutting process
NAA – 1-naphthaleneacetic acid (NAA); CK – control group;; values are means ± SE (n = 3); different letters indicate significant differences among sampling times within the same treatment at $P < 0.05$

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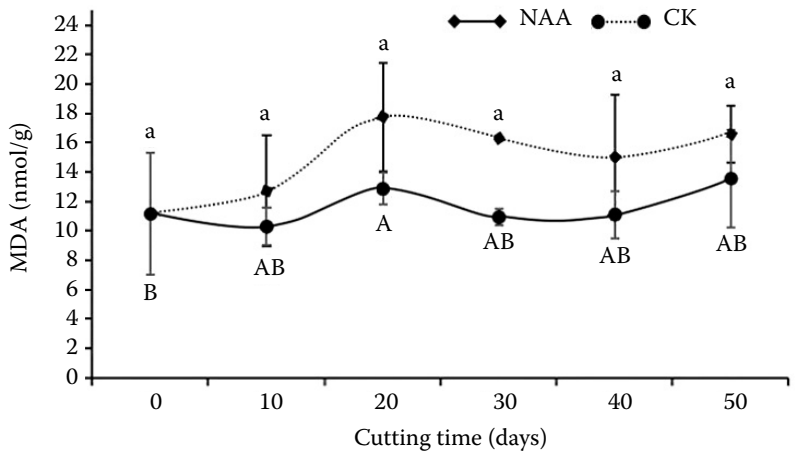

Figure 4. Temporal changes in malondialdehyde (MDA) content in *Acer griseum* cuttings under NAA treatment and water control during the cutting process NAA – 1-naphthaleneacetic acid (NAA); CK – control group;; values are means ± SE (n = 3); different letters indicate significant differences among sampling times within the same treatment at $P < 0.05$

as shown in Figure 4. Both the NAA-treated and the CK exhibited a "rise-fall-rise" trend in MDA levels. This fluctuation is likely attributed to mechanical damage inflicted on the cuttings at the outset of the process, before the wounds had fully healed, causing an initial increase in MDA levels. As the cuttings began to form callus tissue and their physiological state started to stabilise, the MDA levels decreased. However, since the cuttings ultimately failed to root successfully, they were unable to adapt to their surroundings, leading to a subsequent increase in MDA levels. On day 20, the MDA content peaked, likely marking the peak phase of callus tissue formation. Following this phase, as the physiological state of the cuttings improved, the MDA levels decreased. Throughout the entire cutting period, the MDA content in the NAA-treated group remained consistently lower than that in the CK group, suggesting that NAA treatment enhances the cuttings' resilience.

Changes in POD activity. Throughout the cutting process of *A. griseum*, the POD activity within the cuttings demonstrated distinct trends in the CK and the NAA-treated group. The CK group exhibited a pattern of "decline-slow rise-slow decline," whereas the NAA-treated group displayed a "decline-rapid rise-decline-rise" pattern, as shown in Figure 5. During the initial 20 days of the cutting period, POD activity in both groups decreased, with the NAA-treated group experiencing a more rapid decline. Both groups reached their lowest activity levels of the entire period on the twentieth day. From day 20 to day 30 post-cutting, there was a noticeable increase in POD activity, particularly sharp in the NAA-treated group. Between day 30 and day 50 post-cutting, the POD activity in the NAA-treated group exhibited a "decline-rise" trend, whereas in the CK group, the activity levels tended to stabilise.

Changes in CAT activity. Figure 6 illustrates the changes in CAT activity within the cuttings

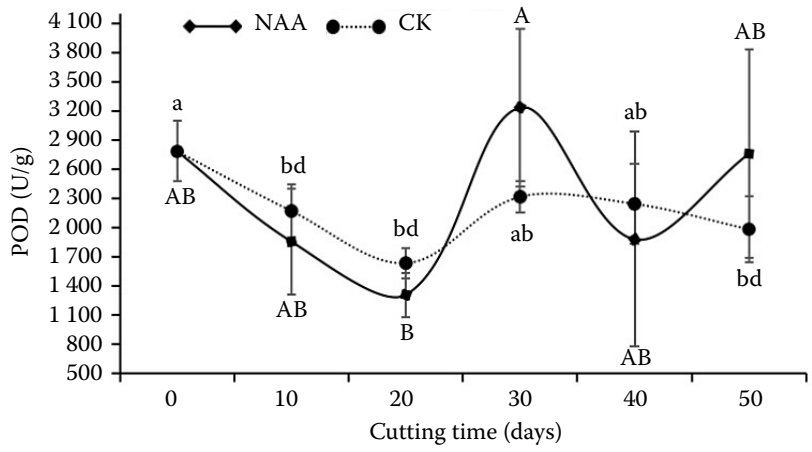

Figure 5. Temporal changes in peroxidase (POD) activity in *Acer griseum* cuttings under NAA treatment and water control during the cutting process NAA – 1-naphthaleneacetic acid (NAA); CK – control group; values are means ± SE (n = 3); different letters indicate significant differences among sampling times within the same treatment at $P < 0.05$

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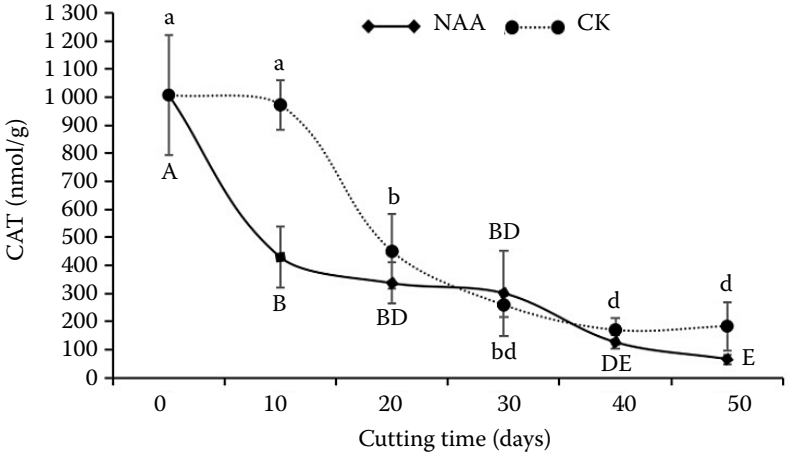

Figure 6. Temporal changes in catalase (CAT) activity in *Acer griseum* cuttings under NAA treatment and water control during the cutting process
NAA – 1-naphthaleneacetic acid (NAA); CK – control group;; values are means ± SE (n = 3); different letters indicate significant differences among sampling times within the same treatment at $P < 0.05$

of *A. griseum* during the cutting process. Both the NAA-treated group and the CK exhibited a general downward trend in CAT activity. During the initial 0–10 days of the cutting period, the CAT activity in the CK group cuttings showed minimal change; however, in the NAA-treated group, CAT activity sharply declined, followed by a gradual decrease to a low point. From day 10 to day 20 post-cutting, CAT activity in the CK group sharply decreased, then slowly declined to a low point. In contrast, after day 10, the CAT activity in the NAA-treated group generally followed a slow declining trend. This pattern suggests that NAA treatment may have accelerated the metabolic rate in the cuttings, thereby precipitating the rapid decrease in catalase activity.

Changes in LPS activity. Figure 7 illustrates the trends in LPS activity within the cuttings of *A. griseum* during the cutting process. Both the NAA-treated group and the CK exhibited a general upward trend in lipase activity. In the initial 0–10 days of the cutting period, lipase activity showed minimal change in both groups. From day 10 to day 30 post-cutting, lipase activity in the CK group cuttings rapidly increased and subsequently stabilised. In the NAA-treated group, lipase activity experienced a significant surge between day 20 and day 30 post-cutting, before stabilising. This indicates that lipase activity within the cuttings intensified, particularly during the 20–30-day interval, with NAA treatment hastening this increase.

Changes in α-AL activity. Figure 8 outlines the trends in α-AL activity within the cuttings of *A. griseum* throughout the cutting process. Both the NAA-treated group and the CK displayed a general downward trend in α-amylase activity. Between days 10 and 20 post-cutting, the decline in α-AL activity was more pronounced in the NAA-treated group, while the CK group experienced a more gradual decrease. From day 10 to day 30 post-cutting, α-AL activity in the CK group decreased

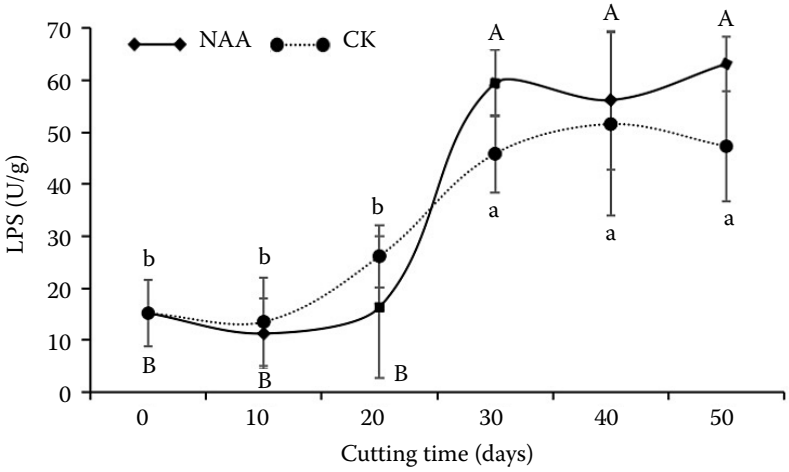

Figure 7. Temporal changes in lipase (LPS) activity in *Acer griseum* cuttings under NAA treatment and water control during the cutting process
NAA – 1-naphthaleneacetic acid (NAA); CK – control group; values are means ± SE (n = 3); different letters indicate significant differences among sampling times within the same treatment at $P < 0.05$

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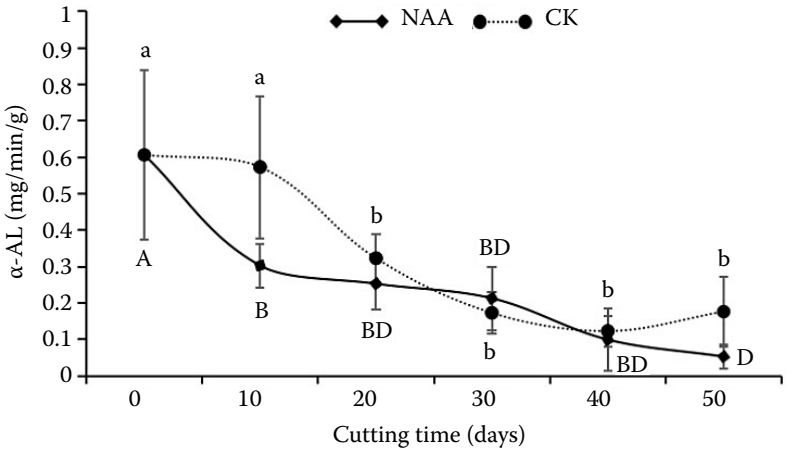

Figure 8. Temporal changes in α-amylase (*α-AL*) activity in *Acer griseum* cuttings under NAA treatment and water control during the cutting process
NAA – 1-naphthaleneacetic acid (NAA); CK – control group; values are means ± SE (n = 3); different letters indicate significant differences among sampling times within the same treatment at $P < 0.05$

sharply, whereas the decline in the NAA-treated group slowed. Notably, the period of rapid decrease in α-amylase activity for the NAA-treated cuttings occurred within the first 10 days post-cutting. After day 30, α-AL activity in both groups remained at lower levels.

DISCUSSION

Relationship between nutrient content and rooting of cuttings. The success of cutting propagation is strongly dependent on the internal nutritional status of the cuttings, particularly the availability of carbon and nitrogen reserves. Adventitious root induction has been shown to be closely associated with carbon supply within cuttings (Liao et al. 2010). In the present study, starch, soluble sugars, and soluble proteins exhibited a continuous decline during the cutting period, indicating sustained consumption of internal reserves. Such depletion may constrain the energy supply and metabolic substrates required for cell division and differentiation during root primordium initiation. Similar patterns of carbohydrate exhaustion have been reported in woody species with poor rooting performance (Shen 2014; Yuan et al. 2024).

In addition, soluble proteins, as major nitrogen-containing compounds, are essential for metabolic activity during rooting. The consistently lower soluble protein content observed in NAA-treated cuttings compared with the control suggests enhanced protein turnover to meet metabolic demands during the cutting process (Xu et al. 2019). Taken together, the progressive exhaustion of carbon and nitrogen reserves, coupled with the absence of successful rooting to replenish these resources, likely represents an important physiological constraint contributing to the persistent failure of adventitious root formation in *A. griseum* cuttings. Future studies could test whether supplemental carbon or nitrogen supply during the middle and late stages of cutting, or regulation of reserve mobilisation, can alleviate nutrient limitation and improve rooting induction.

Relationship between physiological indices and rooting of cuttings. Physiological enzyme activities play important regulatory roles in the induction and development of adventitious roots in cuttings. In the present study, POD activity declined during the early cutting stage in both the NAA-treated and control groups, suggesting a weakened capacity for growth-related metabolic regulation and auxin oxidation. POD is known to participate in auxin metabolism and reactive oxygen species (ROS) scavenging, and appropriate POD activity is considered necessary for root primordium initiation (Zhao et al. 2017). However, toward the later stages of the cutting period, POD activity increased in the NAA-treated group, which may have enhanced endogenous indole-3-acetic acid (IAA) oxidation and thereby limited the maintenance of auxin levels required for sustained root induction. This imbalance in POD activity may partly explain the difficulty in inducing adventitious roots in *A. griseum* cuttings.

In addition, both CAT and α-AL activities exhibited an overall decreasing trend throughout the cutting process. Reduced CAT activity suggests a diminished capacity to decompose hydrogen peroxide, potentially leading to oxidative stress accumulation. Meanwhile, the continuous decline in α-AL activity implies a reduced efficiency

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in starch hydrolysis, which may further restrict the availability of soluble sugars needed to support energy-demanding processes during rooting.

Lipid peroxidation is a common physiological response to stress and tissue ageing in plants, with MDA serving as a key indicator of membrane damage (Shen 2014; Chang et al. 2023). In this study, MDA content showed an initial increase followed by a decline, a pattern similar to that reported in other difficult-to-root species (Xu et al. 2019). Notably, MDA levels were consistently higher in control cuttings than in NAA-treated cuttings, suggesting that NAA application alleviated membrane lipid peroxidation to some extent. Nevertheless, the overall increase in lipase activity observed during the cutting period indicates progressive cellular degradation and physiological ageing, which may further compromise rooting competence.

Taken together, the coordinated decline in antioxidant enzyme activities (POD and CAT), increased lipid peroxidation (as reflected by MDA accumulation), and enhanced lipid degradation suggest that ROS homeostasis could not be effectively maintained during the cutting process in *A. griseum*. Such a physiological imbalance may impair callus development and root primordium formation. Future studies could test whether regulating ROS levels, enhancing antioxidant enzyme activity, or adjusting hormone–antioxidant interactions during critical stages of cutting could improve rooting performance in this species.

CONCLUSION

Although the physiological regulation of adventitious root formation has been extensively studied in many woody species, information remains limited for endangered taxa such as *A. griseum*. This study characterised the temporal dynamics of nutrient status and key physiological indices during the cutting process and demonstrated that persistent depletion of internal reserves and unfavourable physiological conditions were associated with the failure to induce adventitious roots.

Based on these findings, future studies should examine whether improving carbon and nitrogen availability, regulating oxidative stress and antioxidant capacity, and optimising hormone application strategies can alleviate physiological constraints during the cutting period. In addition, the roles of cutting maturity, seasonal timing, and potential endogenous inhibitory factors warrant further investigation to improve vegetative propagation efficiency and support the conservation of this endangered species.

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