Postprint: Variation Characteristics of Tissue Nutrient Content in Malania oleifera Seedlings During Decline

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Date: 2023-08-04T00:00:00+00:00

Abstract

In the absence of host plants, the gradual decline in growth performance of Malania oleifera seedlings with prolonged independent growth is a common phenomenon. Analyzing the changes and distribution characteristics of tissue nutrient content during the decline process of Malania oleifera seedlings can provide theoretical basis and technical reference for developing methods to assess seedling vigor, and offer scientific guidance for rational fertilization during seedling cultivation. This study compared the changes in N, P, and K element concentrations in various tissues of Malania oleifera plants that had grown independently for half a year, two years, and three years, and qualitatively evaluated the distribution and content changes of starch in the roots and stems of these seedlings at different decline levels using tissue section staining methods. The results showed that during the decline process of Malania oleifera seedlings: (1) Except for the gradual increase in K concentration in lateral roots, the concentrations of N, P, and K in tissues of other organs gradually decreased, and the leaf N/P ratio gradually became imbalanced. Seedlings at different decline stages experienced different types of nutrient stress, with seedlings that had grown independently for half a year mainly limited by insufficient N supply (average N/P ratio of 11.33), shifting to P limitation after two years (average N/P ratio of 17.81). After three years, the leaf N/P ratio of Malania oleifera seedlings was severely imbalanced (mean value of 52.46), with extremely low vigor, making them unsuitable for afforestation; (2) The starch content level of the plants gradually decreased, with starch being completely depleted in Malania oleifera seedlings after three years of independent growth. The starch content levels at the stem-root junction, root apex swelling region, main root, and lateral roots all showed significant differences among seedlings at different decline levels, and could serve as important references for evaluating seedling vigor, with lateral roots being an ideal sampling site for minimally invasive detection of seedling vigor.

Full Text

Dynamics of tissue nutrient content in relation to declining seedling growth in *Malania oleifera*

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Abstract

A widespread phenomenon is that *Malania oleifera* seedlings gradually decline in vigor with prolonged independent growth in the absence of host plants. Analyzing the changes and distribution characteristics of tissue nutrient content during the decline process can provide theoretical reference and technical guidance for finding methods to evaluate seedling vigor and for rational fertilization during nursery cultivation. This study compared changes in N, P, and K element concentrations in various tissues of *M. oleifera* seedlings grown independently for half a year, two years, and three years, and qualitatively assessed starch distribution and content changes in roots and stems of seedlings at different decline levels using tissue section staining. The results showed that during the decline process of *M. oleifera* seedlings: (1) Except for gradually increasing K concentration in lateral roots, N, P, and K concentrations in other organs gradually decreased, and leaf N/P ratio became increasingly imbalanced. Seedlings at different decline degrees experienced different types of nutrient stress. Specifically, half-year-old seedlings were mainly limited by insufficient N supply (average N/P ratio 11.33), shifted to P limitation after two years (average N/P ratio 17.81), and three-year-old seedlings had severely imbalanced leaf N/P ratios (mean 52.46) with extremely low vigor, making them unsuitable for afforestation; (2) Starch content levels gradually decreased, being nearly exhausted after three years of independent growth. Starch content levels at the stem-root junction, root apex swelling, main root, and lateral roots showed significant differences among seedlings with different decline degrees and could serve as important references for evaluating seedling vigor, with lateral roots being ideal sampling sites for minimally invasive detection methods.

Keywords: root hemiparasitic plant, macromineral elements, starch, staining of tissue sections, growth vigor of seedlings, rare and endemic plant

Introduction

Malania oleifera is a perennial arbor species in the family Olacaceae, genus *Malania* (Li, 1980). It is a precious oil plant endemic to southwestern China (Ou, 1981) and a rare resource plant rich in nervonic acid (Xue and Shao, 2015), as well as an excellent tree species for ecological restoration and rocky desertification control in karst regions (Lü et al., 2016; Mao et al., 2018).

In the cultivation and afforestation of *M. oleifera*, a common practice involves sowing seeds in nurseries followed by seedling transplantation. However, as *M. oleifera* is a root hemiparasitic plant (Li et al., 2019; Li et al., 2019), its seedlings, despite having numerous lateral roots and certain photosynthetic capacity, exhibit low nutrient absorption efficiency and photosynthetic efficiency (Li, 2021). In the absence of host plants in nurseries, *M. oleifera* seedlings gradually decline in vigor with prolonged independent growth or environmental stress (Li, 2021). Based on previous cultivation experience, severely declining seedlings cannot resume normal growth even with fertilizer application or planting with suitable hosts after transplantation, resulting in extremely low survival rates and significant economic and labor losses. Therefore, a critical challenge in *M. oleifera* cultivation is how to improve seedling growth through rational fertilization before obvious decline symptoms appear, and to efficiently screen vigorous seedlings before transplantation to avoid wasting land, labor, and material resources.

Nutrients are among the most important factors affecting plant growth and development (Zhao et al., 2005; Yang et al., 2021; Zhang et al., 2021). Nutrient absorption efficiency and assimilation capacity are closely related to plant growth (De Graaf et al., 1998; Güsewell & Koerselman, 2002; Luo et al., 2021). *M. oleifera* seeds are large (Guo et al., 2018) and store abundant nutrients (Su et al., 2021), providing essential nutrients for germination and early seedling establishment. Before establishing parasitic relationships with hosts, the nutrient composition of *M. oleifera* seedlings consists of nutrients stored in seeds and those absorbed and synthesized by the seedlings themselves. Since the nutrient content transferred from seeds is fixed, after a period of independent growth, changes in tissue nutrient content are mainly influenced by the balance between total nutrient absorption/synthesis and consumption for growth and development. Therefore, determining nutrient content in various organs can reveal the nutrient budget status of *M. oleifera* seedlings, thereby inferring plant vigor and growth status. Comparing nutrient content changes in tissues of *M. oleifera* seedlings with different independent growth periods can help understand the characteristics of tissue nutrient changes and nutrient limitation types during seedling decline, providing theoretical reference and technical guidance for developing scientific fertilization management strategies and accurate methods for evaluating seedling vigor.

N, P, and K are important macronutrients that play crucial roles in various physiological and metabolic processes (De Graaf et al., 1998; Lucassen et al., 2003; Zhao et al., 2005; Chen et al., 2020; Yang et al., 2021). Plants dynamically allocate nutrients among organs according to environmental changes and growth demands, causing corresponding changes in N, P, and K concentrations in plant tissues (Sun et al., 2023). Understanding the allocation patterns and variation of these elements is important for assessing plant nutrient physiological status and evaluating nutrient limitation levels and types (Tang et al., 2018; Sun et al., 2023). Previous studies have mainly focused on leaf analysis or aboveground parts as a whole, with less research on nutrient concentration changes in stems and roots (Heineman et al., 2016). However, stem and root nutrient concentrations are more sensitive to environmental responses (Tang et al., 2018). Since plant responses to the environment result from collective organ responses, comprehensive analysis of N, P, and K concentration changes in different organs is necessary for fully understanding plant nutrient physiological status (Kleyer & Minden, 2015). Our previous research showed that N, P, and K supply levels affect element concentrations in *M. oleifera* seedling tissues, thereby influencing photosynthetic efficiency and growth (Li, 2021). However, the allocation patterns and concentration changes of these three mineral elements in different organs during *M. oleifera* seedling decline remain unknown. Analyzing the characteristics of N, P, and K changes during seedling decline, particularly the leaf N/P ratio as an indicator of nutrient limitation types (Koerselman & Meuleman, 1996), will help guide timely and rational fertilization to alleviate seedling decline and lay a foundation for understanding the mechanisms of nutrient stress responses in *M. oleifera*.

Starch is an important storage carbohydrate in plants, and its distribution and content directly affect plant resistance to biotic and abiotic stresses (Sauter, 1988; Meletiou-Christou et al., 1992; Johansson, 1993; Zhou et al., 2020). Abundant starch reserves in stems and roots typically enable plants to better resist environmental stress (Sala et al., 2010; Villar-Salvador et al., 2015). While numerous studies have explored the role and variation of starch in normal green plants under environmental stress, research on its role and content changes in root hemiparasitic plants under environmental stress is very limited (Zhang, 2015; Zhou et al., 2020). For root hemiparasitic plants, nutrient stress intensity gradually increases with the consumption of seed storage nutrients before establishing parasitic relationships with hosts. From this perspective, independent growth represents a severe stress condition for root hemiparasitic plants. Limited studies show that starch content changes are strongly affected by seedling age and parasitic status (Zhang, 2015; Zhou et al., 2020). Starch stored in roots and stems plays an important role during the independent growth stage of the root hemiparasitic tree *Santalum album*, providing energy for seedling growth and development and for haustorium initiation and differentiation; starch content in stems and roots decreases with prolonged independent growth in the absence of host plants or after host removal (Zhou et al., 2020). Although *M. oleifera* seedlings contain abundant starch (Lai, 2006), no attention has been paid to the variation characteristics of starch content in different tissues during seedling decline. Compared with other nutrient indicators requiring professional analytical instruments, starch content detection is relatively convenient and can be rapidly assessed through tissue section staining within a short time, making the method more applicable in production practice. Understanding the variation and spatial distribution patterns of starch content during *M. oleifera* seedling decline will help explore a rapid method for evaluating seedling vigor based on starch content changes in different parts, laying theoretical and technical foundations for efficient screening of high-quality afforestation seedlings.

This study used *M. oleifera* seedlings with different independent growth periods (representing different vigor decline levels) as materials. We quantitatively determined N, P, and K concentrations in leaves, stems, main roots, and lateral roots using digestion methods, and qualitatively assessed starch distribution and content changes in roots and stems of seedlings with different decline levels using tissue section staining. The study aimed to address: (1) What are the characteristics of N, P, and K content changes in different organs during *M. oleifera* seedling decline? (2) Do seedlings with different decline degrees experience different types of nutrient limitation? (3) What are the regular patterns of starch content changes and allocation among different parts during seedling decline? The results will provide theoretical reference for rational fertilization and vigorous seedling screening in *M. oleifera* nursery cultivation.

Materials and Methods

1.1 Materials

The experimental seedlings were greenhouse-grown *M. oleifera* seedlings. Seeds were collected from Guangnan County, Wenshan Prefecture, Yunnan Province. After removing the exocarp and mesocarp, seeds were sown in plug trays (length × width × height = 46 cm × 46 cm × 11 cm) and cultivated in a glass greenhouse at Kunming Institute of Botany (25°08 22 E, 102°44 23 N, altitude 1,990 m). To facilitate root observation and control nutrient supply, perlite was used as the cultivation substrate. After seed germination and emergence, Long Ashton standard nutrient solution was applied weekly at 1% of the substrate volume, with watering as needed. The experimental seedlings had grown independently without host plants for half a year, two years, and three years, showing obvious morphological differences [Figure 1: see original paper]. Half-year-old seedlings grew vigorously with numerous lateral roots and strongest new root formation capacity, with an average survival rate of 86.70% six months after transplantation to fertile soil in preliminary experiments and average new root density of 1.04 mg $\cdot$ cm^{-3}. Two-year-old seedlings grew well with developed lateral roots and certain new root formation capacity, with an average survival rate of 83.30% six months after transplantation and average new root density of 0.58 mg $\cdot$ cm^{-3}. Three-year-old seedlings showed obvious decline symptoms such as leaf yellowing, had fewer lateral roots, and difficulty forming new roots, with an average survival rate of only 13.70% six months after transplantation to fertile soil and very few new roots formed.

1.2.1 Determination of N, P, and K concentrations in various parts during seedling decline

Five seedlings were randomly selected from each of the three growth states (half-year, two-year, and three-year-old seedlings). For each seedling, leaves, stems, main roots, and lateral roots were separately collected and placed in paper bags. The collected materials were dried in an oven at 75°C for 24 hours, then ground using an automatic sample grinder (Model: JXFSTPRP-24). N, P, and K concentrations were determined using a Kjeldahl nitrogen analyzer (Model: BUCHI K-360), inductively coupled plasma optical emission spectrometer (Model: PerkinElmer Avio 200), and continuous light source atomic absorption spectrometer (Model: Jena contrAA300), respectively. Leaf N/P ratios were calculated based on N and P concentrations in leaves.

1.2.2 Histological staining observation of starch content levels in stems and roots during seedling decline

Five seedlings were randomly selected from each of the three growth states. From each seedling, 0.5–1 cm long samples were taken from the middle of the stem, stem-root junction, root apex swelling, middle section of the main root, and lateral roots 1 cm away from the main root. The samples were rinsed clean in water and cross-sectioned using a portable sliding microtome to produce 10 m thick sections. Sections were stained in iodine-potassium iodide solution (24 g KI dissolved in 15 mL distilled water, then 1 g iodine added, dissolved, and diluted to 300 mL with distilled water) for 30 minutes, then washed and destained in distilled water for 1 minute. The sections were mounted in glycerin and observed under a stereomicroscope (Model: Olympus SZX7, Japan) at 56× magnification. Starch was stained blue-black, allowing assessment of starch content levels based on staining intensity and area.

1.3 Statistical analysis

SPSS Statistics 26 was used to analyze the three mineral element concentration data in *M. oleifera* seedlings. Two-way ANOVA was employed to analyze differences in N, P, and K concentrations among organs of seedlings with different independent growth periods. GraphPad Prism 8 was used for figure preparation.

Results

2.1 Changes in N, P, K concentrations and leaf N/P ratio in different tissues

Overall, N, P, and K concentrations in *M. oleifera* seedling tissues were affected by both independent growth period and sampling location . Concentrations of all three mineral elements in aboveground parts (leaves and stems) decreased significantly with increasing seedling decline degree, but showed distinct trends in roots [Figure 2: see original paper].

No significant interaction effect between independent growth period and plant part was observed on tissue N concentration . Independent growth period had highly significant effects on tissue N concentration ($F = 17.598$, $P < 0.001$), with N concentrations in all plant parts decreasing as independent growth time extended. Compared with half-year-old seedlings, N concentrations in all parts of three-year-old seedlings decreased significantly. Tissue N concentrations also differed significantly among plant parts ($F = 7.052$, $P = 0.001$), with leaf and stem N concentrations generally lower than those in main and lateral roots [Figure 2A: see original paper].

A strong interaction effect between independent growth period and plant part was observed on tissue P concentration [TABLE:1; $F = 9.477$, $P < 0.001$]. Independent growth period had more significant effects on tissue P concentration ($F = 361.313$, $P < 0.001$) than plant part ($F = 34.798$, $P < 0.001$). With extended independent growth time, P concentrations in all plant parts decreased sharply [Figure 2B: see original paper], particularly in roots. Compared with half-year-old seedlings, P concentrations in all parts of two-year-old seedlings decreased by over 50%, with further substantial reductions in three-year-old seedlings. Overall, leaf and stem P concentrations were significantly lower than those in main and lateral roots.

Leaf N/P ratio was strongly affected by independent growth period, but differences among plant parts were not significant . Based on leaf N and P concentration changes, independent growth period had relatively moderate effects on leaf N concentration [Figure 2A: see original paper] but very strong effects on leaf P concentration [Figure 2B: see original paper], resulting in large differences in leaf N/P ratios among seedlings of different ages. Half-year-old seedlings had average leaf N and P concentrations of 20.69 $mg \cdot g^{-1}$ and 1.86 $mg \cdot g^{-1}$, respectively, with an average N/P ratio of 11.33. Two-year-old seedlings had average leaf N and P concentrations of 17.55 $mg \cdot g^{-1}$ and 0.99 $mg \cdot g^{-1}$, respectively, with an average N/P ratio of 17.81. Three-year-old seedlings had average leaf N and P concentrations of 9.22 $mg \cdot g^{-1}$ and 0.28 $mg \cdot g^{-1}$, respectively, with a dramatically increased average N/P ratio of 52.46.

Changes in tissue K concentration during seedling decline showed different trends from N and P. A strong interaction effect between independent growth period and plant part was observed on tissue K concentration [TABLE:1; $F = 14.057$, $P < 0.001$]. Plant part had slightly stronger effects on tissue K concentration ($F = 17.755$, $P < 0.001$) than independent growth period ($F = 7.142$, $P = 0.002$). With extended independent growth time, leaf and stem K concentrations decreased significantly [Figure 2C: see original paper], while no change was observed in main roots and an increasing trend occurred in lateral roots. In half-year-old seedlings, leaf and stem K concentrations were significantly higher than those in main and lateral roots, but as independent growth time increased, K concentrations in main and lateral roots gradually exceeded those in leaves and stems. In three-year-old seedlings, lateral root K concentration was 3–5 times that in leaves and stems and about twice that in main roots.

Figure 2. The concentrations of N, P, and K in different parts of *Malania oleifera* seedlings. Data are presented as means ± SE of five replicates. Different letters above bars of the same plant part indicate significant differences in element concentration between different seedling ages ($P < 0.05$).

2.2 Changes in starch content in plant tissues

Histological staining results showed that starch content in *M. oleifera* seedlings gradually decreased during the decline process [Figure 3: see original paper]. Half-year-old seedlings had the highest starch content, followed by two-year-old seedlings, while three-year-old seedlings contained extremely limited starch.

Starch was mainly stored in the swollen stem base and fleshy root system of *M. oleifera* seedlings, with abundant starch granules in parenchyma cells of the cortex, phloem rays, xylem rays, and pith [Figure 3: see original paper]. Starch content in seedling stems was generally low, with no significant differences among tested seedlings. Starch content at other sampling locations showed more obvious differences among seedlings with different vigor levels and decreased as seedling vigor declined. In half-year-old seedlings, the stem-root junction, root apex swelling, main root, and lateral root cortex showed the deepest blue-black staining, indicating the highest starch content among all tested seedlings. After two years of independent growth, staining in these locations was lighter than in half-year-old seedlings, indicating significantly reduced starch content. After three years of independent growth, no obvious or only very light staining was observed in the stem-root junction, root apex swelling, and lateral root cortex, with very light staining also in the main root cortex, indicating extremely low starch content.

Figure 3. Starch content and distribution characteristics of cross-sections from different parts of *Malania oleifera* seedlings. S1. Half-year seedlings; S2. Two-year seedlings; S3. Three-year seedlings. S. Cross-sections from the middle part of the stem; CP. Cross-sections from the connecting point of the stem and root; BS. Cross-sections from the basal swelling part of the root; R. Cross-sections from middle parts of the main root; LR. Cross-sections of a lateral root 1 cm away from the main root.

Discussion

3.1 Seedling decline and N, P, K content changes

Element concentration measurements showed that N, P, and K concentrations in aboveground tissues gradually decreased with extended independent growth time of *M. oleifera* seedlings. Half-year-old seedlings had the highest N, P, and K concentrations in all organs, with average leaf N and P concentrations of 20.69 mg · g^{-1} and 1.86 mg · g^{-1}, respectively, higher than the geometric means of leaf N and P concentrations for 753 terrestrial plant species in China (18.6 mg · g^{-1} and 1.21 mg · g^{-1}) (Han et al., 2005). In contrast, three-year-old seedlings had leaf N and P concentrations of only 9.22 mg · g^{-1} and 0.28 mg · g^{-1}. Due to the dilution effect of increasing plant biomass on nutrient concentration, gradual nutrient concentration decrease with seedling growth is common in normal green plants (Greenwood et al., 2008; Tang et al., 2018). In this context, nutrient concentration reduction within a certain range does not necessarily indicate nutrient supply limitation. However, for *M. oleifera*, under conditions without host parasitic relationships, plant growth is slow and the dilution effect of biomass on nutrient concentration is limited. We speculate that the substantial decrease in tissue nutrient concentration mainly results from limited root absorption capacity and photosynthesis levels. As seed nutrients gradually deplete, seedling nutrient absorption and assimilation levels fall far below consumption for life activities. In these seedlings, decreased N, P, and K concentrations indicate that growth is already nutrient-limited. This speculation is validated by leaf N/P ratios in different seedling types.

The leaf N/P ratio reflects plant nutrient limitation status to some extent. When leaf N/P ratio is less than 14, plant growth is typically N-limited, while N/P ratios greater than 16 indicate P deficiency limitation (Koerselman & Meuleman, 1996). Although half-year-old *M. oleifera* seedlings grew vigorously with strong new root formation capacity, their leaf N/P ratio (average 11.33) suggests that growth may already be limited by insufficient N supply. This is consistent with Li (2021), who found that N fertilization significantly promoted growth of independently grown half-year-old *M. oleifera* seedlings, while P and K fertilization had no obvious effects. For two-year-old seedlings, the average leaf N/P ratio of 17.81 suggests P deficiency limitation. In Li's (2021) pot experiments, older *M. oleifera* seedlings showed obvious growth responses to P fertilization, indicating that seedlings at this stage could still improve growth through P fertilization or planting with suitable host plants. However, for three-year-old seedlings, the average leaf N/P ratio of 52.46 indicates extremely severe nutrient stress with severely imbalanced N/P ratios. Such severely declining seedlings hardly form new roots after transplantation and cannot resume normal growth even with fertilizer application or planting with excellent hosts, resulting in extremely low transplantation survival rates. Therefore, to avoid further resource waste in large-scale nursery cultivation, such severely declining seedlings are unsuitable for transplantation. In summary, during independent growth of *M. oleifera*, different degrees of mineral nutrient stress occur with extended growth time. Therefore, it is necessary to increase mineral element supplementation early in the nursery process or plant with suitable host plants to delay or prevent severe seedling decline.

The allocation patterns of N, P, and K concentrations among organs in *M. oleifera* seedlings differ substantially from those in normal green plants. Typically, plants prioritize nutrient allocation to leaves to enhance photosynthetic efficiency and promote biomass accumulation, resulting in leaf nutrient concentrations much higher than those in stems and roots (Tang et al., 2018). However, in independently grown *M. oleifera* seedlings in this study, leaf N and P concentrations were similar to those in stems but significantly lower than those in main and lateral roots, while K concentration showed opposite trends in seedlings with different decline degrees. This may be closely related to the unique survival strategies and physiological characteristics of root hemiparasitic plants. Unlike normal green plants that prioritize biomass accumulation and maintain competitive advantages, root hemiparasitic plants typically experience an extremely slow aboveground growth stage during early seedling stages (Li et al., 2012; Cardona-Medina & Muriel Ruiz, 2015; Li et al., 2019) to ensure sufficient time for establishing parasitic relationships with hosts and avoid excessive nutrient consumption before successful parasitism. This partially explains the conservative N and P allocation patterns among organs in *M. oleifera* seedlings. Additionally, root hemiparasitic plants typically maintain high transpiration rates to drive nutrient acquisition from hosts (Press et al., 1999), and higher leaf K concentration is an effective way to enhance transpiration rates (Li et al., 2021). Thus, higher leaf K concentration in *M. oleifera* appears to be a specific physiological adaptation strategy of root hemiparasitic plants. However, the biological significance of significantly increased K concentration in lateral roots of severely declining *M. oleifera* seedlings requires further study.

3.2 Seedling decline and tissue starch content changes

Starch stored in stem and root tissues sustains plant growth when carbon assimilation capacity deteriorates or is lost, playing an important role in plant resistance to environmental stress (Johansson, 1993; Bollmark et al., 1999; Dietze et al., 2014; Li et al., 2018). Starch content in root hemiparasitic *Santalum album* seedlings is closely related to growth and development, and seedling death occurs when starch content falls below certain levels (Zhang, 2015; Zhou et al., 2020). This study also found that starch content levels in *M. oleifera* plants are closely related to decline degree, with vigorous seedlings having adequate starch reserves while severely declining seedlings have very limited starch content. *M. oleifera* seeds are large and rich in various nutrients (Xue and Shao, 2015; Li, 2021). Abundant starch granules observed in the swollen stem base and fleshy roots of half-year-old seedlings suggest that rich starch in seeds is transferred to the swollen stem base and root system after germination to support early rapid seedling growth. Plants typically prioritize using new carbon and only use stored carbon when stressed or when demand exceeds supply (Dietze et al., 2014). As seedling age increases, independently grown *M. oleifera* seedlings experience gradually intensifying N and P stress due to limited root absorption capacity, leading to progressive decline and gradually decreasing starch content in stems and roots. This indicates that newly synthesized carbon can no longer meet growth demands, requiring mobilization of stored starch to maintain plant growth. Studies show that N and P deficiency reduce plant net photosynthetic rates and hinder carbon assimilation (Zhao et al., 2005). When *M. oleifera* experiences N stress, its leaf net photosynthetic rate decreases significantly (Li, 2021). Li et al. (2015) also found that chlorophyll content in leaves of root hemiparasitic *Santalum album* seedlings decreased significantly under N deficiency. Our results indicate that under N and P stress, starch reserve levels are closely related to growth status in *M. oleifera* seedlings, with adequate starch reserves supporting vigorous growth while starch content decline accompanies seedling decline and death.

Given that starch content levels are closely related to mineral nutrient stress degree and plant vigor levels in *M. oleifera* seedlings, and that starch content assessment is simple and convenient, simple tissue section staining observations can quickly determine starch content status and thus decline degree during large-scale nursery cultivation and afforestation. This could serve as a rapid method for detecting seedling vigor. According to our observations, starch content in *M. oleifera* seedling stems was generally low with no significant differences among tested seedlings, making stems unsuitable sampling sites for vigor detection. Starch content at the stem-root junction, root apex swelling, main root, and lateral roots showed obvious differences among seedlings with different vigor levels and decreased as seedling vigor declined, making these locations suitable for sampling to distinguish seedling vigor. However, considering different damage degrees caused by sampling different locations, cross-sectioning at the stem-root junction, root apex swelling, and main root causes fatal damage (removing aboveground parts), while taking only lateral roots leaves seedlings relatively intact and suitable for continued afforestation use. Moreover, differences in staining intensity and detection sensitivity were more pronounced in lateral root cortex among seedlings with different vigor levels, with higher detection sensitivity than other locations. Therefore, selecting lateral roots as sampling sites represents a minimally invasive sampling strategy. Further research is needed to optimize section thickness, staining and destaining times to develop a rapid, minimally invasive method for detecting *M. oleifera* seedling vigor.

Acknowledgments

We thank Mr. Lang Yu from Yunnan Yuntianhua Modern Agriculture Development Co., Ltd. for assistance in determining tissue content of the three macronutrients, and Ms. Yanqiong Zhao from Yunnan Agricultural University for help with sample grinding.

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Note: Figure translations are in progress. See original paper for figures.