Postprint: Study on Seedling Growth Rhythm of Melia azedarach from Different Provenances

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Abstract

Melia azedarach is widely distributed in China and exhibits extensive genetic variation. To further improve provenance selection and improved variety breeding of Melia azedarach, this study conducted comparative analysis on growth traits and stage-specific growth characteristics of seedlings from different provenances, preliminarily revealing the seedling growth patterns. Using 1-year-old seedlings from 15 provenances as experimental materials, growth traits including seedling height, ground diameter, compound leaf growth, and biomass accumulation were observed and analyzed, and the growth rhythm was fitted using the Logistic equation. The results showed that: (1) Differences in seedling height and ground diameter growth among different provenances reached significant levels, while differences in root biomass, stem biomass, and compound leaf-related traits reached extremely significant levels; (2) Both seedling height and ground diameter growth exhibited a slow-fast-slow S-shaped growth pattern with two growth peaks each; compared with the timing of seedling height growth peaks, ground diameter growth peaks occurred later; (3) The R^2 values of the Logistic fitting equations ranged from 0.976 to 0.994, all reaching extremely significant correlation levels, indicating that the Logistic equation can be used to fit the growth rhythm of Melia azedarach; (4) The duration of the ground diameter fast-growing period was generally 20–30 days longer than that of the seedling height fast-growing period; northern provenances entered and ended the fast-growing period earlier than southern provenances for both seedling height and ground diameter; cumulative growth during the fast-growing period accounted for over 60% of total growth for both seedling height and ground diameter; (5) All growth indices showed negative correlations with latitude; seedling height, root biomass, and compound leaf area showed positive correlations with longitude, while other indices showed negative correlations with longitude. In summary, the results indicate that Melia azedarach is a full-period growth type tree species, with significant differences in growth traits among provenances; growth is controlled by both latitude and longitude, with latitude being the dominant

factor.

Full Text

Annual Growth Rhythm of *Melia azedarach* Seedlings from Different Seed Sources

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Abstract

Melia azedarach is widely distributed across China and exhibits extensive genetic variation. To advance seed source selection and breeding programs for this species, this study compared and analyzed the growth traits and stage-specific growth characteristics of seedlings from different provenances, providing preliminary insights into the seedling growth patterns of *M. azedarach*. Using one-year-old seedlings from 15 provenances as experimental materials, we observed and analyzed growth traits including seedling height, ground diameter, compound leaf development, and biomass accumulation, and fitted growth rhythms using logistic equations. The results showed: (1) Significant differences in seedling height and ground diameter growth among provenances, with highly significant differences in root biomass, stem biomass, and compound leaf-related traits; (2) Both height and diameter growth followed a slow-fast-slow S-shaped pattern with two distinct growth peaks, with diameter growth peaks occurring later than height growth peaks; (3) The R^2 values of logistic fitting equations ranged from 0.976 to 0.994, all reaching highly significant correlation levels, indicating that logistic equations can effectively model *M. azedarach* growth rhythms; (4) The rapid growth period for ground diameter generally lasted 20–30 days longer than that for seedling height. Northern provenances entered and ended their rapid growth periods earlier than southern provenances, with cumulative height and diameter growth during the rapid growth period exceeding 60% of total growth; (5) All growth indices were negatively correlated with latitude, while seedling height, root biomass, and compound leaf area were positively correlated with longitude, and other indices were negatively correlated with longitude. These results demonstrate that *M. azedarach* is a full-cycle growth species with significant inter-provenance variation in growth traits, regulated by both latitude and longitude, primarily by latitude.

Keywords: *Melia azedarach*, seedling stage, logistic equation, growth rhythm

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Introduction

Melia azedarach is a deciduous tree belonging to the family Meliaceae and genus *Melia*, widely distributed across tropical and subtropical regions of Asia (Zheng, 2004). This fast-growing species produces high-quality timber and has an attractive form, making it valuable for both timber production and urban landscaping. Its roots, bark, flowers, and fruits have medicinal properties, earning it the reputation of a "natural insecticide" as an efficient, low-toxicity botanical pesticide (Cheng, 2005). *M. azedarach* tolerates poor soils and exhibits strong salt resistance, capable of normal growth in soils with total salt content below 0.5%, making it suitable for ecological restoration in saline-alkali and rocky desertification areas (Lin, 2004). Consequently, this multifunctional native species has gained increasing attention.

The species shows strong environmental adaptability and extensive natural distribution, typically occurring sporadically and scattered throughout its range. Natural stands are rare, and the complex topography and variable climate within the distribution area, combined with long-term natural selection and reproductive isolation, have generated rich genetic variation among different geographic ecotypes (Cheng and Gu, 2005; Ma et al., 2010). Historically, exploitation of natural resources has neglected *M. azedarach* resource management, particularly the destructive harvesting of bark for azadirachtin extraction, leading to gradual depletion of germplasm resources. Additionally, most planted forests use unselected seedlings of variable genetic quality, severely limiting development and utilization. Therefore, collecting germplasm resources and conducting provenance trials to study seedling growth patterns and compare growth trait differences among provenances represents an effective approach for selecting superior germplasm. Since the 1980s, domestic researchers have conducted numerous studies on provenance selection and breeding, primarily targeting timber production and azadirachtin content, preliminarily identifying superior provenances, families, clones, or individual trees suitable for different regions (Cui et al., 1994; Chen et al., 2008; Jiao et al., 2015; He et al., 2018; Chen et al., 2018). Analyzing annual growth rhythms reveals seedling growth characteristics, growth types, and critical management periods, representing an important technical component for improving seedling quality in production (Yang, 2013). Currently, research on *M. azedarach* seedling growth rhythms and model fitting remains limited, and growth patterns vary significantly among different provenances or geographic sources when cultivated in different regions (Cheng and Gu, 2006; Chen et al., 2014).

This study examined one-year-old seedlings from different provenances, conducting regular observations of height and diameter growth, comparing inter-provenance differences in growth traits, fitting mathematical models, analyzing annual growth rhythms, comparing stage-specific growth characteristics to identify critical periods and determine field management measures for producing high-quality seedlings, and systematically analyzing seedling growth variation among provenances. The study addresses three questions: (1) How do *M. azedarach* seedlings differ in growth among provenances? (2) What are the growth rhythms and stage-specific characteristics of one-year-old seedlings? (3) How do growth traits correlate with geographic and climatic factors of seed sources?

1. Materials and Methods

1.1 Experimental Site The experiment was conducted in Yujia, Maogongpu Village, Hefeng Town, Lishui District, Nanjing (118°59 E, 31°25 N). The site has loose, well-drained soil and is located in a subtropical monsoon climate zone with mild, humid conditions and distinct seasons. Rainfall concentrates between June and September, with an average annual precipitation of 1,037 mm, mean annual temperature of 15.5 °C, and a frost-free period of 222–224 days. The terrain consists of low hills.

1.2 Experimental Materials Seeds were collected from 15 natural *M. azedarach* provenances in East, Central, and South China during November–December 2016. Geographic distribution and climatic conditions of collection sites are shown in Table 1 . At each site, 10–20 healthy, disease-free mother trees at reproductive maturity were selected, spaced at least 300 m apart. Seeds were cleaned and air-dried, then equal quantities from different mother trees were mixed for each provenance.

1.3 Experimental Design Seeds were soaked in warm water for three days on March 15, 2017, and sown on March 18. A randomized block design with three replicates was used. Each provenance was sown in 10 rows per replicate with 30 cm row spacing and 10 seeds per row. Shade nets were applied after sowing to maintain moisture. Around April 25, most seeds germinated and shade nets were removed. Since each fruit stone contains multiple seeds, seedlings emerged in clusters and were thinned to 15–20 cm spacing when reaching approximately 10 cm in height.

1.4 Measurement Methods **Seedling height and ground diameter:** In each provenance and replicate, 10 seedlings were randomly selected and tagged (30 seedlings total per provenance). Height and diameter were measured with a tape measure (precision 0.1 cm) and vernier caliper (precision 0.01 mm) every 15 days from May 15 to November 20, 2017, when growth ceased.

Biomass: After growth cessation and leaf fall, 10 seedlings approximating the mean size were selected per provenance for biomass measurement. Seedlings were excavated with intact root systems, washed free of soil, separated into above- and below-ground parts at the root collar, oven-dried at 105 °C to constant mass, and weighed (precision 0.01 g).

Compound leaf traits: In mid-September 2017, when seedlings reached mature stable growth, the 4th or 5th compound leaf from the apex was collected from each provenance and replicate (5 leaves per replicate). Leaf length and width were measured with a tape measure to calculate length-width ratios, and leaf area was determined using image processing methods (Yu et al., 2012).

1.5 Data Processing Normality of all measured traits was tested using skewness and kurtosis methods, and outlier data for individual samples were removed using the Pauta criterion (3σ criterion) (Zhang et al., 2014). SPSS 23.0 software was used to fit logistic curves for height and diameter data (Chen et al., 2014).

The logistic curve fitting equation is: y = k/(1 + ae^(-bx)), where y represents seedling height or ground diameter growth; k represents growth potential (the theoretical maximum value), calculable using the arithmetic three-point method; x represents growth time; and a and b are coefficients determined through SPSS nonlinear regression analysis. The two inflection points derived from the third derivative of the fitted equation represent the start and end of the rapid growth period, with the interval between them defining the fast-growing stage.

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2. Results

2.1 Differences in Seedling Growth Traits Variance analysis revealed significant differences in cumulative height and diameter growth among provenances ($P < 0.05$), with highly significant differences in root biomass, stem biomass, and compound leaf traits ($P < 0.01$). Coefficients of variation ranged from 13.22% to 44.01%, indicating significant inter-provenance variation in seedling growth traits (Table 2). As shown in Table 2, provenances from Shantou (Guangdong), Nanning (Guangxi), Lin' an (Zhejiang), and Jinggangshan (Jiangxi) showed faster height and diameter growth, while Xinyang (Henan) and Dongying (Shandong) performed poorly. Root and stem biomass accumulation was greater in Shantou, Lin'an, and Jinggangshan provenances, while the poorly performing Xinyang and Dongying provenances showed relatively low biomass. Jinggangshan produced the longest compound leaves and Changsha (Hunan) the widest, whereas Pizhou (Jiangsu) and Dongming (Shandong) had significantly smaller leaf dimensions. Jinggangshan provenance had the largest compound leaf area, while Nanning had the smallest.

2.2 Analysis of Seedling Growth Rhythm Differences As shown in Figures 1 [Figure 1: see original paper] and 2 [Figure 2: see original paper], growth rhythms of height and diameter were generally consistent across provenances, following a typical slow-fast-slow S-shaped curve. Height growth was slow until early July, with minimal inter-provenance differences. Rapid growth began in mid-July, with differences gradually increasing until mid-September, after which growth slowed and increments became less pronounced. Ground diameter growth was also slow until late June, with small inter-provenance differences. Growth rate increased significantly in early July, with differences expanding continuously until mid-October, after which growth gradually slowed or ceased.

Net increments in height and diameter varied substantially over time, with two distinct growth peaks generally occurring at different times among provenances (Figures 3 [Figure 3: see original paper] and 4 [Figure 4: see original paper]).

The first height growth peak occurred in mid-July for all provenances. After a temporary slowdown, Nanjing (Jiangsu), Taixing (Jiangsu), Xinyang (Henan), Hefei (Anhui), Dongying (Shandong), Jingzhou (Hubei), Lin' an (Zhejiang), and Yueqing (Zhejiang) showed a second peak in mid-August, while remaining provenances peaked from late August to early September. The first diameter growth peak was relatively concentrated in late July for all provenances. Except for Changsha (Hunan) and Shantou (Guangdong), most provenances experienced reduced diameter growth in August, followed by a second peak from mid- to late September. Overall, diameter growth peaks occurred later than height growth peaks.

2.3 Growth Model Fitting and Growth Characteristic Analysis Based on height and diameter observations, logistic equations were fitted to model *M. azedarach* growth (Table 3). The R^2 values for height and diameter models ranged from 0.976 to 0.994, all reaching highly significant correlation levels.

The two inflection points derived from each logistic equation define the seedling stage (before the first point), fast-growing stage (between points), and late growth stage (after the second point). Table 4 presents start times, durations, and net growth for each stage by provenance.

Compared with diameter, height entered the rapid growth period later and ended earlier, resulting in a shorter fast-growing period. Theoretical calculations combined with practical observations indicated that the height fast-growing period was generally 20–30 days shorter than that for diameter.

Provenances showed variation in timing and duration of height fast-growing periods. Dongming and Dongying entered and ended their fast-growing periods earliest, while Nanning entered and ended latest, with no clear pattern in duration differences. Diameter fast-growing periods showed more pronounced variation: Dongying entered earliest, Shantou latest, Xinyang had the shortest duration, and Lin' an the longest.

Growth increments varied significantly among stages. During the seedling and late growth stages, cumulative height and diameter growth accounted for 35–39% and 25–38% of total growth, respectively. During the June–September fast-growing period, cumulative height and diameter growth exceeded 60% of total growth.

2.4 Correlation Analysis of Growth Traits As shown in Table 5 , height was highly significantly correlated with stem biomass ($P < 0.01$), while diameter was highly significantly correlated with both root and stem biomass ($P < 0.01$). All compound leaf traits showed significant or highly significant positive correlations with diameter, root biomass, and stem biomass. However, height showed no significant correlation with root biomass or compound leaf dimensions, indicating that seedling height had limited influence on root biomass accumulation and leaf growth.

Numerous studies have documented extensive geographic variation in seedling growth, influenced by both genetic characteristics and local geographic-climatic conditions. As shown in Table 6 , seedling height, root biomass, and leaf area were positively correlated with longitude, while other indices were negatively correlated. All growth indices were negatively correlated with latitude but positively correlated with mean annual temperature, January mean temperature, annual precipitation, and frost-free period. This indicates that in low-latitude regions with higher temperatures, longer frost-free periods, and greater precipitation, *M. azedarach* seedlings have extended growth periods and greater biomass accumulation.

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3. Discussion and Conclusion

As *M. azedarach* primarily reproduces via seeds, studying seedling growth rhythms and comparing inter-provenance performance and trait variation represents an important pathway for superior germplasm selection. This study found significant or highly significant differences in all growth traits among provenances. The species' wide distribution and complex environmental conditions, combined with long-term geographic isolation and natural selection, have generated rich genetic variation among provenances. Since this experiment was conducted at a single site with consistent management, these differences primarily reflect geographic-climatic conditions and inherent genetic variation of seed sources, demonstrating substantial potential for genetic improvement.

The results indicate that both height and diameter growth exhibited two growth peaks with staggered timing, confirming *M. azedarach* as a full-cycle growth species. This pattern is similar to related species including *Chukrasia tabularis* (Wu, 2013) and *Toona ciliata* (Li et al., 2017), and shows comparable trends with *Pistacia chinensis* (Chen, 2009) and *Cyclocarya paliurus* (Yang, 2013). All provenances displayed slow-fast-slow S-shaped growth curves, with logistic equation fits reaching highly significant levels, confirming the suitability of logistic models for analyzing growth rhythms, consistent with findings by Chen et al. (2014) and Li et al. (2017). Theoretical and practical analyses divided growth into three stages: seedling, fast-growing, and late growth periods, with significant inter-provenance differences in stage duration. Generally, northern provenances entered and exited fast-growing periods earlier than southern provenances, similar to results for *Quercus acutissima* (Liu, 2010) and *P. chinensis* (Chen, 2009). This pattern likely relates to temperature and photoperiod—northern provenances require lower temperatures to initiate growth, while southern provenances (e.g., Shantou and Nanning) planted in Nanjing experience relatively extended daylight, prolonging the growth period and delaying growth cessation.

Seedling growth quantity is a key quality indicator, with height and diameter being the most direct morphological reflections of vigor and the most intuitive metrics for seedling quality (Zhai and Ma, 2020). For breeding programs targeting rapid growth, fast-growing period increments provide essential reference data (Kuang et al., 2014). Chen et al. (2013) reported that for *Manglietia insignis*, height and diameter net growth during the peak period accounted for 58.90–69.34% and 67.43–71.49% of annual growth, respectively. For *P. chinensis*, cumulative height growth during the <70-day fast-growing period reached 60% of annual growth (Chen, 2009). This study found that during June–September, with abundant rainfall and sunlight, *M. azedarach* height and diameter cumulative growth exceeded 60% of total growth, demonstrating that fast-growing period increments determine overall growth. This critical period requires adequate nutrient supply to promote rapid growth, while fertilization and irrigation should be reduced during late growth to promote lignification and enhance stress resistance. Under our experimental conditions, Shantou, Nanning, Lin' an, Yueqing, Jinggangshan, and Nanjing provenances showed relatively large net increments during the fast-growing period. However, Shantou and Nanning provenances experienced frost damage at the experimental site, suggesting that for afforestation near the experimental area, priority should be given to seed collection from Lin' an, Yueqing, Jinggangshan, and Nanjing.

Widely distributed species develop genetic differentiation among populations due to diverse environmental influences and restricted gene flow. When transplanted to common environments, these populations exhibit rich geographic variation (Chen and Shen, 2005). Seedling growth traits of *Sorbus pohuashanensis* (Zheng et al., 2012) and *T. ciliata* (Li et al., 2017) are controlled by both latitude and longitude, with longitude being dominant for *T. ciliata* (showing faster east-to-west growth), while latitude primarily controls variation in *S. pohuashanensis*. For *Phoebe bournei*, height shows non-significant negative correlations with longitude and latitude, while diameter is significantly negatively correlated with longitude but significantly positively correlated with altitude (Tan et al., 2019). This study found that *M. azedarach* height, diameter, and dry matter accumulation were all highly significantly negatively correlated with latitude, but showed no clear correlation with longitude, consistent with findings by Chen et al. (2008) and Liao (2015). These negative correlations with latitude actually reflect positive correlations with temperature, precipitation, and frost-free period at seed sources, which correlation analysis confirmed, indicating temperature as the primary factor limiting *M. azedarach* growth. As a full-cycle growth species, longer frost-free periods extend the growing season, facilitating growth and biomass accumulation.

Due to objective constraints, this study sampled only 15 provenances from East, Central, and South China rather than the entire distribution range, limiting the conclusions. To obtain more scientific and robust results, future research should expand sampling scope, conduct multi-site afforestation trials, and perform continuous long-term monitoring to screen for superior germplasm.

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