Analysis and Evaluation of Oleoresin Components from Slash Pine Clones (Postprint)

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Abstract

To select superior slash pine clones with strong resin-producing capacity and high oleoresin quality from existing fast-growing timber clones in slash pine seed orchards, and to efficiently utilize slash pine improved genetic resources, 36 clones from the first-generation slash pine seed orchard were used as experimental materials to determine their resin mass flow rate and diameter at breast height (DBH) growth. GC-MS was further employed to analyze their oleoresin components. Based on these indicators, correlation analysis and cluster analysis were conducted to comprehensively evaluate the 36 tested clones. The results showed: (1) A total of 21 oleoresin components were identified, including 8 monoterpene components and 13 diterpene components. (2) Correlation analysis indicated that resin mass flow rate (RMR) was significantly positively correlated with monoterpene content, weakly negatively correlated with abietic-type resin acids, and showed no significant correlation with pimaric-type resin acids. (3) Cluster analysis of the tested clones based on four dimensions—turpentine content, resin mass flow rate, abietic-type resin acids, and pimaric-type resin acids—classified the 36 clones into three major categories with significant differences among types, with Category 1 performing far better than the other two categories. (4) On the basis of high resin-producing capacity, four clones (6-44, 4-11-1, 1-38, and 3-64) exhibited high monoterpene content; four clones (4-11-1, 3-64, 2-0420, and 3-468) showed high pimaric-type resin acid content; while clone 2-173 had relatively high abietic-type resin acid content. In summary, this study qualitatively analyzed the oleoresin components of slash pine and quantitatively evaluated the resin-producing capacity and component contents of 36 clones, laying a foundation for selecting resin-producing clones of slash pine.

Full Text

Evaluation and Analysis of Resin Composition in *Pinus elliottii* Clones

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Abstract

To select superior clones with strong resin-producing capacity and high resin quality from existing fast-growing timber clones in a *Pinus elliottii* seed orchard and to efficiently utilize elite germplasm resources, we evaluated 36 clones from the first-generation seed orchard. We measured resin mass flow rate and diameter at breast height (DBH) growth, and analyzed resin composition using GC-MS. Based on these indicators, we conducted comprehensive evaluations of the 36 clones using correlation analysis and cluster analysis. The results showed: (1) A total of 21 resin components were identified, including 8 monoterpenes and 13 diterpenes. (2) Correlation analysis revealed that resin mass flow rate (RMR) was significantly positively correlated with monoterpene content, weakly negatively correlated with abietic-type resin acids, and showed no significant correlation with pimaric-type resin acids. (3) Cluster analysis based on four dimensions—turpentine content, resin mass flow rate, abietic-type resin acids, and pimaric-type resin acids—classified the 36 clones into three major categories with significant differences between types, with Category 1 performing far better than the other two. (4) Based on high resin-producing capacity, four clones (6-44, 4-11-1, 1-38, and 3-64) exhibited high monoterpene content; four clones (4-11-1, 3-64, 2-0420, and 3-468) showed high pimaric-type resin acid content; and clone 2-173 had higher abietic-type resin acid content. In summary, this study qualitatively analyzed the resin composition of slash pine and quantitatively evaluated the resin-producing capacity and component content of 36 clones, laying a foundation for selecting resin-use clones in *P. elliottii*.

Keywords: *Pinus elliottii*, resin composition, correlation, cluster analysis, resin-producing capacity

Introduction

Pine resin, a byproduct of pine trees primarily processed into rosin and turpentine, serves as an important raw material in chemical, electronics, aerospace, food, and medical industries, holding significant economic value (Neis et al., 2018). As a major pine resin producer and exporter, China has been researching and improving resin components in slash pine (*Pinus elliottii*)—one of its key resin-tapping species—since the 1980s (Lei et al., 2015a). The main components of slash pine resin are non-volatile rosin and volatile turpentine. Pimaric acid in rosin exhibits bactericidal properties and can be used in anticancer drug preparation (Lai et al., 2020). Turpentine content often serves as a quality indicator, with higher content representing better resin quality. Certain turpentine components, such as β-pinene, show potential as biofuel and can be synthesized into fragrances, making them key research targets (Lei et al., 2015b). Multiple studies have demonstrated that major monoterpene and diterpene components in slash pine are under moderate to strong genetic control, making selective breeding for resin components feasible (Li et al., 2012a; Lai et al., 2020).

Resin-producing capacity, commonly measured as resin yield, is under strong genetic control (Wu et al., 2019; Zhang et al., 2010), making breeding for high yield crucial. Traditional identification of high-yield trees uses downward tapping methods to collect individual yield data, but this approach can affect tree growth and is time-consuming. Neis et al. (2019) developed a method using resin mass flow rate (RMR) for rapid identification of high-yield individuals and validated its reliability, which was further confirmed by Yi et al. (2020). This method measures resin mass flowing from small trunk wounds within a short period (4 h), with high-yield clones showing significantly higher RMR than low-yield clones (Yi et al., 2020). However, previous studies primarily examined correlations between RMR and resin yield without addressing relationships with resin components. Furthermore, past breeding studies on high-yield slash pine focused mainly on yield data while neglecting resin components, particularly lacking targeted breeding research for specific components.

As China's province with the largest slash pine plantation area, Jiangxi currently lacks high-yield resin varieties, with existing elite varieties primarily selected for fast growth and timber use. To develop the resin-use value of existing fast-growing timber varieties, this study used 36 clones from the first-generation slash pine seed orchard at Jiangxia County Forest Tree Seed Farm in Jiangxi Province. We measured resin mass flow rate, resin components, and DBH growth, and employed correlation and cluster analyses to address three main questions: (1) What are the compositional components of slash pine resin? (2) What is the relationship between resin mass flow rate and resin components? (3) Do resin-producing traits vary among slash pine clones?

Materials and Methods

1.1 Experimental Materials The experimental materials were obtained from a first-generation slash pine seed orchard (variety number GLS Gan-Shi No. 1) at Jiangxia County Forest Tree Seed Farm in Jiangxi Province. Established in 1980, the orchard originally contained 55 clones, with 43 currently preserved. We selected 36 clones with good growth and straight stems as experimental materials. Located in Shuibian Town, Jiangxia County, Jiangxi Province (115°24 E, 27°33 N), the seed orchard experiences a mid-subtropical monsoon climate with 1,752 annual sunshine hours, mean annual temperature of 17.7°C, effective accumulated temperature above 10°C for 250 days, annual rainfall of 1,557.9–1,700 mm, and annual evaporation of 1,458.8 mm. The mild climate with abundant rainfall, sufficient light, distinct seasons, and 277 frost-free days annually provides optimal conditions for slash pine growth.

1.2 Resin Collection and DBH Measurement We selected 36 clones with good growth and straight stems from the seed orchard and measured DBH using diameter tapes. On the same day, we collected resin using a resin collection device (Li et al., 2012b). At breast height (h = 1.3 m), we drilled three upward-angled holes and attached 15 mL centrifuge tubes to collect flowing resin. After 4 hours, we removed the tubes, immediately sealed them, and weighed the resin promptly to prevent volatilization. The samples were then stored at 4°C for component analysis.

1.3 Resin Component Analysis Approximately 0.05 g of resin sample was methylated by adding anhydrous ethanol and 25% tetramethylammonium hydroxide solution with phenolphthalein indicator until the solution turned red. The sample was then analyzed using a PE gas chromatograph-mass spectrometer (Clair GC 680-MS 600) with an Elite-5MS quartz capillary column (30 m × 0.25 mm × 0.25 µm). The temperature program started at 60°C for 2 min, increased to 80°C at $5°C \cdot min^{-1}$, then to 230°C at $30°C \cdot min^{-1}$, and finally to 260°C at $5°C \cdot min^{-1}$ for 10 min.

Injection conditions: injection port temperature 250°C, injection volume 1 µL, split ratio 30:1, carrier gas high-purity helium ($1\ mL \cdot min^{-1}$). Electron ionization mass spectrometry conditions: solvent delay 3 min, electron energy 70 eV, ion source temperature 230°C, transfer line temperature 280°C, scan range 35–620 amu.

1.4 Data Analysis Resin component analysis: Qualitative analysis was performed using the GC-MS database and relevant literature. Relative content of each component was calculated by peak area normalization.

Resin mass flow rate (RMR): Expressed as resin mass collected within 4 h.

Correlation analysis: SPSS software was used to test data normality. Pearson correlation coefficients were calculated for normally distributed data.

Cluster analysis: SPSS software with K-means clustering method was used to classify samples based on quantitative data.

Multiple comparisons: t-tests were performed between each cluster group and the overall mean. Differences were considered significant at $P < 0.05$.

Results

2.1 Analysis of Resin Components and Related Traits GC-MS analysis identified 21 resin components, including 8 monoterpenes and 13 diterpenes. Except for limonene and palustric acid, all other components were detected in most clones. Monoterpene content ranged from 13.87% to 21.29%, with a mean of 17.32%. The main components were α-pinene and β-pinene, accounting for approximately 90% of monoterpenes. Diterpenes comprised 68.11%–80.96% of all components (mean 74.20%), including 8.29% pimaric-type resin acids and 64.30% abietic-type resin acids. Components with relatively low content generally showed higher coefficients of variation than abundant components. Among components exceeding 1% content, the three highest coefficients of variation were phellandrene, β-pinene, and dehydroabietic acid, indicating high selection potential. Resin mass flow rate (RMR) showed considerable variation among clones, making it a suitable selection indicator (Table 1).

Table 1 Basic statistics of turpentine composition and related traits

Turpentine composition	Project	Mean (%)	CV (%)
Monoterpenes	α-Pinene	9.45	15.32
	Camphene	0.52	28.85
	Carene	0.31	38.71
	β-Pinene	6.89	20.32
	Myrcene	0.15	33.33
	Limonene	-	-
	Phellandrene	0.12	41.67
	Estragole	0.08	37.50
	Total	**17.32**	**13.86**
Diterpenes	Elliotinoic acid	0.45	31.11
	Sandaracopimaric acid	1.25	24.00
	Isopimaric acid	2.89	18.69
	Pimaric acid	4.15	16.87
	Pimaric-type resin acid	**8.29**	**15.80**
	Levopimaric acid	25.36	12.45
	Palustric acid	-	-
	Dehydroabietic acid	12.58	19.87
	Abietic acid	18.45	14.09
	Neoabietic acid	7.91	16.31
	Abietic-type resin acid	**64.30**	**8.71**

Turpentine composition	Project	Mean (%)	CV (%)
	Total	**74.20**	**5.93**
Resin mass flow rate (g)		10.61	42.32
DBH (cm)		28.35	12.87

Note: Unit of turpentine component is %.

2.2 Correlation Analysis of Resin Components and Related Traits Correlation analysis of all traits in Table 1 revealed that α-pinene, the main monoterpene component, was negatively correlated with carene and phellandrene, while β-pinene showed significant positive correlations with myrcene, limonene, and total monoterpene content. Among diterpene components, dehydroabietic aldehyde, isopimaric acid, palustric acid, levopimaric acid, and dehydroabietic acid were associated with diterpene content, most belonging to abietic-type resin acids. Total diterpene content showed strong positive correlation with total abietic-type resin acid content but weak correlation with pimaric-type resin acid content. No significant association was observed between total diterpene and monoterpene contents. Total pimaric-type resin acid content showed no significant correlation with most monoterpene components, whereas total abietic-type resin acid content was significantly negatively correlated with β-pinene and limonene but significantly positively correlated with phellandrene and estragole.

RMR was significantly positively correlated with monoterpene content (correlation coefficient 0.63) and weakly negatively correlated with diterpene content (coefficient -0.32). Among all resin components, RMR showed significant positive correlations with β-pinene and myrcene in monoterpenes, weak negative correlations with neoabietic acid and total abietic-type resin acids in diterpenes, but no significant correlation with pimaric-type resin acids. DBH showed no significant association with monoterpene components or RMR, only weak correlations with diterpene content, abietic-type resin acid content, and dihydroabietic acid (Figure 1 [Figure 1: see original paper]).

Figure 1 Correlation heat map

2.3 Cluster Analysis We conducted multi-dimensional evaluation of the 36 clones using resin mass flow rate, monoterpene content, abietic-type resin acid content, and pimaric-type resin acid content. K-means clustering based on these four indicators classified the clones into high, medium, and low categories (Category 1 = high, Category 2 = medium, Category 3 = low). Multiple comparisons between categories showed significant differences, indicating reasonable classification (Table 2, Figure 2).

2.4 Comprehensive Evaluation of Resin-Producing Traits in Slash Pine Clones Based on monoterpene content classification, Category 1 included four clones (6-44, 4-11-1, 1-38, and 3-64) with 15.4% higher content than the overall mean. For pimaric-type resin acids, Category 1 comprised clones 1-1-1, 2-191, 4-11-1, 3-64, 2-0420, and 3-468, averaging 9.21% (11.1% higher than the overall mean, significantly different). Category 1 for abietic-type resin acids had a mean of 7.12%, significantly higher than the overall mean by 1.6%, including clones 4-44, 2-173, 2-164, 2-198, 2-126, 3-426, 5-72, and 1-1-1. Classification by resin mass flow rate yielded Category 1 with a mean of 15.15 g, containing 17 clones and exceeding the overall mean by 42.79% with significant differences from other categories, thus representing high resin-producing clones. Among these high-yield clones, four (6-44, 4-11-1, 1-38, and 3-64) showed high monoterpene content; four (4-11-1, 3-64, 2-0420, and 3-468) exhibited high pimaric-type resin acid content; and clone 2-173 demonstrated high abietic-type resin acid content (Table 2 , Figure 2 [Figure 2: see original paper]).

Table 2 Classification of 36 clones

Classification	Monoterpene content	Resin mass flow rate	Pimaric-type resin acid	Abietic-type resin acid
Category 1	6-44, 4-11-1, 1-38, 3-64	6-44, 4-11-1, 1-38, 3-64, 2-142, 0-867, 2-0420, 3-468, 5-21, 2-24, 3-142, 2-150, 3-3-1, 4-11-5, 4-15, 2-173, 6-42	1-1-1, 2-191, 4-11-1, 3-64, 2-0420, 3-468	4-44, 2-173, 2-164, 2-198, 2-126, 3-426, 5-72, 1-1-1
Category 2	2-0420, 1-1-1, 3-468, 2-198, 1-31, 5-21, 2-9, 3-265, 2-126, 2-191, 2-24, 3-55, 2-31, 3-142, 2-150, 3-3-1, 4-11-5, 4-15, 2-173, 6-42, 2-28	4-44, 2-164, 1-1-1, 2-198, 1-31, 2-9, 2-126, 2-191, 3-55, 2-31, 2-28	3-426, 2-113, 7-14, 5-72, 4-47, 2-142, 4-44, 0-867, 8-18, 3-18, 2-164, 2-198, 2-9, 2-126, 3-55, 3-426, 2-113, 7-14, 5-72, 4-47, 8-18, 3-18, 3-265, 6-44, 1-38, 2-142, 0-867, 5-21, 2-24, 3-142, 3-3-1, 4-15	1-31, 2-31, 2-28, 2-150, 4-11-5, 2-173, 6-42

Classification	Monoterpene content	Resin mass flow rate	Pimaric-type resin acid	Abietic-type resin acid
Category 3	3-426, 2-113, 7-14, 5-72, 4-47, 2-142, 4-44, 0-867, 8-18, 3-18, 2-164, 2-198, 2-9, 2-126, 3-55, 3-426, 2-113, 7-14, 5-72, 4-47, 8-18, 3-18, 3-265, 6-44, 1-38, 0-867, 2-24, 3-142, 3-3-1, 4-15, 2-191, 4-11-1, 2-0420, 3-468	2-113, 2-142, 5-21, 3-426, 2-113, 7-14, 5-72, 4-47, 8-18, 3-18, 2-164, 2-198, 2-9, 2-126, 3-55, 3-426, 2-113, 7-14, 5-72, 4-47, 8-18, 3-18, 3-265, 6-44, 1-38, 0-867, 2-24, 3-142, 3-3-1, 4-15, 2-191, 4-11-1, 2-0420, 3-468	2-113, 2-142, 5-21, 3-426, 2-113, 7-14, 5-72, 4-47, 8-18, 3-18, 2-164, 2-198, 2-9, 2-126, 3-55, 3-426, 2-113, 7-14, 5-72, 4-47, 8-18, 3-18, 3-265, 6-44, 1-38, 0-867, 2-24, 3-142, 3-3-1, 4-15, 2-191, 4-11-1, 2-0420, 3-468	2-113, 2-142, 5-21, 3-426, 2-113, 7-14, 5-72, 4-47, 8-18, 3-18, 2-164, 2-198, 2-9, 2-126, 3-55, 3-426, 2-113, 7-14, 5-72, 4-47, 8-18, 3-18, 3-265, 6-44, 1-38, 0-867, 2-24, 3-142, 3-3-1, 4-15, 2-191, 4-11-1, 2-0420, 3-468

Note: ns indicates no significant differences; indicates significant differences ($P < 0.05$); ** indicates significant differences ($P < 0.01$); *** indicates significant differences ($P < 0.001$).*

Figure 2 Multiple comparison among different grades

Discussion and Conclusion

Resin composition is critical for resin quality and an important reference indicator for high-yield breeding. This study detected 21 components in slash pine clones, including 8 monoterpenes and 13 diterpenes. Monoterpene content ranged from 13.87% to 21.29% (mean 17.32%), higher than Zhang et al. (2016) (9.89%) but slightly lower than Wu et al. (2018) (20.16%-25.76%), while Lai et al. (2020) reported 43.42%-45.32% in their study. These differences may relate to geographic environments—Zhang et al. (2016) used materials from Zhejiang, while Wu et al. (2018) collected from Guangxi, where different hydrothermal conditions affect resin biosynthesis (Neis et al., 2018). Genetic factors may also contribute, as Lei et al. (2015b) found higher monoterpene content in high-yield slash pine. Diterpene content in this study ranged from 68.11% to 80.96%, with levopimaric acid as the most abundant component, consistent with most previous studies (Lei et al., 2015b; Zhang et al., 2016).

β-pinene, the main monoterpene component, showed significant positive correlations with myrcene, limonene, and total turpentine content, suggesting these traits could be improved simultaneously. This positive relationship has been confirmed previously—high-yield trees contain higher β-pinene and limonene (Neis et al., 2019), and higher resin yield correlates with increased β-pinene/α-pinene ratios (Yi et al., 2020). Our finding of significant positive correlation between RMR and β-pinene suggests β-pinene could serve as a selection indicator for high-yield clones. Among diterpenes, RMR showed weak negative correlation with neoabietic acid and total abietic-type resin acids but no significant correlation with pimaric-type resin acids, indicating that increasing abietic-type resin acid content may reduce resin yield during component-directed improvement. DBH showed no significant association with monoterpenes or RMR, only weak correlations with diterpene and abietic-type resin acid contents, similar to findings in slash pine families (Li et al., 2012b).

The main monoterpene components α-pinene and β-pinene comprise approximately 90% of total monoterpenes. β-pinene showed higher coefficient of variation than α-pinene, consistent with *Pinus elliottii* × *Pinus caribaea* studies (Liao et al., 2022), indicating greater selection potential. β-pinene exhibits the highest individual heritability among major slash pine components, offering high genetic gain even at 10% selection intensity (Zhang et al., 2016). Its industrial applications and ability to resist bark beetle attacks further enhance its breeding potential. α-pinene, another important monoterpene, has multiple uses including anti-inflammatory and antioxidant effects in medicine, wound healing promotion, blood glucose reduction, and traditional Turkish use for rheumatism pain (Brahim et al., 2018; Santos et al., 2023). As a diesel additive, its unique structure helps reduce harmful emissions (Al ZAABI et al., 2022). Other monoterpenes also have value—carene serves as fragrance and pesticide raw material (Li et al., 2008), while camphene deters pine caterpillar feeding and oviposition (Chun et al., 2007).

Most pimaric and abietic acids in slash pine diterpenes are under moderate genetic control (Lai et al., 2020), enabling effective directional breeding. Although rosin has lower market value than turpentine, individual components have high value when isolated—pimaric acid is a renewable biopolyester material for specialized medical applications (Zia et al., 2016), while abietic acid protects against acetaminophen-induced liver injury by inhibiting activation and increasing Nrf2 expression, and can serve as bio-aviation fuel or additive to improve conventional bio-jet fuel performance (Li et al., 2020; An et al., 2023). As widespread secondary metabolites, terpenoid components show broad application prospects in medicine, chemicals, food, and particularly green environmental protection.

In chemical industry, pine resin is typically separated into different components rather than utilized as polymers. Therefore, slash pine breeding programs for resin traits should not target high yield alone but also emphasize component-directed improvement. Cluster analysis can classify clones by quantitative data. In Caribbean pine breeding for high-value components, researchers identified clones with high α-pinene, high β-pinene, high β-phellandrene, and high total monoterpene content through cluster analysis (Xie et al., 2022). This study classified 36 slash pine clones into high, medium, and low groups for monoterpenes, pimaric-type resin acids, and abietic-type resin acids, with high-content groups exceeding overall means by 15.4%, 11.1%, and 1.6%, respectively, containing 4, 6, and 8 clones. Clustering by resin mass flow rate separated clones into high, medium, and low resin-producing groups, with the high-yield group containing 17 clones significantly different from other groups. Based on correlation results, slash pine breeding can target single traits or combine resin yield with component improvement. Integrating multiple traits yields greater economic benefits in long-term breeding programs. Among the 17 high-yield clones, those emphasizing monoterpene content include 6-44, 4-11-1, 1-38, and 3-64; those favoring pimaric-type resin acid content include 4-11-1, 3-64, 2-0420, and 3-468; and clone 2-173 is suitable for abietic-type resin acid content. These results provide scientific references for targeted breeding of slash pine resin components.

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