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ARTICLE

Comparative Analysis of the Photosynthetic Characteristics and Active Compounds of *Semiliquidambar cathayensis* Chang Heteromorphic Leaves

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ABSTRACT

In the present study, the variation patterns of leaf shape in different populations of individual *Semiliquidambar cathayensis* plants were analyzed to investigate the relationship among leaf shape variation, photosynthetic properties, and active compounds to understand the genetic characteristics of *S. cathayensis* and screen elite germplasms. The leaf shape of 18 offspring from three natural *S. cathayensis* populations was analyzed to investigate the level of diversity and variation patterns of leaf shape. Furthermore, photosynthetic pigment content, physiological parameters of photosynthesis, and the active compounds in leaves of different shapes were determined. Statistical analysis showed that the leaf shape variation in *S. cathayensis* indicated a high level of genetic diversity among and within the populations. Cluster analysis showed that the three natural populations formed two clusters, one whose offspring was dominated by entire leaves and another characterized by palmately trifoliate leaves. The differences in photosynthetic characteristics and active compounds of leaves of three different shapes were comprehensively evaluated using principal component analysis. Two principal components with a cumulative contribution rate of 92.768% were extracted, of which the highest comprehensive score was for asymmetrically lobed leaves. The leaf shape in different *S. cathayensis* germplasms exhibited distinct patterns, and there were some correlations between the photosynthetic properties and active compounds in leaves of different shapes. Thus, the leaf shape can be used to predict active compound content, and in turn, select varieties based on that purpose; it also provides a simple and effective method to classify *S. cathayensis* germplasms.

KEYWORDS

Semiliquidambar cathayensis; leaf shape; photosynthetic pigment; photosynthetic property; active compound

1 Introduction

Semiliquidambar cathayensis belongs to the family Altingiaceae and is endemic to China. It is monoecious and pollinated mainly by wind. It has the following characteristics: straight trunk, attractive tree shape, and variable leaf shape. Its leaves are purplish red in early spring and green, yellow, and purplish red to dark red in late autumn. It is of high value for landscape development. Its roots, branches, and leaves are used as Traditional Chinese Medicine [1,2]. One active ingredient of the root of *S. cathayensis* is oleanolic acid [3], which has anti-hepatitis effect. Various active compounds in the leaves of *S. cathayensis* can be used to treat rheumatism, bruise, swelling and pain, and postpartum paralysis [4]. As harvesting the branches, roots, and nectar of *S. cathayensis* is difficult, people in China often boil the leaves of *S. cathayensis* to prepare a

medicinal solution for bathing, topical application, or ingestion to dispel wind, cold, and dampness, strengthen the body, and achieve reinforcement against the harsh environment in high mountain areas [5].

Mature *S. cathayensis* leaves are clustered at the top of branches and have traits that are a combination of traits of two genera *Liquidambar* and *Altingia*—as single leaves similar to those of *Altingia*, as palmately trifoliate leaves similar to those of *Acer buergerianum*, or asymmetrically lobed leaves known as "evolutionary heterophylly" [4]. For example, the long branches and leaves of young *Populus euphratica* resemble those of *Salix*, but the short branches and leaves of mature plants resemble those of *Populus*, which reflects the relationship between the genera *Populus* and *Salix* [6].

Owing to the harsh growth environment and scarcity of viable seeds, *S. cathayensis* is often a non-dominant species in an ecological community and is often in an unfavorable position when competing for light and thermal resources. Besides over-harvesting, the above factors have contributed to a major loss of the genetic resources of *S. cathayensis*, placing the plant on the verge of extinction. Currently, *S. cathayensis* populations remain only in the mountainous regions of Southern and Southeastern China [7] and thus, artificial breeding and cultivation are urgently required to preserve the species. Using SRAP (Sequence-related amplified polymorphism) marker technology, studies have found that *S. cathayensis* has high genetic diversity [8,9]; therefore, it is important to elucidate the differences among germplasms and screen elite germplasms. The identification and description of plant phenotypic traits are the most appropriate methods for research on germplasm resources. In this study, we analyzed the variation patterns of leaf shape in different populations of *S. cathayensis* and the relationship between leaf shape variation and photosynthetic properties and active compounds. Our results will serve as an important reference and scientific basis for understanding the genetic characteristics of *S. cathayensis* and for screening elite germplasms.

2 Materials and Methods

2.1 Materials

Seeds from individual adult *S. cathayensis* plants in each region were collected between October and November 2018 from naturally distributed populations in Jianghua and Jingzhou Counties, Hunan Province, and Dayu County, Jiangxi Province. In addition, the naturally distributed *S. cathayensis* populations in these three counties were identified as *S. cathayensis* Chang by botanical expert Yan Lihong from Hunan Botanical Garden Plant Conservation Research Institute through morphological identification.

The number of individual plants in each site from which the seeds were collected was determined by the size of the population in that area. The seeds of *S. cathayensis* were collected from 18 individuals and numbered as JH01-JH08 (Jianghua County), JZ01-JZ06 (Jingzhou County), and DY01-DY04 (Dayu County). Detailed geographic distribution data are shown in Suppl. Table S1. In January 2019, the collected seeds were sown in a planting trough at Hunan Botanical Garden nursery and in May, when most of the seedlings had more than four true leaves, 60 young offspring from each parent were randomly selected and transferred to 26 cm × 21 cm plastic nutrient cups with tillage soil as the substrate. The seedling cultures were weeded regularly, and pests and diseases were controlled throughout the experiment.

Ten offspring seedlings from a single JZ01 parent plant were randomly selected for leaf photosynthetic characteristic and active compound analyses. For the analyses, we collected three fully expanded leaves of each of the three leaf shapes from the top to bottom of the second round of healthy and mature branches from each seedling.

2.2 Methods

2.2.1 Semiliquidambar Cathayensis Leaf Shape Diversity

In May 2021, 30 offspring of each parent plant were randomly selected and the leaf shape of each offspring seedling was recorded.

2.2.2 Determination of Photosynthetic Pigment Content in Semiliquidambar Cathayensis Leaves

Photosynthetic pigment content in leaves of different shapes was determined as previously described [10] with slight modifications. Several *S. cathayensis* leaves of different shapes were collected and any dirt on the surface of the leaves was washed off. The midvein was removed and the remaining leaf tissue was cut to ensure that 0.25 g of each sample was obtained; this step was performed for three biological samples. Thereafter, the samples were added into 50 mL centrifuge tubes with 25 mL acetone and absolute ethanol mixture (V:V = 1:1) and placed away from light for 48 h to fully dissolve the pigment. The solution was then added to a quartz colorimeter and the absorbance was measured at 470, 663 and 645 nm using an ultraviolet spectrophotometer (Epoch 2; Biotek, Winooski, VT, USA). Eqs. (1)–(4) were used to calculate photosynthetic pigment content.

$$\text{Chla} = (12.72 \times \text{OD}_{663} - 2.59 \times \text{OD}_{645}) \times \text{V}/(1000 \times \text{W}) \quad (1)$$

$$\text{Chlb} = (22.88 \times \text{OD}_{645} - 4.67 \times \text{OD}_{663}) \times \text{V}/(1000 \times \text{W}) \quad (2)$$

$$\text{Chl (a + b)} = (20.29 \times \text{OD}_{645} + 8.04 \times \text{OD}_{663}) \times \text{V}/(1000 \times \text{W}) \quad (3)$$

$$\text{Car} = (\text{OD470} \times \text{V}/\text{W} - 2.05 \times \text{Chla} - 114.8 \times \text{Chlb})/245 \quad (4)$$

Chla is the content of chlorophyll a, Chlb is the content of chlorophyll b, Chl (a + b) is the content of total chlorophyll, Car is the content of carotenoid, V is the volume of extraction solution (mL), and W is the mass of each sample (g).

2.2.3 Measurement of Photosynthetic Parameters in Semiliquidambar Cathayensis Leaves

Gas exchange parameters were measured using a LI-6400 portable photosynthesis system (LI-COR, Lincoln, NE, USA). Photosynthetic rate–light intensity response curves were plotted as previously described [11]. The tested leaves were induced at a light intensity of 800 $\mu mol \cdot m^{-2}s^{-1}$ for 30 min before each measurement. Next, red/blue light was used to deliver photosynthetic photon flux density of different intensities and the CO_2 concentration was stabilized at 400 ± 0.5 $\mu mol \cdot mol^{-1}$ at a gas flow rate of 500 ± 0.5 $mmol \cdot s^{-1}$. The light response curves of different *S. cathayensis* leaf shapes were fitted with a right-angled hyperbolic correction model. The induction curves of the three replicates were averaged from data collected in triplicate between 08:00 and 10:00 h.

2.2.4 Determination of Active Compound Content in Semiliquidambar Cathayensis Leaves

The fresh leaves of *S. cathayensis* were collected, washed, and dried in shade. The dried samples were powdered and filtered through a 60 mesh sieve. The powder was stored in a sealed bag for subsequent tests.

Determination of Total Flavonoid Content

Total flavonoid content was determined using colorimetric assay according to the method described by Zhang et al. [12] with some modifications. The leaf powder (2 g) was extracted with 50% ethanol (25 mL) for 5 h at 25°C. Thereafter, ultrasonic assisted extraction was performed for 20 min. The extraction process was repeated twice. The extract was passed through filter paper. The filtrate was cooled for 20 min, and then dried using a rotary evaporator. The residue was suspended with methanol (50 mL) and passed through a 0.45-μM membrane (Millipore, USA) for filtration. The extract (5 mL) was added to a 10-mL flask, and then 5% $NaNO_2$ solution (0.3 mL) was added thereto. The samples were mixed well and placed at room temperature for 6 min, and 5% $Al(NO_3)_3$ solution (0.3 mL) was added to the flask. The samples were mixed well and placed at room temperature for 6 min, and 4% NaOH solution (4.4 mL) was added. The samples were mixed well and placed at room temperature for 12 min. Thereafter, the absorbance of the samples was read at 510 nm, and rutin was used as the standard for the calibration curve.

Determination of Total Polysaccharide Content

Polysaccharide content was determined using the phenol-sulfuric acid colorimetric method [13] with some modifications. The leaf powder (0.1 g) was extracted with distilled water (20 mL) at 100°C for 4 h. The aqueous extract was concentrated and then mixed with five volumes of cold absolute ethanol to precipitate the polysaccharides. The precipitate was cooled at 4°C for 12 h before being collected via centrifugation (4000 g, 25 min). The residue was then dried to obtain the desired polysaccharides. The polysaccharides were dissolved in water and diluted to 50 mL in a volumetric flask. The polysaccharide solution (1 mL) was mixed with 5% phenol (1 mL) and concentrated sulfuric acid (5 mL). The mixture was shaken and incubated at 100°C for 15 min. After cooling, absorbance of the samples was read at 490 nm, and D-glucose was used as the standard for the calibration curve.

Determination of Total Polyphenol Content

Total polyphenol content was determined using the Folin-Ciocalteu method [14] with some modifications. The leaf powder (10 g) was added into a 100-mL conical flask; 80% ethanol (50 mL) was into the flask, which was sealed for 3 days. The conical flask was placed in an ultrasonic cleaner for extraction three times. The combined filtrate was concentrated on a rotary evaporator (40°C), alcohol-free. The alcohol-free extract was transferred into a 100-mL volumetric flask, and the volume was made up with distilled water. Thereafter, 1 mL of the solution was transferred to a 10-mL volumetric flask, and the volume was made up with distilled water to prepare the sample solution. The sample solution (1.0 mL) was added into a 10-mL volumetric flask, to which distilled water (3 mL) and FC chromogenic agent (0.5 mL) were added. After shaking, 20% $NaCO_3$ (1.5 mL) was added within 8 min. The volume was made up with distilled water, and the flask was placed in a water bath at 75°C for 10 min. Absorbance of the sample was read at 760 nm, and gallic acid was used as the standard for a calibration curve.

Determination of Total Saponin Content

Total saponin content was determined using the colorimetric assay according to the method described by Xu et al. [15] with some modifications. The leaf powder (2 g) was placed in a Soxhlet extractor, diethyl ether (30 mL) was added to degrease until colorless, and then volatilized the diethyl ether. Thereafter, methanol (30 mL) was used for reflux extraction for 6 h. The methanol solution was recovered at a small amount; the volume of the sample in the volumetric flask was maintained at 10 mL with methanol. The supernatant was centrifuged as the sample solution. The sample solution (0.5 mL) was transferred to a tube with a stopper, the solvent was evaporated, and 50 $g{\cdot}L^{-1}$ vanillin glacial-acetic acid (0.4 mL) and perchloric acid (1.6 mL) were added. The sample was mixed well, heated in a 70°C water bath for 20 min, and cooled in an ice bath for 2 min, glacial acetic acid (5 mL) was added, and shaken well. Absorbance of the sample was read at 545 nm, and ginsenoside Re was used as the standard for the calibration curve.

Determination of Total Triterpenoidic Acid Content

Total triterpenoidic acid content was determined using the colorimetric assay according to the method described by Xia et al. [16] with some modifications. The leaf powder (10 g) was ultrasonically extracted with methanol (50 mL) for 40 min; the solution was cooled and weighed again. Thereafter, the mass loss was compensated with methanol to obtain the sample solution. The sample solution (0.3 mL) was evaporated methanol in a water bath, 5% vanillin glacial-acetic acid solution (0.3 mL) and perchloric acid (1.0 mL) were added, sealed, and mixed well. The sample was allowed to develop color in a 60°C water bath for 45 min and cooled in an ice bath; thereafter, glacial acetic acid was added to a constant volume of 5.0 mL. Absorbance of the sample was read at 550 nm, and ursolic acid was used as the standard for the calibration curve.

2.2.5 Statistical Analysis

MS Excel was used for the statistical analysis of leaf shape diversity. Differences in photosynthetic pigment content, photosynthetic properties, and active compound content were analyzed using the one-way ANOVA function in SPSS 26.0 software (IBM, USA) ($p < 0.05$). Principal component analysis of photosynthetic characteristics and active compound content of heteromorphic leaves was carried out using SPSS 26.0 software.

3 Results

3.1 Variation in Semiliquidambar Cathayensis Leaf Shape

The variation in the leaf shape of the offspring of 18 *S. cathayensis* individuals from three populations was statistically analyzed (Fig. 1, Table 1). The three leaf shapes accounted for different proportions in the offspring of the three populations; the offspring of the JH population was dominated by entire leaves at an average of 50.88%, whereas the proportion of entire leaves observed in the JZ and DY populations was 42.61% and 26.00%, respectively. The offspring of the DY population was dominated by palmately trifoliate leaves, with an average of 56.76%. The differences in leaf shape among the populations were statistically significant ($p < 0.05$), indicating that leaf shape of *S. cathayensis* had a high degree of dispersion at the species level and that geographic population differentiation was either extremely variable or had already occurred. The coefficient of variation (*CV*) represents the variability of phenotypic traits within the population, with a higher *CV* indicating greater dispersion of phenotypic traits. In the three populations, the CVs increased as follows: JZ < JH < DY for entire leaves, JZ < DY < JH for asymmetrically lobed leaves, and JZ < DY < JH palmately trifoliate leaves. The UPGMA (unweighted pair-group method with arithmetic means, UPGMA) clustering map (Fig. 2) showed that the 18 single offspring groups of the three natural *S. cathayensis* populations were divided into two clusters, among which four single offspring groups from the DY population (DY) were clustered in Cluster II and the other 14 single offspring groups were clustered in Cluster I. Cluster I was subdivided into two subgroups; most of the sample plants from the JH population were clustered in subgroup Ib, whereas JH01, JH05, JH08, and the JZ population were clustered in subgroup Ia.

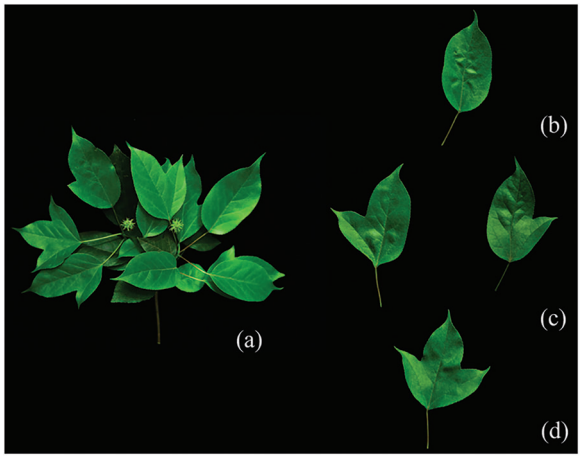

Figure 1: *Semiliquidambar cathayensis* leaf traits found in southern China. (a) The leaves of *S. cathayensis* clustered at the top of a branch. The (b) entire leaf; (c) asymmetrically lobed leaf; and (d) palmately trifoliate leaf traits observed within the tested populations

Table 1: Statistical analysis of *Semiliquidambar cathayensis* leaf shape diversity

No.	Proportion of entire leaves		Proportion of asymmetrically lobed leaves		Proportion of palmately trifoliate leaves	
	Mean ± SD/%	*CV*/%	Mean ± SD/%	*CV*/%	Mean ± SD/%	*CV*/%
JH01	54.96 ± 22.36	40.68	16.97 ± 8.29	48.88	28.07 ± 6.04	21.51
JH02	50.47 ± 15.24	30.20	18.96 ± 7.76	40.92	30.57 ± 8.90	29.11
JH03	47.80 ± 13.28	27.77	16.82 ± 7.95	47.24	35.38 ± 16.73	47.29
JH04	52.36 ± 29.24	55.84	15.42 ± 9.49	61.53	32.22 ± 26.08	80.95
JH05	56.98 ± 17.02	29.87	26.16 ± 4.62	17.66	16.86 ± 8.78	52.08
JH06	45.25 ± 22.13	48.91	17.12 ± 8.37	48.89	37.64 ± 26.84	71.31
JH07	50.68 ± 18.04	35.60	13.81 ± 5.62	40.70	35.51 ± 11.65	32.81
JH08	48.54 ± 16.19	33.35	13.74 ± 9.56	69.59	37.72 ± 17.38	46.07
JZ01	40.23 ± 17.68	43.95	22.31 ± 7.46	33.46	37.46 ± 10.65	28.44
JZ02	42.52 ± 15.81	37.18	26.52 ± 9.32	35.15	30.96 ± 10.25	33.10
JZ03	44.38 ± 16.31	36.75	20.37 ± 9.00	44.16	35.25 ± 10.21	28.96
JZ04	42.16 ± 14.92	35.39	26.25 ± 8.53	32.51	31.59 ± 10.19	32.26
JZ05	40.75 ± 16.44	40.34	18.63 ± 6.37	34.18	40.62 ± 10.17	25.03
JZ06	45.61 ± 14.79	32.43	20.50 ± 8.37	40.82	33.89 ± 10.04	29.63
DY01	25.37 ± 10.92	43.03	18.25 ± 4.94	27.06	56.38 ± 21.08	37.39
DY02	27.51 ± 12.70	46.18	15.08 ± 6.20	41.12	57.41 ± 23.58	41.08
DY03	25.45 ± 9.84	38.66	18.82 ± 7.26	38.56	55.73 ± 20.08	36.03
DY04	25.67 ± 6.94	27.04	16.81 ± 7.81	46.48	57.53 ± 22.31	38.78
JH (Mean ± SD/%)	**50.88 ± 19.19^{a}***	**37.71**	**17.38 ± 7.71^{c}***	**44.36**	**31.75 ± 15.30^{b}****	**48.19**
JZ (Mean ± SD/%)	**42.61 ± 15.99^{a}****	**37.53**	**22.43 ± 8.17^{b}***	**36.45**	**34.96 ± 10.25^{b}****	**29.32**
DY (Mean ± SD/%)	**26.00 ± 10.10^{b}***	**38.85**	**17.24 ± 6.55^{c}***	**38.01**	**56.76 ± 21.76^{a}***	**38.34**

Note: *CV* represents the coefficient of variation. Different lowercase letters within the same line indicate statistically significant differences ($p < 0.05$) in leaf shape among the different single offspring groups from the same population. Asterisks (* and **) indicate statistically significant differences ($p < 0.05$) in the same leaf shape in offspring among the three populations.

3.2 Analysis of Photosynthetic Pigment Content in the Leaves

The photosynthetic pigment content in *S. cathayensis* leaves differed significantly ($p < 0.05$) among the different leaf shapes. The Chla, Chlb, and carotenoid content in the entire leaves were significantly lower than those in the two other leaf shapes. When the palmately trifoliate leaves were used as the control group, the Chl (a+b) content in the entire leaves was only 71.44% of that in the palmately trifoliate leaves, and the carotenoid content was 77.57% that of the palmately trifoliate leaves (Table 2).

3.3 Analysis of Physiological Parameters of Photosynthesis in Semiliquidambar Cathayensis Leaves

A right-angled hyperbolic correction model was used to fit the light response curves of different *S. cathayensis* leaf shapes to a high degree (Fig. 3), with coefficients of determination (R^2) of 0.9993, 0.9986, and 0.9989 (entire leaves, asymmetrically lobed leaves and palmately trifoliate leaves). In general, the net photosynthetic rate of leaves of different shapes increased with increasing chlorophyll

content. When we compared the three fitted light response curves, there were significant differences ($p < 0.05$) in the apparent quantum yield (AQY), maximal net photosynthetic rate ($P_{n\text{-}max}$), light saturation point (LSP), and dark respiration rate (Rd); whereas, the difference in light compensation point (LCP) was not statistically significant ($p > 0.05$). The AQY, $P_{n\text{-}max}$, LSP, and Rd increased with increasing chlorophyll content in each leaf shape, and the LSP of entire leaves, asymmetrically lobed leaves, and palmately trifoliate leaves were 985.374, 1037.858, and 1245.467 μmol·m^{-2}s^{-1}, respectively. As the light intensity increased, photoinhibition was induced and the net photosynthetic rate decreased (Table 3).

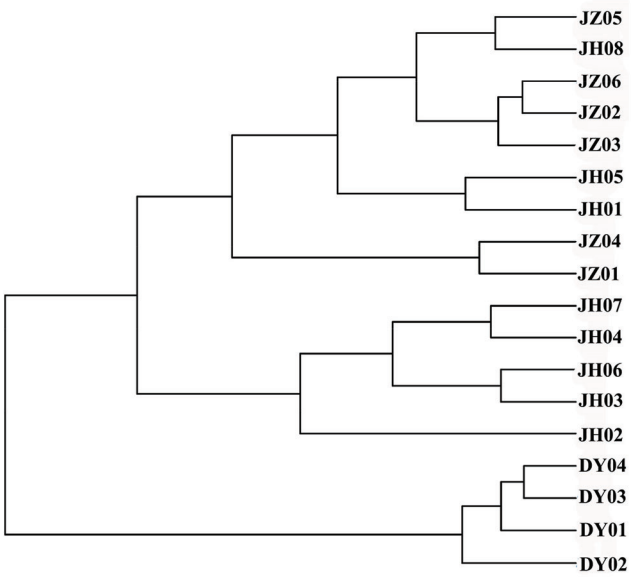

Figure 2: Clustering relationship of different individual *Semiliquidambar cathayensis* plants based on leaf shape traits

Table 2: Photosynthetic pigments in heteromorphic *Semiliquidambar cathayensis* leaves

Phenotypes	Chla/(mg·g^{-1} FW)	Chlb/(mg·g^{-1} FW)	Chl (a+b)/(mg·g^{-1} FW)	Car/(mg·g^{-1} FW)
Entire leaves	1.159 ± 0.003^{a}	0.354 ± 0.001^{a}	1.513 ± 0.004^{a}	0.249 ± 0.001^{a}
Asymmetrically lobed leaves	1.377 ± 0.004^{b}	0.442 ± 0.001^{a}	1.802 ± 0.003^{b}	0.291 ± 0.001^{b}
Palmately trifoliate leaves	1.596 ± 0.000^{c}	0.522 ± 0.002^{b}	2.118 ± 0.001^{c}	0.321 ± 0.002^{c}

Note: FW represents fresh weight. Different lowercase letters in the same column indicate statistically significant differences among different leaf shapes ($p < 0.05$).

3.4 Analysis of Active Compounds in Semiliquidambar Cathayensis Leaves

The content of active compounds, including total flavonoids, polysaccharides, polyphenol, triterpenoidic acids, and saponins, varied considerably among different *S. cathayensis* leaf shapes with some differences in the level of statistical significance (Table 4). The total flavonoid content did not differ significantly among the three leaf shapes, whereas the total polysaccharide, polyphenol, and saponin content in the entire leaves were significantly lower than those in the other two leaf shapes. The total polysaccharide content in the entire leaves was 77.05% of that in the asymmetrically lobed leaves, and 74.53% of that in the palmately trifoliate leaves. The total polyphenol content in the entire leaves was

67.79% of that in the asymmetrically lobed leaves, and 78.25% of that in the palmately trifoliate leaves. The palmately trifoliate leaves had the lowest total triterpenoidic acids content (13.538 $mg \cdot g^{-1}$), which was 67.42% and 68.23% of that in the entire leaves and the asymmetrically lobed leaves, respectively.

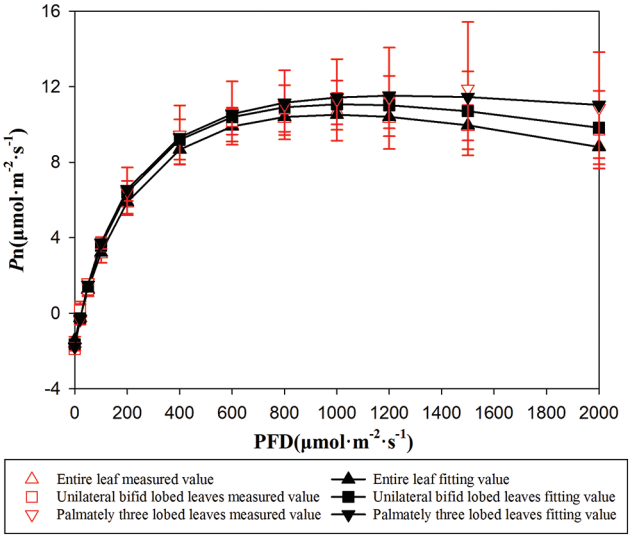

Figure 3: Light response of photosynthesis in heteromorphic *Semiliquidambar cathayensis* leaves

Table 3: Photosynthetic characteristics of heteromorphic leaves of *Semiliquidambar cathayensis*

Phenotype	AQY	$P_{n\text{-}max}$ /(μmol$CO_2 \cdot m^{-2}s^{-1}$)	Light saturation point /(μmol$\cdot m^{-2}s^{-1}$)	Light compensation point /(μmol$\cdot m^{-2}s^{-1}$)	Rd /(μmol$\cdot CO_2 m^{-2}s^{-1}$)	Adjusted factor (R^2)
Entire leaves	0.063 ± 0.004[a]	10.517 ± 0.909[a]	985.374 ± 63.441[a]	24.709 ± 7.566[a]	1.424 ± 0.383[a]	0.999 ± 0.001[a]
Asymmetrically lobed leaves	0.074 ± 0.004[b]	11.075 ± 1.372[b]	1037.858 ± 283.676[a]	24.549 ± 1.363[a]	1.651 ± 0.024[b]	0.999 ± 0.003[a]
Palmately trifoliate leaves	0.081 ± 0.001[c]	11.528 ± 2.576[b]	1245.467 ± 133.601[b]	24.510 ± 2.531[a]	1.774 ± 0.117[c]	0.999 ± 0.002[a]

Note: Different lowercase letters in the same column indicate statistically significant differences among different leaf shapes ($p < 0.05$).

3.5 Relationship among Photosynthetic Pigments, Photosynthetic Properties, and Active Compounds in Semiliquidambar Cathayensis Leaves

SPSS 26.0 software was used to conduct principal component analysis on the photosynthetic characteristics and active components of three different leaf shapes, and two principal component factors were extracted. The results are shown in Table 5. The contribution rate of the first principal component (Y1) was 74.158%, which mainly reflected the photosynthetic pigment content, photosynthetic efficiency, total triterpene acid, total polysaccharide, and total saponin content of leaves. The contribution rate of the second principal component (Y2) was 18.610%, which mainly reflected the content of total flavonoids and total polyphenol in the leaves. The cumulative contribution value of the first two principal

components was 92.768%, which indicates that the first two principal components extracted can represent the nine indexes including the photosynthetic characteristics and active ingredient characteristics of leaves. According to the eigenvalues and contribution rates of the two principal components, the scores of each principal component and the comprehensive scores are shown in Table 6. The high score of principal component Y1 for the three different leaf shapes indicates that it has the advantage of photosynthetic characteristics and the content of total triterpenoidic acids, total polysaccharides and saponins are high. The order of Y1 score was as follows: palmately trifoliate leaves > asymmetrically fallen leaves > entire leaves. The leaf shape with a high score of principal component Y2 indicates that the content of total flavonoids and total polyphenol was high. The order of Y2 score was as follows: asymmetrically lobbed leaves > palmately trifoliate leaves > entire leaves. The comprehensive score (y) was sorted as follows: asymmetrically lost leaves > palmately trifoliate leaves > entire leaves.

Table 4: Differences of the active compounds contents in the heteromorphic leaves of *Semiliquidambar cathayensis*

Phenotypes	Total flavonoids /(mg·g^{-1})	Total polysaccharides /(mg·g^{-1})	Total polyphenol /(mg·g^{-1})	Total triterpenoidic acids/(mg·g^{-1})	Total saponins /(mg·g^{-1})
Entire leaves	159.241 ± 4.402^{a}	0.595 ± 0.012^{a}	23.680 ± 0.155^{a}	20.079 ± 0.913^{a}	105.663 ± 4.658^{a}
Asymmetrically lobed leaves	162.261 ± 7.299^{b}	0.772 ± 0.126^{b}	34.931 ± 4.065^{b}	19.842 ± 0.521^{a}	117.588 ± 13.071^{ab}
Palmately trifoliate leaves	154.764 ± 6.820^{a}	0.798 ± 0.078^{c}	30.261 ± 0.314^{b}	13.538 ± 0.302^{b}	125.869 ± 0.357^{b}

Note: Different lowercase letters in the same column indicate statistically significant differences among different leaf shapes ($p < 0.05$).

Table 5: Component matrix

	Component	
	1	2
Chl (a+b)	0.978	−0.096
Carotenoids	0.992	0.024
AQY	0.949	−0.119
Pn-max	0.971	−0.002
Total flavonoids	−0.240	0.922
Total polysaccharides	0.933	−0.243
Total polyphenol	0.541	0.790
Total triterpenoidic acids	0.934	0.324
Total saponins	0.893	−0.109

4 Discussion

Analysis of the genetic diversity in leaf shape traits can be used to obtain a preliminary assessment of the potential characteristics of germplasm resources [17]. Leaf shape is a structural trait that can remain stable under relatively unstable environmental conditions [18]. In the present study, statistical analysis of leaf shape in the offspring of three natural *S. cathayensis* populations showed that the proportion of the three leaf shapes

varied among these populations, and the variation in leaf shape was maintained at a high level reflecting genetic diversity among and within the populations. In general, the phenotypic diversity observed in populations is related to the number of samples, as populations with a higher number of samples tend to have higher phenotypic diversity [19]. When we compared the sample size and diversity proportions of these *S. cathayensis* populations, we found that the JH population had the highest number of samples and a large CV for the three leaf shapes. Although the DY population had the smallest number of samples, the CV of the asymmetrically lobed and palmately trifoliate leaves was higher than that of the JZ population. In addition, the UPGMA cluster mapping showed that a single offspring of the DY population clustered only in Cluster II, whereas the offspring of the JH and JZ populations were clustered in Cluster I. This indicated that the DY population had relatively high phenotypic diversity and variation. Genetic diversity is the basis of the evolutionary potential of a species to adapt to changes in its external environment [20]. *S. cathayensis* is a natural hybrid between the genera *Populus* and *Salix* with a complex genetic background and high heterozygosity [21,22]. Altingiaceae is the basal taxon of the superrosids, and the high genetic diversity of leaf shape traits in *S. cathayensis* may be the result of the Altingiaceae phylogeny as an ancient tree species, or it may be related to *S. cathayensis* being a natural hybrid. The results of this study provide a scientific basis for the taxonomic conservation of different germplasm resources of *S. cathayensis*.

Table 6: Composite score of heteromorphic leaves of *Semiliquidambar cathayensis*

Phenotype	First principal component score (Y1)	Second principal component score (Y2)	Comprehensive score (y)
Entire leaves	5.500	73.007	19.043
Asymmetrically lobed leaves	6.887	82.143	21.987
Palmately trifoliate leaves	7.333	73.417	20.593

As a basic structural and functional unit, the leaf is the principal organ for photosynthesis and the energy converter for primary producers in an ecosystem [23]. The study of the photosynthetic properties of different *S. cathayensis* leaf shapes is essential for its introduction, cultivation, and processing. Photosynthetic pigments are the foundation of photosynthesis in *S. cathayensis* and are an important indicator of photosynthetic activity [24]. Higher chlorophyll content in plant leaves results in an improved ability of plants to capture light energy at low light intensities, and this facilitates photosynthesis and increases organic matter accumulation. In the light response curve model, the AQY indicates not only increased net photosynthetic rate at low light intensities, but also light energy utilization at low light intensities [25]. The palmately trifoliate leaves had a higher total chlorophyll and carotenoid content and the AQY values were significantly higher than those of entire and asymmetrically lobed leaves, indicating that the light use efficiency (LUE) of palmately trifoliate leaves was significantly higher than that of entire and asymmetrically lobed leaves at low light intensities. The LCP and LSP reflect the light condition requirements and degree of adaptation, whereas $P_{n\text{-}max}$ is a characterization of LUE and photosynthetic capacity in plants [26]. Here, the palmately trifoliate leaves of *S. cathayensis* had significantly higher AQY, $P_{n\text{-}max}$, LSP, and LCP than the other leaf shapes, although this difference was not significant. However, these results suggest that the palmately trifoliate leaves may have higher photosynthetic activity and increased light energy utilization that could improve light energy conversion efficiency and dry matter accumulation. The total chlorophyll and carotenoid content were the lowest and $P_{n\text{-}max}$, LSP, and LCP were significantly lower in entire leaves than in the palmately trifoliate leaves, but not in asymmetrically lobed leaves, indicating there was no significant difference in photosynthetic efficiency

between entire and asymmetrically lobed leaves. Hence, palmately trifoliate leaves are an indicator of higher photosynthetic efficiency in this species.

The quality of *S. cathayensis*, which is not included in Chinese Pharmacopoeia, has not yet been evaluated using uniform standards [27]. From current research on the active compounds in *S. cathayensis* roots and leaves, the known active compounds primarily include flavonoids, polysaccharides, alkaloids, saponins, triterpenoids, and sterols [28]; however, this has not been systematically evaluated. Yang et al. [29] identified 85 chemical constituents from the roots of *S. cathayensis*, including 35 alkaloids, 12 flavonoids, 7 terpenoids, 5 phenylpropanoids, 9 fatty acids, 7 cyclic peptides, and 10 other compounds. Tian et al. [30] identified 38 significantly different metabolites among *S. cathayensis* leaves, stems, and roots. These metabolites were differentially expressed in these tissues; the terpenoids were primarily triterpenoids and most of the flavonoids were significantly enriched in the leaves. In the present study, the analysis of active compounds in *S. cathayensis* leaves of different shapes revealed that the total polysaccharide, phenol, and saponin content in palmately trifoliate leaves were significantly higher than those in the other two leaf shapes, but the total triterpene content was lower than that in the entire leaves and asymmetrically lobed leaves. The utilization of light energy by plants reflects their ability to convert this energy into organic matter [31,32], and the active compounds that determine their use as medicinal herbs are derived directly or indirectly from photosynthesis during plant growth and development [33]. By comparing and analyzing the differences in photosynthetic characteristics and active components among the three leaf shapes of *S. cathayensis*, the principal component analysis was carried out on each characteristic index, and two principal components with a cumulative contribution rate of 92.768% were extracted to comprehensively evaluate the photosynthetic characteristics and active components of the three leaf shapes. The photosynthetic efficiency and active component content of asymmetrically lobed leaves were the highest, followed by those of palmately trifoliate leaves and entire leaves.

5 Conclusions

In this study, we found that the leaf shape of different *S. cathayensis* germplasm resources exhibited some correlation between photosynthetic properties and active compounds. The total proportion of asymmetrically lobed leaves and palmately trifoliate leaves in the DY population was higher than that of the other two populations, suggesting the total active compound content in *S. cathayensis* leaves was higher in the DY population than in the JZ and JH populations. In actual production, we can predict active compound content based on *S. cathayensis* leaf shape to select and breed the required varieties based on medicinal purposes; thus, the use of leaf shape provides a simple and effective method for classifying *S. cathayensis* germplasms and for screening elite germplasms.

Supplementary Materials: Table S1: Geographic distribution information of three natural populations of *Semiliquidambar cathayensis*.

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Table S1: Geographic distribution information of three natural populations of *Semiliquidambar cathayensis*

Location	No.	Longitude	Latitude	Altitude/m
Jianghua County, Hunan Province	JH01	111°54′26″	24°53′15″	849
	JH02	111°56′33″	24°54′5″	747
	JH03	111°56′34″	24°54′5″	744
	JH04	111°49′29″	24°53′3″	509
	JH05	111°50′35″	24°51′36″	625
	JH06	111°50′35″	24°48′43″	564
	JH07	112°1′2″	25°0′34″	878
	JH08	112°1′1″	25°0′36″	747
Jingzhou County, Hunan Province	JZ01	109°23′36″	26°41′54″	418
	JZ02	109°31′22″	26°34′10″	392
	JZ03	109°30′25″	26°32′42″	421
	JZ04	109°47′6″	26°35′36″	524
	JZ05	109°33′48″	26°42′5″	354
	JZ06	109°34′30″	26°29′21″	477
Dayu County, Jiangxi Province	DY01	114°26′05″	25°33′29″	730
	DY02	114°26′05″	25°33′29″	730
	DY03	114°26′05″	25°32′18″	710
	DY04	114°26′05″	25°32′18″	710