

Original Research Paper

# Analysis of Photosynthetic Pigment Content and Leaf Structure of *Pteroceltis Tatarinowii* Mutant with Yellow Leaf

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## Article history

Received: 16-06-2022

Revised: 12-08-2022

Accepted: 26-08-2022

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**Abstract:** The relationship between leaf variation characteristics and leaf structure characteristics of *Pteroceltis tatarinowii* with yellow leaf was explored to provide a theoretical basis for the breeding of other ornamental plants. In this study, the differences in pigment content, leaf structure, and chloroplast ultrastructure in leaves of *Pteroceltis tatarinowii* mutant with yellow leaf 'Huiguang' and its original species 'Lingyansi No. 1' were studied. The results showed that the chlorophyll in 'Huiguang' leaves decreased significantly, and the carotenoids-total chlorophyll ratio increased greatly. The palisade tissue cells in 'Huiguang' leaves are short, thick, and loosely structured, while the sponge tissue cells are irregular in shape and loosely arranged. In addition, the chloroplasts of 'Huiguang' leaves were irregular in shape and the grana lamellae were loosely arranged, broken, and missing, with more osmiophilic granules and fewer starch granules. These anomalies in leaf pigment content and chloroplast structure affect leaf photosynthesis and chlorophyll accumulation, thereby affecting leaf color changes.

**Keywords:** Yellow Leaf Mutant, Pigment Content, Chloroplast, Ultrastructure

## Introduction

Leaf color variation is a common trait of plants. Nowadays, more and more color-leaf plants have been cultivated, such as *Ligustrum lucidum*, *Lagerstroemia indica* L., and *Wisteria sinensis* (Sims), which have greatly enriched the resources of color-leaf plants for horticulture and greening (Davis and Burns, 2016). The color of plant leaves is the comprehensive embodiment of various pigments and is the result of the interaction of internal and external factors. There are 3 types of pigments that affect leaf color in higher plants: Chlorophylls, carotenoids, and anthocyanins. The ratio of these pigments and the selective absorption of light results in different leaf colors.

Plant leaf color mutants come from a variety of sources, including spontaneous mutation, artificially induced mutation, insertion mutation, and gene silencing mutation. Artificially induced mutation mainly obtains leaf color mutants by physical mutagenesis and chemical mutagenesis. Compared with other mutation methods, artificially induced mutation can obtain a mass of mutants in a short time and is widely used in plant breeding. Physical mutagenesis can be carried out by x-ray irradiation,  $\gamma$ -ray irradiation, and space

environment. Chemical mutagenesis can be carried out using alkylating agents, nucleic acid base analogs, and other mutagens. Yan *et al.* (2019) used  $\gamma$ -ray to radiate the seeds of *Rosa beggeriana* to obtain the *Rosa beggeriana* mutant with golden leaves (Yan *et al.*, 2019). Xu *et al.* (2021) used the callus of *Greenable Rice* Xiushui 11 as a material and obtained a low temperature-sensitive yellowing mutant rice X611 with  $^{60}\text{Co}$ - $\gamma$  ray treatment. Yan *et al.* (2019) treated tomato seeds with EMS and obtained mutant plants with yellow leaves during the seed breeding process. Cheng *et al.* (2019) used EMS to mutagenize pepper (*Capsicum annuum* L.) seeds and screened out the light green leaf mutants of pepper with ornamental value during the seed breeding process.

*Pteroceltis tatarinowii* Maxim., also known as Pteroceltis, is a deciduous tree from Pteroceltis and Ulmaceae and is a national third-class key protected plant. It is a unique fiber tree species in China and an important indicator plant of calcareous soil. As an important native and green tree species in China, in landscape construction, the combination of *Pteroceltis tatarinowii* with arbor, shrubs, and grasses cannot only beautify the environment but also improve the urban microclimate and maintain the balance of the urban ecosystem. In recent years, the research on

*Pteroceltis tatarinowii* mainly focuses on stress resistance, community composition and genetic diversity, seed dormancy physiology, bark quality and biomass, and propagation technology, while less research has been done on germplasm resource innovation of *Pteroceltis tatarinowii*. In this study, the mature leaves of artificially mutated *Pteroceltis tatarinowii* yellow leaf mutant 'Huiguang' and its original species 'Lingyansi No. 1' were used as materials to compare the content of relevant pigments, microstructure, and ultrastructure of the leaves, to explore the relationship between leaf variation characteristics and leaf structure characteristics of *Pteroceltis tatarinowii*, and provide a theoretical basis for the breeding of other ornamental plants.

## Materials and Methods

### Experimental Materials

*Pteroceltis tatarinowii* yellow leaf mutant 'Huiguang' and its original species 'Lingyansi No. 1' were all taken from the germplasm resources nursery of Taishan Research Institute of Forestry Science in Tai'an (Fig. 1). In May 2021, the 3-year-old plants were used as the mother plant, and 3 plants were randomly selected, each of which was taken as a duplicate. The mature leaves in the middle of 1-year-old branches were taken as the experimental materials at a height of 1.2 m in the south.

### Determination of Leaf Pigment Content in Leaves

1 g of leaves was taken, washed with deionized water, dried with filter paper, and ground with liquid nitrogen. After 20 mL of 80% acetone was added, the sample was extracted in the dark until the precipitate turned white to centrifuge for 10 min. The supernatant was fixed with 80% acetone, and the absorbance at 663, 645, and 470 nm was measured. The concentrations of Chl a, Chl b, and Chl T were calculated according to the following formula ( $\text{mg}\cdot\text{L}^{-1}$ ), and the photosynthetic pigment content of each sample was calculated according to the constant volume and fresh weight of the samples ( $\text{mg}/\text{g FW}^{-1}$ ).

$$C_a(\text{mg}\cdot\text{L}^{-1}) = 12.70\text{OD}_{663} - 2.69\text{OD}_{645}$$

$$C_b(\text{mg}\cdot\text{L}^{-1}) = 22.88\text{OD}_{645} - 4.67\text{OD}_{663}$$

$$C_T(\text{mg}\cdot\text{L}^{-1}) = 20.29\text{OD}_{645} + 8.05\text{OD}_{663}$$

$$C_{\text{car}}(\text{mg}\cdot\text{L}^{-1}) = (1000\text{OD}_{470} - 3.27C_a - 104C_b)/229$$

### Observation of Mesophyll Tissue Structure

Using the paraffin section method, 5 green leaves, and 5 yellow leaves were cut into square pieces about  $0.5 \times 0.5$  cm, and fixed with a standard fixation solution (V formalin: V acetic acid: V 50% alcohol = 5: 5: 90) for 24 h. After dehydration, wax dipping, embedding, and staining, the sections (thickness 8-10  $\mu\text{m}$ ) were conducted by automatic microtome (LEICA RM2235, Germany), dried and dewaxed, and observed with a Nikon optical microscope (CI-L, Japan).

### Ultrastructural Observation of Chloroplasts

The leaves were cut into 2 mm<sup>2</sup> tissue blocks to put into 2.5% glutaraldehyde, vacuumized to make the samples sink and fixed for 3 h, then rinsed 3 times with 0.1 mol·L<sup>-1</sup> phosphate buffer solution and fixed in 1% osmium acid for 2 h. After rinsing with phosphate buffer solution 3 times, gradients of 30, 50, 70, 80, 90, and 100% acetone were used for dehydration. After embedding with epoxy resin SPURR, the samples were polymerized and trimmed. Slicing was performed using a LEICAUC6 ultramicrotome. The sections were stained with uranium dioxide acetate and lead citrate, and observed and photographed under a JEM-1230 transmission electron microscope. Image J software was used to quantitatively analyze the morphology and structure of chloroplasts in *Pteroceltis tatarinowii* leaves. 30 cells were selected for cell observation, and 30 chloroplasts were selected for chloroplast observation. The number of chloroplasts per unit area and the number of a single cell in leaves, the long diameter, short diameter and ratio of the long axis, the short axis of chloroplast, the number of granules, the osmiophilic granules and starch granules in chloroplasts, the proportion of granule lamellae to the total lamellae number and the thickness of granule lamellae were counted.

### Statistical Analyses

The analysis of variance and significance test was performed by SPSS 19.0 software, and the chart was drawn by sigmaplot10.

## Results and Discussion

### Changes in Photosynthetic Pigment Content in Leaves

There were significant differences in photosynthetic pigment content between *Pteroceltistatarinowii* 'Huiguang' and 'Lingyansi No. 1' (Table 1). It could be seen that the contents of chlorophyll a, chlorophyll b, and total chlorophyll of 'Huiguang' leaves were significantly lower than those of 'Lingyansi No. 1'. The total chlorophyll content of 'Huiguang' was 0.30 mg·g<sup>-1</sup> FW, which was about 49.2% of that of 'Lingyansi No. 1'. The content of carotenoid was 0.17 mg·g<sup>-1</sup> FW, which was about 141.7% of 'Lingyansi No. 1'. The ratio of chlorophyll a to chlorophyll b of 'Huiguang' leaves was significantly lower than that of 'Lingyansi 1', while the ratio of carotenoid to total chlorophyll was significantly higher than that of 'Lingyansi 1'. In terms of pigment composition, chlorophyll content of 'Huiguang' leaves decreased significantly; the carotenoid content increased, and the carotenoid/total chlorophyll ratio increased significantly, which was consistent with the results in corn, soybean (Kong *et al.*, 2017) and millet (Junxia *et al.*, 2021). The ratio of chlorophyll a to chlorophyll b of 'Huiguang' leaves is significantly lower than that of 'Lingyansi No. 1'. It is generally believed that low

chlorophyll a/b is an indicator of shade adaption Xu *et al.* (2021), which shows that 'Huiguang' is more conducive to absorbing limited light energy in the environment under insufficient light. The ratio of carotenoids to total chlorophyll in 'Huiguang' is significantly higher than that in 'Lingyansi No. 1'. This may be due to the decreased photochemical quenching ability caused by the acidification of chloroplast lamellae structure in a high CO<sub>2</sub> environment, which requires supplementation to ensure the normal photochemical quenching function (Khan *et al.*, 2019).

### Comparison of Anatomical Structure Characteristics of Leaves

As shown in Fig. 2, the upper and lower epidermal cells of *Pteroceltis tatarinowii* leaves are closely arranged without intercellular space. The shapes of the upper epidermal cells are regular, and that of the lower epidermal cells are irregular; the leaves are uneven and corrugated. Palisade cells in leaves are slender, perpendicular to the leaf surface, and neatly arranged. The palisade cells of 'Huiguang' leaves are short and thick with a loose structure. The palisade cells located below the upper epidermis are particularly evident, and the palisade tissue consists of 2-3 layers of cells. The palisade cells of 'Lingyansi No. 1' leaves are slender and closely arranged with 3-4 layers of palisade cells. The sponge cells were perpendicular to the leaf surface and arranged neatly, while the sponge cells were irregular and loosely arranged, which is consistent with the results in maize (Lunde *et al.*, 2003), *Arabidopsis thaliana* (Runge *et al.*, 1995), *Oryza sativa* (Murchie *et al.*, 2005) and *avena sterilis* (Aliferis *et al.*, 2006).

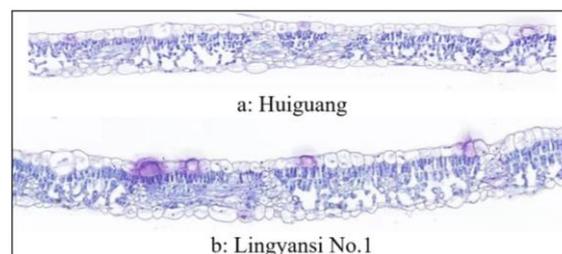
From the perspective of leaf thickness, the thickness of the upper and lower epidermis of 'Huiguang' was significantly lower than that of 'Lingyansi No. 1', which was 13.2 and 9.43μm, respectively. The thickness of palisade tissue and sponge tissue of 'Huiguang' was significantly higher than that of 'Lingyansi 1', which were 2.23 and 18.33μm, respectively. The total leaf thickness of 'Huiguang' was larger than that of 'Lingyansi No. 1' without significant difference (Table 2).

### Distribution and Morphological Differences of Chloroplasts

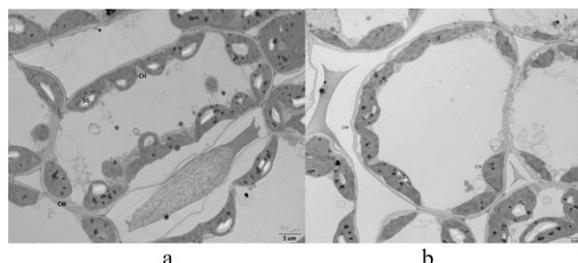
Chloroplasts are the site of photosynthesis in plants, and abnormal development of chloroplasts often affects leaf color. The structural characteristics of chloroplasts are closely related to their functions. The differentiation of stacked and unstacked chloroplast thylakoid membranes promotes the high concentration of light trapping mechanism in chloroplasts and improves the conversion efficiency of light energy from pigment to the reaction center. Figure 3 shows that the chloroplasts of *Pteroceltis tatarinowii* leaves are all arranged in the cell membrane of mesophyll cells, and the shape and number are different.



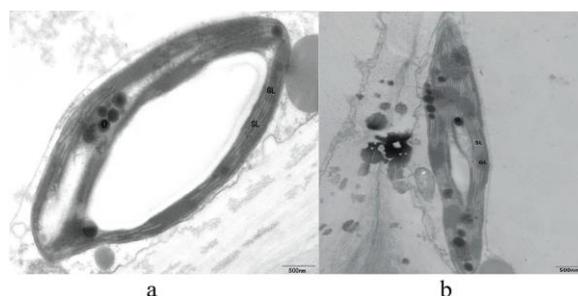
**Fig.1:** Experimental varieties of *Pteroceltis tatarinowii*. Note: 'Lingyansi No. 1' is the original variety of *Pteroceltis tatarinowii*. 'Huiguang' is a new variety of yellow leaves obtained by chemical mutagenesis with 'Lingyansi No. 1' as the experimental material



**Fig. 2:** The anatomical structure of leaves



**Fig. 3:** The ultrastructure of leaf cell of *Pteroceltis tatarinowii* Maxim. Note: The ruler is 5μm. (3a) is 'Lingyansi No. 1' cell; (3b) is 'Huiguang' cell. CH: Chloroplast; SG: Starch Grain; CW: Cell Wall; G: Grana



**Fig. 4:** Ultrastructure of the chloroplasts of *Pteroceltis tatarinowii* Maxim. Note: The ruler is 500 nm; (a) is 'Lingyansi No. 1' chloroplast; (b) is 'huiguang' chloroplast. GL: Grana Lamella; SL: Stroma Lamella; O: Osmiophilic granules

**Table 1:** Comparison of photosynthetic pigment content and composition

Sample	Content of chl a (mg.g <sup>-1</sup> FW)	Content of chl b (mg.g <sup>-1</sup> FW)	Content of total chl (mg.g <sup>-1</sup> FW)	Content of car (mg.g <sup>-1</sup> FW)	Chl a/chl b	Carotenoid/total chl
Lingyansi No. 1	0.42±0.0006a	0.19±0.0021a	0.61±0.0026a	0.12±0.0011b	2.21±0.0066a	0.19±0.0049b
Huiguang	0.19±0.0002b	0.12±0.0003b	0.30±0.0002b	0.17±0.0002a	1.58±0.0072b	0.57±0.0015a

Note: Different small letters in the same column indicate a significant difference at the 5% level

**Table 2:** Comparison of leaf anatomical structures between green and yellow leaves of *Pteroceltistatarinowii*

Sample	Upper epidermis thickness (µm)	Palisade tissue (µm)	Spongy tissue (µm)	Palisade tissue/ (µm)	Lower epidermis Spongy tissue	Blade (µm)
Lingyansi No.1	21.78±0.62a	32.39±1.02b	30.33±0.94b	1.07±0.05a	16.45±0.26a	99.72±1.43a
Hui guang	8.58±1.16b	44.62±3.71a	48.66±3.21a	0.95±0.14a	7.02±0.74b	105.58±3.09a

Note: Different small letters in the same column mean a significant difference at the 5% level.

**Table 3:** The distribution density and morphological parameters of the chloroplasts of *Pteroceltis tatarinowii* Maxim (n = 30)

Materials	The number of chloroplast/mm <sup>2</sup>	The number of chloroplast/cell	Long diameter/ µm	Short diameter/ µm	Long diameter/ Short diameter
Lingyansi No. 1	32514.26±6335.15a	9.05±3.21a	4.99±1.33a	3.02±0.74a	1.65±0.66b
Huiguang	21253.42±279.41b	4.02±1.44b	4.02±1.12b	1.93±0.85a	2.08±1.81a

Note: Different small letters in the same column indicate a significant difference at the 5% level

**Table 4:** Organelle parameters of the chloroplast substructures in *Pteroceltistatarinowii* Maxim's leaves

Sub-cellular structure parameters	Huiguang	Lingyansi No. 1
Number of granules (one/chloroplast)	5.240±1.21a	5.660±4.31a
Number of grana layer (layer/Grana)	9.020±1.47b	15.480±9.66a
Grana lamella thickness (nm)	19.020±2.37b	22.590±1.44a
The proportion of starch granule size of chloroplast (%)	10.240±7.12b	32.650±6.74a
Osmiophilic granules (one/chloroplast)	16.910±2.56a	7.420±3.33b
Grana lamella proportion of the total number of lamellae	0.591±0.47a	0.402±0.23b

Note: Different small letters in the same column indicate a significant difference at 5% level

The chloroplasts of 'Huiguang' leaves are mostly oblong or spindle-shaped, with a few chloroplasts arranged loosely (Fig. 4b). The chloroplasts in 'Lingyansi No. 1' leaves are nearly round or oval, with a large number and orderly arrangement (Fig. 4a). There are many starch granules in the chloroplasts in 'Lingyansi No. 1' leaves, and its photosynthetic ability is optimal. There are few starch grains in the chloroplasts of 'Huiguang' leaves, indicating that their photosynthetic capacity is low. In addition, the chloroplast color of 'Huiguang' leaves was lighter after staining, indicating a relatively low concentration of chloroplast stroma.

Table 3 shows that the chloroplasts in 'Huiguang' leaves are significantly less than that of 'Lingyansi No. 1'. The chloroplasts of 'Lingyansi No. 1' leaves are 32514.26±6335.15/mm<sup>2</sup>, and that of 'Huiguang' is 21253.42±279.41/mm<sup>2</sup>. The chloroplasts per unit area of 'Lingyansi NO. 1' are about 1.5 times that of 'Huiguang'. Taking the number of chloroplasts in a single cell as a statistical index, the number of chloroplasts of 'Lingyansi No. 1' in leaf cells was 9.05±3.21, and that of 'Huiguang' was 4.02±1.44. The number of chloroplasts of 'Lingyansi No. 1' in each cell was about twice as much as that of 'Huiguang'. The long diameter and short diameter of the chloroplasts of 'Lingyansi No. 1' were larger than those of 'Huiguang' chloroplasts, but the ratio of long diameter to the short diameter of the chloroplasts of 'Huiguang' was larger than that of 'Lingyansi No. 1' chloroplasts.

### Subcellular Structure of Chloroplast in Leaves

It can be seen from Fig. 4a that the chloroplast volume of 'Lingyansi No. 1' leaves is larger than that of 'Huiguang', and the chloroplast of 'Huiguang' is slender than that of 'Lingyansi No. 1'. The chloroplast center of 'Lingyansi No. 1' has large starch granules, which are abundant and aggregated. The chloroplast of 'Huiguang' has smaller starch granules, more scattered granules, and more osmiophilic granules.

It can be seen from Fig. 4b that the chloroplast volume of 'Lingyansi No. 1' leaves is larger than that of 'Huiguang', and the chloroplast of 'Huiguang' is slender than that of 'Lingyansi No. 1'. There are larger starch granules in the chloroplast center of 'Lingyansi No. 1', which are abundant and aggregated. The chloroplast of 'Huiguang' has smaller starch granules, more scattered granules, and more osmiophilic granules (the specific structural parameters are shown in Table 4). This is consistent with the results in *Lagerstroemia speciosa* golden leaf mutant 'Jinwei' (Zhang *et al.*, 2014), golden leaf golden bellflower (Sun *et al.*, 2020), and golden leaf forsythia (Sun *et al.*, 2020).

### Conclusion

It is considered that the mutation of yellow leaves in plants is usually caused by chlorophyll deficiency, which is also affected by carotenoids and anthocyanins. In this study,

a new chlorophyll-deficient *Pteroceltis tatarinowii* mutant 'Huiguang' was identified, whose leaves are yellow throughout the growing season. From the microstructure of leaves, the palisade cells of 'Huiguang' leaves are short and thick. The structure is loose, especially a layer of palisade cells under the upper epidermis, which consists of 2-3 layers of cells. The palisade cells of 'Lingyansi No. 1' leaves are slender and closely arranged and composed of 3-4 layers of palisade cells, which are perpendicular to the leaf surface and arranged neatly, while the sponge cells are irregular and arranged loosely.

In this experiment, it was found that the chloroplast structure of 'Huiguang' was different from that of common varieties. The chloroplast of 'Huiguang' is irregular with a basically visible structure, and the grana lamellae are loosely arranged, broken, and missing. There are more osmiophilic granules, fewer starch granules, and light staining in the chloroplast of 'Huiguang'. Fewer starch granules indicate decreased photosynthetic capacity, light staining indicates a lower concentration of cytoplasmic matrix, and more osmiophilic granules indicate destruction of the chloroplast membrane system. These anomalies in chloroplast structure affect the photosynthesis and chlorophyll accumulation of leaves, thereby affecting the variation of leaf colors. It was found that the ratio of grana lamellae to total lamellae of 'Huiguang' chloroplasts was significantly higher than that of 'Lingyansi No. 1', while PSII and LHCII were mainly located on the stacked grana lamellae. The structural differences between them led to the distribution ratio of higher PSII excitation energy of 'Huiguang' chloroplast than that of 'Lingyansi No. 1'. Therefore, it can be speculated that under low light intensity, the chloroplast of 'Huiguang' pays more attention to the light response. It can provide O<sub>2</sub> and carbohydrates for plants in stressful environments, especially under insufficient light.

In this study, we identified the structural differences between *Pteroceltis tatarinowii* and its mutants with yellow leaves. However, due to the low rate of character variation, it is far from enough to identify the functional differences of *Pteroceltis tatarinowii* genes. Therefore, it is of great significance to further study the function of leaf color variation genes to establish a mutant library through a large number of chemical mutagenesis and obtain more mutant materials. In the future, we will use mutants and their wild types as materials to study their physiological, biochemical, and genetic patterns, to further clarify gene functions, and tap useful genetic resources.

## Acknowledgment

This study was supported by "Innovation Driving and Leading Promotion of Seedling and Flower Industry in Mount Tai" of the industrial upgrading project of Science and Technology Park in Shandong Province (2019 YQ 012).

## Author's Contributions

**Yongchang Yu and Chen Lv:** Designed and performed the experiments, and work.

**Min Liu:** Participated to collect the materials related to the experiment.

**Tiantian Cheng:** Designed the experiments and revised the manuscript.

## Ethics

The authors declare their responsibility for any ethical issues that may arise after the publication of this manuscript.

## Conflict of Interest

The authors declare that they have no competing interests. The corresponding author affirms that all of the authors have read and approved the manuscript.

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