

Microscopic Structural Comparison between Epidermal Trichomes in *Blumea balsamifera* (L.) DC. and *Blumea laciniata* (Roxb.) DC

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Abstract

Immature leaves and stems, which were about one to three centimeters nearby the stem tips, of *Blumea balsamifera* (L.) DC. (BB) and *Blumea laciniata* (Roxb.) DC. (BL) were cut into sections under -18°C with a frozen section machine, and observed under an optical microscope. Results show that the most significant difference is that the BB has only one kind of glandular trichomes, while the BL has two. The glandular trichomes found on BB terms were all short glandular hairs (SGH), which were not longer than $100\ \mu\text{m}$. On BL stems, besides the SGH, the long glandular hairs (LGH), which were longer than $200\ \mu\text{m}$, were also found. By the factors pointed out in present study, the BB and BL and be distinguished from each other.

Keywords

Blumea balsamifera, *Blumea laciniata*, Sambong, Ainaxiang, Glandular Hair

1. Introduction

Blumea balsamifera (L.) DC. (BB) [1] and *Blumea laciniata* (Roxb.) DC. (BL) [2] are two medicinal plants belong to Blumea family, distribute in tropical and subtropical zones in Asia, including South China, and produce tiny seeds. They look similar, especially in their seedling stage, making them easily confused. Their seeds are small and also seem alike. The former is the only source of a frequently used traditional Chinese medicine named Aipian, and was of high economic value [3] [4]. The later, were less demanded in the mordent pharmaceutical market. Having mistakenly planted the

wrong seedlings, folks and plantations usually experienced enormous loss. Frozen section technique is a valuable tool used to rapidly prepare slides from tissue for microscopic interpretation [5]. It has been successfully practiced on observation of the morphological development of BB leaves [6]. As a result, micro-examination is worth trying to add more information to help people tell them apart.

2. Materials and Methods

2.1. Materials

BB and BL plants were both collected from outskirts Danzhou (Hainan, China), authenticated by Mr. Ying-Bo Zhang and Dr. Xiao-Lu Chen. Voucher specimens have been deposited at the Traditional Medicinal Plant Germplasm Nursery of South China, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences, Hainan, China.

2.2. Methods

- Frozen section

Fresh and healthy immature leaves and stems about one to three centimeters nearby the stem tips were cut from the BB and BL plants. They were directly cut into 4×4 mm or 5 mm long without fixed. Each pieces of tissues were embedding on a sample table with Jung tissue freezing medium (Leica, German) under -18°C for 10 min, and then cut with Leica CM1900 under -18°C . The thickness ranges from 10 μm to 30 μm . The sections were observed with a computer-equipped microscope.

- Data collection and analysis

Lengths were measuring with a computer equipped microscope with an electronic scale. Statistical analysis of the data was carried out by using the SPSS 19.0 software. The results were expressed as mean \pm SD (standard deviation). Data were analyzed by one-way ANOVA followed by Duncan's multiple range tests or the independent sample *t*-test using Statistical Package for Social Sciences 19.0 (SPSS, Chicago, IL, USA). A *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Types of Epidermal Trichomes

Both BB and BL are covered by epidermal trichomes, including non-glandular and glandular hairs. Results showed in **Table 1** indicated that two types of trichomes were growing on the BB plant, while three were on BL plant.

3.2. Length of Non-Glandular Hairs

According to **Table 2** and **Table 3**, the lengths of non-glandular hairs on BB were usually longer than 500 μm , which were much shorter on BL plants, typically ranges from 100 μm to 500 μm . The lengths of non-glandular hairs in BB and BL were significantly different by the independent sample *t*-test at $P < 0.05$ level.

Table 1. Types of epidermal trichomes.

<i>Blumea balsamifera</i> (L.) DC. (BB)	<i>Blumea laciniata</i> (Roxb.) DC. (BL)
non-glandular hairs	non-glandular hairs
glandular capitate trichome (GCT)	GCT
-	glandular peltate trichome (GPT)

Table 2. Measurement of non-glandular hair Lengths (μm).

BB (n = 12)	BL (n = 12)
1023.11	453.43
1354.67	363.29
1099.87	375.21
789.55	311.27
689.14	102.15
1209.88	290.79
1006.22	132.69
952.11	215.97
890.76	201.3
1355.01	260.12
957.44	196.78
977.23	171.56

Table 3. Length of non-glandular hairs (μm , $n = 12$).

BB	BL
1025.42 \pm 203.64a*	263.91 \pm 107.36b

*Values are expressed as mean \pm SD. Data followed by different letters are significantly different by the independent sample *t*-test at $P < 0.05$ level.

3.3. Morphological Observation of Non-Glandular Hairs

The non-glandular hairs on BB leaf were lender (**Figure 1(A)**) and those on BL leaf were shorter and thicker (**Figure 1(B)** and **Figure 1(C)**). Both of them were composed of several cells arranged in a line. Though the “bell-like” shape was not obvious on BL leaf under stereomicroscope (**Figure 1(B)**), it can be recognized by performing Frozen section (**Figure 1(C)**).

3.4. Length of Glandular Hairs

The length of glandular hairs on BB and BL plants were found to be significantly different. The BB plants grow only SGH with a length of about 56 μm , while the BL plants grow two kinds of glandular hairs, with length of approximately 92 μm , and 257 μm , respectively (**Table 4** and **Table 5**).

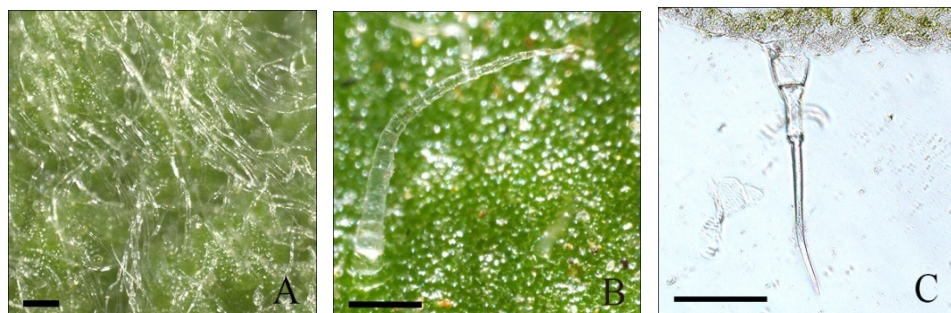


Figure 1. Morphological observation of non-glandular hairs. (A) Non-glandular hairs on BB leaf; (B) Non-glandular hairs on BL leaf; (C) A non-glandular hair on BL leaf.

Table 4. Measurement of glandular hairs (μm , $n = 8$).

BB (GCT)	BL (GCT)	BL (GPT)
56.08	74.77	207.97
47.76	69.02	262.11
43.58	90.69	210.99
56.42	102.28	297.07
56.09	80.14	277.65
48.99	73.68	281.56
60.89	99.63	290.22
78.11	142.00	225.68

Table 5. Length of glandular hairs (μm , $n = 8$).

BB (GCT)	BL (GCT)	BL (GPT)
$55.99 \pm 10.58c^*$	91.53 ± 23.80	256.66 ± 36.38

*Values are expressed as mean \pm SD. Data in the same column followed by different letters are significantly different by Duncan's test at $P < 0.05$ level.

3.5. Morphological Observation of Glandular Hairs

On BB plants, a short glandular hair is comprised of a head with two columns of secretory cells, a short stalk with some smaller cells, and one or two basal cell(s) (**Figure 2(A)**). In another word, it is a glandular capitate trichome. On BL plants, a short glandular hair is similar with the one on BB plant, except the stalk cells are larger (**Figure 2(B)**). The long glandular trichome on BL plant is a glandular peltate trichome (**Figure 2(C)**), which did not found on BB plant. It consists of a head with about four to twenty secretory cells (**Figure 2(D)**), a long stalk with two to eight stalk cells, and one or two basal cell(s).

4. Conclusion

Frozen section has been widely performed nowadays in animal and human researches [7], but not so frequently on plants [8]. The main reason may be the cytoderm were

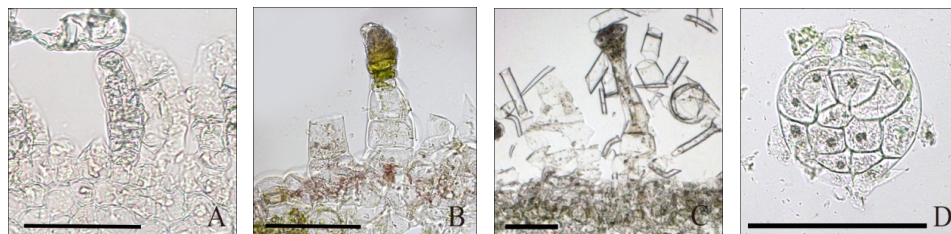


Figure 2. Morphological observations of glandular hairs. (A) A glandular capitate trichome (GCT) on BB stem; (B) A glandular capitate trichome (GCT) on BL stem; (C) A glandular peltate trichome (GPT) on BL stem; (D) A head of a glandular peltate trichome (GPT). Bar = 100 μm .

easily damaged in frozen section. However, this technology can be performed easily and quickly. Present study confirmed the discrimination of the plant of BB and BL is viable by performing frozen section and microscopic observation. These two seem similar plants, though may confused by farmer, could be easily tell apart through the methods and the factors reported in the research. The results showed may help people better discriminate the *Blumea* plants with high economic value and those do not.

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Conflicts of Interest

The authors declare no conflict of interest.

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