Leaf epidermal micromorphology of *Cercis* (Fabaceae: Caesalpinioideae)

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The micromorphology of leaf epidermal cells and stomata of all eight species and one form (11 samples) of *Cercis* were observed by scanning electron microscopy and light microscopy. Both the adaxial and abaxial epidermal cells are polygonal or irregular in shape; the anticlinal walls are straight and arched or undulate. Two types of stomata, which occur only on the abaxial surface of the leaves, are found in the genus. The atypical paracytic type is present in only one species, *Cercis chingii*, and the anomocytic type is present in all other species. Interspecific differences are minor in the genus with regard to leaf epidermal characters, except for *C. chingii*, which is characterized by atypical paracytic-type stomata, a two-lipped outer stomatal rim, the highest stomatal density and undulate and densely pitted anticlinal walls in the adaxial epidermis. © 2008 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2008, **158**, 539–547.


INTRODUCTION

*Cercis* L., with its chromosome number of $x = 7$, is the only genus that has retained the postulated original basic chromosome number in Fabaceae (Polhill, Raven & Stirton, 1981), and has been regarded as having a phylogenetic position of significant antiquity. *Cercis* species are trees or shrubs, with entire or emarginate leaves and cauliflorous flowers in short fasciculate racemes; they are distributed in the warm temperate regions of the Northern Hemisphere (Wunderlin, Larsen & Larsen, 1987). Eight species are currently recognized (Table 1), including five species in China: *C. chinensis*, *C. chingii*, *C. chuniana*, *C. glabra* and *C. racemosa*, and (Wei, 1988). *C. canadensis* and *C. occidentalis* are found in North America and *C.siliquastrum* is found in Europe (Zhang, 1999).

*Cercis* can be distinguished easily from other genera in Caesalpinioideae by its pseudopapillicose flowers, but differences between species of *Cercis* are minor in terms of both morphological and molecular aspects (Hao et al., 2001). However, there have been only limited morphological studies on *Cercis*: leaf venation (Zhang, 1994) and seed coat patterns (Zhang, 1999) are the only aspects that have been investigated. Leaf epidermal characters, especially stomatal types, have long been regarded to be of systematic and evolutionary significance (for example, Heslop-Harrison, 1970; Barthlott, 1981). In this article, the morphology of leaf epidermal cells and the stomata of eight species and one form (11 samples) of *Cercis* were studied as part of an investigation of the evolution and systematics of this genus, exploiting more characters that can be used to identify fossils of this confusing group of plants, which has been widely recorded in the Northern Hemisphere from the Miocene onwards (Owens, Fields & Ewers, 1998).

MATERIAL AND METHODS

All leaf samples were obtained from specimens deposited at the herbarium of South China Botanical Garden (IBSC). A list of investigated materials is given in Table 1. Samples were taken from fully expanded, sun-exposed leaves of about $0.5 \times 0.5$ cm$^2$. The materials for light microscopy were boiled in

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water for about 10 min, followed by maceration in Jeffery's solution (Zheng, 1978). The materials were kept at a temperature of 30–40 °C. After maceration, the adaxial and abaxial epidermal tissue was separated from the leaf tissue; the epidermal tissue was washed with distilled water and stained in a solution of 1% methyl green before mounting in glycerine gel. To check the consistency of the epidermal structure under the light microscope (Olympus Vanox), at least five slides were prepared from different parts of a single leaf or from different leaves of each species; more than ten slides, with materials obtained from different locations, were prepared for C. chingii. Stomata of different types on each slide were counted, and the ratio of the types was calculated. Materials for scanning electron microscopy observation were mounted directly on the stubs without treatment. After gold sputtering using a JEOL JFC-1100 apparatus, the specimens were examined and imaged using a JEOL JSM-T300.

Table 1. Origin of material

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Voucher</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. canadensis L.</td>
<td>University of Richmond, USA</td>
<td>W. J. Hayden 481 (IBSC)</td>
</tr>
<tr>
<td>C. chinensis Bunge</td>
<td>Tai Mountain, Shandong</td>
<td>Forestry Department of Shandong Agriculture College 56-6/76 (IBSC)</td>
</tr>
<tr>
<td>C. chinensis Bunge forma pubescens C. F. Wei</td>
<td>Without precise locality, Anhui</td>
<td>Anhui Exped. 1379 (IBSC)</td>
</tr>
<tr>
<td>C. chingii Chun</td>
<td>Ruyuan, Guangdong</td>
<td>X. Q. Liu 29088 (IBSC)</td>
</tr>
<tr>
<td>C. chinensis Chun</td>
<td>Nanjing, Jiangsu</td>
<td>Y. F. Deng 13291 (IBSC)</td>
</tr>
<tr>
<td>C. chinensis Chun</td>
<td>Hangzhou, Zhejiang</td>
<td>Q. G. Zhu &amp; Q. W. Liu 10 (IBSC)</td>
</tr>
<tr>
<td>C. chiniana F.P. Metcalf</td>
<td>Lechang, Guangdong</td>
<td>X. Y. Wen et al. 13401 (IBSC)</td>
</tr>
<tr>
<td>C. glabra Pamp.</td>
<td>Yiyang, Henan</td>
<td>Survey Exped. 6332 (IBSC)</td>
</tr>
<tr>
<td>C. occidentalis Turr. ex A. Gray</td>
<td>California, USA</td>
<td>B. Bartholomew s.n. (IBSC)</td>
</tr>
<tr>
<td>C. racemosa Oliver</td>
<td>Bijie, Guizhou</td>
<td>Y. Tsiang 8989 (IBSC)</td>
</tr>
<tr>
<td>C. siliquastrum L.</td>
<td>Paris, France</td>
<td>C. M. Hu 9057 (IBSC)</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of the leaf epidermis of Cercis under light microscopy (surface view)

<table>
<thead>
<tr>
<th>Species</th>
<th>Adaxial epidermis</th>
<th>Abaxial epidermis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Shape of cells</td>
<td>Pattern of</td>
</tr>
<tr>
<td></td>
<td></td>
<td>anticlinal walls</td>
</tr>
<tr>
<td>C. canadensis</td>
<td>Polygonal</td>
<td>Straight arched</td>
</tr>
<tr>
<td>C. chinensis</td>
<td>Polygonal</td>
<td>Straight arched</td>
</tr>
<tr>
<td>C. chinensis forma pubescens</td>
<td>Polygonal</td>
<td>Straight arched</td>
</tr>
<tr>
<td>C. chingii</td>
<td>Irregular</td>
<td>Undulate</td>
</tr>
<tr>
<td>C. chiniana</td>
<td>Polygonal</td>
<td>Straight arched</td>
</tr>
<tr>
<td>C. glabra</td>
<td>Polygonal</td>
<td>Straight arched</td>
</tr>
<tr>
<td>C. occidentalis</td>
<td>Polygonal</td>
<td>Straight arched</td>
</tr>
<tr>
<td>C. racemosa</td>
<td>Polygonal</td>
<td>Straight arched</td>
</tr>
<tr>
<td>C. siliquastrum</td>
<td>Polygonal</td>
<td>Straight arched</td>
</tr>
</tbody>
</table>

The classification of the genus by Wei (1988) was adopted, which includes five species and two forms in China: C. chuniana, C. racemosa, C. glabra, C. chinensis, C. chingii, C. chinensis forma alba and C. chinensis forma pubescens. Stomatal terminology was based on the classification proposed by Dilcher (1974), and the terminology for other characters was based on the classification of Wilkinson (1979). All statistical analyses were performed using SPSS11.5 and Microsoft Excel 2002. A confidence level of 0.05 was considered to be significant.

RESULTS

Leaf epidermal characteristics of Cercis are listed in Tables 2 and 3. The anticlinal walls of the epidermis in Cercis are straight arched (Fig. 1A–C, E–I), except in C. chingii, which has undulate and densely pitted anticlinal walls (Fig. 1D). Stomata are found only on the abaxial epidermis (Fig. 2A–K),
and trichomes are found only in C. chinensis forma pubescens (Fig. 2J) and C. racemosa (Fig. 2K). The stomatal density in Cercis leaves is variable, ranging from 861 mm⁻² in C. chingii to 255 mm⁻² in C. siliquastrum.

It appears that stomatal and other epidermal features are constant within species, and thus may be used to elucidate the systematic and phylogenetic relationships between species in the genus.

CHARACTERS OF THE ADAXIAL EPIDERMIS

No stomata were found on the adaxial surface. Under light microscopy, the anticlinal walls of the epidermal cells can be seen to be thickened. According to the shape of the epidermal cells and the patterns of the anticlinal walls, two types of leaf can be distinguished (Table 2).

1. Type I: epidermal cells are polygonal with straight arched anticlinal walls. The cell wall is thickened in C. canadensis (Fig. 1A) and shows single pit thickening in C. chinensis (Fig. 1B); C. chuniana shows variable thickening with a moniliform cell wall (Fig. 2E).

2. Type II: epidermal cells are irregular with undulate anticlinal walls. Only C. chingii belongs to this type (Fig. 1D).

When examined using scanning electron microscopy, the cuticular membrane of the adaxial epidermis (Table 3) under low-power magnification (or focus) is seen to be insular (Figs 3A, G, 4E, I), granular (Figs 3E, M, 4B) or flocculent (Fig. 3K); under high-power magnification, the wax ornamentations of the adaxial epidermis are seen to be acanthoid (Fig. 3D) or finely granular (Fig. 4C).

CHARACTERS OF THE ABAAXIAL EPIDERMIS

The stomatal apparatus (Wilkinson, 1979), or stomatal complex (Dilcher, 1974), occurs only on the abaxial epidermis of Cercis leaves. As shown by light microscopy, the stomata are evenly distributed, and the cell walls of guard cells are variably thickened (cf. Fig. 2B, C, I). In the majority of cases, the stomata are anomocytic; however, they can be divided into two types.

1. Type I (anomocytic stomatal apparatus): the guard cells are surrounded by cells that do not differ in terms of size and shape from other epidermal cells. This type is common in this genus (Fig. 2A–C, E–I), except for C. chingii (Fig. 2D).

In order to be certain of the type of stomatal apparatus in C. chingii, further experiments were carried out by light microscopy. This species is endemic to China. Therefore, herbarium specimens...
were chosen from Guangdong, Jiangsu and Zhejiang provinces. More than ten slides were observed for different leaves from these three locations. As shown in Table 4, the results indicate that the stomatal types from the different localities are not significantly different ($P > 0.05$, Table 4), and the atypical paracytic stomatal type is present at a high percentage (more than 74%). Therefore, the main stomatal apparatus type in *C. chingii* is the atypical paracytic type.

2. Type II (atypical paracytic stomatal apparatus): the guard cells are accompanied by two cells that are similar to the other epidermal cells but are parallel
to the guard cells and aperture. Only *C. chingii* belongs to this type (Fig. 2D).

The outer surface of the cuticle membrane is insular (Fig. 3H, N) or ridged (Fig. 3B, L) in all species (Table 3), and the wax ornamentation is granular (Fig. 4C), scaly (Fig. 3D) or with upright scales (Figs 3I, 4D) in the genus. The positions of the guard cells are raised or level with the epidermis, except for *C. chingii*, where they are somewhat sunken (Fig. 3I).

Figure 2. Characteristics of the abaxial epidermal cells (light microscopy) in *Cercis*: A, *C. canadensis*; B, *C. chinensis*; the arrow shows the guard cell; C, *C. chinensis* forma *pubescens*; D, *C. chingii*; the arrow shows the atypical paracytic-type stomatal apparatus; E, *C. chuniana*; F, *C. glabra*; G, *C. occidentalis*; H, *C. racemosa*; I, *C. siliquastrum*; J, *C. chinensis* forma *pubescens*; the arrow shows the trichome; K, *C. racemosa*; the arrows show the trichomes. Scale bar: D, 20 μm; A–C, E–K, 50 μm.

Figure 3. Characteristics of the epidermal surface (scanning electron microscopy) in Cercis: A–C, C. canadensis: A, the cuticle is insular (arrow), adaxial (Ad); B, the cuticle is ridged (arrow), abaxial (Ab); C, the stomata are level with the epidermis (Ab); D, C. chinensis, acanthoid wax (arrow) (Ad); E, F, C. chinensis forma pubescens: E, the cuticle is granular with many trichomes (arrow) (Ad); F, the stomata are raised above the epidermis and the rim is smooth (Ab); G–I, C. chingii: G, the cuticle is insular (Ad); H, upright scale wax (Ab); the arrow shows the stomata; I, the stomata are somewhat sunken into the epidermis (Ab); the arrow shows the upright scale wax; J–L, C. chuniana: J, the stomata are level with the epidermis (Ab); K, flocculent-granular wax (arrow) (Ad); L, the cuticle is ridged (Ab); the arrow shows the stomata; M, N, C. glabra: M, the cuticle is granular (Ad); N, the cuticle is insular (Ab); the arrow shows the stomata. Scale bar: C, D, 1 μm; A, B, E–N, 10 μm.
Figure 4. Characteristics of the epidermal surface (scanning electron microscopy) in *Cercis*: A, B, *C. glabra*: A, the stomata are raised above the epidermis, abaxial (Ab); B, the cuticle is granular at higher magnification, adaxial (Ad); C, D, *C. occidentalis*: C, the cuticle is granular with upright scale wax (Ad); D, the stomata are level with the epidermis (Ab); the arrow shows upright scales; E, F, H, *C. racemosa*: E, the cuticle is insular with granular wax (Ad); F, the cuticle is insular (arrow) with the stomata level with Ab; H, the trichome on Ab at lower magnification; G, *C. chinensis* forma *pubescens*: the trichome on Ab at lower magnification; I–K, *C. siliquastrum*: I, the cuticle is granular with upright scale wax (Ad); J, the cuticle is insular (arrow) with the stomata level with Ab; K, the stomata are level with Ab. Scale bar: D, 1 μm; A–C, E–K, 10 μm.
Table 4. Comparative results of stomatal apparatus type in Cercis chingii

<table>
<thead>
<tr>
<th></th>
<th>Guangdong province</th>
<th>Jiangsu province</th>
<th>Zhejiang province</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical paracytic (%)</td>
<td>76.0 ± 7.1a (10)</td>
<td>80.0 ± 8.5a (10)</td>
<td>74.0 ± 6.4a (10)</td>
</tr>
<tr>
<td>Anomocytic (%)</td>
<td>23.0 ± 6.4b (10)</td>
<td>20.0 ± 8.5b (10)</td>
<td>26.0 ± 7.2b (8)</td>
</tr>
</tbody>
</table>

Values are means (± standard deviation) of eight or ten replicates. The same letter (a or b) in the same row from different locations indicates no significant difference at P ≤ 0.05. Location difference comparisons were determined using analysis of variance (ANOVA) (least significant difference, LSD).

Cuticular thickening of the common wall between the guard cells, known as T pieces, is absent in all species, and the stomatal rims are generally one-lipped (Figs 3C, F, J, 4A, D, K), except for those of C. chingii which are two-lipped (Fig. 4I). Trichomes occur only in C. chinensis forma pubescens and C. racemosa (Fig. 4E, G, H).

DISCUSSION

Although earlier studies have indicated that subsidiary cells are generally absent in Cercis leaves (Metcalfe & Chalk, 1950), suggesting that the genus Cercis has anomocytic-type stomata, our investigation has revealed that this is not the case for C. chingii. The two cells around the guard cell are not obviously differentiated from the other epidermal cells in this species, and the morphology of the stomatal apparatus is very similar to the paracytic type. Based on the statistical analysis in Table 4, we suggest that the main stomatal apparatus type in C. chingii is the atypical paracytic type rather than the anomocytic type.

Thus, the epidermis of Cercis leaves can be divided into two types: almost all species belong to Type I, except for C. chingii, which is classified as Type II in the present study. Species with Type I leaves are all very similar in terms of the stomatal apparatus, anticlinal walls and cuticular wax ornamentations on the leaf epidermis (Tables 2 and 3). However, C. chingii is different from the other species in the genus, with undulate and densely pitted anticlinal walls in the adaxial epidermis, atypical paracytic-type stomata, high stomatal density and a two-lipped outer stomatal rim. The conclusion that C. chingii is unique in the genus with regard to leaf epidermal morphology is in accordance with Zhang’s extrapolations from studies on seed micromorphology (Zhang, 1999) and leaf venation patterns (Zhang, 1994). The unwinged and more or less woody pods in this species also make it easily distinguishable from other species in the genus. A phylogenetic analysis based on nuclear ribosomal ITS and plastid ndhF sequence data (Davis et al., 2002) has also indicated that C. chingii is sister to the rest of Cercis. On the basis of these characters, we suggest that the phylogenetic position of C. chingii should be reconsidered. Possibly, it should be regarded as the sole species of its own subgenus. Further studies are required to clarify the relationship between C. chingii and the other species in the genus.

Owens et al. (1998) reported that numerous species in over 45 extant genera have leaves that resemble those of Cercis. Five genera have species that closely resemble Cercis: Menispermum, Cocculus (Menispermaeae), Cercidiphyllum (Cercidiphyllaceae), Disanthus (Hamamelidaceae) and Bauhinia (Fabaceae). Leaf epidermal characters have also been shown to be useful for the identification of fossil remains of angiosperms and in the investigation of relationships between extant taxa (Baranova, 1972; Ferguson, 1974; Stace, 1984; Upchurch, 1984). By comparing the epidermal characters in these genera, the other taxa can be differentiated from Cercis. Ferguson (1974) and Hong et al. (2001) found that Cocculus had irregular epidermal cells with sinuous anticlinal walls and a staurocytic stomatal apparatus, and Menispermum had irregular epidermal cells with sinuous anticlinal walls but an anomocytic stomatal apparatus (Hong et al., 2001). Thus, the leaves in these two genera can be distinguished from those of Cercis on the basis of their different leaf epidermal micromorphology. Although the leaves of Disanthus (Hamamelidaceae) more closely resemble those of Cercis than do those of the genera above (Owens et al., 1998), they can also be distinguished from Cercis by the irregular epidermal cells with sinuous anticlinal walls and a paracytic stomatal apparatus (Pan, Lu & Wen, 1990). Bauhinia is a large (c. 300 species) and diverse pantropical genus (Wunderlin et al., 1987). We have found that this genus has multiform epidermal cells and stomatal apparatuses (Zou, 2006), but the main stomatal apparatus type is paracytic, as also reported by Shah & Gopal (1971). Epidermal characteristics can also be used to distinguish Bauhinia from Cercis.

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REFERENCES


