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Leaf epidermal characteristics of Asiatic *Buddleja* L. under scanning electron microscope: Insights into chromosomal and taxonomic significance

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ABSTRACT

The leaf epidermis of all 23 species from Asian *Buddleja* was investigated with scanning electron microscopy (SEM). Characteristics of trichome type, stomatal shape, cuticular membrane on adaxial and abaxial epidermis, inner margin of outer stomatal ledge and ornamentation of outer stomatal ledge are presented in this study. Among the *Buddleja* species, some distinct epidermal patterns were observed. These can be used to characterize and identify different sections among the Asian *Buddleja* species, viz. *Alternifoliae*, *Curviflorae* and *Rectiflorae*. In general, stomatal length of *Buddleja* increases with ploidy level. This character can be used to distinguish diploids from polyploids without preparation of caryograms. Based on this correlation *Buddleja microstachya*, *B. subcapitata* and *B. bhutanica* are characterized to be diploids, as concluded from their stomatal dimensions. Leaf epidermis characters can also be used to differentiate species pairs that are easily confused with each other, viz., *B. asiatica* and *B. bhutanica*, *B. albiflora* and *B. nivea*, *B. candida* and *B. fallowiana*, *B. delavayi* and *B. forrestii*, *B. japonica* and *B. lindleyana*, *B. officinalis* and *B. paniculata*.

1. Introduction

Buddleja is a cosmopolitan genus of Buddlejaceae consisting of about 100 species in the tropical and temperate zones of the world (Leeuwenberg, 1979; Li and Leeuwenberg, 1996). Though taxonomic treatments of this genus have been published since the early 1840s, there still are taxonomic problems about the genus (Leeuwenberg, 1979; Li and Leeuwenberg, 1996; Norman, 2000), especially in the case of some confused Asian species.

Leaf epidermal characters are known to be of great use not only in identifying the fossil remains of angiosperms but also in studying relationships between extant taxa (Baranova, 1987, 1992; Stace, 1984; Kong, 2001; Shi and Li, 2003; Yang and Lin, 2005; Ren et al., 2007; Zoric et al., 2009). The leaf epidermal characters of only a few *Buddleja* species have been studied to identify their taxonomic significance in past decades (Norman, 2000). In this paper, we checked all species in the Asian region with scanning electron microscopy, try to determine taxonomic relationships, and contribute to distinguish some confused Asian species of *Buddleja*.

Our recent work (Chen et al., 2007) reported the different ploidy levels ($2n = 2x$, $4x$, $6x$, $8x$, $12x$, $16x$, $24x$) of *Buddleja* in the Sino-Himalayan region, with a basic chromosome number of $x = 19$. The ploidy level of all the 23 Asian species of *Buddleja* will be known, if this paper's work is included. Compared with some diploids taxa, these polyploids have some distinct morphological characters. Maybe the dosage effect of DNA or C-values (amount of DNA in the unreplicated gametic nucleus) impacts epigenetic characters of polyploids (such as: stomatal length, flower, seed or leaf size, etc.)? Eventually, these characters can really represent the systematic relationships of Asian *Buddleja* species.

In this study, leaf epidermal characters of all the 23 Asian *Buddleja* species were surveyed to: (1) present a comprehensive comparison of taxonomic treatment of complex Asian taxa with different ploidy levels, (2) explore the dosage effect of DNA and speculate on the ploidy level of the last three species in Asian region that are not yet analyzed with respect to their chromosome numbers.

2. Materials and methods

Details of plant material used for the present study are given in Table 1.

Samples of *Buddleja* were taken from cultivated material or from herbarium specimen (Leaf selection was based on leaf position and age. All sampled leaves were homotypic and mature, and from the base of the previous year's branches). They represent 23 species of the genus in Asia. Materials for scanning electron microscope

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Table 1
Species used in the study: name with provenance locality, ploidy level and voucher information.

Taxon	Ploidy level	Locality	Voucher
B. albiflora Hemsl.	6*x*	Henan	K. Q. Fu 114 (KUN)
B. alternifolia Maxim.	2*x*	Lhasa, Tibet	G. Chen 070802 (KUN)
B. asiatica Lour.	2*x*	Wenshan, Yunnan	G. Chen 015 (KUN)
B. bhutanica T. Yamaz.	?	Eastern Himalaya	Yanazaki 14858 (KUN)
B. brachystachya Diels	2*x*	Deqing, Yunnan	G. Chen 073 (KUN)
B. candida Dunn	4*x*	Bomi, Tibet	G. Chen 070817 (KUN)
B. colvilei Hook.f.	8*x*	Gongshan, Nujiang	B. X. Sun 20946 (KUN)
B. crispa Benth.	2*x*	Lhasa, Tibet	G. Chen 070818 (KUN)
B. curviflora Hook. & Arn.	2*x*	Taiwan	W. B. Sun 010 (KUN)
B. davidii Franch.	4*x*	Zhaotong, Yunnan	W. B. Sun 019 (KUN)
B. delavayi L.F.Gagnepain	6*x*	Bingchuan, Yunnan	W. B. Sun 070505 (KUN)
B. fallowiana L.B.Balf.	4*x*	Dali, Yunnan	G. Chen 059 (KUN)
B. forrestii Diels	6*x*	Dali, Yunnan	G. Chen 047 (KUN)
B. japonica Hemsl.	2*x*	Japan	W. B. Sun 017 (KUN)
B. lindleyana Fortune	2*x*	Hubei	G. Chen 053 (KUN)
B. macrostachya Benth.	6*x*	Dali, Yunnan	G. Chen 044 (KUN)
B. macrostachya Benth.	12*x*	Dali, Yunnan	G. Chen 045 (KUN)
B. microstachya E.D.Liu	?	Wuliangshan, Yunan	H. Peng 0859423 (KUN)
B. myriantha Kraenzl.	2*x*	Dali, Yunnan	W. B. Sun 033 (KUN)
B. nivea Duthie	6*x*	Muping, Sichuan	B. X. Sun 39238 (KUN)
B. officinalis Maxim.	2*x*	Kunming, Yunnan	G. Chen 012 (KUN)
B. paniculata Wall.	2*x*	Baoshan, Yunnan	G. Chen 031 (KUN)
B. subcapitata E.D. Liu	?	Yanbian, Sichuan	H. Peng 5153 (KUN)
B. yunnanensis L.F.Gagnepain	2*x*	Simao, Yunnan	W. B. Sun 028 (KUN)

was attached to stubs with some scrape to wipe off some thick trichome cover. After gold sputtering, the specimens were examined and photographed under a Hitachi S-520. To check the constancy of epidermal structure, at least ten fields (416 μm × 312 μm) were investigated from a single leaf and at least three individual leaves of each species were used as repetitions in the analysis. Stomatal length (at random in surface view) was counted according to at least fifty measurements per sample, both on the abaxial or adaxial surface, respectively. Stomatal terminology was based on the classification proposed by Baranova (1972) and the terminology for other characters was based on the classification of Wilkinson (1979). Because of the density of the indumentum and crumpled and distorted thinner leaves it was difficult to study the micro-characteristics of all the species under light microscopy. Therefore, the stomatal index and cell density of these taxa were not calculated in this study.

3. Results

Leaf epidermal micromorphological characters under scanning electron microscope (SEM) are summarized in Table 2 and Figs. 1–81.

Table 2
Leaf epidermal characteristics of *Buddleja* based on scanning electron microscopy (SEM) observations.

Taxon	Adaxial epidermis	Both surfaces	Abaxial epidermis					Figure
	Cuticular membrane	Trichomes Abbrev. see below	Cuticular membrane	Stomatal shape	Inner margin of outer stomatal ledge	Ornamentation of outer stomatal ledge	Stomatal length (μm)	
B. albiflora	Smooth	Can	Ridge	Elliptic	Smooth	Smooth	25.9	1–3
B. alternifolia	Striate	Wide Ste[a]	Smooth	Elliptic	Smooth	Smooth	16.1 (Ad) 18.1 (Ab)	4–7
B. asiatica	Thick striate	Can	Thick striate	Elliptic	Smooth	Smooth	18.6	8–10
B. bhutanica	Thick striate	Absent	Thick striate	Elliptic	Smooth	Smooth	15.7	11–14
B. brachystachya	Smooth	Can and Gla	Striate	Elliptic	Smooth	Smooth	16.6	15–18
B. candida	Striate and wax grains		Ridge	Wide elliptic	Smooth	Striate	23.7	19–21
B. colvilei	Ridge	Absent	Striate	Elliptic	Smooth	Striate	25.0	22–24
B. crispa	Smooth	Can and Gla	Striate	Elliptic	Smooth	Smooth	14.9	25–28
B. curviflora	Ridge	Can	Striate	Wide elliptic	Smooth	Smooth	17.4	29–31
B. davidii	Ridge	Can	Striate	Elliptic	Smooth	Smooth	21.7	32–34
B. delavayi	Ridge	Gla	Ridge	Elliptic	Smooth	Smooth	27.9	35–37
B. fallowiana	Striate	Can	Striate	Elliptic	Smooth	Smooth	28.2	38–40
B. forrestii	Thin ridge	Can	Striate	Elliptic	Smooth	Striate	28.9	41–43
B. japonica	Ridge	Gla	Ridge	Wide elliptic	Smooth	Smooth	16.4	44–46
B. lindleyana	Nearly smooth	Can and Gla[a]	Striate	Elliptic	Smooth	Smooth	16.1	47–50
B. macrostachya	Ridge	Can and Gla	Ridge	Elliptic	Smooth	Smooth	24.3	51–54
B. macrostachya	Ridge	Can and Gla	Ridge	Elliptic	Smooth	Smooth	34.7	55–58
B. microstachya	Striate	Can	Striate	Elliptic	Smooth	Smooth	16.9	59–61
B. myriantha	Nearly smooth	Can and Gla	Striate	Wide elliptic	Smooth	Smooth	16.5	62–65
B. nivea	Thin striate	Can	Striate	Elliptic	Smooth	Smooth	25.3	66–68
B. officinalis	Ridge	Can	Ridge	Elliptic	Smooth	Smooth	16.9	69–71
B. paniculata	Ridge and wax grains	Can and Gla	Ridge	Elliptic	Smooth	Smooth	16.6	72–75
B. subcapitata	Smooth	Can	Ridge	Elliptic	Smooth	Smooth	16.4	76–78
B. yunnanensis	Ridge	Can and Gla	Ridge	Elliptic	Smooth	Smooth	17.9	79–81

Can – candelabra hair, Gla – glandular hair, Ste – stellate hair.
[a] Species with special six-armed hair.