Characteristics of the stomatal apparatus and anatomical structure of the leaf of *Cephalotaxus sinensis* Li.[☆]

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Abstract

This study investigated the characteristics of the leaf stomatal apparatus of *C. sinensis* Li. by using electron microscope, and observated the anatomical structure of the blade by paraffin sectioning technology. The results indicate that the adaxial surface of epidermis is smooth and has no stomatal apparatus, and stomatal apparatus is mainly distributed on both sides of the midribs on the abaxial surface of epidermis, which arranged to form with two white stomatic bands of the rules. The stomatal apparatus density is about 72.48 per mm^2. [Life Science Journal. 2008; 5(2): 41 – 44] (ISSN: 1097 – 8135).

Keywords: *C. sinensis* Li.; leaf epidermis; stomatal apparatus; anatomical structure

1 Introduction

Cephalotaxus sinensis Li. (*C. sinensis* Li.), referred to as *Cephalotaxus sinensis*, belonging to *Cephalotaxus fortune* Hook. f. *Cephalotaxus sinensis* is the unique species in China, and it distributes broadly in 14 provinces in China[1,2]. Because the plant contains esters alkaloid, it was used to treat dyspepsia, smooth lung and vermifuge for a long time, and was used to cure tumour in recent years[3–5]. Academe has engaged in the research on harringtonine of *C. sinensis* Li.[6–8], the author has reported callus induction of *C. Sinensis* Li.[9], but no report about researches on organs of *C. sinensis* Li. There is a very close relationship between plant and its growing environment, and the environment performs an important role on the growth, development and morphosis of the plant. The stomatal apparatus has been affected by the environment very obviously. Generally, the stomatal apparatus exists in *lichen*, *pteridophyte* and *spermatophyte* widely. It is the distinctive apparatus of advanced plant adapted to the land life. Therefore, stomatal apparatus has irreplaceable value in the research on systemic envolvement, especially in the discussion about origin envolvement of archaic terrestrial plant[10]. In this paper, we observed the anatomical structure of the blade by using scanning electron microscope (SEM) and paraffin sectioning technology, in order to comprehend the relationship between stomatal apparatus and environmental factors, to understand ecotype and adaptability, and to provide more datum for protecting *C. sinensis* Li.

2 Materials and Methods

2.1 Materials

The leaves of *C. sinensis* Li. came from Baotianman State Reserve, Nanyang city of Henan province, China. Sampling objects are the leaf of burgeoning branch in the same year. Sampling time was April 12, 2005.

2.2 Methods

2.2.1 Observation of the stomatal apparatus of leaf by SEM. Fixation: selected the plants which grow normally, and chose 15 mature leaves, cut every leaf into pieces with 5 mm wide. All the pieces were fixed by 4% glutaraldehyde, rinsed by 0.2 mol/L phosphate buffer, soaked by 1 mol/L NaOH, 1 h, and rinsed by 0.2 mol/L phosphate buffer.

Dehydration: dehydrated by ethanol grads (30%, 50%, 60%, 70%, 15 seconds respectively), then dehy-

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drated 15 seconds respectively with mixture of absolute ethanol and tert-butanol of their proportion is 3 : 1, 1 : 1, and 1 : 3.

Spraying: deposited in pure tert-butanol – 20 °C, taken out after icing, vacuum dried in JEE-420 vacuum evaporator, gilded in IB-5 ion metallic-membrane plating. Observed and photoed with JSM-5610LV SEM.

Mensuration and statistic analysis: length and width of the stomatal apparatus were measured. These numerical values are mean value of 10 figures, and calculates coefficient of variation (CV).

2.2.2 Observation of the anatomic structure of leaf. All experimental material were fixed by FAA, and cut into 10 μm – 12 μm thickness by paraffin sectioning technology.

3 Results and Discussion

3.1 The structure of the leaf epidermis

The cells of the epidermis on adaxial surface are atactic and compact (Figure 1a), and has no stomatal apparatus and intercellular space, no cutin stick up. The stomatal apparatus are mainly distributed on the epidermis of abaxial surface. The cells are rectangle which arranges compact, and has no intercellular space (Figure 1b).

3.2 The type, structure, scalar property and distributing of the stomatal apparatus

The stomatal apparatus is single stoma type, and each stomatal apparatus consists of two elliptic guard cells, which is surrounded by 2 – 6 assistant guard cells (2 mostly) that arranges polar. The stomatal apparatus sinks slightly (Figure 1b), belongs to euequisetum or equiseta phaneropora type, and there is waxiness on the surface. The stomatal apparatus are mainly distributed on both sides of the midribs on the epidermis of abaxial surface, which arranges regularly to form with two white stomatic bands, and no stomatal apparatus on midrib of the leaf. Each stomatic band arranges about 14 rows.

The size of the hole of the stomatal apparatus is (43.33 × 13.35) μm – (28.55 × 6.64) μm (length × width). The magnitude of the stomatal apparatus is (100.0 × 34.7) μm – (66.6 × 21.44) μm (to the outer edge of guard cells). The shortest distance between two assistant guard cells of stoma is 23.63 μm – 60.10 μm, or the distance is 69.55 μm – 140.11 μm (to the outer edge of two assistant guard cells). The size of assistant guard cells is 27.7 μm – 112.35 μm. CV is less than 10%. The results indicate that the stomatal apparatus density is about 72.48 per mm^2.

3.3 The anatomical structure of the leaf transverse section

The leaf of *C. sinensis* Li. is unequifacial leaf, and the tranverse section anatomical structure shows as Figure 2. There are palisade tissue, spongy tissue, and abaxial epidermis cells from adaxial surface to abaxial surface. There is thick cutin layer in epidermises of the abaxial surface and the adaxial surface respectively, and only one layer cells in both epidermises respectively, and palisade tissue too, which arranges compact. The atactic spongy tissue arranges loose.Vascular bundle was circumfusing with bundle sheath that arranges compact, and xylem on the adaxial surface, phloem on the abaxial surface.

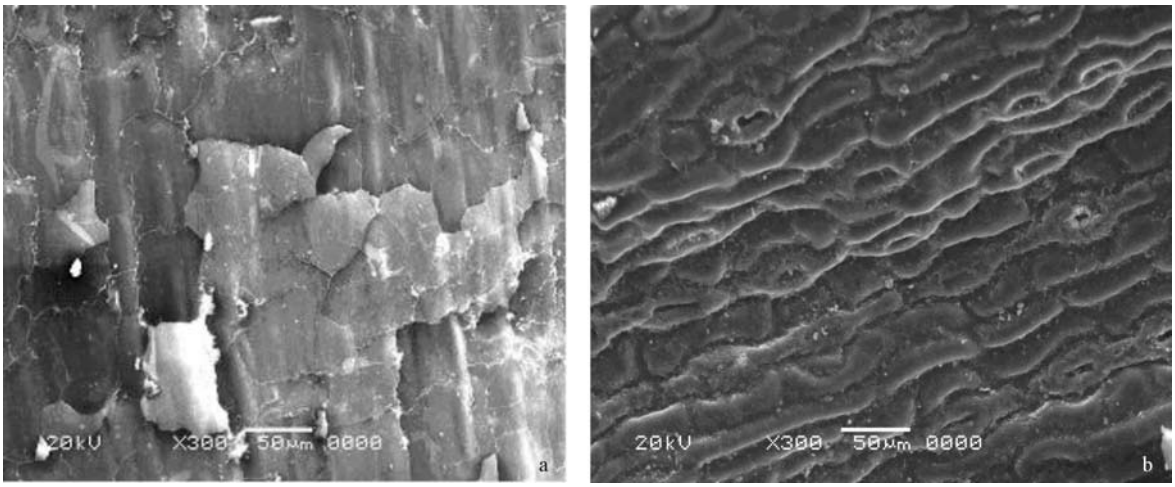

Figure 1. The adaxial and the abaxial surface of the leaf epidermis of *C. sinensis* Li.. a: The adaxial surface (× 300); b: The abaxial surface (× 300).

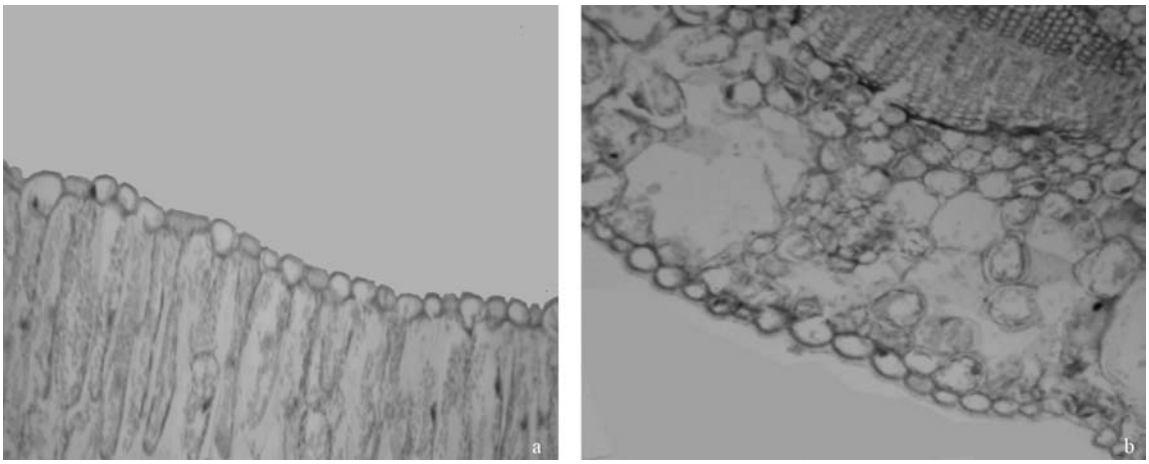

Figure 2. The anatomical structure of the leaf of *C. sinensis* Li. a: Showed the superior epidermis; b: Showed the hypoepidermis and the midribs.

4 Discussion

The stomatal apparatus has a better genetic stability, which is an important trait of botany and the main exchange channel of gases and water between plant and its growing environment. But environment have an impact upon magnitude character of dentisy and size. The stomatal apparatus's switching is correlative with photosynthesis and transpiration. However, photosynthesis and transpiration are intercommunicated and mutual contradiction, and evaporative water loss is ineluctable in the course of photosynthesis, but CO_2 that photosynthesis needs enters the stomatal apparatus when the stomata is opening. So, it is a physiological adaptability such as the stomatal apparatus sinks slightly or grows villus on the surface of leaf. *C. sinensis* Li. is the gymnosperm that grows in humid climates regions, and stomatal apparatus are mainly distributed on the epidermis of abaxial surface. The contradiction between the photosynthesis and transpiration eases by reducing water transpiration .

This experimentation just investigated the distributing, structure and quantitative characteristics of the stomatal apparatus of the leaf of *C. sinensis* Li. The results indicate that environment influenced the distribution and structure of the stomatal apparatus of *C. sinensis* Li. Less density adapts the stomatal apparatus to the moist and muggy habitat. The stomatal apparatus are mainly distributed on the epidermis of abaxial surface, which adapt itself to the droughty growing environment.

In this study, when the leaf stomatal films is prepared by using enamel daub divulsion, we found a mass of particulate matters near the stomata, just guess that paraffin exists in leaf epidermis. Afterward, dealing with dimethylbenzene beforehand, we found no particulate matter, so the guessing validated.

Both *C. sinensis* Li. and *Cephalotaxus hainanensis* are *Cephalotaxus fortunel* Hook. f., relatives' species. In appearance, primary differentiation is that the texture of the leaf of *Cephalotaxus hainanensis* is thinner and inswept, which has an anaclastic edge, columniform in root of the leaf. Du Daolin *et al*[11] have observed the leaf epidermis by using SEM, compared with *C. sinensis* Li. and discovered that both of them are analogical. The epidermis of adaxial surface is smooth and has no stomatal apparatus, and stomatal apparatus are mainly distributed on both sides of the midribs on the epidermis of abaxial surface, which arranges to form with two white stomatic bands regularly. Each stomatal apparatus consist of two elliptic guard cells, which was surrounded by 2 – 6 (2 ecumenic) polar arranges assistant guard cells, stomatal apparatus sinks slightly, and ceraceous epidermis.

The differences between *C. sinensis* Li. and *Cephalotaxus hainanensis* are quantitative characteristics of the structure of the stomatal apparatus. The hole size of stomatal apparatus, the magnitude of the stomatal apparatus (to outer edge of two guard cells) and the distance of the assistant guard cells etc, all these traits of *Cephalotaxus hainanensis* are larger than *C. sinensis* Li., which shows likely that *C. Sinensis* Li. is more tolerant to drought than *Cephalotaxus hainanensis*.

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