Botanica Orientalis – Journal of Plant Science (2009) **6:** 106–108 ISSN 1726-6858

http://www.cdbtu.edu.np/botanica-orientalis

Short communication

Scanning electron microscopic study of leaf surface of some species of *Hopea* Roxb. (Dipterocarpaceae): implications for taxonomy

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Abstract

Since more than two decades, the taxonomy of *Hopea* has been a point for discussion. Time to time, various taxonomists have tried to classify the species of *Hopea* on the basis of morphological and anatomical characters. This type of controversy can be solved by the study of various disciplines. Among them, the Scanning Electron Microscopic (SEM) study may be taxonomically useful to resolve the controversies over the classification of the genus *Hopea*. The SEM study was carried out in *Hopea* of Sri Lanka. The result of SEM indicates that the two taxa (*Hopea jucunda* ssp. *jucunda* and *H. jucunda* ssp. *modesta)* possess different epicuticular appearance justifying to upgrade the subspecies of *H. jucunda to* species level.

Key words: epicuticular wax, SEM, stomata, trichome.

Introduction

Leaf surfaces of the plant exhibit a variety of surface structures that have attracted the attention of taxonomists due to their importance in making primary taxonomic decisions and also for the determination of incomplete plants, e.g. sterile specimens, archaeological remains and fragmentary fossils (Bussotti and Grossoni 1997). Since more than three decades, much progress on the micromorphological works has been made due to the advent of Scanning Electron Microscope (SEM). Based on the micromorphological studies, different workers have given emphasis on various leaf surface characters for the classification of taxa into different categories (Stace 1984). In general, following characteristics are used for describing leaf surface: epidermis, venation systems, trichomes, structure of epicuticular waxes, and stomata. These micromorphoplogical characters of leaf are not only significant in physiological functions of plant but they are also valuable in taxonomic studies and also useful for investigation of phylogenetic relationships (Stace 1984; Joshi 2001).

The genus *Hopea* Roxb. (Dipterocarpaceae) is rich in species diversity with 102 species distributed in Andamans, Bangladesh, Burma, Hainan, India, Indochina, Malaysia, South China and Sri Lanka (Dayanandan *et al.* 1999; Joshi 2002, 2003). In Sri Lanka, there are five endemic taxa of *Hopea* [*Hopea brevipetiolaris* (Thw.) P.S. Ashton, *Hopea cordifolia* Trim., *Hopea discolor* Thw., *Hopea jucunda* Thw. ssp. *jucunda* and *Hopea jucunda* ssp. *modesta* DC.), which are widely distributed in the forest habitats of the wet, intermediate and dry agro-ecological zones of the island and are economically important for timber, resin**,** poles, and firewood (Joshi 2001). The taxonomy of these taxa has always been a point of discussion (Ashton 1980; Trimen 1990; Kostermans 1992). Sometimes some species were categorized into subspecies (Ashton 1980) and sometimes some subspecies upgraded to species level and some species shifted to new genus as synonyms (Kostermans 1992).

In the present paper, an attempt has been made to study the various structures present on the leaf surface of the species of *Hopea* of Sri Lanka and to asses the taxonomic significance of some leaf micromorphological parameters observed under Scanning Electron Microscopy (SEM), and to establish the validity of these parameters in defining species or subspecies entities.

Materials and Methods

Fresh leaves of *Hopea jucunda* ssp. *jucunda*, *H. jucunda* ssp. *modesta* and *H. discolor* were collected from the wet zone forests, Delwala, Sri Lanka. The botanical identity of the plants was determined using relevant literatures. The authenticity of the samples was carefully checked against herbarium specimens from National Herbarium, Royal Botanical Garden, Peradiniya, Sri Lanka. Voucher specimens were lodged in the Department of Botany, University of Colombo, Sri Lanka.

After observing the leaf under dissecting and light microscope, the samples were fixed in 2.5 % glutarldehyde for 2 hrs, and washed with 0.1 M. phosphate buffer and left the samples for over night. The samples were post fixed with 1 % osmic acid, dehydrated through an ethanol series, and processed by critical point drying and further gold coating. After gold coating, the upper and lower surface of each sample was examined under SEM (model top con SEM A B T 32, at 15 kv) and photographed for leaf structures

mainly wax, trichome and stomata in different magnification (Joshi 2001).

Results and Discussion

The micromorphological characteristics observed by SEM showed some variations, which are described as follows:

Hopea jucunda ssp. *jucunda*

The epidermis was single layered with uniseriate trichome and these were distributed mainly along the midrib. The abaxial surface of the leaves was protected by wax. The stomata were raised (Fig. 1).

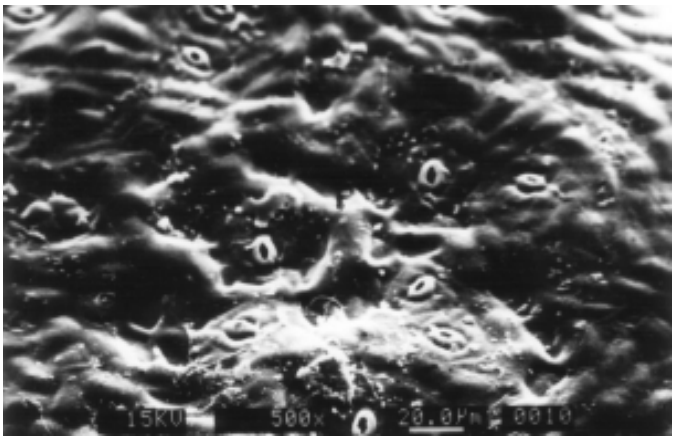

Fig. 1. Micromorphological structure of *Hopea jucunda* ssp. *jucunda* under SEM.

Hopea jucunda ssp. *modesta*

The epidermis was single layered with thread like wax deposition on abaxial surface which was not seen on the above species. Simple uniseriate trichomes were distributed primarily along the midrib. The stomata were sunken (Fig. 2). The wax deposited with somewhat flat depressed areas especially around the guard cells makes it different from the other species.

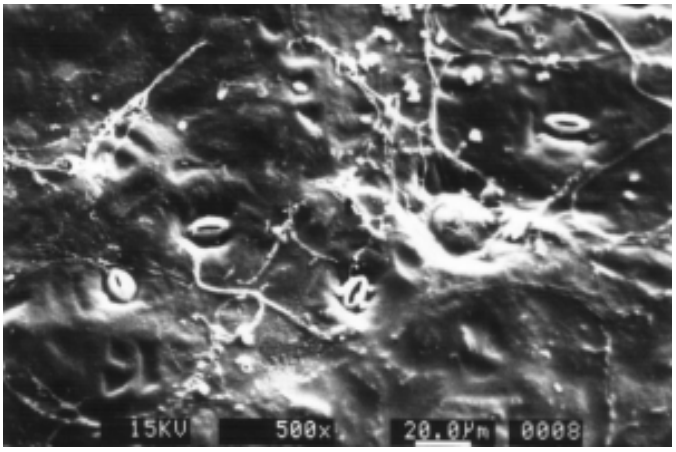

Fig. 2. Micromorphological structure of *Hopea jucunda* ssp. *modesta* under SEM. Wax deposits with flat depressed areas are seen especially around the guard cell.

The results of this preliminary study indicate that the two taxa studied possess different epicuticular apperance. These differences became apparent only after study under SEM which showed variation in the shape and the nature of stomata i.e. sunken stomata in *Hopea jucunda* ssp. *modesta*, and raised stomata in *H. jucunda* ssp. *jucunda*. These variations are notable and are taxonomically useful. In an attempt to compare with other species, *H. discolor* was also examined. The nature and shape stomata of *H. discolor* were different than those of above two sub-species. *H. discolor* had very raised stomata with more laminate surface (Fig. 3). The appearance of thread-like waxes was more clear in *H. discolor.* The epicuticular differences seen in different species of *Hopea* indicates the importance of extending this sort of study for other species of Dipterocarpaceae.

The present study has justified to up-grade the status of sub-species of *Hopea* to species level i.e. *Hopea jucunda* and *H. modesta*. The chemotaxonomic study of the species of Dipterocarpaceae of Sri Lanka carried out by Joshi (2001, 2005) and Joshi *et al*. (2004) also supports the present finding and made recommendation to treat *H. modesta* and *H. jucunda* as two separate species based on flavonoid patterns (Table 1). However, in future, emphasis should also be given to initiate further investigation taking into consideration of other parameters (i.e. DNA sequencing, biogeography, phylogeny, etc) to resolve the controversies over the classification of the plant.

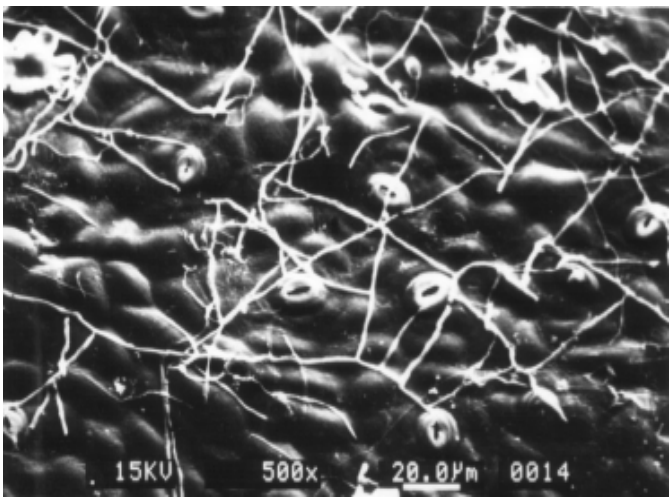

Fig. 3. Micromorphological structure of *Hopea discolor* under SEM showing very raised stomata with more laminate surface.

Table 1. Flavonoid in the leaves of *Hopea* species (Joshi 2001).

Scientific name	Aglycones†							Glycosides‡				
	Flavonol			Flavone		Proathocyanidine						
	M	Q	K	L	A	D	C	1	2	3	4	5
Hopea brevipetiolaris	-	+	+	-	+	-	-	+	+	-	-	-
Hopea cordifolia	-	+	+	-	+	-	-	+	+	-	+	-
Hopea discolor	-	+	-	-	+	-	-	-	-	+	-	+
jucunda ssp. *jucunda*	-	+	+	-	+	-	-	-	-	+	+	+
Hopea jucunda ssp *modesta*	-	+	-	-	+	-	-	+	-	-	-	+

†M = myricetin; Q = quercetin; K= kaempferol; L = luteolin; A = apigenin; D = delphinidin; C = cyaniding

‡1 = Qu 3-glucoside; 2 = Qu 3-rutinoside; 3 = Qu 3- xylosylglucoside; 4 = Km 3,5-glucoside; 5 = Ap 5-glucoside

+ = detected; – = not detected

Acknowledgements

The author is grateful to Dr. G. I. Seneviratne, Department of Botany, University of Colombo, for guidance; and to Professor K. Abeynayake, Dean, Faculty of Science and Professor R.L.C. Wijesundera, Head, Department of Botany, University of Colombo for giving the opportunity to carry out this research and encouragement. Thanks are also due to Dr. Wijesundara, Director, Royal Botanical Garden, Peradeniya and Mr. Upali Dhanasekera, Curator, National Herbarium, Paradeniya, Sri Lanka, for confirming the species identity; and to Dr. Renee Grayer, Royal Botanical Garden, Kew, UK; Prof. P.K. Jha, former Head and Prof. R.P. Chaudhary, Central Department of Botany, Tribhuvan University, Nepal; and Dr. A.R. Joshi, former Director General, South Asia Cooperative Environment Programme (SACEP) for their advice and encouragement.

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