

CHAPTER 8

THE STUDY ON FOLIAR MICROMORPHOLOGY OF *HIPPOBROMUS PAUCIFLORUS* USING SCANNING ELECTRON MICROSCOPE

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The Study on Foliar Micromorphology of *Hippobromus pauciflorus* Using Scanning Electron Microscope

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Abstract: Foliar micromorphology of *Hippobromus pauciflorus* (L.f.) Radlk (Sapindaceae) was investigated by scanning electron microscope examination. The leaves were characterized by anisocytic stomata which were found only on the abaxial surface. The leaves have long unicellular non-glandular trichomes which were distributed over the mid rib and densely populated at the edges of the upper and lower surfaces. Crystal deposits were also observed on the abaxial surface of the leaves. Energy dispersive X-ray spectroscopy SEM of the crystals showed predominant mineral components of Na, Al, Si, K and Ca ions. These micro (Si, Na and Al) and macro (K and Ca) elements are very essential to plant growth. Their presence in the trichomes could contribute to the mechanical stability of the leaf appendages.

Key words: *Hippobromus pauciflorus*, micromorphology, stomata distribution, non-glandular trichome, leaf crystals

INTRODUCTION

Hippobromus pauciflorus (L.f.) Radlk. (Sapindaceae) locally known as *Basterperdepis* or *Ulatihle* in the Eastern Cape province of South Africa, is a resinous tree that grows up to 5 m in height. Several medicinal uses of the plant have been reported. For example, the leaves of the tree are used by the traditional healers for the treatment of malaria (Clarkson *et al.*, 2004). Ethnomedical information from the indigenous people of the Eastern Cape, the leaves are crushed and squeezed into infected eyes. The root is regarded by the Zulus as a love charm and is also used to manage dysentery and diarrhea. In addition, leaves extracts are used for the treatment of livestock diseases and conjunctivitis in the Eastern Cape (Masika and Afolayan, 2003).

Antiplasmodial, anthelmintic and antibacterial properties of *H. pauciflorus* have been reported (Clarkson *et al.*, 2004). Plants of the Sapindaceae family generally contain flavonoids, phenolic acids and tannins (proanthocyanidins) in their leaves (Magid *et al.*, 2005; Voutquenne *et al.*, 2005). Developmental and structural studies of trichomes, according to Franceschi and Giaquinta (1983), can shed light on the nature of the secreted material and their functional significance. It is possible that the antiplasmodial, anthelmintic and antibacterial compounds produced by *H. pauciflorus* are among the saponin, flavonoids, phenolic acids and tannins (proanthocyanidins) exudates on its leaves. This

study was to aim at describing the leaf appendages of *H. pauciflorus* and relating the structures to their possible functional roles.

MATERIALS AND METHODS

Plant materials: The leaves of *H. pauciflorus* were collected in February 2008 from Sikhustwana village near Alice in the Eastern Cape Province of South Africa (latitudes 30°00'-34°15'S and longitudes 22°45'-30°15'E). The plant was identified at the Department of Botany, University of Fort Hare, by Prof DS Grierson and a voucher specimen (Pendota, 2008/1) was prepared and deposited in the Griffen Herbarium of the University.

Scanning electron microscopy: Fresh leaves, 4-6 mm, in were removed from the aerial part of the plant and were fixed in 6% glutaraldehyde in 0.05 M sodium cacodylate for 24 h. After washing with 0.05 M cacodylate buffer (pH 7.5), samples were dehydrated in graded series of ethanol (10-100% ×3) for 15 min per rinse. This was followed by critical point drying with liquid CO₂ in an autosampler 810 critical point dryer and sputter-coated with gold-palladium in a Hummer Vsputter coater. Both the adaxial and abaxial surfaces were observed in JEOL (JSM-6390LV) Scanning Electron Microscope, operating at 10-15 kV acceleration voltage. For energy dispersive X-ray spectroscopy-SEM, the fixing and dehydration

followed the same procedure as in SEM, while a FEI QUANTA 200 Oxford EDX Analyzer was used for the analysis.

RESULTS AND DISCUSSION

The leaves of *H. pauciflorus* were represented as long unicellular non-glandular trichomes which were distributed more on the abaxial than the adaxial surfaces (Fig. 1a-d). The trichomes were enriched distributed more on the edge of the adaxial and abaxial surface than on the midrib. The development of the trichomes from the epidermis usually results from differential enlargement and subsequent divisions of the epidermal cells and their derivatives (Carlquist, 1958). Trichome abundance and distribution over the surface of the leaves are geared, possibly against foraging insects and airborne propagules of fungi. Positive correlation between trichome density and insect resistance has been demonstrated in various plant species (Levin, 1973).

The presence of stomata only on the abaxial epidermis of the leaf of *H. pauciflorus* could be a specific character of the species (Fig. 2a, b). It might be an adaptive component of the plant to the environmental conditions in the Eastern Cape Province, South Africa that experiences very low humidity and erratic rainfall throughout the year. Hypostomatous leaves might not only result in a higher resistance to water deficit but also in a reduced susceptibility of the plant to fungal infections, since most often, fungi infect plants through the stomata.

Some crystal deposits were found on the leaves surfaces and some near the stomata (Fig. 2a-c). The chemical nature of the crystals showed that they were predominantly composed of Na, Al, K, Ca and Si (Fig. 3). These micro (Si, Na and Al) and macro (K and Ca) elements are very essential to plant growth. Their presence in the trichomes most probably increases the mechanical stability of the leaf appendages.

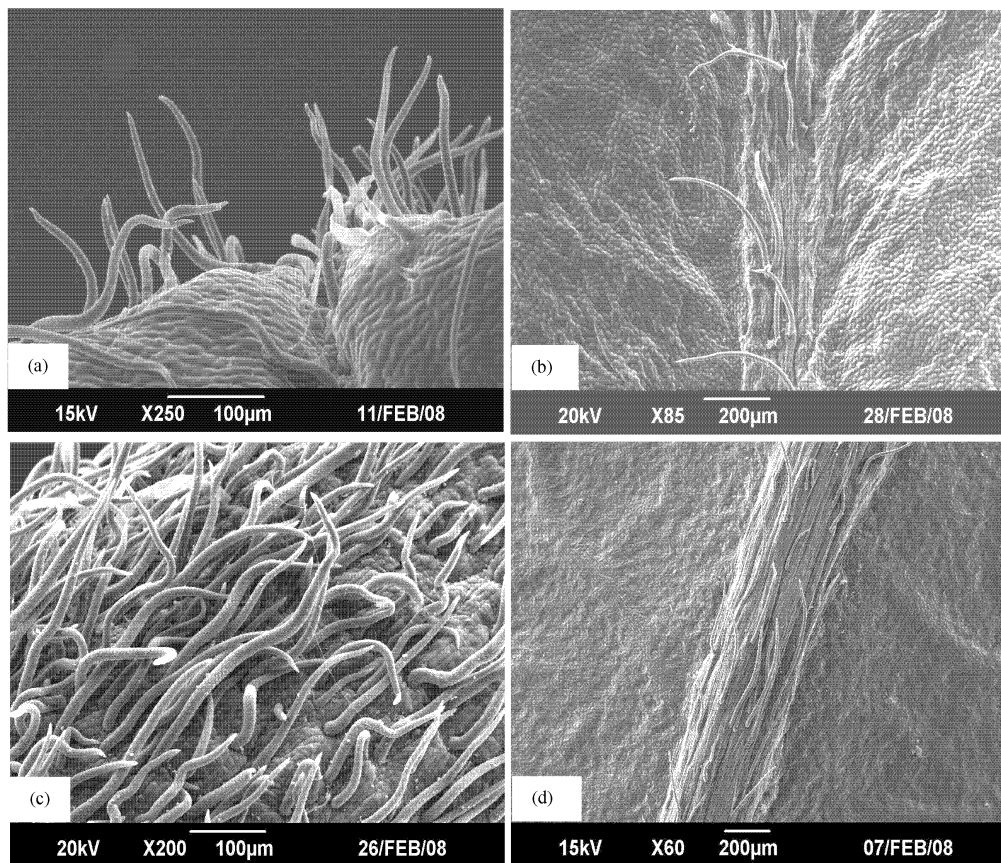


Fig. 1: Micromorphology of non glandular trichomes of *H. pauciflorus* in adaxial epidermal appendages of leaf edge and midrib (a and b) and in abaxial epidermal appendages of leaf edge and midrib (c and d)

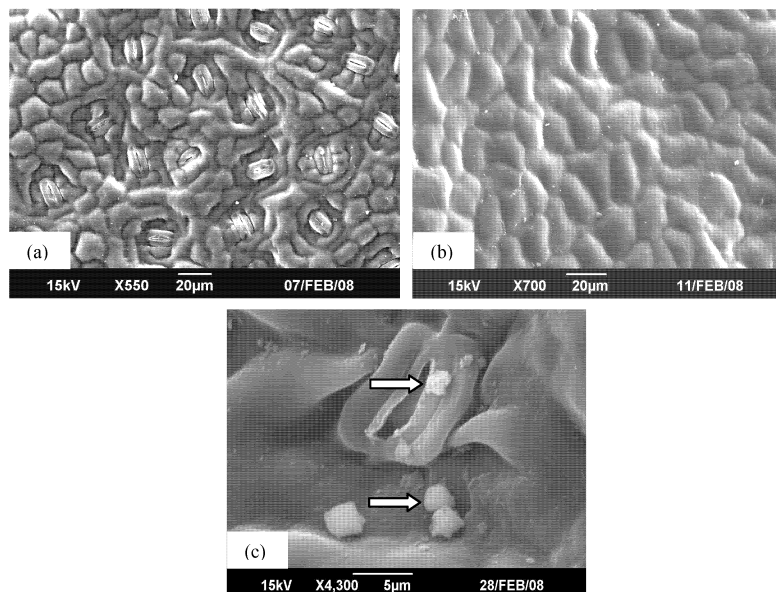


Fig. 2: Stomatal distribution in *H. pauciflorus* in abaxial surface (A) adaxial surface (B). As well as crystal located on the stomatal pore (C)

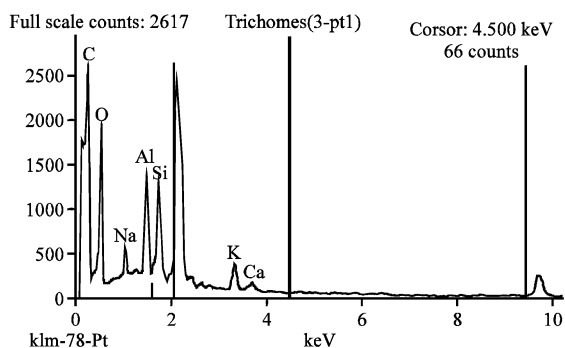


Fig. 3: Energy dispersive X-ray spectroscopy SEM of *H. pauciflorus*. The arrows points to the crystals analyzed

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REFERENCES

Carlquist, S., 1958. Structure and ontogeny of glandular trichomes of Madinae (Compositae). *Am. J. Bot.*, 45: 675-682.

Clarkson, C., J.M. Vinesh, R.C. Neil, M.G. Olwen and P. Pamisha *et al.*, 2004. *In vitro* antiplasmodial activity of medicinal plants native to naturalised in South Africa. *J. Ethnopharmacol.*, 92: 177-191.

Franceschi, V.R. and R.T. Giaquinta, 1983. Glandular trichomes of soybean leaves: Cytological differentiation from initiation through senescence. *Bot. Gaz.*, 144: 175-184.

Levin, D.A., 1973. The role of trichomes in plant defense. *Q. Rev. Biol.*, 48: 3-15.

Magid, A.A., L.V. Nazabadioko, M. Litaudon and C. Lavaud, 2005. Acylated farnesyl diglycosides from *Guioa crenulata*. *Phytochemistry*, 66: 2714-2718.

Masika, P.J. and A.J. Afolayan, 2003. An ethnobotanical study of plants used for the treatment of livestock diseases in the Eastern Cape Province, South Africa. *Pharm. Biol.*, 41: 16-21.

Voutquenne, L., P. Guinot, C. Froissard, O. Thoison and M. Litaudon *et al.*, 2005. Haemolytic acylated triterpenoid saponins from *Harpullia austro-caledonica*. *Phytochemistry*, 66: 825-835.