

THE CALLOSE WALL OF MICROSPORE TETRADS OF *LUFFA CYLINDRICAL*  
(CURCUBITACEAE)

Chih-Hau Tsou

Institute of Botany, Academia Sinica  
Taipei, Taiwan 115, Republic of China  
E-mail: [chtsou@gate.sinica.edu.tw](mailto:chtsou@gate.sinica.edu.tw)

**KEY WORDS:** Aniline blue, callose envelop, calcofluor white, DiOC<sub>6</sub>, freeze fixation, *Luffa cylindrical*, microspore tetrad.

The callose wall enveloping the microspore tetrad during pollen formation in angiosperms has been reported as composing of two or more layers [1, 2, 3]; however, the layered structure is mostly not discernible under LM or TEM when samples are prepared through standard procedures, or under fluorescent microscope when stained with aniline blue. In the present study, fresh anthers of *Luffa cylindrical* were fixed with 2.5% glutaraldehyde for 5-10 minutes, rinsed with phosphate buffer, freeze fixed in nitrogen slush, and then proceeded with freeze substitution. Samples were further transferred to absolute alcohol, embedded in parapalst, sectioned at 8-10 $\mu$ m thick, deparaffined, and stained with three fluorescent dyes. DiOC<sub>6</sub> was able to differentiate three layers within the callose wall (Fig. 1), but aniline blue failed to do so. Calcofluor white, on the other hand, revealed cellulosic component present in the inner most layer.

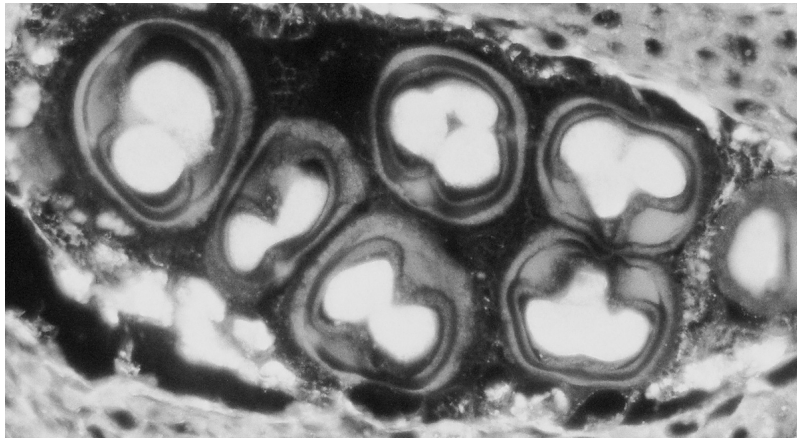


Figure 1: Anther cross section of *Luffa cylindrical*. The section was stained with DiOC<sub>6</sub> and excited by blue light when viewed under fluorescent microscope. The callose wall of microspore tetrad as having three distinct layers is revealed.

- [1] J. Heslop-Harrison, "Cytoplasmic continuities during spore formation in flowering plants," *Endeavour* **25**, 65-72 (1966).  
[2] L. Waterkeyn and A. Bienfait, "On a possible function of the callosic special wall in *Ipomoea purpurea* (L.) Roth," *Grana*, **10**, 13-20 (1970).  
[3] C.-H. Tsou and Y.-L. Fu, Tetrad pollen formation in *Annona* (Annonaceae): proexine formation and binding mechanism, *Amer. J. Bot.* **89**: 734-747 (2002).