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Mechanism of Anther Dehiscence in *Leucaena leucocephala (Leguminosae, Mimosoideae)*

By

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With 2 Figures

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Summary

JAIN S. & VIJAYARAGHAVAN M. R. 1992. Mechanism of anther dehiscence in *Leucaena leucocephala (Leguminosae, Mimosoideae).* Phyton (Horn, Austria) 32 (1): 103–109, 2 figures. – English with German summary.

In *Leucaena leucocephala* (LAMK.) DE WIT, longitudinal type of anther dehiscence occurs. LM and SEM studies reveal the involvement of three major steps in this process that include (i) Lysis of septum, (ii) Formation of stomium, and (iii) Release of pollen grains.

Zusammenfassung

JAIN S. & VIJAYARAGHAVAN M. R. 1992. Der Öffnungsmechanismus der Antheren bei *Leucaena leucocephala (Leguminosae, Mimosoideae).* Phyton (Horn, Austria) 32 (1): 103–109, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Antheren von *Leucaena leucocephala* (LAMK.) DE WIT öffnen sich, indem sie der Länge nach aufbrechen. LM und SEM Untersuchungen ergaben, daß dies in drei Schritten erfolgt: 1. Auflösen des Septums, 2. Bildung des Stomiums und 3. Freisetzen des Pollens.

Introduction

The changes in the anther wall layers and the atmosphere help in anther dehiscence and the ambient release of mature and fully developed

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pollen grains to the external environment. The mechanism of anther dehiscence and pollen release is, however, poorly understood. *Leucaena leucocephala,* possesses single pollen grains (in contrast to polyads usually found in this subfamily) and the present paper focuses on -(i) Opening mechanism of longitudinally dehiscing anthers (ii) Relationships between anther wall layers and pollen development (iii) Role of polyphenols present in epidermal and connective cells.

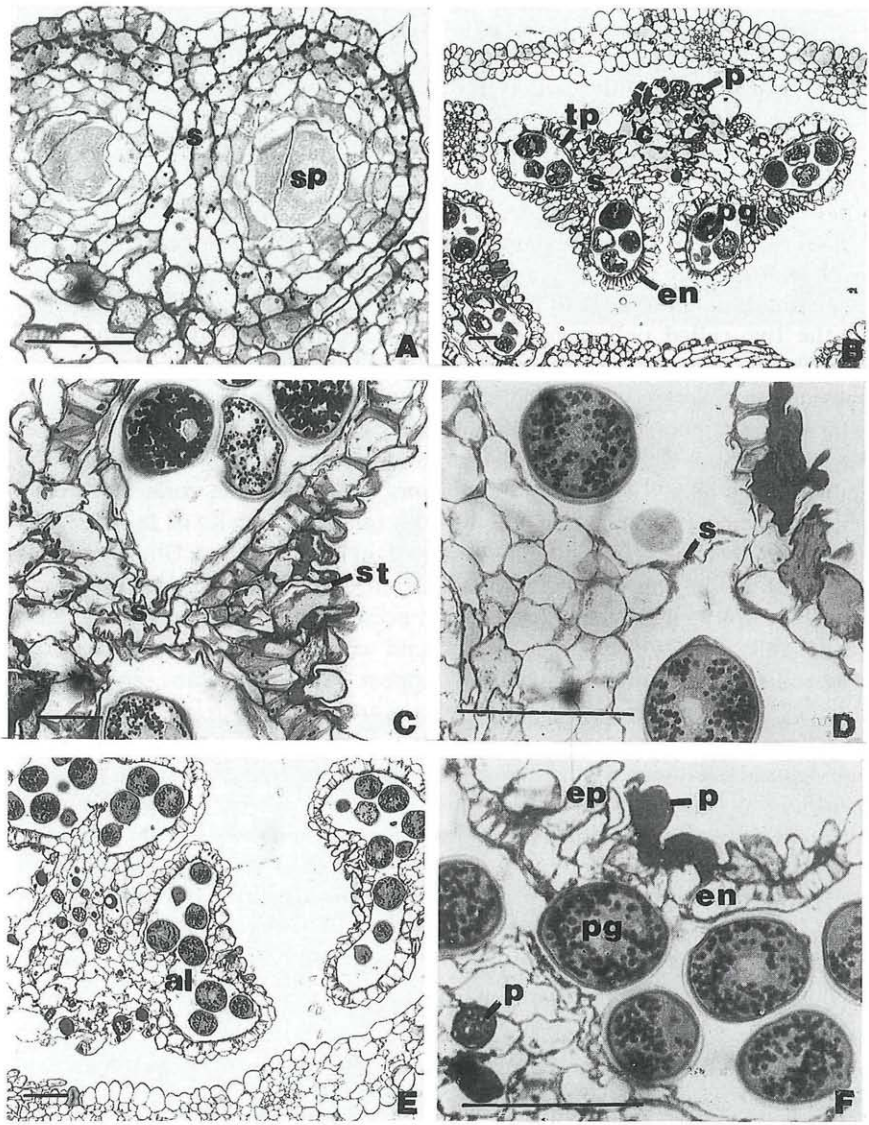
Material and methods

Young buds and dissected anthers of different stages were fixed in an 10 % (v\v) aqueous solution of acrolein, dehydrated in methoxyethanol, propanol, butanol series, infiltrated and embedded in glycol-methacrylate. Two micron thick sections were cut with glass knives. Periodic acid Schiff's reagent (modified after FEDER & O'BRIEN 1968) was used for localizing insoluble polysaccharides, Coomassie blue (modified after FISHER 1968) was used for total proteins and Pyronin Y (modified after TEPPER & GIFFORD 1962) for localizing ribonucleic acid. For scanning electron microscopy, the anthers were fixed in 5 % glutaraldehyde in O.1M phosphate buffer; passed through ascending acetone series and later subjected to critical point drying. The material was mounted on aluminum stubs, sputter coated with silver and scanned under a Philips 501B microscope.

Results

The anther in *Leucaena leucocephala* consists of two thecae bound together by a connective tissue. In each locule, the anther wall consists of four or five layers namely epidermis, endothecium, middle layers (1 or 2) and tapetum. A small zone sandwiched between two adjacent locules is the septum. A few epidermal cells in this region are large, different in morphology than the adjoining epidermal cells and are the presumptive stomium. The presumptive septum differentiates at sporogenous cell stage and is 8 or 9 layered (Fig. 1A) and gradually weakens at about the microspore mother cell and the microspore tetrad stages. The tapetal cells that border the septum region are vacuolate.

Fig. 1. Anther of *Leucaena leucocephala.* A: Transection of a portion of an anther locule at sporogenous cell stage. The septum is 6 or 7 layered. B: A tetralocular anther at uninucleate microspore stage. The septum is 5-layered. The connective cells accumulate polyphenols. The endothecial cells reveal U-shapped thickenings. The tapetal cells remain intact. C: A portion of an anther showing 3-layered septum. The presumptive stomium is noteworthy. D: A portion of an anther showing 1-layered, dissolving, septum). E: A portion of an anther showing completely dissolved septum resulting in confluent anther locule. F: Same, showing accumulation of polyphenols in the enlarged epidermal cells at the stomium region. al = anther locule, c = connective cells, en = endothecial cell, ep = epidermal cell, p = polyphenol, pg = pollen grain, s = septum, sp = sporogenous cell, st = stomium, tp = tapetal cell, index bars = 25 μm.



In the early stages of microsporogenesis, the metabolites like polysaccharides (Fig. 1A), protein and ribonucleic acid are present in the wall layers. With subsequent stages of development, the wall layers show a decrease in the amount of the above metabolites whereas the pollen grains show an increase in the amount of these metabolites (Fig. 1E, F). At the time of anther dehiscence, the pollen grains are gorged with metabolites while wall layers show a depletion (Fig. 2 D).

Detectable morphological changes precede anther dehiscence during microspore tetrad release from callose wall (Fig. 1 B). These are (i) The endothecial cells reveal U-shaped thickenings, (ii) parenchymatous and thin walled septal cells become compressed (iii) contiguous tapetum help septal cells to remain at their original position. At the uninucleate pollen grain stage, the tapetum lysis and concomitantly the septal dissolution initiates. At the two-celled pollen grain stage, the septum is 4-layered and with progressive stages of anther development is further compressed (Fig. 1 C); becomes one layered (Fig. 1 D) and before anther dehisces, the septum totally lysis which results in confluent anther locule (Fig. 1 E). The anther, from this stage onwards, is veneered only by the epidermis and the endothecium (Fig. 1F). The middle layers are lysed. The connective cells accumulate polyphenols (Fig. 1 B, F). The tangential walls of tapetal cells are studded with sporopollenin material in the form of Übisch bodies (Fig. 2 A). In the septum region, two to four epidermal cells enlarge along the presumptive dehiscence furrow and accumulate polyphenols (Fig. 1 F). These cells finally crumble, distort and characteristically demark the longitudinally oriented dehiscence furrow. The important anatomical changes that accompany the above events are:

1. Enlargement of epidermal cells at the stomium site.
2. Accumulation of polyphenols in the enlarged epidermal and connective cells.
3. Sporopollenin in the form of Übisch bodies along the inner tangential wall of the tapetum.
4. Septal lysis resulting in the confluent anther locule.
5. U-shaped thickenings in the endothecium

Tangential swellings of the epidermal and endothecial cells enlarge the circumference of locule walls. The inner tangential walls of the endothe-

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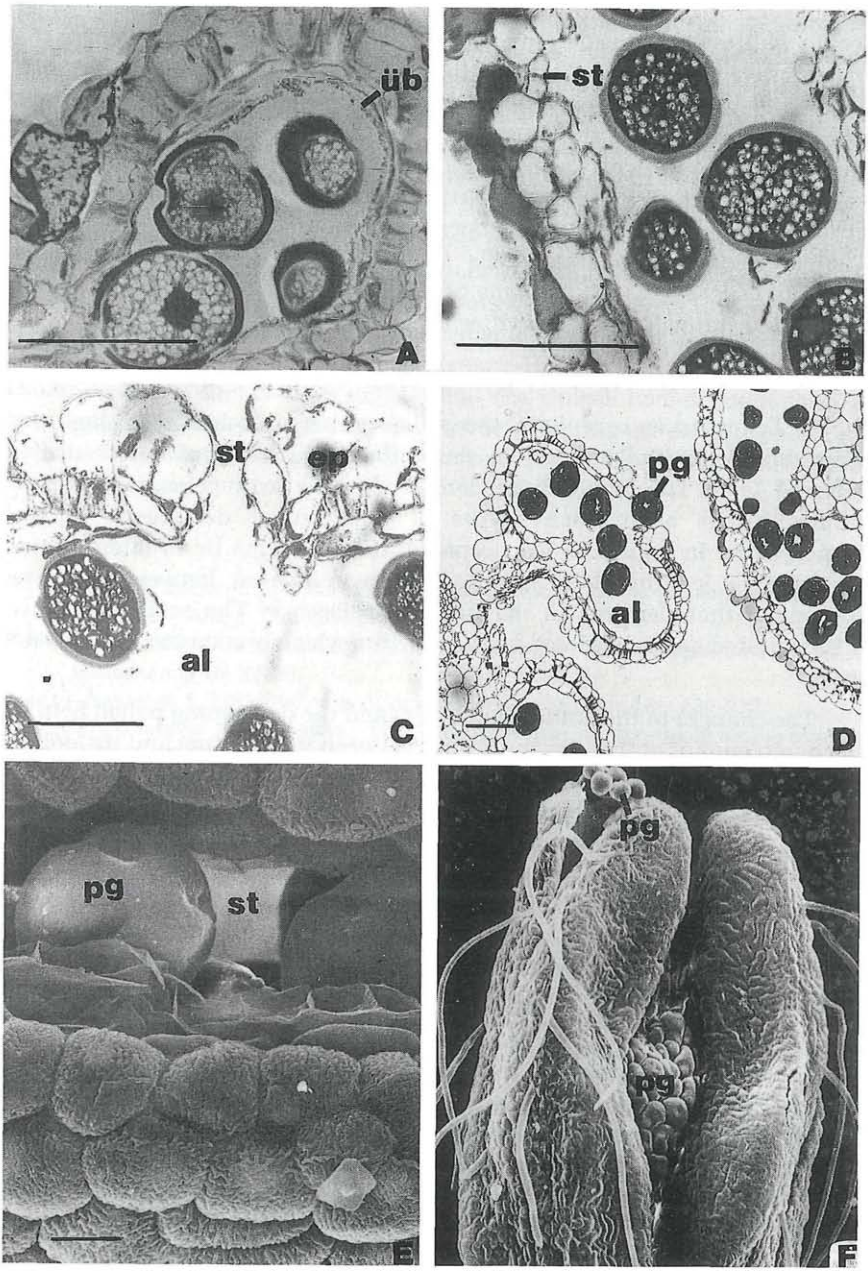
Fig. 2. Anther of *Leucaena leucocephala*. A: A portion of an anther locule at uninucleate microspore stage. The anther locule is bordered by Übisch bodies. B: A portion of an anther locule, showing bridging of stomium by two cells. C: A portion of an anther locule, showing formation of stomium. The outward bending of epidermal cells at the site of stomium is noteworthy. D: An anther locule showing release of pollen grains through the stomium. E: Portion of an anther showing pollen grains inside the anther locule (SEM). F: Longitudinally dehiscing anther (SEM). pg = pollen grain, st = stomium, Üb = Übisch body, index bars = 25 μm.



cium are rigid due to interconnected U-shaped wall thickenings and the dimensions of the rigid inner and outer tangential walls bend the anther locule wall towards inside. Two palisade- like, epidermal cells border the presumptive stomium and act as a fulcrum (Fig. 2 B). The endothecial cells below the presumptive stomium are under tension and the force, thus, created causes lysis of the intermediate cells that span the stomium. The stomium (yet to form an opening), thus, shows a criss-cross arrangement of endothecial cells. The anther dehiscence is brought about by the inwardly directed force in the locule wall opposite to the outwardly directed force. The intermediate epidermal cells finally lyse to form well developed stomium (Fig. 2 C, E). At the time of dehiscence, the mature pollen grains are ejected through the wide orifice (Fig. 2 D, F).

Discussion

In *Leucaena leucocephala,* the pollen grains are released through the longitudinal slit-like opening in the anther. This mode of dehiscence is referred to as the longitudinal dehiscence. The arrangement of anther locules brings about many types of longitudinal dehiscence in the angiosperms. In *Leucaena leucocephala,* the sporangia lie in lateral pairs resulting in longitudinally oriented common furrow, between the two sporangia, that demarcates the line of dehiscence. The septum is fully differentiated and is bordered by the tapetum which becomes vacuolate and weak.

The changes in the anther wall layers and the developing pollen help in the dehiscence of anther. On the border between the tapetum and the pollen grains, the pectins and cellulose are gradually replaced by sporopollenin. This means a shift from hydrophylic into hydrophobic properties (Heslop-Harrison 1968). In *Leucaena leucocephala,* the concentration of metabolites increases in pollen grains with a decrease in the anther wall layers during progressive stages of microsporogenesis. Keijzer 1985 showed a dissolution of starch in both the septum and stomium tissues. The breakdown of insoluble polysaccharides releases ATP's which are utilised for RNA and protein synthesis (Mahler & Cordes 1971).

Woycicki 1924 showed that the collenchyma-like cell walls mainly consist of pectin, indicating a possible role of pectinase in opening the septum. But in *Leucaena leucocephala,* the septum faces a compressive force due to (i) Polyphenols present in the epidermal and connective cells (ii) Übisch bodies deposition on the inner tangential walls of the tapetum. Both these substances are hydrophobic and act towards septum, resulting in its gradual dissolution. Strasburger 1902 reported enzymatic cell wall lysis in the epidermis, helping in the opening of the stomium. Interestingly, in *Leucaena leucocephala,* the epidermal cells that border the stomium enlarge as observed in *Gasteria verrucosa* (Keijzer 1987). In the anther,

the stomium faces both the inward and outward forces which act in the opposite directions. The radial stretch of endothecial cells widens the distance between enlarging epidermal cells and the rigid, inner tangential walls of the endothecial cells. The combination of a flexible outer tangential and a rigid inner tangential walls of the endothecium are responsible for the inward force of the locule walls. At the time of dehiscence, the force outside the anther is strong, resulting in the outward bending of the epidermal cells. In *Leucaena leucocephala,* the inward force of the endothecium opens the stomium, keeps the locule closed and thus prevents premature loss of pollen. Due to an increase of the cytoplasm (WILLEMSE 1972) and the disappearance of the locular fluid (KEIJZER 1983), the pollen mass expands to fill all of the space inside the anther locule. In *Leucaena leucocephala,* the anther locule is bordered by the Übisch bodies. HESLOP-HARRISON 1968 and HESLOP-HARRISON & DICKINSON 1969 suggest that the Übisch bodies might form a non-wettable surface on the locular inside, effecting an easy dispersal of pollen. The endothecium helps to maintain the pollen pressure after the stomium opens.

References

FEDER N. & O'BRIEN T. P. 1968. Plant microtechnique: Some principles and new methods. – Am. J. Bot. 55: 123–142.

FISHER D. B. 1968. Protein staining of ribboned epon sections for light microscopy. – Histochemie 16: 92–96.

HESLOP-HARRISON J. 1968. Pollen wall development. – Science 161: 230–237.

— & DICKINSON H. G. 1969. Time relationships of sporopollenin synthesis associated with tapetum and microspores in *Lilium.* – Planta 84: 199–214.

KEIJZER Ç. J. 1983. Hydration changes during anther development. – In: MULCAHY D. L. & OTTAVIANO E. (eds.), Pollen, biology and implications for plant breeding. – Elsevier Biomedical, New York, pp 197–201.

— 1985. Anther development of *Gasteria.* – In: WILLEMSE M. T. M. & van WENT J. L. (eds.), Sexual reproduction in seed plants, ferns and mosses. – Pudoc Wageningen, p. 20.

— 1987. The process of anther dehiscence and pollen dispersal. I. The opening mechanism of longitudinally dehiscing anthers. – New Phytol. 105: 487–498.

MAHLER H. R. & CORDES E. H. 1971. Biological Chemistry. – Harper and Row Inc. New York.

STRASBURGER, E. 1902. Ein Beitrag zur Kenntniss von Ceratophyllum submersum. – Jahrb. wiss. Bot. 37: 477–524.

TEPPER H. B. & GIFFORD E. M. Jr. 1962. Detection of ribonucleic acid with pyronin. – Stain Technol. 37: 52–53.

WILLEMSE M. T. M. 1972. Morphological and quantitative changes in the population of cell organelles during microsporogenesis of *Gasteria verrucosa.* – Acta bot. neerl. 21: 17–31.

WOYCICKI Z. 1924. Recherches sur la déhiscence des anthères et le rôle du stomium.- Rev. gén. Bot. 36: 196–212.