

## Solubility differences in sporopollenin of pollen and pollinial walls

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Pollen grains of some asclepiads are aggregated and enclosed in a sporopollenin-containing pollinial wall that extends in between the enclosed pollen grain walls but does not fuse with them. The pollinial walls are generally soluble in hot 2-aminoethanol and thus behave like the ectexine of pollen grains in solubility. However, in *Tylophora*, the pollinial wall is soluble only at the bud stage, but as the pollinium matures, the inner region of the pollinial wall becomes insoluble, recalling the character of pollen endexine. The nature and cause of solubility-insolubility changes of sporopollenin related to ageing need further study.

In some members of the Asclepiadaceae, pollen grains are aggregated and enveloped to form a definitive sac called the pollinium. The pollinial wall, like the pollen exine, is composed of sporopollenin<sup>1,2</sup>. Two types of sporopollenin are recognized<sup>3</sup> in pollen exine: hot 2-aminoethanol-soluble groups of the ectexine and insoluble groups of the endexine. With this background we have examined the structure of the wall of pollinia and polyads, and the possible occurrence of 2-aminoethanol-soluble and insoluble regions similar to those of pollen exine.

TEM studies of the pollinia of *Telosma* and *Calotropis* show that the pollinial wall is homogeneous, in contrast to the distinct exine and intine zones of the walls of pollen grains enclosed by them. Interestingly, the pollinial wall extends in between the pollen walls of the aggregated pollen grains but does not fuse with them (Figure 1). As for polyads, there is no separate covering wall around the pollen aggregates but in those examined here the ectexines of component pollen grains are fused to form a composite body.

The action of hot 2-aminoethanol on the pollinial walls of *Asclepias*, *Calotropis*, *Daemia*, *Dregea*, *Telosma* and *Tylophora* (members of the Cynanchoideae) was studied, with pollen grains of four non-asclepiadaceous genera,—viz. *Cosmos*, *Ipomoea*, *Lantana* and *Vinca*, as controls.

The ectexine of the pollen grains of the controls dissolves in 2-aminoethanol at 70–80°C within 10–45 min depending upon the species. Treated pollen grains lose the architectural outer wall and become smooth-surfaced but the continuity of the wall is maintained and the pollen contents are retained. In contrast, the pollinial walls of the asclepiad genera, with the exception of *Tylophora*, dissolve in hot 2-aminoethanol.

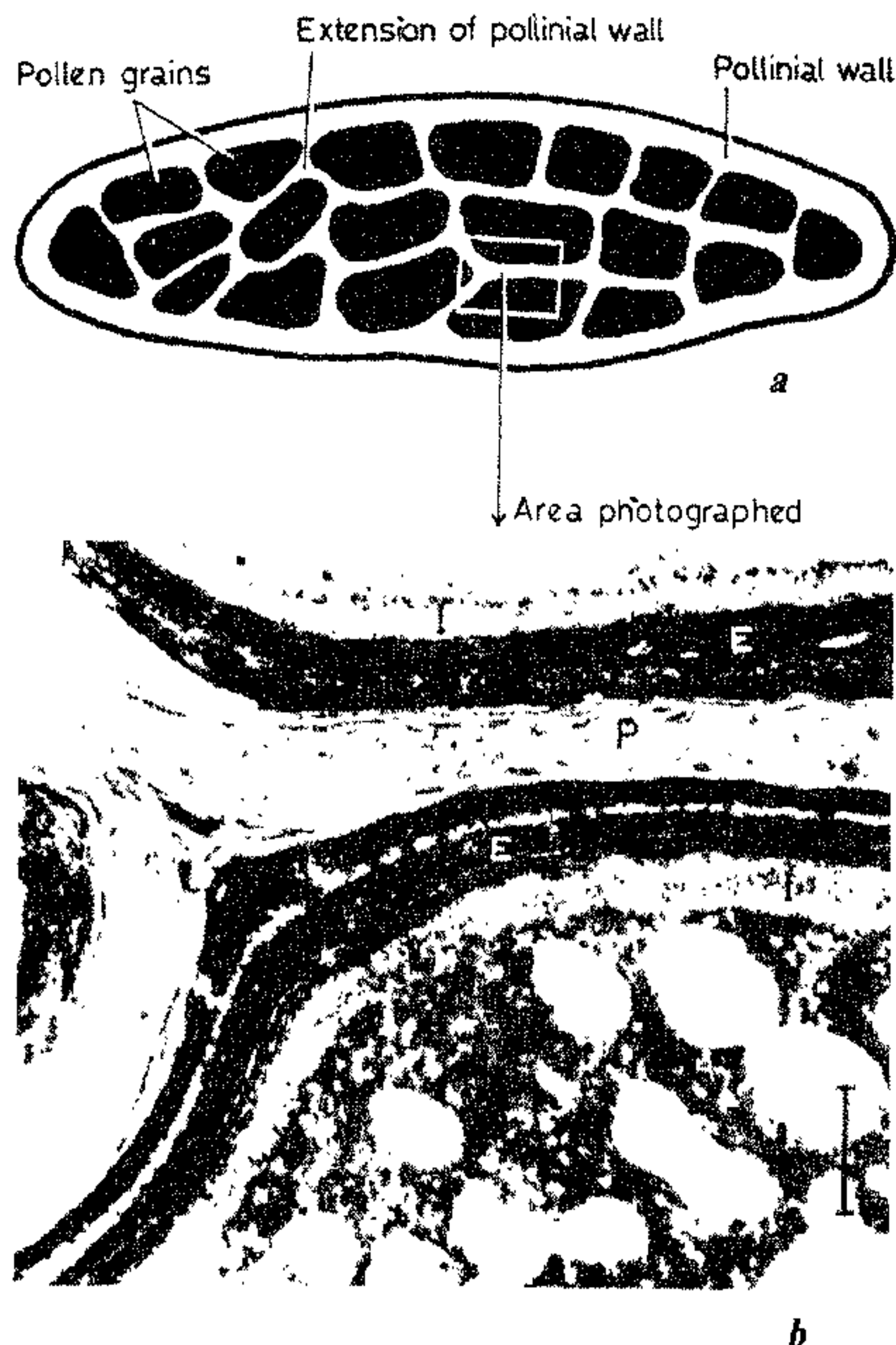


Figure 1. *a*, Drawing of a cross section of the pollinium of *Telosma*. *b*, Electron micrograph of the portion marked in A. [E, exine; I, intine; P, extension of the pollinial wall.]

During treatment, in addition to the pollinial wall, the inner extensions of the pollen grains also dissolve, with the result that individual grains become easily separated.

In regard to solubility, the pollinial wall of *Tylophora* demonstrates an apparent similarity with the pollen exine. The outer pollinial wall materials are lost as globules in the initial stages of treatment. However, even after 45 min in hot 2-aminoethanol, the surviving part of the pollinial wall retains its continuity, like the endexine.

The effect of hot 2-aminoethanol on pollen associations in polyads was also investigated. In *Acacia* and *Calliandra* of the Mimosoideae and in several members of the periplocoideae examined, the pollen grains are aggregated to form polyads, with the number of constituents varying from four in *Cryptostegea* to more than 30 in *Hemidesmus*. Hot 2-aminoethanol generally separates the pollen grains in all these associations. However, in mature pollen associations of *Hemidesmus*, the pollen grains are dissociated up to the level of tetrads only in contrast to young polyads which yield individual pollen grains after treatment.

The present observations confirm that hot 2-aminoethanol dissolves ectexine of pollen grains as

already reported by Southworth<sup>3</sup>. Further, the pollinal walls of asclepiads behave like the ectexines of pollen grains as far as solubility is concerned. Expectedly, the cementing materials of polyads, representing fused ectexines, also dissolve in hot 2-aminoethanol.

In a few systems, solubility of sporopollenins in hot 2-aminoethanol is related to age. In young pollinia of *Tylophora* (up to 4 days before dehiscence) the pollinal wall disintegrates after treatment and the pollen grains are dissociated easily. However, in mature pollinium only the outer portion of the pollinal wall dissolves. A similar situation seems to prevail in the pollen grains of the species used as controls. Hot 2-aminoethanol dissolves the entire wall of pollen grains up to 4 or 5 days before dehiscence. At this stage, ornamentation on walls of pollen grains is discernible and the walls respond positively to tests of sporopollenin-specific solvent of fused KOH. In the mature wall also both ectexine and endexine respond to tests of sporopollenin; but only the ectexine of the mature pollen grain dissolves in hot 2-aminoethanol.

Southworth<sup>4</sup> reported the existence of three different groups of sporopollenin: soluble (ectexine), insoluble (endexine) and soluble when young but insoluble when aged. She had not examined pollinal walls. *Tylophora* illustrates a condition in which the mature pollinal wall has soluble and insoluble regions and in which soluble sporopollenin becomes insoluble as the bud develops to a flower.

The nature and cause of solubility-insolubility changes of sporopollenin of pollen walls associated with ageing are interesting aspects for further study.

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2. Vijayaraghavan, M. R. and Shukla, A. K., *Ann. Bot.*, 1976, 40, 417.
3. Southworth, D., *Am. J. Bot.*, 1985, 72, 1274.
4. Southworth, D., *Am. J. Bot.*, 1974, 61, 36.

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## High yielding preparation of viable protoplasts from hypocotyls of *Sesamum indicum* L.

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Viable protoplasts from hypocotyl explants of *Sesamum indicum* L. var. T-13 were successfully established. Isolation was best done with combination of Cellulase 1.5% (w/v) and Macerozyme 0.5% (w/v) in presence of mannitol (0.6 M) and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.2%) as osmotic stabilizers at pH 5.8. Freshly isolated protoplasts were colourless, spherical and of various sizes.

PLANT protoplasts are becoming an increasingly important experimental system for plant biologists. Currently, such isolated cells find application in basic morphological and physiological studies, plant propagation and in the production of somatic hybrids. Fundamental to all these approaches is the requirement to obtain consistently high yields of viable protoplasts. In the present communication we report successful isolation of viable protoplasts from hypocotyl explants of *Sesamum indicum* L. var. T-13.

Hypocotyl explants were taken from ten-day-old aseptically grown seedlings raised from seeds procured from Agriculture Research Station, Mandore (Jodhpur). Seedlings were grown on 0.6% agar containing half strength salts of Murashige and Skoog's<sup>1</sup> basal medium with sucrose (1%). The release of protoplasts is dependent on a large number of factors which have been standardized for the first time in *Sesamum indicum*, an oilseed crop of major importance in India.

Best results were obtained when hypocotyl segments measuring 1.5 cm were incubated with cell wall-degrading enzymes (Cellulase Onozuka R 10, Kinki Yakult Co., Japan; Macerozyme Onozuka R 10, Kinki Yakult Co., Japan; Drieselase, Hakko Kogyo Co., Japan).

Table 1 enumerates the various enzyme combinations tried. For isolation of protoplasts from hypocotyl segments of *Sesamum indicum*, the best enzyme combination was Cellulase 1.5% (w/v) and Macerozyme 0.5% (w/v), when mannitol (0.6 M) and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (0.2%) were used as osmotic stabilizers. The pH of the enzyme solution was adjusted to 5.8. No pretreatment was given to the explants since it did not prove effective in improving the results. After 4 h of incubation at 30°C in dark, the digested tissue was sieved to remove debris and the filtrate was collected in 15 ml screw cap centrifuge tubes. Centrifugation was done for 5 min at 100 g. After removal of the supernatant with a pasteur pipette, the protoplast pellet was suspended in a washing solution, containing 0.6 M mannitol and 0.2%  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ . This was centrifuged at 100 g for 5 min and the procedure was repeated thrice to remove all the traces of enzyme which proved deleterious for the protoplasts, if left for long. Washed protoplasts were resuspended in 20% (w/v) sucrose solution, to form a density gradient and again centrifuged at 100 g for 5 min. Protoplast band was removed using a pasteur pipette. Purified protoplasts were resuspended in

Table 1. Enzyme combinations tried for release of hypocotyl protoplasts of *Sesamum indicum* L.

Enzyme	Concentration (%)				
Cellulase	3.0	1.5	1.0	1.0	0.5
Macerozyme	1.0	0.5	0.5	1.0	2.0
Drieselase	—	—	0.2	—	—