already reported by Southworth[3]. Further, the pollinial 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 pollinial wall disintegrates after treatment and the pollen grains are dissociated easily. However, in mature pollinium only the outer portion of the pollinial 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[4] 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 pollinial walls. *Tylophora* illustrates a condition in which the mature pollinial 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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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 $CaCl_2 \cdot 2H_2O$ (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[1] 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 $CaCl_2 \cdot 2H_2O$ (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% $CaCl_2 \cdot 2H_2O$. 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	—	—

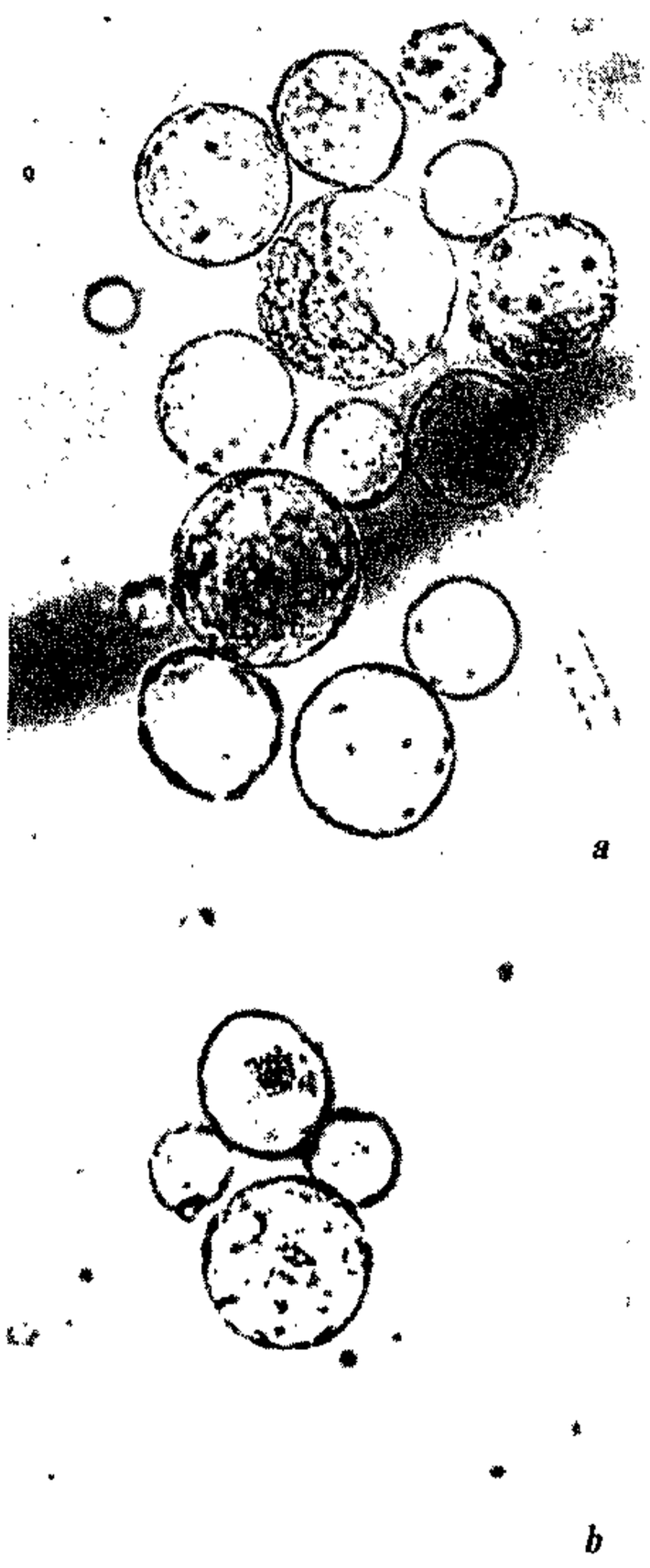

Figure 1. Freshly isolated protoplasts of *Sesamum indicum* L. var. T-13.

different culture media at a density of 1 ml suspension per 2 ml of liquid media.

Freshly isolated protoplasts were colourless spherical and of various sizes with a distinct nucleus and cytoplasmic strands (Figure 1).

In the present investigation, hypocotyl segments were found to be the best source of rapid isolation of protoplasts. Earlier workers have also found hypocotyls to be a good source of stable protoplasts well suited for growth, fusion and regeneration[2-5]. Roy and De[6] also found hypocotyls to be the most ideal material for protoplast isolation with regard to rapid division and high plating efficiency.

Crop improvement using genetic engineering requires the execution of several procedures which are difficult to accomplish in the required sequence in any economic species. Plant protoplasts would undoubtedly play an important role in the rapidly emerging plant biotechnology for crop improvement especially in the case of oilseeds which are known to possess limited genetic variability. Hence further work on the culture of the isolated protoplasts of *Sesamum indicum* would be interesting and rewarding.

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Isolation and characterization of an aflatoxin-inhibiting metabolite from *A. niger*

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The factor from *Aspergillus niger* that inhibits aflatoxin production by *A. flavus* was purified from culture broth and identified as oxalic acid. The inhibition was verified by using authentic samples of oxalic acid.

THE interaction of *A. niger* with *A. flavus* against production of aflatoxin has been reported by several workers[1-3]. Horn and Wicklow[3] observed that lowering of pH is one of the factors suppressing aflatoxin production. They also speculated on the production of an antimetabolite by *A. niger* causing additional inhibition. The isolation, purification and characterization of the aflatoxin-inhibiting metabolite secreted by *A. niger* in liquid synthetic medium are described here.

A. flavus (F_2) and *A. niger* cultures were isolated in this laboratory, and maintained on Czapek-Dox agar and potato dextrose agar slants respectively at 6–8°C. Spores were obtained by subculturing on the agar slants and incubating at 28°C for 5 days. To prepare the crude metabolite, 1 ml distilled water suspension of the *A. niger* culture (0.7×10^6 no./ml) was dispersed into sterile Czapek-Dox-casein[4] medium and incubated at 28°C for seven days under stationary culture conditions. The mycelial mat was filtered off through a cheese cloth. The filtrate was concentrated under reduced