Among the various growth regulators tested 2,4-D (2–4 mg/l) in combination with Kn (0.5–1 mg/l) has been found to be the most effective and the frequency of protoplast division ranged from 2.0% to 3.2% (table 1). Both auxins and cytokinins are required for the protoplasts to divide as cell divisions were either poor or not recorded in media supplemented with auxin or cytokinin singly.

Financial assistance from the Indian National Science Academy, New Delhi is gratefully acknowledged.

11 April 1986; Revised 17 June 1986

1. Bhojwani, S. S., Evans, P. K. and Cocking, E. C., *Euphytica*, 1977, **26,** 343.
2. Gamborg, O. L. and Wetter, L. R. (eds), *Plant tissue culture methods*, NRC Canada, Saskatoon, 1975.
3. Thomas, E., King, P. J. and Potrykus, I., *Z. Pflanzenzuchtg.*, 1979, **82,** 1.
4. Motoyoshi, F., *Exp. Cell Res.*, 1971, **68,** 452.
5. Evans, P. K., Keates, A. G. and Cocking, E. C., *Planta*, 1972, **104,** 178.
6. Maeda, E. and Hatwara, T., *Proc. Crop. Sci. Soc. Jpn.*, 1974, **43,** 68.
7. Koblitz, H., *Biochem. Physiol. Pflanzen.*, 1976, **170,** 287.
8. Nemet, G. and Dudits, D., In: *Use of tissue cultures in plant breeding*, (ed.) J. Novak, Czech. Acad. Sci., Prague, 1977, 145.
9. Chi-Kuei, T., Ying-Ch'ien, C., Yun-Lo, C. and Su-Hsuen, W., In: *Proc. symp. on Plant tissue culture*, (ed.) Hu Han, Science Press, Peking, 1978, p. 317.
10. Potrykus, I., Harms, C. T. and Lorz, H., *Theor. Appl. Genet.*, 1979, **54,** 209.
11. Toriyama, K. and Hinata, K., *Plant Sci.*, 1985, **41,** 179.
12. Deka, P. C. and Sen, S. K., *Mol. Gen. Genet.*, 1976, **145,** 239.
13. Vasil, V. and Vasil, I. K., *Theor. Appl. Genet.*, 1980a, **56,** 97.
14. Bandyopadhyay, S. and Ghosh, P. D., *Life Sci. Advances*, 1986, (In press).
15. Murashige, T. and Skoog, F., *Physiol. Plant*, 1962, **15,** 473.
16. Nagata, T. and Takebe, I., *Planta*, 1970, **92,** 301.

SYNCHRONOUS FORMATION OF COREMIA IN *CHALARA* SP

M. S. S. MOHAN and R. N. SWAMY
Centre for Advanced Studies in Botany, University of Madras, Madras 600 025, India.

COREMIA of fungi are induced by various physical and chemical stimuli. While light is required for induction of coremia in species of *Penicillium*[1], in *Ceratocystis ulmi*, the presence of terpenes or linoleic or other unsaturated fatty acids in the medium is required[2–4]. Watkinson[5] reported that glutamic and aspartic acids are stimulatory in the case of *Penicillium claviforme*. In *Stilbella thermophila*, thiamine and biotin are required for induction of coremia[6].

A method for induction of synchronous formation of coremia in the *Chalara* state of *Ceratocystis fimbriata* isolated from coconut kernel is described in this note. Because of its very high growth rate, this fungus seems to be an ideal material for investigation of the biochemical events associated with the formation of coremia in fungi.

A sterile water suspension of spores was prepared from a 48 hr old culture on potato dextrose agar (PDA). The spore concentration was adjusted to 2×10^6 spores/ml. About 0.5 ml of this suspension was spread on cellophane discs (8 cm dia) overlying PDA medium in 9 cm petridishes. The inoculated plates were incubated for 24 hr at 24°C in the dark. The cellophane discs were then lifted off the medium and transferred to Czapek-Dox agar medium (CDA) (pH 7.0) in petridishes and incubated for 24 hr at 24°C in the dark.

It was observed that initials of coremia as aggregations of hyphae were discernible within 5 hr of transfer to CDA medium all along the edge of the cellophane disc (figure 1a). The initials developed rapidly and a ring of fully mature coremia bearing a glistening globular mass of spores at their tips could be seen after 20–24 hr (figure 1b). The mature coremia were about 1 mm in height.

The CDA medium is not suitable for the growth of this fungus. When the thick-walled spores (those not formed on coremia) of *Chalara* are placed on CDA medium, they do not even germinate. When coremial spores are used, they germinate producing spores straightaway without vegetative growth (microcyclic sporulation). The second medium (CDA as well as the physical act of transferring the growth onto it, both seem to influence formation of

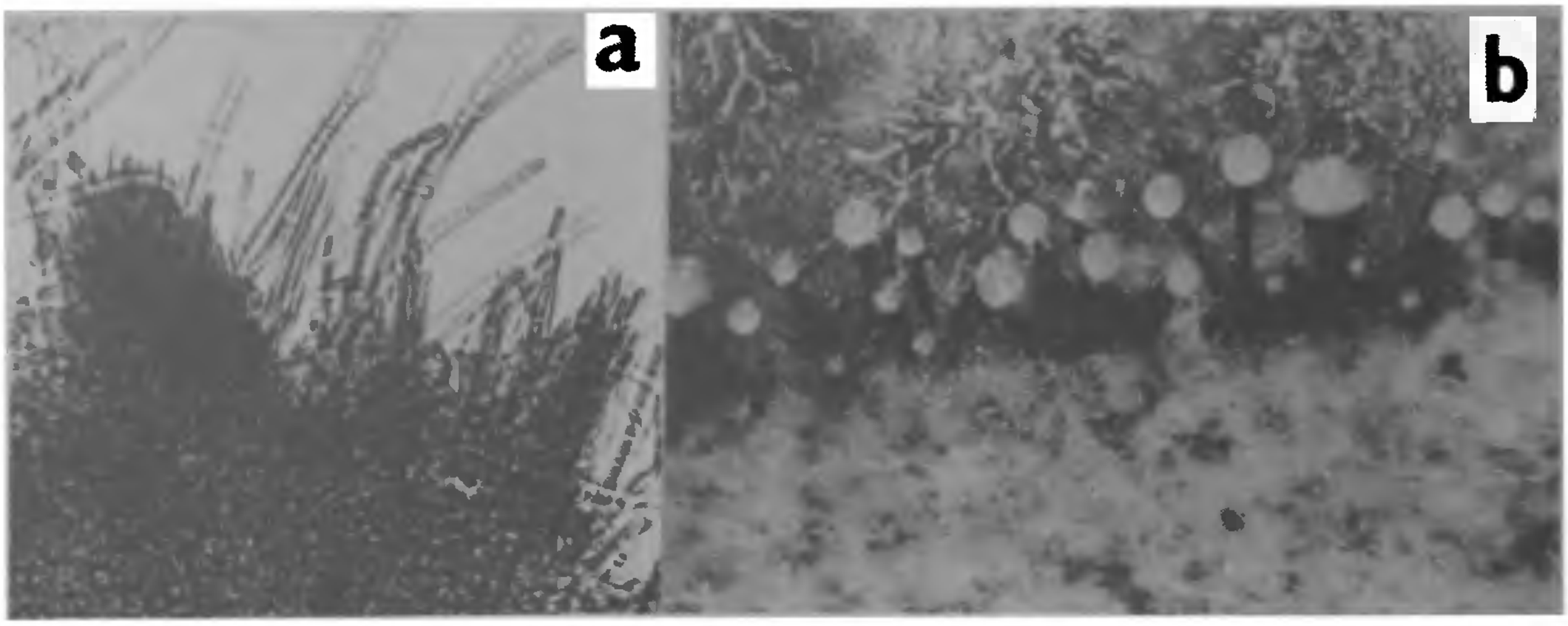

Figure 1. *Chalara* state of *Ceratocystis fimbriata*. **a.** Aggregation of hyphae in preparation for formation of coremia (5 hr after transfer to Czapek medium) (×250). **b.** Ring of coremia along margin of cellophane disc (×10).

coremia. If the transfer is made to a second plate of PDA, coremia are not formed. If the nitrate is replaced by ammonium salts in CDA, more profuse production of coremia occurs but these are loose and feathery and not compact as on nitrate. This shows that the second medium influences the formation of coremia. If a centrally inoculated cellophane disc on PDA is transferred to CDA before the growth has reached the margin of the disc, coremia are not formed suggesting that the disturbance of the growing region of the fungus at the edge of the cellophane also has a role in induction of coremia. Coremia fail to form at temperatures higher than 24–25°C.

11 April 1986; Revised 10 July 1986

1. Carlile, M. J., Dickens, S. W., Mordue, E. M. and Schipper, M. A. A., *Trans. Br. Mycol. Soc.*, 1962, **45,** 457.
2. Hubbes, M., *Eur. J. For. Pathol.*, 1975, **5,** 129.
3. Dalpe, Y. and Neumann, P. J., *Can. J. Bot.*, 1977, **55,** 2159.
4. Bays, D. C. and Hindal, D. F., *Mycologia*, 1982, **74,** 625.
5. Watkinson, S. C., *J. Gen. Microbiol.*, 1977, **101,** 269.
6. Al-Hassan, K. K. and Fergus, C. L., *Can. J. Microbiol.*, 1967, **13,** 351.

CYTOMIXIS DURING MICROSPOROGENESIS IN C_{13} COLCHITETRAPLOID POPULATION OF *MELILOTUS ALBA* DESR

JAYA SINGH and S. S. RAGHUVANSHI
Botany Department, Lucknow University, Lucknow 226 007, India.

As a part of the *Melilotus alba* (sweet clover) breeding programme colchitetraploids were raised by 0.2% aqueous colchicine treatment. Intensive selection has been carried out during subsequent generations. Based on the observation of C_{13} plants, the phenomenon of cytomixis is being reported for the first time in this species.

Cytomixis was observed in 6.7% plants and the mean percentage of cytomictic PMCs in cytomictic plants was 13.6. It was observed at various meiotic stages but frequently at prophase I and metaphase I (figures 1 and 2). At prophase a continuous flow of chromatin was frequently observed through a series of cells (figure 1).

Gates[1] defined cytomixis as the migration of chromatin from one cell to another. It was observed in hybrids, apomicts and chemically treated plants[2–4] besides its reports from normal plants[5, 6]. Despite its occurrence from a large number of genera its cytological causes and significance are still unexplained. Several interpretations like change and disturbances in hydrostatistical state sporogenous tissue[7], disturbances in the nucleocytoplasmic relationships[8] or unknown physiological disturb-