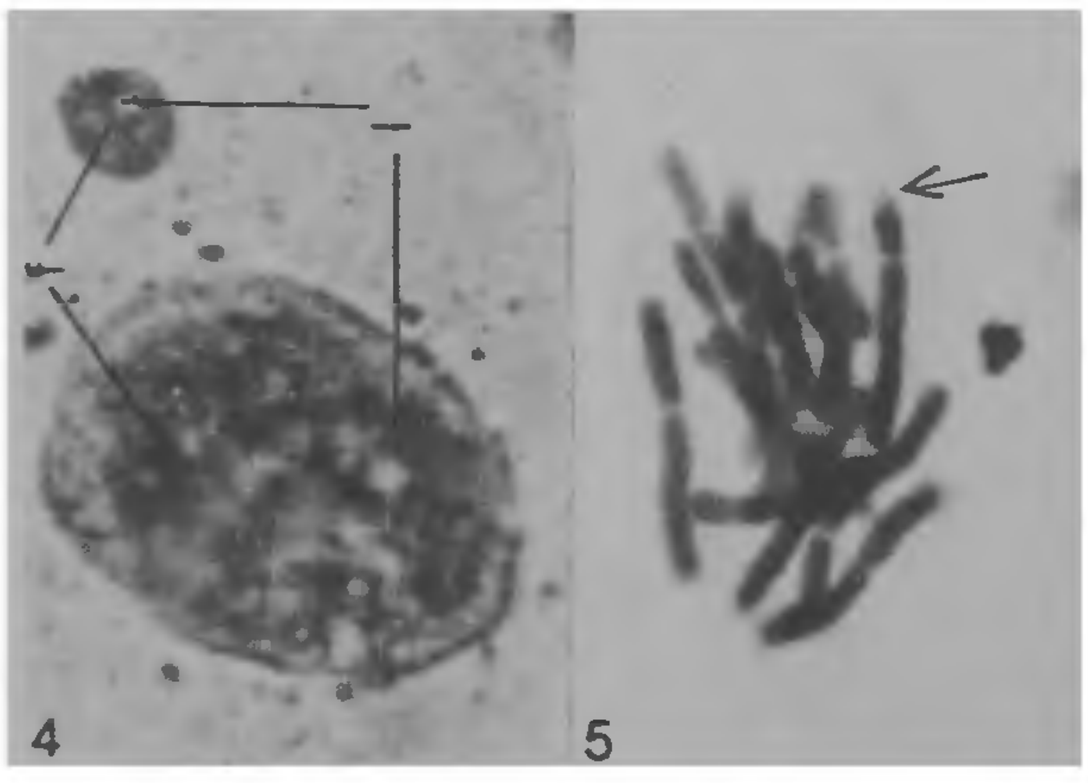
This type of nucleolar chromosome does not belong to any of the six types described in the genus *Allium* by Ved Bratt[6]. Bozzini[1] reported that the nucleolar chromosome present in the variety is of *fistulosum* type. It seems that the *cepa* type nucleolar chromosome is inactive in these cells. However, staining of the nucleolus by the silver staining technique[3] reveals the presence of a big nucleolus having the average area 2.01 μm^2 and a very small nucleoli having the average area 0.19 μm^2 (figure 2). Earlier Bozzini[1] reported the presence of only one nucleolus per cell. Perhaps the small nucleolus could not be detected by the technique used by him. We also failed to detect the small nucleolus by using the AgNOR staining technique of Varley[4] (figure 3). Howell and Black[3] technique seems to be much more sensitive in staining the nucleolus. In some bigger cells, the small nucleolus is relatively bigger in size perhaps due to their polytenic nature[7] and clearly shows the typical nucleolar structure as lacunae, deep and light stained areas (figure 4) after silver staining. If we associate the origin of the big nucleolus with the *fistulosum* type of nucleolar chromosome, the origin of the small nucleolus should then be associated with a *cepa*-type nucleolar chromosome. Indeed in some of the less condensed chromosomes it was possible to detect the *cepa*-type nucleolar chromosome with a rudimentary satellite stalk (figure 5). It is clear that the satellite and possibly a part of the stalk is deleted from this chromosome. As such this chromosome cannot be detected readily (figure 1).



Figures 4 and 5. Nucleoli from polytenic cell after silver staining. Note the presence of lacunae (l), deeply-stained fibrillar regions (f), and light stained regions in both the large and small nucleoli. **5.** *Cepa*-type of nucleolar chromosome with terminal NOR. Note the deletion of the satellite.

Earlier it has been established that ribosomal cistrons are located on the satellite stalk of *A. cepa* nucleolar chromosomes[8]. The development of a small-sized nucleolus may be caused by the deletion of a part of the satellite stalk. These observations on the karyotype, nucleolar chromosomes and nucleoli support the hybrid origin of *A. fistulosum* var *viviparum*. It also indicates that the ribosomal cistrons present in the *cepa*-type nucleolar chromosome are not repressed by the cistrons present in the *fistulosum* chromosomes.

8 April 1986; Revised 16 June 1986

1. Bozzini, A., *Caryologia*, 1964, **17,** 459.
2. Vosa, C. G., *Heredity*, 1976, **36,** 383.
3. Howell, W. M. and Black, D. A., *Experientia*, 1980, **36,** 1014.
4. Varley, J. M., *Chromosoma* (*Berl.*), 1977, **61,** 207.
5. Battaglia, E., *Caryologia*, 1957, **10,** 1.
6. Ved Bratt, S., *Chromosoma* (*Berl.*), 1965, **16,** 486.
7. Chaudhuri, M. and Ghosh, S., *Sci. Cult.*, 1982, **48,** 151.
8. Ghosh, S. and Chaudhuri, M., *Cell Biol. Int. Rep.*, 1982, **6,** 147.

CELL DIVISIONS IN NEWLY FORMED CELLS FROM LEAF MESOPHYLL PROTOPLASTS OF WHEAT (*TRITICUM AESTIVUM* CV SONALIKA)

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PLANT protoplasts have now become an extremely popular tool to plant scientists for their various and diverse nature of applications in studies encompassing both fundamental and applied aspects[1, 2]. But unfortunately only limited success has so far been achieved while culturing the cereal protoplasts, particularly the economically important ones due to their poor response to *in vitro* culture techniques[3]. The perusal of literature reveals that cereal protoplasts can swell, regenerate a cell wall and remain viable for a considerable period or divide occasionally in culture media[4–6]. Callus formation (though mostly from the cultured cells) has been

reported in some species recently[7–11], but there is no further development thereafter. Root/shoot differentiation from calli derived from cereal protoplasts has been reported only in a few cases[12, 13].

Conditions have already been standardized for successful isolation of protoplasts from the leaf mesophyll tissues of wheat (*Triticum aestivum* cv sonalika) and other cereals[14]. The present paper is a report on the studies of cell divisions in newly formed cells from leaf mesophyll protoplasts of wheat (*T. aestivum* cv sonalika).

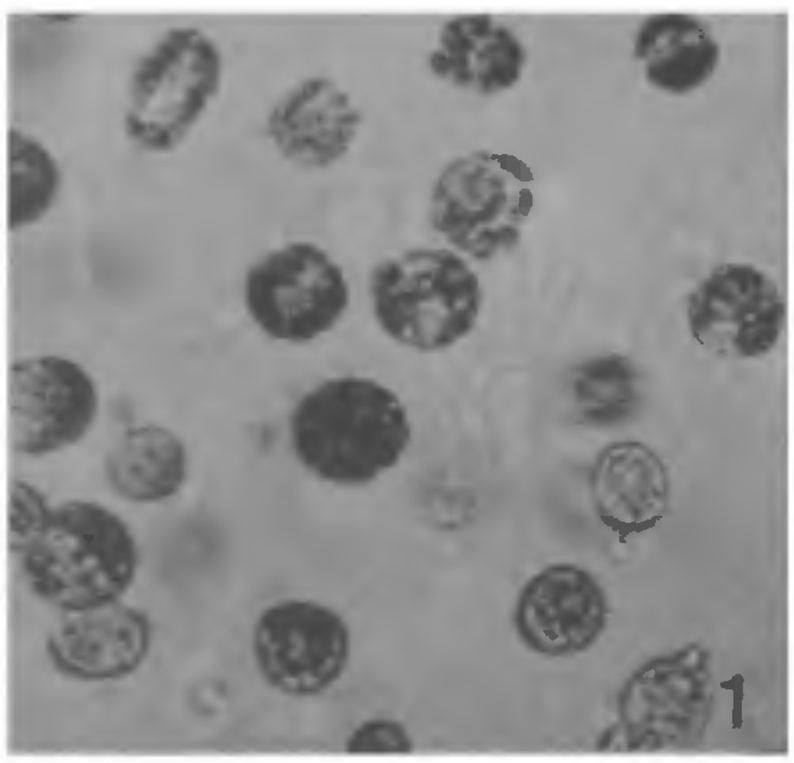
Healthy seeds of *T. aestivum* cv sonalika were surface–sterilized with 0.1% mercuric chloride for 5 min, washed thoroughly with sterile water and germinated in aseptic condition on Murashige and Skoog's (MS)[15] basal medium (minus hormone). The hazardous and time-consuming process of surface sterilization was thus avoided. The ideal isolation procedures should be rapid to minimize the stress conditions experienced by the tissues during isolation. This also probably helps in pre-conditioning the tissues to the 'would-be environment' prior to culture. Initially, 4–6 day old leaves were cut at the base and apical 0.5–1.0 cm region discarded. The rest of the leaves were diced into 0.5 cm^2 pieces, washed in sterile water and pre-plasmolyzed in 0.6 (M) mannitol and 10 mM $CaCl_2$ for about 1 hr. Then the mannitol-salt solution was removed and replaced by a filter-sterile enzyme solution in 0.6 (M) mannitol (pH-5.6) containing 2–2.3% (w/v) Cellulase Onozuka R-10 (Kinki Yakult, Japan), 0.5–1.0% (w/v) Macerozyme R-10 (Kiniki Yakult, Japan) and 0.5–1.0% (w/v) Hemicellulase (Sigma). The digestion was allowed to proceed for about 4–5 hr at 25 ± 1°C in stationary conditions with occassional stirring. Leaf residues were removed by passing the protoplast-enzyme solution through a 100 μm nylon mesh. Protoplasts were recovered by floating the viable ones to the surface in 20% sucrose solution. The remains of the contaminating debris were removed through repeated centrifugation at low speed (50–60 × g) and finally the protoplast suspension (1.5 ml) were plated on an equal volume of MS nutrient medium (pH-5.6) at 40–45°C containing 0.6% agar agar (BDH) fortified with different growth hormones like 2,4-D (2,4-Dichlorophenoxyacetic acid), NAA (Naphthaleneacetic acid), Kn (Kinetin) and BAP (6-Benzyl amino purine) in different concentrations (0.5–4 mg/l) and combinations (table 1) in Falcon petridishes (35 mm) at a density of about $1–3 \times 10^5$/ml. The dishes were sealed and incubated in the dark for 24 hr at 25°C, then for the next 2 days under diffused light and finally under 400–500 lux of continuous light.

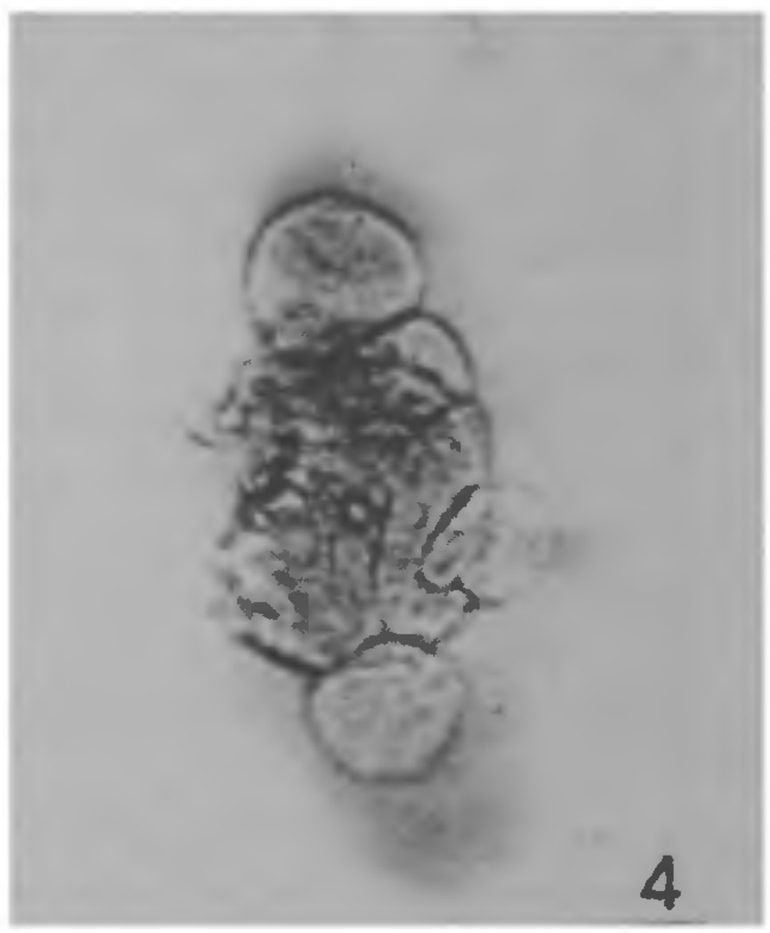
Table 1 *Effect of growth hormones on division of wheat mesophyll protoplasts cultured on MS media*

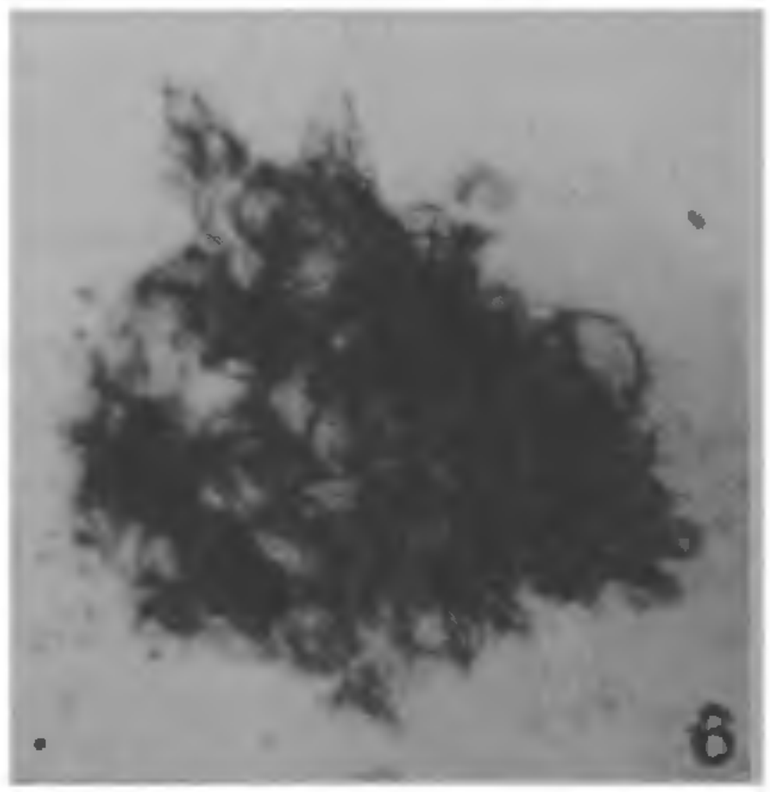
Source	Hormone conc. (mg/l) 2,4-D	Kn	NAA	BAP	Frequency of division* (%)
Triticum aestivum cv sonalika	1	–	–	–	1.8
	2	–	–	–	1.6
	2	0.5	–	–	2.0
	4	1	–	–	3.2
	2	–	–	0.5	0.4
	–	–	–	1	0
	–	–	1	–	0.1
	–	–	1	1	0.23
	2	0.5	0.5	–	2.2

* Estimated as a % of original number of protoplasts after 3 weeks in culture.

Protoplasts freshly isolated (figure 1) showed high percentage (ca. 80–90%) of viability as judged by fluorescein diacetate (FDA) staining. Within 24–48 hr the protoplasts were found to exhibit an initial swelling, reorientation of plastids and regeneration of a new cell wall. A few of them were found to get lysed. Cell wall regeneration was studied by using calcofluor white ST as a stain for cellulose[16]. Within 7–8 days, first typical cell division took place (figure 3), preceded by cell plate formation (figure 2). Some of the dividing protoplasts continued their divisions while some ceased to divide after 1–2 initial divisions and some others exhibited only budding (figure 4). After 2–3 weeks cell colonies, creamy-white in colour, were observed (figure 5). At this stage the colonies were transplanted on a fresh MS medium (with the same hormone/s as before) with lower osmoticum [0.4–0.5 (M)] when some further divisions were observed (figure 6). The cells, however, remained viable for a long time without any further development thereafter. Interestingly, it was noted that only the smaller protoplasts (diameter ranging between 8 and 20 μm) continued proliferation and produced cell colonies/clusters, while the larger protoplasts (diameter 21 μm and above) ceased to divide after the first one or two divisions or only exhibited budding. So the size of the protoplasts showed an inverse correlation with their ability to divide.







Figures 1–6. 1. Isolated mesophyll protoplasts of wheat; **2.** Protoplast showing cell plate formation (×300); **3.** First division of a cell regenerated from a protoplast after 7–8 days in culture (×300); **4.** Protoplast showing budding (×300); **5.** Cell colony after 2–3 weeks in culture (×200); **6.** A clump of cell after 40 days in culture (×100).

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.

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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

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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