SURVIVAL OF ANTHER-, AND OVULE DERIVED COTTON CALLUS FROZEN IN LIQUID NITROGEN

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THE conservation of the germplasm by cryogenic methods[1] especially those of the haploid cultures, which are relatively unstable, is of considerable importance. The significance of haploid cell cultures of cotton[2] in mutation and biochemical genetics is evident. In the present communication results on the survival of the callus of haploid and diploid origin frozen in liquid nitrogen (−196° C) are presented.

The flower-buds obtained from the field-grown plants of two species of cotton (*Gossypium hirsutum* L. cv. F 414 and *G. arboreum* cv. G 27) were cut open under sterile conditions in a laminar flow chamber (Klenzaids, Bombay). Ten to twenty anthers, or 5 ovules were cultured per tube on Murashige and Skoog's[3] and Schenk and Hildebrandt's[4] medium (SH) supplemented with 2,4-D (5 mg/l). After 10-15 days, the proliferating anthers (Fig. 1A) and ovules (Fig. 1B) were again transferred to SH-medium containing 2,4-D (5 mg l) + 3% dimethylsulfoxide (DMSO) and incubated further for 4 days. About 100 of the proliferating anthers, or 20 of the ovules were pooled in a petri dish on a sterile filter-paper moistened with cryoprotectants (mixture of 5% each of sucrose, dimethylsulfoxide and glycerol), and were maintained for 2-3 hr. During this period, the petri dishes were placed in ice. The specimens were then wrapped up in sterile aluminium foils[5] and were subjected to freezing for 5 minutes. The freezing was achieved by using two methods: (i) the sudden immersion of the speciemen in a cylinder containing liquid nitrogen, and (ii) gradual exposure to vapours of liquid nitrogen, and then lowering them into the cylinder of liquid nitrogen.

After the desired freezing treatment, the aluminium wrappers containing the material were thawed in warm water (35–38°C), and the retrieved anthers and ovules were recultured on SH-medium containing 2, 4-D (5 mg/l). The cultures were incubated at 23–25°C in diffused light. The resumption of proliferation in the retrieved cultures was taken as the only criterion for survival.

The proliferating anthers and the ovules of two species of cotton after exposure to ultra-low temperature (−196°C) have been observed to resume growth, and the results are summarized below.

Of all the media tested, the best growth in terms of callus formation was obtained on Schenk and Hildebrandt's medium, supplemented with 2, 4-D (5 mg/l). In the controlled cultures, the anthers and the ovules initiated proliferation within a week, and after about two weeks, a fair amount of callus was formed (figure 1A). The callus obtained by the bursting of the anther, obviously of pollen origin, was compact, whereas that originating from the parts of the anther other than pollen was relatively loose.

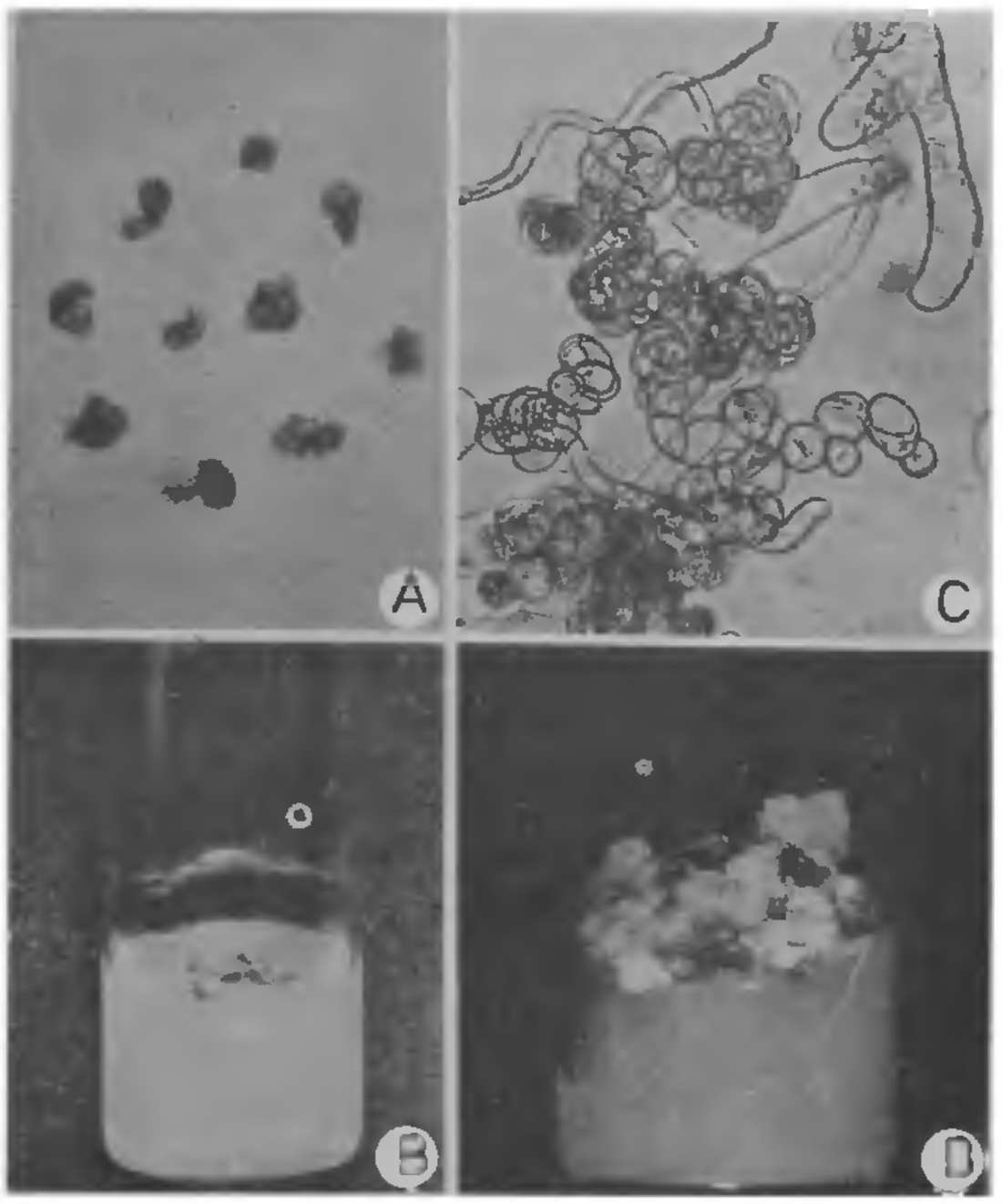

Figure 1A–D. Revival of anther–, and ovule-derived callus of *Gossypium hirsutum and G. arboreum* frozen to –196° C. **A.** Two week-old proliferating anthers at the time of freezing. **B.** Two week-old culture of ovules on a basal medium + 2,4-D (5 mg/ l) at the time of freezing. **C.** A growing suspension of retrieved anther callus cells, showing normal healthy highly cytoplasmic cells. **D.** A mass of callus obtained from proliferating ovule (culture B) of *G. arboreum.*

The growth responses of the callus, as already reported for other species[6], was genotypically oriented. The callus of *G. arboreum* was fast-growing than that of *G. hirsutum.*

The retrieved proliferating anthers and the ovules (figure 1B) turned brown and did not show any sign of growth for the first 3-4 weeks. The callus then underwent localized activity, and at places the cells, especially at the periphery, resumed growth and formed islets of meristems. A mass of callus was formed in 6-7 weeks (figure 1D).

It was observed that the cultures with little callus revived better than the cultures with masses of callus.

In the latter, the callus became spongy and died. The cell suspension (figure 1C) obtained from the retrieved callus resumed active growth, and contained highly cytoplasmic aggregates of cells.

The effect of various cryoprotectants on the survival of cells is shown in table 1. As can be seen, a mixture of 5% each of sucrose, glycerol and dimethylsulfoxide resulted in higher viability than that obtained from chemicals when used singly. The anther-derived callus was more sensitive than that obtained from the ovules as evidenced by 34% and 42% survival respectively.

The present study confirms the earlier work on the freeze preservation of the anther-derived haploid callus cultures[7] and the androgenic anthers of tobacco, petunia[8] and primula[9]. Furthermore, the mixture of various cryoprotectants proved to be better, as they led to higher survival.

TABLE 1

Effect of various cryoprotectants on survival of tissue cultures of Gossypium arboreum frozen in liquid nitrogen. Data based on the resumption of growth

Cryoprotectant	Survival % of control cultures	
	Anther Callus	Ovule Callus
Sucrose 8%	7	9
Glycerol 10%	15	19
DMSO 10%	29	27
Sucrose + glycerol + DMSO (5% each)	34	42

The freeze storage of plant cells and tissues in liquid nitrogen brings down growth and metabolism to the zero level, and thus prevents or delays the process of ageing. Moreover, plant tissue cultures on prolonged storage at ordinary temperatures undergo various genetic changes, and, in this respect, it is envisaged that freeze preservation would prevent these genetic erosions, and thus would help to maintain clones of cultures that are genetically unstable.

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CHARCOAL ROT OF MANGO

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DURING a survey of the local fruit market of Lucknow in May 1980 a severe charcoal rot was observed on mango var. Baganpalli. The disease appeared from the fruit end and later covered the whole surface of the fruit. Rotting was observed from the pedicle portion internally, shrivelled and showed blackening of the internal tissue which finally bore numerous black oval to irregular small size pycnidia.

The fungus was grown on potoato-dextrose agar medium and incubated at 30° C. The pathogenicity of the fungus was established by routine inoculations on healthy fruits typical symptoms of charcoal rot appeared (figure 1). The fungus was identified as *Macrophomina phaseolina* (Tassi) Goid (IMI. 252129). The disease appears to be a new record on mango. Studies on control measures are in progress.

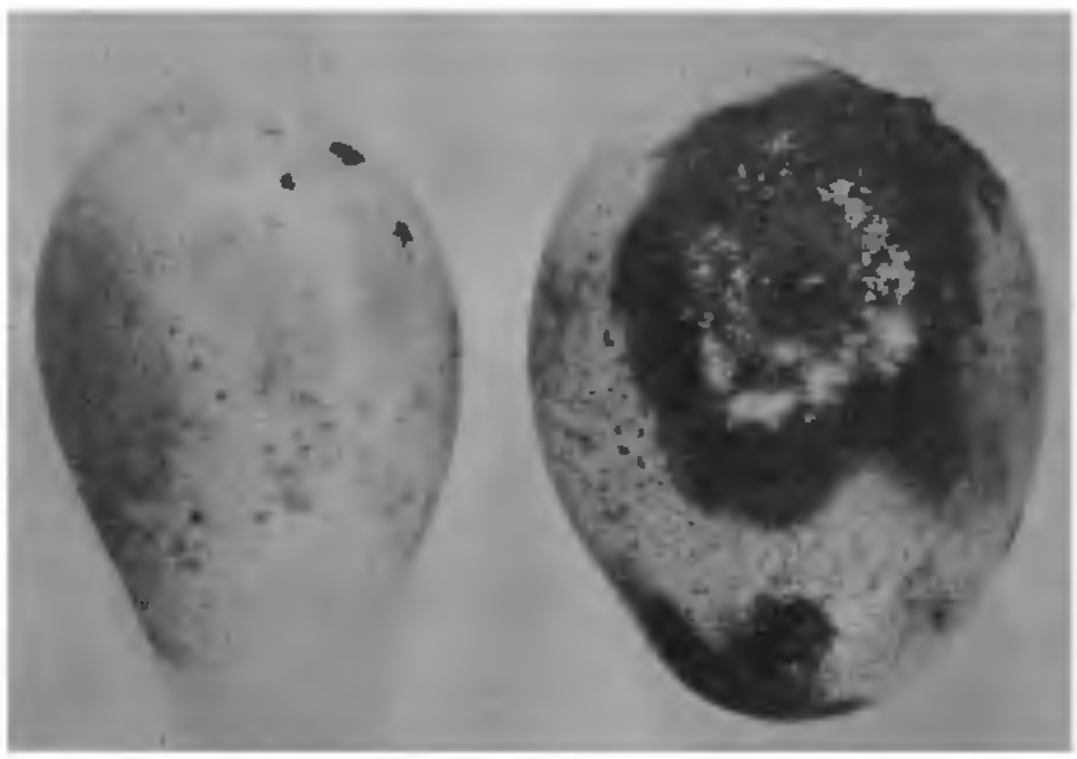

Figure 1. Healthy and *Macrophomina phaseolina* infected mango.

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