GROWTH AND MORPHOGENESIS IN FROZEN (– 196° C) ENDOSPERM AND *EMBRYOS OF RICE*

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THE freeze-preservation of plant cell, tissue, and organ cultures has recently aroused much curiosity for the prevention of the process of ageing and to explore the possibility of long-term conservation of rare and favourable germplasm (Bajaj and Reinert[4]). The subject has been critically reviewed (Bajaj[2]). The present communication deals with the regeneration of complete plants from frozen embryos and endosperm cultures of rice.

Two-year-old seeds of rice (*Oryza sativa* L. cv. B–370—water content 8–11%) were grouped into batches of fifty in plastic ampules, immersed in a cylinder of liquid nitrogen, and were stored. The ampules containing seeds were taken out after 3 weeks and thawed in warm water (35–40° C). To determine the percentage viability of the seeds, some of them were germinated on moist filter-paper in petridishes, whereas the rest were soaked in distilled water for 24 hours. They were then surface-sterilized with chlorine water, dehusked and each seed was transversely cut into halves (one portion contained the embryo along with a portion of the endosperm, the other portion consisted of the endosperm only). The two portions of each seed were cultured on Murashige and Skoog's (MS–1962) medium[5], supplemented with 2,4-D (2 mg/l). All manipulations were conducted under aseptic conditions in a Laminar Flow Chamber (Klenzaids, Bombay), and the cultures were incubated in the dark at 23–26° C. The induction of callus, and the germination were taken as the criteria for survival.

The percentage viability and the growth response of the frozen endosperm and embryo cultures, and of the dehusked seeds are summarized in Table I. As compared with the controls, in the retrieved endosperm, the initiation of proliferation was delayed by a week (Fig. 1). In some cases, callus formation was localised, and a mass of callus (Fig. 2) was formed in 5–9 weeks. When transferred to MS + kinetin (2 mg/l) + IAA (4 mg/l), the callus underwent morphogenesis in about 3% of the cultures to form plantlets (Fig. 3).

The frozen-thawed embryos along with the endosperm started to germinate, and initiated proliferation to form callus (Fig. 4) within a week. The plantlets when transferred to MS medium continued to grow (Fig. 5), and resulted in normal-looking plants (Fig. 6).

There was practically no difference in viability of the frozen seeds preserved in liquid nitrogen and in that of the controls; however, lower survival was observed in the case of the frozen embryo and endosperm (Table I). The capacity to withstand freezing depended on the water content of the seeds. Thus, the 2-year-old seeds (8–11% water content) showed complete revival while the immature and fresh ones (35% water content) survived partially.

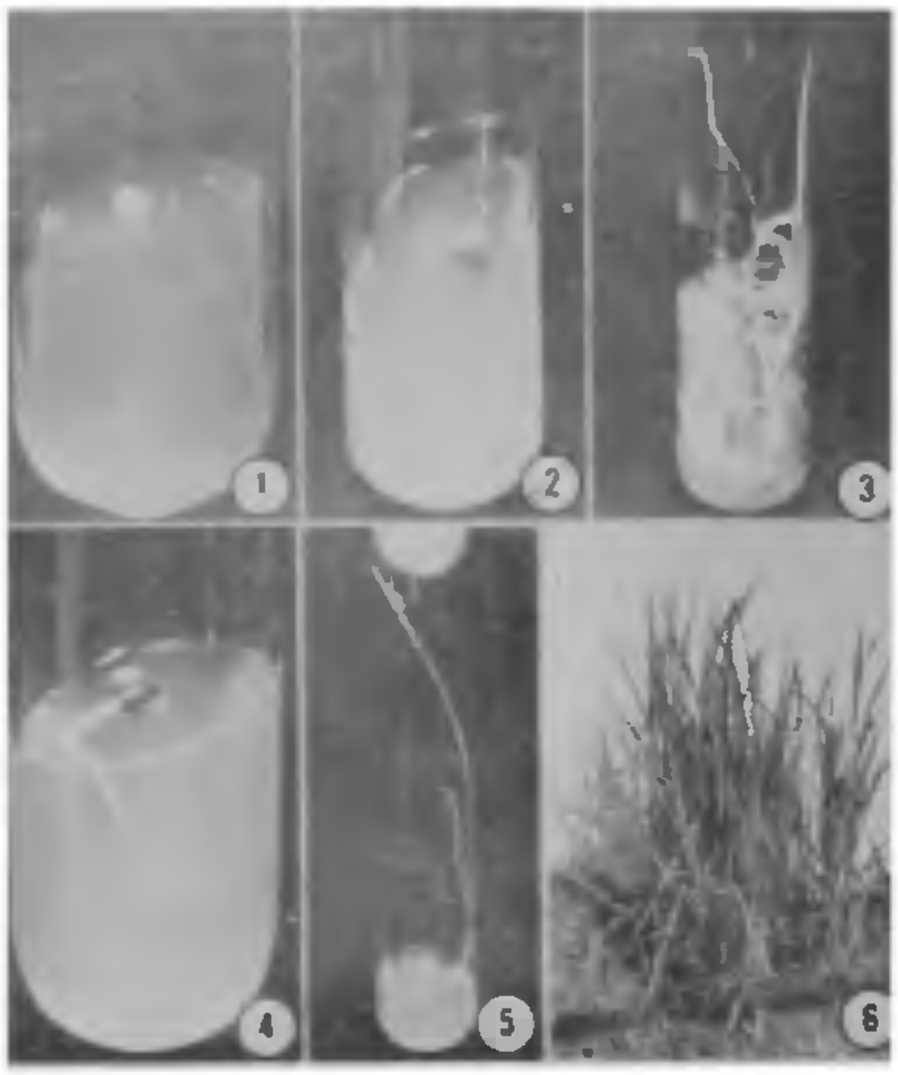
The revival of freeze-preserved cells (Bajaj[1], Sala *et al.*[6]) and the regeneration of complete plants from retrieved pollen (Bajaj[3]), embryo and endosperm (present study) suggest the possibility of using the cryogenic method as a meaningful tool for the conservation of favourable and rare germplasm.

TABLE I

Effect of sudden freezing (–196° C) *on the seeds, excised embryos and endosperm of rice preserved for* 3 *weeks in liquid nitrogen**

	Control	Frozen	
		Growth response (survival)	Growth (percentage of control)
1. Seeds	98% germination	96% germination	98
2. Dehusked seeds	94% germination	82% germination	87
3. Excised embryo with a portion of endosperm	86% grew	71% embryos callused and developed shoots	83
4. Segments of mature endosperm	16% callused	11% proliferated to form callus	68

* The seeds were germinated on moist filter papers in a petri-dish, whereas dehusked seeds, excised endosperm and the embryos were cultured on MS + 2,4-D 2 mg/l. Data based on 350 seeds, 92 dehusked seeds, and 360 cultures of embryos and endosperm.



FIGS. 1–6. Induction of growth and morphogenesis in excised endosperm and embryos of rice frozen in *liquid nitrogen*. Fig. 1. Frozen-thawed segments of endosperm 4 weeks after culture on MS + 2,4-D (2 mg/l); note the initiation of callus. Figs. 2, 3. Differentiation of endosperm callus into shoot and plantlets after transfer to MS + IAA (4 mg/l) + kinetin (2 mg/l). Fig. 4. An embryo (excised from a frozen seed) 3 weeks after culture; note the formation of root, but the growth of the shoot apex is suppressed. Fig. 5. A normal plant obtained from a frozen embryo. Fig. 6. Transfer of test-tube plants to soil.

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USE OF METRONIDAZOLE AS A NEW PRE-TREATING AGENT FOR CHROMOSOME ANALYSIS

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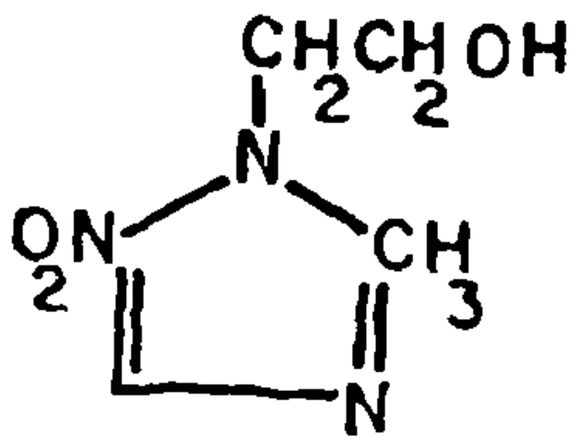
PRE-TREATMENT for the study of chromosomes is effected to bring about the clear scattering of primary and secondary constriction regions[2]. It causes viscosity change in the cytoplasm resulting in the destruction of spindle mechanism. The chromosomes get scattered throughout the cell on solidified plasma, due to pressure applied during squashing. They undergo differential hydration in their segments resulting in the clarification of constriction regions, which are the important landmarks for chromosome analysis. Pre-treatment also results in accumulation of a high frequency of metaphase stages due to the spindle inhibition. A number of chemicals are in use for such a purpose, of which colchicine, para-dichlorobenzene and oxyquinoline are worth mentioning.

While studying the effect of metronidazole on plant chromosomes, it was observed that it could be used as a successful pre-treating agent for chromosome analysis. Metronidazole has the following structural formula:



Metronidazole

The drug is an efficient mitotic poison[1], resulting in the destruction of spindle mechanism. The chromo-