9. Cryopreservation and Germplasm Storage*

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1. Introduction

Cryopreservation is defined as the viable freezing of biological material and their subsequent storage at ultra-low temperatures, preferably at that of liquid nitrogen. The development of cryopreservation strategy for plant cells and organs has followed the advances made with mammalian systems, albeit several decades later. Even for mammalian systems, the discovery of chemicals with cryoprotective properties was a significant step towards the development and refinement of cryopreservation technology. A major breakthrough in this context was the finding that glycerol was capable of protecting avian spermatozoa from freezing injury (Polge et al., 1949). This generated widespread enthusiasm and renewed interest among people interested in low temperature preservation in such fields as biology and medicine. Since the early 1950’s a number of low molecular weight neutral solutes have been identified as potential cryoprotectants, the most commonly recognized ones being dimethylsulfoxide (DMSO or Me₂SO) and glycerol. Dimethylsulfox-
ide, originally used to prevent freezing damage to human and bovine red blood cells and bull spermatozoa (Lovelock and Bishop, 1959), has become a universal cryoprotectant. In recent years, considerable progress has been made in the low temperature preservation of red cells and platelets, leucocytes, bone marrow cells, protozoa, and helminth parasites of man and animals, insects and their cells and microorganisms (Ashwood-Smith and Farrant, 1980). Despite all these advances, unlike plants, most attempts to preserve animal organs at ultra-low temperature have met with limited success.

One of the earliest reports on the survival of plant tissues exposed to ultra-low temperature was made by Sakai when he demonstrated that very hardy mulberry twigs, upon induction of dehydration mediated by extracellular freezing, are capable of survival following immersion in liquid nitrogen provided the frozen samples are subsequently rewarmed slowly at an air temperature of 0°C (Sakai, 1956). Historically, this finding had great significance in the understanding of the mechanism by which plant cells withstand such severe low temperature stress. Later, Sun (1958) achieved partial success when desiccated seedlings of *Pisum sativum* were immersed in liquid nitrogen. Although work continued on the freezing behaviour of tissues of various annual and perennial species, such studies were not extended to cultured plant cells or organs until a decade later when Quatrano (1968) first demonstrated that cultured flax (*Linum usitatissimum*) cells treated with DMSO are capable of survival after freezing to -50°C. This is the first recorded case on the utilization of a cryoprotectant for the freezing of plant cells. This study was followed by a similar kind with cell cultures of *Ipomoea* and *Daucus carota* in which the former survived freezing to only -40°C, while the latter survived immersion in liquid nitrogen (Latta, 1971). The experiments carried out with cell cultures of *D. carota* may thus be considered as the first successful attempt to cryopreserve cultured cells of any plant species. Since *D. carota* was one of the earliest species to be brought into culture, this became a model species to study cryobiology of cultured cells. Nag and Street (1973) successfully regenerated somatic embryos from a cell culture of *D. carota* which had been frozen to the temperature of -196°C, while Dougall and Wetherell (1974) stored cell cultures of wild carrot in a frozen state. In retrospect, it is fortunate that the early investigators chose *D. carota* cell cultures, albeit unknowingly, for their research since even today certain cell cultures are extremely sensitive to freezing injury (Kartha, 1985). Since the report on the successful cryostorage of *D. carota* cell cultures, several types of material such as callus and protoplasts, meristems/shoot-tips, zygotic and somatic embryos, anthers/pollen and even whole seeds have been studied extensively from a cryopreservation perspective. Examples of successful application of cryopreservation technology to a wide array of plant material can be found in Table 1.