

# A Review on the Effectiveness of Cryopreservation as A Germplasm Management Option



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## Abstract

Germplasm management is one of the best methods of conserving wildlife species for future use and to protect extinction of wildlife resources such as trees, animals and plants. The use of cryopreservation has been noted as one of the best options because all resources kept under this method can be used after many years without any problem. Many countries have opted this method compared to keeping of live plant and animals. The only major challenge faced in the use cryopreservation is the effect of climatic changes and mutations which may take place and affect the resources. Some Germplasm resources such as seeds have life span and after that time they may fail to germinate or fail germinate but fail to suit to the climatic conditions due to climate change which causes mutation from live genetic resources. There is need to come up with a viable option of way of making cryopreserved resources suit the climatic conditions after climate change.

**Keywords:** Effectiveness, Cryopreservation, Germplasm, Genetic Resources.

## Introduction

Cryopreservation is a perfect method for long-term conservation of plant, tree and animal genetic resources, using very low temperature (liquid nitrogen,  $-196^{\circ}\text{C}$ ). This method has been recognized as a practical and efficient tool for the long-term storage of Germplasm. Cryopreservation methods may provide the conditions for unlimited conservation of biological materials by reducing metabolic rates. During the cryopreservation all biochemical activities significantly reduced and biological deterioration are stopped. Conservation and subsequent sustainable use of genetic resources are essential to meet the demand for future food security. Several techniques have been developed yet to minimize the damaging effects of desiccation and freezing, ensuring high recovery of plant materials.

## Cryopreservation as a Technique

Techniques like cryopreservation collect and conserve plant genetic resources, especially plants with limited seed storage capability. In agroforestry tree species like *Baikaea plirijuga* need conservation so that they can be available for many years in research. There is only limited number of plants that cryopreservation techniques are used for their germplasm conservation, mainly because the techniques need to be adapted for each species. Therefore, continued efforts are needed in cryopreservation techniques to develop protocols for a wider range of plants. Formation of ice crystal during cryopreservation

is detrimental to cellular structure integrity and causes physical damage to the cells.

Cryopreservation is a part of biotechnology. Biotechnology plays an important role in international plant conservation programs and in preservation of the world's genetic resources [1,2]. Advances in biotechnology provide new methods for plant genetic resources and evaluation (Paunesca, 2009). Cryopreservation, developed during the last 25 years, is an important and the most valuable method for long-term conservation of biological materials. The main advantages in cryopreservation are simplicity and the applicability to a wide range of genotypes [3]. This can be achieved using different procedures, such as pre-growth, desiccation, pregrowth-desiccation, ED, vitrification, encapsulation-vitrification and droplet-freezing [3]. Cryopreservation involves storage of plant material (such as seed, shoot tip, zygotic and somatic embryos and pollen) at ultra-low temperatures in LN ( $196^{\circ}\text{C}$ ) or its vapour phase ( $-150^{\circ}\text{C}$ ). To avoid the genetic alterations that may occur in long tissue cultures storage, cryopreservation has been developed Martin et al. (1998).

## Methods of Cryopreservation

Cryopreservation has conservation methods which includes ex situ and in situ conservation. Today most tissue culture

technologies offer two major options to ex situ conservation of valuable plant germplasm that is slow growth storage and cryopreservation. Cryopreservation is usually called freeze preservation at ultra-low temperature of  $-196^{\circ}\text{C}$  that is the temperature of liquid nitrogen. A temperature of  $-196^{\circ}\text{C}$  arrest biochemical and most physical processes in plants making cryopreservation suitable for conservation of meristematic cells, seeds, pollen seeds, hard wood, softwoods and even embryos [4]. Cryopreservation is a long-term conservation method where plant and tree materials can be stored for unlimited periods whilst in good condition and suitable for use [5]. Besides conserving plant genetic resources, cryopreservation is also useful for safe keeping of medicinal and alkaloids-producing cell lines, hairy root cultures and genetically transformed and transformation-component culture lines [6].

Cryopreservation of biological tissues can be successful if intra-cellular ice crystal formation is avoided because lower temperatures damages cell membrane destroying their partial permeability [4]. Avoidance of crystallization is not possible by plants during cryopreservation. Vitrification is the only method which can prevent crystal formation during cryopreservation [7]. Vitrification is the transformation of physical transition process from aqueous solution to an amorphous and glassy (non-crystalline) [7]. Cryopreservation protocols mostly used are air drying, slow freezing, dehydration and vitrification [8,9]. Air drying is commonly called flash or normal drying and is applicable to orthodox seed, zygotic embryos and pollen of *Zea mays*, *Citrus lemon*, *Mangifera indica* and other horticultural species [10,11]. Some orthodox seeds can even withstand drying below 3 percent moisture content without any damage and reduction of viability. This cryopreservation protocol allows seeds to be kept for long time whilst viable [10]. The protocol proved to be beneficial to recalcitrant zygotic embryos of some plant species [10].

Encapsulation/ dehydration is used to conserve explants such as meristems and embryos [12]. Meristems and embryos are encapsulated in alginate beads which contain mineral salts and organic substances forming synthetic seeds (synseeds / artificial seeds). The synseeds are treated with high sucrose concentration, dried to a moisture content of 20 to 30 percent and rapidly frozen in liquid nitrogen [7]. Drying is done under airflow or using silica gel. The presence of nutritive matrix surrounding synseeds promotes regrowth after thawing since the nutrients provides energy and food for respiration and development of roots and shoots [12].

Vitrification is the most widely used cryopreservation protocol in germplasm management [13,14]. Synseeds are treated with concentrated vitrification solution for variable periods of time and followed by direct plunge into liquid nitrogen. Vitrification solution contains penetrating and non-penetrating cryoprotective substances to preserve both inside and outside of the synseeds so that they remain viable for long period of time

[15]. This protocol can be applied to several tissues and plants species [15]. The most commonly applied solution is plant vitrification solution number 2 (PVS2) which contains glycerol, ethylene glycol and sucrose. These nutrients are used by the synseeds during regrowth so that they quickly grow and prevent loss of viability of synseeds [7,13].

In Herbaceous species cryopreservation is done to conserve specific features which can be lost during normal in vitro maintenance. Cell suspensions and callus cultures are done, and it was noted that morphogenetic potential of embryogenic callus lines is not affected by their storage in liquid nitrogen [16]. Cryopreservation does not affect the expression of foreign sam gene in transgenic *Papaver somniferum* cells and is beneficial for pyrethrin biosynthesis by *Chrysanthemum cinerariaefolium* cell cultures [17,18]. Cryopreseved *Oryza sativum* and *Zea mays* calli proved to be source of regenerable cell cultures for transgenic plant production [17]. Cryopreservation of non-organised cultures is done in coffee, oil palm and banana [19] to make sure specific genes are maintained.

Cryopreservation of pollen facilitates crosses in breeding programmed, distributing and exchanging germplasm among locations, and preserving nuclear genes of germplasm [20]. This also facilitates studies in basic physiology, biochemistry, fertility and biotechnology involving gene expression, transformation and in vitro fertilization [21]. Preservation pollen is very useful for cross-pollination of cultivars differing in flowering periods [3]. Cryopreservation of pollen is very useful because their genetic maintenance and prevents variation in plants caused environment. Same plant can be reproduced for several years without any change.

### Applicability of Cryopreservation

Cryopreservation is suitable in hardwoods, softwoods and other several plant types where seeds, meristems and pollen are stored in liquid nitrogen for use in future. In herbaceous species, cryopreservation of meristematic tissues was the most successful as preservation of non-organised tissues [22]. Shoot meristematic tissues are the most commonly used explants for cryopreservation of vegetative propagated species such as fruit trees, roots and tuber crops. These have lower chances for soma clonal variation [22]. Meristems can be preserved using droplet freezing and vitrification cryopreservation protocol for example cryopreservation of *Solatum tuberosum* uses both protocols [23]. Banana meristems are preserved using droplet vitrification protocol [23], producing about one-quarter of the world's banana collection. The efforts for cryopreservation are also done to preserve cassava meristems so that cassava production increases in future [24]. In Australia endangered species of plants and trees are preserved using cryopreservation so that their genetic material is not lost and production increases in future [22]. Cryopreservation is one of the best options for germplasm management because germplasm can be managed at any stage of plants and trees.

Cryopreservation is also applicable to woody species. Traditionally germplasm preservation of woody species was through seed and field collections [25]. Numerous forest angiosperms such as *Acer* species, *Quercus* species, chestnut and horsechestnut have non-orthodox seeds with very limited storage period. Germplasm management of these species need collection of vegetative material and cryopreservation is done so that the species are obtainable in future [26]. They require conservation of huge numbers of accessions in clonal orchards including old, new, local varieties and wild material to allow choice during reproducing new plants [17]. Cryopreservation should be regarded as a strategically important support to traditional in-field banks. Cryopreservation minimizes risks of accidental loss of woody plant genetic resources [21].

### Conclusion

In woody plant cryopreservation shoot tips, seeds and lines of embryogenic callus are used to maintain genetic resources [27]. The shoot tips are differentiated organs used for preservation of vegetatively propagated plants such as *Citrus* synesis and timber tree cultivars for the maintenance of genetic resources [19]. Shoot tips are obtained from apical or axillary buds and excised from in vitro grown shoot cultures. The bud is kept under sterile conditions to obtain apical meristems which are also kept in liquid nitrogen [28]. Cryopreservation is done for those species characterized by non-or sub-orthodox seeds, which cannot be stored traditional seedbanks [29]. Cryopreservation is the best option for long term conservation of genetic resources for these species [30-34]. Seeds or the isolated embryo axes are preserved for species which produces seeds such as *Eucalyptus camaldulensis*. Lines of embryogenic callus are used in non-orthodox seeds which permit germplasm conservation of these species. This organ type is used to prevent loss of embryogenic potential due to repeated subculturing or allowing the storage of transgenic material Gass et al. (1996).

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