Scientia Horticulturae 168 (2014) 88–107

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Review

Cryopreservation as a tool used in long-term storage of ornamental species – A review

Dariusz Kulus*, Małgorzata Zalewska

University of Technology and Life Sciences in Bydgoszcz, Department of Ornamental Plants and Vegetable Crops – Laboratory of Biotechnology, Bernardyńska St. 6, PL-85-029 Bydgoszcz, Poland

ARTICLE INFO

Article history:
Received 18 March 2013
Received in revised form 9 January 2014
Accepted 11 January 2014

Keywords:
Cryopreservation
Genetic stability
Ornamental plants
Storage

ABSTRACT

The market for ornamental plants is growing every year, becoming an important part of the economy. Every year hundreds of new cultivars, replacing the current assortment, are produced. However, since consumer preferences are changing rapidly, the cultivars considered old-fashioned today may become popular once again. They are also a valuable breeding material source. Bearing that in mind, there is a great need to develop a strategy for their long-term conservation. Storage in gene banks under *in vitro* cultures, although offering many advantages, is expensive and threatened with somaclonal variation and contamination loss. Cryopreservation is believed to be a more promising method. It has been successfully used with many agricultural species. Unlike micropropagation, cryopreservation has not yet found wider employment with ornamental plants. The upcoming years and progress in cryobiology may, however, change this situation and broaden the potential of cryoconservation. Over years several freezing methods have been introduced. The first one developed, based on slow cooling, had limited usefulness in temperate species. Today the encapsulation-dehydration technique is most often used with ornamental plants. In the future, however, combined techniques will probably be the most popular. So far insufficient attention has been paid to the problem of the genetic stability of cryopreserved ornamental species, especially chimeras. The aim of this paper is to present different cryopreservation techniques and their use for the storage, protection and breeding of ornamental plants.

Contents

* Corresponding author. Tel.: +48 0523749522.
E-mail address: dkulus@gmail.com (D. Kulus).

0304-4238/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scienta.2014.01.014

1. Introduction

Ornamental plants are produced mainly for their aesthetic value. They have been an important element of human culture and economy since ancient times (Ozudogru et al., 2010). Thanks to their unique beauty, they accompany us at every stage of our lives. The commercial production of ornamental species is constantly growing. Still the market for ornamental plants is subjected to periodical trend-driven changes. Indeed, every year, hundreds of new cultivars, replacing the current assortment, are produced. However, due to changes in consumer preferences, cultivars unfashionable today may in the future once again be attractive for potential buyers. Furthermore, very often they constitute a great breeding material source. For this reason, the protection and storage of those valuable genetic resources is of great importance in order always to be able to meet market demands. Nevertheless it is difficult for breeders and horticulturalists to provide enough space and funds for traditional cultivation of such numerous cultivars, which is laborious and threatened with biotic and abiotic stresses (Sekizawa et al., 2011). Traditional genetic conservation in the field or greenhouse requires intensive care of pot cultures or carefully separated field plots (Reed, 2006). Haploids (important in breeding) and transgenic cultivars, which are gaining popularity among ornamental plants (*Rosa* L., *Dianthus* L., *Gladiolus* L.), require isolation to protect them from cross-breeding (Joung et al., 2006; Rajasekharan et al., 1994). Additionally many ornamental species (*Orchidaceae*, *Cactaceae*, *Gentianaceae*) are on the brink of extinction. Fast and easy access to high quality gene banks of large material variety is the key for ornamental plant producers and so an efficient method for long-time conservation of the plant material may be extremely valuable for breeding and horticultural production (Halmagyi et al., 2004). Today, cryopreservation, developing rapidly over the last 25 years, is believed to be the most promising and valuable long-term storage method.

Cryopreservation techniques are based on tissue storage (usually with high cell division rates and low water content, e.g. meristems, seeds, zygotic and somatic embryos or pollen but also callus cultures and cell suspensions) at ultra-low temperature of liquid nitrogen (LN, −196 °C/−321 °F) or, seldom, its vapor phase (−150 °C/−238 °F). At this temperature the biochemical metabolic and cell division activities are arrested, allowing for long-term storage. The main advantage of this method is the reduction of *in vitro* culture costs, required space, contamination and somaclonal variation risk. The long-term cryoconservation of embryogenic cell lines could be a valuable tool in genetic transformation. Storage in LN would also help in preserving genetic diversity by storing wild species (e.g. for the purpose of breeding), some of which are endangered already (Winkelmann et al., 2004). Moreover, cryotherapy may be used to reduce the number of pathogens, as proven with *Pelargonium* L'Hér (Gallard et al., 2011; Grapin et al., 2011). Consequently the method is attracting more and more interest among researchers.

Cryopreservation has been successfully used for many agricultural species (Forsline et al., 1998; Xue et al., 2007; Zhao et al., 2005). The first information on cryopreservation of ornamental species was reported by Fukai (1989) and regarded *Dianthus hybrida*. Whit its short history, the significance of cryopreservation for ornamental plants is less, although growing every year.

The aim of this review is to present the previous achievements and difficulties with the cryopreservation of ornamental plants, as well as the popularization of this method for the purpose of breeding, production and protection of these species.

2. Material selection

It is possible to freeze biological material (e.g. buds of *Chrysanthemum* × *grandiflorum*/Ramat./Kitam., spores of *Cyathea australis* (R. BR.) Domin, or seeds and bulblets of *Lilium ledebouri* [Baker] Boiss) excized directly from *ex vivo* conditions (Fukai, 1990; Kaviani et al., 2009, 2010; Mikuła et al., 2009a). Such material, due to its greater size, is easier to isolate and very often has a better survival rate (Mikuła et al., 2009a). It is, however, more exposed to loss due to contaminations for disinfection is performed after thawing (Fukai, 1990). Furthermore, because nowadays the commercial production of ornamental plants is based mainly on micropropagation (about 160 genera [Rout et al., 2006]), the use of *in vitro* derived explants seems well-founded.

The selected material should be young, demonstrating meristematic potential (or regeneration potential, in the case of non-meristematic explants, such as callus cultures and cell suspensions, which should be in the linear growth phase 7–10 days after subculture) since only cells with dense cytoplasm and small vacuoles can survive freezing. Moreover, the use of a meristematic explant provides a greater chance of avoiding any variation.

2.1. Cryopreservation of shoot and root tips

Different explants are used for cryostorage e.g. apical or axillary shoot tips, seeds, spores, gametophytes, rhizomes or even protoplasts (Yamazaki et al., 2009; Table 1).

Among them, shoot tips are used most often. As for vegetatively propagated, sterile plants (e.g. *Crocus* L., *Chrysanthemum* L. – crops with a high economic value) shoot tips constitute the best initial material (Fukai, 1990; Zadeh et al., 2009). As a result it is necessary to prepare the shoot tips first. As for plants of elongated growth (e.g. *Dianthus*, *Chrysanthemum*), they are very easy to obtain by inoculating single-node explants on the Murashige and Skoog (MS; 1962) medium for 14 days. The *in vitro* phase cannot be a source of any variation at that point. The optimal age of the cryopreserved buds should be between 2 (for shoot apices) and 7 weeks (for axillary buds) (Popova et al., 2010). Shoot tips of older plants are more difficult to isolate, due to the development of covering leaves, although Takagi et al. (1997) reported better results when using 2–3-month-old mother plants of *Colocasia esculenta* L. Schott.

Many authors emphasize the significance of the frozen explant size for protocol success. This phenomenon, however, seems to be genotype-dependent in the case of crocus (*Crocus sativus* L.) and chrysanthemum (*Chrysanthemum* × *grandiflorum*); satisfying results were reported for both smaller (0.5–1.0 mm) and bigger (3–4 mm) shoot tips (Fukai, 1990; Zadeh et al., 2009) using different techniques. With rose (*Rosa* × *hybrida* L.) smaller explants (1–2 mm) were less efficient than bigger ones (3–4 mm) with 2–4 leaf primordia after applying the droplet-vitrification technique (Halmagyi and Pinker, 2006). Similar results were observed with encapsulated protocorms of orchids (Yin et al., 2011). Still, the latter are easier to isolate and less susceptible to injury (Shuhaida et al., 2009). However, as for vitrified *Colocasia esculenta*, smaller (0.8 mm) shoot tips with one leaf primordium provided a 5× higher survival than the bigger ones (2.0 mm) with two leaves primordia (Takagi et al., 1997). It could be assumed that when