Review Article

In vitro conservation of ornamental plants [1]

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ABSTRACT

The market of flowers and ornamental plants is dependent on the diversification of species and the availability of high quality propagation materials. Actually, *in vitro* culture techniques performance a prominent role in the multiplication and maintenance of commercially propagated ornamental plant species, and are promising for the production of thousands of high quality plants in relatively short term. In addition, when market demand for a particular species is low or zero in a specific period of the year, *in vitro* culture techniques allow the conservation of cultures under aseptic conditions, by Slow Growth Storage (SGS), from a few weeks to one year (or more), without affecting their viability and potential regrowth. This can be achieved by modifying the constitution of the culture medium and the maintenance conditions of *in vitro* cultures. Obviously, the success of the technique depends on greatly on the physiological characteristics of the species to be conserved, as well. Once a SGS protocol is optimized, the expenses labor, the possibility of contamination and the probability of somaclonal variation can be reduced markedly.

Keywords: Flowers, Shoot cultures, Slow Growth Storage

1. INTRODUCTION

The ornamental plant industry is expanding every year around the world (BOTELHO et al., 2015). This is due to the availability of high quality regenerative and phytosanitary propagation materials, allowing the diversification of ornamental plants used in the various segments of the business chain, as garden plants, cut flowers and foliage, dried flowers, edible flowers, and others. The use of biotechnological tools, such as *in vitro* culture techniques, is, undoubtedly, one of the key components that allowed the increase of this diversification of quality materials available to the actual market of ornamental plants.

Recent developments in *in vitro* culture techniques allow the medium-term storage of plants under aseptic conditions, without requiring any periodic operation, such as subculturing to fresh medium. The storage is done by reducing the metabolism of plants, and although highly species-specific, it can be extended for several months or even to several years. The induction of a slower metabolism in the plants allows the reduction of the frequency of periodic subcultures, therefore it also reduces labor and costs, and the risk of contamination during the subcultures. Plant metabolism may be reduced by (i) reduction of temperature and / or light intensity, (ii) addition of osmotic compounds such as mannitol or sucrose, thus reducing the availability of water and (iii) addition of growth retardants in storage medium, especially inhibitors of gibberellin biosynthesis (GROUT, 1995; RADEMACHER, 2000; CASTRO and HILHORST, 2004; SILVA and SCHERWINSKI-PEREIRA, 2011). These approaches can be applied alone, or in combination. For instance, the reduction of temperature, in combination with reduced or absence of light intensity is the one mostly applied. These factors reduce significantly the respiration, water loss, and ethylene production, and thus the cell metabolism and growth (OZUDOGRU et al., 2010). The optimized conservation protocols also allow an immediate access to germplasm of interest when the market request arrives again and maintain genetic diversity without compromising its stability (ENGELMANN, 2004; SHIBLI et al., 2006; RAI et al., 2009). It should be noted that, also the age, size and physiological status of the plant material affect markedly the success and the maximum storage time of SGS (ORLIKOWSKA, 1992).

Although routine use of this technique is still limited, examples are increasing where *in vitro* conservation is used both in research laboratories and in ornamental plant biofactories. For instance, the germplasm conservation of native species with high commercial potential as ornamental, or for the alimentation and pharmacological use, has been used to preserve genetic variability and allowed the study of its properties in a controlled environment through the use of *in vitro* culture techniques (FORD-LLOYD and JACKSON, 1991; VILLALOBOS et al., 1991; BERTONI et al., 2010). The establishment of an *in vitro* protocol for the conservation of a wild species allowed the creation of reference databases for future studies of other species (CORDEIRO et al., 2012).

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In vitro conservation can refer to medium- or long-term conservation. Long-term conservation of the germplasm is possible only through a technique called ‘cryopreservation’, where plant material is stored at ultra-low temperatures (usually of liquid nitrogen, at -196°C). This review, however, will deepen into the technique of Slow Growth Storage for the medium-term conservation of ornamental plants.

2. SLOW GROWTH STORAGE OF ORNAMENTAL PLANTS

The method of slow growth storage consists in decelerating or suppressing the the plants physiological metabolism. Differently of cryopreservation, in which for long periods, the plant material is stored at ultra-low temperatures, suppressing growth, in order to avoid deterioration of plant (GROUT, 1995; SILVA and SCHERWINSKI-PEREIRA, 2011). This method has been used for conservation in short- and medium- term, mainly for meristems and/or shoot tips of several species. It consists in reduction of the growth and increase the intervals between the subcultures, without affecting significantly the viability of the explants (ENGELMANN, 2011).

This can be achieved modifying the potentially osmotic from the culture medium, thereby reducing the availability of water, or using plant growth retardants (especially inhibitors of gibberellin biosynthesis). It is also possible to reduce the light and temperature of the incubation room, in such a way as to achieve the minimum *in vitro* growth (RADEMACHER, 2000; CASTRO and HILHORST, 2004; SILVA and SCHERWINSKI-PEREIRA, 2011). However, the success of this technique depends greatly on physiological characteristics of the species to be conserved.

The use of low temperature (04 to 18ºC) and nutrient medium supplementation with osmoregulators (sucrose for example) have been used to conserve explants for clonal propagation such as tubers, roots of fruit and ornamental species (OZUDOGRU et al., 2010).

Thus, a widely approach used has been the addition of osmotic agents and the reduction of temperature together, in which the species are maintained. The low temperature increases the transfer interval to new medium and often regardless of the type and concentration of carbohydrate used (LIMA-BRITO et al., 2011).

There are a few studies in Slow Growth Storage (SGS) of ornamental plants and most of them use temperature reduction to obtain slower rates of growth during the storage. The storage temperatures range from 2-6°C to 23-24°C especially for the species of tropical and subtropical climates (Table 1).

Table 1. Slow Growth Storage of ornamental plants

Species	Storage Temp (°C)	Storage Light/ Radiance	Storage Period (Months)	Recovery (%)	Explant Type	References
Acanthostachys strobilacea	10	12 h (30 µmol m^{-2} s^{-1})	3	100	*Seedling*	CARVALHO et al, 2014
Anthurium andreanum	6-10	Darkness	8	56	*Shoot cultures*	BENELLI et al., 2012
Camellia japonica	4	Darkness	2	30	*Synthetic seeds*	JANEIRO et al. 1997
Camellia japonica (cv Alba Plena)	2–4	16 h (8 µmol m^{-2} s^{-1})	12	100	*Shoot cultures*	BALLESTER et al., 1997
Camellia reticulata (cv Mouchang)	2–4	16 h (8 µmol m^{-2} s^{-1})	12	100	*Shoot cultures*	BALLESTER et al., 1997
Cedrela fissilis	25	16 h (20–25 µmol m^{-2} s^{-1})	9	8	*Synthetic seeds*	NUNES et al., 2003
Deutzia scabra	24-4	Darkness	12	96.67	*Shoot cultures*	GABR and SAYED, 2010
Dianthus ingoldbyi	4	Darkness	6	58	*Shoot cultures*	ARDA et al., 2016
Drimiopsis kirkii	15	16 h (3000 lux)	4	64.4	*Synthetic seeds*	HAQUE and GHOSH, 2014
Epidendrum chlorocorymbos	23	16 h (1500 lux)	6	100	*Seedling*	LOPEZ-PUC et al., 2013
Gerbera (cv Marleen)	4	Darkness	3	100	*Shoot cultures*	HEMPEL and HEMPEL, 1987

Table 1. cont.

Heliconia champneiana	25	16 h LED [100 % blue, 100 % red , 70 % red + 30 % blue and control white (25 µmol m^{2} s^{-1})]	3	100	*Seedling*	RODRIGUES et al., 2018
Hibiscus moscheutos (cv Lord Baltimore)	5	Darkness	19.5	80	*Synthetic seeds*	WEST et al, 2006
Humulus spp.	4	12 h (3 µmol m^{-2} s^{-1})	36	NR*	*Shoot cultures*	REED et al., 2003
Lilium longiflorum	4	Darkness	6	92	*Synthetic seeds*	STANDARDI et al,1995
Metrosideros excelsa Soland. ex Gaertn.	4-10	Darkness	4	58	*Synthetic seeds*	BENELLI et al., 2017
Morus spp	5	Darkness	3	18	*Synthetic seeds*	PATTNAIK and CHAND, 2000
Nandina domestica	4-8	Darkness	6	100	*Shoot cultures*	OZUDOGRU et al., 2013
Nerium oleander	4	Darkness	3	30	*Synthetic seeds*	OZDEN-TOKATLI et al., 2008
*Paulownia elongate*e	4	Darkness	2	32	*Synthetic seeds*	IPEKCI and GOZUKIRMIZI, 2003
Photinia × fraseri Dress	4-10	Darkness	4	65	*Synthetic seeds*	BENELLI et al., 2017
Photinia fraseri	4	Darkness	3	91	*Synthetic seeds*	OZDEN-TOKATLI et al., 2008
Polygala myrtifolia	4-10	Darkness	8	68.8	*Synthetic seeds*	BENELLI et al., 2017
Ranunculus asiaticus	4-10	Darkness	9	100	*Shoot cultures*	BENELLI et al., 2012
Rosa cvs	4	Darkness	6	>80	*Shoot cultures*	PREVIATI et al., 2008
Rosa hybrida ‘King's Ransom	4	Darkness	1.33	30	*Synthetic seeds*	JAYASREE and DEVI, 1997
Splachnum ampullaceum	5	Darkness	30	50	*Synthetic seeds*	MALLÓN et al., 2007
Syringa vulgaris	5	Darkness	1.5	83	*Synthetic seeds*	REFOUVELET et al., 1998
Turbinicarpus	4	Continuous light (54 µmol m^{-2} s^{-1})	12	NR*	*Shoot cultures*	BALCH et al., 2012
Vriesea inflata	15	12 h (55 µmol m^{-2} s^{-1})	24	100	*Seedling*	PEDROSO et al., 2010

* NR: not reported

Storage under dark conditions is more commonly applied approach for conservation of ornamental plants, however shorter photoperiods (i.e., 8-12 h), instead of 16 h that is commonly used as standard in *in vitro* plant cultivation (e.g., REED et al, 2003; PEDROSO et al., 2010; CARVALHO et al., 2014), or the use of low light intensities (3 to 8 µmol m^{-2} s^{-1}) have also been shown to be effective in reducing plant metabolism (e.g., BALLESTER et al., 1997; REED et al., 2003). Rodrigues et al. (2018) tested different light spectrum of LED light to reduce the plant development.

Another strategy for SGS is the use of substances such as sucrose, mannitol, and sorbitol that reduce the osmotic potential of the culture medium (GROUT, 1991). For example, the use of mannitol provided 4 month-storage of *Epidendrum chlorocorymbos* (LOPES-PUC, 2013). In *Nandina doméstica,* storage up to 6 months was possible using a relatively high concentration of sucrose (60 g L^{-1} instead of 30 g L^{-1}) (OZUDOGRU et al., 2013).

Decreasing the salt concentration of the culture medium, in other words reducing the absorption of nutrients

responsible for plant growth and development, can also be used as a strategy to reduce plant growth during the SGS (ENGELMANN, 1991). Indeed, for *Epidendrum chlorocorymbos,* reducing the salts of the culture medium to 50% provided a better SGS, being able to store for 4 months (LOPES-PUC, 2013).

Regarding the maximum storage time, *Hibiscus moscheutos* was stored for approximately 20 months (WEST et al., 2006) and *Splachnum ampullaceum* for 30 months (MALLÓN et al., 2007). Reed et al. (2003) reported the possibility of storing *Humulus spp* at low temperature for up to 3 years.

The type of the culture container to be used in storage is another important factor to be taken into account in SGS (OZUDOGRU et al., 2010). For instance, in *Gerbera* it was observed that when larger pots were used, greater rooting and survival rates were obtained at the end of 3 months compared to the smaller pots (HEMPEL and HEMPEL, 1987).

For *in vitro* conservation of *Nandina domestica*, effects of different storage temperatures (4 and 8°C) in dark and different concentrations of sucrose (30, 45, 60 g L^{-1}) in the storage medium were evaluated (OZUDOGRU et al., 2013). The results showed that the plants had a maximum survival (100%) after 6 months when they were stored at 8°C in the storage medium containing the highest concentration (60 g L^{-1}) of sucrose. On the contrary, storage at 4°C in the medium containing 30 g L^{-1} sucrose provided the lowest (75%) survival rate after 6-month storage (Figure 1).

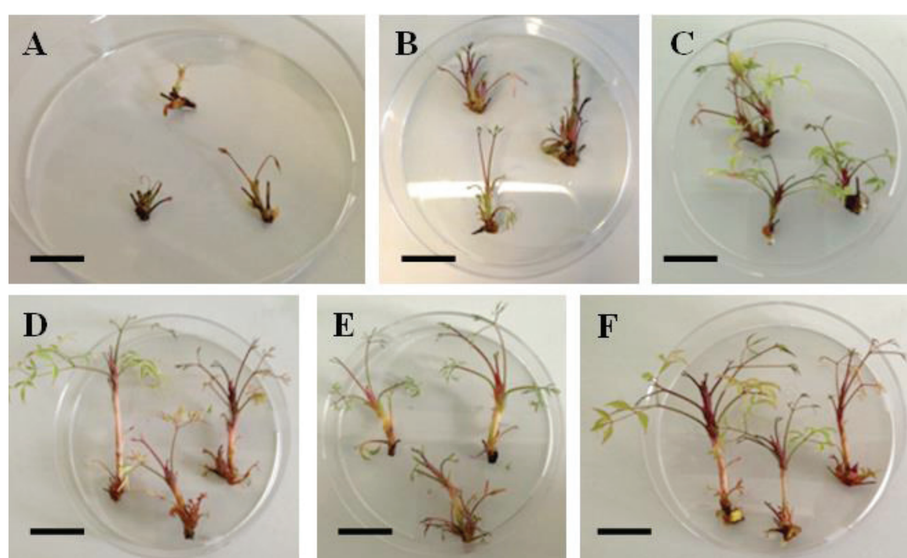

Figure 1. *In vitro* conservation of *N. domestica* by slow growth storage. Shoot recovery following 6 months of conservation at 4°C in darkness on storage medium containing 30 (A), 45 (B) or 60 g L^{-1}(C) sucrose, or at 8 °C in darkness on 30 (D), 45 (E) or 60 g L^{-1} (F) sucrose. A, bar 15 mm; B-F, bar 20 mm (OZUDOGRU et al., 2013).

Although only a limited number of studies use an *in vitro* conservation of ornamental species, the development of the technique, such as the use of new tools for the reduction of plant metabolic, can lead to an increase in the number of species conserved using the Slow Growth Storage technique.

3. CONCLUSIONS

In recent years, great progress has been observed in the development of new propagation and conservation techniques for ornamental species, especially in the area of slow growth, thus demonstrating a market increase in the use of these biotechnological advances. But it is important to emphasize that the technique of slow growth storage is one of the complementary alternatives for the conservation of ornamental plants, without taking away the importance of the other forms of conservation like the conservation *in situ* or *ex situ*.

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AUTHORS CONTRIBUTIONS

All authors: writing and proofreading the text.

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