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In vitro culture as a powerful method for conserving Iranian ornamental geophytes

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Abstract

Never before in history has the issue of preserving biodiversity been of such significance than today, to the extent that it has become an important global issue. Nowadays, humans have a massive impact on biodiversity whether in rural, urban, or wilderness settings. Besides, biodiversity today is also being significantly constrained by climate change and the introduction of new varieties of plants. Plant tissue culture is a method that can be useful for collecting, storing, and multiplicating of plant germplasms. Nowadays, many valuable germplasms are in danger of extinction, especially in Iran. Thus, there is a necessity to preserve valuable Iranian ornamental geophytes such as *Oxalis articulata*, *Eminium jaegeri*, *Muscari kurdicum*, *Leopoldia tijtijensis*, *Gagea calcicola*, *Tulipa faribae*, *Gagea alexii*, and *Allium*. In this study, plant tissue culture as well as *in vitro* conservation techniques as means for medium- and long-term conservation of Iranian ornamental geophytes is reviewed. There are only few studies on plant tissue culture of Iranian ornamental geophytes despite their great importance in Iran. To our knowledge, there is no report on cryopreservation of the abovementioned plants. In conclusion, the methods presented in this review can be utilized for conserving these valuable germplasms for future generations.

Key words: plant tissue culture, cryopreservation, medium-term conservation, long-term conservation, plant genetic resources

Introduction

Flowers are of great importance for all civilizations across the world. Throughout history, flowers have been used in expressing emotions, decorating graves, and celebrating life events. Many cultures use flowers as an expression of art and beauty (Farahmand and Nazari, 2015). Among flowers, geophytes have a flourishing history and play a significant role as garden plants. There are many paintings and vases found in the relics of ancient Crete from 1600–1800 BC that are decorated with lily flower motifs. History shows that in ancient Egypt, narcissi, and lilies were used in funerals, and Pharaohs grew anemones in their gardens (Farahmand and Nazari, 2015; Jalali et al., 2012). Gardens containing crocuses and lilies belonging to Solomon were mentioned in the Old Testament. Also, there is evidence proving the importance of crocuses, lilies, and hyacinths in ancient Greece (as early as 380 BC). Theophrastus – a botanist and a philosopher – mentioned the impact of *Allium*, *Anemone*, *Crocus*, *Cyclamen*, *Gladiolus*, *Ranunculus*, and *Scilla* species for religious ceremonies in ancient Rome in 340 BC (Farahmand and Nazari, 2015).

A vast number of bulbous plants are cultivated in the Middle East (Istanbul, Syria, Lebanon, Afghanistan, and Iran) and the southeastern region of the main island of Malta. Interestingly, other parts of the world have a smaller contribution to the growing of these species (Genders, 1973; Kamenetsky and Okubo, 2012). Growing bulbous plants in these regions might prove that these can grow in various climatic conditions such as alpine, xeric, and mesic climates. This adaptation arises from the law of nature that is omnipresent and creates, as a result, a vast biodiversity among the organisms. The Convention on Biological Diversity offers a formal defini-

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tion of biodiversity as "the variability among living organisms from all sources including *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems."

Maintaining biodiversity is an important task, especially now, when humans, whether in rural, urban, or wilderness settings, have a massive impact on it that frequently leads to a decrease in biodiversity (Kamenetsky and Okubo, 2012). According to the International Union for Conservation of Nature (IUCN), roughly 34 000 plant species worldwide (12.5% of the estimated world flora) are threatened by extinction. Furthermore, the world's biodiversity is declining abruptly, for example between 1996 and 2004, the IUCN added 8321 plant species to the endangered species (red) list (IUCN, 2004). The aim of the IUCN red list was to characterize the species that are exposed to the risk of global extinction. At that time, over 60% plants were exposed to a risk of extinction. Thus, there is a dire need of preserving and conserving endangered species to maintain biodiversity in the world (Sarasan et al., 2006). The necessity to protect biodiversity for its sustainable use was the main subject of the International Convention on Biological Diversity (ICBD) and UNCED's Agenda 21 that has been signed by representatives of 150 countries, who participated in the Earth Summit in 1992 (Lomba et al., 2010).

A vast number of plants have orthodox seeds, which means that they can be dehydrated to a low water content and stored in low temperatures for extended periods of time (Roberts, 1973). The "problematic" plants, in terms of seeds, are categorized into three groups. The first group contains plants that do not produce seeds, and they are propagated via asexual methods. The second group covers plants that produce orthodox seeds, such as sugarcane; however, these seeds are highly heterozygous and pose limited interest for conservation (Engelmann, 2011). Therefore, these species are mainly propagated via cloning. The third group is composed of a vast number of species such as forest trees and ornamental geophytes growing in tropical zones that produce recalcitrant seeds that cannot survive at low moisture and temperature conditions (Ellis et al., 1990; Roberts, 1973; Salmi and Hesami, 2016). Beside these three groups, there is a vast number of species called intermediate (Ellis et al., 1990) as their preservation via seeds is difficult. The conservation methods in use to preserve these species is called field collection (*ex situ* conservation) in which botanical gardens play critical roles (Gonzalez-Arnao et al., 2008a). This approach, however, has numerous drawbacks that limit its efficiency and exert a negative impact on plant genetic resources. This method is often used for crop species. However the conservation of rare, wild, and endangered plant species is a pressing issue because biodiversities around the world have been declining abruptly (Sarasan et al., 2006). Thus, for conserving wild species, *in situ* methods (*in vitro* methods) are more efficient than *ex situ* methods (Engelmann, 2011). It is also important to introduce and develop new methods to ensure sufficient storage and rapid multiplication of species.

Ornamental geophytes in Iran

Iran is the second largest country in the Middle East and the 16th largest in the world (FAO, 2005) that has a high plant diversity (Salmi and Hesami, 2016; Yavari and Shahgolzari, 2010). Iran is located in the Middle East and surrounded by Pakistan and Afghanistan in the east, the Persian Gulf and Oman Sea the in the south, Turkey and Iraq in the west, and Azerbaijan, the Caspian Sea, Turkmenistan and Armenia in the north. It consists of 30 provinces and the population of Iran is about 70 million with a 1.7% growth rate (Akbari et al., 2017; Heshmati, 2012). The important mountain chains of Iran are Alborz and Zagros. These mountain chains have formed a nonuniform spatial area and are responsible for the distribution of temperature and precipitation in the entire country. The area between these two mountain chains is a high plateau where another mountain chain begins (and continues toward the border with Pakistan and southern parts of Afghanistan). The highest mountain in the Alborz Range is called Damavand peak and reaches 5670 m above the sea level (Farahmand and Nazari, 2015; Heshmati, 2012). Except northern and part of western Iran, the rest of the country has arid and semi-arid climate. Thus, these regions have hot and dry summers and freezing winters. The maximum annual rainfalls in Iran are found in the Caspian Sea plains, Alborz and Zagros slopes with more than 1800 and 480 mm, respectively. Interestingly, the average annual rainfall in most of Iran area is roughly 240 mm (Heshmati, 2012), except the central parts that is less than

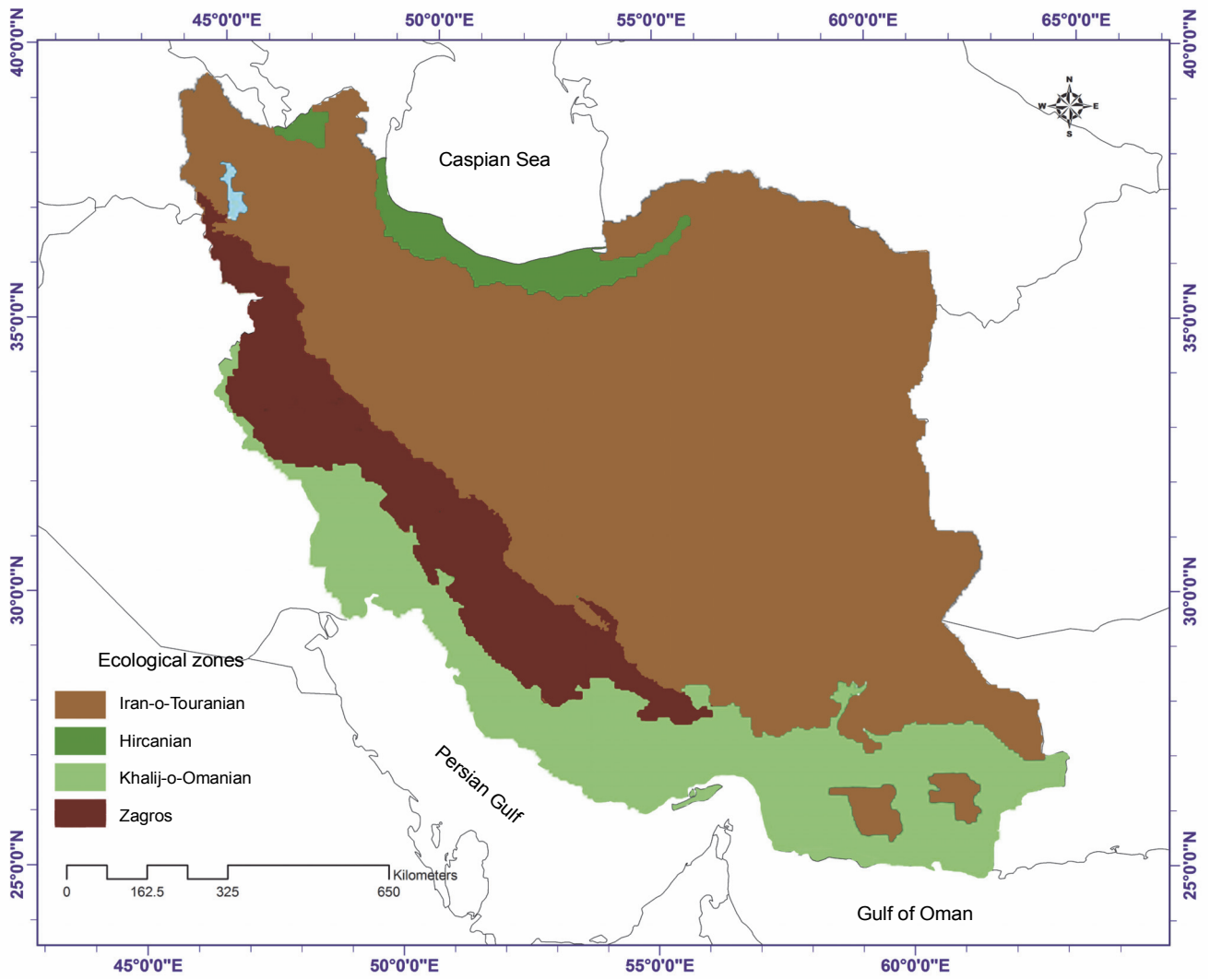

Fig. 1. The distribution map of four ecological zones in Iran

100 mm (Amiraslani and Dragovich, 2011). Many biologists and orientalists are attracted to Iran by the affluent flora and fauna, distinctive landscapes, and honorable ancient civilization of Iran (Yavari and Shahgolzari, 2010). Over 8000 flowering plant species that belong to 167 families and 100 genera are found in Iran; 1700 of these species are known as being endemic to this country (Farahmand and Nazari, 2015; Samiei et al., 2010). All these plant species (Fig. 1) can be grown in four ecological zones (Fig. 1). Over 200 geophytes from Liliaceae, Amaryllidaceae, and Iridaceae families are grown in Iran, and all of these species have a massive impact on the creation of a unique scenery (Wendelbo, 1977). However, some species such as *Biarum* spp. Schott and *Arum* spp. L. from Araceae, *Dactylorhiza* spp., *Gymnadenia* spp., *Steveniella* spp., and *Orchis* spp. from Orchidaceae (Mozaffari, 1996), *Leontice* spp. (Polyphyllaceae), *Geranium tuberosum* (Geraniaceae), *Corydalis* spp. (Fumariaceae), *Ophrys kurdistanica* Renz, *Epipactis rechingeri* Renz, and *Ophrys turcomanica* Renz (Farahmand and Nazari, 2015) are not in the Wendelbo (1977) category. The introduction of new species, especially bulbous plants such as *Oxalis articulata* (Ghahremaninejad and Gholamian, 2006), *Eminium jaegeri* (Bogner and Boyce, 2008), *Muscari kurdicum* Maroofi (Maroofi, 2007), *Leopoldia tijtijensis* (Jafari, 2012), *Gagea calcicola* (Zarrei et al., 2010), *Tulipa faribae* (Ghahreman et al., 2007), *Gagea alexii* (Ajani et al., 2010), and *Allium* (Razyfard et al., 2011), increased the richness of geophytic species in Iran. These species have a high potential for being employed in floriculture industries and serve as cut, garden and landscape flowers, and pot flo-

wering plants (Farahmand and Nazari, 2015; Mohammadi-Dehcheshmeh et al., 2008; Nazari et al., 2014; Nazari et al., 2007; Samiei et al., 2010).

In vitro conservation

The advancements in biotechnology allowed the production of a new category of germplasms containing clones of elite genotypes, cell lines possessing desired attributes, and genetical transformation (Engelmann, 1991) of species with high values. The advancements of these new techniques are being used to ensure safe conservation of important plant species. Therefore, in the early 1970s, biotechnology, and especially *in vitro* tissue cultures, attracted significant attention as a way of solving plant conservation problems. Plant tissue culture is a method useful for collecting, storing, and multiplicating of plant germplasms (Bunn et al., 2007; Engelmann, 1991, 2011; Jalali et al., 2012). Using this method, plants can be propagated in an aseptic environment, with high propagation rates. Importantly, plants free of diseases can be achieved by culturing meristems in combination with thermotherapy (high temperatures). This method, known as a quarantine method, produces virus-free stocks which are very important for the international germplasm exchange. Furthermore, owing to the use of this method, the labor costs and space requirements for explants have dramatically decreased and what is very important, the general costs of maintaining the germplasms has also significantly decreased. Many researchers have established *in vitro* regeneration protocols for many plants (Azadi and Khosh-Khui, 2007; Engelmann, 1997; Hesami et al., 2017a; Jafari et al., 2017; Jalali et al., 2012; Rahimi et al., 2013), especially for endangered species (Fay, 1992; Sarasan et al., 2006). Importantly, for different storage durations, various *in vitro* regeneration protocols can be employed. The primary goal for short (Engelmann, 1991) and medium (Engelmann, 2011) term storage is to decline the growth rate and increase intervals between subcultures. However, for long-term storage, a new method called cryopreservation allows to store the explants at ultra-low temperatures, usually at −196 °C (Bhojwani and Dantu, 2013; Engelmann, 1997; Fay, 1992). At this temperature, all metabolic and cell division processes are stopped, and so the plant material can be stored without any modification for an unlimited period of time. Additionally, explants are stored in tiny volumes, protected from contamination, viruses, and other diseases (Engelmann, 1991, 1997, 2011; Fay, 1992; Jalali et al., 2012). Nowadays, cryopreservation is known as an important tool for storing valuable plant germplasms for a long time. It is also a very reliable alternative method to the conventional ones such as *ex situ* conservation. Usually, the "slow cooling" method is applied for ornamental plants to promote cell tolerance to storage at −196 °C, while more innovative "one-step freezing" methods such as vitrification and encapsulation-vitrification are preferred (Bhojwani and Dantu, 2013; Engelmann, 2011). In the method of slow cooling, the plant sample should be first cooled at a controlled rate of 0.5–2 °C min^{-1} down to −30 °C to −40 °C, and then it is held at this terminal prefreezing temperature for 30 minutes before transferring to liquid nitrogen (Bhojwani and Dantu, 2013). The controlled slow cooling is performed using a computer programmable cooling system, manufactured, for instance, by Planner Products Co., UK and Cryomed, USA (Bhojwani and Dantu, 2013; Engelmann, 2011). In the vitrification-based method, the plant material is desiccated first, and then it is directly saturated in liquid nitrogen (Engelmann, 1997). The solute concentration of the protoplasm increases remarkably and becomes viscous during desiccation. When such material is directly immersed into liquid nitrogen the water undergoes a phase change from liquid to a glassy state. This physical process is known as vitrification (Engelmann, 1991). Vitrification based cryopreservation protocols are very simple and more efficient for complex organs, such as embryos and shoot tips, and they do not need programmable freezers (Engelmann, 2011; Fay, 1992; Sarasan et al., 2006). The section below is a further explanation and analysis of cryopreservation methods and slow growth patterns in *in vitro* cultures.

In vitro medium-term conservation

In vitro medium-term conservation includes maintaining the cultures under growth limiting conditions to extend the intervals between subculturing. Some plant species that have a slow growth pattern can survive for several months in standard culture conditions, while others, requiring medium-term storage, usually need alternate media conditions to reduce the growth of the culture (Engelmann, 2011). The most efficient technique

for the medium-term storage of plants is to store cultures at low temperatures (Fay, 1992). Other methods that are being applied in medium-term storage are modification by adding osmotically active compounds to the culture medium, such as mannitol or reducing the nutrition, plant growth regulators (PGRs), oxygen levels, and desiccating the tissues (Engelmann, 1991, 1997).

In vitro long-term conservation

Cryopreservation as a long-term conservation for germplasms is a method based on freezing live material at the temperature of liquid nitrogen (−196°C) to suspend all metabolic activities of the cells. Therefore, using this method, cells can theoretically be stored for an unlimited time without the need of subculturing. However, increased attention is critical to protect cells from cryogenic injury during freezing, storage, and retrieval (Keller et al., 2006). In the last three decades, for the conservation of germplasms, remarkable studies have been performed to introduce effective protocols for cryopreservation of tissues, plant cells, and organized structures such as embryos or shoot tips (Ashmore and Engelmann, 1997; Engelmann, 2011; Fay, 1992; Gonzalez-Arnao et al., 2008b). Thus, many cryopreservation protocols for over 200 plant species have been introduced to date (Engelmann, 1991; Fay, 1992; Jain and Ochatt, 2010; Mérillon, 2013). The most important goal in cryopreservation is to prevent or to limit ice crystal formation in the cells during the cooling process. This can be achieved by two primary methods: first, slow or step-wise freezing method (traditional), and second, ultra-rapid freezing method (new and more popular). Regarding both methods, cells must be adequately dehydrated during or before freezing so that the protoplasms become concentrated and ice crystal formation is limited (Fay, 1992). A preculture step is helpful in increasing the desiccation tolerance of the cells. There are four major steps in cryopreservation of plant materials: 1) preculture, 2) freezing, 3) thawing, and 4) reculture. The success of a cryopreservation protocols depends also on the proper choice of the tissue to be frozen (Engelmann, 2011).

Cryopreservation of vegetatively propagated plants

There are numerous studies that provide a list of plants, which have been successfully cryopreserved (Bhojwani and Dantu, 2013; Engelmann, 1991, 1997, 2011; Fay, 1992; Gonzalez-Arnao et al., 2008a; Reed, 2008). Cryopreservation can be used broadly for vegetatively propagated species due to successfully established protocols for roots, tubers, and fruit trees, especially for tropical plants that grow all around the world in tropical regions (Pacheco et al., 2016). The survival rate for the cryopreservation method of vegetatively propagated plants is high. Some species such as *Allium* (Pandey et al., 1992), yam (Mandal et al., 1996), and potato (Pennycooke and Towill, 2000) have a 100% survival rate in this method. The meristematic zone of apices, from which the growth derives, constitutes a homogenous population of small and actively dividing cells, with a high nucleocytoplasmic ratio and little vacuoles. Also, the meristematic zones are tolerant to desiccation due to the presence of very few vacuoles. The vitrification-based methods do not form ice crystals, while in the classical approach ice formation is frequently observed. In cryopreservation methods, it is necessary to avoid the considerable damage caused by these ice crystals (Fay, 1992; Gonzalez-Arnao et al., 2008a).Thus, entire meristematic zones have been preserved by the vitrification-based methods that allow for a direct, organized regrowth (Reed, 2008).

Many vegetatively propagated plants are successfully cryopreserved. Explants that are obtained from *in vitro* regeneration are used for freezing because of their relative homogenous size, physiological state, growth response, and cellular composition. Ultimately, samples of relatively large sizes (shoot tips of 0.5 to 2–3 mm) can regrow directly without difficulties when using the vitrification-based method. Nowadays, cryopreservation approaches are easily applicable for large-scale experimentation with a vast number of plants. Based on a broad range of efficient and operationally simple methods that are available today, any vegetatively propagated plant should be responsive to cryopreservation (Engelmann, 2011; Sarasan et al., 2006).

In vitro culture

Various plant tissue culture techniques are applied for propagating plants vegetatively using tiny parts of living tissues such as shoot and root tips, leaf tissues, anthers, nodes, meristems, and embryos on artificial growth mediums under sterile environment (Hesami and Daneshvar, 2016b; Jafari et al., 2016 *In vitro* culture is

an asexual propagation of plants under *in vitro* conditions and is broadly used for commercial purposes worldwide (Bhojwani and Dantu, 2013; Hesami and Daneshvar, 2016a). Some of the plant tissue culture methods are discussed below.

Shoot induction

The shoot induction method is better than the calli method for clonal propagation of plants (Hesami et al., 2017a; Jafari et al., 2017) because callus cultures may produce abnormal plants while direct organogenesis produces uniform diploid individuals (Arab et al., 2014b; Bhojwani and Dantu, 2013).

Callus culture

Callus is an amorphous and coherent tissue that forms when plant cells multiply in a disorganized manner (Hesami and Daneshvar, 2016b; Jalali et al., 2012). However, when the callus is placed in suitable and controlled conditions, the differentiation of shoots, embryos, and roots can be achieved. Based on these culture systems, various studies on biochemical or cytological processes of growth attributed to cell enlargement, differentiation, and cell division have been conducted (Bakhshaie et al., 2016; Hesami and Daneshvar, 2016a, 2016b; Jalali et al., 2012; Niazian et al., 2017). Moreover, various reactions of genotypes from one species to *in vitro* culture conditions have been observed regularly and has been explained to be due to variations in endogenous phytohormone levels or abilities to react differently to exogenously applied plant growth regulators (PGRs) (Bhojwani and Dantu, 2013; Hesami et al., 2017b).

Somatic embryogenesis

In vitro somatic embryogenesis is a vegetative form of plant regeneration from somatic tissues; embryos can form through the developmental approach of zygotic embryogenesis (Pilarska et al., 2016). This is an important technique for genetic enhancement and an efficient asexual propagation approach for geophytes (Bhojwani and Dantu, 2013; Jalali et al., 2012). Regeneration of true-to-type plants by somatic embryogenesis contributed largely to the production of elite and new genotypes in a short time in comparison with other approaches (Cardoso et al., 2016). Somatic embryogenesis is however a double-edged sword which has advantages and disadvantages in large-scale plant regeneration. The major advantage of somatic embryogenesis is the possibility to produce the large-scale somatic embryo in encapsulation, bioreactors, genetic transformation, clonal propagation, and cryopreservation (Bhojwani and Dantu, 2013; Cardoso et al., 2016). Major limitations are genotypic dependence resulting often in poor germination rate and somatic embryo production (Jalali et al., 2012; Pilarska et al., 2016).

Some of the main advantages of multiplication over the traditional methods of clonal propagation are 1) small pieces of explants are required to initiate aseptic cultures, 2) a large number of plants can be produced in a short time and small space, starting from a single individual plant, 3) it is useful for many genotypes for which *in vivo* vegetative multiplication is difficult or impossible, 4) the rate of *in vitro* propagation is usually much faster than in the *in vivo* methods of asexual propagation, because in cultures it is feasible to manipulate the nutrient and PGRs' levels, temperature, and light conditions, 5) the plants remain protected from reinfection if they are derived from virus-free stock, 6) multiplication can be performed at any time throughout the year, 7) in the season of low market demand, *in vitro* production can be better planned by storing the cultures at low temperatures, 8) the plants are free of microorganism infestation, and the last but not the least 9) multiplying plants might gain new desirable traits via somacolonal variation such as increased number of runners in strawberry and bushy habit in ornamental plants (Arab et al., 2014a; Bhojwani and Dantu, 2013; Hesami et al., 2017b; Jalali et al., 2012).

Many methods such as micropropagation, seed germination, and regeneration from callus, embryo rescue, and cryopreservation are introduced for conserving rare and endangered plants' germplasms. *In vitro* methods for the propagation and preservation of geophytes have many advantages such as fast multiplication and relatively low costs, and thus they attract much more attention than traditional methods such as vegetative propagation (Bakhshaie et al., 2016; Hesami and Daneshvar, 2016a, 2016b; Hesami et al., 2017a, 2017b; Jalali et al., 2012; Mohammadi-Dehcheshmeh et al., 2007, 2008).

Despite various results being obtained for the survival of explants recovered from cryostorage at the beginning of establishing this new technique, efficient cryoprotocols have only been established and developed over time (Pacheco et al., 2016). According to a previous study, Banciu and coworkers (2010) investigated the *in vi-*

Table 1. Studies of Iranian ornamental geophytes using *in vitro* culture techniques

Species	Explant type	Regeneration method	References
I. petrana	flower, leaf, anther	indirect organogenesis	Al-Gabbiesh et al. (2006)
I. atrofusca	flower, leaf, anther	indirect organogenesis	Al-Gabbiesh et al. (2006)
Scilla autumnalis	*in vitro* grown seedling	bulblet formation	Banciu et al. (2010)
Lilium ledebourii	scale	bulblet formation	Azadi and Khosh-Khui (2007)
Fritillaria persica	leaf disk, leaf scale, and bulblet	regeneration and bulblet formation	Cakmak et al., (2016)
Fritillaria persica	petal	bulblet regeneration	Mohammadi-Dehcheshmeh et al., (2008)
Fritillaria persica	scale, leaf primordial, and petal	indirect organogenesis	Rahimi et al. (2013)
Cyclamen spp.	cotyledon, tuber, petiole, and root	indirect organogenesis	Jalali et al., (2012)

tro propagation of endangered plant, *Scilla autumnalis* L. This plant is protected by the National Red List of higher plants in Romania. They established that the protocol of cryopreservation of *Scilla autumnalis* as the best way for conserving this plant. In another study, *in vitro* culture was developed for two endangered *Iris* species (*I. petrana* and *I. atrofusca*) by Al-Gabbiesh and coworkers (2006). A tissue culture protocol was also established and developed for the mass multiplication of *Lilium species* Thunb. var. *gloriosoides* Baker, a native and rare perennial bulbous plant that only exists at the 150–660 m altitudes in the northern part of Taiwan (Bakhshaie et al., 2016; Chang et al., 2000). Many propagation methods were employed to find a fast and applicable method of propagation for *Fritillaria persica* (Cakmak et al., 2016; Ebrahimie et al., 2006; Genders, 1973; Mohammadi-Dehcheshmeh et al., 2007; Rahimi et al., 2013) and such studies were conducted after a notice (warning) had been issued informing that this species should be protected (Uluğ et al., 2010). Ebrahimie and coworkers (2006) reported that *Fritillaria* species such as *F. imperialis* are in danger of extinction in Iran. Thus, somatic embryogenesis via callus phase from petals was used for micropropagation of this endangered plant in Iran (Mohammadi-Dehcheshmeh et al., 2008). Indirect regeneration of *F. imperialis* was indicated by Rahimi and coworkers (2013). A protocol for the *in vitro* propagation of "Susan-e-Chelcheragh," *Lilium ledebourii* (Baker) Boiss has been developed and growth regulators and sucrose concentrations, harvesting season, and cold treatment have been optimized (Azadi and Khosh-Khui, 2007). Also, a somatic embryogenesis protocol was established and developed to propagate *Lilium ledebourii* by employing new vegetative microscale methods and using the transverse thin cell layer protocol (tTCL) of the young bulblet roots as the sources of explants (Bakhshaie et al., 2010). Farahmand and Khosh-Khui (2006) investigated three methods namely twin-scaling, chipping, and micropropagation for propagating two *Narcissus tazetta* L. species, locally known as Narges-e-Shahla and Narges-e-Meskin. The authors developed and optimized vegetative propagation methods with specific protocols for *in vitro* regeneration of researched populations. Fortunately, Narges-e-Shahla and Narges-e-Meskin are protected by the government in some provinces of Iran such as Khuzestan and Fars. The studies of Iranian ornamental geophytes in plant tissue cultures are summarized in Table 1.

Conclusions

Iran is known as the national and international heritage resource of ornamental geophytes. The natural propagation rate for many ornamental geophytes is low while the rate of extinction is high. Therefore, certain measures must be undertaken and steps formed regarding the preservation of these invaluable natural resources at the local, national, and international levels, to save them for the current and future generations.

Plant tissue culture together with cryopreservation methods are of great importance for medium- and long-term conservation of valuable plant germplasms, and geophyte species, in particular. However, different problems have to be coped with before such methods can routinely be used. Furthermore, the germplasms have to be measured for long-time storage.

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