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Research review paper

Current status and conservation of *Pistacia* germplasm

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ABSTRACT

The genetic erosion of *Pistacia* germplasm has been highlighted in many reports. In order to emphasize this and to focus more attention on this subject, national and international (especially IPGRI and IFAR) institutions have initiated projects proposing to characterize, collect and conserve *Pistacia* germplasm. Therefore, this paper reviews recent research concerning conventional (*in situ* and *ex situ*) and unconventional biotechnological conservation strategies applied to the preservation of *Pistacia* germplasm. As regards conventional conservation, the majority of germplasm collections of *Pistacia* species are preserved on farms (*in situ*) and in seed and field genebanks (*ex situ*), as well as in the wild, where they are vulnerable to unexpected weather conditions and/or diseases. Hence, complementary successful unconventional *in vitro* methods (organogenesis, somatic embryogenesis and micrografting) and slow-growth storage conditions for medium-term preservation of *Pistacia* are presented together with the morphological and molecular studies carried out for the characterization of its species in this review. Moreover, special attention is additionally focused on cryopreservation (dehydration- and vitrification-based one-step freezing techniques) for the long-term preservation of *Pistacia* species. Possible basic principles concerning the establishment of a cryobank for the successful conservation of *Pistacia* germplasm are also discussed.

Contents

1. Introduction

Pistacia genus belongs to the *Anacardiaceae* family and consists of at least 11 species of dioecious trees and shrubs, among which pistachio (*Pistacia vera* L.) is the most important species of the genus with its commercially valuable edible nuts. Pistachios are generally

Abbrevations: ABA, abscisic acid; AFLP, amplified fragment length polymorphisms; BA, 6-benzyladenine; DMSO, dimethylsulfoxide; ISSR, inter simple sequence repeat; LN, liquid nitrogen; LS, loading solution; MC, moisture content; MS, Murashige and Skoog; *P.*, *Pistacia*; PVS2, plant vitrification solution 2; RAPD, randomly amplified polymorphic DNA; RFLP, restriction fragment length polymorphisms; SSR, simple sequence repeat.

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consumed as a luxury table nut and it is also widely used in the pastry confectionary industry, confectionery and ice-cream industries for its pleasing flavour and green color. In addition to being a valuable nut tree, the pistachio tree has other uses, including tannin production for the leather industry and resin containing mastic and its volatile oils. The colorful wood of the pistachio is also used in the production of high charcoal (Onay and Jeffree, 2000).

Pistachio and other *Pistacia* species cultivation play a vital role in the nutrition and agricultural economy of many poor communities living in the arid and semi-arid regions of Iran, Turkey, Syria and other pistachio producing countries due to the tree's adaptation to harsh desert conditions where only a limited number of other Mediterranean species can be cultivated (Padulosi et al., 1996). In addition, the cultivation of pistachio and other drought resistant *Pistacia* species is important as wind breaks, especially for some Mediterranean countries, in the conservation of soil against erosion (Batlle et al., 1996). Furthermore, seeds of *P. vera*, *P. lentiscus* and *P. terebinthus* are also used for oil and soap production in the cosmetic and pharmaceutical industries (Kamangar et al., 1975), while *P. integerrima*, *P. khinjuk*, *P. atlantica*, *P. lentiscus*, *P. terebinthus* and *P. palaestina,* even in some countries *P. vera,* are used as rootstocks for pistachio (Joley, 1969).

Despite their long-term cultivation and their important contribution to the economics of especially arid and semi-arid countries, the genetic erosion of *Pistacia* species has been occurring across Central and West Asia, North Africa (CWANA) and the Mediterranean (Padulosi et al., 1996; Padulosi and Hadj-Hassan, 2001). The erosion has primarily been due to the long life-span of the trees, the propagation systems (Barone and Caruso, 1996), the abandonment of local varieties due to the specialization of the pistachio (*P. vera*) orchards on a few commercial cultivars and the destruction of the tree's natural habitat (Padulosi and Hadj-Hassan, 2001) by severe anthropogenic pressures such as fires and unregulated forest clearance for agricultural purposes (Kaska et al., 1996). Therefore, an international network for the collection, characterization and conservation of *Pistacia* genetic resources was initiated in 1994 within an IPGRI Project entitled "Conservation and Use of Underutilized Mediterranean Species (UMS)". One of the main objectives of this project was the conservation of *Pistacia* germplasm in seed banks and clonal collections in order to preserve its biodiversity (Padulosi et al., 1996). By means of these initiatives, successful attempts were initially achieved concerning the characterization of the genus *Pistacia* via morphological and molecular markers; however, some confusion still exists in the classification of the *Pistacia* genus, which requires further investigation.

Besides characterization studies, conservation of plant germplasms can be conventionally accomplished *in situ*, in place, or *ex situ*, by removing the plant material from its original location (Hummer, 1999). In recent years, concerns have been overridden about the long-term conservation of plant germplasms by using both conventional *in situ* and *ex situ* facilities, as germplasms preserved this way can be lost due to unexpected weather changes and/or diseases. Hence, unconventional biotechnological methods, including the development of *in vitro* micropropagation methods (organogenesis, somatic embryogenesis and micrografting) and slow-growth storage conditions (including the modifications of both medium components and culture conditions) for medium-term and cryopreservation (dehydration- and vitrification-based one-step freezing techniques) for the long-term preservation of *Pistacia* species, should be used to complement conventional methods.

Micropropagation in which shoot multiplication is achieved by axillary or adventitious shoot proliferation can be used as an initial attempt for unconventional *in vitro* germplasm preservation. Moreover, it plays a vital role in the re-establishment of endangered plant species (i.e., *Cypripedium calceolus* L., Ramsay and Steward, 1998). However, *in vitro* techniques require periodic transfer (generally once every 3–4 weeks) of cultures to fresh media for the renewal of the gaseous atmosphere of the vessels and the inclusion of plant growth regulators and organic, and inorganic components of the culture media. These requirements increase the cost of conservation, but more importantly, increase the risk of the occurrence of somaclonal variation, which means that the genetic fidelity of the stored germplasm is not ensured.

The risk of the occurrence of somaclonal variation can be decreased by using other unconventional conservation techniques like slow-growth storage or, more safely, cryopreservation. In order to extend the subculture period for the maintenance of the genetic fidelity of *in vitro* conserved material, cultures are stored at low temperatures (always above freezing) sometimes together with modifications in the culture medium and light conditions in the former. Differently, plant cells, tissues or organs are conserved in liquid nitrogen (LN) at ultra-low temperatures (−196 °C) in the latter. As all cellular divisions and metabolic activities cease at this temperature, plant materials can be stored without any genetic alteration or modification for a theoretically unlimited period of time (Engelmann, 2004). Thus, cryopreservation provides a long-term storage method for the conservation of plant genetic resources which cannot be maintained by using conventional preservation methods (Benson, 1999).

Up to now, the majority of studies carried out on *Pistacia* genus are devoted especially to the initiation and optimization of organogenesis and somatic embryogenesis in *in vitro* culture, while slow-growth storage and cryopreservation studies are still very limited and mainly concern only *P. vera*. Thus, concerning the importance of the genus, this review aims not only to summarize recent studies carried out on morphological and molecular characterization together with *in vitro* culture, slow-growth storage and cryopreservation techniques applied to *Pistacia* for conservation of its germplasm, but also, to highlight the importance of the application of conservation studies and the establishment of cryobanks for *Pistacia* germplasm.

2. Conventional (*in situ* and *ex situ*) conservation strategies applied to *Pistacia*

Today, pistachio is cultivated primarily in Iran, the US (through importation of the nut to California by Charles Mason, who distributed the seed for experimental plantings in California, Texas and some southern states in 1854), Turkey, Tunisia, China, Syria, Greece and Italy (Table 1). According to FAOSTAT (2007), a total of approximately 595,000 ha of pistachios were harvested worldwide in 2007; however, it is accepted that probably less than 100 cultivars have been described worldwide (Maggs, 1973). The major cultivars of some producer countries are also listed in Table 1, however because of the genetic erosion of the pistachio germplasm, some relict varieties such as "Natalora", "Rappa di sessa", "Hinnulina" and "Agostina" may have already been lost as they can no longer be found in orchards (Barone and Caruso, 1996).

Conventional conservation studies of genetic resources require the proper maintenance of the gene pool diversity both for *in situ* (on farm) and *ex situ* facilities. In Turkmenistan, for instance, *P. vera* grows in the wild in Badghyz, especially around Kepele, in south-eastern Turkmenistan, and around Agachli, along the Afghan border southeast to Badghyz. These places are among the last remaining places on earth in which natural populations of pistachio are protected in national reserves in their natural habitat (Barazani et al., 2003). In addition, there is a forest of *P. atlantica* on the European side of Istanbul, Turkey which has been properly preserved (Kaska et al., 1996).

Although *in situ* conservation of plant species is carried out mostly through the management of wild populations and natural habitats, *ex situ* techniques can also be used to complement *in situ* methods (Sarasan et al., 2006). In addition to *in situ* conservation, research