

Review Article

Contribution of Plant Biotechnology in the Conservation of Threatened Plant Genetic Resources in the Mediterranean

BENMAHIOUL Mohammed El Hadi

Department of Forest Resources, Faculty of Natural Sciences and Life, Earth Sciences and the Universe, University of Tlemcen, Algeria.

***Corresponding Author:** Benmahiouel Mohammed El Hadi, University of Tlemcen, Algeria; Email: benmahiouel_mohammed@yahoo.com

Article history: Received: May 26th 2026 ; Revised: June 17th 2026; Accepted: June 17th 2026

Abstract

The regions of the Mediterranean basin are subject to significant anthropogenic pressures, resulting in overexploitation and a marked decline of natural resources. Conventional conservation techniques present numerous challenges. They are generally labor-intensive and difficult to fund. Currently, strategies for conserving plant genetic resources are focused on biotechnological techniques, which have yielded very encouraging results at low cost. *In vitro* culture is widely used for the medium-term conservation of plant species. However, plant material can be preserved for the very long term through cryopreservation in liquid nitrogen at -196°C.

Keywords: Mediterranean Basin, plant biotechnology, plant genetic resources, endangered species, *in vitro* culture, cryopreservation.

المخلص

تتعرض منطقة حوض البحر الأبيض المتوسط لضغوط بشرية هائلة، مما يؤدي إلى استغلال مفرط وتراجع ملحوظ في الموارد الطبيعية. وتواجه أساليب الحفظ التقليدية العديد من المشاكل، فهي عموماً شاقة ويصعب تمويلها. أما حالياً، فتتجه استراتيجيات حفظ الموارد الوراثية النباتية نحو التقنيات الحيوية، التي حققت نتائج مشجعة للغاية بتكلفة منخفضة تستخدم زراعة الأنسجة النباتية على نطاق واسع لحفظ أنواع النباتات على المدى المتوسط. مع ذلك، يمكن حفظ المواد النباتية لفترات طويلة جداً عن طريق التجميد العميق في النيتروجين السائل عند درجة حرارة -196 درجة مئوية.

الكلمات الرئيسية: حوض البحر الأبيض المتوسط، التقنية الحيوية النباتية، الموارد الوراثية النباتية، الأنواع المهددة بالانقراض، زراعة الأنسجة، التجميد العميق

Introduction

Biodiversity constitutes the Earth's natural wealth and provides the elements necessary for human life and prosperity. It underpins food security, environmental resilience, and the provision of essential goods and services upon which societies depend. However, this biological diversity is now seriously threatened by human activity and the effects of climate change. It is disappearing at an alarming rate across the globe.

The Mediterranean region is home to significant biological diversity. It is one of the world's richest regions with a high rate of endemism. Its unique geographical position, climatic heterogeneity, and long evolutionary history have contributed to the development of a highly diverse flora and fauna. However, the current status of plant genetic resources is alarming. Indeed, human activities, often aggressive toward a fragile natural environment, have significantly altered nearly all natural ecosystems. These pressures, combined with the effects of global climate change, are accelerating biodiversity loss and threatening the long-term survival of numerous plant species and populations.

The process of degradation in the Mediterranean region has accelerated over the past several decades due to the onset of regressive processes resulting from climate change, soil degradation through erosion and salinization, as well as declining soil fertility and shrinking land areas. Such processes not only diminish ecosystem functioning and resilience but also erode the genetic diversity that is essential for species adaptation to changing environmental conditions.

Biodiversity conservation plays an essential role in the fight against poverty and contributes to sustainable development. In particular, the conservation of plant genetic resources is essential for ensuring agricultural productivity, food security, and the adaptive capacity of crops and wild relatives in the face of environmental and climatic challenges. Today more than ever, all nations need plant genetic resources to sustainably increase their production in order to address the challenges of environmental changes and climate change. This is why the conservation and rational use of these resources remain necessary.

Plant biotechnology plays a vital role in addressing some of these issues. This article provides a brief overview of natural resources and the causes of their degradation. This review briefly presents the main conservation techniques and places particular emphasis on modern processes used in plant biotechnology, namely *in vitro* culture and cryopreservation, and their applications in the preservation of plant genetic resources.

Causes of the Degradation of Mediterranean Plant Genetic Resources

Plant genetic resources are a fundamental component of biodiversity. They make a significant contribution to the economic growth of every country. They represent an invaluable reservoir of genetic diversity that supports crop improvement, ecosystem resilience, and the sustainable provision of ecosystem services (FAO, 2019; IPBES, 2019). Mediterranean ecosystems possess many distinctive characteristics (a prolonged dry season, narrow coastal plains, mountain ranges, etc.), which account for the exceptional biological diversity of the Mediterranean region. It is one of the world's richest regions (25000 species of higher plants) and has a high rate of endemism, approximately 50% (Quezel, 1995; Médail and Myers, 2004). This significant biodiversity, combined with this endemism, makes this region one of the most fragile on the planet (Médail, 2017). Conversely, demographic pressure, rural exodus, tourism, industrialization, and the development of intensive industrial agriculture - a source of multiple forms of pollution - have accelerated the process of degradation in the Mediterranean region (Ramade, 1996; Médail and Diadema, 2006). Habitat fragmentation and land-use change are now recognized among the principal drivers of biodiversity loss throughout the Mediterranean Basin (IPBES, 2019; UNEP/MAP, 2020). Thus, the effects of climate change have begun to exacerbate existing pressures and ecosystem degradation, causing significant and sometimes irreversible changes to the environment such as soil erosion and desertification (Garavaglia and Besacier, 2014; Cramer et al., 2018; MedECC, 2020). This process of desertification, which began long ago on the southern shore of the Mediterranean (Maghreb), is intensifying day by day and causing significant losses of soil and plant genetic resources.

The degradation of natural resources in the Mediterranean basin is not a recent phenomenon. Some forests, for example, have been transformed or deforested over the course of several millennia. According to Devisse and Vallauri (2009), more than 75% of the forests and scrublands on the European shore of the Mediterranean no longer exist today. Long-term deforestation, recurrent land-use changes, and increasing human pressure have significantly reduced habitat connectivity and ecological integrity throughout the region (Médail, 2017). Mediterranean forests are currently under particular threat from wildfires, excessive logging, and overgrazing, which risks reducing forest cover by a quarter (Antoine, 1996; FAO and UNEP, 2020). Climate-induced increases in fire frequency and intensity further threaten the conservation of forest genetic resources and ecosystem resilience (Pausas and Keeley, 2021).

Industrialization plays a role in the degradation of plant genetic resources in the Mediterranean basin. Indeed, it causes further degradation, both directly through the physical destruction of ecosystems and indirectly through various forms of pollution. In industrialized countries, air pollution leads to forest decline and also threatens the genetic heritage of forest tree species. Chronic exposure to pollution may also decrease genetic diversity within plant populations, thereby limiting their adaptive potential under changing environmental conditions (Taylor and Pitelka, 1992 ; Konrad et al., 2025).

The expansion of industrial agriculture in the Mediterranean basin, particularly in coastal areas, also poses a serious threat to plant genetic resources. Indeed, the excessive use of chemicals (fertilizers and pesticides) causes soil and groundwater pollution. According to Ramade (1996), the use of herbicides

and fertilizers has impoverished the flora and had negative impact on populations of ruderal and field plants, which are of considerable species richness in the Mediterranean basin.

Biological invasions constitute an additional and increasingly important driver of biodiversity loss in Mediterranean ecosystems. Invasive alien plant species can alter ecosystem structure and functioning, compete with native species, and contribute to the decline of endemic plant populations (Vilà et al., 2011; IPBES, 2023).

The loss of biodiversity and the degradation of ecosystems pose a threat to the functioning of our planet, our economy, and humanity as a whole. Indeed, the ongoing depletion of natural resources remains one of the most urgent conservation issues at the international level. Recent global assessments indicate that biodiversity is declining at an unprecedented rate, with profound consequences for ecosystem services and the achievement of sustainable development goals (IPBES, 2019). Currently, the need to implement effective strategies for the management and preservation of these resources is recognized in many countries. Integrated approaches combining *in situ* conservation, *ex situ* conservation, ecological restoration, sustainable land management, and climate-adaptation measures are increasingly recognized as essential for safeguarding Mediterranean plant genetic diversity and ensuring its long-term availability for future generations (FAO, 2019; MedECC, 2020; IPBES, 2023).

Conservation of Plant Genetic Resources

Methods for conserving plant genetic resources vary both in terms of overall strategy and the techniques employed. Indeed, they can be carried out in various ways, ranging from the preservation of ecosystems to the conservation of organs and/or cells in liquid nitrogen, all with the common aim of maintaining maximum genetic and e al diversity across all species. Regardless of the method used, the primary objective is to maintain the highest possible levels of genetic diversity and evolutionary potential within and among species (Maxted et al., 2020).

Generally, there are two main conservation strategies: *in situ* conservation and *ex situ* conservation. *In situ* and *ex situ* methods are complementary and are not exclusive. They offer different alternatives for conservation, but selection of the appropriate strategy should be based on a number of criteria, including the biological nature of the species and the feasibility of applying the chosen methods (Engelmann, 2012). Current international conservation policies emphasize the importance of combining both approaches to maximize the long-term preservation of plant genetic resources (FAO, 2019; CBD, 2022).

The first type of conservation involves the management and monitoring of taxa. It enables the genetic heritage to be conserved *in situ*, where the population in question is found, that is, within its native ecosystem. This conservation is also referred to as dynamic because plant genetic resources are subject to the natural process of evolution, which over time causes species to disappear or emerge. Field gene banks are beneficial for species that do not produce seeds or that are difficult to preserve in seed form: this is the case for many perennials, plants that reproduce vegetatively, and certain woody species.

In situ conservation maintains not only existing genetic diversity but also the evolutionary processes that generate new diversity over time (Maxted et al., 2020). Field gene banks represent an important component of *in situ* conservation strategies, particularly for species that produce few or no viable seeds, exhibit recalcitrant seed behavior, or are propagated vegetatively. This is especially relevant for perennial crops, woody species, and many fruit trees. These collections provide valuable opportunities for characterization, evaluation, breeding, and research while ensuring the maintenance of unique genotypes (Volk et al., 2021).

In situ conservation can be implemented by establishing protected areas such as reserves and national parks. These measures are effective but cumbersome and costly (Nanson and Jacques, 1998). According to Ramade (1996), there are approximately two hundred protected areas with a surface covering 50 hectares or more, spread across the 14 countries bordering the Mediterranean. Since then, considerable progress has been made in expanding protected area networks; however, important

conservation gaps remain, particularly in biodiversity-rich coastal ecosystems and areas harboring endemic plant species (UNEP/MAP, 2020).

In North African countries, *in situ* conservation of forest resources is limited to the static protection of certain forest populations or species within national or regional parks and nature reserves (Harfouche et al., 2005). According to the same authors, this conservation technique is more akin to a large-scale protection effort, the effectiveness of which remains to be proven. Furthermore, it is essential to supplement these *in situ* conservation measures with modern *ex situ* conservation methods. Recent studies further emphasize the need for genetic monitoring programs capable of assessing the adaptive potential and long-term viability of conserved populations under climate change scenarios (FAO, 2025).

The second method, *ex situ* conservation or ‘off-site’ conservation encompasses the maintenance and management of biological diversity components outside their native habitats. Its principal objective is to enhance conservation outcomes by securing the long-term survival of threatened taxa and conserving their genetic resources. *Ex situ* techniques should always be seen as complimentary to *in situ* conservation, aiding species recovery and reintroduction. This is referred to as static conservation, which encompasses several techniques such as field collections, pollen conservation, seed banks, *in vitro* tissue culture, cryopreservation, and DNA repositories. *Ex situ* collections play a critical role as safeguards against genetic erosion, habitat destruction, and species extinction (Engels and Ebert, 2021). Nevertheless, *ex situ* conservation has, in some cases, limitations in its application depending on the nature of the plant species. Indeed, the costs of maintaining collections are generally high (labor, phytosanitary treatments, etc.), and the plant material involved in conservation is often exposed to various pathogens and pests as well as climatic and edaphic stresses (frost, drought, salt and water stress, etc.). Furthermore, the capacity of these collections to preserve a significant amount of diversity is generally limited (Engelmann, 1997; Engelmann and Engels, 2002). In addition, prolonged *ex situ* maintenance may occasionally result in genetic drift or unintentional selection, potentially altering the genetic composition of conserved populations (Volk et al., 2021).

Pollen conservation is considered a special case. Indeed, pollen storage, often of limited duration, is aimed much more at artificial fertilization and variety development than at the preservation of plant genetic resources (Ferreira et al., 2007). Advances in pollen cryopreservation have significantly improved long-term storage possibilities for numerous economically important species (Rajasekharan and Ganeshan, 2019 ; Rajasekharan et al., 2025).

Seed storage remains one of the most widely used and least expensive conservation measures. This technique involves placing seeds in conditions that inhibit germination in order to maximize their shelf life. Seed longevity is partly determined by water content and temperature, but also by environmental interactions during the maturation phase (Walters et al., 2010). Globally, seed banks currently conserve millions of accessions and constitute the backbone of *ex situ* conservation programs (FAO, 2019). However, seed conservation is not suitable for all plant species. The seeds of certain plants can be preserved for several months, or even several years, when storage conditions are favorable. However, some species produce sterile seeds, generate limited quantities of seeds, exhibit complex dormancy mechanisms, or possess seeds with inherently short longevity (Pence, 2005).

Depending on their response to desiccation, seeds are generally classified as orthodox or recalcitrant (Roberts, 1973). Orthodox seeds tolerate substantial dehydration and can usually be stored at low temperatures for extended periods without significant viability loss. Examples include wild cherry (*Prunus avium*) and ash (*Fraxinus excelsior*), whose seeds can tolerate moisture contents of approximately 8–9% and remain viable for several years under sub-zero storage conditions (Muller, 1992).

In contrast, recalcitrant seeds contain high levels of water and cannot survive severe dehydration (Bewley and Black, 1994). Their storage therefore requires relatively high moisture content and carefully controlled environmental conditions. Even under optimal conditions, viability generally declines within a few weeks or months (Engelmann, 1992). This category includes numerous tropical and woody species, such as coconut, coffee, oil palm, walnut, hazelnut, and chestnut. The

conservation of recalcitrant-seeded species remains one of the major challenges in plant genetic resource management worldwide (Pritchard et al., 2022). Similarly, the conservation of vegetatively propagated plants presents significant difficulties because conventional seed storage is often not possible. Alternative approaches are therefore required to ensure the long-term preservation of these valuable genetic resources.

Modern biotechnological tools have revolutionized plant genetic resource conservation. Their objective is not only the storage of genes but also the reliable propagation of rare and valuable genotypes threatened with extinction. *In vitro* culture techniques enable the medium-term conservation of vegetatively propagated species, while cryopreservation allows the long-term storage of tissues, embryos, shoot tips, pollen, and somatic cells at ultra-low temperatures (-196°C) in liquid nitrogen with minimal genetic alteration (Engelmann, 2011; Reed et al., 2011).

Today, cryobiotechnology is increasingly regarded as one of the most reliable approaches for the long-term conservation of endangered species, crop wild relatives, elite cultivars, and rare genotypes threatened by habitat loss and climate change. Beyond simple storage, these technologies support germplasm exchange, restoration programs, genetic improvement, and the preservation of evolutionary potential for future generations (FAO, 2025).

4. Plant biotechnology tools developed to conserve plant genetic resources

Plant biotechnology encompasses a wide range of technological approaches, including plant tissue culture, molecular marker technologies, genomics, cryopreservation, and genetic engineering, which are used to improve plant species and support their sustainable utilization. These techniques facilitate the propagation, characterization, conservation, and genetic enhancement of plant genetic resources.

Biotechnology currently offers promising prospects in the field of genetic improvement in agriculture, horticulture, and forestry. Advances in plant biotechnology, especially those associated to *in vitro* culture and cryopreservation, have also provided powerful tools to support and improve conservation and management of plant diversity (Withers, 1995). These technologies complement conventional *ex situ* and *in situ* conservation strategies by enabling the long-term preservation of germplasm, the rapid multiplication of threatened taxa, and the recovery of rare or endangered species with limited reproductive capacity (Engelmann, 2011; Reed et al., 2011). They are essential for preventing the extinction of very specific species in the context of conserving the biological diversity of Mediterranean ecosystems and enhancing their value.

4.1. *In vitro* conservation of plant germplasm

In recent decades, plant biotechnology has undergone substantial technological advancements, providing innovative tools for the conservation and sustainable management of plant genetic resources worldwide. Among these technologies, *in vitro* plant tissue culture has become an essential component of conservation programs aimed at safeguarding plant biodiversity and supporting the genetic improvement of economically and ecologically important species.

Tissue culture techniques are widely used for the collection, multiplication, and preservation of plant germplasm. They are particularly valuable for the conservation of species producing recalcitrant seeds, vegetatively propagated crops, as well as rare, endemic, and threatened plant species that are difficult to maintain through conventional seed banking approaches (Bunn et al., 2007; Engelmann, 2011). *In vitro* conservation also provides a secure alternative for preserving genotypes vulnerable to habitat loss, climate change, invasive species, and emerging pests and diseases, thereby contributing to global biodiversity conservation efforts (Li and Pritchard, 2009; Reed et al., 2011). Furthermore, *in vitro* techniques offer several advantages, including high multiplication rates, the production of genetically uniform and vigorous plant material, the generation of pathogen-free plants and rapid large-scale propagation throughout the year. These systems facilitate the establishment of specialized germplasm collections under controlled environmental conditions, minimizing risks associated with environmental fluctuations and biological contamination (Kaviani, 2011 ; Pence, 2014). In addition, *in*

vitro collections generally require less space and lower operational costs than field gene banks, making them particularly suitable for the conservation of valuable or difficult-to-maintain genetic resources.

In vitro conservation is primarily considered a medium-term preservation strategy. It has been successfully applied to numerous woody perennials, forest trees, fruit crops, medicinal plants, and vegetatively propagated species, including those producing roots, tubers, bulbs, and rhizomes. The technique is based on the reduction of plant growth and metabolic activity through the modification of culture conditions. Growth reduction can be achieved by manipulating environmental factors such as low temperatures, reduced light intensity, shortened photoperiods, or complete darkness depending on the species. Nutritional adjustments, including the reduction of mineral salt concentrations and the incorporation of osmotic agents, activated charcoal, or growth regulators such as abscisic acid, may further contribute to growth suppression and prolonged storage periods.

The slow-growth storage approach aims to extend subculture intervals while maintaining the viability and genetic stability of conserved material. This strategy significantly reduces labor requirements, operational costs, and the risk of somaclonal variation associated with repeated subculturing (Engelmann, 2011; Kaviani, 2011). Growth retardation under *in vitro* conditions can also be achieved by limiting oxygen availability within culture vessels. However, following prolonged storage, explants often exhibit delayed regrowth, reduced vigor, and, in some cases, tissue necrosis (Engelmann, 1992).

Despite its effectiveness, minimal-growth storage remains a medium-term conservation strategy because the regenerative capacity of vegetative tissues may decline progressively during extended storage periods. Moreover, prolonged *in vitro* maintenance can increase the risk of physiological disorders, microbial contamination, and genetic or epigenetic alterations that may compromise the integrity of conserved germplasm (Harding, 2004; Wang et al., 2021). Consequently, for long-term conservation, cryopreservation is currently the most widely used and effective technique (Engelmann, 2011; Benmahioul et al., 2015 ; Reed et al., 2011; Bettoni et al., 2018). The use of cryopreservation is now considered a best-practice strategy for the management of ex situ plant germplasm collections, particularly for species that cannot be effectively conserved through conventional seed banking methods (FAO, 2025; Maxted, 2025).

4.2. Long-Term Conservation through Cryopreservation

Cryopreservation is regarded as the safest and most cost-effective strategy for long-term conservation of the germplasm of economically-important plant species as well as endangered plants. It provides a secure means of preserving a wide range of plant materials, including species producing recalcitrant (Engelmann, 2009; Kaviani and Kulus, 2022). Many species produce seeds that cannot be stored for more than a couple of weeks, and some species rarely or never produce seed. Within the framework of global biodiversity conservation, cryopreservation plays a pivotal role in *ex situ* conservation programs by ensuring the maintenance of genetic diversity, minimizing the risks of genetic erosion, and safeguarding plant resources against habitat loss, climate change, emerging pests, and anthropogenic disturbances.

During recent years, a significant progress has been made with the development of new cryopreservation techniques for non-orthodox and vegetatively propagated species. Currently available technologies offer the possibility of cryopreservation for many plants.

The principle of cryopreservation consists of storing viable plant material in liquid nitrogen (LN) at -196°C or in its vapor phase (approximately -150°C). At these ultra-low temperatures, cellular division and metabolic activities are effectively arrested, allowing biological materials to be preserved for theoretically unlimited periods without genetic alteration or physiological deterioration (Engelmann, 2011). Unlike conventional *in vitro* conservation methods, cryopreservation eliminates the need for frequent subculturing, thereby reducing labor costs, contamination risks, somaclonal variation, and the accumulation of genetic changes over time. However, the efficiency of cryopreservation protocols depends on the genotypes of the plant and may vary not only for the individual plant species but also for cultivars and genetic lines.

The physiological and developmental status of the starting plant material is a critical determinant of cryopreservation success. Plant tissues selected for cryogenic storage should ideally be juvenile, small in size, highly cytoplasmic, poorly vacuolated, and characterized by low water content. Such characteristics enhance cellular tolerance to dehydration and freezing stresses. Prior to storage, samples are placed in cryovials specifically designed to withstand extreme cryogenic conditions.

A major challenge during cryopreservation is preventing the formation of intracellular ice crystals, which can cause irreversible cellular damage and loss of viability. Therefore, the preparation of plant material before freezing constitutes one of the most crucial stages of the protocol. This preparation aims to induce cellular hardening, reduce freezable water content, and increase tolerance to ultra-low temperatures.

Certain types of plant material, such as orthodox seeds and naturally cold-hardened dormant buds, can be cryopreserved with minimal or no pretreatment. For example, *Sorbus latifolia* dormant buds have been successfully cryopreserved following direct transfer from field-grown trees to liquid nitrogen with limited preparatory treatment (Harvengt & Dumas, 2003). This approach is particularly valuable for the conservation of woody perennial species and forest genetic resources, where seed storage may not adequately preserve the full genetic diversity of natural populations.

In contrast, highly hydrated and freezing-sensitive tissues such as shoot tips, meristems, embryogenic cultures, somatic embryos, calli, and zygotic embryos require controlled dehydration before freezing. Artificial dehydration minimizes the amount of free intracellular water and thereby reduces the risk of lethal ice crystal formation. Dehydration may be achieved through physical methods, including exposure to silica gel (Benmahioul et al., 2015) or controlled air desiccation under sterile laminar airflow conditions.

Alternatively, intracellular ice formation can be avoided through vitrification-based cryopreservation techniques, which are now among the most widely used approaches in plant cryobiology (Sakai and Engelmann, 2007). Vitrification refers to the transition of cellular water from the liquid state directly into a metastable amorphous glassy state without crystallization. During this process, explants are treated with highly concentrated cryoprotective solutions and subsequently cooled at extremely rapid rates. As a result, cellular contents solidify into a glass-like matrix, preventing mechanical damage associated with ice crystal growth.

Recent advances in plant cryobiotechnology have led to the development of several vitrification-derived methods, including encapsulation-dehydration, encapsulation-vitrification, droplet-vitrification, and cryo-plate techniques. These approaches have significantly improved post-thaw recovery rates across a broad spectrum of plant species, including many endangered and economically important taxa.

Cryopreservation has also been successfully applied to a variety of cellular systems, including protoplasts and suspension cultures (Langis et al., 1989; Uragami et al., 1989; Langis & Steponkus, 1990). Prior to freezing, these materials undergo osmotic dehydration through incubation in media supplemented with cryoprotective agents. Common cryoprotectants include dimethyl sulfoxide (DMSO), glycerol, ethylene glycol, sucrose, and plant vitrification solutions (PVS2 and PVS3) (Kim, 2023), which reduce freezing injury by stabilizing cellular membranes and promoting glass formation during cooling.

Following cryogenic storage, successful recovery depends on rapid thawing and the implementation of optimized regeneration protocols. Post-thaw assessments typically include measurements of survival, regrowth, genetic stability, and physiological integrity.

Numerous studies have demonstrated that properly cryopreserved plant materials retain their genetic fidelity after long-term storage, confirming the value of cryopreservation as a cornerstone technology for the conservation of plant biodiversity and the sustainable management of genetic resources.

Conclusion and recommendations

In recent decades, the degradation of natural resources in the Mediterranean Basin has accelerated considerably as a result of climate change, land-use transformation, habitat fragmentation, and increasing human pressure. These factors have contributed to unprecedented rates of species decline and extinction (Gaston, 2005; Díaz et al., 2006). Current projections indicate that climate warming, increasing aridity, and the growing frequency of extreme climatic events may further exacerbate biodiversity loss and reduce the adaptive capacity of Mediterranean ecosystems during the coming decades. Addressing these challenges constitutes one of the most important environmental priorities for Mediterranean countries and requires the development of scientifically sound, integrated, and long-term conservation strategies.

At the same time, financial constraints associated with the maintenance of field collections, together with the difficulties involved in conserving species producing recalcitrant seeds or those propagated vegetatively, have increased the importance of plant biotechnology in biodiversity conservation. Modern biotechnological approaches now provide powerful complementary tools for safeguarding threatened plant genetic resources, particularly for species that cannot be efficiently conserved through conventional seed banking. Techniques such as *in vitro* culture, slow-growth storage, cryopreservation, molecular characterization, and genomic analyses offer effective solutions for the long-term preservation of genetic diversity under increasing environmental and anthropogenic pressures. Nevertheless, these technologies should not be viewed as substitutes for conventional conservation approaches. Rather, they should be integrated into a comprehensive conservation framework combining *in situ*, *ex situ*, and restoration-based strategies. Such an integrated approach maximizes the preservation of evolutionary processes, adaptive potential, and genetic diversity while enhancing the effectiveness of biodiversity conservation programs. Biotechnology therefore provides conservation practitioners and gene bank managers with additional tools for securing plant genetic resources and improving the resilience of conservation systems.

Given the exceptional biodiversity value of the Mediterranean Basin, recognized as one of the world's major biodiversity hotspots, urgent and coordinated actions are required to strengthen conservation efforts and ensure the sustainable management of natural resources. The implementation of science-based policies, supported by regional cooperation and adequate financial investment, will be essential to mitigate biodiversity loss and enhance ecosystem resilience under future environmental change. To achieve these objectives, the following recommendations are proposed:

i. Strengthen Integrated Conservation Programs

Conservation efforts should promote a balanced integration of *in situ* and *ex situ* approaches. This complementary strategy enhances the preservation of both genetic diversity and evolutionary potential while reducing the risk of species extinction.

ii. Establish Regional Mediterranean Germplasm Networks

Mediterranean countries should develop coordinated regional germplasm conservation networks and strengthen existing gene bank infrastructures. Regional collaboration would facilitate the sharing of expertise, harmonization of conservation protocols, and establishment of safety duplication systems for valuable genetic resources.

iii. Increase Research on Endemic, Rare, and Threatened Species

Additional financial and institutional support should be allocated to research programs focused on endemic and endangered Mediterranean flora. Priority should be given to studies addressing species ecology, population genetics, reproductive biology, climate-change vulnerability, restoration ecology, and conservation genomics, which are critical for evidence-based conservation planning.

iv. Adaptive Wildfire Management

As climate change is expected to increase wildfire frequency and severity throughout the Mediterranean region, conservation policies should prioritize adaptive fire management based on prevention, risk reduction, ecological restoration, and long-term ecosystem monitoring. Integrating climate projections into management frameworks will be critical for maintaining biodiversity,

reducing ecosystem vulnerability, and strengthening landscape resilience under future environmental change.

v. **Promote International and Multidisciplinary Cooperation**

Enhanced collaboration among Mediterranean countries is necessary to address shared conservation challenges. Joint research programs, transboundary conservation initiatives, and scientific exchange networks would facilitate the transfer of knowledge, technologies, and genetic resources. Furthermore, stronger partnerships between governmental institutions, research organizations, non-governmental organizations, local communities, and the private sector are crucial for achieving long-term conservation objectives.

vi. **Strengthen Policies for the Protection of Genetic Resources**

Mediterranean countries should reinforce legal and institutional frameworks aimed at protecting biodiversity and genetic resources. Effective regulations are needed to control the overexploitation of natural resources, combat habitat destruction, and support ecological restoration and reforestation programs. The incorporation of biodiversity conservation objectives into national climate adaptation and sustainable development policies should also be prioritized.

vii. **Expand the Use of Plant Biotechnologies in Conservation Programs**

The integration of biotechnology into national conservation strategies should be actively encouraged. *In vitro* conservation, cryopreservation, molecular marker technologies and genomic tools can significantly improve the management and long-term preservation of plant genetic resources. These technologies are particularly important for conserving threatened species with limited populations, low reproductive capacity, or complex propagation requirements.

viii. **Build Scientific Capacity and Technological Infrastructure**

The successful implementation of advanced conservation technologies requires substantial investments in human resources and infrastructure. Mediterranean countries, particularly those in the southern region, should prioritize the training of specialists in plant biotechnology, conservation genetics, cryobiology, and restoration ecology. Strengthening laboratory facilities, improving access to modern equipment, and fostering international scientific partnerships will be essential for reducing technological disparities and enhancing regional conservation capacity.

In conclusion, the future conservation of Mediterranean biodiversity will depend on the successful integration of ecological knowledge, innovative biotechnological tools, effective governance, and international cooperation. By combining traditional conservation practices with emerging technologies and ecosystem-based management approaches, Mediterranean countries can significantly improve the protection of their unique biological heritage and strengthen ecosystem resilience in the face of global environmental change.

References

- Antoine, S., 1996. Nature, environnement et développement durable en Méditerranée. *Revue Aménagement et nature*, n° 121 - Méditerranée et Développement durable, 15–23.
- Benmahioul, B., Kaid-Harche, M., Daguin, F., 2015. Cryopreservation of *Pistacia vera* embryonic axes. *J. FOR. SCI.*, 61(4): 182-187. DOI: 10.17221/63/2014-JFS
- Bettoni, J.C.; Costa, M.D.; Souza, J.A.; Volk, G.M.; Nickel, O.; Da Silva, F.N.; Kretzschmar, A.A., 2018. Cryotherapy by encapsulation-dehydration is effective for *in vitro* eradication of latent viruses from ‘Marubakaido’ apple rootstock. *J. Biotechnol.*, 269, 1–7.
- Bewley, J.D. & Black, M., 1994. Seeds: physiology of development and germination. New York: Plenum Press, p445
- Bunn, E., Turner, S.R., Panaia, M. & Dixon, K.W., 2007. The contribution of *in vitro* technology and cryogenic storage to conservation of indigenous plants. *Austr. J. Bot.*, 55, 345–355.
- CBD, 2022. *Kunming-Montreal Global Biodiversity Framework*. Convention on Biological Diversity (CBD). <https://www.cbd.int/article/cop15-final-text-kunming-montreal-gbf-221222>

- Cramer, W., Guiot, J., Fader, M. *et al.* 2018. Climate change and interconnected risks to sustainable development in the Mediterranean. *Nature Clim Change* 8, 972–980. <https://doi.org/10.1038/s41558-018-0299-2>.
- Devisse, J.S. et Vallauri, D., 2009. Les forêts de la Méditerranée : un capital nature, une nature capitale. *Forêt méditerranéenne* t. XXX, n° 4, 355–358.
- Diaz, S., Fargione, J., Chapin, F.S., Tilman, D., 2006. Biodiversity Loss Threatens Human Well-Being. *Pl Biology*, 4(8): 1300-1305.
- Engelmann, F., 1992. Les nouvelles méthodes de conservation ex situ. Actes du colloque en hommage à Jean Pernès, 435–445.
- Engelmann, F., 1997. *In vitro* conservation methods. In: Ford-Lloyd BV, Newbury JH, Callow JA, (Eds.). *Biotechnology and Plant Genetic Resources: Conservation and Use*. Wellingford : CABI.
- Engelmann, F., 2007. Vitrification, encapsulation vitrification and droplet- vitrification: a review. *Cryoletters*. 28(3) : 151 – 172.
- Engelmann, F., 2009. Biotechnologies et conservation des ressources phytogénétiques. *Cah. Agric.*, 18(6) : 481–485.
- Engelmann, F., 2011. Use of biotechnologies for the conservation of plant biodiversity. *In Vitro Cell. Dev. Biol. Plant*, 47 : 5–16.
- Engelmann, F., 2012. Germplasm Collection, Storage and Conservation. In *Plant Biotechnology and Agriculture* (Altman & Hasegawa, Eds.), Academic Press: Oxford, UK : 255–268.
- Engelmann, F., Engels J.M., 2002. Technologies and strategies for ex situ conservation. *Managing Plant genetic diversity. Guide to effective management of germplasm collection*, 89–103.
- Engels, J.M.M. & Ebert, A.W., 2021. A Critical Review of the Current Global Ex Situ Conservation System for Plant Agrobiodiversity. I. History of the Development of the Global System in the Context of the Political/Legal Framework and its Major Conservation Components. *Plants*, 10, 1557. <https://doi.org/10.3390/plants10081557>
- FAO, 2019. The State of the World's Biodiversity for Food and Agriculture. Ed. by J Bélanger and D Pilling. FAO Commission on Genetic Resources for Food and Agriculture Assessments. Food and Agriculture Organization of the United Nations. <http://www.fao.org/3/CA3129EN/CA3129EN.pdf>.
- FAO & UNEP, 2020. *The State of the World's Forests 2020*. Forests, biodiversity and people. Food and Agriculture Organization of the United Nations Rome, 188p. Accessed on 20.12.2025. Available on the website : <https://openknowledge.fao.org/server/api/core/bitstreams/dfb12960-44ee-4ddc-95f7-bec93fbb141e/content>
- FAO, 2025. *The Third Report on the State of the World's Plant Genetic Resources for Food and Agriculture*. FAO Commission on Genetic Resources for Food and Agriculture Assessments, 2025. Rome. <https://doi.org/10.4060/cd4711en>
- Ferreira, C.A., Pinho, E.V. de Von, Alvim, P de O., Andrade, V de, Silva, T.T. de A., Cardoso, D.L., 2007. Conservation and determination of viability of maize pollen grain. *Revista Brasileira de Milho e Sorgo*, 6: 159-173.
- Garavaglia, V. et Besacier, C., 2014. L'état des forêts méditerranéennes en 2013. *Unasylva* 65(1): 3–13.
- Gaston, K.J., 2005. Biodiversity and extinction: species and people. *Progress in Physical Geography*, 29(2): 239-247.
- Harfouche, A., Nedjahi, A., Ellatifi, M. et Daly-Hassen, H., 2005. Les ressources génétiques forestières nord-africaines et leur conservation. *Rev. For. Fr.* LVII, 15-32.
- Harding K., 2004. Genetic integrity of cryopreserved plant cells: a review. *CryoLetters* 25(1): 3-22
- Harvengt, L. et Dumas, E., 2003. La cryoconservation des ligneux, une technique au service de la gestion des ressources génétiques sauvages et domestiquées. *Informations-Forêt*, 1: 1–6.

- IPBES, 2019. *Global Assessment Report on Biodiversity and Ecosystem Services*. Accessed on 13.11.2025. Available on the website : https://files.ipbes.net/ipbes-web-prod-public-files/inline/files/ipbes_global_assessment_report_summary_for_policymakers.pdf
- IPBES, 2023. *Assessment Report on Invasive Alien Species and their Control*. Accessed on 20.12.2025. Available on the website : <https://www.ipbes.dk/wp-content/uploads/2023/12/Hroy12.12.23.pdf>
- Kaviani B., 2011. Conservation of plant genetic resources by cryopreservation. *Australian Journal of Crop Science*, 5(6) :778-800
- Kaviani, B., Kulus, D., 2022. Cryopreservation of endangered ornamental plants and fruit crops from tropical and subtropical regions. *Biol.*, 11, 847. <https://doi.org/10.3390/biology11060847>
- Kim H., 2023. Optimizing the Droplet-Vitrification Procedure by Balancing the Cryoprotection and Cytotoxicity of Alternative Plant Vitricification Solutions Based on the Nature of Donor Plant Vigor. *Plants*, 12(23) :4040. <https://doi.org/10.3390/plants12234040>
- Konrad, H., George, J.P., Szukala, A., 2025. The State of Forest Genetic Diversity: Anthropogenic Impacts and Conservation Initiatives. In: Lapin, K., Oettel, J., Braun, M., Konrad, H. (eds) *Ecological Connectivity of Forest Ecosystems*. Springer, Cham. https://doi.org/10.1007/978-3-031-82206-3_4
- Langis, R., Schnabel, B., Earle, E.D., Steponkus, P.L., 1989. Cryopreservation of *Brassica campestris* L cell suspensions by vitrification. *Cryo-Letters* 10: 421-428.
- Langis, R. and Steponkus, P.L., 1990. Cryopreservation of rye protoplasts by vitrification. *Plant Physiol*, 92: 666–671.
- Li, D.Z., & Pritchard, H.W. (2009). The science and economics of ex situ plant conservation. *Trends in Plant Science*, 14, 614–621.
- Maxted, N., 2025. A significantly enhanced role for plant genetic resource centres in linking in situ and ex situ conservation to aid user germplasm access. *Genetic Resources*, (S2), pp. 203–223. Doi: 10.46265/genresj.UNVV5571
- Maxted N., Hunter D, Ortiz Rios R. O., 2020. *Plant genetic conservation*. Cambridge: Cambridge University Press, 560 p.
- Médail, F., 2017. The specific vulnerability of plant biodiversity and vegetation on Mediterranean islands in the face of global change. *Reg Environ Change* 17, 1775–1790. <https://doi.org/10.1007/s10113-017-1123-7>
- Médail, F. et Diadema, K., 2006. Biodiversité végétale méditerranéenne et anthropisation : approches macro et micro régionales. *Annales de géographie* 651 : 618–640.
- Muller, C., 1992. Conservation des graines et les problèmes de levée de dormance chez les feuillus précieux. *Rev. For. Fr.* XLIV - n° sp. : 39–46.
- MedECC, 2020. Climate and Environmental Change in the Mediterranean Basin – Current Situation and Risks for the Future. First Mediterranean Assessment Report [Cramer, W., Guiot, J., Marini, K. (eds.)] Union for the Mediterranean, Plan Bleu, UNEP/MAP, Marseille, France, 632p. Doi: 10.5281/zenodo.4768833
- Nanson, A. et Jacques, D., 1998. Les ressources génétiques forestières. *Biotechnol. Agron. Soc. Environ.* 2(1) : 59–64.
- Pausas J.G. & Keeley J.E. 2021. Wildfires and global change. *Frontiers in Ecology and Environment* 19(7): 387-395. <https://doi.org/10.1002/fee.2359>
- Pence, V.C., 2005. *In vitro* collecting (IVC). I. The effect of collecting method and antimicrobial agents on contamination in temperate and tropical collections. *In Vitro Cell. Dev. Biol. Plant*, 41 : 324–332
- Pence V.C., 2014. *In vitro* methods and cryopreservation: Tools for endangered exceptional species preservation and restoration. *Acta Hort* 1039:73–79
- Pritchard, H.W.; Sershen; Tsan, F.Y.; Wen, B.; Jaganathan, G.K.; Calvi, G.; Pence, V.C.; Mattana, E.; Ferraz, I.D.K.; Seal, C.E., 2022. Regeneration in recalcitrant-seeded species and risks from

- climate change. In *Plant Regeneration from Seeds: A Global Warming Perspective*; Baskin, C.C., Baskin, J.M., Eds.; Elsevier: New York, NY, USA, p.15
- Quezel, P., 1995. La flore du bassin méditerranéen : origine, mise en place, endémisme. *Ecologia Mediterranea* XXI (1/2) : 19-39.
- Ramade, F., 1996. La conservation des écosystèmes méditerranéens. *Revue Aménagement et nature*, n° 121 - Méditerranée et Développement durable, 24–32.
- Rajasekharan PE, Ganeshan S., 2019. Current perspectives on pollen cryopreservation in horticultural species. In III international symposium on plant cryopreservation. *Acta Hort* 1234:47–56
- Rajasekharan, P.E., Gowthami, R., Magudeeswari, P., 2025. Cryopreserved Pollen: Applications in Biotechnology and Crop Improvement. In: Rajasekharan, P.E., Gowthami, R. (eds) *Pollen Cryopreservation Protocols*. Springer Protocols Handbooks. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-4686-1_2
- Reed, B.M., Sarasan, V., Kane, M., Bunn, E. and Pence, V.C., 2011. Biodiversity Conservation and Conservation Biotechnology Tools. In *Vitro Cellular & Developmental Biology-Plant*, 47, 1-4. <https://doi.org/10.1007/s11627-010-9337-0>
- Roberts, E.H., 1973. Predicting the viability of seeds. *Seed Sci. Technol.*, 1: 499–514.
- Sakai, A., Engelmann, F., 2007. Vitrification, encapsulation-vitrification and droplet-vitrification: a review. *Cryo-Letters*, 28(3): 151-172.
- Taylor, G.E., Pitelka, L.F., 1992. Genetic Diversity of Plant Populations and the Role of Air Pollution. In: Barker, J.R., Tingey, D.T. (eds) *Air Pollution Effects on Biodiversity*. Springer, Boston, MA. https://doi.org/10.1007/978-1-4615-3538-6_7
- UNEP/MAP, 2020. State of the Environment and Development in the Mediterranean [en ligne]. Accessed on 07.12.2025. Available on the website: <https://planbleu.org/wp-content/uploads/2020/11/SoED-Full-Report.pdf>
- Uragami, A., Sakaï, A., Nagaï, M., Takahashi, T., 1989. Survival of cells and somatic embryos of *Asparagus officinalis* cryopreserved by vitrification. *Plant Cell Rep* 8: 418-421.
- Walters, C., Ballesteros, D., Vertucci, V.A., 2010. Structural mechanics of seed deterioration: Standing the test of time. *Plant Science* 179(6): 565-573.
- Vilà M., Espinar J.L, Hejda M., Hulme P.E., Jarošík V., Maron J., Pergl J., Schaffner U., Sun Y. and Pyšek P., 2011. Ecological Impacts of Invasive Alien Species: A Meta Analysis of Their Effects on Species, Communities and Ecosystems. *Ecological Letters*, 14, 702-708. <https://doi.org/10.1111/j.1461-0248.2011.01628.x>
- Volk G. M. Byrne P. F. Coyne C. J. Flint-Garcia S. Reeves P. A. Richards C., 2021. Integrating genomic and phenomic approaches to support plant genetic resources conservation and use. *Plants* 10, 2260. Doi: 10.3390/plants10112260
- Wang, M.; Lambardi, M.; Engelmann, F.; Pathirana, R.; Panis, B.; Volk, G.M.; Wang, Q., 2021. Advances in cryopreservation of in vitro-derived propagules: technologies and explant sources. *Plant Cell Tiss Organ Cult* 144, 7–20 (2021). <https://doi.org/10.1007/s11240-020-01770-0>
- Withers, L.A., 1995. Collecting *in vitro* for Genetic Resources Conservation. In *Collecting Plant Genetic Diversity* (Guarino et al, Eds.), Centre for Agricultural Bioscience International: Wallingford, UK, 511–515.