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The RIBES strategy for *ex situ* conservation: conventional and modern techniques for seed conservation

Abstract

Magrini, S. Bonomi, C., Bacchetta, G., Bedini, G., Borzatti, A., Boscutti, F., Carasso, V., Carta, A., Casavecchia, S., Casolo, V., Ceriani, R., Cristaudo, A., Di Cecco, V., Di Martino, L., Digangi, I., Fabrini, G., Guglielmo, F., Mariotti, M., Negri, V., Porceddu, M., Villani, M., Zappa, E. & Salmeri, C.: The RIBES strategy for *ex situ* conservation: conventional and modern techniques for seed conservation. — Fl. Medit. 32: 395-401. 2022. — ISSN: 1120-4052 printed, 2240-4538 online.

The Italian seed bank network (RIBES) aims to improve the quality and safety of the germplasm reserves of native plant species in Italy to ensure the long-term conservation of endangered and/or endemic flora. The strategy includes traditional methods to secure seed conservation. A comprehensive priority list for seed collection is being defined, it was prepared by crossing data of various checklists (red lists, endemics) and will soon be cross-referenced with an updated list of accessions of the whole network. A safety-backup program of duplicates will quickly be implemented to secure the conservation of the most threatened species in at least two seed banks of the network. On the other hand, the RIBES strategy also includes research by applying modern techniques. In collaboration with the Millennium Seed Bank, research on the storage behaviour of seeds and spores through thermal analysis is ongoing to inform conservation. Using the Differential Scanning Calorimetry (DSC), we could evaluate seed lipid properties such as glass transition temperature, melting, crystallization, oxidation behaviour, and thermal stability.

Finally, RIBES participates as a co-funder in the LIFE Nature project SEEDFORCE, coordinating 11 seed banks of the network for collecting seeds/spores of 29 threatened species of EU interest.

Key words: conservation strategy, Italian flora, priority lists, seed banks, seed/spore research.

Introduction

The Convention on Biological Diversity (1992) recognised the importance of *ex situ* conservation and the relevance of networks for conservation (CBD Article 18).

In 2002, the Global Strategy for Plant Conservation (GSPC) has set out specific targets to be met by 2010, among them: Target 8: “60% of threatened plant species in accessible *ex situ* collections, preferably in the country of origin, and 10% of them included in recovery and restoration programmes” and Target 16: “Networks for plant conservation activities established or strengthened at national, regional and international levels” (CBD 2003). These GSPC targets stimulated the establishment of several networks in Europe for *ex situ* conservation: e.g. REDBAG - *Red Española de Bancos de Germoplasma de Plantas Silvestres* in Spain (2002); the network of the *Conservatoires botaniques nationaux* in France; GENMEDA - *Network of Mediterranean plant conservation centres* (whose members implemented the development of GENMEDOC and SEMCLIMED projects); ENSCONET - *European Native Seed COnservation NETwork* (2004) in Europe.

Considering this proliferation of initiatives at the international level and the lack of institutional coordination at the national level in Italy, in December 2005, 18 groups involved in the field of *ex situ* conservation of native plants (mostly belonging to public institutions, but also private or non-profit organizations) founded the association RIBES – *Rete Italiana Banche del germoplasma per la conservazione Ex Situ della flora minacciata* (Italian network of germplasm banks for *ex situ* conservation of threatened flora) (Rossi & al. 2012). RIBES was founded to promote and, as far as possible, ensure a) the *ex situ* conservation of Italian native flora at risk of extinction, also for reintroduction programs, and b) the *ex situ* conservation of native species of biogeographical and ecological significance and potential interest for renaturalization actions, such as environmental restoration and requalification (art. 2 of the Statute). The activities were organized into four working groups governed in a participatory manner and dedicated to very specific areas of action: (1) the collection of germplasm, (2) its treatment in seed banks, (3) data management, (4) promotion and dissemination (Rossi & al. 2012).

Today, RIBES includes 18 members representing 14 Italian regions and it is an active member/partner of other wider networks so providing an active connection with the international context: ENSCONET – *European Native Seed Conservation Network* (<http://ensconet.maich.gr/>), GENMEDA – *Network of Mediterranean Plant Conservation Centres* (<http://www.genmeda.net/>), *Millennium Seed Bank Partnership*, Royal Botanic Gardens, Kew (<http://brahmsonline.kew.org/msbp>); INSR – *International Network for Seed-based Restoration* (<https://ser-insr.org/>).

The ex situ conservation

According to the last surveys (2015, 2018), more than 25,000 accessions of seeds and spores are conserved *ex situ* in the RIBES seed banks, with about 3,200 taxa representing approx. 40% of the Italian native vascular flora.

Cross-checking the list of seed/spore accessions with the Italian Red Lists (Rossi & al. 2013, 2016, 2020; Orsenigo & al. 2018) and the list of endemics (Peruzzi & al. 2014) provided us with a detailed picture of the consistency of *ex situ* conservation in Italy. It showed that 303 threatened and near-threatened taxa are long-term conserved in seed banks, in particular, 32% of the Critically Endangered, 41% of the Endangered and 27% of the Vulnerable species. Moreover, 90 taxa, 41% of the so-called Policy Species (species listed in Annexes of the Habitat Directive or Bern Convention; Rossi & al. 2013), are secured in the Italian seed banks.

In 2018, a specific survey focused on endemic species was carried out, based on the list of Italian endemics (Peruzzi & al. 2014) and the new red list of species endemic to Italy (Orsenigo & al. 2018). The survey revealed that the RIBES network conserves seed/spore accessions of 554 taxa, 41% of the Italian endemics, stored in 16 seed banks, mainly in Sicily (300 taxa), Sardinia and Tuscany (>110). These taxa belong to 48 families, among them, *Asteraceae* with ca. 120 taxa, *Brassicaceae*, *Fabaceae*, and *Caryophyllaceae* with more than 40 taxa, are the most represented families. It is noteworthy that 48% of the threatened endemic taxa are stored in 15 seed banks, mainly in Sicily and Sardinia, including 52% of the Critically Endangered, 46% of the Endangered, and 42% of the Vulnerable taxa.

Another specific survey carried out in 2015 and focused on Crop Wild Relatives (CWRs), wild plant species that share a common ancestor with cultivated crop plants, showed that 37% of the Italian CWRs listed in the FAO Treaty (FAO 2001) are stored in 14 seed banks, mainly in the seed bank of Perugia, for a total of 6029 seed-lots of 229 taxa belonging to 11 families, mainly *Fabaceae* (75 taxa), *Poaceae* (68) and *Brassicaceae* (49). Among them, 294 accessions of 14 CWRs endemic to Italy (50%), mainly in 5 seed banks: Catania, Sardinia, Perugia, Padova, and Palermo (Magrini & al. 2016).

The strategy for an effective ex situ conservation

The ultimate goal of the RIBES network is the general improvement of the quality and safety of the germplasm reserves of native plant species in Italy to ensure effective long-term conservation and protection of the endangered and/or endemic flora. To achieve this goal, the RIBES strategy includes mainly traditional methods, such as the definition of priority lists to optimize the collection of seeds and spores, and the implementation of a safety-backup program of duplicates of the accessions, together with the use of modern techniques to define seed behaviour during storage and secure seed conservation.

Toward a comprehensive priority list for seed collection for the Italian flora

Prioritization of endemic and threatened species is a crucial point of conservation actions, particularly in species-rich areas like Italy. Priority lists are a fundamental and useful tool to identify the target species for *ex situ* conservation, optimizing collection efforts and conservation costs (Gauthier & al. 2010; Bacchetta & al. 2012a, 2012b).

A priority list of 43 Italian CWRs listed in the FAO Treaty (FAO 2001) was drawn up in 2016 to identify the target species for seed collection and *ex situ* conservation measures, selecting all the endemic taxa (28), the policy species (4), the threatened (CR, EN, VU) and near threatened (NT) taxa at both Italian and European level (19) (Rossi & al. 2013; Peruzzi & al. 2014; Bilz & al. 2011), and investigating their occurrence in *ex situ* collections. In the priority list, 20 taxa were considered of highest priority (HP) for collection as not preserved at all in seed banks and other 5 taxa of priority (P) for further collections being stored in seed banks as a single accession (Magrini & al. 2016). After the recent publication of the first comprehensive national inventory of CWRs (Ciancaleoni & al. 2021), such list and the list of the accessions stored in the RIBES seed banks have already been cross-referenced with the existing checklists mentioned above (Rossi & al. 2013, 2016, 2020; Peruzzi & al., 2014; Orsenigo & al. 2018) to have a complete set of data to update the previous priority list (Villani & al. 2021).

A preliminary priority list for the threatened species and the endemics is in progress. We populated a database which is continuously updated with all the available data on the endemics, threatened species, and all the relevant published papers. The analysis of the database of the seed bank collections (obtained with the last surveys in 2015-2018) highlighted many collection gaps concerning the coverage of threatened taxa, endemics and their geographic representativeness. Then, a further survey across the network is required soon to update the accession database and to be able to draw up a comprehensive updated priority list for the Italian flora.

Safety-backup program for duplicates

A duplicate security backup program will soon be implemented to ensure the conservation of the most endangered species. A safety duplicate is a sub-sample of an accession made to mitigate the risk of its partial or total loss caused by natural or man-made catastrophes. The first step will foresee the selection of the priority species to duplicate, according to updated priority lists. Safety duplicates, including both the duplication of material and its related information, will be deposited in at least another seed bank in the network. Moreover, one duplicate for each species will be sent to the Millennium Seed Bank, Royal Botanic Gardens, Kew (UK). This activity will bring benefits in terms of the safety of the collections of the most important species and will also stimulate research collaboration between seed banks.

Thermal analysis: a new tool to inform seed conservation

Under conventional storage conditions, dry orthodox seeds stored at low temperatures (-20°C) can be expected to live for tens or hundreds of years. However, longevity is known to be extremely variable, depending on the species and seed traits (Liu & al. 2018). Seed lipids have long been thought to be a determinant of seed ageing, with lipid composition impacting different susceptibility to oxidation and variation in thermal behaviour (Priestley 1985; Walters 1998; Mira & al. 2019).

Research has been ongoing since 2019 in collaboration with the Millennium Seed Bank, Royal Botanic Gardens, Kew (UK), to better understand the storage performance of seeds through Differential Scanning Calorimetry (DSC). DSC is a powerful technique that can be used to characterize the physical properties of seeds and has the potential as a rapid, non-invasive tool to understand variability in the storage performance of seeds stored in seed banks by observing lipid phase transitions (Hamilton & al. 2009).

Understanding the balance of seed lipid composition and the thermal characteristics of their melting and crystallisation behaviours could prove to be a useful tool for identifying long-term cold storage problems in seeds and potentially predicting the optimal storage temperatures, likely by avoiding freezing damage promoted by lipid crystallization and melting (Mira & al. 2019). The first DSC analyses of seeds of 15 Mediterranean terrestrial orchids showed useful results suggesting different optimal storage temperature ranges for distinct genera (Magrini & al. 2022).

The “Mediterranean plant germination reports” column

Since 2019, a new series of germination accounts for the Mediterranean flora (*sensu* Med-Checklist), proposed by RIBES, has been published annually in *Flora Mediterranea*

journal (Magrini & Salmeri 2019). In the initial three issues (2019-2021) of the “Mediterranean plant germination reports” edited by Sara Magrini & Cristina Salmeri, 23 papers were published for a total of 85 germination reports for 80 taxa belonging to 25 families. Seed germination is an important step for an effective conservation activity, thus, making these and other published germination data available to a wider audience, we are building an online, open-access database within the RIBES website, that typically includes sections on taxonomy, location data, storage behaviour, besides germination results, as part of the RIBES conservation strategy.

The LIFE SEEDFORCE project

In 2021, the LIFE20 NAT/IT/001468 “Using SEED banks to restore and reinFORCE the endangered native plants of Italy” – SEEDFORCE was funded by the European Commission. The project goal is to improve the conservation status of 29 Annex II species with an Unfavourable-Inadequate or Unfavourable-Bad conservation status, according to the last Report ex Art.17, occurring in 76 SCI/SACs mainly in Italy, and in France, Slovenia and Malta. RIBES participates in the project as a co-financier ensuring: 1) the long-term germplasm conservation according to international standards, 2) the implementation of a duplicate security system of the accessions which will be kept in at least 2 banks of the network.

Conclusions

The development of a national plan aiming at the safe and successful conservation of seeds of the Italian plants comprises the following steps:

- to optimize the long-term conservation of seeds by using traditional and modern analytical methods;
- to assess the conservation of the threatened/endemic species stored in seed banks to update the national inventory;
- to define an updated national priority list for seed collection, filling gaps in *ex situ* conservation and optimizing collection efforts;
- to implement a safety-backup program of duplicates to secure the conservation of the most threatened species in at least two seed banks of the network;
- to share information on germination behaviour and any other requirements for long-term seed storage.

We expect the conservation strategy planned by the RIBES network will be able to ensure the rapid achievement of the conservation targets for the Italian native flora by optimizing seed storage efforts at a national scale, fostering collaboration between research bodies and local authorities, and increasing public awareness on the risks of biodiversity loss.

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