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## Seed conservation actions for the preservation of plant diversity: the case of the Sardinian Germplasm Bank (BG-SAR)

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### Abstract

The Sardinian Germplasm Bank (BG-SAR) is a facility of the *Hortus Botanicus Karalitanus* (HBK), which belongs to the University of Cagliari (Italy). Its main objective is the conservation, study and management of the germplasm of Sardinian endemic, threatened and policy species (i.e., species inserted in the Habitat Directive 92/43/EEC, CITES and Bern convention), *taxa* from insular territories of the Mediterranean region, as well as Crop Wild Relatives (CWR), landraces, useful plants and plant remains. A summary of the procedures implemented by BG-SAR for the *ex situ* conservation, some international scientific results achieved, and some research projects at regional, national and international level in which the bank is involved, are reported in this work, with the main aim to highlight how a germplasm bank can be considered an important tool for the preservation of plant biodiversity. This paper allows to make a reflection about the importance of the germplasm banks, as well as their staff members, who constantly and daily work in order to preserve and conserve the planet's biodiversity.

Key words: archaeobotany, endangered species, *ex situ* conservation, invasive species, research activity, seed germination.

### Introduction

The Mediterranean Basin, with ca. 11,700 endemic plant species, has been recognized as the second largest hotspot of the 36 hotspots in the world and the largest of the world's five Mediterranean-climate regions (CEPF, 2016). Mediterranean islands and islets are singular for their species richness and high endemism rates. Among them, Sardinia (and its ca. 300 circum-Sardinian islets, including four archipelagos; Fenu *et al.*, 2014), situated in the western Mediterranean Basin and covering 24,090 km<sup>2</sup>, is the second largest island in the Mediterranean Sea, and it could be classified as a meso-hotspot within the Tyrrhenian macro-hotspot and the Mediterranean mega-hotspot of biodiversity (Cañadas *et al.*, 2014). The particular geological and human history of Sardinia has been a determining factor in the development of its floristic peculiarities (Fenu *et al.*, 2014).

The Sardinian flora consists of 2,494 *taxa* (Conti *et al.*, 2007), and 290 of them are considered as Sardinian endemic (e.g., narrow endemics, Sardinian endemics, Corso-Sardinian endemics, Corso-Sardinian-Balearic endemics; Bacchetta *et al.*, 2012; Fenu *et al.*, 2014), and 183 as exclusive Sardinian plant species; moreover, Sardinia is a priority region for the biology conser-

vation due to its high number of endemic plant species (Bacchetta *et al.*, 2012; Fenu *et al.*, 2015), and most of them facing several threatening factors, e.g. land-use and land-cover change, habitat fragmentation, climate warming and introduction of alien species.

In this context, from 1997, the research center named Sardinian Germplasm Bank (BG-SAR) plays a central role in the conservation of Sardinian plant diversity and in the protection of the most sensitive ecosystems of the island. Since 2015, BG-SAR is a facility of the *Hortus Botanicus Karalitanus* (HBK), which belongs to the University of Cagliari. The Bank preserves, studies and manages the germplasm of Sardinian endemic, threatened and policy species inserted in the Habitat Directive 92/43/EEC, CITES and Bern convention, *taxa* from insular territories of the Mediterranean region, as well as Crop Wild Relatives (CWR), landraces, useful plants and archaeological plant remains (Porceddu *et al.*, 2015; Uccesu *et al.*, 2016a).

The main research activities of BG-SAR mainly concern the *ex situ* conservation, seed germination ecology and seedling development studies, archaeobotany and studies on the potential invasiveness of the "Invasive Alien Species" (IAS), mainly dangerous in Sardinian coastal habitats. The staff and researchers belonging to BG-SAR are constantly involved

in several initiatives with the aim to promote local awareness, involving schools at all levels, to spread the results at local, national and international levels. In addition, BG-SAR offers free learning courses and/or practical activities in the field of plant biodiversity characterization, management and conservation (e.g., HEI-PLADI Erasmus+ project; <https://dibt.unimol.it/HEI-PLADI/elearning/>), as well as practical training for *ex situ* conservation techniques (IPAMed project by CARE-MEDIFLORA; <http://www.medplantsnetwork.net/training-for-ex-situ-conservation-by-care-mediflora-partners-in-collaboration-with-iucn-med-in-progress/>).

The research center participates in international seed conservation consortia, such as the European Native Seed Conservation Network (ENSCONET), the Network of Mediterranean plant conservation centres (GENMEDA) and, at national level, it is a founding member of the Italian Network of Germplasm Banks for the *Ex situ* Conservation of Native Flora (RIBES).

A summary of the procedures implemented by BG-SAR for the *ex situ* conservation, some international scientific results achieved during the last years, and some research projects at regional, national and international level, are reported in this work, with the main aim to highlight how a germplasm bank can be considered an important tool for the preservation of plant biodiversity.

### Seed conservation and preservation at BG-SAR

The seed conservation processes carried out at BG-SAR follow internationally recognized protocols and guidelines for the gene bank standards (Bacchetta et al. 2006, 2008a). All information are recorded and managed in IrisBG (Botanical Software Ltd © 2016), a suitably designed database to manage botanical and seed bank collections.

The collected germplasm is subject to a quarantine and post-maturation period, and only afterwards, the seeds are introduced in the bank. Subsequently, each accession is cleaned, quantified, selected and processed. The seed lots are gradually dried at 15°C and 15% of relative humidity (RH), in order to reach ca. 3-5% of internal seed moisture content, and stored at -25°C (as base collections under long-term conservation) and/or at +5°C (as active collections under medium-term conservation). Actually, BG-SAR preserves approximately 2,800 seed lots, many of which endemics of Sardinia. In particular, the bank preserves the germplasm referable to 47% of policy species and to 41% of exclusive Sardinian endemics (Fenu et al., 2015). It is important to highlight that the bank preserves most of the *taxa* included in the Annexes of the Habitats Directive [e.g., *Astragalus maritimus* Moris, *A. verrucosus* Moris, *Brassica insularis* Moris, *Gen-*

*tiana lutea* L. subsp. *lutea*, *Helianthemum caput-felis* Boiss., *Lamyropsis microcephala* (Moris) Dittrich & Greuter, *Linum muelleri* Moris, *Ribes sardoum* Martelli, Rouya polygama (Desf.) Coincy and *Silene velutina* Pourr. ex Loisel.], species considered, *sensu* Bacchetta et al. (2012), the ten most threatened exclusive endemic species of Sardinia (*Anchusa littorea* Moris, *Aquilegia barbaricina* Arrigoni & E.Nardi, *Aquilegia nuragica* Arrigoni & E.Nardi, *A. maritimus*, *A. verrucosus*, *Centranthus amazonum* Fridl. & A.Raynal, *Dianthus morisianus* Vals., *L. microcephala*, *Polygala sinisica* Arrigoni and *R. sardoum*), and several Sardinian *taxa* listed in the Italian national and regional red lists resulting threatened according to the criteria of the IUCN (Conti et al., 1992, 1997; Rossi et al., 2013) or inserted in attention list as the IUCN Top50 species of the Mediterranean islands (Montmollin de et Strahm, 2005; Pasta et al., 2017). In addition, BG-SAR preserves several accessions referable to Crop Wild Relatives (Ucchesu et al., 2016a).

### Seed germination ecology studies

Studies on the germination ecophysiology are constantly carried out to increase the biology knowledge and define the optimum germination protocol for each of the preserved *taxa*. For example, specific studies on seed germination ecology of the Sardinian endemic *Ribes multiflorum* Kit. ex Roem. & Shult. subsp. *sandalioticum* Arrigoni (Mattana et al., 2012) and *R. sardoum* (Porceddu et al., 2017), the only two members of *Ribes* genus present in Sardinia, were carried out, demonstrating that these *taxa* needed (after dormancy release) low temperatures for seed germination, highlighting an increasing risk from global warming for both *taxa*. Recently, it was demonstrated that seeds of *Paeonia corsica* Sieber ex Tausch exhibited differential temperature sensitivity for the different sequential steps in the removal of dormancy and germination processes, that resulted in the precise and optimal timing of seedling emergence (Porceddu et al., 2016). More recently, Cuenca Lombrana et al. (2016, 2017), in their studies on *Gentiana lutea* L. subsp. *lutea*, underscored the importance of studying the germination behaviour in the field and identified the type of seed dormancy in order to define the real thermal requirements of a species with high conservation value. In addition, studies on thermal time, niche modeling and the effect of global warming on the seed germination of Mediterranean species were carried out (e.g., Orrù et al., 2012a; Porceddu et al., 2013; Picciau, 2016).

Moreover, germination tests for some endemic and endangered *taxa* [e.g., *Phleum sardoum* (Hackel) Hackel, *Rouya polygama* (Desf.) Coincy, *B. insularis*, *Lavatera triloba* L. subsp. *triloba*, *L. triloba* subsp. *pallescens* (Moris) Nyman (Santo et al., 2014a,b,

2015a,b)], and IAS species [*Acacia saligna* (Labill.) Wendl., *Carpobrotus edulis* (L.) N.E.Br., *Lycium ferocissimum* Miers (Meloni *et al.*, 2015; Podda *et al.*, 2015a,b)] belonging to coastal habitats were conducted to detect the effect of environmental abiotic stresses such as salinity (e.g. NaCl), or the nutrient availability (e.g. KNO<sub>3</sub>) on the seed germination behaviour.

### Germplasm identification by image analysis

Image analysis technique carried out at BG-SAR is developed in order to identify and characterize the autochthonous germplasm in entry to the bank. Basically, digital images of germplasm are acquired by a flatbed scanner and successively processed and elaborated with specific software as KS-400 V. 3.0 (Carl Zeiss Vision, Oberkochen, Germany) and ImageJ v.1.9 producing macros and plugins able to detect morphometric and colorimetric measurements on seeds (Bacchetta *et al.*, 2008b; Bacchetta *et al.*, 2010; Grillo *et al.*, 2010; Ucchesu *et al.*, 2016b).

Applying this technique, Bacchetta *et al.* (2008b) and Grillo *et al.* (2010) characterized and discriminated seeds of wild plants typical of the Mediterranean vascular flora, referable to the *Apiaceae*, *Asteraceae*, *Boraginaceae*, *Brassicaceae*, *Caryophyllaceae*, *Cistaceae*, *Fabaceae*, *Lamiaceae*, *Poaceae* and *Scrophulariaceae* families. These studies stimulated further researches in many areas, including agronomical field (e.g., Grillo *et al.*, 2011; Orrù *et al.*, 2012b, 2015; Lo Bianco *et al.*, 2015; Sarigu *et al.*, 2017). In recent years, the morphometric analyses were applied to archaeological plants remains. These materials, such as seeds and fruits, were studied with the aim to investigate the evolution of crops and wild plants, explore agrarian practices harvesting, crop processing and determining the role of species with high cultural and economic value, such as cereals and fruits [e.g., *Cucumis melo* L., *Ficus carica* L., *Malus domestica* Borkh., *Olea europaea* L., *Prunus domestica* L., *Pyrus communis* L. and *Vitis* sp. (Orrù *et al.*, 2013; Sabato *et al.*, 2015; Ucchesu *et al.*, 2014, 2016a,b)]. For example, in the case of the *Vitis* genus, it was possible to establish that the seeds found in the archaeological site of Sa Osa (Cabras, Central-Eastern of Sardinia) in a Nuragic well dated to Bronze Age (about 3000 Before Present “BP”; Orrù *et al.*, 2013) belonged to grape cultivar (Ucchesu *et al.*, 2014) allowing to establish that in Sardinia viticulture was present from to Bronze Age. Recently, archaeological samples of *Prunus* sp. of Santa Giusta (Oristano, Central-Eastern of Sardinia) were compared through morphometric analysis with different traditional varieties and wild species from Sardinia. This study allowed to identify correctly the archaeological samples referable to *P. spinosa* and *P. domestica* (Ucchesu *et al.*, 2017).

### Projects

An important part of financing for BG-SAR comes from regional, national and international projects. Below, some of them are presented. For example, at regional scale, the project denominated “Morpho-colorimetric, ecophysiology and omic analysis of *Vitis vinifera* and *Vitis sylvestris* in Sardegna” funded in September 2015 through the “Legge Regionale 7 agosto 2007 RAS”. The expected results will consist to draw phyletic relationships between Sardinian wild grapes, cultivated grapes, and the archaeological seeds (found in archaeological contexts).

The “Pilot project of *Gentiana lutea* L. *in situ* and *ex situ* conservation, genetic characterization, populational reinforcement and reintroduction of *Gentiana lutea* L.” was supported by the Autonomous Region of Sardinia. The project allowed to assess the actual distribution range of *G. lutea* in Sardinia, to investigate the species phenology and the reproductive biology, and to identify the main threatening factors for this *taxon*; in addition, it allowed the implementation of the *ex situ* and *in situ* conservation measures, the identification of the optimal requirements for seed dormancy release and germination, and the investigation of the genetic diversity.

At international level, the LIFE+ PROVIDUNE project “Conservation and restoration of habitats dune in five Site of Community Importance (SCI) areas of the Provinces of Cagliari, Matera and Caserta” (2009-2014; <http://www.lifeprovidune.it>), financed by the European Union, was aimed to protect and restore the priority coastal dunes habitat with *Juniperus* ssp. and other related habitats characterizing the Mediterranean sandy coasts.

The LIFE+ project RES MARIS “Recovering Endangered Habitats in the Capo Carbonara MARIne Area, Sardinia” (2014-2018; <http://www.resmaris.eu>) aims at the conservation and recovery of marine and terrestrial ecosystems of the emerged and submerged beach system, in particular of priority habitats (DIR. 92/43/EEC) included in the marine SCI ITB040020 “Isola dei Cavoli, Serpentara, Punta Molentis e Campulongu” (Acunto *et al.*, 2017). Within the terrestrial conservation actions, the germplasm of the structural species of each habitat was collected and the best protocols for germination were investigated to produce plants for the restoration actions.

The ENPI CBC MED project ECOPLANTMED “ECOLOGICAL use of native PLANTs for environmental restoration and sustainable development in the MEDITERRANEAN region” (2014-2015; <http://www.ecoplantmed.eu>) was aimed to the *ex situ* conservation, duplication and germination experiments of the target plant species, the publishing of the “Manual for the propagation of the target native plant species” (Bal-

lesteros *et al.*, 2015) and the “Guide of good restoration practices in Mediterranean habitat” (Marzo *et al.*, 2015), the creation of a germplasm bank in Tunisia and finally, two pilot restoration actions of Mediterranean habitats in Lebanon and Tunisia.

Since April 2016, BG-SAR is responsible of the scientific management of the project CARE-MEDIFLORA “Conservation Actions for Threatened Mediterranean Island Flora: *ex situ* and *in situ* joint actions”, funded by MAVA Foundation (<http://www.care-mediflora.eu/>). The project aims to improve the conservation status of threatened Mediterranean plant species. It is implemented by institutions of six Mediterranean islands and the IUCN/SSC Mediterranean Plant Specialist Group.

## Conclusions

In the latest years, good progress has been made at BG-SAR relating to understanding the mechanisms of seed behaviour, dormancy, germination, viability and longevity of several *taxa*, as well as ensuring the preservation of plant biodiversity. In addition, through the experimental researches carried out, further knowledges are provided on the possibility of efficient preservation of seeds over long periods. All these approaches allow BG-SAR, not only to attain an effective *ex situ* conservation and to deepen the knowledge of the endangered Mediterranean species, but also to face actual and important problems such as global warming and invasiveness of alien species. The evaluation of salt stress allows us, in particular for endemic and rare species, to acquire information regarding their germination ecology that might be useful in the case of population reintroduction. The knowledges obtained for IAS, for example, give an important contribution in developing a wide management strategy for the control of the biological invasions; in particular, we could predict the potential ability of each species to successfully colonize via seeds some coastal habitats.

Concerning the image analysis, it has proved to be a repeatable, reliable and non-destructive method. It is fully accepted and utilized, not only for archaeological studies and/or taxonomic investigations of wild *taxa*, but also for studies on cultivated plants; this method can be useful to compare different varieties, contributing to the cataloguing and conservation in germplasm banks, or allowing the definition of objective parameters for the typifying of particular landraces in the attribution of European trademarks such as protected designation of origin (PDO) and protected geographical indication (PGI). In addition, it can be a valid helpful tool to discover false attributions (synonyms/homononyms) and origin of cultivars in different areas. The study of plant remains provides the opportunity to explore agrarian practices and crop domestication processing, enabling, therefore, a deeper insight into the way farming com-

munities manage their crops and use plants for their subsistence going beyond the mere identification of plant species. Furthermore, scientific archaeobotanical works are encouraged, which may have wider implications, as the identification of wild plants and to facilitate the study of the role of wild resources in human subsistence as a complement to the diet.

Consistently with these observations, BG-SAR continues to promote further researches and projects that are of fundamental importance for diversity plant conservation in the Mediterranean area, due to the position of the island in the middle of the Mediterranean Sea, the inclusion in the Mediterranean mega-hotspot of biodiversity and the high concentration of endemism.

Future researches may be focused, for example, on nursery and plant production systems for wild plant species that maximize genetic diversity, so that introduced seeds and plant materials have the resilience to cope with future environmental stresses (Hay and Probert, 2013). Regarding future perspectives, the bank is going to expand the working area to genetic studies. A laboratory dedicated to genetic analysis is in the planning, design and construction phase; it will allow BG-SAR to make a survey on genetic diversity of the studied *taxa* (e.g., endemic and threatened ones), thus reconstructing the best estimates of the historical and evolutionary relationships among the various taxonomic entities.

As well, concerns about the costs of *ex situ* conservation compared with *in situ* conservation have been allayed, the *ex situ* conservation revealed to be excellent value for money. Scientists, government departments and non-governmental organisations increasingly appreciate seed banking as an effective and economic conservation tool because of its complementarity to *in situ* approaches (Li and Pritchard, 2009). Especially in this time of financial crisis where the most scientific works are self-funded, the regional, national and international projects provide the resources necessary to continue in this important direction for the seed banking conservation. Regarding the critical aspects, as already mentioned in a previous paper regarding the BG-SAR (Atzeri *et al.*, 2012), the bank has no special management problems but it is necessary to highlight the difficulties due to the precarious work of most of the staff. Even the high management costs are not covered by a safe and consistent revenue. According to this vision, we argue that BG-SAR can be considered an important tool for the preservation of plant diversity, as well as the several germplasm banks that constantly and daily work to preserve and conserve the planet's biodiversity.

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