Israel Journal of Plant Sciences

ISSN: 0792-9978 (Print) 2223-8980 (Online) Journal homepage: http://www.tandfonline.com/loi/tips20

Assessing the conservation value of ex situ seed bank collections of endangered wild plants

Inmaculada Ferrando-Pardo, Pablo Ferrer-Gallego & Emilio Laguna-Lumbreras

Israel Journal of Plant Sciences, 2016
http://dx.doi.org/10.1080/07929978.2015.1125676

Taylor & Francis
Taylor & Francis Group

Assessing the conservation value of *ex situ* seed bank collections of endangered wild plants

Inmaculada Ferrando-Pardo[a,b]*, Pablo Ferrer-Gallego[a,b] and Emilio Laguna-Lumbreras[a]

[a]*Servicio de Vida Silvestre, Generalitat Valenciana, CIEF (Centro para la Investigación y Experimentación Forestal), E-46930, Quart de Poblet, Valencia, Spain;* [b]*VAERSA, E-46015, Valencia, Spain*

(*Received 12 May 2015; accepted 7 November 2015*)

In order to achieve certain targets of the Global Strategy for Plant Conservation (GSPC), countries should aim to have at least 75% of their threatened species preserved in *ex situ* genetically representative collections, preferably in the country of origin, and at least 20% of the threatened species must be available for recovery plans and restoration programs (Target 8). Assessing the conservation value of *ex situ* collections is needed in order to identify the gaps in gene banks and to determine the baseline situation. Selection of the target species for conservation followed the Regional Responsibility criterion, as the first-order of priority at the local level with highest ranking given to species whose distribution is endemic to the study area. Data analysis was performed on 85 species listed in the Valencian Catalog of Threatened Plant Species (VCTPS) stored in the Germplasm Bank Collections of Valencian Flora, Spain. Our results show that conservation value of an *ex situ* collection will depend on whether the analysis is based on the representation of species (i.e. the presence or absence of species in an *ex situ* collection) or based on their representativeness (i.e. how well the collected and stored natural populations represent the "theoretical" species genetic diversity and what is the potential to produce plants for recovery actions, summarized in the proposed index R4). For 85 species listed in VCTPS the existing *ex situ* seed bank collections keep samples of 91.8% (78 species), indicating good representation. However, the criteria accounting for number of populations collected and quality of the stored germplasm revealed that less than 50% of the listed species are properly preserved in the *ex situ* collections.

Keywords: Valencian Community; *ex situ* conservation; endangered plants; seed bank collections; species representativeness; Global Strategy for Plant Conservation

1. Introduction

Currently it is recommended to have centers dedicated to the development of *ex situ* conservation programs for endangered species in order to achieve the targets formulated by the Global Strategy for Plant Conservation (GSPC), a program of the Convention on Biological Diversity (CBD). The strategy was originally adopted in 2002 and updated in 2010 (Sharrock & Jones 2011), focusing on the conservation of plants and fungi (CBD 2002, 2012) to halt the loss of biodiversity. Revised and updated, this last version (2011−2020) provides a legal framework and targets for accessing, conserving, and using biodiversity in a fair and equitable manner. In response, in 2011 Spain adopted the Strategic Plan for Natural Heritage and Biodiversity 2011−2017 (in Spanish: Plan Estratégico del Patrimonio Natural y de la Biodiversidad 2011−2017), which established the basic legal framework for the conservation, sustainable use, improvement, and restoration of Spain's natural heritage and biodiversity. This Plan established a series of specific Goals, Targets and Actions for the government to fulfill. In 2014, the Sectorial Conference on Environment approved the Spanish Strategy for Plant Conservation 2014−2020 (in Spanish: Estrategia Española de Conservación Vegetal 2014−2020), which aimed to meet the targets of the GSPC (MAGRAMA 2014).

Target 8 of the GSPC aims to achieve a comprehensive program of *ex situ* conservation that complements *in situ* conservation through the development of genetically representative collections of threatened species. Such a program would support ecological restoration initiatives and strengthen responses to the impacts of climate change, unsustainable land use, and overharvesting of plant resources. The target requires that at least 75% of threatened plant species be conserved in *ex situ* collections, with high representative genetic variability, preferably in the country of origin, with collections of at least 20% of these species be available for recovery plans and restoration programs (CBD 2012).

Several in-depth reviews of progress towards implementation of Target 8 were based on varying interpretations of how well one species is effectively represented in an *ex situ* collection. In some reviews, a species is considered well represented if at least one seed per accession is

*Corresponding author. Email: ferrando.inma1@gmail.com, endemica_cief@gmail.com

stored or when a living plant collection is established (Hernández-Bermejo 2007; Sandev et al. 2013; Sharrock et al. 2014). However, for species that have more than one viable population, and in the absence of genetic studies, a single sample most probably will not be representative of the species genetic diversity (Hernández-Bermejo 2009). Godefroid et al. (2011) used two variables as surrogates for genetic diversity: (1) the number of accessions per species, and (2) the number of seeds per accession; they considered the threshold values recommended by the Seed Collecting Manual for Wild Species (ENSCONET 2009; i.e. minimum conserving from five populations per species if there are more than five, and 5000 seeds per accession). Krigas et al. (2014) proposed to modify this recommendation to reduce the risk of loss when seed accessions are held in a single collection by depositing accessions in at least two collections.

In addition, to assess whether a species is efficiently preserved and available for restoration programs, as required in Target 8 (2011−2020), it is essential to know the methods of seed germination and plant propagation. The focus and challenge for this Target is not only to increase the number of threatened plants in *ex situ* collections, but also to ensure the conservation value of such collections (BGCI 2012; Mattana et al. 2012). The critical limitation to objectively assessing implementation success is the lack of methodology for measuring and monitoring progress in meeting the target (Paton & Lughadha 2011).

The Mediterranean basin hosts flora of around 25,000−30,000 plant species (Heywood 2003) and has been recognized as one of the Global Biodiversity Hotspots (Myers et al. 2000; Mittermeier et al. 2004). Threatened plant species are concentrated in the Mediterranean and Balkan regions with Italy, Spain and Greece being the countries with the greatest numbers of threatened plants. In the European Red List of Vascular Plants (Bilz et al. 2011), 90% are single-country endemics (Sharrock & Jones 2011). The Valencian Community of Spain is known as one of the richest biodiversity regions for vascular plants in Europe (see Castroviejo 1986−2014).

The conservation of plant species in the Valencian Community (Spain) has been undertaken using an integrated approach developed over several decades. From the 1980s to the present, a great effort has been made by the Community administration and other organizations, all of which led into the publication of the Valencian Catalog of Threatened Plant Species (VCTPS) (in Spanish: Catálogo Valenciano de Especies de Flora Amenazada; see Aguilella et al. 2010). The conservation includes two components: *in situ* and *ex situ* applied in a series of steps (see Ferrer-Gallego et al. 2013). The *in situ* (first step) activities focus on diagnosis and actual status of the species in the wild (threat detection, assessment of real situation in natural populations, georeferenced network, search of new individuals, etc.); *ex situ* conservation (the second step) is done by creating long-term collections, located at Botanical Garden of University of Valencia, and short-term or active collections, located at Center for Forestry Research and Experimentation (CIEF) and Freshwater Species Research Center, the latter's aim being the production of plants to be used in restoration programs; and *in situ* actions (the third step) is focused on direct management of wild populations, population translocations, etc. The first step resulted in the creation of several Natural Protected Areas (Valencia's Natural Park) and the microreserve networks (Laguna et al. 2004; Fos et al. 2014). However, passive *in situ* conservation through area protection is not enough due to high anthropogenic pressure in the Valencian region, characteristic for the whole Mediterranean Basin (Heywood 2014), and more active conservation *in situ* is necessary for natural populations' maintenance and survival (Volis & Blecher 2010; Heywood 2015; Volis 2015).

Setting conservation priorities and creating a priority species list is a necessary and crucial component of efficient conservation in general and in the Valencian region in particular. For this purpose, several authors identified the Regional responsibility as the first order of priority at the local level, with highest ranking given to species whose distribution is endemic to the study area (Gauthier et al. 2010; Bacchetta et al. 2012; Mattana et al. 2012). Subsequently, using this list, the sampled collections have been organized into three groups according to their category of threat: the first group includes 'Catalogued' species, which constitute the VCTPS, and the two other groups are the 'Non-catalogued Protected' species and 'Watched' species (CITMA 2013). Then the sampled accessions were stored in two germplasm bank collection: active or short-term conservation and base or long-term conservation.

The aim of this paper is to assess these *ex situ* collections in their effectiveness in meeting GSPC Target 8. For the latter we used two assessment criteria: (1) the degree of representativeness, based on the number of known wild populations preserved per species to ensure a high genetic diversity in absence of genetic studies, and (2) the capacity of obtaining plants needed for *in situ* actions (such as reinforcement, reintroduction and relocation) based on the results of viability testing of seeds stored.

2. Materials and methods

2.1. Study area. Natural populations and Operational Conservation Units: target species

The Valencian Community hosts more than 3200 vascular plants (Mateo & Crespo 2014), with 370 (11%) of them being endemic to the Iberian Peninsula or Iberian-Balearic Islands, and 64 (17%) being strictly endemic to the Valencian area (Laguna 1998). In the Valencian territory there are 389 species protected by legal Decree 70/2009 and Order 6/2013, which are divided into three annexes: (i) 85 'Catalogued' species, which constitute the VCTPS and are subdivided into 35 'In danger of Extinction' (IE) and 50 'Vulnerable' (V); (ii) 141 'Non-catalogued Protected' species; and (iii) 163 'Watched' species (CGV 2009; CITMA 2013). Note that these legal categories have no correspondence with the IUCN Red List Categories, despite the fact that they are sometimes homonymous (e.g. Vulnerable).

Criteria for the target species and conservation priorities in this paper followed regional responsibility. Therefore, 139 species present in the Valencian Community included in threatened lists (see section 2.4 and Table 2) have been analyzed. In this sense, only endangered categories have been considered here. For the Valencian (VCTPS) and Spanish Catalog of Threatened Plant Species (SCTPS; in Spanish: Catálogo Español de Especies de Flora Amenazada; MAGRAMA 2014): In danger of Extinction (IE) and Vulnerable (V) categories (see VV. AA. 2004), and for assessments IUCN (Spanish Red List of Vascular Plants, European Red List of Vascular Plants and IUCN Red List): Critically endangered (CR), Endangered (EN) and Vulnerable (VU) categories.

Determination of the conservation status of threatened species in the Valencian Community is based on the identification and characterization of all natural populations of a species, which are considered in this work as Operational Conservation Units (OCU). The latter is motivated by the importance of every extant population for collecting and propagating material and population demographic studies, among other tasks (Adams et al. 2005; Iriondo et al. 2009). These OCUs are characterized, recorded and updated in the Biodiversity Data Bank of the Valencian Community (BDBCV, http://bdb.cth.gva.es/).

For botanical nomenclature of species we have used the checklist of Mateo and Crespo (2014), which mostly follows the nomenclature of *Flora iberica* (Castroviejo 1986–2014).

2.2. Material origin and storage conditions

We analyzed data on collection and conservation activities for the period 1994–2014 (Ferrer-Gallego et al. 2014).

Seeds or fruits were collected when fully mature from as many individuals as possible from natural populations or from living plant collections (the latter method was only performed in exceptional cases in which it was strictly necessary to preserve germplasm). In highly threatened populations with less than 50 individuals, all individual plants were sampled (Bañares et al. 2011; Hoban & Schlarbaum 2014). Accessions were georeferenced, characterized (using such seed quality parameters such as physical purity, moisture content, seeds number per accession, seed morphological structures, etc.), and recorded in a database to ensure the traceability of germplasm as described by Bacchetta et al. (2008) and Ferrer-Gallego et al. (2013). Storage conditions followed the international standard for long-term seeds conservation (FAO 2013). First, seeds were progressively dried in a room with controlled atmosphere until the moisture content decreased to 10–16% (over 2–3 months), and then seeds were ultra-dried with silica gel in airtight conditions (1–2 months) to 5–7%. These conditions are considered optimal for the majority of orthodox seeds. Once dried, seeds were placed in glass containers stored at 4 °C in airtight conditions in a cold room (active collection) and/or −20 °C (base or long-term collection).

In addition, for species that rarely reproduce sexually under natural conditions, or have low fecundity, vegetative materials were collected and then cultivated in the CIEF's nursery fields and greenhouses (i.e. *Aristolochia clematitis*, *Cistus heterophyllus* subsp. *carthaginensis*, *Frangula alnus* subsp. *baetica* and *Narcissus perezlarae*) to obtain the necessary amount of seeds.

2.3. Viability testing and germination capacity

Tests were performed at least three months after seed collection and before storage. Viability testing consisted of a germination test (see ISTA 2006) followed by non-germinated seed analyses. Testing was applied to 100 apparently viable seeds (well-shaped versus seeds that are misshapen, empty, immature, or present signs of predation) per accession (or less depending of the availability of seeds) and divided into two or four samples. Seeds were soaked in distilled water for 24 h and then placed into glass Petri dishes (9 cm Ø) lined with a moistened deionized water filter paper disk. Seeds were visited daily and germination was recorded when the radicle was visible. Germination testing was terminated one month after last seed germinated. After each count, germinated seeds were removed and transplanted to pots for development and growth. Seeds that did not germinate were subjected to a cut test to identify the number of fresh, moldy (dead), or empty (undeveloped) seeds. Pre-treatments and germination conditions were first based on standard testing (20 °C; 12/12 h, darkness/light). If seeds failed to germinate or the results were below 75%, new treatments were applied based on the recommended literature for the same genus or based on climate conditions experienced by the species at the time of germination in the field (Ferrer-Gallego et al. 2013). Pre-treatments required for some species included mechanical or sulfuric acid scarification of seeds, application of gibberellic acid (GA3; 100–500 mg/l), soaking and scalding seeds, cold stratification at 4 °C (30–90 days), or alternating warm/cold stratification.

Germination capacity (GC) was calculated as the total number of seeds germinated divided by the total number of tested seeds minus unviable (empty or dead) seeds (Gosling 2003). Each value obtained was expressed as the mean of four replicates and standard deviation.

Seed viability (SV) was determined as the number of germinated seeds plus the number judged viable from the cut-test, which is expressed as a percentage of the total (Offord et al. 2004; Crawford et al. 2007). SV not only provides information about the proportion of viable seeds in the collection, but can be a more objective criterion for assessing quality of the seed collections in comparison with GC for species with strong seed dormancy. The results shown are from germination tests conducted according to the most appropriate protocol for each species. Germination data were obtained as a part of the routine seed bank testing and therefore are not amenable to analytic study due to heterogeneity of testing conditions.

2.4. Data analysis

Databases of germplasm bank collections from the Center for Forestry Research and Experimentation (CIEF), Wildlife Service's Freshwater Species Research Center, and Botanical Garden of the University of Valencia were compared with the following local, national, and international threatened plant species list:

(1) Valencian Catalog of Threatened Plant Species (CITMA 2013)
(2) Spanish Catalog of Threatened Plant Species (MAGRAMA 2014)
(3) Spanish Red List of Vascular Plant (Bañares et al. 2011)
(4) Annex II of the Habitat Directive 92/43/EEC
(5) European Red List of Vascular Plant (Bilz et al. 2011)
(6) IUCN Red List (IUCN 2014)

We calculated several different indices to assess the representation and the representativeness of species in *ex situ* collections to measure the progress made on meeting Target 8:

R1: Representation of threatened species conserved in* ex situ *collections. Determined as the number of species with at least one germplasm accession preserved divided by total number of target species expressed as percentage.

R2: Representativeness of threatened species conserved in* ex situ *collections. Determined as the number of Operational Conservation Units conserved per species with at least one germplasm accession genetically representative (OCUg) divided by the number of Operational Conservation Units known per species (OCUw); R2 = OCUg/OCUw. We have not considered setting a minimum number of seeds stored per species as indicated by Godefroid et al. (2011).

R3: Representativeness of threatened species conserved according to Godefroid et al. (2011). The number of adequately conserved in *ex situ* collection species divided by total number of species with wild populations. One species is adequately conserved if all its known populations are conserved through seedlots (at least five populations represented by at least one accession each with 5000 seeds/accession).

R4: Representativeness of threatened species, an indicative approach to monitoring progress on meeting Target 8 as we propose here. Determined by two assessment criteria: (1) the degree of representativeness of species by means of parameter R2, being R2 $\geq$ 0.75 and (2) the capacity of potential plants production (CPP) based on the results of viability testing and germination capacity of stored seeds.

CPP: Capacity to produce potential plants. An indicative approach to the capacity to obtain plants from a stored seed accession for species. Determined as the seed viability (SV) multiplied by the germination capacity (GC) and divided by 100; CPP (%) = (SV $\times$ GC) / 100.

3. Results

Regarding the representation of threatened wild plants from the Valencian Community, seed bank collections hold 680 seed accessions of 78 species, originating from material collected in the wild or derived by propagation directly from an original wild source species, but only 288 accessions have more than 5000 seeds stored (Table 1). Thus, 91.8% of Valencian threatened species have representation (R1) and are presently conserved (see Table 2 and Figure 1). However, in terms of representativeness, only 47.1% (40 species) of species had all their known natural populations conserved (R2 = 1; Figure 2), and the average R2 = 0.8 $\pm$ 0.4. If we assess representativeness as proposed by Godefroid et al. (2011) (R3), the results are different, and only 31.8% of the species (27 species) are well represented (Figure 1).

The index of representativeness of threatened species to monitor target progress as we propose here (R4) showed that 40.0% of species (34 species) had at least 75% of their OCU conserved with its stored material available to carry out effective recovery and restoration works whenever needed. However, for nine species that had all of their OCU preserved, all tested germination protocols failed, and no seeds germinated.

Germination capacity (GC) and seed viability (SV) of the target species on average is 83.1 $\pm$ 26.0 and 76.6 $\pm$ 28.8%, respectively. For 23 species (27.1%), the germination protocols are in process currently. The capacity to obtain new material (CPP) from seed accessions is on average 42.2 $\pm$ 46.3% for all threatened species

Table 1. Results from threatened species of Valencian flora for different parameters calculated in order to approach their representation and representativeness in germplasm bank collections.

Species	Valencian Catalog of Threatened Plant Species	Spanish Catalog of Threatened Species	Assessment IUCN	Hab. Dir. 92/43/EEC	No. seed accessions	No. accessions with > 5000 seeds	OCUw	OCUg	R2	GC	SV	CPP	GCT
Acis valentina	V		VU (Spanish Red List)		5	0	21	4	0.19	92.0 ± 4.0	75	69.0	20 °C; 12/12 h d./l.
Ajuga pyramidalis subsp. *meonantha*	V				5	0	3	1	0.33	56.5 ± 8.6	85	48.0	15 °C; 24 h d. PT
Allium subvillosum	IE				5	0	2	1	0.50	82.2 ± 6.9	90	74.0	15 °C; 24 h d.
Althenia orientalis	V				0	0	1	0	0	n.a.	n.a.	n.a.	n.a.
Antirrhinum valentinum	V		VU (Spanish Red List)		15	10	17	5	0.29	96.6 ± 3.0	88	85.0	20 °C; 12/12 h d./l.
Apium repens	V			Present	8	0	4	2	0.50	91.0 ± 7.2	91	82.8	20/25 °C; 10/14 h d./l.
Aristolochia clematitis	IE				3	1	1	1	1	0.0 ± 0.0	0	0	5, 10, 15, 20, 25, 30 °C; 24 h d. PT
Armeria fontqueri	V		VU (Spanish Red List)		3	0	2	2	1	100.0 ± 0.0	70	70	15/20 °C; 12/12 h d./l.
Asplenium celtibericum	V				2	2	1	1	1	n.a.	n.a.	n.a.	n.a.
Asplenium marinum	IE				6	6	1	1	1	n.a.	n.a.	n.a.	n.a.
Astragalus oxyglottis	V		VU (Spanish Red List)		4	1	3	2	0.67	96.7 ± 9.2	91	87.9	20 °C; 12/12 h d./l. PT
Athyrium filix-femina	V				2	2	2	1	0.50	n.a.	n.a.	n.a.	n.a.
Berberis hispanica subsp. *hispanica*	IE				3	0	1	1	1	91.7 ± 7.1	92	84.4	5 °C; 24 h d.
Boerhavia repens	IE		CR (Spanish Red List)		5	2	1	1	1	70.7 ± 5.0	75	53.0	20/25 °C; 12/12 h d./l.
Callipeltis cucullaria	V				3	0	5	3	0.60	0.0 ± 0.0	90	0	20 °C, 25 °C; 12/12 h d./l.
Campanula mollis	V				1	0	1	1	1	100.0 ± 0.0	100	100	20 °C; 12/12 h d./l.
Carex digitata	V				1	0	2	1	0.50	22.6 ± 7.4	85	19.2	20 °C; 10/14 h d./l.
Carex elata	V				4	2	2	2	1	16.3 ± 6.3	75	12.2	20 °C; 10/14 h d./l.
Centaurea alpina	IE				1	0	1	1	1	100.0 ± 0.0	1	1.0	20/25 °C; 12/12 h d./l.

(*continued*)

Table 1. (*Continued*)

Species	Valencian Catalog of Threatened Plant Species	Spanish Catalog of Threatened Species	Assessment IUCN	Hab. Dir. 92/43/EEC	No. seed accessions	No. accessions with > 5000 seeds	OCUw	OCUg	R2	GC	SV	CPP	GCT
Centaurea lagascae	V				4	0	2	2	1	90.8 ± 5.0	76	69.0	20/25 °C; 12/12 h d./l.
Ceratophyllum submersum	IE				3	0	2	1	0.50	62.5 ± 13.0	78	48.7	20/25 °C; 10/14 h d./l.
Cistus heterophyllus subsp. *carthaginensis*	IE	IE	CR (Spanish Red List)		1	0	1	1	1	84.4 ± 5.7	86	72.6	20 °C; 12/12 h d./l. PT
Clematis cirrhosa	V				7	0	4	2	0.50	78.9 ± 14.4	90	71.0	15 °C; 24 h d. PT
Coeloglossum viride	IE				0	0	4	0	0	n.a.	n.a.	n.a.	n.a.
Corema album	IE				1	0	1	1	1	75.5 ± 2.5	84	63.4	10/20 °C; 12/12 h d./l.
Cotoneaster granatensis	IE				2	0	2	1	1	86.8 ± 5.2	76	65.9	15 °C; 24 h d. PT
Dactylorhiza incarnata	V				0	0	6	0	0	n.a.	n.a.	n.a.	n.a.
Dianthus carthusianorum	V				1	1	1	1	1	100.0 ± 0.0	98	98.0	20 °C; 12/12 h d./l.
Diplotaxis ibicensis	V	IE		Present	6	6	7	5	0.71	98.0 ± 2.0	98	64.0	10/20 °C; 24 h d.
Elatine brochonii	V		VU (European Red List)		1	0	2	1	0.50	n.a.	n.a.	n.a.	n.a.
Epipactis fageticola	V				0	0	3	0	0	n.a.	n.a.	n.a.	n.a.
Equisetum moorei	IE				0	0	6	0	0	n.a.	n.a.	n.a.	n.a.
Euphorbia nevadensis subsp. *nevadensis*	V	IE			2	0	2	1	0.50	6.0 ± 3.0	15	0.9	20 °C; 12/12 h d./l.
Euphrasia salisburgensis	IE				0	0	1	0	0	n.a.	n.a.	n.a.	n.a.
Frangula alnus subsp. *baetica*	IE		VU (Spanish Red List)		4	2	4	2	1	83.9 ± 7.3	93	78.0	10/20 °C; 24 h d. PT
Fumaria munbyi	V		EN (Spanish Red List)		2	0	1	1	1	0.0 ± 0.0	96	0	5, 10, 15, 20, 25, 30 °C; 24 h d. PT
Garidella nigellastrum	V				14	2	6	6	1	96.7 ± 3.9	92	88.9	5 °C; 24 h d. PT
Genista umbellata	V				3	2	1	1	1	89.8 ± 5.0	88	79.0	20 °C; 12/12 h d./l. PT
Halimium atriplicifolium	IE				7	5	3	3	1	34.1 ± 7.7	41	13.9	15/20 °C; 12/12h d./l. PT

(*continued*)

Table 1. (*Continued*)

Species	Valencian Catalog of Threatened Plant Species	Spanish Catalog of Threatened Species	Assessment IUCN	Hab. Dir. 92/43/EEC	No. seed accessions	No. accessions with > 5000 seeds	OCUw	OCUg	R2	GC	SV	CPP	GCT
Halopeplis amplexicaulis	V		VU (Spanish Red List)		3	2	3	2	0.67	100.0 ± 0.0	98	98.0	10/20 °C; 24 h d.
Helianthemum caput-felis	V	VU	EN (Spanish Red List) EN (European Red List)	Present	12	8	18	6	0.33	89.9 ± 8.6	89	80.0	20 °C; 12/12 h d./l. PT
Isoetes velatum	V				2	2	1	1	1	n.a.	n.a.	n.a.	n.a.
Kernera saxatilis subsp. *boissieri*	V		VU (Spanish Red List)		5	0	2	2	1	100.0 ± 0.0	89	89.0	15/20 °C; 12/12 h d./l.
Launaea arborescens	IE				5	0	3	2	0.67	100.0 ± 0.0	7	7.0	20/25 °C; 12/12 h d./l.
Launaea lanifera	IE				1	0	2	1	0.50	100.0 ± 0.0	5	5.0	20/25 °C; 12/12 h d./l.
Leucanthemum arundanum	IE				5	0	2	1	0.50	100.0 ± 0.0	7	7.0	20 °C; 12/12 h d./l.
Limonium bellidifolium	IE				6	3	1	1	1	100.0 ± 0.0	84	84.0	15/20 °C; 12/12 h d./l.
Limonium dufourii	IE		CR (Spanish Red List)		48	16	8	8	1	100.0 ± 0.0	100	100	15/20 °C; 12/12 h d./l.
Limonium lobatum	IE				5	3	1	1	1	100.0 ± 0.0	99	99.0	20 °C; 12/12 h d./l.
Limonium mansanetianum	V		CR (Spanish Red List)		28	17	13	13	1	100.0 ± 0.0	98	98.0	20 °C; 12/12 h d./l.
Limonium perplexum	IE	IE	CR (Spanish Red List)		15	5	1	1	1	100.0 ± 0.0	100	100	15/20 °C; 12/12 h d./l.
Littorella uniflora	IE				1	0	1	1	1	n.a.	n.a.	n.a.	n.a.
Lupinus mariae-josephae	V		CR (Spanish Red List)		39	0	9	9	1	98.0 ± 2.0	100	98.0	20 °C; 12/12 h d./l. PT
Marsilea strigosa	V		VU (Spanish Red List) VU (European Red List)	Present	7	0	2	1	0.50	n.a.	n.a.	n.a.	n.a.
Maytenus senegalensis subsp. *europaea*	V				2	0	2	1	0.50	n.a.	n.a.	n.a.	n.a.

(*continued*)

Table 1. (*Continued*)

Species	Valencian Catalog of Threatened Plant Species	Spanish Catalog of Threatened Species	Assessment IUCN	Hab. Dir. 92/43/EEC	No. seed accessions	No. accessions with > 5000 seeds	OCUw	OCUg	R2	GC	SV	CPP	GCT
Medicago citrina	V	VU	CR (Spanish Red List) CR (IUCN Red List)		46	5	4	4	1	100.0 ± 0.0	94	94.0	20 °C; 12/12 h d./l. PT
Myriophyllum alterniflorum	V				1	0	1	1	1	n.a.	n.a.	n.a.	n.a.
Narcissus perezlarae	IE				1	0	6	1	0.17	n.a.	n.a.	n.a.	n.a.
Notoceras bicorne	V				3	1	1	1	1	90.0 ± 10.0	100	90.0	20/25 °C; 12/12 h d./l.
Nymphaea alba	IE				18	0	8	6	0.75	98.0 ± 2.5	100	98.0	20 °C; 10/14 h d./l.
Odontites valentinus	V		EN (Spanish Red List)		5	3	4	3	0.75	100.0 ± 0.0	92	92.0	10/20 °C; 24 h d.
Orchis conica	V				1	1	13	1	0.01	n.a.	n.a.	n.a.	n.a.
Orchis papilionacea	V				2	2	10	2	0.20	n.a.	n.a.	n.a.	n.a.
Parentucellia viscosa	IE				8	8	8	4	0.50	97.8 ± 10.5	93	90.9	10/20 °C; 12/12 h d./l.
Phyllitis sagittata	IE				2	2	1	1	1	n.a.	n.a.	n.a.	n.a.
Polystichum aculeatum	V				2	2	6	1	0.17	n.a.	n.a.	n.a.	n.a.
Pteris vittata	V				14	14	14	9	0.64	n.a.	n.a.	n.a.	n.a.
Reseda hookeri	IE		CR (Spanish Red List)		7	3	1	1	1	43.1 ± 50.9	58	24.9	10/20 °C; 12/12 h d./l. PT
Reseda lanceolata	IE				2	1	1	1	1	25.0 ± 8.9	100	25.0	20 °C; 12/12 h d./l. PT
Ribes uva-crispa	V				2	0	2	1	0.50	100.0 ± 0.0	47	47.0	15/20 °C; 12/12 h d./l. PT
Rumex roseus	IE				3	0	3	2	0.67	100.0 ± 0.0	98	98.0	15/20 °C; 12/12 h d./l.
Salsola soda	V				3	1	2	1	0.50	100.0 ± 0.0	100	100	20 °C; 12/12h d./l.
Silene cambessedesii	IE		VU (Spanish Red List)		10	7	2	2	1	60.3 ± 8.9	63	37.9	20/25 °C; 12/12 h d./l. PT

(*continued*)

Table 1. (*Continued*)

Species	Valencian Catalog of Threatened Plant Species	Spanish Catalog of Threatened Species	Assessment IUCN	Hab. Dir. 92/43/EEC	No. seed accessions	No. accessions with > 5000 seeds	OCUw	OCUg	R2	GC	SV	CPP	GCT
Silene hifacensis	IE	IE	EN (Spanish Red List) EN (European Red List) EN (IUCN Red List)	Present	82	43	4	4	1	100.0 ± 0.0	98	98.0	15 °C; 24 h d.
Solenopsis laurentia	V				5	1	2	2	1	9.1 ± 3.8	77	7.0	10/20 °C; 12/12h d./l.
Teucrium lepicephalum	V	IE	EN (Spanish Red List) EN (European Red List) EN (IUCN Red List)	Present	17	15	10	8	0.80	91.3 ± 10.5	80	73.0	10/20 °C; 12/12 h d./l.
Thalictrum maritimum	V				14	8	8	4	0.50	86.0 ± 1.4	90	77.4	20/25 °C; 8/16 h d./l.
Thelypteris palustris	IE				7	7	3	3	1	n.a.	n.a.	n.a.	n.a.
Thymus ricardii subsp. *vigoi*	V				2	0	1	1	1	100.0 ± 0.0	85	85.0	15 °C; 24 h d.
Thymus webbianus	V		CR (Spanish Red List)		8	1	3	2	0.67	56.1 ± 5.0	38	21.3	20 °C; 12/12 h d./l.
Tilia platyphyllos	V				72	3	62	10	0.16	92.5 ± 5.2	67	62.0	4 °C; 24 h d. PT
Ulmus glabra	V				23	0	20	4	0.20	99.0 ± 2.0	23	22.8	20/25 °C; 16/8 h d./l.
Utricularia australis	IE				0	0	2	0	0	n.a.	n.a.	n.a.	n.a.
Vitaliana primuliflora subsp. *assoana*	V		VU (Spanish Red List)		1	0	1	1	1	18.0 ± 5.4	75	13.5	5/10 °C; 12/12 h d./l. PT
Zannichellia contorta	V				2	0	4	2	0.50	n.a.	n.a.	n.a.	n.a.

Protection category: Valencian and Spanish Catalog: In danger of Extinction (IE) and Vulnerable (V); Assessment IUCN: Critically endangered (CR), Endangered (EN) and Vulnerable (VU).
OCUw, number of known Operational Conservation Units for a species;
OCUg, ,umber of known Operational Conservation Units represented in *ex situ* collection by one or more accessions;
R2, representativeness per species, i.e. how well species are conserved through the conservation of the largest number of known populations. R2 = OCUg / OCUw;
GC, germination capacity (accumulated germination), mean ± SD, expressed in %, for the best results obtained in germination tests;
SV, seed viability, calculated as the number of germinated seeds plus the number judged viable from the cut-test expressed as a percentage of the total fresh seeds;
CPP, capacity to produce plants. An indicative approach to the capacity to obtain plants for *in situ* actions from one accession. CPP = (SV × GC) / 100;
GCT, germination conditions tested (treatment for temperature and light); d., darkness; l., light; PT, pre-treatment required.
n.a., data not available.

Table 2. Summary of results obtained by comparing databases of germplasm bank collections with species listed in different catalogues of threatened species of Valencian flora (local, national and international) to assess their representation (R1) and representativeness (R2, R3, and R4). *Only endangered categories have been considered [Valencian and Spanish Catalog: In danger of Extinction (IE) and Vulnerable (V); Assessment IUCN: Critically endangered (CR), Endangered (EN) and Vulnerable (VU)].

	Protection category*					
	Valencian Catalog of Threatened Plant Species (IE + V)	Spanish Catalog of Threatened Plant Species (IE + V)	IUCN Spanish Red List of Vascular Plants (CR + EN + VU)	Annex II of the Habitat Directive 92/43/EEC	IUCN European Red List of Vascular Plants (CR + EN + VU)	IUCN Red List v. 2014.3 (CR + EN + VU)
No. of threatened species in wild	85	146	1,192	572	412	10,584
No of threatened species present in Valencian Community	85	9	65	7	5	8
No. of threatened species with *ex situ* reproductive material conserved	78	9	61	7	5	8
R1_Representation (%)	91.76	100.00	93.80	100.00	100.00	100.00
R2_Representativeness (here are expressed the average values of R2 for species) (%)	85.11	79.25	64.80	67.10	62.60	87.88
R3_Representativeness according to Godefroid et al. 2011 (%)	31.80	75.00	33.32	57.10	60.00	50.00
R4_Representativeness as an indicative approach to monitoring progress on meeting Target 8 (%)	40.00	66.67	26.15	28.57	40.00	50.00

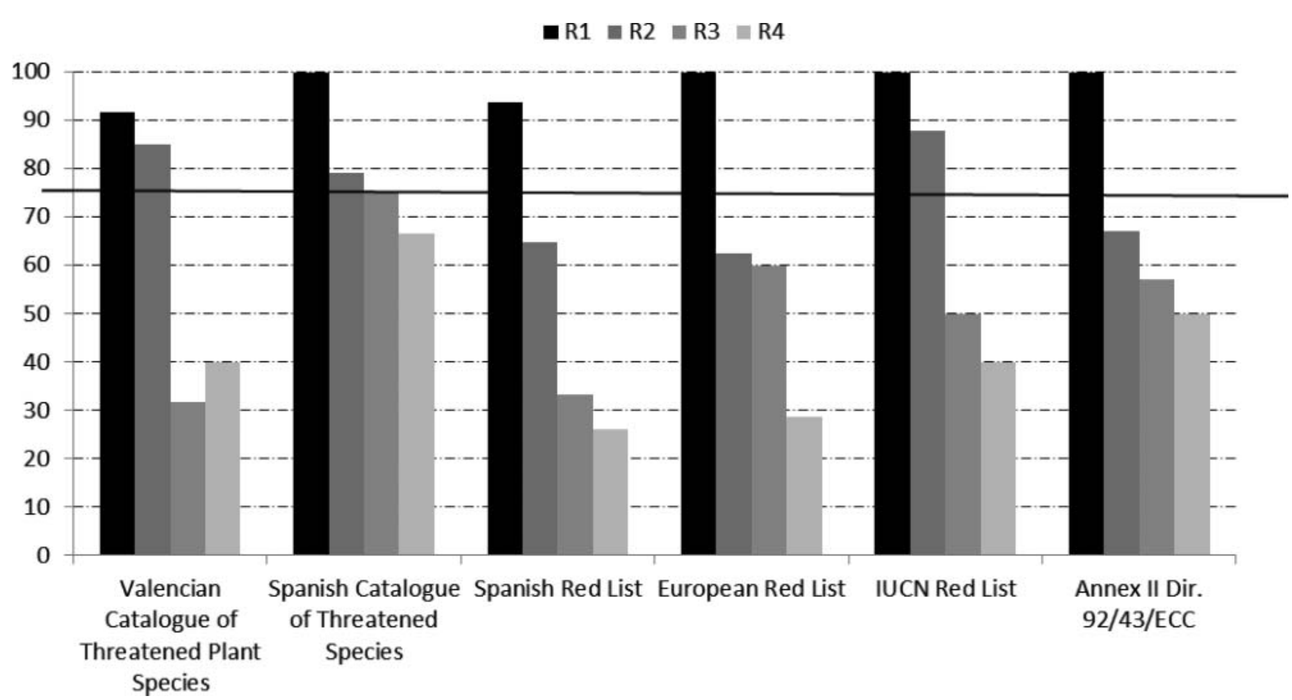

Figure 1. Percentage of the threatened species that can be considered conserved in the germplasm bank collections of Valencian flora using four different criteria.

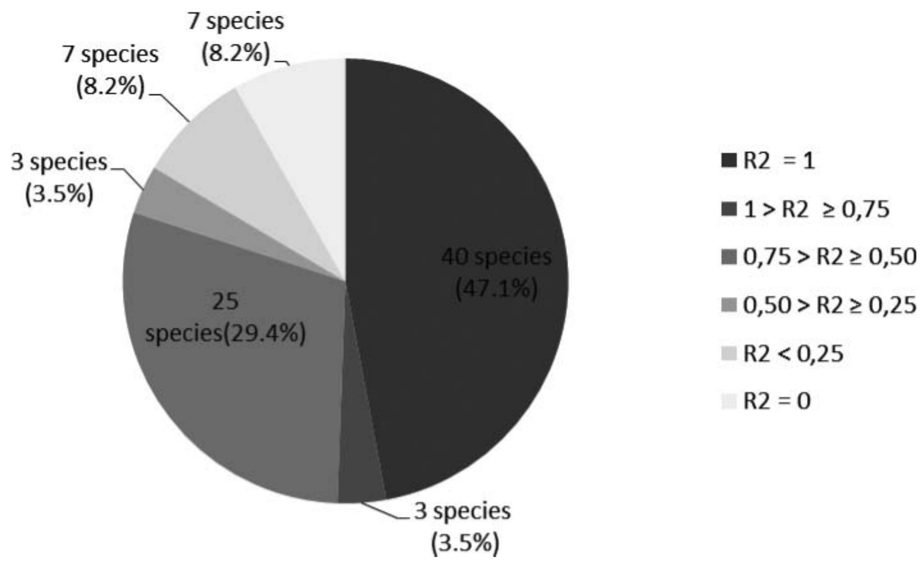

Figure 2. The frequency distribution of six categories of the degree of representativeness (R2) in the threatened Valencian flora. The R2 is a ratio of known for the species Operational Conservation Units represented by one or more accessions in *ex situ* collections.

(VCTPS), and seven species have a value between 0% and 10% (Table 1).

From the SCTPS there are nine species represented in the germplasm bank collections (R1 = 100%), and the representativeness values for these species are R2 = 79.2%, R3 = 75.0% and R4 = 55.6%. For the three IUCN lists, IUCN Spanish Red List, IUCN European Red List and IUCN Red List, the species representation indices are 93.8%, 100% and 100%, respectively. However, representativeness is lower (R2: 64.8%, 62.6% and 87.9%; R3: 33.3%, 60.0% and 50.0%, respectively). All the threatened plant species present in Valencian Community listed in Annex II of DIR. 92/43/EEC are conserved in the *ex situ* collections (R1 = 100%), and R2 and R3 are 67.1%, and 57.1%, respectively (Table 2 and Figure 1).

Assessment of the *ex situ* conservation value of threatened species using the proposed R4 index yielded the values 66.7% for the SCTPS, 26.1% for IUCN Spanish Red List, 40% for IUCN European Red List, 50% for IUCN Red List and 28.6% for species present in Annex II DIR 92/43/EEC (Table 2 and Figure 1).

4. Discussion

Our results show clear differences between representation (R1) and representativeness (R2, R3, or R4) when assessing the conservation value of existing *ex situ* collections of endangered wild plants. In terms of representation alone, the *ex situ* collections preserve 91.8% of the whole of VCTPS, greatly exceeding the 75% proposed by the GSPC. However, if we analyze the representativeness (i.e. how well collected and stored natural populations represent the "theoretical" species genetic diversity and what is the potential to produce plants for recovery actions, summarized in the proposed index R4), the values are much lower – 36.5% – and well below the required 75%. We use the term "theoretical" because the information on the structure of genetic variation needed for working out an efficient collecting design for each species is rarely available (i.e. Guerrant et al. 2004; Godefroid et al. 2011; Cires et al. 2013; Odong et al. 2013; Alonso et al. 2014; Hoban & Schlarbaum 2014; McGlaughlin et al. 2014).

Collections that are to be used in conservation applications (e.g. *in situ* actions such as recovery or reintroduction programs) must be representative of the species' genetic diversity, and ensure that the material is available for research and conservation activities over the long term (Baccheta et al. 2008; BGCI 2012; Ferrer-Gallego et al. 2013; IUCN/SSC 2014; Volis 2015). Therefore, the assessment of the utility of the *ex situ* collections for restoration programs requires considering not only the number of accessions stored, but also knowledge of the germination capacity (GC), seed viability (SV), and the overall capacity to produce plants (CPP). Perhaps the most important component is seed viability testing (Godefroid et al. 2010). It should be emphasized that the viability values obtained for the most threatened species (IE) are lower than for species of the V category (see Table 1). Some endangered species, e.g. *Aristolochia clematitis*, *Berberis hispanica*, *Boerhavia repens*, *Centaurea alpina*, *Cistus heterophyllus* subsp. *carthaginensis*, *Euphorbia nevadensis* subsp. *nevadensis*, *Launaea arborescens* or *Launaea lanifera*, produce very few fertile seeds, which can be a consequence of small population size and low genetic diversity. As reduced seed production can result from a variety of reasons including self-incompatibility, inbreeding depression, lack of pollinators, etc., collecting fertile seeds in natural populations can be impossible without understanding the cause and providing the necessary conditions for successful sexual reproduction. Given the heterogeneity of the source data and potential importance of such unanalyzed effects such as variation in seed viability among populations and among years for the same population, the values reported here appear to be primarily informative. The observed mean value of 42.2 ± 46.3% (CPP) indicates that on average only about half of the collected and stored plant material can be useful for the future *in situ* actions.

According to Godefroid et al. (2011), the criteria to assess the value of *ex situ* conservation of threatened species are impossible to apply in some cases. They used the number of accessions and seeds per accession as surrogates for genetic diversity, and a species was considered well represented in an *ex situ* collection when at least five populations are stored in the bank with 5000 seeds per accession. We believe that this recommendation must be nuanced and refined because it does not take into account the reproductive biology of the species, and such parameters as the number of seeds per effective population (reproductive adults). Examples for the Valencian flora are *Armeria fontqueri* or *Allium subvillosum*, species with all of their populations conserved in germplasm bank, but seedlots never reached more than 5000 seeds due to the characteristics mentioned above; however, the quality of this germplasm reaches 100% germination levels. On the other hand, there are species with few individuals per population but with many populations, i.e. *Antirrhinum valentinum* (see Mateu-Andrés & Segarra-Moragues 2000), and for these species sampling only five populations does not appear to be an adequate collecting strategy.

The conservation of Valencian threatened species in local *ex situ* collections, as demonstrated in this paper, is still far from reaching the percentages indicating by GSPC Target 8 (2011−2020) if two assessment criteria are performed as we proposed here: the degree of representativeness of species and the capacity to produce future plants. Although we certainly understand that these criteria are very difficult to achieve and require large investments, they are necessary to ensure the implementation of *ex situ* conservation programs and guarantee that the collection and storage of germplasm is not done to create a Noah's Ark. This means that the final aim of *ex situ* programs should be back *in situ* through genetically representative plants from natural populations produced in *ex situ* conditions, thereby restoring or improving the damaged native populations. Considering this interpretation, the actual data fulfillment for achieving Target 8, and the data expressed by Sharrock et al. (2014), we believe that the GSPC goals proposed may be very difficult to accomplish. This difficulty may be even greater in countries with high species diversity, as occurs in the Mediterranean basin.

Our study shows that it is really essential to identify gaps in existing *ex situ* collections and to establish quantitative and qualitative indicators for measuring collections' conservation utility. Defining clear criteria for evaluating how well the threatened species are preserved in *ex situ* collections and how useful the collections are for recovery programs is complex, with currently no consensus on the proper criteria for assessment (Odong et al. 2013). As a result, inappropriate analyses that lead to erroneous conclusions can be made, with conservation attempts not reaching the proposed objectives, as shown here.

In this study, we provide insight on criteria used for evaluating collections utility for species conservation (index R4). Our findings should provide gene bank curators and researchers with the tools for making informed choices when creating, comparing and using collections to carry on future effective *in situ* actions. Assessment must take into account seed germination and viability; if seed viability is low and/or germination is poor or absent, the focus of *ex situ* conservation for this species must be on studying the cause of low seed viability or poor seed germination instead of collecting seeds that are of no use.

Acknowledgments

The authors would like to acknowledge the collaboration of the Botanical Garden of University of Valencia, the Wildlife Service's Freshwater Species Research Center and the working team of the CIEF, especially Antoni Marzo, Francisco José Albert, Mari Carme Picher, Jesús Martínez, Víctor Martínez, Albert Navarro and Mª Carmen Escribá. We would like to thank Dr Sergei Volis (Chinese Academy of Sciences, China), Dr Vernon H. Heywood (School of Biological Sciences, University of Reading, UK) and Dr Laura A. Weingartner (Department of Biology, Indiana University, USA) for their great help.

Funding

This study was partially financed by European funds FEDER and FEADER.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Adams VM, Marsh DM, Knox JS. 2005. Importance of the seed bank for population viability and population monitoring in a threatened wetland herb. Biol Conserv. 124:425−436.

Aguilella A, Fos S, Laguna E, editors. 2010. catálogo Valenciano de Especies de Flora Amenazadas. [Valencian catalog of threatened plant species]. Valencia: Generalitat Valenciana. Spanish.

Alonso MA, Guilló A, Pérez-Botella J, Crespo MB, Juan A. 2014. Genetic assessement of population restorations of the critically endangered *Silene hifacensis* in the Iberian Peninsula. J Nat Conserv 22(6):532−538.

Bacchetta G, Bueno A, Fenu G, Jiménez-Alfaro B, Mattana E, Piotto B, Virevaire M, editors. 2008. Conservación ex situ de plantas silvestres [Ex situ conservation of wild plants]. Oviedo: Principado de Asturias and La Caixa. Spanish.

Bacchetta G, Farris E, Pontecorvo C. 2012. A new method to set conservation priorities in biodiversity hotspots. Plant Biosyst. 146(3):638−648.

Bañares A, Blanca G, Güemes J, Moreno JC, Ortiz S, editors. 2011. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Adenda 2010. Madrid: Dirección General de Medio Natural y Política Forestal y Sociedad Española de Biología de la Conservación de Plantas; 170 pp. Spanish.

[BGCI] Botanic Gardens Conservation International. 2012. Implementing *ex situ* conservation programmes. Richmond, UK: Botanic Gardens Conservations International; [cited 2015 Apr 7]. Available from: http://www.plants2020.net/target-8-ex_situ/

Bilz M, Kell SP, Maxted N, Lansdown RV. 2011. European red list of vascular plants. Luxembourg: Publications Office of the European Union.

Castroviejo S, editor, 1986-2014. Flora iberica. Plantas vasculares de la Península Ibérica e Islas Baleares. [Flora iberica. Vascular plants of the Iberian peninsula and Balearic islands]. 19 vols. Madrid: Real Jardín Botánico-CSIC. Spanish.

CBD. 2002. Global Strategy for Plant Conservation. Montréal, Canada and Richmond, UK: The Secretariat of the CBD and BGCI.

CBD. 2012. The Global Strategy for Plant Conservation: 2011−2020. Richmond, UK: Botanic Gardens Conservation International.

[CGV] Consell de la Generalitat Valenciana. 2009. Decreto 70/2009, de 22 de mayo, del Consell, por el que se crea y regula el Catálogo Valenciano de Especies de Flora Amenazadas y se regulan medidas adicionales para su conservación. [Decree 70/2009, passed 22 May 2009, of the Council, to create and regulate the Valencian Catalog of Threatened Plant Species, as well as to regulate additional measures for their conservation]. DOCV [Official Gazette of the Valencian Community]. 6021:20143−20162. Spanish.

Cires E, De Smet Y, Cuesta C, Goetghebeur P, Sharrock S, Gibbs D, Oldfield S, Kramer A, Samain MS. 2013. Gap analyses to support ex situ conservation of genetic diversity in Magnolia, a flagship group. Biodivers Conserv. 22:567−590.

[CITMA] Consellería de Infraestructuras, Territorio y Medio Ambiente. 2013. Orden 6/2013, de 25 de marzo, de la Conselleria de Infraestructuras, Territorio y Medio Ambiente, por la que se modifican los listados valencianos de especies protegidas de flora y fauna. [Regulation 6/2013, passed 25 March 2013, of the Department of Infrastructures, Territory and Environment, to modify the Valencian lists of protected species of flora and fauna]. DOCV. 6996:8682−8690. Spanish.

Crawford AD, Steadman KJ, Plummer JA, Cochrane A, Probert RJ. 2007. Analysis of seed-bank data confirms suitability of international seed-storage standards for the Australian flora. Aust J Bot. 55:18−29.

[ENSCONET] European Native Seed Conservation Network. 2009. ENSCONET seed collection manual for wild species. Kew and Madrid: RBG Kew and Universidad Politécnica de Madrid.

FAO. 2013 Draft revised genebank standards for the conservation of orthodox seeds. Rome: Food and Agriculture Organization of the United Nations; [cited 2014 Nov 30]. Available at: www.fao.org/fileadmin/templates/agphome/documents/PGR/genebank/Genebankstandards-Nov2011english_FINAL.pdf

Ferrer-Gallego PP, Ferrando I, Gago C, Laguna E, editors. 2013. Manual para la conservación de germoplasma y el cultivo de la flora valenciana amenazada [Handbook for germplasm conservation and cultivation of the Valencian threatened flora]. Valencia: CITMA-Generalitat Valenciana. Spanish.

Ferrer-Gallego PP, Ferrando I, Albert FJ, Martínez V, Escribá MC, Navarro A, Laguna E. 2014. Conservación y distribución de accesiones del Banco de Germoplasma de Flora Silvestre Valenciana en la colección CIEF [Accessions conservation and distribution in the CIEF collection of the Valencian Germplasm Bank of Wild Flora]. Cuad Biodiversidad. 46:9−18. Spanish.

Fos S, Laguna E, Jiménez J. 2014. Plant Micro-Reserves in the Valencian Region (E of Spain): are we achieving the expected results? Passive conservation of relevant vascular plant species. Fl Medit. 24:153−162.

Gauthier P, Debussche M, Thompson JD. 2010. Regional priority setting for rare species based on a method combining three criteria. Biol Conserv. 143:1501−1509.

Godefroid S, Van de Vyver A, Vanderborght T. 2010. Germination capacity and viability of threatened species collections in seed banks. Biodivers Conserv. 19:1365−1383.

Godefroid S, Rivière S, Waldren S, Boretos N, Eastwood R, Vanderborght T. 2011. To what extent are threatened European plant species conserved in seed banks? Biol Conserv. 144:1494−1498.

Gosling PG. 2003. Viability testing. In: Smith RD, Dickie JB, Linington SL, Pritchard HW, Probert RJ, editors. Seed conservation turning science into practice. Kew: Royal Botanic Gardens; p. 445−481.

Guerrant EO, Fiedler P, Havens K, Maunder M. 2004. Revised genetic sampling guidelines for conservation collections of rare and endangered plants. In: Guerrant EO, Havens K, Maunder M, editors. Ex situ plant conservation: supporting species survival in the wild. Washington DC: Island Press; p. 419−441.

Hernández-Bermejo JE. 2007. Jardines botánicos y bancos de germoplasma: la conservación ex situ frente a la perspectiva de una estrategia para la conservación de las plantas [Botanic gardens and germplasm banks: ex situ conservation facing the challenge of a strategy for plant conservation]. Ambienta. 65:40−47. Spanish.

Hernández-Bermejo JE. 2009. Biodiversidad: del patrimonio a la soberanía [Biodiversity: from heritage to sovereignty]. Ambienta. 88:63−72. Spanish

Heywood VH. 2003. Mediterranean plant collections: needs and options. Setting the scene: what we have inherited. Bocconea. 16(1):283−287.

Heywood VH. 2014. An overview of *in situ* conservation of plant species in the Mediterranean. Fl Medit. 24:5−25.

Heywood VH. 2015. In situ conservation of plant species − an unattainable goal? Isr J Plant Sci. http://dx.doi.org/10.1080/07929978.2015.1035605

Hoban S, Schlarbaum S. 2014. Optimal sampling of seeds from plant populations for *ex-situ* conservation of genetic biodiversity, considering realistic population structure. Conserv Biol. 177:90−99.

Iriondo JM, Albert MJ, Gimenez L, Domínguez F, Escudero A, editors. 2009. Poblaciones en Peligro: Viabilidad Demográfica de la Flora Vascular Amenazada de España. [Populations in peril: demographic viability of threatened Spanish vascular flora]. Madrid: Dirección General de Medio Natural y

Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino); 242 pp. Spanish.
ISTA. 2006. International rules for seed testing. Edition 2006. Bassersdorf, Switzerland: International Seed Testing Association, ISTA.
IUCN. 2014. The IUCN red list of threatened species. Version 2014.3. Cambridge, UK: IUCN Global Species Programme Red List Unit; [cited 2015 Apr 19]. Available from: http://www.iucnredlist.org. Downloaded on 19 April 2015.
IUCN/SSC. 2014. Guidelines in the use of *ex situ* management for species conservation. Version 2.0. Gland, Switzerland: IUCN Species Survival Commission.
Krigas N, Menteli V, Vokou D. 2014. Analysis of the ex situ conservation of the Greek endemic flora at national European and global scales and of its effectiveness in meeting GSPC Target 8. Plant Biosyst. http://dx.doi.org/10.1080/11263504.2014.988194
Laguna E, editor. 1998. Flora rara, endémica o amenazada de la Comunidad Valenciana. [Rare, endemic or theatrened flora in the Valencian Community]. Consellería de Medio Ambiente. Generalitat Valenciana; 445 pp. Spanish.
Laguna E, Deltoro, VI, Pérez-Botella J, Pérez-Rovira P, Serra L, Olivares A, Fabregat C. 2004. The role of small reserves in plant conservation in a region of high diversity in eastern Spain. Biol Conserv. 119(3):421−426.
[MAGRAMA] Ministerio de Agricultura, Pesa y Alimentación. 2014. Estrategia Española de Conservación Vegetal 2014−2020: principios y orientaciones para la conservación de la diversidad vegetal silvestre en España [Spanish Strategy for Plant Conservation 2014−2020: principles and orientations to conserve the wild plant diversity in Spain]. Madrid: MAGRAMA. Spanish. http://www.magrama.gob.es/es/biodiversidad/planes-y-estrategias/estrategia_ce_vegetal_2014-2020_tcm7-332576.pdf
Mateo G, Crespo MB. 2014. Claves Ilustradas para la Flora Valenciana [Illustrated guidelines for the Valencian flora]. Monografías de Flora Montiberica. Jolube Consultor y Editor Botánico; 503 pp. Spanish.
Mateu-Andrés I, Segarra-Moragues JG. 2000. Population subdivision and genetic diversity in two narrow endemics of Antirrhinum L. Mol Ecol. 9:2081−2087.
Mattana E, Fenu G, Bacchetta G. 2012. Regional responsibility for plant conservation: the 2010 GSPC Target 8 in Sardinia. Plant Biosyst. 146:649−653.
McGlaughlin ME, Riley L, Brandsrud M, Arcibal E, Helenurm MK, Helenurm K. 2014. How much is enough? Minimum sampling to capture extant genetic diversity in ex situ seed collections: examples from the endangered plant *Sibara filifolia* (Brassicaceae). Conserv Genetics 16:253−266.
Mittermeier RA, Robles P, Hoffmann M, Pilgrim J, Brooks T, Mittermeier CG, Lamoreux J, Da Fonseca GAB, editors. 2004. Hotspots revisited. Mexico: CEMEX.
Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J. 2000. Biodiversity hotspots for conservation priorities. Nature. 403:853−858
Odong TL, Jansen J, Van Eeuwijk FA, Van Hintum TJL. 2013. Quality of core collections for effective utilization of genetic resources: review, discussion and interpretation. Theor Appl Genet. 126:289−305.
Offord CA, McKensy ML, Cuneo PV. 2004. Critical review of threatened species collections in the New South Wales Seedbank: implications for *ex situ* conservation of biodiversity. Pac Conserv Biol. 10:221−236.
Paton A, Lughadha EN. 2011. The irresistible target meets the unachievable objective: what have 8 years of GSPC implementation taught us about target setting and achievable objectives? Bot J Linn Soc. 166:250−260.
Sandev D, Mihelj D, Kovačić S. 2013. Meeting Target Eight − *Ex situ* conservation of Croatian threatened and statutorily protected plant species in the Botanical Garden of the Faculty of Science, University of Zagreb (Croatia). Nat Croat. 22(2):343−362.
Sharrock S, Jones M. 2011. Saving Europe's threatened flora: progress towards GSPC Target 8 in Europe. Int J Biodivers Conserv. 20:325−333.
Sharrock S, Oldfield S, Wilson O. 2014. Plant Conservation Report 2014: A review of progress and implementation of the Global Strategy for Plant Conservation 2011-2020. Secretariat of the Convention on Biological Diversity, Montréal, Canada and Botanic Gardens Conservation International, Richmond, UK. Technical Series 81; 56 pp.
Volis S. 2015. Species-targeted plant conservation: time for conceptual integration. Isr. J. Plant Sci. http://dx.doi.org/10.1080/07929978.2015.1085203
Volis S, Blecher M. 2010. Quasi in situ − a bridge between ex situ and in situ conservation of plants. Biodiversity and Conservation. 19:2441−2454.
VV.AA. 2004. Criterios orientadores para la inclusión de taxones y poblaciones en catálogos de especies amenazadas. Comisión Nacional de Protección de la Naturaleza. Madrid: Ministerio de Medio Ambiente. Spanish.