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Identification and assessment of the crop wild relatives of Spain that require most urgent conservation actions.

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Abstract. Crop Wild Relatives (CWR) are receiving significant attention over the last decades. Numerous conservation plans and guidelines to better manage these resources have been developed lately at both national and international levels. In this sense, Spain is following a similar path to that followed by other countries and has included CWR in the National Strategy for Plant Conservation of Spain and invested in scientific projects dealing with their conservation.
In this work, we present a preliminary assessment of the conservation status (both *in situ* and *ex situ*) of the Spanish CWR that are in a most urgent need of conservation. Crossability to crops, endemicity, threat status according to IUCN standards and high-quality georeferenced occurrence data were the criteria applied to select the target species, generating a list of 47 CWR species. Eleven of them, classified as Critically Endangered or Endangered by IUCN criteria are not, and should be, included included in the National Catalogue of Threatened Species of Spain; however 35 of them are included in at least one autonomous catalogue. Seventy-five per cent of the species are represented in protected areas, but if a minimum of five populations inside protected areas is sought the representation decreases to a 37%. The preliminary assessment of *ex situ* conservation shows that a high percentage of the species (81%) has at least one accession in national or international germplasm banks. However, additional studies are needed to determine if the accessions included in germplasm banks provide an adequate representation of the genetic variability of the species.
Keywords: Threatened; endemic; conservation status; crop wild relatives.

Identificación y evaluación de las especies silvestres emparentadas con cultivares que requieren acciones urgentes de conservación

Resumen. En los últimos años se están desarrollando numerosos planes y guías de conservación para Parientes Silvestres de Cultivos (PSC), debido al interés que estas especies están despertando. En este sentido, España se ha sumado a las últimas tendencias en conservación de PSC, por ejemplo, incluyéndolos en la Estrategia Nacional de Conservación Vegetal o invirtiendo en proyectos de investigación que buscan su conservación.
En este trabajo se presenta una evaluación preliminar sobre el estado de conservación (tanto *in situ* como *ex situ*) de los PSC en España que se encuentran en una necesidad más urgente de conservación. Como criterios para seleccionar especies se ha tenido en cuenta su potencial de cruzamiento con cultivos, endemicidad, grado de amenaza de acuerdo a los criterios de la UICN y la disponibilidad datos corológicos de alta calidad de georreferenciación, generándose una lista de 47 especies. Se comprobó la presencia de estas especies en el Catálogo Nacional de Especies Amenazadas de España y en todos los catálogos autonómicos, encontrando que 11 de ellas clasificadas como en Peligro Crítico y en Peligro de acuerdo a los criterios de la UICN no están recogidas en el catálogo nacional; además, 35 de ellas están incluidas en al menos un catálogo autonómico. El 75% de las especies se encuentran representadas en áreas protegidas, sin embargo, si se establece un mínimo de cinco poblaciones este porcentaje baja hasta el 37%. La evaluación *ex situ* preliminar muestra un alto porcentaje de especies representadas en bancos de germoplasma nacionales o internacionales (81%). No obstante, aún es necesario profundizar en la evaluación del estado de conservación *ex situ* de estas especies y determinar si su diversidad genética está representada de manera adecuada en los bancos de germoplasma.
Palabras clave: Amenazadas, endémicas, estado de conservación, parientes silvestres de cultivos.

Introduction

Crop Wild Relatives (CWR) are species closely related to crops (Heywood & *al*., 2007) and their utilization as useful gene donors in crop breeding is well recognized (Ford-Lloyd & *al*., 2011; Hajjar and Hodgkin, 2007). Their evolution in natural conditions makes them really valuable, as natural selection pressures may have provided them with adaptation traits to different conditions (Hawtin & *al*., 1996). Thus, CWR are not only worth for conservation as components of biodiversity but should be also seen as inexorable future starring elements for food security under the climate change context (Maxted & *al*., 2010).

Their conservation has attracted the interest of scientists, institutions and governments over the last years. Thus, they are specifically mentioned in the targets

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of the Global Strategy for Plant Conservation outlined by the Convention on Biological Diversity (UN CBD). The generation and publication of multiple lists and inventories of CWR all over the world endorses this assertion; among others, there are inventories for the United Kingdom (Maxted & *al*., 2007), Venezuela (Berlingeri & Crespo, 2012), the United States (Khoury & *al*., 2013), China (Kell & *al*. 2014), Italy (Landucci & *al*., 2014), Cyprus (Phillips & *al*., 2014), England (Fielder & *al*., 2015a); Scotland (Fielder & *al*., 2015b), Norway (Phillips & *al*., 2016), The Netherlands (van Treuren & *al*., 2017), the Czech Republic (Taylor & *al*., 2017), Spain (Rubio Teso & *al*., 2018) and even a global one (Vincent & *al*., 2013).

As any other wild species, CWR populations are threatened by habitat fragmentation, loss of habitat or genetic erosion (Heywood, 2011; Kell & *al*., 2012; Maxted & *al*., 2010, 2012), therefore conservation measures are needed to maintain their genetic diversity and avoid extinction. In Spain, the creation of the National Catalogue of Threatened Species promoted by Royal Decree 139/2011 (BOE n. 46, 23/02/2011), provides the ultimate framework to design and implement a conservation plan for endangered species. Additionally, the autonomous communities in which Spain is structured have enacted legislation comprising Regional Catalogues of Threatened Species that confer protection within their territorial limits. The inclusion of a species in these catalogues implies legal protection and the commitment by the administrations to elaborate periodic assessments of its conservation status and implement conservation measures. The *in situ* conservation of Spanish CWR could be approached using the Natura 2000 network. This network was designed in 1992 under the Habitats Directive (Council Directive 92/43/EEC) seeking the creation of a transnational system in Europe to protect both species and their habitats. Consequently, it may provide an effective way to confer passive conservation to CWR populations in Spain. In addition, it could facilitate the drafting of CWR genetic reserves in which their genetic diversity could be more actively preserved (Iriondo & *al*., 2008; Maxted & *al*., 2008). On the other hand, the *ex situ* conservation of seeds in germplasm collections can prevent the loss of genetic diversity of plant species (Bacchetta & *al*., 2008). Thus, it should be considered as a complementary system to the *in situ* conservation.

The compilation of CWR information on threat status, endemicity and crossability with crops can help in implementing conservation plans and directing efforts in the right way. In this sense, Rubio Teso & *al*. (2018) generated a prioritized CWR list for Spain containing 578 species. Still 578 species is a large number of species to consider for the implementation of conservation measures. Hence, it arises the need of identifying the CWR species which most urgently need conservation actions and assessing their conservation status.

The aim of this paper is to generate information that may help in ordering priorities for CWR conservation in Spain and implementing conservation actions. Thus, we pose the following questions: Which are the CWR in most urgent need of conservation? Are these species legally protected? What is the *in situ* conservation status of their populations? Are these species conveniently represented in germplasm banks?

Materials & Methods

Selection of species

The selection of species aimed at identifying the crop wild relatives that were in most urgent need of knowing their conservation status and of implementing conservation actions. Thus, using as reference the Prioritized Spanish Checklist of Crop Wild Relatives (578 species; Rubio Teso & *al*., 2018), a strict filtering was made to include just those species which simultaneously: a) were threatened under any of the IUCN categories according to the Spanish Red List of Vascular Flora (Moreno, 2008), b) were endemic to Spain, and c) had high crossability potential with crops of reference, belonging to genepool concept levels 1 or 2 (Harlan & de Wet, 1971) or taxon group concept levels 2 or 3 (Maxted & *al*., 2006). These three criteria, call attention to three key factors: threat, uniqueness and facility of use for breeding purposes.

Distribution data for the resulting species were downloaded from the GBIF data portal (GBIF, 2011-2013), filtering by scientific name and country (Spain). Synonyms were taken into account and included in the search. Quality of the georeferencing data was evaluated to be able to provide an accurate estimate on whether the populations of the target species fell within limits of protected areas. Consequently, data lacking locality description, geographic coordinates or with geographic coordinates with less than two decimals of decimal degrees (around 1 km accuracy) were eliminated from the analysis. Duplicates based on geographic coordinates were also eliminated. Only species with distribution data with the minimum quality standards established were selected and taken into account for further analysis.

Legal protection of the target species

To assess whether any of the target species were under legal protection in Spain, the Spanish National Catalogue of Threatened Species promoted by Royal Decree 139/2011 (BOE n 46, 23/02/2011) was checked. In addition, the Regional Catalogues of Threatened Species from all seventeen autonomous communities in Spain were consulted in order to verify their protection at the subnational level.

In situ and *ex situ* conservation preliminary assessment of the target species

A gap analysis (Scott & *al*., 1993) is a useful approach used to assess the representation of biological components in protected areas. This analysis provides a rough estimation of the *in situ* conservation status of a given species. However, it must be noted that while occurrence data confirms the presence of a species in a given territory, the lack of occurrence data does not necessarily mean the absence of the species. Once this premise was established, a gap analysis was performed using the distribution data of the selected species and the layer of Sites of Community Importance constituting Natura 2000 network in Spain. The analysis was performed using ArcGIS software, v. 10.1 (ESRI, USA). The number of populations for each species after georeferencing quality data assessment and of those within the Sites of Community Importance were added to the database of the study.

Brown & Briggs (1991) considered that the adequate preservation of the genetic diversity of an endangered species requires conservation of a minimum of five populations. On the other hand, Whitlock & *al.* (2016) established that 35% of the populations of a species are needed to conserve 70% of its genetic diversity. Consequently, these two thresholds were considered for the conservation assessment of this study.

Simultaneously, *ex situ* conservation status was assessed consulting different national and international databases. Again, the absence of data in the searched databases does not necessarily mean that there are no accessions preserved anywhere else, but that data are not available or public. Databases consulted were: I) the Spanish network of autochthonous plant genetic resources and wild plant germplasm banks (REDBAG), which belongs to the Iberian-Macaronesian Association of Botanical Gardens; II) the European Search Catalogue for Plant Genetic Resources (EURISCO), and III) the GRIN-USDA database belonging to the United States National Plant Germplasm System (GRIN-USDA). Information on number of accessions were not accessible in all sources consulted. Thus, the assessment focused on the presence/absence of accessions of the target species in germplasm collections.

Results

Selection of target species

The selection of species according to the established criteria, including the georeferencing quality criterion, generated a list of 47 species. If this last criterion had not been taken into account, 26 additional species would have been included. Results indicate that the CWR species in most urgent need of conservation assessment belong predominantly to the ornamental category use (Table 1) and to the Plumbaginaceae (40%), Lamiaceae (19%) and Amaryllidaceae (15%) families. The species were not evenly distributed among the three most endangered IUCN categories, as almost half of the species belonged to the Vulnerable category (22 species), followed by the Critically Endangered category (12 species), the Endangered category (eight species) and finally by the Near Threatened category (five species). A database was generated containing information on the scientific name of the each of the target CWR species, taxonomic family, use category, IUCN threat category, number of populations recorded with minimum georeferencing quality data, number of these populations within the Sites of Community Importance of the Natura 2000 network, presence of accessions in germplasm collections, category of legal protection according to the Spanish National Catalogue of Threatened Species and categories of legal protection according to the Regional Catalogues of Threatened Species of the 17 autonomous communities. All this detailed information is shown in Table 2.

Table 1. First two columns show the distribution of target CWR genera and species across use categories. In last two columns, number of genera and species per category of use in the Prioritized Spanish Checklist of Crop Wild Relatives (Rubio Teso & *al.*, 2018) is shown.

Category	N. genera	N. species	N. genera CWR	N. species CWR
Food	3	3	32	137
Forage & fodder	2	3	12	185
Ornamental	4	32	5	161
Industrial	2	9	10	95
TOTAL	11	47	59	578

Legal protection of target species

Ten of the 47 target CWR species are included in the Spanish National Catalogue of Threatened Species, which represent around 21% of the species of this study. Four of them are classified in this catalogue as “in danger of extinction”, four as “protected” and two as “vulnerable”. Thirty-five species (around 74% of the species of this study) are included in at least one of the regional catalogues. From these, six species are present in two regional catalogues. Eleven species from our list are classified into the highest IUCN threat categories (Critically Endangered and Endangered) but not included in the National Catalogue of Threatened Species; however, all of them except for *Sideritis reverchoni* Willk., are included in the regional catalogues (see Table 2).

Preliminary *in situ* and *ex situ* conservation assessment of target species

The application of the georeferencing data quality criteria produced a final occurrence dataset for 47 species, with 699 records in total. Figure 1 depicts the distribution of these occurrences in Spain.

The *in situ* gap analysis showed that 39% of the recorded populations of the target species were inside protected areas (Table 3). On the other hand, 36 target species (74%) have at least one of their populations within the limits of the Sites of Community Importance, and 18 species (38%) five or more populations. The application of the threshold involving the conservation of 35% of the populations showed that 27 species (57%) would comply with this requisite.

Regarding *ex situ* conservation, 40 species (85%) have at least one accession preserved in national and international germplasm collections (Table 3). The coverage of the *ex situ* conserved species was quite akin along the IUCN categories.

Table 2. List of species associated to category, family and threat category according to IUCN standards (Th.), number of populations (NP), number of populations inside the network of Sites of Community Importance of Natura 2000 (NP SCI) and percentage in relation to total number of populations (%SCI), presence of accessions preserved in national and international germplasm banks (Germ. banks), inclusion of the species in the National Catalogue of Threatened Species (Nat. Cat.; R.D. 139/2011) and inclusion of the species in the catalogues of the autonomous communities of Spain (Aut. Cat.). Abbreviations are: CR: Critically Endangered; EN: Endangered; VU: Vulnerable; NT: Near Threatened; DE: Danger of Extinction; PR: Protected; IE: Interest for Ecosystems of Canary Islands; MS: Monitored Species; RP: Included in Regime of Protection; SH: Sensitive to Habitat Alteration; SI: Special Interest; SP: Special Protection; AND: Andalucía; ARA: Aragón; BAL: Baleares; CAN: Canarias; CAT: Cataluña; CLM: Castilla-La Mancha; MUR: Región de Murcia; VAL: Comunidad Valenciana.

Category	Family	Species	Th.	NP	NP SCI	% SCI	Germ. banks	Nat. Cat.	Aut. Cat.
Ornamental	Asteraceae	*Argyranthemum broussonetii* (Pers.) Humphries	VU	3	0	0	yes	no	no
		Argyranthemum callichrysum (Svent.) Humphries	VU	3	2	66.7	yes	no	no
		Argyranthemum foeniculaceum (Willd.) Webb ex Sch. Bip.	VU	1	0	0	yes	no	no
		Argyranthemum maderense (D. Don) Humphries	VU	2	1	50	yes	no	yes(CAN/IE)
		Argyranthemum winteri (Svent.) Humphries	CR	1	0	0	yes	yes/VU	yes(CAN/VU)
	Caryophyllaceae	*Dianthus toletanus* Boiss. & Reut.	NT	10	5	50	no	no	no
	Plumbaginaceae	*Limonium album* (Coincy) Sennen	VU	12	6	50	no	no	yes(MUR/VU)
		Limonium aragonense (Debeaux) Font Quer	CR	5	2	40	no	no	yes(ARA/SH)
		Limonium arborescens (Brouss) Kuntze	EN	1	0	0	yes	yes/PR	yes(CAN/IE)
		Limonium carthaginense (Rouy) C. E. Hubb. & Sandwith	VU	2	0	0	yes	no	yes(MUR/VU)
		Limonium catalaunicum (Willk. & Costa) Pignatti	CR	28	3	10.7	yes	no	yes(ARA/SI) (CAT/DE)
		Limonium dufourei (Girard) Kuntze	CR	6	1	16.7	yes	no	yes(VAL/DE)
		Limonium erectum Erben	EN	3	2	66.7	yes	no	yes(CLM/DE)
		Limonium estevei Fern. Casas	CR	12	10	83.3	yes	no	yes(AND/DE)
		Limonium fruticans (Webb) Kuntze	EN	2	0	0	yes	yes/PR	yes(CAN/IE)
		Limonium grosii L. Llorens	VU	2	1	50	yes	no	no
		Limonium puberulum (Webb) Kuntze	EN	1	0	0	yes	no	yes(CAN/IE)
		Limonium revolutum Erben	VU	1	0	0	yes	no	yes(CAT/VU)
		Limonium rigualii M.B. Crespo & Erben	VU	4	1	25	yes	no	yes(VAL/MS)
		Limonium ruizii (Font Quer) Fen. Casas	VU	44	16	36.4	no	no	yes(ARA/VU)
		Limonium santapolense Erben	VU	9	1	11.1	yes	no	yes(VAL/MS)
		Limonium subglabrum Erben	EN	5	0	0	yes	no	yes(AND/SP)
		Limonium tabernense Erben	VU	15	11	73.3	yes	no	yes(AND/RP)
		Limonium thiniense Erben	VU	12	5	42	yes	no	yes(VAL/MS)
		Limonium tremolsii (Rouy) Erben	NT	4	3	75	yes	no	yes(CAT/VU)
	Amaryllidaceae	*Narcissus alcaracensis* Ríos & al.	EN	4	3	75	yes	no	yes(CLM/VU)
		Narcissus bugei (Fern. Casas) Fern. Casas	VU	10	1	10	yes	no	yes(AND/RP)
		Narcissus eugeniae Fern. Casas	VU	2	1	50	yes	no	no
		Narcissus longispathus Pugsley	EN	10	6	60	yes	yes/DE	yes(AND/DE)
		Narcissus nevadensis Pugsley *enemeritoi* Sánchez-Gómez & al.	CR	5	5	100	yes	yes/DE	yes(AND/DE) (MU/DE)
		Narcissus tortifolius Fern. Casas	VU	16	10	62.5	yes	no	yes(AND/VU) (MU/VU)
		Narcissus yepesii Ríos & al.	VU	5	4	80	yes	no	no
Forage & fodder	Fabaceae	*Astragalus cavanillesii* Podlech	CR	3	0	0	no	no	yes(CLM/VU) (MUR/VU)
		Astragalus tremolsianus Pau	CR	6	6	100	yes	yes /PR	yes(AND/DE)
		Medicago citrina (Font Quer) Greuter	CR	4	2	50	yes	yes /VU	yes(VAL/VU)
Food	Asteraceae	*Cynara alba* Boiss. ex DC.	VU	21	9	42.9	yes	no	no

Category	Family	Species	Th.	NP	NP SCI	% SCI	Germ. banks	Nat. Cat.	Aut. Cat.
	Rosaceae	*Prunus ramburii* Boiss.	VU	17	13	76.5	yes	no	no
	Solanaceae	*Solanum lidii* Sunding	CR	2	1	50	yes	yes /DE	yes(CAN/DE)
Industrial & other uses	Lamiaceae	*Sideritis chamaedryfolia* Cav.	VU	34	11	32.4	yes	no	yes(CLM/VU) (VAL/VU)
		Sideritis glauca Cav.	VU	21	6	28.6	yes	yes /PR	yes(MUR/VU)
		Sideritis lasiantha Pers.	NT	200	119	59.5	yes	no	yes(MUR/VU)
		Sideritis reverchonii Willk.	EN	17	0	0	no	no	no
		Sideritis serrata Lag.	CR	6	0	0	yes	yes /DE	no
		Sideritis stachydioides Willk.	VU	20	16	80	yes	no	no
		Thymus herba-barona Loisel. subsp. *bivalens* Mayol, L. Sáez & Roselló	CR	2	1	50	no	no	yes(BAL/DE)
		Thymus moroderi Pau ex Mart. Mart.	NT	97	11	11.3	yes	no	yes(MUR/VU)
		Thymus willkommii Ronniger	NT	9	7	77.8	yes	no	yes(CAT/VU) (VAL/MS)

Finally, two species (*Astragalus cavanillesii* Podlech and *Sideritis reverchonii* Willk.) have no populations within the limits of the Sites of Community Importance of the Natura 2000 network, nor accessions in germplasm banks. It is remarkable that these two species classified respectively as Critically Endangered and Endangered in the Spanish Red List of Vascular Flora are not included into the National Catalogue of Threatened Species of Spain, although *A. cavanillesii* is included in the catalogues of Castilla La Mancha and Región de Murcia (see Table 2).

Table 3. *In situ*, *ex situ* and legal conservation status of target CWR species of Spain (T CWR). For abbreviations on the rest of variables see Table 2.

Th.	T CWR	NP SCI	% SCI	Germ. banks	Nat. Cat.	Aut. Cat.
CR	12	9	42	9	6	11
EN	8	4	25	7	3	7
VU	22	18	39	20	1	13
NT	5	5	55	4	0	4
TOTAL	47	36	39	40	10	35

Discussion

Selection of species

The Mediterranean area is a region with high speciation rates and endemicity (Medail & Quezel, 1999; Thompson, 2005) and the Iberian Peninsula shelters more than 30% of European endemic species (Araújo & *al*., 2007). Thus, it is not surprising that 13% of the prioritized CWR of Spain (Rubio Teso & *al*., 2018) fulfilled the targeted criteria of being both threatened and endemic. The lack of available high-quality data for 26 of these species reduced this percentage to 8%. This lack of publicly available data does not mean it does not exist and can be simply explained by the zeal of some administrations in sharing sensitive data that could menace the survival of the populations. Thus, it has been a common procedure that projects focused on the study of threatened plants provide low-resolution occurrence data to chorological databases to preserve the location from unwanted visits (e.g. AFA project (Bañares & *al*., 2001)). We must also highlight that some of the occurrence data may be outdated as sources used include very old records. Nevertheless, the selection carried out of georeferencing data of high quality eliminated most of the old records. In any case, the work with endemic and threatened species requires up-to-date data that reflect the real distribution status of the analysed species. Thus, records over ten-years old should be revisited and their occurrence confirmed, at least for the most endangered species.

The selection of target species in this study clearly favours species included in the Ornamental category (32 species). This can be explained by the fact that genera selected from this category (particularly *Limonium* Mill. and *Narcissus* L.) are highly diversified and narrowly distributed and thus, with higher number of endemics and threatened species. In addition, these genera have their centers of diversity in the Mediterranean basin (Crespo, 2009; Raimondo, 1993; Roselló & *al*., 1994; Santos-Gally & *al*., 2012; Simón & *al*., 2010), and are probably responsible for the Eastern distribution of the occurrences shown in Figure 1.

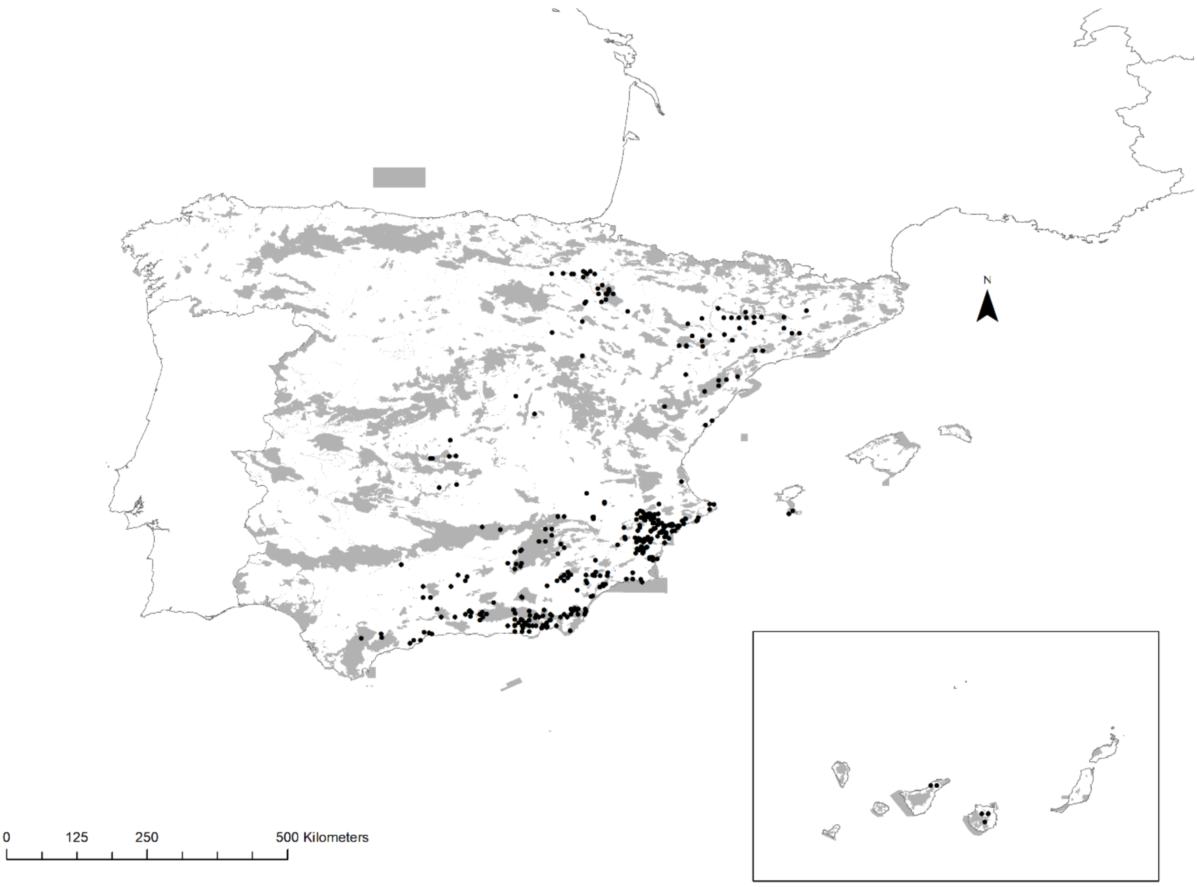

Figure 1. Distribution of the occurrence data of target threatened and endemic CWR of Spain. Grey areas correspond to Sites of Community Interest – Natura 2000.

Legal protection of target species

Thirty-seven target species considered to be in different levels of threat according to the Spanish Red List of Vascular Flora (Moreno, 2008) are not included in the National Catalogue as scientific information concerning threatened species is made available. However, results show that more than 74% of the CWR target species are included in the regional catalogues which provide further protection. On the other hand, eleven of the CWR species classified in the two highest IUCN threat categories are not found in the National Catalogue although 10 of them are in the regional catalogues. The inclusion of these species into regional catalogues demonstrate the engagement of the autonomous administrations in preserving their autochthonous flora, as a first step to start protecting our flora. Still, the inclusion of these species into the regional catalogues does not suppress the need to include them in the National Catalogue. The national administration is also giving steps in this sense and committed to increment knowledge about threatened plants, as manifested through the concatenation of different projects dealing with the study of threatened plant species in Spain, i.e., the Atlas and Red Book of Vascular Flora of Spain (Bañares & *al.*, 2004), the collection of germplasm and development of management protocols for protected plants of Spain project (ref: TEC0004223-TRAGSATEC) or the SEFA project (http://www.conservacionvegetal.org/proyectos.php). Results from our study stress the need of implementing conservation actions for the eleven species in the highest IUCN categories and not included in the National Catalogue, particularly focusing on *Astragalus cavanillesii* Podlech and *Sideritis reverchonii* Willk., which have no known populations within protected areas or seed accessions in germplasm banks. Special attention should be given to *S. reverchonii*, which is not included in any of the regional catalogues of threatened flora either.

***In situ* and *ex situ* conservation preliminary assessment of target species**

Different assessments of the Natura 2000 network have been reported concerning the conservation of different biological entities in Spain (Martínez & *al*., 2006; Araújo & *al*., 2007; Rubio-Salcedo & *al*., 2013). Whilst Araújo

& *al.* (2007) found acceptable representation (73-98% depending on the used criteria) of plant and animal species (pteridophytes, gymnosperms, dicotyledons, monocotyledons, reptiles, amphibians, birds and mammals), Rubio-Salcedo & *al*. (2013) concluded a poor coverage of lichen species based on the percentage of the potential distribution area present in the network. The representation of the target CWR of our study in the Natura 2000 network was around the lower range of values presented by Araújo & *al.* (2007). This can be explained by the much higher resolution of the data we have used (1 km vs. 50 km) and the stricter criterion applied to assign a grid cell to a protected area. On the other hand, the percentage of distribution area present in the network of our target species was in the same range of values found by Rubio-Salcedo & *al.* (2013) for lichens. Determining whether the actual coverage of endangered and endemic CWR in the Natura 2000 network is acceptable depends on which thresholds are set as a reference base. Thus, if the threshold is the species representation in the network by at least one population, the assessment is favourable, as that reported by Araújo & *al*. (2007). When the criteria is based on having in the network a higher number of populations, e.g., five (Brown & Briggs, 1991), or a substantial representation of its populations, e.g. 35% (Whitlock & *al*., 2016), the percentage of targeted CWR species that comply with these requirements is much lower and the appropriateness of the Natura 2000 network for their passive *in situ* conservation becomes arguable.

Concerning the choice of the optimal threshold to assess the conservation status of endangered endemic CWR in Spain, it is clear that the drafting of conservation measures should not stop with the simple representation of targeted CWR species in protected areas networks or in germplasm banks. Following this approach, it is likely that the genetic diversity component of threatened species will be neglected, being this especially serious in the case of CWR. Attempts to incorporate this component to conservation efforts have been made over the last decades (Brown & Briggs, 1991; Hamilton, 1994; Whitlock & *al*., 2016) and recently implemented in CWR conservation through the use of ecogeographical land characterization maps as a proxy to estimate genetic diversity (Maxted & *al*., 2012; Parra-Quijano & *al*., 2012; Phillips & *al*., 2016; Taylor & *al*., 2017). Thus, the problem of assessing the conservation status of the genetic diversity of a species could be approached by following Whitlock et al. (2016) and including 35% of known populations or proportionally representing populations from each of the ecogeographical units where the species is found (Parra-Quijano & *al*., 2012).

The high percentage of target CWR species found in national and international germplasm collections (81%) highlights the concern of Spanish conservationists in preserving threatened and endemic flora, and the high activity of seed collecting that has taken place by the REDBAG network in order to *ex situ* preserve at least 60% of Spanish threatened plant species (REDBAG) as targeted by the Global Strategy for Plant Conservation. The latest update of the objectives of the Global Strategy for Plant Conservation raises to 75% the percentage of threatened species to be *ex situ* preserved (UN CBD, 2010), a goal which is still met for the targeted CWR species. In any case, the nine target CWR species without representation in germplasm banks should be a priority for *ex situ* collecting missions.

In order to assess whether the genetic diversity component of the target species is being conserved, it is essential to gather information including the number of accessions of each species preserved in germplasm collections. Their origin and collection dates are also important data that should be retrieved. All this information would allow a more precise assessment of the *ex situ* conservation status of the species and the design of collecting actions to improve the quality of germplasm collections holdings. García & *al*. (2017) provide an example of this approach. In this study, they identified 88 Spanish CWR species from legumes and cereals crops and assessed their *ex situ* conservation status, proposing an optimized harvesting design for their collection.

Conclusions

To integrate these species into the national conservation programmes, we suggest an expert conservation assessment for the 11 species that are Critically Endangered and Endangered according to the IUCN criteria but not included in the National Catalogue of Threatened Species of Spain. The case of *Sideritis reverchonii* Willk., which is not included in any regional catalogue either, should be immediately addressed. These particular assessments would require gathering detailed information on field occurrences, exact number of accessions and their origin in germplasm collections and an ecogeographical evaluation. Authorities in Spain should be informed of results from these assessments and encouraged to design and implement the corresponding conservation plans.

The threatened and endemic CWR of Spain are adequately represented at the species level both *in situ*, in the Natura 2000 network, and *ex situ,* by national and international genebanks. However, the *in situ* conservation of their genetic diversity by the Natura 2000 network is deficient, while additional information is needed to be able to make the assessment at the *ex situ* level.

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References

Araújo, M.B., Lobo, J.M. & Moreno, J.C. 2007. The effectiveness of Iberian protected areas in conserving terrestrial biodiversity. Conserv. Biol. 21: 1423-1432.

Bacchetta, G., Bueno, Á., Fenu, G., Jiménez-Alfaro, B., Mattana, E., Piotto, B. & Virevaire, M. (Eds.). 2008. Conservación ex situ de plantas silvestres. Principado de Asturias. La Caixa. 378 p.

Bañares, A., Bermejo, E., Blanca, G., Domínguez, F., Güemes, J., Moreno, J.C. & Ortiz, S. 2001. La conservación de la flora amenazada en España y el Atlas de Flora Amenazada. In: S.E.C.F.-Junta de Andalucía (Eds.). Actas III Congreso Forestal Español 3. Pp. 161-168. Granada.

Bañares, A., Blanca, G., Guemes, J., Moreno, J. & Ortiz, S. 2004. Atlas y Libro Rojo de la Flora Vascular Amenazada de España. Dirección General de Conservación de la Naturaleza, Madrid. 1069 pp

Berlingeri, C. & Crespo, M.B. 2012. Inventory of related wild species of priority crops in Venezuela. Genet. Resour. Crop Evol. 59(5): 655-681.

Anonymous. 2011. Desarrollo del Listado de Especies Silvestres en Régimen de Protección Especial y del Catálogo Español de Especies Amenazadas. BOE 46, Sec. I, Pp 20912-20951. Real Decreto 139/2011, 4 de febrero (in Spanish).

Brown, A.H.D. & Briggs, J.D. 1991. Sampling strategies for genetic variation in ex situ collections of endangered plant species. In: Falk, D.A. & Holsinger, K.E. (Eds.). Genetics and Conservation of Rare Plants. Pp. 99-199. Oxford Univ. Press, New York.

Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora. Official Journal of the European Communities No L 206/7.

Crespo, M.B. 2009. A new coastal species of Limonium (Plumbaginaceae) from Southeastern Spain. Folia Geobot. 44: 177-190.

Fielder, H., Brotherton, P., Hosking, J., Hopkins, J.J., Ford-Lloyd, B. & Maxted, N. 2015a. Enhancing the Conservation of Crop Wild Relatives in England. PLoS One 10(6): e0130804.

Fielder, H., Smith, C., Ford-Lloyd, B. & Maxted, N. 2015b. Enhancing the conservation of crop wild relatives in Scotland. J. Nat. Conserv. 29: 51-61.

Ford-Lloyd, B. V., Schmidt, M., Armstrong, S.J., Barazani, O., Engels, J., Hadas, R., Hammer, K., Kell, S.P., Kang, D., Khoshbakht, K., Li, Y., Long, C., Lu, B.-R., Ma, K., Nguyen, V.T., Qiu, L., Ge, S., Wei, W., Zhang, Z. & Maxted, N. 2011. Crop Wild Relatives Undervalued, Underutilized and under Threat? Bioscience 61: 559-565.

García, R.M., Parra-Quijano, M. & Iriondo, J.M. 2017. A multispecies collecting strategy for crop wild relatives based on complementary areas with a high density of ecogeographical gaps. Crop Sci. 57: 1059-1069.

Hajjar, R. & Hodgkin, T. 2007. The use of wild relatives in crop improvement: A survey of developments over the last 20 years. Euphytica 156: 1-13.

Hamilton, M.B. 1994. Ex situ conservation of wild plant species: time to reassess the genetic assumptions and implications of seed banks. Conserv. Biol. 8: 39-49.

Harlan, J.R. & de Wet, J.M.J. 1971. Toward a rational classification of cultivated plants. Taxon 20: 506-517.

Hawtin, G., Iwanaga, M. & Hodgkin, T. 1996. Genetic resources in breeding for adaptation. Euphytica 92: 255- 256.

Heywood, V. 2011. Conservation strategies for species/populations occurring outside protected areas. In: Hunter, D. & Heywood, V. (Eds.). Crop Wild Relatives. A Manual of In Situ Conservation. Pp. 253-294. Earthscan, London.

Heywood, V., Casas, A., Ford-Lloyd, B., Kell, S. & Maxted, N. 2007. Conservation and sustainable use of crop wild relatives. Agric. Ecosyst. Environ. 121, 245-255.

Iriondo, J.M., Ford-Loyd, B., De Hond, L., Kell, S.P., Lefèvre, F., Korpelainen, H. & Lane, A. 2008. Plant Population Methodologies for the In Situ Genetic Conservation of CWR. In: Iriondo J.M. Maxted, N. & Dulloo, M.E. (Eds.). Conserving Plant Genetic Diversity in Protected Areas. Pp. 88-123. CAB International, Wellingford.

Kell S.P., Maxted, N. & Bilz, M. 2012 European Crop Wild Relative Threat Assessment: Knowledge gained and lessons learnt. In: Maxted, N., Ford-Lloyd, B., Kell, S., Iriondo, J., Dulloo, M.E. & Turok, J. (Eds.). Agrobiodiversity Conservation: Securing the Diversity of Crop Wild Relatives and Landraces. Pp. 218-242. CAB International, Wallingford.

Kell, S., Qin, H., Chen, B., Ford-Lloyd, B., Wei, W., Kang, D. & Maxted, N. 2014. China's crop wild relatives: Diversity for agriculture and food security. Agric. Ecosyst. Environ. 209: 138-154.

Khoury, C.K., Greene, S., Wiersema, J., Maxted, N., Jarvis, A. & Struik, P.C. 2013. An inventory of crop wild relatives of the United States. Crop Sci. 53: 1496-1508.

Landucci, F., Panella, L., Lucarini, D., Gigante, D., Donnini, D., Kell, S., Maxted, N., Venanzoni, R. & Negri, V. 2014. A prioritized inventory of crop wild relatives and wild harvested plants of Italy. Crop Sci. 547: 1628-1644.

Martínez, I., Carreño, F., Escudero, A., & Rubio, A. 2006. Are threatened lichen species well-protected in Spain? Effectiveness of a protected areas network. Biol. Conserv. 133: 500-511.

Maxted, N., Ford-Lloyd, B.V., Jury, S., Kell, S. & Scholten, M. 2006. Towards a definition of a crop wild relative. Biodivers. Conserv. 15: 2673-2685.

Maxted, N. Kell, S.P. & Ford-Lloyd, B.V. 2008. Crop wild relative conservation and use: Establishing the context. In: Maxted, N., Ford-Lloyd, B., Kell, S., Iriondo, J., Dulloo, M.E. & Turok, J. (Eds.). Crop Wild Relative Conservation and Use. Pp. 1-30. CAB International, Wellington.

Maxted, N., Kell, S., Ford-Lloyd, B., Dulloo, E. & Toledo, Á. 2012. Toward the systematic conservation of global crop wild relative diversity. Crop Sci. 52: 774-785.

Maxted, N., Kell, S., Toledo, Á., Dulloo, E., Heywood, V., Hodgkin, T., Hunter, D., Guarino, L., Jarvis, A. & Ford-Lloyd, B. 2010. A global approach to crop wild relative conservation: Securing the gene pool for food and agriculture. Kew Bull. 65: 561-576.

Maxted, N., Scholten, M., Codd, R. & Ford-Lloyd, B. 2007. Creation and use of a national inventory of crop wild relatives. Biol. Conserv. 140(1-2): 142-159.

Medail, F. & Quezel, P. 1999. Biodiversity Hotspots in the Mediterranean Basin: Setting Global Conservation Priorities. Conserv. Biol. 13: 1510-1513.

Moreno, J.C. coord. 2008. Lista Roja 2008 de la Flora Vascular Española. Dirección General del Medio Natural y Política Forestal (Ministerio de Medio Ambiente, y Medio Rural y Marino, y Sociedad Española de Biología de la Conservación de Plantas), Madrid. 86 p.

Parra-Quijano, M., Iriondo, J.M. & Torres, E. 2012. Ecogeographical land characterization maps as a tool for assessing plant adaptation and their implications in agrobiodiversity studies. Genet. Resour. Crop Evol. 59: 205-217.

Phillips, J., Asdal, Ä., Magos Brehm, J., Rasmussen, M. & Maxted, N. 2016. In situ and ex situ diversity analysis of priority crop wild relatives in Norway. Divers. Distrib. 22: 1112-1126.

Phillips, J., Kyratzis, A., Christoudoulou, C., Kell, S. & Maxted, N. 2014. Development of a national crop wild relative conservation strategy for Cyprus. Genet. Resour. Crop Evol. 61: 817-827.

Raimondo, F.M. 1993. Limonium optimae, a new species from central Silicy. Flora Mediterr. 3: 13-18.

Roselló, J.A., Mus, M. & Soler, J.X. 1994. Limonium ejulabilis, a new endangered endemic species from Majorca (Balearic Islands, Spain). An. Jard. Bot. Madrid 51: 199-204.

Rubio-Salcedo, M., Martínez, I., Carreño, F. & Escudero, A. 2013. Poor effectiveness of the Natura 2000 network protecting Mediterranean lichen species. J. Nat. Conserv. 21: 1-9.

Rubio Teso, M.L., Torres Lamas, E., Parra-Quijano, M., de la Rosa, L., Fajardo, J. & Iriondo J.M. 2018. National inventory and prioritization of Crop Wild Relatives in Spain. Genet. Resour. Crop Evol. 65 (4): 1327-1253.

Santos-Gally, R., Vargas, P. & Arroyo, J. 2012. Insights into Neogene Mediterranean biogeography based on phylogenetic relationships of mountain and lowland lineages of Narcissus (Amaryllidaceae). J. Biogeogr. 39: 782-798.

Scott, J.M., Davis, F., Csuti, B., Noss, R., Butterfield, B., Anderson, H., Caicco, S., Erchia, F.D., Edwards, T.C. & Ulliman, J. 1993. Gap analysis: A Geographic approach to protection of Biological Diversity. Wildl. Monogr. 123: 3-41.

Simón, V.I., Xavier Picó, F. & Arroyo, J. 2010. New microsatellite loci for Narcissus papyraceus (Amarillydaceae) and cross-amplification in other congeneric species. Am. J. Bot. 97: 10-13.

Taylor, N.G., Kell, S.P., Holubec, V., Parra-Quijano, M., Chobot, K. & Maxted, N. 2017. A systematic conservation strategy for crop wild relatives in the Czech Republic. Divers. Distrib. 23(4): 448-462.

Thompson, J.D. 2005. Plant Evolution in the Mediterranean. Oxford Univ. Press, New York. 293 p.

van Treuren, R., Hoekstra, R. & van Hintum, T.J.L. 2017. Inventory and prioritization for the conservation of crop wild relatives in The Netherlands under climate change. Biol. Conserv. 216: 123-139.

Vincent, H., Wiersema, J., Kell, S., Fielder, H., Dobbie, S., Castañeda-Álvarez, N.P., Guarino, L., Eastwood, R., Lén, B. & Maxted, N. 2013. A prioritized crop wild relative inventory to help underpin global food security. Biol. Conserv. 167: 265-275.

Whitlock, R., Hipperson, H., Thompson, D.B.A., Butlin, R.K. & Burke, T. 2016. Consequences of in situ strategies for the conservation of plant genetic diversity. Biol. Conserv. 203: 134-142.

Websites

EURISCO Catalogue. European search catalogue for plant genetic resources. https://eurisco.ipk-gatersleben.de/apex/f?p=103:1. Accessed 27 Feb 2018.

GRIN-USDA. U.S. National Plant Germplasm System. https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomyquery.aspx. Accessed 27 Feb 2018.

GBIF. 73 Datasets downloaded. www.gbif.org. Accessed 1 May 2017

REDBAG. Red Española de Bancos de semilla. https: www.redbag.es. Accessed 27 Feb 2018.

UN CBD 2010. United Nations Convention on Biological Diversity. Global Strategy for Plant Conservation: Technical rationale, justification for updating and suggested milestones and indicators. https://www.cbd.int/gspc/strategy.shtml