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The conservation of the Italian Crop Wild Relatives in the RIBES seedbanks: first data to establish national inventories and conservation priorities

*Sara Magrini¹, Paolo Atzeri², Gianluigi Bacchetta², Gianni Bedini³,
Valentina Carasso⁴, Angelino Carta³, Roberta Ceriani⁵,
Simona Ciancaleoni⁶, Luciano Di Martino⁷, Marco Di Santo⁷, Giuseppe
Fabrini⁸, Luigi Forte⁹, Loretta Gratani⁸, Valeria Negri⁶, Marco Porceddu²,
Cristina Salmeri¹⁰, Roberto Sarigu², Anna Scialabba¹⁰, Fabio Taffetani¹²,
Mariacristina Villani¹³, Elena Zappa¹⁴ & Mauro Mariotti¹⁴*

¹Banca del Germoplasma della Toscana, Viterbo, Italy; ²Banca del Germoplasma della Sardegna, Cagliari, Italy; ³Banca del Germoplasma di Pisa, Italy; ⁴Banca del Germoplasma del Piemonte, Chiusa Pesio (CN), Italy; ⁵Banca del Germoplasma delle Piantе Lombarde, Italy; ⁶Banca del Germoplasma dell'Università degli Studi di Perugia, Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Perugia, Italy; ⁷Majella Seed Bank - Parco Nazionale della Majella, Italy; ⁸Banca del Germoplasma di Roma, Italy; ⁹Banca del Germoplasma del Museo Orto Botanico dell'Università di Bari, Italy; ¹⁰Banca del Germoplasma di Palermo, Italy; ¹¹Banca del Germoplasma di Catania, Italy; ¹²Orto Botanico "Selva di Gallignano", Ancona, Italy; ¹³Centro di Ateneo Orto Botanico dell'Università di Padova, Italy; ¹⁴Laboratorio per la conservazione della diversità vegetale ligure, Genova, Italy. E-mail: reteribes@gmail.com

Introduction

Crop Wild Relatives (CWR), a component of Plant Genetic Resources for Food and Agriculture (PGRFA), are wild species closely related to crops, including their progenitors, defined by their potential ability to contribute beneficial traits for crop improvement (Maxted et al., 2006; Vincent et al., 2013). CWR populations are particularly likely to contain the adaptive genes necessary to develop new varieties because of the wide variety of habitats in which they grow and broad range of conditions they are adapted to, so their genetic diversity offers an insurance against the predicted harmful impacts of climate change on biodiversity and food security, together with the growing world

population (FAO, 2008; Vincent et al., 2013).

On the other hand, CWRs, which are intrinsically no different to any other group of wild species, are subject to an increasing range of threats in their host habitats, then a more systematic attention to their conservation is required (Maxted & Kell, 2009; Bilz et al., 2011; Vincent et al., 2013). Particularly, a concerted effort devoted to improving the conservation and availability of CWRs for crop improvement is thus timely both for biodiversity conservation and for food security objectives, as the window of opportunity to resolve these deficiencies will not remain open indefinitely (Vincent et al., 2013; Castañeda-Álvarez et al., 2016).

In such contest, a census of CWRs of species of food and forage interest listed in the Annex I 'Priority crops' of the *International Treaty on Plant Genetic Resources for Food and Agriculture* (FAO, 2001) was carried out in 2014 by the *Italian National Institute for Environmental Protection and Research* (ISPRA), based on Landucci et al. (2014) and the CWR and Wild Harvested Plants published at <http://vnr.unipg.it/PGRSecure> (Andreella et al. 2015), in order to quantify the extent of CWR representation in the Italian *ex situ* collections.

Here, we present the updated results of such census in the *Italian Seed Bank Network for native species conservation* (RIBES) with an analysis of the contribution to the conservation of the Italian FAO priority CWR. Finally, a national priority list for conservation of CWRs was drawn up and proposed here.

Materials and methods

In order to quantify the extent of CWR representation in the seed-banks of the RIBES network, data of 620 crop wild relatives of species of food and forage interest listed in the Annex I 'Priority crops' of the *International Treaty on Plant Genetic Resources for Food and Agriculture* (FAO, 2001) have been collected within the census carried out by ISPRA in 2014 and updated by RIBES in 2015. The number of Italian CWRs and of their accessions stored in each seed-bank were determined. The richness of CWR taxa and their accessions in *ex situ* collections was graphically displayed in maps.

In order to draw up a priority list for the Italian CWRs listed in the FAO Treaty identifying the target species to address conservation measures the following taxa were selected:

- the endemic taxa (Peruzzi et al., 2014), according to the "Regional responsibility" criterion proposed by Gauthier et al. (2010) and Bacchetta et al. (2012);
- the species listed in the Annexes of the Habitats Directive 92/43/EEC, the so-called Policy Species;
- the threatened (CR, EN, VU) and near threatened (NT) taxa at both Italian (Rossi et al., 2013; 2016; Perrino & Wagensommer, 2013a,b,c) and European level (Bilz et al., 2011).

The distribution of Italian endemic CWRs and of threatened/near threatened in the Italian regions were graphically displayed in maps (Conti et al., 2005; Peruzzi et al., 2014).

TABLE 1. Families and most represented genera (> 5 taxa) among the Italian CWRs preserved in the seed-banks

Family	N. of taxa	Genus	N. of taxa
Fabaceae	75	<i>Brassica</i>	30
Poaceae	68	<i>Trifolium</i>	17
Brassicaceae	49	<i>Vicia</i>	16
Solanaceae	11	<i>Solanum</i>	11
Asparagaceae	7	<i>Lathyrus</i>	8
Rutaceae	5	<i>Aegilops</i>	8
Apiaceae	4	<i>Medicago</i>	7
Rosaceae	4	<i>Triticum</i>	7
Asteraceae	3	<i>Lotus</i>	6
Chenopodiaceae	2	<i>Asparagus</i>	6
Convolvulaceae	2	<i>Lupinus</i>	6

Investigating the completeness of the *ex situ* collections for such priority CWRs, a priority for collection (P) was assigned to taxa with less than 5 accessions and a higher priority (HP) for taxa with no current representation in seed-banks.

Results

Ex situ collection of CWRs

According to the 2015 census, the RIBES network preserves 37% of the Italian CWRs listed in the FAO Treaty. Particularly, in 14 out of 16 seed-banks are preserved 6,029 accessions of 229 CWR taxa, belonging to 11 families and 57 genera. The most represented families are Fabaceae (75 taxa with 3545 accessions), Poaceae (68 taxa with 1602 accessions) and Brassicaceae (49 taxa with 725 accessions) (Tables 1 and 2); the genera *Brassica*, *Trifolium* and *Vicia* are represented by the highest number of

Family	N. of access.	Genus	N. of access.	Seed Banks
Fabaceae	3545	<i>Phaseolus</i>	1295	2
Poaceae	1602	<i>Medicago</i>	1018	6
Brassicaceae	725	<i>Brassica</i>	623	12
Solanaceae	91	<i>Lolium</i>	494	5
Asparagaceae	17	<i>Hordeum</i>	477	4
Rutaceae	7	<i>Trifolium</i>	454	7
Apiaceae	16	<i>Lotus</i>	200	7
Rosaceae	7	<i>Dactylis</i>	183	7
Asteraceae	9	<i>Solanum</i>	91	7
Chenopodiaceae	4	<i>Poa</i>	77	3
Convolvulaceae	2	<i>Triticum</i>	50	5

TABLE 2. Families and genera among the Italian CWRs with the higher number of accessions preserved in the seed-banks

TABLE 3. Species among the Italian CWRs with the higher number of accessions (>100) preserved in the seed-banks

Species	N. of access.	Seed Banks
<i>Phaseolus vulgaris</i> L.	1195	2
<i>Medicago sativa</i> L.	746	3
<i>Hordeum vulgare</i> L.	474	3
<i>Lolium perenne</i> L.	466	3
<i>Medicago rigidula</i> (L.) All.	188	1
<i>Lotus corniculatus</i> L.	182	4
<i>Trifolium repens</i> L.	178	1
<i>Dactylis glomerata</i> L.	177	4
<i>Onobrychis viciifolia</i> Scop.	169	3
<i>Schedonorus arundinaceus</i> (Schreb.) Dumort	144	1
<i>Brassica rupestris</i> Raf. subsp. <i>rupestris</i>	140	3
<i>Brassica oleracea</i> L. var. <i>italica</i> Plenck	116	1
<i>Vigna unguiculata</i> (L.) Walp.	109	2

taxa (Table 1), while the genera *Phaseolus* and *Medicago* have the highest number of accessions (more than 1,000, see Table 2).

Thirteen taxa have more than 100 accessions, and four species more than 400, particularly, *Phaseolus vulgaris* (1,195) and *Medicago sativa* (746) having the highest numbers (Table 3). On the other hand, 140 taxa are represented by fewer than ten accessions and 81 taxa by only one (Figure 1).

Accessions of the genus *Brassica* are stored in quite all the seed-banks (12 out of 14), followed by the genera *Dactylis*, *Lotus*, *Solanum*, *Trifolium* and *Vicia* (7), and *Medicago* and *Asparagus* (6) (Table 2). The 1,295 accessions of the genus *Phaseolus* are stored only in two seed-banks (see Table 2).

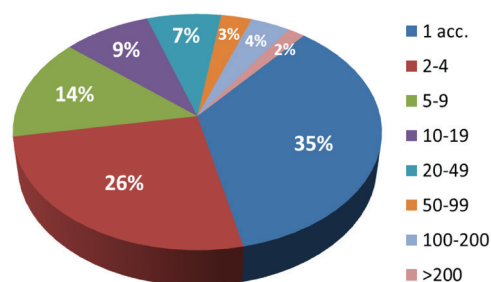


FIGURE 1. Number of accessions for each species preserved in the seed-banks

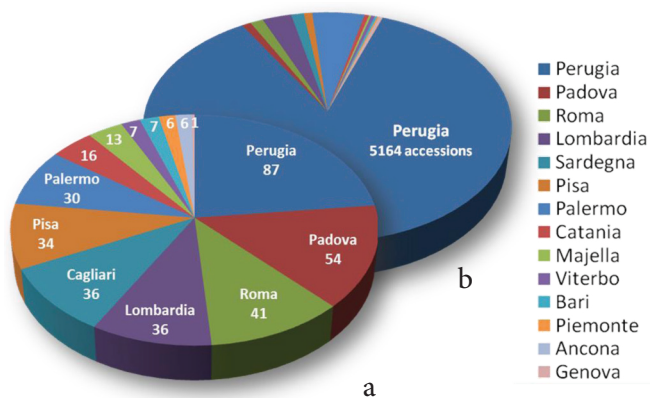


FIGURE 2. Number of a) CWR taxa and of b) their accessions stored in each seed-bank

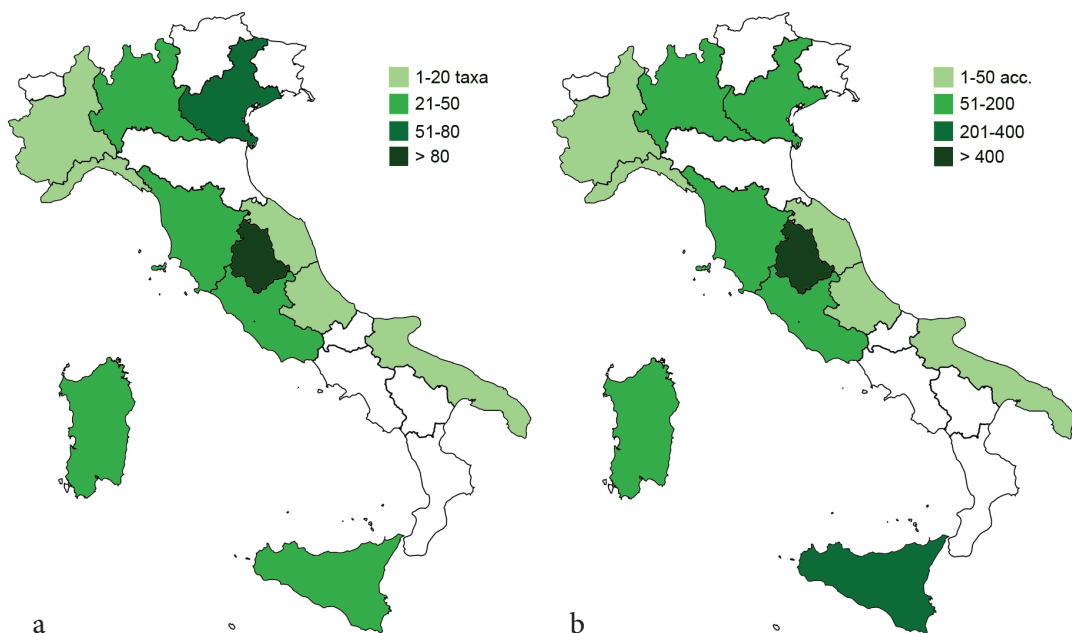


FIGURE 3. Number of a) CWR taxa and of b) their accessions stored in seed-banks in the Italian regions

Among the species, *Brassica nigra* (L.) W.D.J. Koch is preserved in the higher number of seed-banks (5) with seven accessions.

The CWR conservation in Italy is concentrated in Umbria (Figures 2 and 3) where the seed-bank of Perugia, which is devoted to this group of wild plants, preserves 38% of the taxa and 86% of the accessions (Figure 2). Seven out of fourteen seed-banks preserve more than 30 taxa, among these, more than 40 only in the seed-banks of Padova and Rome; five seed-banks preserve less than 10 taxa (Figure 2).

Regarding the consistence of the collections, beside Perugia, in the two Sicilian seed-banks are stored the highest number of accessions (342), with the main contribution given by the Palermo seed-bank (315) (Figures 2 and 3).

Towards a national priority list for the Italian Crop Wild Relatives

Endemic species richness

According to Peruzzi et al. (2014), in the reference list 28 endemic taxa occur (Table 4), 20 of them exclusive of one Italian region and, particularly, 12 exclusive of Sicily, especially belonging to the genus *Brassica* (11 taxa). Figure 4a shows the distribution and richness in the Italian regions of the endemic CWRs and highlights how endemism is highest in Sicily (16 taxa), Calabria (6), Latium (6), and Abruzzo (5). No endemic CWRs occur in four regions of Northern Italy.

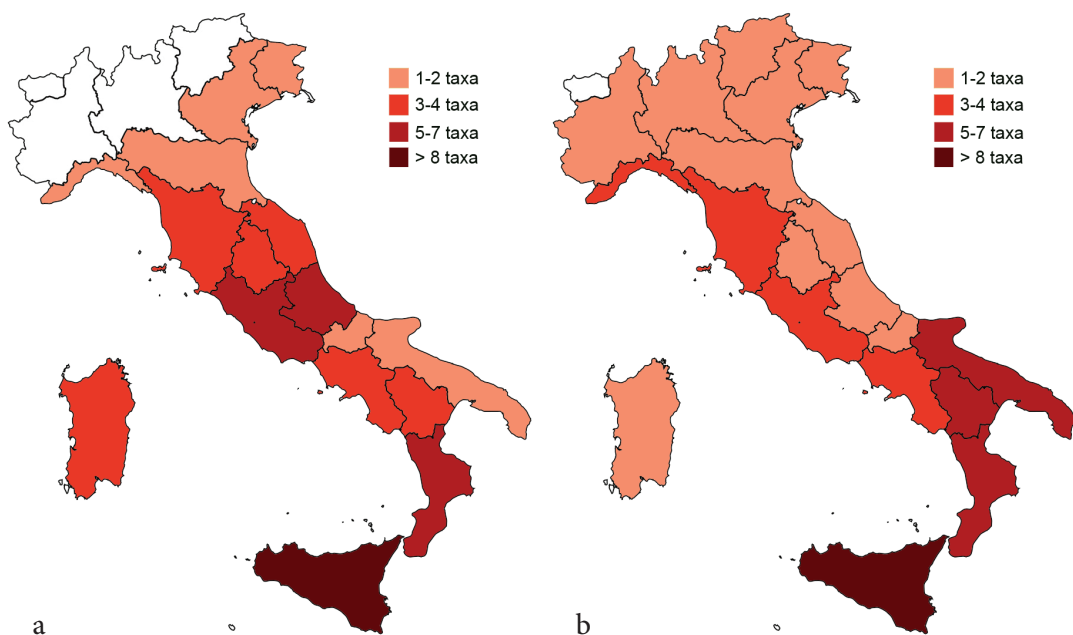


FIGURE 4. Spatial distribution of a) Italian endemic CWRs (Peruzzi et al., 2014) and b) threatened (CR, EN, VU) and near threatened (NT) CWRs (Bilz et al., 2011; Perrino & Wagensommer, 2013abc; Rossi et al., 2013; 2016) in Italy (Conti et al., 2005)

Three endemic CWRs are threatened with extinction (Rossi et al., 2013): *Brassica macrocarpa* [CR], a Sicilian endemic, *Brassica glabrescens* [VU], endemic of Friuli Venezia Giulia, and *Vicia giacominiiana* [CR], endemic of Apulia and Calabria.

The assessment of the occurrence of endemic CWRs in the RIBES *ex situ* collections has shown a total of 306 seed accessions belonging to 14 out of 28 taxa preserved in five seed-banks, Catania, Sardinia, Perugia, Padova and, especially, Palermo with 257 accessions of nine taxa. On the other hand, no accessions at all are preserved in the seed-banks for the other 14 taxa, among them also a threatened species as *Vicia giacominiiana*.

Policy Species

Among the CWRs of the reference list, 4 taxa are listed in the Annex II of Habitats Directive 92/43/EEC: *Brassica macrocarpa*, designated in the Annex as a priority species in view of the threats it face, *B. glabrescens*, *B. insularis*, and *Crambe tataria* (Table 4).

Regarding their conservation, all these species are represented in the *ex situ* collections, for a total of 40 accessions in seven seed-banks, particularly: *B. insularis* with 27 accessions in the seed-banks of Sardinia and Perugia, and *B. macrocarpa* with 12

11 accessions in four seed-banks. On the other hand, *B. glabrescens* and *C. tataria*, two Friulan species, are preserved only in Padova each with one accession.

Threatened species richness

In the reference list, 12 threatened (CR, EN, VU) taxa occur, besides seven near threatened, for a total of 19 priority taxa (Bilz et al., 2011; Rossi et al., 2013; Perrino & Wagensommer, 2013a,b,c) (Table 4). Two species show the highest extinction risk, *Critically Endangered* at both European and Italian level: *Brassica macrocarpa* and *Vicia giacominiiana*, both endemics. Two species were assessed as *Endangered*: *Beta macrocarpa* at European level and *Lathyrus palustris* at Italian level. Other eight taxa are *Vulnerable*.

Six out of these 19 priority taxa belong to the genus *Brassica*, three to the genera *Aegilops* and *Lathyrus*, and two to *Vicia* and *Crambe*.

Figure 4b shows the geographic distribution of threatened and near threatened CWRs in Italy (Conti et al., 2005). Threatened CWRs occur in quite all the Italian regions, except Val d'Aosta. The top regions in terms of richness of threatened taxa occur all in South Italy: Sicily (17), Apulia (7), Calabria (6), and Basilicata (5). Seven taxa are exclusive of one region and, among them, three of Sicily and two of Friuli Venezia Giulia.

Eleven taxa are represented in the *ex situ* collection with a total of 91 accessions in nine seed-banks; only the seed-banks of Perugia, Bari and Padova preserve accessions of more than one species (five, four and three taxa, respectively). The higher number of accessions are preserved in the seed-bank of Perugia (27), Sardinia (23 accessions of *Brassica insularis*), and Genova (17 accessions of *B. montana*).

Eight out of 19 taxa were found to be not represented in *ex situ* collections, among the most threatened: *Vicia giacominiiana* (CR), *Beta macrocarpa* and *Lathyrus palustris* (EN).

A first priority list for the Italian Crop Wild Relatives

A priority list of 43 Italian CWRs listed in the FAO Treaty was drawn up (Table 4) in order to identify the target species for conservation measures, selecting all the endemic taxa (28), the policy species (4), and the threatened (CR, EN, VU) and near threatened (NT) taxa at both Italian and European level (19).

Investigating the completeness of the *ex situ* collections for these 43 CWR taxa (Table 4), a total of 30 of them should be considered of priority for collection from their natural habitats. Particularly, 20 should be considered of highest priority (HP) for collection as not represented at all in seed-banks: 14 endemic taxa and six taxa threatened with extinction, besides two near threatened taxa. Among them, five taxa belong to the genus *Vicia*, four to *Lathyrus*, and two to *Daucus*. Other 10 taxa should be considered of priority (P) for further collection as represented in *ex situ* collection with fewer than five accessions, five of them with only one: six endemics, two policy species, and five threatened taxa.

Priority list of Italian Crop Wild Relatives	Endem.	Habitats Direct.	Italian Red List	European Red List	Italian distribution	Seed-Banks	N. of acc.	Collection Priority
<i>Aegilops biuncialis</i> Vis.	-	-	VU	-	PUG CAM	Bari	2	P
<i>Aegilops uniaristata</i> Vis.	-	-	VU	-	PUG BAS	Bari	4	P
<i>Aegilops ventricosa</i> Tausch	-	-	VU	-	UMB ABR BAS PUG	Bari, Pisa	4	P
<i>Arrhenatherum elatius</i> (L.) P. Beauv. ex J. Presl & C. Presl subsp. <i>nebrodense</i> (Brullo, Miniss. & Spamp.) Giardina & Raimondo	E	-	-	-	SIC	-	-	HP
<i>Asparagus pastorianus</i> Webb & Berthel.	-	-	-	VU	SIC	-	-	HP
<i>Barbarea rupicola</i> Moris	E	-	-	-	SAR	Sardinia	2	P
<i>Beta macrocarpa</i> Guss.	-	-	-	EN	LIG CAM BAS SIC	-	-	HP
<i>Brassica baldensis</i> (Prosser & Bertolli) Prosser & Bertolli	E	-	-	-	VEN	-	-	HP
<i>Brassica glabrescens</i> Poldini	E	II	NT	VU	FVG	Padova	1	P
<i>Brassica insularis</i> Moris	-	II	NT	NT	SAR SIC	Perugia, Sardinia	27	-
<i>Brassica macrocarpa</i> Guss.	E	II*	CR	CR	SIC	Catania, Lombardy, Palermo, Perugia	11	-
<i>Brassica montana</i> Pourr.	-	-	VU	LC	LIG EMR TOS MAR LAZ CAM BAS CAL	Genova, Perugia	28	-
<i>Brassica rupestris</i> Raf. s.l.	E	-	-	NT	CAL SIC	Perugia	4	-
– subsp. <i>hispida</i> Raimondo & Mazzola	E	-	-	-	SIC	Catania, Palermo, Sardinia	33	-
– subsp. <i>rupestris</i>	E	-	-	-	CAL SIC	Catania, Palermo, Sardinia	140	-
<i>Brassica tyrrhena</i> Giotta, Piccitto & Arrigoni	E	-	-	-	SAR	Sardinia	3	P
<i>Brassica villosa</i> Biv. s.l.	E	-	-	NT	SIC	Perugia	4	-
– subsp. <i>bivonana</i> (Mazzola & Raimondo) Raimondo & Mazzola	E	-	-	-	SIC	Catania, Palermo, Perugia, Sardinia	17	-
– subsp. <i>brevisiliqua</i> (Raimondo & Mazzola) Raimondo & Geraci	E	-	-	-	SIC	Catania, Palermo, Sardinia	14	-
– subsp. <i>drepanensis</i> (Caruel) Raimondo & Mazzola	E	-	-	-	SIC	Catania, Palermo, Perugia, Sardinia	59	-
– subsp. <i>tinei</i> (Lojac.) Raimondo & Mazzola	E	-	-	-	SIC	Catania, Palermo, Sardinia	10	-
– subsp. <i>villosa</i>	E	-	-	-	SIC	Catania, Palermo	5	-
<i>Crambe hispanica</i> L.	-	-	VU	LC	PUG CAL SIC SAR	Bari, Padova	5	-
<i>Crambe tataria</i> Sebeók	-	II	NT	LC	FVG	Padova	1	P
<i>Daucus broteri</i> Ten.	E	-	-	DD	LIG EMR TOS MAR UMB LAZ ABR MOL PUG CAM BAS CAL	-	-	HP
<i>Daucus carota</i> L. subsp. <i>rupestris</i> (Guss.) Heywood	E	-	-	-	SIC	-	-	HP
<i>Daucus nebrodensis</i> Strobl	E	-	-	-	SIC	-	-	HP
<i>Diplotaxis scaposa</i> DC.	E	-	-	-	SIC	Palermo	1	P
<i>Elytrigia corsica</i> (Hack.) Holub	E	-	-	-	SAR (and Corse)	Sardinia	1	P
<i>Festuca centroaenninica</i> (Markgr.-Dann.) Foggì, F. Conti & Pignatti	E	-	-	-	TOS MAR UMB LAZ ABR	-	-	HP
<i>Ipomoea sagittata</i> Poir.	-	-	-	VU	LAZ PUG CAL SIC	-	-	HP
<i>Lathyrus amphicarpos</i> L.	-	-	-	NT	LAZ PUG SIC	-	-	HP
<i>Lathyrus apenninus</i> F. Conti	E	-	-	-	MAR UMB LAZ ABR	-	-	HP
<i>Lathyrus odoratus</i> L.	E	-	-	NT	PIE TOS LAZ ABR MOL CAM BAS CAL SIC SAR	-	-	HP

<i>Lathyrus palustris</i> L.	-	-	EN	-	LOM TAA VEN LIG TOS	-	-	HP
<i>Malus crescimannoi</i> Raimondo	E	-	-	-	SIC	-	-	HP
<i>Phalaroides arundinacea</i> (L.) Rauschert subsp. <i>rotgesii</i> (Foucaud & E. Mandon ex Husn.) Valdés & H. Scholz	E	-	-	-	SAR	-	-	HP
<i>Vicia cosentina</i> Spreng.	E	-	-	-	CAL	-	-	HP
<i>Vicia cusnae</i> Foggi & Ricceri	-	-	VU	-	EMR	-	-	HP
<i>Vicia giacominiiana</i> Segelb.	E	-	CR	CR	PUG	-	-	HP
<i>Vicia ochroleuca</i> Ten. subsp. <i>ochroleuca</i>	E	-	-	-	TOS LAZ ABR CAM BAS CAL SIC	-	-	HP
<i>Vicia serinica</i> R. Uechtr. & Huter	E	-	-	-	BAS	-	-	HP
<i>Vicia tenuifolia</i> Roth subsp. <i>elegans</i> (Guss.) Nyman	E	-	-	-	LAZ CAL SIC	Catania	1	P

TABLE 4. Priority list of Italian Crop Wild Relatives listed in the FAO Treaty and their distribution in the Italian regions. Taxa are reported in alphabetical order with data about: endemism (Peruzzi et al., 2014); Policy Species, indicated with the number of the Annex of the Habitats Directive 92/43/EEC, where * indicates priority species; IUCN Red List status (Bilz et al., 2011; Perrino & Wagensommer, 2013abc; Rossi et al., 2013; 2016), and their distribution (Conti et al., 2005; Peruzzi et al. 2014). Italian regions are reported as follows: ABR: Abruzzo; BAS: Basilicata; CAL: Calabria; CAM: Campania; EMR: Emilia Romagna; FVG: Friuli Venezia Giulia; LAZ: Latium; LIG: Liguria; LOM: Lombardy; MAR: Marche; MOL: Molise; PIE: Piedmont; PUG: Apulia; SAR: Sardinia; SIC: Sicily; TAA: Trentino Alto Adige; TOS: Tuscany; UMB: Umbria; VEN: Veneto. Indication of collection priority are reported as follows: HP: high priority (0 accessions); P: priority (< 5 accessions)

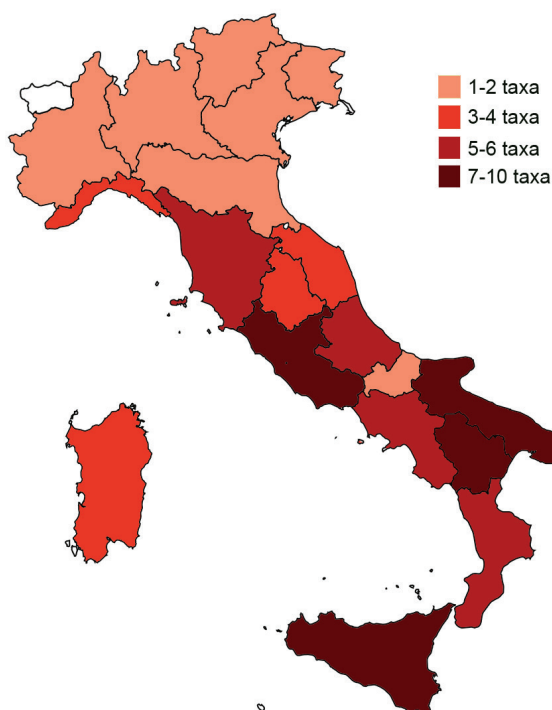


FIGURE 5. Spatial distribution in Italy of the Italian CWRs with priority (HP and P) for further germplasm collection

The regions identified as the highest priority for further germplasm collection are Sicily (12 taxa), Latium (8), Basilicata and Apulia (7) (Figure 5).

Conclusion

For the first time the contribution of RIBES to the conservation of CWR in Italy has been quantified. Even if, most of the RIBES seed-banks are not specialized in CWR conservation, 37% of the Italian CWRs listed in the FAO Treaty are preserved in 14 seed-banks with 6,029 accessions of 229 CWR taxa.

However, these results highlight that CWRs are currently under-represented in RIBES seed-banks as for 391 taxa (63% of total), no germplasm accessions exist at all, a further 140 taxa are represented by fewer than five accessions and 81 taxa by only one. Then a systematic effort to improve their comprehensiveness in seed-banks is critically needed.

Here we outline priorities for the conservation of CWRs (Table 4) and also provide a list of priority taxa to address the germplasm collection on the basis of their current representation in seed-banks, recommending filling gaps in *ex situ* conservation first for the CWRs endemic and threatened with extinction (Table 4). Finally, we identified geographic hotspots where considerable richness of priority CWRs is concentrated. Such sites represent particularly valuable targets, both for efficient collecting for *ex situ* conservation in gene banks and for *in situ* conservation in protected areas (Castañeda-Álvarez et al., 2016).

Even if limited to the CWRs listed in the FAO Treaty, this group of taxa can represent the starting point of the building up of a more comprehensive national priority list for the Italian CWRs, which must guarantee the *in situ* and *ex situ* conservation of wild species of interest for their agronomic and economic value.

Prioritizing crop wild relative species for collection and conservation is essential, given the limited resources available for these activities. CWR taxa may be categorized with a shared priority score system based on the economic importance of the crop to which they are related, on the degree of relationship of the wild relative to the crop, on the level of extinction risk, as well as on the “Regional responsibility” biogeographic criterion proposed by Gauthier et al. (2010) for the endemic taxa (e.g. Gauthier et al., 2010; Bacchetta et al., 2012; Castañeda-Álvarez et al., 2016).

Finally, there is the need of the development of a national plan aiming at successful conservation of CWR diversity that comprises a series of steps:

- to define a shared checklist of CWR, which allows us to preserve all the Italian CWR diversity;
- to assess their conservation in seed-banks in order to prepare national inventories;
- to define shared criteria in order to categorize taxa with a priority score;
- to define a national priority list to ensure an effective CWR conservation, filling gaps in *ex situ* conservation, and optimizing collection efforts.

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