

NATIONAL INVENTORY AND PRIORITIZATION OF CROP WILD RELATIVES FROM ROMANIA

Marius Dan ȘANDRU

Genebank "Mihai Cristea" Suceava, 17 1 Mai Blvd, Suceava, Romania

Corresponding author email: dan.sandru@svgenebank.ro

Abstract

The paper aimed to present a checklist of wild plant species for use in agriculture, which are native in Romania and contains 525 species. Following an additional selection based on the prioritization criteria (gene traversability, importance and economic use, IUCN status, etc.), the National Priority List containing 275 species was compiled and about 10% of them are preserved in the Suceava Gene Bank. The crop wild relatives represent an important element of the vegetal genetic resources of a nation through their availability for the conservation and sustainable use of their diversity in order to ensure the food security of the country. This national inventory represents the technical support, through which the institutions that manage plant genetic resources strengthen their capacities to implement the national program of conservation of plant genetic resources in accordance with ITPGRFA, FAO, CBD strategies.

Key words: conservation of plant genetic resources, crop wild relatives, checklist, food security, national inventory.

INTRODUCTION

The Convention on Biological Diversity (CBD), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), the Global Plant Conservation Strategy and the Strategic Plan on Biological Diversity, all of which emphasize the need for efficient conservation of plant genetic resources for food and agriculture for counteract the current rate of biodiversity loss at global, regional, national and local levels.

Although farmers have always adapted their cropping systems to unfavorable climatic and environmental conditions, the speed and complexity of the latter indicate another scale of the problems. Thus, a new diversity of cultivated species will be needed to adapt to future extreme environmental conditions.

In this context, farmers need to change their agricultural practices to effectively adapt to climate change, if they are to maintain and improve crop quality and yields. Such practices include adjusting planting times to avoid drought or heat stress and adopting new crop varieties, amongst others (Howden et al., 2007). However, these measures may not be sufficient (Turner and Meyer, 2011), as modern cultivars may lack the ability to adapt to environmental change due to their narrow genetic base, resulting from selection applied in

previous domestication and breeding processes (Stamp and Visser, 2012).

A functional definition of a crop wild relative (CWR) is based on two concepts, the first being the gene pool (Gene Pool) and in the absence of cross-information (gene transferability) and genetic diversity, the second concept is used, that of taxonomic group (Taxon Group).

Thus, Maxted et al. (2006) promoted the following definition: "A crop wild relative of crop plants is a wild plant taxon that has a use derived from its genetic relationship, relatively close to a crop plant; this relationship is defined as belonging to gene groups 1 or 2 or to taxon groups 1 to 4 of the culture species".

Taking into account the genetic variability of these taxa, they can be used as a genetic resource useful in mitigating the effects of climate change on cultivated species, thus helping to maintain and improve productivity and ensure food security (Brozynska et al., 2016).

Factors that affect the entire world biodiversity have a negative impact on the wild relatives of crops and among the most relevant are: genetic erosion, expansion of the anthropogenic area, destruction, degradation, homogenization and fragmentation of natural habitats, use of pesticides and herbicides, changes in practices competition with invasive species and a lack of awareness of the need for conservation and sustainable use of these plant taxa.

Accordingly, it is urgent to take actions to reduce genetic erosion or species extinction.

In situ conservation of CWR in protected areas (Hunter and Heywood, 2011), establishment of genetic reserves (Pinheiro de Carvalho et al., 2012; Fielder et al., 2015a), identification of priorities and efficient collection of samples for ex situ conservation (Khoury et al., 2015; Garcia et al., 2017) are some of the recently approached procedures for CWR conservation. Contextually, compiling the national inventory of CWR is the first essential step in developing the national strategy for the conservation of plant genetic resources, thus ensuring first of all the identification of information needs and the coordination of efforts to conserve and sustainably use these crop wild relatives.

In this regard, a floristic approach was used to generate this national inventory, which initially involved comparing the list of national flora with the list extracted from the PGR Forum Catalog of crop wild relatives for Europe and the Mediterranean (Kell et al., 2007) and selection of those CWRs present in Romania.

To date, as far as we know, seventeen national inventories have been developed by the United Kingdom (Maxted et al., 2007), Portugal (Magos-Brehm et al., 2008), Russia (Smekalova, 2008), Israel (Barazani et al., 2008), Denmark (Bjørn et al., 2011), Spain (Maria Luisa Rubio Teso et al., 2018), Finland (Fitzgerald, 2013), Benin (Idohou et al., 2013), Italy (Panella et al., 2014), Cyprus (Phillips et al., 2014), the Czech Republic (Taylor et al., 2017), the Netherlands (van Treuren et al., 2017), England (Fielder et al., 2015a) and Scotland (Fielder et al., 2015b).

In addition, a global inventory was generated and published (Vincent et al., 2013), two European Catalogs (Heywood and Zohary, 1995; Kell et al., 2005) and a priority checklist of North Africa (Lala et al., 2017).

In Romania, at the moment, there is no database that can be digitally harmonized with the databases of cultivated species, thus, the composition of the national inventory of CWR was done manually.

Romania's flora is estimated at 3795 species and subspecies of higher plants (623 cultivated species and 3136 spontaneous species) (Ciocârlan, 2000), and 37% of plant species are found in meadow habitats, over 700 plant

species are in marine and coastal areas. 4% of plant species are endemic, 75% of them being in the mountain area.

Although, at European level, Romania has the most diversified and valuable natural heritage; the area of protected natural areas of national interest, relative to the area of the country, is 7%. There is no clear evidence of CWR existing in protected areas or outside them.

Regarding the cultivated species, in 2020 the catalog of plant varieties (varieties) that are cultivated on the Romanian territory was made and according to this catalog the number of plant varieties is 2118.

In Romania, are currently cultivated 60 species of plants with human food potential, 22 species with fodder potential, 27 medicinal and aromatic species, 6 species of ornamental plants, 2 species of ornamental shrubs, 25 species of trees and fruit shrubs.

However, high-productivity varieties are known to have a narrow genetic basis and, in many cases, lack the long-term mechanisms of adaptation to extreme environmental conditions (Stamp & Visser, 2012).

These CWRs are often associated with high genetic variability and the idea of food security because they represent the progenitors of today's crop plants, which have in their genetic background, gene sets or even gene complexes that have beneficial traits that confer tolerance to biotic and abiotic factors, which improve nutritional quality and quantity and increase productivity.

Table 1. Some examples of the use of crop wild relative in breeding and the traits they provide. (Mihai D.Cristea, Danela Murariu, 2018)

Crop wild relative	Crop	Target traits
<i>Aegilops triuncialis</i> L.	Wheat	Sources of disease and pest resistance
<i>Avena fatua</i> L.	Wheat	Sources of resistance to drought, disease and
<i>Elymus repens</i> (L.) Gould	Wheat, Barley	Sources of salinity resistance
<i>Sorghum halepense</i> (L.) Pers.	Johnson grass	Sources of disease and pest resistance
<i>Beta trigyna</i> Waldst. & Kit.	Beet	Sources of disease and pest resistance
<i>Linum flavum</i> L.	Flax	Sources of cold resistance
<i>Brassica elongate</i> Ehrh.	Cabbage	Sources of cold resistance
<i>Lathyrus aphaca</i> L.	Grass pea	Sources of disease resistance and productivity

Moreover, CWRs are vital plant genetic resources that, if efficiently conserved and used sustainably, can increase food security, alleviate poverty and improve the stability of natural and semi-natural ecosystems, which have a special role to play in their functioning. and therefore ensures the sustainability of the environment and the continuity of the natural processes within it.

As CWRs have already been used and have been shown to be useful in growing crops (Hajjar & Hodgkin, 2007), increasing knowledge about them and improving their conservation is becoming urgent nowadays.

The purpose of this paper was to develop a CWR checklist of importance in Romania and a priority list of CWR for the implementation of conservation plans and introduction in plant breeding programs to create new varieties and native hybrids that provide increased resistance in relation to climate change, but also to add economic value in response to current consumer market requirements.

To achieve this goal, we have advanced some questions: (i) What criteria should be applied to prioritize CWR in Romania? (ii) What is the degree of national and European threat of CWR from the national priority list?

(iii) Are these species under legal protection in Romania? (iv) What is the degree of endemism of the priority CWR? (v) What is the distribution of their populations and their situation in ex situ conservation in Romania?

This approach has recently been successfully implemented for the implementation of the national inventory of cwr for Spain (María Luisa Rubio Teso, Elena Torres Lamas, Mauricio Parra-Quijano, Lucía de la Rosa, Juan Fajardo, José M. Iriondo, 2017).

MATERIALS AND METHODS

The whole process of generating the checklist and the priority list of wild relatives of crop plants is presented in Figure 1.

The process involves four stages (two for compiling information and two for setting priorities) thus generating four distinct lists: (1) Basic list of cultivated species genera, (2) List of selected cultivated species, (3) Checklist of cultivated species of crop wild relatives for

Romania (4) Checklist of crop wild relatives of priority for Romania.

1. Basic list of genera of cultivated species

According to the PGR Forum project (Kell et al., 2008), in Romania there are over 3000 CWR which represent over 90% of the Romanian Flora, this number being too large to be managed for conservation or sustainable use.

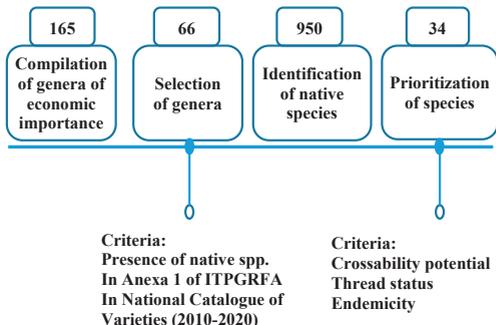


Figure 1. Complete process depicting the steps followed for the generation of the Prioritized Romanian Checklist of Crop Wild Relatives. The process involves four steps (two of compilation of information and two of prioritization) and provides four distinct products: (a) Baseline List of Crop Genera, (b) List of Selected Crop Genera, (c) Romanian Checklist of Crop Wild Relatives and (d) Prioritized Romanian Checklist of Crop Wild Relatives

In this sense, the first step in generating the CWR national checklist was to identify cultivated species that contribute to global food security and are of economic importance, thus obtaining the list of genera that include species cultivated in Romania.

This was done in two stages: (a) compilation of the basic list of cultivated species genera and associated information and (b) selection of cultivated species genera in Romania.

The cultivated species considered were classified into 4 use categories: (1) food, (2) fodder, (3) ornamental and (4) industrial and other uses

The classification of species according to their usefulness was carried out in accordance with Appendix 1. of the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2010) and in the Official Catalog of Cultivated Plant Varieties in Romania (of which more information on

production and cultivated area for each species).

To verify the economic importance of other species, compared to those already included, were used as sources of documentation: Union by the International Union for the Protection of New Varieties of Plants (UPOV 2011) and the Germplasm Resources Information Network database of the United States Department of Agriculture (GRIN-USDA 2017) and for ornamental it was used The Community Plant Variety Office (CPVO) (Kwakkenbos pers. comm. 2004).

The UPOV database was also used to collect data on the number of species, infraspecific taxa and / or hybrids associated with a particular crop, specialized publications, plant breeding trends, and other inventories were verified. national CWR.

Thus, the database was compiled with the genera corresponding to these cultivated species and with the information adjacent to them.

2. Selection list of cultivated species genera

Following the in-depth analysis of all data, a list of genera was selected based on the following criteria: a) the selected genus must contain at least one species native to Romania; b) the genus must meet at least one of the following conditions: - it contributes to overall food security (found in Annex 1. of the ITPGRFA), - it includes at least one crop species which has at least one variety registered during the 1980s- 2020 in the Official Catalog of cultivated plant varieties in Romania, to highlight the economic importance for the country (Romania).

The resulting list of genera including crop species was evaluated by experts from agricultural institutions who validated all selected genera as well as by consulting databases:

- Global Biodiversity Information Facility, GBIF (www.gbif.net);
- The Mansfeld's World Database of Agriculture and Horticultural Crops (www.mansfeld.ipkgatersleben.de).

3. Romanian checklist of crop of wild relatives

The initial list of CWR in Romania was developed, in the first stage, by selecting geographic criteria (Romania) from the Catalog

of crop wild relatives for Europe and the Mediterranean developed by PGR Forum (Kell et al., 2005).

In order to ensure that all the genera that represent the species cultivated in the country as well as the species studied with them that are found spontaneously on the Romanian territory were included, and were used several documents.

For the identification of plant species from the spontaneous flora of Romania, reference sources were consulted, such as:

- regarding the name of the taxa, it was taken over, in the case of the species from Romanian flora (Ciocârlan, 2000), Atlas of Romanian flora (Mohan and Ardelean, 2011) and Flora RSR, vol. I-XIII, for hybrids and infrataxons;
- Flora Europaea (<http://rbgweb2.rbge.org.uk/FE/fe.html>) and Euro + Med PlantBase (2005) were also consulted to establish the taxonomic rank and synonyms of the taxa (<http://www.euromed.org.uk>);

- the flora lists that can be found in all the management plans of the protected areas (435 sites of community importance, Natura 2000 sites etc);

- articles, studies and monographs of spontaneous and cultivated species.

- articles and books from libraries (public and private), research institutes and botanical gardens were consulted.

The priority-setting process is a first step in any conservation strategy (Maxted and Kell, 2009a,b)

To make this list, the following criteria used proposed by Brehm et al., (2010):

1. Native status. This national inventory gives a higher priority to native species in Romania but Non-native species were also included in this list, this decision being based on the importance of these species in the development of the national economy, by increasing the diversity and availability of genetic resources for food and agriculture.

2. Economic value. The main use of CWR is to improve the genetic quality of existing crop plants and / or varieties or can be used in the creation of new varieties.

3. Ethnobotanical value. Assessment of local knowledge on species uses, thus giving priority to species that are of high importance to local communities.

4. Global distribution. The priority is inversely proportional to the distribution, so species that have a low distribution (national or regional) have high priority over species that have a global distribution.

5. National distribution. A species that occurs in several areas has been considered rarer compared to a species that occurs throughout the country, so the former will be a priority for active collection and conservation.

6. In situ and ex-situ conservation status. If there are species that do not have sufficient genetic diversity conserved in both conservation systems, then these species are a priority for active collection and conservation.

7. Legislation. If a species is protected by law then it is a priority for conservation because national governments are responsible for their protection.

8. Threat assessment. The status of a species in accordance with the IUCN Red List is probably the most widely used criterion for determining conservation priority. Thus endangered, threatened species are a priority for active conservation and collection.

The following documentation materials were used in compiling the Romanian checklist of crop of wild relatives and for applying the selection criteria:

- IUCN threat category (www.iucnredlist.org).
- number of infraspecific taxa belonging to species included in the Red List of Endangered Species in Romania (Oltean et al., 1994), Red Book of Vascular Plants in Romania (Dihoru and Negrean, 2009), Carpathian List of Endangered Species (Krzysztof Kukuła et al., 2003), Carpathian red list of forest habitats and species carpathian list of invasive alien species (Ján Kadlečík, 2014),
- cross-breeding potential (gene transmission) was evaluated according to the concept of genofond issued to Harlan and de Wet (1971) (possibility of gene transfer between wild and cultivated species) and the concept of the taxon group by Maxted et al., (2006) (assimilation of the taxonomic hierarchy to the concept of the genetic basis).

For the identification of native species in Romania were used:

- Flora RSR, vol. I-XIII. (<https://www.cwrdiversity.org/checklist/>),

-the Germplasm Resources Information Network database of the United States Department of Agriculture (<https://npgsweb.arsgrin.gov/gringlobal/taxon/taxonomysearchcwr>) or additional references.

- The Cwr Catalog For Europe And The Mediterranean (Kell, 2005).

For the compilation of cross-breeding potential and genetic background data, a complementary list of cultivated species was generated for each selected genus using as a reference the database of the US Department of Agriculture's Germoplasmic Resource Information Network (GRIN-USDA, 2017).

Same database (<https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysearchcwr>) was used to determine the degree of kinship (at the level of the gene pool and / or the taxonomic group) between the CWR and the cultivated species.

4. List of crop wild relatives priority for Romania

Regarding the compilation of the priority list of the CWR, there is some consensus in combining three criteria: a) the potential for economic capitalization of the related crop species, b) the potential for crossing (degree of kinship of the CWR with the crop species / ease of passage CWR in culture, in relation to the genofond and / or group of taxa), c) the relative level of threat and endemism (for food, feed, industrial and ornamental plants).

However, regardless of the priority setting methodology and criteria used, the total number of priority CWRs should be adjusted to a number that can be actively conserved using available financial and human resources. There is no precise way to estimate the number of priority CWRs because the estimate would be subjective.

A more flexible approach would be to assign different levels of priority for conservation, depending on the priorities of the institutions that will carry out the CWR conservation and the use of a reasonable number of taxa for each of these institutions, to implement active conservation.

Continuing to use this approach, some taxa, which are not yet an immediate priority for conservation, may appear in the same sites as

those with high priority, so they may be included in the same

In view of the above, it has been established that the priority CWR species are those found in the primary gene pool 1 (in which gene transfer is free, where the cultivated and wild forms of the crop plant are found) and those in secondary gene pool 2 (gene transfer is possible using conventional breeding techniques) (Harlan and de Wet, 1971) or CWR species belonging to group of taxa category 2 (same series or section as crop plant) and 3 (same subgenus as a crop plant) (Maxted et al., 2006). In prioritizing CWR, the concept of gene pool has always been a priority over the concept of the taxon group, but when information on cross-species was not available, the taxon concept concept was applied.

Another specific element in compiling the CWR priority list was the selection of all species for direct food use, forage potential species, industrial species, which are found in any of the IUCN threat categories (critical, endangered species, vulnerable and almost threatened) or endemic to Romania.

Thus, the resulting list was verified in relation to: Red List of higher plants in Romania, Directive no. Council Directive 92/43 / EEC of 21 May 1992 on the conservation of natural habitats and of wild flora and fauna (Annexes II and IV), European Red List of Medicinal Plants, European Red List of Vascular Plants and Convention on the conservation of European wildlife and natural habitats (Bern, 1979).

The inclusion of species in this national inventory provides these species with legal protection that involves the design and implementation of appropriate conservation plans as well as the constant assessment and monitoring of their conservation status.

Finally, all the priority species were verified, worldwide, in the databases of plant genetic resources: - the database of the Bank of Plant Genetic Resources "Mihai Cristea" Suceava; - the EURISCO catalog (EURISCO 2020); - the Germplasm Resources Information Network database of the United States Department of Agriculture (GRIN-USDA, 2020); - the GENESYS Global Portal on Plant Genetic Resources (GENESYS, 2017).

The development and improvement of the national "ex situ" collection of the CWR requires an urgent collection, the priority species in this respect are those found in gene pool 1b and 2 or taxon group 2, which is under any threat category according to the International Union for Nature Conservation (IUCN), species that have the status of endemic to Romania as well as those species that have less than five entries (germplasm samples) in the Plant Genetic Resources Bank "Mihai Cristea" Suceava. This threshold of five entries is considered to be the minimum number of populations needed to conserve intraspecific genetic diversity (Brown and Briggs, 1991). The priority for CWR collection provides the following criteria:

- (1) Urgent priority. They are CWR species present in the primary or secondary genetic background as well as in the group of taxa of the crop species, are endemic or threatened and have no representation in gene banks;
- (2) Urgent. The species is not represented in gene banks;
- (3) To be collected. The species has less than five populations represented in Gene Bank;
- (4) Not a priority for collection. The species has over five Gene Bank entries for each species.

RESULTS AND DISCUSSIONS

The basic list of genera associated with cultivated species comprises 165 genera.

The genus selection list associated with cultivated species used in the generation of the CWR Checklist contains 66 genera.

The distribution of the genera in this list according to the category of use of the species is as follows:

- food species include 41 genera included in 12 families (*Brassicaceae*, *Fabaceae* - 26 species, *Liliaceae* - 27 species, *Poaceae* - 20 species, *Rosaceae* - 30 species);
- fodder species include 20 genera included in 2 families (*Poaceae* - 54 species, *Fabaceae* - 71 species);
- ornamental species include 1 genus included in a family (*Rosaceae* - 16 species);
- industrial and other uses include 6 genera included in 4 families (*Lamiaceae* - 18 species).

The families Fabaceae and Poaceae are the most important, having 15 and 17 genera respectively, followed by *Brassicaceae* with 8 genera.

The checklist of wild relatives of crop plants comprises a total of 940 species. The category of food species includes 356 species, the category of feed species has 350, the ornamental ones 97 species, and the industrial and those with other uses are 131 (Figure 2).

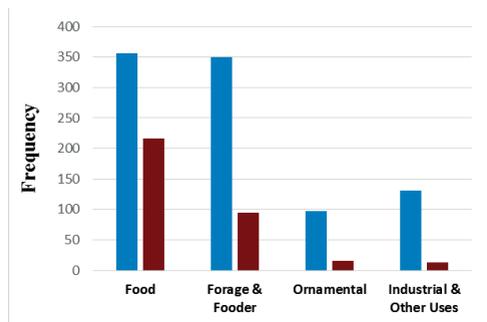


Figure 2. Number of species in the Checklist of wild relatives of crop plants and Priority list of wild relatives of crop plants, ordered by categories of use

This checklist of species together with information on priority use, taxonomic classification, concept of genofond group of taxa, threat status, endemism and number of chromosomes are to be integrated into a database, which will later be available online. Because the gene pool information was found for 128 of the 350 species, the others were assigned the group of taxa corresponding to the category.

Romania's priority list of wild relatives of cultivated plants

Applying the agreed criteria to each use category has reduced the number of species from the list to 328 (34% of the original checklist).

The Romanian priority list of wild relatives of crop plants contains 162 species related to crop species for food use, 124 species with forage value, 15 ornamental species and 23 industrial species and other uses (Figure 2).

All priority species, together with all the information gathered during the selection process will be available online.

According to the prioritization criteria used, the selected species belonged mainly to the primary (60 species) or secondary genes (42

species), but also to the group of taxa (28 species to the primary group of taxa and 39 species to secondary group of taxa - the same section or subsection as the crop species) (Figure 3a).

Three of the priority species are endemic in Romania. Over 8% of the prioritized species (27 species out of 328) are classified in one of the IUCN threat categories at the national level (including the almost endangered category Fig. 3b), and 15 species are threatened in the Carpathian area (Table 2).

In addition, 65 species from the Romanian Priority List of wild relatives of cultivated plants are included in the Red List of higher plants in Romania (Oltean, Negrean et al. 1994), The Red Book of vascular plants in Romania (Dihoru, Negrean, 2009).

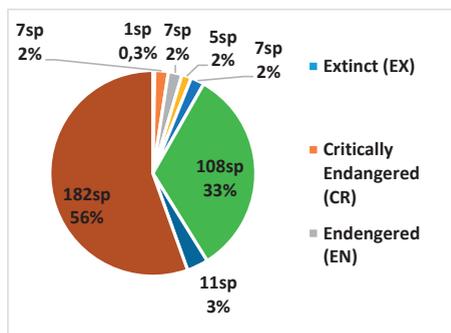
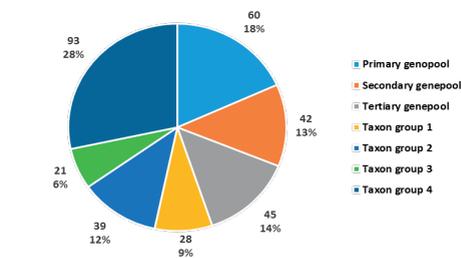


Figure. 3: a) Classification of priority species according to the genetic background or group of taxa of Harlan and Wet (1971) and Maxted and colab. (2006); b) The number of species in any of the threat categories according to Red list of superior plants from Romania: Oltean, M. (coord.), According to the Union Criteria International Organization for Conservation of Nature (IUCN

Regarding the *ex situ* conservation, Suceava Gene Bank has 105 species in the asic collection (+ 4 °), approximately 32% of the

priority CWR, so 219 species are not represented in the national collection of plant genetic resources.

Analyzing this situation, less than 89% of the species represented in the national collection of plant genetic resources managed by the Plant Genetic Resources Bank "Mihai Cristea" Suceava have 20 or more entries, while 69% of these species have between 1 and 4 entries.

A total of 58 species are of primary importance, because they are endemic to Romania, they are threatened according to the IUCN, they belong to the primary or secondary genofond or group 2 of taxa, as well as represented by less than five entries in the BRGV Suceava collections.

Of these, twenty-eight have no representation in BRGV Suceava (Priority 1).

One hundred and eight (108) species are found in priority collection category 2 (Emergency. Species are not represented in gene banks), 21 in priority collection category 3 (Need for collection. Less than five populations represented in gene bank) and finally 141 in priority collection category 4 (no priority for collection) All this information will be available for each species in the Romanian priority list of the CWR.

Romania's national strategy for CWR conservation aims at the long-term active conservation of CWR taxonomic and genetic diversity but, at the same time, promotes the use of these resources, because experience has shown that sustainable conservation is achieved through use.

In particular, with regard to the *in situ* conservation of CWR, once conservation sites have been established (genetic reserves and informal *in situ* conservation sites), they can be grouped into a coherent national network, thus providing the opportunity to monitor and evaluate short- and long-term changes in CWR diversity.

This national CWR conservation strategy is a major objective of the CBD 2020 Strategic Plan, COP 10 decision X / 2 (Nagoya, Japan, October 2010).

Development of the Romanian Checklist a wild relatives of crop plants was made following the "list of crop species", although many countries use the national floristic list.

This method was approached because the European Catalog of the CWR indicates about 3,000 CWR are present in Romania (Kell et al., 2008), which represents more than 80% of flora in the country.

Many species in this catalog are already administered by other public administration interest groups, e.g. forest species, which have their own national inventory and conservation program, as well as medicinal and aromatic plants. With this in mind, the CWR checklist was generated directly from a list of crop species that are important for the efficient use of economic resources and to avoid duplicates and overlaps with species that are already managed by the public administration.

In order to simplify the procedure for identifying CWR, a list of crop species has been compiled in accordance with national and regional socio-economic criteria. Thus, we focused on the most important CWR for Romania and, at the same time, without neglecting the crop species that contribute to the country's economy and food security worldwide. This approach was also followed by Berlinger and Crespo (2012) in Venezuela, Idohou et al. (2013) in Benin, Maria Luisa Rubio Teso et al. (2017) in Spain, it can be a valid alternative to extract the most important CWRs from a wide list of species present in a nation. Non-native species were excluded from the checklist of wild relatives of cultivated species in Romania because a large number of CWRs are naturally present in our country, requiring the establishment of strict criteria for the prioritization of these species.

On the other hand, in this list of CWR, species that are far from their centers of diversity - centers of origin (geographical areas where the species has a higher degree of variation and where there is significant genetic variability) have been introduced. represented by alleles " (Corinth, 2014)] because these species lack high genetic variability, which is of fundamental importance in reproduction.

However, non-native species with a source of genetic variation have been included in the Romanian CWR checklist, noting that these taxa have only been introduced at the species level.

Table 2. CWR species on the Romanian Prioritized Checklist (Food and Forage & Fodder categories) threatened at the Carpathian level and their corresponding status at the national level

Species	Family	Red List Carpathian Status	Red List Romanian Status
<i>Castanea sativa</i> Mill.	Fagaceae	EN	NT
<i>Astragalus pseudopurpureus</i> Gusul.	Leguminosae	EN	EN
<i>Trifolium lupinaster</i> L.	Leguminosae	CR	EN
<i>Astragalus roemeri</i> Simonk.	Leguminosae	EN	NE
<i>Lactuca aurea</i> (Vis. & Pančić) Stebbins	Asteraceae	EN	NE
<i>Barbarea vulgaris</i> W. T. Aiton	Brassicaceae	CR	DD
<i>Phleum subulatum</i> (Savi) Aschers. & Graebn.	Poaceae	EN	DD
<i>Ribes spicatum</i> E. Robson	Grossulariaceae	CR	NE
<i>Festuca filiformis</i> Pourr.	Poaceae	EN	DD
<i>Barbarea stricta</i> Andr. ex Besser	Brassicaceae	CR	DD
<i>Pisum elatius</i> L.	Leguminosae	CR	DD
<i>Astragalus depressus</i> L.	Leguminosae	CR	DD
<i>Agropyron cristatum</i> subsp. <i>sabulosum</i> (L.) Gaertn.	Poaceae	VU	VU
<i>Aegilops triuncialis</i> L.	Poaceae		
<i>Armoracia macrocarpa</i> Baumg.	Brassicaceae	VU	DD
<i>Carthamus lanatus</i> L.	Asteraceae	EN	DD
<i>Allium obliquum</i> L.	Alliaceae	EN	DD
		CR	NE

Red List of Superior Plants in Romania (Oltean, Negrean et al., 1994), Red Book of Vascular Plants in Romania (Dihoru, Negrean, 2009), Carpathian List of Endangered Species (Krzysztof Kukula et al. 2003), Carpathian red list of forest habitats and species carpathian list of invasive alien species (Ján Kadlecík, 2014).

Consequently, the CWR checklist, with reference to Romania (940 species) is higher than the list generated by Spain (926 species), but lower than those generated for other countries, such as Finland (1905 taxa), the Sea Great Britain (1955 species), Portugal (2261 taxa), USA (2495 taxa), Czech Republic (3283 species) or China (almost 24,500 species). Generating an initial list identifying CWRs also involves further prioritizing them in order to take direct action on the conservation and sustainable use of these plant genetic resources. These data will be available on the web, which will allow you to make potential taxonomic updates / changes and changes in the evaluation of the list of the most important wild relatives of crop plants in Romania.

This lack of available information on direct crossover experiments is consistent with the results found in previous studies (Kell et al., 2014; Fielder et al., 2015a, 2015b).

Although the concept of the taxon group can be a useful decision-making proxy when genepool information is not available, these results clearly show that cross-breeding experiments between cultivated species and their wild relatives are essential to assess and facilitate the potential use of CWR, namely in the introgression of genes useful in the genome of cultivated species.

The criteria for prioritizing the CWR in Romania were the same as those used by other countries, namely those related to the concept of crossability, degree of threat and endemism.

In the CWR priority list, only 17% of all identified species have high crossability, these species are present in the primary gene pool of crop forms, data normally provided by plant breeders. However, these data are often considered confidential information and are not available as published material.

Where information on direct cross-breeding experiments between CWR and crop plants, which are essential for the evaluation and potential use of CWR in plant breeding, is not available, data on the taxonomic group of crop species in relation to CWR may be used.

Evaluating the degree of threat of CWR from the priority list, we find that approximative 20% of them fall into a category of threats described in the IUCN Red List.

This percentage is higher compared to other countries such as Cyprus (9%), Germany (16%), Lithuania (16%), Norway (13%), Great Britain (12%) and even compared to the large flora of China (17%), but lower compared to countries such as the Czech Republic (54%), Finland (71%), Jordan (32%), Portugal (65%) or Spain (23%).

However, endangered species at European level that have not been assessed in the Romanian Red List should be assessed in future editions of the Romanian Red List of Vascular Flora.

The publication of this list can also be very useful in subsequent reviews of the National Catalog of Threatened Species to include all priority CWRs that are endangered and ensure their legal protection.

The inclusion of CWR in the Romanian Strategy on the Conservation of Plant Genetic Resources will be an important step in recognizing the importance of this category of plants, at national level, even if the implementation of active conservation plans is the responsibility of local communities.

For the genetic diversity of a species to be well conserved, that species should have a germplasm in a Gene Bank of at least five entries from five different populations (according to Brown and Briggs (1991) and Maxted et al., (2008).

However, recent studies suggest that this criterion of at least five populations per species should be replaced, in the long run, with a more ambitious goal in which the number of entries to be collected is estimated on the basis of species, in proportion to genetic diversity of that species. Based on this premise, in addition to prioritizing the collection of the 168 species on the priority list that do not have accessions in Gene Bank, CWR collection should also focus on improving the representation of species that are already conserved to obtain the minimum population sampled to represent their genetic diversity.

Of these, 58 species on the priority list that are endemic, threatened and have less than five gene bank accessions should have the highest priority.

Given the above criteria, Whitlock et al., (2016) propose that over 35% of populations be kept in line with the recommendations of the Convention on Biological Diversity (CBD).

Although this study aims to implement active *in situ* conservation plans, it could also be applied in *ex situ* conservation, as its major objective is to preserve sufficient genetic diversity to adequately represent the species. The safest method of preservation is the *ex situ*, in Gene Bank, where the germplasm is stored in optimal conditions of temperature and humidity. The optimal genetic diversity of a species stored in

Gene Bank is different from species to species, taking into account the type of multiplication of the species and the distribution and size of populations along with their environmental conditions (Brown & Marshall, 1995).

In addition, the use of existing molecular data can also help in correctly determining the minimum number of accessions that may represent genetic diversity within a species (Camadro, 2012).

ACKNOWLEDGMENTS

This research and development activity of its structure and content National Inventory of CWR would not have been possible without the financial support of the project - DECIDE - Development through entrepreneurial education and innovative doctoral and postdoctoral research, project code POCU / 380/6/13/125031, project co-financed from the European Social Fund through the Operational Program Human Capital 2014-2020.

CONCLUSIONS

Romania's biodiversity, which also includes existing CWR species, must be conserved using strategies and action plans based on the establishment and management of protected areas and sustainable human use of the rest of the territory, as well as species-specific approaches.

Therefore, the correct identification and setting of the priority CWR is essential. This List of wild relatives of crop plants in Romania must be managed coherently and coordinated by the agriculture and environment departments of the public administration and continuously reviewed in a participatory way to include species with real potential to meet the needs and ever-changing trends in agriculture and plant growth.

REFERENCES

- Arora, R.K., Nayar, E.R. (1984). *Wild relatives of crop plants in India*. New Delhi, India.
- Mohan, A., Ardelean, Gh. (2011). *Atlas. Flora Romaniei*. Editura All.
- Brown, A.H.D., Briggs, J.D. (1991). *Sampling strategies for genetic variation in ex situ collections of endangered plant species*. In: Falk DA, Holsinger KE

- (eds) Genetics and conservation of rare plants. Oxford University Press, New York, pp 99–199.
- Brozynska, M., Furtado, A., Henry, R.J. (2016). Genomics of crop wild relatives: expanding the gene pool for crop improvement. *Plant Biotechnol J.*, 14(4), 1070–1085. <https://doi.org/10.1111/pbi.12454>
- Ciocârlan, V. (2000). *Flora Ilustrată a României* (in Romanian). Editura Ceres.
- Allen, D., Bilz, M. (2014). *European Red List of Medicinal Plants*. DOI 10.2779/907382
- Dihoru, G., Parvu, C. (1987). *Plante endemice in flora Romaniei*. Editura Ceres, Bucuresti.
- Dihoru, G., Negrean, G. (2009). *Cartea rosie a plantelor vasculare din Romania*. Editura Academiei Romane.
- 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:e0130804. <https://doi.org/10.1371/journal.pone.0130804>
- Garcia, 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(3), 1059–1069. <https://doi.org/10.2135/cropsci2016.10.0860>
- Howden, S.M., Soussana, J-F, Tubiello, F.N., Chhetri, N., Dunlop, M., Meinke, H. (2007). Adapting agriculture to climate change. *Proc Natl Acad Sci USA* 104(50), 19691–19696. <https://doi.org/10.1073/pnas.0701890104>
- Hunter, D., Heywood, V. (2011). *Crop wild relatives. A manual of in situ conservation*. Earthscan, London
- Stamp, P., Visser, R. (2012). The twenty-first century, the century of plant breeding. *Euphytica*, 186:585–591. <https://doi.org/10.1007/s10681-012-0743-8>.
- Ján Kadlečík et al. (2014). *Carpathian red list of forest habitats and species carpathian list of invasive alien species*. BioREGIO Carpathians project.
- Khoury, C.K., Heider, B., Castañeda-Alvarez, N.P., Achicanoy, H.A., Sosa, C.C., Miller, R.E. (2015). Distributions, ex situ conservation priorities, and genetic resource potential of crop wild relatives of sweetpotato (*Ipomoea batatas* (L.) Lam., I. series Batatas). *Front Plant Sci.*, 6, 251. <https://doi.org/10.3389/fpls.2015.00251>
- Krzysztof Kukuła et al. (2003). *Carpathian List of Endangered Species*. EUROPRESS, Kraków, Poland.
- Magos-Brehm, J., Maxted, N, Ford-Lloyd, B.V., Martins-Louçã, M.A. (2008). National inventories of crop wild relatives and wild harvested plants: case-study for Portugal. *Genet Resour Crop Evol.*, 55, 779–796. <https://doi.org/10.1007/s10722-007-9283-9>
- Maria Luisa Rubio Teso, Elena Torres Lamas, Mauricio Parra-Quijano, Lucía de la Rosa, Juan Fajardo, José M. Iriondo. (2018). National inventory and prioritization of crop wild relatives in Spain. *Genet Resour Crop Evol*, <https://doi.org/10.1007/s10722-018-0610-0>
- Maxted, N., Scholten, M., Codd, R., Ford-Lloyd, B. (2007). Creation and use of a national inventory of crop wild relatives. *Biol Conserv*, 140, 142–159. <https://doi.org/10.1016/j.biocon.2007.08.006>
- Melanie Bilz, Shelagh P. Kell, Nigel Maxted and Richard V. Lansdown (2011). *European Red List of Vascular Plants*. doi:10.2779/8515
- Oltean, M., Negrean, G., Popescu, A., Roman, N., Dihoru, G., Sanda, V., Mihăilescu, S. (1994). Lista roșie a plantelor superioare din România. In: Oltean, M. (coord.), *Studii, sinteze, documentații de ecologie*. 1. Academia Română, Institutul de Biologie, București: 1-52.
- Pinheiro de Carvalho MA, No'brega H, Freitas G, Fontinha S, Frese L (2012). Towards the establishment of a genetic reserve for *Beta patula* Aiton. In: Maxted N, Dulloo ME, Ford-Lloyd BV et al (eds), *Agrobiodiversity conservation: securing the diversity of crop wild relatives and landraces*. CAB International, Wallingford, pp 36–44
- Turner N, Meyer R (2011). Synthesis of regional impacts and global agricultural adjustments. In: Yadav S, Redden R, Hatfield J et al (eds), *Crop adaptation to Climate Change*.