



Novel characterization of crop wild relative and landrace resources as a basis for improved crop breeding



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Crop wild relative

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Conserving plant genetic resources

for use now and in the future

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Borago officinalis L., one of the priority crop wild relative species of Spain. Cultivated forms are grown along the Ebro Valley in the northeast. Its basal leaf petioles are used as a vegetable. Borage is also a valuable medicinal plant with a high content of gamma linolenic acid.

Photo:

Cristina Mallor, Agrifood Research and Technology Centre of Aragon (CITA), Spain



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Contents

Editorial	3
Systematic crop wild relative conservation planning for the Czech Republic <i>N.G. Taylor, S. Kell, V. Holubec, M. Parra-Quijano, K. Chobot and N. Maxted</i>	5
Prioritization of crop wild relatives in Finland <i>H. Fitzgerald, H. Korpelainen and M. Veteläinen</i>	10
Strategic work on crop wild relatives started in Sweden <i>J. Weibull</i>	13
Development of a national crop wild relative conservation strategy for Cyprus <i>J. Phillips, A. Kyrtziz, C. Christoudoulou, S. Kell and N. Maxted</i>	17
Boost for crop wild relative conservation in Norway <i>Å. Asdal, J. Phillips and N. Maxted</i>	20
National Park <i>Bashkiria</i> as a reserve for crop wild relative conservation <i>S. Miftakhova, T. Smekalova and L. Sultangareeva</i>	22
Prioritized crop wild relatives in Spain: status on the National Inventory of Plant Genetic Resources for Agriculture and Food <i>L. De la Rosa, E. Aguiriano, C. Mallor, M.L. Rubio Teso, M. Parra Quijano, E. Torres and J.M. Iriondo</i>	23
Are soil seed banks relevant for agriculture in our day? <i>A. Horovitz, S. Ezrati and Y. Anikster</i>	27
Utilization of <i>Aegilops biuncialis</i> (goatgrass) to widen the genetic diversity of cultivated wheat <i>A. Schneider, I. Molnár and M. Molnár-Láng</i>	30
Status of collections of <i>Aegilops</i> spp. in Bulgaria and opportunities for their use in breeding programmes <i>G. Desheva, Z. Uhr, K. Uzundzhaliyeva and E. Valchinova</i>	34
Crops and wild species from the Apiaceae family – perspectives from the Nordic region <i>S. Øivind Solberg, M. Göransson and S. Jeppson</i>	37
Collecting and preliminary evaluation of grape wild relatives in China <i>J. Jiang, X. Fan, Y. Zhang, H. Sun, D. Cheng and C. Liu</i>	39
Diversity of crop wild relatives in the Egyptian flora <i>W.M. Amer</i>	42
<i>In situ</i> conservation of rare wild perennial chickpea (<i>Cicer montbretii</i> Jaub. & Spach) found in Strandja mountain, Bulgaria <i>S. Petrova and S. Angelova</i>	44

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Editorial

Recent advances in our understanding of crop wild relative (CWR) diversity, as well as in planning for their complementary conservation, has provided a solid foundation for the development of a strategic approach to CWR conservation based on a range of commonly agreed and widely tested scientific concepts and techniques. However, achieving effective conservation and utilization of CWR diversity as a means to promote food and economic security will require a coherent, coordinated policy at national, regional and global levels, and the appropriate resources to fund policy implementation. Within Europe, to achieve sustainable conservation of CWR and maximize their sustainable exploitation, the time is right to develop EU-led CWR policy and to harmonize their conservation, characterization and evaluation with existing biodiversity conservation and agricultural initiatives, and to develop new initiatives where necessary.

The *In Situ* and On-farm Conservation Network of the European Cooperative Programme for Plant Genetic Resources (ECPGR) has recently produced a concept for *in situ* conservation of CWR to guide EU and national policy development which can be used as a blueprint to drive concerted actions throughout the region and as a guide for similar action in other regions. The Concept was developed by the *In Situ* Conservation of CWR in Europe Task Force which was established under the guidance of members of the ECPGR *In Situ* and On-farm Conservation Network and the ECPGR Secretariat in response to a mandate provided by the ECPGR Steering Committee. The objective is to agree and adopt the Concept with a view to offering it to the European Commission (EC) for its consideration when formulating future European policy on *in situ* conservation of CWR diversity. This policy would substantially aid and secure the implementation of the wider EU strategy for the conservation of genetic resources in food, agriculture and forestry in Europe, and help underpin the diversification of EU agriculture required in the renewed Common Agricultural Policy.

The Concept includes: a vision of how a CWR conservation strategy for Europe would integrate national and regional (pan-European) priorities and actions; rationale for the new policy required to establish and support the ongoing operation of an integrated strategy; proposals for enhancing the utilization of conserved CWR resources in Europe; and options to promote

awareness and raise additional funding for *in situ* CWR conservation with complementary management of *ex situ* germplasm samples. The Concept has been endorsed by the members of the ECPGR *In Situ* and On-farm Conservation Network and represents the Network's vision of how *in situ* conservation

of CWR diversity can be achieved in Europe, as well as forming the basis of an action plan for the Network's activities on CWR conservation over the next ten years. The Concept, which can be downloaded from www.pgrsecure.org/documents/Concept.pdf, has been submitted to the ECPGR Steering Committee, together with its sister concept for on-farm conservation of landraces in Europe, and will be offered to the EC for consideration.



Monitoring a population of *Crambe maritima* L. on the island of Stråholmen, Norway (Photo: Nigel Maxted)



Cicer montbretii Jaub. & Spach, a close wild relative of *C. arietinum* L. which is under threat in its native locality in Strandja Mountain, southeastern Bulgaria (see p. 44). (Photo: Institute of Plant Genetic Resources, Sadovo, Bulgaria)

National CWR conservation strategies are central to the concept for *in situ* conservation of CWR diversity because all *in situ* conservation actions are necessarily implemented at national level, recognizing that nations have sovereignty over the genetic resources within their borders and the corresponding responsibility to for their conservation. This issue of *Crop wild relative* highlights the considerable progress made in CWR conservation strategy planning in a number of European countries, including the Czech Republic (p. 5), Finland (p. 10), Sweden (p. 13), Cyprus (p. 17) and Norway (p. 20). Other countries that have made significant progress include Spain, Italy, the UK and Bulgaria, and discussions are underway in Greece and Turkey. This progress has been enabled through the EC-funded project, PGR Secure (www.pgrsecure.org) which provides a Helpdesk facility for national PGR programmes who are embarking on CWR (and landrace) conservation strategy planning (see www.pgrsecure.org/helpdesk), as well as one to one technical assistance through in-country visits. The project has also financed work for four national CWR conservation strategies case studies, as well as travel and subsistence costs for in-country technical meetings and research support. This initiative has raised awareness of the need for CWR

conservation and we are pleased to report has stimulated several national governments to invest additional resources in CWR conservation and use.

The perceived value and impact of an integrated CWR conservation strategy for Europe ultimately depends on successfully channelling conserved germplasm from *in situ* and *ex situ* conservation facilities to the user community for crop improvement. The strategy needs to meet the interests of public

and private plant breeding research institutes, breeding companies, plant genebanks and agro-NGOs. Therefore, parallel concerted actions to enhance the utilization of conserved plant germplasm by the conservation and user communities are required. The four main aspects of this challenge that need to be addressed are: (a) strengthening the interface between *in situ* and *ex situ* conservation, (b) increasing efforts to characterize and evaluate conserved germplasm, (c) improving the availability of conservation, characterization and evaluation data to end users, and (d) addressing issues of access by the user community to *in situ* and *ex situ* conserved germplasm. As noted by Schneider *et al.* (p. 28), “the conservation of the genetic variability of wild species and the utilization of available accessions are important for the future of wheat production.” However, the authors also note that “many *Aegilops* species remain unexploited despite the availability of many accessions in genebanks.” One of the key foci of the PGR Secure project is the facilitation of greater engagement of the stakeholder community in the use of conserved CWR genetic diversity. The project has organized the workshop, ‘On the conservation and sustainable use of plant genetic resources in Europe: a stakeholder analysis’ (www.nordgen.org/index.php/en/content/view/full/2481/), which for the first time will bring together the main stakeholder groups in Europe (public and private plant breeding companies and research institutes, genebanks, government and agro-NGOs) with the aim of identifying constraints in the use of PGRFA and proposing solutions to overcome the barriers. This workshop will also provide an opportunity to launch the Plant Genetic Resources Diversity Gateway, an information system that aims to bridge the gap between agrobiodiversity conservation and use by providing characterization, evaluation and conservation data to the user community.

Turning to CWR conservation at global level, readers of *Crop wild relative* may already be aware of the project ‘Adapting agriculture to climate change: collecting, preparing and protecting the crop wild relatives’ led by the Global Crop Diversity Trust in partnership with the Millennium Seed Bank, Royal Botanic Gardens Kew, the International Centre for Tropical Agriculture (CIAT) and the University of Birmingham, which has generated the ‘Harlan and de Wet Crop Wild Relative Inventory’ of global priority CWR taxa (www.cwrdiversity.org/ch

[ecklist/](#)). The objectives of the project are to sample and actively conserve representative germplasm of the priority taxa *ex situ* and to promote the utilization of the material in plant breeding for crop improvement to sustain food security. The team at CIAT who are currently engaged in the *ex situ* gap analyses of the priority CWR taxa have recently created an interactive map to aid conservation planning (the Crop Wild Relatives Global Atlas – www.cwrdiversity.org/distribution-map/). The map displays the results of gap analyses of 29 food and forage crop gene pools; further gene pools will be added in the coming months. The map allows the user to explore taxon richness, potential distribution, hotspots and *ex situ* conservation gaps using a Google Map interface. Those interested in individual or multi-crop gene pool conservation and use can for the first time in one location find information on where are the best locations to find novel genetic diversity not already held *ex situ*. The map also displays the ‘global summary’ of taxon richness for all crop gene pools assessed thus far and the geographic areas with the greatest concentration of species considered of high priority for collecting (Fig. 1). The Atlas is dynamic in that it will change, perhaps significantly, as additional data on CWR distribution and *ex situ* conservation priorities are added and the ‘gaps’ are filled. However, even the initial results are a fascinating insight into where collecting activities need to be focused at national, regional and global levels.

Finally, a major upcoming event for readers’ calendars is the conference, ‘ENHANCED GENEPOOL UTILIZATION – Capturing wild relative and landrace diversity for crop improvement’ which will take place in Cambridge, UK, June 17–20 2014 (see www.pgrsecure.org/conference). A call for abstracts will be circulated in early November when registration will also open. We look forward to your participation at this important event which we hope will generate a vision for future PGRFA conservation and use.

Issue 10 of *Crop wild relative* will be published in late summer 2014. We look forward to reporting on progress in efforts to conserve and utilize our valuable CWR diversity as a contribution to food and economic security, as well as to publishing news and research from across the globe.

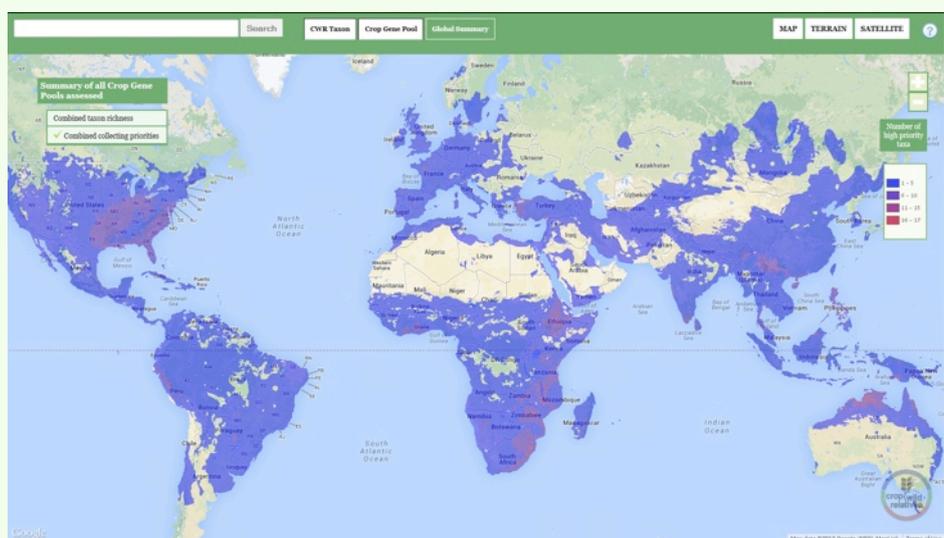


Figure 1. Geographic areas with the greatest concentration of global priority CWR species considered of high priority for collecting (source: www.cwrdiversity.org/distribution-map/)

Systematic crop wild relative conservation planning for the Czech Republic

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The importance of crop wild relatives (CWR) for future food security is widely recognized. CWR contain considerable genetic diversity and many desirable traits—especially tolerance to biotic and abiotic stresses—that may be useful for improvement of existing crops (Tanksley and McCouch, 1997; Hajjar and Hodgkin, 2007; Maxted *et al.*, 2007; Maxted and Kell, 2009; Maxted *et al.*, 2012). If this utility is not an argument for conservation in itself, like other taxa, many CWR face threats from which they require protection (Maxted, 2003). In the Czech Republic, land use change is the primary threat to wild plants (Miko and Hošek, 2009).

Across Europe, important practical conservation actions are being researched and implemented under the EU FP7-funded PGR Secure project (www.pgrsecure.org). These include the development of CWR conservation strategies for individual nations because it is their responsibility to conserve and sustainably use their national CWR diversity; any practical conservation actions will be implemented within national borders, even when driven by policy at the European level (Kell *et al.*, in prep.). Within the PGR Secure project, national strategies are being developed for Finland (Fitzgerald, this issue), Spain (Rubio Teso *et al.*, 2012), Italy (Landucci *et al.*, 2012) and the United Kingdom (Fielder *et al.*, 2012). With technical and partial financial support from PGR Secure, strategies have also been/are being developed in Cyprus (Phillips *et al.*, this issue), Norway (Asdal *et al.*, this issue), Sweden (Weibull, this issue) and Bulgaria. These complement CWR conservation plans developed in other countries outside of Europe, including Armenia, Bolivia, Madagascar, Sri Lanka and Uzbekistan (Hunter and Heywood, 2011).

“In the Czech Republic, land use change is the primary threat to wild plants”



Figure 1 *Papaver hybridum* L., the only species in our analysis to occur solely outside of protected areas. Photo: Hans Hillewaert (Wikipedia Commons)

Here, we outline the development of a CWR conservation strategy for the Czech Republic. The strategy provides a reasoned tool to protect the nation's CWR genetic resources, as well as being the first exemplar national strategy for Central and Eastern Europe.

Methods

First, we developed a checklist of CWR taxa in the Czech Republic which contains all plants in the Czech Republic that are congeneric with crops cultivated anywhere in the world (Taylor *et al.*, in prep.). From the checklist we selected CWR in priority categories which were defined by consultation with local experts and literature review: a) CWR of grass and fodder crops and of the most economically important food crops in the Czech Republic, and b) CWR taxa endemic to the Czech Republic. We excluded alien, cultivated, weedy or widespread taxa to yield a final list of priority CWR to include in the CWR inventory of the Czech Republic to form the basis of the development of the national CWR conservation strategy.

For all species in the CWR inventory, we compiled a database of observations in the Czech Republic from a variety of sources. Our primary source was the Species Occurrence Database of the Nature Conservation Agency of the Czech Republic (AOPK ČR, 2012). We also included locations of georeferenced genebank accessions of material originating from wild Czech plants, primarily from the Czech National Genetic Resource Information System (EVIGEZ, 2012).

We then carried out spatial analyses in the open-source geographic information system (GIS) software DIVA GIS (Hijmans *et al.*, 2011) to inform the development of a complementary (*in situ* and *ex situ*) conservation strategy (Maxted *et al.*, 1997; MZP, 2005; CBD, 2010). We considered conservation at both the species and genetic levels, recognizing the importance of conserving the gene pools of entire species as well as the genetic variation within those species. In the absence of explicit data on genetic variation, we considered spatial and ecogeographic data as proxies. Spatial data are used on the assumption that genetic variation is related to geographic distribution, with disjunct populations following different re-

Table 1 Summary of the CWR inventory of the Czech Republic. Use codes for the Czech Republic (EVIGEZ 2012): A – aromatic and medicinal; C – cereals; D – flowers; F – fruit (woody); G – grasses; H – vegetables; O – oil plants; T – fodder; W – ornamental woody plants; X – industrial plants

Family	Use 1	Use 2	Use 3	Use 4	No. of genera	No. of species	No. of taxa	No. of endemic taxa
Alliaceae	D	H			1	13	15	–
Apiaceae	H				1	1	1	–
Asparagaceae	D	H			1	1	1	–
Asteraceae	A	D	H	G	5	9	9	2
Brassicaceae	H	O			7	12	13	–
Campanulaceae	D				1	4	4	4
Cannabaceae	X				1	1	1	–
Caryophyllaceae	D				1	3	3	3
Fabaceae	T	A	D		19	68	73	–
Grossulariaceae	F				1	3	4	–
Iridaceae	A	D			1	1	3	3
Lentibulariaceae	A				1	1	1	1
Malvaceae	T	D	A		2	5	5	–
Papaveraceae	O	D			1	5	5	–
Plantaginaceae	A				1	1	1	1
Poaceae	C	G			19	59	64	2
Polygonaceae	T	D			1	3	3	–
Primulaceae	A	D			1	1	1	1
Ranunculaceae	A				1	1	1	1
Rosaceae	A				7	25	27	8
Rubiaceae	A				1	1	1	1
Saliaceae	W	X			1	1	1	1
Saxifragaceae	D				1	1	1	1
Totals					76	220	238	29

gimes of selection and drift (Heywood, 1991; Ferguson *et al.*, 1998; Hargreaves *et al.*, 2010). Although this assumption does not always hold true for all taxa, in the absence of genetic variation data, spatial data provide a useful proxy, especially when combined with ecogeographic data. The use of ecogeographic data as a proxy for genetic variation is made on the assumptions that there is a relationship between the environmental characteristics of a site and the genetic features of the populations occurring at that site (Greene and Hart, 1999) and that all plants in our inventory will have a similar evolutionary response to a core set of environmental parameters. To this end, we generated a generalized ecogeographic land characterization (ELC) map (Parra-Quijano *et al.*, 2011; Taylor *et al.*, in prep.) containing 22 ecogeographic categories.

Results

Czech CWR checklist and inventory

The CWR checklist for the Czech Republic contains 3195 species (3443 taxa, including subspecies and varieties), which represent approximately 89% of the taxa in the entire Czech Flora (Kubát *et al.*, 2002). The inventory (containing the list of priority taxa and associated data) contains 238 taxa in 220 species and is summarized in Table 1. Eighty-one of these species are related to food crops, 59 to fodder crops and 56 are grasses. There are other uses (e.g., aromatic and medicinal plants) represented by the 29 endemic taxa included in the inventory. Some wild relatives are related to crops with more than one use type or to multiple species with different use types.

In situ conservation

We carried out an *in situ* gap analysis to determine the extent to which the inventory taxa are found within existing protected areas. All but one species, *Papaver hybridum* (Fig. 1), have been recorded in existing protected areas. *P. hybridum* is therefore a high priority for further *in situ* protection. A further 24 priority species occur in only one protected area. For all of these species, further active conservation is recommended as a matter of urgency. This may involve population management outside of protected areas rather than through the creation of new protected areas (Hunter and Heywood, 2011).

A complementarity analysis (Rebello, 1994) of all 220 inventory species was then undertaken to identify a network of populations in 100 km² grid cells that, if protected and managed, would conserve a high percentage of priority CWR species. Results indicate that all species could be conserved in 29 grid cells, although with vastly diminishing returns such that the top ten cells contain 195 (88.6%) of these species



Figure 2 View of Pálava Protected Landscape Area during the cold winter of 2012. Pálava is one of the hottest hotspots of priority CWR diversity in the Czech Republic. (Photo: Nigel Taylor)

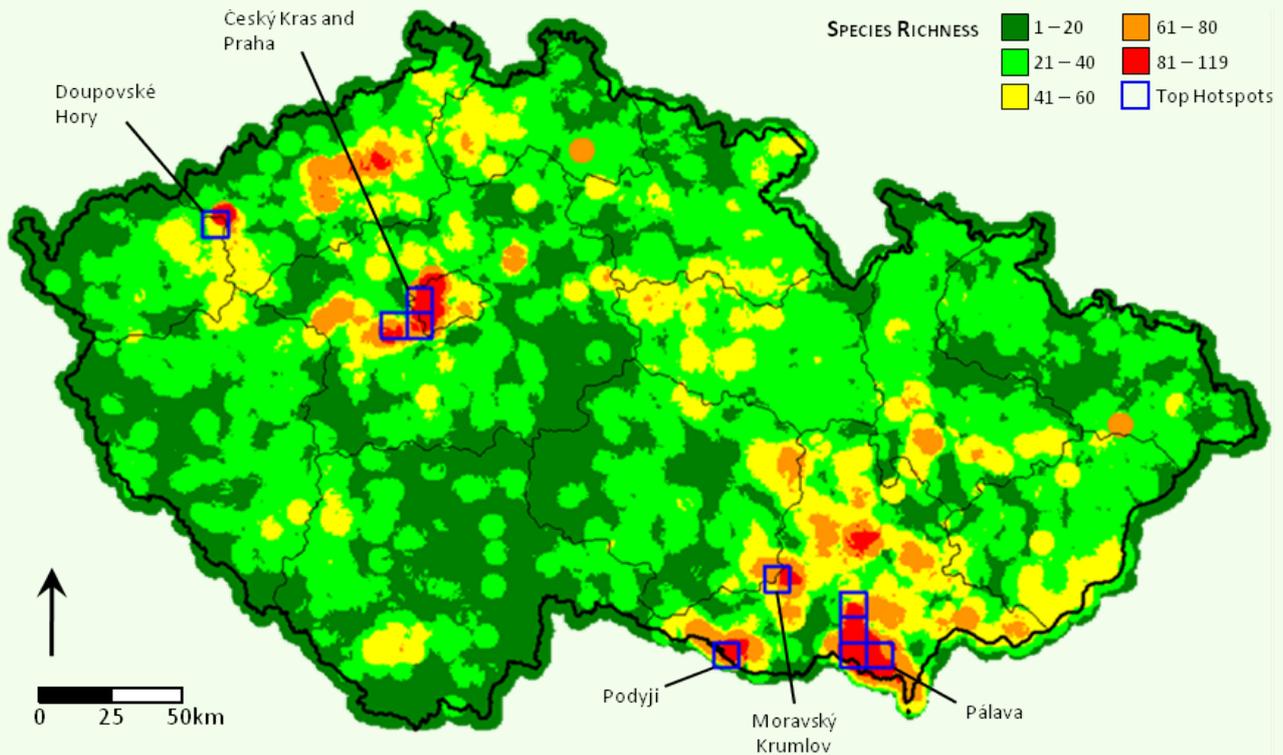


Figure 3 Proposed CWR *in situ* conservation strategy for the Czech Republic. Solid squares show priority cells and hatched areas show priority protected areas. Comp – based on complementarity analysis; EG – based on ecogeographic analysis.

(Fig. 3). In fact, the first selected cell is the outstanding priority, containing 116 (52.7%) of all inventory species. This cell overlaps with the Pálava Protected Landscape Area (PLA) in South Moravia (Figs. 2 and 3), which is generally recognized as a species diversity hotspot.

Populations within the top ten cells from our complementarity analysis can be augmented with a further ten areas containing priority CWR populations to ensure that populations from all 22 botanical ecogeographic categories of the Czech Republic are conserved *in situ*. These ten additional areas (within the blue cells in Figure 3) represent the most species rich areas of the ecogeographic categories that were not (incidentally) included in the complementarity analysis. We assume that inclusion of these extra populations in the strategy will increase the conserved genetic diversity of the priority species. Note that where cells extend beyond the border of the Czech Republic, only land within Czech territory is included in the strategy as our location records only originated from Czech territory. However, this does not negate the opportunity for coordinated trans-boundary conservation action. Indeed, this already occurs in many protected areas which straddle the Czech border, including the Czech-Polish Krkonoše/Karkonosze UNESCO Trans-boundary Biosphere Reserve (Štursa, 2011). A collaborative international approach to *in situ* conservation of priority CWR in these locations would be highly beneficial.

Together, the 20 areas identified as a result of the complementarity and ecogeographic analyses are the optimum sites for *in situ* CWR conservation in the Czech Republic (Fig. 3). These 20 areas perform better in terms of the number of species conserved and spatial coverage of the country than an alterna-

tive strategy of conserving hotspots of species richness (Taylor *et al.*, in prep.). We also highlight existing protected areas that correspond to the 20 priority areas (Fig. 3), acknowledging that where possible it may be most practical and pragmatic to incorporate CWR conservation into the remit of existing protected areas (Maxted *et al.*, 2008a).

Ex situ conservation

We identified 599 genebank accessions of 63 priority CWR species. Taxonomically, the vast majority of these were of *Lactuca serriola* (129 accessions) and spatially, collection was centred on South Moravia (65.2% of all accessions). There are 157 species without any known *ex situ* conservation action in the Czech Republic. Collecting material of these species is the top priority for *ex situ* conservation action. The examination of spatial patterns of species richness for these 157 species (both observed and predicted, based on species distribution models created in Maxent – Phillips *et al.*, 2006) identified areas in which collections would be most efficient

“There are 157 species without any known *ex situ* conservation action in the Czech Republic. Collecting material of these species is the top priority for *ex situ* conservation action.”

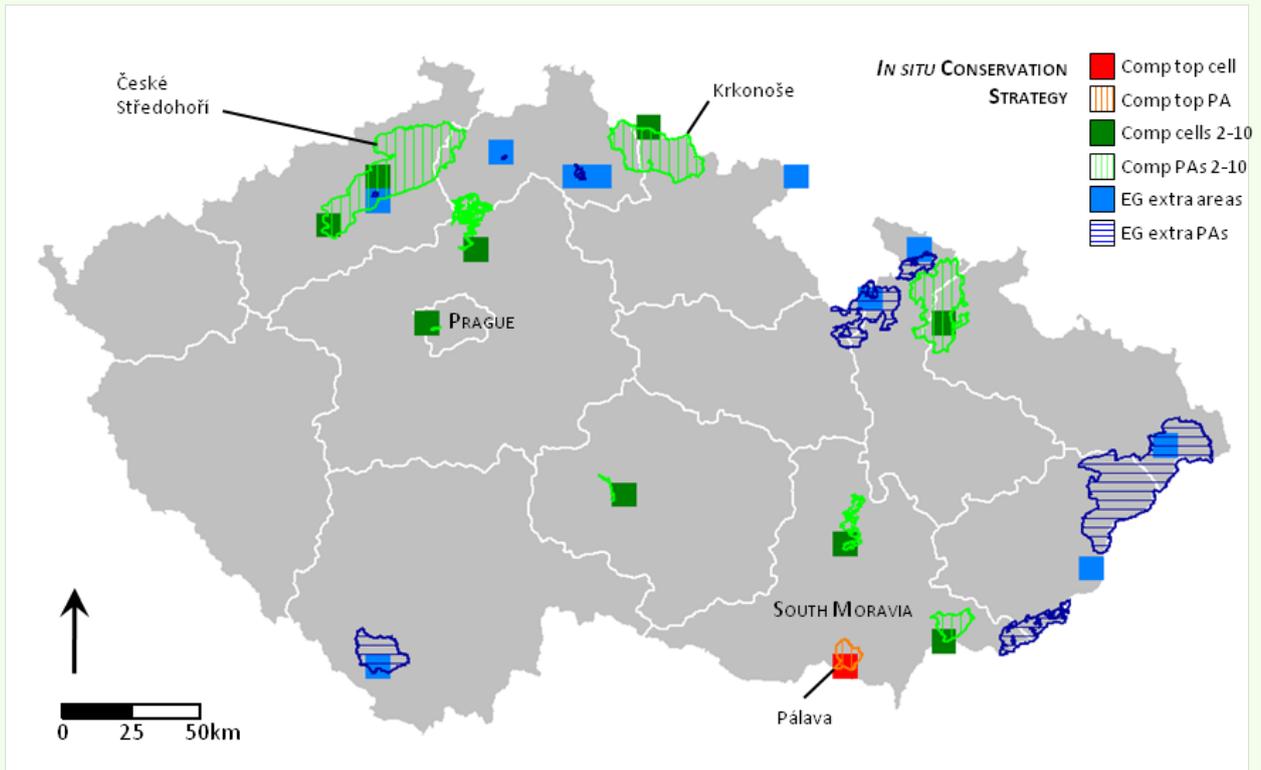


Figure 4 Observed species richness of the 220 priority species in the CWR inventory of the Czech Republic based on circular neighbourhood analysis. Blue boxes show the 10 hottest 100 km² grid cells, based on analysis using a grid of this resolution. Labels refer to the closest protected area and/or town to each hotspot.

(i.e., collecting the most species with the least travelling involved). These are South Moravia (especially Pálava) and southwest of Prague (Český Kras)—not surprisingly matching areas of total species richness (Fig. 4).

We also prioritized collection of further material of the 63 priority CWR species of which some accessions already exist, considering both spatial and ecogeographic representativeness of existing collections (Ramírez-Villegas *et al.*, 2010). The species with the lowest representativeness in existing collections are the highest priorities for further collection of material, targeted away from areas in which collections have already been made (Taylor *et al.*, in prep.).

Discussion

The CWR conservation strategy for the Czech Republic (Taylor *et al.*, in prep.) suggests both *in situ* and *ex situ* conservation actions to be implemented simultaneously as complementary measures (MZP, 2005; CBD, 2010). Implementation will involve both legislative changes and practical action. Active *in situ* conservation measures (e.g., demographic and genetic monitoring, maintenance of population size and habitat management) should be implemented (see Dulloo *et al.*, 2008; Iriondo *et al.*, 2008; Maxted *et al.*, 2008b) and minimum quality standards adhered to (see Iriondo *et al.*, 2012; <https://sites.google.com/site/qualitystandardsforcwrs/home>). *Ex situ* conservation must initially focus on the 157 species that require urgent collection of material for genebank storage.

The CWR conservation strategy for the Czech Republic is fluid and amenable to modification on the availability of additional data or changing priorities. Importantly, the recommended sites

for *in situ* conservation need to be ground-truthed to confirm the presence of populations of the target taxa since the analysis is based on genebank and herbarium specimen locations. Further, its recommendations should be reviewed in the light of climate change predictions for the Czech Republic. Does the proposed strategy still function effectively in the novel climates of 2050 or 2080?

“The CWR conservation strategy for the Czech Republic is fluid and amenable to modification on the availability of additional data or changing priorities.”

After further refinement of the strategy, taking it forward to the implementation stage is the next challenge which will involve the key stakeholders from both the PGRFA and nature conservation communities—the latter who will ultimately be responsible for *in situ* conservation of the country's priority CWR resources. The strategy has been developed in collaboration with Czech experts, stakeholders and authorities from the outset. We hope this collaboration will continue as the plan is implemented and will be instrumental in its success.

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Prioritization of crop wild relatives in Finland

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Crop wild relatives (CWR) harbour genetic resources and variability that has great potential in plant breeding. In the face of climate change this resource may turn out to be instrumental for future food security. CWR have potential to enhance agricultural production and the growing world population with sustainable production of crops. However, since CWR grow in wild habitats, they are often not included in conservation programmes; yet they may be susceptible to habitat destruction and degradation and may require urgent conservation actions. The need to conserve CWR taxa has been identified by policy-makers by including them in policy instruments, such as the European Strategy for Plant Conservation (Planta Europa, 2008), the Global Strategy for Plant Conservation (CBD, 2010a), CBD Strategic Plan for Biodiversity 2011–2020 (CBD, 2010b), and the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2001). To conserve CWR, however, we need to first identify the CWR taxa within a country and decide on efficient methods to conserve their genetic resources.

The Finnish CWR checklist and its prioritization create a starting point for the Finnish CWR conservation strategy. This work is part of the EU FP7-funded PGR Secure project of which one element is to create national CWR strategies. The main organizations involved in the project in Finland are the Finnish Museum of Natural History, University of Helsinki and the MTT Agrifood Research Finland. There is also cooperation with other institutes, such as the Finnish Environment Institute, National Advisory Board for Genetic Resources in Finland, Finnish Plant Genetic Resources Programme, Finnish Forest Research Institute, Botanic Gardens of Helsinki, Oulu and Turku, Ministry of Agriculture, National Board of Forestry, Government of Åland and NordGen.

The CWR conservation strategy will enable the conservation of the maximum taxonomic and genetic diversity of Finland's

CWR. Finland does not have many close relatives of the globally important crop species, but due to its remote northern location, it has been noted that many species are found at the edge of their distribution and species with generally wide distribution have distinct subspecies or races (Hämet-Ahti *et al.*, 1998; Miranto *et al.*, 2012). These may contain valuable genetic variation and adaptations to the European flora in general as the peripheral populations can often be genetically and morphologically diversified from central populations and have distinct traits allowing adaptation to environmental change (Lesica and Allendorf, 1995). It is therefore important to conserve their genetic resources.

CWR checklist for Finland

Approximately 2503 vascular plant species are found in Finland (Hämet-Ahti *et al.*, 1998; Lampinen *et al.*, 2012). A draft checklist of the CWR taxa for Finland was obtained from the CWR Catalogue for Europe and the Mediterranean (Kell *et al.*, 2005, 2008) to form the basis of the Finnish CWR checklist. This list of 2334 CWR taxa was based on Euro+Med PlantBase (Euro+Med PlantBase, 2005), Mansfeld's World Database of Agricultural and Horticultural Crops (Hanelt and IPK Gatersleben, 2001) and additional data for forestry, ornamental, medicinal and aromatic species. The Finnish draft checklist was taxonomically harmonized with the Field Flora of Finland (Hämet-Ahti *et al.*, 1998) and its update (Hämet-Ahti *et al.*, 2005). For some taxa, the status and synonyms were checked with The Plant List (2010). Native, archaeophyte, neophyte and alien species were all included in the checklist. The nationally threatened or protected subspecies which were not already on the draft list were added. Hybrids and synonymic taxa were removed along with the apomictic species such as *Taraxacum* ssp., *Hieracium* ssp., *Ranunculus auricomus* Group, due to the difficulties with their identification and changing taxonomy. The CWR checklist for Finland contains 1905 CWR taxa.



Figure 1 *Malus sylvestris* Mill. (Photo: Jouko Lehmuskallio, Luontoportti)



Figure 2 *Malus sylvestris* Mill. fruits (Photo: Jouko Lehmuskallio, Luontoportti)

Box 1 Prioritization criteria applied to the Finnish CWR checklist

1. Relative threat

- a. Finnish Vascular Plant Red List Category 2010
- b. National protection status (Nature Conservation Decree, 1997)
- c. Species listed in the EU Habitats Directive Annexes II, IV and V and EU Annex Priority species European Communities (1995–2007)
- d. Socio-economically important European endemic species (Bilz *et al.*, 2011)
- e. OEK species (European species which Finland is responsible for conserving since a minimum of 20% of their European distribution is within the country) (Rassi *et al.*, 2001)

2. Value

- a. Yield of CWR-related main crops in Finland in 2010 (Tike, 2010)
- b. Yield of CWR-related herb crops in Finland (Galambosi, 2007)
- c. High priority human food crops in Europe (Kell *et al.*, 2012) based on production quantity in Europe.

3. Use

- a. Medicinal/pharmaceutical use, food, forage/fodder plants (Hanelt and IPK Gatersleben, 2001)
- b. Species listed in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO, 2001)
- c. Nordic mandate species including root, oil and fibre plants, vegetables, fruits and berries, cereals and forage plants listed by former NordGen thematic working groups (formally by the Nordic Gene Bank). Data were sourced from www.ngb.se and can be acquired from NordGen.
- d. Species in GP1b, GP2 (Harlan de Wet, 1971) and TG1b, TG2 and TG3 (Maxted *et al.*, 2006) derived from The Harlan and de Wet Crop Wild Relative Inventory (Vincent *et al.*, 2013; www.cwrdiversity.org/checklist/).

Prioritization to form the national CWR inventory

The CWR checklist was prioritized to create a workable list of taxa for further analysis. To achieve this, the first task was to select indigenous and archaeophyte taxa. Alien and neophyte taxa were removed. The categorization of alien, native, archaeophyte and neophyte species was based on the Field Flora of Finland (Hämet-Ahti *et al.*, 1998) and its update (Hämet-Ahti *et al.*, 2005), which draw a line between archaeophytes and neophytes at the 17th century. Additional information of the native status of plant species found in Finland was obtained from Euro+Med PlantBase (Euro+Med PlantBase, 2005). Neophytes were removed during the prioritization process. However, it is recognized that the neophyte group in Finland includes many relatives of crop species that have already formed adaptations to the Finnish climate and could therefore contain important material for breeding. For this reason, they have been kept on the national CWR checklist.

The second task was to create the prioritization criteria. In all, three main criteria and twelve subcriteria were selected (Box 1). Firstly, the taxa having IUCN threatened categories (Vulnerable, Endangered and Critically Endangered) in the 2010 Red List of Finnish Species (Rassi *et al.*, 2010) were selected into the priority CWR list. This included the 150 most threatened CWR taxa in Finland. Secondly, the taxa classified Near Threatened in the 2010 Red List of Finnish Species were se-



Figure 3 *Fragaria vesca* L. (Photo: Jouko Lehmuskallio, Luontoportti)

lected if they had at least one point in the four use subcriteria. Thirdly, the taxa which were not classified as threatened in the 2010 Red List of Finnish Species but had more than three points in the 12 subcriteria were included in the prioritized list. Finally, those subspecies which are not threatened were removed from the list. This resulted in the prioritized CWR national inventory list of 205 taxa.

Results and discussion

The CWR checklist for Finland contains 1905 CWR taxa. This includes 1381 species, 492 subspecies and 32 varieties. The majority of the total Finnish flora (76%) consists of CWR species. A high percentage of these (71%) are indigenous or archaeophyte taxa. The Finnish CWR taxa are found in 101 plant families, the main ones being Poaceae, Rosaceae, Cyperaceae, Brassicaceae and Asteraceae, all with more than 100 taxa represented. In all, 13% out of the total CWR in Finland are threatened, and 18% of the indigenous and archaeophyte taxa are threatened. Among them, five taxa are classified as Regionally Extinct, 21 Critically Endangered, 62 Endangered, 62 Vulnerable and 96 Near Threatened. Altogether, 117 taxa are under national protection by the Nature Conservation Decree (MoE, 1997) and 47 are Finnish 'responsibility' species with more than 20% of their European distribution within Finland.

The prioritized national inventory of CWR for Finland consists of 205 taxa, which is 11% of the Finnish CWR diversity. Out of the prioritized CWR species, the main threat factors are (Rassi *et al.*, 2010): overgrowing of meadows and open habitats (affecting 31% of priority species); construction on land (16%); random factors (including small population or area/short-term climate change) (13%); chemical disturbances (12%); peatland drainage and peat harvesting (11%); forest management activities (10%); and construction of waterways (7%). Habitats of the prioritized CWR are: Baltic Sea shores, lake shores or river banks (23% of species); rural biotopes or cultural habitats (21%); forest environments (18%); mires (10%); rocky outcrops (10%); alpine heaths and meadows above treeline (9%); and aquatic habitats (9%). From this it can be perceived that one of the main actions to conserve CWR taxa in Finland would be to prevent overgrowing of shoreline meadows and open habitats, such as rural biotopes

and cultural habitats, and restore already changed habitats when needed.

Out of the Critically Endangered CWR species, several also score points in the use and value subcriteria (Box 1), including *Anthyllis vulneraria* subsp. *polyphylla* (DC.) Nyman, *Artemisia campestris* subsp. *bottnica* Kindb., *Hypericum montanum* L., *Pimpinella major* (L.) Huds., *Rosa canina* L. and *Sorbus meinichii* Hedl. The following Critically Endangered species score points in the threat subcriteria (Box 1): *Asperula tinctoria* L., *Bromopsis benekenii* (Lange) Holub, *Dianthus superbus* L., *Erica tetralix* L., *Liparis loeselii* (L.) Rich, *Melica ciliata* L., *Polygonum oxyspermum* Lebed., *Puccinellia phryganodes* (Trin.) Scribn. & Merr., *Salix pyrolifolia* Lebed., *Silene furcata* Raf. and *Sium latifolium* L. Thus, these species may have additional value compared to the other threatened taxa. Out of the CWR species classified as Regionally Extinct (RE) or Extinct in the Wild (EW)—*Carex maritima* Gunnerus, *C. montana* L., *Sparanium neglectum* Beeby, *Stellaria humifusa* Rottb. (all RE) and *Rubus humulifolius* C.A. Mey (EW)—the last one, *R. humulifolius*, has points also in other prioritization criteria and it is already in a reintroduction programme. Out of the Near Threatened species which have at least one point in the use subcriteria, the following species score highest: *Malus sylvestris* Mill. (Figs. 1 and 2), *Fragaria vesca* L. (Fig. 3) and *F. viridis* Duchesne. Out of the not threatened taxa which have at least three points in the 12 subcriteria, the following score highest: *Alisma wahlenbergii* (Holmb.) Juz., *Allium schoenoprasum* L. (Fig. 4), *Lathyrus sylvestris* L., *Ribes nigrum* L., *R. spicatum* E. Robson, *Vicia cracca* L., *V. hirsuta* (L.) Grey, *V. sepium* L., *V. tertrasperma* (L.) Schreb. and *V. villosa* Roth. The genera *Phleum* and *Fragaria* are relevant for current plant breeding programmes in Finland. Others remain to be utilized in the future by plant breeders from other countries or in arising novel breeding programmes in Finland.

In general, the prioritized list of species divides the group into either highly threatened taxa with a narrow distribution or not threatened and more common taxa. The next stage is to undertake *in situ* and *ex situ* gap analysis to identify key *in situ* areas containing CWR and to identify key components of Finnish CWR diversity not already conserved *ex situ* to make feasible, practical suggestions for the conservation of Finnish priority CWR taxa. Successful conservation and sustainable



Figure 4 *Allium schoenoprasum* L. (Photo: Jouko Lehmuskallio, Luontoportti)

use of CWR taxa and their genetic resources are highly important for both the maintenance of biodiversity and for the improvement of plant production.

The Finnish CWR checklist and the prioritized national CWR inventory will be published with the national CWR strategy report for Finland (Fitzgerald, 2013) which will be available via the PGR Secure website. The data will also be uploaded to and be accessible via the 'PGR Diversity Gateway' online information system which is under development within the context of the PGR Secure project and will be launched early in 2014.

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Strategic work on crop wild relatives started in Sweden

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Sweden—a 1572 km long land mass stretching over 14 latitudes and 13 longitudes—is not exactly known for its richness in crop wild relatives (CWR). Situated in the northern temperate zone, the country can roughly be divided into a southern third dominated by agriculture and deciduous forests while the two northern thirds are mainly forested and belong to the taiga belt of the northern hemisphere. Due to the Gulf Stream, agriculture is possible up to the Arctic Circle, beyond which a sixth of the country stretches.



Figure 1 Timothy grass, *Phleum pratense* L. is the most economically important Swedish fodder grass. It has several wild relatives in the country (Photo: Peder Weibull)

Being part of the forest belt, the occurrence of forest genetic resources such as Norway spruce, Scots pine, birch and other important tree species is evident. When it comes to genetic resources for food and agriculture, the species diversity is perhaps less apparent. For most of its agricultural and horticultural crops, Sweden depends upon genetic resources from outside the region. Some plant groups however—notably forage grasses (Fig. 1), legumes, fruits and berries, and certain conspicuous vegetable CWR (Fig. 2)—are indigenous to the country. However, when the strategy for inventory of indigenous genetic resources was drawn up in 2001, CWR were not that exhaustively elaborated. The following quote (Jordbruksverket, 2002), however, represents an attempt to assign priority to some CWR for *ex situ* conservation:

“A point of departure for the conservation of the wild relatives should be their life status. Endangered species, whose range is known through regional inventories, shall be collected for further ex situ conservation within the mandate of the NGB¹ on behalf of the national programme. Responsibility for in situ conservation should henceforth be on the Environmental Protection Agency. Examples of species that should urgently be subject to additional collections under NGB's operations include wild sea-beet (Beta maritima), sea kale (Crambe maritima), wild celery (Apium graveolens) and meadow barley (Hordeum secalinum).

A second point of departure is the (anticipated) commercial value where relatives of known crops should primarily be inventoried, collected and described with respect to the genetic variation. Examples of this include Ribes species, wild carrot (Daucus carota), prickly lettuce (Lactuca serriola), Japanese rose (Rosa rugosa), caraway (Carum carvi), thyme (Thymus

¹ Nordic Gene Bank – After 1 January 2008: Nordic Genetic Resource Centre (NordGen)

serpyllum) and others. Inventory and collection of such species should preferably be done within the framework of individual externally funded projects conducted for plant breeding purposes. No priority between species is made within this group.”



Figure 2 Some sea-kale (*Crambe maritima* L.) plants grow to impressive size! (Photo: Jens Weibull)

As it turned out, this anticipation was a bit too optimistic. Although the issue on CWR was brought up several times by the advisory board of the national programme, the work never really took off. Complementary collecting of threatened species was made to a limited extent, and no targeted *in situ* activities were implemented. The lack of progress in this important field was indeed frustrating. With this as a background, a group of people representing relevant government agencies (the Swedish Board of Agriculture and the Swedish Forest Agency), the Swedish Species Information Centre, NordGen and the Swedish Association of Botany got together with the aim of invigorating the work and establish both criteria for prioritizing species and a list of targeted CWR. The Swedish Environmental Protection Agency (EPA) was also invited but unfortunately declined to participate. This article briefly describes how this process was carried out, some results and further anticipations.

On process

We first agreed upon how to proceed with the work and concluded the terms of reference for the group. We also set up quite an ambitious agenda including: (1) the establishment of a national CWR Priority List and (2) identification of national needs concerning: a) complementary inventories; b) identification of areas for *in situ* conservation; c) development of management plans; and d) complementary collecting of selected species for *ex situ* conservation at NordGen.

We completed the work on a priority list of CWR species for Sweden by February 2012. An article describing the work and the list was published in the journal of the Swedish Association of Botany (regrettably only in Swedish; available as pdf from Jens Weibull). We have, however, so far not managed to identify the national needs as outlined under (2); they were seen as essential components of a full report of our conclusions and recommendations, and that work has yet to be carried out (see below).

Historically, sporadic initiatives for complementary collecting of seeds of Red Listed CWR (item 2d), have been executed by NordGen and others to the extent that project money have been available. Thus, accessions of wood barley (*Hordelymus europaeus* (L.) Harz) and meadow barley (*Hordeum secalinum* Schreb.) were collected during the early 2000s as well as sea beet (*Beta vulgaris* subsp. *maritima* (L.) Arcang.), wild lettuce (*Mulgedium quercinum* (L.) C. Jeffrey) and *Elymus mutabilis* (Dobrow) Tzvelev. These accessions are now stored at NordGen.

Points of departure

Firstly, we decided to include all CWR of plants for food and agriculture, including those of ornamental plants and forest tree species. We then began the work by discussing whether to work through a Gene Pool or a Taxon Group perspective and concluded that the latter, following the definition by Maxted *et al.* (2006), would better serve the purpose of the project. The reasons for our decision was either that not all of the priority crop gene pools in the Swedish CWR inventory have had the Gene Pool concept applied or it was not possible to apply the concept due to a lack of genetic data. An example of how the Taxon Group concept can be applied is shown in Table 1.

Secondly, we needed to compile a ‘full’ list of taxa as a starting point. The list for Sweden extracted from the Crop Wild Relative Catalogue for Europe and the Mediterranean (Kell *et al.*, 2005) was indeed very comprehensive, yielding a total of 2361 taxa of worldwide crops and their CWR. From the point of view of this project, however, we decided to compile the list based upon available domestic literature. For this purpose, we used a number of sources but most notably the Swedish Cultivated and Utility Plants Database (SKUD – www.slu.se/sv/centrumbildningar-och-projekt/skud/) (Aldén and Ryman, 2009). The database, unfortunately only available in Swedish for the time being, currently contains information on more than 81,600 botanical names based on more than 9000 references. We systematically went through all taxa and noted those that could be considered to be native in the country. Swedish botanists use two different time limits for the determination of

Table 1 An example of the application of the Taxon Group concept (Maxted *et al.*, 2006) for the barley genus, *Hordeum*

Taxon Group	Defined as	Taxon example	Common name
1a	Crop	<i>Hordeum vulgare</i> subsp. <i>vulgare</i>	Barley
1b	Same species as crop	<i>H. vulgare</i> subsp. <i>spontaneum</i>	Wild barley
2	Same series or section as crop	<i>H. bulbosum</i>	Bulbous barley
3	Same subgenus as crop	<i>H. murinum</i>	Wall barley
4	Same genus	<i>H. secalinum</i>	Meadow barley
5	Same tribe but different genus to crop	<i>Hordelymus europaeus</i>	Wood barley

native plants: the years 1800 for Red Listed taxa (Gärdenfors *et al.*, 2010) and 1700 for all other taxa (Karlsson and Ages-tam, 2013).

This exercise yielded a total of 1478 taxa of which the majority (almost 95%) are true species and the remaining subspecies/varieties or spontaneous hybrids (very few). In addition to listing the taxa we also noted their primary type of usage (as recorded in SKUD) according to the following:

- Food and feed
- Ornamental
- Medicinal
- Aromatic
- Wood/timber
- Multiple use (noted as 'outdoor ornamental' or 'former medicinal' but listed for other uses as well)
- Other (e.g. water or aquarium plants)

On criteria

The next step of the project included an evaluation of various criteria that would help us in prioritizing among all the 1478 taxa. We began by identifying the 'relatedness' and 'threat' criteria as being highly essential, the 'relatedness' criterion linking to Taxon Group and the 'threat' criterion to the classification according to the Swedish Red List. The latter follows closely the IUCN classification.

We then went on to discuss other tentative criteria such as the following:

- 'Responsibility' – the taxon has most or a large part of its distribution within Sweden

- 'Classification' – the taxon represents an important indicator for threatened habitats
- 'Usage' – the taxon is particularly important or useful for research and/or breeding
- 'Value' – its domesticated and cultivated species represents a high economic value

Finally, we also dwelled upon the option to include a criterion that reflects the extent of 'genetic variability' of a taxon. The risk of applying a strict species approach is that subpopulations containing particularly valuable genetic variation might be overlooked. For example, local Scandinavian populations of Norway spruce representing refuges that survived the last glacial period have been shown to contain unique genetic variability that may prove valuable in a climate change context (Parducci *et al.*, 2012). The problem is, however, that we generally know very little about the genetic variation of species. This is true also for important crops such as fodder grasses and legumes. The 'genetic variability' criterion was therefore abandoned and we decided to include the aspects of 'responsibility' and 'usage'. All criteria were given equal weighting.

Some results

Using the four different criteria (i.e., 'relatedness', 'threat', 'responsibility' and 'usage'), altogether 84 taxa out of the total of 1478 are deemed priority taxa. Whereas our full list contained 104 plant families and 403 genera, the priority list includes 23 families and 62 genera. The order among plant families changed as priority taxa were selected with Lamiaceae (mint family) ranking highest (Fig. 3). This can be explained by the fact that many species are influenced by two important criteria: usage and threat. Members of the mint family are often used either as ornamentals or as aromatic plants, and many of them are in the Red List.

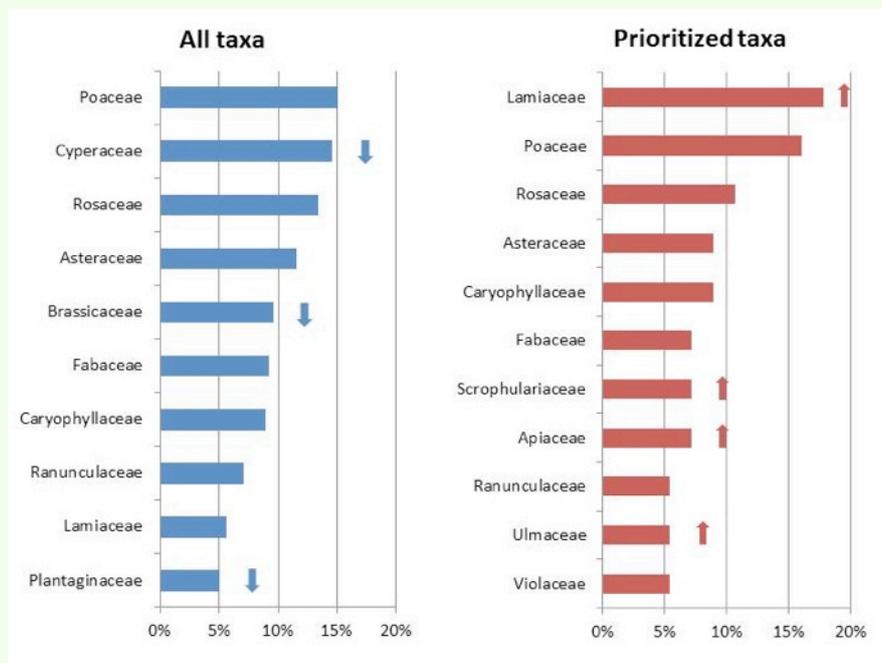


Figure 3 The ten highest ranked families over all 1478 taxa (blue bars) and the 84 prioritized taxa (red bars), respectively. Blue arrows denote previously dominating families that fell low in rank following prioritization. The sedge family (Cyperaceae) contains the species-rich genus *Carex*, but no sedge species are both threatened (CR, EN or VU) and closely related to cultivated sedge. Similarly, red arrows indicate plant families that climbed in rank. Many species within the mint family (Lamiaceae) combine these particular criteria.

Table 2 The distribution (in %) of 84 prioritized CWR taxa using three different selection criteria. The two most dominating groups for each criterion are highlighted (modified from Aronsson *et al.*, 2012)

Taxon Group	%	Red List category	%	Use category	%
1a	35.7	VU	32.1	Food and feed	6.0
1b	56.0	NT	4.7	Ornamental	57.1
4	7.1	EN	41.7	Medicinal	4.8
5	1.2	CR	17.9	Aromatic	0.0
		(none)	3.6	Tree and timber	2.4
				Multiple use	20.2
				Other (e.g. water plants)	9.5

The elm family (Ulmaceae) enters the list of priority species because of three Red Listed taxa—wytch elm (*Ulmus glabra* Huds. subsp. *glabra* and subsp. *montana*) and European white elm (*Ulmus laevis* Pall.)—though this particular family represents only a fraction of all taxa on the full list. The same is true for the figwort family (Scrophulariaceae) which contains several Red Listed species of the genera *Euphrasia* and *Verbascum*.

Almost 92% of the taxa belong to Taxon Group 1 (a + b) (Table 2) which means there are unlikely to be any crossing barriers between populations and transfer of desirable genetic traits would be easily achieved. The observation that 32.7% of the prioritized taxa include the crop itself is explained by the fact that many species (particularly ornamental species) include both wild and cultivated forms that are not split into formally recognized subspecific taxa. Their genetic variability can therefore be utilized immediately. Several of the species belonging to Taxon Group 4 (e.g., *Elymus kronokensis* (Kom.) Tzvelev, *E. mutabilis* (Drobow) Tzvelev and *Lactuca quercina* L.) have significant populations in Sweden.

The distribution among various threat categories does not follow any particular pattern (Table 2). However, altogether 59.6% of the priority species belong to the highest categories Critically Endangered (CR) and Endangered (EN), emphasizing the need to develop strategies for their long-term conservation and implementing procedures for continuous follow-up of their population status.

Concerning usage a clear majority of the taxa (57.1%) are classified as ornamentals, many of which are commercially very important (genera *Rosa*, *Iris*, *Dianthus*, *Aconitum* and several Pteridophyta). Whereas a fifth (20.2%) are taxa of documented multiple use, only five represent taxa for food and feed: field brome (*Bromus arvensis* L.), smooth brome (*B. racemosus* L.), [wild] celery (*Apium graveolens* L.), *Brassica rapa* subsp. *campestris* (L.) A.R. Clapham, and *Mentha x gracilis* Sole.

Priority tree taxa include large-leaved lime (*Tilia platyphyllos* Scop. nom. cons.) and ash (*Fraxinus excelsior* L.), apart from the previously mentioned elms. The ash has recently been reclassified as Vulnerable (VU) due to the rampant ash die-back disease.

Final words

We have yet to prepare the final report which is planned to include detailed information on each taxon in the priority list including, among others: current status, distribution and occur-

rence within and outside of protected areas, a gap analysis with respect to conservation measures, and management responsibility. We hope to be able to carry out this work jointly with the Swedish EPA in order to provide for a solid strategy of sustainable CWR conservation and use. It is indeed time for us to uphold the legacy of Linnaeus.

Acknowledgements

The author gratefully acknowledges the input of Mora Aronsson/Swedish Species Information Centre, Sanna Black-Samuelsson/Swedish Forestry Agency, Margareta Edqvist/Swedish Association of Botany, Erik Persson/NordGen (currently at the Swedish University of Agricultural Sciences) and David Ståhlberg/Swedish Board of Agriculture, without whom this work would not have been accomplished.

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Development of a national crop wild relative conservation strategy for Cyprus

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Despite its small size, Cyprus is a floristically diverse country due to both its varied climate and geology and the presence of man since 8200 AD. Cyprus has the highest concentration of threatened plants in Europe (Tsintides *et al.*, 2007). It also has 139 endemic taxa (108 species and 31 subspecies) and a total of 1625 indigenous species and subspecies (Hand *et al.*, 2012), of which 1613 taxa can be considered crop wild relatives (CWR). Due to the country's location within the Mediterranean and Near East (where it abuts the Fertile Crescent), which are two of the global regions with the highest number of CWR taxa (Vincent *et al.*, 2013), Cyprus is

rich in CWR that are adapted to unique environments. A recent surge in research on CWR has highlighted their value for crop improvement and research has shown that breeders' use of CWR taxa has increased year on year (Maxted and Kell, 2009), particularly to combat the impacts of climate change on agro-environments and crop production, and ultimately to contribute to global food security. This justifies the need for the development of a national strategy for the *in situ* and *ex situ* conservation of CWR in Cyprus.

Table 1 Summary of the CWR inventory of Cyprus (N = native, E = endemic, C = cultivated, W = wild)

Family	Genus	No. species	No. infra-specific taxa	Native / endemic	Occurrence status
Amaryllidaceae	<i>Allium</i>	17	4	N & E	W
Asteraceae	<i>Lactuca</i>	7	0	N & E	W
Brassicaceae	<i>Brassica</i>	3	0	N & E	W
Brassicaceae	<i>Crambe</i>	1	0	N	W
Brassicaceae	<i>Diplotaxis</i>	1	0	N	W
Brassicaceae	<i>Eruca</i>	1	0	N	W
Brassicaceae	<i>Lepidium</i>	4	1	N	W
Brassicaceae	<i>Sinapis</i>	3	2	N	W
Chenopodiaceae	<i>Beta</i>	1	1	N	W
Fabaceae	<i>Lathyrus</i>	11	0	N	W & C
Fabaceae	<i>Lens</i>	3	0	N	W
Fabaceae	<i>Lupinus</i>	1	0	N	W
Fabaceae	<i>Medicago</i>	21	0	N	W
Fabaceae	<i>Melilotus</i>	2	0	N	W
Fabaceae	<i>Pisum</i>	1	1	N	W
Fabaceae	<i>Trifolium</i>	34	6	N	W & C
Fabaceae	<i>Vicia</i>	22	2	N	W
Poaceae	<i>Aegilops</i>	10	3	N	W
Poaceae	<i>Agrostis</i>	1	0	N	W
Poaceae	<i>Avena</i>	8	4	N	W
Poaceae	<i>Crithopsis</i>	1	0	N	W
Poaceae	<i>Dactylis</i>	2	1	N	W
Poaceae	<i>Elymus</i>	4	1	N	W
Poaceae	<i>Hordeum</i>	7	2	N	W
Poaceae	<i>Lolium</i>	5	2	N	W & C
Poaceae	<i>Phalaris</i>	1	0	N	W & C
Poaceae	<i>Poa</i>	4	0	N	W & C
Poaceae	<i>Taeniatherum</i>	2	2	N	W

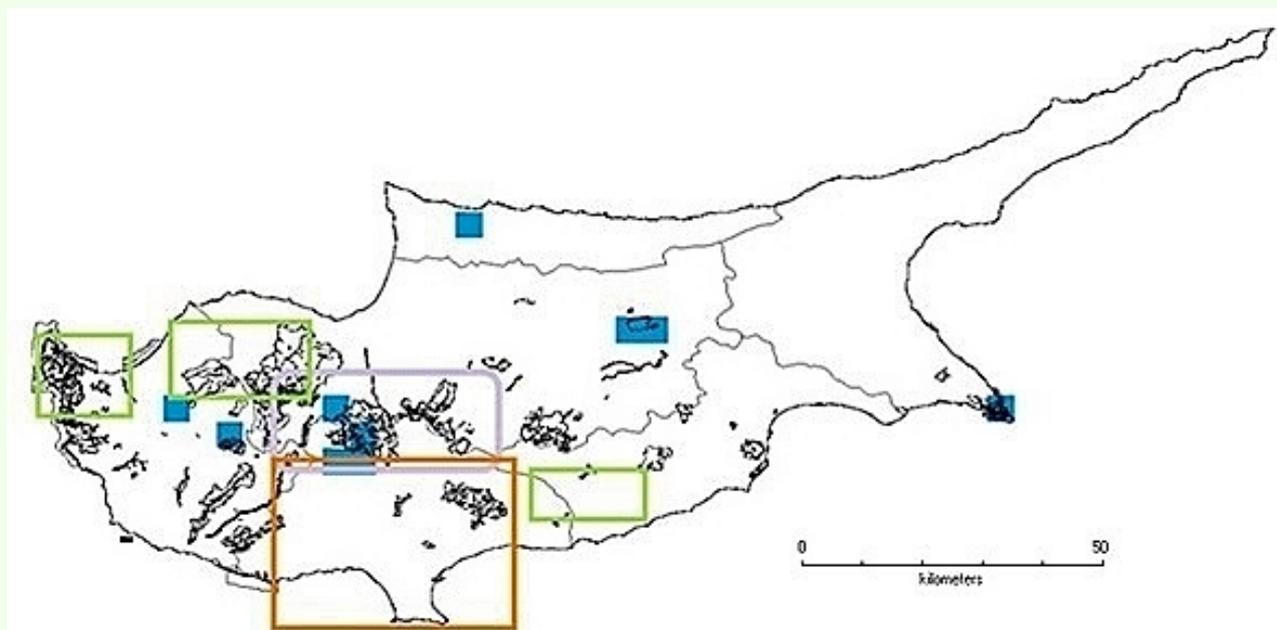


Figure 1 Summary of the CWR conservation strategy for Cyprus. The blue grids are the top ten priority sites for genetic reserve establishment, the area enclosed by the brown rectangle requires CWR surveying, the areas enclosed by the green rectangles are areas for further *ex situ* collecting, and the area enclosed by the grey rectangle is the area with the highest CWR richness. Existing protected areas are outlined with black lines.

In a recent study funded by the EU FP7 project PGR Secure (www.pgrsecure.org) and the University of Birmingham, UK, a list of 178 priority CWR native to Cyprus was developed using a systematic approach. The inventory of priority CWR was created following discussions with Cypriot stakeholders (which composed of the Agricultural Research Institute, Department of Forests and the Ministry of Agriculture, Natural Resources and Environment) and used the following criteria: a) CWR whose centre of diversity is within Cyprus, the Near East or the Mediterranean region; b) CWR of economic importance to Cyprus; c) CWR of economic importance to Europe; d) CWR listed in Annex 1 of the ITPGRFA (FAO, 2001); and e) CWR selected by local experts and plant breeders as being of high importance to their current or future work. The 178 priority taxa represents approximately 9% of the total CWR taxa within Cyprus (see Table 1 for a summary) and is comparable to other countries which have undertaken the same exercise: Finland – 205 priority taxa (~11% of total CWR) (Fitzgerald, this issue); UK – 250 priority taxa (~16% of total CWR) (Fielder *et al.*, 2012) and Spain – 400 priority taxa (~6.6% of total CWR) (Rubio Teso *et al.*, 2012).



Aegilops triuncialis L. (Photo: Charalampos Christodoulou)

Using the complementarity analysis method described by Scheldeman *et al.* (2010), ecogeographic data for the 178 priority taxa were analysed using DIVA-GIS and Maxent to

suggest a minimum network of ten *in situ* genetic reserves that conserve 74.7% of the taxa (Figure 1). It is a testament to the extensive coverage of the current protected areas in Cyprus that nine of the ten target grid cells contain a currently protected area in which the management plan could potentially be amended to incorporate the active genetic conservation of CWR diversity. The Troodos Mountains and the Pafos region stand out in all analyses as containing high numbers of the priority CWR. A review of current *ex situ* conservation shows that the National Cyprus Genebank contains accessions of 100 of the priority taxa and the other 78 priority taxa are identified for immediate collection. Furthermore, geographic representativeness studies highlight areas in the Pafos forest and Akamas peninsula as targets for germplasm collection to increase the genetic representativeness of genebank collections (Figure 1).

Having made such specific recommendations for *in situ* and *ex situ* CWR conservation, it is necessary to ground truth the existence of the predicted presence of target populations (Maxted *et al.*, 2008). The *in situ* complementarity analysis aims to conserve the highest number of taxa within the genetic reserve network; it does not necessarily locate those populations that will maximize genetic diversity conservation overall. However, from the *ex situ* analyses, we see overlap between target *ex situ* and *in situ* conservation sites; therefore we can



Trifolium dasyurum C. Presl (Photo: Angelos Kyratzis)



Lotus tetragonolobus L. (Photo: Angelos Kyratzis)

say with reasonable certainty that using a complementary *ex situ* and *in situ* approach, maximum genetic diversity is likely to be conserved. Furthermore, the conservation of CWR in Cyprus is a dynamic process and therefore as more data are gathered, the national strategy is likely to be enhanced by the availability of genetic diversity studies or through the use of the method proposed by Parra-Quijano *et al.* (2012) which uses ecogeographic land characterization (ELC) as a more accurate proxy for genetic diversity to generate a more tightly focused conservation strategy.

“The establishment of a national system of complementary genetic reserves together with systematic *ex situ* seed conservation will place Cyprus at the forefront of PGR conservation”

The establishment of a national system of complementary genetic reserves together with systematic *ex situ* seed conservation will place Cyprus at the forefront of PGR conservation and will help to ensure the long-term protection of a significant Cypriot and global resource for future generations. This is only possible due to the close involvement of local authorities and experts, and the continuous involvement of stakeholders within Cyprus. The continued cooperation between all involved is essential for the full implementation and sustainability of the Cyprus national CWR conservation strategy.

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Boost for crop wild relative conservation in Norway

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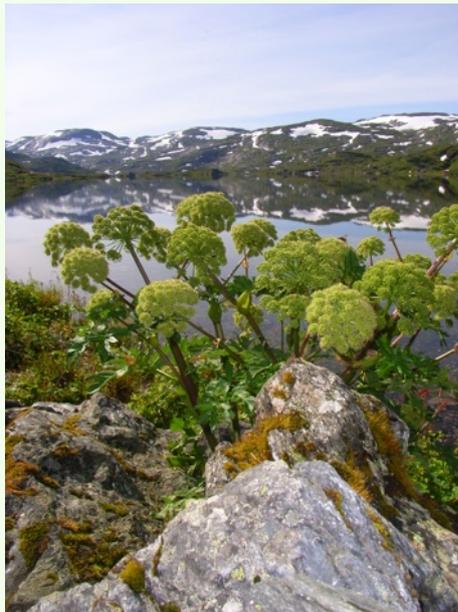
Starting from a list of 174 prioritized CWR species, initial analysis shows that 85% of these species are present in one or more of 19 10 x 10 km grid cells, each of which contains protected areas and these sites form the basis of a strategy for further in situ conservation measures in Norway. These initial achievements and a further three year collaborative project with the University of Birmingham will review the current state of conservation of CWR in Norway and produce a national strategy for Norwegian CWR conservation and use.

The Norwegian Genetic Resource Centre and its Advisory Board for Plant Genetic Resources have in recent years been discussing how to implement real *in situ* conservation of crop wild relatives (CWR) in Norway. But due to a lack of resources and skilled personnel the progress has been slow. However, last year a former student from the University of Birmingham volunteered for three months (working for six weeks in Norway during October and November 2012) helping to investigate Norwegian CWR *in situ* conservation and the results from the study gained significant attention among national authorities in Norway.

The first part of this pilot project was to identify an appropriate number of prioritized CWR species in the native flora of Norway which contains 2923 taxa of vascular plants. The prioritization was carried out in cooperation with the Norwegian Advisory Board for Plant Genetic Resources. The criteria used were: economic value of associated crops on a global, regional and national level; taxa listed in Annex 1 of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA); and taxa highlighted by national experts and plant breeders. Also, IUCN Red Listing was taken into account and some flagship species were included (i.e., species that have a certain role in promoting public awareness of the value of CWR diversity and providing good exemplars for *in situ* conservation for the authorities and public alike).

Animal production predominates in rural farming in Norway and the majority of the prioritized list of 174 species comprises grass and legume forage species, but also includes species that are either used directly as edible fruits, berries, vegetables and spices (i.e., collected from the wild), or they are closely related to such crops. The berries include species in the genera *Fragaria* L., *Ribes* L. and *Rubus* L., among them the traditional and highly appreciated cloudberries (*Rubus chamaemorus* L.) and a group of Red Listed blackberry species. Among edible plants, the Norwegian flora contains a few valuable and

not very common species and subspecies of *Allium* L., *Crambe maritima* L., *Asparagus officinalis* L. and *Angelica archangelica* L., the latter having a long and interesting record in Norwegian agriculture and cuisine for at least for the last 1000 years. Hop (*Humulus lupulus* L.) has been included in the list in accordance with a rapidly growing interest for home brewing and demand for indigenous genotypes of hop. Among the medicinal and aromatic plants (MAPs) on the priority list, *Arnica montana* L., *Mertensia maritima* (L.) Gray, *Origanum vulgare* L., *Rhodiola rosea* L. and *Thymus* L. spp. are included.



Angelica archangelica L. has long traditions in Norwegian cuisine and agriculture, and through medieval times it was exported in significant volumes. Current interest for the plant and its products is increasing (Photo: Åsmund Asdal)

With help from Dag Terje Endresen, who was recently engaged as the Node Manager for GBIF (Global Biodiversity Information Facility) Norway, it was revealed that quite significant data on presence and distribution of plant species in Norway were available in formats appropriate for data analysis. The ecogeographic data analysis in the initial pilot study showed the presence and distribution of the 174 priority species within existing protected areas, and it was revealed that 85% have been observed within 19 10 x 10 km grid cells that contain at least one protected area. Further, the analyses showed in which protected areas the concentration of priority species is the highest and indicated where conservation activities would be most

cost effectively put in place. Some potential hotspots for conserving populations of a significant number of the species have been highlighted. The pilot study also gathered more detailed knowledge for the 22 highest priority species of forage crops, edible plants and MAPs or their relatives. This information could be used in order to define and discuss implementation of concrete *in situ* measures in these areas. This study has established a firm foundation for implementing specific *in situ* conservation measures in cooperation with the nature management authorities.



Botanist Kristina Bjureke explains how *Arnica montana* L. is dependant on continued agriculture and why the species with medicinal properties has been included in the Norwegian Red List (Photo: Åsmund Asdal)

“The project will provide results and recommendations for actions to be implemented and conducted by NordGen”

The work carried out so far has been primarily a desk study, although some ground truthing in the field has occurred. The initial study was able to leverage funding for a more detailed follow-up project which will start in September 2013. The project, ‘Establishment of PGR *in situ* conservation in protected areas in Norway’ will be carried out over three years from 2013 with earmarked funding from the Norwegian Ministry for Agriculture and Food. The project will partly be carried out as PhD research in cooperation with the University of Birmingham and connected to the ongoing PGR Secure project and similar initiatives in other European countries. Other partners and contributors to the three year project will be GBIF Norway and the Natural History Museum (both located at the University of Oslo), the Directorate for Nature Management, Nordic Genetic Resource Centre (NordGen) and the county authorities in relevant counties as they are the managers of protected areas. The specific goal of the project will be national implementation of the provisions for the *in situ* conservation of plant genetic resources in relation to the FAO ITPGRFA and Global Plan of Action (GPA). The project shall further contribute to Norwegian compliance to the CBD Aichi Target 13 which is by 2020 to have sustained genetic diversity within crops and their wild growing relatives.

The main tasks in the project are:

- a) To choose 5–10 relevant hotspots with occurrence of many of the important CWR, carry out field work and ensure the quality of data on occurrence of populations and develop the scientific basis for the establishment of formal *in situ* conservation.
- b) Gather and secure the quality of occurrence data for a number of prioritized CWR in protected areas. Management authorities (counties) and the Directorate for Nature

- c) Carry out analyses based on available data in order to find the optimum number of *in situ* conservation sites to conserve a maximum part of the genetic diversity within the most important CWR and clarify how many *in situ* conservation sites are needed to conserve 99% of the alleles coding for adaptive traits of the five most important CWR in Norway.
- d) Based on national legislation on nature diversity and the ITPGRFA, develop guidelines, procedures and practical routines for collection, storage and use of seeds in *in situ* localities, and rules for the use of such genetic material. When these guidelines are in place, carry out practical seed collection in order to back up *in situ* conservation with *ex situ* conservation in seed genebanks.



Allium fistulosum L. has its habitats only on grass roofs on farm houses in the valleys in the middle of eastern Norway. The number of suitable roofs are decreasing and the plant is now Red Listed (Photo: Åsmund Asdal)

The project will provide results and recommendations for actions to be implemented and conducted by NordGen. These will include:

- a) A national action plan for *in situ* conservation of CWR diversity in Norway.
- b) Selection of pilot sites for practical *in situ* conservation.
- c) Suggestions to and cooperation with the Ministry of Agriculture and Food and relevant authorities about political issues related to *in situ* conservation (i.e., follow-up of the Norwegian Nature Diversity Act, development of legislation, adaptation of provisions for protected areas etc.).
- d) Development of options for cooperation for *in situ* CWR conservation in Norway.
- e) Promotion of links between national Norwegian CWR conservation and regional, European and global conservation initiatives.

We expect to be able to present results in meetings and conferences for national PGR programmes and for the CWR conservation community.

National Park *Bashkiria* as a reserve for crop wild relative conservation

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National Park *Bashkiria* is located on the western outskirts of the southern Urals (southeast of Bashkortostan), between Belaya and Nugush rivers (Fig. 1). It is a scientifically justified and suitable place for crop wild relative (CWR) *in situ* conservation. The Park was founded in 1986 to protect the mountain forests of the southern Urals; the forests now cover 80% of the Park's territory. Further, it was necessary to preserve the unique geological complex with numerous caves and limestone sinks named *Kutuk-Sumgan*.



Figure 1 Location of Bashkiria National Park. Left: [Wikimedia Commons](#), Right: Adapted from [Maps.at.ua](#) (2009–2010) and [INLER.ru](#) (2013).

The Park is located on the border between forest and steppe natural zones and between European and Siberian biogeographic realms. This determines the flora and vegetation diversity. The zonal vegetation type is broad-leaved (primarily lime, *Tilia cordata* Mill.) forests. There are a few fragments of Siberian pine and pine–birch forests on the Ural Mountain slopes. Steppe communities are represented by *Helictotrichon-Stipa* and shrubby steppes with a great deal of petrophytes. There are rich wet meadows along the riverbanks and steppe meadows on hillsides (www.npbashkiria.ru).

There are 765 species of higher vascular plants in the *Bashkiria* National Park flora and 160 of them are CWR. Five CWR

species are included in the regional Red Book (Mirkin, 2011): *Allium obliquum* L., *Crambe tataria* Sebeók, *Glycirhiza korschinskyi* Grig., *Trifolium alpestre* L. and *Linum uralense* Juz.. To conserve a large population of *Allium obliquum* L. (Fig. 2), the nature reserve named *Bear Glade* was established.

One of the important components of CWR conservation activities is the creation of *ex situ* collections, complementary to *in situ* conservation. For this purpose it was decided to organize a living collection for the most valuable, typical and endangered species in the National Park. In addition, these types of collection have much significance in ecological education. In 2012 the authors took part in developing the collection and in CWR investigations, starting with steppe species. Some of them can be recommended to be included in the collection. Examples include:

- *Amygdalus nana* L. (Fig. 3), a wild relative of almond. It can be used in breeding for drought resistance, salt tolerance and winter-hardy almond cultivars. It is also a honey and ornamental plant and the seeds contain useful almond oil. *A. nana* occurs in the stony steppes with *Stipa capillata* L., *S. pennata* L. and *Cerasus fruticosa* Pall. The National Park territory is the north border of its area of distribution.
- *Crambe tataria* is a European steppe species, also sometimes cultivated as a vegetable. It is being collected from the wild by the local population as a spice (roots) and a salad (edible stalks and leaves). Wild plants are a source of frost hardiness for their cultivated counterparts. The species grows on the steppe slopes with limestone and chalk exposure. An enormous *C. tataria* population is situated in the *Bashkiria* National Park on the east boundary of its area of distribution (Fig. 4).
- *Asparagus officinalis* L. (Fig. 5) is a wild relative of cultivated asparagus and can be used as a spring vegetable, for medicinal purposes (excellent remedy for blood pressure decreasing and heart strengthening) and ornamen-



Figure 2 *Allium obliquum* L. in National Park *Bashkiria* (Source: National Park Images Bank)



Figure 3 *Amygdalus nana* L. flowering (Source: National Park Images Bank)



Figure 4 Collecting seeds of *Crambe tataria* Sebeók (Photo: Snezhana Miftakhova)



Figure 5 *Asparagus officinalis* subsp. *officinalis* with fruits (Photo: Yulia Kanygina)

tal plant. Young shoots contain a lot of vitamins and microelements. Wild asparagus from *Bashkiria* has the potential to contribute drought resistance to cultivated asparagus. *A. officinalis* can be found in the Park in the steppes, steppe meadows and forest edges.

CWR growing in the National Park *Bashkiria* will be collected and all accessions will be included in the National Genebank of Russia within the partnership agreement between the Park and N. I. Vavilov Institute of Plant Industry.

Reference

Mirkin, B.M. (ed.) (2011) *Red Book of the Republic of Bashkortostan*. MediaPrint, Ufa. 384 pp.

Prioritized crop wild relatives in Spain: status on the National Inventory of Plant Genetic Resources for Agriculture and Food

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This paper aims to report progress in the prioritization of crop wild relatives (CWR) to be achieved by 2020, with the objective of establishing national conservation action plans, filling *ex situ* conservation gaps and enhancing CWR utilization, as proposed by Maxted and Kell (2012). In order to know the status of the *ex situ* conservation of CWR in Spain, we have reviewed the passport data in the database of the Spanish National Inventory of Plant Genetic Resources for Food and Agriculture (PGRFA NI) of the species included in the crop genera selected in the preparation of the prioritized Spanish CWR checklist in the context of the PGR Secure project (Rubio Teso *et al.*, 2012).

In Spain, the collection and the *ex situ* conservation of CWR, together with their *in situ* conservation in protected areas, have been key factors in the preservation of these species, particularly in those cases where the habitat had clearly been disturbed. The best example of this was the reintroduction of *Diplotaxis siettiana* Maire in Alborán Island with germplasm stored in a genebank after the only

natural population became extinct (Martínez-Laborde, 1998). Although PGRFA databases have usually been ignored in biodiversity catalogues, national inventories of plant genetic resources provide a good data source that complements those of floristic and vegetation databases (Landucci *et al.*, 2012), helping attain a global view of the status of CWR conservation. Knowledge of the CWR maintained in genebanks and their availability allows the identification of CWR species that are not conserved and is useful for making decisions related to collection and regeneration/multiplication activities. Together with the study of the National Inventory of PGRFA, the quality assessment of georeferenced passport data of the included species will be a good support to prioritize actions in order to efficiently conserve CWR species in Spain as done in other countries

(e.g., Magos Brehm *et al.*, 2008; Berlingeri and Crespo, 2012; Idohou *et al.*, 2013). We have mainly used the information from three Spanish genebanks belonging to the National Network of Plant Genetic Resources: the CRF-INIA (National Centre of Plant Genetic Resources), the BGHZ (Vegetables



Daucus carota L. (Photo: BGHZ)

Table 1 Status of prioritized CWR genera and the prioritized checklist of Spanish National Inventory of PGRFA

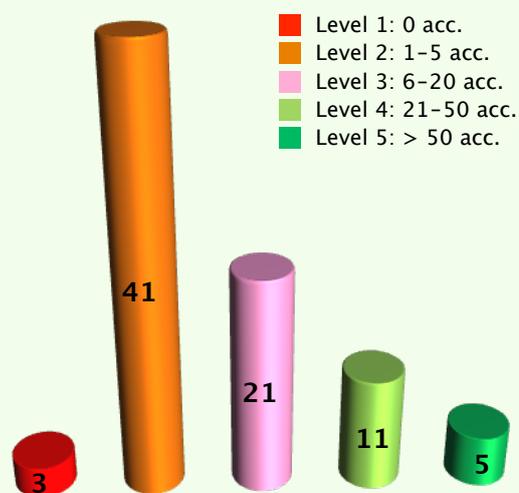
Genera	N° acc. with passport data	N° species in PGRFA NI	N° acc. in collection	N° of prioritized species
<i>Aegilops</i>	527	17	423	5
<i>Allium</i>	179	11	163	3
<i>Apium</i>	7	3	5	1
<i>Asparagus</i>	16	7	15	4
<i>Avena</i>	391	8	330	4
<i>Beta</i>	33	5	26	2
<i>Borago</i>	15	1	15	1
<i>Brassica</i>	784	36	762	7
<i>Capsella</i>	22	3	21	1
<i>Cicer</i>	4	3	4	1
<i>Cichorium</i>	18	1	13	1
<i>Cynara</i>	6	5	4	4
<i>Daucus</i>	24	4	23	1
<i>Diplotaxis</i>	311	27	311	3
<i>Erucastrum</i>	145	16	145	2
<i>Fragaria</i>	42	4	42	0
<i>Hordeum</i>	178	10	146	2
<i>Lactuca</i>	29	20	28	6
<i>Lathyrus</i>	100	13	55	7
<i>Lens</i>	65	3	36	2
<i>Moricandia</i>	83	8	82	1
<i>Patellifolia</i>	1	1	1	1
<i>Pisum</i>	10	2	8	1
<i>Raphanus</i>	87	2	87	1
<i>Secale</i>	5	1	2	1
<i>Sinapis</i>	113	7	113	2
<i>Solanum</i>	549	34	539	2
<i>Vicia</i>	504	22	412	15
Totals	4248	274	3811	81

Genebank of Zaragoza) of the Aragón Autonomous Government and the “César Gómez” genebank of the Polytechnic University of Madrid. CRF responsibilities include the conservation of safety duplicates of all Spanish seed collections, the documentation and National Inventory of the network of PGRFA collections under the National Programme and the characterization and evaluation of grain legumes, winter cereals and some industrial crops in active collections. BGHZ is responsible for one of the largest Spanish vegetable active collections, including neglected and underutilized crops. Finally, “César Gómez” Bank holds the largest Spanish collection of wild plant species.

In this study, genera that reproduce by seeds (with the exception of the genus *Fragaria* which is mainly clonally reproduced) and included in the category ‘food genera’ of Rubio Teso *et al.* (2012) were studied, whereas the remaining vegetatively reproduced crops (*Malus* Mill., *Prunus* L., *Pyrus* L., *Vitis* L. and *Olea* L.) were excluded.

A total of 4248 accessions of CWR species were identified (Table 1) in the Spanish National Inventory of PGRFA. In a first approach the accessions at genus level were analysed (column 1). The number of records by genus in the PGRFA NI passport database is shown in the second column. All prioritized genera are represented in *ex situ* collections, ranging in the number of accessions from one in *Patellifolia* A.J. Scott, Ford-Lloyd & J.T. Williams to 784 in *Brassica* L. The third column shows the number of CWR species by selected genus found in the PGRFA NI. A total of 274 species were identified. The genera *Brassica* L. and *Solanum* L. displayed the greatest diversity with 36 and 34 species, respectively.

In order to perform a more realistic approach to the conservation status of the prioritized CWR, in the fourth column, ‘N° acc. in collection’, the number of accessions by genus currently preserved in active collections is indicated. The difference between the second and the fourth columns indicates the loss of accessions over time due to poor initial quality seeds or problems in the multiplication process.

**Figure 1** Distribution of PGRFA NI species by number of accessions according to their multiplication/regeneration needs

The initial checklist of CWR of food crops was further used to generate a prioritized checklist of Spanish CWR species, which presently comprises 140 taxa (Rubio Teso *et al.*, in prep). Thus in a second approach, the species contained in the above-mentioned genera in the National Inventory of PGRFA were checked, resulting in the identification of 81 common species (column 5).

Data analysis shows that 38% of species of the prioritized CWR checklist are not included in the PGRFA NI. This means that 48 CWR prioritized species are not conserved *ex situ* (Table 2). Similar results have been previously observed in wider studies (Jarvis *et al.*, 2008). The collection and *ex situ* conservation of representatives of these species seems like a crucial step, which would be quicker and more feasible than the establishment of *in situ* reserves. Likewise, some additional species belonging to the prioritized genera, but not included in the prioritized checklist of Spanish CWR species, are included in PGRFA *ex situ* collections, particularly the genera *Diplotaxis* DC. with eight and *Vicia* L. with seven species, respectively. These already conserved accessions must be taken into account when the number of prioritized species enlarges with the inclusion of species which presently are considered to be of secondary priority (Maxted *et al.*, 2010), since some of these species are included in ongoing CWR global inventories (Vincent *et al.*, 2013).

Based on the number of accessions per species, five categories of CWR species were established according to their need of multiplication/regeneration in the Spanish genebanks (Fig. 1). According to this classification the first two levels, which include species with zero or up to five accessions, were considered as a priority for multiplication/regeneration or collection. On the first prioritization level (no accessions), *Allium ampeloprasum* L., *Cynara scolymus* L. and *Lathyrus sativus* L. were found. In the case of the latter species, there are available landraces not considered in this study, since only the wild



Vicia pannonica Crantz (Photo: L. De la Rosa)

Table 2 Prioritized CWR species not included in the Spanish genebanks

<i>Allium ampeloprasum</i> L.	<i>Cynara scolymus</i> L.
<i>Allium commutatum</i> Guss.	<i>Daucus arcanus</i> García Martín & Silvestre
<i>Allium melananthum</i> Coincy	<i>Hordeum zeocriton</i> L.
<i>Allium palentinum</i> Losa & P. Montserrat	<i>Lactuca singularis</i> Wilmott
<i>Allium pruinatum</i> Link ex Spreng.	<i>Lathyrus bauhini</i> Genty
<i>Allium pyrenaicum</i> Costa & Vayr.	<i>Lathyrus cirrhosus</i> Ser.
<i>Allium rouyi</i> Gaut.	<i>Lathyrus nudicaulis</i> (Willk.) Amo
<i>Allium schmitzii</i> Cout.	<i>Lathyrus pisiformis</i> L.
<i>Allium sphaerocephalon</i> L.	<i>Lathyrus pulcher</i> J. Gay
<i>Allium stearnii</i> Pastor & Valdés	<i>Lathyrus sativus</i> L.
<i>Allium subhirsutum</i> L.	<i>Lathyrus sylvestris</i> L.
<i>Apium bermejoi</i> L. Llorens	<i>Lathyrus vivantii</i> P. Monts
<i>Asparagus albus</i> L.	<i>Patellifolia patellaris</i> (Moq.) A. J. Scott, Ford-Lloyd & J.T. Williams
<i>Asparagus aphyllus</i> L.	<i>Patellifolia procumbens</i> (C. Sm. ex Hornem.) A. J. Scott, Ford-Lloyd & J. T. Williams
<i>Asparagus arborescens</i> Willd.	<i>Vicia altissima</i> Desf.
<i>Asparagus fallax</i> Svent.	<i>Vicia argentea</i> Lapeyr.
<i>Asparagus maritimus</i> (L.) Mill.	<i>Vicia bifoliolata</i> J. J. Rodr.
<i>Asparagus nesioties</i> Svent.	<i>Vicia glauca</i> C. Presl
<i>Asparagus plocamoides</i> Webb ex Svent.	<i>Vicia lathyroides</i> L.
<i>Avena lusitanica</i> (Tab. Morais) R. Baum	<i>Vicia leucantha</i> Biv.
<i>Brassica montana</i> Pourr	<i>Vicia nataliae</i> U. Reifengerber & Reifengerber
<i>Cichorium spinosum</i> L.	<i>Vicia pyrenaica</i> Pourr.
<i>Cynara algarbiensis</i> Coss. ex Mariz	<i>Vicia sepium</i> L.
<i>Cynara humilis</i> L.	<i>Vicia chaetocalyx</i> Webb & Berthel.

forms were under study. This is not an exception as other prioritized species also have landraces in the *ex situ* collections consulted (e.g., *Vicia sativa* L., *Apium graveolens* L., *Brassica oleracea* L. and *Pisum sativum* L.).

Nearly half of *ex situ* conserved species are grouped at the prioritization level 2: *Aegilops biuncialis* Vis, *A. geniculata* Roth, *Allium grossii* Font Quer, *A. schoenoprasum* L., *Apium graveolens* L., *Asparagus acutifolius* L., *A. officinalis* L., *A. pastorianus* Webb & Berthel., *A. stipularis* Forssk., *Avena murphyi* Ladiz., *A. prostrata* Ladiz., *Brassica bourgeauii* (Webb ex Christ) Kuntze, *Cicer canariense* A. Santos & G. P. Lewis, *Cynara alba* Boiss. ex DC., *C. cardunculus* L., *C. tournefortii* Boiss. & Reut., *Erucastrum canariense* Webb & Berthel., *Hordeum bulbosum* L., *H. distichon* L., *Lactuca livida* Boiss. & Reut., *L. palmensis* Bolle, *L. perennis* L., *L. saligna* L., *L. serriola* L., *L. virosa* L., *Lathyrus annuus* L., *L. latifolius* L., *L. ochrus* (L.) DC., *L. tuberosus* L., *Lens ervoides* (Brign), *Patellifolia webbiana* (Moq.) A.J. Scott, Ford-Lloyd & J.T. Williams, *Secale montanum* Guss., *Solanum lidii* Sunding, *S. vespertilio* Aiton, *Vicia articulata* Hornem., *V. bithynica* (L.) L., *V. cirrhosa* C. Sm. ex Webb & Berthel., *V. ervilia* (L.) Willd., *V. filicaulis* Webb & Berthel., *V. hybrida* L. and *V. cordata* Hoppe.

Twenty-one species are included at the level of prioritization 3 (6–20 accessions): *Aegilops triuncialis* L., *A. ventricosa* Tausch, *Beta macrocarpa* Guss., *B. vulgaris* subsp *maritima* (L.) Arcang., *Borago officinalis* L., *Brassica balearica* Pers., *B. barrelieri* (L.) Janka, *Capsella bursa-pastoris* (L.) Medik., *Cichorium intybus* L., *Daucus carota* L., *Diplotaxis muralis* (L.) DC., *D. tenuifolia* (L.) DC., *Erucastrum gallicum* (Willd.) O.E. Schulz, *Lathyrus clymenum* L., *L. tingitanus* L., *Pisum sativum* L., *Vicia lutea* L., *V. narbonensis* L., *V. pannonica* Crantz, *V. peregrina* L. and *V. scandens* R. P. Murray.



Lupinus mariae-josephae H. Pascual (Photo: L. De la Rosa)

Species with more than 20 accessions (levels 4 and 5) have a lower risk. However it must be pointed out that a representative sample of the diversity of each species might not be preserved. Therefore the representativeness of the prioritized CWR accessions included in the PGRFA NI should be assessed in future studies. Considering that ecogeographic diversity can be a good proxy of adaptive genetic diversity, the availability of ecogeographic characterization land maps and ecogeographic gap analysis can be a useful alternative to study which species are well sampled, when characterization or evaluation information on the accessions is not available (Parra-Quijano *et al.*, 2008).

“ecogeographic diversity can be a good proxy of adaptive genetic diversity”

In spite of the difficulties associated with the regeneration and multiplication of wild species in a different site than that of provenance, mainly related to the risk of genetic erosion, in some cases their regeneration could be recommended. In this case, the knowledge of the exact site of collection will be very useful. The quality evaluation of the georeferenced passport data of CWR included in the PGRFA NI was conducted following the procedure GEOQUAL described by Parra-Quijano *et al.* (2013). GEOQUAL produced three parameters that measured different aspects of precision and accuracy of the locality description and coordinates from passport data. These three parameters are summarized in the TOTALQUAL100 parameter which offers a global quality value in a 0100 scale. For the complete database of PGRFA NI, the average value of TOTALQUAL100 is 53.23 while the value for the subset of CWR accessions is 45.30. The most limiting quality factor detected by the GEOQUAL method for the CWR accessions was the level of accuracy of the coordinates, many of them obtained up to minutes. The explanation of the lower accuracy of wild species geographic information, especially for the oldest accessions, could be the lack of good georeferencing methods at the time when they were collected, and to the difficulties to improve data later.

In conclusion, although the Spanish PGRFA NI contains a large number of CWR species, the collection has been established taking into account the conservation of biodiversity, but not the presently selected criteria for the prioritization of CWR. This may be the main reason for the existence of vast gaps in the current *ex situ* conservation of these species. In the near future, the regeneration of existing collections, together with collecting missions for less represented CWR conserved *ex situ*, will be prioritized. Although the quality of the georeferencing of the collecting sites described in passport data of CWR included in the Spanish PGRFA NI is higher than that of the complete NI, additional efforts are required to improve their quality so appropriate gap and representativeness analysis can be undertaken.

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Are soil seed banks relevant for agriculture in our day?

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Does the botanically fascinating ability of some annual crop wild relatives (CWR) to produce waiting seed reservoirs in the soil have any relevance for modern agriculture? And should this ability be studied in connection with modern crop breeding programmes? In nature, these reservoirs aid the survival of competing genotypes in the wild-growing population by excluding them for periods of time from uncertain or harmful environmental selection. Undeniably modern crop cultivars have no need of nature for their survival, but we, the modern consumers, need an assurance of yields. *In situ* seed reservoirs would have been of no avail to the failed summer 2012 maize crop in the USA because of the cost of land. But seed reserves that have already been planted at a cost could perhaps aid in replenishing yields in certain areas of the world in which hunger epidemics are frequent and land is cheap or in any case marginal. If so, failed crops could be left to occupy the ground for a second or even third season to catch the rare environment in which the seed reserve can produce yields.

In current demographic and ecological studies, soil seed reserves are approached in banking parlance and referred to as 'banks', and the protection provided by prevention of germination as 'bet hedging' (Venable, 2007; Childs *et al.*, 2010). One way in which reserves of viable non-germinating seed in the ground are formed is passive as far as the plant is concerned. Seeds with an innate longevity remain buried in the soil until the environment supplies the necessary germination requirements. It is not really known how widespread such natural seed banks are in areas that are environmentally marginal for the survival of a respective plant species. But even less is known about autonomous banks in which the plant itself develops inhibitors to curtail germination. To a large extent, the study of such autonomous seed banks has become the pre-

rogative of weed research. The researcher is confounded by staggered germination that prevents him from exposing an entire weed population simultaneously to herbicides. *Aegilops cylindrica* or jointed goatgrass, a noxious introduced weed in North American winter wheat-growing areas and elsewhere, is such a species on which a great deal of research is invested (see Donald and Zimdahl, 1987 as an early reference).

As a test case and bid for more exhaustive studies, the present authors use this issue of *Crop wild relative* to summarize their first insight into the autonomous soil seed bank of wild emmer, *Triticum turgidum* subsp. *dicoccoides* some 20 years ago (Horovitz, 1998). This *par excellence* CWR, one of the ancestors of cultivated wheat, forms wild populations in western Iran, northern Iraq, southeast Turkey, Syria, Lebanon, Israel, Palestine and Jordan (Fig. 1). This is a plea to scientists living in those areas to investigate delayed germination in wild emmer and perhaps other related species in natural local populations. For if the idea of bet hedging is in some way applicable to modern wheat breeding, the mechanisms operating in nature need to be understood.

We studied wild emmer in the catchment area of the Upper Jordan Valley. In that area with rainless summers and winter rains averaging 600, or in Upper Galilee, 800 mm, the wild wheat forms dense stands in rocky oak-park-forest grasslands that have been cattle pastures for millennia. At maturity, the wheat spike disarticulates into arrow-shaped propagules (Fig. 2) consisting of a 2- or rarely 3-grained spikelet and a sharp-edged portion of the rachis. The pointed arrow-head enables the spikelet to penetrate dry baked ground after the rains cease (Fig. 3). Air currents cause awn movements and help insertion. The upper grain in the spikelet is usually well developed, while the lower grain is thin and often aborted. Seedling

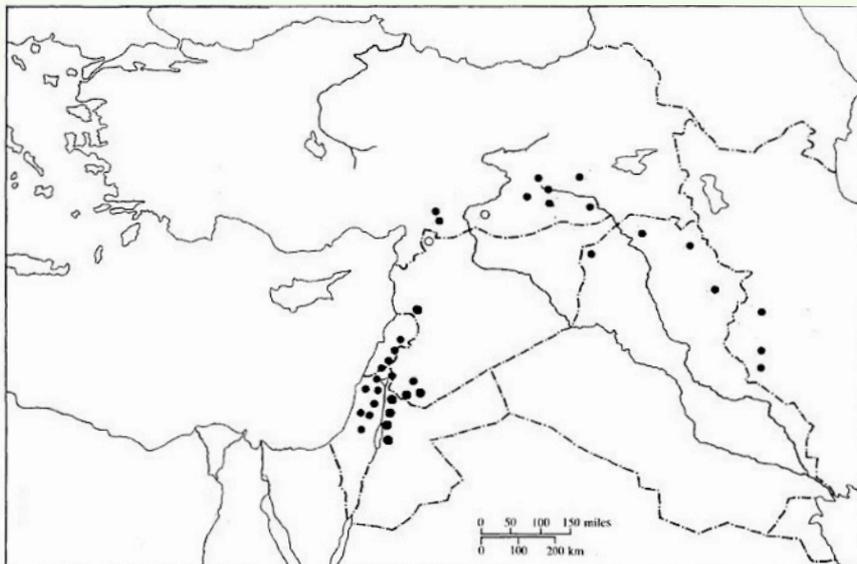


Figure 1 World distribution of wild emmer (Source: Zohary and Hopf, 2000)

Table 1 Germination frequencies of upper and bottom grains of wild emmer spikelets in the years 1993 to 1995

	Intact spikelets (703)		No germination	Separated grains	
	Upper grains	Bottom grains		Upper grains (441)	Bottom grains (547)
First year	0.80	0.06	0.20	0.86	0.16
Second year	0.01	0.35	0.19	0.01	0.43
Third year ^a	0.09	0.19			

^a Subsample of 70

emergence from the upper grain proceeds in cohorts, with partial germination after the first rains in October and complementary waves after rainfall during November and December. While rainfall in that area allows for germination in the majority of viable spikelets, it is not always sufficient for maturation of the ensuing plants. Yet populations are long lived.

In a search for the source from which populations are replenished, we monitored germination in container grown spikelets derived from the Ammiad site in Eastern Galilee. This was done at the instigation of the late botanist, conservationist and plant explorer Calvin Sperling. The spikelets and later seedlings were irrigated from planting time in October until the following March. The partially developed plants were then dried, and irrigation was resumed in October. The cycle of irrigation and drying continued through a second and third year. The same regime was employed for separately sown naked upper and bottom grains of the spikelets. These tests showed that the upper grain germinates in the autumn and winter following its separation from the spike, whereas germination in the lower grain occurs mainly in the second year or later (Table 1) and at a reduced rate.

In all, about 44% of the bottom grains formed the seed bank that produced yields in the second and third year. When naked upper and bottom grains were sown separately, 16% of the bottom grains germinated already in the first year, indicating that a part of the now removed inhibiting factors is present in the spikelet axis, the glumes and lemmas. Returning to bank-

ing parlance, each individual is endowed with its own hedge fund protecting it from exposure to hazards (mainly seasonal drought) that can prevent it from reaching maturity.

To verify this germination sequence in nature, study plots were set up in different sites in a nature reserve in the Golan Heights and were kept under observation for three years, in parallel with the *ex situ* study. Eight 4 x 4 m plots were each divided into a rim area and a central circular 0.6 m diameter observation area. The rim areas were set up to protect the observation areas from invasion by alien spikelets.

All plots were stripped of wheat spikes prior to dispersal every year. In Table 2, the studied plants in the central plots are divided into control and test micro-populations. In the latter, all plants that had flowered or at least produced a shoot in the first year were excavated before dispersal, together with the spikelet from which they had sprouted. The control populations were wiped out within two years, after the bottom grains (the seed bank) had produced their seedlings. In the test plot populations, eradication was achieved within a year. Plants flowering in 1993 had been characterized by their high molecular weight glutenin genotype, and their similarity to or identity with possible sister plants from the seed bank in 1994 or 1995 had been verified. While the appearance of single plants in test populations in the second year is ascribed to incomplete excavation of parent spikelets, two plants that appeared in test plot T2 in the third year were found to differ from other genotypes found in the area. It is assumed that their spikelets were introduced by grazing cattle.

In summary, our 1993 study indicates that a mechanism of delayed germination is present in wild emmer and extends life spans of individuals in each population. The short-term seed bank enables these plants to survive post-emergence hazards of their unstable environment, mainly droughts but also overgrazing and fire. A catastrophe that prevents seedlings from reaching maturity has to strike twice in two consecutive years to cause extinction of a population. This may be fatal for isolated patches but, if the catastrophe is local and wheat stands are continuous, re-invasion of the denuded patch by seeds from unaffected sites will occur. Such invasions, rather than recruitment from an old seed bank, may lead to changes in the resident gene pool.

It is noteworthy that the surviving seed is the least vigorous of the pair produced by the spikelet. Wild plant strategies based on delayed development and absence of vigour, have been lost in domesticated crops and are ignored in current plant breeding.



Figure 2 Mature disarticulating wild emmer plants (Photo: S. Ezrati)

Table 2 Slow extinction of micro-populations in plot centres through removal (stripping) of all spikes that could have supplied seed (control plots) and rapid extinction through removal of entire plants including the basal parent spikelet (test plots) after the first year

Year	Control plots				Test plots			
	C1	C2	C3	C4	T1	T2	T3	T4
1993 untouched	32	16	98	36	51	16	12	52
1994 stripped	5	19	46	7	5	1	0	2
1995 stripped	0	1	1	3	0	2	0	0

In a way parallel to the situation of wild tetraploid wheat in the Mediterranean climate of our study area, modern wheat cultivars in areas of hunger epidemics have enough water for germination but may suffer from or succumb to water shortage before reaching maturity, because much larger amounts of water are needed at these later stages. If the option of creating seed banks in the sown fields is deemed feasible, the inhibitors at work in delaying germination would need to be studied. The mechanism of partitioned germination in each individual is unlikely to be applicable to modern free-threshing wheat cultivars with only residual traces of modularity within the spike. Inhibitors preventing germination altogether would have to be incorporated into a percentage of each grain shipment sold. Densities of sowing would have to be adjusted, etc. Actually, inhibitors that lead to staggered germination and their times of action were studied or searched for in the CWR species *Aegilops kotschy* and *Ae. geniculata* in their native area in the 1970s.

Datta *et al.* (1970) showed a delayed germination position effect in the dispersal unit of *Ae. geniculata*, and Lavie *et al.* (1974) extracted monoepoxy linanolid from the glumes of this species, which acted as a germination inhibitor of lettuce seed. Mechanisms underlying differential seed dormancy in *Ae. kotschy* were studied by Wurzbürger *et al.* (1974). A shortage of endogenous gibberellins was found to be a key factor in delaying germination. Inhibitors causing this shortage were presumed to be present in glumes and lemmas. Wurzbürger and

Koller (1973) showed that position effects are induced during a limited period at anthesis. If the floret that produces the germinating grain is excised during that period, other florets that normally produce grains in which germination is inhibited, give rise to grains that germinate readily. It appears then that not only maternal factors but also rivalry among sibling seeds within the dispersal unit can induce dormancy. This sociological riddle was investigated by Dyer (2004) in barbed goatgrass, *Ae. triuncialis*, which has become an invasive weed in northern California.

Both environmentally imposed and autonomously created seed banks in key CWR species warrant a better understanding for their own conservation and the protection of desirable genotypes. Data should be assembled on longevity of seed buried in the soil and in different soils, the impact of deep-freeze storage on inhibition of germination, the specificity, chemistry and physiology of inhibitors, and the differences between after-ripening requirements and a state of inhibition. With regard to artificial inhibition of germination, let us learn from the wild species and perhaps imitate their bet hedging.

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Figure 3 Spikelets of wild emmer inserting themselves in the ground (Photo: S. Ezrati)

Utilization of *Aegilops biuncialis* (goatgrass) to widen the genetic diversity of cultivated wheat

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A*egilops biuncialis* Vis. ($2n = 4x = 28$, $U^bU^bM^bM^b$) could play an important role in broadening the cultivated wheat gene pool (Van Slageren, 1994). *Ae. biuncialis* is a wild species closely related to cultivated wheat (*Triticum aestivum* L.), showing a great number of agronomically useful features such as salt and drought tolerance, and disease and rust resistance (Van Slageren, 1994; Molnár *et al.*, 2004; for review see Schneider *et al.*, 2008). These useful genes can be transferred into cultivated wheat by first developing wheat–*Ae. biuncialis* amphiploids (Molnár *et al.*, 2009) followed by addition or substitution lines (Schneider *et al.*, 2005) and by inducing intergenomic translocations (Molnár *et al.*, 2009). An amphiploid is an interspecific hybrid having at least one complete set of chromosomes derived from each parent species. Addition lines carry a pair of additional alien chromosomes. Substitution line refers to a line in which one or more chromosome pairs are replaced with an alien chromosome pair(s). A translocation line is the transfer of part of a chromosome to a different position. The great genetic adaptability of *Ae. biuncialis* may be due to the natural cross between *Ae. umbellulata* ($2n = 2x = 14$, UU) and *Ae. comosa* ($2n = 2x = 14$, MM), allowing it to carry useful traits from both ancestor species. The great genetic variability of the *Aegilops* species causes polymorphism in the fluorescence *in situ* hybridization (FISH) patterns of the individual chromosomes (Schneider *et al.*, 2005; Molnár *et al.*, 2011).

In situ hybridization (ISH) is a powerful tool for the detection and identification of alien chromosomes or chromosome segments. In order to identify *Ae. biuncialis* chromosomes in different genetic materials it is necessary to use FISH, while possible chromosome rearrangements between different genomes can be detected using genomic *in situ* hybridization (GISH). GISH is a powerful technique to visualize alien chromatin in wheat–alien hybrids (Molnár-Láng *et al.*, 2000), while the multicolour GISH (mcGISH) technique enables three or more different genomes to be detected simultaneously, providing a more detailed genome analysis (Molnár *et al.*, 2009). The sequential application of FISH with several repetitive DNA probes and GISH with differentially labelled U- and M-genomic DNA allowed the characterization of wheat–*Ae. biuncialis* translocations in irradiated wheat–*Ae. biuncialis* amphiploids (Molnár *et al.*, 2009).

However, high genetic variability within *Aegilops* species causes substantial polymorphism in the FISH patterns of the individual chromosomes (Schneider *et al.*, 2005; Molnár *et al.*, 2011). This complicates the detection of the *Ae. biuncialis* chromosomes in the wheat genome, making the alien chromosomes difficult to identify. On the grounds of the high level of FISH polymorphism, it is useful to support the identification of the *Ae. biuncialis* chromosomes with the help of molecular (microsatellite, SSR) markers.

“*Ae. biuncialis* is a wild species closely related to cultivated wheat (*Triticum aestivum* L.), showing a great number of agronomically useful features such as salt and drought tolerance, and disease and rust resistance”

Materials and methods

The plant material consisted of one accession each of *Ae. umbellulata* and *Ae. comosa*, 32 accessions of *Ae. biuncialis*, 19 accessions of *Ae. geniculata* ($2n = 4x = 28$, $U^aU^aM^aM^a$), *Triticum aestivum* Martonvásári9kr1 wheat line (Molnár-Láng *et al.*, 1996), *T. aestivum* (Mv9kr1)–*Ae. biuncialis* amphiploids, 2M^b, 3M^b, 7M^b, 1U^b, 2U^b, 3U^b, and 1U^b/6U^b, and *T. aestivum* (Mv9kr1)–*Ae. biuncialis* (MvGB642; Martonvásár GeneBank No. 642) addition lines produced in Martonvásár (Logojan and Molnár-Láng, 2000; Molnár-Láng *et al.*, 2002; Schneider *et al.*, 2005).

Wheat–*Ae. biuncialis* amphiploids were produced and irradiated with ⁶⁰Co γ rays. The irradiated and control seeds were germinated and the plants were allowed to self-pollinate. Both the radiated and the non radiated (self-pollinated) generations were analysed by mcGISH to identify chromosome rearrangements involving wheat and the U^b and M^b genome chromosomes of *Ae. biuncialis*.

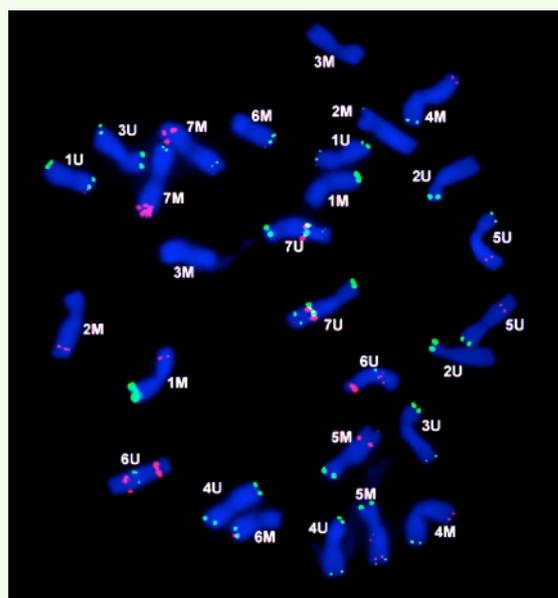


Figure 1 Fluorescence *in situ* hybridization (FISH) pattern of parental *Aegilops biuncialis* Vis. accession No. MvGB642 using pSc119.2 (green) and Afa family (red) repetitive DNA probes.

The 2M^b, 3M^b, 7M^b and 3U^b Wheat–*Ae. biuncialis* disomic addition lines produced in Martonvásár (Schneider *et al.*, 2005) were crossed with the wheat cv. Chinese Spring *ph* mutant line (Sears, 1977) in order to induce pairing of wheat and *Ae. biuncialis* chromosomes. The hybrids were grown in the nursery and their chromosome-pairing were analysed in pollen mother cells.

Root-tip and anther squash preparations were made in 45% acetic acid according to Jiang *et al.* (1994). FISH was carried out according to Szakács and Molnár-Láng (2007) and GISH following the instructions of Molnár *et al.* (2009). The repetitive DNA probes used for FISH were: pSc119.2, a 120 bp long highly repeated sequence amplified from rye genomic DNA using polymerase chain reaction (PCR), labelled with a green fluorescence dye according to Contento *et al.* (2005), and Afa family, a tandem repetitive sequence, labelled with a red fluorescence dye using PCR according to Nagaki *et al.* (1995). The clone pTa71 was labelled with 50% green fluorescence dye and 50% red fluorescence dye. The (ACG)_n microsatellite probe was amplified from the genomic DNA of *T. aestivum* and labelled with a red fluorescence dye using the same PCR conditions as applied for the production of the (GAA)_n probe (Vrána *et al.*, 2000). To carry out GISH experiments, total genomic DNA of *Ae. umbellulata* (2n=2x=14, UU) and *Ae. comosa* (2n=2x=14, MM) was labelled with biotin or digoxigenin and detected with streptavidin-FITC (green) or antidig-rhodamine (red) according to Molnár *et al.* (2009). FISH and GISH images were taken using an image analyser software and a CCD camera attached to a Zeiss epifluorescence microscope.

A total of 108 wheat SSR markers were analysed (Schneider *et al.*, 2010). PCR reactions were carried out according to Nagy *et al.* (2003) with minor modifications. Agarose gel electrophoresis was carried out using 2% gels. The bands were visualized by ethidium bromide staining. Images were taken with the help of a gel documentation system.

Results and discussion

In order to identify the *Ae. biuncialis* chromosomes in the amphiploids, addition and translocation lines, it was necessary to analyse the FISH patterns of *Ae. biuncialis* and *Ae. geniculata* (Fig. 1) carrying U and M genomes, to compare them with their progenitors (*Ae. umbellulata* and *Ae. comosa*). The FISH technique was applied using the pSc119.2, Afa family, (ACG)_n and (GAA)_n DNA probes on different accessions of *Ae. biuncialis*, *Ae. geniculata*, *Ae. comosa* and *Ae. umbellulata*. All the U and M genome chromosomes could be distinguished according to their standard FISH karyotypes (Badaeva *et al.*, 1996; Schneider *et al.*, 2005; Molnár *et al.*, 2011). Comparison of *Ae. biuncialis* and *Ae. geniculata* with their ancestors revealed differences in morphology and FISH patterns in many chromosomes belonging to the U^b, U^g, M^b and M^g genomes. The hybridization patterns of the M genomes were more variable than those of the U genomes and the identification of the M chromosomes required consideration of both the FISH pattern and chromosome morphology (i.e., the position of the centromere). U/M translocations were detected in four accessions of *Ae. biuncialis* and in two accessions of *Ae. geniculata*. Chromosome 7U was found to be involved in five of the six translocations detected between the U and M genomes of these species (Molnár *et al.*, 2011).

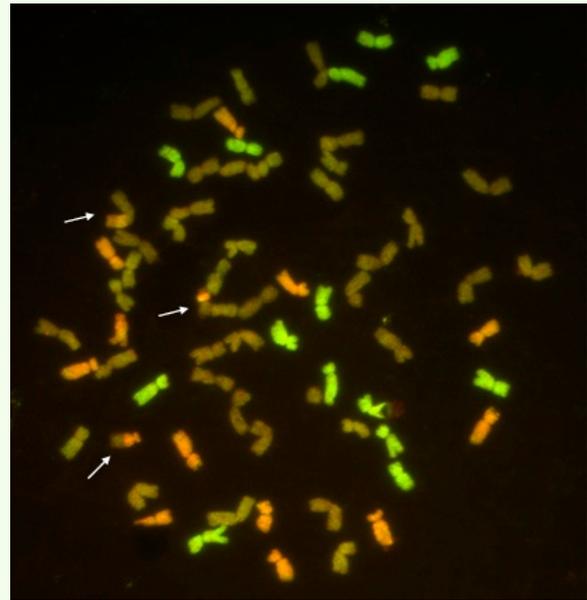


Figure 2 Multicolour GISH discrimination of U^b genome (red), M^b genome (green), and unlabelled wheat chromosomes (brown) in irradiated wheat–*Ae. biuncialis* Vis. amphiploids. Wheat–*Ae. biuncialis* translocations are indicated with arrows.

Chromosome rearrangements were induced by irradiation in wheat–*Ae. biuncialis* amphiploids to create intermediate materials valuable for the transfer of agronomically valuable traits from *Ae. biuncialis* to bread wheat. In the irradiated amphiploids, different types of translocations and chromosome fragments were the most frequent chromosomal aberrations, while the formation of insertions was rare (Fig. 2). Sequential FISH and mcGISH were used to identify chromosome aberrations in the selfed generations of the irradiated amphiploids (Molnár *et al.*, 2009). Based on the hybridization pattern of the pSc119.2, Afa family, and pTa71 probes, most of the U^b and wheat chromosomes could be identified. The identification of M^b chromosomes was unambiguous in the case of 1M^b and 6M^b, which showed pTa71 hybridization signals, but quite problematic in the remainder. The combination of mcGISH and FISH demonstrated the presence of three wheat–M^b translocations, and a wheat–wheat translocation involving chromosome 2D was also detected (Molnár *et al.*, 2009).

Seven different disomic addition lines were produced and analysed using FISH and GISH (Fig. 3) (Schneider *et al.*, 2005; Schneider and Molnár-Láng, 2012). FISH was carried out using the repetitive DNA probes pSc119.2 and Afa family. All *Ae. biuncialis* chromosomes have unique hybridization patterns in the wheat–*Ae. biuncialis* addition lines produced (Fig. 3) (Schneider *et al.*, 2005; Schneider and Molnár-Láng, 2012). GISH was applied to all of the addition lines and the *Ae. biuncialis* chromosomes were differentiated from the wheat chromosomes, but no wheat–*Ae. biuncialis* translocations were detected. Each of the disomic addition lines had different morphological features (Fig. 3), but all spikes of the Mv9kr1–*Ae. biuncialis* disomic addition lines appeared to bear a greater resemblance to those of Mv9kr1. Wheat–*Ae. biuncialis* addition lines carrying chromosomes 6M^b, 6U^b, 3U^b/5U^b/7U^b, and 5M^b/6M^b/7M^b were also selected, but the progenies of these plants exhibited a dwarfism defect or sterility. However, their parents are still available, allowing the selection of new lines carrying different *Ae. biuncialis* chromosomes.

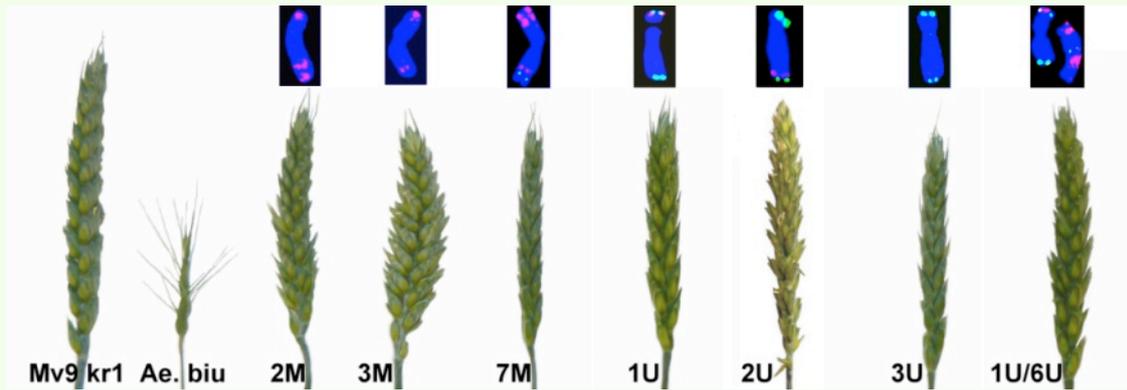


Figure 3 From left to right: spikes of Mv9 kr1 wheat genotype, *Ae. biuncialis* Vis., wheat–*Ae. biuncialis* disomic addition lines 2M^b, 3M^b, 7M^b, 1U^b, 2U^b, 3U^b and 1U^b/6U^b. The FISH pattern of one of the pair of *Ae. biuncialis* chromosomes in the addition line, obtained with the repetitive DNA probes pSc119.2 (green) and Afa family (red), is shown above each spike.

The wheat–*Aegilops biuncialis* disomic addition lines 2M^b, 3M^b, 7M^b and 3U^b were crossed with the wheat cv. ‘Chinese Spring’ *ph1b* mutant genotype in order to induce chromosome pairing between wheat and *Ae. biuncialis* chromosomes, with the final goal of introgressing *Ae. biuncialis* chromatin into cultivated wheat. Wheat–*Aegilops* chromosome pairing was studied in the hybrids. Using U and M genomic probes, GISH demonstrated the occurrence of wheat–*Aegilops* chromosome pairing in the case of chromosomes 2M^b, 3M^b and 3U^b, but not in the case of 7M^b (Molnár and Molnár-Láng, 2010). The wheat–*Aegilops* pairing frequency decreased in the following order: 2M^b > 3M^b > 3U^b > 7M^b, which may reflect differences in the wheat–*Aegilops* relationships between the examined *Aegilops* chromosomes (Molnár and Molnár-Láng, 2010). The selection of wheat–*Aegilops* recombinations could be successful in later generations.

A total of 108 wheat SSR primers were tested on wheat line Mv9kr1 and *Ae. biuncialis* in order to help the exact identification of the *Ae. biuncialis* chromosomes in wheat background. Some wheat SSR markers give bands on *Ae. biuncialis* due to the close relationship between wheat and *Ae. biuncialis*. Out of the 108 SSR markers, 79.62% gave PCR products on *Ae.*

biuncialis, 51 markers (47.22%) were non-polymorphic between Mv9kr1 and *Ae. biuncialis*, while for 22 markers (20.37%), bands were obtained on wheat line Mv9kr1, but no PCR product was obtained with *Ae. biuncialis*. A further 35 SSR markers (32.40%) were polymorphic (i.e., PCR products with different fragment lengths were obtained on Mv9kr1 and *Ae. biuncialis*). These markers were tested for *Ae. biuncialis* chromosome specificity on the wheat–*Ae. biuncialis* addition lines. Three markers (8.57% – GWM44, GDM61 and BARC184) gave specific PCR products on the addition lines. Presumably the remaining 32 polymorphic markers were located on *Ae. biuncialis* chromosomes for which no addition lines were available (Fig. 4) (Schneider *et al.*, 2010).

The U and M genomes are present in 13 of the 23 *Aegilops* species, most of which are valuable gene sources for wheat improvement. The present results showed that a combination of the FISH technique, using several DNA probes [pSc119.2, Afa family, pTa71, (ACG)_n and (GAA)_n], and mcGISH leads to the more accurate identification of U and M chromosomes in the wheat background (Molnár *et al.*, 2011). The level of FISH polymorphism was higher in the M^b genome, than in the U^b genome. These results strengthen the pivotal-differential evo-

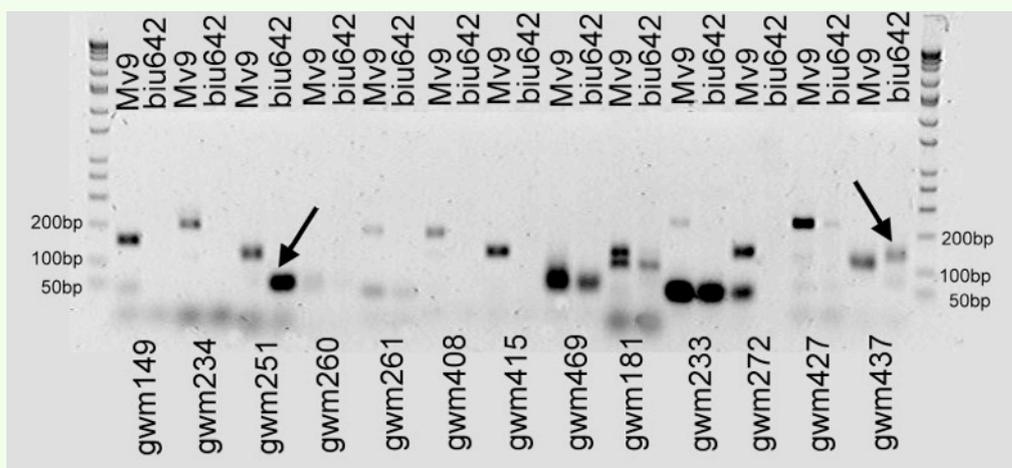


Figure 4 Band patterns obtained for the wheat SSR markers GWM149, GWM234, GWM251, GWM260, GWM261, GWM408, GWM415, GWM469, GWM181, GWM233, GWM272, GWM427 and GWM437 on wheat line Mv9 kr1 (Mv9) and on the *Ae. biuncialis* Vis. genebank accession MvGB642 (biu642). Bands specific to *Ae. biuncialis* are indicated with arrows. Among the two markers seen to give polymorphic bands on *Ae. biuncialis* (GWM251 and GWM437), none exhibited chromosome specificity on wheat–*Ae. biuncialis* addition lines.

lution theory, which suggests that *Aegilops* species having 28 chromosomes and sharing the U genome, but differing in their second pair of genomes, can explain the dissimilarities between the modified M genomes present in various UM species (Zohary and Feldman, 1962). In *Ae. biuncialis* and *Ae. geniculata* the U genome is the pivot (stable genome), while M genome is differential.

“The conservation of the genetic variability of wild species and the utilization of available accessions are important for the future of wheat production”

The aim of this study was to produce wheat–*Ae. biuncialis* amphiploids, addition and translocation lines, and to identify them using different molecular cytogenetic techniques. The development of wheat–*Ae. biuncialis* addition lines allows the study of the genetic effects of individual chromosomes added to the wheat genome, the tracing of *Ae. biuncialis* chromosomes in the translocation lines produced, and the determination of the chromosomal location of any resistance genes transferred from *Ae. biuncialis* into wheat. The importance of wild wheat genetic resources, such as *Aegilops* species, is widely recognized. Therefore, pre-breeding is a promising alternative to involve *Aegilops* genetic resources (e.g., for salt and drought tolerance, and leaf rust resistance) in breeding programmes. The conservation of the genetic variability of wild species and the utilization of available accessions are important for the future of wheat production. Alien chromosome additions and translocations are useful tools in plant genetics research and breeding, as they serve as a bridge for the transfer of agronomically useful traits from wild species into cultivated wheat. Although many wheat genetic materials containing genes from *Aegilops* species have been developed, the transfer of useful traits from *Aegilops* species into cultivated wheat cannot be regarded as complete. Continued effort is currently underway to transfer genes from various *Aegilops* species, so new genetic materials containing favourable traits from these species should be available in the future. Many *Aegilops* species remain unexploited despite the availability of many accessions in genebanks.

Acknowledgements

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Status of collections of *Aegilops* spp. in Bulgaria and opportunities for their use in breeding programmes

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Conservation of plant genetic resources (PGR) of cultivated and wild flora is a priority worldwide. It is encapsulated in the Convention on Biodiversity (CBD) which Bulgaria ratified in 1996. The Institute of Plant Genetic Resources in Sadovo is the Coordinator of the National PGR Programme and a member of the European Cooperative Programme for Plant Genetic Resources (ECPGR). On the territory of the Institute is located the National Seed Genebank where 57,684 seed samples are preserved (<http://eurisco.ecp-gr.org>). The plant species diversity is represented by more than 2670 species of crops and their wild relatives.

Crop wild relatives (CWR) are an important resource for plant breeding and the focus of genetic and biological research (Ko-

eva *et al.*, 1994; Zaharieva, 1998). Species from the genus *Aegilops* are increasingly important sources for extending the genetic basis of common and durum wheat. As a result of work in the period 1988–1991, a programme of collection and study of *Aegilops* species from Bulgaria was developed.

A new species of the flora of Bulgaria was determined—*Aegilops comosa* Sm.—and was identified in a location of rich species diversity (Angelova *et al.*, 2002). Rich *ex situ* collections are established and maintained (Zaharieva, 1998) with 648 accessions of 30 *Aegilops* species registered in the National Genebank (Table 1). The main species in the collection are *Ae. triuncialis* L. (151 accessions), *Ae. cylindrica* Host (89), *Ae. ovata* L. (82) and *Ae. biuncialis* Vis. (70). Foreign

Table 1 Status of the *Aegilops* collection maintained in the National Genebank of Bulgaria

Species	No. of accessions (accessions in long-term storage are shown in parentheses)	Status of the germplasm		
		Bulgarian origin	Received from abroad	Unknown
<i>Ae. aucheri</i> Boiss	1	–	1	–
<i>Ae. bicornis</i> (Forssk.) Jaub. & Spach	3	–	3	–
<i>Ae. biuncialis</i> Vis.	70 (41)	59 (35)	8 (3)	3 (3)
<i>Ae. caudata</i> L.	12 (4)	6 (3)	5 (1)	1
<i>Ae. columnaris</i> Zhuk.	9 (4)	–	5 (1)	4 (3)
<i>Ae. comosa</i> Sm.	6 (2)	3 (2)	3	–
<i>Ae. crassa</i> Boiss.	8 (1)	–	6 (1)	2
<i>Ae. cylindrica</i> Host	89 (34)	53 (22)	28 (9)	8 (3)
<i>Ae. geniculata</i> Roth	11 (9)	–	10 (9)	1
<i>Ae. juvenalis</i> (Thell.) Eig	4 (1)	–	3 (1)	1
<i>Ae. kotschyi</i> Boiss.	5 (1)	–	3 (1)	2
<i>Ae. longissima</i> Schweinf. & Muschl.	7	–	3	4
<i>Ae. lorentii</i> Hochst.	1 (1)	–	1 (1)	–
<i>Ae. macrochaeta</i> Shuttlew. & A. Huet ex Duval	1	–	–	1
<i>Ae. markgrafii</i> (Greuter) K. Hammer	1	–	1	–
<i>Ae. mutica</i> Boiss.	1	–	1	–
<i>Ae. neglecta</i> Bertol.	21 (14)	18 (13)	3 (1)	–
<i>Ae. ovata</i> L.	82 (69)	42 (38)	38 (30)	2 (1)
<i>Ae. peregrina</i> (Hack.) Maire & Weiller	2 (2)	–	2 (2)	–
<i>Ae. searsii</i> Hammer	6	–	1	5
<i>Ae. speltooides</i> Tausch	22 (5)	3	12 (4)	7 (1)
<i>Ae. squarrosa</i> L.	16 (4)	–	8 (2)	8 (2)
<i>Ae. tauschiei</i> Coss.	44 (11)	1	33 (8)	10 (3)
<i>Ae. triaristata</i> Willd.	40 (32)	22 (17)	10 (8)	8 (7)
<i>Ae. triuncialis</i> L.	151 (103)	96 (70)	48 (27)	7 (6)
<i>Ae. umbellulata</i> Zhuk.	6 (2)	–	4 (1)	2 (1)
<i>Ae. uniaristata</i> Vis.	3 (1)	–	3 (1)	–
<i>Ae. variabilis</i> (Maire & Weiller) Eig	1	–	–	1
<i>Ae. vavilovii</i> (Zhuk.) Chennav.	1 (1)	–	1 (1)	–
<i>Ae. ventricosa</i> Tausch	9 (4)	–	9 (4)	–
<i>Ae. spp.</i>	15 (5)	11 (5)	2	2
	648 (351)	314 (205)	255 (116)	79 (30)

– = No accessions



Aegilops caudata L. (Photo: Institute of Plant Genetic Resources, Sadovo, Bulgaria)

material is represented by samples originating from Morocco (59), Azerbaijan (49), Armenia (26), Syria (18), Russia (17) and France (16). There are 79 accessions of unknown origin (Table 2). Bulgarian material constitutes more than 48% of the samples in the collection. They are represented entirely by local forms and populations collected from different regions of the country (Table 3). Ten species have been collected: *Ae. triuncialis*, *Ae. biuncialis*, *Ae. cylindrica*, *Ae. ovata*, *Ae. triaristata*, *Ae. neglecta*, *Ae. caudata*, *Ae. comosa*, *Ae. speltoides* and *Ae. tauschii*. Samples of *Ae. triuncialis* account for more than 30% of the collection, with *Ae. biuncialis*, *Ae. cylindrica* and *Ae. ovata* accounting for more than 18%, 16% and 13% respectively. The other species are represented by a smaller number of samples.

Chapman (1989) found that wild species are present in the pedigree of only 10% of modern crop varieties. According to Monneveux *et al.* (2000), distal hybridization is one of the most effective ways to obtain a plant with an alternative type of physiological process, which may be the basis for research efforts to improve the yield of crop plants and their resistance to stress factors. Reasons for the increasing interest in including the genetic diversity of wild species in crops are: the development in recent years of methods that allow rapid screening of large numbers of accessions in a variety of physiological and agronomic traits; development methods using embryo cultures (*in vitro*); and the rapid advance of biochemical and molecular methods that allow the precise identification of the genes and markers that provide high inheritance of agronomically useful attributes.

The wide range of distribution of the species of the genus *Aegilops* and adaptability to different climatic conditions are factors determining their valuable agronomic characteristics. The wild progenitors of wheat are the main source of valuable genes that increase the tolerance of wheat to fungal diseases, insect pests, salt tolerance, increased tolerance to copper and aluminium, drought and cold resistance, and other stresses

Table 2 Origin of accessions of *Aegilops* spp. maintained in the National Genebank of Bulgaria

Country of origin	No. of accessions
Bulgaria	314
Unknown	79
Morocco	59
Azerbaijan	49
Armenia	26
Syria	18
Russia	17
France	16
Iran	8
Romania	8
Turkey	8
Germany	7
Jordan	7
Other countries	32
Total	648

(Amri *et al.*, 1992; Schneider *et al.*, 2008; Stoyanov *et al.*, 2010; Plamenov and Spetsov, 2011). Further, the wild progenitors provide greater genetic diversity of the endosperm proteins gliadins and glutenins which are associated with the baking qualities of bread wheat. Several studies have shown that many gliadin alleles from the wild progenitor have a positive influence on the strength of the gluten (Sosinov and Popereya, 1982; William *et al.*, 1993). Therefore gliadins and high molecular weight glutenins are subject to transfer from *Ae. tauschii* in hexaploid wheat for quality improvement (Yan *et al.*, 2003a,b; Xu *et al.*, 2010).

The collection of local germplasm of *Aegilops* spp. which are maintained in IPGR-Sadovo carry genes for resistance to abiotic and biotic factors. Results of a study on resistance to powdery mildew and rusts suggest that the majority of populations of *Aegilops* species from Bulgaria are characterized by a high level of resistance to powdery mildew (Dimov *et al.*, 1993; Zaharieva, 1998; Zaharieva *et al.*, 2003). Under field conditions, 54.7% of the studied samples are shown as sustainable. Four diploid (*Ae. caudata*, *Ae. speltoides*, *Ae. comosa*, *Ae. umbellulata*) and two tetraploid forms (*Ae. ovata*, *Ae. neglecta*) are characterized by major resistance to powdery mildew, while samples of *Ae. cylindrica* are highly sensitive. Further, 60% of *Ae. biuncialis* and 32% of *Ae. triuncialis* accessions included in the study show resistance to powdery mildew.

The majority of accessions of *Ae. biuncialis* and *Ae. triuncialis* are resistant to brown rust (*Puccinia recontita*) and those from *Ae. cylindrica* are highly sensitive. Many of the accessions of *Ae. neglecta*, *Ae. ovata* and *Ae. triuncialis* are characterized by resistance to yellow rust (*Puccinia striiformis*), while good cold resistance is exhibited by *Ae. crassa* and *Ae. tauschii* (containing chromosomes of the D genome) and *Ae. speltoides* (G genome). In the group which are most sensitive to cold are samples of *Ae. caudata* (Uhr *et al.*, 2007a). Information

Table 3 Collecting regions of *Aegilops* spp. in Bulgaria

Species	Collecting regions
<i>Ae. cylindrica</i> , <i>Ae. neglecta</i>	Central and south
<i>Ae. speltoides</i>	Trakian Valley, Rodopi mountain
<i>Ae. biuncialis</i> , <i>Ae. triuncialis</i>	Central and southwest
<i>Ae. ovata</i>	Trakian Valley, Mesta and Strandja Valley
<i>Ae. umbellulata</i> , <i>Ae. comosa</i>	Trakian Valley
<i>Ae. caudata</i> , <i>Ae. tauschii</i>	Trakian Valley



Aegilops triaristata Willd. (Photo: Institute of Plant Genetic Resources, Sadovo, Bulgaria)



Aegilops cylindrica Host (Photo: Institute of Plant Genetic Resources, Sadovo, Bulgaria)

about high levels of cold tolerance of different accessions is published by other authors (Limin and Fowler, 1981; Barashkova, 1991; Tahir and Ketata, 1997). Accessions from the collection of IPGR-Sadovo are included in breeding programmes for cold tolerance in wheat.

Studies have also found that populations of *Ae. tauschii*, *Ae. crassa*, *Ae. caudata* and *Ae. umbellulata* are beneficial sources of genes for tolerance to osmotic stress and may be used as a source of drought resistant genes for common and durum wheat (Stankova *et al.*, 1995; Uhr *et al.*, 2007b). Due to their exceptional adaptability to different climatic conditions, these four *Aegilops* species are included in interspecific hybridization programmes.

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Crops and wild species from the Apiaceae family – perspectives from the Nordic region

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The Apiaceae family includes economically important vegetables such as carrot (*Daucus carota* L.), celery (*Apium graveolens* L.) and parsnip (*Pastinaca sativa* L.), but also a wide range of spices and medicinal plants, where caraway (*Carum carvi* L.) and angelica (*Angelica archangelica* L.) are of historical importance for the Nordic region. The Nordic Genetic Resource Centre (NordGen) works on various aspects of conservation and use of plant genetic resources and holds an *ex situ* collection of c. 32,000 accessions. Out of these, 7600 are classified as wild or semi-wild material, with the grasses as the dominant group. Wild or semi-wild material from the Apiaceae family is limited to 69 accessions of *C. carvi*, 35 of *A. archangelica*, 16 of *D. carota*, 4 of *P. sativa*, and a few from other species. The global database FAO WIEWS (2013) reports 228 wild/semi-wild accessions of *C. carvi* and 73 of *A. archangelica*, while the European database EURISCO (2013) reports 182 and 38 accessions respectively (Fig. 1).

Caraway

Caraway (*C. carvi*) is used primarily as a spice in bread, cheese and aquavit and is an important aroma in the Nordic kitchen. Caraway grows wild, but almost all the commercial use is based on cultivated caraway and commercial varieties. The regional food industry relies to some extent on caraway produced in the Nordic countries, but does also import it from Central Europe and other regions. Wild growing caraway could be originally wild material, but could also be semi-wild natural-

ized caraway from earlier cultivations. For example, the introduction of caraway to Iceland is known to have occurred in the mid-17th century by Gísli Magnússon (1621–1696) who settled in Hlíðarendi in the south of Iceland and allegedly brought seeds from Denmark or the Netherlands (Benediktsson, 1939). In Hlíðarendi, caraway can still be found growing semi-wild in the meadows (recently collected and conserved as accession NGB20109). Later it spread throughout Iceland.

The importance of quality is stressed by most actors in the food system in our region, but few studies have been carried out to analyze the quality of caraway. The studies of chemical content have revealed variation both in the amount of essential oils and their ratio (e.g. Galambosi and Peura, 1996; Børtnes and Mordal, 2010). The effect of composition and concentration of essential oil components of caraway seeds on subtle taste properties in aquavit has as far as we know not been investigated. Together with a company we started a pre-study in 2009 (unpublished), where three cultivars and eight wild/semi-wild accessions were grown in two different locations: Iceland and Sweden. The aim was to find material with an optimal quality for aquavit production, but also to find any 'genotype by environment' effects.

The examined cultivars had an average thousand seed weight that was higher than the wild/semi-wild accessions, and the plants were in general bigger and had a more upright growth

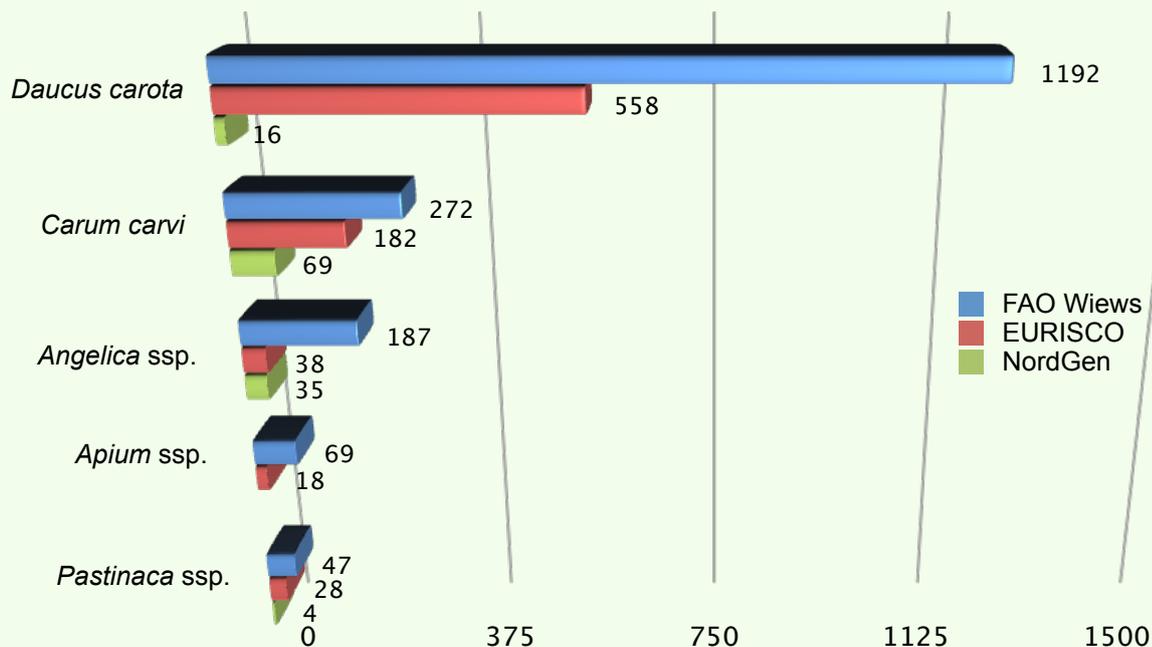


Figure 1 Numbers of wild/semi-wild accessions of selected Apiaceae species reported by FAO WIEWS, EURISCO and NordGen



Figure 2 Collecting angelica seeds in Iceland (Photo: S.Ø. Solberg)

pattern than the wild/semi-wild material. The content of the two main components of caraway essential oil, carvone and limonene, was highest in the cultivar 'Polaris'. Location had no significant effect on the mentioned oils; however, minor essential oil components differed between accessions as well as between the growth locations.

Angelica

Angelica (*A. archangelica*) is one of the oldest cultivated plants of origin in the Nordic and the Arctic region. The tall herbaceous plant has been used as a vegetable and medicine by the Sami as well as the Inuit people by collecting plants from the wild. Cultivation started a long time ago. Written records from the 11th century describe angelica and it was part of the food of the Vikings travelling between Iceland, Norway and in the north Atlantic region. Several hundred year old Norwegian records use terms such as "angelica gardens". A local variety of angelica called "Vossakvann" is found in the western parts of Norway. This variety is unique, with its solid petioles and sweet taste. The history of its origin is unknown, but most likely "Vossakvann" is a result of selection during cultivation (Fægri, 1951). Today angelica is not commonly used, but initiatives have been made on local food culture and within the concept of "the New Nordic food" which highlights traditional and food and knowledge in new settings. Angelica is part of this food culture.

Over the last years NordGen has collected angelica seeds from wild populations in Greenland, Iceland (Figure 2), Faroe Islands, Norway, Sweden and Finland. An initial study using ISSR molecular markers was published by Göransson *et al.* (2011). The study analyzed and discussed the genetic diversity within and between angelica populations. Furthermore, the study indicated relatedness between the Norwegian traditional cultivar "Vossakvann" and the Icelandic wild populations. Wild angelica populations from the eastern part of Norway showed closer relationship to the material from southern Sweden. Further studies are in progress. Work has also been carried out on germination protocols on wild species (Leo, 2013). Many of the species from the Apiaceae family have dormant seeds. The seeds need some kind of stratification or cold treatment to germinate. Angelica needs at least 4–6 weeks of stratification. Such information is crucial to maintain *ex situ* conserved accessions.



Figure 3 Characterization of wild carrot. Two collections of wild carrot (left and right) compared to cultivated carrot of the Nantes type (centre) – NGB547. (Photo: S.Ø. Solberg)

Wild carrot

Cultivated and wild carrot (*D. carota*) differ in a lot of characters. Wild carrot has the potential of being used in breeding to introduce new disease and pest resistance genes (GenRes, 2002). NordGen is a partner in the European Cooperative Programme for Plant Genetic Resources (ECPGR) Umbellifer working group project on wild carrot. The project includes studies on resistance to the pathogen *Alternaria dauci* carried out at the Julius Kühn Institut Bundesforschungsanstalt für Kulturpflanzen (JKI), Germany, but also characterization of genebank accessions (Figure 3) and work related to taxonomy and molecular markers. The funding has so far been based on self-financing from participatory institutions and a small contribution from the ECPGR.

There are two subspecies of wild carrot in the Nordic region: *D. carota* subsp. *carota* which is common in the southern parts of the region, and *D. carota* subsp. *gummifer* (Syme) Hook. f., which is rare and only found in coastal areas of parts of Denmark (Poulsen, 2009). Currently only one of the accessions in the NordGen collection is of subsp. *gummifer*.

Other Apiaceae species

Wild relatives of other crops from this family can be found in the Nordic region, such as *Pastinaca sativa* that is very common in southern Scandinavia, and *Apium graveolens* L. that is rare and weakly naturalized. The same is to be said for *Levisticum officinale* W.D.J. Koch, while *Myrrhis odorata* (L.) Scop. tends to be invasive. The Nordic region also hosts five different species of *Chaerophyllum* L., four subspecies of *Aethusa cynapium* L., and the very poisonous *Conium maculatum* which is believed to have been used as medicine or to kill people in the old days.

In situ conservation

NordGen is not involved with *in situ* conservation actions, but facilitated two workshops focusing on CWR in protected areas. A Nordic environmental group was established. However, the policy-makers have not prolonged the mandate for the group and *in situ* conservation is now fully in the hands of each country. In Sweden a priority list of CWR has been made (Weibull, this issue), and *Apium graveolens* is on this list. The same species is also Red Listed as Critically Endangered (CR) in Sweden (Artsfakta, 2013). Another Red Listed CWR species of the Apiaceae family is *Chaerophyllum prescottii*

that grows in northern Sweden. Work on CWR is reported from Denmark (Poulsen, 2009). Inventories have been made but targeted conservation of CWR is not carried out. In Norway an action plan for CWR is under development (Asdal *et al.*, this issue).

Concluding remarks

Breeding of vegetables, spices and medicinal plants is (with few exceptions) not carried out in the Nordic region any more. When priorities are made, the minor crops risk being put on hold, giving focus for more economically important crops. Our contribution is an example of the opposite. The increasingly important local and regional food culture highlights the need for access to diversity and authenticity. Genebanks may have a role to play in serving these networks with material and information. With access to genebank material, the plants can be cultivated instead of collecting them from the wild with the risk of destroying natural habitats. Genebanks do not usually work with *in situ* conservation but we may act as distribution channels and a back-up for material conserved in protected areas.

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Collecting and preliminary evaluation of grape wild relatives in China

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Wild relatives of grape (*Vitis* spp.) are potentially important sources to provide valuable traits for the improvement of cultivated grape varieties. They possess significant characters resistant to biotic and abiotic stresses, such as cold, drought, pests and diseases, and are useful for grape breeding. In addition, many fine varieties were selected from wild relatives of grape and have been applied in the field production (Jiang *et al.*, 2011). China is one of the major centres of diversity of grape and is therefore one of the most abundant sources of *Vitis* germplasm in the world. There are 39 species, one subspecies and 14 varieties of grape wild relatives native to China (Kong, 2004), which are distributed in all the provinces except Xinjiang Uygur Autonomous Region (Fig. 1). According to the China Species Red List, *V. yunnanensis* C.L. Li, *V. wenchouensis* C. Ling ex W.T. Wang and *V. hui* Cheng are highly threatened with less than five localities each, and moreover, their populations are in continuous decline. There is only one known locality of both *V. bashanica* He P.C. and *V. mengziensis* C.L. Li



Figure 1 The number of grape wild relatives distributed in each province of China

and the populations are under serious threat (Wang and Xie, 2004).

We have collected grape wild relatives from their main distribution areas since 2002, which covered Beijing, Henan, Gansu, Hunan, Hubei, Jiangxi, Guangxi, Zhejiang, Yunnan, and Tibet Autonomous Region of China (Figs. 2 and 3). By the end of 2012, as the Zhengzhou national repository for grapevine, we have collected 23 species, one subspecies and one variety, while the national repository for grapevine in Zuoqia has collected and conserved 380 accessions of *V. amurensis* Rupr. and the national repository for grapevine in Taigu has collected and conserved two species (Ren *et al.*, 2012). Nearly half of grape wild relatives need further collection and protection.

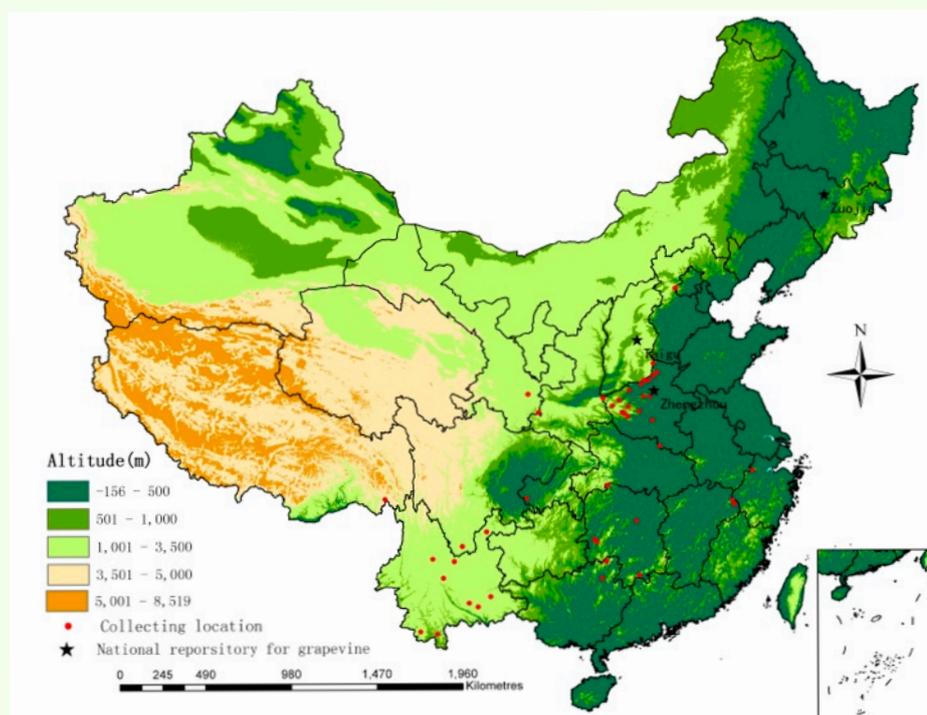


Figure 2 Locations where we collected the wild relatives and the three national repositories for grapevine

Table 1 Main characteristics of 14 grape wild relatives and one cultivar (results of preliminary evaluation)

Taxon	No. of accessions evaluated	Time of bud burst (M.D)	Time of full flowering (M.D)	Time of berry ripening (M.D)	Berry growth period (D)	Bunch size (cm ²)	Bunch weight (g)	Berry size (cm ²)	Berry weight (g)
<i>Vitis adenoclada</i> Hand. Mazz.	6	4.14–4.19	5.29–6.06	8.30–9.14	73–85	36.5–127.7	27.4–42.8	1.4–1.5	0.8–1.0
<i>V. amurensis</i> Rupr.	5	3.15–4.01	4.23–4.26	7.25–8.06	72–82	36.1–72.5	22.9–56.7	1.2–1.6	0.7–1.2
<i>V. betulifolia</i> Diels & Gilg	3	3.28–4.05	4.22–4.26	7.26–8.27	79–98	20.3–61.3	8.4–12.0	0.8–1.0	0.4–0.5
<i>V. bryoniaefolia</i> Bge.	7	3.24–3.28	4.25–4.27	7.02–7.18	59–63	55.0–81.8	27.3–57.5	0.6–1.4	0.6–0.9
<i>V. davidii</i> (Rom. Caill.) Foëx	22	3.16–4.17	4.27–5.14	8.09–9.04	74–94	59.8–210.7	45.2–283.3	2.3–4.1	1.8–4.8
<i>V. flexuosa</i> Thunb.	1	4.05–4.10	5.07–5.09	7.29	83	7.5	3.3	0.6	0.3
<i>V. hancockii</i> Hance	1	3.26	4.23	6.28	53	5.6	2.5	0.4	0.2
<i>V. heyneana</i> Roem. & Schult.	2	4.15	6.03	9.16	81	80.5	57.5	0.2	1.5
<i>V. heyneana</i> subsp. <i>ficifolia</i> C.L. Li	8	4.08–4.16	5.17–5.25	8.11–9.08	65–79	44.2–108.2	28.9–110.9	0.8–1.4	0.5–1.0
<i>V. piasezkii</i> Maxim.	11	3.27–4.05	4.23–5.07	7.15–7.26	63–65	12.8–43.7	4.9–14.0	0.4–1.1	0.4–0.7
<i>V. pseudoreticulata</i> W.T. Wang	6	4.10–4.19	5.12–5.21	7.11–7.29	54–59	53.6–91.7	28.1–73.5	0.7–0.9	0.4–0.5
<i>V. romanetii</i> Rom. Caill.	3	3.27–3.30	4.22–4.27	7.17–7.21	68–70	84.1–85.5	16.1–23.1	0.8–1.3	0.4–0.9
<i>V. vinifera</i> L. 'Muscat Hamburg'	1	4.04	5.19	8.02	75	240.5	517.1	5.3	4.2
<i>V. wilsoniae</i> Veitch	2	4.02–4.05	5.05–5.07	8.02–8.05	73–74	47.5	14.3	1.4	0.9
<i>V. yeshanensis</i> D.Z. Lu et H.P. Liang	1	3.27–4.06	4.30–5.06	7.29–8.06	63–67	52.2	7.8	0.7	0.3

M = month; D = day



Figure 3 Collecting grape wild relatives and recording detailed information in Sanqingshan Nature Reserve, Jiangxi province (Photo: Jianfu Jiang)

As one of the national repositories for grapevine (Zhengzhou), in 2012 we studied 78 accessions of 13 grape wild relative species, one subspecies, and one cultivar. A preliminary evaluation of the phenophase and fruit traits of those accessions was carried out following the standards of Liu *et al.* (2006) (Table 1).

The time for phenophase varies among and within species. For example, the species with the earliest time of bud burst is *V. amurensis*, which is also the most cold-hardy species and is mainly concentrated in northeast China (Fig. 4). *V. hancockii* Hance has the earliest berry ripening date which is 80 days earlier than *V. heyneana* Roem. & Schult., and both of the two species can be used as early-maturing and late-maturing material for grape breeding, respectively. The berry size and weight of most grape wild relatives is small, but that of *V. davidii* (Roman. Du Caill.) Foëx is large, of which the biggest size and weight is 4.1 cm² and 4.8 g, respectively. It is used for making wine or as a table fruit in southern China (Figs. 5 and 6). At same time, it has been reported that *V. davidii* possesses genes for resistance to anthracnose and a wet climate.

In conclusion, China has a high diversity of grape wild relatives with great potential for improving cultivated grapevine. We recommend that firstly, grape wild relative germplasm—especially of the threatened species—must urgently be collected and conserved to capture diversity before further genetic erosion takes place. Secondly, all materials must be characterized and made available for use in grapevine breeding. Finally, techniques for the identification and utilization of stress-resistant genes from grape wild relatives using modern molecular biology techniques need to be developed.

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Figure 4 *V. amurensis* Rupr. can tolerate severe below zero temperatures down to -40 C. It is mainly used locally for making red wine. (Photo: <http://icgr.caas.net.cn/32pu/aa-szjspt.htm>)



Figure 5 *V. davidii* (Rom. Caill.) Foëx has large berries and strong resistance to anthracnose (Photo: Jianfu Jiang)



Figure 6 Selling *V. davidii* in a fruit shop in Huaihua, Hunan province (Photo: Dawei Cheng)

Diversity of crop wild relatives in the Egyptian flora

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The interaction between a given crop and its wild relatives has become an issue of great global concern, especially the wild relatives of the economic food and fodder species. The Egyptian flora comprises a considerable number of crop wild relatives (CWR) which still exist in the wild. This work will throw light on the potential CWR species within the Egyptian borders.

The Egyptian flora has a special interest: the desert and weed species diversity arose thousands of years ago from a unique mixture of African and Asiatic species (Ghabbour, 1997). The Egyptian flora comprises about 2076 species of vascular plants (Boulos, 2000). A considerable number of species are CWR that may provide genes for improvement of cultivated fodder, food, medicinal, oil and fibre crops (Hepper, 1998; Amer, 2005, 2006, 2008), especially in this harsh environment (Ghabbour, 1997). The study of CWR is an urgent priority needed to overcome the production–consumption gap. The potential traits of CWR (e.g., productivity, salt, disease and/or drought resistance) are targets of global interest. This trend prompted the author to carry out several studies to deduce the inter-specific relationships between some cultivated crops in Egypt and their wild relatives within the Egyptian flora. Among these were studies of cotton, *Gossypium barbadense* L. (Amer, 1999) and barley, *Hordeum vulgare* L. (Amer *et al.*, 2013) and their wild relatives. In addition, studies of a number of underutilized species and their wild relatives included: henbane, *Hyoscyamus muticus* L. (Amer,

“The study of CWR is an urgent priority needed to overcome the production–consumption gap”



Hordeum murinum subsp. *leporinum* (Link) Arcang. (Photo: Wafaa Amer)



Avena fatua L. growing in the wild with *Onopordum* L. sp. (Photo: Wafaa Amer)

2004), coffee senna, *Senna occidentalis* (L.) Link (Amer and Sheded, 1998), tobacco, *Nicotiana tabacum* L. (Amer and Fawzy, 2005), papyrus, *Cyperus papyrus* L. (Amer and Serag, 2003) and desert-date, *Balanites aegyptiacus* (L.) Delile. (Amer *et al.*, 2002). Current research is focused on grey-leaved saucer berry, *Cordia sinensis* Lam. and tigernut, *Cyperus esculentus* L. and their wild relatives. Important CWR in Egypt have been grouped based on the utilization of the related crop species as follows: I – food; II – fodder (both legumes and grasses); III – paper; IV – fibre; and V – medicinal ([supplementary Table 1](#)).

In addition to their potential to contribute beneficial traits to crops, many of Egypt's CWR species have potential for cultivation but are currently underutilized (e.g., species of *Hordeum*, *Lolium*, *Panicum*, *Setaria* and *Sorghum*). The establishment of a national research programme to propagate, improve and utilize some of these species to produce grain would help to reduce the country's dependence on imported food such as wheat (around 50% of which is bought from other countries) and contribute to national food security.

Furthermore, many of Egypt's CWR are utilized in their wild form to provide food to local people, particularly in hunger gaps and times of famine. The author recommends that the government encourages the increase of the use of wild food species to 5% of the national food basket by 2015. However, while some sustainable use of CWR can be allowed, some species are collected

(and sometimes traded) in large quantities. These consumption and trade pressures are putting many wild species at risk of genetic erosion and even extinction. Research and concerted actions are needed to assess the status of Egypt's priority CWR species and develop a national strategy for their conservation and sustainable utilization.

“National policy, legislation and programmes are needed to enhance the conservation and utilization of CWR for poverty alleviation and to reduce hunger in Egypt”

To conclude, despite the contribution of wild species to food and nutritional security, the sustainability of this contribution is threatened by many issues, including:

- Lack of national programmes to enhance CWR conservation;
- Insufficient CWR characterization and evaluation data;
- Intangible governmental efforts directed to public awareness for sustainable utilization of these resources;
- Exclusion of CWR from official statistics and economic values of natural resources on both national and international scale.

National policy, legislation and programmes are needed to enhance the conservation and utilization of CWR for poverty alleviation and to reduce hunger in Egypt, as well as in many other developing countries.

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Hyoscyamus muticus L. (Photo: Wafaa Amer)



Closeup of *Hyoscyamus muticus* flower (Photo: Wafaa Amer)

In situ conservation of rare wild perennial chickpea (*Cicer montbretii* Jaub. & Spach) found in Strandja mountain, Bulgaria

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The main aim of many international programmes and projects is finding and rescuing rare wild relatives of crops such as wheat, rice, barley, lentil, bean and chickpea. Some of these are of great importance for improving crops and for strengthening future food security (Fowler and Hodgkin, 2004). The wild relatives of cultivated species contain genetic diversity that could be incorporated into domestic species in order to become more resistant and more environmentally adapted. Many authors have reported that wild species of *Cicer* L. possess resistance to biotic and abiotic stress factors (Juan *et al.*, 2003; Knights *et al.*, 2003; Nguyen *et al.*, 2005).

The southern region of Bulgaria should be considered as one of the centres of origin of cultivated chickpea, *C. arietinum* L. In Strandja Mountain in the most southern part of Bulgaria, Koinov (1968) discovered the wild species *C. montbretii* Jaub. & Spach, a close relative of *C. arietinum*. Based on information published by Kaiser *et al.* (1998), the Bulgarian locality of this rare species was confirmed by the authors during an expedition to Strandja Mountain.

The species is protected under the Biodiversity Act. The populations are within protected areas (reserves 'Ropotamo' and 'Uzunbudzhak'; Natural Park 'Strandja' and protected area 'Silistar'). These zones are also included in the protected areas of the Natura 2000 network.

During an expedition performed by our team in Strandja Mountain, one locality of *C. montbretii* was found near to the village of Gramatikovo, not far away from Malko Tarnovo town (Fig. 1). This trip was supported financially by the European project SEELEGUMES - 168/01 - SEE-ERA.NET Plus Joint Call. No



Figure 1 Locality of the newly discovered population of *Cicer montbretii* Jaub. & Spach in southeast Bulgaria (Adapted from mappery.com)

other locations of chickpea species rare in Bulgaria were found during this survey.

The newly discovered locality of *C. montbretii* was situated on an eroded slope with leached cinnamon forest soil, near an oak forest at 198 m. The observed subpopulation of *C. montbretii* consisted of single plants and small groups of plants sparsely distributed over an area of 10–20 m². The discovered locality is near to the road.

Unfortunately, there are several serious threats to this subpopulation: 1) possible reconstruction of the road; cleaning the side of the road or sanitary cleaning of the forest; 2) the increase of invasive species such as wheat grasses, the main competitor, as well as populations of *Amorpha fruticosa* L. There is therefore an urgent need to protect and to preserve the discovered locality. In view of the small population it is necessary to collect seeds and to preserve them *ex situ* (Jarvisa *et al.*, 2008). To this end, seeds from *C. montbretii* were collected during our last expedition. We will attempt germination and if successful, cultivate plants in our botanical garden.

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