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Crop wild relative

Issue 7 April 2009

The newsletter of the Crop Wild Relative Specialist Group

Conserving plant genetic diversity
for use now and in the future

Crop wild relative

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Above: Nikolai Vavilov. Photo: N.I. Vavilov Research Institute of Plant Industry (VIR).

Editorial

Welcome to issue 7 of *Crop wild relative*—the newsletter of the Crop Wild Relative Specialist Group (CWRSG) of the IUCN Species Survival Commission (SSC). These are exciting times for CWR science—CWR conservation and use is a complex, interdisciplinary, global issue that is increasingly being addressed by a number of national, regional and international initiatives; notably two Global Environment Facility projects ('*In Situ* Conservation of Crop Wild Relatives through Enhanced Information Management and Field Application' and 'Design, Testing and Evaluation of Best Practices for *In Situ* Conservation of Economically Important Wild Species'), the European Community-funded projects, 'European Crop Wild Relative Diversity Assessment and Conservation Forum (PGR Forum)' and 'An Integrated European *In Situ* Management Work Plan: Implementing Genetic Reserve and On Farm Concepts (AEGRO)', the FAO commissioned study, 'Establishment of a global network for the *in situ* conservation of crop wild relatives: status and needs', and activities of the European Cooperative Programme for Plant Genetic Resources (ECPGR) '*In Situ* and On Farm Conservation Network'. Several of these major initiatives have reached or are currently reaching fruition, but publication of their conclusions is very timely owing to the growing concern over the predicted devastating impacts of climate change, the massively growing world population, and the negative implications for biodiversity and food security.

CWR diversity will undoubtedly be negatively impacted by the pressures resulting from the increasing human population and climate change; yet, at the same time, this diversity is likely to be critical for crop improvement and food security in the future. It is therefore surprising that the importance of CWR has not been widely recognized, particularly in the agricultural community and that they have not been given a higher profile. The effective conservation and use of CWR diversity is no longer an option—it is clearly a priority. They are a critical component of plant genetic resources for food and agriculture (PGRFA), have already made major contributions to crop production and are vital for future food security. Their systematic conservation in ways that ensure their continuing availability for use is therefore imperative.

In the first quadrennium (2005–2008), the members of the CWRSG have made a significant contribution to addressing the challenge facing CWR diversity, not least by raising the value and profile of CWR diversity within the IUCN Species Programme, first in a meeting of the Specialist Group Chairs in Al Ain, Abu Dhabi in February 2008 and secondly at the World Conservation Congress held in Barcelona in October 2008, where the CWRSG and CWR-related issues were given high visibility. During the Congress, CWR featured prominently, with a knowledge café, an exhibition and several Alliance workshops dedicated to discussions of CWR conservation and use. Members of the CWRSG who were present in Barcelona also had the opportunity to meet and discuss the activities of the CWRSG and many suggestions have been made to take the group forward.

Looking ahead to future activities in 2009, we await the formal reconstitution of the Specialist Groups. We had a productive meeting to discuss the CWRSG and its future with the new SSC Chair, Simon Stuart, and accepted his invitation to continue as co-Chairs of the CWRSG. Our next objective is to formally reconstitute the membership of the new CWRSG for the new quadrennium (2009–2012). We hope you will be willing to join us in continuing to contribute to achieving the vision and goals of the CWRSG. A major initiative that we will be looking to members to assist with over the coming year is the Red Listing of 500–600 European CWR species—a project funded by the EU. Red Listing is a key role of the Specialist Groups and this project provides an important boost to raise the profile of CWR, both within and outside of Europe.

Some important meetings are coming up that will provide an opportunity to spread the word about the importance of CWR; notably 'Plant conservation for the next decade: a celebration of Kew's 250th Anniversary', 12–16 October 2009, Royal Botanic Gardens, Kew and the FAO 12th Regular Session of the Commission on Genetic Resources for Food and Agriculture (19–23 October 2009), where CWR will be firmly on the agenda. 2010 will be the year of biodiversity and the ECPGR *In Situ* and On-farm Conservation Network, together with the EC-funded project AEGRO will be organizing a joint meeting in Madeira, Portugal on CWR and landrace conservation methodologies at which we expect many CWRSG members to offer papers on their research. Watch the CWRSG website for further developments (http://www.cwrsg.org/) and please remember to send us news and updated information to keep the website current.

In this issue we review the extent of use of CWR in crop improvement (p. 7), with a special feature on the potential for *Triticum boeoticum* as a gene donor for salt tolerance in wheat (p. 4). A regional report from Asia and the Pacific describes the significance of the region as the centre of diversity and domestication of a range of important food crops (p. 9), while articles on three national CWR projects in Australia, Denmark and Switzerland illustrate the increasing recognition of the importance of CWR by national agencies and NGOs, many of whom are now bringing CWR to the forefront of their conservation action plans (pp. 10–16). Pages 17–24 cover a range of CWR features from around the globe: tree tomato in Bolivia, coffee in Mauritius, sainfoin in Armenia and wild olive in Malta. Many thanks to all the contributors.

We hope you will enjoy this issue of *Crop wild relative* and we look forward to receiving contributions to the next issue, both from members and non-members alike.

CWR in crop improvement

Determination of salt tolerance in wild einkorn wheat (*Triticum boeoticum* Boiss.) under *in vitro* conditions

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Salinity is a global problem and one of the major environmental stresses that largely affects plant growth and development (Greenway and Munns, 1980). Soil salinity affects a substantial portion of the Earth—as much as 25% of the total arable land available in the world can be described as being saline (Abrol *et al.*,1988). There are different types of salinity according to their causes, including irrigation, dryland and urban. Salts originate from mineral weathering, inorganic fertilizers, soil amendments (e.g., gypsum, composts and manures), and irrigation waters. Soil salinity affects many parts of Armenia's ecosystems, both rural and urban. The Ararat valley, which represents 13% of Armenia's arable lands, provides almost half of total agricultural production and about 42,000 ha in the Ararat valley have soil salinity problems, of which 9,000 ha are classified as severe (soil pH > 9) (Haykazyan and Pretty, 2006) (Fig. 1).

Figure 1. Saline soils (yellow region) of Ararat valley in Armenia.

Wheat (*Triticum* spp.) is the world's major cereal crop, with annual production of over 627 million t in 2004 (http://faostat.fao.org/). Wheat is grown under irrigated and rain-fed conditions—both types of agriculture are threatened by salinization (Ghassemi *et al*., 1995; Mujeeb-Kazi and Diaz de Leon, 2002). If cropping is to continue on these salt-affected soils, substantial increases in the salt tolerance of crops are needed. Wild relatives, including some halophytes, might be sources of tolerance to improve wheat. There is no precise definition of a halophyte. In ecological terms, halophytes are plants that are able to complete their life cycle in salt concentrations approaching those found in sea waters (around 500 mM NaCl).

The identification of suitability of cultivars and their wild relatives to saline conditions will contribute to genetic improvement of the crops and help to increase their yield and quality. For these purposes an efficient screening method is required—one that identifies plants tolerant to saline or alkaline soils. The screening of genotypes under *ex vitro* conditions is difficult as it entails a large amount of resources and space. The determination of absolute salt tolerance under *ex vitro* is also difficult because of the complex interactions that exist between the plant and different soil components. *In vitro* culture is an ideal system for screening and evaluation of saline-tolerant plants as it can be carried out under controlled conditions with limited space and time (Cano *et al*., 1998). Moreover, studies at cellular level provide better knowledge to deepen our understanding of the mechanism of salt tolerance.

Armenia is considered to be part of the centre of origin of cereals. Its flora has 13 wild wheat species and more than 360 cultivated varieties (Fig. 2). Some of the wild species are known to be salt- and drought-resistant, which is particularly important in Armenia due to its climate and frequent water shortages.

The availability of wheat wild relatives in Armenia offers an excellent opportunity for screening of salt tolerant species and genotypes. Therefore, the aim of this study was to screen wild einkorn wheat (*T. boeoticum*) populations for response to salinity and to develop an early diagnostic protocol that depends on reliable parameters using *in vitro* techniques.

Methodology

The effects of NaCl treatments on growth parameters were determined in wild einkorn wheat (*Triticum boeoticum* Boiss.) and the wheat cultivar Bezostaja 1.

Figure 2. *Triticum boeoticum* population in Erebuni Reserve (Armenia).

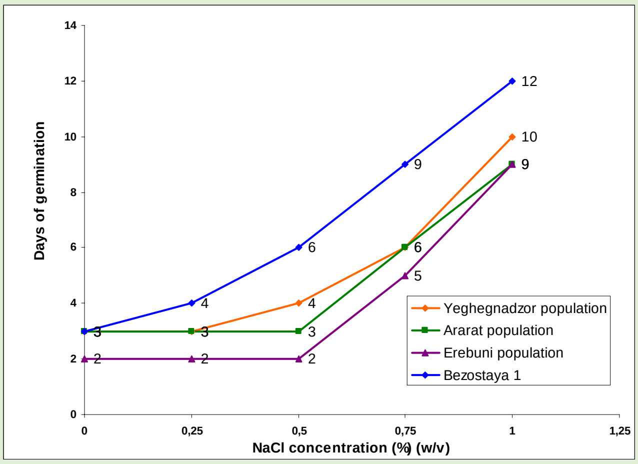

Figure 3. Effect of different concentrations of NaCl on three *T. boeoticum* populations and Bezostaja 1 germination under *in vitro* conditions.

Seeds from different populations of T*. boeoticum* and Bezostaja 1 were germinated in test tubes on half-strength Murashige and Skoog salt solution, pH 5.5, added with 0 (control), 0.25, 0.50, 0.75, 1.00 and 1.50 % (w/v) NaCl. After three weeks seedlings were removed from their media and evaluated for growth and chlorophyll content analysis. A tolerance index was calculated to summarize the general effect of five different NaCl concentrations and to compare *T. boeoticum* populations and cultured wheat Bezostaja 1 (La Rosa *et al*., 1989). Total chlorophyll content was calculated according to Holden (1976).

Results and discussion

The differences with respect to their response to different levels of salinity were observed among the populations of *T. boeoticum* and cultivar Bezostaja 1. An increase in salinity did not affect the germination ratio. In general, germination was delayed with increased salinity in the medium (Fig. 3).

NaCl at concentrations of 0.25 and 0.5% did not delay germination of *T. boeoticum* seeds from populations from Ararat, Yeghegnadzor and Erebuni, but for Bezostaja 1, seed germination was delayed by two days in 0.5% NaCl. The addition of extra salt to the medium delayed germination by three days in 0.75% NaCl and by more than five days in 1.0% NaCl for all of the three studied populations of *T. boeoticum*, while for Bezostaja 1, germination was delayed by four and nine days respectively. This delay in germination in higher salt media (0.75% and 1.0%) may be due to the increased osmotic potential of the saline medium—the higher osmotic potential of the medium affects water and nutrient uptake, which may in turn inhibit the metabolic activities necessary for seed initiation and growth. However, *T. boeoticum* is able to germinate well under higher saline conditions. Research of salt tolerance in plants was focused primarily on tolerance in post-germination seedlings and mature plants. Less information exists on the ability of seeds to germinate under saline conditions. It is known that mannitol-synthesizing plants have been shown to grow well under osmotic and salt stress. One of the possible mechanisms of the ability of *T. boeoticum* seeds to germinate under osmotic stress is the conversion of starches to glucose or other simple sugars during germination. It is known that the enzyme α-amylase releases monomeric sugars from starch. For example, a drought and salt tolerant cultivar of chickpea, *Cicer arietinum* L., shows higher α-amylase activity in cotyledons of germinating seed than a drought- and salt-sensitive cultivar (Gupta *et al*., 1993). The greater concentration of monomeric sugars acts to adjust osmotic potential. The role of carbohydrates in salt tolerance of *T. boeoticum* is an important point for further investigations.

In the Yeghegnadzor population, relative growth did not decrease due to the increase in NaCl concentrations in the 0.5% NaCl treatment. However, the 0.75 and 1.00% NaCl treatments inhibited relative growth and decreased total chlorophyll content of explants. At 0.75% NaCl, the average decline in relative growth and total chlorophyll content was 43.4 % and 41.5 % respectively, and at 1.00% NaCl, the reduction was 68.8% and 62.3% (Fig. 4).

During three week salt treatments (0.25–1.00% NaCl) of *T. boeoticum* from the Ararat and Erebuni populations, significant decreases were determined in the relative growth and total chlorophyll content at 1.00 % of NaCl treatment. At 1.00 % NaCl, the average decline in relative growth and total chlorophyll content was 91.2% and 67.7% in the Ararat population, and 78% and 75% in the Erebuni population, respectively (Fig. 2). The total chlorophyll content did not change during a three week treatment with 0.25–0.75% NaCl—it remained stable at a high level. The response in extra salt treatment of these two populations of *T. boeoticum* was similar.

The results obtained suggest that the Ararat and Erebuni populations are more tolerant to extra concentrations of salt in comparison with the Yeghegnadzor population. These differences between populations may be a result of the long-term adaptation of Erebuni and Ararat populations of *T. boeoticum* to specific ecological factors (clay soil, semi-desert climatic conditions etc.) of the ecosystems where they are growing.

Differences were also observed in response to extra salt concentrations in the widely distributed cultured wheat Bezostaja 1 (Fig. 2). Although explants maintained their relative growth in 0.25%, 0.5% and 0.75% NaCl treatments over three weeks, decreases in total chlorophyll content were significant in 0.75% and 1.00 % NaCl treatments. In contrast, in wild wheat *T. boeoticum*, the total chlorophyll content did not decrease in treatments of 0.25%–0.75% NaCl.

In conclusion, in experimental conditions, wild wheat *T. boeoticum* is more tolerant to salinity stress than cultured wheat Bezostaja 1.

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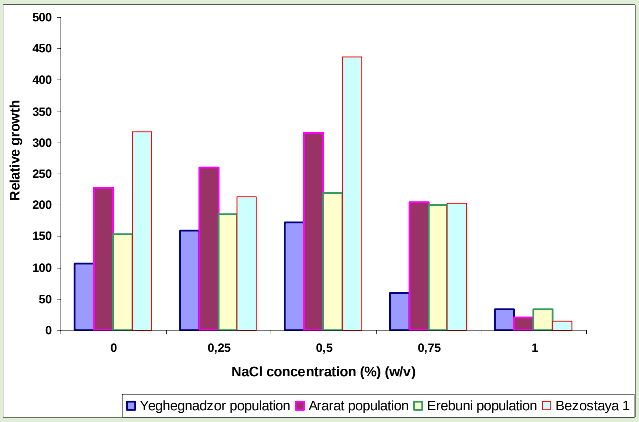

Figure 4. Relative growth of different populations of *T. boeoticum* and Bezostaja 1 under salt treatment.

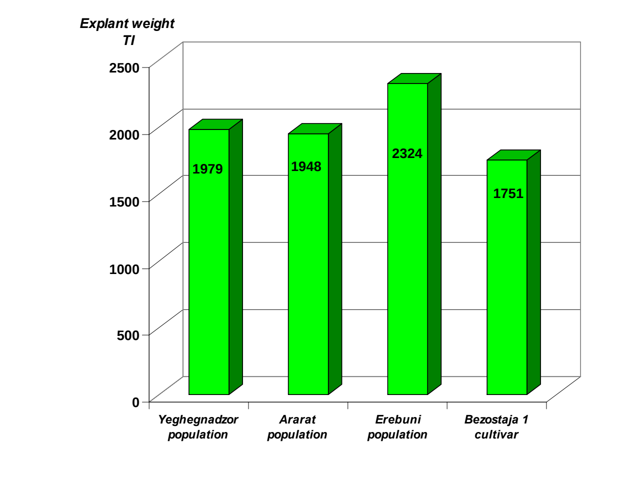

Figure 5. Tolerance indexes of *T. boeoticum* and Bezostaja 1 on the basis of explant weight.

The tolerance mechanism of *T. boeoticum* to salt stress has to be further investigated, but our results correlate with other studies in which the relative growth parameters for halophytic and potentially halophytic plants are described. The basic mechanism of a potential halophyte's salt tolerance is its ability to use the controlled uptake of Na^+ (balanced by Cl^- and other anions) into cell vacuoles for osmotic adjustment. It is known that more tolerant plants are osmoconformers, maintaining an osmotic pressure in the shoot approximately two to three times higher than the osmolality of the external solution (Farrukh, 2002). This is the result of a metabolic response to salt stress in tolerant plants. The cells of such plants synthesize compatible osmolytes (sugars, polyols, amino acids and tertiary and quaternary ammonium, and sulphonium compounds) (Hare *et al.*, 1998; Hong *et al.*, 2000).

Concerning the tolerance indexes (TIs) calculated on the basis of fresh weight of explants, differences between populations of *T. boeoticum* and cultured Bezostaja 1 were shown (Fig. 5).

At the end of the three week NaCl treatments, the highest TIs (2324) were obtained from the Erebuni population of *T. boeoticum*. The minimum TIs (1751) were determined in Bezostaja 1. So, *T. boeoticum* has a high potential of tolerance to salinity.

The results of the present study show the advantages and usefulness of salt tests conducted under *in vitro* conditions using plantlet culture. Therefore, *in vitro* salt tests could be used as an early diagnostic method to screen wild wheat and their cultivars for response to salinity or to identify salt tolerant wild wheat species, genotypes and cultivars for genetic improvement.

The utilization of tolerance indexes was found to be a good evaluation method for classifying different cultivars and their wild relatives. These parameters can be used to give a coefficient in evaluated plants species. Thus, further investigation of salt tolerance mechanisms of *T. boeoticum* will help us to understand the potential of wild einkorn wheat for genetic improvement of existing cultivars and development of more salt tolerant crops. This research is in line with other works on *in vitro* and *in vivo* studies of NaCl treatments on wild and cultured wheat and may contribute to the development of high salinity agriculture through sustainable use of the rich breeding potential of crop wild relatives (Farrukh, 2002; Colmer *et al.*, 2006; Munns *et al.*, 2006).

Acknowledgements

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CWR in crop improvement

CWR in crop improvement: to what extent are they used?

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CWR are defined by their potential ability to contribute beneficial traits to crops, such as pest or disease resistance, yield improvement or stability. They were first routinely used by agricultural scientists to improve major crops in the 1940s and 50s, and by the 1960s and 70s, this practice was leading to some major breeding improvements (Meilleur and Hodgkin, 2004), resulting in the fact that now almost all modern crop varieties contain some genes derived from a CWR. Development in the biotechnology industries has also allowed the transfer of genes from more distantly related species, further enhancing the value of CWR—both those closely and more distantly related (see Hajjar and Hodgkin, 2007; Hodgkin and Hajjar, 2008).

CWR present a tangible resource of actual or potential economic benefit for humankind at national, regional and global levels. Prescott-Allen and Prescott Allen (1986) calculated that the yield and quality contribution to US grown or imported crops was over US$350 million a year, while Phillips and Meilleur (1998) estimated that potential losses associated with endangered food crop relatives was worth about US$10 billion annually in wholesale farm values. Further, Pimentel *et al.* (1997) estimated that if the contribution of genetic resources to yield increase is about 30% of production and that a significant amount of this is due to wide crosses with wild accessions, then the introduction of new genes from wild relatives contributes approximately $20 billion toward increased crop yields per year in the United States and $115 billion worldwide.

Despite their known value as gene donors, Tanksely and McCouch (1997) argued that breeders were not fully exploiting the potential of CWR because historically, they relied on searching for specific beneficial traits associated with particular CWR taxa, rather than searching more generally for beneficial genes; further, they avoided transfer into polyploid crops where transfer was more difficult (e.g., rice, sorghum and sweet potato).

Although it would be very difficult to give a precise estimate of CWR use by breeders because the data are likely to be commercially sensitive and therefore not readily available, Maxted and Kell (2009) recently reviewed the use of CWR in crop improvement and cited 291 articles reporting the identification and transfer of useful traits from 185 CWR taxa in 29 crop species. They found that the degree to which breeders had used CWR diversity varies markedly between crops, being particularly prominent in barley, cassava, potato, rice, tomato and wheat; but of these, rice and wheat are the crops in which CWR have been most widely used, both in terms of the number of CWR taxa used and successful attempts to introgress traits from the CWR to the crop (Figure 1). The most widespread CWR use has been and remains in the development of disease and pest resistance, with the references citing disease resistance objectives accounting for 39%, pest and disease resistance 17%, abiotic

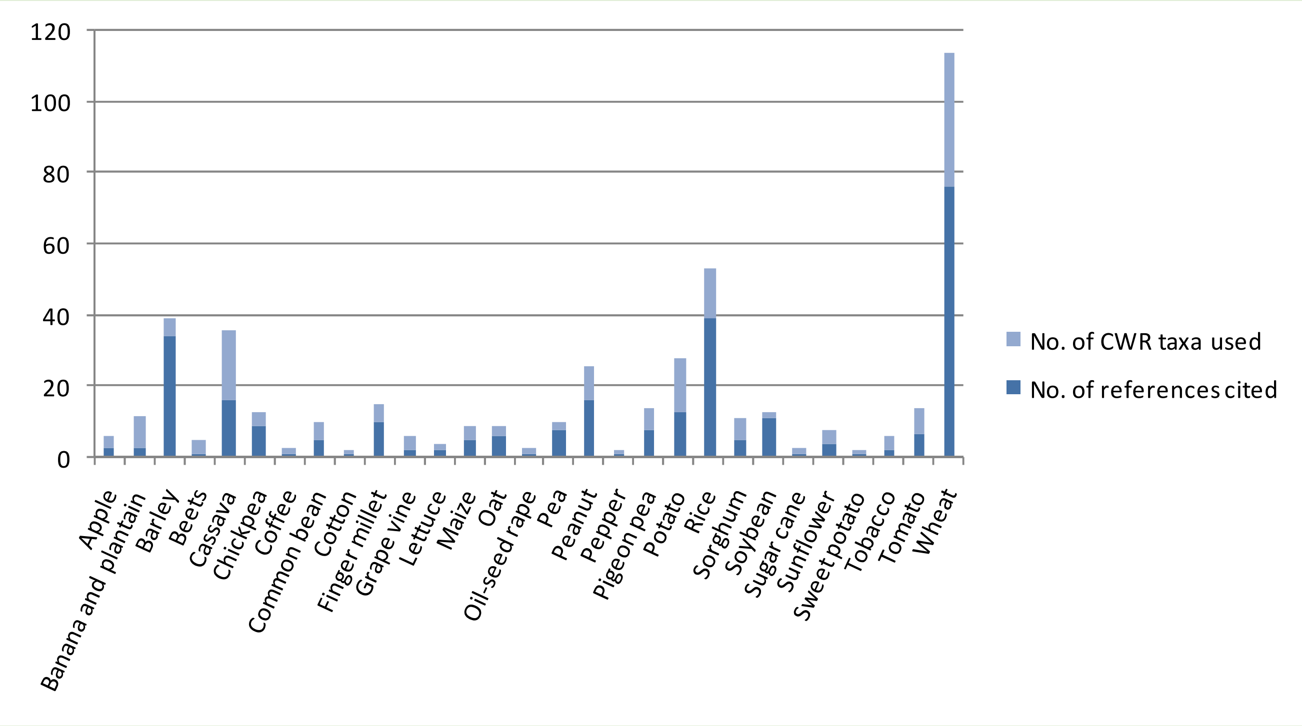

Figure 1. The number of references reporting the identification and transfer of useful traits from 185 CWR taxa to 29 crop species, showing the number of CWR taxa used in each crop.

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"The most widespread CWR use has been and remains in the development of disease and pest resistance"

stress 13%, yield increase 10%, cytoplasmic male sterility and fertility restorers 4%, quality improvers 11% and husbandry improvement 6% of the reported inter-specific trait transfers. It is also notable that the number of articles detailing the use of CWR in breeding has increased gradually over time—presumably as a result of technological developments for trait transfer—with 2% of citations recorded prior to 1970, 13% in the 1970s, 15% in the 1980s, 32% in the 1990s and 38% after 1999 (Maxted and Kell, 2009). Further it can also be seen that since the year 2000, the number of attempts to improve quality, husbandry and end-product commodities has increased substantially.

The exploitation of the potential diversity contained in CWR species remains hit and miss as the approach by breeders to CWR use has not been systematic or comprehensive; therefore, the vast majority of CWR diversity remains untapped for utilization. Hajjar and Hodgkin (2007) comment that although quantitative trait loci have been identified in many CWR species, the potential to exploit them as a breeding resource using new molecular technologies has yet to be fully realized. Although this is likely to improve with time, it does underpin the need for the continued availability of a broad range of CWR diversity, also emphasizing the conservation–use linkage and the need for the conservation community to meet the evolving needs of the users.

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Upcoming conferences and meetings

June 2009

- 3rd Session of the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), 1–5 June 2009, Tunis, Tunisia (http://www.planttreaty.org/meetings/gb3_en.htm)

October 2009

- Plant conservation for the next decade: a celebration of Kew's 250th Anniversary, 12–16 October 2009, Royal Botanic Gardens, Kew, UK (http://www.kew.org/science/anniversary-conference/)
- Second DIVERSITAS Open Science Conference – Biodiversity and society: understanding connections, adapting to change, 13–16 October 2009, Cape Town, South Africa (http://www.diversitas-osc.org/)
- FAO 12th Regular Session of the Commission on Genetic Resources for Food and Agriculture (CGRFA), 19–23 October 2009, FAO, Rome, Italy (http://www.fao.org/ag/cgrfa/meetings.htm)

March 2010

- 13th OPTIMA Meeting, Antalya, Turkey, 22–26 March 2010 (http://www.flora2010.org/)

Summer 2010 (date to be announced)

- AEGRO (An Integrated European *In Situ* Management Work Plan: Implementing Genetic Reserve and On Farm Concepts – http://www.bafz.de/aegro/) Final Dissemination Conference, in association with the ECPGR *In Situ* and On Farm Conservation Network

Regional report

CWR in Asia and the Pacific

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In the tropical regions of Asia, Pacific and Oceania, the diversity of tropical fruits plays a very important role in people's livelihoods. They are important for human nutrition and for their significant role in environmental protection and income generation. They are valuable as a source of medicines, timber, fuel and livestock feed. Wild species that occur in this region include citrus *(Citrus* L. spp*.)*, mango (*Mangifera* L. spp.*),* rambutan (*Nephelium* L. spp.), jackfruit (*Artocarpus* Forst. spp.*)*, litchi (*Litchi* Sonn. spp.), mangosteen (*Garcinia* L. spp.) and papaya (*Carica* L. spp.).

In the East Asian centre, a region of plant domestication dominated by southern and eastern China, some 97 different agricultural plants have been domesticated, including several important crop species such as soybean, adzuki bean, foxtail millet*, citrus,* tea and rice, among others. The centre of origin and diversity of citrus is in Southeast Asia and it is in this region where the greatest amount and diversity of citrus genetic resources can be found.

Asia is also the centre of diversity of rice. Cultivated Asian rice, *Oryza sativa L.*, represents the world's most important agricultural species, feeding more people since the time of its domestication than any other crop. With increasingly unpredictable climate patterns, drought tolerant rice cultivars are desperately needed to stabilize production. Wild species of *Oryza* L. may serve as sources of superior drought tolerance alleles for cultivated rice. Genes from wild relatives are being used to improve tolerance to drought in wheat (Faroq and Azam, 2001) and have been tested for heat tolerance in rice (Sheehy *et al*., 2005).

The Pacific is the centre of diversity and origin for a small number of crops. Due to its history of colonization, crop genetic diversity in the mostly vegetatively propagated crops of the region declines markedly from the west to east. The countries or territories in the region that have relatively high crop diversity are mainly Papua New Guinea and other Melanesian Islands, as well as New Caledonia. Polynesia and Micronesia are minor zones in the region for the diversity of crop wild relatives. A number of countries in Micronesia are small atolls and a marked feature of plants growing on these islands is that they are relatively salt and drought tolerant.

The Pacific and Asia region is rich with wild and cultivated diversity of *Musa* L. (banana and plantain). *Musa acuminata* subsp. *banksii* N.W Simmonds is believed to be the ancestral parent of the majority of the edible banana cultivars. While the centres of diversity of the AA genome are Indonesia–Philippines and Melanesia, there is an exceptionally high diversity in New Guinea. Cultivar type and wild species Maia, Maoli-Populu and Iholena with the genome group AAB have their centres of diversity in Polynesia, Melanesia and Micronesia. The semi-wild type, Fe'i is mainly found in the Pacific. The wild type Australimusa is found in Asia and also in Melanesia (INIBAP/IPGRI, 2006). Two wild banana species, *Musa acuminata* Colla and *M. balbisiana* Colla can be found in Sri Lanka. The main group of edible bananas and plantains is derived from these two species.

Domestication of breadfruit (*Artocarpus altilis* Fosb.), sugarcane (*Saccharum* L. spp.), taro (*Colocasia esculenta* (L.) Schott) and the greater yam (*Dioscorea alata* L.) occurred in the Pacific region. Domestication of taro is believed to have first occurred in New Guinea and further east in Melanesia (Lebot, 1999). Other crops that that were domesticated in the Pacific region are: giant swamp taro (*Cyrtosperma merkusii* Schott) (Figure 1); giant taro *Alocasia macrorrhiza* (L.) G. Don (of major importance in some Polynesian islands); coconut (*Cocos nucifera* L.); sago palm (*Metroxylon sagu* Rottb.); kava (*Piper methysticum* G. Forst.); pandanus (*Pandanus* Rumph. ex L.f. spp.); and aibika/bele or island cabbage (*Abelmoschus manihot* (L.) Medik.).

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Tevita Kete

Figure 1. Giant swamp taro, *Cyrtosperma merkusii* Schott

National projects

Development of conservation technologies for Australian rain forest fruits and CWR

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Australia has crop wild relatives (CWR) of many worldwide economically important species including citrus, banana and rice. Over 100 rare and threatened Queensland edible plants (bush foods) and / or CWR were identified in a recent study (Hamilton, 2007). Many of these species are of rainforest and / or tropical origins and may have non-orthodox seed storage behaviour, thus excluding standard seed banking approaches for long-term *ex situ* conservation. There is an urgent need to develop alternative *ex situ* conservation technologies to conserve this diversity. Table 1 lists some of the nationally and internationally significant CWR and bush foods of Australia. These include rainforest genera that contain species that are commercially cultivated (e.g., *Macadamia integrifolia*), CWR of commercially important species (e.g., *Macadamia* spp., *Citrus* spp. and *Musa* spp.), or of local importance as bush foods (e.g., *Diploglottis campbellii* (small-leaved tamarind), *Citrus australasica* (finger lime) and *Davidsonia jerseyana* (Davidson's plum – Figure 1). Table 1 also provides a summary of the percentage of species under threat (protected under the Queensland Nature Conservation (Wildlife) Regulation 1994) in each genus in Queensland.

Conservation of Australian rainforest fruits and CWR

One of the key risks of projected climate change in Australia is its effect on rainforests, which are one of five natural systems predicted to be vulnerable to damage (Hennessy *et al*., 2007). Rainforest plant diversity is best conserved by utilizing strategies that combine both *in situ* and *ex situ* approaches.

The seed of orthodox-seeded species can be routinely stored *ex situ* in seed banks using standard desiccation (5% moisture content) and freezing (-20°C) protocols. However, not all species are amenable to these procedures and require the development of alternative conservation technologies, particularly *in vitro* and cryopreservation approaches (i.e., storage at ultra-low temperatures), before long-term *ex situ* conservation can be achieved (Pritchard, 2004; Ashmore *et al*., 2007). Conservation of these species is thus currently restricted to *in situ* approaches or field

Table 1. Some Australian genera with likely non-orthodox seed and of socio-economic importance as crop wild relatives (CWR) or bush foods (BF). Numbers and percentages of species in each genus under threat in Queensland are given. Table modified from Ashmore *et al*. (2007)

			Fraction of QLD species under threat[1]	
Genus	Common name	Category	Fraction	%
Alpinia	Native ginger	CWR	1/5	20
Capparis	Australian caper	CWR	2/22	9
Citrus	Wild limes	CWR/BF	2/5	40
Elaeocarpus	Quandong	BF	4/27	15
Davidsonia	Davidson's plum	BF	1/3	33
Diploglottis	Native tamarind	BF	3/10	30
Garcinia	Wild mangosteen	CWR	1/6	17
Macadamia	Macadamia	CWR/BF	6/7	86
Musa	Wild banana	CWR/BF	2/3	66
Myristica	Australian nutmeg	CWR/BF	0/2	0
Passiflora	Wild passionfruit	CWR/BF	0/1	0
Piper	Wild pepper	CWR/BF	1/7	14
Syzygium	Lilly Pilly, rose apple	BF	10/49	20

[1]From Henderson (2002). Threatened species are protected under the Queensland Nature Conservation (Wildlife) Regulation 1994.

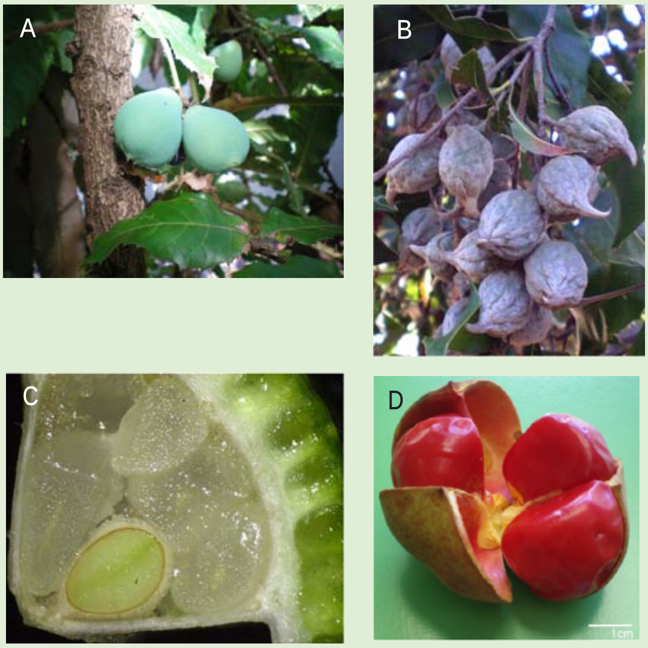

Figure 1. Rare and threatened rainforest fruits of Australia (protected under the Queensland Nature Conservation (Wildlife) Regulation 1994). (A) *Davidsonia jerseyana* (Davidson's plum) (endangered) and (B) *Macadamia ternifolia* (small-fruited Queensland nut) (vulnerable) shown in cultivation at the Mount Annan Botanic Garden. Cut fruits of (C) *Citrus garrawayi* (Mount White lime) (rare) and (D) *Diploglottis campbellii* (small-leaved tamarind) (endangered). Photos: K. Hamilton

collections *ex situ*, making them particularly vulnerable to loss. The numbers of Australian species with non-orthodox seeds is unknown. Recent studies have estimated that it may be up to 30% of flowering plants or >80,000 species worldwide that have desiccation sensitive seeds and thus are not amenable to standard seed banking protocols. Tweddle *et al.* (2003) estimate that 48% of species in non-pioneer evergreen rainforest will have seeds that display desiccation sensitivity.

Target 8 of the Global Strategy for Plant Conservation recognizes the importance of the development of new approaches to long-term *ex situ* conservation for recalcitrant (i.e., non-orthodox) seeded species, stating the need for 'additional resources, technology development and transfer, especially for species with recalcitrant seeds' (GSPC, 2002). Thus, there is an urgent need to develop conservation technologies (e.g., *in vitro* and cryopreservation – see Figures 2 and 3) to conserve the diversity of Australia's rainforest fruits and CWR. Research on the development of conservation technologies in Australia is being undertaken by the Australian Rainforest Seed Project (Botanic Gardens Trust, Mount Annan Botanic Garden) in partnership with Griffith University and the Millennium Seed Bank Project (Royal Botanic Gardens Kew, UK).

Case study: Australian wild relatives of citrus

The International Treaty on Plant Genetic Resources for Food and Agriculture has identified citrus as one of 35 food crops important to humanity for conservation and development of crop diversity (www.planttreaty.org/). Australia has six native species of citrus, the largest number of indigenous citrus species of any country worldwide, and these represent an important source of genetic diversity (Mabberley, 2004). All of these species are limes and five are endemic to Australia. *Citrus inodora* and *C. garrawayi* are native to Northern Queensland (QLD). They are listed as vulnerable and rare, respectively, and protected under the 2000 schedule of the QLD Nature Conservation Act 1992 (QLD Nature Conservation (Wildlife) Regulation 1994) (Forster, 2002). Australian wild limes have breeding compatibility with commercial cultivars and some species, such as finger limes (*Citrus australasica*), are eaten as a popular 'bush food' and are currently being commercialized.

Citrus germplasm has traditionally been conserved in *ex situ* field collections of botanic gardens and research stations because of its non-orthodox seed storage behaviour. These collections are vulnerable to pests, disease and natural disasters so complementary long-term storage options are needed. Cryopreservation has been reported for seeds of cultivated species of citrus, but has not been fully researched or developed for routine use in seedbanks (Lambardi *et al.*, 2004; Hor *et al.*, 2005).

A recent PhD studied *ex situ* conservation options for three Australian wild *Citrus* species (Hamilton, 2007). Both micropropagation and cryopreservation protocols were developed for these species and can now be used to conserve this important genetic diversity. An example of the findings is given for the rare listed species, *Citrus garrawayi* (Mount White lime). *C. garrawayi* is an edible lime with unique fruits that grows in the monsoon forests and rainforests of the Cape York Peninsula of Australia and in Papua New Guinea. Several complexities hinder the *ex situ* storage and use of seeds of *C. garrawayi*. These include seed availability (i.e., limited access and supply), quality (e.g., maturity) and some desiccation sensitivity (Hamilton, 2007; Hamilton *et al.*, 2007). It was found that seeds at different maturities were amenable to cryopreservation and could also be coupled to a straightforward *in vitro* propagation system (Hamilton, 2007; Hamilton *et al.*, 2008). This example illustrates the use of conservation technologies to create *ex situ* storage options and facilitate propagation for utilization of valuable Australian plant diversity (e.g., horticultural and restoration purposes).

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Figure 2. *In vitro* storage and propagation of *Citrus inodora* (Russell River lime) (vulnerable—protected under the Queensland Nature Conservation (Wildlife) Regulation 1994).

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A. Briggs

Figure 3. Cryopreservation of seeds offers an *ex situ* storage option for important rainforest diversity. Australian native fruits, wild limes and Davidson's plum are pictured in the forefront.

Conservation of *Caesalpinia bonduc* (L.) Roxb. in Benin, West Africa

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The project entitled 'Developing conservation and domestication strategies for the endangered scrambling shrub, *Caesalpinia bonduc* (L.) Roxb. in Benin, West Africa' is financed by the People's Trust for Endangered Species (United Kingdom). It will be coordinated by Dr. Ir. Achille E. Assogbadjo, researcher and lecturer at the Faculty of Agronomic Sciences in Benin and IUCN/SSC CWRSG Regional Leader for Sub-Saharan Africa. *C. bonduc* is a key economic, cultural and medicinal tree naturally found in the tropics and subtropics. The long-term viability of the species is threatened by overexploitation of its roots, which are mainly used against prostate gland disease in traditional pharmacopoeia in Africa. *C. bonduc* faces a very high risk of extinction and was classified as an endangered species in the IUCN Red List of Threatened Species 2000. Unfortunately, there are no known scientific studies from Africa on the species despite its extensive use by rural populations for medicinal purposes. The project aims to provide scientific studies on genetic diversity, ethnobotany and propagation methods for the species conservation and domestication in the parkland agroforestry systems of Benin. At the end of the project, scientific articles will be published in peer reviewed journals and guidelines related to the conservation strategies and propagation methods on *C. bonduc* will be developed for the benefit of local people.

National projects

Conservation of CWR in Denmark

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Conservation of Danish plant genetic resources for food and agriculture has until now been focussed on *ex situ* seed banking and clonal archives in the Nordic collaboration in the former Nordic Gene Bank—presently NordGen. This collaboration was established about 30 years ago. In 1994, a Danish programme was established for forest and landscape species (Graudal *et al*., 1995). It comprises 75 species of which 15 are coincident with species interesting for food and agriculture. In this programme, ten *in situ* populations of each species are preserved throughout the country to cover diversity in adaptive traits due to environmental diversity. Furthermore, plantations for breeding and seed production were established. The forestry business has a long tradition for genetic work and thus has a good genetic knowledge of the populations in the forests. In conservation of crop wild relatives (CWR) we do not have this advantage and must rely on diversity represented as adaptation to the diverse ecological and geographic conditions which prevail in different areas of the country. Here, the forestry programme has also been helpful as they have developed a map of gene ecological zones of the country. The map is based on previous work on natural geographical regions of Denmark (Jacobsen, 1976) and a good knowledge of forest genetics. This map will be adapted to the needs of conservation of CWR, with a small amendment that takes into consideration that the Great Belt Region is an especially arid zone which influences the flora considerably.

Wild turnip, *Brassica rapa* L. subsp. *campestris* was previously considered a noxious weed. Due to effective weed control this species is becoming extremely rare. In Denmark it is adapted to cultivated fields and thus must be preserved on farm. Photo: G. Poulsen

The national inventory of CWR was developed on the backbone of the Nordic Gene Bank Taxon database (Hulden *et al*., 1998) by combining different listings previously prepared (only in Nordic languages). Subsequently, these species were evaluated for:

- Present or previous cultivation in Denmark
- Present or previous breeding activities in the country
- Future breeding and cultivation potential
- Is it a crop wild relative
- Is it exploited as a wild species
- Is it at spice or medicinal plant (Asdal *et al*., 2006)

A list of 450 species was the result of the first compilation, and looking at the occurrence of the taxa from a national flora handbook we ended up with 100 species that are not common. These must be assessed for their presence in nature. It must be stressed that we are not dealing with species conservation as such—we want to preserve the genetic diversity within each species.

The conservation approach outlined in the forestry conservation programme is being adapted to our needs so that we aim for ten *in situ* populations of each prioritized mandate species with a good ecological and geographic distribution covering the country. The populations shall preferably be located in nature conservation areas.

The target species in the project include ten woody species, primarily with fruit production potential. The largest group is grasses and forages with 45 species, vegetables 18, and nine taxa of spice or medicinal plants. The list will need some adjustments before it is published. Data management for the CWR taxa and populations will be achieved in collaboration with NordGen.

The countryside in Denmark is exploited for agricultural use and hardly any original nature now exists. The natural flora has developed under these conditions for centuries, and it is this agricultural landscape that we want to conserve. This implies that the wild relatives exist under some kind of semi-cultural conditions. Many survive in isolated nature protection areas where some kind of maintenance takes place, such as grazing. It is anticipated that more common plants are safe in their natural habitats, which is obviously not a fact for many species. In spite of growing in a protected area, many species are stressed by high levels of nitrogen carried by water or air, no matter whether they are common or rare. Many weeds are adapted to the old cultivation systems and new agricultural practices make it hard for them to survive. Species like rye brome (*Bromus secalinus* L.), corn cockle (*Agrostemma githago* L.),

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G. Poulsen

The wild form of celery, *Apium graveolens* L. is native to Denmark but it is not very common.

Wild asparagus *Asparagus officinalis* L. appears here and there on the coastal cliffs. Photo: G. Poulsen

The sea carrot *Daucus carota* L. subsp. *gummifer* is considered spontaneous along the long coastlines of Denmark. Photo: G. Poulsen

wild radish (*Raphanus raphanistrum* L.) and wild turnip (*Brassica rapa* subsp. *campestris* (L.) A. R. Clapham) are heading towards extinction in the wild.

A particular group of wild relatives represented by the wild turnip can only survive as pioneer plants directly in the cultivated fields of spring crops. The fields are not protected areas, therefore in this case some kind of on-farm conservation must be established to conserve such species *in situ*. Alternatively, *ex situ* seed storage is a solution. It is only justified to look after the species that are strict relatives to the agricultural crops.

Some wild species in protected areas live in rather small and isolated populations and are thus in danger of suffering genetic erosion. In such cases a solution can be the establishment of artificial populations comprising a larger diversity than found in the natural populations.

In April 2008, the Ministry of Food, Agriculture and Fisheries granted a three year programme to initiate a scheme for conservation of plant genetic resources in wild relatives to agricultural crops. The project is being carried out by the faculty of agricultural sciences of the University of Aarhus.

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National projects

First step towards CWR conservation in Switzerland

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Context of CWR conservation and their use in Switzerland

The National Action Plan for conservation and sustainable utilization of plant genetic resources (NAP) for Food and Agriculture is a program conducted by the Swiss Federal Office of Agriculture (FOAG; http://www.blw.admin.ch/themen/00010/00071/00127/index.html?lang=de) in which private as well as public organizations are financially supported in their actions to conserve and use plant genetic resources (PGR). This program, based on the report about the implementation of the Global Plan of Action for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (GPA) (FOAG, 1997; http://www.fao.org/ag/AGP/AGPS/GpaEN/gpatoc.htm) started in 1999. Although the GPA (Activity 4, Assessment) and the NAP included crop wild relatives (CWR) as part of PGR for food and agriculture (PGRFA), eight years passed before the CWR topic was really raised in Switzerland. As a matter of fact, in 2007 the Federal Office of Agriculture requested the elaboration of a CWR report and highlighted its importance. As a result, the Swiss Commission for the Conservation of Cultivated Plants (CPC) (see Box 1) initiated a one year project intended to carry out a CWR inventory and identify the priority CWR species in Switzerland.

An interdisciplinary approach

CWR conservation and use is an interdisciplinary topic that has been largely described in literature (e.g., Valdes *et al*., 1997; Azzu and Collette, 2008; Iriondo *et al*., 2008; Maxted *et al*., 2008) with numerous case studies (e.g., Zohary, 1997; Frese and Nothnagel, 2008), proving the value of CWR and their importance as a component of PGRFA. To get an interdisciplinary, as well as an integral approach of this subject in Switzerland,

Box 1: Swiss Commission for the Conservation of Cultivated Plants
(http://www.cpc-skek.ch)
To coordinate the decentralized approach of the NAP Program a coordinating office is required. The Swiss Commission for the Conservation of Cultivated Plants (CPC) is executing that task on behalf of the Federal Office of Agriculture. Today, the CPC has 29 active, public and private member organizations. This network is collaborating to conserve and use PGR. The CPC has created a powerful internet database (http://www.bdn.ch/) to achieve that goal. Every organization that works on PGR or has a conservation collection of PGR has access to that database and can introduce its data. The descriptors used conform to EURISCO (http://eurisco.ecpgr.org/documents/eurisco_descriptors-update-feb2008.pdf) standards. A further development of the database will be the implementation of the future CWR data.

experts from different disciplines (population genetics, breeding, conservation) and institutions (government, NGOs and research institutes) discussed the project frame in three workshops and established both the methodology of the CWR inventory and the criteria to define a priority list of CWR. In addition, this project is based on the definition of a CWR (Maxted *et al*., 2006) and the CWR Catalogue for Europe and the Mediterranean (Kell *et al*., 2005), produced in the context of the PGR Forum project (http://www.pgrforum.org). It is important to highlight that without the framework of the methodologies produced by the PGR Forum project, the current project would not have achieved its goal in such a short time. The described method takes into account the PGR Forum approach as well as the specific context of Switzerland.

CWR inventory in Switzerland

Identification of the CWR of Switzerland is the first step towards a national CWR strategy (Maxted *et al*., 2007) and is known as the CWR inventory. Even when the elaboration of a national CWR strategy for Switzerland is a long-term objective, a CWR inventory can be used to sustain the first CWR conservation activities. The definition of a CWR proposed by Maxted *et al*. (2006) was used to establish this inventory. This broad definition considers a CWR as a wild plant that belongs to the same genus as a cultivated plant. The Swiss CWR inventory can be found online at: http://www.bdn.ch/cwr/inventory/view.

Methodology

Figure 1 shows the methodology used. It is basically the result of the overlay of the three CWR lists within the Swiss Flora (Aeschimann and Heitz, 2005; http://www.zdsf.ch). If some taxa appear in at least one of the three lists, it can be considered as a CWR.

1. The Swiss part of the CWR Catalogue for Europe and the Mediterranean (Kell *et al*., 2005) was linked to the actual Swiss Flora. As a result, 82% of the taxa of the Swiss list of crops and CWR from the CWR Catalogue for Europe and the Mediterranean could be identified in the Swiss Flora. The difference (18%) can be explained mostly by missing subspecies in the Swiss Flora.
2. Ornamental plants of Switzerland: because ornamental plants were not yet integrated in the NAP, but were considered as part of the CWR, they were evaluated according to five Swiss cultivators' catalogues.
3. Socio-economically important cultivated plants of Switzerland: a list of threatened cultivated plants (food, fodder, forage and industrial crops, condiment and medicinal plants) in the NAP was established by considering achieved projects during the past eight years. It has to be noted that conservation actions for cultivated plants on this list are not all at the same level. For example, conservation of grapevine is more advanced than conservation of strawberries.
4. The broad definition of a CWR was applied to the list of socio-economically important cultivated and ornamental plants to define their CWR.

According to some studies conducted in other countries using the same approach defined by Maxted *et al*. (2007), the results obtained in Switzerland were expected to be in the same range. As a matter of fact, 2749 taxa, or 83% of the Swiss flora could be considered as CWR. Finland, for example, defined 60% of their national flora as CWR (Korpelainen *et al*., 2008) and the Netherlands 83% (Hoekstra *et al*., 2008).

Table 1 shows that the three CWR lists have contributed differently to the final CWR list. For example, the CWR Catalogue for Europe and the Mediterranean contains 98% of the taxa also present in the CWR list of Switzerland. Furthermore, the CWR of the socio-economically important cultivated plants, which is based on the actual activities of the NAP, contains 53% of the taxa of the CWR list.

Priority CWR species of Switzerland

The definition of conservation priorities was strongly influenced by the specific context of the conservation of PGR in Switzerland given by the NAP. As already explained, specific conservation actions on cultivated plants have been realized since 1999 within the NAP. The first conservation actions of CWR can therefore be linked with those already achieved for the related cultivated plants. In Switzerland, priority will be given to this category of plants.

As shown in Table 1, 53% (1447 taxa) of the CWR inventory was chosen to be evaluated as being potential priority CWR taxa. Specific analyses on these groups, such as extinction risks (Moser *et al*., 2002), the distribution of different crops and CWR of the major and minor food crops (Groombridge and Jenkins, 2002), were made. To prioritize the list of these 1447 taxa, a

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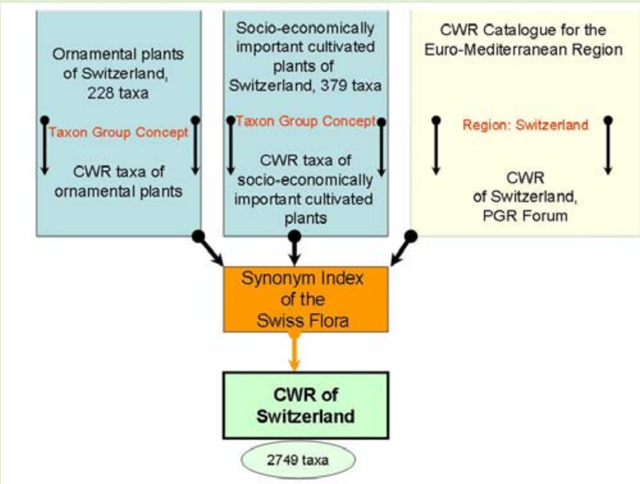

Figure 1. Methodology for creation of the CWR inventory of Switzerland

questionnaire concerning the estimation of their potential (breeding, utilization, genepool conservation, etc.) was prepared and sent to 20 experts (the questionnaire is available in French and German on the project website: http://www.bdn.ch/cwr/umfrage/umfrage_cwr/view). Discussions on the results of that survey revealed that a pragmatic solution had to be found. Only CWR selected by the experts showing a high potential in breeding and in utilization are included in the priority list of CWR species. 143 species were proposed as priority CWR.

Aster alpinus Photo: R. Häner

The survey revealed a high potential of the CWR of fruit trees. Within the priority list, fruit trees are over-represented, compared with other crops such as forage crops, berries or vegetables. In addition, the high diversity of aromatic and medicinal plants in Switzerland was highlighted. The list of the priority CWR taxa of Switzerland can be found at: http://www.bdn.ch/cwr/inventory/cwr/view.

More information about both the survey and the criteria to define the CWR priority lists will be published soon. This information is already available in German and/or French on the project's website.

The CWR priority list of Switzerland is a starting point for CWR conservation within the NAP. This list should expand soon, thanks to the activities of other programs and to the collaboration of further institutions, such as the Federal Office of the Environment (FOEN).

Conclusions and perspectives

It is pleasing to know that CWR conservation is a topic considered positively within the NAP and that this project will contribute to make subsumable (CWR list for Switzerland and priority CWR species) the CWR topic for conservationists, as well as for decision-makers. This project has established a small guideline to develop the first conservation and use activities within the NAP. We hope that the institutions get together to develop an interdisciplinary approach and to carry out the first examples of CWR conservation and use. Nevertheless, further investigations will be necessary to increase the awareness of decision-makers about the importance of CWR in Switzerland. Other federal offices will have to be convinced, private sponsors will have to be found and research institutions motivated to undertake further studies about CWR.

As proposed by Maxted *et al*. (2007) and Maxted and Kell (2009), a national CWR strategy has to be developed and further conceptual and strategic work needed. In order to achieve this goal, the CPC suggests the following actions:

1. *In situ* CWR conservation within protected areas.
2. Further investigation with the flagship species, wild grapevine (*Vitis sylvestris* C.C. Gmel.).
3. Evaluation of the actual *ex situ* conservation situation of the priority CWR taxa.
4. Evaluation of the conservation approaches of endangered CWR species.

For more information, visit the project's website: www.bdn.ch/cwr (in German and/or French)

Acknowledgements

The CPC thanks members of the CWR *ad hoc* group for their voluntary participation, experts for their input of know-how, the FOAG for the financial support, M. Schwartz-Seale, J. Velasquez and S.P. Kell for their assistance for this publication, and O. Viret for the critical review of the manuscript.

Wild pear, *Pyrus pyraster* Photo: R. Häner

Table 1. Contribution of the three CWR lists to the final CWR inventory of Switzerland

List name	Number of taxa	% of the Swiss flora	% of the CWR taxa
Swiss Flora	3299 taxa	100	–
CWR Catalogue for Europe and the Mediterranean	2693 taxa	82	98
CWR of socio-economically important cultivated plants	1447 taxa	44	53
CWR of ornamental plants	1339 taxa	41	49
CWR Inventory (total)	2749 taxa	83	100

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On some wild relatives of cultivated sainfoin (*Onobrychis* L.) from the flora of Armenia

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Sainfoin (*Onobrychis* L.), a traditional fodder legume, has been known in Armenia from ancient times. Interest in this valuable plant has renewed recently. In the archives of Armenian manuscripts one can find references on the cultivation of sainfoin that are dated earlier than the time that officially landmarks domestication of this crop in Europe. For example, Ghevond Alishan, in his book of Armenian botany 'Haibusak' (Alishan, 1895), cited Grigor Narekatsi (an Armenian poet and theologian of the 10th century), describing sowings and harvest of sainfoin haystacks in Armenia.

Onobrychis transcaucasica and *O. altissima* Grossh. are wild sainfoin species that are taxonomically close to the sainfoin cultivated in Transcaucasia—particularly in Armenia. It is also believed that they are ancestors of the varieties currently cultivated in this region. Comparative studies of these two crop wild relatives and the cultivars of sainfoin currently grown by local populations in Armenia indicate that there is no significant difference between wild and cultivated forms. Their biological characteristics, particularly winter-resistance, earliness and drought-resistance, are also close to those of cultivated varieties.


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Figure 1. *Onobrychis transcaucasica* in the wild, showing a close-up of the inflorescence (inset)

In the wild, *O. transcaucasica* is 60 cm tall and has a loose, many-flowered inflorescence (Fig. 1). The ends of young shoots are pubescent and silvery in colour. The corolla is bright pink, up

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J. Akopian

Figure 2. Variation in seed morphology of the wild relatives of sainfoin: 1) *O. altissima,* 2) *O. transcaucasica* 3) *O. hajastana.*

to 10–12 mm long and the seed has teeth on the crest (Fig. 2). *O altissima* is taller, up to 90 cm long, and is different from *O. transcaucasica* in that it has semicircular smooth seeds with no teeth (Fig. 2). Sainfoins are pollinated by insects (mainly bees) and pollination boosts their yield. All sainfoins are very good honey plants. Plants of *O. transcaucasica* from the population (ecotype) of Talin region are notable for the highest nectar content, whereas those from Sisyan are low in nectar.

Onobrychis transcaucasica was domesticated more than 1000 years ago. Centuries-long cultivation in Armenia has led to the selection of populations with economically and biologically valuable characteristics, such as plant height, leafiness and high productivity. Matevosyan (1950), who studied local sainfoins, wrote that it was a common practice for the peasants in old times to select the best seeds from that year's harvest to increase the area cultivated. Raised with care throughout generations they evolved into local landraces.

Figure 4. *Onobrychis hajastana* grows on stony slopes on yellow and red gypsum clays in Yerevan region, Armenia, where it is adapted to extremely xerophytic environments. Photo: I. Gabrielyan

In Armenia, *O. transcaucasica* and *O. altissima* are cultivated as pasture grasses as a source of green animal fodder and hay, and for seeds (Fig. 3). As a fodder it is suitable for any type of livestock. When harvested for the hay, the sainfoin is collected before mid-phase of flowering. Ripening of seeds is not synchronic. The seeds on the lower branches of the inflorescence ripen earlier. When harvested for seeds, sainfoin is usually collected when seeds on the lower inflorescence turn brown, while those on the top are still green. This is done to avoid seed losses. Sainfoin is drought-resistant, frost-resistant and very productive. Due to its ability to assimilate inaccessible nutrients from the soil, there is almost no need for fertilization during their cultivation. Both species prefer soils rich in lime. They can grow on rocky limy soils and steep slopes. Hay and green raw material of the sainfoin are rich in proteins and vitamins and have a very high voluntary intake by livestock. One hundred kg of grass contains 22 nutritional (fodder) units, 3.1 kg of proteins and 6.5 g of carotene, whereas the nutritional composition of 100 kg of hay is 10.1 kg of proteins and 2.5 g of carotene. The hay of the plant is also notable for its anti-parasitic effect, which is due to the presence of tannins.

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Figure 3. A cultivated field of *Onobrychis transcaucasica*, Kotayk region, near Zar, Armenia.

The sainfoins cultivated in Armenia yield two or three harvests during the growing season. In Armenia they are usually harvested starting from the second year of cultivation—however, flowers are formed in the first year and are suitable for harvesting. The most remarkable in terms of raw material quality, attained height (it can be up to 1m high) and exuberant growth, is the Sisyan ecotype of *O. transcaucasica*, which was used as an initial source of material in breeding the cultivar, Sisyanskij 34.

Up-to-date information on the current status of sainfoin populations (both in the wild and cultivated), as well as their traditional uses was collected during field surveys conducted within the framework of UNEP/GEF project on '*In situ* conservation of crop wild relatives through enhanced information management and field application'.

Another remarkable representative of the genus, which is taxonomically close to the cultivated and aforementioned wild species, is *O. hajastana* (Fig. 4). Unlike *O. transcaucasica* and *O. altissima*, it is less common in Armenia (Tamamshyan, 1962). It grows on stony slopes on yellow and red gypsum clays in the floristic region of Yerevan, where it was first described. It is distinguished by its low-growing habit (20–40 cm), linear leaflets, small corollas and small, extremely toothed fruits (Fig. 2). Adapted to extremely xerophytic environments, it can be used as initial material for breeding drought-resistant sainfoin cultivars. Two new sites were discovered for this species within the framework of the project on '*In situ* conservation of crop wild relatives through enhanced information management and field application'. The natural range of this species is continuously shrinking as a result of urbanization. Hence, there is an urgent need to conserve it.

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The wild relatives of the tree tomato (*Cyphomandra betacea*) in Bolivia: distribution, ecology and uses

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The state of knowledge about the diversity, distribution, ecology and conservation status of the species of the genus *Cyphomandra* has been poorly studied in Bolivia. We only had some taxonomic reviews that include this information in a limited way (Bohs, 1991, 1994a, 1994b, 1995; Nee, 1993). The UNEP/GEF project '*In situ* conservation of crop wild relatives through enhanced information management and field application' is carrying out several activities focused on generating information that contributes to improve the state of knowledge and conservation of these species in the country. The present project has as its main goals: a) to determine the distribution of the species of *Cyphomandra* in Bolivia and b) to determine its main uses.

Methods

We have registered 151 records of occurrence (Table 1), which were systematized from different sources: national herbaria (BOLV, LPB, USZ, HSB); taxonomic reviews (Bohs, 1991, 1994a, 1994b, 1995; Nee, 1993); http://www.nhm.ac.uk/research-curation/research/projects/solanaceaesource/; virtual herbaria (NY, MO, F). This information was supplemented with field records carried out during the years 2007 and 2008. We used the program DIVA-GIS to generate the maps of the current distribution of the species of *Cyphomandra*. We used 27 x 27 km grids to identify the places with highest species richness in Bolivia by means of the use of the Chao 1 richness estimator (S1). We used semi-structured interviews to know the ethnobotanic uses of the species by local people.

Diversity and geographic distribution in Bolivia

Ten species of *Cyphomandra* are found in Bolivia (Table 1), four of which are endemic (*C. acuminata, C. benensis C. maternum and C. uniloba*). One species is cultivated and marketed locally (*Cyphomandra betacea*). This species, although cultivated, occurs as wild populations in the central-south of the country. This is an important fact because it could be the centre of diversity of the cultivated tree tomato (Bohs, 1989). Five of all the species found in Bolivia produce edible fruits that are occasionally consumed by local communities (Table 2).

The ten species of *Cyphomandra* are distributed from the lowlands of Amazonia to the high Andean areas of the Boliviano-Peruano Yungas, occupying 15 of the 39 biogeographical areas of Bolivia (Navarro & Ferreira, 2004) (Fig. 1a). The highest richness of species is concentrated between the areas of the Boliviano-Peruano Yungas and the Sub-Andean Amazonia of the departments of La Paz and Cochabamba. This richness is localized, especially in disturbed areas of humid forests and borders of cultivated areas. A few species are distributed in areas of North Amazonia of Bolivia, the Chiquitania and the plains of the Beni department where they appear at the interior of humid forests to subhumid forests. The area of the Tucumano-Boliviano represents an important place of conservation since here we found wild populations of the cultivated species, *C. betacea*. Furthermore, there are wild populations of *C. maternum* that can form natural hybrids with the cultivated species of *C. betacea*.

Through the models of species distribution, three important areas are recognized where the highest species diversity is concentrated in Bolivia (Fig. 1b – blue circles). The first and largest one is located at the department of La Paz, in the biogeographic area of the Peruano-Boliviano Yungas at the river Beni basin. The second one is located in the central part of the department of Cochabamba in the biogeographic province of the Peruano-Boliviano Yungas at the basin of the river Ichilo, and the third one is located in the region of the elbow of the Andes in the east part of the department of Santa Cruz.

Table 1. General data about the wild relative species of the tree tomato

Species	No. of occurrence records	Distribution[1]	Elevation (m asl)
Cyphomandra betacea	33	1, 2, 3, 5, 6	190–2750
Cyphomandra acuminata	22	1, 2	950–2450
Cyphomandra benensis	5	3, 4	235–700
Cyphomandra tenuisetosa	3	3	170–400
Cyphomandra maternum	36	1, 2, 5, 6, 7,8	1300–2550
Cyphomandra oblongifolia	24	3, 4, 9, 11, 12, 13,14	130–360
Cyphomandra uniloba	22	1, 4,15	250–2400
Cyphomandra pendula	2	3	315–500
Cyphomandra pilosa	1	15	400–500
Cyphomandra hartwegii	3	1,13	200–800

[1] 1: Yungas Peruano-Boliviano of the high basin of the river Beni; 2: Yungas Peruano-Boliviano of the high basin of the river Ichilo; 3: Amazonia of the Sub-Andean of the North Andes; 4: Amazonia of the Sub-Andean of the Central Andes; 5: Boliviano-Tucumano formation of the North; 6: Central Boliviano-Tucumano formation; 7: Xeric Interandean-Valleys; 8: Subhumid Puna; 9: Moxos plains; 10: Subandean transitional Chiquitania; 11: North Chiquitania; 12: Amazonia of the Itenez; 13: Central-Occidental Amazonia of Pando; 14: Amazonia of the Oriental Pando; 15: Santa Cruz region.

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Conclusions

Bolivia is a country that harbours a high diversity of the species of *Cyphomandra* in the neotropics (10/35). This represents an important fact considering that the cultivated species could have had its origin in the south of Bolivia and north of Argentina. This high diversity is concentrated in mountainous humid forests of the Boliviano-Peruano Yungas between the departments of La Paz, Cochabamba and Santa Cruz.

The use of *Cyphomandra* species generate new hypotheses about the potential fruiting of these species in the context of the genetic resources important for food.

Acknowledgments

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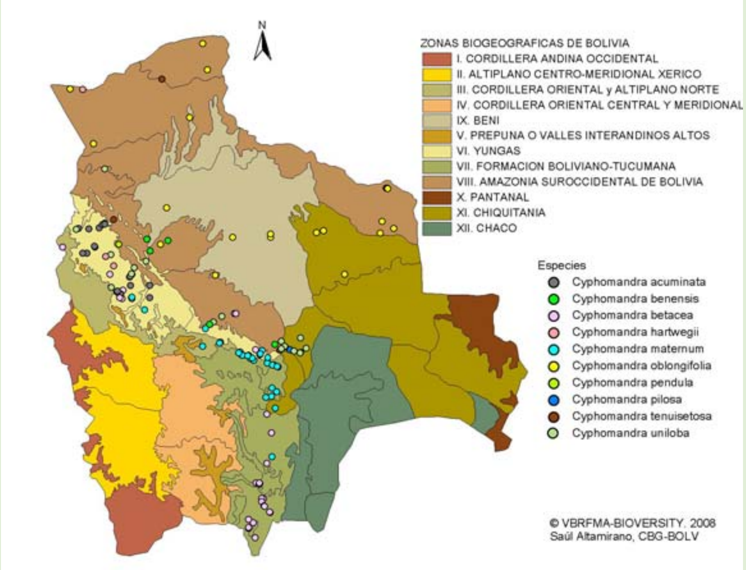

Figure 1a. Current distribution of *Cyphomandra* species in Bolivia.

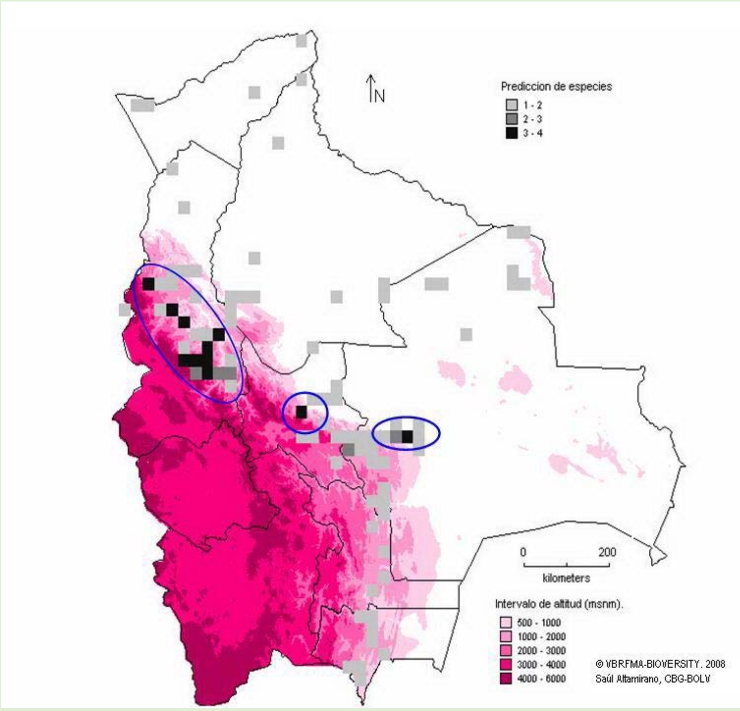

Figure 1b. Distribution of *Cyphomandra* species in Bolivia based on prediction models.

Table 2. Common uses of the *Cyphomandra* species in Bolivia.

Species	Local uses
Cyphomandra betacea	– The fruits are consumed previously cooked in different types of foods. The leaves are used as medicine to cure ailments of the throat (Bohs, 1994b)
Cyphomandra acuminata	– The leaves are used to cure blows and wounds (this study).
Cyphomandra benensis	– The fruits are consumed, previously toasted and prepared in form of sauce. The leaves are used in traditional medicine to combat scabies and anaemia (crushed leaves) (this study).
Cyphomandra tenuisetosa	– No use of this species is known (this study).
Cyphomandra maternum	– The fruits are consumed previously cooked (this study).
Cyphomandra oblongifolia	– The fruits are consumed in a direct way by the indigenous T'simane and the leaves are used by the ethno Chacobo to cure problems of the liver (this study).
Cyphomandra uniloba	– The mature fruits are consumed in a direct way; in some cases the fruits are boiled and they are prepared as sauces (Bohs, 1989).
Cyphomandra pendula	– Reports that apparently the fruits are consumed in their mature state. Specimen information of herbarium of LPB, Quintana number 397.
Cyphomandra pilosa	– In some countries like Ecuador and Peru, the leaves are used in traditional medicine (Bohs, 1994b); the fruits are not consumed (this study).
Cyphomandra hartwegii	– The fruits are edible in their mature state and they are consumed in a direct way without cooking them (this study).

Collecting wild coffee species in Mauritius

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Coffee is the most popular beverage in the world and represents the world's most valuable agricultural export commodity. It belongs to the genus *Coffea* L. which is composed of 96 species, endemic to Africa, Madagascar, Comoros and the Mascarene Islands (Davis *et al*., 2006). The coffee beverage comes from four main species: *C. arabica* L., *C. canephora* Pierre ex Froehner, *C. liberica* Bull ex Hiern. and *C. excelsa* Chev. In addition, there is a wide diversity of wild *Coffea* species described by various botanists (Davis *et al*., 2006).

Coffee species are distributed across West and Central Africa to southwestern Ethiopia and East Africa. The forests of Madagascar and to a lesser extent the Mascarene Islands (Mauritius and Réunion) are the home of the relatively isolated *Mascarocoffea* section of the genus characterized by low levels or even absence of caffeine. These areas, together with farmers' fields containing traditional coffee varieties, are the ultimate sources of coffee genetic diversity. Unfortunately, deforestation, encroachment by agricultural activities, population pressures and economic hardships threaten all these reservoirs of great genetic diversity and with them comes the danger of significant erosion of the *Coffea* genepool. In the 1960s to 1980s, FAO, ORSTOM (now IRD – L'Institut de Recherche pour le Développement), CIRAD (French Agricultural Research Centre for International Development) and Bioversity International organized a number of collecting missions (Meyer *et al*., 1968; Guillaumet and Hallé, 1978; Berthaud and Guillaumet, 1978; Berthaud *et al*., 1980, 1983; Anthony *et al*., 1985; De Namur *et al*., 1987; Eskes, 1989) to ensure that coffee genetic resources are safely conserved in *ex situ* collections.

These collections are primarily being conserved in field genebanks in African countries such as Côte d'Ivoire, Ethiopia, Cameroon and Madagascar (Dulloo *et al.,* 2001). IRD established a safety duplicate core collection of the coffee genetic resources it helped to collect in Africa, initially in Montpellier. Since 2003, this collection has been transferred to Réunion Island where the environment in better adapted for maintaining it. This transfer was performed in the framework of a specific project co-financed by IRD, the Région Réunion and the European Union. The collection comprises 460 genotypes and includes 31 species represented by 86 diversity groups, to which genotypes of *C. mauritiana* from endemic populations of Réunion and of several coffee species which have been introduced in the island have been added. In order to improve the representativeness of this collection, a collecting mission was organized in Mauritius to collect the wild coffee species.

According to the Flora of the Mascarene Islands, there exist three species of *Coffea*: *C. mauritiana* Lam., *C. macrocarpa* A. Rich and *C. myrtifolia* (A. Rich. ex DC.) Leroy. *C. mauritiana* (Figure 1 and front cover) is known from Mauritius (where it is restricted to a couple of populations) and Réunion where it has a much wider geographical distribution and a high degree of polymorphism (Leroy, 1963). There also seem to be distinct differences between the plants from the two islands. *C. macrocarpa* (Figure 2) and *C. myrtifolia* are endemic to Mauritius. *C. macrocarpa* is mainly located within the native upland forest. It has a rather broad distribution extending from the southwest of the island to the east of the island at a location called Montagne des Creoles, where a form somewhat different from *C. macrocarpa sensu lato* exists and which was once named *C. bernardiniana* Leroy (Leroy, 1962, 1963). *Coffea myrtifolia* was probably first collected in 1772 by Commerson at a locality in the east, near Montagne Bambou. The species was believed to be extinct in the wild until it was re-discovered in 1950 by Dr. R.E. Vaughan in a different locality, Les Trois Mamelles (Leroy, 1968, 1971). *C. myrtifolia* is now known from at least five different localities and is still relatively common in the lowland native forests.

In July 2008, IRD in collaboration with Bioversity International organized a mission to collect samples of the main populations of the wild coffee species to include in the international collection established in Réunion Island (Figure 3, Table 1). Permission to collect the germplasm samples was obtained from the Mauritius National Parks and Conservation Service (NPCS) and staff of the NPCS and the Mauritian Wildlife Foundation (MWF) helped to organize field visits to the native forest.

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Figure 1. *Coffea mauritiana* with green and ripe fruit at Plaine Champagne, Black River Gorges National Park, Mauritius

Figure 2. *Coffea macrocarpa* at le Petrin, Black River Gorges National Park, Mauritius
Photo: M. Allet

Table 1. Wild *Coffea* spp. collected in Mauritius in 2008

Taxon	No. of seeds collected	No. of plants sampled	Locality
C. mauritiana	229	16	Plaine Champagne
C. macrocarpa	61	3	Plaine Champagne
C. macrocarpa	109	4	Le Pétrin
C. macrocarpa (different form, previously described as *C. bernardiniana*)	209	5	Montagne des Creoles
C. macrocarpa x *C. mauritiana*	21	1	Le Pétrin
C. myrtifolia	880	6	Mont Brise

The main wild populations for *C. macrocarpa* and *C. mauritiana* were located in the southwest within the island's only National Park, Black River Gorges. The other form of *C. macrocarpa* (Montagne des Creoles form) and *C. myrtifolia* were collected from the eastern mountains of the island. Many of the seeds were ripe, but also fully grown green seeds were taken. These seeds are able to ripen post harvest for successful viability (Figure 4) .

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Figure 3. Emmanuel Couturon, geneticist at IRD (right) and Asha Poonith, Plant Manager of the MWF (left) collecting seeds from *C. mauritiana* at Plaine Champagne, Black River Gorges National Park, Mauritius.

E. Couturon

Figure 4. Variation in seed size and shape of four wild coffee species. Clockwise from top left: *C. bernardiniana*, *C. macrocarpa*, *C. myrtifolia*, *C. mauritiana.*

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Wild olive, *Olea oleaster* in the Maltese Islands: history, current status and conservation measures

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Situated within a strategically central Mediterranean location, the Maltese Islands have been subject to a long history of anthropogenic influence, invasions and colonization. Phoenicians, Carthagenians, Romans, Turks, Arabs, and more recently the British, have all laid claim to these small islands. A history that is as unique as the native language of Maltese citizens: *Il-Malti*. Before human settlement, the Maltese Islands were covered in lush mixed woodland, consisting of Aleppo pine (*Pinus halepensis* Mill.*)* and holm oak (*Quercus ilex* L.), reaching a forest climax in specific localities (Falzon and Sultana, 2002). Only isolated remnants remain, whilst much of the tree cover is composed of secondary maquis and woodland.

The historical direct link between olive trees and the Maltese people could not have been better manifested than by the actual names of several towns and villages! In Maltese the olive is called *żebbuġ,* (formerly known as *żejtuna*); therefore, towns and villages such as Birżebbuġa, Ħaż-Żebbuġ, Żejtun, and Żebbuġ in Gozo are clear evidence of the historical affinity of the olive tree within the Maltese Islands. Even some valleys are named after olive trees: Wied ta' Għajn Żejtuna in Mellieħa, and Wied iż-Żebbuġ in Rabat.

Surprisingly, despite the intimate connections between settlers and the olive tree, the actual history of wild olive, *Olea oleaster* Hoffmanns. & Link in the Maltese Islands is ambiguous. Even its taxonomic status is uncertain, with some authors believing that it does not warrant species status but that it belongs within the species *O. europaea* L., wherein it has been variously classified as *O. europaea* subsp. *sylvestris* (Mill.) Rouy, *O. europaea* var. *sylvestris* Brot., *O. europaea* subsp. *oleaster* (Hoffmgg. & Link) Negodi, *O. europaea* var. *oleaster* (Hoffmanns. & Link) DC. and *O. europaea* subsp. *europaea* var. *sylvestris* (Mill.) Lehr.

The true wild olive has very small leaves, spines and small drupes—overall, not unlike the olive-leaved buckthorn (*Rhamnus oleoides* L.). Indeed, in some cases the two species are only distinguished by their foliage: opposite in *O. europaea* s.l., alternate in *R. oleoides.* Olive trees have a particular tendency to revert back to their original wild traits if left uncultivated from one generation to another. The pattern of reversion could be associated with a history of cultivation and land abandonment, and quite likely a succession of both, over a considerable period of time. But to add to its taxonomic ambiguity, sometimes a single tree can have both cultivated (large long leaves, no spines, and large, oily drupes) and 'wild' features, including spines and small leaves (D.T. Stevens, pers. comm., 2009), presenting a complex yet interesting genetic composition.

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Wild olive, *Olea oleaster* at Mellieħa, Malta.

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Remnant holm oak forest at Wardija, Malta with interspersed olive trees in the background and contiguous arable land in the foreground.

Geographical isolation and anthropogenic factors are likely to have had a marked impact on the wild olive tree within the Maltese Islands. It could very well be the case that the original wild stock underwent a genetic bottleneck following extensive land clearance for arable farming, cotton plantations and citrus groves, which concurrently almost exterminated the native oaks and pines. Thus, in the process, the wild species could have lost certain traits whilst others became fixed over time. It is also very likely that such selective process was influenced by the introduction of cultivars from other parts of the Mediterranean and North Africa, providing an opportunity for the local stock to interbreed with foreign *Olea europaea s.l*. Therefore, what little is left of the wild stock may in effect be the result of:

- generations of interbreeding between the true wild type and cultivars;
- abandoned cultivars reverting back to their wild phenotypic characters; and
- intermittent mixtures of both scenarios.

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In recent years, several cultivated varieties have been imported, particularly from France and Italy (Drinkwater, 2008), thus extending the possibility of further outcrossings. If Malta is to safeguard the genetic composition of its native olive trees, such imports should be kept under strict control and more efforts placed on safeguarding both the remnant wild population as well as our own cultivated indigenous stock.

There is some legislation for the protection of olive trees in the Maltese Islands, but its effectiveness for wild olive is limited. Olive trees are listed in Schedule II of Legal Notice (LN) 12 of 2001 (MEPA, 2001) as *Olea europaea s.l.* The national legal framework protects all olive trees by establishing specific precautionary and protective measures to safeguard them from damage (intentional and unintentional) and prohibits uprooting, unless authorized by the Director of Environment Protection through a formal environmental permit.

LN 12/2001 does not differentiate between CWR and cultivated varieties and while there is no reference to the potential use of genetic material of CWR, the objective of this legal instrument is to safeguard the "biological identity" of the protected trees, bearing the same definition as that established by the Convention on Biological Diversity (i.e., "the variability among living organisms from all sources including, among others, terrestrial, marine and other aquatic ecosystems, and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems") (MEPA, 2001).

The current conservation strategy is restricted to the protection of indigenous trees and their overall integrity, based on *in situ* conservation objectives. Importation of trees is also regulated by this legislation. Section 14 of LN 12/2001 specifies that "The Director may stop the importation of trees which in his opinion may endanger the biological identity of Malta, or for any other reason in the national interest" (MEPA, 2001). However, to date, importation of foreign varieties of olive trees into the Maltese Islands is still allowed and plantations might consist of a mixture of indigenous stock and varieties from neighbouring countries. There is insufficient awareness of the threats foreign varieties may pose not only on local varieties but more significantly on the scarce wild relative.

This situation does not look promising for the future of wild olive trees in the Maltese Islands. The possibility of outcrossing with imported varieties is a great concern, particularly where such planting occurs within the range of wild specimens. The situation is more perturbing considering that propagation of wild olive trees has, to date, never been carried out (this is subject to an environmental permit). Nurseries only cater for the propagation of local cultivars since these yield bigger drupes—hence they are more productive and in demand, unlike the wild relative. Notwithstanding, one particular nursery produces pure *O. oleaster* oil from a single specimen located within their grounds, not surprisingly on a very small non-commercial scale (D. Agius, pers. comm., 2009). Sadly, the drupes are only used for olive oil production, with no attempt made at germinating the seeds. Clearly, such important genetic material is being wasted.

Given the scarcity of *O. oleaster* within the Maltese Islands, there is a dire need for the legal framework not only to extend specifically to the *in situ* conservation of wild olives, but also to highlight and address *ex situ* conservation measures for this rapidly eroding CWR.

On a more positive note, efforts to propagate the local varieties are carried out, and rightly so. After all, there is no benefit in changing what is already proven to be just right: such trees are known for their relatively low input–high yield value and their exceptional taste!

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Typical maquis habitat, Malta

New CWR publication

'Establishment of a global network for the *in situ* conservation of crop wild relatives: status and needs' (Maxted and Kell, 2009). Commissioned by the FAO CGRFA, this comprehensive report addresses: 1) assessment of the global importance of CWR diversity; 2) estimating global CWR numbers; 3) development of national CWR conservation strategies; 4) threats to CWR species and genetic diversity and the likely impacts of global change; 5) the extent and effectiveness of current *in situ* and *ex situ* conservation of CWR diversity at national, regional and global levels; 6) CWR conservation outside of formal structures; 7) systematic approaches to *in situ* and *ex situ* CWR conservation, including the establishment of a global network of CWR genetic reserves for high priority CWR taxa; 8) enhancing the use of CWR diversity; and 9) increasing awareness of CWR importance and policy development. The report can be downloaded from http://www.cwrsg.org/Publications/Reports/Global_in_situ_CWR_conservation_network.pdf and will be formally published by FAO later this year.