A.11. Establishment and implementation of *ex situ* conservation

A.11.1. Overview

What are the* ex situ *conservation goals of a National management plan for CWR conservation?

A *National management plan for CWR conservation* aims at the development and implementation of a systematic and complementary action plan for the active conservation and sustainable use of CWR within a country. This will include parallel *in situ* and *ex situ* conservation action but it is the *ex situ* collections that primarily facilitate access to these materials for crop improvement and research.

The Convention on Biological Diversity[86] changed the relative focus of conservation efforts so that following its inception, *ex situ* conservation was seen primarily, at least for the broader biodiversity conservation community, as a safety back-up strategy to provide security for the favoured *in situ* approach. While recognising that it would be foolish to implement a *National management plan for CWR conservation* and establish key national conservation areas without a safety back-up to help guarantee long-term conservation of the populations, the policy change fails to recognise the fact that CWR diversity has historically been almost exclusively conserved *ex situ* and it can be argued that *ex situ* collections provide the most practical means of access for the germplasm user community. At least in the short term, how many plant breeders or researchers are likely to approach PA managers for germplasm to use in their breeding programmes? As *ex situ* conservation provides the practical route for germplasm access for the user community; even if populations are adequately conserved *in situ* there is still an imperative to duplicated diversity *ex situ* for the benefit of the user community.

However, *in situ* conservation has unique importance in maintaining the process of adaptation to changing environments which cannot happen with *ex situ* conservation – each *ex situ* accession is a snapshot of that population's diversity at the time of sampling. Therefore both *ex situ* and *in situ* techniques have their advantages and disadvantages, and they should be seen not as alternatives or subservient to one another but as complementary strategies.

There are a range of *ex situ* conservation techniques available, but because the vast majority of CWR have orthodox seeds (i.e., they can be effectively dried and stored at -18^{0}C without loss of viability) seed storage in gene banks predominates as the most practical *ex situ* conservation technique applied. The establishment and implementation of *ex situ* conservation priorities includes three steps (Figure): (i) review of *ex situ* conservation gaps, (ii) selection of CWR and sites for

[86] CBD (1992)

targeted collecting, (iii) gene bank seed processing, and (iv) post-storage seed care.

Box 52. *Ex situ* conservation techniques

CWR diversity can be stored as seed, explants, living plants and genomic samples using the following *ex situ* techniques:

Seed Storage – The collection of seed samples at one location and their transfer to a gene bank for storage. The samples are usually dried to suitably low moisture content and then kept at sub-zero temperatures;

In Vitro Storage – The collection and maintenance of explants (tissue samples) in a sterile, pathogen-free environment;

Field Gene Bank – The collecting of seed or living material from one location and its transfer and planting at a second site. Large numbers of accessions of a few species are usually conserved;

Botanic Garden / Arboretum – The collecting of seed or living material from one location and its transfer and maintenance at a second location as living plant collections of species in a garden or for tree species an arboretum. Small numbers of accessions of a large number of species are usually conserved.

DNA / Pollen Storage – The collecting of DNA or pollen and storage in appropriate, usually refrigerated, conditions.

Source: Hawkes *et al.* (2000).

Collecting seeds of *Convolvulus fernandesii* P. Silva & Teles, a CWR endemic to Cabo Espcichel (Portugal), for *ex situ* conservation (photo: Carlos Ferreira Silva).

Ex situ seed conservation. Photo: ICARDA.

Box 53. *Ex situ* seed conservation

Ex situ conservation is the conservation of biological diversity outside their natural habitats. It involves the location, sampling, transfer and storage of samples of the target taxa away from their native habitat to be conserved at a remote site. Examples of major *ex situ* seed collections include the International Maize and Wheat Improvement Centre (CIMMYT) gene bank with more than 160,000 accessions (i.e., samples collected at a specific location and time), the International Rice Research Institute (IRRI) with 108,925 accessions, the world's largest collection of rice genetic resources, and the Millennium Seed Bank at the Royal Botanic Gardens, Kew, which holds the largest seed collection of 24,000 wild species. Important national/regional collections include: coffee in Côte d'Ivoire, Ethiopia, Cameroon, Kenya, Madagascar and Tanzania; sesame in Kenya; cassava in Malawi, Zambia and Tanzania; and sweet potato in Mauritius, Zambia, Swaziland and Tanzania, as well as China's largest seed bank, the Germplasm Bank of Wild Species (GBWS).

Source: Global Crop Diversity Trust (2007).

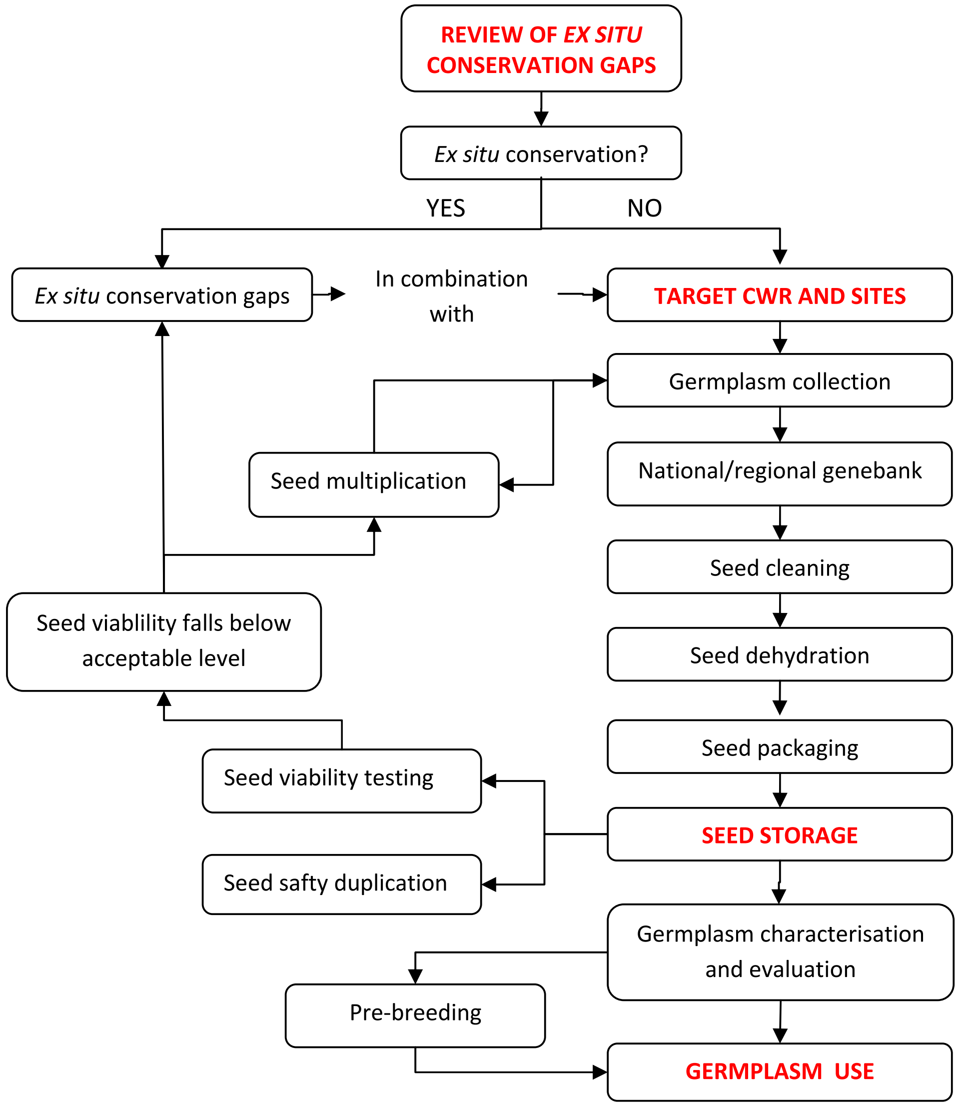

Figure 22. *Ex situ* conservation of CWR

A.11.2. Methodology

(i) Review of *ex situ* conservation gaps. *Ex situ* conservation gaps that resulted from the gap analysis should be the foundation of the planning of the national *ex situ* collection programme to ensure systematic *ex situ* conservation of priority CWR species (see Section 8). Due to the potentially very large number of CWR species it is unlikely that sufficient resources will be available to conserve all national CWR species. As is mentioned above *ex situ* collections are often the 'market stall' through which the germplasm user community access the germplasm they require, therefore another important consideration when formulating the *ex situ* collection programme is meeting the users demands. Further ideally the germplasm curator should anticipate the demand and have germplasm ready to meet that demand whether as directly sampled germplasm or pre-bred lines before the user requests the germplasm.

(ii) Selection of CWR and collecting sites for targeted collecting. Priority should be given to collecting individual CWR that are not conserved *ex situ* or *in situ*, as well as CWR populations (within the same CWR) (identified by undertaking gap analysis of ecogeographic, trait or genetic diversity) that are not represented in gene banks. It may not always be necessary to collect fresh CWR if the necessary gap filling germplasm is held by a sister gene bank then material may be obtained from inter-gene bank exchange or even knowledge that the germplasm is held by a sister gene bank may fill the gap. Note all CWR collection should be undertaken legally with the appropriate national permission and ensuring the collection is not counter to international conventions (e.g. CITES http://www.cites.org; International Treaty on Plant Genetic Resources for Food and Agriculture http://www.planttreaty.org/). Collectors are also referred to the FAO International Code of Conduct for Plant Germplasm Collecting and Transfer (http://www.fao.org/ag/agp/agps/PGR/icc/icce.htm) for further guidance.

CWR will be collected from natural or semi-natural habitats bearing in mind 6 basic field sampling factors:

- Distribution of sites within the target area – using either the cluster (site close together to pick up micro-habitat associated genetic diversity) or transect approach (site along line to pick up diverse ecosystem associated genetic diversity);
- Number of sites sampled – maximum possible with the resources available;
- Delineation of a site – related to the size of the interbreeding unit the edges of the site may also be delineated by dominant habitat changes;
- Distribution of the plants sampled at a site – randomly throughout the site or if there are distinct habitats stratified random that encourages sampling from each habitat type, collecting off-types or interesting material selectively;
- Number of plants sampled per site – 2,500 seeds sampled from 40-50 plants but preferably 5,000 seeds from 100 individuals;
- Indigenous knowledge held by local community – field collectors should note knowledge held by local people on the CWR found in their area, this may relate to population locations, threats, habitat associations and uses.

Each of these factors may vary depending on the nature of the target CWR being sampled and also assumes it is possible to apply the ideal sampling strategy; many CWR are, for instance, found as individual plants or small clumps of plant not dense stands and further ripening is not uniform so all the potential fruit is unlikely to available during one sampling visit. A further important point to consider is that germplasm is virtually worthless unless it has detailed passport data associated with the collection location, so this data must be collected in the field (including GPS location), placed in a database and made available to the user community. With CWR collections it is also advisable to collect voucher specimens so the accessions identification can be checked post-collection.

(iii) Gene bank seed processing. Following collection the sample arrives at the gene bank and is processed in the standard manner, which is likely to include: seed cleaning (to separate chaff and fruit debris from seed and ensure the accession is sample of a single species), seed health evaluation (inspection for seed borne diseases and pests), dehydration (normally to around 5-6% relative humidity), packaging (which most often take the form of glass vials, metal cans or laminated aluminium foil packets), registration (entering an associated record in the seed bank management system and making the accession available to the users) and storage (usually in a -18°C cold room). When field collecting CWR species it may not always be possible to obtain a sufficiently large seed sample to be banked directly so there may need to be a seed multiplication cycle before the seed can be processed and incorporated into the gene bank. See 'Additional materials and resources' for detailed gene bank methodologies.

(iv) Post-storage seed care. Once the seed is incorporated into the gene bank the seeds viability will gradually decrease over time and there will be a need to extract a sample of seed and test its germination viability at approximately 10 year intervals. Viability is a measure of how many seeds are alive and can develop into normal plants. It is usually expressed as percentage germination and above 75% is an acceptable level of viability. Viability is usually determined before the seeds are packed and placed into storage, and subsequently at regular intervals during storage. When germination falls below 75% the accessions requires regeneration.

The aim of regeneration is to increase the quantity of seed of any accession but while doing so it is very important to ensure that the original genetic characteristics of the accession are retained as far as possible. Each multiplication / regeneration cycle contains hazards to maintenance of the genetic integrity of the accession, such as: (a) contamination from foreign pollen during fertilisation, (b) contamination through seed adulteration during harvesting, threshing and packaging, (c) changes due to gene mutation, (d) genetic drift due to random loss of alleles, particularly when regenerating from small numbers of individuals, and (e) genetic shift due to unconscious natural or artificial selection (related to diverse environmental conditions during regeneration)[87]. The risks involved with regeneration will vary considerably according to the crop species, but it is also a costly operation, therefore, the most efficient and cost effective way of maintaining genetic integrity is to keep the frequency of regeneration to an absolute minimum.

A.11.3. Examples and applied use

Box 54. *Lathyrus belinensis*: a CWR discovered and almost lost

In 1987 while collecting legume species near Cavus, Antalya province, Turkey a new species of the genus *Lathyrus* was discovered and described as *Lathyrus belinensis*. The single population was growing alongside a new road that was just then being cut through fields between Kumluca and Tekirova. The population appeared to have its greatest concentration in and around an ungrazed village graveyard in the village of Belin. The new species was most closely related to *L. odoratus* (sweet pea), being just as scented as sweet pea but with more hairy vegetative parts. The most striking and economically interesting distinguishing feature of *L. belinensis* is the flower colour which is yellow with conspicuous red veins, which contrasts with *L. odoratus* flowers which can be purple, blue, pink or cream, but never yellow. Thus the discovery of *L. belinensis* was an opportunity for horticulturalists to breed a yellow sweet pea—a goal of many contemporary sweet pea breeders.

The type population was found over an area of only 2 km^2 and although the species was published in 1988, no further populations have subsequently been reported. The only known population was threatened the new road construction and the planted of conifers at the time of original collection. On returning to collect more seed in 2010 the original type location had been destroyed by earthworks associated with the building of a new police station. Although a few plants were found in the area and seed is held ex situ, the richest area within the site had been lost. L. belinensis has recently been assessed using IUCN Red List Criteria as Critically Endangered—the most highly threatened category, only time will show if field conservation will save this species in the wild!

Maxted (2012)

[87] Sackville-Hamilton and Chorlton (1997)

Box 55. *Ex situ* conservation of the world's major CWR

The Global Crop Diversity Trust has recently initiated a large scale global project concerned with "Adapting Agriculture to Climate Change: Collecting, Protecting, and Preparing Crop Wild Relatives". Although the bulk of the project will focus on the utilisation of CWR diversity, it includes the first systematic attempt to collect and conserve priority CWR diversity at a global scale. This is only feasible now due to 1) the taxonomic and genetic relationships between CWR becoming increasingly clarified, 2) ease of access to large on-line ecogeographic data resources, 3) better knowledge and tools for modelling and mapping the distribution of plant species through geographic information systems (GIS), and 4) a concerted global desire to implement the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). The priority CWR species were identified by combining the ITPGRFA Annex 1 and the major and minor food crops listed in Appendix 2 of the *World Atlas of Biodiversity* (Groombridge and Jenkins 2002). This resulted in a list of approximately 10,500 CWR species. To produce a reduced list of priority CWR, only those species present in Gene Pools 1b and 2 or Taxon Group 1b, 2 and 3 were included, as these are the taxa that can most easily be used in plant breeding using conventional techniques. The priority list contains 1,392 CWR species from 109 genera. *Ex situ* gap analysis is being undertaken to identify the locations of genetic diversity un- or under-secured in *ex situ* collections in order to inform planning of germplasm collecting for *ex situ* conservation. The project is currently gathering and geo-referencing species occurrence and conservation data from on-line resources, herbarium and gene bank databases, and following the gap analysis, extensive CWR collection and *ex situ* storage is planned so that for the first time the CWR diversity most important to underpin global food security will be available to the user community. Collected CWR accessions will be stored in relevant national and international gene banks, and will be safely duplicated for long-term security at the Svalbard Global Seed Vault, in Norway. Following collection, traits of value for adaptation to climate change will be transferred into cultivated lines through pre-breeding, and the results will be evaluated in the field. The wild species accessions and the promising lines generated will be collected and made available to the global community for breeding and research under the terms of the ITPGRFA.

Source: Khoury *et al.* (2011)

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WWW Bioversity International training modules on plant collecting: http://www.bioversityinternational.org/training/training_materials.html#c10721

WWW Bioversity International training modules on *ex situ* conservation/genebank management: http://www.bioversityinternational.org/training/training_materials.html#c10715

WWW European Native Seed Conservation Network (ENSCONET) (with collecting and curation manuals, database, germination recommendations, etc.): http://ensconet.maich.gr/

WWW FAO International Code of Conduct for Plant Germplasm Collecting and Transfer (http://www.fao.org/ag/agp/agps/PGR/icc/icce.htm)

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