Chapter 12

Complementary Conservation Actions

Adopting a complementary conservation strategy means that a range of methods are employed, each appropriate to a specific component part of the overall conservation programme and taken together, these methods complement each other in order to achieve the most efficient and safest conservation in the long term (Sharrock and Engels, 1996).

Aim of this chapter

As this book often makes clear, *in situ* conservation is the favoured approach to CWR conservation, as it has the distinct advantage that target species are continuously exposed to a changing natural environment that allows new diversity to be generated. However, such exposure can often dramatically threaten the very existence of these species. For this reason, *in situ* conservation approaches will often need to be supported by complementary conservation approaches for the sake of security. Such complementary conservation approaches also have the advantage that they help facilitate access by plant breeders to important genetic materials for crop improvement. Some level of complementary conservation will need to be practised for the optimal conservation of CWR. It is beyond the scope of this manual to provide an in-depth examination of the various complementary conservation approaches available for CWR. Moreover, it is the aim of this chapter to provide the reader with a general overview of the types of approaches and techniques that are available and to highlight how these might be used to complement *in situ* conservation, such as the provision of a safety net for genetic diversity, which is difficult to conserve *in situ* or threatened in the wild. Further, the potential role of *ex situ* collections in facilitating the recovery and reintroduction of CWR populations *in situ* is highlighted.

Introduction

Conserving CWR *in situ* is not sufficient in itself. While *in situ* conservation is essential to maintain the evolution of the species and allow new diversity to be created through natural selection processes, it presents many disadvantages for conservation and has severe limitations in facilitating the use of CWR for crop improvement (see Box 12.1) (for reviews see Maxted et al, 1997 and Engels et al, 2008). Although *in situ* conservation is an efficient tool for conservation of CWR, in order to make CWR more accessible for crop improvement and other human uses and to ensure that the maximum genetic diversity of target species is safely conserved, other approaches will need to be applied. It is important to back up any *in situ* interventions with complementary *ex situ* conservation in genebanks as seed, pollen, living plants (in field genebanks or in botanic gardens), tissue culture, or cryopreservation, depending upon the biology of species to be conserved.

As discussed in Chapter 1, CWR have increasingly provided new genes for crop improvement (Hajjar and Hodgkin, 2007) when they are readily accessible to plant breeders and, in such cases, have been extensively used as a source of useful genetic traits for disease resistance as well as abiotic (temperature and drought) stress tolerance, crop yield and improved quality. With the anticipated impacts of climate change on agricultural production, climate-adapted traits most likely to be found within CWR will become even more in demand by plant breeders. Consequently, back-up samples of CWR in *ex situ* collections to facilitate access and use in plant breeding programmes is becoming a high priority. However, wild relatives of some crops are still poorly represented in *ex situ* collections, despite the fact that over the last decade there has been an increase of 3 per cent in their collection, as shown in the recent update of the *State of the World Report for Plant Genetic Resources for Food and Agriculture* (FAO, 2010).

It should be noted that the *ex situ* conservation of some CWR presents major challenges for genebank managers both from technical and management aspects. Often, the storage conditions which have been established mainly for major crops are not well adapted for some of their wild relatives, on which limited research has been undertaken to refine their conservation *ex situ*. Some may have dormancy problems or be simply difficult to germinate, while other species may have recalcitrant seeds. In fact, the seed storage behaviour can vary among, and even within, species, and different provenances may not adapt to the field condition in the case of field collection. For example, among *Coffea* species, a range of storage behaviours from orthodox to recalcitrant may be found. Protocols may not exist for specific CWR to be considered for *in vitro* or cryopreservation conservation. Some CWR may be easier to store *ex situ* than the crop species, e.g. seed-bearing *Musa* species. There can also be restraints in accessing germplasm from genebanks due to government policies relating to the exchange of germplasm, property rights, access and benefit-sharing regulations, or phytosanitary regulations. Further, the cost of maintaining a genebank should be taken into account as it can be prohibitive in many countries and lack of sustained funding in such instances can threaten collections.

Box 12.1 Advantages and disadvantages of *in situ* and *ex situ* conservation of CWR

Advantages	*Disadvantages*
***In situ* conservation**	
• Avoids storage problems associated with field genebanks and recalcitrant seeds • Allows evolution to continue through exposure to pests and diseases and other environmental factors • Indirect benefits, including ecosystem services • Sustainable use by local people	• Requires extensive areas for effective conservation • Exposes natural populations to a wide range of natural catastrophic events (storms, hurricanes, cyclones) and other threats • Materials cannot be readily used and are very difficult to access • Subject to conflict with management by landowners (CWR may not have high priority) • Expensive to maintain
***Ex situ* conservation**	
• Rescue of threatened germplasm • Requires limited space to conserve large numbers of accessions • Conserves an adequate representative sample of CWR populations • Ease of accessibility and exchange of germplasm; use can be promoted • Evaluation facilitated • Ease of documentation • No exposure to pests, diseases and other hazards (except for field collections and botanic gardens) • Indefinite maintenance of germplasm • More cost-effective compared to *in situ* conservation	• Freezes the evolutionary process • Difficult to ensure adequate sampling (intra-specific variability) • Total genetic integrity cannot be ensured due to human error, selection pressure during regeneration • Only limited accessions can be conserved in field genebanks • Natural catastrophes in field genebanks • *In vitro*-somaclonal variation

What do we mean by a CWR complementary conservation strategy?

The concept of a complementary conservation strategy for CWR involves the combination of different conservation actions, which together lead to an optimum sustainable use of genetic diversity existing in a target gene pool, now and in the future (Dulloo et al, 2005). Complementary conservation strategies are also

known as integrated or holistic and the principle is that the full range of conservation options available should be considered and the appropriate combination applied in particular situations (Falk and Holsinger, 1991; Given, 1994). The two main conservation approaches (*ex situ* and *in situ*) are both important in the conservation and use of genetic diversity. In addition, it may be appropriate to attempt other techniques such as *inter situs* (see below) and assisted migration or colonization (see Chapter 14).

The ultimate purpose of germplasm conservation is use and, consequently, any conservation strategy should include mechanisms that will also ensure access to the germplasm by relevant stakeholders. Other important issues that must be addressed in a conservation strategy include issues related to policy and legal frameworks, documentation, socio-economic aspects, infrastructure and networks. Since the needs of users and the conservation technologies may change over time, a complementary conservation strategy should be flexible enough to allow such changes to be taken into consideration. Dulloo et al (2005) proposed a framework for developing a complementary conservation strategy using coconuts as an example. The process involves first defining the options for conservation of the target species, taking into account the feasibility of conserving it *in situ*, its seed storage behaviour, whether or not the species can be conserved as seeds, whether or not protocols for *in vitro* or cryopreservation are developed or whether they can only be conserved as live plants in field genebanks or botanic gardens and, finally, if options such as translocation or *inter situs* approaches are necessary.

The choice of the complementary conservation actions should also take into account the intended use of the conserved germplasm, available infrastructure and human resources, space availability, accessibility and so on. Nevertheless, in the case of CWR, one must keep in mind that their conservation is not always based on their availability for immediate use. Based on these elements, state of knowledge and the options available to date, a framework for a complementary conservation strategy can be developed. Thus a complementary conservation strategy can be seen as a logical process and not just a selection of appropriate conservation methods. The framework can be seen as a series of steps (see Figure 12.1); at each step information is gathered, specific actions taken and/or decisions made. It is important that proper consultation be held with all stakeholders in developing the complementary conservation strategy (see Chapters 4 and 5 for in-depth discussion on how to involve stakeholders). This could be done by establishing a network of stakeholders, facilitated by a lead agency. It would be the role of this network or committee to then define the complementary conservation strategy objectives and sub-objectives. These could be, for example, the necessity for creating a back-up of the *in situ* population, for implementing a reintroduction/recovery programme, carrying out research, use in evaluation/breeding programmes or increasing the awareness of the public on the importance of CWR (see Chapter 16) or for training and education (see Chapter 15). For each specific objective, the complementary conservation strategy options available should then be analysed in terms of their feasibility and requirements in infrastructure, human resources, land, costs, accessibility and the risks involved. The pros and cons of

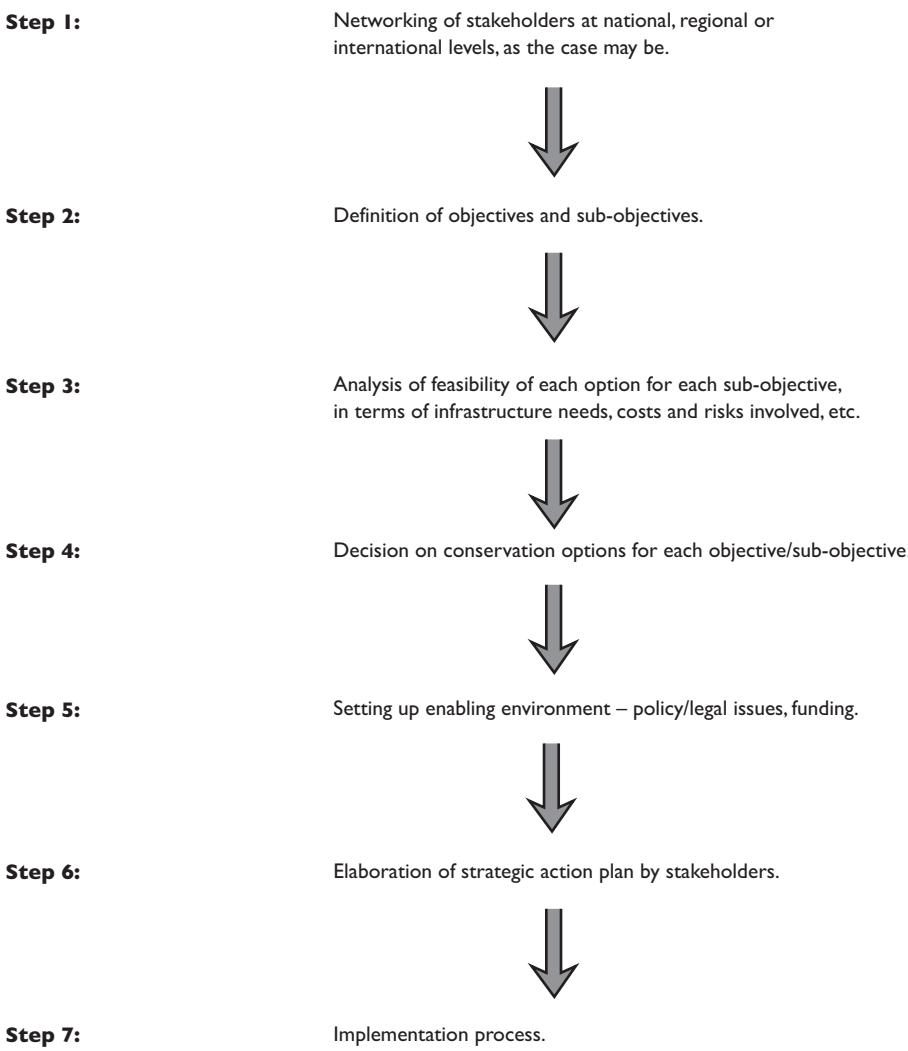

Figure 12.1 *Framework for developing a complementary conservation strategy*

each of the possible options must be weighed against each other and decisions made on the complementary conservation strategy options to be followed for specific objectives.

The next important step in the process would be to ensure there is an adequate policy and regulatory framework in place, which will allow implementation of the complementary conservation strategy options. This would involve an analysis, and possible revisions, of policy issues in terms of legislation, germplasm exchange and benefit-sharing. Consideration must also be given to sources of funding. Once these issues are addressed and put into place, a strategic action plan can be developed and implemented (steps 6 and 7 in Figure 12.1). For each

step, the stakeholder network should be consulted before relevant decisions are made and responsibilities assigned to the various relevant players.

Ex situ conservation options

This section provides some technical guidelines for establishing an *ex situ* collection and a brief description of the different *ex situ* conservation options. For a general review of complementary *ex situ* methods see Guerrant et al (2004), Thormann et al (2006) and Engels et al (2008) .

Guidelines for seed collecting

Collecting seeds or other propagules is obviously the first activity for establishing an *ex situ* collection. This needs to be well planned and well prepared to maximize the genetic diversity of the population. It is not intended here to provide a detailed account of collecting. There are a number of excellent technical guidelines written about how to plan and prepare for collecting for *ex situ* conservation (Guarino et al, 1995; Schmidt, 2000; Smith et al, 2003; Guerrant et al, 2004; ENSCONET, 2009). Given that seeds are the easiest and most amenable material to collect and conserve, most of these guidelines focus on seeds. However, Guarino et al (1995) also provide guidelines on how to collect vegetatively propagated germplasm (see their Chapters 21 and 22). They also provide guidelines for collecting *in vitro* and pollen material (see their Chapters 24 and 25). In addition, there is much information available for download on the internet. For example, there are good seed collecting summaries and field manuals for download from the Millennium Seed Bank site.[1] A documentary on wild chickpeas seed collecting by Ken Street, available to view at http://www.seedhunter.com/, provides an excellent insight into the practical realities of collecting for diversity.

It may be desirable to target collecting sites that contain the highest species and genetic diversity. The use of predictive tools (such as FloraMap, DIVA-GIS (Hijmans et al, 2001)) based on geographic information systems (GIS) can help identify such potential collecting sites (see Chapter 8). Guarino et al (2001) provides a general discussion on the application of species distribution models in the conservation and use of plant genetic resources. Many of the GIS methods use the climatic variables as the principal drivers of geographic distribution and can be used to predict sites of high species diversity. For example, Hijmans and Spooner (2001) used DIVA-GIS to describe the geographical distribution of wild relatives of potatoes and identified Peru as a location for high numbers of wild potato species, including rare wild species. Their study also allowed the identification of areas of high species richness which facilitated the design of *in situ* conservation reserves to protect them. Another good example is the study by Jarvis et al (2005), where GIS was used to optimize a collecting mission for a rare wild pepper species (*Capsicum flexuosum* Sendtn.) in Paraguay. The species was found at five out of the seven points predicted to harbour the species and was not found at four of the five points predicted not to harbour the species. Such

approaches allow the collecting of germplasm to be carried out in a much more systematic and efficient manner.

The GapAnalysis website, http://gisweb.ciat.cgiar.org/GapAnalysis/, developed by Bioversity International, the International Rice Research Institute (IRRI) and the International Centre for Tropical Agriculture (CIAT), is a useful tool to enable plant collectors to target areas that contain traits and taxa that are under-represented in *ex situ* collections. A detailed methodology on how to carry out a crop wild relative gap analysis is provided at http://gisweb.ciat.cgiar.org/GapAnalysis/?p=139.

In addition, Maxted et al (2008) use a gap analysis methodology to identify areas for conservation, taking into consideration ecogeographic characteristics of the target taxon, as well as elements of the diversity effectively represented by existing *in situ* and *ex situ* conservation actions. The methodology is illustrated by its application to the African *Vigna* species.

In the context of this manual, some key activities that must be implemented when collecting samples include the following:

- Gather the information about the species to be collected so as to develop the *ex situ* conservation strategy. This should include information about seed storage behaviour, plant phenology (flowering, fruiting times) and reproductive biology, as well as ecogeographic information including botanical nomenclature, synonyms, historical location data and as full a data set as possible from local and regional herbaria. This is discussed in more detail in Chapter 8.
- Liaise with the relevant stakeholders and organize a stakeholder network.
- Undertake a gap analysis to identify the populations most in need of collecting due to threats, but also to identify areas rich in diversity.
- Obtain the necessary authorization for collection. Collection should be undertaken in line with national and international laws and regulations.
- Devise a sampling strategy for collecting that optimizes the highest level of genetic diversity, including the number of plants to be sampled. According to ENSCONET (2009), it is recommended to collect from five populations across the range of the species and to try and collect from at least 50 plants (preferably 200 plants) per population, but this should be used solely as a guide. The actual number to collect may depend on local circumstance and the plant collector needs to use his or her judgement so that the maximum genetic diversity can be captured while not endangering the population. Another consideration is the proposed use of the material, e.g. long-term safety back-up or reintroduction. See also the Guidelines on the Conservation of Medicinal Plants (1986), jointly published by the WHO, IUCN and WWF: http://apps.who.int/medicinedocs/documents/s7150e/s7150e.pdf (accessed 23 November 2010).
- Collect seeds and other materials in the field, including a herbarium specimen to verify the taxonomic identity. This is important since, very often, seeds of unknown species are collected and remain in collections as such for a long

time. Such collections then have very limited value and use (see Miller and Nyberg, 1995).

Guidelines for the proper handling of the seeds in the field include:

- Seeds should be extracted from fruits where possible and pre-cleaned.
- Seeds should be prepared for safe transportation in a paper bag, envelope or cloth bag.
- If transportation to the genebank is expected to take a long time, it is best to dry seeds over silica gel or other appropriate desiccant in plastic containers.
- Avoid exposing seeds to direct sunlight and high humidity (especially at night).

For more details see Smith, 1995; Schmidt, 2000 (see Sections 3 to 5); Smith et al, 2003 (see Section 1); and ENSCONET, 2009.

Ex situ conservation methods

Box 12.2 summarizes the different methods available for *ex situ* conservation.

Seed genebanks

Very few seed genebanks are dedicated to wild species, such as CWR (Heywood, 2009). The first to be established, in 1966, was the seed bank (ETSIA-UPM, now BGV-UPM) for native Spanish species at the Polytechnic University of Madrid, founded by the late Professor C. Gómez Campo; it now contains samples of 350 threatened Spanish species and subspecies, representing almost a quarter of the threatened flora of Spain. An even more notable exception is the Millennium Seed Bank (MSB) at Wakehurst Place, Royal Botanic Gardens, Kew, UK, which aims to house up to 10 per cent of the world's seed-bearing flora, principally from arid zones, by 2010. Recently, the MSB celebrated this target by the collection of a crop wild relative of banana from China, *Musa itinerans*, which may provide valuable genetic material for breeding new varieties of banana with resistance to diseases. The National Center for Genetic Resources Preservation (NCGRP) of the United States Department of Agriculture, based in Fort Collins, Colorado, also aims to systematically preserve a national collection of genetic resources including many CWR.

The representation of CWR in agricultural seed banks is very patchy and often the accessions are very small and collected incidentally rather than as part of a deliberate policy. The second *State of the World Report on Plant Genetic Resources for Food and Agriculture* (FAO, 2010) reports 10 per cent of the global germplasm holdings are wild species. Of these, forages and industrial crops account for a relatively high proportion of CWR. However, Maxted and Kell (2009), highlight that only between 2 and 6 per cent of global genebank *ex situ* collections are CWR, and of the total number of CWR species, only about 6 per cent have any accessions conserved *ex situ*. The discrepancy between these figures may also be a consequence of how CWR are defined.

Box 12.2 *Ex situ* conservation methods

Seed genebanks: This involves the drying of seeds to low moisture content (generally between 3 and 7 per cent) and storing in moisture-proof containers at low temperature (4°C for short-term conservation and –20°C for long-term conservation; FAO and IPGRI, 1994). Only taxa with orthodox seeds that can support drying to low moisture content and are cold-tolerant can be conserved in seed genebanks.

Field genebanks: This consists of growing living plants in a field, or very often in pots, in a screen or greenhouse. Field genebanks offer easy access to the plant material for characterization, evaluation and subsequent utilization, but are often difficult and expensive to maintain, time-consuming, labour-intensive, vulnerable to bad weather conditions, may mingle with adjacent plants, hybridize and blend with each other and can only conserve limited genetic material because of space issues.

Botanic Gardens: This covers the maintenance of (usually) small numbers of living plants in the garden collections and landscapes; extensive samples grown in field plots or under glass as conservation collections or as temporary collections for use in reintroduction experiments. Many botanic gardens have a strong focus on growing wild origin material, including CWR. They also play an important public awareness and education function.

Tissue culture: This involves the maintenance of explants in a sterile, pathogen-free environment with a synthetic nutrient medium. Different *in vitro* conservation methods are available: (1) slow growth conservation by limiting the environmental conditions and/or the culture medium; (2) synthetic seed technique, which aims to use somatic embryos as true seeds by encapsulating embryos in alginate gel, which can then be stored after partial dehydration and sown directly.

Cryopreservation: This involves the storage of a range of living tissues, including cell suspension, calluses, shoot tips, embryo and even whole seeds, at extremely low temperatures, usually at –196°C in liquid nitrogen, at which cell metabolism is effectively suspended. The material has to survive the freezing procedure before storage and the thawing procedure after storage. A range of cryopreservation techniques, including controlled rate cooling, vitrification, encapsulation-dehydration, encapsulation-vitrification, dormant bud preservation, pre-growth desiccation and droplet freezing.

Pollen storage: Pollen can be stored in the same way as described above for seeds and used as a conservation method for genetic resources, especially for perennial species of fruit and forest trees. It has a relatively short viability when conserved under classical storage conditions (partial desiccation followed by storage at sub-zero temperatures) and has therefore been used only to a limited extent in germplasm conservation.

Over 200 botanic gardens around the world also have seed banks (Laliberté, 1997; BGCI, 1998), ranging from small numbers of accessions stored in a domestic or commercial deep freezer to large-scale custom-built facilities, such as the Germplasm Bank of the Environmental Agency of Andalucía (Banco de

Germoplasma Vegetal Andaluz de la Consejería Andaluza de Medio Ambiente) at the Jardín Botánico de Córdoba, Spain, which stores more than 7000 accessions or propagules, mainly seeds, of more than 1500 different species of Andalusian plants and about 500 other Iberian endemic species. The Fletcher Jones Education Centre for the Preservation of Biodiversity complex at Rancho Santa Ana, California, USA, includes cold storage for seeds, climate-controlled growth chambers that facilitate germination studies and graduate programme research, seed-processing equipment and ample laboratory space.

There is an extensive amount of literature on the state of the art of *ex situ* seed conservation. Among them, the key information sources include Engels and Wood (1999); Hawkes et al (2000); Engels and Visser (2003); Smith et al (2003); Rao et al (2006); Thormann et al (2006); Engels et al (2008). An interactive self-learning module on seed handling in genebanks has been prepared by Bioversity International to help genebank technicians to process and prepare seeds for conservation (http://cropgenebank.sgrp.cgiar.org/images/flash/seed_handling_elearning_module/index.htm). In addition, the MSB technical information sheets (http://www.kew.org/msbp/scitech/publications/info_sheets.htm) contain information of relevance to *ex situ* conservation of CWR.

Field genebanks

For many species that produce no seeds (clonally propagated) or have seeds that are desiccation- and cold-sensitive, such as cacao, rubber, oil palm, coffee, banana and coconut, field genebanks are the best means for their conservation. For example, in Madagascar, the wild relatives of coffee are conserved in an important field genebank located at Kianjavato, first established in the early 1960s; to date, the collection holds 171 accessions (Dulloo et al, 2009). One of the advantages of field genebanks is that materials can be easily characterized and evaluated. For example, a project on tropical fruit trees in the Philippines led to the establishment of field collections of wild relatives of four tropical fruits trees including durian (*Durio zibethinus* Murray), mangosteen (*Garcinia mangostana* L.), jackfruit (*Artocarpus heterophyllus* L.) and pili nuts (*Canarium ovatum* Engl.). The germplasm collected were characterized and evaluated and, as a result, two new commercial varieties, a jackfruit variety, officially named 'Baybay Sweet', and a mangosteen accession, named 'UPLB Sweet', have been approved and registered with the National Seed Industry Council (NSIC) of the Philippines and are being marketed.

Key references for the management of field collection include those of Engelmann, 1999; Hawkes et al, 2000; Reed et al, 2004; Thormann et al, 2006.

Living conservation collections in botanic gardens

Historically, botanic gardens have played a key role in the collection and exchange of seed and other propagules with other gardens (Heywood, 2009). The roles played by botanic gardens such as Bogor, Howrah (Calcutta), Pamplemousses (Mauritius) and Singapore in introducing and developing

plantation crops such as tea, oil palm, rubber, coffee and various spices are fully recognized (Heywood, 1991). Botanic gardens are now much more involved in the conservation of plant genetic resources, particularly for non-crop, medicinal and wild species, focusing on rare and endangered species (Du Puy and Wyse Jackson, 1995; Maunder et al, 2004). For example, the Royal Botanic Garden in Edinburgh, UK, developed an International Conifer Conservation Programme in 1991, and has been engaged in activities on assessing the conservation status of endangered conifers and has developed a Conifer Action Plan for the IUCN. It has also been active in carrying out applied research activities on conifers and on establishing a network of *in situ* and *ex situ* sites to protect the threatened species.

The conservation role of botanic gardens has often been the subject of much debate. Given that botanic gardens have limited amounts of space, the number of accessions of individual species is limited and, thus, their value for genetic diversity conservation is often questioned. However, it has been demonstrated that for rare species, botanic garden collections may help to conserve a higher genetic diversity than wild populations and can be used to augment the genetic diversity of wild populations. An example is the wild populations of *Brighamia insignis* A. Gray, an endemic species of Hawaii, which is represented by only 20 individuals in the wild but is widely cultivated in botanic gardens. Using isozymes, Gemmill et al (1998), were able to show that the collections held at the National Tropical Botanic Garden (NTBG) in Hawaii, were a good representation of the diversity found in the wild and would therefore serve as suitable stock population to augment natural populations. Botanic gardens also have considerable horticultural expertise that can help with the propagation of rare species and subsequently their reintroduction back into the wild. An example of the use of botanic gardens for *ex situ* conservation in Sri Lanka is given in Box 12.3.

Tissue culture

The problems associated with field genebanks as described above have prompted much research into the development of alternative techniques, notably *in vitro* culture or tissue culture techniques for recalcitrant seed and vegetatively propagated species (see Box 12.2). The major prerequisites for conservation by tissue culture are the availability of skilled personnel and reasonably equipped laboratory facilities (see Reed et al, 2004, for the physical requirements for a plant tissue culture laboratory). Examples of crop wild species that are conserved *in vitro* include the Global *Musa* Germplasm Collection under the management of the International Network for the Improvement of Banana and Plantain (INIBAP)/Bioversity hosted by the Katholieke Universiteit Leuven (KULeuven). The collection contains nearly 1200 accessions and represents the single centralized holding of a large proportion of the known gene pool. About 15 per cent of the collection includes wild *Musa* species (INIBAP, 2006). Other examples of tissue culture collection containing wild relatives include the cassava collection at CIAT, potatoes at CIP, Peru, and wild apples (NCPGR-USDA).

Box 12.3 *Ex situ* conservation initiatives in botanic gardens of Sri Lanka

Under the National Botanic Gardens (NBG), are the Royal Botanic Gardens (RBG) at Peradeniya, Hakgala, Gampaha (Henerathgoda), Sitawake (Awissawella) and Mirijjawila (Hambantota District), which provide coverage of all major climatic zones. The medicinal plant gardens at Ganewatte (on 23ha) in the North-Western Province and a Biodiversity Complex at Gampola, also function under the NBG. The RBG at Peradeniya, located on 59ha has over 4000 species under cultivation. It is mandated for *ex situ* conservation and has pioneered floriculture in Sri Lanka. However, only a fraction of the species in the botanic gardens at present are endemic to Sri Lanka, and the role of these institutions as reservoirs of indigenous biodiversity is not well established, due to historical reasons. This trend has been reversed somewhat in recent times, and the RBG now has 1471 specimens from local species, while the more recently developed herbarium at the Hakgala Botanic Gardens has about 2000 specimens from local species. One of the main objectives of the NBG is for development of technologies related to exploitation of lesser-known and under-utilized plants and development of ornamental and amenity horticulture. There are several medicinal plant gardens located in the wet zone of Sri Lanka (i.e. in Navinna and Meegoda). The Ayurvedic Garden in Navinna harbours around 200 species of medicinal plants, with more than 1500 individual plants.

Source: Fourth Country Report from Sri Lanka to the United Nations Convention on Biological Diversity, 2009

Cryopreservation

Cryopreservation is one of the most promising conservation methods for long-term conservation. One of its most important advantages is that it requires very little space as compared to *in vitro* and field genebanks. It is also the more cost-effective method for long-term conservation requiring very little maintenance (Dulloo et al, 2009). The maintenance of the collection is reduced to mainly topping up the liquid nitrogen, as there is no need for re-culturing, as is the case for *in vitro* conservation. However, cryopreservation protocols, like *in vitro* culture techniques, need to be developed for each and every species, a factor that limits application to a wide diversity of CWR. To date, very few, if any, cryo-collections exist for CWR. Kew has developed protocols for cryopreservation of wild plants, especially ferns, mosses, orchids, shrubs and herbs (see: http://www.kew.org/ksheets/pdfs/K31_cryopreservation.pdf).

Research on cryopreservation has made much progress and protocols for conserving over 200 plant species are now available (Engelmann and Takagi, 2000; Engelmann, 2004). Research work involving CWR undertaken in Australia has led to the development of cryopreservation protocols for *Carica papaya* and a wild relative *Vasconcellea pubescens* (Ashmore et al, 2007) and for certain *Citrus* species (Hamilton et al, 2005, 2008). For a review of cryopreservation techniques, see also Engelmann (2000), Thormann et al (2006) and Reed (2008). Of these,

Reed (2008) provides a practical guide on plant cryopreservation and gives step-by-step instructions for the transfer of cryopreservation technology in conservation of important plants materials.

Pollen storage

Pollen is another plant material that can be stored and used as a conservation method for genetic resources, especially for perennial species of fruit and forest trees, and can be of much interest for CWR. It is commonly used by plant breeders, particularly for production of haploids in breeding programmes, to bridge the gap between male and female flowering time and to improve fruit setting in orchards (Towill, 1985; Alexander and Ganeshan, 1993). For example, the main use of coffee pollen is for breeding, since crosses may have to be made between trees that do not flower simultaneously or that grow far apart (Walyaro and van der Vossen, 1977). Collection and storage of pollen could be a way to obtain a more representative sample of genetic diversity in wild populations (Panella et al, 2009). For this reason alone, pollen can be an effective way of conserving, as well as using, CWR in breeding activities.

Pollen is also used for distributing and exchanging germplasm among locations, since transfer of pests and diseases through pollen is rare (except for some virus diseases) and is subjected to less stringent quarantine restrictions. Other uses are preserving nuclear genes of germplasm, studies in basic physiology, biochemistry and fertility, and studies for biotechnology involving gene expression, transformation and *in vitro* fertilization (Towill and Walters, 2000).

Pollen storage also has several disadvantages. Many species produce small amounts of pollen, which are not sufficient for effective pollen collection and processing. Because of its low viability, pollen needs to be replenished periodically. In this context, it is obvious why pollen preservation is supplemental: the seed or clone must also be conserved to yield the pollen. Multiple generations introduce the risk of population genetic problems, such as loss of alleles through random drift or splitting of adaptive complexes. Only paternal material is conserved and regenerated, and in order to utilize the germplasm, a recipient female plant must always be available for fertilization.

Use of *ex situ* collection in the recovery and reintroduction of CWR populations

Wild populations of CWR are often depauperate genetically to the point of near extinction as a result of habitat degradation and other threats. *In situ* conservation of these populations would require the development of a recovery plan and active interventions to reconstitute these populations. It is important to ensure a broad genetic base of the wild populations to guarantee its survival in the long term, especially with rapidly changing environmental conditions including climate change.

Ex situ collections can be used in recovery programmes in two main ways:

1 To reintroduce a species that has disappeared from its natural site. While the species may have become extinct from one of its sites, if accessions from the same site have been collected in the past and conserved in genebanks or in botanic gardens, these can provide valuable materials for restoration. However, the reintroduction of *ex situ* materials to the wild can be a complex activity and needs to be undertaken with great caution. One must ensure that the stock or accessions introduced are really native to the site, that the plants are free of diseases and that they have adequate genetic diversity to ensure their survival, etc. To assist conservationists in thinking through and taking all factors into account, the IUCN/SSC Re-introduction Specialist Group have developed policy guidelines for reintroduction (IUCN/SSC, 1995). These guidelines are applicable to both animals and plants and are therefore rather general. The IUCN technical guidelines on the management of *ex situ* populations for conservation (IUCN, 2002), also discusses the increasing value of *ex situ* conservation in *in situ* ecosystem and habitat conservation. The *Handbook for Botanic Gardens on the Reintroduction of Plants to the Wild* (Akeroyd and Wyse Jackson, 1995) published by BGCI contains plant-specific guidelines and provides botanic garden managers with guidance on the reintroduction of plants materials from botanic gardens to the wild and explores the issues of reintroduction and challenges of the reintroduction process.
2 *Ex situ* collections can be used in enrichment planting or reinforcement or supplementation if the population is threatened and is not regenerating in the wild. New plant material may be obtained from *ex situ* collections and planted to reinforce the population at the site. Again, it is important to observe precautions in such practices so as not to disrupt and threaten the genetic integrity of the natural population. In recovery programmes, it is important to consider the provenance of the material, the use of genetically variable reintroduction stock, as well as the potential of loss of genetic diversity (IUCN/SSC, 1995; IUCN, 2002; Guerrant et al, 2004; Kell et al, 2008).

In both these cases, it is important to ensure that the provenance of the materials introduced comes from the same site or as close to it as possible, in order to ensure the genetic integrity of the population. It is also most likely that material from the site would be locally adapted to it and this would ensure higher probability of success of the reintroduction. Often, however, such materials may not be available. In such cases, it is recommended that plant materials come from environments that have matching ecogeographic characteristics.

In practical terms, when there is a need to use *ex situ* collections for *in situ* intervention, the following steps should be followed and be included in the recovery plan:

1 **Site assessment** – a thorough examination of the site should be carried out, documenting not only the status of the target population, including popula-

tion size of the target species, patterns of distribution at the site, competitive plants, associated plants, pollinators, dispersers and predators, but also any threats affecting the population. The latter would need to be resolved prior to any reintroduction of the species. The site assessment would determine the strategy to adopt for replanting, in terms of planting density, pattern of planting, revegetation methods required (see below), etc.

2 **Revegetation method** – there are a number of revegetation methods that can be used to reintroduce the species back into the wild. These could be through direct seeding, planting using naked-rooted seedlings, potted seedlings or planting under nurse crops.

3 **Identification of source material** – the source of the material from *ex situ* collection must be chosen with a great deal of attention. Accessions coming from the same site, or as close to it as possible, should be selected.

4 **Sampling to ensure genetic diversity** – samples from the genebank accession(s) should be taken so as to represent the maximum genetic diversity present in the accessions. It is recommended to sample seeds from as many accessions as possible.

5 **Propagating of materials** – the planting materials (seeds or cuttings) should be multiplied in a nursery, taking into account dormancy and germination difficulties and an equal number of plants from each accession raised to the required number of plants needed for the replanting. It is important to clearly label all the plants with scientific names and accession number for long-term monitoring.

6 **Site preparation and replanting** – the success of the reintroduction will depend on good site preparation. As mentioned above, if there are any competing factors (competing alien plants, predators) that would affect the regeneration of the plants, they would need to be controlled prior to planting. Methods used could be as simple as weeding out competing plants to more elaborate treatments using chemical or biological control agents, depending upon the nature of the problem.

7 **Post-planting treatment** – once planted, the seedlings should be monitored and measures taken to ensure their survival. This may include mulching and weed control, either by hand or using herbicides. If they die, they need to be replaced from the nursery stock. It is important that a nursery stock of the *ex situ* accessions continue to be maintained to provide for some gap filling after planting in the wild.

Inter situs and other conservation approaches

In addition to *in situ* and *ex situ* conservation strategies, a number of other approaches have been developed recently, some of which blur the distinction between *ex situ* and *in situ*. For tree species, for example, the concept of 'forest genebanks' has been introduced (Shaanker et al, 2002): these are *in situ* sites that act as repositories of genes from as many diverse populations as possible, so as to

maximize the representation of genes captured. Other strategies involve maintaining *ex situ* populations in artificially created simulations of the ecosystems in which they occur naturally.

The term *inter situs*[2] conservation has been applied to the reintroduction of species to locations outside their current range but within the known recent past range of the species[3] (Burney and Burney, 2009). It contrasts with the 'assisted migration' discussed in Chapter 14 and has been practised with apparent success to save rare Hawaiian plants. It is a procedure which involves considerable risks and should not be practised except in very urgent cases.

Sources of further information

Akeroyd, J. and Wyse Jackson, P. (1995) *A Handbook for Botanic Gardens on Reintroduction of Plants to the Wild*, Botanic Gardens Conservation International (BGCI), p31.

ENSCONET (2009) *ENSCONET Seed Collecting Manual for Wild Species*. ISBN: 978-84-692-3926-1, available at: http://www.maich.gr:9000/PDF/Collecting_protocol_English.pdf.

Engels, J.M.M., Maggioni L., Maxted N. and Dulloo, M.E. (2008) 'Complementing *in situ* conservation with *ex situ* measures', in J. Iriondo, N. Maxted and M.E. Dulloo (eds) *Conserving Plant Genetic Diversity in Protected Areas,* Chapter 6, pp169–181, CAB International, Wallingford, UK.

Guarino, L., Ramanatha Rao, V. and Reid, R. (1995) 'Collecting plant genetic diversity technical guidelines, CAB International, Wallingford, UK.

Notes

1. http://www.kew.org/msbp/scitech/publications/03-Collecting%20techniques.pdf; http://www.kew.org/msbp/scitech/publications/fieldmanual.pdf
2. Usually referred to, incorrectly and ungrammatically, as *inter situ*.
3. This usage differs from that of Blixt (1994) who applies it to the maintenance of domesticates in farmers' fields, more commonly referred to as on-farm conservation.

References

Akeroyd, J. and Wyse Jackson, P. (1995) *A Handbook for Botanic Gardens on Reintroduction of Plants to the Wild*, Botanic Gardens Conservation International (BGCI), Richmond, UK

Alexander, M.P. and Ganeshan, S. (1993) 'Pollen storage', in K.L. Chadha and J.E. Adams (eds) *Advances in Horticulture, vol 1, Fruit Crops: Part I*, Malhotra Publishing House, New Delhi, India

Ashmore S.E., Drew, R.A. and Azimi-Tabrizi, M. (2007) 'Vitrification-based shoot tip cryopreservation of *Carica papaya* and a wild relative *Vasconcellea pubescens', Australian Journal of Botany*, vol 55, pp541–547

BGCI (1998) *Seed Banks*, Botanic Gardens Conservation International (BGCI), http://www.bgci.org/resources/Seedbanks/ (accessed 30 May 2010)

Blixt, S. (1994) 'Conservation methods and potential utilization of plant genetic resources in nature conservation', in F. Begemann and K. Hammer (eds) *Integration of Conservation Strategies of Plant Genetic Resources in Europe,* IPK and ADI, Gatersleben

Burney, D.A. and Burney, L.P. (2009) '*Inter situ* conservation: Opening a 'third front' in the battle to save rare Hawaiian plants', *BGjournal,* vol 6, pp17–19

Dulloo, M.E., Ramanatha Rao V., Engelmann F. and Engels J. (2005) 'Complementary conservation of coconuts', in P. Batugal, V.R. Rao and J. Oliver (eds) *Coconut Genetic Resources,* pp75–90, IPGRI-APO, Serdang, Malaysia

Dulloo, M.E., Ebert, A.W., Dussert, S., Gotor, E., Astorg, C., Vasquez, N., Rakotomalala, J.J., Rabemiafar, A., Eira, M., Bellachew, B., Omondi, C., Engelmann, F., Anthony, F., Watts, J., Qamar, Z. and Snook, L. (2009) 'Cost efficiency of cryopreservation as a long-term conservation method for coffee genetic resources', *Crop Science*, vol 49, pp2123–2138, doi:10.2135/cropsci2008.12.0736

Du Puy B. and Wyse Jackson P. (1995) 'Botanic gardens offer key component to biodiversity conservation in the Mediterranean', *Diversity,* vol 11, no 1 and 2, pp47–50

Engelmann, F. (ed) (1999) *Management of Field and* In Vitro *Germplasm Collection,* Proceedings of a consultation meeting, 15–20 January 1996, CIAT, Cali, Colombia, International Plant Genetic Resources Institute (IPGRI), Rome, Italy

Engelmann, F. (2000) 'Importance of cryopreservation for the conservation of plant genetic resources', in F. Engelmann and H. Tagaki (eds) *Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Application*, Japan International Research Center for Agricultural Sciences, Tsukuba, Japan/International Plant Genetic Resources Institute, Rome, Italy

Engelmann, F. (2004) 'Plant cryopreservation: Progress and prospects', *In Vitro Cellular and Developmental Biology – Plant,* vol 40, pp427–433

Engelmann, F. and Takagi, H. (eds) (2000) *Cryopreservation of Tropical Plant Germplasm: Current Research Progress and Applications,* Japan International Research Centre for Agricultural Sciences, Tsukuba, Japan/IPGRI, Rome, Italy

Engels, J.M.M. and Visser, L. (eds) (2003) *A Guide to Effective Management of Germplasm Collections,* International Plant Genetic Resources Institute Handbooks for Genebanks 6, International Plant Genetic Resources Institute (IPGRI), Rome, Italy

Engels, J.M.M. and Wood, D. (1999) 'Conservation of agrobiodiversity', in D. Wood and J.M. Lenné (eds) *Agrobiodiversity: Characterization, Utilization and Management,* pp355–385, CAB International, Wallingford, UK

Engels, J.M.M., Maggioni, L., Maxted, N. and Dulloo, M.E. (2008) 'Complementing *in situ* conservation with *ex situ* measures', in J. Iriondo, N. Maxted and M.E. Dulloo (eds) *Conserving Plant Genetic Diversity in Protected Areas,* Chapter 6, pp169–181, CAB International, Wallingford, UK

ENSCONET (2009) *ENSCONET Seed Collecting Manual for Wild Species,* European Native Seed Conservation Network (ENSCONET), ISBN: 978-84-692-3926-1, http://www.ensconet.eu/Download.htm, accessed 31 May 2010

Falk, D.A. and Holsinger, K.E. (eds) (1991) *Genetics and Conservation of Rare Plants*, Oxford University Press, New York and Oxford

FAO (2010) S*econd Report on the State of the World's Plant Genetic Resources for Food and Agriculture,* Food and Agriculture Organization of the United Nations (FAO), Rome, Italy

FAO and IPGRI (1994) *Genebank Standards*, Food and Agriculture Organization of the United Nations (FAO)/International Plant Genetic Resources Institute (IPGRI), Rome, Italy

Gemmill, C.E.C., Ranker, T.A., Ragone, D., Pearlman, S.P. and Wood, K.R. (1998) 'Conservation genetics of the endangered endemic Hawai'ian genus *Brighamia* (Campanulaceae)', *American Journal of Botany,* vol 85, no 4, pp528–539

Given, D.R. (1994) *Principles and Practice of Plant Conservation,* Timber Press, Portland, OR, USA

Guarino, L., Ramanatha Rao, V. and Reid, R. (1995) *Collecting Plant Genetic Diversity Technical Guidelines,* CAB International, Wallingford, UK

Guarino L., Jarvis A., Hijmans R.J. and Maxted N. (2001) 'Geographic information systems (GIS) and the conservation and use of plant genetic resources', in J. Engels, V. Ramanatha Rao, A.H.D. Brown and M.T. Jackson (eds) *Managing Plant Genetic Diversity,* pp387–404, CAB International, Wallingford, UK

Guerrant Jr., E.O., Havens, K. and Maunder, M. (eds) (2004) *Ex Situ Plant Conservation. Supporting Species Survival in the Wild*, Island Press, Washington, DC

Hajjar, R. and Hodgkin, T. (2007) 'The use of wild relatives in crop improvement: A survey of developments over the last 20 years', *Euphytica,* vol 156, pp1–13

Hamilton, K.N., Ashmore, S.E. and Drew, R.A. (2005) 'Investigations on desiccation and freezing tolerance of *Citrus australasica* seed for *ex situ* conservation', in S.W. Adkins, P.J. Ainsley, S.M. Bellairs, D.J. Coates and L.C. Bell (eds) *Proceedings of the Fifth Australian Workshop on Native Seed Biology,* pp157–161, Australian Centre for Minerals Extension and Research (ACMER), Brisbane, Queensland, AUS

Hamilton, K.N. (2008) 'Protocol 19.7.2 – Cryopreservation of wild Australian citrus seed', in H.W. Pritchard and J. Nadarajan 'Cryopreservation of orthodox (desiccation tolerant) seeds', in B.M. Reed (ed) *Plant Cryopreservation: A Practical Guide,* Springer, Berlin, Germany

Hawkes J.G., Maxted N. and Ford-Lloyd, B.V. (2000) *The* Ex Situ *Conservation of Plant Genetic Resources,* Kluwer Academic Publishers, Dordrecht, The Netherlands

Heywood V.H. (1991) 'Developing a strategy for germplasm conservation in botanic gardens', in V.H. Heywood and P.S. Wyse Jackson (eds) *Tropical Botanic Gardens – Their Role In Conservation and Development,* pp11–23, Academic Press, London, UK

Heywood, V.H. (2009) 'Botanic gardens and genetic conservation', *Sibbaldia* guest essay, *Sibbaldia, The Journal of Botanic Garden Horticulture,* no 7, pp5–17

Hijmans, R.J. and Spooner, D.M. (2001) 'Geographic distribution of wild potatoes species', *American Journal of Botany,* vol 88, no 11, pp2101–2112

Hijmans, R.J., Guarino, L., Cruz , M. and Rojas, E. (2001) 'Computer tools for spatial analysis of plant genetic resources data:1 DIVA-GIS', *Plant Genetic Resources Newsletter,* vol 127, pp15–19

INIBAP (2006) *Global Conservation Strategy for* Musa *(Banana and Plantain)*, International Network for the Improvement of Banana and Plantain (INIBAP), www.croptrust.org/documents/web/Musa-Strategy-FINAL-30Jan07.pdf, accessed 23 March 2010

IUCN (2002) *IUCN Technical Guidelines on the Management of* Ex-Situ *Populations for Conservation,* approved at the 14th Meeting of the Programme Committee of IUCN Council, Gland, Switzerland, 10 December 2002, International Union for Conservation of Nature (IUCN), http://intranet.iucn.org/webfiles/doc/SSC/SSCwebsite/Policy_statements/IUCN_Technical_Guidelines_on_the_Management_of_Ex_situ_populations_for_Conservation.pdf

IUCN/SSC (1995) *IUCN/SSC Guidelines for Re-introductions,* SSC Re-introduction Specialist Group, approved by the 41st Meeting of the IUCN Council, Gland, Switzerland, May 1995. International Union for Conservation of Nature (IUCN),

Gland, Switzerland, http://iucnsscrsg.org/policy_guidelines.php

Jarvis A, Williams, K., Williams,D., Guarino, L., Caballero, P.J. and Mottram, G. (2005) 'Use of GIS in optimizing a collecting mission for a rare wild pepper (*Capsicum flexuosum* Sendtn.) in Paraguay', *Genetic Resources and Crop Evolution*, vol 52, no 6, pp671–682

Kell, S.P., Laguna, L., Iriondo, J. and Dulloo, M.E. (2008) 'Population and habitat recovery techniques for the *in situ* conservation of genetic diversity', in J. Iriondo, N. Maxted and M.E. Dulloo (eds), *Conserving Plant Genetic Diversity in Protected Areas,* Chapter 5, pp124–168, CABI Publishing, Wallingford, UK

Laliberté, B. (1997) 'Botanic garden seed banks/genebanks worldwide, their facilities, collections and network', *Botanic Gardens Conservation News*, vol 2, pp18–23

Maunder M., Guerrant Jr, E.O., Havens, K. and Dixon, K.W. (2004) 'Realizing the full potential of *ex situ* contributions to global plant conservation', in E.O. Guerrant Jr., K. Havens and M. Maunder (eds) Ex Situ *Plant Conservation. Supporting Species Survival in the Wild,* Island Press, Washington, DC

Maxted, N. and Kell, S.P. (2009) *Establishment of a Global Network for the* In Situ *Conservation of Crop Wild Relatives: Status and Needs*, FAO Commission on Genetic Resources for Food and Agriculture, Rome, Italy

Maxted, N., Ford-Lloyd, B.V. and Hawkes, J.G. (1997) 'Complementary conservation strategies', in N. Maxted, B.V. Ford-Lloyd and J.G. Hawkes (eds) *Plant Genetic Conservation: The* In Situ *Approach,* Chapman and Hall, London, UK

Maxted, N., Dulloo, M.E., Ford-Lloyd, B.V., Iriondo, J. and Jarvis, A. (2008) 'Gap analysis: A tool for complementary genetic conservation assessment', *Diversity and Distributions*, vol 14, no 6, pp1018–1030

Miller, A.G. and Nyberg, J.A. (1995) 'Collecting herbarium vouchers', in L. Guarino, V. Ramanatha Rao and R. Reid (eds) *Collecting Plant Genetic Diversity Technical Guidelines,* Chapter 27, pp561–573, CAB International, Wallingford, UK

Panella L., Wheeler, L. and McClintock, M.E. (2009) 'Long-term survival of cryopreserved sugarbeet pollen', *Journal of Sugar Beet Research,* vol 46, pp1–9

Rao N.K., Hanson, J., Dulloo, M.E., Ghosh, K., Nowell, D. and Larinde, M. (2006) *Manual of Seed Handling in Genebanks,* Handbooks for Genebanks No 8, Bioversity International, Rome, Italy

Reed, B. (ed) (2008) *Plant Cryopreservation: A Practical Guide,* Springer, New York, USA

Reed, B., Engelmann F., Dulloo M.E. and Engels J.M.M. (2004) *Technical Guidelines on Management of Field and* In Vitro *Germplasm Collections*, Handbook for Genebanks No 7, IPGRI, Rome, Italy

Schmidt, L. (2000) *Guide to Handling Tropical and Subtropical Forest Seed,* Danida Forest Seed Centre, http://en.sl.life.ku.dk/Publikationer/Udgivelser/DFSC/DFSCBook1.asp.

Shaanker, Uma R., Ganeshaiah, K.N., Nageswara Rao, M. and Ravikanth, G. (2002) 'Forest gene banks – a new integrated approach for the conservation of forest tree genetic resources', in J.M.M. Engels, A.H.D. Brown and M.T. Jackson, (eds) *Managing Plant Genetic Diversity*, pp229–235, CAB International, Wallingford, UK

Sharrock, S. and Engels, J. (1996) 'Complementary Conservation', INIBAP Annual Report 1996, pp 8–9, INIBAP, Montpellier.

Smith, R.D. (1995) 'Collecting and handling seeds in the field', in L. Guarino, V. Ramanatha Rao And R. Reid (eds) *Collecting Plant Genetic Diversity Technical Guidelines,* Chapter 20, pp419–456, CAB International, Wallingford, UK

Smith R.D., Dickie, J.B., Linington, S.H., Pritchard, H.W and Probert, R.J. (2003) *Seed Conservation: Turning Science into Practice*, Royal Botanic Gardens Kew, Richmond, UK

Thormann, I., Dulloo, M.E. and Engels, J. (2006) 'Techniques for *ex situ* plant conservation', in R.J. Henry(ed), *Plant Conservation Genetics,* pp7–36, Haworth Press, AUS

Towill, L.E. (1985) 'Low temperature and freeze-/vacuum-drying preservation of pollen', in K.K. Harthaa(ed) *Cryopreservation of Plant Cells and Organs,* pp171–198, CRC Press, Boca Raton, FL, USA

Towill, L.E. and Walters, C. (2000) 'Cryopreservation of pollen', in F. Engelmann and H. Takagi(eds) *Cryopreservation of Tropical Plant Germplasm – Current Research Progress and Applications,* pp115–129, Japan International Centre for Agricultural Sciences, Tsukuba/International Plant Genetic Resources Institute, Rome, Italy

Walyaro, D.J. and van der Vossen, H.A.M. (1977) 'Pollen longevity and artificial cross-pollination in *Coffea arabica* L', *Euphytica,* vol 26, pp225–231