5.2 Conservation methods

It is now widely accepted that conservation can be done on-site (in situ) and off-site (ex situ). In this section these and other conservation approaches and methods will be briefly described.

In situ conservation

The CBD (UNEP, 1992), covering both wild and domesticated species, uses a complex definition for in situ conservation: "the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticates or cultivated species, in the surroundings where they have developed their distinctive properties." There may be substantial differences in approach for the conservation of wild species and domesticates. For example, for wild species conservation, the introgression of alien genes into populations of the target species would be avoided. In contrast, for crops, it has been argued that introgression of genes from wild species into crop populations is an evolutionary event and one advantage of in situ conservation and thus should be allowed to occur (Altieri and Merrick, 1987, and many others).

With the conclusion of the CBD and Agenda 21 in 1992, and with the adoption of the GPA by the participating countries in the Fourth International Technical Conference on Plant Genetic Resources (FAO, 1996), a significant impetus has been given to in situ conservation. In recent years on-farm conservation activities have become closely linked with development work, including the farmer empowerment (Jarvis and Hodgkin, 2000a).

Protected areas: Protected areas are widely regarded as instrumental for in situ conservation of wild relatives. Wild relatives of crops and domestic animals may occur beyond the influence of farming, in natural and semi-natural ecosystems and their conservation may well fit into the existing system of nature reserves. Many proposals relied on this approach (Ingram, 1984; Prescott-Allen, 1984; Prescott-Allen and Prescott-Allen, 1984; Wilcox, 1990) but, until recently, few of these proposals were funded. Currently the conservation of agrobiodiversity in protected areas is largely unplanned and this component of biodiversity is usually not specifically addressed. A feature of this form of conservation is that evolutionary processes continue to operate and that entire populations can undergo changes, and can become extinct. A disadvantage of protected area conservation is that the conserved material is not readily available for agricultural use. Also, with limited opportunity for management, little characterization and evaluation can be done on the germplasm, restricting its use as a genetic resource (Maxted, et al., 1997b).

Conservation on-farm: Farmers worldwide have been practising on-farm conservation for as long as agriculture has existed, as a necessary part of crop production. For them, the most effective management practices have been those that combined highest yields with the greatest food security. Usually, these practices are based on within- and among-species diversity, surviving in areas that are not served by modern high-input agriculture. In addition to crops, wild and weedy species occur that are associated with farming. Suggestions have been made for intervention to boost the effectiveness of this age-old process. Jarvis et al. (2000b) provided detailed suggestions and procedures for the management of these resources on-farm in the framework of traditional farming systems, that allow for continued maintenance and evolution of traditional landraces and wild and weedy species that depend on traditional agricultural practices for their survival. Potential advantages and disadvantages of conservation on-farm will need to be weighed for suitability for application to conservation, as well as for impact on farm livelihoods.

Home gardens: Home gardens are a reservoir of diversity for fruits, vegetables and small domestic livestock. Proximity to the home allows detailed selection, for example, of colour variants of most plants and animals, as well as generation of the vast morphological variation that exists in many domesticated species. Several authors (Maxted et al., 1997a; Damania, 1996; and Engels, 1995) list the conservation of plant genetic diversity in home gardens separately. As for on-farm conservation, the method is dynamic. A community of gardens may need to be included, as the intraspecific diversity within an individual garden is often limited, whereas the variation among gardens is often substantial (Engels, 2002b).

Many ideas and proposals have been put forward for in situ conservation of agrobiodiversity, ranging from 'mass reservoirs' (Simmonds, 1962; Frankel and Bennett, 1970; Frankel et al., 1995) to recommendations of ethnobotanists (Brush, 1986 and 1999; Oldfield and Alcorn, 1987; Altieri and Merrick, 1987). Others proposed to contribute to on-farm conservation by genetic base broadening through decentralized multi-site adaptation of composite populations. A good overview of lessons learned from on-farm conservation can be found in Jarvis et al. (2000b).

Ex situ conservation

Seed storage: Storing genetic diversity as seed is the best researched, most widely used and most convenient method of ex situ conservation. Much is known about the optimum treatment of the seed of most of the major food crops. For an early review, see Harrington (1970). Requirements include adequate drying, i.e. seed moisture contents as low as 3% for oily seeds and 5% or more for starchy seeds; appropriate storage temperature (-18°C is recommended for long-term storage); and careful production of quality seed to ensure the greatest longevity (Rao and Jackson, 1996). Recent research shows that very low moisture contents could be sub-optimal and care is needed.

However, the seeds of many crop species, especially tropical shrubs and trees, will lose viability if dried (so-called 'recalcitrant' seeds). Seeds of some species can be dried to some extent but cannot survive low-temperature storage and are intermediate in storage characteristics. This category includes coffee, citrus species, rubber and others. In addition, seeds of wild relatives do not always behave similarly to the seed of domesticates, and optimal storage conditions have to be individually determined.

An IPGRI protocol to determine the precise seed storage characteristics of little researched species (Hong and Ellis, 1996) and a compendium of available data on storage behaviour of approximately 7000 species, including references to individual species, is available (Hong et al., 1996; Engels et al., 2001).

Most national genebanks now rely on cold storage facilities for seed maintenance. However, these depend on a reliable electricity supply, which can represent a problem in some countries. To overcome this problem, alternative approaches to low temperature storage have been developed, including the so-called 'ultra-dry seed' technology. Drying seeds to a moisture content as low as 1% (in the case of oily seeds) or approximately 3% (starchy seeds) and hermetic packaging allows storage for long periods at room temperature. Care must be taken to prevent over-drying of the seeds (Walters and Engels, 1998).

Some genebanks have also experimented with storing seeds in liquid nitrogen. Besides the already mentioned danger of over-drying the (orthodox) seeds, seed size is important for economic cryopreservation. Furthermore, it has been agreed that this approach might have advantages under circumstances where electricity supply is unreliable.

Pollen storage: The technique for pollen storage is comparable with that for seed storage, since pollen can be dried (less than 5% moisture content on a dry weight basis) and stored below 0°C. There is limited experience on the survival and fertilizing capacity of cryopreserved pollen more than five years old (Towill, 1985). Hoekstra (1995) using information on more than 1500 plant species failed to determine a clear correlation between the storability of pollen and of seed of the same species. Pollen might represent an interesting alternative for the long-term conservation of problematic species (IPGRI, 1996). However, pollen has a relatively short life compared with seeds (although this varies significantly among species), and viability testing can be time-consuming and uneconomical. Pollen has, therefore, been used to a limited extent in germplasm conservation (Hoekstra, 1995). Other disadvantages of pollen storage are the small amount produced by many species; the lack of transmission of organelle genomes via pollen; the loss of sex-linked genes in dioecious species; and the general inability to regenerate into plants (Hoekstra, 1995). An advantage is that pests and diseases are rarely transferred by pollen (excepting some virus diseases). This allows safe movement and exchange of germplasm as pollen.

Field genebanks: Field banks are used for the conservation of clonal crops; where seed is recalcitrant; and for crops that rarely produce seed. The rule of thumb is to use the same propagation techniques as the farmer, for example not disrupting adapted clones through genetic segregation in a seed cycle. Many temperate and tropical fruit trees fulfil one or more of these conditions, as do many commodity crops such as cocoa, rubber, oil palm, coffee, banana and coconut as well as most root and tuber crops. An example of the scale of management of field genebanks is that oil palm genetic resources in Malaysia are planted at a density of 140 palms per hectare, and the collection from Nigeria alone occupies 200 ha. Since oil palm seed cannot be stored for more than two years, and pollen only for three years, a living collection, although expensive, is currently the only practicable conservation method. Similarly, the coffee genebank in Jima, Ethiopia contains over 1600 accessions of coffee trees from the centre of diversity of the crop.

Management may be the same as used during routine farming, and cultivation methods can be adapted to local circumstances. Conserved material can be readily characterized and evaluated and then accessed for research and use. Some natural selection may take place within and between accessions, but management is designed to prevent it. Major constraints faced by field genebanks include costs and all the natural hazards of farming, including pests and diseases, drought, flood, cyclones etc. (Engelmann and Engels, 2002).

In vitro conservation: When a conservation method is susceptible to unavoidable hazards, as with field genebanks, an alternative, complementary method should also be used. In vitro conservation involves maintenance of explants in a sterile, pathogen-free environment and is widely used for the conservation and multiplication of species that produce recalcitrant seeds, or do not produce seeds (Engelmann, 1997). Although research on in vitro techniques only started some 20 years ago the technique has been applied for multiplication, storage and, more recently, for collecting germplasm of more than 1000 species (Ashmore, 1997).

Various in vitro conservation methods are used. For short- and medium-term storage the aim is to increase the intervals between subcultures by reducing growth. This is achieved by modifying the environmental conditions, including the culture medium, to realize so-called slow-growth conservation. The most widely applied technique is temperature reduction (varying from 0–5°C for cold tolerant species to 9–18°C for tropical species) that can be combined with a decrease in light intensity or storage in the dark

(Engelmann, 1997) and adjustment of the growth medium. Alternatives to standard slow-growth conservation include modification of the gaseous environment of cultures, desiccation and encapsulation of explants. The latter is termed synthetic seed where the idea is to use somatic embryos as true seeds. Embryos encapsulated in alginate gel can be stored after partial dehydration and sown directly in vivo (Janick et al., 1993).

For small volumes, long-term storage is practicable through storage of cultures in cryopreservation at ultra-low temperature, usually by using liquid nitrogen (-196°C). At this temperature all cellular divisions and metabolic processes are virtually halted and, consequently, plant material can be stored without alteration or modification theoretically indefinitely (Engelmann, 1997).

Botanical gardens and arboreta: Botanical gardens have played a historical role in the exchange and introduction of crop genetic resources. Usually botanical garden collections consist only of one or a few individuals per species (FAO, 1998), although in recent years there has been a tendency towards the establishment of conservation units, including seed banks (Laliberté, 1997). Unfortunately, most botanical gardens have limited interest or expertise in crop genetic resources, although efforts are being made to change this (Heywood, 1998).

DNA storage: This more recently developed technique is increasing in importance. DNA from the nuclei, mitochondria and chloroplasts is now routinely extracted and stored. For the purpose of analysis, DNA is often immobilized on nitrocellulose sheets where it can be probed, including with cloned genes. With the development of PCR (polymerase chain reaction) specific oligonucleotides and genes can now be routinely amplified. DNA cloning technology has further facilitated efficient use of DNA sequences. These advances have led to the formation of an international network of DNA repositories for genomic DNA (Adams, 1997). The advantage of storing DNA is that it is efficient and simple and overcomes many physical limitations and constraints that characterize other forms of storage. The disadvantage lies in problems with subsequent gene isolation, cloning and transfer, but, most importantly, it does not allow the regeneration of live organisms (Maxted et al., 1997a; for recent updates see also www.cgn.wageningen-ur.nl/pgr/).

Complementarity of conservation strategies

Farming itself is the original method of conservation, linked directly with utilization. But farming is changing, rendering conservation of diversity at the farm superfluous given development of specialized crop breeding. Most farmers cannot afford and would not wish to be curators of living museums of agrobiodiversity (as suggested by Wilkes, 1971). Fortunately, the wide spectrum of conservation methods can meet a wide range of conditions. With the range of genetic diversity included in conservation, security and accessibility can be balanced against feasibility and cost-efficiency. The choice of a single method of conservation will often not be enough: different and complementary methods of conservation have advantages and disadvantages. In making choices it is important to take a holistic view of the intended conservation effort and to place it in a wider context of current and potential future user groups, whenever applicable. It is also important to examine carefully the technical and human resources available as well as the administrative and political environment in which the conservation will be done in order to minimize problems (Engels, 2002a).

In choosing alternative or complementary methods of conservation, the most obvious contrast is between in situ and ex situ approaches. The dynamic processes of in situ conservation could be combined with the usually more secure approach of ex situ conservation, and improve accessibility to the germplasm. As a result of disease pressure and natural selection, continuous adaptation is likely to occur, possibly enhancing the value of on-farm populations as a source of variability for breeding for disease resistance. This potential for exploiting the evolutionary process during on-farm conservation was noted by Allard (1990) for disease resistance (of the barley-scald pathosystem). However, the rate of this adaptation is unknown, and methods of sampling or evaluation in the field have not yet been thoroughly developed to monitor this process (Maxted et al., 1997a).

Many minor but locally important crops have been neglected by collectors and ex situ genebanks. For these crops and their wild relatives, in situ (including on-farm) conservation is appropriate. Notwithstanding the advantages of continuing evolution on farm, and the substantial diversity of material that can be conserved, there will be limited access to those resources; a lack of adequate characterization and evaluation; and the danger that farmers abandon the cultivation of traditional landraces under economic pressures. Careful monitoring will always be needed. Conservation through use in situ might run the risk of losing specific alleles or genotypes as a result of continuous adaptation and a backup system through ex situ conservation will be required. This was emphasized by Hammer et al. (1996) who found that 96.8% of the samples collected in Albania in 1941 were still intact in the Gatersleben genebank in Germany, whereas a survey 50 years later in the same region in Albania showed genetic erosion of about 50%. The authors concluded that this “is an amazing result as the material had to survive the Second World War and two translocations”.

The choice between conservation methods may be dictated by the biology of the species. For instance, if the cultivated species does not produce seeds (as for bananas) the choice includes on-farm conservation, maintenance in field genebanks, in vitro slow growth and cryopreservation (Sharrock and Engels, 1997). Cassava and potato represent examples of extensively studied genepools used to develop in vitro techniques, for which a broad range of conservation options is now available.