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Ex Situ and *In Situ* Conservation of Agricultural Biodiversity: Major Advances and Research Needs

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Abstract

The effective conservation and use of agricultural biodiversity is vital for creating and maintaining sustainable increases in the productivity of healthy food for mankind, as well as contributing to the increased resilience of agricultural systems. Major advances in the two main complementary strategies for agricultural biodiversity conservation, namely *ex situ* and *in situ*, over the last decade are presented to reflect on their current global status and trends. The FAO Second State of the World Report on Plant Genetic Resources for Food and Agriculture reports that the total number of accessions conserved in *ex situ* collections is about 7.4 million, in over 1750 genebanks around the world. There has also been increasing awareness of the importance and value of conserving crop wild relatives (CWR) *in situ* and a greater understanding of the scientific issues surrounding on farm management of genetic diversity. Recent research outputs produced by Bioversity International to ensure the effective and efficient conservation and use of genetic diversity are cited. These have involved development of best practices for genebank management and the development of enhanced technologies and methodologies for conserving and promoting the use of the genetic diversity. Bioversity International has led the development of methodologies for on farm conservation, and promoted the drafting of policies and strategies for the *in situ* conservation of crop wild relatives and their management inside and outside protected areas. Also an outlook of the research priorities and needs for conservation and use of agricultural biodiversity is described.

Keywords: Agricultural biodiversity, genetic diversity, ex situ conservation, in situ conservation, crop wild relatives

Introduction

Agricultural biodiversity is an important component of biodiversity, which has a more direct link to the well being and livelihood of mankind than other forms of biodiversity. In fact it is one of our most fundamental and essential resources, one that has enabled farming systems to evolve since the birth of agriculture about 10,000 years ago. Food plant and animal species have been collected, used, domesticated and improved through traditional systems of selection over many generations (Plucknett, 1987). The resulting diversity of genetic resources developed by early farmers now forms the basis on which modern high yielding and disease resistant varieties have been produced to feed the growing human population, expected to reach 9.1 billion by 2050. According to the Convention on Biological Diversity (CBD), "agricultural biodiversity includes all components of biological diversity of relevance to food and agriculture, and all components of biological biodiversity that constitute agro-ecosystems: the variety and variability of animals, plants and micro-organisms, at the genetic, species and ecosystem levels, which are necessary to sustain key functions of the agricultural ecosystem, its structure and processes" (COP decision V/5, appendix - http://www.cbd.int/decision/cop/?id=7147 (last accessed 25 August 2010). The effective conservation and use of agricultural biodiversity is very important in ensuring sustainable increases in the productivity and production of healthy food by and for mankind as well as contributing to increased resilience of agricultural ecosystems.

There are many threats or drivers of changes on biodiversity that have been recognized and intensified in recent years (Millennium Ecosystem Assessment, 2005). With regard to agriculture the most important ones include changes in land use, replacement of traditional varieties by modern cultivars, agricultural intensification, increased population, poverty, land degradation and environmental change (including climate change) (FAO, 2010; van de Wouw *et al.*, 2009). It is predicted that climate change will have a significant impact on agriculture with temperatures rising on average by 2-4°C over the next 50 years, causing significant changes in regional and seasonal patterns of precipitation (IPCC, 2007; Burke *et al.*, 2009). Climate change will also impact agricultural biodiversity in a major way. Model projections carried out by Lane and Jarvis (2007) based on global distribution of suitable cultivated areas of 43 crops, highlight that more than 50% may decrease in extent. Evidence based on bioclimatic modelling suggests that climate change could cause a marked contraction in the distribution ranges of CWR. In the case of wild populations of peanut (*Arachis spp.*), potato (*Solanum spp.*) and cowpea (*Vigna spp.*), studies suggest that 16-22% of these species may go extinct by 2055, with most species possibly losing 50% of their range size (Jarvis *et al.*, 2008). These threats or drivers of change are leading to large scale degradation and loss of agricultural biodiversity and consequently its genetic variability (Millennium Ecosystem Assessment, 2005, van de Wouw *et al.*, 2009). Information regarding the threat and rate of genetic erosion among various components of agricultural biodiversity is important, yet very little work has been carried out to quantify the magnitude of any trends. The availability of large gene pools, including CWR, is becoming even more important as farmers will need to adapt to changing conditions that result from these pressures. It is likely that many of the genetic traits which will be necessary to adapt our crops to changing climate will be found in CWR.

There are two main strategies for conserving agricultural biodiversity, namely *ex situ* and *in situ* conservation, both of which are equally important and should be regarded as complementary (Thormann *et al.* 2008; Engelmann and Engels, 2002; Dulloo *et al.*, 1998; Maxted *et al.*, 1997). *Ex situ* conservation is the conservation of components of biodiversity outside their natural habitats (CBD definition, UNCED, 1992). It is generally used to safeguard populations that are at present or potentially under threat and need to be collected and conserved in genebanks in the form of seeds, live plants, tissues, cells and/ or DNA materials. Article 2 of the CBD defines *in situ* conservation as "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 domesticated or cultivated species, in the surroundings where they have developed their distinctive properties" (UNCED, 1992). It thus refers to the maintenance of a species in its natural habitat. This can be either on farm, requiring the maintenance of the agro-ecosystem along with the cultivation and selection processes on local varieties and landraces, or in the wild, which involves the maintenance of the ecological functions that allow species to evolve under natural conditions.

This paper will review the status and trends on agricultural biodiversity, some of the recent advances in the *ex situ* and *in situ* conservation (with emphasis on CWR) including an outlook of the research priorities and needs for conservation and use of agricultural biodiversity.

Status and Trends of Agricultural Biodiversity

Little is known about the global status of agricultural biodiversity. Although the CBD recognize genetic diversity as one of the fundamental levels of biodiversity, actions to protect genetic diversity are lacking (Laikre *et al.*, 2010). Laikre *et al.* (2010) provides some examples of empirical work that demonstrates how populations and even species can collapse due to loss of genetic diversity (Cited examples include: Newman and Pilson, 1997; Briskie and Mackintosh 2004; Frankham, 2005). It also provides evidence supporting the importance of maintaining genetic variation to sustain species and ecosystems (Cited: Wimp *et al.*, 2004; Crutsinger *et al.*, 2006; Whitham *et al.*, 2006). Policy makers and scientists require a better understanding of how the intraspecific diversity is changing over time and space in order to make informed decisions for their conservation. However there is no routine global scale monitoring of genetic diversity over time (Frankham, 2010, Laikre *et al.*, 2010), except for a few target species at national level (Laikre *et al.*, 2008). A major challenge remains to develop simple inexpensive means to monitor genetic diversity at a global scale (Frankham, 2010). Several efforts under the 2010 Biodiversity Indicators Partnership (http://www.twentyten.net) have been made to identify indicators useful to detect changes in species and ecosystem diversity, but there are only two initiatives that are explicitly working on developing indicators that deals with genetic variation for agricultural biodiversity (Laikre, 2010; Walpole *et al.*, 2009). These include an indicator on *ex situ* crop collections and the number of food production breeds of domestic animals. These initiatives are still under development (Walpole *et al.*, 2009) under the 2010 Biodiversity Indicators and the Pan European initiative "Streamlining European 2010 Biodiversity Indicators," which deals exclusively with the number of domestic livestock breeds within countries and not plants (Bubb *et al.* 2005; EEA 2007 cited in Laikre, 2010).

The only authoritative account of agricultural biodiversity status at the global level is represented by the First and Second reports on the *State of the World's Plant Genetic Resources for Food and Agriculture* published by the Food and Agriculture Organization of the United Nations (FAO) (FAO, 1998, 2010). The Second Report mention that there are about 7.4 million accessions conserved in over 1750 genebanks around the world in either seed banks, field collections, and *in vitro* and cryopreservation conditions (Fig. 1) (FAO, 2010). This represents an increase of more than 1.4 million accessions added to *ex situ* collection since publication of the First Report on the *State of the World's Plant Genetic Resources for Food and Agriculture*. Although reportedly over-represented, a large part of the genetic diversity of major food crops is stored in *ex situ* collections. The exact proportion is still uncertain, but estimates suggest that more than 70% of the genetic diversity of some 200-300 crops is already conserved in genebanks (SBSTTA, 2010). In addition there are over 2,500 botanic gardens maintaining samples of some 80,000 plant species (FAO, 2010). However, regeneration of genebank accessions remains a major problem, threatening collections (FAO, 1998). In the past decade there have been significant advances made in regenerating collections at risk, in part due to efforts made by the Global Crop Diversity Trust (CGDT) in supporting regeneration programmes of globally important priority genebank collections for 22 priority crops for which crop specific regeneration guidelines have recently been produced (Dulloo *et al.*, 2008). Another major achievement has been the creation of the Svalbard Global Seed Vault (SGSV) in 2008, established to serve as the ultimate safety net for seeds samples from the world's most important collections (GCDT, 2010).

Great efforts for the conservation of many CWRs and wild species have been made by the Millennium Seedbank (MSB) at Wakehurst Place, Royal Botanic Gardens, Kew, UK which aims to house up to 10% of the world's seed-bearing flora, principally from arid zones by 2010. Genetic erosion has also been prevented by the significant amount of crop genetic diversity in the form of traditional varieties and neglected and underutilized species (NUS) that continues to be maintained on-farm. Yet, in spite of these advances, important reservoirs of adaptive variation such as CWR, landraces and NUS, which are increasingly recognized by the global scientific community as key resources for the maintenance of agrobiodiversity, remain under-represented (FAO, 2010).

CWR in particular, which have avoided the genetic bottleneck of domestication, contain greater genetic variation than their cultivated relatives and represent an important reservoir of genetic resources for breeders (Maxted and Kell, 2009). Yet to retain the genetic characteristics that make them so valuable for crop improvement, it is now widely recognized that populations of CWR are best conserved *in situ*, in their wild habitats, where they can continue to adapt and evolve along with their natural surroundings, thus ensuring new variation is generated in the gene pool and the continued supply of the novel genetic material critical for future crop improvement. The underpinning of the conservation strategy of most countries is a protected areas system and this is reflected in the CBD, where the main thrust of biodiversity conservation is *in situ*, through the development of such protected systems. Populations of many CWR occur in existing protected areas, but this alone does not in many cases represent effective *in situ* conservation without some degree of management or intervention targeted at the populations of the particular target species, particularly if the species is threatened. Despite protected areas being in existence for many years we still have not been able to undertake significant actions to conserve the CWR they contain, except a few cases.

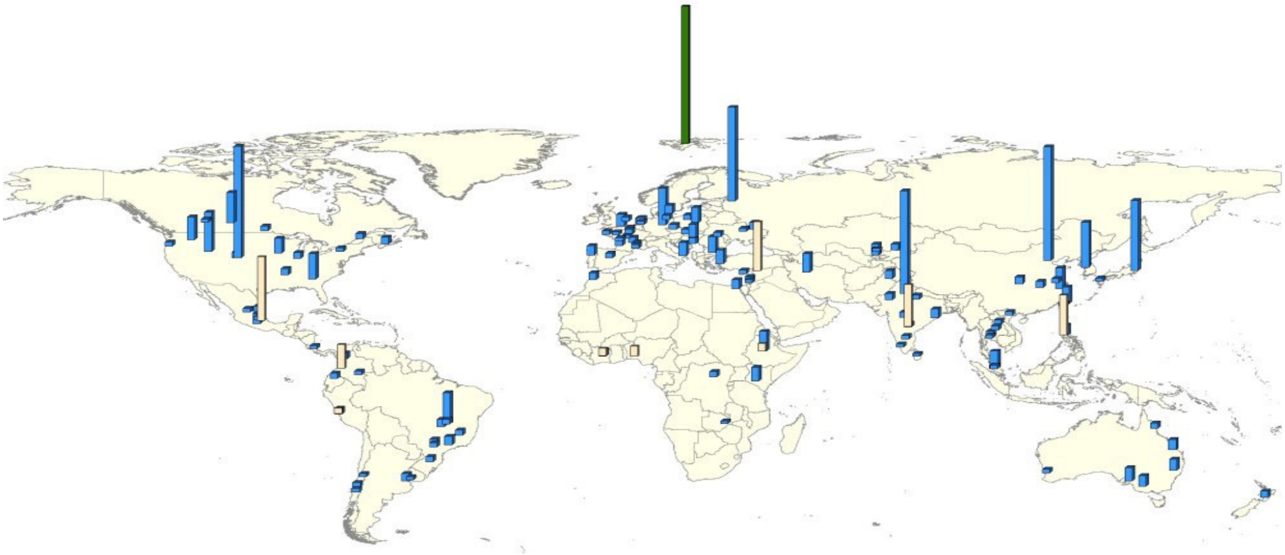

Fig. 1. Geographic distribution of genebanks with holdings of >10,000 accessions (*Source:* FAO, 2010)

Despite this, the *in situ* conservation of CWR has gained increasing attention in many countries, as demonstrated by their inclusion in the many national reports drafted for the Second report on the *State of the World's Plant Genetic Resources for Food and Agriculture* (FAO, 2010). Unfortunately, little quantitative data were provided by countries on the changing status of CWR, but several reports indicated that specific measures had been taken to promote their conservation. The Second report also mentions that surveys and inventories of CWR were carried out in at least 28 countries and many new priority sites for conserving CWR *in situ* have been identified over the last decade. There is also evidence that public awareness of the importance of CWR, and neglected and under-utilized species such as traditional vegetables and fruits, is growing both in developing and developed countries (FAO, 2010). This has been furthered by a number of global initiatives aimed at conserving CWR, such as the proposed establishment a global network for the *in situ* conservation of CWR (Maxted and Kell, 2009), and more concretely by the creation of web-based international platforms for the exchange of CWR information and data. These include the European platform "An Integrated European In Situ Management Work Plan: Implementing Genetic Reserves and On Farm Concepts" (AEGRO) (http://aegro.bafz.de/index.php?id=95 - last accessed September 2010) and the CWR Global Portal (www.cropwildrelatives.org), developed as part of the UNEP/GEF Crop Wild Relative Project, that provides access to CWR information and data at the global level (Thormann *et al*., 2010). The significant increase in number of scientific articles published on CWR and on specific actions targeting their conservation is also a testimony to the renewed interest in CWR, however, to the best of our knowledge few of the recommendations have been implemented, largely due to a lack of funds and capacity.

Over the last decade, the number and coverage of protected areas has increased by approximately 30% (United Nations, 2010), yet limited efforts have been made to target CWR, whose conservation remains unplanned and largely an indirect effect of protecting flagship species or threatened habitats. For example, despite the increase in isolated activities targeting CWR conservation, the formal recognition and/or the adoption of appropriate management regimes to protect CWR are largely lacking. Furthermore, considering that national parks and other conservation areas cover only 12-13% of the earth's surface, it is clear that these areas alone will not be able to ensure the continued existence of CWR species, the majority of which occur in marginal lands outside protected areas, where no form of legal protection is offered. If protected areas are to ensure the long-term survival of CWR they will need to become more flexible in size and scale and a connected network of habitats will need to be established to allow species to migrate and adjust their ranges in response to global change and anthropogenic disturbances, along with the development of effective management strategies targeting their conservation (i.e. off-reserve management). The success of this strategy will depend largely on promoting more biodiversity-sensitive management of ecosystems outside protected areas, and successfully engaging private landowners and local communities living around protected areas in the conservation process. Finally, more effective policies, legislation and regulations that take into account the impacts of global changes on future species distribution and that govern the *in situ* conservation of CWR, both inside and outside of protected areas, are needed, along with closer collaboration and coordination between the agriculture and environment sectors.

Ex Situ Conservation Research

The principal aim of *ex situ* conservation is to maintain seeds and other germplasm materials alive as long as possible and to reduce the frequency of regeneration that may cause the loss genetic diversity. In the past, research on *ex situ* methods focused mainly on conserving seeds of major cereals and legume crops since seeds are considered the easiest materials to conserve in genebanks. Seed banking techniques rely on the storage of dried seeds at low temperatures and thus the most important factors influencing seed longevity are temperature, seed moisture content (SMC) and relative humidity (Ellis and Roberts 1980; Dickie *et al*., 1990). Genebank Standards of conserving seed at -18C and 3-7% seed moisture content (SMC) have been established governing most seed bank procedures (FAO/IPGRI, 1994). However this "gold" standard is being reviewed in light of new research which shows that these may not be the most optimum storage conditions. Recently a Crop Genebank Knowledge Base has been published on line (http://cropgenebank.sgrp.cgiar.org/) that provides crop specific information on genebank procedures for nine different crops, including three clonally propagated crops (Jorge *et al*., 2010).

Current research is showing that there exists variability in seed longevity for different species being conserved under similar conditions (Probert *et al*., 2009; Nagel and Borner, 2009; Crawford *et al*., 2007; Walters *et al*., 2005). These findings have significant implications for the management of multi-species genebanks which make up the majority of national genebanks and some of the Consultative Group on International Agricultural Research (CGIAR) genebanks (e.g. ICARDA, ICRISAT, ILRI, and CIAT). As the focus shifts, as the focus shifts to collecting CWR and NUS, which comprise a wide diversity of species, this issue would become increasingly important, even for single crop genebanks. Recent advances in *ex situ* research have shown that some families of plants and the localities where they originate may be a key factor in determining their shelf life (Probert *et al*., 2009; Nagel and Borner 2009; Crawford *et al*., 2007; Walters *et al*., 2005). In addition it has been found that type of seed (endospermic v/s non endospermic) (Probert *et al*., 2009) and intraspecific variation (Nagel and Borner, 2009) may also affect accessions longevity. These factors are not well studied and need be investigated further in a coordinated manner. One of the reasons why CWR are not well represented in *ex situ* collection is because little is known about their storage behaviour and many are difficult to conserve in seed banks. Standard procedures often do not work for these species. Thus the research focus of *ex situ* conservation should be to enhance our understanding of the responses of a wide diversity of species (in particular CWR and NUS, including those bearing recalcitrant seeds) to both single and different storage conditions and methods, with the aim of providing conservationists with information on suitable options for conserving given species.

High initial quality seeds are a major pre-requisite for ensuring seed longevity in seed banks, as factors operating during post harvest treatment of seeds can adversely affect the initial seed quality and undermine the value of genebank accessions ultimately leading to genetic drift and erosion. It is recognized that conventional cold storage has many constraints – in terms of personnel, costs, and reliance on electric power sources (especially in many developing countries where electricity power can be unreliable). It is known that the response to drying can vary according to drying method used, physiological state of seeds, type of seeds and species (Probert *et al*., 2007; Vodouhe *et al*., 2008). Among these post harvest treatments, seed drying poses a major problem for many resource-poor genebanks in Africa, Asia and Latin America. It is often assumed that SMC and temperature act independently and that SMC is more important for long term conservation than temperature and that seed longevity increases with decreasing moisture content following Harrington's rule (Harrington, 1972) and viability equations (Ellis and Roberts, 1980). Several empirical studies, initiated in the 1980's have shown that seeds of various species store well at seed moisture contents (SMC) below 3% and when kept in moisture proof containers at room temperature for long periods (Ellis *et al*., 1986, 1988, 1989). However recent studies have demonstrated that drying seed beyond a critical moisture content provides no additional benefit to longevity and may even accelerate seed aging rates (Vertucci and Roos 1990; Walters, 1998). Furthermore, it has been shown that lowering the storage temperature increased the optimum seed moisture content level, suggesting that there may be a danger in over-drying seeds (Walters and Engels, 1998). This question still remains debatable (Ellis and Hong, 2006; Vertucci and Roos 1990; Walters, 1998) yet findings stemming from a recently concluded 15 year study commissioned by Bioversity International and involving USDA-ARS-NCGRP, ICRISAT and CAAS have shown the limited benefits of drying and low temperature on seed longevity (Walters *et al*., 2009). More research in this area is warranted to develop cost effective methods to help national genebanks in resource poor countries.

Plants that cannot be conserved as seeds because of their recalcitrant nature (i.e. seeds that are desiccation and/or cold sensitive) or are clonally propagated are traditionally conserved as live plants in field genebanks (Reed *et al*., 2004; Dulloo 2001). But field genebanks present real logistical challenges; they require large areas and are costly, they are vulnerable to pests and diseases, natural disasters, political unrest, extreme weather, fire, vandalism and theft, and often are at risk due to policy changes on land use (Dulloo *et al*., 2001; Hawkes *et al*., 2001; Engelmann and Engels, 2002; Parfitt, 2010). For example, a single cyclone in Madagascar destroyed a unique field collection of *Mascarocoffea* species that are important because many contain little or no caffeine, a trait of great interest to coffee breeders (Dulloo *et al*., 2009). There are similar examples of the impacts of cyclones in the Pacific. Recently the world renowned Russian fruit field collection at Pavlovsk, the largest repository of European fruits and berries in the world established by Nicolai Vavilov in 1926, is being threatened by a property development project (Parfitt, 2010). Research on finding solutions to better conserve these difficult-to-store seeds has focused on the use of biotechnology (Engelmann and Engels, 2002). *In vitro* slow-growth conservation methods, involving culturing different parts of the plant (meristem, tissues, cells) into pathogen-free sterile culture in a synthetic medium with growth retardants have been cited as good ways of complementing and providing backup to field collections. It has long been known that *in vitro* slow growth method suffers high risks of somaclonal variation (Withers, 1993) and also from the need to develop individual maintenance protocols for the majority of species (Engelmann, 1991; Thormann *et al*., 2006).

Cryopreservation, in which living tissues are conserved at very low temperatures (−196°C) in liquid nitrogen (LN) to arrest mitotic and metabolic activities, provides a more promising option (Engelmann and Engels, 2002; Thormann *et al*., 2006). Significant progress has been made in cryopreservation research over the past twenty years and much of that research has been focusing on understanding the desiccation sensitivity of recalcitrant seeds and on the underlying mechanism of desiccation tolerance (Engelmann and Panis, 2009; Berjak and Pammenter, 2008; Berjak, 2005). Much has been done also on the development of a number of analytical tools in the framework of the EU-funded FP5 research project "CRYMCEPT" which allowed a more scientific and rational approach to establishment cryopreservation protocols compared with the more empirical approach followed previously (Dussert *et al*., 2003; Zhu *et al*., 2006; Carpentier *et al*., 2007; Ramon *et al.*, 2003). Such tools, including thermal (Differential Scanning Calorimetry (DSC)), biochemical (sugars, lipids, proteins) and histo-cytological analyses, have led to the development of cryo-protocols for conserving more than 200 plant species, including *Musa*, coffee, and citrus (Engelmann and Takagi, 2000; Engelmann, 2004; Dussert and Engelmann, 2006; Engelmann and Panis, 2009; Panis *et al.,* 2005). It is now realized that cryopreservation methods can offer greater security for long term, cost effective conservation of plant genetic resources, including orthodox seeds. Studies carried out by Walters *et al*. (2004) on the ageing of lettuce seeds stored at temperatures between 50 and −196°C have shown that the viability of orthodox species, even when stored under optimal conditions of low temperature and low moisture content, is much shorter than anticipated previously when using the available seed viability equations. The storage in liquid nitrogen clearly prolonged shelf life of lettuce seeds with half-lives projected as 500 and 3400 years for fresh lettuce seeds stored in the vapour and liquid phases of liquid nitrogen, respectively. However the same study also established that cryogenic temperatures did not restrict degradative reactions and resulted in measurable changes in germination after more than 10 years, especially if the initial stages of aging were allowed to progress at higher storage temperatures (Walters *et al*., 2004). The cost effectiveness of cryopreservation as a long term conservation method over field collections has also been demonstrated in a study on coffee genetic resource (Dulloo *et al*., 2009). This study compared the costs of maintaining one of the world's largest coffee field collections with those of establishing a coffee cryo-collection at the Centro Agronómico Tropical de Investigación y Enseñanza (CATIE) in Costa

Rica. The results indicated that although the per-accession establishment costs of 300 accessions cryo-collections (US$95.00 per accession) were more expensive than establishing a field collection (US$69.62 per accession), the per-accession costs for maintenance of the cryo-collection (US$8.00 per accession) was significantly less that the field collection of 1992 accessions (US$15.00 per accession), and that the cost forecasted for 2000 accessions (comparable to current field collection) was even further reduced (US$3.00 per accession) (Dulloo *et al*., 2009). Future research is needed on interactions between storage temperature and longevity in orthodox seeds and to improve the understanding of the mechanisms involved in tolerance to desiccation and freezing using available analytical tools for developing cryo-protocols for recalcitrant seeds. In addition the potential of cryotherapy as a way of eliminating pest and diseases (Wang *et al.,* 2008) needs to be investigated and standards for management of cryopreservation collections need to be developed.

With the rapid development in the field of molecular genetics and genomics, DNA material is becoming more and more in demand for molecular studies and is one of the most requested materials from genebanks (Andersson, 2006). The establishing of a DNA storage facility as a complementary "back-up" to traditional *ex situ* collections has been suggested (Dulloo *et al*., 2006), but little effort has been made to collect and conserve DNA as a genetic resource. Some efforts have been made to establish DNA banks for endangered animals (Ryder *et al*., 2000) and a few plant DNA banks including Missouri Botanic Garden, Royal Botanic Gardens - Kew, Australian Plant DNA Bank and Trinity College Dublin (TCD) (Rice *et al*. 2006, Hodkinson *et al*., 2007). Many research groups are already developing their own archives of extracted genomic DNA (Rice *et al*., 2006). Recently, The Global Biodiversity Information Facility (GBIF) in Germany has establish a DNA Bank Network in 2007 (http://www.dnabank-network.org/Index.php , last accessed 22 September 2010) and offers a worldwide central web portal, providing DNA samples of complementary collections (microorganisms, protists, plants, algae, fungi and animals). DNA bank databases of all partners of the network are linked and are accessible via this portal. What is lacking is a concerted effort to standardize, organize and document existing and future information on DNA storage. The GBIF Germany DNA network would provide a good mechanism to link both to the scientific community conserving genotypes in genebanks and to breeders and molecular biologists that use the resources for genetic improvement.

The regeneration process is one of the most critical steps and a major challenge in genebank management, during which there is the highest probability for genetic erosion. Basic information on the extent of genetic diversity in collections and factors contributing to changes of allele frequencies and loss is largely missing for the vast majority of CWR and NUS. Also the impact that different methods of conservation and the regeneration of accessions within collections have on genetic diversity of collections is not well documented and is largely unquantified. The sample size and the effective population size (Ne) are key attributes for the preservation of genetic variability in genebanks. If samples are too small valuable alleles may be lost through random changes in allele frequency (Crossa, 1995). Information on the genetic composition and spatial genetic structure (of genebank accessions) is an important starting point to develop monitoring for genetic erosion (Laikre *et al.*, 2008). A key research questions is to establish the optimum size of genebank accessions for new species (CWR and NUS) in order to reduce genetic drift that can result from too small a sample size. It is equally important to understand how different conservation methods (seed, field, cryopreservation) and their management can affect or change the gene make up, thereby reducing the effective population size (Ne). This will also contribute to decision-making process for determining which methods to use for conservation of the wide diversity of CWR and NUS.

In Situ **Conservation Research**

According to the Second Report on the *State of the World's Plant Genetic Resources for Food and Agriculture* (FAO, 2010) significant progress has been achieved in developing tools to support the assessment, conservation and management of on-farm diversity in domesticated species, with countries reporting increasing numbers of national surveys and inventories documenting the status of conservation efforts targeting these genetic resources and priorities for further action. The role of farmers and of traditional knowledge in understanding and managing crop diversity has increasingly been recognized as essential for the maintenance of PGRFA, with a significant amount of crop genetic diversity in the form of traditional varieties continued to be maintained on farm (Besançon *et al*., 2009; Rana *et al*., 2007; Sadiki *et al*., 2007). More often than not these are poor, small-scale farmers (Kontoleon *et al*., 2009) who rely on traditional crop varieties to meet their livelihood needs.

Within the framework of the project *Strengthening the Scientific Basis of In Situ Conservation of Agricultural Biodiversity* (Jarvis and Hodgkin, 1998) and in line with the CBD's Programme of Work on Agricultural Biodiversity, Bioversity International worked with partner organizations from 8 countries and 27 crops to ensure the maintenance of on farm crop genetic diversity, with particular emphasis on landraces (Jarvis and Hodgkin, 2008). Studies have focused mainly on the maintenance of diversity in home gardens (e.g. Eyzaguirre and Linares, 2004; Watson and Eyzaguirre, 2002), on conserving neglected and underutilized crops (Padulosi *et al*., 2008) and on nutrition and biodiversity maintenance and use (Frison *et al*., 2006).

In recent times, a highly-adaptable range of tools and methodologies has become available to help farmers maintain and benefit from the use of traditional crop genetic diversity growing in their fields (Friis-Hansen and Sthapit, 2000; CIP/UPWARD, 2003; Sthapit *et al.*, 2006; Jarvis and Hodgkin, 2008; Kontoleon *et al.*, 2009; Lipper *et al.*, 2010). A set of key actions has also been developed by Bioversity International (Jarvis and Hodgkin, 2008) to address some of the main constraints that are currently limiting the effective conservation of these important species, namely: the lack of sufficient diversity of local crop varieties maintained on farm; limited access by farmers to available diversity and to information on different varieties, and, the lack of marketing mechanisms to ensure that farmers continue to benefit from on farm conservation of agrobiodiversity (Jarvis *et al.*, 2004).

A range of tools for the in depth-assessment of on-farm diversity, which need to be carried out to prioritize species for conservation, are now available to quantify the amount of diversity within farmers' fields, for instance using the names and descriptions given by farmers to distinguish their varieties (Sadiki *et al.*, 2007). "Community Biodiversity Registers" in Nepal, which record the cultivars and areas of production of the different crops grown by farmers in the community, have also been developed to strengthen indigenous knowledge systems (Rijal *et al.*, 2000). These studies are further assisted by the advancement of molecular techniques, such as single nucleotide polymorphisms (SNPs), phylogenetic analysis and functional genomics (Brown and Hodgkin, 2007). In Nepal, for instance, SSR markers were used to establish landrace diversity of over 20 rice cultivars identified by farmers (Bajracharya *et al.*, 2006).

Efforts to provide farmers with access to information on local germplasm, on the other hand, have resulted in the promotion of Diversity Fairs, which allow farmers to appreciate the range of diversity available in a region, whilst providing an informal platform for the exchange of seed materials, strengthening local knowledge and seed supply systems (Sthapit *et al.*, 2002). Improved communication technologies have further enabled farmers to access timely information on agricultural information to fit their needs (Kesavan and Swaminathan, 2008).

Bioversity also assisted countries in developing national plans or strategies targeting the on farm maintenance of traditional varieties and the sustainable use of agrobiodiversity, promoting their integration in sectoral and cross-sectoral plans and programmes (Visser and Jarvis, 2000). An example is the establishment in Nepal of a National Agrobiodiversity Coordination Committee to pilot good practices of on farm management of agricultural biodiversity in 29 districts (Jarvis and Hodgkin, 2008). Public awareness activities were also carried out and sustained action has led to a growing interest in farmer-based conservation initiatives (Jarvis and Hodgkin, 2008).

It has to be said that lesser progress has probably been made in regards to the assessment, conservation and management of CWR *in situ*. Although the number of countries reporting carrying out CWR inventories has risen from four to 28 in the last decade (FAO, 2010), a study of the International Union for the Conservation of Nature's (IUCN) Red List of Threatened Species in 2008 showed that assessments have been carried only for 45 wild species related to important food security crops, the majority of which are relatives of the potato (IUCN, 2008). Furthermore, despite renewed appreciation of the importance of CWR and the designation of dedicated areas for their conservation, the second Report on the *State of the World's Plant Genetic Resources for Food and Agriculture* (FAO, 2010) points out that the distribution across regions of reserves that include populations of CWR remains uneven, and several major regions, such as Sub-Saharan Africa (SSA), are still under-represented.

The very limited practical experience in conserving CWR *in situ* to date means that there are no generally agreed protocols that can be recommended and good practice is limited by the shortage of successful examples to draw upon. Despite this, some progress has been made in relation to prioritization of species and areas, assessments of distributions, diversity and threat status, *in situ* management in protected areas, development of CWR national plans and strategies and raising awareness and understanding of their importance. Most recently, the UNEP/GEF-supported project, *In situ* conservation of crop wild relatives through enhanced information management and field application' (CWR Project), implemented by Bioversity International in collaboration with international (FAO, BGCI, IUCN and WCMC) and national partners (Armenia, Bolivia, Sri Lanka, Madagascar and Uzbekistan) with financing from the Global Environment Facility (GEF) and implementation support from the United Nations Environment Programme (UNEP) – has expanded substantially the previously limited body of knowledge on *in situ* CWR conservation in developing countries. Aside from countries assessing more than 310 CWR species according to IUCN guidelines and Red List criteria, and Bolivia producing the first ever Red List of CWR (VMABCC-BIOVERSITY, 2009), the project undertook what is one of the largest bodies of work on ecogeographic surveys of CWR and this has added substantially to the global knowledge base. The project prioritized for action 36 different CWR genera in the five countries, and developed tools and methodologies to assist other countries in different geographical regions to develop adaptation and mitigation strategies for the effective *in situ* conservation of CWR, further contributing to Objective 7 of the GSPC (i.e. develop effective means of conserving and using CWR *in situ*).

Meilleur and Hodgkin (2004) point out that few CWR conservation activities will be successful without effective planning. At the international level the conservation and sustainable use of CWR are addressed in both the agriculture and environment sectors through the ITPGR-FA and CBD. The CBD requires the Parties to develop national strategies, plans or programmes for the conservation and sustainable use of biodiversity. However, most countries' biodiversity strategies and action plans have been shown not to specifically refer to CWRs or even to the *in situ* conservation of individual species in general. Such is the importance of CWR that it is clearly desirable for countries to develop a separate National Action Plan (NAP) or strategy for their conservation and sustainable use, including reviews of relevant national policy and legislation documents and strategies for capacity building and communication. With assistance from the UNEP/GEF CWR Project, Bioversity International has assisted Armenia, Bolivia, Madagascar, Sri Lanka and Uzbekistan draft national action plans or strategies and these represent a unique resource. Prior to this project, very few countries had developed such NAP or strategies, so there are few examples that offer guidance. However, the general absence of such CWR national action plans and strategies in other countries represents a significant gap in practice.

The numbers and diversity of CWR species are huge while the resources, both human and financial, available for their conservation is insufficient to satisfy all the demands and needs. As a consequence some form of priority setting is particularly relevant to the field of CWR conservation and some progress has been made in this area (Hunter and Heywood, 2010; Brehm *et al.,* 2010). There is no precise or agreed methodology for selecting CWR species or populations that should be given priority as targets for *in situ* conservation and a great deal will depend on local requirements and circumstances. In practice, the selection made will be influenced by the priorities and mandate of the institution or agency involved commissioning the conservation actions (Ford-Lloyd *et al.*, 2008).

Populations of many CWR occur in existing protected areas, although the lack of inventories means that detailed information is not always available, and it may be assumed that this may afford some degree of protection provided the area is well managed. But this passive conservation alone does not in many cases represent effective *in situ* conservation without some degree of active management or at least recurrent monitoring targeted at the populations of the particular target species, particularly if the species is threatened (Maxted *et al.*, 1997). Through the involvement of protected area authorities and other relevant stakeholders such as indigenous and local communities, Bioversity has facilitated the development of CWR species management plans for implementation in protected areas, as well as the adaptation of protected area management plans so as to take into account the needs for CWR conservation. The work has highlighted the considerable challenges and obstacles facing CWR conservation in protected areas (Hunter *et al.*, in prep.). As a result of this Bioversity-led work we now have for the first time comprehensive CWR species management plans prepared for: wild yams (*Dioscorea maciba, D. bemandry, D. antaly, D. ovinala and D. bemarivensis)* in Ankarafantsika National Park, Madagascar; wild cinnamon (*Cinnamomum capparu-coronde)* Kanneliya Forest Reserve, Sri Lanka; wild almond (*Amygdalus bucharica*) in Chatkal Biosphere Reserve, Uzbekistan; wild wheat (*Triticum araraticum*, *T. boeoticum*, *T. urartu* and *Aegilops tauschii*) in Erebuni State Reserve, Armenia; and wild cacao (*Theobroma* spp.) in Parque Nacional y Territorio Indigena Isiboro-Secure, Bolivia. More importantly a process has been facilitated which brought together agriculture, protected area staff and sometimes local and indigenous communities to establish effective working partnerships and considerable trust and confidence. These and their corresponding lessons learned (Hunter *et al.*, in prep), are useful examples for other countries to follow and to guide future work in this area.

As previously stated, protected areas may no longer be sufficient to guarantee the survival of these species under future climate-change scenarios and new methods for assessing the ecogeographic status and threats to CWR need to be developed. Moreover, reliance on the continued existence of protected areas in their current location is a risky strategy for CWR conservation in the face of global, especially climatic, change (Hunter and Heywood, 2010). Using bioclimatic modelling, possible scenarios of climatic change in Mexico were used to analyze the distribution patterns of eight wild Cucurbitaceae closely related to cultivated plants (Lira *et al.*, 2008). The possible role that the Mexican system of protected areas might have in the conservation of these taxa was also assessed. The results showed a marked contraction of the distributions of all eight taxa. The authors also found that, under a drastic climatic change scenario, the eight taxa will be maintained in just 29 out of the 69 natural protected areas where they currently occur. This emphasises again the future need for greater linkages between protected and agricultural landscapes to facilitate gene flow and dispersal. It also highlights the urgent need for effective linkages to *ex situ* conservation.

In situ conservation of CWR will mostly take place in some form of protected area so the effects of global change on such areas are of major concern. It is clear that the projected impacts on protected areas in many parts of the world will force us to rethink their role in biodiversity conservation. The political boundaries of protected areas are fixed but the biological landscape is not (Lovejoy, 2006). It is clearly difficult for a fixed system of protected areas to respond to global change and considerable rethinking in the design of such areas will be needed if they are to survive and remain effective. Climate change therefore has major implications not only for protected areas but for protected area management and managers (Schliep *et al.,* 2008).

Given that protected areas and other conservation areas cover in all only 12-13% of the earth's surface, it is clear that they cannot alone ensure the survival of species and ecological communities, even without the impacts of accelerated global change. It follows logically that many CWR will be included in the species that grow outside protected areas. It is crucial, therefore that lands outside protected area networks be managed in ways that allow as much biodiversity as possible to be maintained. The *in situ* conservation of species outside protected areas, where the majority of them occur, is a seriously neglected aspect of biodiversity conservation and in the face of global change it demands greater attention from governments and conservation agencies. On the one hand, we need to address what actions may be proposed so that many areas which are not protected as such and that are found to house target species will be maintained in such a way as to ensure their protection at the ecosystem or landscape level both by positive management policies or by the prevention of certain forms of activity. On the other hand it may be possible to take actions to ensure that such areas outside formal protection, whether on public or private lands, can provide a sufficient degree of protection to target species so as to ensure maintenance of viable populations, through some form of agreement with the landowner. However, we have very little experience so far of how to safeguard CWR in such a context (Hunter and Heywood, 2010)

Meilleur and Hodgkin (2004) propose managing 'small sacred sites' within an informal network as a possible viable approach to CWR conservation. Such sacred sites and their natural vegetation are likely to contain numerous CWR and it is envisaged these could be inventoried as part of the conservation process which would build on the sustainable indigenous management practices which have been in existence for hundreds of years. Another approach is to promote CWR in situ conservation in the increasingly recognised indigenous and community conserved areas (ICCAs), such as the Parque de la Papa (Potato Park) in Peru, many different kinds of which occur across the world but have so far remained outside the scope of formal conservation policies and programmes and often conventional designation of protection status. A considerable part of the world's biological diversity is located in such areas whose ownership, control and use is in the hands of indigenous and local communities, including nomadic peoples (Hunter and Heywood, 2010).

Outputs from the project have helped bridge some of the gaps identified by FAO Second Report on the *State of the World's Plant Genetic Resources for Food and Agriculture* as limiting the effective conservation of CWR, namely: involving farmers and indigenous communities in developing sustainable CWR management practices; drafting policies and legislation governing the *in situ* conservation of CWR; establishing effective cross-sectoral partnerships among the agriculture and environment sectors for the common goal of CWR protection; developing strategies for the conservation of select CWR inside and outside protected areas; strengthening research capacity in project countries and creating CWR inventories. These results are already being shared with the global conservation community through the CWR Global Portal (www.cropwildrelatives.org). The FAO report also identified the following gaps for further research:

- Develop early warning systems for genetic erosion;
- Measures to counter the threat of alien invasive species;
- Strengthened research capacity, particularly taxonomy using new molecular tools;
- Need for characterization data on land races, and CWR
- Studies on the reproductive biology and ecological requirements of CWR and other useful wild species;
- Ethnobotanical and socio-economic studies, including the study of indigenous and local knowledge, to better understand the role and limits of farming communities in the management of PGRFA;
- Studies of the effectiveness of different mechanisms for managing genetic diversity and how to improve them;
- Studies of the dynamic balance between *in situ* and *ex situ* conservation.
- Studies on the mechanisms, extent, nature and consequences of geneflow between wild and cultivated populations;

Further research to provide information to underpin the development of appropriate policies for the conservation and use of genetic diversity, including the economic valuation of PGRFA.

Conclusions

The central challenge facing agricultural biodiversity in the future involves identifying when and in what ways diversity makes key contribution to sustainable production. Determining what is the optimum diversity of different components in different situations and systems is a major challenge. It is also important to fully understanding the extent, distribution and functions of genetic diversity, and trends in genetic erosion and vulnerability. The maintenance (and conservation) and use of diversity will need to be addressed in a holistic manner and need to meet the demands of the users of germplasm which does not include only breeders, researchers, but also farmers and rural communities, and of those concerned with improving agricultural production and of maintaining healthy ecosystem functions. Achieving these aims will involve both investigative aspects and the identification of specific interventions that can support improved diversity management and agricultural production. Agricultural biodiversity has a key role to play in these processes.

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