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Plant Genetic Resources: Selected Issues from Genetic Erosion to Genetic Engineering

K. Hammer [*1] and **Y. Teklu** [2]

Abstract
Plant Genetic Resources (PGR) continue to play an important role in the development of agriculture. The following aspects receive a special consideration:
1. *Definition*. The term was coined in 1970. The genepool concept served as an important tool in the further development. Different approaches are discussed.
2. *Values of Genetic Resources*. A short introduction is highlighting this problem and stressing the economic usfulness of PGR.
3. *Genetic Erosion*. Already observed by E. Baur in 1914, this is now a key issue within PGR. The case studies cited include Ethiopia, Italy, China, S Korea, Greece and S. Africa. Modern approaches concentrate on allelic changes in varieties over time but neglect the landraces. The causes and consequences of genetic erosion are discussed.
4. *Genetic Resources Conservation*. Because of genetic erosion there is a need for conservation. PGR should be consigned to the appropriate method of conservation (*ex situ*, *in situ*, on-farm) according to the scientific basis of biodiversity (genetic diversity, species diversity, ecosystem diversity) and the evolutionary status of plants (cultivated plants, weeds, related wild plants (crop wild relatives)).
5. *GMO*. The impact of genetically engineered plants on genetic diversity is discussed.
6. The *Conclusions and Recommendations* stress the importance of PGR. Their conservation and use are urgent necessities for the present development and future survival of mankind.

Keywords: Plant Genetic Resources (PGR), crop plants, genetic erosion, genetic resources conservation, GMO

1 Introduction

World population is expected to increase by 2.6 billion over the next 45 years, from 6.5 billion today to 9.1 billion in 2050. The world needs astonishing increase in food production to feed this population. Plant genetic resources (PGR) constitute the foundation upon which agriculture and world food securities are based and the genetic diversity in

[*] corresponding author
[1] Prof. Dr. Karl Hammer, Department of Agrobiodiversity, University of Kassel, Steinstrasse 19, 37213 Witzenhausen, Germany
[2] Dr. Yifru Teklu, Institute of Plant Genetics and Crop Plant Research, Gene and Genome Mapping Unit, Corrensstr 3, 06466 Gatersleben, Germany

the germplasm collections is critical to the world's fight against hunger. They are the raw material for breeding new plant varieties and are a reservoir of genetic diversity. Genetic adaptation and the rate of evolutionary response to selective forces depend on inherent levels of genetic diversity present at the time a species experiences a threat to its survival. Genetic diversity gives species the ability to adapt to changing environments, including new pests and diseases and new climatic conditions.

Over the millennia, traditional farmers have given us an invaluable heritage of thousands of locally adapted genotypes of major and minor crops that have evolved because of natural and artificial selection forces (MYERS, 1994). The genetic base of landraces, wild and weedy relatives in which future breeding is based have been threatened by various factors of genetic erosion. Erosion of these genetic resources along with accompanying practices and knowledge that farmers use to develop, utilize and conserve crop genetic resources could pose a severe threat to the world's food security in the long term. Loss of genetic variation may decrease the potential of species to persist in the face of abiotic and biotic environmental change as well alter the ability of a population to cope with short-term challenges such as pathogens and herbivores. Detecting and assessing genetic erosion has been suggested as the first priority in any major effort to arrest loss of genetic diversity. Generally, nevertheless, many national programs have not regarded quantification of genetic erosion as a high priority, as apparent from the paucity of information in the State of the World Report (FAO, 1996b).

With the further development of scientific and technical possibilities, the need for various plant genetic resources will increase. Therefore, the results of unabated gene erosion must by all means be reversed. Urgent action is needed to collect and preserve irreplaceable genetic resources (FRANKEL, 1974). All effort should be made to cover this future need by utilizing both *in situ* as well as *ex situ* maintenance. In situ means the setting aside of natural reserves, where the species are allowed to remain in their ecosystems within a natural or properly managed ecological continuum. This method of conservation is of significance to the wild relatives of crop plants and a number of other crops, especially tree crops and forest species where there are limitations on the effectiveness of *ex situ* methods of conservation. The *ex situ* form of conservation includes, in a broad sense, the botanic gardens and storage of seed or vegetative material in gene banks. Biotechnology has generated new opportunities for genetic resources conservation. Techniques like *in vitro* culture and cryopreservation have made it possible to collect and conserve genetic resources, especially of species that are difficult to conserve as seeds. DNA and pollen storage also contribute to *ex situ* conservation. No single conservation technique can adequately conserve the full range of genetic diversity of a target species or gene pool. Greater biodiversity security results from the application of a range of *ex situ* and *in situ* techniques applied in a complementary manner, one technique acting as a backup to the other techniques.

Advances in biotechnology have offered a new arsenal of methods to effectively utilize genetic resources. Gene technology increased the possible use of distantly related trait carriers as donors for the desired characteristics. However, the movement of genes across species boundaries presents many opportunities for both expected and unexpected risks.

In addition to food safety, other concerns involve ecological risks, such as new or increased resistance to insecticides and weed resistance to herbicides due to hybridization or excessive selection pressure, changes in the ecological competitiveness of crops, and the possible loss of genetic diversity in areas of crop origin (St Amand *et al.*, 2000). Transgenes conferring novel traits that enhance survival and reproduction may inadvertently disperse from cultivated plants to wild or weedy populations that lack these traits and might generate similar but unwanted effects in their weedy relatives through gene flow.

2 Plant Genetic Resources

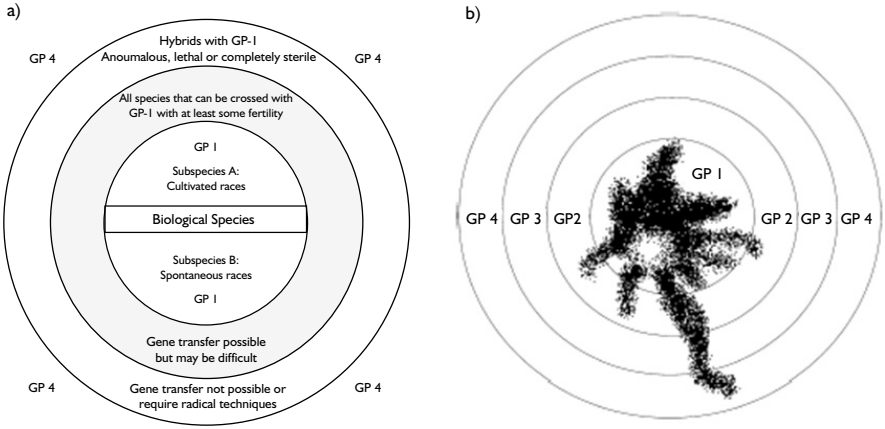
2.1 Definition of Plant Genetic Resources

The term "genetic resources" was first used at a conference which took place under the International Biological Program (Hawkes, 1997). The conference papers were published in 1970 (Frankel and Bennett, 1970). Since then various attempts were made to define plant genetic resources. According to the revised International Undertaking 1983 of the FAO, plant genetic resources were defined as the entire generative and vegetative reproductive material of species with economical and/or social value, especially for the agriculture of the present and the future, with special emphasis on nutritional plants. Brockhaus and Oetmann (1996) defined PGR as "plant material with a current or potential value for food, agriculture and forestry". A correlated definition that appends a value of aggregation to PGR was given by FAO (1989). According to this definition, plant genetic resources refer to the economic, scientific or societal value of the heritable materials contained within and among species. They include materials used in cytogenetic, evolutionary, physiological, biochemical, pathological or ecological research on one hand, accessions evaluated for their agronomic or breeding propensities on the other.

The work of Harlan and de Wet (1971) that starts with gene pools (Figure 1) has formed a valid scientific basis for the definition of plant genetic resources. Plant breeders recognized three major gene pools based on the degree of sexual compatibility (Harlan and de Wet, 1971; Gepts, 2000). All crop species belong to a primary gene pool together with such material with which they produce completely fertile crosses through hybridisation. In contrast, all those plant groups that contain certain barriers against crossing belong to the secondary gene pool. The tertiary gene pool includes groups that can only be crossed with the help of radical new techniques. Plant breeders have traditionally emphasized closely related, well-adapted domesticated materials within the primary gene pool as sources of genetic diversity (Kelly *et al.*, 1998). More recently, however, plant transformation and genomics have led to a new quality which has been defined by Hammer (1998) and Gepts and Papa (2003) as a fourth gene pool, whereas Gladis and Hammer (2002) concluded that information and genes from other species should be a special case for the third gene pool. The fourth gene pool should contain any synthetic strain shown with nucleic acid frequencies, DNA and RNA, that do not occur in nature (Figure 1). Transgenesis allows us to bypass sexual incompatibility barriers altogether and introduce new genes into existing cultivars. It should be emphasized here

that the major function of transgenic technologies is not the creation of new cultivars but the generation of new gene combinations that can be used in breeding programs (GEPTS, 2002).

Figure 1: The gene pool concept, established by HARLAN and DE WET (1971) and an example of an organismoid or a hypothetically designed crop.



a) The gene pool concept, established by HARLAN and DE WET (1971), modified. GP 1 The biological species, including wild, weedy and cultivated races. GP 2 All species that can be crossed with GP 1, with some fertility in individuals of the F1 generation; gene transfer is possible but may be difficult. GP 3 Hybrids with GP 1 do not occur in nature; they are anomalous, lethal, or completely sterile; gene transfer is not possible without applying radical techniques. Information from other genes refers to comparative genomic information on gene order and DNA sequence of homologous genes. GP 4 Any synthetic strains with nucleic acid frequencies (DNA or RNA) that do not occur in nature.

b) Example of an organismoid or a hypothetically designed crop with a genome composed of different gene pools and synthetic genes [for the explanation of this complicated matter, see GLADIS and HAMMER (2002)].

2.2 Values of Genetic Resources

Human civilizations have benefited greatly from the domestication, conservation and use of plants species used for agriculture and food production. For thousands of years, farmers have used the genetic variation in wild and cultivated plants to develop their crops. Genetic diversity is the basic factor of evolution in species. It is the foundation of sustainability because it provides raw material for adaptation, evolution, and survival of species and individuals, especially under changed environmental, disease and social conditions (HAMMER, 2004), and it will allow them to respond to the challenges of the next century (HAMMER *et al.*, 1999). The future food supply of all societies depends on the exploitation of genetic recombination and allelic diversity for crop improvement, and many of the world's farmers depend directly on the harvests of the genetic diversity they sow for food and fodder as well as the next seasons seed (SMALE *et al.*, 2004).

The considerable genetic diversity of traditional varieties of crops is the most immediately useful and economically valuable part of global biodiversity. Subsistence farmers use landraces as a key component of their cropping systems. Such farmers account for about 60% of agricultural land use and provide approximately 15-20% of the world's food (FRANCIS, 1986). In addition, landraces are the basic raw materials used by plant breeders for developing modern varieties.

Over the last few decades, awareness of the rich diversity of exotic or wild germplasm has increased. This has lead to a more intensive use of this germplasm in breeding and thereby yields of many crops increased dramatically. Domesticated tomato plants are commonly bred with wild tomatoes of a different species to introduce improved resistance to pathogens, nematodes and fungi. Resistance to at least 32 major tomato diseases have been discovered in wild relatives of the cultivated tomato. Genes responsible for promoting resistance to 16 of these have been bred into commercial cultivars, allowing tomato production in areas where they could not otherwise have grown. Lodging was one of the major constraints limiting further increases in yields in wheat production since it prohibits the application of high amount of fertilizer. A search was therefore made among wheat from different areas of the world to locate a suitable source of genetic dwarfness to overcome this barrier. Norin 10, an extremely dwarf wheat landrace from Korea found in Japan's collections, proved to be a suitable source because of two genes, Rht1 and Rht2, that caused dwarfing. Norman Borlaug speculated that by breeding these genes into Mexican wheat lodging would be reduced and the plants would respond to fertilizer application. As it turns out, these genes not only reduce lodging through reduced height, they have direct effects on yield as a result of more efficient nutrient uptake and enhanced tillering. Despite decades of active efforts by plant breeders to control potato late blight, the disease continued to cause the loss of billions of dollars for growers each year (KAMOUN, 2001). The exploitation of genetic resistance remains the most promising approach for the long-term control of late blight. The wild potato species *Solanum bulbocastanum* is a source of genes for potent late blight resistance. Similarly, the use of landraces and wild species in rice breeding has had an enormous impact on rice productivity in many countries. For example, of the 6723 accessions of cultivated rice and several wild species of *Oryza* screened for resistance, only one accession of wild species *O. nivara* was found to be resistant and used to introduce resistance to grassy stunt virus into cultivated rice (LING *et al.*, 1970). The use of Turkish wheat to develop genetic resistance to diseases in western wheat crops is valued in 1995 at US $ 50 million per year. Ethiopian barley has been used to protect Californian barley from dwarf yellow virus, saving damage estimated at $160 million per year. Mexican beans have been used to improve resistance to the Mexican bean weevil, which destroys as much as 25% of stored beans in Africa and 15% in South America (PERRINGS, 1998). The diversity of plants in different ecosystems brings a lot of pleasure and inspiration to people with cultural and/or religious significance and the potential for income generation through eco-tourism. Thus, it is important to appreciate the contribution to human welfare and environmental sustainability made by all the three levels of biodiversity: (i) ecosystems, (ii) species, and (iii) genetic diversity (IPGRI, 1993).

3 Genetic Erosion

From the beginning of agriculture, farmers have domesticated hundreds of plant species and within them genetic variability has increased owing to migration, natural mutations and crosses, and unconscious or conscious selection. This gradual and continuous expansion of genetic diversity within crops went on for several millennia, until scientific principles and techniques influenced the development of agriculture (Scarascia-Mugnozza and Perrino, 2002). The impact of humans upon biodiversity has gradually increased with growing technology, population, production and consumption rates. The quest for increasing food production and the ensuing success achieved in several crops has begun to replace landraces by uniform, true-breeding cultivars. N.I. Vavilov and even Jack Harlan are sometimes proposed as the first researchers that became aware of genetic erosion in the 1920s and 1930s (Scarascia-Mugnozza and Perrino, 2002). In fact, this phenomenon was postulated for the first time by Baur (1914, pp. 104-109), see also Flittner (1995) and Hammer and Teklu (2006). So far, the American plant breeders H. V. Harlan and M. L. Martini (Harlan and Martini, 1938) have been credited with first recognizing the problem of genetic erosion in crops (Brush, 1999). The concept emerged forcefully between 1965 and 1970, in a period when crop improvement had clearly demonstrated its power to transform local crop populations in industrialized countries and in certain less developed regions (Brush, 1999) and the term gene erosion was coined (Bennett, 1968). Brush (1999) defined genetic erosion in crops as the loss of variability from crop populations. Variability refers to heterogeneity of alleles and genotypes with their attendant morphotypes and phenotypes. Genetic erosion implies that the normal addition and disappearance of genetic variability in a population is altered so that the net change in diversity is negative.

3.1 Cases Studies

Several approaches have been employed to estimate the degree of genetic erosion that a particular taxon faces in a certain region over a given time. Methods usually rely on either the analysis of molecular data (Provan *et al.*, 1999) and allozyme analysis (Akimoto *et al.*, 1999), or comparison between the number of species/cultivars still in use by farmers at present time to those found in previous studies (Hammer *et al.*, 1996) or using the genetic assessment model presented by Guarino (1999) or using a checklist of risk factors (de Oliveira and Martins, 2002). The most widely used figures in estimating genetic erosion are indirect, i.e., the diffusion of modern crop varieties released from crop breeding programs. The two case studies conducted by Hammer *et al.* (1996) to estimate genetic erosion in landraces revealed that genetic erosion was found to be 72.4% in Albania and 72.8% in South Italy. Genetic erosion up to 100% was detected in *T. durum* and *T. dicoccon* in some districts of Ethiopia (Teklu and Hammer, 2006). Hammer and Laghetti (2005) used temporal comparison method to examine the loss of genetic diversity in Italy. In the early years (from the 1920s to the 1950s), a relatively high genetic erosion was observed (13.2% p.a.). From the 1950s until the 1980s erosion rates between 0.48 and 4% p.a. were estimated. In the little island of Favignana there was an erosion rate of 12.2% p.a. leading to the extinction of the last wheat landraces

of this island. The study of 220 land races with 147 forms in South Korea (Ahn *et al.*, 1996) showed a medium gene erosion of 74%. Akimoto *et al.* (1999) evaluated the threat of genetic erosion faced by Asian wild rice in Thailand and reported that the wild rice population was seriously destroyed and fragmented. Between 1949 and 1970, the number of wheat varieties cultivated in China dropped from 10000 to only 1000 (Thrupp, 1998). The upland rice varieties in the Jinuo community of southern Yunnan have been decreased from over 100 before 1980 to 65 in 1994. Recent statistics have shown that variety numbers of crops in Swidden agro ecosystems in the community have dropped since several improved varieties were introduced to the area within the past 10 years (Long *et al.*, 1995). In India rice varieties have declined from an estimated 40,0000 before colonialism to 30,000 in the mid-19th century with several thousand more lost after the green revolution in the 1960s. Also Greece is estimated to have lost 95% of its broad genetic stock of traditional wheat varieties after being encouraged to replace local seeds with modern varieties developed by CIMMYT (Lopez, 1994). The widespread adoption of high-yielding rice varieties has led to biological impoverty of rice germplasm, as local rice varieties are abandoned for modern varieties (Gao, 2003). IUCN has developed a system of categories of conservation status, the so-called IUCN Red Data List Categories (IUCN, 1994). A review of the situation in southern Africa using this system revealed that of the 23,000 species in the flora, 58 were extinct, 250 endangered, 423 vulnerable, and 1141 rare (Hilton Taylor, 1996). Hammer and Khoshbakht (2005) have also used Mansfeld's Encyclopedia (2001) and the IUCN Red List of threatened plants (2001) to document the current genetic resources status of agricultural and horticultural plants (excluding ornamentals) in Iran. About 200 threatened cultivated plants are considered and presented in the respective lists, among them completely extinct crop plants such as *Anacyclus officinarum* and *Bromus mango*. According to their report, there is even loss in crop plants on the species level.

In an attempt to determine the changes produced on genetic diversity as a result of modern plant breeding, Khlestkina *et al.* (2004) compared the diversity of cultivated wheat (*Triticum aestivum*) gene bank accessions collected up to 80 years ago in four divergent geographical regions with materials that entered the gene bank about 50 years later but originating from the same areas. They used a set of microsatellite markers and reported a non-significant difference in both the total number of alleles per locus and in the polymorphic information content when the material collected in the repeated collection missions in all four regions were compared. They reported that an allele flow took place during the adaptation of traditional agriculture to modern systems, whereas the level of genetic diversity was not significantly influenced. Khlestkina *et al.* (2004) only investigated the allelic changes occurred, over time, in the conserved materials. Hence, their studies couldn't fully address the actual genetic erosion occurred in the field.

3.2 Major Causes of Genetic Erosion

The manifest cause of genetic erosion is the diffusion of modern varieties from crop improvement programs (Brush, 1999). Much of the evidence for genetic erosion pre-

sented in the 1970/71 FAO survey (Frankel, 1973) is data on the diffusion of modern cultivars (Kjellqvist, 1973). Landraces adapted to optimal local agronomic conditions are probably the crop plant genetic resources that are most at risk of future loss through habitat destruction or by replacement by introduced elite germplasm (Brush, 1995). With the development of scientific plant breeding, high-quality and homogenous new varieties were quickly and widely distributed suppressing landraces. Yield (or yield potential), which is the characteristics of most modern varieties, is the most important criterion for the choice of a variety by a farmer (Heisey and Brennan, 1991). The "Green Revolution" contributed and still undoubtedly contributes to the loss of genetic diversity, even if the issue is not as cut and dried asWood and Lenné (1997) state it in the equation "Green revolution = Loss of genetic diversity". Population growth, urbanization, developmental pressures on the land resources, deforestation, changes in land use patterns and natural disasters are contributing to abundant habitat fragmentation and destruction of the crops and their wild relatives. The famine of the mid-1980s seriously threatened Ethiopia's biological resources (Worede and Mekbib, 1993). The study of Stephen *et al.* (2002) showed a marked reduction in rice diversity in the northeastern Philippines from 1996 to 1998 as a result of drought due to the El Niño phenomenon in 1997 and flooding due to two successive typhoons in 1998. According to Erskine and Muehlbauer (1990), droughts of just a single season could result in people consuming seed stocks, while successive years of drought can prompt changes in cropping patterns and the geographic distribution of crops. Social disruptions or wars also pose a constant threat of genetic wipeout of such promising diversity. Overexploitation and also the introduction of invasive alien species are the other factors contributing to the loss of genetic resources. More recently, global warming and a high degree of pollution have also been recognized as further causes for the loss of biodiversity (Myers, 1994).

The modern world is placing a range of pressures on wild areas and on traditional agricultural communities, and external interests (often dominated by economic or political issues) strongly impinge (Tunstall *et al.*, 2001). The major external forces advocate the introduction of high-yield varieties, accompanied by mechanization and major chemical inputs, as the means to increase total production and economic return. These forces change the nature of the decision-making process dramatically; the farmer is encouraged to grow high-yield varieties in monoculture using inputs of fertilizer and pesticides. In many parts of the world, farmers were given several socio-economic incentives to replace varieties that evolved within their agro-ecosystem with improved/introduced varieties (Louette *et al.*, 1997; Teklu and Hammer, 2006).

Often there are relationships of substitution between ecological functions of agrobiodiversity and external input (for example fertilizer or pesticides) (Hammer, 2004). That means that external inputs can take over functions of agrobiodiversity and vice versa. In homogenous, high-input agricultural systems, ecosystem functions that are missing because of low agrobiodiversity are replaced with intensive management and external inputs. Because of this, those components of agrobiodiversity whose functions can be substituted at lower cost are particularly endangered. For example, in former years many different fodder plants were grown in German fields (oats, barley, beans, clover,

Leucerne, fodder beets and potatoes). Now, corn is usually the only fodder plant, possibly supplemented by soybean meal as a protein component. Each of the species has lost the race in its own fashion. Indigenous crops are adapted to the conditions of less developed agriculture such as "crude land preparation and low soil fertility" (HARLAN, 1975). As these conditions change with improved traction and fertilizer, the existing adaptation of landraces turns from asset to liability. TUNSTALL *et al.* (2001) described that landraces, which are grown because of their high resistance to pests during seed storage, may become less important if improved storage systems are introduced.

Two types of genetic erosion can be distinguished in wild rice: the extinction of populations and the drastic change of genetic structure of populations (GAO *et al.*, 2001). The first type means the total loss of genetic resources, which results from complete destruction of habitats, and all genotypes and/or alleles being lost, while the second one originates from isolated local populations due to the deterioration of habitats. For plants and some animals, area measurements of habitat patch sizes will provide a reasonable basis to estimate population size (BROWN *et al.*, 1997), an important factor determining survival of individuals. HAWKES (1983) reported that smaller area in traditional crops reduces diversity. The frequency distribution of the sizes of individual populations is likely to reflect the way in which genetic variation is partitioned within and among populations, with small populations being at increased risk of loss of alleles, reduced heterozygosity, increased uniformity, enhanced inbreeding or possible extinction. BROWN *et al.* (1997) also indicated that the size and number of individual populations are related to their ability to cope with both random (stochastic) fluctuations in the environment and steady (systematic) long-term change. In some cases the loss of particular crop varieties is not complete, but instead reduces surviving members of a landrace to a few isolated populations (VAN TREUREN *et al.*, 1990). In such cases there is significant risk of the ultimate loss of diversity, because smaller populations are vulnerable to demographic and environmental stochasticity and the decline in fitness associated with genetic drift and inbreeding (FRANKEL and SOULÉ, 1981). Allozyme genetic diversity, inversions and visible mutations all declined more rapidly in smaller than large populations (MONTGOMERY *et al.*, 2000). Two genetic consequences of small population size are increased genetic drift and inbreeding. Genetic drift is the random change in allele frequency that occurs because gametes transmitted from one generation to the next carry only a sample of the alleles present in the parental generation. Genetic drift changes the distribution of genetic variation in two ways: (i) the decrease of variation within populations (loss of heterozygosity and eventual fixation of alleles), and (ii) the increase of differentiation among populations. Every finite population experiences genetic drift, but the effects become more pronounced as population size decreases (FALCONER, 1989).

The problem of genetic erosion through inappropriate maintenance of *ex situ* collections is widely recognized. Genetic erosion can occur at many stages in the preparation, sub-sampling, exchange, storage and regeneration of seed (SACKVILLE HAMILTON and CHORLTON, 1997). They also highlighted loss of diversity through genetic shifts and convergent selection during regeneration as a potentially severe and often under-

acknowledged problem. In the world collection, beyond the problem of duplication among accessions, the security of *ex situ* conservation as a whole is endangered. About half of all gene bank accessions urgently require rejuvenation, and in several countries the percentage is even higher (Hammer, 2004). However, the different institutes are suffering from financial problems, lack of staff and shortage of farms. The long-term storage strongly reduces the metabolism and therefore highly limits viability and seed vigor. According to Tsehaye (2002), durum wheat materials from the Ethiopian gene bank have showed poor germination potential and vigor in the field, which is an indicator of genetic erosion. Considerable evidence indicated that damage to chromosomes, some of it resulting in heritable changes, takes place as seeds loose their viability. Studies in barley and wheat showed that as storage age increases, chromosome aberrations (per cell) increase (Gunthardt *et al.*, 1953). Changes in the properties of DNA associated with loss of viability in rye seeds, namely the loss of DNA-template activity (Holden and Williams, 1984) and decreases in the molecular size of extractable DNA (Cheah and Osborne, 1978), also have been observed.

Genetic erosion can also be caused by limited support for gene banks and in appropriate focus or change in institutional policies. The work of gene banks in Eastern Europe towards the end of the last century was reduced due to lack of money and employees. Only international help was able to prevent catastrophic breakdowns (Frison and Hammer, 1992). New technological developments allow us to change agricultural products during the processing phase so much that only a few basic raw materials are necessary. It is possible, for example, with the aid of biotechnological methods to produce iso-glucose from starch. In the USA, a large part of the present demand for sugar is met with iso-glucose made from cornstarch. This has led to a strong decrease in the importance of cane sugar (Knerr, 1991). Another approach attempts to supply widely differing quality products with a regionally well- adapted variety. For example, different oil qualities are produced from canola (rapeseed, *Brassica napus*) in order to avoid importing oils or growing other oil plants. Transgenic canola with high laurin acid oil content can be used to substitute coconut or palm oil (Sovero, 1996), and reduce the demand for these oils in the industrial countries. Buerkert *et al.* (2006) have reported genetic erosion in *T. turgidum* L. and *T. aestivum* L. (including *T. compactum* Host) in Afghanistan because of 23 years of war. They reached to this conclusion after they compared their survey studies conducted in 2002 with the survey results of Vavilov (1997) and Vavilov and Bukinich (1929). Other prominent causes of genetic erosion include the market preferences of consumers for uniform grains, vegetables or foods (Myers, 1994), pest and disease outbreaks, urbanization, population pressure, lack of recognition of current or future value of genetic resources; poor monitoring and management, and lack of sustainable breeding program.

3.3 Consequences of Genetic Erosion

Genetic uniformity leaves a species vulnerable to new environmental and biotic challenges and causes heavy damage to the society. The Irish Potato famine was a dramatic example of the dangers of genetic uniformity. The Irish population had reached about

8.5 million by 1845. Potatoes were the only significant source of food for about one third of the Irish population. Farmers came to rely almost entirely on one very fertile and productive variety known as 'Aran Banner'. Unfortunately, this particular variety was highly sensitive to the fungal disease late blight (*Phytophthora infestans*), which had spread from North America to Europe. The blight destroyed the potato crop of 1845. Consequently, the Irish Famine of 1846-50 took as many as one million lives from hunger and disease, and changed the social and cultural structure of Ireland in profound ways. The famine also caused emigration of between 1.5 and 2.0 Million Irish.

By 1970 roughly three-quarters of the corn acreage in the US was planted in "Texas T cytoplasm" corn. The Texas T cytoplasm results in individuals that are male-sterile. This makes production of hybrid corn far less labor intensive, as there is no need of detassleing. However, this maize is highly sensitive to host selective toxin (T toxin) produced by race T of *Cochliobolus heterostrophus*, the casual organism of southern corn leaf blight (Hooker *et al.*, 1970). In 1970 this blight swept through fields of "Texas T cytoplasm" corn and yield was reduced by approximately 710 billion bushels. The cost to farmers was about $1 billion (Ullstrup, 1972). Browning (1988) argued that the epidemic was "the greatest biomass loss of any biological catastrophe" and that it was "a man-made epidemic caused by excessive homogeneity of the USA's tremendous maize hectarage.' The loss of a significant fraction of the Asian rice crop to grassy stunt virus also illustrates the same point. The catastrophic outbreak of coffee rust in 1970 caused great losses in Brazil with higher coffee world market prices as a consequence. In 1916 a rust fungus destroyed about 3 million bushels of wheat in the United States, roughly one-third of the crop. Other examples include the coffee rust epidemic in Ceylon in the 1870s, the tropical maize rust epidemic in Africa in the 1950s and the blue mould epidemic on tobacco in the USA and Europe in the 1960s (Marshall, 1977).

The loss of one species is estimated at being worth $203 million (Farnsworth and Soejarto, 1985). These authors have calculated a total financial loss for the USA through the loss of plant species at $3,248 billion dollars up to the year 2000. Presently, 33,730 plant species are characterized as being extinct or strongly endangered (Lucas and Synge, 1996).

4 Genetic Resources Conservation

4.1 The Need for Conservation

Many species and varieties are becoming extinct and many others are threatened and endangered. To reverse these unabated gene erosion, conservation of genetic diversity is a fundamental concern in conservation and evolutionary biology, as genetic variation is the raw material for evolutionary change within populations (Frankel and Soulé, 1981). Conservation is the process that actively retains the diversity of the gene pool with a view to actual or potential utilization Maxted *et al.* (2002). Utilization is the human exploitation of that genetic diversity. The aim of conservation is to collect and conserve adaptive gene complexes. Collection can be seen as a subject in its own right and is not reviewed here – recently a first review appeared in this respect on one of Vavilov's gene centres (Vavilov, 1997) -– the Mediterranean (see Laghetti

and HAMMER (2004). The raw materials of plant genetic resource conservation are genes within gene pools, the total genetic diversity of the particular plant taxon being conserved (MAXTED *et al.*, 2002). The product of gene pool conservation is utilised or potentially utilisable genetic diversity.

The conservation of plant diversity is of critical importance because of the direct benefits to humanity that can arise from its exploitation in improved agricultural and horticultural crops, because of the potential for development of new medicinal and other products and because of the pivotal role played by plants in the functioning of all natural ecosystems. A great diversity of plants is indeed to keep the various natural ecosystems functioning stably. No organism exists alone but all depend on a magnitude of interactions that relate them together such as pollination and depend on a multitude of interactions that relate them together (PRANCE, 1997). No doubt that primitive and wild gene pools will continue to serve as important sources of genes for resistance to parasites or for characteristics indicated by advances in science or technology or by changing demands of the consumer. In the case of species, which are already used by human beings as crops, it is very important to have a broad genetic base, to improve existing genotypes when necessary.

4.2 A methodology for plant genetic resource conservation

Methods for germplasm conservation are determined by a number of factors. MAXTED *et al.* (1997a) proposed a model of plant genetic conservation, which summaries the entire process of plant genetic conservation from selection of a target crop gene pool through to its utilization (Figure 2). One of the first factors to be considered when conserving botanical diversity is the efficient and effective selection of the target taxa. The decision must have been taken that the target taxon is of sufficient importance to warrant active conservation and that the gene pool is not currently adequately conserved MAXTED *et al.* (1997b). A practical approach to select target species could be the use of the gene pool concept of HARLAN and DE WET (1971). This concept is based on the ease with which species hybridize with each other. The availability of information on different gene pools enables the priority setting of target species to be incorporated into the different conservation strategies. After selecting a taxa, a form of commission in a form of formal statement containing the actual conservation activities including the objectives of the conservation and justification for selection, how the material is to be utilized, where the conserved material is to be safely duplicated, etc., and perhaps indicating which conservation technique is to be employed should be formulated. A clear and concise commission statement will help to focus subsequent conservation activities MAXTED *et al.* (1997a).

In formulating strategies for the conservation of any crop, it is essential to know its areas of distribution, and identify regions where both collecting for conservation activities could usefully be initiated. This will be due to a combination of high levels of genetic diversity at the site(s), interest the user community in the specific genetic diversity found at or believed to be found at the site, lack of previous conservation activities, and imminent threat of genetic erosion (MAXTED *et al.*, 1997a). Hence, an ecogeographic survey

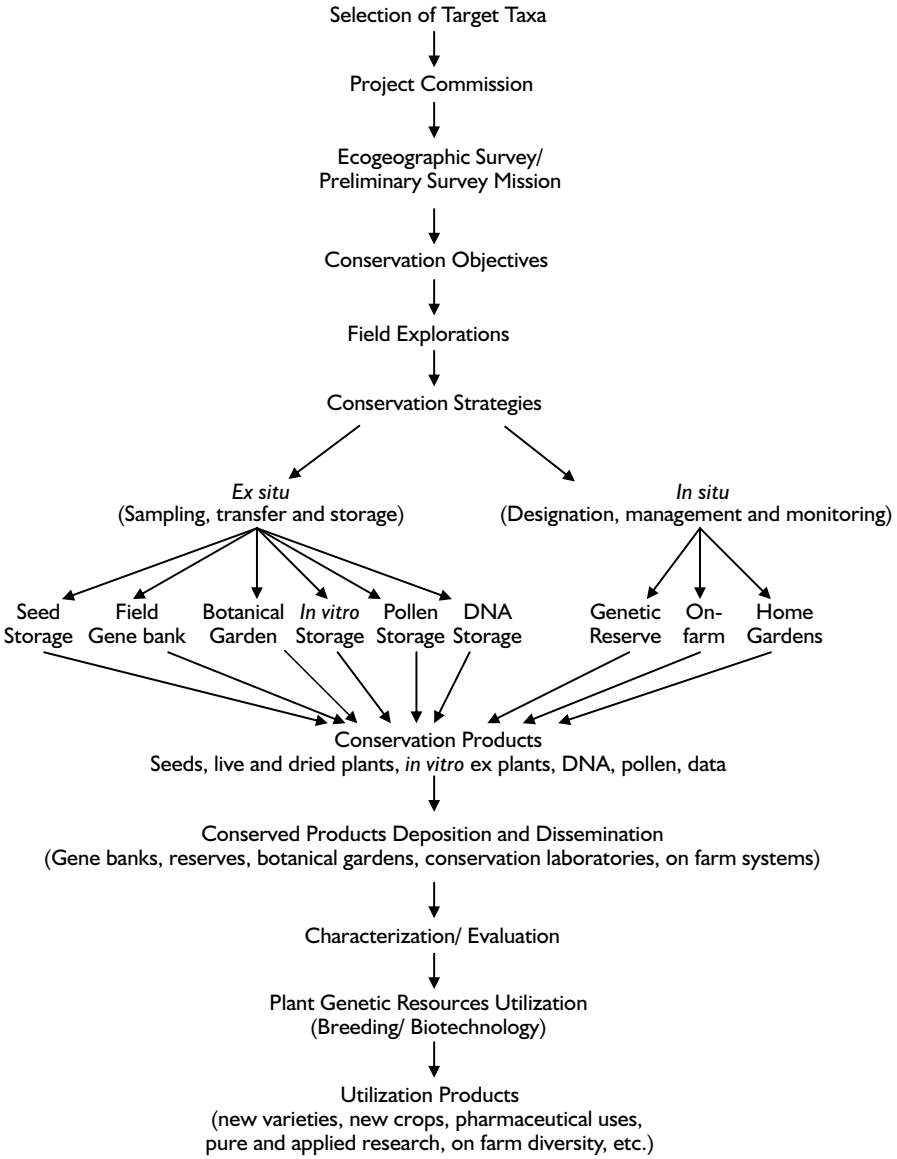
has to be undertaken to define the most appropriate conservation strategy (Maxted et al., 1995), and specific conservation objectives should be formulated, involving both *ex situ* and *in situ* components. The synthesis and analysis of ecogeographic data enable conservationist to make vital decisions concerning, for example, which taxa to be included in the target group, where to find these taxa, which combination of *ex situ* and *in situ* conservation to use, what sampling strategy to adapt, and where to store the germplasm or site the reserve (MAXTED *et al.*, 1995). Because the ecogeographic data will rarely be sufficiently comprehensive to locate actual populations precisely; the preparatory element of conservation activities should be followed by field exploration, during which the actual populations are located (MAXTED *et al.*, 1997a). There are two primary complementary conservation strategies, *ex situ* and *in situ*, each of which includes a range of different techniques that can be implemented to achieve the aim of the strategy. The products of conservation activities are primarily conserved germplasm, live and dried plants, cultures, and conservation data. The conservation products are either maintained in their original environment or deposited in a range of *ex situ* storage facilities. To ensure safety, conservation products should be duplicated more than one location.

4.2.1 *Ex situ* conservation

Ex-situ conservation is defined as the conservation of components of biological diversity outside their natural habitat. In a broad sense, *ex situ* conservation of germplasm is a practice that humans have used since the beginning of agriculture, to expand cultivation and/or to colonize new lands and to ensure the spread of agriculture around the world plants have traveled, during human migrations and along the ancient caravan routes, from continent to continent. Moving from the Old to the New World and vice versa, PGR have made many important contributions to agricultural production, diversification and eating habits around the planet (FAO, 1959). Starting from the beginning of agriculture, man has stored plants and seeds from one cycle of cultivation to the next in different ways. Storage of germplasm also took place during migration.

The great genetic diversity to be found in the traditional stocks of peasant agriculture in the centres of genetic diversity, where the wild or weedy relatives of crop species can be found, were called gene centres or centres of diversity (VAVILOV, 1926). Wild and primary gene pools constitute the genetic resources available for the adaptation of present-day cultivars, or for initiating new and potentially valuable pathways of crop evolution (FRANKEL, 1974). As agriculture progressed with the beginning of scientific plant breeding and human population increased, modern varieties were widely distributed displacing landraces from cultivation. This increased the need to formally store plants and seeds *ex situ*. Land races were then gathered together, which resulted in fairly large collections, above all in the USA and in Russia (PLUCKNETT *et al.*, 1987). In particular, the Russian scientist N. I. Vavilov amassed an unbelievable collection of diversity in a Leningrad Institute (now St. Petersburg) by systematically collecting material. With the rapid advancement of biotechnology in recent years, the ex-situ conservation of living and genetic resources has increased in importance. At present, over 6 million accessions are stored *ex situ* throughout the world (PLUCKNETT *et al.*,

Figure 2: Proposed model of plant genetic resources conservation (taken from MAXTED *et al.* (1997a)

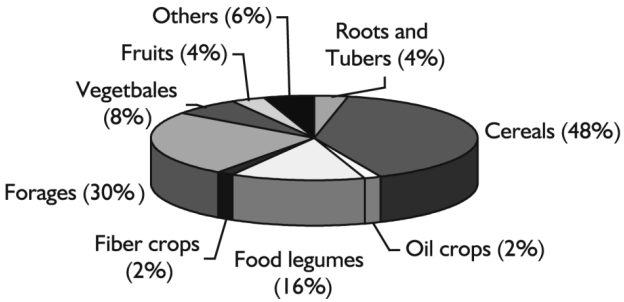


1987). Of these, some 600,000 samples are maintained within the Consultative Group on International Agricultural Research (CGIAR), the remaining 5.4 million accessions are stored in national or regional gene banks (Table 1). Figure 3 shows the representation of different crops in gene banks.

Table 1: Number of worldwide *ex situ* collections and their material (according to FAO (1996b))

Region	*Number of gene banks*	*% world*	*Number of accessions*	*% world*
Africa	124	10	353,523	6
Asia	293	22	1,533,979	28
Europe	496	38	1,934,574	35
Near East	67	5	327,963	6
North America	101	8	762,061	14
Latin America and Caribbean	227	17	642,405	12
Sum	1,308	100	5,554,505	100
CGIAR system			593,191	
Total sum			6,147,696	

Figure 3: Major crop groups in *ex situ* gene banks (Source: HAMMER (2004))



Only 30 crops make up the major part of the conserved plant material indicating that most of the remaining 7,000 species of cultivated plants and many other valuable genetic resources species have only been included on a limited scale in the gene bank collections (HAMMER, 2004)

4.2.1.1 Ex situ conservation techniques

Among the various *ex situ* conservation methods, seed storage is the most convenient for long-term conservation of plant genetic resources. Traditionally, many crops are conserved as seed in gene banks. This involves desiccation of seeds to low moisture contents and storage at low temperatures (KAMESWARA RAO, 2004). However, there is a large number of important tropical and sub-tropical tree species, which produce recalcitrant seeds that quickly lose viability and do not survive desiccation, hence conventional seed storage strategies are not possible (ROBERTS, 1973). For vegetatively propagated and recalcitrant seed species, living plants can be stored in field gene banks and/or botanical gardens. Major disadvantages of field gene banks, such as high maintenance costs, the limited amount of genetic variation that can be stored and vulnerability to natural and human disasters have led to efforts to develop *in vitro* conservation methods. *In vitro* conservation is also used by botanical gardens for the reproduction of rare species. It guarantees freedom from pest infestation and diseases. However, it is extremely labor and cost intensive and can therefore only be used for special material as a long-term storage possibility. The rapid developments in the field of biotechnology have opened up new avenues for the conservation of germplasm in the form of tissue culture, cryopreservation, pollen storage and DNA banks (CALLOW *et al.*, 1997). Cryo-conservation (storage in extreme deep freeze situations) is accomplished with liquid nitrogen at -196 °Celsius (HAMMER and HONDELMANN, 1997). It is also suitable for seeds and leads to a dramatic prolongation of germination rates. It allows for an extremely long storage of many species. For *in vitro* maintenance cultures, it is the choice of preference because somaclonal variation can be prevented. The problem with cryo-conservation is its high cost, especially for technical equipment. A constant supply of liquid nitrogen also has to be available at all times (HAMMER, 2004). The methods of *ex situ* conservation have been summarized in Table 2.

The *ex situ* conservation of large numbers of cultivated plants depends on the longevity of the seeds. Most species belong to the orthodox seed type with a logarithmical progression of shelf life as humidity and storage temperature are reduced (HAMMER and HONDELMANN, 1997). The duration of seed viability can be estimated fairly precisely by taking these aspects into account (ELLIS and ROBERTS, 1980). The life expectancy is determined through genotype. Care should be taken that viability not sink under 85% (if the original rate is set at 100%), so that gene mutations will not occur in the seed during storage.

4.2.2 *In situ* conservation

Storing genetic resources in collections as back-up seed stocks in *ex situ* collections does not substitute for the evolution of crop plants in the fields of farmers. Plant populations on farms have the capacity to support a greater number of rare alleles and different genotypes than accessions in gene banks (BROWN, 2000). As a result, *in situ* approach was proposed in the early 1970's for strictly agricultural purposes (KUCKUCK, 1974), but it has been scarcely utilized in the international crop germplasm system. *In situ* conservation is defined 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

Table 2: Methods of *ex situ* conservation for various plant genetic resources (according to FAO (1996a))

Storage technology	*Storage material*	*Function*
Low temperature (-18°C), 3-7% moisture content	Orthodox seeds	Long-term storage (basic collection), working collection
Dried seeds at cool temperatures	Orthodox seeds	Active and working collections, medium-term storage
Ultra-dried seeds at room temperature	Orthodox seeds with long-term viability	Medium to long-term storage (active and working collections)
Field gene banks	Vegetatively-reproduced species, species with recalcitrant seeds, species with long reproduction cycles and minimal seed production	Short or medium-term storage, active collections
In-vitro culture under slow-growth conditions	Vegetatively-reproduced species, some species with recalcitrant seeds	Medium-term storage, active collections
Cryo-conservation at -196°C with liquid nitrogen	Seeds, pollen, tissue or embryos that are suitable for in-vitro regeneration after freeze drying	Long-term storage

have developed their distinctive properties (UNEP, 1992). This definition encompasses two distinct concepts (and techniques), which may be distinguished as "genetic reserve conservation" and "on-farm conservation." Both involve the maintenance of genetic diversity in the locations where it is encountered (i.e., *in situ*), but the former primarily deal with wild species in natural habitats/ecosystems and the latter with domesticated species in traditional farming systems. MAXTED *et al.* (1997a) provide the following working definitions for the two activities. Genetic reserve conservation is the location, management and monitoring of genetic diversity in natural wild populations within defined areas designated for active, long-term conservation. On-farm conservation is the sustainable management of genetic diversity of locally developed crop varieties (land races), with associated wild and weedy species or forms, by farmers within traditional agricultural, horticultural or agricultural systems.

On-farm conservation is dynamic and is aimed at maintaining the evolutionary processes that continue to shape genetic diversity. It is based on the recognition that farmers have improved and grown genetic diversity and that this process still continues among many farmers in spite of socio-economic and technical changes. Farmers play a big role through their selection of plant material which influences the evolutionary process and through their decisions to continue with a certain landrace or not (BELLON, 1996). Each sea-

son the farmers keep a proportion of harvested seed for resowing in the following year. The farmer makes a conscious decision about which sample to retain for seed. For a successful implementation of on-farm conservation, a fuller understanding of both crop populations on the farming systems that produce them is needed to create active co-operation between farmers and conservationists (BRUSH, 1995). On-farm conservation assumes planned conservation in the framework of agricultural or garden production; conservation must therefore take place during agricultural production. Modern varieties, which often are more productive than the landraces, compete for this space with landraces or wild plants. Therefore, financial or other incentives have to be built into the system to safeguard future conservation. These requirements can be more easily attained in developing countries. Subsistence farming tolerates a multitude of cultivated plant species and forms in mixed culture and should be considered a living conservation reservoir (ESQUIVEL and HAMMER, 1988).

Table 3: Number of species of wild plants, plant genetic resources (PGR) and cultivated plants in the world (after HAMMER (2004))

	Higher plants	*PGR among higher plants*	*Cultivated plants among higher plants*
Number	250,000	115,000	7,000

Wild plant material is usually conserved in its natural habitat. Compared with the relatively small number of cultivated plant species, the plant genetic resources of wild plants are quite numerous (HAMMER, 2004, see Table 3). General protective measures can therefore be of great importance to a large number of plant genetic resources. Protected areas include national parks, biosphere reserves and Nature parks, which can be divided into areas of varying protection, as well as riparian or wetland areas (SCHLOSSER *et al.*, 1991). Policy strengthening, establishment of nature reserves and protected areas with rich wild populations, management promotion, environmental education and training (especially in local communities), adoption of indigenous knowledge, and local peoples' participation can strongly support *in situ* conservation of agro-biodiversity (LONG *et al.*, 2003).

4.3 Comparison of the different conservation measures

Conservation measures have often been critically evaluated. Many supporters of the *in situ* strategy consider *ex situ* methods to be at best a transitional method leading to further *in situ* maintenance (LANDE, 1988). The differing standpoints have been formulated in various international documents and treaties.

It is important that criticism of *ex situ* maintenance includes the limited possibilities of evolution available with this method. In gene banks, conservation of the material is handled in such a manner as to exclude natural evolution. This has to do with long-term seed storage on the one hand, which strongly reduces the metabolism and therefore strongly limits evolution. On the other hand, gene banks often have to grow the plant

material in areas that are far away from its place of origin, and this can easily result in changes in population composition. Additional points of criticism are that insufficient equipment and facilities are available to gene banks, that long-term storage is overrated, and that the necessity of reproduction is underrated. *Ex situ* collections are not going to be the universal means of preventing the results of gene erosion. The collections will always be limited and gene banks will only be able to include a portion of all genetic resources (HAMMER, 2004).

The advantages of *in situ* conservation are undisputed in order to maintain a large wealth of species, at the same time guaranteeing further evolutionary adaptation. The possibilities of easily gaining access to the material are positive aspects of *ex situ* maintenance. Also, a vast amount of material of the most important plant groups, mostly in the infraspecific area, can be safely conserved. Above and beyond this, systematic documentation and characterization can be carried out more easily. From the side of the user, the major criticism of *in situ* conservation lies in the difficulty of obtaining access to the material for basic and breeding research. Furthermore, in many cases, it is easier to conserve a viable population *in situ* than *ex situ*. This is certainly true of tree species. Further comparison of the advantages and disadvantages of the different conservation methods is presented in Table 4.

Table 4: Advantages and disadvantages of the different conservation methods (KJAER *et al.*, 2001).

Maintenance method	*Advantages*	*Disadvantages*
In situ	• Interactions with other species and organisms are possible • Interspecific and infraspecific variations can be combined • Can also be used for vegetatively reproducible species or those with recalcitrant seeds	• Requires large area for maintenance • Only a small number of genotypes can be managed this way. Does not protect against epidemics, diseases, etc., possible losses • Access to the material is difficult
In situ or On-farm	• Further evolution through natural evolution and choice of varieties is possible	• No conservation of the status quo, selection • Gene erosion is possible

(Table 4 continuation)

Maintenance method	*Advantages*	*Disadvantages*
Ex situ Seed banks	• Genetic status quo of the stored seeds can be maintained with appropriate reproduction strategy • Propagules ready for use (although the amount of seed typically is too limited to serve as input to commercial use) • Little space required (at least for species with small seeds) • Intra- and inter-population can be easily conserved provided species range adequately sampled • Seed can be conserved far away from the *in situ* environment if requested	• No further evolutionary development dependent on the surrounding environment • Problems with the maintenance of recalcitrant and vegetatively reproducible species • Facilities and large amount of space necessary for storage (large seeds) • The original surrounding flora is not conserved as well • Regeneration needs space and is money and labor intensive • Only a limited portion of the variability is collected and maintained • Change of population structure through reproduction of populations that are too small • 'Short term storage rather than conservation' for the majority of species
Field Gene banks	• Can conserve genetic resources in the habitats of expected use • Can develop into multiple population conservation programmes where new intrapopulation variation is developed as response to different conditions of growth or selection criteria • Can be combined with utilization • Can function as seed sources allowing rapid procurement of seed in commercial scale in early domestication	• Many areas required • Spatial isolation to conserve population identity required • Lack of pollinators may cause problems • Relatively expensive if not combined with utilization
Botanical gardens and arboreta	• Botanical gardens are often part of very stable institutions and likely to be continuously maintained by trained staff • Can be combined with demonstration and education.	• Suitable site(s) required • Difficult to collect seed due to hybridization • In general not apt for conservation of inter and intra- population variation (requires a larger number of individuals than usually planted in botanical gardens/arboreta).
Tissue culture	• Little space needed • Good for vegetative material and recalcitrant species • Aseptic conservation (minimizes disease risk) • Short time required to produce propagules for use	• High technical outlay • Expensive facilities required • Sampling problems (representative individuals and within individual) • Difficult to conserve adequate number of genotypes • Protocols are specific for species and often even for genotypes • Problems of soma-clonal variation and early maturation

(Table 4 continuation)

Maintenance method	*Advantages*	*Disadvantages*
DNA	• Little space needed • Can be used anywhere • Future method of last resort in isolated cases	• Is not a germplasm conservation method *per se*

From the viewpoint of plant genetic diversity for food and agriculture, the diversity of (1) cultivated plants, (2) wild relatives of cultivated plants, (3) introgressions between cultivated plants and their relatives, and (4) weeds should be differentiated. Although a different conservation strategy may be the most appropriate modus operandi for each of these categories and for each specific group within these categories, the integrated use of the different methods for conservation is necessary depending on the categories of diversity and diversity groups. It is also necessary to consider different economics of the countries. The argumentation scheme, which was based on is based on the methods of conservation (*ex situ*, *on-farm*, *in situ*) as well as on the type of diversity, species diversity and diversity of the ecosystem, has been proposed by HAMMER *et al.* (2003) (Table 5).

Table 5: Conservation methods for different categories of diversity rated by their importance for specific groups of diversity (HAMMER *et al.*, 2003).

Category of diversity	*Method of conservation*			
	ex situ (genebanks)	*on-farm (agro-ecosystems)*		*in situ (other ecosystems)*
		Developing countries	*Developed countries*	
Infraspecific diversity	C**	C***	C**	C°
	R*	R***	R*	R***
	W**	W***	W*	W*
Diversity of species	C*	C***	C**	C°
	R*	R***	R*	R**
	W**	W***	W**	W*
Diversity of ecosystems	C°	C***	C*	C°
	R°	R***	R**	R**
	W°	W***	W***	W*

The number of stars indicates the relative importance of the methods for the various diversity groups. C= Crop species, R = Wild Relatives of Crop Species, W = Weeds
° = no importance, * = low importance, ** = important, *** = very important

4.4 The economics of plant genetic resources conservation

It looks much different when we talk about costs. The high costs for *ex situ* maintenance are visible, and it is possible to obtain an overall picture from the concrete figures for material and equipment listed in the global report. According to PLÄN *et al.* (1994), the conservation of one seed sample costs approximately 0.50 German marks a year (calculated according to SMITH (1984) and PAREZ (1984). The entire volume of finances for the gene bank Gatersleben in the year 1992 (payroll, investment costs, overhead) came to 4,790,800 German marks (THOROE and HENRICHSMEYER, 1994). Taking 100,000 samples into account, the costs for the maintenance of one sample comes to approximately 50 German marks. Included in this estimation are not only the costs for the maintenance of the material, but also research, without which the collection cannot be vitally maintained over a longer period. The case studies made to estimate annual cost of maintaining different crops is given in Table 6.

The economics of plant genetic resources, with relation to gene banks, is going to establish itself as new research area (VIRCHOW, 1999). The basis for these considerations is usually the search for larger budget-cutting possibilities. But since gene banks have often already been degraded to the role of harvest silos, such examples are highly unsuited for a general estimate of costs. The economic conclusions reached by such studies could further burden the already unstable situation of global *ex situ* conservation.

Table 6: Annual costs of maintaining cassava, wheat and maize germplasm in field gene bank, *in vitro* and seed conservation.

Conservation Technique	*Crop*	*CG Centre*	*Total cost/accession (US $)*
Field	Cassava	CIAT	17.09
In vitro	Cassava	CIAT	26.22
Seed	Wheat	CIMMYT	0.05
Seed	Maize	CIMMYT	0.33

Source: EPPERSON *et al.* (1997).

5 The Impact of Genetically Engineered Plants on Genetic Diversity

Genetic engineering has potential to solve problems that have proved intractable using conventional breeding approaches, such as developing crop varieties with in-built resistance to key pests and diseases and tolerance to stresses such as drought. Genetically modified (GM), or transgenic, organisms are created through genetic engineering techniques that allow genetic material to be moved between similar or vastly different organisms with the aim of changing their characteristics for a purpose. In developing an engineered plants, genes are introduced into a genome using *Agrobacterium tumefaciens*, a pathogenic bacterium that naturally transfers DNA to plants during the disease process (GELVIN, 2000) and using biolistics or a variant, particle discharge (BIRCH, 1997). Another method associated with transgenic technology is the process of nuclear

transfer, which is a cloning technique. Since the birth of the first successful transgenic plant in the beginning of 1980's, tremendous accomplishments associated with transgenic biotechnology have been achieved and rapid application of the biotechnology in agriculture has substantially benefited crop genetic improvements. As a consequence, a great number of genetically modified crops (GMC) have been released and commercialised (e.g. HUANG *et al.* (2002). New cultivars of maize (*Zea mays* L.), soybean (*Glycine max*), cotton (*Gossypium* spp.), papaya (*Carica papaya*), tomatoes (*Lycopersicon esculentum*), canola (*Brassica napus*), and others have been developed that carry additional genes conditioning traits as herbicide tolerance, insect resistance, or virus tolerance. From 1996 to 2004, the global area of biotech crops increased more than 47 fold, from 1.7 million hectares in 1996 to 81.0 million hectares in 2004, with an increasing proportion grown by developing countries. More than one-third (34%) of the global biotech crop area of 81 million hectares in 2004, equivalent to 27.6 million hectares, was grown in developing countries where growth continued to be strong. The estimated global area of approved biotech crops for 2004 was 81.0 million hectares up from 67.7 million hectares in 2003. Approximately 8.25 million farmers in 17 countries grew biotech crops in 2004 (JAMES, 2005).

Domesticated plants and their wild relatives usually belong to the same biological species and they often hybridize and give rise to viable and fertile progenies (HARLAN and DE WET, 1971; ELLSTRAND *et al.*, 1999), if wild and domesticated forms are sexually compatible, grow within pollinator flight distance (in the case of insect-pollinated species), and their flowering times overlap at least partially. Such hybridization may lead to gene flow: 'the incorporation of genes into the gene pool of one population from one or more populations' (FUTUYMA, 1998). Many cultivated plants hybridize spontaneously with wild or weedy relatives (SMALL, 1984; ELLSTRAND *et al.*, 1999). For example, cultivated rice and its wild relatives *O. rufipogon* have sympatric distribution and overlapping flowering times, which meets the spatial and temporal conditions for transgene escape from cultivated rice to its wild relatives (LU *et al.*, 2003). Though cultivated sorghum is largely self-pollinated with outcrossing rate of 2-30% (SCHMIDT and BOTHMA, 2006), analyses of progeny segregation, allozymes, and RFLPs reveal crop-specific alleles in wild S. bicolor when it co-occurs with the crop in Africa, suggesting that intraspecific hybridization and introgression are common (ALDRICH and DOEBLEY, 1992). Studies carried out to measure spontaneous hybridisation between wild radish (*Raphanus sativus*) and cultivated one (KLINGER *et al.*, 1991) and with *Sorghum bicolor* and *Sorghum halepense* (a widespread weed) (ARRIOLA and ELLSTRAND, 1996) demonstrated that spontaneous hybridisation does take place (for a recent review see ELLSTRAND (2003). With transgenic plants, the problem of gene flow, which may ultimately cause possible ecological risks, has acquired special significance.

5.1 Consequences of gene flow

Transgenes coding for novel traits such as resistance to biotic and abiotic stresses could have the potential to enhance ecological fitness of wild and weedy genotypes (ELLSTRAND and ELAM, 1993; SNOW, 2003) and thus cause ecological problems. For

example, wild sunflower populations host many of the same herbivores and diseases as cultivated ones (Seiler, 1992) and crop genes can easily backcross into wild sunflower populations (Whitton *et al.*, 1997). Snow (2003) studied a crop-developed *Bacillus thuringiensis* (Bt) transgene, *cry1Ac*, in backcrossed wild sunflower populations and found that a transgene derived from a crop has the potential to increase the fitness of wild plants, and an increase in frequency in wild populations. The spread of transgenic herbicide resistance is likely to pose challenges for controlling weeds and unwanted "volunteer" crop plants (Snow *et al.*, 1999). Other possible risks are that transgenic phenotypes with altered fitness could change in abundance in the ecosystem, with unwanted effects on other species and on ecosystem integrity, or that the ecosystems are affected indirectly by the transgenic plants (Jorgensen *et al.*, 1999).

Both genetic and geographic barriers to gene flow from crop to wild sunflower are minimal (Snow *et al.*, 2002). Hence, simple co-existence of GE and non-GE crops might be impossible. For instance, there are now scientific evidences for cultivated rice outcrossing to non-GE rice (Lu *et al.*, 2003; Gealy *et al.*, 2003; Chen *et al.*, 2004). Cultivation of GE rice will therefore cause weedy strains of *O. sativa* such as "red rice" and the wild relative, *O. rufipogon* to become contaminated with the GE transgenes (the GE DNA insert). There are concerns that if red rice becomes tolerant to the herbicide used in conjunction with a GE herbicide-tolerant rice, it will become more difficult to control (Gealy *et al.*, 2003; Chen *et al.*, 2004). The GE contaminated populations of wild and weedy species of rice are likely to be persistent, becoming reservoirs of GE transgenes for further contamination. A loss of genetic diversity of domesticated and wild relatives is cited among the potential drawbacks of the introduction of transgenic crops (Berthaud and Gepts, 2004). When alien transgenes escape to and express normally in wild relatives and weedy species, the transgenes will persist and disseminate within the wild or weedy populations. This will lead to contamination of the original populations of the wild relatives, and even to the extinction of endangered populations of the wild relatives in local ecosystems (Ellstrand and Elam, 1993). Examples of genetic assimilation or extinction by displacement of native allelic diversity are provided in date palm (*Phoenix dactylifera*), olive (*Olea europaea*), and coconut (*Cocos nucifera*) (Bronzini *et al.*, 2002). Furthermore, natural hybridisation with cultivated rice has been implicated in the near extinction of the endemic Taiwanese taxon, *O. rufipogon* ssp. *formosana* (Small, 1984). Small-scale farmers generally maintain a range of genetic diversity on their farm to meet their various needs and to be self-reliant. Genetic assimilation and displacement of genetic diversity may constrain the livelihood of subsistence farmers. The presence of transgenic volunteers in crop fields would contaminate harvest with transgenic seeds and prevent the farmer from obtaining a 'non-genetically modified crop' label for this product. Transgene contamination has been found in local traditional varieties of maize in Mexico (Quist and Chapela, 2001). The escape and persistence of transgenes in environment will also make effective *in situ* conservation of wild genetic resources more difficult. Genetically modified crops cause much trouble particularly in centres of diversity where crops are grown along with their wild relatives.

Most alien genes carried by genetically modified agricultural products are not from crops, instead, they are from other organisms or microorganisms, even from an artificially synthesized origin. These genes may completely alter the natural habit of crop species and significantly change wild relatives of the crop species when transgene escape happens. As a consequence, the environmental safety, particularly the agricultural ecosystems might be under their negative influence (Ellstrand *et al.*, 1999). The insertion of transgenic DNA may bring about small-scale rearrangements of the transgene and native DNA sequences at the insertion site (Windels *et al.*, 2001). The interactions of transgene with other genes in the genome ("background effect") may affect the overall level of expression of the trait. Through recombination, genes belonging to a specific variety can migrate into new genetic backgrounds where new linkages and gene interactions may modify the expression of transgenes in an unpredictable fashion (Berthaud and Gepts, 2004). When more than one sequence is introduced or if a transgene is similar to a native sequence in the genome, then gene silencing can take place (Comai, 2000).

Some crop plants have been genetically engineered to produce pharmaceuticals and industrial chemicals (GE "pharm" crops). These pharm crops are not intended to be eaten by humans and animals, but to be used by drug companies or in industrial processes. The compounds produced by these plants are often biologically active chemicals and all are potentially toxic to animals and humans.

Apart from the specific problems with GMOs, there is a more general effect. The use of GMOs speeds up the breeding process and consequently leads to high in the agricultural production, which results in a high genetic erosion. But this is the consequence of all modern technology.

6 Conclusions and Recommendations

Thousands of genetically distinct varieties of our major food crops owe their existence to years of evolution and to careful selection and improvement by our farmer ancestors. Nevertheless, processes that once took hundreds or thousands of years to develop could then be carried out within decades or even years under human influence (Hammer, 2004). There has been a significant loss of genetic diversity during the last l00 years and the process of gene-erosion continues (Hammer *et al.*, 2003). With genetic erosion it is not only genetic resources that are under threat of disappearance but also the indigenous knowledge of selecting, utilizing, and conserving these materials that has been accumulated for thousands of years. Erosion of crop genetic resources could pose a severe threat to the world's food security in the long term since loss of genetic variation may decrease the potential for a species to persist in the face of abiotic and biotic environmental change as well alter the ability of a population to cope with short-term challenges such as pathogens and herbivores. It is also threatening the genetic base of many important crops in which future breeding is based. As a result, the loss of biodiversity belongs to one of the central problems of mankind, next to other important matters such as climate change and securing an adequate supply of drinking water. Paradoxically, in many parts of the world, although it is generally accepted that

significant amount of genetic erosion has occurred and is still occurring, there is little data on its amount and extent. Without remedial action, genetic erosion will inevitably increase, and the costs of replacement of diversity needed in the future by the community will be much greater (HAMMER, 2004). Future progress in the improvement of crops largely depends on immediate conservation of genetic resources for their effective and sustainable utilization. It is widely agreed that the primary solution to the genetic impoverishment of crop germplasm is genetic conservation and utilization in breeding of the vast genetic variation found in populations of the wild progenitors and landraces of cultivated plants (FRANKEL and BENNETT, 1970; TANKSLEY and MCCOUCH, 1997). The discovery, collection, and conservation of potentially valuable but endangered plant genetic resources is a primary obligation of all countries and institutions adhering to the FAO international undertaking on plant genetic resources (HAMMER *et al.*, 2003). A better characterization and understanding of genetic diversity and its distribution is essential to efficiently exploit the available resources in more valuable ways. It is crucial to efficiently design collecting trips and conservation projects (TEKLU and HAMMER, 2006). The value of diversity is in its use (GAO, 2003).

Since the beginning of the 70s of the last century an effective program has been developed for collecting, conserving and using of plant genetic resources, which was called "plant genetic resources movement" (PISTORIUS, 1997). But step by step parts of this program have become outdated. Recently, a paradigm shift in the discipline of plant genetic resources has been observed (HAMMER, 2003) which includes 1) the maintenance of material (*in situ* instead of *ex situ*), 2) the enforced inclusion of neglected and underutilized cultivated plants, 3) the methods of quantifying genetic diversity between different cultivated plant taxa, 4) the methods of analyzing genetic diversity among the different cultivated plant taxa, 5) their evaluation and 6) their reproduction.

The best method of conservation is the use of complementary approach of the different *ex situ* and *in situ* conservation techniques. Since part of the worldwide *ex situ* collections is endangered, priority should be placed on securing and providing financial support for existing collections. The regular regeneration of material is essential and must be made possible (HAMMER, 2004). The expansion of strong national programs should be supported as an important basis for a functional global plan. Support for networks of cooperation in the area of plant genetic resources must also be improved. It is always necessary to conserve a large number of collections of a particular taxon. The classic example of this was the screening for resistance against the grassy stunt virus of rice at the International Rice Research Institute in the Philippines. Under the 6723 accessions of cultivated rice and several wild species of *Oryza* screened for resistance, only one accession of wild species *O. nivara* was found to be resistant and used to introduce resistance to grassy stunt virus into cultivated rice (LING *et al.*, 1970). The principle of prevention (Noah's Ark principle) tells us to maintain as much material as possible. There is presently no scientific method, except the identification of duplicates, which can give us a secure assessment as to which parts of the collections are expendable. Apart from conservation, creation of sustainable agricultural systems that actively use as much biodiversity as possible must remain the major goal. Only in use can diversity

be appreciated enough to be saved, only in use it can continue to evolve, and thus retain its value (PARTAP, 1996). Hence, there should be an ultimate linkage between conservation and utilization.

Biotechnology offers us a new arsenal of methods for the study of genetic resources, but also for certain conservation techniques. Gene technology increases the possible use of distantly related trait carriers as donors for the desired characteristics. Most of the crop cultivars that are developed are compatible *inter se* but they are also compatible with related wild or weedy relatives, suggesting that gene flow has always taken place. The possibility of transgene flow from engineered crops to other varieties, to their wild relatives or to associated weeds is one of the major concerns in relation to the ecological risks of the commercial release of transgenic plants (MESSEGUER, 2003). It is currently impossible to prevent gene flow between sexually compatible species in the same area. Pollen and seeds disperse too easily and too far to make containment practical (SNOW, 2002). It is therefore necessary to understand genetic relationships and actual gene flow frequencies between the transegenic crop and wild/weedy relatives or landraces, to know geographic distribution patterns and flowering habits of cultivated and wild crop species, and to understand other factors influencing the gene flow. This will facilitate the effective prediction of transgene escape and its potential ecological risks, and the development of strategies to minimize the escape of alien transgenes. Empirical research is also needed to evaluate the persistence of transgenes in the recipient populations and its effect on fitness should be measured to fully assess the impact of gene flow of transgenes.

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