Strategies for conservation of genetic resources in relation with their utilization

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Abstract:

The general interest for genetic resources is based on the opportunities offered by their utilization. Genetic resources are a necessary starting point for plant improvement. Up to now, reflection on the management of genetic resources considered a three-step linear model :

conservation → evaluation → utilization

In this system, it is expected that genetic resources are kept in a gene bank, in the best possible way, i.e. using methods maintaining over time the initial genetic state of the accessions and conferring them the longest life possible. In this system, important characteristics of conservation of genetic resources are stability and availability. We present a choice of methods of conservation directed to the maintenance of genetic stability of accessions. We present also methods of conservation in relation with the availability of the genetic material for the various utilizations by the plant breeder.

Complementary methods are now proposed for the conservation of genetic resources. These methods correspond to a modified version of the linear model, with interactions between conservation, evaluation and utilization. These new methods place less emphasis on the conservation of genetically well defined accessions but promote genetic mechanisms allowing evolution of these accessions. This evolutionary conservation is obtained by management of experimental populations, or by " on-farm " management of landraces, relying on knowledge and activity of farmers, and on local breeding. For the wild relatives of crops, an *in situ* management is to be considered. Using some examples, we discuss the constraints of these new modes of genetic resources conservation and utilization.

Introduction

Three steps in the reflection on and interest in the genetic resources have been noted (Frankel and Brown, 1984). Pionner works, as those of Vavilov (Crow, 1993), H.V. Harlan (Harlan, 1957).. have led to the development of the first phase, i.e; interest of the utilization of biodiversity for plant breeding. Starting in the seventies (Frankel and Bennett, 1970) and emphasizing on the concept of genetic erosion (IPGRI; 1993), the reflection has then been orientated towards the limited availability of genetic resources. Surveys were carried out, and collections established. Investigation was dedicated to surveying, sampling and conservation methods for this genetic material. A strong impulse to this research certainly came from the discovery of the susceptibility to *Helminthosporium maydis* of the hybrid maize with cytoplasmic male sterility from " Texas " cytoplasm and the epidemic that destroyed part of the maize crop in the USA in 1970. This mobilization led to the collection of many samples for most of the cultivated plants and the establishment of gene banks in many countries and in the centers of the CGIAR system (consultative group for international agricultural research) (IPGRI, 1993). The current questions addressed by investigation on genetic resources are now related to management of genetic resources. It is necessary to conserve successfully the collected material as long as possible, to regenerate this material when needed and to establish a more systematic utilization of the genetic resources by the breeders. Genetic resources are still by large an untapped

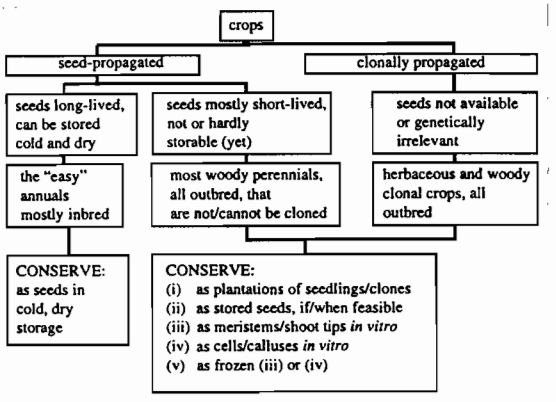

Figure 1 : Modes of conservation of plants according to their biological characteristics (Adapted from Simmonds, 1980)

reservoir of diversity.

A typical presentation of activities in genetic resources (IPGRI, 1993) is based on their two main components: conservation and utilization, with the conservation function played by *ex situ* gene banks, very often seed banks, managed by curators, and utilization under the responsability of breeders (public institutions, seed companies, national agricultural research systems). As a complement, it has been proposed for a long time that conservation be also organized *in situ*, in the eco- and agrosystems where the current diversity has been maintained. This proposal was often linked to a freezing of the system (Benz, 1988; Iltis, 1974). For a few years now, this position has evolved (Brush, 1995). The concept of variety for example takes now into account that a variety is not only a set of genes resulting from the impact of natural selective forces or from the breeder, but is also the result of a volontary intelectual construction (Bellon et al, 1996). The farmer becomes one of the actors of the creation and maintenance of the present diversity. Beyond the cultural aspect, it is the concept of conservation of genetic resources that has to be modified to integrate also the possibility of maintenance of the processes that lead to the creation and maintenance of varieties as well as the maintenance of genes of these varieties (Worede and Mekbib, 1993).

The classic model proposes a linear system that goes from conservation to utilization, but other options already exist and could be implemented that would link conservation and utilization. In our presentation, we will describe various combinations of conservation-utilization, try to show advantages, disadvantages, implications and complexity of these solutions. We have tried to group the options according to three models that we call: linear model, triangle model, and the circular model. They will be described in the next paragraphs.

Linear model

This linear model can be seen as classic, as it is the most widely used, especially in all the international centers of the Consultative Group on International Agricultural Research (CGIAR). It is conceived on a linear mode, i.e. from the conservation with the hierarchy of base collections, active collections and working collections to evaluation and utilization. This model relies on an *ex situ* conservation and puts emphasis on two points :

1) stability of the conserved material: the techniques used are aimed at reducing to a minimum the genetic variation, either at the level of genome of individual accessions, or at the level of the populations conserved. As shown in Fig. 1, different strategies of conservation will be followed according to the biological characteristics of the plants (Simmonds, 1980). Most useful will be the distinction between orthodox and recalcitrant seeds (Roberts, 1973).

2) Availability of the material. The genetic resources are to be easily available to the breeders, who will be in charge of their utilization. The modalities of utilization are evolving as breeders have more and more access to new tools from molecular biology and the level of utilization become closer to the gene than the genotype.

The *ex situ* collections have now reached impressing sizes, (table 1, IPGRI, 1993). A reflection started more than 10 years ago (Frankel and Brown, 1984) to work with

Table 1 : Accessions of plant genetic resources conserved in the CGIAR system (IPGRI, 93)

Centre	Crop	Number of accessions	Centre	Crop	Number of accessions
CIAT	*Phaseolus vulgaris*	23 711	ICRISAT	sorghum	32 890
	Phaseolus lunatus	1 836		pearl millet	21 919
	other *Phaseolus* species	1 305		chickpea	16 443
	cassava	5 035		pigeonpea	11 910
	cassava (*in vitro*)	4 788		groundnut	12 841
	wild cassava			finger millet	3 220
	forages-grasses	2 092		foxtail millet	1 452
	forages-legumes	17 927		proso millet	831
				little millet	423
CIMMYT	breadwheat	52 839		sawa millet	424
	durum wheat	13 448		barnyard millet	188
	barley	7 991		kodo millet	544
	Triticale13268				
	rye	194	IITA	cowpea	15 185
	primitive wheat	4 523		wild *Vigna* (cowpea)	1 620
	wild wheats	2 984		yam	2 250
	teosinte	80		soyabean	1 500
	maize	10 893		bambara groundnut	2 000
	Tripsacum	80		cassava	1 704
				wild cassava	52
CIP	potato	3 955		*Musa*	440
	sweet potato	4 895		maize	1 343
	other Andean root & tuber	468		misc. food legumes	329
	wild potato species	1 500		multipurpose trees	20
	wild sweet potato species	768		sweet potato *(in vitro)*	1 000
				taro *(in vitro)*	60
ICARDIA	barley	20 379		rice	12 355
	breadwheat	6 806			
	durumwheat	17 496	ILCA	forages-legumes	6 759
	Aegilops	1 897		forages-grasses	1 775
	wildwheats	1 329		forages-browse species	1 466
	chickpea	8 256			
	wild chickpea	254	INIBAP	banana and plantain	563
	lentil	7 126			
	wild lentils	331	IRRI	*Oryza sativa* (Asian rice)	78 381
	faba bean	4 124		*Oryza glaberrima* (African)	2 398
	forages-legumes	19 771		wild rice species	1 887
			WARDA	rice	6 076

collections of reduced size, the core collections (C.C.). These collections maintain a level of diversity representative of the reference collection but with a limited number of accessions and then become more manageable.

Enforcing stability

The selection of techniques used for the conservation relies only on biological traits of the plants. As shown in table 1, this will depend on availability of seed for the plants to be conserved, and behavior of seeds in relation with temperature and humidity factors. According to Roberts, (1973) seeds are orthodox when their preservation is favored by a lowering of temperature and humidity, and recalcitrant when they react differently. For orthodox seeds, preservation is carried out in cold rooms. Stability of conservation will depend on the species. Using positive temperature in the cold rooms will allow a conservation for no more than 50 years for most of the species, and in many cases, regeneration of seeds will be needed after some 20 years in conservation. The LAMP

(Latin America Maize Project, Salhuana, 1989) was started in 1983-1985 with the first objective of regenerating seeds for the many samples that had been collected in the fifties in this region and were in need of regeneration. About 15000 accessions were involved in the process.

Using negative temperature, the viability of seeds can be extended. At the present time, experience is still limited with this technique, and it is difficult to have more than estimate of the duration of viability of seeds. An experiment with onions (Stanwood and Sowa, 1995) showed no decline in germination for seeds stored at -18°C and -196°C for a ten year period but a loss for seeds stored at +5°C. However this method of storage requires specific facilities that can be difficult to maintain in some countries.

These methods are used in the international centers, with negative temperature for the base collections and positive for the active collections. At ORSTOM, we keep at 4°C 3500 accessions of sorghum and 3500 of pearl millet. These accessions are landraces collected in West Africa from 1975 to 1980 (Clement, 1985). Now their germination rate ranges in average between 85 and 90% (Dussert, pers.com.)

For plants with recalcitrant seeds or usually clonally propagated as potato, yam, cassava, banana, other methods are used : field gene bank, *in vitro* culture, cryopreservation.

Field gene banks can have a high maintenance cost but are very effective for perennial plants with long generation cycle. An example is given by the field gene banks of coffee (Anthony, 1992). For perennial crops with a shorter life as yams and cassava, there are more problems as much more labor is requested for the repeated plantings.

Possibilities and technical constraints of *in vitro* culture have been reviewed by Engelmann (1991, 1992). The technique is available for many species. However, as the specific purpose of a genetic resources conservation is to preserve many different genotypes, we are faced with the problem of genotypes reacting differently to standard protocols of *in vitro* culture. Collections maintained *in vitro* are usually of small size. For example, a pilot conservation of cassava has been established for 100 genotypes (IPGRI/CIAT, 1994). At ORSTOM a 200 genotype collection of yam is maintained in these conditions, with a transplanting every 6 months and 12 plantlets (replications) per genotype (Malaurie et al, 1993)

Cryopreservation is a method of conservation that is receiving much attention. Various plant material, mainly *from in vitro* culture, as apices or embryos have been used for cryopreservation Fine tuning of the technique is still required to be effective for most of the genotypes of many species. Engelmann et al (1995) show a list of 38 species for which successful techniques are available to preserve somatic or zygotic embryos. This technique has become routine for 80 genotypes of oil palm trees preserved as somatic embryos. Stem apices are used to preserve yam genotypes (Malaurie and Trouslot, pers. com.). Cryopreservation will become even more useful when it is possible to deep-freeze seeds, the technique becoming far more simple in this case. Success has already be reached with onion for example, and coffee trees (Normah and Vengadasalam, 1992, Dussert, pers. com.). As the coffee trees have seeds considered as recalcitrant or intermediate (Ellis et al, 1990), cryopreservation could be another route to maintain recalcitrant seed material.

These preservation methods, in vitro and cryopreservation are confronted with the general problem of plant tissue culture which is the stability of genome of plant going through this process. Studies directed to this problem are in progress, for example in rice (Xie et al, 1995) and tomato (Bogani et al, 1995). Keeping in mind this problem, the cryopreservation methods offer the most efficient long term conservation. However, to take advantage of this possibility, one has to invest in establishing the protocols most appropriate for the considered species, and to set up the Laboratory facility. At the present time, these requirements restrict the utilization of cryopreservation to small sized collections.

Enforcing availability

In our linear model, the role of conservation is to distribute to the users, i.e. breeders, a genetic material quite similar to the material that was placed in storage, in order for them to have access to a material identical to the material used in the evaluation process. Utilization of the material is based on extraction of the interesting accessions from the gene bank and inclusion in a process of plant breeding. The simplest and most linear relation between conservation and utilization is represented by the case of *Panicum maximum* (Jank et al, 1989). From surveys in East Africa, a field gene bank (base collection) had been established in Ivory Coast. A working collection was extracted from this base collection and set up in Brazil. After evaluation in this country, genotypes have been selected and distributed. As apomixis is the reproductive mode of this species, no recombination has been involved in the whole process of conservation-utilization. The genotypes distributed as varieties are genetically identical to those collected in the wild and preserved in the original collection. In this case, it is quite important to ensure stability in the process of conservation, as availability of useful genotypes will directly depend on it.

Usually, genetic resources are used for transfer of genes to varieties already improved for many traits. It is the most often cited use for genetic resources. For example, in the case of tomato, *Lycopersicon esculentum*, many wild species have been used to introduce resistance traits and traits of technological interest into the cultivated form (Rick, 1987).

When we consider genetic resources as a source of genes more than genotypes, we can think of other ways of conservation that would offer a more easily available genetic material for this purpose.
It would be beneficial to conserve pollen, especially for those species that have a long juvenile period. This would also help in getting through quarantine regulations. This method has already be proposed (Charrier et al, 1984) but is still limited in its applications. Examples of short and medium term conservation have been published, based on a conservation at -18°C. Oil palm tree pollen has been preserved for 6 to 12 months (Benard and Noiret, 1970). Van der Vossen and Walyaro (1981) have kept coffee pollen viable for 2 to 6 years. Using cryopreservation techniques would help in extending the storage life of this material., Barnabas and Rajki (1981) were successful in conserving maize pollen at -196°C. At CIMMYT (Inagaki and Mujeeb-Kazi, 1994) results were obtained by freezing the pollen grains at -196°C and storing them at -80°C. We were able to preserve *Tripsacum* pollen by freezing and storing it at -80°C after a slight dehydration. For all these plants, the dehydration step is crucial. On the disadvantage side, the pollen conservation could be difficult because some species produced a very limited amount of pollen, or it is very difficult to collect. It is also very difficult to go through the dehydration process as the range of optimum humidity for pollen conservation is quite narrow. An adavantage is that pollen can be easily transported, and when used, one produces directly a F1 generation, shortening the process of gene transfer by recombination.

The new techniques of molecular biology and genetics now offer new opportunities of gene isolation and gene transfer through genetic engineering. Resistance genes have been isolated from plants and transferred into other plants. A recent example is the gene *Xa21* conferring resistance to *Xanthomonas oryzae* (Song et al, 1995). These techniques extend the availability of the genetic material as it is expected that genes from one given species will be useful when transferred into another species. These techniques make use of YAC and BAC (yeast and bacterial artificial chromosomes). Genome of plants can be available as DNA fragments (Woo et al, 1994, Wing, 1995). Conservation of these artificial chromosomes can be a genetic resource activity, offering an easy access to many interesting genes. This method is limited in the strict sense of conservation of diversity. The number of alleles per locus maintained through this method will be very small. However, it could be a very important step to a wider use of natural genes, especially as new technologies offer possibility to identify other

versions of known genes, using a combined transposon and PCR technique (McElver et al, 1995) for example.

Another entry into the collections: the core collections

We already mentioned the problem of continuously expanding number of accessions in the gene banks, making more difficult the conservation, management and utilization of the genetic resources. A solution to this problem has been proposed by Frankel and Brown (1984) and Brown (1989) : the core collections (C.C.). The principle is quite simple: a sampling is made in the base collection, conserving most of the diversity. The result is a smaller collections representative of the existing diversity, that is easier to manage, evaluate and utilize. Various methods have been proposed for this sampling. They rely on the available information, either on morphological and adaptive traits or on neutral traits as molecular markers. Efficiency of these methods have been evaluated. A review on the C.C. has been presented recently by Hamon et al (1995). Designing a core collection will make the best use of the diversity studies that are now available for most of the crops preserved in gene banks.
This C.C. approach is tested and applied in different ways in many collections. In some cases, it is an entry to the main collection, based on selected criteria, some of them being user-defined. It is the case in CIMMYT for the maize collection for example (Taba, 1995). For others, it is a fixed entry to the main base collection, the sampling being done only once, an example is the US perennial *Medicago* collection (Basigalup et al, 1995). In some other cases, after completion of sampling, only the core collection will be maintained. To our knowledge, there is no published report of examples of such a possibility.

Core collections offer an opportunity to work with a number of accessions quite manageable. Breeders will then be more willing to evaluate these collections for specific traits. As information builds up, utilization will become more active. C.C. are an excellent tool for improving the utilization of genetic resources collections and do not modify the approach of

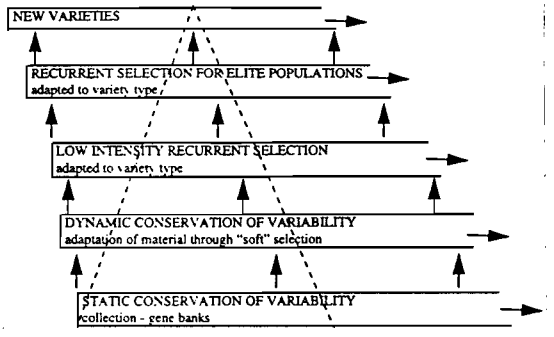

Figure 2 : Strategy proposed to move from static conservation of genetic resources to creation of new varieties. Dash line triangle indicates the restriction of variability as the genetic material is moved towards new varieties (After Gallais, 1989)

conservation-utilization we called the linear model, but C.C. are a more efficient way to extract interesting genes from the main collections to be used for transfer to varieties under improvement.

To summarize, the linear model is a mode of conservation-utilization adapted to the large collections and for an utilization that could be named gene by gene. It is well adapted to a biotechnological utilization of genetic resources, the techniques of genetic engineering permitting a gene by gene approach. The linear model has proved to be effective for the improvement of many crops, for example, wheat, tomato (Rick, 1987), and many more. Thanks to the establishment of core collections, the exploration of the main collections become easier and lead to interesting results, especially when looking for disease resistance genes. An example is offered by the *Pisum* core collection in the UK, that was used to identify new resistance genes (Matthew and Ambrose, 1994)

The triangle model

After presenting the linear model, one characteristic of which is a gene by gene use, we will present the model we called triangle. It is based on a reflection to utilize the wide range of diversity available in gene banks for

building new varieties minimizing the loss of diversity at each step of the process of improvement. We called the model triangle to remind the progressive restriction of diversity. The gene banks are not used for extracting only genes but many favorable genetic combinations.

The model, as proposed by Gallais (1989), Fig. 2, is a multi-step procedure. Each step corresponds to a phase of selection and restriction of variability. We will present two examples on maize, that are very different but we consider as representative of this model, the Latin American Maize Project (LAMP) and the INRA/PROMAIS project.

The LAM project (Salhuana, 1989, Sevilla et al, 1994) has been established at a large scale, covering all of Latin America. It was based on the need to regenerate the accessions maintained in many collections in Latin America, proceeding from surveys made mainly from 1950 to 1960 and on the idea that this process could advantageously be used to extract more interesting varieties through several steps of selection and reach the production of elite varieties.

The project started with the increase of 15000 accessions. The accessions were grouped by regions, 32, and 5 homologous areas, from lowland to high land and temperate). In the first phase of increase, and selection on *per se* value, 20% of the accessions have been kept. In the second phase, only 5% of the increased accessions has been selected. This represents a selection of roughly 1% and actually 270 elite varieties have been selected. The selection process was based on a series of 16 descriptors, involving most of the agronomic traits, including yield. The third step in the selection process was based on the combining abilities of the elite lines. In this project, the genetic gain has been obtained through several steps of selection, restricting the diversity existing in the base collection, and keeping the most promising accessions. The genetic constitution of the material actually used for the creation of new varieties is in fact very reduced when compared to the material available at the beginning of the project. The possibility offered by the recombination process for genetic gain has been implemented only in the third phase. In fact, in this example, we are very close to the linear model we proposed in the first paragraph.

The second example, the INRA/PROMAIS project is closer to the model proposed by Gallais. The recombination phenomenon has been used at several steps of the process leading to the creation of new varieties. This project (INRA/PROMAIS, 1994) started with a collection of 1236 accessions of maize, adapted to temperate climate. A first phase of increase of this material was used to test the material for its *per se* value and its value in test cross, with 3 testers. Various traits were recorded, including yield. During the first step of this study, it was noted that geographical origin did not relate with agromorphological traits estimated in topcross trials (Lavergne, 1988). From these results, the collection has been structured in pools on the basis of:

1) utilization criteria: earlyness, grain versus silage;
2) two levels of diversity: restricted base pool with 10 best accessions per pool; broad base pools with more than 30 accessions per pool;
3) results of combining ability tests with several testers.

Several accessions were used in various pools, and a total of 630 accessions was included in the organization of these pools (half the size of the original collection). A step of improvement of the pools by crossing with elite material from the same heterotic groups has also been tested; these pools were called improved broad base pools. A large amount of the original collection is used in the successive steps of improvement. However, the creation of the different pools has led to a subdivision, limiting genetic exchanges to sub-samples of the whole collection for the following steps of improvement. After a generation of increase by random mating, pools have been evaluated. It was observed that the genetic gain is variable and depends on the pools considered, but the main improvement came from the crosses with elite material. The strong selection that led to the restricted base pools was not sufficient to produce a genetic material coming close to the level of the improved broad base pools.

A phase of recurrent selection has been established for the improved broad base pools. After a cycle of recurrent selection, the yield level reached was close to commercial checks.

The various steps of this project have been documented by analysis of the evolution of the many quantitative traits monitored and by analysis of molecular markers. It has been observed that the intra-accession diversity is large, and represents about 75% of the total diversity, estimate based on isozyme diversity (Lefort-Buson et al, 1991). This result gives another reason to base the improvement on pools organized according to their utilization.

The genetic material produced during the project corresponds to several levels of improvement, with several levels of restriction of diversity. All this material can be a base for further improvement and participates in a process of reorganization of diversity. In this example, there is no longer a clear cut between the base collection from which gene can be extracted and the process of breeding this material. It is a stepwise process that combines conservation and utilization.

Other models have been proposed (Kannenberg, 1981, Namkoong et al, 1980) that are close to those already described and can be of more general application, but still fall under the triangle model. The model, MPBS: Multiple Populations Breeding System from Namkoong et al (1980) can be presented briefly.
It is designed to maintain a high diversity, relying on subdivided populations maintained in several different environmental and/or agronomic conditions. Within each subpopulation, an improvement is proposed by recurrent selection, to reduce the pace of restriction of diversity. The level of improvement is the same in all the subpopulations. If we consider also the possibility of establishing different types of subpopulations and gene exchanges between subpopulations at some steps of the conservation process, this system becomes quite flexible, and can be adapted to forest tree breeding as well as to crop breeding.

The examples presented explored ways of conservation-utilization that allows a progressive shift from conservation to utilization, with a certain loss of diversity and gain of adaptation to designed objectives. However, this restriction of diversity that justifies the name of triangle model, does not prohibit new introduction of foreign accessions and genes. The INRA/PROMAIS project showed that the cross between elite material (from other improvement programs) and landraces is an effective way to improve genetic material.
This conservation is dynamic. New genetic structures are created by grouping accessions from various origin in pools. Within pools, an evolution is favored by recurrent selection. The genetic material proceeding from these various steps of improvement is available for inclusion in other improvement projects and is conserved on the same basis as the base collection.

This conservation-utilization system could become quite complex if it is organized at a global level, but is well adapted to projects of a limited size, i.e. for the needs of a country or a region.

The circular model

In the two first models presented, the relation between conservation and utilization is a one way process. Conservation is followed by utilization. The utilization is linked to a reduction of diversity and when new needs appear they will satisfied by returning to the base collection. In this case, the key stage is static conservation of the broadest diversity available in genetic material. But other systems have been proposed to take into account the evolution and evolutionary potential of the genetic material (Eriksson et al, 1993). Evolution is favored by conserving the plants *in situ*, i.e. in the same conditions as they have been for a long time, facing diseases and pests, and participating in gene flow between populations. For crops, the *in situ* conservation would be experienced on-farm, as the landraces, have been created and maintained by the farmers themselves, with their own selection pressure and process, and with gene

flow controlled between wild and cultivated forms or between landraces.

One can imagine that when this system is well established, there is no need for more static conservation *ex situ*, conservation function relying only on the *in situ* activity. However, it can be thought that these varieties maintained *in situ* represent a source of valuable material for other areas of the concerned crop or species, under other agro-ecological conditions, and that their *ex situ* conservation will bring a benefit. Accessions from gene banks can also be increased and tested in new sites, and become adapted to these new sites. It is this relation between *in situ* conservation and *ex situ* conservation, and between genetic material corresponding to genetic resources and material used as varieties that we consider when we present the circular model, basically a shuttle between conservation and utilization.

The purpose is to find a system that will maintain or enhance the evolutionary potential of the plants to be conserved, by exploiting the mechanisms at the origin of the present diversity. At our time scale, these mechanisms are selection, migration, drift, and recombination.

In the experiments found in the literature, two situations are distinguished :

1) Populations are maintained in natural conditions, that can be field conditions in the case of crops. The evolutionary mechanisms are maintained and the action of farmer in the process is limited to a low level comparing with the natural forces of selection. This system is called dynamic conservation. A precursor project of dynamic conservation was started in 1928 by H.V. Harlan (Harlan, 1975) for barley and conducted for more than 60 generations (Allard, 1988). We report another example, for bread wheat in France (Henry et al, 1991, Le Boulc'h et al, 1994).

2) Diversity is maintained in the farmers' fields by the farmers. Evolutive constraints are imposed by the farmers, in their search for varieties better adapted to their changing needs. At the present time, this situation is more a subject of reflection than experimentation. However, we will present some examples of activities related to this type of evolutive on-farm or *in* situ conservation.

The dynamic conservation of bread wheat

This experiment started in 1984 (Henry et al, 1992) with 3 synthetic populations, built from 16 parents and established on several sites in France. Seeds are not exchanged between sites. One of these synthetic populations includes a proportion of nuclear male sterile plants. In this population, only the male sterile plants are harvested and their seeds used for the next generation. In this case, the reproductive strategy of the population is strictly allogamous, while the other populations reproduce naturally with a high rate of autogamy.

The 3 populations have been observed for a set of morphological and resistance traits for 10 generations. Their evolution has also been documented by using molecular markers. It was noted through the generations that the evolution was directed towards different states depending on the sites. This evolution involved all the traits that have been documented including the disease resistance genes for powdery mildew, *Erysiphe graminis*. The frequency of the resistance gene has been modified as well as the number of resistance genes associated in a genotype. These parameters have been very different between sites (Le Boulc'h et al, 1994). It was also shown by these studies that competition between genotypes play a large role. Genotypes corresponding to the tallest plants are favored by the competition. Conversely, the frequency of dwarfing genes is diminishing (Goldringer et al, 1994). But these gene contribute to the genetic constitution of the most efficient modern varieties. In this case, the natural selection conferred a higher selective value to plants that are not agronomically interesting in the current cropping environment.

We deduce from this experiment that these populations, placed in natural conditions were genetically flexible and capable to react to different conditions of cultivation. The

experiment presented was simple in its design but it could be easily modified and become more complex when gene flow are organized through the different sites. This model could also be used for other plants and integrate the relationship between wild, weed and cultivated forms of a crop.

At different steps of evolution of these populations, an *ex situ* phase of conservation could also be implemented to:
1) conserve and distribute this material to interested breeders;
2) create a shift in the evolution of the material for resistance to diseases. Specially when the reaction between pathogen and host is of the gene for gene type, the pathogens develop races adapted to the cultivated varieties. When cultivated varieties are replaced by new ones, races of the pathogen can disappear and former resistance genes in the plants become useless and disappear also. Conserving *ex situ* these varieties, or these populations under dynamic conservation, will allow an accumulation of resistance genes in the collection, ready for new cycles of selection in the field.

Conserving diversity on-farm

Very often, it has been reported that modern varieties that bring with them a higher productivity are displacing the traditional landraces, reducing the available diversity in the process as these modern varieties have a lower genetic diversity (IPGRI, 1993). The on-farm conservation has been presented as a response to this genetic erosion phenomenon by freezing the evolution of agricultural activities (Iltis, 1974; Benz, 1988). Under this acception, it is not desirable as there is no reason to impose this constraint to a social group. However, Brush (1995) shows that there is at least some examples when farmers maintain modern and traditional varieties in the same areas. The examples given are taken from wheat, potato, and maize. One can imagine that on-farm conservation is linked with a local improvement and breeding, limiting the need for modern varieties to be introduced directly.

We present a case study of management of traditional landraces by farmers, in a rural community, Cuzalapa, in the State of Jalisco in Mexico (Louette, 1994).

In this rural community the main crop is maize, cultivated under a rainfed and an irrigated cycles. Six cycles were observed. The author noted that these farmers maintained 6 local varieties and 3 varieties considered as foreign, i.e. from recent introduction. But during this period, 17 other varieties were also introduced, tested or maintained at a lower scale. All these varieties cover different cycle length and grain color. This diversity is conserved not only by farmers sowing their own seeds (53% of the planted plots were sown with seeds from this origin), but also by seed exchange between farmers within the community (36%) and from outside (11%). This village is typical in Mexico, and such a diversity is also found in other countries for other crops (Bush, 1995). For conservation purposes, a village can be considered as a real laboratory for evolution, where natural selection pressures and farmers'selection pressures are applied.

Maintenance of such a diversity is obtained by partly subdivided populations (equivalent to varieties). Varieties planted side by side in the same fields, exchanges with other communities, and alternating irrigated and rainfed cycles are situations that bring a high gene flow and very distinct selection pressures to act on these populations. In this system, varieties that are conserved do not correspond to commercial criteria of stability and uniformity . What is maintained is more a process of evolution through gene flow and mechanisms of recombination, selection and drift than a fixed content of genes. This system correspond to an open genetic system, i.e. relying continuously on exchanges with the outside, testing new varieties in the village, going through a process of adoption or rejection and eventually diffusion of new forms outside the village. As there is no formal distinction between genetic resources and varieties, the entries for this system can be elite material and/or landraces from other environments, the output of the system is genetic resources and varieties. Stability in the system is only observed for gene flow and for traits that are selected for by the farmers,

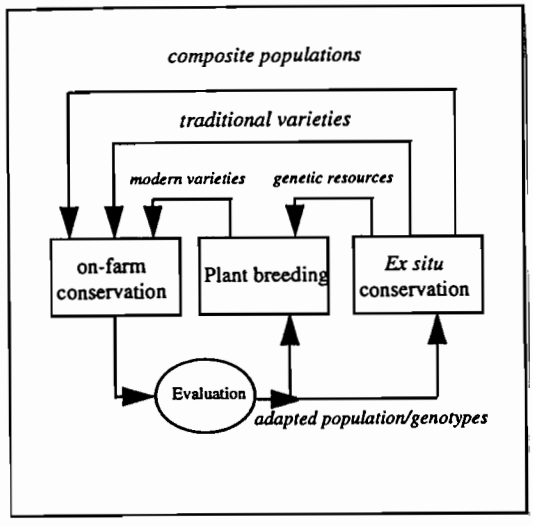

Figure 3 : Possible exchanges of genetic material between on-farm conservation, plant breeding and *ex situ* conservation (Bellon *et al.*, 1996)

mainly ear and grain traits (Louette, 1994). This example is a good illustration of how a " simple " farmer's management of seeds can correspond to a very complex and effective management of varieties and genetic resources, where there is not clear distinction between genetic resources and varieties, utilization and conservation.

We consider this system a circular model for the management of genetic resources because the model of evolutionary conservation should be coupled with a static conservation process, allowing the distribution of the material maintained in the village (and in others) and then permitting access to this material for other potential users. The static conservation step could play a buffering role and could be a backup against some unpredictable evolution. In fact, if this system presents several advantages, it is not protected against rapid and local evolution of the rural societies involved. In the example we described, in the village of Cuzalapa, it could be decided to plant sugar cane in place of maize as a sugarmill will be built in this area and this commodity could offer a higher return.

The phase of static conservation should be conceive only as connected with the dynamic, on-farm conservation. What should be established is a short or medium term *ex situ* conservation. Varieties conserved *ex situ* could be reintroduced in the fields when needed, and replaced with a high turn-over. With such a system of exchanges, evolution for the material conserved *in situ* and *ex situ* will be maintained at the same place. As this system is quite open, it is still possible to integrate new genetic material at each step, bringing new sources of potential improvement. This integration of new genetic material could be realized by activities of local breeding. An example could be the size of the maize plant. In his backcross experiments between landraces and elite material in Mexico, Marquez-Sanchez (1993) showed that important genetic gains can be produced. He obtained a 28% increase in yield in BC1. In the INRA/PROMAIS project, crosses between elite material and landraces brought also an improvement for the observed traits. This local breeding would bring less drastic changes to the genetic structure of the landraces and the resulting improved landraces could compete efficiently against modern varieties. It could also make use of lines specially developed by large breeding institutions for this purpose, or use of varieties from other areas of cultivation conserved *ex situ*. There is a possibility of a coupling between conservation and utilization that is quite different from the linear model we described at the beginning of this article.

A future for the circular model

The model we described, accepts many variations, from dynamic conservation to on-farm conservation, including various types of actors, playing with different types of genetic material, wild, weed, cultivated, with different mode of reproduction, with different rate of gene flow between populations and varieties. This model has to be tested now on a larger basis, to evaluate its feasibility, efficiency and stability. Proposals to apply this model exist, for example for rice in South East Asia (Bellon et al, 1996, Fig. 3). It would be of special interest to document genetically this new process of conservation. The methodological framework requires experiments that are not easily set up without perturbing too much the system, and there many intervening actors

responding to constraints that are only partly genetic. It is a real challenge.

This on-farm and *in situ* conservation and utilization is conceivable at the field or village level. It is interesting at a higher level but could become rapidly quite complex if it had to be promoted as an institutional activity. We think it is more appropriate to a participatory approach, limiting the relations with institutions to exchange of genetic material.

Conclusion

The models we presented, linear, triangle, circular, show that conservation and utilization strategies are multiple. We can imagine more variations from the examples we gave. The utilization of new tools, as the marker assisted selection introduces a higher efficiency in the creation of new varieties, in the transfer of genes by genetic recombination and allows a broader use of genes available in the gene banks.

Other new and useful techniq especially genetic engineering, offer new ways of utilization of genetic resources and conversely new forms of gene storage should be made available. The genetically transformed plants, harboring new gene should become also valuable for gene conservation.

Realizing that *ex situ* conservation is not the unique way of conserving genetic material for the improvement of future varieties but that *in situ* and on-farm conservation have a role to play in the process of conservation, introduces new relationships between farmers and breeders, between users and managers of genetic resources (Berg, 1993, Hardon and de Boef, 1993). It is a field wide open for new investigations and experiments, for new relations between scientists and users, and a different distribution of responsibility in conservation of genetic resources.

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