Methodologies for *in Situ* Conservation of Plant Genetic Resources

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Abstract

In recent times, conservation efforts have typically emphasized habitat protection as a crucial means of maintaining the fast-eroding biological diversity in the face of climatic change and the subsequent migration of plants to new habitats. There are two basic methods for conservation of crop plant genetic resources, *in situ*, in nature without disturbance, and *ex situ*, in gene banks at subzero temperatures and low humidity. Presently the latter method has been exclusively used to conserve crop germplasm in genetic resources centers. Biologists are of the opinion that further evolution of the germplasm is arrested when preserved in gene banks and hence relegates it to a sort of museum piece. To counter this line of thought, *in situ* methods have been suggested where evolutionary processes continue to operate in nature, thereby keeping the germplasm in tune with the changing environment. In this article we describe the biological diversity of wild germplasm to be found in some of the countries within the Fertile Crescent, including archaelogical evidence of widespread cultivation of brittle-rachis *Triticum* spp., and suggest a mehtodology for their *in situ* conservation with the use of ethnobotanical information among others. A brief account of four ongoing and future projects aimed at *in situ* conservation of wild populations of close relatives of our most important food crops in the region under ICARDA's mandate is presented also.

Introduction

The protection of wild relatives and rare species of our cultivated crops and the conservation of biological diversity in more general terms have emerged as key concerns of modern biology. While the international scientific community grapples with the shaping of complex legal conventions to regulate and monitor the environment, funding mechanisms are being tested to assist developing countries in addressing global dimensions of national environmental protection programs. The preservation of biodiversity of cultivated species and their wild relatives is of paramount importance in present times of rapid and permanent genetic erosion and degradation of the environment.

The capability of plant species to disperse to suitable new sites before they become extinct in their original habitat is one of the crucial questions facing conservationists and plant ecologists (Primack and Miao, 1992). The Fertile Crescent, which traverses Jordan, Palestine, Syria, Lebanon, southern Turkey, northern Iraq and western Iran, is one of the world's principal centers of crop plant biodiversity where naturally occurring populations of the wild progenitors of some our most important crop plants can still be found.

The International Center for Agricultural Research in the Dry Areas (ICARDA) being located in the Fertile Crescent, considers *in situ* conservation of the crop wild relatives an integral part of its genetic resources conservation strategy. The Center holds nearly 100,000 accessions of cultivated and wild species of cereals and legumes in the gene bank as *ex situ* collections. However, recent studies at ICARDA and

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elsewhere have demonstrated extensive genetic diversity in the original populations of the wild gene pool in the natural habitat which is impossible to preserve by the standard *ex situ* collection procedures. Also, it is generally accepted that, during field collection, usually only about 50–150 plants per site are sampled and many genotypes may be left out. On the other hand, under *in situ* conservation a much larger and continuously evolving genetic diversity is preserved. We believe that *ex situ* and *in situ* methods are complementary methods of genetic resources conservation, especially for a wide range of exotic characters that are not of immediate interest to plant breeders. However, *in situ* conservation may be more practicable for forest species and wild relatives of our cultivated crops (Frankel, 1977 ; Swaminathan, 1988), although it also has been suggested as a suitable conservation method for obsolete cultivated species, such as *Triticum dicoccum* in Italy (Perrino and Hammer, 1982).

Biologists are of the opinion that *ex situ* conservation arrests further evolution of the germplasm and hence relegates it to a sort of museum piece. Also, it is claimed that seed gene banks are static, since they do not allow further natural development of the original diversity (Brown, 1982 ; Myers, 1983). To counter these claims, *in situ* methods have been suggested where evolutionary processes continue to operate in nature, thereby keeping the germplasm in tune with the changing environment. Germplasm could be repeatedly collected for off-site evaluations from time to time. Also, to overcome the limitations of the *ex situ* collections, preservation of crop wild relatives populations in the natural habitat is important for long-term benefits of national programs and the international community as a whole. The germplasm so conserved is not only adequate for fulfilling current research needs but also those for future, such as responding to possible changed climatic conditions due to global warming, changing rainfall patterns, acid rain, and habitat destruction (Davis, 1989). National Agricultural Research Services (NARS) have neither the incentive nor the means to conserve the resources that may not be useful for their own country. Hence, in order to succeed, *in situ* conservation projects must be politically viable and share broad national development goals, such as increased farm income, as well as straightforward conservation. Political viability depends on acceptance of the project by several interest groups other than genetic resources scientists and nature conservationists, i. e., farmers, consumers, and government officials.

Despite the recent interest in environment preservation generated worldwide there has been limited experimental verification of the potential value of *in situ* conservation of wild relatives of crop plants. Indirect evidence from experimental plant population genetics, such as studies in heterogeneous composite cross populations carried out by Allard (1988, 1990 ; Allard and Jain, 1962) and later by Allard and Hansche (1964), Acharya and Jana (1982) and Jana and Khangura (1986) suggest that, under certain agricultural conditions, dynamic evolutionary forces are capable of maintaining sufficient genetic variability in wild annuals to meet both short-term and long-term evolutionary needs. It has been shown that conditions for the *in situ* preservation of wild barley, *Hordeum spontaneum* C. Koch. (Holwerda *et al*., 1986 ; Jana and pietrzak, 1988) and wild wheats *Triticum urartu* and *T. dicoccoides* are feasible in the Near East Fertile Crescent (ICARDA, 1993).

In situ methods of conservation or the encouragement given to farmers to continue to grow traditional varieties are, understandably, looked at with skepticism by the breeders. As long as conservation and crop improvement are directly linked, any form of conservation will be judged by its short-term benefits to breeders, and *in situ* methods will attract considerable opposition (Brush, 1991). However, on-site conservation is more plausible if these two goals are decoupled, making biodiversity conservation an end in its own right.

It is essential to investigate the association of genetic diversity with ecological and environmental variables, the distribution pattern of the genetic diversity, and human activities at or near the site in order to determine optimal conditions in suitable locations for *in situ* conservation of the target species. In several cases *in situ* conservation may actually be less expensive than *ex situ* maintenance of collections, especially if methods other than direct financial subsidies to farmers are implemented (Brush, 1991).

An efficient *in situ* germplasm conservation strategy requires a joint effort of all parties, i. e., national programs, governmental and non-governmental organizations, International Agricultural Research Centers (IARCs) and United Nations agencies. In some countries, such as India, Seychelles, Mauritius, and Ecuador (Galapagos Islands), germplasm conservation *in situ* has been successful through inclusion of the sites in so-called "nature reserves" or "biological parks" which link conservation of both flora and fauna with cultural practices, archaeology and tourism encompassing large areas. The Man and the Biosphere (MAB) program of UNESCO has successfully taken a lead in the right direction with regard to this kind of project.

ICARDA has been involved in and/or has initiated several activities in recent years relating to *in situ* conservation of wild relatives of cereals and legumes which are described below.

In situ conservation of genetic diversity in wild progenitors of wheat and barley

West Asia has been not only a cradle of civilization but also includes areas where agriculture first began about 10,000 years ago. The region includes two of the most important Vavilovian centers of origin of food crops, viz. the Near East and the Mediterranean (Vavilov, 1951). The countries involved fall within the pattern of genetic diversity also described by Harlan (1970, 1971). Although Harlan did not agree with Vavilov's concept of the center of origin, he recognized the importance of the region as a center of diversity. However, the rich regional biodiversity in crop plants and their wild relatives is threatened by urbanization, overgrazing, and agricultural development which is based on introduction of new high-yielding varieties with a narrow genetic base and modern technologies.

A project proposal was developed with the overall objective of preserving the genetic diversity of wild progenitors of wheat and barley in their native habitat. The project would complement existing comprehensive *ex situ* collections of wild wheats and barley. The specific objectives were the following:

1. to identify areas with significant genetic diversity of wild progenitors of wheat and barley in their original habitats in the Fertile Crescent,
2. to develop a methodology for monitoring the nature and magnitude of change in genetic composition of populations of the target species over long periods of time,
3. to assess the genetic diversity in populations of wild progenitors under *in situ* conservation and identify characters or character associations which could be utilized in breeding or other studies,
4. to identify the socio-economic factors involved in *in situ* conservation and monitor their effect on farmers and the local community,
5. to complement the current Global Environment Facility (GEF) project on *in situ* conservation in Turkey, which covers the northern part of the Fertile Crescent.
6. to determine feasibility and costs required to maintain wild populations under *in situ* conservation so that the project can serve as a model for similar endeavors in the future.

National programs of Syria, Jordan and Lebanon would be involved in the project, as well as advanced institutions in Europe or North America. The project has been planned for three years with a total budget of $1. 5 million. Currently, external sources of funding are being sought.

In situ conservation of wild progenitors and relatives of cereals and food legumes on uncultivated and arable land

The ability of species to establish new populations at unoccupied sites is a critical feature in the maintenance of biological diversity, and it has taken on new importance as a result of global climate change, alteration in species distribution patterns and destruction of original habitats due to increasing human pressures.

A project to study the colonization of certain wild relatives of field crops on fresh as well as occupied land has been set up at ICARDA. The main objective of this project is to establish populations of indigenous crop wild progenitors and relatives and conserve them as *in situ* collections for monitoring and analyzing the population dynamics. The experiment, which is supported through core funding, is planned for an initial period of three years and will be located at the principal ICARDA experimental farm at Tel Hadya, Syria. The species involved are as follows: *H. spontaneum, T. urartu, T. boeoticum, T. dicoccoides, Lens orientalis, L. odemensis, Cicer reticulatum, C. echinospermum, Medicago rotata* and *M. rigidula.*

Plots of individual species and species mixtures of different complexity are planted in two different environments: a) an uncultivated grazing land, and b) arable land. Wherever possible seeds used in the experiments were taken from two types of sources, viz., the nearest occurring wild population as well as from those outside the province. The main factors which affect survival and competitive ability in populations of target species conserved *in situ* will be determined and the possibility of reintroducing the crop wild progenitors and relatives into original habitats where genetic erosion has taken place will be thus tested.

Monitoring of the native populations of wild *Triticum* spp. in Syria

In exploration expeditions conducted jointly with the national program of Syria in 1991 and 1992, populations of *T. urartu, T. boeoticum* and *T. dicoccoides* from the southern provinces of Sweida and Damascus and the nothern province of Aleppo were collected and analyzed for genetic diversity by means of seed storage protein polymorphism. Extremely high genetic diversity was found in populations collected from the high plateau in the province of Sweida, while populations found in valleys of the Anti-Lebanon mountains showed lower diversity (Valkoun and Damania, 1992). Further information on this activity has been given in the annual reports of the Genetic Resources Unit of ICARDA (1992, 1993).

Based on these preliminary results, an ICARDA core-funded research project was developed for monitoring native populations of wild *Triticum* spp. This project will assess the suitability of these species and the methodology for their *in situ* conservation.

During a monitoring trip conducted jointly with the NARS scientists of Syria in 1993, information was gathered on the site (land use, farming systems and socio-economic background, botanical composition of the vegetation, etc.) and target species populations (phenology, reproductive ability, vigor and health). Results of this study are being used to identify the most suitable populations and to develop a sustainable strategy for *in situ* conservation of indigenous wheat progenitors in Syria and elsewhere.

In situ conservation of wild relatives of wheat in Armenia

In 1992 ICARDA conducted a collection mission for wild wheat relatives to Armenia in cooperation with the national counterparts. The trip included a visit to the Erebuni Nature Reserve, northeast of Yerevan, which represents a classic case of *in situ* conservation. In the 1930s Vavilov recommended protection of this site because of its unique richness of the wider *Triticum* genepool. A new wild wheat species, *T. urartu*, was discovered there in 1935 by Tumanyan and the scientific description of the species was given later by Gandilyan in 1972. Other wild wheat species, *Triticum boeoticum* and *Triticum araraticum*, grow in the protected area together with *Aegilops* species. *Amblyopyrum muticum*, a species of *Aegilops* (such as *Ae. mutica*) and considered the taxonomic intermediate between *Aegilops* and *Agropyron*, also was found near this nature reserve. Hence, this is the only site outside Turkey where the uncommon species of the Anatolian highlands are found. The actual size of the reserve is about 100 ha but protection of a much wider area, ca. 400 ha, is needed to include rare populations growing at the periphery of the protected area.

For the preservation of the wild wheat genepool a more interesting site than the Erebuni Reserve cannot be imagined. At present, however, the reserve is not fenced and the site is under growing pressure of human disturbance from the surrounding villages. There is also an imminent danger that the land on which the reserve exists may be sold to commercial developers if the current trend at privatization, in the wake of the dissolution of the USSR, cotinues. ICARDA is making efforts to develop a project proposal for possible external funding, jointly with the NARS of Armenia, to safeguard this unique site for future research and exploration, and thus save the genetic diversity in this *in situ* reserve.

In conclusion, it is our opinion that genetic resources conservationists should develop methods not only to understand and preserve existing populations of wild relatives and rare species, but also to provide technical knowledge for future projects on *in situ* conservation aimed at creating new populations at sites in the original habitats where genetic erosion has taken place. Such activity will perhaps reverse the trend of species extinction occurring at an alarming pace throughout the world as a result of rapid urbanization and habitat destruction due to growing human population pressures. ICARDA, while accepting the challenge to comprehensively conserve genetic resources of its mandate crops under *ex situ* conditions, will also do more to preserve biodiversity through *in situ* conservation projects with the active involvement of the NARS, other organizations, and funding agencies.

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Discussion

Rana, R. S. (India): We are interested in *in situ* conservation of wild rices and wild relatives of other crop plants. We are mainly interested in monitoring changes. What is the minimum population size that you consider to be adequate for *in situ* conservation allowing free evolutionary processes?

Answer: We consider that an area of 30–70 ha would be sufficient. There was a lower diversity in the mountain populations with few mutations in single bands. On the other hand, in the large populations there was a wide diversity and we observed gene recombination as well as single mutations, suggesting that these populations are very old.

Sakamoto, S. (Japan): Could you find evidence of natural interspecific or intergeneric hybridization, hybrid swarm formation or introgression in your very large sympatric populations of several species of wild wheat and *Aegilops?*

Answer: Yes, we could find evidence of gene introgression from diploid *Triticum urartu* to tetraploid *T. dicoccoides* using electrophoresis of gliadins. The former species predominates in high plateaus and the latter in humid areas. There was morphological evidence of hybridization between *Aegilops* and *T. dicoccoides* and in a population from Lebvanon the rate of outcrossing was very high. Crosses between *T. durum* or *T. dicoccoides* and *Aegilops* were observed in some areas.

Nakagahra, M. (Japan): Regaring *in situ* conservation of genetic resources, monitoring activities could be hampered under a high density of grazing pressure. Are there any efforts or policies to preserve these areas from grazing pressure?

Answer: Overgrazing is the major factor that affects the survival of the populations. The case I illustrated is peculiar as the area is inhabited by Druzes who came to Syria from Lebanon in the 19th century. They cater these areas and do not allow nomads to penetrate in the region before cereals are harvested (before shattering of spikelets). Such areas could be protected only in close collaboration with government officials, national programs, local communities and through funding to maintain traditional systems of agriculture that are sustainable. Such areas are almost equivalent to natural reserves.

Yonezawa, K. (Japan): Change in genetic composition or persistence of a population depends on many factors, among which the size of the population and the size of the reserve field are key factors. What is the rationale in selecting a size of 30–70 ha for your reserve fields?

Answer: This is the size proposed by the CEF project in Turkey. In the case of autogamou crops like the wild relatives of wheat there is often a mosaic pattern of distribution of genetic diversity. Therefore, the larger the better. The area previously indicated (30–70 ha) is a pragmatic compromise.

Morishima, H. (Japan): In the case of wild rice (*O. sativa*), perennial populations persist at the same site unless the habitat is disturbed. Annual populations which are adapted to disturbed habitats tend to colonize frequently. Did the natural populations of wild relatives of wheat and barley persist at the same site for a long time?

Answer: We assume that some of the populations of wild wheat are old and there is biochemical evidence to support this assumption. Some of these populations are sympatric populations of *T. urartu*, *T. dicoccoides* and *Aegilops* showing a wide range of diversity and occasional mutations.

Singh, B. B. (IITA): Since *in situ* preservation is rather difficult, what criterion do you use to decide when to undertake "*in situ preservation*"?

Answer: It is a difficult question to answer. In the case of annual wild relatives of cereals the criteria may be, species growing in the original habitat, high genetic diversity, sufficient size (more than 10 ha), danger of genetic erosion, etc. Since wheat, barley, lentils are the oldest crops, it is recommended to keep them in their original habitat for future evolution.