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Complementary Conservation

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Although traditionally *Musa* germplasm has always been conserved in field genebanks, a whole spectrum of techniques and methodologies are available for the conservation of such material. Each technique or method has particular advantages and disadvantages in relation to the type of material to be conserved and the aims of the conservation programme. In developing a strategy for conserving the whole *Musa* genepool, the most appropriate methods must be determined. Adopting a complementary conservation strategy means that a range of methods are employed, each appropriate to a specific component part of the overall conservation programme and taken together, these methods complement each other in order to achieve the most efficient and safest conservation in the long term.

The two basic approaches to conservation are *in situ* (on site) and *ex situ* (off-site) methods. *In situ* conservation refers to the maintenance of a species in its natural habitat, which may include in the case of *Musa*, natural forests for wild species, but also farmers fields and backyards for cultivated varieties. These are the sites where the species/varieties acquired their distinctive characters. *In situ* conservation effectively requires the conservation of the whole eco-system of which that species/variety is a part. Such conservation allows natural evolution to continue, providing breeders with a dynamic source of resistance and other traits. It also facilitates research on species in their natural habitats.

Ex situ conservation, by contrast, is the maintenance of the material outside its original habitat, in facilities such as seed, *in vitro* and field genebanks, and in botanic gardens. *Ex situ* conservation facilitates the study, distribution and use of plant genetic resources but the reproductive material is conserved in a static non-evolutionary state.

This paper describes the various methods, both *in situ* and *ex situ*, available now, or with potential for use in the future, for the conservation of the *Musa* genepool.

In situ conservation

There are three main types of *in situ* conservation:

- conservation in protected areas
- habitat management outside protected areas
- on-farm conservation

Conservation in protected areas

Protected areas are pieces of land set aside as biological reserves, where untended plant communities can continue to exist and evolve. This type of conservation is particularly appropriate for the conservation of wild *Musa* species and such areas would serve as reservoirs of traits outside the plant breeder's immediate interests, but which may be of interest in the future. Until now, most of the world's national parks and protected areas have been set up to conserve wildlife and almost none have the specific aim of conserving crop genetic resources. Any conservation of crop wild relatives in such areas therefore generally occurs as an unplanned result of nature protection. In relation to *Musa*, there are no known records of wild *Musa* species being conserved in existing protected areas. The method must surely have potential however for the conservation of species known to exist in the rainforests of South-east Asia and the Pacific, such as *Musa ingens*. Before embarking on such *in situ* conservation programmes for *Musa* however, there is a need for more information on the distribution of wild *Musa* species, on minimum habitat size and on population dynamics. The establishment of good links between those responsible for the management of protected areas (typically Ministries of the Environment or Forestry), and those responsible for the conservation of crop genetic resources (usually Ministries of Agriculture) will also be essential.

Habitat management outside protected areas

Most *Musa* genetic resources are located outside protected areas, in ecosystems such as farms, cleared land and forests. Many wild *Musa* species survive in human-made habitats, such as on roadsides and on the edges of cleared land. In the areas where they occur, wild bananas are often among the first colonizers when natural regeneration follows forest clearance. In order to conserve these species *in situ*, a habitat management strategy would be required. The communities living in and around such habitats obviously need to participate in this type of conservation, which should allow the continued preservation and evolution of the wild species, while at the same time allowing local communities access to the land. In fact it is only through human's activities that such habitats can be maintained. It may be, that by encouraging the harvesting of certain useful products from the wild bananas, leaves, male bud etc., in a sustainable manner, local communities could be encouraged to support and participate in such programmes.

On-farm conservation

Many farmers are already practicing *de facto* on-farm conservation of *Musa* genetic resources through the continued cultivation of landraces or old varieties of bananas and plantains. Such varieties will be conserved *in situ* as long as they have productive potential and continue to be cultivated by farmers. It is, in effect, conservation through use. The conservation of such traditional varieties by farmers differs in some important aspects from the *in situ* conservation of wild material. In the case of *Musa*, these varieties are sterile and do not reproduce sexually. There is thus no exchange of genetic material between individuals or populations as occurs within and between wild populations. Thus, whereas the conservation of wild species is focused on conserving diversity at the population-level, conservation of cultivated varieties focuses on individual genotypes in which some limited variation may occur as a result of somaclonal variation.

Traditional varieties have generally been selected by man to suit the environment in which they are cultivated and the particular needs of the grower. They are the result of domestication, followed by constant development through farmer selection. Traditional varieties do not however remain static, they continue to evolve and develop.

Banana garden in Papua New Guinea, Western province

In the case of *Musa*, new, beneficial mutations occur from time to time. These are recognized by the farmer who consciously selects such individuals and continues to maintain them. It is important that such farmer-selection process are allowed to continue, through the on-farm management of genetic diversity. This calls for the design of programmes which simultaneously increase income and productivity, but do not rely on the displacement of genetic diversity. Such programmes should also take into account the valuable knowledge that farmers hold about the resources they are handling. It is important that this information is not lost and that the decision making processes involved in selecting traditional varieties for maintenance and continued cultivation are understood. Such traditional, indigenous knowledge should also be complemented with a sound scientific understanding of the underlying genetic process and environmental interactions taking place in the farmers field.

It is only in recent years that specific projects and programmes have been initiated to promote, support and develop on-farm conservation, and to date, none of these focus on *Musa*. With the recent advances that have been made in breeding new banana hybrids, and the initiation of widespread distribution of these hybrids, this is a particularly opportune time for the *Musa* community to recognize the importance of conserving farmers' traditional varieties 'on-farm' and to thus respond to the challenges presented.

Natural stand of Musa itinerans *and bamboo in China*

Typical house with its garden in Eastern Highlands, Papua New Guinea

Ex situ conservation

Ex situ conservation conserves alleles, genotypes and populations, at any location, usually within easy access for breeders and other users, and safeguards them from loss (due to habitat destruction, disease, replacement by new varieties etc.) and also from changes resulting from evolutionary pressures. The various types of *ex situ* conservation include seed, field and *in vitro* genebanks, pollen and DNA storage and cryopreservation

Seed storage

For a crop such as *Musa*, seed storage has limited applicability, as it is only the wild species that produce significant numbers of seeds. However, for the conservation of such species, seed storage is an area which requires further investigation. It is known that *Musa* seeds are orthodox in their storage behaviour, i.e. they can be dried and stored for long periods at reduced temperatures. *Musa* seeds appear to enter a period of dormancy once dried, and further work is required in order to fully understand the mechanisms involved in breaking this dormancy. Further information is also required on methods for handling seeds prior to storage, and on optimum storage conditions to achieve maximum longevity.

Field genebanks

Field genebanks are the most common means of conserving diversity in crops such as *Musa*, which do not normally produce seeds. Field genebanks are plants assembled and grown in the field as a living collection of accessions. Field genebanks of *Musa* exist in most countries where banana is an important crop, although the size and diversity represented in these collections varies considerably. Plants in field genebanks can be readily characterized and evaluated but they are also vulnerable to attack by pests and diseases. Similarly, accessions can be lost from field collections as a result of natural disasters, such as hurricanes and floods. In the case of *Musa*, virus diseases are a particular threat to the maintenance of collections in some areas. Field genebanks are labour intensive and costly to run, and the long-term security of accessions cannot be assured. Nevertheless, despite the drawbacks of field genebanks, INIBAP recognizes their importance in the overall *Musa* conservation effort, particularly in relation to germplasm characterization. In field genebanks accessions can be fully characterized and duplicates identified. It is the network of field genebanks at the national and regional level that underpins INIBAP's efforts to develop an international *Musa* conservation network.

In vitro storage

In vitro storage is now being developed as an alternative method which is complementary to field genebanks for the storage of vegetatively propagated crops. For *in vitro* storage, germplasm accessions are maintained as sterile plantlets on a nutrient medium under suitable environmental conditions. The largest *in vitro* collection of *Musa* germplasm in the world is maintained by INIBAP and it consists of some 1089 accessions. Several other institutions, such as the International Institute of Tropical Agriculture (IITA), the Queensland Department of Primary Industries (QDPI) and the Taiwan Banana Research Institute (TBRI), also hold *in vitro* collections of *Musa*. Techniques for slowing the growth of material stored *in vitro* (low temperature / low light levels) have been developed by INIBAP and plantlets can be kept, on average, for 12 months before requiring transfer to fresh growth medium. The advantages of *in vitro* storage are that the collection is maintained in a controlled environment, free from most pests, diseases and environmental extremes. In addition, material can be readily propagated and disseminated when required. *In vitro* culture is an essential requirement for the safe movement of vegetatively propagated germplasm and is used by INIBAP in combination with virus indexing procedures to ensure that all *Musa* germplasm movement is carried out in an appropriate manner to prevent the spread of pests and diseases. It is for this very reason that INIBAP maintains its *Musa* germplasm collection *in vitro*. *In vitro* techniques can also be used in virus therapy programmes aimed at the elimination of viruses from plant material.

One drawback of *in vitro* storage is the possibility of genetic instability due to somaclonal variation which can occur during the culture process. However, the storage of cultures under slow growth conditions, whereby the rate of cell division is reduced, may help to reduce the frequency with which such mutations occur.

Fruits of Musa acuminata *ssp.* banksii *with seeds*

Cryopreservation

Cryopreservation involves the storage of plant material at very low temperatures (–196°C) in liquid nitrogen. At this temperature, cell division and metabolic processes stop and the plant material can therefore be stored without modification or alteration for long periods of time. It is thus a promising option for the safe, long-term storage of germplasm of vegetatively propagated crops such as *Musa*. The method also requires limited space, protects the material from contamination, involves little maintenance and over a long time period, is cost-effective.

Research supported by INIBAP at KUL, Belgium has resulted in the development of a simple cryopreservation technique for proliferating *in vitro Musa* meristems and embryogenic cell suspensions. Meristem culture is the method of choice in relation to cryopreservation as the production of embryogenic cell lines is highly-genotype specific. However, the embryogenic cell lines which may be produced as a result of genetic transformation research can also be used for cryopreservation. This improved technique has proved to be suitable for a range of *Musa* genotypes and is presently being further tested at the laboratory. The use of cryopreservation should ensure the long-term security of conserved material and should overcome the potential problem of genetic instability presently associated with *in vitro* conservation.

Pollen storage

This technique is not presently used for *Musa* conservation, and is only likely to be of use in the conservation of wild species and some male-fertile cultivars. The technique is at an early stage of development, but has several potential advantages. These include the small sample size required for storage per accession and the fact that pollen is less likely than seed to be infected by disease. Pollen storage alone cannot however conserve the cytoplasmic genetic diversity of a species and much further research is required before this is likely to become a useful tool in conservation.

Conservation of DNA or DNA sequences

This is another technique in the early stages of development, and which is not currently applied to *Musa*. The principle use of “DNA-libraries” is for the isolation of specific, useful genes, which can then be used in genetic engineering. It is not a technique likely to be considered as an alternative conservation strategy in the near future.

Conservation in botanic gardens

Many botanic gardens conserve *Musa* genetic resources, especially the wild species with an ornamental appearance. However, although botanic gardens may conserve considerable amounts of inter-species diversity, their role in conserving intra-specific diversity is limited because most conserve only a few accessions per species or taxon. The work carried out by botanical gardens in relation to *Musa* taxonomy may therefore prove to be more important than their role in conservation *per se*.

Complementary strategies

It is clear that many methods of conservation are relevant to the conservation of *Musa*. *In situ* conservation, both through protected areas and habitat protection are methods which can be used for conserving the diversity of wild species, while allowing natural evolutionary process to continue. This is of particular importance in areas where the species has co-evolved with the major *Musa* pathogens. *Ex situ* seed storage should be used as a back-up method for conserving wild species, particularly for those species known to be under threat in the wild. For cultivated varieties, on-farm conservation is important in allowing the continuation of farmer selection of natural variation and promoting the use of diversity in farming systems. *Ex situ* conservation in field genebanks is essential as it facilitates characterization and evaluation of varieties by researchers. However as the long-term security of material in field genebanks cannot be assured, conservation of duplicate material using *in vitro* techniques is important as a back-up. *In vitro* techniques are also essential for the safe movement of *Musa* germplasm and underpin INIBAP’s activities in germplasm distribution. Cryopreservation is the most reliable *in vitro* technique for the long-term storage of vegetative material, and this method should therefore be pursued as the method of choice for the long-term *ex-situ* conservation of *Musa* cultivars.