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Conservation Strategies for *MUSA*

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Wild and cultivated diversity of *Musa* (banana and plantain) is at its richest in the Asia and Pacific region. The genus *Musa* represents a group of approximately 25 forest dwelling species, divided into four sections, distributed between India and the Pacific, as far north as Nepal and extending to the northern tip of Australia. The genus belongs to the family Musaceae, which also comprises some seven species of *Ensete* and a third, monospecific, genus *Musella*, which is closely related to *Musa*. Taxonomists estimate that there are at least 1000 recognizable *Musa* cultivars distributed pantropically. Edible forms have been selected by farmers from the progeny of either one or two wild parent species: *Musa* acuminata ssp. banksii is believed to be the ancestral parent of the majority of edible banana cultivars, contributing what is called the 'A' genome while *Musa* balbisiana contributed the 'B' genome to several banana cultivar groups and all plantain.

Collecting missions to the Asia and Pacific region in recent decades give reason for confidence that no or few new major cultivar groups remain to be discovered. However, new wild species and varieties continue to be described are inadequately represented in collections. Threats posed by habitat destruction and the replacement or loss of traditional cultivars intensify the urgency for collection and conservation efforts.

NEED FOR *MUSA* DIVERSITY

Genetic improvement presents a potentially cost-effective mechanism to address current constraints in smallholder production by providing high-performing varieties adaptable to diverse environments. The products of existing improvement programmes, drawing on sources of resistance from wild and edible genotypes, are not meeting several important criteria, such as widely-acceptable fruit-pulp quality, and only a fraction of the genetic diversity in diploid *Musa* is being used. Yet variation among wild and edible *Musa* species offers a wide spectrum of fruit and bunch qualities. For instance, the ecology of various wild species suggest that sources of resistance to abiotic stresses exist in *Eumusa* along the northern periphery of its distribution, including mechanisms for tolerance to cold (*M. sikkimensis, M.basjoo, M.thomsonii*), water-logging (*M. itinerans*), and drought (*M.balbisiana, M. nagensium*).

CONSERVATION OF *MUSA* DIVERSITY

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 gene banks, 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.

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. 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 rain forests of South-east Asia and the Pacific, such as *Musa ingens*.

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.

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.

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.

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.

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. *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.

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 intraspecific 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*.