Papers Presented

Meeting the challenge of conserving tropical tree species with recalcitrant and intermediate seeds

A.S. Ouédraogo[1], J.M.M. Engels[1], L. Kraak[2] and F. Engelmann[1]

[1] International Plant Genetic Resources Institute (IPGRI), 00145 Rome, Italy
[2] Centre for Plant Breeding and Reproduction Research (CPRO-DLO), PO Box 16, 6700 AA Wageningen, The Netherlands

The International Plant Genetic Resources Institute, in collaboration with the International Centre for Research in Agroforestry (ICRAF) and the Food and Agriculture Organization of The United Nations (FAO), proposes to coordinate the development of a 4-year research project in close collaboration with relevant experienced research institutes in developing and developed countries. The project will focus on seeds of valuable tropical forest tree species with recalcitrant or intermediate storage behaviour. Groups of participants will study various aspects of problems imposed by the limited storability and difficulty in handling seeds of these species. It is anticipated that the combined research efforts will result in (1) improved scientific knowledge on the storage of recalcitrant and intermediate tree seeds, (2) improved medium- and long-term conservation of the genetic resources of the species, and (3) improved availability of recalcitrant and intermediate seed species for use in reforestation programmes,.

The knowledge and technology generated by the project will be exchanged among all participating institutes and will be used to develop practical guidelines for the handling of seeds of tropical forest tree species. A network will be formed among researchers in developing and developed countries. Through contributing to the sustainable conservation management and use of the selected high-value species in key tropical forest ecosystems, the project will provide the basis for an improved standard of living for local communities. Valuable species such as *Azadirachta indica* (neem) are included in the project; the planned research efforts on this species will contribute to the overall objective of the International Neem Network coordinated by FAO.

Background

The problem

Many forestry and agroforestry tree species in tropical and temperate countries are of high economic importance. Many of them are multipurpose trees, including fruit trees, of which the products have manifold uses. A major problem in using the seeds of many trees in reforestation programmes is the short storage life of their seeds. Sometimes it is not even possible to store the seeds from harvest until the next sowing season. Indeed, for some species it is hardly possible to maintain viability during collecting and transportation. Improved methods for short- or medium-term storage and for handling are required to enable the use of these species in reforestation programmes. Long-term storage for genetic conservation presents even greater problems.

State of knowledge

Seeds of many species can be dried. Under favourable storage conditions (low temperature and low moisture content), these seeds remain viable for many years. Such seeds are called **orthodox** (Roberts 1973). However, many forest and fruit tree species from temperate and especially tropical regions produce seeds that are damaged by desiccation and are often sensitive to low temperatures. Accordingly, these so-called **recalcitrant** seeds have a short storage life. Seeds of some species have shown **intermediate** storage behaviour, surviving desiccation to a fairly low moisture content, but suffering injury by low temperature.

Recent studies suggest that several mechanisms are involved in desiccation tolerance. The presence of sugars like sucrose, raffinose and stachyose appears important for stabilization of membranes (Leopold and Vertucci 1986; Crowe *et al.* 1992), proteins (Carpenter *et al.* 1987) and other macromolecules conferring cryoprotectant properties (Withers and King 1980). Another mechanism by which sugars may act to protect cells during desiccation is formation of an intracellular glassy state (Bruni 1993). Apart from sugars, some proteins which are synthesized at the end of the seed maturation process (Late Embryogenesis Abundant - LEA proteins or dehydrins) may play a protective role during dehydration. These proteins are induced by ABA, a hormone involved in the development of desiccation tolerance in orthodox seeds (Koornneef *et al.* 1989). Furthermore, mechanisms to avoid damage by free radicals (oxidative damage) during drying (Benson 1990; Hendry *et al.* 1992) and the degree of vacuolization of cells in the seed may play a role in the acquisition of desiccation tolerance. Attempts to induce desiccation tolerance in recalcitrant seeds by manipulating any one of these mechanisms have not been successful as yet. Further research appears necessary to achieve success in making entire seeds and/or their component parts more desiccation tolerant and consequently easier to store.

For medium-term storage, *in vitro* conservation methods involving slow growth of cultures are being developed for both reproductive and somatic tissues (Withers and Engels 1990). For long-term genetic conservation, techniques for cryopreservation of partially dehydrated embryonic axes (Chin 1992) and other plant tissues such as somatic embryos and shoot-tips (Withers and Engelmann 1996) are under development for a number of species. However, further studies involving more species and refinement of emerging techniques are required.

Programme approach and coherence

The intention is to conduct collaborative research on intermediate and recalcitrant forest tree species in developing and developed countries over a period of 4 years. A combination of applied and fundamental approaches is considered essential for the development of methods for handling and storage of tropical forest tree seeds. Whilst developing countries have knowledge of and access to the local trees and their seeds and possibilities for applied studies, developed countries have equipment and research capacity available for strategic and fundamental studies. Therefore, applied studies will be carried out, as far as possible in developing countries, while more fundamental studies will be performed by scientists of developing countries and within PhD programmes involving developing country students.

The planning of experiments with very short-lived seed species is dependent upon their time of harvest. However, as seeds of various species and different origins and, consequently different harvest times, will be used in the project, seed material will be available throughout the year. It is foreseen that IPGRI will facilitate and coordinate the project through an informal research network institutions interested in applied seed research on forestry species, taking into account practicalities such as

seed availability. The basic functions of the network will be to facilitate technology transfer and information exchange among all participating scientists. Over time, it is expected that a wider range of species will gradually be covered and more institutes, especially in developing countries, will be encouraged to participate and to thereby contribute to the conservation and use of forest genetic resources.

Long-term objectives

1. To improve the effectiveness of sustainable forest management and conservation of biodiversity through adequate conservation, use and handling of forest genetic resources.
2. To increase the availability of diversified forest tree species with poor seed storability for use in reforestation programmes and breeding (seed supply).
3. To improve medium- and long-term conservation technologies of tropical forest genetic resources (genetic conservation).
4. To build and strengthen research capabilities in developing countries through training and transfer of knowledge and technology and the establishment of an informal network of researchers on forest tree seeds from developing and developed countries (technology transfer and sharing of experience).

Immediate objectives

1. **Scientific aspects:** To characterize the seed storage behaviour (recalcitrant, intermediate or short-lived orthodox) of specific, valuable tropical forest trees and to recommend regimes for their short- and medium-term storage.
2. **Seed supply aspects:** To develop effective technologies/methods for seed collecting, transport, storage, testing and seed health aspects.
3. **Genetic conservation aspects:** To develop guidelines for genetic conservation of seeds of forest species (or groups of species).
4. **Technology transfer:** To produce a publication and practical guidelines for the handling of forest tree seed species, dealing with all aspects from ripeness, harvest and storage to testing and sowing. To develop and publish guidelines for genetic conservation of valuable forest genetic resources.

Outputs

1. Publication of an updated list of tropical tree species with specific information about recalcitrant, intermediate seed and recommended regimes for their short- and medium-term storage. This information will be included in the IPGRI/CIFOR/ICRAF Forest Genetic Resources database.
2. Guidelines for the practical handling of forest tree species, dealing with all aspects from ripeness, harvesting and storage to testing.
3. An informal research network with researchers from Forest Research Institutes from developing and developed countries.
4. Staff from cooperating countries trained; technology and know-how transferred.
5. An international workshop on seed aspects of forest tree species, with special reference to intermediate or recalcitrant seeds for conservation and use.

Development of international cooperation on seed storage of forestry and agroforestry tree species

As a result of a workshop held in 1992 in Rome, a CIFOR/ICRAF/IPGRI project was developed with the objectives of defining a clear international forestry and agroforestry genetic resources research agenda and of establishing an information system on forestry and agroforestry genetic resources. As a result of this project, three reports have been prepared, one of which is specifically devoted to germplasm collecting, *ex situ* conservation, *in vitro* culture, seed and pollen storage, germplasm health and disease indexing (Tompsett 1993).

Among the priority areas identified for CGIAR attention, in close collaboration with relevant research groups, were seed handling and storage of intermediate and recalcitrant forest tree seeds, as well as alternative methods to enhance the conservation and use of these genetic resources.

Priority research issues and activities

The project's research and training involve the following major topics. For detailed information, see Engels, this volume.

1. Studies on physiological characteristics: the storage behaviour (desiccation and chilling sensitivity) and germination requirements of seeds and/or embryonic axes of the various forest tree species
2. Physical and biochemical studies on the causes of desiccation sensitivity and chilling sensitivity
3. Treatments of seeds to induce desiccation tolerance and chilling tolerance
4. Studies on (inhibiting growth of) microorganisms (mainly for recalcitrant species)
5. Alternative germplasm conservation methods (for recalcitrant and intermediate species).

All topics also include training components, either through group training or on-the-job training. Close collaboration between advanced and less advanced research institutes will facilitate this process.

Work programme

General project management

The project will be coordinated by IPGRI. The various tasks will be carried out by teams of participants according to their area of interest and expertise. The teams are based on geographical distribution, on existing cooperation between developed and developing country institutions and on specialization of institutions. In each team of participants, one team leader will be responsible for the coordination of the work. For each participant, a detailed timetable will be defined on a yearly term.

Five to ten PhD students from developing countries will carry out research work in collaboration with researchers from developed country institutes. In addition, a total of approximately 20 students will perform MSc studies in developing countries.

An informal international research network among researchers on forest tree seeds (including researchers who do not participate directly in the project) from developing and developed countries will be formed and will implement experiments on seed physiology/storability/handling, following agreed protocols resulting from the research work of the present proposal.

Project impact

The project is envisaged as having environmental impact, impact on development and impact on research capacity and achievement as detailed below.

Environmental impact

The project is aimed at solving seed storage and handling problems which hamper the use of various valuable tree species in afforestation programmes. The project will lead to an increase of genetic diversity in the environment through improved availability of seeds of these forest species.

Relevance for development

Results of the project will be of utmost importance for reforestation programmes in developing countries. A good supply of high-quality forest tree seeds is of great advantage to nursery operators and to local people who make economic use of the trees and their products. Developing countries from the project will benefit through international exchange of forest tree genetic resources and through transfer of knowledge and know-how via the informal network. Security of forest tree genetic resources is essential for maintaining and increasing forest biodiversity.

Innovation

New methods are needed in the field of recalcitrant species. Simple methods and techniques for practical, large-scale handling of intermediate/ recalcitrant tree seeds in developing countries will be produced. Various innovative techniques such as FTIR (Fourier Transform Infra-Red) microspectroscopy and field emission scanning electron microscopy will be used by researchers from developing countries in collaboration with researchers from developed countries. Application of such techniques to recalcitrant and intermediate seeds from tropical tree species opens a new and interesting field to the research of developed countries.

Exploring artificial seed techniques and other alternatives for genetic conservation of recalcitrant/intermediate seeds of forest tree species and the development of complementary conservation strategies form other innovative approaches.

The joint research project will lead to strengthening of research capacity in both developing and developed countries. Results of the project will improve sustainable use and management of forest tree genetic resources and improve the use of more diversified planting material, thus increasing the stability of the forest.

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