## A Global Strategy for the conservation and use of Coconut Genetic Resources 2018-2028

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trees in field genebanks, in farmers' fields and gardens, and in some protected areas such as natural reserves and public places. Other technologies like *in vitro* plantlets or cryopreserved embryos, pollen or tissue could be used but are not yet available and need further refinement.

Emerging powerful new technologies such as molecular genetics, genomics, proteomics and eco-geographical remote-sensing techniques are greatly expanding the methodologies supporting the conservation, management and utilization of genetic resources. Advances in informatics and communication technologies have also markedly increased our capacity to use, analyse and communicate relevant data and information, but are not yet in use in the coconut genebanks.

## 2.2.1 Ex situ conservation methods

*Ex situ* conservation plays a crucial role in preserving many varieties, particularly those that are disappearing from farmers' fields. It forms an essential buffer between users and the fast evolving *in situ* genetic diversity in nature and farms.

Effective conservation and management of coconut genetic resources includes the following routine activities: targeted collecting actions; establishing and maintaining field collections; regenerating old accessions using controlled hand-pollination; effective characterization and identity verification studies; evaluation for important priority traits; information management; safe exchange of germplasm; and sometimes germplasm pre-breeding. Most of these methods have been described in the numerous books available on the COGENT website<sup>5</sup>.

Presently, *ex situ* coconut conservation relies solely on the 24 field genebanks as described in Chapter 1, table 1.1. Conservation in the form of field collections has the advantage that the growing material can readily provide seednuts or pollen. The material remains available for distribution, characterization, evaluation, as well as training and demonstration. The field collections however, are highly vulnerable to pests and diseases, to other natural disasters such as floods, typhoons hurricanes and fires, and to land pressure.

The "Stantech Manual" was published in 1996 (Santos et al. 1996) after an extensive consultation involving researchers from COGENT and International institutions. It provides agreed standardized research techniques in coconut breeding and conservation. In coconut *ex situ* genebanks, the recommended sample size for an accession ranges from 72 to 96 palms for a heterogeneous allogamous Tall population. A lower sample size of 45 palms could be used for autogamous homogeneous dwarfs. These minimum numbers per accession were calculated to represent accurately the genetic diversity of the populations and to allow both consistent characterization and workable regeneration (Ramanatha 2005).

<sup>&</sup>lt;sup>5</sup> Available from the URL: <u>http://www.cogentnetwork.org/manuals-and-handbooks</u>

The use of standardized descriptors contributes to the development of consistent databases and increases the uniformity of documentation and the ability to work with other partners. It also efficiency greater enables in collection management by helping to identify and reduce duplication (Gotor et al. 2008). The last publication of the full list of the international coconut descriptors dates back to 1996 in the Stantech Manual. According to Laliberté et al. (1999) on average, each set of descriptors should be revised approximately every ten years. Some coconut researchers and curators believe that present standard descriptors for coconut do not



Diversity of coconut faces.

allow comprehensively identifying most of the allogamous tall coconut cultivars. There are still no coconut descriptors for roots, inflorescence or pollen morphology, the upper part of the fruit, the three eyes of the nut, and the top of the canopy.

In 2007, a list of 17 minimum descriptors was extracted by COGENT from the previous document. This strategic set of descriptors, together with passport data, is the basis for the global accession-level information system being developed at international level (Yao et al. 2015).

## **About pollination**

Making controlled hand-pollination (CHP) is costly, time-consuming and complex. The visits conducted in many countries by the COGENT Secretariat highlighted that existing written guidelines are not sufficient for a research centre to develop de novo the laboratories and skills needed for making CHPs. For the rejuvenation of a Tall-type accession, the CHPs are implemented over a four-month period; the mature seednuts are harvested one year later, also over four months; then the old accession is removed from the field and replaced by a new one. For regenerating an accession, or for creating a new hybrid between two accessions, researchers often use a minimum of 48 female parents crossed with 24 male parents. Each female parent is pollinated three times with pollen from three distinct male parents. A CHP gives only 1 to 2 seedlings, so this will allow the production of about 200 seednuts within a 4-month period. Production of the seednuts needed for the duplication of an accession will demand one and half years' preparation; and will cost more than US\$2000. Only scientists with healthy research budgets can afford ordering varieties from coconut genebanks.



Emasculation is one of the steps of controlled hand pollination. (R. Bourdeix, CIRAD)



It has long been recognized that sexual reproduction (in absence of vegetative propagation) in an allogamous species variety requires isolation to exclude other varieties. For regeneration of allogamous Tall-types, controlled hand-pollination with inflorescence-bagging is strongly recommended. Regenerating autogamous Dwarf-type is much easier: the stem is shorter and seeds can be produced by open pollination if the variety is sufficiently isolated, or with a simple bagging to isolate the whole inflorescence and ensure selfing.

The Stantech manual gives a detailed description of the controlled hand-pollination process. In 2008, the same subject was developed in a shorter version as a part of the regeneration guidelines for coconut (Konan et al. 2008).

A major constraint that the curators face is the accessibility of the inflorescence for safe and effective hand-pollination<sup>6</sup>. Until recently, the crucial techniques of climbing the palm crown were not discussed in COGENT documents. In 2013, a compilation of 23 videos on palm-climbing was released on the website<sup>7</sup>.

## 'Friends of coconuts'



Coconut palm climbing mechanical device in India. (A. Prades, CIRAD)

One of the major problems experienced in the coconut sector of India particularly in the major producing states like Kerala is the critical shortage of palm climbers. The number of traditional palm climbers is consistently in decline due to the disinterest of the younger generation for this traditional profession. Consequently, the farmers experience difficulty in arranging timely harvesting and also plant protection. To overcome this problem, the Coconut Development Board initiated a novel scheme to provide intensive one-week training courses in palm climbing using a mechanical device, in nursery management and in the control measures for common pests and diseases to groups of educated but unemployed rural youth.

This scheme named 'Friends of Coconut' has already trained hundreds of young men and women, whose services are now available to the farmers for harvesting and plant protection.

Although palms can survive for more than 100 years, the average useful lifespan of coconut accessions in most of *ex situ* genebanks is only 25 to 30 years. For instance, for climbing the palms in Côte d'Ivoire, workers of the international coconut genebank<sup>8</sup>

<sup>&</sup>lt;sup>6</sup> Several climbing devices exist to reach the top of the stem and harvest the fruits but, for hand pollination, it is necessary to reach young inflorescences in the centre of the crown.

<sup>&</sup>lt;sup>7</sup> Available at the URL: <u>http://www.cogentnetwork.org/videos/climbing-the-coconut-palm</u>

<sup>&</sup>lt;sup>8</sup> International Coconut Collection for Africa and the Indian Ocean, located at the Marc Delorme Research Centre, National Centre for Agronomic Research (CNRA) in Côte d'Ivoire.

use large triple aluminium ladders which can reach a maximum height of 14 meters. Thus, palms must be regenerated within 25 to 30 years, before their stems extend beyond 14 meters. If taller, it will be impossible to conduct controlled handpollinations, unless new techniques are developed. A better, safer technique could enable casualty-free regeneration to be conducted only every 60 years.

DNA studies show that about 50% of total coconut genetic variation is presently found within heterozygous Tall-type cultivars. Whether the germplasm is transferred (or regenerated) by seednuts, by *in vitro* culture of zygotic embryos, or by any other means, the traceability of the planting material genealogy is crucial for three main reasons and thus, needs to be properly recorded. Such traceability allows:

- Curators to know the numbers of female and male parents used at each generation and to control the genetic drift;
- Checking the reliability of progenies by using DNA markers;
- Conducting genetic studies and comparing the progenies of different parents from the same variety. For instance, within a cultivar, the progeny of a given palm can be tolerant to a particular disease while the progenies of the other palms will die.

For each coconut palm conserved in a genebank, at least its mother palm needs to be known. Presently, less than 20% of accessions conserved at global level meet this requirement<sup>9</sup>.

*In vitro* collections are not yet used for safety duplication of the field collections or for rapid multiplication and dissemination of disease-free planting material. A benefit of the approach is that it can act as a barrier to the transmission of many diseases. A drawback of *in vitro* conservation is that the material demands regular sub-culturing and might be subject to somaclonal variation. Therefore, rejuvenation and verification of the trueness-to-type of the conserved germplasm will have to be performed periodically. Another major drawback is that today, very few of the coconut *ex situ* genebanks are equipped to use this technique or have appropriately skilled staff.

Cryopreservation offers a complementary means to enhance the security of germplasm collections (Nguyen et al. 2015). Storage of frozen pollen samples may offer an additional way to conserve coconut, though the parent's genetic identity would not be maintained as a whole. Frozen pollen is already used in some of genebanks, such as in India.

<sup>&</sup>lt;sup>9</sup> Two different cases exist:

<sup>-</sup> Open-pollinated seednuts collected from farmers' fields: In this case, for allogamous varieties such as Tall-type coconuts, the father palm is unknown. A unique number must be allocated to each mother palm harvested in farmers' fields. Ideally maps of farmers' and genebanks' fields are documented, using a geographical system and/or satellite image. The unique number given to each harvested palm features on both types of map.

<sup>-</sup> Seednuts or embryos harvested in ex situ genebanks. In most of the cases, for allogamous varieties such as Tall-type coconut palms, both female parents and male parents are located in the genebank. The seednuts are obtained by controlled pollination. In this case, the female parent number and the male parent number must be kept carefully even after the progeny palms have been planted.