Manual on Standardized Research Techniques in Coconut Breeding

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International Plant Genetic Resources Institute
IPGRI

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FOREWORD

Coconut is planted in over 11 million hectares in more than 85 countries with 96% of this hectarage in the hands of smallholders cultivating 0.4-5.0 hectares. World yields have been declining in recent years from 710 kg copra per hectare in 1976 to 430 kg in 1984 and 400 kg in 1992. Farm productivity has also declined, decreasing the competitiveness of the coconut and the incomes of millions of coconut farmers.

One of the ways to increase farm productivity and competitiveness of this important smallholder crop is to develop improved varieties that possess the capacity for high productivity, resistance to pathogens and adaptation to specific growing environments. It is also important to conserve coconut genetic resources which could serve as the fundamental basis for a strong breeding programme. Admittedly, these are big tasks because of the perennial nature of the coconut research and the lack of well-trained researchers.

To overcome the above constraints, the International Plant Genetic Resources Institute (IPGRI), upon the recommendation of 15 coconut-growing countries, established the International Coconut Genetic Resources Network (COGENT) to coordinate research activities of national, regional and global significance particularly in germplasm exploration, collecting, conservation and enhancement and to establish a basis for collaboration on the broader aspects of coconut research and development.

One of the first activities of COGENT was a workshop to standardize coconut breeding research techniques. This was held at the Station Marc Delorme Cocotier, IDEFOR in Côte d'Ivoire on 20-25 June 1992 through the support of GTZ, BUROTROP and IPGRI. During this workshop, coconut breeders from 16 coconut-producing countries and experts from CIRAD and IPGRI formulated the first draft of this manual. The draft was pre-tested and revised during the Trainers' Course on Coconut Breeding Research Techniques which was held at the Research Institute for Coconut and Palmae, in Manado, Indonesia which was attended by 21 coconut researchers through the support of the Asian Development Bank. The manuscript was then edited and forwarded to IPGRI for review and publication.

The STANTECH Manual, as this document has been called, will enable coconut breeders and germplasm researchers worldwide to use standardized techniques as guidelines in breeding and germplasm conservation. It is hoped that this manual will help coconut researchers to obtain better and comparable results to accelerate the development of improved varieties for millions of coconut farmers.

COGENT is indebted to the GTZ, BUROTROP and ADB for providing financial support in the formulation of this manual, to the ODA for funding its publication and to CIRAD, IPGRI and the researchers in the Côte d'Ivoire workshop and Indonesia trainers' course (Appendix 1) for their technical inputs. Finally, COGENT wishes to thank the editing team for executing a difficult task, Mr. F. Bonnot of CIRAD for advice on the statistical content of the manual and Dr. V. Ramanatha Rao of IPGRI, who critically reviewed the manuscript.

Basil Been
Chair, COGENT Steering Committee

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Chapter 1

BOTANY OF THE COCONUT PALM

Coconut (*Cocos nucifera L.*) is the sole species of the genus *Cocos* belonging to the subfamily Cocoideae which includes 27 genera and 600 species. It is a diploid with 32 chromosomes (2n=32). As such, hybridization is mainly intraspecific.

The major classification of coconut based on stature or height is as follows:

(1) **Tall palms,** sometimes referred to as var. *typica* (Nar). They are widely planted both for household and commercial use and grow to a height of 20-30 m. They are slow maturing and flower 6-10 years after planting. They are long-lived with an economic life of about 60-70 years, although much older palms are known to exist and yield well. They are normally cross-pollinating and therefore considered to be heterozygous.

(2) **Dwarf palms,** sometimes referred to as var. *nana* (Griff). These are believed to be mutants from tall types with short stature, 8-10 m when 20 years old. They begin bearing about the third year at less than 1 meter high. They have a short productive life of 30-40 years. They are normally self-pollinating and therefore considered to be homozygous.

The Roots

The palm has adventitious roots continually produced from the basal 40 cm or so of the trunk, which is the swollen part or what is termed 'bole', in tall types and in some dwarf hybrids. It has no taproot or root hairs but has lots of primary roots which bear large quantities of rootlets.

The main roots grow out somewhat horizontally from the bole and are mostly found within the topsoil. The main branches grow deeper and may extend laterally to as much as 10 m.

The roots, having no carnbium, are noticeably uniform - the main roots reaching a maximum diameter of about 1 cm. The root tip is the actively growing region and behind it is the absorbing area whose epidermis is a single layer of thin-walled cells that gradually thicken and become impervious with age. In old roots, the epidermis disintegrates and exposes the hard hypodermis which is generally red.

The root centre has a stele surrounded by a single-celled pericycle sheath from which rootlets and aerenchymatous (respiratory exchange) protuberances or pneumatophores arise. The respiratory exchange occurs more abundantly nearer the sod surface to allow easy diffusion of oxygen into and carbon dioxide out of the root.

The Stem

The stem develops from the single terminal bud called the 'cabbage' which is the palm's only vegetative growing point. Under favourable conditions, the foundation of the trunk of a young palm reaches full development within 3-4 years.

In the tall types, the base of the trunk is up to 0.8 m in diameter, tapering quickly to about 0.4 m (Child 1974). Once formed, the trunk does not change much in diameter. If variation occurs from base to crown, this is not caused by biological factors but by climatic conditions and cultural practices.

Stem growth is fastest at early stages, which can be as much as 1.5 m per year. The incremental growth rate levels off as the palms grow old; up to 10-15 cm per year at about the 40th year and over.

The coconut stem has no cambium. Hence, it cannot regenerate damaged tissues. However, a mature palm may have as much as 18 000 vascular bundles which help it to withstand significant physical damage to its trunk, provided pest entry is prevented.

The Leaf

The first leaves of a coconut seedling have the pinnae fused together and appear as entire leaves. After eight to ten have been formed, subsequent leaves tend to split into leaflets. After about 3-4 years, the stem starts to form with a single terminal growing point where new leaves develop. Generally, a normal adult palm produces 12-16 new leaves annually, each bearing a corresponding flower cluster (inflorescence). There are about 30-40 leaves in a healthy crown with a similar number of leaf primordia, each differentiated about 30 months before it emerges as a 'sword leaf'. A mature leaf is 3-4 m long and has 200-250 leaflets. A leaf remains on the palm for about 3 years and thereafter, shed leaving a permanent scar on the trunk.

The age of an adult palm is correlated with the number of leaf scars. The number of scars on the stem, divided by 13, gives the approximate age of the palm in years (Mahindapala 1991). This may be important in estimating the age of existing palms used as parent materials in breeding.

The Inflorescence

The coconut inflorescence is enclosed in a double sheath or spathe, the whole structure known as a 'spadix' which is borne singly in the axil of each leaf. The palm is monoecious, i.e. its inflorescence carries both male and female flowers. The male flowers are more numerous than the female flowers. The former are borne on the top portion of spikelets which are attached to a main axis or peduncle. The female flowers are situated at the base of the spikelets.

The inflorescence primordium can be detected about 4 months after the first leaf primordium is differentiated; the male and female flowers, 22 months thereafter. The opening of the fully grown spathe occurs 1 year later.

The male flowers are the first to open, beginning at the top of each spikelet and proceeding towards the base. After each flower opening, the pollen is shed, and male flowers abscise, the whole process taking just 1 day. The male phase, however, takes about 20 days in most palms but this may vary according to season and variety.

A female flower remains receptive from 1 to 3 days. Depending on the environmental conditions and variety, the female phase may begin a few days or later after the spathe has opened and lasts 3-5 days in tall palms and about 8-15 days in dwarfs. A normal inflorescence may have 10-50 female flowers. With natural pollination, 50-70% usually abort and fall off, especially those which emerge during severe dry weather. The remaining flowers develop into fruits, which take about 12 months to mature.

The length of the male and female phases is affected by climatic environment and usually do not overlap in the tall types, such that self-pollination rarely occurs. In some dwarfs, particularly the Malayan Dwarf, overlapping of the male and female phases and between spadices usually takes place, promoting selfing. Hence, these dwarfs are reasonably homozygous.

The Fruit

Once pollination and fertilization occur, fruits set and develop to maturity in about 12 months, or less than 1 year for some dwarf cultivars. A count of bunch and fruit set can give a reasonable estimate of yield.

The fruit is a fibrous drupe but with a smooth outside skin (exocarp), which may vary from green to red brown or even ivory. The coat (mesocarp) in the young coconut is white and firm. On the other hand, the ripe nut has a fibrous mass, the husk, from which coir is obtained. Within this fibrous mass is the nut with a hard shell (endocarp) enclosing the kernel (endosperm). Between the shell and the kernel is a thin brown seed coat (testa). It adheres firmly to the kernel which is the white flesh, about 12 mm thick lining the central cavity containing the nut water. Towards the end of maturation, the volume of water in the cavity decreases considerably which may be due to absorption by the endosperm tissue or to evaporation. Matured nuts have a sloshing sound of water inside when shaken. Yield is usually estimated in terms of the number of nuts produced per palm or unit area and weight of equivalent copra.

Chapter 2

STUDYING GENETIC DIVERSITY IN SITU

There are several methods of studying genetic diversity. These include morphometric, biochemical and molecular methods. For this manual, only the morphometric method will be discussed as procedures for use of other methods are yet to be standardized.

Studying genetic diversity *in situ* and the exploration and survey of new sources of germplasm are two of the four main activity areas arising from the principal objectives of the International Coconut Genetic Resources Network (COGENT). These activities include taking palm and nut samples within the identified sampling sites following the International Plant Genetic Resources Institute's *(1PGRI) Coarse Grid Sampling Strategy* (Chapter 10). The most ideal practice is for the identified populations to be observed for six months to one year *in situ* following a regular schedule. *In situ* work requires the observation of the populations based on an individual palm performance. Hence, all the observed sample palms could also serve as source palms for collecting seednuts for the genebank. The latter is described in Chapter 3.

Guide to sampling

An accurate description of the populations *in situ* is crucial because this will be the only guide for their use in breeding programmes in the intervening period from the time the surveyed populations are collected to over a decade of complete evaluation and characterization. This description involves two aspects'.

(a) Identification of the populations in accordance with the IBPGR list of descriptors as discussed in Chapter 11, and

(b) Characterization of a certain number of plant and fruit components. These measurements should be taken from a random sample of 30 normal palms (discarding diseased or atypical palms) in the chosen site. Wherever and whenever possible, one ripe nut should be analyzed per studied palm. Otherwise, 30 nuts are taken from the heap.

Depending on field situation, it is necessary to consider the following sampling techniques:

Random palm sampling - *This* is the most ideal technique. The collector chooses at random 30 sample palms and obtains one representative nut per palm.

Random heap sampling - *This* is done when nuts from the sampled palms are already harvested and heaped or piled at the time of the visit. Sample nuts are picked at random from the heap. Only undamaged, fully ripened ungerminated nuts are taken.

Characters to be observed

The following list of parameters is not exhaustive and should be seen as a basis for minimum comparison. Details of procedure are found in Chapter 7.

(a) Stem morphology (figs. 1 & 2)

- Girth measurement at 20 cm above soil level (cm)
- Girth measurement at 1.5 m height (cm)
- Length (m) of stem with 11 leaf scars, measured starting from the bottom of the first leaf scar to the bottom of the 11th leaf scar

(b) Overall appearance/shape of crown (fig. 3)

1 Spherical
2 Semi-spherical
3 X-shaped 'silhouette'
4 V-shaped
5 Other (specify)

(c) Leaf morphology (figs. 4 & 5)

The observation is normally made on leaf #14, which is the leaf subtending the bunch with fist-size nuts. For practical reasons, however, in old coconut palms and if age is not precisely known, take the oldest fully mature green frond by detaching the entire leaf (petiole included) from the stem using a sharp machete or bolo. The following data should be gathered from the sample leaf.

- Colour of petiole

1 Yellow	7 Green
2 Yellow-Red	8 Green-Yellow
3 Red-Yellow	9 Yellow-Green
4 Red	10 Brown
5 Red-Green	11 Other (specify)
6 Green-Red	

- Petiole length (cm) - from base to the most proximal leaflet
- Petiole thickness (cm) - measure at insertion of first leaflet
- Petiole width (cm) - measure as above
- Rachis length (cm) - from the base of the petiole to the tip
- Number of leaflets - count on one side of the frond that has the first leaflet closest to the base
- Leaflet length (cm) - use four leaflets (two on each side) near the middle of the rachis and record average of four measurements
- Leaflet width (mm) - use the same leaflets as above and record average (at maximum width) of four measurements

d) Inflorescence and flower morphology (fig. 6)

Preferred samples are inflorescences with male flowers open (may be used for pollen collection if necessary), one inflorescence per palm.

Type

1 Normal
2 Spicata (full or partial)
3 Androgena
4 Additional spathes or bracts
5 Other (specify)

- Overlapping of male and female phases:

  - Presence of receptive female flowers on palm
  - Presence of open male flowers on palm
  - If both are present, are they in the same inflorescence?

- Length of peduncle (cm) - distance between the point where the bunch is attached to the palm and the base of the first spikelet

- Length of the central axis (cm) - measure from the first spikelet to the end of the axis

- Diameter of the peduncle (cm) - at the insertion of first spikelet

- Number of spikelets with female flowers

- Number of spikelets without female flowers

- Length of first spikelet bearing female flower

- Total number of spikelets

- Number of female flowers can be counted from scars if flowers have already been shed

- Female flower distribution per spikelet: number of female flowers divided by total of number of spikelets

(e) Fruit appearance

All fruits analyzed should be mature, i.e. when changing from fresh to dry fruit and water sloshes when fruit is shaken.

- Fruit set (visually estimate number of fruits bigger than a fist in each sample palm)

  1 0 to 10
  2 11 to 20
  3 21 to 50

4 51 to 80
5 81 and above

- Fruit colour (less than 6 months old) - follow colour code description for petiole

- Shape of fruit (polar view, figs. 7a & 7b)

  1 Round
  2 Egg-shaped
  3 Pear-shaped
  4 Elliptic
  5 Other (specify)

- Shape of fruit (equatorial view, fig. 8)

  1 Round
  2 Angular
  3 Flat
  4 Other (specify)

- Appearance/shape of husked nut (fig. 9)

  1 Flat
  2 Pointed
  3 Ovoid
  4 Almost round

(f) Fruit component analysis (FCA)

- Fruit weight (g)
- Nut weight (g)
- Weight of split nut (g)
- Shell weight (g)
- Meat weight (g) - difference of shell weight and the weight of the split nut

(g) Endosperm

Measure (mm) the endosperm thickness, at the equatorial portion of 10 mature fruits (more or less 12 months old) and take the mean. Do not include makapuno (gel-endosperm) types.

Data analysis

Statistical analysis of the palm and nut/fruit characters observed in the survey situation is complicated because the characters observed may have been influenced by the environment. Hence, any information available on the soil and climatic conditions and the probable origin of the populations has to be taken into account when interpreting these results.

It should be borne in mind that the characters to be studied are those that are highly heritable. In some cases it may be even better to consider the ratio between two measurements, as in number of nuts, copra per nut and copra per palm, rather than the basic data. Studies conducted within the experimental station could provide clearer answers to these questions.

Simple analysis of variance (ANOVA) is sufficient for determining coefficient of variance when dealing with effect of treatments (e.g. cultivars) on individual parameters (e.g. yield). However, to compare several parameters or characters, a multivariate analysis is required.

There are several methods of conducting multivariate data analysis. The choice of a method depends on the problem to be worked out. If the problem is to represent a set of individuals without preliminary grouping, *principal component analysis* can be used. The purpose of this technique is to condense the information given by the original variables into a smaller set of new independent variables.

Comparison of populations can be made with homogeneity tests. *Hotelling's T^2 test*, for instance, can be used to test the equality of the means of two populations with several variables. For more than two populations, *analysis of dispersion* (multiple analysis of variance) can be used. Discriminant analysis can be used to plot points representative of the populations on main axes taking into account within-population variability, and to compute *Mahalanobis distances* between populations. Mahalanobis generalized distance between two populations is presented in Appendix 2.

Representation of distances between individuals can be defined with classification methods. *Dendograms* built with clustering techniques can help to define groups of individuals. *Additive trees* can be interpreted as phylogenetic representations to relations between individuals. All these methods can be applied to populations as well as individuals.

Another problem is to allocate individuals to groups previously defined. In this case, discriminant analysis or *Fisher's discriminant functions* can be used.

A biometrician is particularly helpful in analyzing quality data (colour, fruit shape, etc.) or discontinuous variables (e.g. fruit set) which require special coding. The use of a data management system software to facilitate analysis should be explored.

Chapter 3

GERMPLASM EXPLORATION AND COLLECTING

Choice of population

The success of a germplasm survey, either for studying genetic diversity or for collecting nuts for a germplasm collection, is largely governed by the choice of sample population. Ideally, it is best to identify and collect as much genetic variability as possible. However, the available area, the financial resources and the ability of the organizations involved in the conservation, evaluation, characterization and use of genetic materials impose certain limitations on the number of populations to be observed and collected.

In the absence of an expressed selection criterion, it is often best to set the number of sample populations based on a well conceived and balanced collecting scheme that takes into account the limiting factors mentioned. The coarse and fine grid sampling strategies can be used. An effective use of available data based on a rational approach prior to actual collecting, e.g. multivariate analysis, should increase survey efficiency.

In some instances, outright collection of seednuts may be done provided that the area is free from diseases and pests, and if the population possesses highly distinctive traits or features which would discriminate it from other populations. Transport of seednuts should be done following the technical guidelines for safe movement of coconut germplasm (Frison *et al.* 1993). The approach proposed below is intended to guide researchers in their choice of population.

1. Set the objectives of the collecting survey.

   The collecting strategy will differ depending on the objective, as follows:

   - If it is to describe and exploit the genetic diversity existing in a given region without a preconceived idea of the characters sought, a systematic sampling of the population should be attempted;

   - If the purpose is to collect genes coding for particular characteristics (sometimes called a target collection), focus should be on the population most likely to contain the desired characters; and

   - If the aim is to safeguard threatened populations for their conservation and subsequent use, population disappearance risk factors should then be identified (cyclones, pests and diseases, urbanisation, crop shifting or conversion, etc.) to pinpoint the collection area.

2. Identify all the populations from which the samples will be collected.

   Depending on collecting strategy, the geographical zones to be covered should be fixed precisely and an inventory of the population involved should be made. Prior reconnaissance of the zones is useful at this stage.

3. Obtain information about the population

Apart from odd exceptions, coconut is historically and closely associated with human activities, whether this involves the setting up of large estate plantations, or traditional small-scale cultivation. Its geographical distribution is probably reflected in historical records related to its establishment and adaptation to the environment, which usually take many generations. The search for written documentation or information by word of mouth should therefore cover such diverse fields as ethnobotany, ecology, agronomy, cropping systems, plant pathology and, of course, genetics. In this way, maximum information can be obtained on the probable origin, adaptation characters, yields and uses of the target population.

These data should be sought not only in scientific literature such as lists of flora, books, studies and survey reports, but also from other related sources like agriculture services, agricultural research organisations, plantation companies, farmer cooperatives, botanical gardens, local people, etc.

4. Gather pre-prospection data *in situ.*

In situ measurements of growth habit and of fruit components, as described in Chapter 2, are valuable tools for the choice of population to be collected. These measurements should be carried out before the actual collecting. However, if this is impossible, these observations are still useful to form an essential guide for collection management, e.g. numbers to be kept, breeding objectives and logistical requirements.

Collecting samples

Once the choice of population has been made, sample type (nut, pollen and/or embryos) and the have to be fixed, along with the number of sample palms. The ultimate target is to obtain sample size representative samples that possess the genetic image of the surveyed population.

When one is dealing with seednuts corning from several populations and/or origins, a great bulk of the errors in their identification are committed when nuts arrive at the receiving station without proper seednut and population dispatch information. A flow diagram for proper handling of seednut collections and seednut dispatch is shown in Appendix 3.

Although transport costs for nuts can become prohibitive under most circumstances, seednuts are the simplest and often the cheapest form of collection. Seednuts enable the quick reproduction of the population for characterization, and genetic evaluation and utilization. Care must be taken to ensure that the source palms are free from any disease or pest.

Transfer to genebanks

The coconut fruit takes more or less 12 months to ripen from the time of pollination. However, 11-month old nuts are considered physiologically mature and can be harvested. These nuts can be easily identified when the husk of one or two nuts in the bunch start turning brown.

In theory, sample size for dwarf types can be smaller than that of the tall types since dwarfs are relatively homogeneous. However, as long as the mode of multiplication is through the seednut, dwarfs will often be used as female parents or mother trees for hybrid production, hence they should be well represented.

To conserve and at the same time characterize the sampled population as effectively as possible, they should be planted following an appropriate design. Tall and dwarf types should be planted separately, using one control cultivar for each type, with at least three replications. Planting distance is 9 m triangular for tall and 7.5 to 8.0 m for dwarfs. A single accession comprising three replicates and elementary plots of 5 x 6 palms (i.e. 90 palms per accession) is appropriate. This design can be modified if a sufficiently large plot is not available. However, each accession should be represented by at least 72 palms, with a minimum of two replications, set out in square or rectangular plots or blocks of at least three rows per replicate. Failing a block design, it is essential for the control to be sufficiently represented (one control plot for every four collection plots).

Under certain circumstances such as when population size is inadequate, or when only a small quantity of nuts is available, or the germination of the nuts is poor, the sampled population may be preserved while additional collecting is undertaken to meet the desired population size.

If collecting cannot be repeated, the preserved material risks being a biased and incomplete sample of a population study. However, if the population has been chosen for precise breeding purposes as in a target collection, its preservation as a gene source is justifiable.

General approaches to collecting coconut germplasm

Collecting of nuts for ex situ *conservation.* Choose 100 normal palms at random towards the middle of the population and take a sample of two nuts per palm to generate a total of 200 nuts. If the number of palms in the population is too small, the number of sample nuts per palm can be increased. Between 80 and 100 plants should be planted in the genebank. Sample nuts from heaps should only be used when there is no alternative.

Pollen collecting. When pollen and seednut collecting are combined, the value of both the pure population and its hybrids can be studied at the same time, thereby gaining a breeding cycle. It quickly enables a study of a population when hybridized and/or recombined with another more advanced material.

Pollen collecting involves harvesting male flowers from 25 to 30 palms chosen at random towards the middle of the plot. If the collecting site is near the research station, the male flowers could be packed separately. Otherwise, pollen should be taken from all the inflorescences and mixed to produce a pack of bulk pollen representative of the sampled population.

Embryo collecting. The technique of embryo culture was first developed by Dr E.V. de Guzman at the University of the Philippines at Los Baños in the early 1960s. Aside from rescuing embryos from the lethal effects of the endosperm as in the makapuno coconut, it has been used for wider applications such as in the safe transport of coconut genetic materials (in bulk) over long distances. It merits the same applications as for nut collecting, with the additional possibility of cryopreservation and experimentation under laboratory conditions. Over the years, the technique has undergone a series of refinements and modifications to serve today's requirements.

Embryo samples should be taken according to the method recommended by 1PGRI (Assy Bah *et al.* 1987) which is described in detail in Chapter 9. Up to 600 embryo samples (200 for field collection, 200 for cryopreservation and 200 for exchange) can be taken from at least 100 palms.

Chapter 4
NURSERY MANAGEMENT

The rearing of coconut seedlings in a well-maintained nursery facilitates efficient selection of normal uniform seedlings. It permits equal and unbiased application of treatment and evaluation. Care must be taken in choosing the seedlings to start a plantation since plantings will be in the field for many years. The nursery management techniques described herein can be applied for both the genebank and hybrid trials.

Nursery site selection

A good nursery should: (a) be open, level and well-drained; (b) have light or loose-textured soil to facilitate nursery operations; (c) have a good source of water without possibility of being flooded; (d) be accessible to transportation; and (e) be far from existing potential sources of coconut insect pests and diseases, e.g. sawmills, pile of decaying logs, dump site of animal manure, etc. A nursery site with a minimum area of 3,600 m^2 is needed to accommodate about 12,000 seednuts for 50 ha.

To be fully operational, the nursery should have a fence for security; a shed to house the implements and supplies; farm implements and small equipment; a source of water for irrigation; and sufficiently trained manpower.

The seedbed

Seedbed preparation - The seedbed (fig. 10a) should preferably be in the centre of the nursery. To facilitate sowing of nuts, it should be cleared, plowed and harrowed to a fine tilth. Seedbeds are prepared with the following dimensions.

elevation:	10-20 cm high to provide drainage
width:	1 m to avoid stepping on seednuts during maintenance and transfer operations
length:	a 2 m long seedbed is ideal for easy inspection, management and maintenance
pathway:	1 m between seedbeds should be provided to facilitate inspection, selection, pricking, maintenance and seedling transfer activities.

Sowing of seednuts - Nuts are planted by firmly setting them either upright or slightly tilted with the germ end at the top (fig. 10b). The nuts are set close to one another to prevent them from floating in case of heavy rains. The nuts are then covered with soil, with about 2/3 of their size buried. In addition to keeping a record file, a signboard, placed in front of each bed, provides the following information:

Name of variety/ type
Date of sowing
Number of nuts sown
Seedbed number
Date when nuts are harvested, if available

Maintenance of seedbed - This activity involves daily watering except when it is raining; weeding, if necessary; partial shading, when needed; and inspection for disease and pest incidence.

Pricking - *When* the sprout emerges through the husk to a height of 4-6 cm, seedlings are planted in the field nursery either directly in the soil or in polybags, to allow them more space to grow. At this stage, some roots, already out of the husk, might have been injured in the process of pricking. It is therefore necessary to trim them before transferring in the field or polybag nursery. The trimming of these roots assists the seedling to establish quickly as it will induce the seedling to produce more roots. Seedlings of the same age are pricked on the same day and immediately planted in the field or polybag nursery. It is very important that pricking is done only when the field or polybag nursery is already prepared. Pricking can be scheduled once a week. The date of seed germination is recorded in a field book as well as the date of each transfer to the field or polybag nursery. After pricking the germinated seednuts from the seedbed, the vacated plot is refilled with sod so as not to destabilize the remaining ungerminated seednuts. The optimum waiting period for ending the observations of germination in each seedbed is around 16 weeks from the date of sowing or when 85% germination had been achieved, whichever comes first.

The polybag nursery

As mentioned above, there are two types of nurseries for rearing coconut seedlings: polybag nursery and field nursery. A polybag nursery (fig. 11) makes use of black polyethylene bags, hence its name. It is preferred over the field nursery because: a) transplanting shock is greatly minimized, thereby promoting early establishment of transplanted seedlings; b) seedlings can be retained longer in the nursery when conditions for field planting are not yet favourable; and c) age-wise, seedling selection is easily accomplished.

Land preparation - Depending on the area, this involves either heavy or light cultivation of the soil and clearing the area of weeds and other existing vegetation and obstructions to facilitate operations like staking and placement of nuts.

Polybagging of germinated nuts - A polybag, preferably black, UV resistant for durability and measuring 40 x 40 x 0.015 cm (for smaller nuts) or 45 x 45 x 0.015 cm (for bigger nuts) with 8 - 10 holes at the bottom sides, is half filled with soil and compost mixed at 50:50 ratio. Decomposed sawdust, corn cobs, rice hull and other organic materials can be used. This will reduce the weight of the half-filled polybag and improve soil fertility. If polybags are not gussetted (Note: polybags with folds at the bottom may be ordered), the bottom corners should be folded inward to make the bottom of the bag round and for it to stand firmly. The open edge of the bag is also folded back (about 3 cm) to prevent it from tearing easily. The germinated nut is then placed in the half-filled bag with the sprout in an upright position in the centre of the bag. Next, the bag is filled with soil with the sides slightly pressed to keep the nut firm until it is fully covered. As the soil settles, it will cover up to 2/3 of the nut after some time. When the polybagged seedlings are ready, they are laid out in the polybag nursery.

Laying out of the polybag nursery (staking) - Equal setting of the seedlings at optimum distance dows them to grow and develop normally. The technique follows a. triangular system with equal spacing of 60 em. The materials needed are the following:

- about 30 m of rope or twine for establishing a straight line and making a 3,4,5 triangle
- 100 pieces bamboo pegs or equivalent, 30 cm long
- a measuring tape or stick
- twine for marking 30-cm spaces, about 10 m long
- pieces of stick, 52 cm long
- a sharp bolo, machete or its equivalent
- compass
- marking pen.

Figure 12 shows how these materials are to be used to attain a 60-cm spacing in a triangular manner. To minimize shading, rows should be oriented in a North-South direction.

As a start, about 10 m of twine (preferably cotton) is marked every 30 cm along its entire length. First, establish a straight line and a 90º corner with the longer side towards the North. This is done easily by making a 3,4,5 triangle using the 30 m rope. Boundaries are set by means of rope or twine to guide the setting of rows. This is made to establish four straight boundary lines and four 90º corners.

The next step is to lay the 10 m string in the direction of the North and then mark the 30 cm spaces. This becomes line 1 or row 1. To make line 2 or row 2, measure 52 cm down towards the East (or West) in both ends of row 1. Then align the string marked every 30 cm and subsequently peg the 30 cm marks. Repeat the procedure to make rows 3,4,5, etc. To establish 60 cm, remove the pegs every other 30 cm starting from the second mark in line 2. These are marked (x) in Figure 12. As the work progresses, and as the workers gain experience, the marks are pegged every 60 cm by estimation.

The size of a polybag nursery could be 3 x 6 m with about 1.5 m spaces between plots. Each plot will easily accommodate 115 seedlings. While only 72-96 palms will be used, extra seedlings might be needed for future use as replacements.

Setting the polybagged seedlings - The polybagged seedlings are placed in front of the stake, set firmly and with the erect shoot aligned at the stake. Since centering of the sprout in the polybag may not be precise, the emerging shoot should be set in only one orientation at a point closest to the stake.

Set the polybagged seedlings in the same order as they germinated. The earliest germinating seedlings are placed in the first row in the eastern side of the area. The last ones to germinate are placed at the western section of the area. This practice reduces competition for sunlight from among the earliest and latest germinating seedlings. Most importantly, selection of vigorous seedlings is facilitated and since the first pricked seedlings are placed in rows, culling or judging by age is easily accomplished. A signboard indicating the type/variety, the number of seedlings and date of sowing is installed in front of each plot.

Maintenance of the polybagged nursery - in order of priority, this activity involves watering, weeding, and inspection for pest and disease incidence. Fertilizer application for each seedling is recommended as follows:

Age after germination (months)	**Ammonium sulphate ($(NH_4)_2 SO_4$ 21-0-0 (g)**	**Potassium chloride (KCI) 0-0-60 (g)**	**OR**	**Sodium chloride (NaCI) (g)**
2	20	25		20
5	40	45		40

The fertilizers are mixed and applied directly to the soil around the nuts. Afterwards, the soil is lightly cultivated to promote faster dissolution and absorption of the fertilizer.

At 6-8 months after polybagging, leaf splitting occurs, indicating that the seedlings are ready for field planting.

Chapter 5

EXPERIMENTAL DESIGNS AND DATA ANALYSIS

The *in situ* and *ex situ* evaluation of genetic diversity, the techniques for obtaining or producing the seednuts, and the nursery management of the seedlings have been described in earlier Chapters. This Chapter will focus on the experimental design, the methods used for data collection and analysis for coconut field genebank and for breeding trials.

Basic principles

Three basic principles necessary to provide valid and efficient control of experimental error should be followed in the design and layout of coconut experiments. These are:

Replication - Replication provides an estimate of experimental error; improves the precision of the experiment by reducing standard error of the mean, and increases the scope of inference of the experimental results.

Randomization. This is practised to avoid bias in the estimate of experimental error and to ensure the validity of the statistical tests.

Control of experimental error - Experimental error can be controlled and generally reduced by blocking.

In addition to these basic principles, it is recommended to include a local check variety, which provides the means of comparing the 'gains' or performance of the test materials or treatments with the locally adapted cultivar.

Choice of sample size

Three factors have to be considered when choosing a sample size, to measure a character:

(1) The desired confidence interval **(CI):** it is the interval $[x-b_\alpha, \mathbf{x}+b_\alpha]$ around the average (**x**) of the sample which will include the 'true average' (***m***) of the population with probability $1-\alpha$ (generally, $\alpha = 0.05$).

(2) The coefficient of variation (**CV**) of a single measurement. It is determined by the variability of the population, and the experimental error.

(3) The cost of the sample, which is related to the sample size, **N**.

Let $\mathbf{CI}_\alpha \times b_\alpha/m \times 100$, then these factors are linked by the following formula:

$$\mathbf{Cl}_{0.05} = \mathbf{1.96 \times CV/\sqrt{N}}$$

where factor 1.96 corresponds to α=0.05. This allows us to calculate the sample size as

$$N = \{1.96 \times CV / \mathbf{CI_{0.05}}\}^2$$

Table 1 shows the calculated values for the optimal sample size according to **CV** and the desired $CI_{0.05}$.

Table 1. Optimal sample size according to CV and desired $CI_{0.05}$

Coefficient of Variation (%)									
$CI_{0.05}$	5	7.5	10	12.5	15	17.5	20	22.5	25
5.0%	4	9	16	25	35	48	62	78	97
7.5%	2	4	7	11	16	21	28	35	43
10.0%	1	3	4	7	9	12	16	20	25
12.5%	1	2	3	4	6	8	10	13	16
15.0%	1	1	2	3	4	6	7	9	11

Fruit component characters are generally more variable than vegetative measurements. For this reason and because of their economic importance, the sample size should not be inferior to that of vegetative measurements. At least 30 palms are needed to obtain a CI ranging from ±5% to ±7.5% for FCA.

Experimental design for germplasm collection

Population size - The recommended sample size for each population or variety in a coconut field ranges from 72 to 96 palms for a heterogeneous tall population. Lower sample size could be used for homogeneous dwarfs, but maintaining the same number is advantageous.

Layout - Besides the standard characterization of the accessions in a genebank, assessment of the genetic variability within and between populations is very important. A simple randomized complete block design can be used since environmental differences among blocks are minimized. In a field genebank, it is advisable to use plots of 6 rows of 5 palms replicated three times. Single-row design should be discarded because it is subject to a lot of errors.

Check cultivar or control population - A proper evaluation of cultivars in a coconut collection is conducted in relation with a well-known population used as a control. A dwarf control should be used for the dwarf ecotypes while a tall population should serve as a check for tall varieties.

The frequency of the control cultivar depends on the experimental design chosen. If it is a randomized complete block design, the control should occur in each block. .

In cases where the treatment materials or research area are limiting, treatments can be set in a single block of unreplicated trials, provided the control occurs more frequently, i.e. one for every four entries. The control cultivars are planted at the start and end of every four treatment blocks to enable the comparison of their growth and development. At the Marc DELORME Coconut Research Station in Côte d'Ivoire, as described by De Nuce de Lamothe (1977, 1979, 1981), Sangare *et al.* (1984), N'Cho *et al.* (1988), the Malayan Yellow Dwarf (MYD) is the control for the dwarfs while the West African Tall (WAT) is used for the talls. On the other hand, at the PCA Zamboanga Research Centre, MYD is so use or the dwarfs while the Baybay (BAY) is used for the talls.

Planting density - The optimum planting density for dwarf ecotypes is 180 palms/ha (8 m triangular); for the tall, a density of 143 palms/ha (9 m triangular) is suggested. This density could be increased up to a maximum of 210 palms/ha (7.5 m triangular) for dwarf ecotypes with small crowns or when the land is limiting.

Management conditions for accurate germplasm characterization and evaluation

Any source of heterogeneity which can increase experimental error and reduce accuracy must be avoided to allow a better evaluation of the germplasm. This requires efficient management of the field genebank through an effective interdisciplinary collaboration among breeders, agronomists and plant protection specialists.

Labelling and sampling - To facilitate the evaluation, the labelling system by field number, row and rank of the palm in the row should be adopted. The sample palms for the evaluation of the different varieties should have a specific mark. The use of aluminium labels where marks are embossed and the use of copper wire as tying material ensure the tag is durable for many years in the nursery and in the field. In areas where field workers are not highly qualified, a very simple labelling method is more appropriate. Simple labels are generally preferred since size for studying morphological traits and FCA is 30 palms per variety.

A regular harvesting method (monthly on the dwarfs and d x d hybrids; and bimonthly on the d x t and t x t hybrids, and tall) should be followed for yield data collection. For the fruit component analysis the frequency is bimonthly which is described in greater detail in the FCA section.

Experimental designs for hybrid tests

This section presents the layout of the breeding trials and the conditions for efficient gathering and analysis of field data.

Optimum population size - For population or hybrid trials, 24 male parents crossed with a sufficient number of female parents (minimum of 48 palms) are enough to allow the production of sufficient seednuts (144) required for each combination within 3 months.

Given a selection rate of about 67% at the seedbed nursery stage, these seednuts will give 96 seedlings which is the optimum size to use per hybrid entry (t *x* t; d x d or d x t). These estimates may vary based on specific research station conditions.

Experimental layout - The overall objective of the experimental design is to reduce the experimental error and increase the accuracy when comparing treatments (materials tested for yield, disease and other environmental stress tolerances). Randomized complete block, the latin square, and balanced incomplete block designs (Cochran and Cox 1957; Fisher 1960; Addleman 1970) are often used by coconut breeders. The choice of a design will depend on the objective of the trials, the genetic structure of the test material, the number of entries and homogeneity of the experimental field.

For example, in a yield trial involving a general combining ability test between ecotypes (d *x t or t x t),* a simple randomized complete block design is adequate. The latin square design is more suitable if two gradients are to be controlled. On the other hand, a balanced incomplete block design is preferred when a specific trait of the individual palm is the point of concern because this kind of trial involves a large number of half-sib families. The design for disease resistance study under natural exposure conditions is similar to the layout for field trials. Aside from these block designs, the completely randomized design (CRD) with a single-palm-per replicate arrangement can be used under uniform field conditions (no gradient) and when the competition between the materials tested is minimum.

The suggested planting density for the t x t hybrids is 143 palms/ha, 180 for the dwarfs and d x d, and 160 for d x t hybrids, arranged in a triangular system.

Plot size - There is a xenia effect (Influence of the pollen genotype on the albumen of the nut) and high degree of outcrossing which occurs in most tall and hybrid materials. Hence, large plots are needed to estimate the component copra per nut for each hybrid test. In the inter-ecotype tests with high heterogeneity, a plot size of 24 palms (4 x 6) is suggested while for the performance test between half-sib families, a total of 16 palms (4 x 4) is adequate.

Number of replications - *Standard* number of replications based on variability, mean comparison or variance test, and other statistical tests to detect differences, can be found in the literature. However, the coconut planting material is bulky and land is often limiting, requiring a more realistic approach in determining the number of replications. For example, four replications with a plot size of 24 palms or 16 palms with six replications are enough in a randomized complete block design for yield trials. Multilocation trials could also be adopted for increasing the number of replications.

Guard rows am] palms - Two guard rows (the first and the last) can serve to protect the experimental area. Within the row, the first or the last palm is used as a guard. The guard rows should be planted with the same type of material. Palms in guard rows should not be sampled to eliminate external or border effect in gathering data.

Control - A proper breeding trial requires the use of a locally adopted cultivar as a control. The PB 121 (Malayan Yellow Dwarf x West African Tall), cultivated worldwide, could be used as an international check for d x t hybrid yield tests; WAT x RIT for t x t; in addition to another local hybrid. For reference, the local check variety can be planted at both ends of the trial for relative comparisons.

Fertilizer lion and disease control - Hybrids express their genetic potential better under optimum nutritional conditions. Therefore, it is very important to monitor the nutritional status of the test materials through foliar analysis. A standard rate of recommended fertilizer (Manciot *et al.* 1979) should be applied on all the treatments. Further, uniform plants should be maintained through proper pest and disease control when yield is the primary objective. When disease tolerance is the main focus, the natural exposure to infection is of course necessary for screening the test materials.

Labelling - Coconut breeding is long and tedious work which requires special care in the recording system to avoid errors that could occur at any stage of the experimentation; from hybrid seed production, nursery management, field planting to evaluation. Accession books giving information on the origin and pedigree of the tested materials, the nursery records and the field designs must be kept. The palms should be labelled following the system specified earlier in this Chapter. Moreover, the sample palms should be specifically marked. A file detailing the status of every palm in the trial (replacement, producer, abnormal, dead, border) should be available and updated every year.

Sampling - The vegetative and reproductive characters are observed using 30 random palms for every hybrid entry. For the yield components, number of bunches and nuts are counted on each individual palm during every harvest which is conducted bi-monthly (in the case of tall, t x t, and d x t hybrids), or monthly (in the case of dwarfs and d x d hybrids). For the fruit component analysis, one nut per palm will be taken and pooled samples analyzed on a per plot basis.

Data collection - The precise steps are described in detail in Chapter 7.

Data analysis - Suggested methods of analyzing data were presented in Chapter 2. However, this section puts emphasis on data management to facilitate the analysis. Several data files are required for the efficient management of data in a coconut breeding programme: a) information on the palm's status; b) origin and identity of the combinations tested in each trial; c) number of bunches and nuts; d) copra and oil content, data on fruit component; and e) other qualitative characters. For data analysis, a common software should be developed for breeding and genetic trials.

Chapter 6

FIELD PLANTING

When the proper site for a germplasm collection or hybrid trial planting has been selected, the amount of work in land preparation will depend on the nature of the land to be opened. Recommended land preparation practices should be carried out to allow optimum planting and subsequent application of treatments in the field. The field must be laid out according to experimental design before planting.

Land preparation

Like other crops, coconut cannot be established in thick vegetation. Clearing of debris from thick vegetation is primarily necessary to eliminate possible breeding sites for the destructive rhinoceros beetle. Hence, the area should be cleared of felled trees/shrubs, stumps, weeds and other obstructions and then ploughed and harrowed to improve soil tilth.

Staking of field layout

Staking is done following the triangular system as shown in fig. 12. Planting density per hectare for each type of material is shown below.

	Tall, T x T	**D x T**	**Dwarf, D x D**
Density (palms/ha)	143	160	180
Distance between palms	9 m	8.5 m	8 m
Distance between rows	7.8 m	7.35 m	6.9 m

Access roads and surface drainage

Access roads are needed in delivering seedlings and supplies, and later in hauling the produce to and from the research or germplasm blocks. These facilitate inspection, data gathering and evaluation of entries. The design of the experimental and or genetic block determines the size and length of access roads. Surface drainage is essential to avoid waterlogging.

Soil conservation measures

Roads, paths and blocks should be carved such that sloping or undulating terrain are taken into account to avoid soil erosion and permit optimum mobility.

Hole preparation

Prior to digging of holes, planting guides are put in place by using two pegs placed at equal distances from the stake. This indicates the centre of the hole where the sprout of the seedling to be planted later on will have to be aligned. It must be noted that by using a stick marked at the centre, and using the planting guides at planting time, the relocation of the stake in the hole can be easily done. Holes should be dug at 50 x 50 x 50 cm size. This operation commences as early as 2 months before planting to allow for weathering of the soil on the sides and bottom of the holes. Weathering is encourage to promote early root-soil contact.

Seedling selection

Selection is an indispensable process in any crop improvement work. In coconut plantations, seedling selection aims to produce high-quality planting materials which, when properly done could easily increase uniformity and production by 10% or higher. However, for hybrid trials where test materials should possess representative 'genetic image' of the chosen germplasm, pest- and disease-free seedlings showing good germination, vegetative development and vigour should be selected. Unless the number of 'abnormal-looking' seedlings significantly exceeds the average number of 'normal-looking' seedlings per population, the selection should be towards the normal-looking seedlings. If an unusually high incidence of abnormal seedlings is observed, it is advisable to check this out from the pre-prospection records.

Unless found to be significant, e.g. number is sizeable, trait is distinct and uniform, the following types of seedlings are culled right away: multiple shoots, thin or leggy and etiolated, and albinos or seedlings which are devoid of chlorophyll.

Records and layout of palms

After laying out the field, a planting plan or map should be prepared. In this map, the plots are identified to show the spots where specific palms are to be planted. This facilitates the identification of palm pedigrees.

Planting

The best time to transplant seedlings is at the onset of the rainy season. Hence, timing of the nursery should be practised in accordance with the seasonal changes. Palms should be 8-10 months old but 6-month old seedlings can be planted if and when the timing of planting warrants it, i.e. if the seedlings will be 8 months by the start of dry season. Eight-month old transplants give a better idea of their general growth and development. However, differences in vigour are best seen when the seedlings are still too young to be moved, with the majority of their leaves still very succulent.

Field nursery seedlings should be planted immediately or at the latest 3 days after removal from the nursery to reduce mortality. Before transplanting, each hole should be applied with fertilizers mixed with soil. Alternatively or in addition, a small amount of organic matter, e.g. seaweeds, husks or any other compost materials, can be placed at the bottom of the hole and covered with soil leaving about one-third free for the seedling nut to 'sit'.

For polybagged seedlings, remove the polybag first, then transplant the seedling. The hole should be covered with loose topsoil, slightly firmed at the base of the crown. The top of the nut must be about 5-8 cm below the ground level. Deep planting might suffocate the bud while shallow planting might cause the planting material to bend, sway or lean during heavy rains and windy days. A slight depression towards the base of the crown must be provided to trap rainwater.

Chapter 7

DATA GATHERING

One of the most difficult phases in coconut research is gathering data in the field over long time intervals. This is compounded by the size of the experimental field which often covers an area of at least several hectares. A coconut researcher must, therefore, be prepared to be exposed to the most trying field conditions where patience, good common sense and perseverance are required.

This Chapter describes in detail the various steps to be taken for both germplasm collection and hybrid trials. A pro-forma is suggested in Chapter 11. The sections are subdivided into pre-planting (nursery or seedling stage); pre-flowering juvenile) and post-flowering or reproductive phases.

Seedling data

Speed of germination - The average speed of germination is usually indicated as the number of days from the date of sowing to attain: a) initial; b) 25%; c) 50%; d) 75%; and e) maximum germination rate based on the number of nuts sown per accession or population. This character is important in coconut since it is positively correlated with flowering. (Note: For practical reasons, observations are made at weekly intervals and the number of days are determined by interpolation.)

Final germination - It is expressed as a percentage of the final number of germinated nuts 4 months after sowing over the total number of nuts sown. It is difficult to study germination characters of newly introduced materials given shipment delay and the disturbances that this brings to the seednuts. In such cases, observations on germination speed can be made either in their country of origin and/or later, in the Importing country, as soon as the introduced palms start bearing.

Number of leaves emitted (leaf production data) - Leaf production is defined as the number of leaves produced or emitted by a palm over a definite period of time. This is taken following the steps outlined below prior to the planting of the palms in the field.

1 . Marking of leaf - This activity is very important because it serves as the sole basis for subsequent counting of leaves in the field. Leaf number 1 is the youngest open leaf and exhibits or shows the last inserted leaflet that is found closest to the spear. The leaf is marked by painting a small portion of the rachis to serve as a guide for the counting of emitted leaves in the succeeding periods (fig, 13).

2. Counting - Count the number of leaves starting from the oldest leaf up to leaf number 1. In a coconut seedling, the first or oldest leaf which is seen to emerge from the 'eye' or which comes out of the husk is normally devoid of any leaf blade. Thus, this leaf is called a 'false' leaf or pseudoleaf Since this could have already been detached at counting time, start counting from the first true leaf or the oldest leaf which exhibits the leaf blade, no matter how small. Record the number of leaves counted.

Girth - In a seedling, the girth is a narrow portion located between just above the germination point and the base of the oldest frond. The girth is determined using a measuring tape strapped around the base of the seedling.

Observations on girth are carried out at specified intervals on 30 randomly selected plants, e.g. on 3 to 6-month old seedlings for genebanks and on all experimental palms in hybrid trials.

Field data gathering: Vegetative growth before flowering

As soon as palms have been planted, data gathering shifts from the nursery to the field. Since this phase would depend on the previous data taken at planting time, it is necessary to take a look at the initial data on leaf production.

Number of leaves emitted (leaf production data) - Leaf production is a very simple character to observe. However, as with any other observation done in coconut, the time interval between observations and the size of the experimental area to be covered make it rather difficult. For this reason, the following steps must be taken to avoid confusion and ensure regular and precise observation:

1. Mark leaf number 1 (fig. 13), the youngest fully open leaf found closest to the spear that exhibits or shows the last inserted leaflet. The leaf is marked by painting a small portion of rachis to serve as a guide for the counting of emitted leaves in the succeeding period. Note that the leaf marked number 1 will become leaf number 6 or 7, six months after the last counting.

2. Count the number of leaves starting from the leaf following the previously marked leaf up to leaf number 1. Follow the direction of the phylotaxy of the palm while counting.

3. Record the number of leaves produced in a prescribed form (Chapter 11).

Frequency of data gathering for leaf production - Leaf production is recorded by counting the leaves produced every 6 months for the first 5 years or until 50% of the palms are flowering, whichever comes last. The total of two consecutive counts is taken as the annual rate of leaf production.

Girth - The girth of the stem of a palm is a narrow portion below the oldest living frond which when measured 20 cm from the ground, is called the bole in a tall palm. As a palm matures, its girth gradually swells or expands until it attains a maximum span. However, since the basis for measuring the girth, i.e. base of the oldest frond, moves with the upward development of the crown through the bud, girth size will definitely decrease with time or age. Afterwards, the palm starts to grow upward, forming the stem. Although this character is strongly correlated with vigour, under normal environment, and after about 2.5-3.5 years, the size of the girth practically remains constant and therefore need not be measured. Once the stem is formed, it stops expanding or thickening. This is due to the absence of a cambium layer.

The girth is measured by using a measuring tape strapped around the base of the stem just below the oldest living frond every 6 months during the first 3-4 years after planting.

Leaf measurements - At the young or juvenile stage (up to 2 years old), leaf measurements are taken from the previous leaf # 1 and observed every 6 months.

The following characters are recorded:

Petiole length - taken from the point of attachment to the base of the first leaflet insertion (fig. 5), average of 30 samples or palms.

Total length of the leaf - taken from stem attachment or base of the petiole to the tip of the topmost leaflet, average of 30 samples or palms.

Number of leaflets - taken by counting the number of leaflets from one side of the rachis. Counting is done in sets of five leaflets (e.g. 5 x 10 sets = 50 leaflets) using the side bearing the leaflet at its lowest insertion or point in the petiole as the reference side. It is not necessary to count both sides of the leaf frond. A practical way of counting leaflets in a mature frond is by taking one leaflet per set of five leaflets.

Flowering and reproductive data

A comprehensive recording of flowering, Yield and other reproductive data is required in coconut to ascertain yield stability, uniformity and distinctiveness. Although 10 years of yield data is accepted as a means of assessing the value of a certain genetic material, longer periods of data gathering are encouraged for better accuracy. Through the years, recording of yield data will certainly become more regular, particularly when product and other by-product utilization of coconut are developed.

Initial flowering and flowering distribution - More or less 30 months after field planting, coconut palms, especially the hybrids, pass the transition stage and initiate flowering. This phenomenon should be recorded since it marks the onset of the reproductive phase. Barring unforeseen problems in cultural management and climatic conditions, it is expected that, as soon as the initial flowering of an individual palm is seen, others belonging to the same variety or population also start flowering.

Since this cannot be predicted in advance, apart from the knowledge that some palms could flower in 30 months, a regular interval schedule of determining the number of flowering palms should be made after this period. The number of flowering palms with initial spathe appearance should be recorded. This provides information on which population/accession shows a narrower distribution of flowering or more uniform flowering as well as the average time to reach 100% flowering.

To determine flowering distribution, the following steps must be conducted:

(a) Observe every palm monthly from the second year or earlier after field planting onward for initial spathe appearance.

(b) As soon as the first spathe appears, mark on the leaf supporting the spathe the number 1 and the date of its emergence, with a marking pen.

(c) Record the palm number of the population/accession involved and the date of its initial spathe emergence in appropriate form (Chapter 11).

(d) To determine the flowering distribution of each population, count the total number of palms with initial flowering quarterly. Indicate the year and the quarter covered by the observation.

Frequency of *observation* - For dwarfs and d x d hybrids, the recommended frequency of observation is monthly from the first year and for tall, d x t and t x t hybrids, monthly from the second year after planting.

Inflorescence and flower morphology (fig. 6)

In addition to the characters to be observed as listed in Chapter 2, floral biology should be studied for one whole year on 12 sample palms 2 years after the first flowering to determine the following:

- Duration of male phase (days)
- Duration of female phase (days)
- Period between phases (days) in one inflorescence
- Period between successive inflorescences.

Counting of the female flower - In undertaking this activity, which is done during harvesting of nuts, the following procedures should be observed.

(a) With the use of pruning shears or secateurs, trim and count the individual button scars borne on the spikelets. This procedure is implemented in order to avoid repetition in the process of counting the button scars.

(b) The total number of button scars observed on the bunch plus the number of nuts harvested should represent the total number of female flowers on a particular palm.

Counting the spikelets - After counting the female flowers, count the total number of spikelets borne by the bunch by trimming individually the spikelets closer to the rachis and simultaneously counting them. Refer to Chapter 11 for guide forms in recording data.

Vegetative data after flowering

Flowering behaviour as observed is not uniform in coconut. Generally, however, initial flowers may appear within 2-5 years or earlier after planting, after which the following vegetative data should be recorded using appropriate guide forms presented in Chapter 11:

Stem measurement (circumference) - Taken only once at two points from 30 randomly selected normal palms per population or hybrid. Stem circumferences at 20 cm and 1.5 m from ground level are taken using a measuring tape strapped around the stem.

Plant height - Measured from the ground level to the base of the oldest living frond from the same 30 randomly selected normal palms. Data are taken at 6 and 10 years after planting.

Length of stem with 11 leaf scars - Measured from 1.5 m above the ground level at year 10. Take measurement from the bottom of scar no. 1 to the bottom of scar no. 11, as shown in figure 2.

Leaf measurements - Taken from the oldest living frond from 12 randomly selected palms per population. This is done by detaching the chosen leaf from the crown.

The measurements (in cm to one decimal place, where applicable) are done only once for the following characters:

(a) Length of petiole - measured from stem attachment to the base of the first leaflet insertion.
(b) Thickness of petiole - using a vernier calliper with extended thickness measuring tip, it is measured at the base of the first leaflet insertion towards the centre of the width of the petiole
(fig. 5).

(c) Width of petiole - using the same calliper, measured at the base of the first leaflet insertion.
(d) Length of rachis - measured from the base of the first leaflet insertion to the point of last leaflet attachment.

(e) Number of leaflets - taken by counting the number of leaflets from one side of the rachis with the first leaflet insertion. To get the total, the data are multiplied by 2.

(f) Width of leaflet - measured at the middle portion of each leaflet. Use four leaflets, two on each side, where the frond shows the widest span.

(g) Length of leaflet - measured on the four leaflets used in

Fruits and bunch return (FBR)

About a year after initial flowering, when the palms are expected to yield nuts, the interest is focused on the number of nuts, bunches, female flowers and spikelets produced by each population/accession.

Harvesting - Harvesting frequency is 2 months for tall, t x t and d x t hybrids, and monthly for dwarfs and d x d hybrids. Harvesting should be done on 30 fixed sample palms per population/accession and on all test palms in hybrid trials. Set aside fruit samples as described in the FCA section below.
Harvest only those bunches with at least one or two fruits already turning brown. Record in the FBR form (Chapter 11) the total bunches harvested on individual sampled palms.

Counting of fruits - This activity involves the following steps:

(a) Detach the fruits from the bunch and count the total number of fruits harvested;

(b) Shake all the fruits to find out if abnormal or barren fruits are present; and

(c) Record the total number of fruits harvested including barren or abnormal ones (indicate in the remark column the number of barren or abnormal fruits observed).

Fruit component analysis[1] (FCA)

As soon as 90% of the palms in an accession reach productivity, fruit component analysis can be started. The frequency of FCA is six times a year for all types, done for 4 consecutive years and periodically thereafter as needed.

Fruit sampling - At random, take a maximum of two fruits &from each of the 30 sample palms. For hybrid trials, one fruit is taken per palm per entry. However, analysis is done in lots on a per plot basis.

Identify sample fruits by palm serial number and harvest date and collect together the samples taken from the same palm.

Handling and storage - Haul sample fruits immediately to the nursery shed. Allow samples to ripen further until all fruits turn brown (about 1-2 weeks).

Weighing of fruit components - *The* following steps are followed in determining the weight of fruit components (in grams up to one decimal place, where applicable):

(a) Weigh fruit samples in batches by population. Each group should be identified properly.
(b) Weigh the fruit, Record the fruit weight (FW) and the number of samples.
(c) Dehusk fruit and weigh. Record the nut weight.
(d) Split the nut at the equatorial zone to remove the water and record the split nut weight (SNW).
(e) Deshell split nut and weigh. Record weight of shell.

Determination of copra

Choice of firesh meat or albumen sample - At random, select four nuts from each population or hybrid. Split the nut and take fresh meat sample from the half of the nut without the embryo near the equatorial zone. Sample about 15-20 g of fresh meat from each nut.

Preparation of fresh meat sample - Cut meat sample into 1 x 2 cm pieces and put them in a plastic bag. Label properly and record weight of fresh meat.

[1] *For purposes of recording yield, the STANTECH Workshop participants suggested the use of nut, e.g. Number of nuts/palm/year; Nuts/ha,- etc. It is also used in referring to the dehusked fruit. When referring to samples and the analysis* of *components like husk, shell, water, fresh meat and copra, the term fruit is used. Likewise, fresh meat and albumen are the terms used for the fresh solid endosperm while water refers to the liquid endosperm. The term copra refers to the dry solid endosperm and kernel could either be fresh or dry, e.g. fresh kernel =fresh meat or albumen dry kernel = copra. Coconut water refers to the liquid endosperm while coconut milk refers to the white substance which is expressed or extracted from the fresh meat.*

Oven-drying - Put individual samples in separate aluminium trays and place them inside an oven for drying at 105^0C for 9-10 hours with an overnight cooling-off period after the first 4-5 hours of drying to avoid hardening of the fresh meat.

Weighing of oven-dried meat - Allow samples to cool to room temperature after drying and record the weight of dry matter.

Modification of residual moisture determination - A copra sample having 6% moisture content is ideal. This can be easily determined using standard moisture meters. However, in the absence of a moisture meter, take three samples of oven-dried meat. Slice them into 'paper-thin' pieces. Record the weight of oven-dried samples. Be sure that all pieces of sample are kept. Place samples inside the oven for further drying at 105^0C for 3-4 hours. Record the weight of the oven-dried sample. Repeat the same procedure until the weight of the oven-dried meat becomes constant. The difference between the original oven-dried meat weight and the final weight is the residual moisture content. Take the average residual moisture of three samples to get the mean residual moisture.

Calculation of copra recovery and fmit component characters - This is discussed in detail in Appendix 4.

Units of measurement for yield data

To compute yield, 5% is deducted ftom the total palm density to make way for abnormalities. Yields of nut and copra are determined as follows:

Nut yield - Nut yield is expressed as the number of nuts/palm per year and as the number of nuts/ha per year.

For tall populations, the number of nuts/palm per year is recorded as the average nut production per population based on the 30 sample palms. For hybrids, number of nuts/palm per year is taken from all the sample palms.

Nuts/ha per year is obtained by multiplying the number of nuts/palm per year by 135 (for tall, t x t and d x t; planted at 9 m triangular) or by 171 (for dwarf types planted at 8 m triangular) or by its plant density rninus 5% if planted at other spacings.

Copra yield - The yield is expressed either as kg copra/palm per year or kg copra/ha per year. Copra/nut in grams is taken from the average copra per nut of the summarized FCA per population. Copra/palm per year is determined by multiplying copra/nut by nuts/palm per year. Copra/ha per year is estimated by multiplying copra/palm per year by the planting density (minus 5%).

Chapter 8

CONTROLLED HAND-POLLINATION

The coconut is a monoecious plant (fig. 6). Succession of inflorescence is more or less monthly, but it may be more rapid during the dry than in the wet season. Duration of the male phase is about 20 days but this may vary according to variety and season. The female phase ranges from 3-5 days in tall and averages 8 days in the dwarfs. In extreme cases, the female phase may extend up to 15 days. Each female flower (also called 'button') stays receptive for 1-3 days. Pollination is assured naturally by insects and wind. Once pollination is over, the stigma necroses. If fertilization is successful, the flower develops into a fruit which is botanically classified as a drupe. Some female flowers do not reach the stage of maturity. During the first 6 weeks from pollination, up to 70% of flowers fall off. At harvest time or after 11 - 12 months from fertilization, an average fruit set of 3 0% is common.

Coconut pollen is continuously shed from inflorescence opening until the 24th day in a basipetal pattern. It can be dispersed by wind up to 200 m in most cases and up to 315 m (Child 1974) in some. Pollen Yield per inflorescence varies from a little over 1 g in some dwarfs to about 10 g in tans. It can remain viable for several days at ambient temperature. Pollen succumbs easily to exposure to alcohol and to a temperature of 1501 C. Naturally, shed pollen is no longer viable after 6 days owing to alternating wet and dry regimes, and alternating low night and high day temperatures.

Selection of male and female parents

Parental materials are chosen on the basis of their phenotypic characters and genetic potential as well as vegetative and reproductive growth performance. Desirable traits like tolerance to adverse conditions, resistance to pest and diseases are likewise taken into consideration. Knowledge of the basic characteristics of dwarf and tal.1 cultivars, desired ideotypes, results of genetic studies and combining ability tests, as well as specific objectives of the breeding programme are valuable guides in the selection of male and female parents.

Dwarfs, because of their precocity and slow upward growth, are mostly utilized as female parents. On the other hand, tall populations possessing stable yield, tolerance to adverse conditions and resistance to pest and diseases are usually used as pollen source.

Pollen collection

The conventional method being followed in the collection of coconut pollen involves isolation of the inflorescence; harvesting of male flowers; preparing and conditioning of the pollen; and pollen quality control. The materials/equipment needed to carry out these procedures are listed in Appendix 5.

Male flower collecting bag - The collecting bag, which is patterned after the pollen-collecting bag for oil palm, facilitates the harvesting of male flowers. If used as directed, pollen contamination will be avoided. Its features include: the main pollen collecting bag with a spout in front, a detachable spikelet receptacle; and a polyvinyl chloride (PVC) pipe (fig. 14).

Filling the components - *The* spout below the plastic window is reinforced with the PVC pipe. The mouth of the spikelet receptacle is fitted to it and tied with a rubber band. The gartered end of the armholes is also tied with a rubber band.

Care of the collecting bags - *After* each use, the bags are washed with soap and water, and gently scrubbed with the palm of the hand on both sides. Afterwards, they are dipped in 5% formalin solution and then dried by hanging under the sun. When not in use, the bags are sprayed with lysol an stored in dry rooms.

In the field, bags are sometimes damaged by rats which often are attracted by kapok *(Ceiba pentadra)* seeds. The latter should therefore be removed and the peduncle area of the inflorescence cleaned of stipules. Should heavy infestation persist, baiting on the crown and on the ground must be done. If needed, rat bands may be applied to palms used as parents.

Isolation of inflorescence for pollen collection - *Isolating* the inflorescence for male flower collection is important to obtain pollen of the highest genetic purity. This technique is useful as pollen competition can occur to favour one or another contaminating pollen. Bagging, however, considerably reduces the length of the male phase owing to the higher temperature inside the pollen-collecting bag.

As the pollen has a life span of 6 days under field conditions, the inflorescence - from which male flowers are to be collected - must be bagged at least 6 days before the spikelets are collected.
Depending on the type of coconut and the qualities of pollen needed, bagging should be done either on the day of or a day after the natural opening of the spathe. The bagging technique is described below.

Placing the bag - The spathe of the inflorescence to be isolated is removed. The base of the peduncle is wrapped with kapok or cotton pad previously treated with powder insecticide. A small amount of a weak solution of water-based insecticide is then sprayed on the inflorescence to chase off insects (fig. 15). The pollen-collecting bag is then slipped over the inflorescence, taking care not to damage the spikelets and male flowers and ensuring that the open end of the bag reaches the peduncle. This base of the bag is pleated over the kapok pad and secured with a rubber band (fig. 16). The date of bagging is tagged and then recorded in a record book.

Harvesting of male flowers - The prescribed duration of isolating the inflorescence is 6-8 days, after which the spikelets are harvested/collected (fig. 17). Pruning shears or secateurs, the collector's arms and hands should be decontaminated with 95% ethyl alcohol before carefully opening the sleeve of the bag. Cut the spikelet one by one inside the bag, about 5 cm above the female flowers, if any, and drop them through the spout into the spikelet receptacle. After the desired number of spikelets have been gathered, withdraw arms and pruning shears carefully from the bag sleeve. The receptacle, filled with spikelets, is then gently detached from the spout and its open end tied immediately with a rubber band. The pollen-collecting bag is then removed for washing. The bag of spikelets is labelled with the palm number and date of collecting and then brought to the laboratory for processing.

To avoid error in labelling, the label indicating the palm number should be placed inside the receptacle bag before bagging.

In case two collecting rounds are desired from one inflorescence, cut the desired number of spikelets, withdraw the sleeve and tie the sleeve with a rubber band to seal it. Carefully detach the spikelet receptacle and tie its mouth. Then immediately get another spikelet receptacle with label inside and tie it to the spout.

Preparing and conditioning the pollen

Newly harvested, fresh male flowers are too humid and give off very small quantities of pollen. Also, a large proportion of these flowers is immature with non-viable pollen. This explains the relatively low viability percentage of coconut pollen. It is therefore necessary to subject the male flowers to a series of treatments so that large quantities of viable pollen can be extracted.

Considering that under natural condition, the life span of fresh pollen is only a few days, the pollen must be conditioned to prolong and maintain high viability. At present, the most effective technique is to keep dehydrated pollen (5%. moisture) in the freezer and condition it under high vacuum. The preparation and conditioning of the pollen follows a series of steps as described below:

Preparations - Insert a paper bag into a small canvas bag and place them inside the pollen manipulation box or PMB together with small pieces of paper bearing the date of collecting, the palm number and some paper clips before sterilization (fig. 18).

Stripping male flowers from the spikelets - Before handling the spikelet-filled bags, decontaminate arms and hands with 95% ethyl alcohol. Open the mouth of the bag and fit it in one of the armholes of a pre-sterilized PMB. From inside the bag, draw the spikelets with one hand and transfer them all into the PMB (fig. 19). When all of the spikelets have been transferred, withdraw the bag from the hole and keep it in a closet for used bags. Decontaminate arms with alcohol and proceed to detach the male flowers from the spikelets inside the PMB (fig. 20).

When all male flowers have been detached, put them in paper bags (fig. 21 a). It is advisable to use only a sufficient amount of male flowers, e.g. a handful per bag to facilitate drying. The identity of the pollen and date of collecting are then indicated on each canvas bag (fig. 21b) using the paper labels which are fastened to the canvas bag using paper clips. In case more than one collection are being processed, each kind of male flowers must be handled in separate isolation boxes.

Cracking of male flowers - The small canvas bag protects the male flowers during the crushing operation which is done by rolling a bottle or a rolling pin on top of the canvas bag to open the petals (fig. 22). This assists the drying process.

Drying of male flowers - The crucial factor in the production of highly viable pollen is the drying of the male flowers. The cracked male flowers while still inside the canvas bags are put inside the hot-air dryer (fig. 23) to reduce moisture content to a level ensuring prolonged conservation. Drying temperature must be maintained at no more than 40ºC to avoid damage to the pollen grains. Total drying period varies from 24 to 36 hours. This brings the pollen moisture content down to about 5%. The palm numbers and the corresponding number of bags per palm are recorded in an oven registry book (Chapter 11).

Sieving and packing in ampoule - After oven-drying or prior to sieving, the canvas bags containing the dried male flowers are lightly shaken or beaten to release the pollen grains. Then, the paper bags are withdrawn from the canvas bag and transferred into the PMB through one of the sleeves. The contents are emptied into a sterilized sieve that has been place previous y inside t e PMB together with paper labels and cotton plugs.

Sieving is done inside the PMB (fig. 24). All dried/cracked male flowers from one particular palm are placed in a sieve, gently shaken to extract and sift the pollen from its floral parts and collected in a bottom pan. The pollen is collected in quantities of 0.4-0.5 g per glass ampoule, plugged with sterile cotton wad for freeze-drying purposes and labelled properly using a paper tag indicating the pollen identity and the date of collection. The pollen must be lightly packed in the ampoule to facilitate freeze-drying. In case no glass ampoules are available, the pollen may be collected in glass vials and similarly sealed.

Constriction of ampoules - This is necessary to facilitate the sealing of the ampoules after freeze-drying. Using a special kind of equipment, called ampoule constrictor, the ampoules containing the pollen are constricted at about 2 cm from its open end (fig. 25).

Freeze-drying -After constriction, the ampoules are tightly fitted one by one to the rubber teats in the freeze-drying apparatus (fig. 26). Freeze-drying preserves the pollen longer because it is eventually dried with a minimum residual moisture content while fully retaining its original properties, e.g. protein, viability, enzymes, etc. A vacuum is created inside the ampoule. In the end, the pollen retains its full quality despite long storage.

Desiccation of pollen - If a freeze-dryer is not available, coconut pollen can be put in vials with porous covers and placed in a desiccator. A bag of silica gel (amount depends on the size of the desiccator) which is colour indicative may be used to maintain the desired relative humidity in the desiccator.

The desiccated pollen can be kept viable for hand-pollination of short duration, i.e. 2-3 months. It is advisable, however, to provide a device to isolate each kind of pollen in the desiccator to prevent pollen contamination.

Sealing of ampoules and testing for vacuum - After running the freeze-dryer for 15-20 minutes at 10^{-1} torr, the ampoules are sealed by heating them at the constricted end with an air-gas sealing torch (fig. 27). The seal ampoules are then tested for vacuum by means of a spark tester. The spark is seen to concentrate and pass through any crack or small hole on the ampoule to give a clear indication of a leak. In any case, improperly sealed ampoules are the first ones that should be used.

Storage of pollen - The sealed ampoules are kept in the freezer (fig. 28) for a period of up to 6 months or longer. Experience at the Philippine Coconut Authority (PCA) - Zarnboanga Research Center, has shown that properly prepared and conditioned pollen which is stored in the freezer can retain its viability even after 5 years. Nonetheless, it is necessary to regularly test pollen viability before using, particularly if the age of the pollen is over 6 months.

Pollen should be taken out *of* the freezer only when it is needed because frequent alternate freezing and thawing are detrimental to pollen. All stored pollen must be recorded in a pollen registry (Chapter 11) to facilitate withdrawal and identification.

Pollen quality control

Testing of pollen viability - A sample of each pollen batch is set aside for viability testing and moisture content determination. If the results are not good, a second sample is tested. The whole lot is destroyed in case of contamination. The germinability of the pollen is tested *in vitro* on an agar-sucrose medium supplemented with a trace amount of boric acid.

The following steps are observed in preparing the pollen-germinating medium:

(a) Dissolve 0.5 g of agar in 100 ml distilled water by heating gently with continuous stirring until the agar is completely dissolved;

(b) Let the dissolved agar cool for a while and add 10 g of sugar little by little with continuous stirring until it is completely dissolved;

(c) Add 1 n-d of 100 ppm boric acid while stirring; and

(d) Pour 10 n-d of the medium in a clean petri dish and allow it to cool to room temperature. To prevent cracking of the solidified medium, the cover of the petri dish should be lined with a wad of wet cotton. The cover must not be removed except when dusting the pollen in the medium and when examining the sown pollen.

Dusting of pollen - With a small ball of cotton or a brush, a small amount of pollen grains is collected from an ampoule or vial. This representative pollen sample is gently tapped to form a cloud of pollen over the medium. The dish is then covered and labelled. One petri dish is used per sample.

Determination of percent pollen germination - After having activated pollen germination by exposing it for 2 hours at ambient temperature, pollen viability is observed with the use of an ordinary light microscope (fig. 29). Germinated, abnormal and ungerminated pollen grains are counted, and the reading expressed as percentages of total number of counted pollen. To facilitate counting, a hand tally counter is used. Counting is done until at least a total of 100 pollen grains are counted. Percent pollen germination (PPG) is calculated as follows:

$$PPG = \frac{\text{Number of germinated pollen}}{\text{Total number of pollen}} \times 100$$

The readings are recorded opposite the sampled population in a pollen registry sheet (see guide form in Chapter 11).

For pollen to be considered viable, the accepted quality norm is at least 25% germination for a particular batch of pollen. Pollen with poor viability is appropriately disposed.

Moisture content of pollen - Drying the freshly collected pollen (after sieving) in an oven at 105ºC for 24 hours should reduce its moisture content to 4-8%. If moisture content is more than 8%, further drying is needed.

Isolation of female flowers

Emasculation or removal of male flowers - The time of emasculation depends primarily on the expected start of the female phase. This is where the knowledge of floral biology of the female parent is important.

Initially, the spathe is removed followed by all the male flowers in the inflorescence. The male flowers are collected in a bag and disposed of properly (fig. 30). Record the date the work was performed in the record book and on the frond. Cutting of the spikelets to facilitate emasculation is not advisable because it triggers the production of ripening hormones which promote abscission of female flowers. Besides, by leaving the spikelets intact, accidental rubbing of the female flowers against the isolation bag is prevented (figs. 31 a & 31 b).

Time of bagging - To serve as a guide in determining the time of bagging, the floral biology of the variety used as female parent should have been studied earlier. Bagging is done at least 6 days before any of the female flowers in an emasculated inflorescence become receptive. The receptivity of a female flower is denoted by the splitting of the white stigma and the secretion of nectar.

Placing the bag - The bag is slipped over the emasculated inflorescence with its opening coming down over the peduncle (fig. 32). Then, a packing of kapok or cotton which had been previously treated with powder insecticide is placed around the peduncle where the opening of the bag is pleated over. A small amount of water-based aerosol insecticide is sprayed inside the bag before finally securing the enclosure with a rubber band. The date of this operation is recorded on the frond and in the record book.

Carrying out the pollination - Pollination is done when the female flowers are receptive.
Receptivity is manifested by the parting of the stigma and the simultaneous secretion of nectar. To verify this, the isolated inflorescence should be inspected daily or (depending on the skill of the pollinator) every other day, from the time of bagging to determine the precise date of receptivity.

Labels and records - A day before pollination, the crossing supervisor checks the female parents to be pollinated either by directly going to the field with the pollinator or by looking at the activity control board. The supervisor determines which specific palms are to be crossed by referring to the previously prepared crossing plan and prints them in individual crossing sheets (Chapter 11). Each pollination is given a number and to avoid duplications, numbering is done consecutively.

On the day of pollination, the pollinator gets from the supervisor the prepared sheets and submits them to the pollen processor who withdraws the pollen from the freezer. From the printed pollination number in the sheet, the pollen processor prepares a label using plain sheet aluminium with a piece of copper wire and gives it to the pollinator. Then, the pollinator proceeds to the pollen talc mixing room.

Preparation of pollen for pollination - *Viable* pollen from only one donor is used for each pollination except when two or more female parents simultaneously require the same pollen as stated in the crossing plans.

Sterilization of equipment and premises - At the end of each day, all PMBs are cleaned with alcohol and sterilized by heating at 150ºC for 10 minutes or longer. The puffer bottles used for the day are washed with alcohol, spouts scaled with masking tape and placed inside a pre-sterilized PMB which is kept closed at all times.

Rehydration of pollen - The ampoules/vials are opened without removing the cotton plug and labels. They are half-suspended in water and left to stand for about 1 hour inside a chamber of high relative humidity. However, this step may be omitted in the case of freshly collected or non-freeze dried pollen.

Filling of spray flasks or puffer bottles (fig. 33) - Since rehydrated pollen tends to stick together, it must be mixed with talcum powder before using to attain better dispersal. Owing to the hydrophilic (water affinity) and thermophilic (temperature stability) properties of talcum, pollen is subsequently protected from heat and from being too moist. While the pollen is allowed to rehydrate, disinfect hands and arms with alcohol and fill each flask with a teaspoon of talcum powder. At this rate, the mixture is more or less one part pollen to eight parts talcum. This operation must be done inside a PMB. Take out the flask and write the specific number of parents to cross on a piece of sticking tape previously provided in each flask. Wipe the outside portion of the flask with alcohol and distribute each flask to individual PMBs.

Afterwards, disinfect arms and hands with alcohol and mix the pollen and talcum, one flask at a time. Before moving to the succeeding box, see to it that the spout and cap are properly sealed. Disinfect arms and hands with alcohol again and proceed to the other box. The same process is followed until mixing is finished. After completing this operation, clean and wipe the boxes with alcohol.

To facilitate pollen-talc mixing, it is advisable to have an adequate number of pollen manipulation boxes. If this is not possible, a box may be re-used, but only after it has been properly decontaminated by heating the PMB at 150'C for 10 minutes or longer. Before leaving the pollen laboratory, re-check the pollination numbers and the specific crosses to be done. Then proceed to the field to do the following:

(1) Carefully place the ladder (if needed) close to the palm to be pollinated;

(2) Take the right flask, the tag bearing the pollination number and the aerosol/insecticide can, and climb;

(3) Spray a little amount of water-based aerosol insecticide around the bagged inflorescence to chase off insects. Afterwards, tear the plaster covering the small hole at the window of the bag and then remove the seal in the spout of the flask and insert it into the hole (fig. 34). Make three or four puffs of the pollen-talc mixture into the bag, beating the bag while puffing the pollen-talc mixture. Remove the flask afterwards and stick back the plaster to cover the hole;

(4) After the first pollination, tie the label to the peduncle of the bunch, climb down and write the pollination number in the manifold book. If there are more palms to be pollinated, repeat the above steps;

5) Step 3 is repeated for several days using the same kind of pollen. In general, pollination is carried out for not more than four rounds. In some cases, pollination is done every day, e.g. for NW. For seed parents of the tall population, pollination can be done twice because of the more or less simultaneous receptivity of the female flowers;

(6) At the end of the day, discard all leftover pollen-talc mixtures, wash all flasks and disinfect them with alcohol. All boxes are sterilized and prepared for use the following day. The crossing sheet is submitted to the supervisor for checking and recording in the crossing plan; and

(7) Before the end of the day, indicate in the activity control board the operation performed for the day (fig. 3 5).

Removal of the isolation bag

As soon as the stigma of 0 female flowers necrose or turn brownish-black, the bag is removed (fig. 36). Usually, this occurs on the fifth or sixth day after the last pollination. To be sure, the pollinated inflorescence is visited on the third or fourth day from the last pollination. After careful removal of the bag, the label is firmly tied to the peduncle around the first basal spikelet. The operation is then recorded.

Registration of pollination - *As* soon as the pollination is completed and the bag has been removed, the pollination is entered in a registry book. Likewise, the corresponding block in the crossing plan is crossed out.

Monthly report - *To* know the progress made in hand-pollination, a monthly report is drawn from the record books of individual pollinators, and then compared with the records in the pollination register. Because of the injuries inflicted on the inflorescence during emasculation and the unfavourable atmosphere created by bagging, fruit set by hand-pollination is usually very low, i.e. only 1-4 nuts per bunch.

Blank pollination

Pollinators and baggers must be closely monitored for efficiency aside from routine checking by an inspector. During the preparation of the crossing plan, the plant breeder must include some blank pollination as a means of checking the quality of work. A few ampoules of dead pollen could be issued but this should not be known to the pollinators. Pollen is killed by boiling the ampoules in a double boiler for 30 minutes. Dead pollen when issued and applied properly would check bagging efficiency as there will be no fruit setting a week after debagging. The pollinators should know that there are blank pollinations but they should not know which one is with live pollen or with dead pollen. If fruit set is observed from a blank pollination after 10-12 weeks, based on a previous monthly report for hand-pollination, take appropriate action to prevent repetition of the same. Checking and reassessing the system should be employed and each step rectified as needed.

Harvesting and handling of hybrid seednuts

Apart from all the careful work done by the personnel involved in the production of hand-pollinated nuts, equal care must be taken in harvesting and hauling of hybrid seednuts. Any accidental mixing or wrong labelling of the nuts will destroy all the careful work done over a year.

Time of harvesting - As already mentioned, the fruit of the coconut takes about 12 months to mature. If allowed to stay longer on the paIn-i, some nuts or the whole bunch may fall and scatter all over, which could lead to confusing identity of hybrid nuts. To avoid this, harvest when they are about 11 months old or at the colour break stage. At this stage, the nuts are mature enough to germinate. This practice also eliminates the possibility of in-transit germination in case of a hybrid seednut exchange programme.

Harvesting - Based on the pollination registry book, a list of the bunches to be harvested is prepared. The fruits are harvested by bunch and the pollination number is printed on each fruit as soon as they are on the ground. The corresponding number of fruits is recorded opposite the pollination number in the registry book. Then, the fruits are tied together by means of a sack needle and the pollination tag secured properly to the tied fruits. It must be remembered that henceforth the only means of identifying the fruits is through their respective pollination numbers so extra care must be maintained throughout. To avoid confusion, harvesting must be done on one crossing plan at a time.

Handling of hybrid seednuts in the nursery - The fruits are hauled to the nursery right away and should never be allowed to stay in the plantation overnight to avoid accidental mixing or losses of valuable materials. When all the fruits have been hauled, re-check the harvest and see to it that all fruits are accounted for. For fruits belonging to different crossing plans, a colour-coding system in the painting of the pollination numbers should be followed for easier identification once they are set to germinate in the nursery beds. A specific colour is used for each crossing plan.

Harvest report - When the colour coding and piling of hybrid seednuts are completed, prepare a report of the harvest. State the number of nuts harvested per pollination number, the female palm numbers, and the respective colour codes used per crossing plan.

Chapter 9

EMBRYO CULTURE

Collecting and conservation of coconut genetic resources are important priorities for breeding programmes (WPGR 1985). The coconut seednut is characterized by considerable weight and volume. The lack of dormancy renders its transport conditions very difficult and costly, and poses phytosanitary problems. The use of *in vitro* techniques can facilitate the transport and offer some phytosanitary guarantees (Assy Bah *et al.* 1987). In addition, the cryopreservation of zygotic embryos can also play a major role in the conservation of coconut germplasm and in the exchange of genetic resources (Bajaj 1984; Assy Bah and Engelmann 1992b).

A summary of the 10 years of research work conducted by Institut de Development de Foret/Department du Plante Oleagineous (IDEFOR/DPO) in collaboration with ORSTOM and CIRAD for collecting and culturing zygotic embryos is presented.

Collecting fruits using in vitro techniques

Fruit collecting through zygotic embryos involves sampling, disinfection and transferring of embryos to the culture medium. The ideal age of the fruit is about 10- 11 months. Embryos of over-ripe nuts may grow prematurely and disinfection becomes difficult at this stage.

Sampling - Sampling consists of cutting the albumen cylinder with the embryo. This operation is done in non-sterile conditions. The nut without the husk is split open. The part with the embryo is placed on the table and a cylinder of albumen around the embryo is cut using a 2.0-cm cork borer. Gouging of the albumen around the embryo serves to protect this organ during storage and disinfection. This piece of albumen is removed just before culturing.

Disinfection - The cylinders are put in a 500-rril flask containing calcium hypochlorite solution (8% active chlorine). During the imbibition, stir the disinfectant in the flask once or twice. After the disinfection, the cylinders can either be stored for subsequent transfer to the growth medium or the embryos can be cultured right away.

Embryo transfer in the field - In the laboratory, the transfer is done in a laminar flow cabinet. In the field, the different operations are conducted inside a field inoculation box (fig. 37) equipped with an alcohol lamp for flame-disinfection of the scalpel. The scalpel is heated on the flame, then cooled off inside the jar containing the albumen cylinders with the calcium hypochlorite solution.

Using the scalpel, the albumen core or cylinder surrounding the embryo is carefully cut to isolate the embryo which is then placed in a sterile petri dish, washed with distilled water, and placed in a sterilized 30-ml flask containing the prepared culture medium. The tubes of cultured embryos can either be put directly inside a culture room (at 25-27ºC), or conserved under a shade before the transfer to the culture room.

If a tissue/embryo culture laboratory is near the collecting site, the albumen cylinders can be sampled in the field and brought to the laboratory. The cylinders should then be put temporarily in a KCl solution, before the excision of the embryos.

Storage of albumen cylinder

After the disinfection, the albumen cylinder with the embryo is transferred into a 30-ml flask of storage solution containing 16 g/L KCI. The scalpel should be regularly disinfected by heating. The different operations are conducted over a flame to avoid any contamination by microorganisms. The cylinder with the embryo can be transferred this way to the laboratory for culturing. After the storage period, maximum 14 days, the cylinder is disinfected with calcium hypochlorite solution for 20 minutes. After the excision, the embryo is then washed with sterile distilled water and put in the culture medium (Table 1).

Direct culturing in the field gives a higher contamination rate (10%) than culturing after storage of the cylinders (5%). Both methods are acceptable since their contamination rates vary only slightly from that of the control cultured in the laboratory. The latter has a contamination rate of 3-8%.

Table 1. Equipment and materials needed in collecting coconut embryos in the field

Material	Storage of albumen cylinders with embryos	Direct culturing in the field
4 liters of hypochlorite solution (8% chlorine)	+	+
2 scalpels	+	+
20 sterile petri dishes	-	+
Plastic film	-	+
100 tubes containing 20 mi of culture medium	-	+
100 pc - 30 mI flasks containing 15 ml sterile water	-	+
100 pc - 30 mI flasks containing 15 ml KC1 solution	+	-

The basic equipment is composed of. 1 sponge, 1 small camping gas burner, 1 hammer, 2 pairs of forceps (30 cm), *2 cork borers, 1 soap, 4 - 500 ml bottles, 1 portable table and one box. For the culture of 100 embryos, the complementary equipment detailed above is necessary.*

Culture medium and condition applied

A very simple culture medium that ensures adequate development of the embryo in vitro and enhances its subsequent growth in natural condition can be used. This medium is composed of the mineral solution of Murashige and Skoog (1962) enriched with 41 mg/L of iron-EDTA, 100 mg/L of sodium ascorbate, 60 g/L of sucrose, 2 g/L of active charcoal and 7 g/L of agar at pH 5.5. The culture is placed in the dark at 27 ± 1 ºC until the appearance of the plumule. It is then transferred to another room and cultured under the light (35EMm-2S-1) with a 12-hr photoperiod. Subculturing is done every month.

Acclimatization

One month before the transfer into the nursery, the in vitro plantlets are subcultured on a medium with a higher dose of sucrose (60, 90, 120 g/L). After 1 month in this medium, the plantlets are washed with tap water and transferred by batches to sand which was previously sterilized by autoclaving. The use of a plastic cover during the first 2 weeks allows a saturated moisture condition. The plantlets in the batches are watered regularly (3 times/week) to keep the sand moist for 1 month and then they are uncovered and exposed in the open air. A nutritive solution (Table 2) is added to the batch every 2 days. After 3 months, plantlets are transferred from the sandy batches to polybags with compost. The nutritive solution is regularly applied to the plantlets to stabilize growth.

Table 2. Nutritive solution used for acclimatization of plantlets (mg/L)

$KN0_3$	274
$Ca(N0_3)_2\ 4H_20$	1095
KH_2P0_4	137
$MgS0_4$ - $7H_20$	274
$(NH_4)_2SO_4$	137
KCL	2.74
H_3B0_3	3
$MnS0_4\ H_20$	1.7
$ZnS0_4.7H_20$	2.74
$(NH_4)_62M0_70_{24}\ 4H_20$	2.74
H_2S0_4	0.137
$CuS0_4.5H_20$	1.37
EDTA	26.1
$FeS0_4\ 7H_20$	24.9

Chapter 10

GENERALIZED SAMPLING STRATEGY

A basic problem in coconut germplasm collecting arises from the fact that the crop has a cosmopolitan distribution with an uncertain centre of origin.

Guided by the sampling methods described in Chapter 2, the coarse and fine grid sampling strategies outlined below would ensure that the areas to be sampled are carefully scrutinized and that a minimum area is skipped. Since there is no existing information on natural geneflow between coconut populations (apart from the studies conducted on polyphenols, electrophoresis, and more recently, DNA patterns among coconut varieties obtained from various origins, all of which yield variable, inconclusive results), the coarse grid sampling strategy as described in a practical course initially organized by the 1PGRI (then I13PGR) in 1978 and 1979 in Bogor, Indonesia, has been tried leading to a potential systematic coverage of the coconut areas in the Philippines (Santos 1987) and Malaysia (Jamadon 1987). When combined with fine-grid and biased sampling method, this strategy ensures that no important diversity is missed, and that the widest possible array of conditions is covered. Another feature of this strategy is that surveys can be suspended and resumed as necessary by the explorer/collector because areas are pre-identified.

Coarse grid sampling method

The basic requirements are as follows:

1. Knowledge of the extent of coconut-growing areas and distribution;

2. Knowledge of the degree of environmental diversity in the areas where the crop is grown.

To implement the coarse grid sampling procedure, the following steps which are followed by the Breeding and Genetics Division of the Philippine Coconut Authority (PCA), are discussed for purposes of illustration.

1. A suitable sized map of the Philippines was obtained (Scale: 1: 1,000,000) and grids of approximately 40 x 40 km were marked (fig. 38), following latitude and longitude divisions/degrees.

2. All grids which are coconut-growing areas were then identified and according to relative size or hectarage, the number of sampling sites was determined per grid. (See item 6 below).

3. Travel arrangements were then made by the leader of the survey team with the local PCA Regional and Provincial Offices seeing to it that all of the needed equipment, materials and supplies were prepared in advance.

4. The two-person team (e.g. the leader, a technical staff of the division, and a climber/technician) set out to travel to the pre-identified sites.

5. Local labour was hired to assist in FCA and vegetative measurements following the mininum list of descriptors, and passport data.

6. Each grid was surveyed using 5 to 6 sampling sites (SS) with intervals between SS determined according to any noticeable changes in ecological conditions, e.g. coastal to upland.

7. A local guide (normally the coconut development officer) assists the team in explaining the usefulness of the survey to the fanners/owners and to the local population.

Biased sampling/outright collecting

1. If the technical person encounters something new, one which he/she feels is not yet represented in the genebank, the supervisor is notified and will determine whether the finding is unique. If it is, and no disease or important pest is evident, the decision is made to collect 200 nut samples of the said population or variety. These are sent to the Research Centre for conservation in the genebank.

   As good information of the distribution of diseases in the country exists, the above step is possible. But it is emphasized that this is only done when one is sure that the risk is negligible. Otherwise collecting is not made until fully certain that the transfer of the material is safe and sound.

2. The passport data are brought to the station when about 4 to 5 grids have been surveyed. This means that data from 20-30 sampling sites have been collected. The data are subjected to Mahalanobis Generalized Distances Method Analysis and Cluster Analysis.

3. The survey is always coordinated with local officials and extension workers because, depending on the result of the multivariate analysis, field collecting of seednuts from the identified farms are coordinated with the local guides.

Collecting of seednuts

Depending on the results of statistical analysis, nut samples are collected in such a way that the widest array of genotypes in the identified population(s) is covered. If a certain grid is noted to be different in ecological condition from the other grids but without any significant differences noted between sites, the same number of samples, as in section 6 above, are collected for better coverage. The resultant collection or accession is therefore identified in the genebank as Accession No ____, Grid# _ , SS# 1, 2, 3, 4 ... planted at random within the designated block with each palm properly identified as to its exact origin, e.g. Grid # A14, SS # 3.

Chapter 11

GUIDE FORMS FOR DATA RECORDING

I	Passport data:	IBPGR list of descriptors (may be obtained from IPGRI)
II	Collecting data:	IBPGR list of descriptors
III	Characterization data:	IBPGR list of descriptors
IV	Nursery	Form Code
	1. Germination data	Form 1
	2. Seednut record	Form 2
V	Vegetative growth	
	1. Semi-annual growth observations	Form 3
	2. Leaf measurements	Form 4
VI	Flowering and reproductive data	
	1. Semi-annual growth observations	Form 3
	2. Flowering observations	Form 5
	3. Fruit and bunch returns	Form 6
	4. Monthly harvest records	Form 7
	5. Fruit compnent analysis	Form 8
	6. Data on fresh meat sample and copra analysis	Form 9
VII	Mature Palm Measurements	
	1. Stem measurements	Form 10
	2. Plant height measurements	Form 11
VIII	Hand Pollination	
	1. Pollen collecting sheet	Form 12
	2. Oven registry sheet	Form 13
	3. Processed pollen receipt record	Form 14
	4. Field inventory of pollen collection	Form 15
	5. Crossing plan sheet	Form 16
	6. Pollen registry sheet	Form 17
	7. Pollen dispatch record	Form 18

Form 1 - GERMINATION DATA

Population: ______________________________ Date of Sowing: ________________________

Location: ______________________________

PALM NO	NUT NUMBER	DATE OF GERMINATION	PIGMENTATION

PALM NO	NUT NUMBER	DATE OF GERMINATION	PIGMENTATION

Submitted by: ____________________________

Date: ____________________________

Form 2 - SEEDNUT RECORD

Population: _____ Total nuts sown: _____

Location: _____ Total nuts germinated: _____

Date sown: _____ Percent germination: _____

N°	GERMINATION DATE	PIGMENTA-TION	NO	GERMINATION DATE	PIGMENTATION	NO	GERMINATION DATE	PIGMENTATION
1			31			61		
2			32			62		
3			33			63		
4			34			64		
5			35			65		
6			36			66		
7			37			67		
8			38			68		
9			39			69		
10			40			70		
11			41			71		
12			42			72		
13			43			73		
14			44			74		
15			45			75		
16			46			76		
17			47			77		
18			48			78		
19			49			79		
20			50			80		
21			51			81		
22			52			82		
23			53			83		
24			54			84		
25			55			85		
26			56			86		
27			57			87		
28			58			88		
29			59			89		
30			60			90		

Submitted by: _____

Date: _____

Form 3 - SEMI-ANNUAL GROWTH OBSERVATIONS

Population: _____ Observation date: _____
Location: _____ Date planted: _____
Plot number: _____
Replication: _____

PALM NO.	LEAF PRODUCTION (NO)	GIRTH (CM)	REMARKS

Submitted by: _____
Date: _____

Form 4 - LEAF MEASUREMENTS

Population: _____ Observation date: _____

Location: _____

PALM NO	PETIOLE			RACHIS LENGTH (cm)	LEAFLETS			REMARKS
	LENGTH (cm)	WIDTH (cm)	THICKNESS (cm)		NO	WIDTH (cm)	LENGTH (cm)	

Submitted by: _____

Date: _____

Form 5 - FLOWERING OBSERVATIONS

Population: Observation date: ____________________

Location: ____________________ Date of planting: ____________________

PALM NO	DATE OF FIRST SPATHE EMERGENCE	PALM NO	DATE OF FIRST SPATHE EMERGENCE	PALM NO	DATE OF FIRST SPATHE EMERGENCE

Submitted by: ____________________

Date: ____________________

Form 6 - FRUITS AND BUNCH RETURNS

Population: _____ Year: _____
Location: _____ Month: _____
Observation date: _____ Block: _____

PALM NO	NUMBER OF					REMARKS
	BUNCH	NUTS	F.FLOWERS	SPIKELETS	NUTS SAMPLED	

Submitted by: _____
Date: _____

Form 7 - MONTHLY HARVEST RECORDS

Population: ______________________ Date of observation: ______________________

Location: ______________________

PALM NO	NUMBER OF				REMARKS
	BUNCHES	F. FLOWERS	NUTS	NUTS SAMPLED	
TOTAL MEAN					

Submitted by: ______________________

Date: ______________________

Form 8 - FRUIT COMPONENT RECORD (grams)

Month of : _____ , 199 Variety: _____

Date analyzed: _____ , 199 Form: _____

Location:

PALM NO	NO. OF SAMPLES	WEIGHT OF		WEIGHT OF HUSK		SPLIT NUT WEIGHT	WEIGHT OF MEAT		WEIGHT OF SHELL		WATER	COPRA
		FRUIT	NUT	TOTAL	PER NUT		TOTAL	PER NUT	TOTAL	PER NUT		

Submitted by: _____

Date: _____

Form 9 - DATA ON FRESH MEAT SAMPLE AND COPRA

Month of: _____ , 199 ___ Population: _____

Date analyzed: _____ , 199 ___ Location: _____

TRAY NO.	WEIGHT OF					
	TRAY	TRAY + FM	FM	TRAY	TRAY + DM	DM

FM - Fresh Meat
DM - Dried Meat

Submitted by: _____

Date: _____

Form 10 - STEM MEASUREMENTS

Population: Observation date: _____

Location: _____

PALM NO	CIRCUMFERENCE OF GIRTH		LENGTH OF STEM (WITH 11 LEAF SCARS)	REMARKS
	AT 20 cm	AT 1.5 m		

* Measured from ground level

Submitted by _____

Date : _____

Form 11 - PLANT HEIGHT MEASUREMENTS

Population: Observation date: ____________________

Location: ____________________

PALM NO	HEIGHT	PALM NO.	HEIGHT	PALM NO.	HEIGHT	PALM NO.	HEIGHT

Submitted by: ____________________

Date : ____________________

Form 12 - POLLEN COLLECTING SHEET

POLLEN COLLECTION		POLLEN COLLECTION	
POPULATION	PALM NO.	POPULATION	PALM NO.
NO. OF SPIKELETS	DATE	NO. OF SPIKELETS	DATE
TIME	COLLECTOR	TIME	COLLECTOR

POLLEN COLLECTION		POLLEN COLLECTION	
POPULATION	PALM NO.	POPULATION	PALM NO.
NO. OF SPIKELETS	DATE	NO. OF SPIKELETS	DATE
TIME	COLLECTOR	TIME	COLLECTOR

POLLEN COLLECTION		POLLEN COLLECTION	
POPULATION	PALM NO.	POPULATION	PALM NO.
NO. OF SPIKELETS	DATE	NO. OF SPIKELETS	DATE
TIME	COLLECTOR	TIME	COLLECTOR

Form 13 - OVEN REGISTRY SHEET

POLLEN DRYING RECORD

PALM NO.	NUMBER OF BAGS ENTERED	TIME/DATE

PALM NUMBER	NUMBER OF BAGS ENTERED	TIME/DATE

Submitted by: _____

Date: _____

Form 14 - PROCESSED POLLEN RECEIPT RECORD

Population: _____

Location: _____

PALM NO.	DATE OF COLLECTION	NUMBER OF AMPOULES*	GERMINATION (%)	REMARKS

*In units of grams

Submitted by: _____

Date: _____

Form 15 – FIELD INVENTORY OF POLLEN COLLECTION

Population: _____

Location: _____

PALM NO.	DATE OF COLLECTION/SPATHE NUMBER								
	1	2	3	4	5	6	7	8	9

Submitted by: _____

Date: _____

Form 16 - CROSSINIG PLAN SHEET

FEMALE PARENT	CROSSING PLAN	FEMALE PARENT	CROSSING PLAN
POLLINATION DATE	POLLEN AGE	POLLINATION DATE	POLLEN AGE
MALE PARENT	POLLINATION NO.	MALE PARENT	POLLINATION NO.

FEMALE PARENT	CROSSING PLAN	FEMALE PARENT	CROSSING PLAN
POLLINATION DATE	POLLEN AGE	POLLINATION DATE	POLLEN AGE
MALE PARENT	POLLINATION NO.	MALE PARENT	POLLINATION NO.

FEMALE PARENT	CROSSING PLAN	FEMALE PARENT	CROSSING PLAN
POLLINATION DATE	POLLEN AGE	POLLINATION DATE	POLLEN AGE
MALE PARENT	POLLINATION NO.	MALE PARENT	POLLINATION NO.

Form 17 - POLLEN REGISTRY SHEET

CROSSING PROGRAM (Indicate Hybrid Cross, i.e. A x B)

POLLINATION NUMBER	PROGENITORS		NO OF FEMALE FLOWERS	AGE OF POLLEN	DATE OF			NO OF NUTS HARVESTED	REMARKS
	FEMALE	MALE			POLLINATION	DEBAGGING	HARVEST		

Submitted by: _____

Date : _____

Form 18 – POLLEN DISPATCH RECORD

Origin: _____ Pollen Dispatch No.: _____

Sender _____ Year Series: _____

Shipment to: _____

Destination:

Kind of pollen: _____

Quantity: _____

POLLINATOR NUMBER	COLLECTION DATE	NO. OF AMPOULES*	GERMINATION (%)	REMARKS

Submitted by: _____

Date: _____

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Appendix 2

MAHALANOBIS GENERALIZED DISTANCE

INTRODUCTION

In order to characterize the relations between multivariate populations, several problems have to be worked out: test of conformity of the mean of a population, comparison of the means of two populations, definition of groups of populations. Each of these problems requires the definition of a distance between the mean values $\mathbf{m}_1$, and $\mathbf{m}_2$ of two *p*-variate populations, where

$$\boldsymbol{m}_1 = \begin{bmatrix} m_{11} \\ m_{12} \\ . \\ . \\ . \\ m_{1p} \end{bmatrix}, \quad \boldsymbol{m}_2 = \begin{bmatrix} m_{21} \\ m_{22} \\ . \\ . \\ . \\ m_{2p} \end{bmatrix},$$

m being the mean value of the f' variable in the i' population. Many types of distance between M_1, and M_2 can be defined, for instance the euclidian distance d is defined by

$$d^2 = (\boldsymbol{m}_1 - \boldsymbol{m}_2)'(\boldsymbol{m}_1 - \boldsymbol{m}_2) = \sum_{i=1}^{p} (m_{1i} - m_{2i})^2$$

But distances of this type do not take into account the correlations between variables. For this reason, *d* increases indefinitely with the number of variables, even if the new variables do not add any information useful for a better discrimination between the two populations.

DEFINITION OF MAHALANOBIS GENERALIZED DISTANCE

Let us consider two *p*-variate populations; with mean values $\boldsymbol{m}_1$, $\boldsymbol{m}_2$, and with the same dispersion matrix S. The element on the i^{th} row and the j^{th}column of S is the covariance between the i^{th} and the j^{th} variable within populations; if $i=j$, it is the variance of the j^{th} variable within populations. Mahalanobis generalized distance is defined as

$$\boldsymbol{D}^2 = (\boldsymbol{m}_1 - \boldsymbol{m}_2)'\boldsymbol{S}^{-1}(\boldsymbol{m}_1 - \boldsymbol{m}_2) = \sum_{i=1}^{p}\sum_{j=1}^{p} s_{ij}(m_{1i} - m_{2i})(m_{1j} - m_{2j})$$

where s_{ij} is the element on the i^{th} row and the column of $\mathbf{S}^{-1}$, inverse matrix of **S**. The main interest of this distance is that it takes into account the correlations between variables. A new variable always increases D^2, but this variation is small if the new variable is redundant with others. The distance D^2 is independent of any change of origin or scale of the variables.

AN APPLICATION OF MAHALANOBIS GENERALIZED DISTANCE

Let us consider two p-variate samples of sizes N_1, N_2 from the previously defined two populations. Let $\boldsymbol{x}_1$, $\boldsymbol{x}_2$ be the means of the samples and $\boldsymbol{A}_1$, $\boldsymbol{A}_2$ the matrices of the corrected sums of squares and products. The dispersion matrices of the samples are $\boldsymbol{S}_1 = \boldsymbol{A}_1/\boldsymbol{N}_1$, and $\boldsymbol{S}_2 = \boldsymbol{A}_2/\boldsymbol{N}_2$. Under the hypothesis that both populations have the same dispersion matrix S, this matrix can be estimated by

$$\boldsymbol{S} = \frac{\boldsymbol{N_1 S_1} + \boldsymbol{N_2 S_2}}{\boldsymbol{N_1} - \boldsymbol{N_2} - \boldsymbol{2}}$$

Then the Mahalanobis generalized distance is estimated by

$$\boldsymbol{D}^2 = (\boldsymbol{x}_1 - \boldsymbol{x}_2)'\boldsymbol{S}^{-1}(\boldsymbol{x}_1 - \boldsymbol{x}_2)$$

It can be shown that under the hypothesis that $\boldsymbol{m}_1 = \boldsymbol{m}_2$,

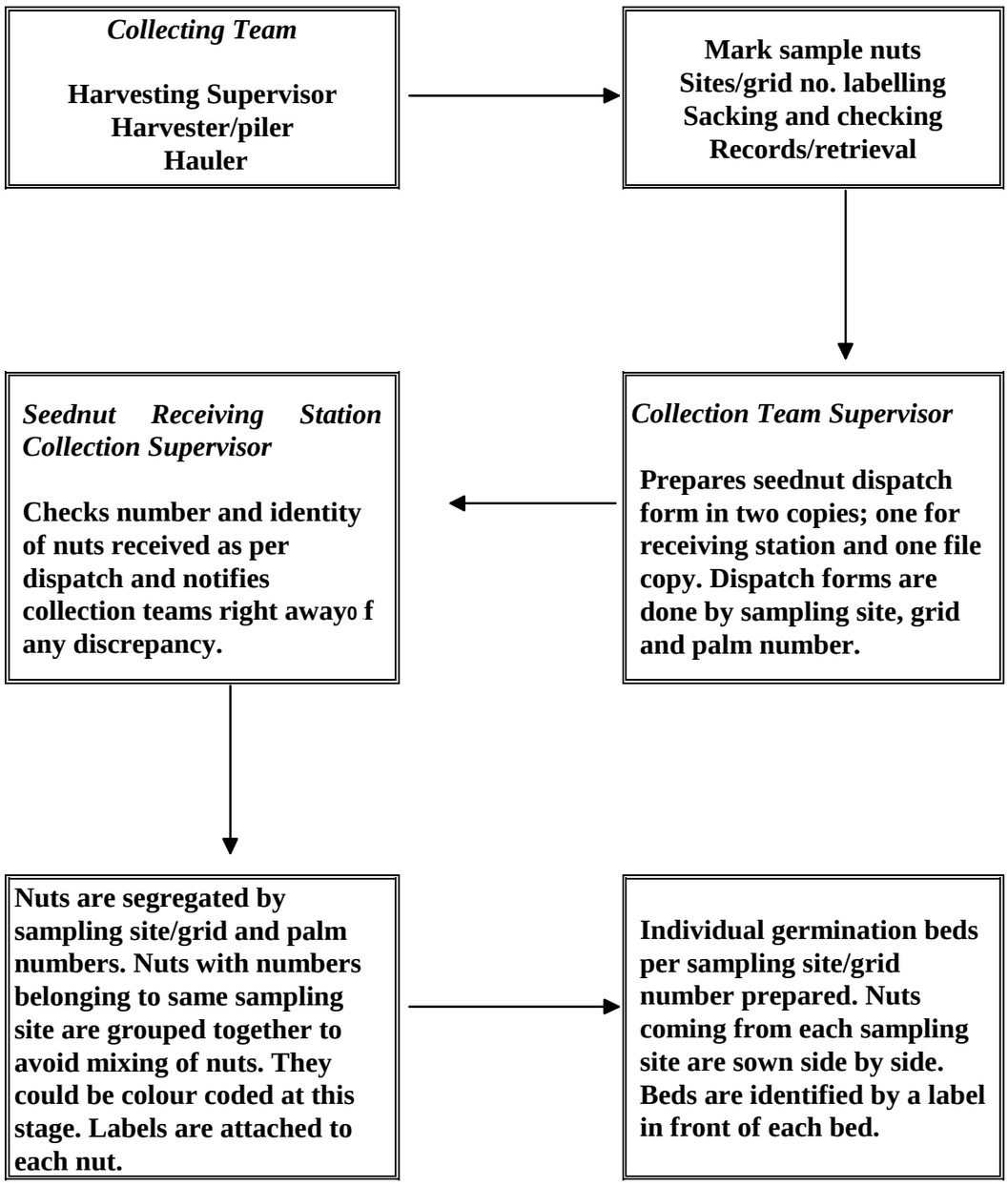
$$\boldsymbol{F} = \frac{\boldsymbol{N_1 N_2}\,(\boldsymbol{N_1} + \boldsymbol{N_2} - \boldsymbol{p} - \boldsymbol{1})}{\boldsymbol{p}(\boldsymbol{N_1} + \boldsymbol{N_2})\,(\boldsymbol{N_1} + \boldsymbol{N_2} - \boldsymbol{2})}\ \boldsymbol{D}^2$$

has a Fisher's distribution with p and $N_1 + N_2 - p - 1$ degrees of freedom. Then m, and $\boldsymbol{m}_1 = \boldsymbol{m}_2$ be declared different at level α if F is greater than the tabular value of a Fisher's variable with p and $N_1 + N_2 - p - 1$ degrees of freedom, at the corresponding level α. This test is known as Hotelling's T^2 test.

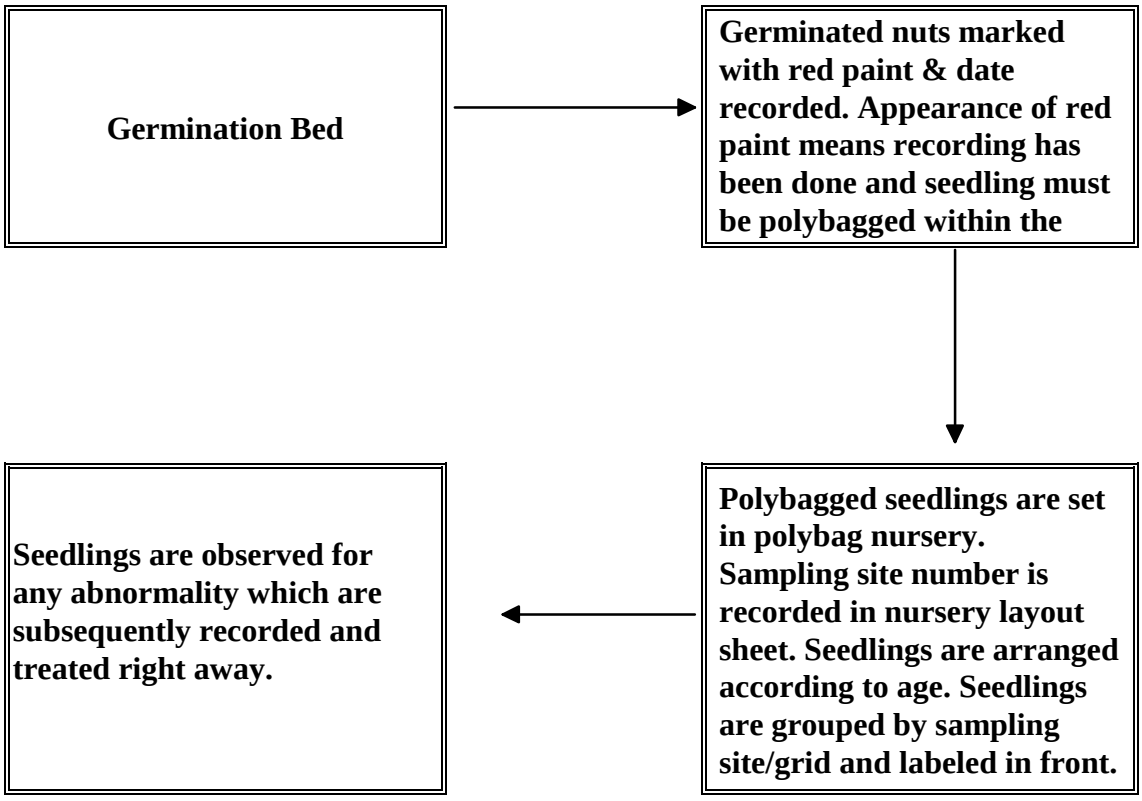
Appendix 3

FLOW DIAGRAM FOR THE HANDLING OF COLLECTED SEEEDNUTS AND SEEDLINGS

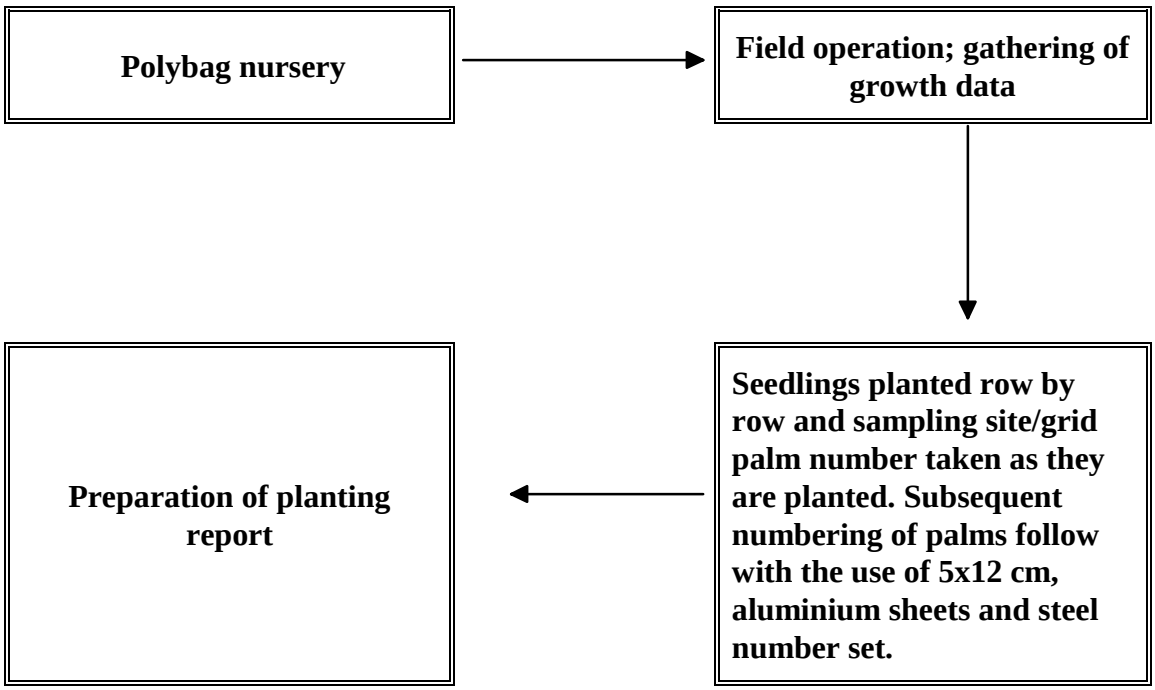
A. FROM HARVESTING TO THE GERMINATION BED



B. FROM PRICKING TO POLYBAG NURSERY



C. FROM NURSERY TO PLANTING IN THE FIELD



Appendix 4

CALCULATION OF COPRA RECOVERY AND FRUIT COMPONENT

To obtain the weights of the various components, the following calculations are used (note: all weights are in grams per fruit):

Weight of fruit (M)	$\frac{\text{total weight of fruits (A)}}{\text{total number of samples (B)}}$
Weight of husk (HW)	$\frac{\text{(A) - total weight of nuts (NW)}}{\text{(B)}}$
Weight of water	$\frac{\text{(NW - total wt. of split nut (SNW)}}{\text{(B)}}$
Weight of fresh meat (FMW)	$\frac{\text{(SNW - total weight of shell (SW)}}{\text{(B)}}$
Dry matter	$\frac{\text{(oven-dried wt- res. moisture)}}{\text{(FMW)}} \times 100$
Weight of copra	$\frac{\text{(FMW) x DM}}{94 \times 100}$

Appendix 5

EQUIPMENT AND MATERIALS NEEDED IN POLLEN COLLECTION, POLLEN PROCESSING AND POLLINATION

1 . Isolation bag - The bag used is made of thick, closely-woven canvas or thick denim material. It must be pollen-proof but allow air exchange. It is 90 cm long and 60 cm wide. A plastic window with a dimension of 16 x 27 cm is provided on one side, 10 to 13 em from the sealed end of the bag. For pollen collection, a sleeve, 40 cm long and 14-16 cm wide, is provided. For pollination, the sleeve is omitted but a small hole is punched at the upper mid-portion of the plastic window. Some bags could be designed for left handed individuals.

2. Male flowers collection bag - This bag has a dimension of 22 x 32 em with overlap, made of thick, closely-woven canvas or denim material or thick plastic. This is where the spikelets are placed when collected.

3. Pollen manipulation box - The box is made of plain aluminium, 1.75 mm thick equipped with 2 detachable, 1,000-watt infrared quartz lamps or chromium wire for heat sterilization and decontamination purposes.

4. Oven - The oven (Memmet or Hereaus) is provided with a fan to allow free air circulation, a thermostat, and a temperature knob. The capacity of the oven depends on the expected volume of male flowers to dry per day. The oven should be capable of attaining and maintaining 40T to dry the flowers in 36 hours or less.

5. Pollen processing - Through the years, the technology in the processing of coconut pollen has undergone tremendous modifications. Today, the most suitable technique is employed using sophisticated equipment to ensure prolonged pollen viability. Some equipment and supplies that could be used include:

   (a) Freeze-Dryer - This could either be the refrigerated or non-refrigerated type. In the former, freeze-drying is carried out by providing a suitable amount of colour indicated desiccant and is good for less than 1,000 cc of material. The latter is good for quantities more than 1,000 cc

   (b) Air-gas sealing torch, (e.g. Type 3A Blowpipe, VERIFLO Corp. Richmond, CALIF)

   (c) Air-vacuum pump, (e.g. EDWARDS EGB 1)

   (d) Glass ampoule constrictor, (e.g. EDWARDS Model 2)

   (e) Vacuum/spark tester (e.g. EDWARDS, ST4M Spark Tester)

   (f) Brass sieves - 21 em diameter, fitted with bronze wire gauze No. 200, with cover and bottom pan

   (g) Glass ampoule - length - 100 mm; thickness = 0.5 to 1 mm, 0.5 em diameter.

   (h) Small canvas bags - Same material as isolation bags, 22 x 23 em with overlap. Number depends on volume of male flowers dry

   (i) Pruning shears or secateurs - Number depends on the number of pollen collector and emasculators

(j) Paper bags - 21 x 31 em, brown or white

6. Chemicals, other supplies and equipment

(a) Trigger sprayer, half litre capacity

(b) Plastic puffer bottles, 100 or 200 ml capacity

(c) Ampoule cracker

(d) Ethyl alcohol, 95%

(e) Cotton or kapok *(Ceiba pentandra)*

(f) Sticking tape, 12 inch wide

(g) Insecticide, WP

(h) Aerosol insecticide, water-based

(i) Talcum powder

(j) Rubber band

(k) Detergents, lysol

(1) Pipettes, beaker, petri-dish, agar, analytical sucrose. H3B03 (Boric acid)

(m) Tally counter

(n) Pollen trays

(o) Map pins, assorted colours

7. Analytical balance

8. Compound microscope

9. Deep freezer, upright type

10. Hot plate or gas stove

11. Ladders, preferably 4, 8, 12, 14 ft long, could be aluminium or bamboo.

12. Embossing machine for 13 mm aluminium or stainless steel labelling tape

13. Dymo label maker - for 10 mm vinyl tape

14. Tagging wire, copper, 1 mm thick

15. Labelling tapes - aluminium, no adhesive, 13 mm or coloured vinyl tapes, 10 mm.

16. Marking pens, glass pencil

Disclaimer - Mention of trade names of equipment and products in this manual is done only for illustrative purposes and does not constitute an endorsement from COGENT/IPGRI.

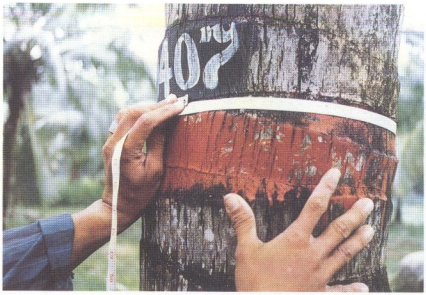


Fig.1. Measuring stem diameter, 1.5m from ground level

Fig.2. Measuring stem length of 11 leaf scars

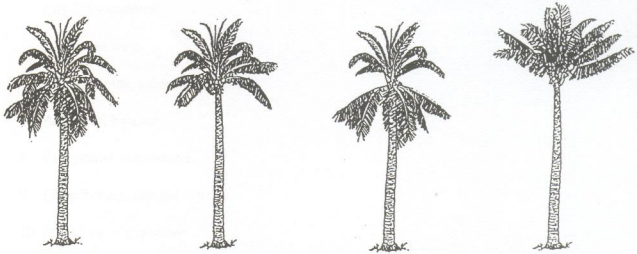


Fig. 3. Over-all appearance/shape of crown

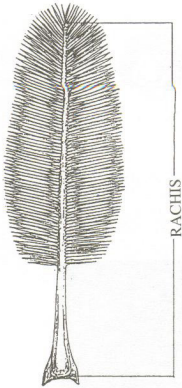


Fig. 4. Coconut leaf

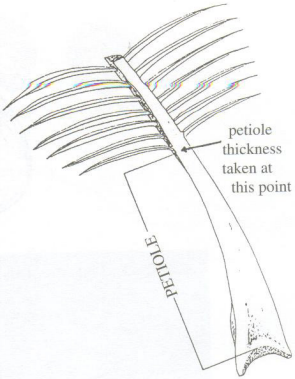


Fig. 5. Petiole of coconut leaf showing first leaflet insertion

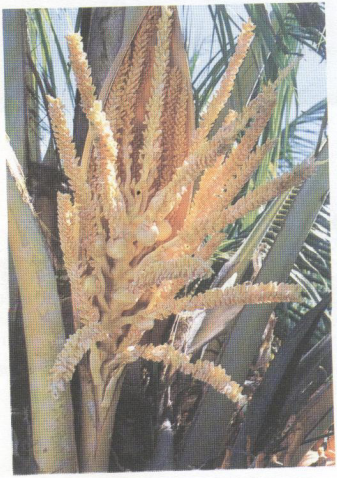


Fig.6. A newly opened coconut inflorescene

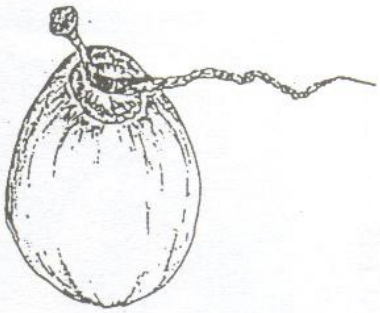


Fig. 7a. Polar view of coconut fruit

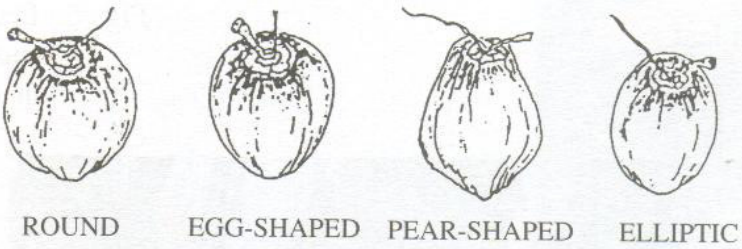


Fig. 7b. Shape of fruit, polar view

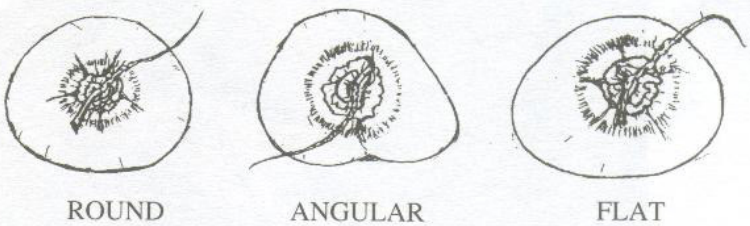


Fig. 8. Equatorial view of fruit

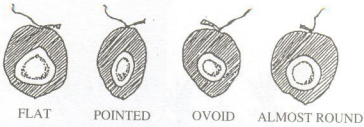


Fig. 9. Appearance/shape of nut cavity

Fig.10a. Coconut seedbed

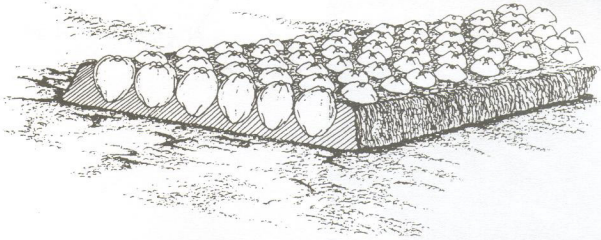


Fig. 10b. Sowing of seednuts in a germination bed

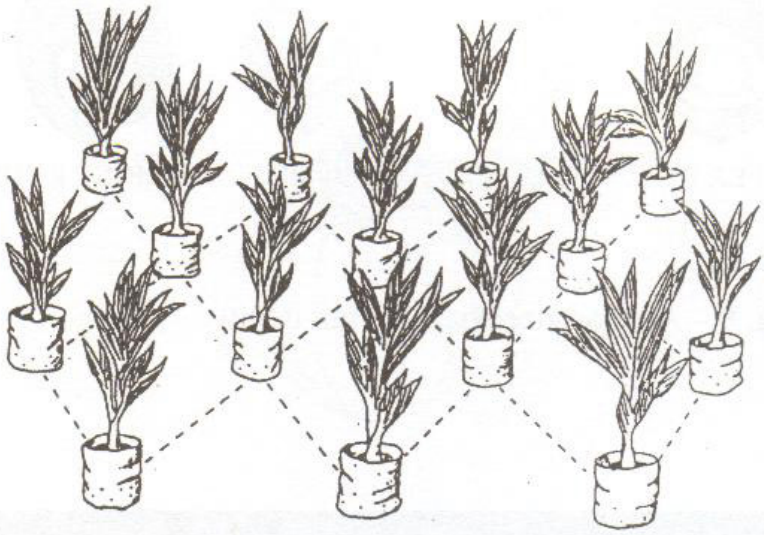


Fig. 11. Arrangement of seedlings in polybag nursery

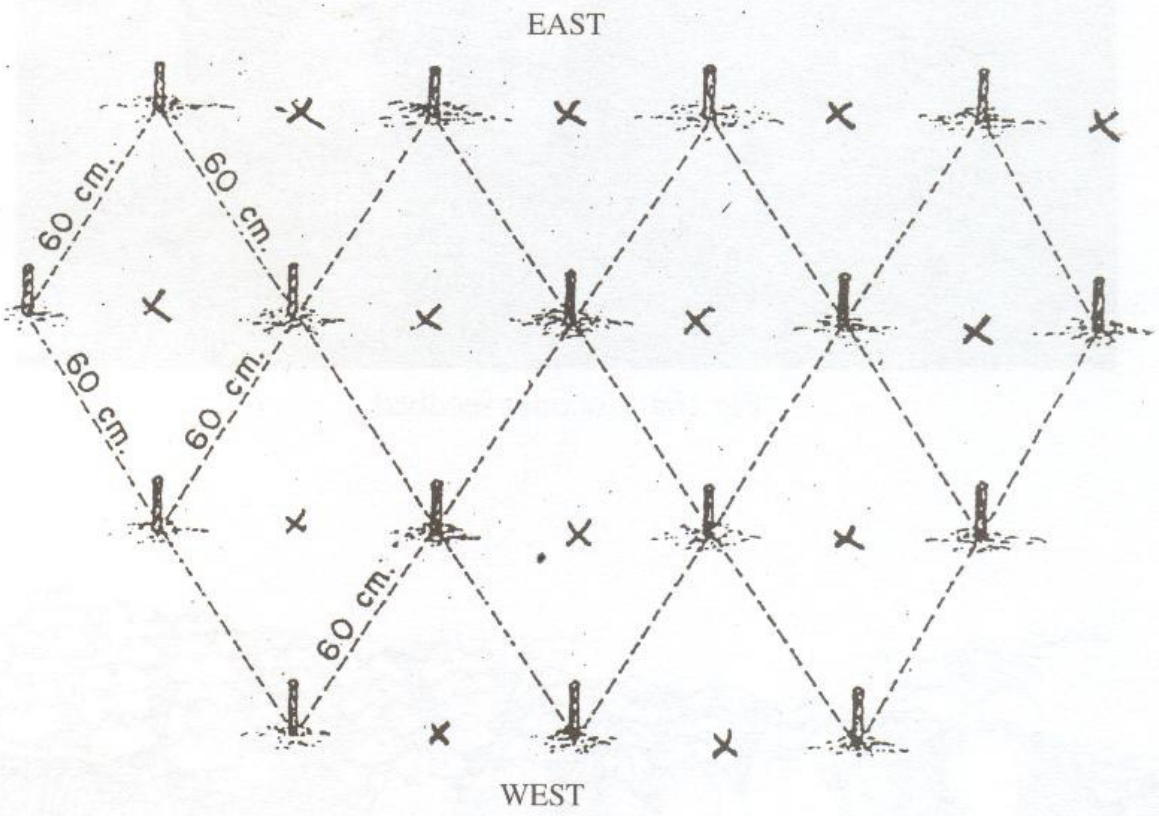


Fig. 12. Staking of polybag nursery

Fig.13. Marking of leaf no. 1

Fig. 14 located after Fig. 15

Fig.15. Spraying weak insecticide on the inflorescence just before bagging for pollen collection

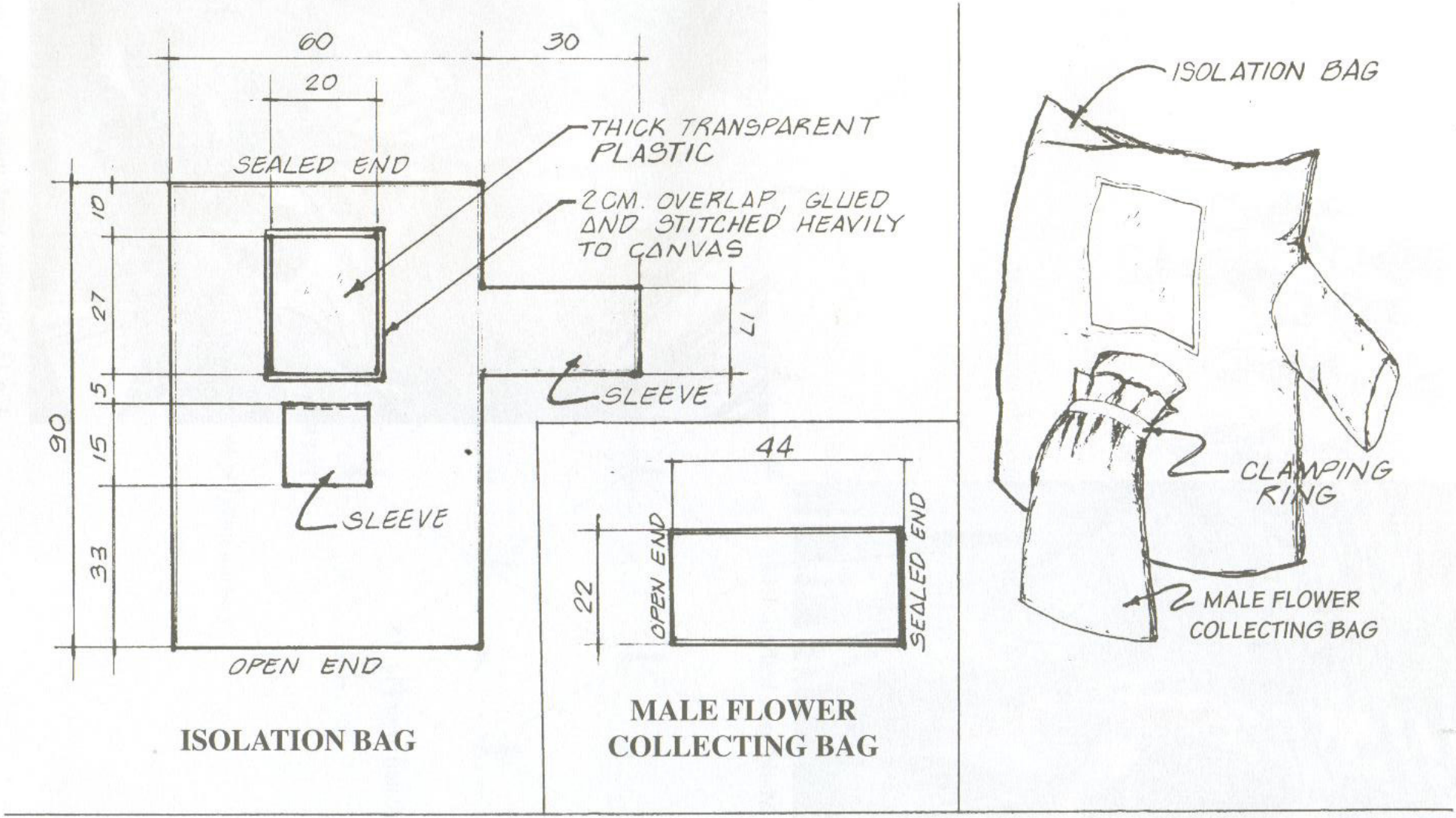


Fig. 14. Male flower collecting bag

Fig.16. Bagging of inflorescence for pollen collection. Note sealed arm sleeve.

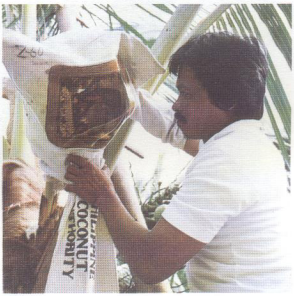


Fig.17. Cutting spikelets with male flowers

Fig.18. Sterilization of Pollen Manipulation Box (PMB) using two infra-red lamps (1000 watt per bulb)

Fig.19. Transferring spikelets with male flowers into the PMB

Fig.20. Detaching male flowers from spikelets inside PMB

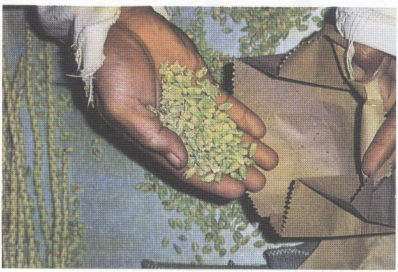


Fig.21a. Placing male flowers in paper bag

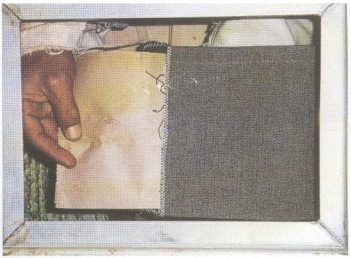


Fig.21b. Placing male flowers in canvas bags

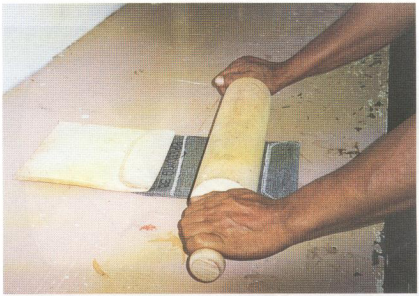


Fig.22. Cracking male flowers to free the pollen grains

Fig.23. Drying male flowers

Fig.24. Sieving male flowers to extract and sift pollen

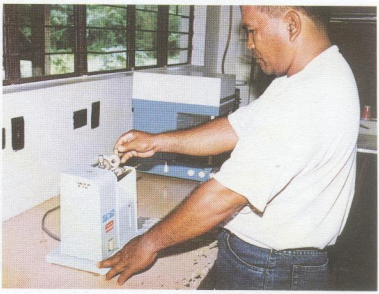


Fig.25. Ampoule constrictor

Fig.26. Fitting ampoules on the teats of the freeze-dryer

Fig.27. Sealing ampoules with a blow torch

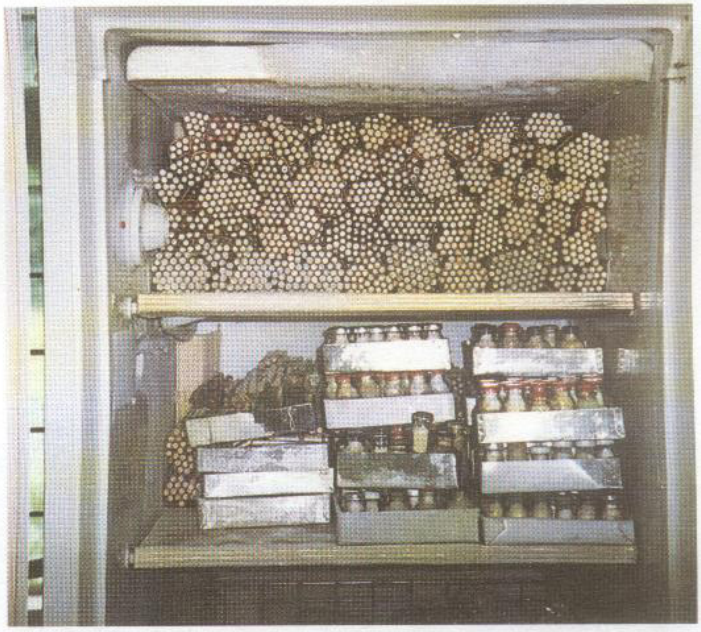


Fig.28. Processed pollen stored in an upright freezer

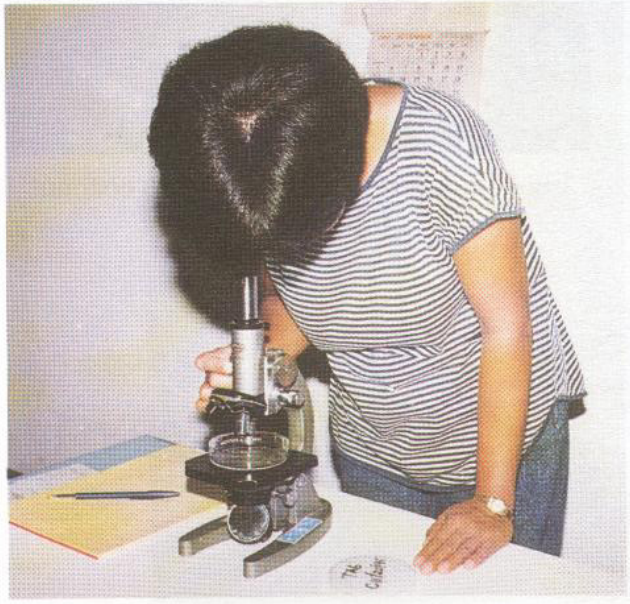


Fig.29. Checking pollen viability

Fig.30. Emasculation. Male flowers are collected in a bag

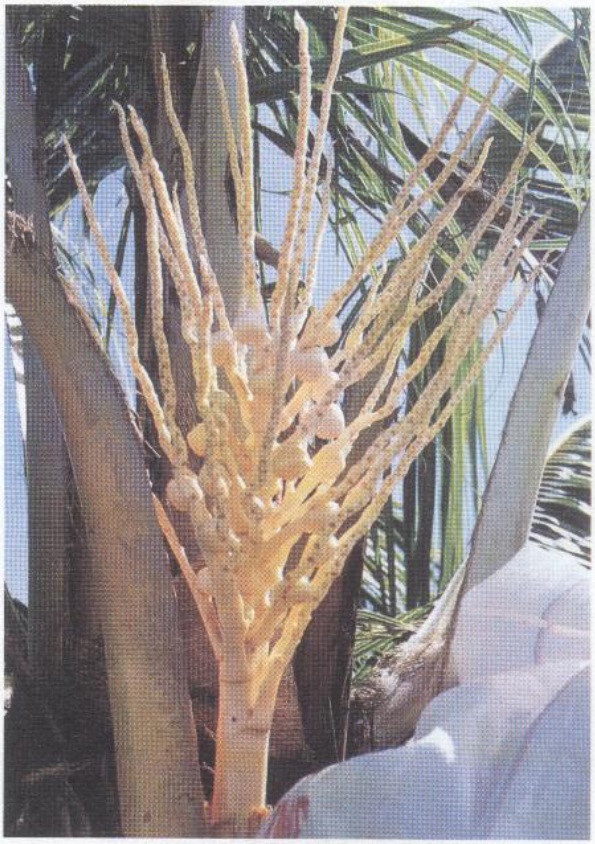


Fig.31a. A properly emasculated coconut inflorescence

Fig.31b. A properly isolated emasculated inflorescence

Fig.32. Slipping the isolation bag on the emasculated inflorescence

Fig.33. Pollen-talc mixing inside PMB

Fig.34. Pollination of isolated female flowers

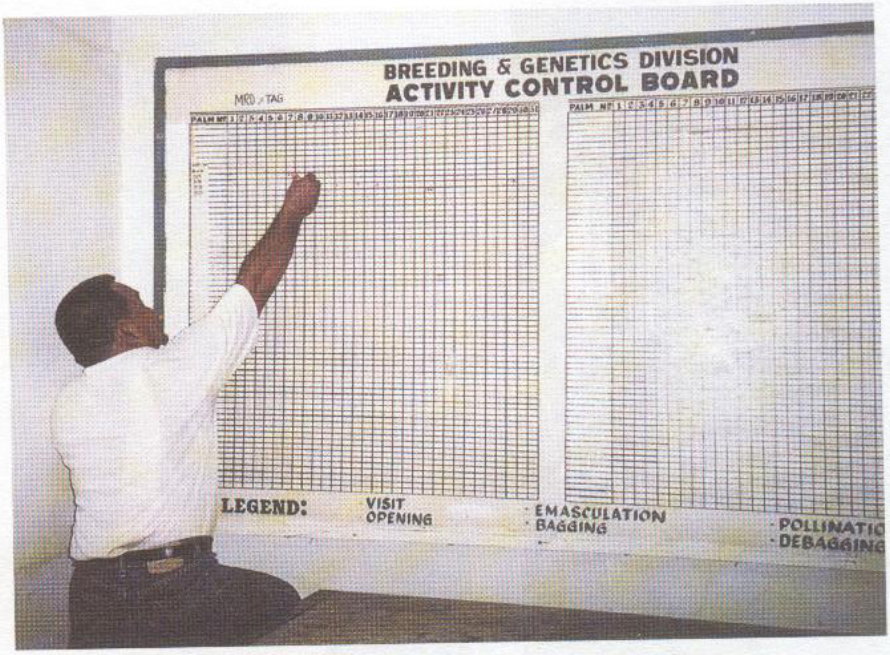
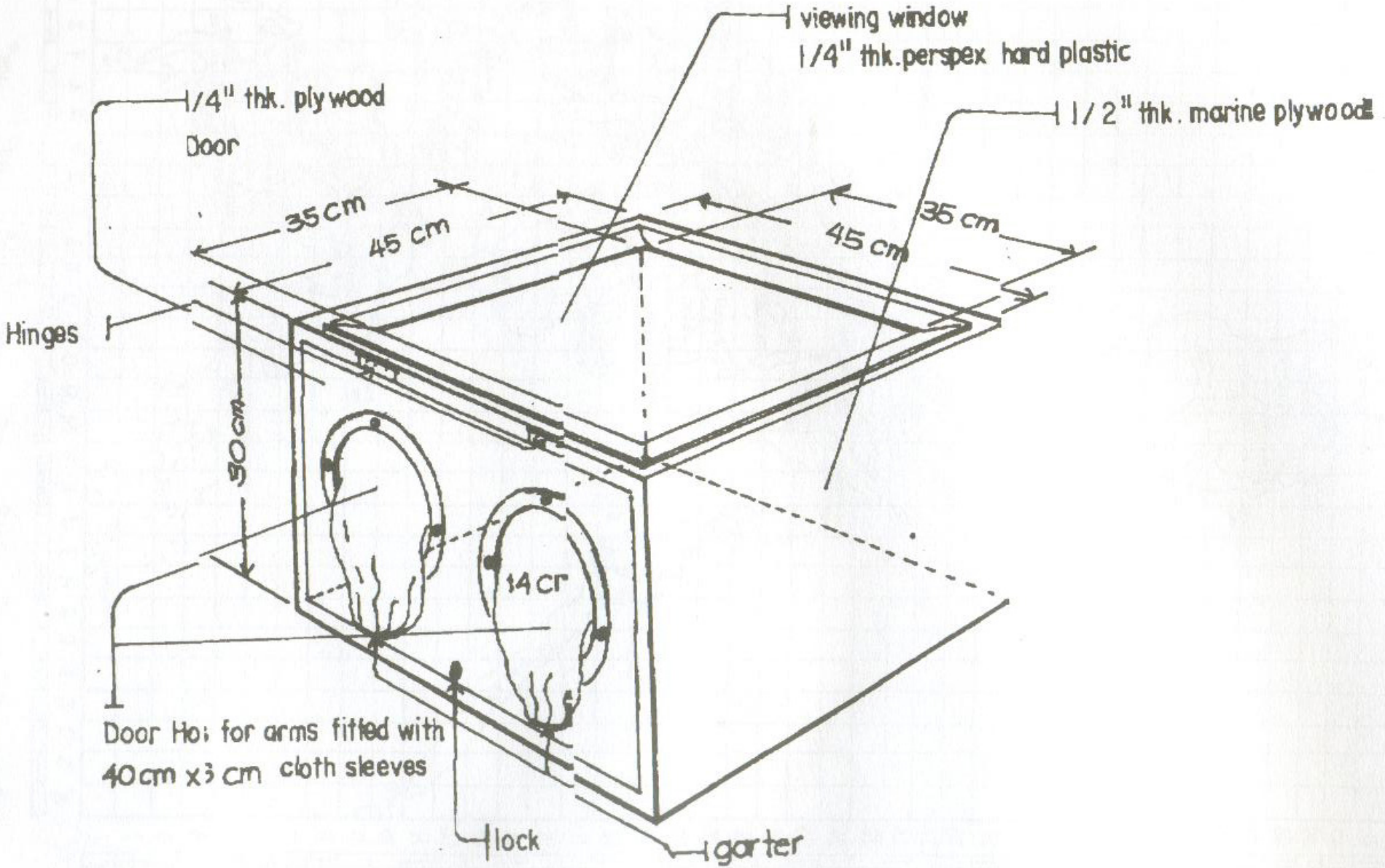


Fig. 35. Activity control board indicating operations

Fig. 36. A newly de-bagged hand-pollinated inflorescence. Note label bearing pollination number attached to the bunch

Fig. 37 FIELD INOCULATION BOX



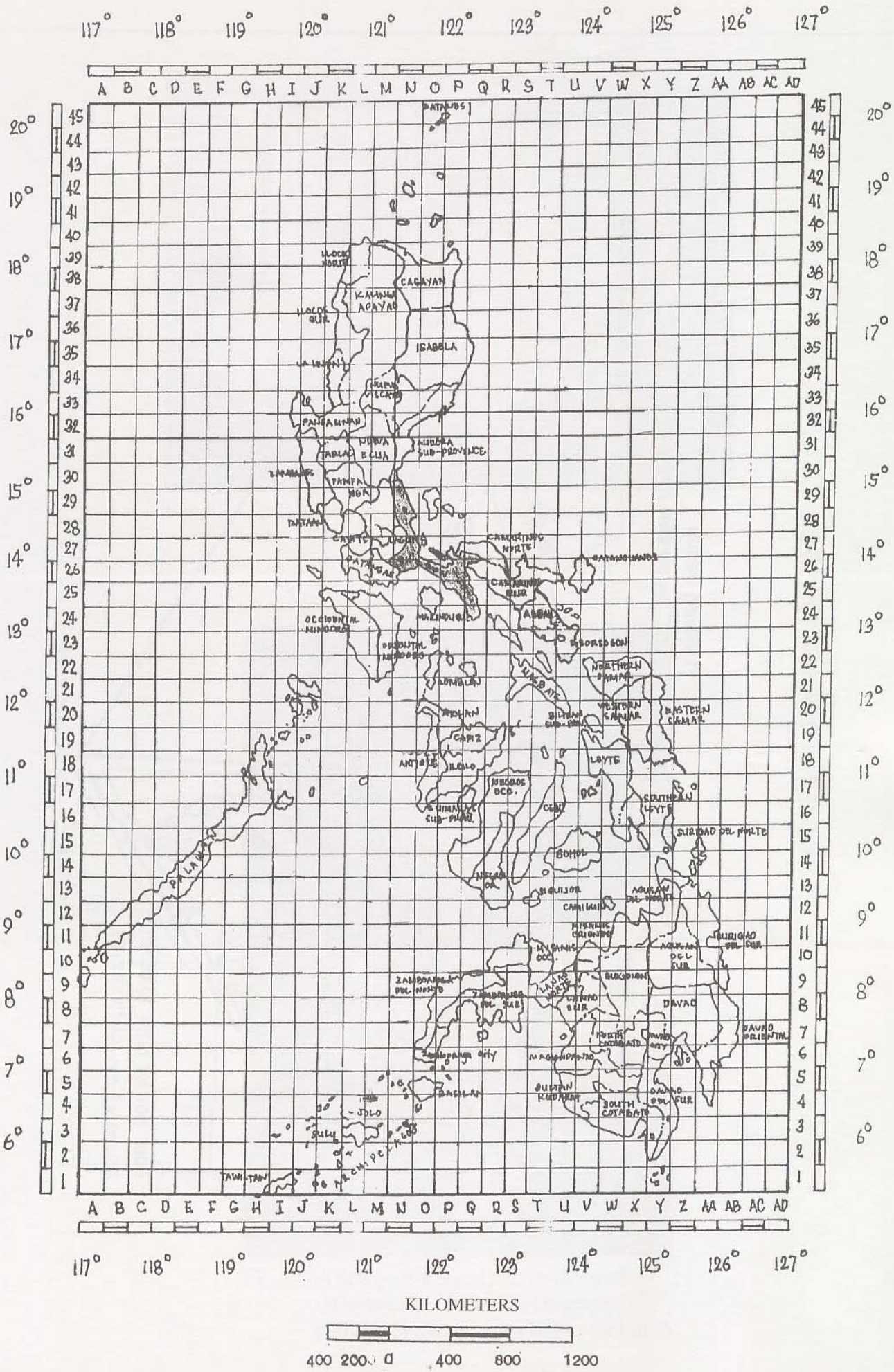


Fig. 38. Map of the Philippines, with grids

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