

PROCEEDINGS  
OF THE  
SYMPOSIUM ON THE SCIENCE  
OF OIL PALM BREEDING



**PALM OIL RESEARCH INSTITUTE OF MALAYSIA**  
**MINISTRY OF PRIMARY INDUSTRIES, MALAYSIA**

**PROCEEDINGS OF THE SYMPOSIUM**  
**ON**  
**THE SCIENCE OF OIL PALM BREEDING**

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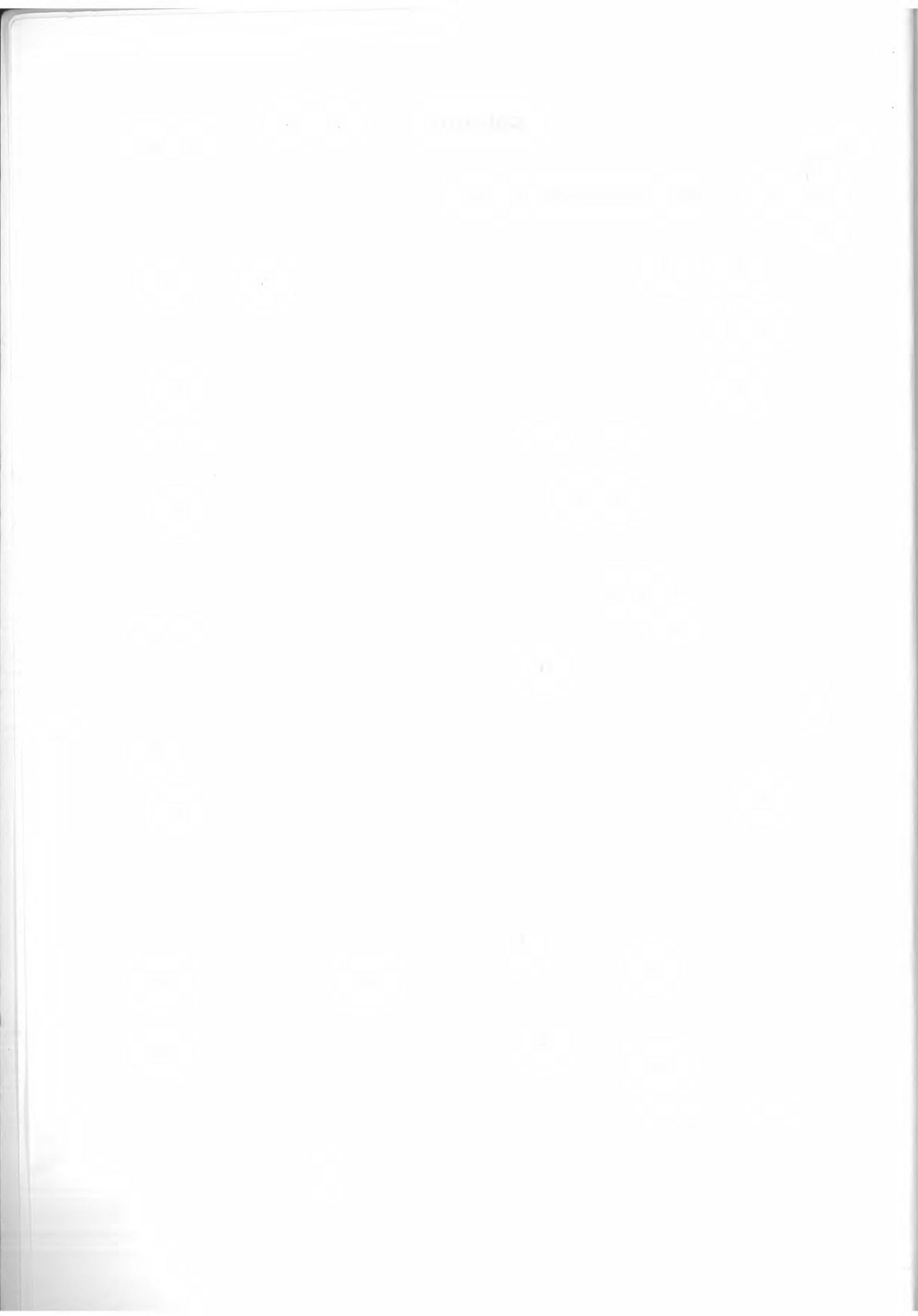
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## **Preface**

The symposium on 'The Science of Oil Palm Breeding' was organised by ISOPB, CIRAD and PORIM. It was held from 30 June - 2 July 1992 at Montpellier, France.

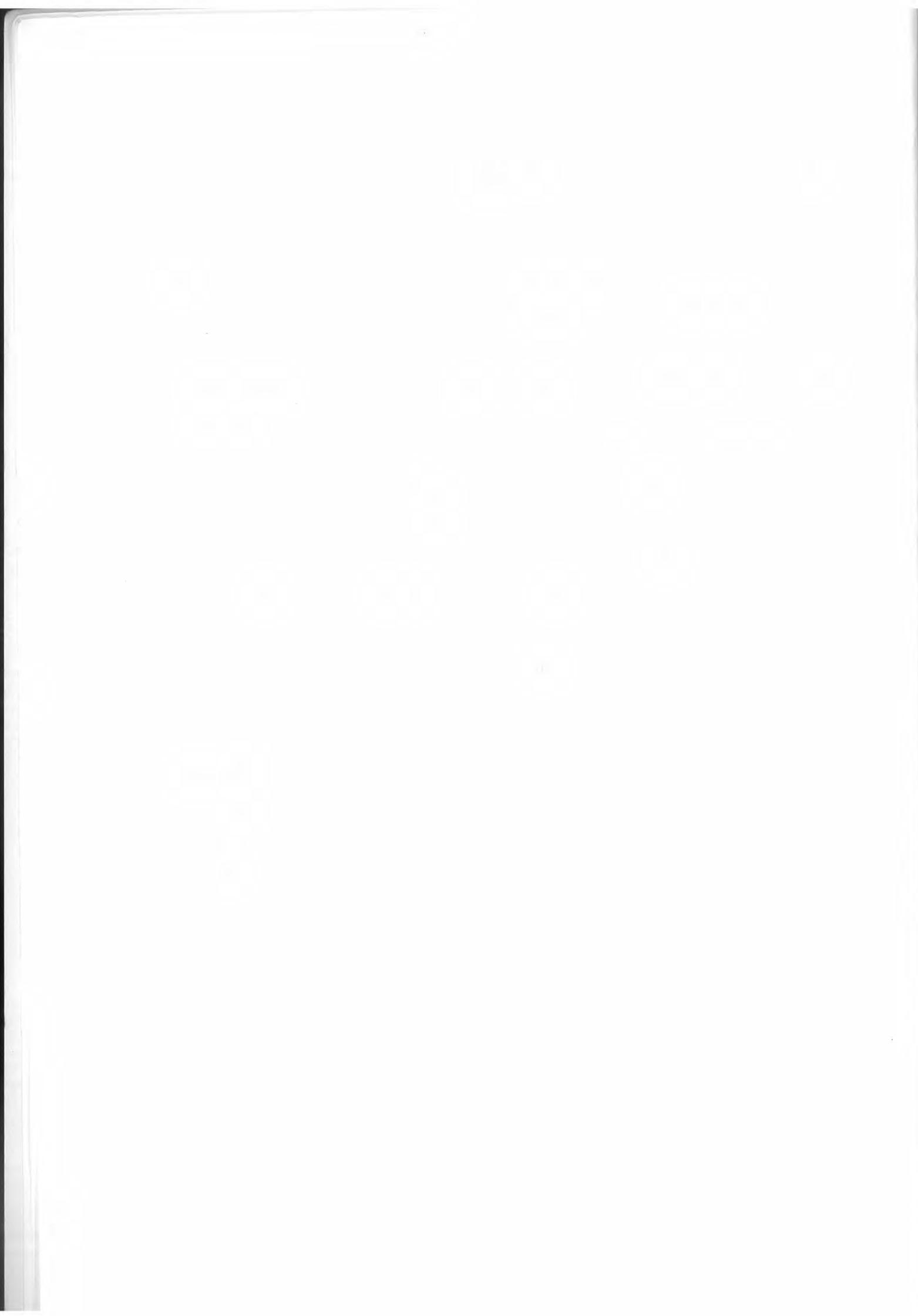
The proceedings contain 15 chapters covering fields such as oil palm genetic resources, application of biotechnology to oil palm breeding, breeding plans, selection methods, oil palm breeding programmes and future prospects for oil palm breeding. We have attempted to bring together in one volume the main topics of interest in oil palm breeding. The proceedings will give an excellent introduction on oil palm breeding to current and future oil palm breeders. Although there is a delay in publishing the proceedings, the contents are still relevant to the present time.

### **Editors**

Rajanaidu N.

Jalani B.S.

June, 1999



## **DEVELOPMENTS IN THE FIELD OF BIOTECHNOLOGY AND THEIR APPLICATIONS TO OIL PALM BREEDING**

J. Meunier<sup>1</sup>

Mr. Chairman, Ladies and Gentleman,

I would first like to thank the ISOPB for inviting me to present this talk on breeding and its application to oil palm.

My first reaction as a "classical" geneticist would be to lament the lack, over the past ten years, of major developments in plant breeding compared with the remarkable steps forward in cellular and molecular biology. It is clear that the tools developed by these disciplines will no doubt rapidly revolutionize the work of breeders.

I shall therefore talk less of breeding as such than of these impressive techniques, generally known as biotechnology, and their applications to breeding.

You are familiar with the three fields of particular importance in which recent developments have opened the way for practical applications in the near future, and which universal applicability means that oil palm will not be spared them. Although they may appear independent, the techniques are largely complementary. They are:

- genome analysis
- micropropagation
- genetic engineering

### **The Breeders' Dream: Knowing The Genome**

Only 10 years ago, this almost unattainable dream, particularly for perennial plants, was limited to knowing only a few Mendelian genes, often of little interest. It is now becoming realized with molecular markers.

Several molecular biology techniques, such as Restriction Fragment Length Polymorphism (RFLP) and Polymerase Chain Reaction (PCR) now make it possible to produce genetic and physical genome maps. Their areas of application are vast, as it is now possible to gain access

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<sup>1</sup> CIRAD, Montpellier, France

into nuclear and cytoplasmic genes and structural and regulatory genes to understand their roles and modes of function. I shall not go into details of this range of tools, which are constantly developing, particularly under momentum from the Human Genome Project (see Annex).

Numerous species have benefited from these methods - maize, tomato, tobacco, wheat, barley, rice, etc. In our Montpellier laboratories, (BIOTROP), we have begun genome mapping for cocoa, banana, rubber and sugar cane.

I feel that oil palm genome analysis is one of the major priorities today for several reasons:

Analysis of the genetic diversity of materials in collection and checks on progeny legitimacy are now possible using enzymatic markers.

It is obvious that these tools, quite easily applicable to oil palm, are insufficiently used by research centres. In these fields, RFLP and PCR do not offer much in the way of further advances, except perhaps for clone identification, where enzymes may be insufficient to differentiate between all clones. However, molecular biology is of obvious value for:

- locating genes of major interest. Work under way on the shell thickness gene can be applied to other genes governing qualitative resistance or quality characters.
- monitoring the transmission of certain genes in backcrossing programmes (vascular wilt tolerance, growth, oil composition in *E. oleifera* and *E. guineensis* hybridization programmes) and in recombining schemes.
- generally speaking, finding early breeding markers, including those for quantitative characters, by detecting Quantitative Trait Loci (QTL) groups.
- in the longer term, understanding genome functioning (at nuclear, but also mitochondrial and chloroplastic, levels) and associated physiological characters, and preparing for controlled genome manipulation (genetic engineering).

The rapid development of these techniques means that they can now be realistically used for some of the above aims. RFLP studies remain complex and costly, needing well-equipped laboratories and broad cooperation, but PCR should rapidly become a tool for use by breeders.

## **From Multiplication to Improvement, An Indispensable Tool: *in vitro* regeneration**

I shall not go into detail on these techniques, which have been studied for more than 20 years in oil palm and which are described in many publications.

True-to-type vegetative propagation by somatic embryogenesis is one of the major turning points in the history of oil palm breeding. This technique may not lead to genetic progress but makes it possible to valorize and pass on to breeders genetic progress that is almost unusable in materials produced from seeds.

Molecular biology tools will shortly be able to guarantee reliability of the procedure. Some efforts are still required to improve output and lower the cost for commercial ramet production. Culturing isolated embryogenic structures in liquid medium is a technique that also allows for genetic engineering.

The use of *in vitro* culture for varietal creation has not generated much interest among oil palm researchers. This is understandable, given the priority accorded to trueness-to-type to help overcome the difficulties of conventional breeding in oil palm.

However, wheat, rice and asparagus varieties created from haploids by anther or ovule culture are already on the market. Haploid production is still a somewhat neglected field for oil palm. Even if dihaploid production did not lead to new varieties, it would still be of great interest for analyzing and understanding the genome.

Protoplast production is still a complex operation, of largely theoretical interest for oil palm. It would become an important tool if protoplasts become an essential step in genetic engineering.

## **Routine Genetic Transformation In The Future**

The most spectacular development over the past few years has been the transformation of cultivated species by genetic engineering. The recent releases into the market of slow-ripening tomatoes and cotton resistant to certain *Lepidopteras* are striking examples of this.

The obvious success of this approach can be put down to several factors: the universal nature of the biological phenomena involved which have been studied to great depth in animals and humans, the understanding of plant function and regulation gained by concentrating research on models (for example, *Arabidopsis thaliana*), the simultaneous development of analysis and transformation tools, and the theoretical simplicity of the methodologies.

The scope for application is limitless as, by introducing foreign genes or by modifying gene expression, it is possible to intervene in all aspects of plant functioning. There are now many examples of modified plants tested in the laboratory or field.

The best known cases are modifications of plant responses to the environment by introducing insect resistant genes:

- coding *Bacillus thuringiensis* genes for endotoxins in tobacco (by Plant Genetic System, Agracetus) and tomato (by Monsanto) in 1987, and many other species since,
- protease-inhibitive genes in tobacco (by Agricultural Genetics, 1987).

also genes controlling their responses to herbicides phosphinotricine = Basta, glyphosate = Round-Up, Atrazine), viruses (capsid protein genes, ribozymes) and fungi (chitinases).

It is also possible to intervene in biosynthesis by modifying the amounts of metabolites produced in the different organs of the plant and their compositions:

- increasing starch levels in potato by the expression of an ADP glucose pyrophosphorylase bacterial gene controlled by a patatine promoter.
- modifying stored fatty acid composition (triacylglyceros)
- synthesizing new proteins.

The genes controlling morphogenesis and floral biology are becoming better known and accessible.

- A series of genes from a very clearly defined region, the MADS box, have been cloned from *Arabidopsis* and *Antirrhinum*. These genes affect the different floral organs, and if expressed early in the floral meristems, the FLO gene can control floral expression.
- The promoter of a gene expressed specifically in anthers has been transferred to tobacco, rape, lettuce, chicory, cotton, tomato and maize. The tapetum of the transformed plants is destroyed, and they become sterile males (PGS). Introducing an RNase inhibition gene in restorative families makes it possible to obtain fertile progenies.

The genes governing apomixis will shortly be available. Their transfer can considerably modify cultivated crop production strategies.

It is also possible to influence plant physiology. The most advanced work concerns:

- controlling ripening in tomato by reducing polygalacturonase activity using a counter-sense RNA
- improving drought and salt tolerance by introducing a glycinebetaine biosynthesis chain.

Lastly, it is now possible to intervene in a controlled way in chloroplastic and mitochondrial DNA.

The identification, using biolistics, of the *chIN* and *chIB* genes involved in chlorophyll synthesis in darkness can lead to improved photosynthetic efficiency.

This brief overview, which is far from complete, gives an inkling of the many possibilities offered by genetic engineering for plant breeding. What are the possible applications to oil palm?

In theory, the universal nature of the techniques means that any application is possible. It is theoretically possible to envisage creating insect and fungus resistant, drought tolerant oil palms with reduced growth that are easier to harvest. It is easy to imagine the implications of introducing male sterility or apomixis in oil palm.

To quote a well-known researcher, "the important thing is to develop a transformation system; the applications will be self-evident". However, two fields seem to be of particular interest for the medium term:

- it should be quite easy to transfer some of the valuable characters of *Elaeis oleifera* without any major disturbance of the *E. guineensis* genome. The genes involved in resistance (to bud rot, vascular wilt), growth and oil composition would be good targets.
- modifying lipid biosynthesis to increase uses in the food industry (access to the market for fluid oils), particularly for high value-added industrial fatty acid production (hydroxyl groups, ricinoleic acid derivatives, esters, epoxy, dicarboxylic acids C8 - C12, etc.). Pressure from environmentalists to replace fossil fuels with renewable oils and the production potential of oil palm mean that the plant has a promising future.

## CONCLUSION

Recent progress in biotechnology has been such that its application to oil palm now seems to be a major priority.

To conclude, I would like to return to two passages in this talk which may appear somewhat daring, not to say provocative:

- The relative ease with which the techniques can be implemented is a reality. Current labeling methods mean that it may be possible to produce usable genetic maps within 2 to 3 years. Transformation tools are developing rapidly and are gradually being mastered, even for monocotyledons, which were long considered recalcitrant. The main issues are only the resources and degree of collaboration required. The only real bottleneck in applying these techniques remains the regeneration of transformed plants. We therefore have to continue and step up our research on *in vitro* vegetative propagation, which is the key to possible future progress via genetic engineering.

- The second aspect is "the lack of major developments in the plant breeding field" as such. It would be wrong to believe that oil palm breeding in the future will be wholly ensured by a few biologists working in isolation in well-equipped laboratories. The most consistent and significant progress will still come from conventional breeding for some time to come. We would insist on the fact that it is essential to create the right genotype before multiplying it. Similarly, promising genes will only come into their own in the right genome.

We must guard against placing too much faith in the magic of transformation. In-depth knowledge of populations, heredity studies and breeding schemes integrating biotechnology will remain the most important factors in genetic progress, coupled with essential cooperation between all protagonists.

### ANNEX

The wide range of genome sequencing and labeling techniques has given rise to a multitude of acronyms. The following list, although far from complete, gives the acronyms for the techniques most commonly used or currently being improved.

- CSC :** Coincident Sequence Cloning: enables sequence isolation in complex DNA mixtures
- FISH :** Fluorescence *In Situ* Hybridization: enables the specific detection of single sequences of different lengths.
- OHS :** Oligonucleotide Hybridization Sequencing: enables the sequencing of long DNA fragments from small sequences (also FS = Fragmentation Sequencing, and SBH = Sequencing by Hybridization).
- PCR :** Polymerase Chain Reaction: a technique for enzymatic amplification of specific nucleotide sequences, enables sequencing by chromosome walking.
- PGFE :** Pulsed-Fields Gel Electrophoresis: makes it possible to produce physical maps by separating large DNA fragments containing a marker.
- RAPD :** Randomly Amplified Polymorphic DNA: a range of techniques enabling the amplification of small nucleotide sequences (FS, OHS, PCR, SBH, etc.)

- RFLP :** Restriction Length Fragment Polymorphism. Makes it possible to produce genetic maps and monitor the heredity of specific genes using bonding groups.
- STM :** Scanning Tunneling Microscopy: DNA sequencing by direct observation under a tunneling microscope.
- STS :** Sequence Tagged Site: short non-repeated DNA sequences characterizing a region of the genome and detectable by PCR (also SCP = Single Copy Probe).
- YAC :** Yeast Artificial Chromosome: cloning vector enabling the insertion of large DNA fragments (up to 2 mb) and the cloning of large genes.

For more details, see 1992 - Trends in Biotechnology - Vol. 10, 1/2 - Special Issue: Genome mapping and sequencing.

## SOME ANCESTRAL PALMS AND THEIR DESCENDANTS

Rosenquist E.A.<sup>1</sup>

### ABSTRACT

*Three oil palm breeding populations of restricted origin are discussed:*

- i) *The Serdang Avenue palms which were introgressed with the unrelated Ulu Remis population and have since been distributed to many countries.*
- ii) *The SP540-AVROS population which has been distributed as a "pure" breeding population of restricted origin to many countries and which is still being widely used as a source of pisifera.*
- iii) *The Dumpy palm which has not fulfilled early expectations but which is of renewed interest as it appears to be very resistant to vascular wilt (Fusarium Oxysporum).*

### INTRODUCTION

The tree breeding populations to be described have been chosen because I have worked with them. The last of the Serdang Avenue palms were still alive when I started work at Serdang in 1949 as "Botanist", but I did not then appreciate their importance. At that time Jagoe was the Senior Botanist and the Dumpy progeny received much of my attention for five years. Although the "AVROS SP540" population was already well known I did not work with it until during my first visit to Dami Oil Palm Research Station in 1972.

It is sometimes important to know the origin of the material in a breeding programme but too often early records get lost. Since 1972 I have been fortunate enough to visit many oil palm breeding stations. This paper attempts to draw together historical information for the benefit of oil palm breeders working with the three populations in many parts of the world.

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<sup>1</sup>Consultant Agronomist, England.

## I. THE CONTRIBUTION FROM SERDANG AVENUE PALMS

Oil palm breeders in Malaysia, and elsewhere, often refer to “the Ulu Remis Deli population” when discussing Deli palms that originated at Ulu Remis. It is sometimes forgotten that in many cases more than half of the genes came from the Serdang Avenue palms.

The lineage of the Serdang Avenue palms and the Ulu Remis “Parent Palms” are shown in figure 1. Both strains can be traced back to the Economic Garden, Bogor, where a population of palms derived from the original four palms in the Botanic Garden was planted in 1878.

From the Economic Garden seed reached the “Public Garden” in Kuala Lumpur in 1905 by a route that is not known. These were fruiting in 1909 (Bunting *et. al.*, 1934). Seed from the Public Garden was used to establish palms in the “Experimental Plantation KL” and this was the source of seed for the Serdang Avenue Palms. Selected Serdang Avenue palms were crossed and the progenies were planted on *Elaeis* estate in 1931 where selected palms were integrated with the Ulu Remis breeding programme in 1937/38.

The Ulu Remis strain also originated in the Economic Garden in Bogor but passed through an unknown number of generations in ornamental avenues in Sumatra until it was established at Marihat Baris Estate in 1915. Seed from Marihat Baris was planted at Ulu Remis in 1929/30 where “Parent Palms” were selected and used in breeding programmes from 1935.

The first crosses between the Serdang Avenue strain and the “Ulu Remis P.P.” strain were planted in GB IIIA in September 1938. The two strains were therefore separate from 1878 (or earlier) for 60 years or more, during which time they each passed through at least three generations.

### **The Serdang Avenue**

The “Guide to the Government Experimental Plantations, Serdang: FMS Jan 1931” (Anon 1930) records on page 18 that the Serdang Avenue palms were planted in May 1922. The avenue runs east and west from near the entrance to the station and in front of the former College of Agriculture. There were 43 palms north of the avenue and 43 south of the avenue. Artificial pollination was applied to one inflorescence per month to the palms on the south site only. This increased the weight of bunches by 153% and the weight of clean fruit by 330%. But, it was to be many years before it was realised that the absence of a small insect pollinator common in

Africa was the explanation. Most, but not all, observers in Africa had been looking for bees and butterflies.

Yields of the Avenue palms were recorded from 1925 to 1929. The highest yielding palms all had "odd" numbers and were, no doubt, on the south side of the Avenue. The "Guide" lists ten selected palms and gives annual yields. The average annual yield of these palms increased from 42 kg/palm in 1925 to 121 kg in 1929. Hartley (1967) records that the average fruit to bunch ratio was 64.9% and the average mesocarp to fruit 61%. There has never been any doubt that these were "Deli" palms and the latter figure helps to confirm it.

The "Guide" lists 20 legitimate progenies that were planted in November 1930 in Block 10A with an open pollinated progeny in every fifth row as a control. It was when the progenies though to be legitimate failed to outyield the controls that interest in this planting declined. Serdang Av 23 was crossed with 8 of the palms listed by Hartley and these progenies were planted in Block 17-19 in October 1930. There are no records to show that these second generation palms at Serdang were further developed in Malaysia.

Fortunately legitimate seed from Serdang Avenue crosses was sent to WAIFOR (now NIFOR) and to Elaeis Estate and these descendants will be discussed in turn.

#### At WAIFOR (NIFOR)

The WAIFOR Deli Trial (Expt S-1) was planted in June 1941. (Toovey and Broekmans, 1955). It contained 10 Serdang Avenue Dx D crosses and 5 local progenies (3 selfs and 2 crosses). The Serdang parents included only S.AV 7 and 65 of the original 10 selections. The parents did include 6 Avenue palms not previously selected and 4 from the Serdang Avenue F2 in Block 10A but not S.AV 5 whose progenies did so well on *Elaeis*.

One Malaysian cross yielded well (S.AV 19 x 65) but the others were inferior to local strains.

	<u>Fruit bunches kg/palm/annum</u>	
	1946 - 49	1950 - 53
Malaysian average (10)	31	41
Nigerian (5)	29	68

The better mesocarp on bunch of the Malaysian material (37.4% v 32.5%) did not compensate for the lower yield. Toovey thought the large bunches and low bunch number of the Deli may make them unsuitable for the local climate with large seasonal variations. The conclusion has been confirmed elsewhere in Africa but the Deli makes a good female parent and is used for example by IRHO to produce seed for Africa.

In the large genetic blocks at Binga (Rosenquist *et. al.*, 1990) the Deli has very good GCA for yield when crossed with “African palms” (Corley and Dumortier private communication).

The Serdang Avenue population has been maintained at NIFOR and four second generation selections of S.Av19 x S.Av65 have been intercrossed (intra Deli crosses) and compared with Deli selections obtained by intercrossing five different sources (inter-Deli crosses) and with five unrelated African *dura* parents (Okwuagwu - Oil Palm Conference 1991). In the abstract to her paper she explains how quantitative genetic estimates of total genetic variance for bunch yield, bunch number and average single bunch weight were calculated. The levels of total and heritable genetic variation in the intra-Deli population were either very low or absent. For the inter-Deli populations the estimates were high and comparable with estimates for the population derived from unrelated *dura* parents. She concludes “It is evident from the results of this study that intercrossing among Deli selections from different breeding programmes or sources for which generations of selection have resulted in the fixation of different groups of genes, is comparable to mating among unrelated parents”. This conclusion may help to explain the unexpectedly large variability found when the Serdang Avenue population was introgressed with the Ulu Remis Deli population as explained below.

### **Ulu Remis Parent Palms (PP)**

The “parent palms” on Ulu Remis estate came as open pollinated seed from Sumatra in about 1929. (Yield recording started in 1933). The first large scale selection programme in Sumatra started on Marihat Baris estate when 2000 palms which had been planted in 1915 were recorded from the start of production (Hartley 1967). It is believed that the open-pollinated seed received by Ulu Remis came from Marihat Baris. It is possible that the seed came from palms that had been yield recorded.

The first crosses between Ulu Remis “Parent Palms” were planted in GBIA in September 1935. The crosses must have been made in early 1934 soon after recording started. Genetic blocks I to V which contained progenies of 129 Ulu Remis parent palms were recorded for six years and

the yields per palm increased with successive genetic blocks from 105 kg/palm/yr in GB IA to 167 kg/palm/yr in GB VB planted in April 1941.

These figures are distorted because the ages at which the palms were recorded varied and because of improved agronomic practices in later genetic blocks especially increasing use of fertilisers. Nevertheless there is little doubt that the later blocks contained better genetic material because they had been selected over a longer period.

### **The Serdang Avenue/Ulu Remis Population**

Legitimate progenies from selected Serdang Avenue palms were planted on Elaeis Estate in 1931. Recording started as soon as they came into production and selections were made based on bunch yield and components. The first progenies of these selections were planted in GB IIIA in September 1938. Detailed records from Elaeis have been lost but palms were numbered by row and number in the row. The highest row number was 211 and the highest palm number 31 so the population must have been fairly large although only 46 were selected for progeny testing.

### **Results from GBI-V**

Based on the bunch yields of their progenies over a six year period the best parents were identified. These were referred to as Grade A parents and are listed in the Chemara 1954 annual report. From that report the following table has been constructed:

Summary of data from Ulu Remis GB I-V

<u>Source of parents</u>	<u>Number of parents</u>	<u>No. crosses involving the parents</u>	<u>No. Parents Grade A</u>	<u>% parents Grade A</u>
UR PP	129	600	19	14.7%
S.Av via Elaeis	46	114	13	28.3%

A greater proportion of Grade A parents came from the Serdang Avenue palms which had been selected in legitimate progenies on Elaeis Estate.

Subsequently in GB VII (1950) and VIII (1951) a few more Ulu Remis parent palms were tested and selected. Populations of pure UR.PP origin survive but it is thought that no pure Serdang Avenue populations survive in Malaysia (I should be pleased to receive information to the contrary).

## **Distribution of Introgressed Populations**

The Banting breeding programme started in 1957 when 18 "Ulu Remis" DxD progenies were planted at Klanang Bharu (Trial A). All 18 progenies contained genes from Serdang Avenue palms with 6 being 75% Serdang Avenue and the remainder 50%.

The breeding programme at Dami (Papua New Guinea) started in 1968 when 12 DxD progenies received from Banting were planted. The content of Serdang Avenue genes varied from 25% to 75% and averaged 54.2%.

Bah Lias in Indonesia received 8 progenies from the introgressed population at Dami and the average content of Serdang Avenue genes was 62.5%. These were planted in 1983.

Coto in Costa Rica received three crosses from Banting and five from Ulu Remis which were planted in 1969 followed by 20 crosses from Dami planted in 1979 - all contained a high proportion of Serdang Avenue genes.

Las Flores, Colombia, planted 20 crosses from Dami in 1985 and more have been planted since. Again all contained Serdang Avenue genes.

The above list is certainly incomplete and does not include distributions within Malaysia.

Overall the Serdang Avenue palms have played an important part in oil palm breeding in Malaysia but this has been overlooked because it was often not realised that Deli seed coming from Ulu Remis often contained many Serdang Avenue genes.

In 1962, IRHO at La Me received five Ulu Remis progenies, (Gascon *et. al.*, 1976). From the UR progeny codes given it appears that only one contained Serdang Avenue genes. In 1960-63, IRHO received progenies from four Deli palms at NIFOR which originally had come from Serdang. It is possible that at La Me there may be a pure Serdang Avenue as well as a pure Ulu Remis population in addition of course to Dabou Deli which has a completely different origin.

## **Changing Selection Criteria**

In the early years selection was based on the yield of bunches and their oil content.

By 1954 Chemara had established that although within progenies the tallest palms gave the highest yield this was not true when progeny means were compared.

In crops such as rice and wheat modern selection criteria include Harvest Index which is a measure of the proportion of the products that go into yield rather than growth. Hardon *et. al.*, 1969 and Corley *et. al.*, 1971 developed non-destructive techniques for estimating oil palm dry matter production in the oil palm. Bunch Index became an important selection criterion.

Dami Oil Palm Research Station is located on soils where there is a serious magnesium deficiency problem. Breure *et. al.* (1982) established a strong correlation at Dami between progeny yield and the magnesium status. Furthermore he showed that magnesium status was strongly inherited. This became a selection criterion.

### **Selection for Oil with A High Iodine Value Seems Desirable**

Other criteria can be used to supplement yield and dry matter production such as mitochondrial activity (Kouame, 1978) and photosynthetic activity (Gerritsma, 1988).

A successful breeding population should contain sufficient variability to permit selection for changing selection criteria. At Dami the Serdang Avenue/Ulu Remis population has been carried into a fourth generation since introgression. Expt 210 contains 24 progenies derived from 12 palms for which GCA estimates have been calculated by Breure (1990). Using GCA data Table 1 gives the mean, maximum, minimum and coefficient of variation for a range of parameters. There is a good range of variability especially for oil yield, kernel yield and frond weight. Clearly progress can be made in the desired direction at least for those parameters. In view of the paper by Okwuagwu (1993) it is suggested that part of this variability may be because, although derived from the original four Deli palms at Bogor, the two populations were separate from at least 1878 to 1938. Over these 60 years, they passed through at least three generations, probably four and possibly more.

### **Genetic Improvement of the Deli Population**

In her paper Okwuagwu (1993) suggests that further genetic improvement of the Deli population may be obtained by intercrossing divergent Deli sub-populations.

The same thought occurred to Lubis *et. al.* (1990) who chose Deli parents “from different progenies which may have differentiated through independent selection cycles in different research centres”. The degree of divergence between the populations in Indonesia may however not be very great.

The Serdang Avenue population is perhaps the most divergent from other Deli populations but other divergent populations will include the Dabou Deli in the Ivory Coast, the Lancetilla Deli from Honduras, an old Deli population in Colombia and the Lofindi population in Zaire. A programme to intercross these very divergent Deli populations may be rewarding.

## II. AVROS S.P. 540T

In 1921-22 AVROS (later RISPA now BPPM) imported 38 consignments of seed from various parts of Africa (Janssen, 1959). One of the consignments came from the “Djongo” palm in the Eala Botanic Gardens and was planted at Aek Pancur in 1922. Seed from the famous Djongo palm was also extensively used in the Yangambi breeding programme from where it was distributed very widely.

Schmole (1930) presented a paper to the 4th Pacific Sciences Congress in 1929 and regarding the imports of seed he wrote: “Investigations will only be carried out further in a few palms especially in the case of one palm the seed of which was obtained from the Belgian Congo. The fruits of this palm contained only about 6% of shell .... and the palm oil content calculated on the weight of the fruit amounted to approximately 40%. If the production in weight of the fruit bunches per annum on this palm proves satisfactory then it will make a very good starting point for further selection”. He cannot have imagined that SP540 would have millions of descendants in Indonesia, Malaysia, Papua New Guinea and in most countries where the oil palm is grown. Many members of the ISOPB will have seen SP540 carefully fenced near a padi field at Aek Pancur. It merits conservation for as long as possible and will no doubt be cloned.

Schmole selfed SP540 in March 1930 and the derived family No. 820 was planted in the Polonia genetic block in late 1931. There were 123 palms but the segregation ratio is not known.

As shown in Fig. 2 two *tenera* in family 820 were sib crossed to give family 945 and another *tenera* was crossed with *pisifera* Pol.632.3184P to give family 1019. This *pisifera* is described by Hartley (1967) as “largely fertile, but with the usual low fruit to bunch ratio”. A *tenera* in family 945 was crossed with a *pisifera* in 1019 to give family that was planted in Indonesia as

KB1316 and in Malaysia as BM119. Great use has been made of *pisifera* in this family and they are often referred to as AVROS or SP540 *pisifera*.

Lubis *et. al.*, 1990 have described experiments involving 290 DxP crosses and 170 DxT crosses which compared eight *tenera* populations. The following figures have been abstracted from their table of "mean characteristics":

	Yield oil t/ha Year 3-5 (Rank)	After year 6 (Rank)	Height at 8½ years
RS	5.88 (1)	5.98 (3)	4.44
LM	5.45 (2)	6.30 (1)	3.63
YA	5.26 (3)	6.14 (2)	3.43
Trial Mean (8)	5.04	5.71	3.96

RS = RISPA or 'AVROS'                      LM = LaMe                      Ya = Yangambi

This is the usual experience. The 'AVROS' population is precocious and gives high early yields but although remaining high yielding it may be overtaken by other material at full maturity. Also the 'AVROS' material is tall.

As shown in Fig. 3 another *tenera* 3520 in family 820 was also crossed with *pisifera* 3184. A resulting *tenera* was selfed to give family 1276 within which *pisifera* 71P was selected. A population of Deli palms on Gunung Melayu Estate is very short and a selection programme has shown that the best palm, based on GCA for yield and height is GM35/17. This was crossed with 1276.71P to give family BL238 which was compared with 36 DxP progenies from various sources in three experiments. Family BL238 gave the highest yield of bunches on the shortest palms. This suggests that it may be possible to find high yield on short palms in SP540 derived populations. This makes the Aek Pancur 1973 plantings to be described below very interesting.

The ancestry of four families planted at Aek Pancur in 1973 is shown in Fig. 4. Three *tenera* selections in KB1316 (= BM119) were back crossed with 945.1107T and the selfing of SP540 was repeated. (There must have been some difficulty in getting up to the crown of this 50 year old palm). The four families were planted as Nos. 1391 to 1394 at Aek Pancur. Brief records are shown below for the 1973 planting:

Family (see Fig.,3)	No. palms	FFB kg/palm/year 1976 - 1980	Oil to bunch %
1391 SP540 shelved	43	65.5	25.6
1392 1107 x 17T	65	87.0	27.2
1393 1107 x 35T	65	84.1	26.7
1394 1107 x 12T	58	74.3	26.3

Some inbred Deli populations are high yielding, but this population as is usual for inbred derivatives of SP540 is very low yielding.

*Tenera* palms in these four progenies have been selected for good growth characters and TxT crosses were planted in 1988 in the hope that they will contain *pisifera* with the potential of producing high yielding short progenies.

#### **'AVROS' material in Malaysia**

Family BM119 was planted at Banting in 1959 and consisted of 36 palms including 9 *pisifera* which were progeny tested in PT38 planted in 1968 and in other trials (Lee and Yeow, 1985). There were a few significant differences between their progenies and the best four *pisifera* were used for seed production over many years.

Numerous sib matings were made between the *tenera* in BM119 and *pisifera* in these families were also progeny tested.

In the DxP trials described by Lee and Yeow, no other types of material were included for comparison but yield records from commercial areas are presented. On six estates on coastal clays yields exceeded 20 tonnes of bunches in year 4 and over 30 tonnes at full maturity.

In the progeny trial planted in 1968 to compare 9 *pisifera* annual height measurements averaged 58.8 cm from 1976 to 1979. In an attempt to reduce height increment BM119 *tenera* were crossed with semi-dumpy *tenera* - Fig. 5. Records calculated from Lee and Yeow, Table 6 can be summarised:-

<i>Pisifera</i>	No. Progenies	FFB/p/yr	O/B %	Oil Yield tonnes/ha	Height
BM824	6	193.8	24.1	6.4	1.27
BM822	3	223.5	23.7	7.2	1.51
Commercial DxP	-	202.8	24.9	6.9	1.44

With BM824 there was a height advantage but a yield disadvantage. With BM822 there was a yield advantage and a height disadvantage. Selected Dumpy AVROS *pisifera* in BM822 and 824 are being used for seed production by the dumpy genes have not contributed as much as had been hoped.

From Banting, the AVROS population was distributed to PORIM and to other oil palm breeding stations. 'AVROS' *pisifera* are now being used in Malaysia by stations other than Banting.

#### 'AVROS' material in Papua New Guinea

Dami Oil Palm Research Station was started in 1968 as a private enterprise project although now it is a joint project with the Government. In 1968 Dami planted five TxT progenies in a trial using commercial DxP as a control. There was only one pure AVROS family which was a sib cross between two *tenera* in BM119:

$$\text{DM742} = \text{BM119/31T} \times \text{BM119/20T}$$

Other pure AVROS families reached Dami but growth in the nursery was so poor that they were not field planted. This may have been a mistake.

The other TxT family in which *pisifera* were selected for progeny testing was the equivalent of BM822 (see Fig. 5).

$$\text{DM743} = \text{BM119/20} \times \text{BM29/42 (s semi-dumpy tenera)}$$

The following table compares DM742, DM743 which were TxT and commercial DxP using data from Breure (1977):

**Dami Expt. 204**

	Yield (D+T) kg/palm/yr 1972-76	Mesocarp (T only) on fruit %	Oil to (T only) Bunch %	K %	FronD 17 (D+T+P) Mg %	Ca %	Total bases %
DM742	121.4	83.0	26.6	0.838	0.250	0.970	2.058
DM743	164.6	80.0	25.0	0.778	0.203	0.935	1.916
DxP	173.2	(79.1)	25.3	0.848	0.198	0.822	1.868
LSD 5%	12.9	-	-	0.050	0.020	0.076	-

The inbred AVROS progeny (DM742) gave very low yields but had good bunch characters. An important feature is the very high leaf magnesium level in comparison with other material. The segregation ratio in DM742 was 26 *dura*, 48 *tenera* and 23 *pisifera*. The appearance of the progeny was poor and about half of the *pisifera* could be discarded because of magnesium deficiency symptoms, orange spotting, boron deficiency symptoms and spear rot. The most promising six were tested in the first *pisifera* progeny trial at Dami planted in 1976 (Breure, 1977).

This trial also tested six *pisifera* from DM743 and two of the *pisifera* from BM119 which had been tested at Banting.

<i>Pisifera</i>	FFB	O/B	Oil Yield	Ht increment	FronD 17 Mg
DM742 Mean of 6	177	26.4	46.7	78	0.182
DM743 Mean of 6	164	24.4	39.9	76	0.167
BM119 Mean of 2	176	25.1	44.2	76	0.194

There were only small differences between the DM742 and BM119 *pisifera* but both were clearly superior to the DM743 *pisifera*. The DM743 *pisifera* had been included because it was hoped that the dumpy genes would lead to a reduced height increment but this did not happen.

Many more *pisifera* have now been tested but the four selected from the 1976 trial are still in use.

## AVROS *pisifera* in Costa Rica

Two BM119T crosses were planted at Coto in 1969 and these have been used to create more pure AVROS families. There was a second importation planted in 1980 consisting of one TxT cross and three TxP crosses.

### Fertile *pisifera* with AVROS genes

AVROS *pisifera* are usually very sterile and even with the standard hormone treatments it is often difficult fruit formation.

However when BM119.2T was used in TxT crosses at Banting the progenies often contained some fertile *pisifera*. BM119/27 was crossed with a fertile *pisifera* from Serdang SP29/36 to produce family BM555. This was planted in 1966 and all the *pisifera* produced were fertile.

BM555	No. of Palms	FFB kg/palm/an 1970 - 75	Fruit to Bunch %	Oil to Bunch %
<i>Tenera</i>	19	189	60.1	23.5
<i>Pisifera</i>	18	164	46.9	22.4

Pollen was collected from *pisifera* BM555/36 which had yielded an average of 192 kg/year in the same period. Part of the pollen was sent to Dami and crossed onto a selected *tenera* in DM735 which was itself derived from BM119/27 (see Fig. 6). The selected *tenera* DM735.408 had been progeny tested and GCA estimates were good for most characters. The cross DM735.408 x BM555.36 is DM960 which was also planted at Bah Lias as BL158.

This progeny contains some high yielding *pisifera* with good bunch characters. At Dami the three highest yielding *tenera* and *pisifera* are:

<i>Tenera</i>		<i>Pisifera</i>	
Palm No.	kg/palm/an 85/88	Palm No.	kg/palm/an 85/88
115	191	105	184
216	188	401	174
315	181	114	162

The best *tenera* have given higher yields than the best *pisifera* but the *pisifera* yields are high yielding.

At Bah Lias bunch analysis data are available for both the *tenera* and *pisifera*. The three best in each case are:

<i>Tenera</i>					
Palm No.	F/B	M/F	OWM	O/B	No. of Analyses
1.8	61.9	81.6	56.9	28.6	4
1.2	65.0	76.2	50.6	25.0	5
1.12	63.1	73.3	54.7	25.3	9
<i>Pisifera</i>					
1.11	58.4	100	60.0	35.0	7
2.5	49.9	100	57.9	28.9	5
2.13	49.6	100	58.4	28.9	9

The fertility of these *pisifera* is such that they could be selected in the same way as *dura* and *tenera*. If a *pisifera* is low yielding it may be because of some residual sterility. If it is high yielding there can be no objection on this score. The oil to bunch figures may be less useful but the oil to wet mesocarp figure should be meaningful.

The best *pisifera* in the family (DM960/BL158) will be progeny tested and it is hoped to make P x P crosses using unrelated fertile *pisifera* to create a fertile *pisifera* population. The initial objective is to make *pisifera* selection easier.

It will be recalled that the original ancestral *pisifera* used to create family 820 was a fertile *pisifera* (Fig. 2). Genes for *pisifera* fertility appear to have survived in some *tenera* in the AVROS population.

## Conclusion

When Schmole predicted in 1929 that SP540 may be the starting point for further selection he could not have imagined how much work would be done on its descendants in many parts of the world. And the story is not yet finished.

### III. BREEDING HISTORY OF THE DUMPY PALM (Elmina E206)

Elmina estate obtained Deli seed from Rantau Panjang (N. Sumatra) in 1911-12. It was at Elmina that Jagoe found the dumpy palm. The story has been told by Hartley 1988 and Jagoe 1952.

The selfed progenies of Elmina selections were planted in replicated trials at Serdang Experiment Station field 4 in 1937 (4 progenies) and 1939 (5 progenies). Some results from these replicated trials were published by Coulter *et. al.*, 1955. In addition two rows of E206 selfed were planted amongst other progenies at the end of the field.

Then in 1940 three small plots (about 40 palms) were planted in the adjacent field 3A. These plots contained E206 selfed, E152 x E206 and E268 x E206. Some results from these plantings are given in Table 2.

The F1 self of E206 in field 3A had rather uniformly short trunks with a larger girth. The yield was the highest amongst the selfs but it was realised that the dumpy plot was not in the same field and that 16 of the 40 palms were at the end of the row or on the edge of the field with no guard row. Despite the unreliability of the data the high yield on such short palms created much interest. In 1956 the "Co-operative Breeding Scheme" was formally created and involved four estates - Klanang Batu (Selangor), Ulu Remis (Johore), Johore Labis (Johore) and Jenderata (Perak), (Haddon *et. al.*, 1959). Much material had been distributed earlier than this.

The breeding history of the dumpy palm is summarised in Figure 7. The dumpy was exploited using four different routes which will be briefly described:

1. The semi dumpy hybrid route (Soh *et. al.*, 1981).
2. The  $\frac{1}{4}$  dumpy *pisifera* route (Lee and Yeow, 1985).
3. Using F2/F3 dumpy for DxP seed production (Soh *et. al.*, 1981).
4. Wilt testing F3 material in Cameroon and Zaire.

#### **The semi-dumpy hybrid route**

The original E206 was felled during the 1939-45 war so that a true backcross was not possible. Instead selections in E152 x E206 were crossed with selected dumpies in the E206 selfed progeny. The resulting "semi dumpy backcross" palms (75% dumpy genes) were crossed with *pisifera* to produce commercial seed containing 37.5% dumpy genes. Some records from the

resulting palms adapted from the paper by Soh *et. al.*, 1981 are shown in Table 3. The yield was only a little less than Deli x AVROS.P commercial seed. The height differences was irregular but the dumpy derived material was on average about 5% shorter. The slightly reduced height was not adequate compensation for the reduced yield and larger girth, frond area and frond weight.

The semi-dumpy backcross route has not been perused.

### **The $\frac{1}{4}$ dumpy *pisifera* route**

In the early 1950s there were two aspects of oil palm breeding that created much interest (I) the dumpy palm and (ii) the Beirnaert hypothesis on shell thickness inheritance which made possible the creation of *tenera* plantations by the use of DxP seed. It seemed logical to combine these together and to produce dumpy Deli *dura* x *pisifera* seed. Much seed of this " $\frac{1}{2}$  dumpy *tenera*" type was distributed for trials.

This dumpy DxP material was fairly successful and yielded 8 tons/ac (19 tons/ha) in the fifth year at Jerangau (Haddon *et. al.*, 1959). This encouraged more work on similar lines.

Several Malaysian organisations used their  $\frac{1}{2}$  dumpy *tenera* to cross with *pisifera* to create their own source of  $\frac{1}{4}$  dumpy *pisifera* - some of the programmes are summarised in Fig. 8. The  $\frac{1}{4}$  dumpy *pisifera* have been used for seed production with varying degrees of success.

Data from field trials have been published by Lee *et. al.*, 1985 and others. Data from Papua New Guinea has been used in Table 4 because comprehensive growth data were available (Breure *et. al.*, 1982). The experiment compared six 'AVROS' *pisifera* with six  $\frac{1}{4}$  dumpy *pisifera* with an ancestry as shown in Fig. 8 under Banting. The results from PNG are rather similar to those reported from Malaysia.

The progenies of the  $\frac{1}{4}$  dumpy *pisifera* have given a smaller crop of bunches because the sex ratio was lower and the abortion rate higher. As the oil to bunch was lower the average oil yield from the  $\frac{1}{4}$  dumpy *pisifera* progenies was around 15% lower than that from the AVROS *pisifera* progenies.

On the average neither the yield nor growth characters are attractive. However the best of the six  $\frac{1}{4}$  dumpy *pisifera* is attractive and is being used as a parent.

### Using "pure" F2/F3 dumpy for seed production

Various Malaysian organisations have planted F2 dumpy material but only BPPM in Medan have reported using such material for seed production. Soh *et. al.*, 1981 have published some results which are briefly summarised in Table 5. It should be noted that the Dumpy x P was planted in 1957 and the Deli x P in 1968. The results are not from one experiment.

The indications are that the yield of bunches of the Dumpy DxP is less than from ordinary Deli x P and despite better oil to bunch the oil yield still appears to be 15% less. The Dumpy DxP expresses the growth characters of the dumpy which is not surprising as 50% of the genes are from the dumpy. The trunks are shorter but the fronds are heavier.

There are two density trials on an estate in North Sumatra where five densities are tested each at three fertiliser levels. One trial is planted with Dumpy D x AVROS P and the other with ordinary Deli x AVROS P. The two trials are adjacent and were planted at the same time. Average yields over six years in tons/ha/yr were Deli P 33.0 and Dumpy DxP 31.2. Although the Dumpy x P is the lower yielding there is still a demand for the seed because of the shorter trunk.

The field trials used seed from F2 dumpy but F3 palms are now available with an ancestry as in Fig. 9. The use of advanced generation pure dumpy as the female parent for DxP seed production is perhaps the most promising route for the commercial exploitation of the dumpy.

### Wilt resistance in dumpy material

In 1968 Lobe Plantation in Cameroon received two dumpy x dumpy DxD crosses as part of an exchange programme. As a routine procedure a nursery wilt test was carried out using the technique described by Prendergast (1963). In one cross there were no losses and in the other only one loss. Many hundreds of tests had been carried out in the past and none had shown such a high degree of resistance.

Later in 1972, Binga (Zaire) received two crosses from Chemara. The crosses were:

Bg258 UR558/1474 x UR555/1301 planted in Expt 73/42

Bg259 UR555/1545 x UR555/1301 planted in Expt 73/38

UR555 is E206.3/8 x E206.1/9 and Bg259 is a sib cross within UR555.

Detailed records were not kept of Expt 73/42 which was not well replicated but in Bg259 there was only one wilt loss in the experiment where the average loss was 22.5% - with 72 palms per progeny. In Expt 73/38 Bg 259 gave the lowest yield but had the biggest bunches.

In view of the apparent wilt resistance of dumpy material some *in vitro* testing has started at Bath University. From preliminary results Dr Flood confirms that there appears to be exceptionally good resistance. Work is continuing.

An inter-origin trial comparable to that at Marihat (Syukur and Lubis, 1982) was planted at Binga where it included some Malaysian material. Losses in the United Plantation material were only 5.5% which was far below the trial average (17.2%) while other Malaysian material suffered over 33% losses. At that time the UP male parent was a  $\frac{1}{4}$  dumpy *pisifera*. It is possible that only a small content of dumpy genes may give resistance.

Dumpy palms in Bg259 have been crossed with selected *tenera* palms in wilt resistant progenies. In 1989 35 dumpy x T crosses were planted in a wilt infected area at Binga and most crosses were also planted at Yaligimba. The crossing design makes it possible to get GCA estimates for 17 dumpy palms. If the wilt resistance of the dumpy is confirmed these palms could be used as the basis for an F4 breeding programme. International collaboration would be desirable to bring together the best F2/F3 dumpy genes from all over the world.

## DISCUSSION

In the early 1950s there was much enthusiasm about the dumpy. It was expected that it would have a major impact on oil palm breeding. Despite a great deal of attention and many breeding programmes the dumpy has only had moderate success.

The short trunk is desirable both economically and physiologically but other growth characters are undesirable. Breeding better dumpy palms for seed production may be the best approach to exploiting the dumpy character. However short trunks can be obtained from other populations and the dumpy is now of little interest.

If it can be confirmed that the dumpy population is exceptionally wilt resistant it could become important for seed production in Africa.

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Table 1: Variability in the Serdang Avenue/Ulu Remis Population in the Fourth Generation After Introgression

Based on GCA estimates determined for 12 Deli *dura* palms in Dami experiment 210

Parameter	Mean	Max	Min	CV
Yield FFB/p/yr (5 yrs)	136	163	104	11.0
Oil on bunch %	19.2	22.0	16.6	9.6
Oil yield kg/p/yr	26.1	32.0	18.4	16.4
Kernel yield kg/p/yr	2.5	3.2	1.7	18.9
Leaf of 17 mg%	0.169	0.196	0.152	12.2
Height cm	217	262	184	11.1
Fronde weight kg	3.30	4.51	2.80	16.7
Fronde production p/yr	31.9	34.5	30.3	4.9
Bunch Index	0.408	0.442	0.318	10.4
Leaf Area m <sup>2</sup>	8.07	8.85	7.04	8.4
Rachis length m	4.93	5.25	4.75	4.2

Table 2: Elmina Selves at Serdang

Trial and Year Planted	Elmina Number Selves	Original Parent 8-13 yrs kg/palm/an	Selfed progeny 1946-50 kg/palm/an	Height to harvest point inches	Girth at 4ft inches	Fruit to bunch %	Pericap on Fruit %
Field 4	211	240	95	177	86	61.7	60.5
Trial 01	200	160	68	166	85	62.8	61.8
1937	93	158	56	148	93	62.5	67.5
	45	160	55	219	80	63.3	57.9
Trial 02	268	208	100	195	93	58.5	63.7
1939	216	196	87	159	87	58.4	63.4
	207	184	67	165	84	63.3	61.1
	152	197	64	164	81	59.6	67.5
	97	171	62	147	78	59.9	61.1
Unreplicated	206	154	101	114	107	54.2	64.5
Field 3a							
1940	cross						
	152x206	-	NA	165	87	67.8	62.3
	cross						
	206 x 268	-	NA	160	98	55.3	64.7

Table 3 Semi-Dumpy backcross in DxP crosses (37.5% dumpy)  
Based on data from Soh *et. al.*, 1981

Year Planted	Field	FFB kg/palm/yr		LSD 0.5	O/B	
		Deli x P	SDB* x P		Deli x P	SDB x P
69	1	209	174	15	26.8	26.1
72	2	188	189	21	26.9	26.1
73	3a	170	162	-	23.7	23.9
73	3b	171	171	-	23.0	23.2
74	4	139	143	17	27.5	26.8
74	5a	150	150	-	24.6	26.2
74	5b	135	128	-	23.3	24.7
74	5c	133	125	-	22.5	21.5
	Mean	162	155		24.8	24.8

	Field	Height m			Girth		LSD 0.5
69	1	4.84	4.45	0.35	2.30	2.68	0.15
72	2	2.68	2.33	0.18	2.11	2.42	0.11
73	3a	1.45	1.38	0.10	2.38	2.59	0.06
73	3b	1.45	1.57	0.10	2.48	2.63	0.06
74	4	0.54	0.55	0.07	2.51	2.61	0.11

	Field	Froned Area (m <sup>2</sup> )			Froned Weight (kg)		
69	1	22.0	21.4	1.9	4.47	4.68	0.32
72	2	17.5	18.6	1.3	3.26	3.60	0.25
73	3a	13.9	14.6	1.0	2.62	2.93	0.16
73	3b	13.8	15.8	1.0	2.62	3.25	0.16
74	4	10.8	11.8	-	2.07	2.52	-

Note: Yield data refers to various periods (see Soh *et. al.*, 1981).

Growth data refers to 1979

\*SDB - Semi-dumpy backcross

Table 4: Progenies of 1/4 Dumpy *Pisifera* in Papua New Guinea

	DM742	DM743
"AVROS" <i>pisifera</i> 1/4 Dumpy <i>pisifera</i>		
Dami Expt. 207 planted 1976		
Number of <i>pisifera</i>	6	6
Palms per main plot	16	16
Palms per <i>dura</i> sub-plot	4	4
Replicates 3 x 2 densities	6	6
Total palms per <i>pisifera</i>	96	96
Data from Breure <i>et. al.</i> , 1982		
Yield kg/palm/yr 478-382	177	164
Oil to bunch O/B	26.4	24.4
Kernel to bunch	8.3	7.5
Oil yield kg/palm/yr	47	40
<u>Growth data 78-92</u>		
Bunch Index	0.436	0.421
Harvest Index	0.276	0.245
LAR	2.35	2.25
Height increment cm/yr	78	77
FronD 17 Mg%	0.182	0.167

After Breure *et. al.*, 1982Table 5 Pure dumpy x *pisifera* (50% dumpy)

Year planted	Deli x AVROS P	Dumpy x AVROS P
	1968	1957
FFB yield kg/palm/yr (8 yr mean)	195	160
Bunch No.	19.7	12.3
Bwt (kg)	11.0	16.4
Oil to bunch (%)	23.9	24.6
FFB x O/B (%)	46.6	39.4
Height m at 6 years	2.00	1.02
FronD length at 6 years	4.80	5.01
FronD area m <sup>2</sup>	6.50	7.38
FronD production p year	28.5	28.5
Estate x Density Trial tonnes/ha	33.0	31.2
At above O/B% tonnes oil/ha	7.89	7.68

Data from BPPM Soh *et. al.*, 1981

Fig. 1

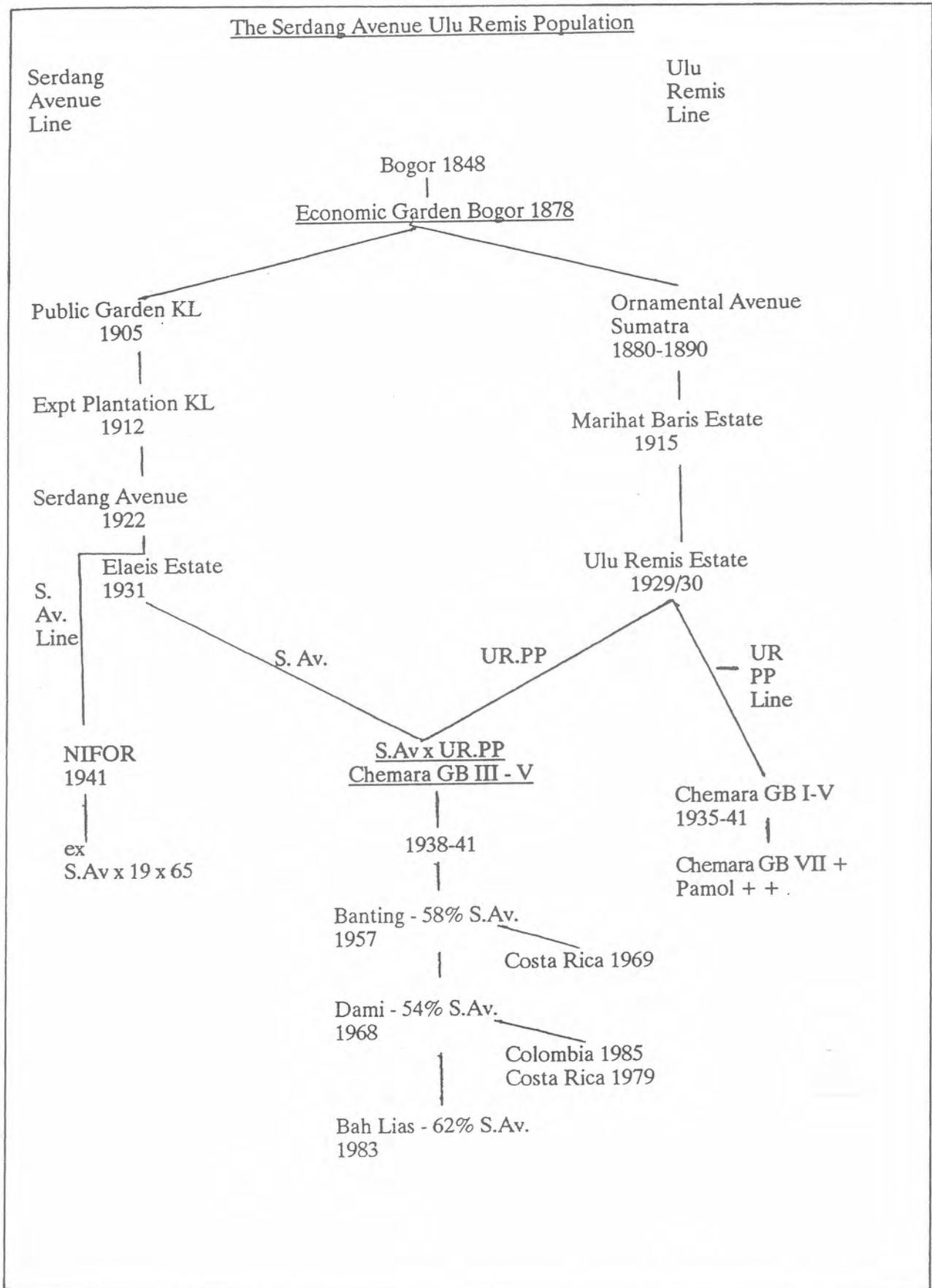


Fig. 2

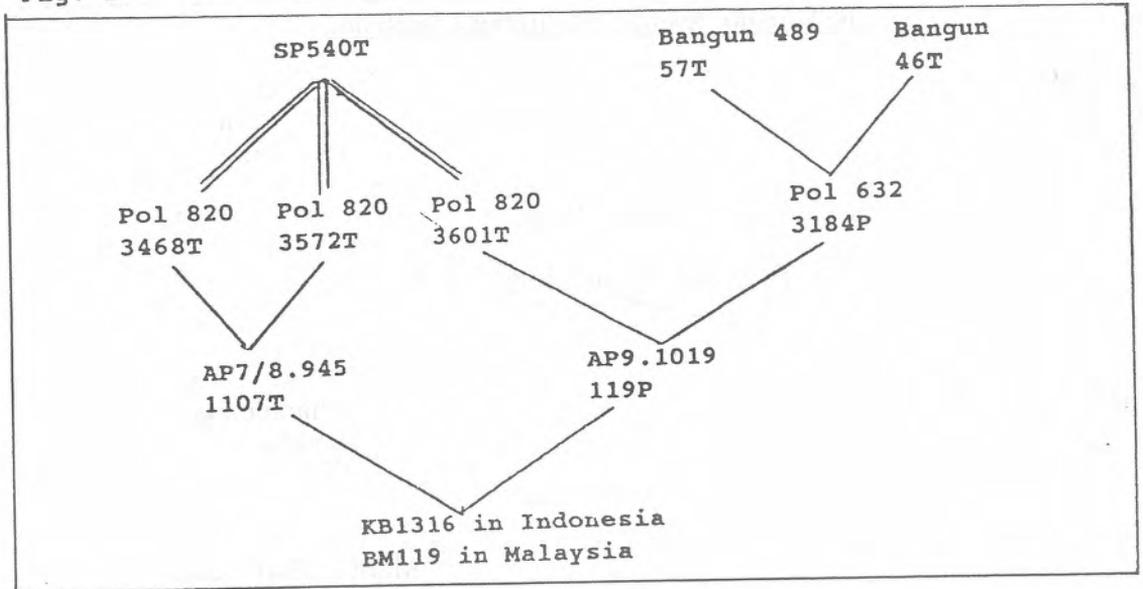


Fig. 3

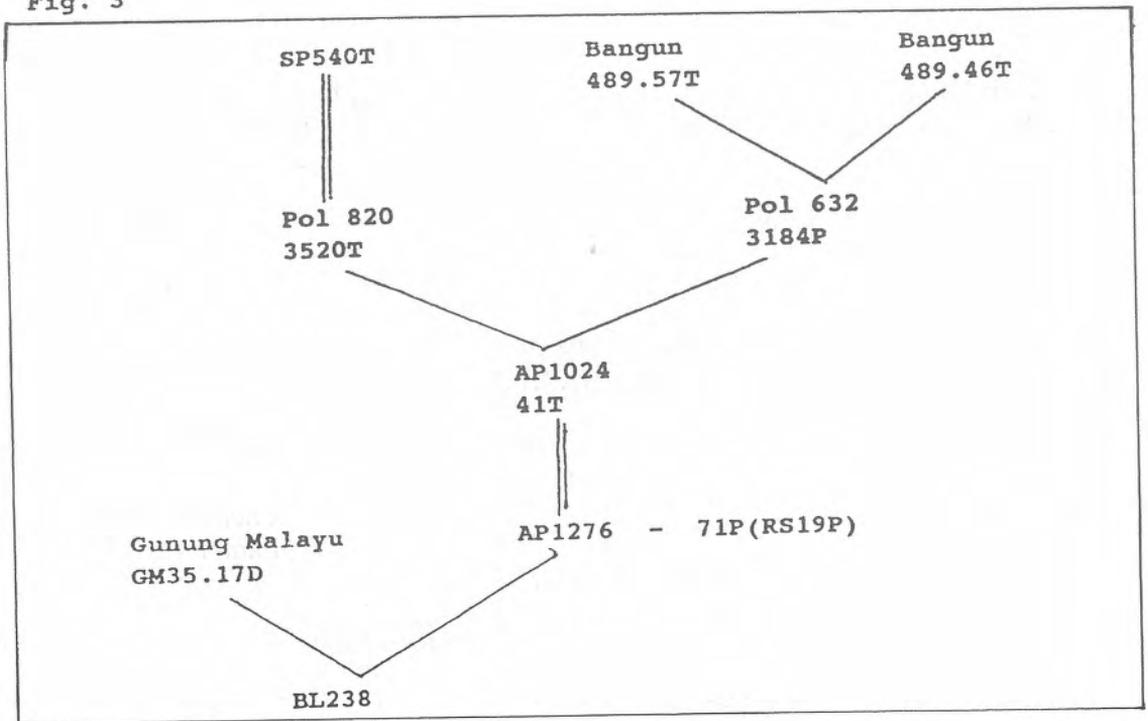
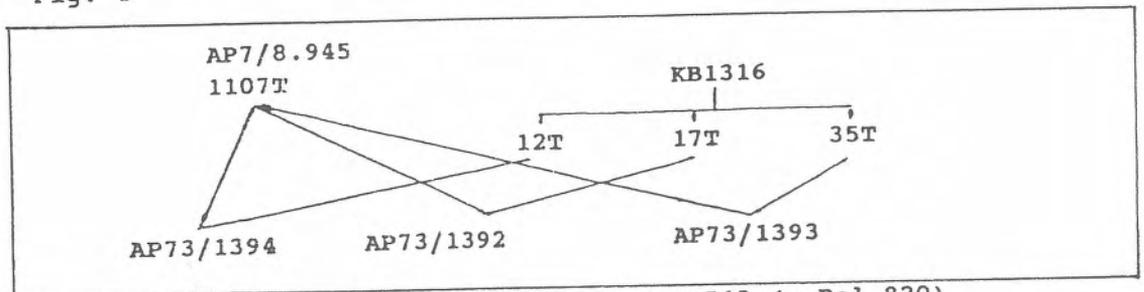


Fig. 4



Note AP73/1391 is the repeated self of SP540 (= Pol 820)

Fig. 5

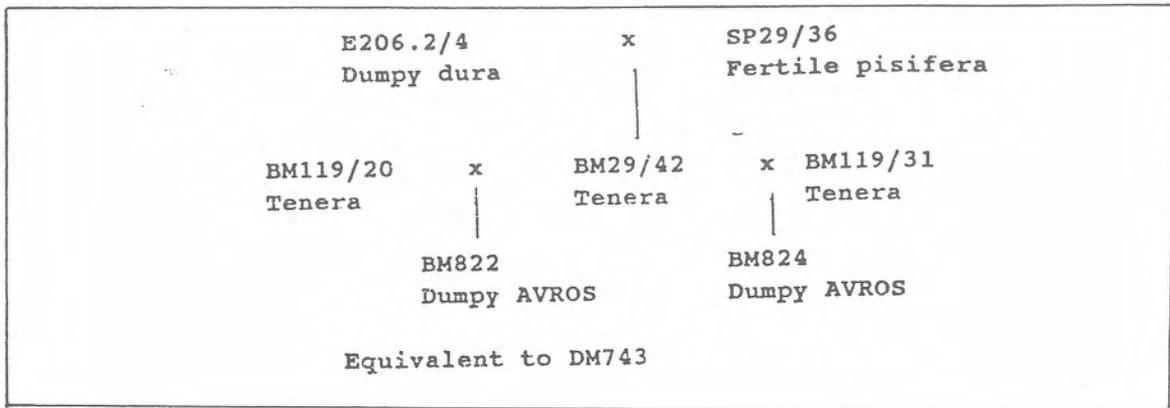
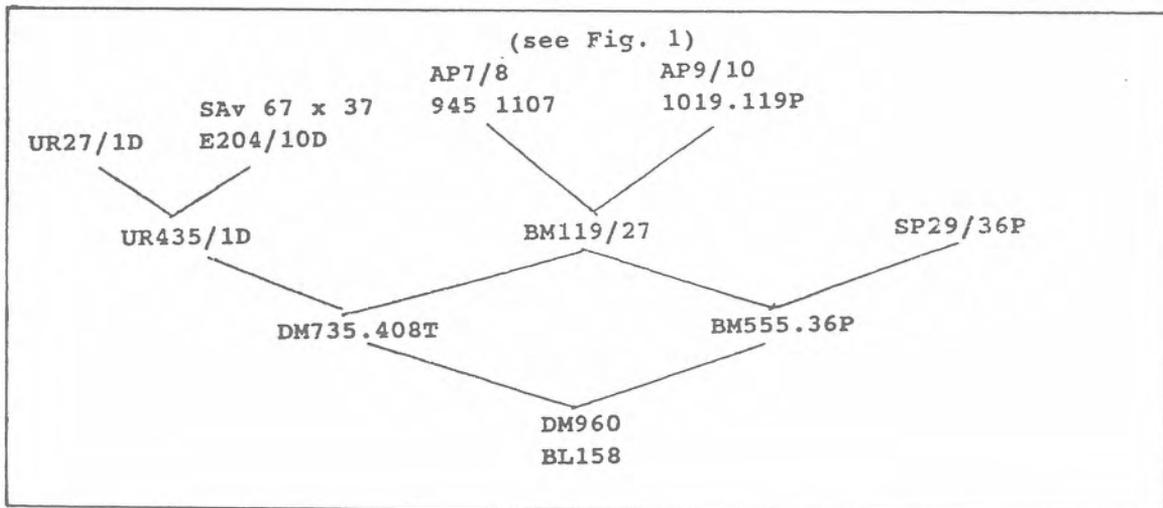


Fig. 6



Breeding History - The Dumpy

Rantau Panjang

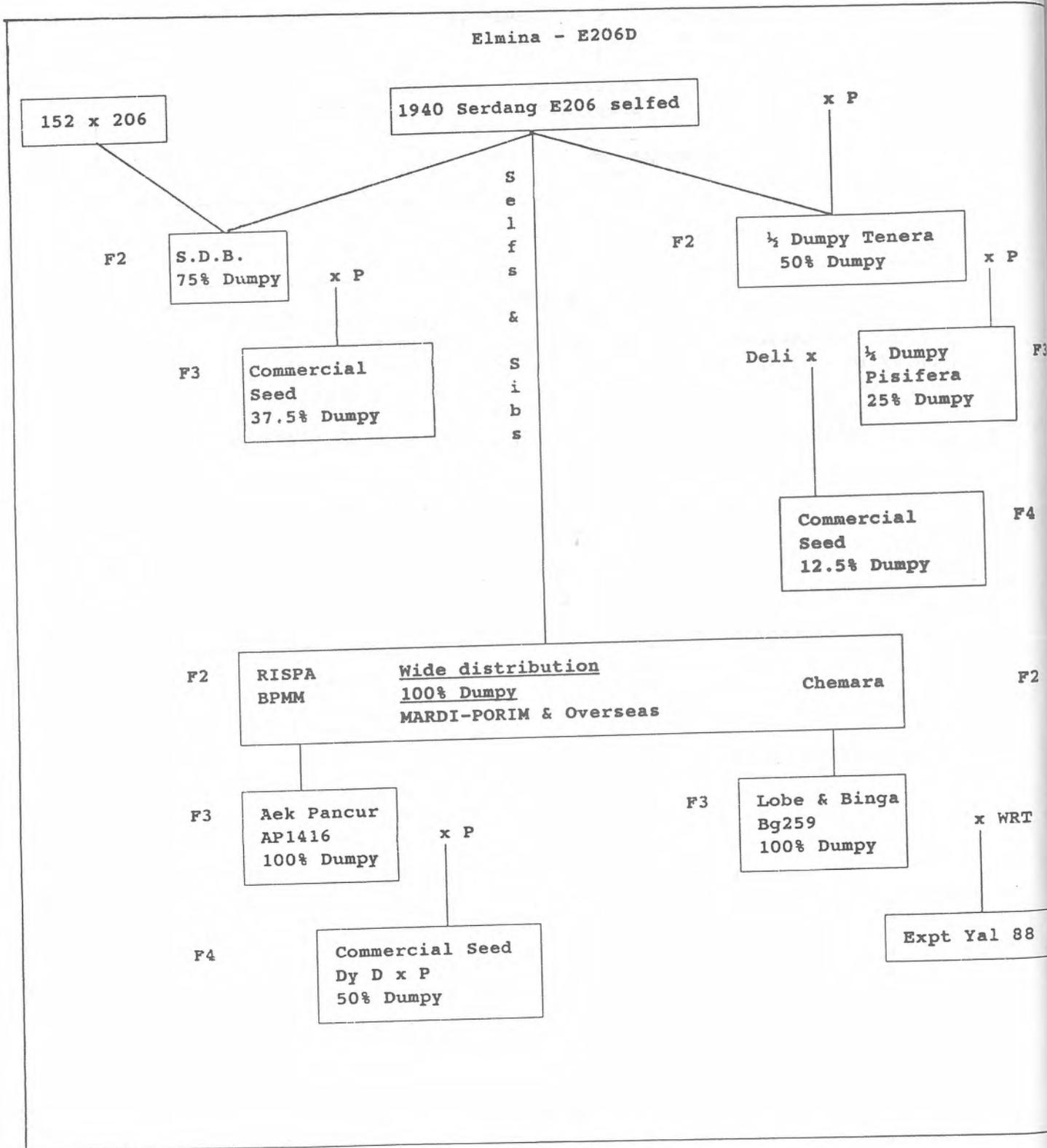


FIGURE 8

Some  $\frac{1}{4}$  dumpy pisifera  
which have been used for  
commercial seed production

United Plantations  
Jenderata ( - in 1977)

E206.3/5 x Serdang 27B.P

$\frac{1}{2}$  Dumpy Tenera x Serdang 36/21 P

$\frac{1}{4}$  Dumpy Pisifera

H.R.U. (from Sales brochure)

E206.1/7 x Serdang 27B.P

$\frac{1}{2}$  Dumpy Tenera x AVROS P

$\frac{1}{4}$  Dumpy Pisifera

Banting (Lee C.H. x Yeok K.H. 1985)

E206.2/4 x SP29/36P

$\frac{1}{2}$  Dumpy Tenera

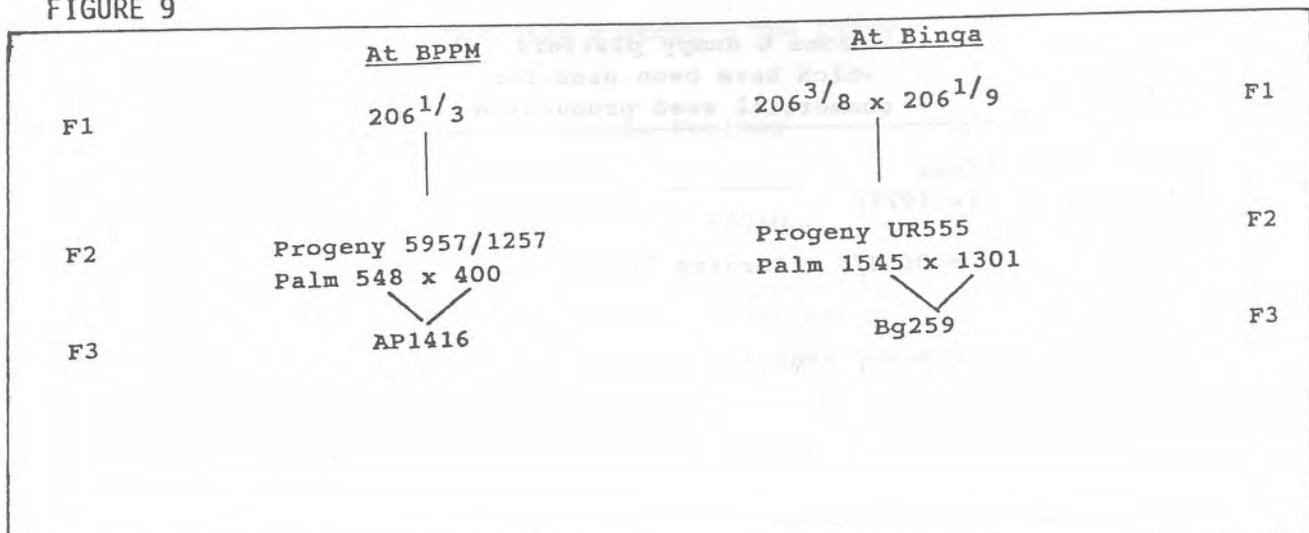
BM119/20 x BM29/42 x BM119/31

BM822

BM824

(equivalent to DM743  
at Dami in Papua New Guinea)

FIGURE 9



## USE OF DNA MARKERS (RFLPs) IN OIL PALM BREEDING

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### ABSTRACT

*DNA molecular markers (especially RFLPs) have become widely used in the plant sciences in a number of ways, including genetic fingerprinting, determination of genetic distances, genome analysis and more recently identification of markers linked to desirable breeding traits. We have been exploiting such DNA marker systems in oil palm for the last 6 years. Early work was concerned with confirming the identify of tissue culture-derived clones with the original selected palms (genetic fingerprinting). For this purpose we have selected DNA markers revealing high levels of polymorphism, for example one probe (pOPg54) generates at least 50 distinct banding patterns amongst 124 genotypes. A further hypervariable clone (pSMP6) has been identified and in this case DNA sequence analysis reveals a complex mosaic of interspersed repetitive domains. More recently we have initiated a programme aimed at the construction of an oil palm RFLP-linkage map with the intention of identifying markers to characters such as shell thickness, Fusarium wilt resistance and more long term, oil quality and yield. Mapping is being accomplished in a number of populations including selfed material, crosses between distinct E. guineensis accessions and possibly E. guineensis x E. oleifera inter-specific crosses, should there be sufficient breeding interest in introgressing desirable E. oleifera characters. Widely differing values of DNA polymorphism have been obtained in each of these crosses, ranging between 15% (for selfings), 50% (for distinct crosses) and 95% (for inter-specifics).*

### INTRODUCTION

Over the last ten years, DNA markers have become widely used in a variety of biological fields, including genetic fingerprinting and analysis of inherited diseases in humans, as well as breeding and population studies in animals, however one of their most powerful applications arises within plant breeding. This is reflected in the fact that complete DNA marker genetic maps have now been constructed for at least 10 major crop species and many more have been characterised at a less detailed level (see Range of Plants Studied below). This paper is in two parts, the first being

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a review of the use of DNA markers in plants and the second an account of our work in developing oil palm DNA markers and how we are integrating them within breeding and tissue culture cloning programmes.

## BACKGROUND REVIEW

### Range of Plants Studied

Broadly speaking, DNA markers have been utilised in two distinct though related ways: the first being genotype characterisation (for example genetic fingerprinting and germplasm evaluation), and the second being identification of markers linked to desirable breeding traits. The first application requires a relatively modest research effort, since all that is required for the crop of interest is the availability of probes capable of revealing differences between genotypes. Thus, over the last few years a huge number of plant species have been examined. In some cases heterologous probes have been used i.e. DNA elements cloned from other plant, animal or viral species. Examples include the use of maize probes in sugarcane (Lu *et al.*, 1991), or wheat and rice ribosomal probes in banana (Lanaud *et al.*, 1991) and pearl millet (Hilu and Johnson, 1992) respectively; human fingerprinting (Jeffreys') probes have been used in ornamentals such as carnations and roses (Tzuri *et al.*, 1991), as well as rubber (Besse *et al.*, 1991) and rice (Dallas, 1988) and viral M13 DNA probes have been successfully used in apples (Nybom and Schaal, 1990). Even synthetic oligonucleotide DNA probes such as (GATA)<sub>4</sub> have proven discriminatory in chickpea (Weising *et al.*, 1989) and barley (Beyerman *et al.*, 1992).

Increasingly though workers are cloning DNA probes from the genera under study. This has two major advantages: firstly stronger and cleaner signals and secondly simpler and more readily scorable patterns. Some recent examples include sugar beet (Nagamine *et al.*, 1989), *Vicia faba* (van de Van *et al.*, 1990), sunflower (Perez *et al.*, 1988 and Gentzbittel *et al.*, 1991), bamboo (Friar and Kochert, 1991), cocoa (Laurent *et al.*, 1991 and Wilde *et al.*, 1992), yams (Terauchi *et al.*, 1992) and banana (Faura *et al.*, 1991), as well of course as all the mapped species described below.

The second major application of DNA markers i.e. the identification of markers closely linked to desirable monogenic and polygenic characters, requires the development of an ordered set of linked markers covering the entire genome in order to screen for linkage efficiently. This requires a significantly larger effort and consequently the number of plant species so far mapped

is much smaller, nevertheless most of the major temperate crop species have been mapped including maize (Helentjaris, 1987); lettuce (Landry *et al.*, 1987); tomato (Bernatzky *et al.*, 1986) and derived pepper (Tanksley *et al.*, 1988) and potato (Bonierbale *et al.*, 1988); rice (McCouch *et al.*, 1988); lentil (Havey *et al.*, 1989); soyabean (Diers *et al.*, 1992); *Brassica oleraceae* (Slocum *et al.*); peas (Ellis, pers. comm.), barley (Graner *et al.*, 1991 and Heun *et al.*, 1991) and wheat (Liu and Tsunewaki, 1991 and Gale, pers. comm.). It should be noted that for some of these crops, mapping has utilised very wide or even inter-specific crosses to achieve sufficient levels of polymorphism; in such cases only a minority of mapped probes are likely to be informative between varieties within the species.

### **DNA Marker Technology**

Marker systems of one form or another have been available to breeders for many years - these include morphological characters, disease resistance genes, seed storage protein genes and isozymes, however DNA markers differ from these other systems in two major respects. Firstly, from a technical point of view: any tissue can be examined (e.g. leaf, root, pollen, callus tissue culture etc.) and many loci can be scored on the same DNA preparation - which itself can be stored indefinitely. The second major difference is that the genotype is scored directly rather than through expressed genes; thus one is not restricted to relatively-abundantly expressed structural genes (as required for isozymes) - any genetic element can, in principle, be scored - so that instead of being restricted to perhaps 20 or 30 markers one has many thousands available, making complex polygenic traits amenable for the first time. It should however be remembered that DNA markers complement, rather than replace, other marker systems e.g. where close linkage of an inexpensively-scored isozyme to a desirable trait has been identified, this will continue to be the method of choice for some time.

### **Applications of DNA Markers in Plants/Opportunities for Oil Palm**

Section Range of Plants Studied listed some of the many plant species which have now been examined with DNA markers and divided applications into genotype characterisation and trait-marker linkage. We will now consider some of these applications in more detail, paying particular attention to those of relevance to the oil palm.

#### **Genotype characterisation**

At its simplest level this involves identification of probes which are capable of distinguishing

different individuals of the same species. Given information concerning the variability of such probes within a population (allele frequencies) one can use such markers to identify individuals, as has been achieved for genetic fingerprinting in humans (Jeffreys *et al.*, 1985). Current human fingerprinting technology is approaching the point where, in theory, the entire human population ( $5 \times 10^9$ ) could be assigned data base-compatible unique genetic fingerprints (Jeffreys *et al.*, 1991).

Genetic fingerprinting can be used in an entirely analogous fashion in plants i.e. it can be used to unambiguously identify a plant variety or clone. Currently the major limitation in plants is the variability in discriminatory power of DNA probes and that in itself is directly related to the fertilisation behaviour of the species and its breeding history.

Thus self-pollinating crops derived from restricted gene pools (e.g. wheat and tomato) have very low levels of polymorphism, whilst out-pollinators (e.g. maize) have much more diversity. Our experience with oil palm is that with the exception of self-pollinated populations (see below), levels of polymorphism are high and it is relatively straightforward to distinguish individual palms from one another. Thus we are using genetic fingerprinting as a quality control to ensure that clones are not misassigned and are beginning to use it in breeding programmes to assess progeny for spurious pollination events in specific crosses.

Genetic fingerprinting, where individuals are compared for identity, requires relatively few markers, however if more markers are available then it is possible to develop this genotyping to the point where one can estimate genetic distance between individuals or species. Over relatively wide distances (e.g. between *tenera* or widely-separated species), relationships may be determined using highly conserved sequences such as chloroplast DNA (e.g. in determining phylogenies of cultivated yams - Terauchi *et al.*, 1992), however within a species, or for very closely related species, such variability is generally insufficient, as we have found for oil palm. In these circumstances low copy-number nuclear DNA probes are used, as for bamboo (Friar and Kochert, 1992), soyabean (Keim *et al.*, 1989), brassicas (Song *et al.*, 1988) and tomato (Miller and Tanksley, 1990). It is likely that such approaches will be increasingly applied to characterising germplasm collections, particularly where there are few conventional genetic markers available and/or where maintenance of collections is time-consuming and expensive. In this way the most distantly-related individuals can be identified to ensure that as broad a genetic base as possible is maintained. Such an approach can also be used to characterise genotypes as a predictor of heterotic potential, for example in maize it has been shown that genetic distance, as measured by RFLPs, is the best indicator of heterotic performance, even

exceeding in value the extensive pedigree information available for this crop (Smith *et al.*, 1990). It would be of interest to search for such correlations within oil palm breeding programmes. Of particular interest might be where inbreeding depression appears to be absent. We have examined one particular selfing with low reported in-breeding depression though in this case it appears that a high level of inadvertent out-crossing is responsible.

### **Genetic mapping and trait linkage**

This is likely to be the most productive area for DNA markers in plant breeding, especially for long-lived perennial crops with long breeding cycles, poorly characterised genotypes and high individual value, typified by oil palm. As described in Range of Plants Studied above, detailed DNA linkage maps have been constructed for many important crop species. Using such maps, linkage of DNA markers has been established to important traits such as soyabean hard seededness (Keim *et al.*, 1990), soyabean seed protein and oil content (Diers *et al.*, 1992), vernalisation requirement in barley (Chojecki *et al.*, 1989), and plant height in maize (Helentjaris, 1987). We have initiated a programme aimed at providing a complete linkage map for oil palm (see Unilever Oil Palm DNA Marker Programme below). One of the early markers we intend to screen for is one linked to the shell-thickness gene; such a marker may prove useful in identifying *pisiferas* and *duras* at an early (nursery) stage prior to planting out. Other relatively simply inherited characters of interest in oil palm include disease resistance genes, such as those to *Fusarium* wilt; crosses have been constructed to confirm the genetics of this resistance as reported by Franqueville and deGreef (1988). Of longer term interest is the identification of markers associated with complex characters such as yield and oil quality. Here the intention is to use a RFLP linkage map to scan the genome for regions containing genetic components contributing to that character (interval mapping). Such an approach has been used to identify quantitative trait loci (QTL) for characters such as soluble solids content in tomato (Paterson *et al.*, 1991). In the above example, anonymous mapped probes were used, however for characters such as yield and especially oil quality, a number of relevant genes have already been cloned; allelism within such genes may contribute to these characters and hence they might be especially informative as probes.

Having established tight linkage between markers and traits it might then be possible to walk along the genome and molecularly clone the genes responsible - as has been attempted for both TMV resistance (Ganal *et al.*, 1989) and root knot nematode resistance (Messeguer *et al.*, 1991) genes in tomato. However such research efforts are considerable and it is unlikely that marker-assisted cloning would be productive in oil palm for some time yet with current technology.

For further information on this and other applications, see the review of Tanksley *et. al.*, 1989.

### **Future Technology Development**

Until recently DNA marker technology relied almost entirely upon RFLPs. Although powerful and robust, RFLPs do suffer from the disadvantage that they involve a number of labour-intensive steps including DNA purification, restriction enzyme digestion, gel electrophoresis, blotting, probe preparation and hybridisation. This means that conventional RFLP analysis is slow, expensive and not readily amenable to analysis of large numbers. Recently however, major advances have been made in our ability to amplify specific DNA elements *in vitro* using the polymerase chain reaction (PCR), see Saiki *et. al.* (1988). If polymorphic technology can be adapted to include PCR then many of the above time-consuming and expensive steps are eliminated. Thus instead of being able to handle perhaps 50 - 100 samples per week one might be able to analyse that many in a day. Having identified a polymorphic region using RFLPs, this could be made PCR-compatible through DNA sequencing of short flanking regions and in the case of major DNA rearrangements (deletions, insertions and amplifications), polymorphism could be scored directly through length variation of products. For more subtle polymorphic changes e.g. point mutations, a sequence-sensitive technique is required e.g. DGGE (Riedel *et. al.*, 1990) or SSCP (Orita *et. al.*, 1989). Although of considerable promise, such procedures are currently rather difficult to use on a routine basis and cannot be guaranteed to identify all events. For this reason it may be more productive to select polymorphisms which are associated with length variation. Microsatellites i.e. relatively short tandem arrays (20 - 200 bases) composed of very simple repeats (2, 3 or 4 bases) offer one such possibility and are being widely used in animal and human genome mapping (Love *et. al.*, 1990). Alternatively, sequence variants within human minisatellite arrays (about 30 base repeats, duplicated 20 to many hundreds of times) are now detectable using PCR (Jeffreys *et. al.*, 1991), though this does require rather stringent requirements in the nature of the array. Much of this technology development is being driven by animal and human genome mapping studies and it is likely that before long, techniques as robust as RFLPs, but orders of magnitude faster and easier to use, will be available. Thus DNA markers are poised to become a standard analytical tool for use in any reasonably well equipped laboratory.

## UNILEVER OIL PALM DNA MARKER PROGRAMME

We have been exploiting DNA marker technology in oil palm for the last 6 years, initially through the development of genetic fingerprinting systems for use in confirming the genotypic identity of tissue culture-derived clonal material with the original explanted palm samples, and more recently, for assessing progeny from defined breeding crosses. Our current programme involves the construction of a genetic map for oil palm based upon RFLP-linkage data for use in identifying DNA markers to simply-inherited traits (including shell thickness and *Fusarium* wilt resistance), as well as more complex characters (such as yield and oil quality). Additionally we wish to assess genotypes for genetic distance, both as a predictor of heterosis between inbred lines and to evaluate different gene pools for genetic diversity.

### Genetic Fingerprinting

Two EcoRI genomic DNA clones have been examined in some detail using a set of 124 palms (some closely related). One probe (pOPg95) gave 12 different RFLP patterns, whilst a second probe (pOPg54) yielded over 50 distinct band patterns. The combination of just these two probes gave about 100 different variants within the set of 124 genotypes. Thus most palms can be distinguished from each other with only two probes (many thousands more being available if necessary). More recently, we have identified a further highly variable DNA probe (pSMP6) from a PstI genomic library. Sequence analysis reveals that it is composed of a complex mosaic of repetitive domains. It seems likely that the high level of variability at this locus arises through DNA slippage events upon replication and/or unequal crossing over events.

### Genetic Mapping

Plasmid genomic (Pst I) DNA libraries have been constructed and screened for low copy DNA clones and levels of variability assessed against a panel of genotypes. Approximately 95% of clones are polymorphic in inter-specific (*E. guineensis* x *E. oleifera*) crosses but this drops to 50% between unrelated *E. guineensis* genotypes and only 15% between siblings in a selfed population. Mapping populations segregating for yield components and shell thickness have been identified and linkage blots are being established. Additionally, multiple crosses have been made to assess the genetics and to identify markers to components of *Fusarium* wilt resistance.

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## **OIL PALM BREEDING TECHNIQUES**

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### **ABSTRACT**

*Breeding techniques for (1) collection, storage, and utilization of pollen, (2) bagging and controlled pollination of female inflorescences, (3) seed processing and germination, (4) yield recording, (5) bunch analysis, (6) fatty acid composition, (7) height measurement, and (8) vegetative measurements are reviewed. Stringent quality control measures for bagging of both male and female inflorescences are essential to ensure legitimate crosses. New techniques need to be developed for early confirmation of the legitimacy of the crossings.*

### **INTRODUCTION**

Techniques for the breeding of oil palms have evolved since various organizations began their oil palm breeding programmes. Except for yield recording, which has remained basically unchanged, much modifications and improvements have been made in techniques for bagging and controlled pollination, bunch analysis and seed germination in response to changing conditions over the years.

The following major techniques will be examined in:-

- (1) Collection, storage, and utilization of pollen;
- (2) Bagging and controlled pollination of female inflorescences;
- (3) Seed processing and germination techniques;
- (4) Yield recording;
- (5) Bunch analysis;
- (6) Fatty acid composition;
- (7) Height measurement;
- (8) Vegetative measurements;

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## Collection, Storage and Utilization of Pollen

Basically the male inflorescence selected for pollen collection must be bagged at least five days before anthesis. The inflorescence is cleaned and sterilized with dilute (4 per cent) formalin spray.

After this, the inflorescence is covered with a bag which is open at both ends and which has inspection windows. Insecticide treated cotton wool is then put around the stalk and the bag tied at this point with wire. Similarly at the top of the bag, insecticide treated cotton wool is folded at the opening which is then also tied with wire. For additional security, another bag is used to cover the "inner" bag and the ends are also tied with wire. Wire-netting is also used to enclose the bagged inflorescence, to prevent squirrel and rat damage to the bag. At anthesis, the stalk is cut and the bagged inflorescence is then taken for pollen processing, which is normally carried out in a special room which is sanitized prior to each pollen processing session.

Before the pollen is processed, the inflorescence has first to be dried for about four hours in an air conditioned room or in the heat-room at 40°C. Then the inflorescence is well shaken to dislodge the pollen from the spikelets. The empty stalk is then discarded and the pollen further dried before being sieved and collected into test tubes. The pollen thus collected is tested for viability and normally only pollen with viability exceeding 60 per cent is retained for pollination purposes. Two methods exist for testing pollen viability, one using agar-agar media and the other boric acid media. Viability is estimated from the percentage of pollen grains which germinate within a set period on either agar-agar or boric acid media. Boric acid media gives faster results (within two hours) compared with four hours for agar-agar media. The pollen is normally kept in the freezer at -5°C and the moisture content of the pollen should be less than 8 per cent on a dry weight basis. If the pollen is expected to be stored for periods exceeding six months, then it should be stored in vacuum sealed ampoules after freeze drying. Otherwise the quality of the pollen deteriorates with time and such pollen, if used for pollination purposes, can result in seeds with defective embryos. Poor germination rates and high nursery culling rates can arise (Noiret and Ahizi Adiapa, 1970).

Quality control is of utmost importance during the pollen collection and processing process, to ensure that only legitimate pollen is obtained. Systematic checks have to be carried out at all stages to ensure this. This include inspecting the bagged inflorescence for tears or holes, or loose tyings, or presence of weevils within the bag. If weevils are seen (dead or alive) in the processed pollen, then the whole lot of pollen has to be discarded. Utensils must be sterilized before use, such as by cleaning with formalin, or heating in the oven at 105°C for three hours.

## Bagging and Controlled Pollination of Female Inflorescence

Female inflorescences are bagged at least seven days before anthesis. The sheath covering the inflorescence is removed and dilute formalin (4 per cent) sprayed onto the inflorescence to kill any stray pollen which may be present. The inflorescence is also examined for the presence of small male anthers at the end of the spikelets. Inflorescence with such features normally have stalked spikelets. Such inflorescences are rejected for controlled pollination purposes as there is a slight chance that natural self-pollination of a few flowers may occur. The confirmed inflorescence is then bagged. Both openings of the bag are padded with insecticide-soaked cotton lints which are then tied to the stalk with wire on one end and folded and tied at the other. For additional security, another bag is used to cover the "inner" bag and the ends are also tied with wire. To hold the bag in an upright position, the top of the bag is linked with wire to a supporting frond base. Bags used for bagging can be canvas, terylene, or paper. The choice depends on the confidence the respective organization has on the quality of the bagging material to prevent entry of the pollination weevil and/or stray pollen. An additional security feature commonly used nowadays is protecting the bag with wire mesh netting, to prevent rats or squirrels from damaging the bags and thereby opening the way for illegitimate pollination.

The bagged inflorescence is inspected once in two days for receptivity. When this occurs, controlled pollination is then carried out. The pollen to be used for controlled pollination is normally diluted with talcum powder in the ration 1:4 by weight. The equipment and place where this is done must first be sterilized to avoid contamination with any foreign pollen. The pollen-talcum powder mixture is kept in a test-tube with two L shaped glass tubes attached to the stopper. Both the exterior ends of the glass tubes are sealed prior to use.

Prophylactic sprays of insecticides and diluted formalin are made to kill any stray insects (especially weevils) and foreign pollen respectively on and around the bagged inflorescence before the actual controlled pollination is carried out. For bags with windows, a small hole is made on the window and one end of the L tube put in and the pollen is then puffed in by blowing from the end of the other L-tube. The hole is then sealed back. The sides of the bag are then tapped to ensure an even distribution of the pollen over the whole receptive inflorescence. Bags without windows are pollinated by opening slightly the top end of the bag after the appropriate prophylactic sprayings have been done. The bag is resealed after the pollen has been puffed in, and the sides of the bag also tapped for even distribution of the pollen. Any excess pollen-talcum powder mixture not used up on the same day is discarded. The glasswares used are then washed, cleaned, and sterilized by putting into the oven at 105°C for three hours. For *Elaeis guineensis*

bunches, one controlled pollination is normally sufficient to ensure good fruit set. However, for *E. oleifera* and hybrids, two controlled pollinations may be required as the period of receptivity is generally longer than that of *E. guineensis*.

The receptivity period of the female inflorescence presents the greatest danger of illegitimate pollination and stringent measures must be taken to prevent such occurrences. Among the measures done nowadays are to have additional insecticides and even crushed naphthalene balls applied around the bunch stalk to deter weevils from approaching the bagged receptive female inflorescence. What is really needed is a chemical which can mask the scent of the female inflorescence at receptivity as this scent attracts the presence of weevils. Inspections are also mandatory especially during this period to ensure that there are no tears on the bag, or the tied ends are loosen. Any bagging or controlled pollination which is found not meeting the quality control checks should be rejected immediately.

About four weeks after the controlled pollination, the bag and wire netting are removed to allow the bunch to develop normally. As a quality control check, the interior of the bag is then checked for the presence of weevils. The bunch is discarded if weevils are found within the bag.

Other quality control checks on the bagging and controlled pollination process which are done monthly include bagging without controlled pollination, and also controlled pollination using dead pollen (dummy check). The bunches are subsequently checked for fruit formation and whether the fruits have kernel (i.e. pollination had occurred). If contamination is detected, the whole process of bagging and controlled pollination must be re-examined.

Another quality control measure is the monthly random harvest of bagged inflorescences at the time of receptivity, for inspection of the presence of weevils inside the bag. If weevils are detected, the whole system must be re-examined.

The importance of ensuring that the controlled pollination is a legitimate cross cannot be over emphasized. At present there are no practical methods to identify and discard illegitimate seedlings in the nursery, and results of illegitimate crosses will only be apparent when the palms start to bear fruits in the field. This will be about five years after the crossing was carried out. As this will result in tremendous losses in terms of time, effort, land, money and reputation, it is imperative that baggings and controlled pollinations must meet stringent quality control standards.

## Seed Processing and Germination Techniques

Ripe fruits of controlled pollinated bunches of *dura*, *tenera*, and *oleifera* origins are normally processed using the depericarper developed for processing commercial DxP seeds. *Pisifera* seeds cannot be similarly processed due to their shell-less nature. Although a method was developed by Arasu (1970), a more practical method used by Chin (1983, unpublished), which was adapted from germinating *pisifera* seeds in Ghana by Wonkyi-Appiah (1973), is to keep the *pisifera* fruits under sand cover with a moist environment for about two weeks. By this time the mesocarp has softened and can be easily peeled off the seeds. Seeds are then cleaned and processed further in the usual manner. This technique avoids nicking injuries which can happen when knives are used to depericarp the fruits manually.

Germination techniques for *dura* seeds have been well established, especially for DxP seed production. Basically this involves dry heat treatment at 18 per cent moisture content in a heat-room at a temperature of 39-40°C for up to 60 days. The seeds are kept in moisture proof polythene bags. At the end of the heat treatment, the seeds are resoaked to raise the moisture content to 22 per cent for germination.

*Tenera* seeds are more difficult to germinate. The main problem appears to be moisture content control due to the varying thickness of the shell, which can range from thin to moderate. To maintain the moisture control, air drying after processing should be as short as possible (Rao and Rajanaidu, 1992, per. comm.). The dry heat treatment used on *dura* seeds is also used on *tenera* seeds to break dormancy for germination.

*Pisifera* seeds are also very difficult to germinate. Many apparently normal *pisifera* seeds have defective or absent embryos. As it is not possible to eliminate such defective seeds before germination, the germination rate for *pisifera* seeds is generally much lower than that of *dura* seeds. Due to the spread of fungal diseases from such dead seeds upon soaking for germination, germination in polythene bags such as practised for *dura* seeds is not practical. The seeds need to be separated. Germination on sand and charcoal media have been attempted with some degree of success (Chin, 1983, unpublished). A practical method of germinating *pisifera* seeds has also been reported (Wonkyi-Appiah, 1973).

With the advent of tissue culture technology, embryo rescue and subsequent germination *in vitro* is also another technique for getting *pisifera* plantlets. However success rates are also low (Soh, 1992, per.comm.).

*Oleifera* seeds are also a problem to germinate, as there is a high proportion of parthenocarpy. Only the larger sized seeds are retained for germination. A method reported to give satisfactory germination in Colombia was by adopting the dry-heat treatment with 22 per cent moisture content for an initial 15 days, then soaking in warm water at 43°C for 15 minutes and following this with the wet-heat treatment for 65 days (Chew, 1976, quoted by Hartley, 1988).

### **Yield Recording**

Yield recording is one of the major technique used in breeding programmes. It measures the number of ripe bunches and the individual weights of each of the these bunches. From these records, the productivity of the palm as measured by the fresh fruit bunch (FFB) or yield of the palm over a given period of time is obtained.

Basically yield recording is carried out two to three times a month, whereby ripe bunches are harvested and weighed together with the loose fruits for every valid identified individual palm under recording. The yield records obtained are then transferred to yield record databases, which are usually stored on computer.

The period of yield recording normally begins when harvesting starts and lasts for a minimum of four to six years. The advantage of six years of yield recording is that the first three years measure the productivity during the juvenile period while the second three years measure the productivity of the mature period.

As for the number of palms to be recorded for comparative breeding trials, studies by Soh *et al.* (1989) have shown that, under Malaysian coastal clay soils, plot sizes of 12 and 16 palms replicated four and three times respectively are needed to detect treatment differences of about 10-15 per cent. For inland trials, however, at least five replications for both plot sizes are necessary to be able to detect treatment differences of less than 15 per cent.

As breeding trials are recorded on individual palm basis, yield data is normally expressed as kg/palm, which is understood by all. However, when such values are converted to yield per hectare basis, the figures obtained will depend on the conversion factor used. For instance, the IRHO and associated organizations use 135 palms per hectare to estimate the yield when 148 palms per hectare are planted, allowing 10 per cent for unproductive palms or other field losses. Other organizations choose to use the full stand of 148 palms per hectare for the yield estimate. As there are no fixed standards for expressing yield per hectare, care must be exercised when comparing yield per hectare data.

## Bunch Analysis

Basically bunch analysis is a technique to know bunch, fruit, and oil components. Most organizations follow the method developed by Blaak *et al.* (1963). Essentially this involves chopping the bunch to separate the stalk and spikelet components. The spikelets which contain the fertile and parthenocarpic fruits are then stripped. Samples of fertile fruits are taken and the mesocarp is scraped to determine the mesocarp content. The nuts are dried before being cracked to determine the shell and kernel components. For oil determination, a sample of the mesocarp is taken for oil content through solvent extraction.

In recent years this technique has been critically examined by Malaysian bunch analysis laboratories (Rao *et al.*, 1983). Recommended procedures have accordingly been established with respect to ripeness standard, stalk length, spikelet sampling, spikelet and fruit storage, mesocarp drying, sieving for mesocarp samples, oil extraction, and nut drying.

The recommendations are summarised in the following table:-

Item	Recommendation
1) Ripeness standard	<ul style="list-style-type: none"><li>- Ripeness standards affected bunch components, but practical constraints do not permit standardization.</li><li>- Desirable to specify the ripeness standard with the results.</li><li>- Proper specification would be the number of loose fruit, counted immediately after bunch harvest, per kg of bunch weight.</li></ul>
2) Stalk length	<ul style="list-style-type: none"><li>- Standardize by close cutting the stalk to the point of lowest spikelets.</li></ul>
3) Effect of delay between harvesting	<ul style="list-style-type: none"><li>- Delays of even five hours between bunch harvesting and analysis are critical insomuch as analysis bunch weights are reduced.</li><li>- Where delays are unavoidable and a regular feature, field balances should be used.</li><li>- Usage of gunny sacks/clean fertilizer bags may be useful to reduce damage in transit.</li></ul>

Item	Recommendation
4) Sampling spikelets for fruit to bunch weight ratio estimation	<ul style="list-style-type: none"> <li>- Random methods are superior to spiral/stratified sampling.</li> <li>- The revised PAMOL method gave overall best estimates. Here the bunch is chopped on a table with a movable wooden surround on three sides. After all spikelets are removed, they are individually separated. The spikelets are then thoroughly mixed on the table with a shovel. A sample of spikelets is then taken by pulling the moveable surround forward so that the spikelets fall off the front of the table into a collection box. The collecting box is designed in such a way that a strip of spikelets from front to back along one side of the table is collected, the rest being discarded.</li> </ul>
5) Storage of spikelets	<ul style="list-style-type: none"> <li>- Storage of spikelets for up to three days results in a large number of the fruits abscising and the remaining adhering fruits are more easily detached manually.</li> <li>- However there is loss of moisture, resulting in slight bias in fruit to bunch estimates. If stored fruit weight and fresh spikelets weight are used in fruit to bunch computations, an underestimate of about five per cent is obtained. Stored fruits and stored spikelets weight give an over estimate of about 2.5 per cent.</li> </ul>
7) Storage of fruit sub-sample	<ul style="list-style-type: none"> <li>- Keeping fruits in a tied polythene bag of fairly thick gauge has been found to reduce drying considerably, especially at reduced temperatures (refrigeration) for even up to two days.</li> </ul>
8) Depericarping drying of mesocarp	<ul style="list-style-type: none"> <li>- Flat, large but thin sections and ("waters") are best suited for even drying.</li> <li>- Mincing, especially when producing a lump of smaller cubic pieces, should be avoided.</li> <li>- Sub-sampling of mesocarp for moisture determination is not recommended.</li> <li>- No mesocarp should be lost between depericarping and weighing of dried mesocarp.</li> </ul>
9) Sieving of mesocarp	<ul style="list-style-type: none"> <li>- Sieving using 0.125 inch mesh gave more complete extraction and shorter extraction times are possible.</li> </ul>

Item	Recommendation
10) Oil extraction	<ul style="list-style-type: none"> <li>- Results justify the present practice of using 5g but there is little argument against 2.5 or 10g i.e. half or double present sample size. Below 2.5g, differences between duplicate samples are more marked while samples of more than 10g gave poorer extraction. Using 5g samples, the necessity for duplication was examined.</li> <li>- Results show no significant difference between duplicates. Hence it is recommended that duplication, provided mesocarp is sieved and a random sample taken, is unnecessary.</li> </ul> <p>No significant differences exist between normal 16 hours and shorter 12 or 14 hours of extraction. Hence assuming an eight hour day, extraction may proceed for 1½ to 2 days.</p>
11) Nuts drying	<ul style="list-style-type: none"> <li>- Complete drying in oven at 105°C for 24 hours is recommended. It may be necessary to recompute kernel weight to commercial moisture levels.</li> </ul>

For oil determination, besides Soxhlet extraction, cold solvent extraction is also available (Blaak, 1970). Another alternative, practised by the IRHO, is oil determination using specific gravity measurements. This is the "oleometre" method. Results are more rapidly obtained. Calibration checks with Soxhlet extraction are also routinely carried out. In this method a given weight of solvent of known specific gravity is added to a given weight of dried mesocarp and the mixture then finely ground and mixed. The solvent/oil mixture is filtered out and its specific gravity recorded. This specific gravity is dependent on the amount of oil in the filtrate and hence, in the mesocarp samples. Density/oil content tables, corrected for temperature, are constructed for easy reference (IRHO Bunch Analysis Procedure, IGK 9, June 1980).

For *oleifera* bunch analysis, the presence of both large and small parthenocarpic fruits necessitates separate analyses for such fruits in addition to the fertile fruits. Due to wide spread infertility within and between palms of *oleifera* hybrids progenies, individual palm bunch analyses may give rise to non-representative results if only fertile bunches are selected for analysis.

Hence the IRHO has adopted the "global analysis". For one progeny, all bunches at maturity on the same day are harvested (minimum of six bunches). All bunches are weighed and spikelets and fruits removed. Only empty spikelets and white or dry parthenocarpic fruits are eliminated.

All other fruits are mixed and analysed as a single sample (500g drawn from a sampler). For a progeny one analysis is done every three months - (IRHO Bunch Analysis Procedure, IGK 16, August 1980).

The number of analyses for a good characterization of bunch and fruit components has been calculated with reference to bunch analysis data of several crosses in Cote de I'voire by the IRHO. This number is 80 (40 palms analysed twice) for a cross and 15 for an individual palm (Gascon, 1983).

Except when selecting for ortets, Malaysian breeders carry out a minimum of five bunch analyses for each individual palm which is analysed (SIRIM, 1988). Nevertheless, as pointed out by Rao (1986), more than 50 analyses are required per palm to detect significantly a 2 percent difference in the oil to bunch ratio of palms. Out of the four components (fruit to bunch, mesocarp to fruit, dry mesocarp/wet mesocarp, oil to dry mesocarp) making up the calculation for oil to bunch, mesocarp to fruit has the least variation, requiring only five analyses for significant detection whereas fruit to bunch required 23 analyses.

For expression of oil extraction rate estimates, the IRHO and associated organizations use a conversion factor of 0.855 on the oil to bunch ratios obtained from bunch analyses. This takes into account mill efficiency and the selected bunches used for analysis. The value obtained for oil extraction rate is useful as an indication of the rate at the mill.

### **Fatty Acid Composition**

Breeding palms with higher unsaturated fatty acid composition is desirable nowadays. Regarding the sampling required for fatty acid composition, the IRHO's research has shown that one analysis per palm is sufficient, using a sample of 10 to 20 external ripe fruits from one bunch (Meunier, per com. 1987). To know the average composition of a progeny, one analysis is performed on a sample made of a mixture of oils from the maximum number of palms. The technique used is as follows:-

- (i) Three times a month, remove 5 to 10 fruits from 4 bunches obtained from 4 different palms.
- (ii) Extract 15 ml of oil and keep in a 500 ml plastic bottle with sodium sulphate in a deep freezer.
- (iii) Pour each sample into the same bottle.
- (iv) After six months, 72 palms would have been taken.
- (v) Shake the mixture and take 10-15 ml for analysis.

The actual procedure involves autoclaving the fruit sample for 90 minutes after which the mesocarp is depericarped and stored at room temperature for 12 hours. The mesocarp is then put into the oven at 105°C for two hours. After this, the mesocarp is ground using a food blender. A sample of the mesocarp of known weight is then mixed with n-hexane of known volume. The mixture is then filtered using filter paper containing anhydrous sodium sulphate. The filtrate of oil mixed with n-hexane is then placed into the Buchi evaporator for 5 minutes to separate out the n-hexane and thus getting a sample of pure oil. This sample of oil is then despatched for fatty acid composition using gas liquid chromatography.

### **Height Measurement**

Height of palm is a measure of how fast the palm grows and is an important criterion when selecting for slower growing progenies, which will extend the economic life of the palms.

There are differences in the technique used to measure height of palms by various organizations. Most breeders in Malaysia measure the height from ground level to the base of frond number 41. Height increment rate is the height measured divided by the age of the palm. On the other hand, the IRHO and associated organizations measure the height from ground level to the base of frond number 33. Height increment rate is the height measured divided by the age of palm less three (assuming no height increase for the first three years after planting). Normally the height is measured once during the juvenile period (Year 3 to Year 5 after planting) and once during the mature period (Year 6 after planting and beyond).

### **Vegetative Measurements**

Hardon *et al.* (1969) and Corley *et al.* (1971) developed methods of estimating growth parameters, like leaf area and leaf area index (LAI), vegetative dry matter production (VDM), crop growth rate (CGR) and net assimilation rate (NAR) from non-destructive measurement and showed that under Malaysian conditions significant progeny differences existed for most of the growth parameters. These methods enable comparative growth analysis work covering leaves, trunk and bunches to be carried out. These organs were found to constitute over 96 percent of total annual dry matter production.

The actual technique involves cutting frond number 17 for measurement of the following characters:-

- (i) rachis length
- (ii) number of leaflets on one side of the frond

- (iii) petiole cross-section
- (iv) length and breadth of six leaflets in the centre of the frond.

The number of fronds produced per year is known from marking frond number 1 and is  $n-1$ , where  $n$  is the frond position of this frond number 1 at the same time the following year.

The various formulae for these physiological characters are summarized in Appendix 1.

Recently Henson (1991) re-examined the relationships between (i) petiole cross-section size (width x depth) and frond dry weight, and (ii) leaflet dimensions x numbers and frond area for oil palms during the first five years after field planting. The previously established 'standard' regression parameters were found to overestimate both the dry weight and area of fronds of young palms. Discrepancies in frond dry weight were greatest in younger palms and diminished with age, disappearing by the fifth year. Discrepancies in leaf area showed little consistent age trend. He concluded that productivity during the early years after field planting will be overestimated by non-destructive measurement using the 'standard' formulae, and that some destructive sampling of fronds is necessary in order to obtain reliable estimates of both dry matter production and leaf area development in young field palms.

## DISCUSSION AND CONCLUSION

The ability to identify the fruit type of a palm before the palm begins to bear bunches is useful for the breeder. So far, there is no practical technique to screen for potential fruit type during the nursery stage. If such a technique can be developed, nursery palms can then be screened to identify those palms which the breeder desires to plant in the field. For instance, in TxT crosses, the *dura* palms are not desired as they cannot be used for further breeding work. If they are identified in the nursery, then there will be savings of at least 25 per cent in terms of land requirement as these palms need not be planted out. In addition, such a technique can also be used as an early test for legitimacy of the cross. For instance, the presence of *dura* seedlings detected in a DxP cross will mean that the cross is illegitimate and should not be field planted.

With labour for the agricultural sector becoming increasingly scarce, such as in Malaysia, there may come a time when finding workers to do yield recording may be a problem. Techniques may need to be developed to provide reliable estimates of FFB production. Counting the number of bunches is relatively easy, but estimating bunch weight reliably is more difficult.

Similarly bunch analysis is also labour intensive, with a significant amount of time required to depericarp the fruits into thin slices manually. So far no new reliable techniques which are simpler or more rapid have been developed to replace the present process.

To conclude, oil palm breeding technique will continue to evolve with time, with refinements or modifications to suit ever changing conditions. New techniques are also needed for early confirmation of the legitimacy of crossings.

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## FORMULAE : PHYSIOLOGICAL CHARACTERS

### Character Known

FFB	-	Fresh Fruit Bunch
OBN	-	Oil to Bunch
BK	-	Kernel to Bunch
FRD	-	FronD Production
RL	-	Rachis Length
NLEAF	-	No. of Leaflet
HT	-	Height
CDW )	-	Collar Diameter (petiole cross-section)
CDD )		

### Physiological Characters Calculations

1. Leaf Area,  
AREAL = 
$$\frac{[\sum (L \times W) \times NLEAF \times 2 \times 20.57]}{6} \div 10000$$
2. Leaf Area Index,  
AREALI = 
$$(40 \times AREAL \times 148) \div 10000$$
3. Leaf Dry Wt,  
DWLEAF = 
$$(0.1023 \times CDW \times CDD) + 0.2062$$
4. Leaf Dry Wt/Ha,  
DWLEAFH = 
$$(DWLEAF \times 148) \div 1000$$
5. Trunk Dry Wt,  
TRDW = 
$$3.142 \times (DIAMETER \div 2)^2 \times (HT \div AGE \text{ of Tree}) \times 1000 \times 0.17$$
6. Trunk Dry Wt/Ha,  
TRDWH = 
$$(TRDW \times 148) \div 1000$$

7. Frond Dry Wt,  
FRDW = FRD x DWLEAF
8. Frond Dry Wt/Ha  
FRDWH = (FRDW x 148) ÷ 1000
9. Vegetative Dry Matter,  
VDM = 148 x (FRDW + TRDW) ÷ 1000
10. Fractional Interception,  
F =  $1 - e^{-0.47} \times (\text{AREALI} - 0.3)$
11. Bunch Dry Wt,  
BDW =  $0.53 \times \text{FFB} \times [(1 - (\text{OBN}/100)) + (1.2 \times (\text{OBN}/100))] \times 148 \div 1000$
12. Adjusted Bunch Index = BDW ÷ (VDM + BDW)
13. Conversion Efficiency = (VDM + BDW) ÷ (31 x F)
14. Total Dry Matter = VDM + BDW
15. Bunch Index =  $(0.53 \times \text{FFB}) \div [90.53 \times \text{FFB} + (\text{FRDW} + \text{TRDW})]$
16. Harvest Index =  $\frac{(\text{BDW} + ((\text{FFB} \times \text{BK})/100) \times 1.5)}{\text{VDM} + \text{BDW} + ((\text{FFB} \times \text{BK})/100) \times 1.5}$
17. Frond Index = AREAL ÷ DWLEAF

## **BREEDING PLANS AND SELECTION METHODS IN OIL PALM**

SOH A. C.<sup>1</sup>

### **ABSTRACT**

*The scientific basis and experimental results of the two main basic breeding plans in oil palm - modified reciprocal recurrent selection (RRS) and modified recurrent selection (MRS) were reviewed. Results of the relative efficacy of the two plans appeared to be stronger for the former. Backcross breeding, recombinant inbred breeding and breeding for clonal propagation were considered as special side programmes to exploit as commercial cultivars materials generated from the main breeding programme.*

*Use of the selection techniques commonly practised in animal breeding such as best linear prediction (BLP), selection index and best linear unbiased prediction (BLUP) of breeding values in oil palm is illustrated and encouraged. Breeding towards an ideal or optimum hybrid genotype using such techniques is suggested.*

*Developments in animal and forest tree breeding besides molecular biology, will have much relevance to oil palm breeding and computer-assisted selection will be indispensable. Breeders should draw up breeding plans which will be able to exploit emergent materials and techniques to the fullest.*

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## INTRODUCTION

It is often claimed that plant breeding is an art as well as a science. Today, of course, we are dealing with the science of oil palm breeding, and to me, at least, real art is founded on good science in modern plant breeding.

My role today is to review the scientific developments in breeding plans and selection methods in oil palm.

### BREEDING PLANS

Central to the objective of a breeding programme is the setting up of a breeding plan or scheme. In oil palm, major breeding programmes have gravitated towards two basic plans:

#### **Modified Reciprocal Recurrent Selection (RRS, Figure 1)**

This scheme, practised in Ivory Coast, NIFOR, Marihat and Socfindo, is adapted from the RRS developed by Comstock *et al.* (1949) to exploit both general (GCA) and specific combining abilities (SCA). It has been particularly developed for, well tested (e.g. Moll and Stuber, 1971, Eberhart *et al.* 1973 Eyherabide and Hallauer, 1996) and practised in maize, and also adopted in other cross-pollinated crops. It has also been evaluated in laboratory animals (e.g. Kojima and Kelleher, 1963) and practised in animal breeding e.g. poultry.

The RRS in oil palm breeding (Meunier and Gascon, 1972) is a very attractive scheme. Firstly both the selection of parents which go into the next recombination cycle of breeding and choice of parents for commercial hybrid production are based on the progeny-tests of the prospective commercial interpopulation hybrid.

Secondly, the plan is divided into two distinct phases: One allows shorter term commercial exploitation of the best test-cross and its improvement by recurrent selection with the selfs of the selected parents. This within hybrid improvement (WHI) phase would exploit SCA to its fullest. The other or recombinant phase allows accumulation of favourable alleles (additive

and non-additive) and maintenance of genetic variability for a sustained longer term improvement. Thirdly, the commercial hybrid can be reproduced utilising D's (*duras*) and P's (*pisiferas*) from the selfs of the parents, once the interpopulation test results are known.

## Results

Jacquemard *et al.* (1981) showed that the DxT (*tenera*) cross can be reproduced by about 15 D's and 15 P's from the selfs of the parents involved in the cross.

Gascon *et al.* (1988) reported that by selecting 15 hybrids from 529 tested, an 18% improvement in oil yield (OY) was achieved in the first cycle. In the second cycle (WHI) trials, selecting the best crosses would provide another 10-15% improvement (Nouy *et al.* 1988). The reproductions of the best crosses from these second cycle trials for commercial production were expected to be around now. It would be interesting to know whether large scale commercial seed-production is hampered by inbreeding depression in the second selfs of the parent palms.

In Marihat, from their first cycle trials of about 500 crosses, selecting the best 40 crosses would result in 25% improvement in OY (Lubis *et al.*, 1990).

It is interesting to note that in the trials to test the reproductions of the first cycle crosses (Jacquemard *et al.*, 1982) non-additive effects (although likely to be overestimated) predominated in the hybrids. Similarly from the results of the second cycle trials (Nouy *et al.*, 1990) selection improvement in OY appeared to be through improvement from oil to bunch (O/B) rather than from fresh fruit bunch (FFB) yield or bunch number (BNo) as compared to the first cycle. These implied the exhaustion of additive variation for FFB and BNo and that improvement in subsequent WHI cycles would have to be through exploitation of SCA. These results are consistent with experiences with RRS in maize (Eberhart *et al.*, 1973, Moll and Stuber, 1971, Eyheribide and Hallauer, 1991b).

It is also interesting to note that in the Marihat first cycle trials, although there were no significant differences in the hybrid due to D families, they were able to identify certain D lines and parents with high GCA. Similarly in the second cycle trials of Nouy *et al.* (1988) they found that although the GCA's of the D parents appeared to be less than those of the T's nevertheless they can be substantial. In many DxP trials in Malaysia, (Rajanaidu *et al.*, 1988; differences among DxP families were largely due to D parent contributions. These indicate that substantial improvement in the DxP can be achieved by progeny - testing the D parents. Results of the first recombinant cycle tests have yet to be reported.

The main disadvantage of this RRS scheme is the size of the programme required. To be able to produce at least 3-4 million seeds from the reproductions of the top 15% of the crosses, about 500 crosses (~ 362 ha.) and 180 parental selfs from the populations, assuming one parent crossed to three parents from each side, (~ 260 ha.) would be needed.

The other disadvantage is from the point of seed production where fertility in the later selfs (particularly the D mother palms, although the P's too) drops severely to limit seed production. To get around this sib-crosses among parents can be made to produce 3-way or 4-way cross hybrid seeds. This may be at the expense of full exploitation of SCA and seedling uniformity, although Gascon *et al.* (1988) found that 3-way crosses gave even better improvements.

### **Modified Recurrent Selection (MRS, Figure 2)**

Breeding programmes in Zaire, Papua New Guinea and Malaysia tended to favour family and individual phenotypic selections in the parental populations for recombination breeding and for seed-production, with interpopulation DxP progeny - tests conducted mainly for the identification of P parents for seed production. (Hardon *et al.* 1985). Rosenquist (1990) used the acronym, "FIPS", but since it is a breeding plan or scheme, rather than just a method of selection and that selected parents are interbred for each cycle, I prefer to call it a modified recurrent selection scheme (MRS).

The central premise or assumption in this scheme is that the additive effects or GCA effects expressed within population crosses (determined by family means or combining ability analyses) are also reflected in the interpopulation crosses. This assumption has yet to be verified. It must be stressed that the GCA of a line or individual is relative to and dependent on the particular populations or lines to which it has been crossed (Sprague and Tatum, 1942, Henderson, 1952), an important principle often forgotten.

In maize (Moll and Stuber, 1971), correlations between intra and inter-varietal additive gene effects were 0.63 and 0.72, and response of the variety hybrid to RRS was 1.3 times greater than the response to full-sib family (FS) selection. Heterosis in the variety hybrid increased markedly after RRS but showed little change after FS selection. In another example, Eberhart *et al.* (1975) reported that there was little improvement in the parental populations but a great response in the population cross after RRS.

In MRS also, the separation of within population crosses into non-related crosses for the recombinant cycle and related crosses (to concentrate favourable alleles) for exploitation in commercial hybrid production were not so well delineated or emphasised. In the latter, interpopulation progeny-testing is necessary as there is likely inbreeding and that selecting parents based on within population family means or combining abilities is inadvisable as the more heterozygous (for the favourable alleles) parents will likely be chosen and would not translate into a heterotic interpopulation hybrid.

## Results

Evidences of the efficacy of MRS pertaining to interpopulation hybrid improvement have not been as strong as for RRS. Although MRS has contributed to the oil yield improvement of about 15% per generation for four generations in the Deli D (Hardon *et al.* 1987), how much of this improvement has been translated into DxP improvement was not stated. Similarly it was not known how effective T-sib selection (for choice of P's for progeny-testing) has been, although selecting the best P's gave 7% oil yield improvement. The authors stated that D selection has not been effective. Lee *et al.* (1990) revealed that comparison of third and

fourth generations of Deli D crossed with BM119 P's indicated no selection progress for FFB yield but a 6.4% increase in OY mainly due to an improvement in the OB. Rajanaidu *et al.* (1990) claimed that there was an improvement of 9% FFB and 7% OY when comparing DxP materials using first and second generation *duras*.

Yong and Chan (1992) hinted that MRS in the Ulu Remis Deli D's were not effective, as they found that when these D's were crossed to one P (BM119) the differences in yield attributed to D families, to D's within family and to D's across families would be as high as 10%, 22% and 30% respectively. Similarly Breure (1992) admitted that GCA estimates in DxD crosses (Breure and Konnimor, 1992) might be imprecise due to much inbreeding.

### **Comparison of RRS and MRS Plans**

It is really difficult to compare objectively the relative efficacy of these two breeding schemes based on the above results because of differences in the starting base populations in terms of size and genotypes and standard control crosses. It would also be unwise to initiate a proper experiment to test this because of the tremendous and likely futile effort required. The choice of which scheme to adopt depends very much on one's bias, objectives e.g. relative emphasis on short and long term gains and stage of breeding programme.

Undoubtedly without having to expend much effort and time in interpopulation progeny-testings and selfings as required by the RRS, more and quicker turnover of recombinant crosses can be made with the MRS. However, unless one practises rigorous selection in the recombinant cycle, one is merely postponing the effort needed in progeny-testing. To confidently practise vigorous selection in this phase, it will be useful to know how far intrapopulation selection can be translated into interpopulation improvement.

Personally I would prefer to use MRS or FIPS to develop relatively unimproved populations and to reduce the size of recombinant families to go into the RSS programme.

## Other Breeding Plans

I would regard backcross breeding and recombinant inbred line breeding (Pooni *et al.* 1989) as rather specific programmes directed towards commercial exploitation of a specific hybrid.

Backcross breeding would be useful in the *Elaeis oleifera* x *Elaeis guineensis* hybrid development programme to improve the fertility of the hybrid while retaining the oil quality, disease resistance and low height attributes of the *oleifera*. Similarly, it would be a useful approach to incorporate a desirable trait into an otherwise superior parent e.g. incorporating short stature character into the high oil yielding AVROS P to give the Dumpy-AVROS P (Soh *et al.*, 1981).

The recombinant inbred approach would also be useful to develop improved Deli dura breeding lines through introgression.

I have not discussed breeding plans for clonal propagation as I do not envisage any breeder presently drawing up a main programme to breed specifically for clones. I would regard it as a side programme (Soh, 1986) as in WHI, backcross breeding and recombinant inbred breeding, to exploit into cultivars what has been generated in the main breeding programme.

## Selection Methods

Many of us are at the stage where we are embarking on the recombinant cycle and have to make critical choices of parents to go into the cycle in order to achieve our breeding goal while keeping the programme within manageable size.

Predicting breeding values of parents is a key step in the process.

### A) Predicting parental breeding values

The most commonly practised methods are:

- Phenotypic selection - mass selection  
family and individual selection (Rosenquist, 1990)
- Genotypic selection - combining ability (least squares) selection  
(Meunier and Jacquemard, 1988; Breure and Konimor, 1992)

There are a number of new methods originally developed in animal breeding which are now finding use in plant breeding, particularly forest tree breeding (Henderson, 1984; White and Hodge 1989) which I believe will be of much use in oil palm breeding.

These are the Best Linear Prediction (BLP), Selection Index (S.I) and Best Linear Unbiased Prediction (BLUP) techniques.

The advantages of these methods over the combining ability or least squares method are:

1. Can handle highly unbalanced (mating) designs.
2. Can utilize information from a number of experiments even without standard check varieties.
3. Can utilize information from other relatives or sources.
4. Allows a more directed approach in breeding.
  - a. breeding towards an ideal or optimum genotype.
  - b. breeding towards a target environment.
5. Allows refinement as better genetic information becomes available.
6. Can handle multiple traits at one time.

In all these methods in contrast to least squares method replicate and environment effects are considered as fixed and "nuisance" effects to be removed (or estimated) in order to predict the genetic effects which are random effects.

### Best Linear Prediction (BLP, White & Hodge, 1989)

The objective is to develop a function of the  $n \times 1$   $y$  vector of data which predicts both accurately and precisely  $g$ , a  $q \times 1$  non-observable random vector of genetic (breeding) values

$g = a + b'y$  where  $g$  = vector of genetic values, its dimension reflects the number of traits and genotypes for which predictions are required

$b'y$  = linear function for observed values.

The approach is to find that linear combination of the data that minimizes the expected value of the squared difference between the true and predicted breeding values.

i.e. find  $g = a + b'y$ . such that  $E(g - g)^2$  is minimum.

By algebraic manipulation and partial differentiation setting equations to zero, the result:

$$\text{BLP : } g = r + C' V (y - \hat{\theta})$$

where  $g$  = BLP breeding value.

$r$  = expected value of  $g$ , usually assumed 0 so that prediction are expressed as deviation from zero. With selected population,  $r$  may reflect expected progress from selection.

$V$  =  $n \times n$  matrix of variances and covariances among the observations.

$C$  =  $\text{Cov}(y, g')$ ,  $n \times g$  matrix of covariance between the observation and the genetic values being predicted.

$y$  = observed value.

$= E(y) = n \times 1$  vector of expected values of the observed data.  $\hat{\theta}$  may represent block mean, trial mean and other fixed effects.

The formula can be 'viewed' as a multiple regression relating  $g$  to  $y$ . A different set of coefficients is developed for each trait and genotype depending upon the exact trait being predicted and the data observed for that genotype.

### Properties of BLP's

1. The predictions are unbiased.
2. The error variance of the predictions is minimized.
3. The correlation between the predicted and true genetic values is maximized
4. A multivariate normal joint distribution results in three other properties.
  - a) BLP is the best (minimum error variance) predictor among all possible functions and transformations (not just linear function) of the data.
  - b) BLP maximises the probability of selecting the better of two candidates.
  - c) BLP maximizes expected genetic progress for a fixed number of selections made.
5. BLP of a linear transformation of genetic values is that function of the predictions. Any linear function of the predicted genetic values is the best linear prediction of the same linear functions of the underlying true genetic values. This is very important when economic weights are used to combine multiple traits into aggregate genetic value. It means that the economic weights can be applied after the predictions and still result in BLP of the aggregate genetic value.
6. The variance among the predictions can be calculated.
7. The covariance between predicted and true genetic values can be calculated.
8. The error variance of the predictions can be calculated.
9. The correlation between true and predicted genetic values can be calculated.

BLP is a generalisation of classical selection index developed by Smith (1936) and Hazel (1943). The classical selection index approach assumes equal amounts and quality of data available for all genotypes. Hence a single vector of weights (coefficients) is developed and used for all genotypes. Whereas BLP develops a different vector of coefficients for each genotype to reflect the nature of the data available for that genotype.

## Selection Index (S.I)

The S.I. is set up as follows:

If,  $y = m \times 1$  vector of phenotypic observations pertaining to a single candidate for selection (may include several types of observations such as individual measurements and family means on several traits).

- $\mu = E(y)$ ,  $m \times 1$  vector of expected values of the observed data pertaining to each candidate.
- $b = m \times 1$  vector of index coefficients to be estimated
- $g = q \times 1$  vector of unobservable genetic values.
- $a = q \times 1$  vector of economic weights.

We want to predict for each candidate its genetic worth,

$$w = a_1 g_1 + a_2 g_2 + \dots + a_q g_q \\ = \underline{a}'g$$

The selection index,  $I$ , which is a linear function of the observed data is :

$$I = b_1(y_1 - \mu_1) + b_2(y_2 - \mu_2) \dots + b_m(y_m - \mu_m)$$

We want to find  $\underline{b}$  such that the  $\text{Corr}(W, I)$  is maximised or  $E(w - I)^2$  is minimized.

The result of algebraic manipulations:

$$\underline{b} = \underline{V}^{-1} \underline{C} \underline{a}$$

$$\underline{w} = \underline{a}' \underline{C}' \underline{V}^{-1} (\underline{y} - \underline{\mu})$$

which is identical to  $\underline{g} = \underline{r} + \underline{C}' \underline{V}^{-1} (\underline{y} - \underline{\mu})$  - BLP

except premultiplication by  $\underline{a}$  creates a linear combination of the predicted genetic values into a single aggregate genetic worth, the vector matrices apply to a single candidate and are therefore much smaller than those of BLP which apply to all observations in all candidates.

An example of this approach utilising plot and family information in selecting oil palm for cloning is given below (Soh & Chow, 1993).

### Selection Index (S.I.) Example

The example illustrates selection for a single trait case and a multiple-aggregate trait case.

#### a) Single Trait

The observed data:

$$y = y_{ij}AE_{lm} = \text{individual observation}$$

$y_{ij}AE_l$	plot mean
$y_iAE$	full-sib family mean
$y_iAF$	half-sib family mean 1
$y_iAG$	half-sib family mean 2

The model for an individual true observation.

$$y_{ijklm} = U + E_i + B_{ij} + f_k + m_l + p_{ijkl} + w_{ijkln}$$

where:

- $U$  = general mean
- $E_i$  = fixed effect of  $i$ th test environment
- $B_{ij}$  = fixed effect of the  $j$ th block in  $i$ th test
- $f_k^*$  = random effect of  $k$ th female in  $i$ th test (includes  $f_e + f_m + f_{me}$  effects)
- $E(f_k^*) = 0$      $\text{Var}(f_k^*) = \sigma^2_{f^*}$
- $m_l$  = random effect of the  $l$ th male (includes the effects)
- $E(m_l^*) = 0$      $\text{Var}(m_l^*) = \sigma^2_{m^*}$

$p_{ijkl}$  = random plot error of  $k$ th family in  
 $j$ th block of  $i$ th test.

$$E(p_{ijkl}) = 0 \quad \text{Var}(p_{ijkl}) = \sigma_p^2$$

$w_{ijklm}$  = random tree error of with tree in  $ijkl$ th  
 plot  $E(w_{ijklm}) = 0$

$$\text{Var}(w_{ijklm}) = \sigma_w^2$$

V-matrix : variance and covariance of observations.

$$\text{Var}(\text{indiv mean}), VI = \sigma_f^2 + \sigma_m^2 + \sigma_p^2 + \sigma_w^2$$

$$\text{Var}(\text{plot mean}), VP = \sigma_{f^*}^2 + \sigma_{m^*}^2 + \sigma_p^2 + \sigma_w^2/n$$

$n$  = no. palms per plot

$$\text{Var}(\text{full-sib mean})VF = \sigma_{f^*}^2 + \sigma_{m^*}^2 + \sigma_p^2/b + \sigma_w^2/bn$$

$b$  = no. of blocks.

$$\text{Cor}(\text{indiv. plot}) = \text{Var}(\text{plot mean})$$

$$\text{Cor}(\text{indiv. F.S.}) = \text{Var}(\text{F.S. mean})$$

$$\text{Cor}(\text{plot, F.S.}) = \text{Var}(\text{F.S. mean})$$

$$\text{Cor}(\text{indiv, H.S.}) = \sigma_{m^*}^2$$

$$\text{Cor}(\text{plot, H.S.}) = \sigma_{m^*}^2$$

$$\text{Cov}(\text{FS/HS, H.S.}) = \sigma_{m^*}^2$$

$$V = \begin{matrix} VI & VP & VF & \sigma_{m^*}^2 & \sigma_{m^*}^2 \\ VP & VP & VF & \sigma_{m^*}^2 & \sigma_{m^*}^2 \\ VF & VF & VF & \sigma_{m^*}^2 & \sigma_{m^*}^2 \\ \sigma_{m^*}^2 & \sigma_{m^*}^2 & \sigma_{m^*}^2 & VF & \sigma_{m^*}^2 \\ \sigma_{m^*}^2 & \sigma_{m^*}^2 & \sigma_{m^*}^2 & \sigma_{m^*}^2 & VF \end{matrix}$$

**C - matrix: Covariance between observation and genetic value**

$$\text{Cov. (indiv. obsv, genetic value)} = \sigma^2 A$$

$$\text{Cov. (plot mean, genetic value)} = \frac{1}{2} \sigma^2 A (1+1/n)$$

$$C = \text{Cov. (F.S. mean, genetic value)} = \frac{1}{2} \sigma^2 A (1+1/bn)$$

$$\text{Cov. (H.S. mean, genetic value)} = \sigma^2 m^*$$

$$\text{Cov. (H.S. mean, genetic value)} = \sigma^2 m^*$$

$$\text{Selection index equation } Vb = Ca$$

$$\text{Solution: } b = V^{-1} Ca; a = 1 \text{ or } -1 \text{ (single trait)}$$

Correlation between true and predicted genetic worth i.e. precision of prediction.

$$\text{Corr (w w)} = a' C' V^{-1} C a / a' G a; G = \sigma^2 A$$

The results for index selection for single trait for height increment are given in Table 1.

**b) Multiple traits:**

Only three traits, oil yield, bunch number and height increment and individual, plot and full-sib family information were considered.

**V - matrix**

The following were the additional items included Cov.(trait u, trait v, same tree)

$$= \sigma_{f^* \underline{u,v}} + \sigma_{m^* \underline{u,v}} + \sigma_{p, \underline{u,v}} + \sigma_{w, \underline{u,v}}$$

Cov.(u, indiv, v, plot)

$$= \sigma_{f^* \underline{u,v}} + \sigma_{m^* \underline{u,v}} + \sigma_{p, \underline{u,v}} + \sigma_{w, \underline{u,v}} / n$$

Cov.(u indiv. v FS)

$$= \sigma f^*_{uv} + \sigma m^*_{u,v} + p_{uv}/b + \sigma_{w,uv}/bn$$

Where  $\sigma_{uv}$  are the covariance components of traits  $u$  and  $v$ .

### C - matrix

The following additional items were needed.

$$\text{Cov}(y^u \text{ indiv, } g^v \text{ indiv.}) = \sigma_{A,uv}$$

$$\text{Cov}(y^u \text{ plot, } g^v \text{ indiv.}) = \sigma_{1/2 A,uv} (1 + 1/bn)$$

$$\text{Cov}(y^u \text{ FS, } g^v \text{ indiv.}) = 1/2 \sigma_{A,uv} (1 + 1/bn)$$

### a - vector

The relative economic values for oil yield (1) bunch number (0) and height increment (-1) were assigned.

The results are given in Table 1.

As shown, inclusion of other sources of information plot, family and correlated traits can improve greatly the efficiency of selection.

### Best Linear Unbiased Selection (BLUP)

In BLP and SI, the means, variances and covariance of the joint distribution of  $g$  and  $y$  are assumed known although in practice they are not and are estimated. Estimates of the fixed effects (block, trial etc) have been obtained by simple arithmetic coverages (ordinary least squares method prior to predicting the genetic values). The estimates of  $g$  are assumed to be both accurate and precise and treated as known constants throughout the whole process.

For many "messy" or very unbalanced data (dairy cattle, oil palm) ordinary least squares estimates of fixed effects are unsatisfactory. The BLUP procedure (Henderson 1984)

incorporates best linear unbiased estimates of the fixed effects through generalised least squares with best linear unbiased prediction of the random genetic effects.

Linear mixed model:  $y = X\beta + Z\mu + e$

where  $y = n \times 1$  vector of observed data records.

$\beta = t \times 1$  vectors of fixed effects due to blocks, environment, genetic groups.

$X = n \times t$  design or incidence matrix containing

0's and 1's that relate the fixed effects in

$\beta$  to the elements

$\mu = S \times 1$  vector of random genetic effects

$Z = r \times$  incidence matrix containing 0's and 1's that relate the random effects in  $u$  to the elements in  $y$ .

$e = n \times 1$  vector of random effects associated with components of experimental error as plot effects, within plot tree effects.

It can be shown:

$$g = C'V^{-1}(y - XB) - BLUP$$

$$\text{Where, } B = (X'V^{-1}X)^{-1}(X'V^{-1}y)$$

$$\text{Compare, } g = C'V^{-1}(y - ) - BLP$$

i.e. BLUP substituted known fixed effects, with estimated fixed effects  $XB$

An example illustrating this method in oil palm is given below (Soh 1992).

**BLUP Example:** Estimation of breeding values for AVROS and Dumpy AVROS *pisiferas* from three unbalanced DxP experiments. The data structure is given in Table 1.

The linear mixed model (Henderson, 1984):

$$y = X\beta + Qg + Z\mu + e$$

Where,  $y$  = vector of observed data records.

$\beta$  = vector of fixed effects for experiments.

$X$  = incidence matrix relating  $\beta$ 's to  $y$ 's

$g$  = vector of *pisifera* group fixed effects.

$Q$  = incidence matrix relations record to each *pisifera* effect.

$\mu$  = random genetic (breeding value) effects.

$Z$  = incidence matrix relating  $\mu$ 's to  $y$ 's.

$e$  = random error effects.

The mixed model equation:

$$\begin{pmatrix} X'X & X'Q & X'Z \\ Q'X & Q'Q & Q'Z \\ Z'X & Z'Q & Z'Z + A^{-1} \end{pmatrix} \begin{pmatrix} \beta \\ g \\ \mu \end{pmatrix} = \begin{pmatrix} X'y \\ Q'y \\ Z'y \end{pmatrix}$$

Where  $A$  = the additive relationship matrix among the *pisiferas* (Figure 4) computed from the pedigree diagram (Figure 3)

$$4 - h^2$$

$$\lambda = \sigma^2_e / \sigma^2_s = h^2 ; \sigma^2_s = \text{pisifera group variance}$$

$$h^2 = \text{heritability}$$

The solution:

$$\begin{pmatrix} \mathbf{b} \\ \mathbf{g} \\ \boldsymbol{\mu} \end{pmatrix} = \begin{pmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Q} & \mathbf{X}'\mathbf{Z} & -\mathbf{1} & \mathbf{X}'\mathbf{y} \\ \mathbf{Q}'\mathbf{X} & \mathbf{Q}'\mathbf{Q} & \mathbf{Q}'\mathbf{Z} & & \mathbf{Q}'\mathbf{y} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Q} & \mathbf{Z}'\mathbf{Z} + \mathbf{A}^{-1} & & \mathbf{Z}'\mathbf{y} \end{pmatrix}$$

The solutions and relative breeding values for height increment for the various *pisiferas* are illustrated in Table 3.

Although the correlation between tree and predicted breeding values ( $r_{\Gamma\Gamma}$ ) were low, (owing to the highly unbalanced data) the rankings are still valid.

BLUP can also incorporate multiple traits and relative economic values as in SI.

All these methods can be used for selecting parents or individuals from data obtained from intrapopulation or interpopulation crosses. If the genetic covariance between a trait in one intrapopulation cross and the same trait in the interpopulation cross (treating them as different traits) can be established, the breeding value of an individual in a interpopulation cross can be predicted from just the intrapopulation data. When both inter and intrapopulation cross data are available they can be combined to give an even better prediction (Moreno-gonzalez and Hallauer, 1982).

Similarly if the genetic covariance or correlation for the same trait of the same population planted in two different situations (e.g. locations, nursery and field evaluations for disease resistance) can be established, it is possible to predict the breeding values for the target environment (White and Hodge, 1991).

## Selection of Parents For Recombination Programme

As mentioned earlier, selecting parents for a recombination programme is a very important step in the recurrent breeding programme. One is usually faced with a big number of potential parents at this stage. How can one best combine the parents to give the maximum genetic progress at the shortest time while at the same ensuring maintenance of sufficient genetic variability for continuous selection, and maintenance of the size of the programme within manageable proportions. Meunier (1988) has suggested picking the best general combining parents mainly from the better combining families and also some from not so good combining families to prevent too rapid loss in genetic variability. Pairs of selected parents differing in genetic origins were crossed in a partial diallel manner.

The following more systematic approach is suggested.

### 1. Specify the ideal genotype

Specifying the ideal genotype which is the objective of the breeding programme expressed in a succinct manner, allows a more directed approach in the selection programme.

Firstly the ideal hybrid genotype is specified. The required ideal genotypes for the combining parents are specified (based on additive model). The ideal genotypes for the two parental genotypes should be complementary especially for traits which are highly negatively correlated, e.g. bunch number and bunch weight, mesocarp to fruit and kernel to fruit. It will be more expedient to select for high bunch number and mesocarp to fruit in one population and high bunch weight and kernel to fruit in the other and achieve the ideal combination in the hybrid rather than select for all four traits on both sides.

After specifying the ideal genotype of the recombination programme the desired gains index method (Yamada *et al.*, 1975; Sasaki *et al.*, 1985; Soh *et al.*, 1994) can assist in

the selection. Alternatively Grafius's (1965) vector analysis and Pederson's (1981) least square analyses may be used.

## 2. Choice of matings

The actual mating combinations to make in practice is a subjective mixture of matings for long term and short term gains. Matings for long term gains are to generate within cross variability and will entail matings of selected individuals from divergent genetic origins. This will need knowledge of the history of the materials, which may be reinforced by studying genetic polymorphism by RFLP or isozyme analyses (Ghesquiere and Meunier, 1986) while matings for short term gains will involve matings from selected individuals from high performing families with complementary and enhancing traits. Incomplete diallels or factorials are the likely mating designs, whether by design or default.

The latter matings will branch off into the WHI programme of progeny-testing, inbreeding and seed-production, while the former continues in the main breeding programme.

If adequate check crosses are included in the trials, it is possible to estimate the selection progress made, reassess the estimates of the genetic variances and covariances and plan another more refined round of recombination breeding.

## CONCLUDING REMARKS

Breeding progress culminates from the procedures of the choice of traits of interest, generation of genetic variability for them and selecting in the best way to maximize improvement or fixation of these desirable traits in the resultant cultivars. Although much of the talk dealt with the latter two procedures, critical choice of traits for improvement cannot be overemphasised for a perennial tree crop. Inclusion of too many traits will hinder selection progress for the important traits while wrong choice of desirable traits will result in much loss of valuable time and effort. This is particularly relevant as we move into the next

century where social and end-use technological developments and breakthroughs are expected to result in a more discriminating and competitive demand particularly on the quality characteristics of palm oil. Similarly new or novel traits will be available from current germplasm collections and from transgenic palms or genetic engineering technology. The challenge to the oil palm breeder facing these new market demands and new sources of genetic variability is to identify correctly the likely desirable traits in the future and to devise selection methods from the large amount of information available from current trials, to maximize genetic progress. It will be of advantage to the oil palm breeder to keep abreast with developments in animal and forest tree breeding which have much in common oil palm breeding besides interacting with the molecular biologist for marker assisted selection for quantitative trait loci (QTL). In all these, computer assisted selection is indispensable to handle the mass of data. This should not pose a problem as computers are becoming cheaper, more powerful and user-friendly and soft-ware packages for the various selection techniques are readily available. The breeder can then practise his art in the final choice of candidates short-listed by the computer.

Finally there are indeed exciting and challenging times ahead and breeders should draw up their breeding plans which will be able to exploit emergent materials and techniques to the fullest.

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TABLE 1  
Yield performance of Guthrie DXP planting material on inland soils in Malaysia

Treatment	Yield (kg/ha)			
	F1	F2	F3	F4
1	10	10	10	10
2	10	10	10	10
3	10	10	10	10
4	10	10	10	10

**Table 1: Estimated to coefficients, correlation between the index and the aggregate genetic worths [Corr (ww)] and selection gains ( $\Delta$  g) for the various traits in the hybrid progeny - test trial.**

Traits	Index Coefficients							$\Delta$ % g
	Relative economic value (a)	Indiv. Obsv.	Plot mean	Full sib mean	Half sib mean1	Half sib mean2	Corr (ww)	
y+	1	1						100.0
HINC+	-1	-1						
HINC+	-1	-0.4117	0.3271	-0.6284	-0.0748	-0.0748	0.77	110.6
Y	1	0.1023	-0.0645	0.6494				157.1
+BNo	0	0.2565	-0.0952	0.0285			0.77	
+HINC	-1	-1.3344	0.9749	-1.1226				68.2

+ = mass selection

Y = oil yield

HINC = height increment

BNo = bunch number

\* = expressed over  $\Delta$  g for mass-selection of the corresponding trait

**Table 2 : Summary of data structure (number of families in each *pisifera*, in each genetic group and in each trial) in the BLUP analysis.**

Trial	AVROS <i>Pisiferas</i>				Dumpy-AVROS <i>Pisiferas</i>					Total
	P1	P2	P3	P4	P5	P6	P7	P8	P9	
1		10		7						17
2	1	1	10	2						14
3		4			3	3	2	3	3	18
Total	1	15	10	9	3	3	2	3	3	49

**Table 3: Estimates of fixed effects and relative breeding values for the various *pisifera* (p) parents for height increment.**

Effect		Estimate/Breeding Value	$r_{TT}^+$
L1	(Trial)	5.48	
L2		4.24	
L3		5.58	
G1	(AVROS)	0.00	
G2	(Dy-AVROS grp)	-0.99	
P1		0.01	-0.10
P2	AVROS	-0.11	0.22
P3		-0.53	0.18
P4		0.15	0.23
P5		-1.84	0.20
P6	Dy-AVROS	-2.12	0.20
P7		-2.02	0.18
P8		-2.00	0.20
P9		-1.95	0.20

+  $r_{TT}$  = correlation between true and predicted breeding values

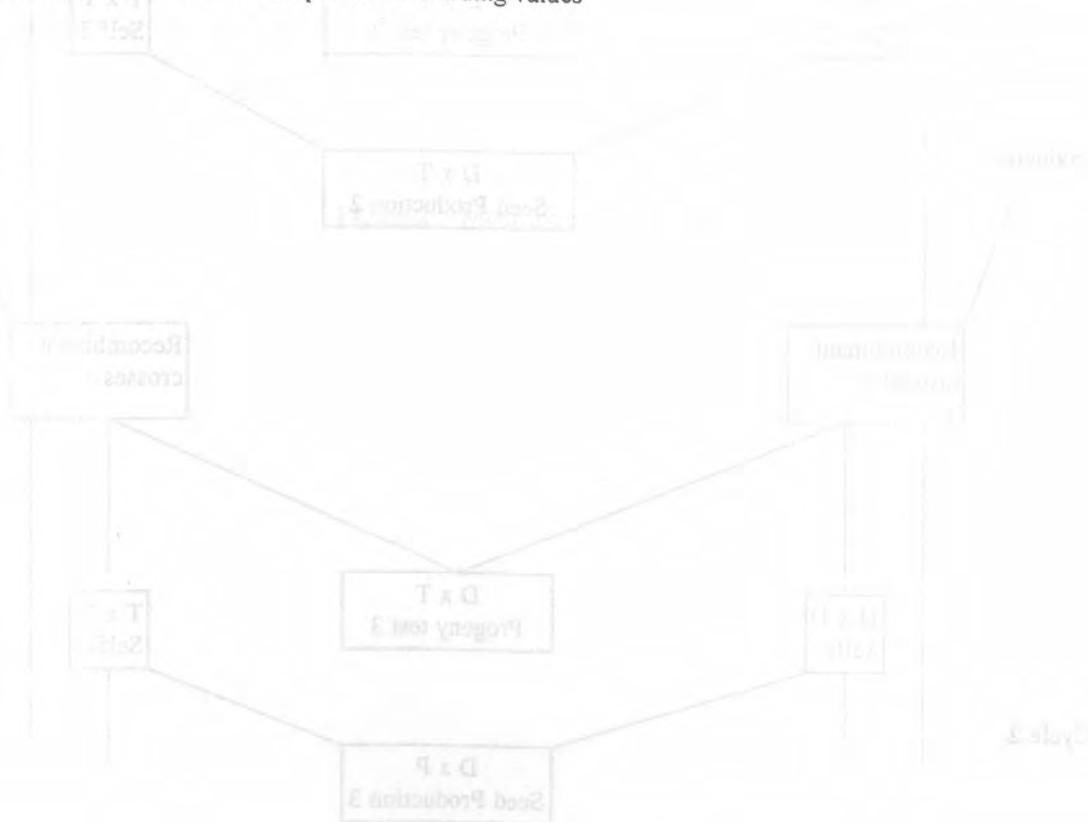


Figure 1. Annual Recurrent Selection in Oil Palm Breeding.

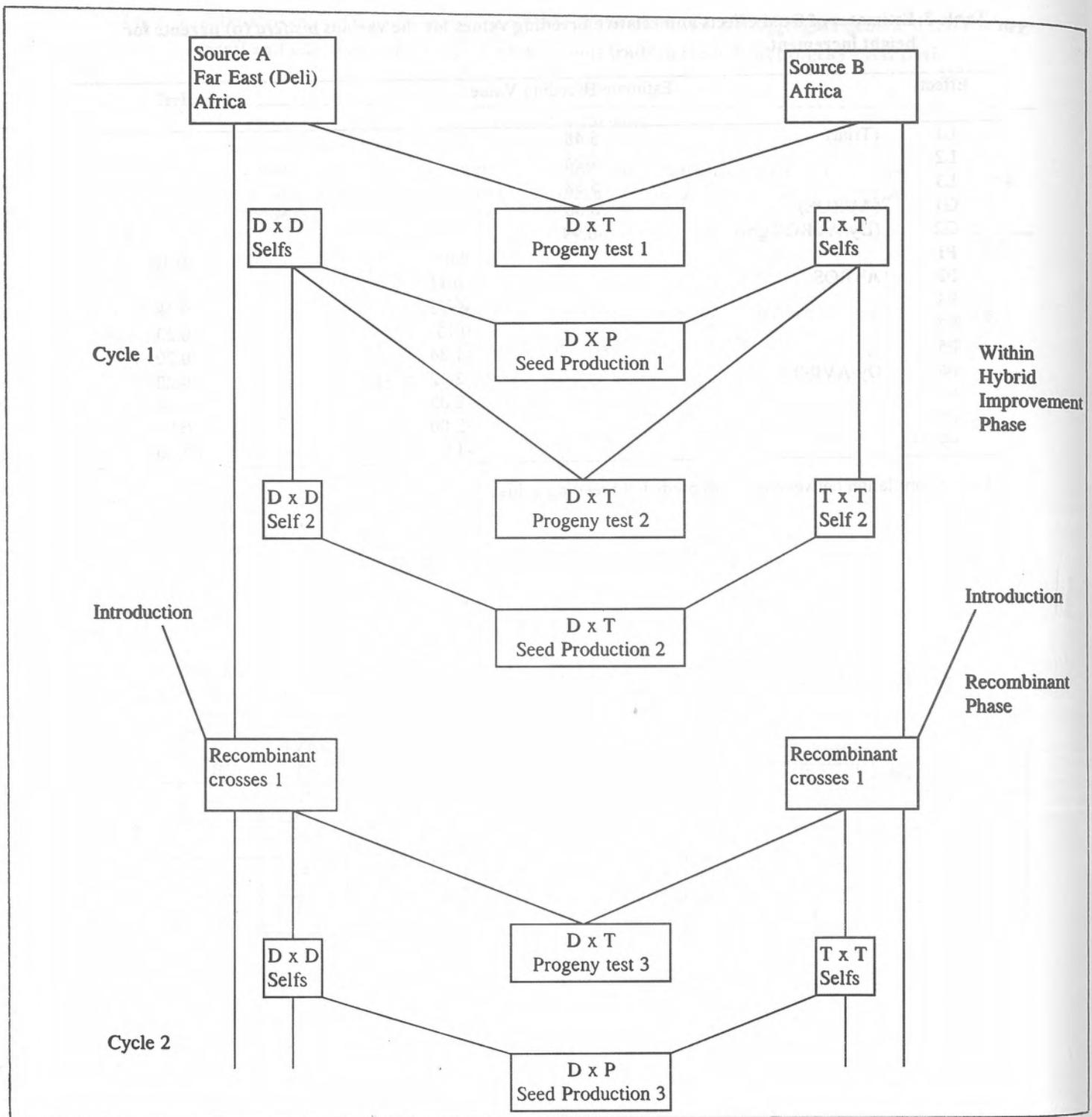


Figure 1 : Modified Recurrent Selection in Oil Palm Breeding.

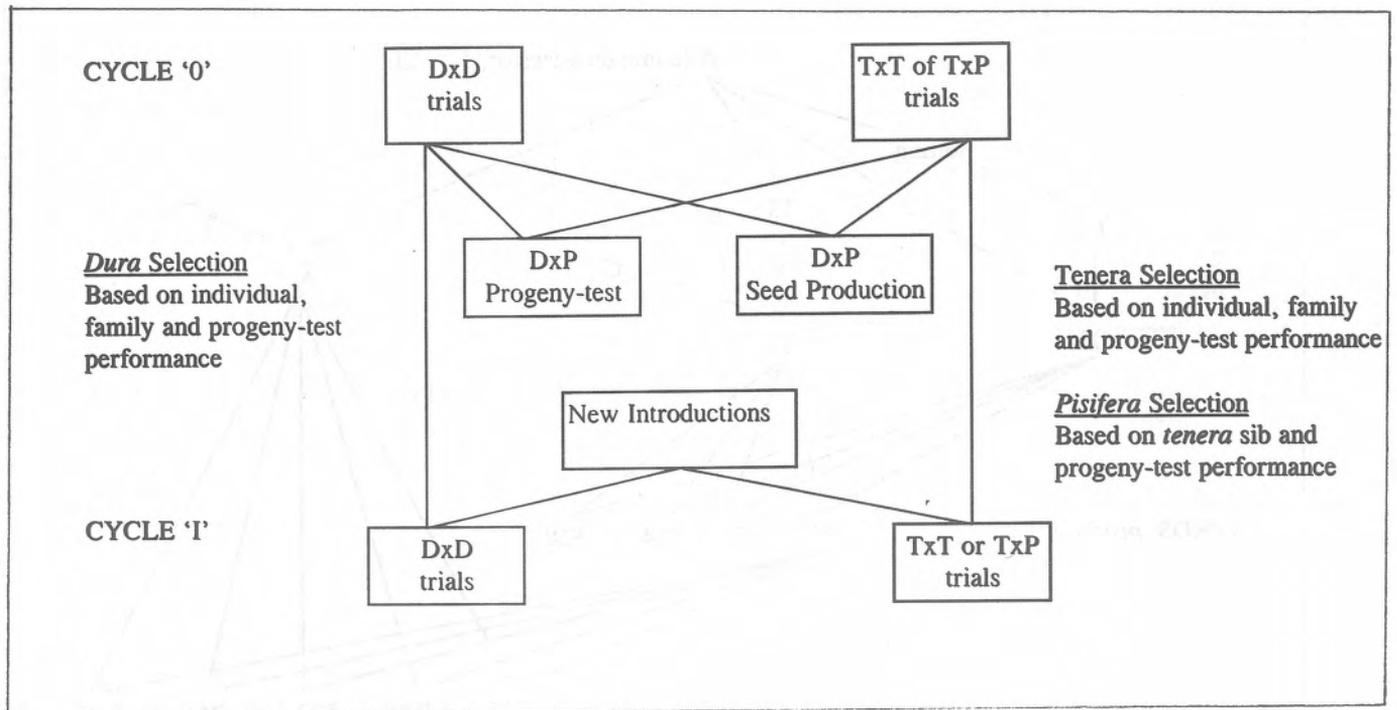


Figure 2 : Modified recurrent selection scheme

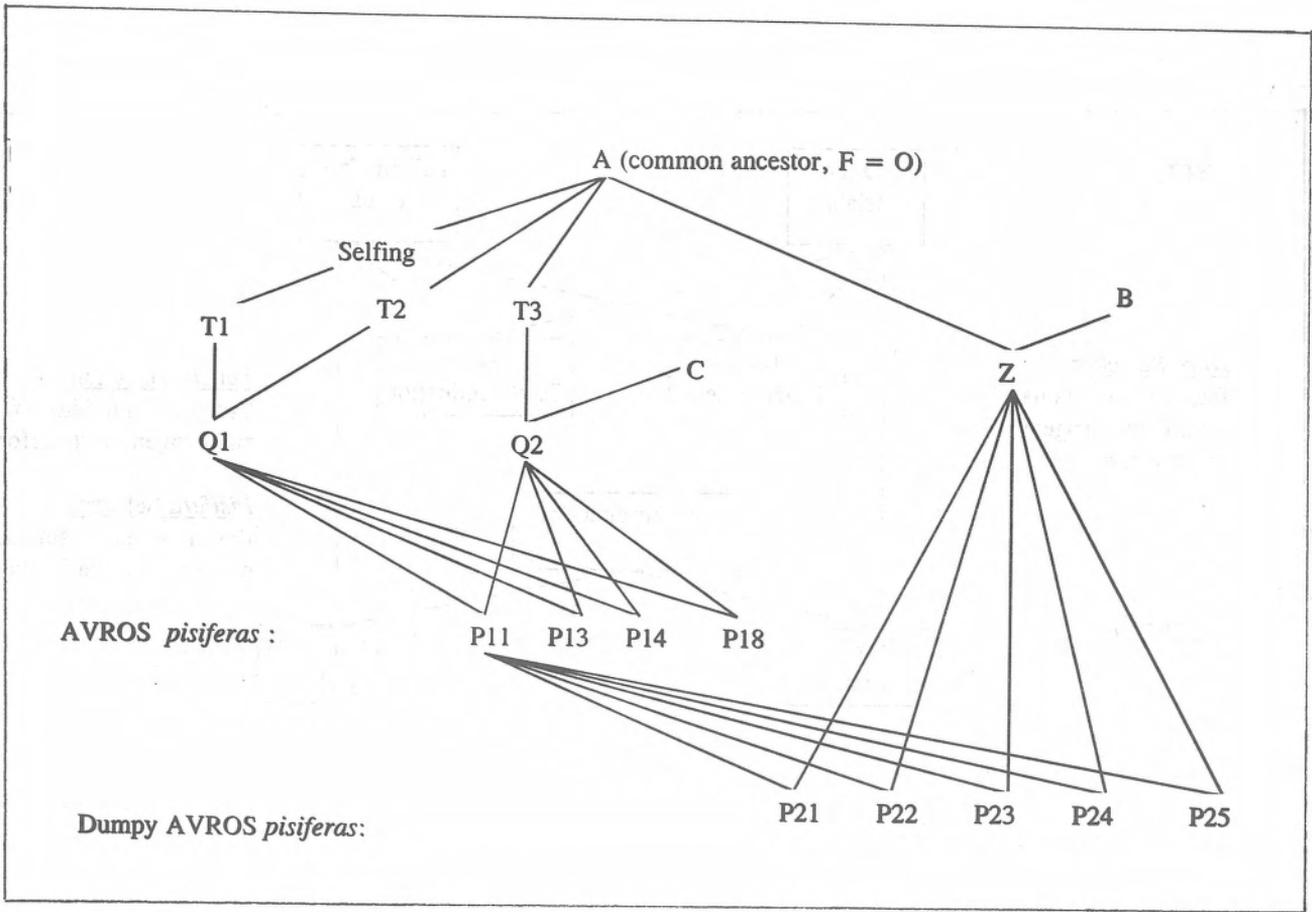


Figure 3: Pedigree diagram for AVROS and Dumpy-SVROS *pisifera* male parents. (Adapted from Lee and Yeow, 1985, and Soh *et al* 1981.)

1/16)	20	14	14	14	13	13	13	13	13
	14	20	14	14	13	13	13	13	13
	14	14	20	14	13	13	13	13	13
	14	14	14	20	13	13	13	13	13
	13	13	13	13	19	12	12	12	12
	13	13	13	13	12	19	12	12	12
	13	13	13	13	12	12	19	12	12
	13	13	13	13	12	12	12	19	12
	13	13	13	13	12	12	12	12	19

Figure 4: The additive relationship matrix (A) for AVROS and Dumpy-AVROS *pisiferas*.

## GENOTYPE ENVIRONMENT INTERACTION IN OIL PALM

Lee C.H.<sup>1</sup> and Rajanaidu N.<sup>2</sup>

### INTRODUCTION

Oil palm cultivars are usually planted over a range of environments with their yields varying according to the environment. Genotype-environment (GxE) interaction has long been known by plant breeders. It occurs when cultivars perform differently, relative to each other, in different environments.

GxE interaction is important in plant breeding. With it, it no longer suffices to test, for selection, genotypes in one environment. This interaction reduces the correlation between phenotypic and genotypic values, and reduces progress from selection (Comstock and Moll, 1963).

The plant breeder, therefore, has to develop cultivars which yields well over the range of environments. Yield stability becomes a desirable quality, and cultivars with it are said to be "well buffered". According to Allard and Bradshaw (1964), there are two kinds of buffering – "individual" and "population". "Individual buffering" is the property of a genotype and denotes its ability to produce an acceptable phenotype in different environments. "Population buffering" is the property of a population and derives from the possession, in its diversity, of several genotypes adapted to the range of environments.

There are several ways to study GxE interaction - analysis of variance, linear regression, principal component analysis, multivariate analysis, cluster analysis, ranking and other non-parametric tests. Linear regression is the most frequently used. According to Caligari (1991), this approach is favoured because of its inherent appeal – it can provide a visual picture and a summary overview. Yates and Cochran (1938) first proposed regression analysis for estimating G x E interaction, and this was modified later by Finlay and Wilkinson (1963). This was further refined by other workers, including Eberhart and Russell (1966) and Perkins and Jinks (1968). Lin *et al.* (1986) reviewed the methods used to identify stable genotypes in the presence of GxE interaction.

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In oil palm, the study of GxE interaction is recent. Nevertheless, several papers have been published in the last 10 years. This paper reviews the studies on GxE interaction on oil palm and discusses its impact on oil palm breeding.

## GXE STUDIES

### FFB Yield and Yield Components

Rosenquist (1982) compared D x D, T x D and D x P progenies at Dami, Papua New Guinea and Banting, Malaysia. After excluding progenies with exceptionally high and low frond magnesium levels, he found little evidence of G x E interaction for yield as both environments were conducive to yield. At Dami, magnesium deficiency was common but not at Banting, especially in the trials. Perhaps, G x E interaction could have been detected if the progenies with high/low frond magnesium levels had not been excluded. However, the fact that the author found no change in ranking of the palms did not necessarily mean no GxE interaction, as differential responses to environmental changes can also indicate GxE interaction.

In a trial evaluating D x P oil palm progenies from seven origins, planted on three locations, Rajanaidu *et al.* (1986) found that the origins (of progenies) performed consistently over the years and locations. Origin x location and origin x year interactions were non-significant for FFB yield and its components, indicating the absence of G x E interaction (Appendix 1). It should be noted that for each origin, the materials planted were mixtures of 24 - 30 D x P progenies which probably would have provided better buffering than individual progenies. In addition, the environments of the three locations (two on coastal clays, one on Rengam series soil) were probably not sufficiently different.

The studies mentioned so far did not detect GxE interactions in oil palm. However, significant GxE interactions were found in several other studies. The results are summarised and presented in Appendix 1.

Obisesan and Fatunla (1983) evaluated 49 D x P hybrid families over 14 years on one location at NIFOR. They treated years as environments as yearly differences in the environmental factors - rainfall, sunshine hours, number of dry days, temperature, humidity and heat units - were large enough to differentiate between the families. Their results showed significant G x E interactions for FFB yield, bunch number and bunch weight. Evaluating five *duras* and one D x P over nine years, Obisesan and Parimoo (1985) found significant G x year interaction for FFB yield and bunch number, but not for bunch weight. Rajanaidu *et al.* (1990), evaluating two trials of D x P hybrids (generated with the North Carolina Model 1 crossing design) on one location, found significant progeny x year interaction for FFB yield and its components.

In studying G x year interaction, it must be borne in mind that oil palm has age trends for bunch number and bunch weight. Bunch number decreases with age due to a lower frond production and reduction in the sex ratio. However, mean bunch weight increases until the product of the two levels out at "maturity" (Hartley, 1977). The age characteristics of oil palm are, therefore, confounded with changes in the yearly climate to contribute to the "effects" of years and genotype x year interactions.

In studying G x location interaction for FFB yield and its components, Ong *et al.* (1986) found significant effects in seven NIFOR and four Malaysian D x P hybrids on four locations. Chan *et al.* (1986) found similar results in two sets of trials. However, in three other sets, G x location interaction was not significant for FFB yield, although significance was detected for bunch number and bunch weight in two out of three sets of trials.

Rajanaidu *et al.* (1990) evaluated 34 D x P hybrids on five locations. A combined analysis of variance showed significant G x location interaction for FFB yield and its components. Data from individual locations were analysed to study G x year interaction on FFB yield. The interaction was non-significant except for one location where it was significant at the 5% probability level.

Rajanaidu *et al.* (1991) reported on two sets of trials. In the first, 33 D x P progenies were evaluated on six locations (the same study reported by Rajanaidu *et al.*, 1990, with an extra location) and, in the second, 50 open-pollinated families (from the Nigerian prospected materials) on three locations. Significant G x location interaction was detected only for FFB yield in the first set of trials; in the second, G x location interaction was significant only for FFB yield and bunch number, but not for bunch weight. Oil and kernel yields were also studied in Set 1, but the G x location interaction was not significant.

They also investigated genotype x year interaction for FFB yield in Set 1 for each location separately, and found this generally absent except on one location. In Set 2, G x year interaction was studied on one location, and found non-significant for FFB yield and bunch number but significant for bunch weight.

Corley *et al.* (1991) also studied inter-origin trials using data from Lobe (Cameroons), Binga (Zaire) and Jenderata Estate (Malaysia). Correlations of yields from the three locations suggested that G x E interaction was important. The correlation of yield between Binga and Lobe was improved by excluding crosses susceptible to vascular wilt and progenies with the heaviest bunches. In the case of Lobe and Jenderata, the correlation was improved by excluding progenies with a bunch weight/number ratio of <1.0 and those with below average leaf areas. The correlations between locations for bunch number and bunch weight were high, suggesting little G x E interaction.

Combined analyses of variance over locations and years were also studied and reported. Lee *et al.* (1988) evaluated 23 D x P hybrids from an incomplete factorial crossing design on two locations over four years. Hybrid x location, hybrid x year and hybrid x year x location interactions were highly significant for FFB yield and its components. In producing the D x P hybrids for this study, the parents used were 10 Deli *dura* palms (female) crossed with three *pisiferas*, of which two were derived from Yangambi and one from NIFOR. In comparing the different D x P hybrid groups by *pisifera* source, two of the three groups did not show significant interaction with location for FFB yield. This implies that, averaged over the four years, the two groups were consistent in performance at both locations. Interactions of the three groups with year, and with year x location for FFB yield were significant.

In a combined analysis over locations and years, Yong and Chan (1990) found significant hybrid x location interaction for FFB yield and mean bunch weight, but not bunch number, from 12 D x P hybrids planted on two locations and recorded for 5 years. Hybrid x year was non-significant for FFB yield but significant for bunch number and bunch weight.

The studies on G x E interaction in oil palm have been mainly done on D x P hybrids with only a few studies on other oil palms. As oil palm can be propagated vegetatively by tissue culture, there is great interest in multi-locational evaluation of clones. Lee and Donough (1991) reported on two series of trials with several oil palm clones from selected/unselected ortets. In the first series, five clones and three D x P hybrids were evaluated on three locations for six years. There was no significant G x E interaction for the hybrids for FFB yield. However, highly significant clone x location, clone x year and clone x year x locations interactions were found for FFB yield and its components. In the second series of trials, six clones were evaluated at two planting densities and on three locations over six years. Significant clone x location, clone x year, clone x year x location and clone x density x location interactions were observed for FFB yield and, generally, for its components as well. Clone x density interaction was not significant in this study.

A study on interspecific hybrids (*E. oleifera* x *E. guineensis*) was also done Yong *et al.* (1991). Fifty two interspecific hybrids on five locations were analysed, showing G x location interaction to be highly significant for FFB yield, bunch number and bunch weight. This study was the first report of a large scale multi-locational evaluation of interspecific hybrids through the joint efforts of several plantation companies. Unless it is economically viable to plant interspecific hybrids commercially, it is unlikely that future oil palm breeding programmes will involve any multi-locational evaluation of these materials.

## Variance Components for FFB Yield and Yield Components

The variance components for FFB yield, bunch number and bunch weight were computed in several papers. The proportion of G x E to total genetic variation reflects its importance to the materials evaluated. Rajanaidu *et al.* (1989), after detecting significant progeny x year interaction for FFB yield and its components, obtained a low level of GxE interaction in one trial (1% - 5%) and a somewhat higher level in another (8% - 16%) (Table 1). The contribution of G x E interaction to total genetic variation was also low in two other studies evaluating oil palm on several locations (Rajanaidu *et al.*, 1990 and Rajanaidu *et al.*, 1991).

**Table 1. GxE interaction (%) for FFB yield, bunch number and bunch weight (estimated from variance components).**

Source	Interaction	FFB Yield	Bunch No.	Bunch wt.
Rajanaidu <i>et al.</i> (1989)	GxY	0.9	1.6	5.2
	GxY	15.8	11.4	8.3
Rajanaidu <i>et al.</i> (1990)	GxL	2.35	4.48	2.01
Rajanaidu <i>et al.</i> (1991)	GxL	2	3	4
	GxL	4	2	1

The results of Yong and Chan (1990) showed that GxE interaction variances are low relative to the other variances for FFB yield and bunch number, but relatively high for bunch weight (Table 2). For FFB yield, the G x location interaction is more important than G x year interaction.

**Table 2. Variance Components for FFB Yield and its Components**

Component	FFB Yield	Bunch Number	Bunch Weight
$\sigma^2G$	95.06	1.57	0.08
$\sigma^2GY$	11.68	0.47	0.06
$\sigma^2GL$	29.07	0.12	0.05
$\sigma^2GLY$	4.05	0.11	0.06
$\sigma^2$	260.76	3.02	0.30

(Yong and Chan, 1990)

Evaluating clones, Lee and Donough (1990) found that G x E interaction variances contributed much to the total genetic variances for FFB yield, bunch number and bunch weight (Table 3). In FFB yield, clone x year x location was the largest interaction component. Clone x year x location

and clone x year contributed the major proportion of the G x E interaction for bunch number and bunch weight.

**Table 3. Estimates of Variance Components for FFB Yield and its Components in Oil Palm Clones**

Component	FFB Yield	Bunch Number	Bunch Weight
S <sup>2</sup> C	6.115	12.484	3.282
S <sup>2</sup> CD	0.050	0.061	0.004
S <sup>2</sup> CL	1.793	0.719	0.000
S <sup>2</sup> CY	2.263	6.649	1.472
S <sup>2</sup> CDL	1.508	0.277	0.005
S <sup>2</sup> CYL	14.396	9.804	0.957
S <sup>2</sup> CYD	0.014	0.072	0.063
S <sup>2</sup> CYDL	0.438	0.034	0.000
O <sup>2</sup> E	5.952	2.747	0.645

S<sup>2</sup>C = variance component due to clones, O<sup>2</sup>E = error variance  
(Lee and Donough, 1991)

### Bunch and Fruit Characters

Yield is the main interest in most GxE studies in oil palm, and few studies have been made on other characters. Nevertheless, several authors have provided data on bunch and fruit characters. The data are summarised in Table 4 for bunch and fruit components. There were varying degrees of GxE interaction.

**Table 4. GxE Studies on Bunch and Fruit Characters**

Source	Interaction	Significance of GxE Interactions							
		F/B	M/F	K/F	S/F	O/WM	O/DM	O/B	K/B
Ong <i>et al.</i> (1986)	G x L	ns	*	**	ns	-	-	***	-
Lee <i>et al.</i> (1988)	G x L	**	*	**	*	*	ns	**	-
Rajanaidu <i>et al.</i> (1991)	G x L	**	**	ns	**	ns	ns	ns	ns
Lee & Donough (1991)	DP x L	ns	ns	ns	-	ns	-	ns	-
	C x L	**	ns	ns	-	ns	-	ns	-
	C x L	**	**	***	-	*	-	*	-
	C x D	ns	ns	ns	-	ns	-	ns	-
	C x D x L	ns	ns	ns	-	ns	-	ns	-

G x L = Genotype x Location  
C x L = Clone x Location

DP x L = DxP Hybrids x Location  
C x D = Clone x Density

Studying oil palm progenies, Ong *et al.* (1986) and Lee *et al.* (1988) found significant G x location interaction for oil/bunch ratio, while Rajanaidu *et al.* (1991) and Lee and Donough (1991) did not find any. With clones, Lee and Donough (1991) found no clone x location interaction for oil/bunch ratio in one set of trials, but a significant level in another. For clone x density and clone x density x location, no significance was detected for oil/bunch ratio.

### Vegetative Characters

There are few studies on GxE interaction on vegetative characters in oil palm. What little data available on some of the main vegetative characters are summarised in Table 5. Significant G x location interactions were reported for frond production, frond length, petiole cross-section area, leaf area and trunk height by Rajanaidu *et al.* (1983), Ong *et al.* (1986) and Lee *et al.* (1988).

**Table 5. GxE Studies on Some Vegetative Characters in Oil Palm. Significance of GxE Interaction.**

Source	Interaction	Frond Production	Frond Length	Petiole Cross-section	Leaf Area	Trunk Height
Rajanaidu <i>et al.</i> (1983)	G x L	**	**	**	**	-
Ong <i>et al.</i> (1986)	G x L	-	-	***	**	***
Lee <i>et al.</i> (1988)	G x L	-	**	**	*	**

Rajanaidu *et al.* (1983) included other vegetative characters - rachis length, leaflet number/length/width petiole length/width/depth and total leaf area - in their study. They were highly significant for G x location interaction except for petiole depth and leaflet width. Variance components of the vegetative characters were also reported by Rajanaidu *et al.* (1983) and GxE (location) interaction accounted for 0.29% - 3.57% of the total variation, which is relatively small.

### Yield Stability

Analysis of variance only provides information on the presence and magnitude of GxE interaction. It is still necessary to identify the stable genotypes. There are several ways to do this.

Obisesan and Fatunla (1983), Obisesan and Parimoo (1985), Ong *et al.* (1986), Lee *et al.* (1988), Yong and Chan (1990), Rajanaidu *et al.* (1990) used stability parameters proposed by Eberhart and Russell (1966). In some of the studies, each year within a location was considered an environment. The stability parameters are the linear regression (b) of the genotype mean on the mean of all the genotypes in each environment, and the mean square deviation from regression for each genotype (S d). The linear regression, b, measures the linear response to environmental

changes, and the mean square deviation from regression,  $s_d$ , the stability or consistency of the response. According to Eberhart and Russell, a desirable cultivar would have high yield, unity regression coefficient ( $b = 1$ ) and a low deviation from regression ( $s_d = 0$ ). Stable and high yielding DxP progenies were identified using these criteria.

The genotype-grouping technique, proposed by Francis and Kannenberg (1978), was used by Lee *et al.* (1988) and Lee and Donough (1991) to identify high yielding and stable genotypes of oil palm. The method subdivides genotypes into four groups based on their yields and coefficients of variation (CV). The four groups are:

- High mean yield and low CV - Group I
- High mean yield and high CV - Group II
- Low mean yield and low CV - Group III
- Low mean yield and high CV - Group IV

Group I genotypes are desirable as they have high mean yields and consistent performances in different environments, or low CVs. This technique is attractive for classifying genotypes for yield stability as it is simple and does not need laborious calculations. Furthermore, the position of a selected high yielding and stable genotype relative to the others is easily visualized. However, statisticians object to using the CV as it is calculated from the mean. To overcome this objection, the standard deviation can be used instead. Yong *et al.* (1991) used this approach to classify *Elaeis oleifera* x *E. guineensis* hybrids in their study.

### **Other GxE Studies**

GxE studies usually involve multi-locational evaluation of genotypes over several years. According to Comstock and Moll (1963), the environment is defined as “all the things, other than genotype of the plant, that affect its development”. As such, GxE studies should extend beyond evaluation of genotypes over locations and years. There are few publications with this approach.

In a study where palms were severely pruned to simulate high density planting, Corley (1976) reported significant progeny x pruning interaction for FFB yield. As the effects of pruning are somewhat similar to high density planting, progeny x pruning interaction is likely to be similar to progeny x density interaction.

The performances of seven oil palm clones in a systematic fan design density trial was reported by Corley and Donough (1990). They found significant differences in optimal density between clones from four years' yield data. However, in the study by Lee and Donough (1991) reported earlier, clone x density interaction was not significant in six of the same clones. Similarly, Rao *et al.* (1990) reported the absence of progeny x density interaction effects for FFB yield in their study on open-pollinated oil palm progenies.

## DISCUSSION

In the past, there was little interest in GxE interaction in oil palm as it was assumed to be unimportant. It is only recently that studies on it are gaining importance in oil palm breeding. The results have so far not been consistent. This is to be expected as different planting materials were used, and the locations and years also differed. With only a few locations not sufficiently contrasting in their environments, it would have been difficult to elicit differential responses from the materials evaluated. In addition, it has been shown by Lee *et al.* (1988) and Lee and Donough (1991) that some planting materials perform consistently on different locations. These may have been some of the reasons why GxE interaction was not detected in the early studies.

Studies in the last 10 years have shown the presence of GxE interaction. This means that the oil palm breeder cannot conclude from the results of a single trial on one location and year as the they would have been biased upward by the GxE interaction. It is, therefore, necessary to evaluate progenies/clones on several locations over several years. With a large GxE interaction, Sprague and Federer (1951) recommended that there be less replications in favour of more locations and years.

GxE interaction, if present, is likely to influence all stages of the breeding programme - from DxD, TxT or TxP to DxP progenies. Ideally, programmes should be devised to take into account G x E interaction at every stage. However, this will inflate research costs tremendously with additional burdens on the time, effort and resources of the breeder. A sound and practical approach generally practised is to ignore the interaction at the early stages of breeding. Only at the final stage is progeny testing/clone evaluation done on different locations over several years. Only if it is shown that G x E interaction affects the effectiveness of early selection should breeders revise their programmes to include thorough early testing and evaluation in target environments. An example is the selection for disease resistance or tolerance.

The plant breeder should decide whether to go for a genotype that does well over a range of environments, or one adapted to a specific environment. The first choice is favoured by a small GxE interaction, and the second by a large one. With the current practice of seed production - *dura* mother palms selected phenotypically are crossed with *pisiferas* progeny-tested for good general combining ability - it is not practical to produce these selected DxP hybrids. However, the parental palms of D x P hybrids with broad adaptability or specific adaptability can be propagated by tissue culture for the subsequent mass production of clonal seeds.

Lee and Donough (1991), evaluating seven oil palm clones, found that Clone 54A outyielded DxP hybrids in FFB by an average 14.1% on both coastal and inland soils. It was high yielding and stable over the environments tested. This is a case of "individual buffering" as defined by Allard and Bradshaw (1964). Clone 90A was higher yielding than DxP hybrids on coastal soils

but lower yielding on inland soils. It is therefore an example of specific adaptation to a target environment. As the GxE interaction was large relative to the total genetic variation for FFB yield (Lee and Donough, 1991), clonal evaluation should cover more target environments, if possible. Breeders can then select for specific adaptability which can be important for clones, as well as broad adaptability. If progeny testing is done on different locations, it would allow breeders to select ortets and eventually produce clones for planting in specific environments.

Stability in yield has always been the main emphasis of oil palm breeders. This is understandable as data on FFB yield and yield components are readily available. The other important component contributing to the final oil yield - oil/bunch ratio – also deserves attention. However, not much attention has been given to although what little data available suggest that GxE interaction occurs for it in some of the trials. If GxE interaction is important in oil/bunch ratio, then it should be considered in breeding strategies as for FFB yield. Studies to-date are restricted to G x location interaction because of the difficulty in getting oil/bunch data. Other interactions which may be even more important than G x location interaction, and for which no data is available, are G x year and, possibly, G x seasons. Bunch analysis is expensive and laborious, and a laboratory can only handle a few bunches a day. Perhaps an easier, cheaper and faster method can be developed for bunch analysis.

Studies of GxE interaction involve the use of statistics and analytical methods to detect and quantify it. However, there is little understanding of its biological basis. Expression of the economic trait of a plant is the result of a series of physiological activities in its life.

GxE interaction occurs if some of the physiological activities respond differently to changes in the environment. Studies should be designed to study the physiological activities affected, perhaps even to the molecular and cellular level. A change in the environment constitutes a stress to the plant which then responds according to its genetic make-up. To begin understanding the biological basis for GxE interaction, studies on the responses of different genotypes to different stresses should be done. The stress on oil palm depends on its location, climatic variations and management inputs, for example, water, nutrient and light stresses, and pests and diseases. Studies by Corley (1976), Corley and Donough (1990), Rao *et al.* (1990), and Lee and Donough (1991) on G x pruning and G x density interactions are steps in the direction. Plant breeders, agronomists and physiologists should work together to examine some of the GxE interactions important for oil palm, e.g. G x water stress and G x nutrient stress. With this approach, breeders will be able to improve their testing and selection strategies and limit evaluation to specific key locations.

## CONCLUSIONS

GxE interaction is gaining importance in oil palm breeding. This is more so with the prospects of planting clones and clonal seeds commercially in the future. Plant breeders generally prefer developing planting materials with broad adaptability to yield well over a range of environments. Nevertheless, materials for specific environments should also be developed, especially for studying and understanding the biological basis for G x E interaction. This will enable breeders to set more precise breeding objectives and develop better testing and selection strategies to increase genetic gain from selection.

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Appendix 1. Results of GxE Studies on FFB Yield and Its Yield Components in Oil Palm

Source	Genotypes	Location	Years	Interaction	Significance of GxE Interactions		
					FFB Yield	Bunch No.	Bunch Wt
Obisesan and Fatunla (1983)	49	1	14	G x Y	**	**	**
Obisesan and Parimoo (1985)	1+5(D)	1	9	G x Y	**	**	Ns
Rajanaidu <i>et al.</i> (1986)	7	3	3	G x L	ns	ns	ns
				G x Y	ns	ns	ns
Ong <i>et al.</i> (1986)	11	4	(5)	G x L	***	***	***
Chan <i>et al.</i> (1986)	21	2	(4)	G x L	ns	***	***
	30	2	(4)	G x L	ns	*	*
	23	2	(4)	G x L	**	***	***
	14 (DxD)	2	(5)	G x L	ns	ns	ns
Lee <i>et al.</i> (1988)	11	4	(5)	G x L	***	***	***
	23	2	4	G x L	**	**	**
				G x Y	**	**	**
				G x Y x L	**	**	**
Rajanaidu <i>et al.</i> (1989)	3-4 + x 13 o	1	4	G x Y	**	**	**
	3-4 + x 14 o	1	4	G x Y	**	**	**
Rajanaidu <i>et al.</i> (1990)	34	5	(4)	G x L	**	**	**
	53	(1)	5	G x Y	ns	-	-
	52	(1)	5	G x Y	ns	-	-
	53	(1)	4	G x Y	**	-	-
	43	(1)	4	G x Y	ns	-	-
	47	(1)	4	G x Y	ns	-	-
Yong and Chan (1990)	12	2	5	G x L	**	Ns	**
				G x L	ns	**	**
				G x Y x L	ns	ns	*

Source	Genotypes	Location	Years	Interaction	Significance of GxE Interactions			
					FFB Yield	Bunch No.	Bunch Wt	
Rajanaidu <i>et al.</i> (1991)	33	6	(4)	G x L	**	**	**	
	53	(1)	5	G x Y	ns	-	-	
	52	(1)	5	G x Y	ns	-	-	
	53	(1)	4	G x Y	*	-	-	
	43	(1)	4	G x Y	ns	-	-	
	47	(1)	4	G x Y	ns	-	-	
	50 (OP)	3	(4)	G x L	**	*	ns	
	Lee and Donough (1991)	3 + 5 (C)	(1)	4	G x Y	ns	ns	**
			3	6	G x L	**	**	**
					(DP x L)	ns	*	*
				(C x L)	**	**	*	
				G x L	**	**	**	
				(DP x Y)	ns	ns	**	
Yong <i>et al.</i> (1991)	6 (C)	3	6	(C x Y)	**	**	**	
				G x Y x L	**	**	**	
				(DP x Y x L)	ns	ns	**	
				(C x Y x L)	**	**	**	
				C x L	**	**	ns	
				C x Y	**	**	**	
	52 (E.o x E.g)	5	(6)	C x D	ns	ns	ns	
				C x Y x L	**	**	**	
				C x Y x D	ns	ns	**	
				C x D x L	**	*	ns	
				C x Y x D x L	ns	ns	ns	
				G x L	***	***	***	

NB: Genotypes refer to DxP hybrids unless stated otherwise (DxD = dura x dura, OP = open-pollinated, C = clones, E.o x E.g. = interspecific hybrid)  
 (6) indicates years or recording and analysis was based on mean over years  
 DP x L = DxP hybrids x location interaction, CxD = Clone x density interaction

**PRODUCTION OF IMPROVED OIL PALM  
(*ELAEIS GUINEENSIS* JACQ.) PLANTING MATERIALS  
FROM SEED AND CLONAL METHODS**

Jacquemard J.C.<sup>1</sup> and Durand-Gasselín T.<sup>1</sup>

**ABSTRACT**

*After a brief history of the production of improved planting materials and accounts of the most commonly used techniques in oil palm breeding, the principles and practical details of three production methods are given: seed production, biclonal seed production and clone production. Their outputs are analyzed by the following criteria - investment, reproduction security, response time and actual improvement obtained. Cloning to produce planting materials is the most effective in both production and economic terms.*

**INTRODUCTION**

Over the past 50 years or so, oil palm has become one of the world's major sources of vegetable fats and oils. The extraordinary increase in its share of the world oils and fats production (from 4% in 1958/62 to 17% (forecast) for 1998/2002, that is, by almost 13 million tonnes (Chone, 1989) stems from remarkable gains in its productivity. Productivity has improved 15% every 8-10 year breeding cycle (Soh *et al.*, 1989) and, since 1960, growers have grown improved planting materials that have effectively guaranteed profits.

Two main breeding strategies are used. They are similar and involve recurrent selection (Soh, 1990). Reciprocal Recurrent Selection (RRS) is used by NIFOR, IRHO-CIRAD and its partners in Africa (IDEFOR/DPO, POBE Oil Palm Research Station and IRA LA DIBAMBA) and Southeast Asia (SOCFINDO and MARIHAT), and Family/Individual Selection (FIPS) in Malaysia (PORIM and its partners) and Papua New Guinea (OPRS) (Rosenquist, 1989).

RRS exploits the heterosis in D x P hybrids resulting from crossing Deli *duras* with *pisiferas* of African origin (Benard and Malingraux, 1965). FIPS also crosses D x P to exploit the good phenotypic traits in the parental palms, and their general combining abilities and those of their families (Lee and Yeow, 1965; Hartley, 1967; Breure *et al.*, 1982).

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## PRODUCTION

The early 1970s saw the first use of vegetative propagation by *in vitro* culture of oil palm (Jones, 1974; Lioret and Ollagnier, 1981). With the encouraging development, studies were done to assess the gains in time and productivity that were achievable this way.

Given the knowledge (in the broad sense) of character heritability at the time, several strategies were used to test the clones:

- Where heritability was low, clones were chosen a posteriori (Soh, 1986; Soh *et al.*, 1989). Corley (1981) suggested the criteria necessary for effective selection of clones - ortets have to be selected on their most heritable characters.
- Meunier *et al.* (1988) obtained relatively high estimates of yield heritability from comparative trials. So cloning the best palms from the best crosses in progeny tests, as proposed by Noiret *et al.* (1985), is the best strategy. The results obtained recently have confirmed this (Baudouin and Durand-Gasselín, 1991).

## BREEDING SCHEMES AND VARIETAL OUTPUT

### General Principles

As seen above, the breeding strategies, by whatever name, all involve reciprocal recurrent selection (Fig. 1). In effect, two populations are kept separate and successive cycles of selection is carried out (Meunier and Gascon, 1972; Soh, 1990).

Both methods need estimates of the combining abilities of parents and families from progeny tests. Given the perennial nature of oil palm, parental palms are grown for a long time and can be used permanently for commercial seed production and breeding.

The basic difference between the two strategies lies in whether all the crosses are planted. Taking the example by Rosenquist (1989), and including the parental selfs necessary for commercial production, the following is necessary for 35 crosses planted:

- in RRS, two groups, five parents/group, 25 crosses (5 x 5) and 10 selfs.
- in FIPS, also two groups, seven parents/group, 21 crosses (7 x 3) and 14 selfs. 49 crosses are assessed, including 28 virtually.

### Commercial Production

Once the best crosses are identified, there are several ways to reproduce them. Two techniques can be used for seed production and cloning. The first, which has been used for some time, is generally well mastered, whereas the second, which is still being developed, has given encouraging results (Le Guen *et al.*, 1991). Figure 2 shows the most commonly used methods.

### **Conventional seeds**

A good A x B cross can be reproduced exactly by crossing palms obtained by selfing A with those from selfing B (Jacquemard, *et al.*, 1981). This way, it is possible to reproduce a progeny with benefits from both general combining ability (GCA) and specific combining ability (SCA). Most breeders agree that GCA is more important for the oil characters (Rosenquist, 1989; Soh, 1990). However, SCA cannot be overlooked.

There are variations to the methods - crossing can be done between groups instead of selfing (Fig. 3). For example, it is possible to use two crosses at once (A1 x B1) x (A2 x B2). This is often done if the B parent is a *pisifera*.

### **Clonal Seeds**

As above, the aim is to reproduce an actual or theoretical progeny from seed. Rather than crossing parental selfs with each other, their clones from *in vitro* culture - are used. This method is extremely useful if the group B parent is a *pisifera*, but needs selfing if it is a *tenera* (Fig. 4). Either biclonal (clone x clone) or semiclonal (clone x self) crosses can be made.

### **Cloning**

Figure 5 gives a general diagramme for cloning. The differences between crossing by cloning and cloning of elite individuals are described below:

- Cross reproduction by cloning  
The cross, is repeated until there is a sufficient number of zygotic embryos or seedlings for cloning. The clones obtained are representative of the cross. This is that first stage of mass production. Generally, the clones are then compared with each other in a trial, and only the best distributed (Fig. 5).
- Cloning elite individuals  
Cloning for high yield is only possible with palms that have been individually yield recorded.

The theory (discussed below) requires 20% of the palms to be cloned. Initially, only clones from the best 5% of ortets are distributed. The clones are then compared and only the best distributed.

## **ADVANTAGES AND LIMITATIONS OF THE DIFFERENT PRODUCTION METHODS**

The methods for producing commercial planting materials can be assessed by certain criteria, the most important of which are:

- expected genetic progress and time management
- investment required

- risks incurred
- operational flexibility

### **Expected Genetic Progress and Time Management**

It is reasonable to expect the best crosses to yield 15% more than the current planting materials. But how long would it take for the improvement to permeate through into commercial planting materials? Faster progress is possible with cloning. The data used were deduced from broad heritability values calculated from clonal trials (Baudouin and Durand-Gasselín, 1991) and estimates of expected progress (Meunier *et al.*, 1988; Soh, 1990). Tables 1 and 2 summarize and compare the different strategies.

#### **Conventional seeds**

To reproduce crosses from seed, it is possible to prepare the parental selfs at the time the trial is planted. All the selfs can be planted, but it may be wiser to wait until the trial is 5 - 6 years old in order to limit observation to the most promising parents and plant only half the selfs.

As soon as the trial is complete (10 years after planting), the *dura* selfs can be used for seed production. But it takes another 3 - 4 years before the *pisiferas* can be used. The fairly common occurrence of *tenera* abortion means that *pisiferas* must be identified by their lack of shell around the kernels in their fruits. It is also necessary to wait for pollen production which does not occur much in young palms.

The genetic progress obtained will be:

- 11 years after planting the trial, the benefit from *dura* exploitation is realized:  $15\% \times 0.5 = 7.5\%$ .
- 15 years after planting the trial, the benefits from *dura* and *pisifera* exploitation are realized: 15%.

#### **Clonal seeds**

When a cross is to be reproduced by cloning the parents, this can be done out as soon as the trial is planted. It takes about 6 years to obtain clones from 85% of the parents (commercial production is not the issue here). Once the trial is complete, the *dura* clones will already be bearing and, 3 or 4 years later, pollen will be available from the *pisifera* clones. This is also about the time when it is possible to obtain pollen from the *pisifera* produced by selfing *tenera* parents, to produce semiclinal seeds.

Genetic progress is exactly the same as with conventional seeds.

### **Gross reproduction by cloning**

Gross reproduction by cloning zygotic embryos or seedlings is somewhat cumbersome. In view of the success rate for cloning, around 80 embryos or palms have to be cultured. Under these conditions, it is best to delay cloning until the merits of the cross have been confirmed in the field.

As soon as the trial is complete and embryos or young palms available, samples are taken. It is easier to clone young palms than adult ones; hence within 4 to 5 years, a sample of clones representative of the cross can be obtained.

Their fidelity (particularly in floral morphogenesis) has to be ascertained in the field (for 2 to 3 years) before distribution. In the meantime, a comparative trial can be planted, and 6 to 9 years later the best clones distributed. The maximum progress is made this way - 25% compared to with the initial cross (Baudouin and Durand-Gasselín, 1991).

The genetic progress obtained is therefore:

- 18 years after the trial is planted, clones representative of the cross are available, for which flowering has been observed in the field. Exact reproduction of the cross: + 15%.
- 25 years after the trial is planted, only the best 5% of clones are distributed:  $15\% + 25\% = 40\%$ .

### **Cloning elite individuals**

Cloning particularly high-yielding individuals from a cross is carried out as soon as the trial is complete. As the palms are adult, marketable clones will be available 6 years later, although their flowering has to be first observed before distribution. To date, it has been possible to market one in every two trees sampled (T. Durand-Gasselín - personal communication). Only clones from the best 5% of ortets are distributed, even if the plan was to sample the best 20% of palms from a cross tested in a clone comparative trial. In this case, the progress achieved is 15% compared to the initial cross (Meunier *et al.*, 1988), with an oil production heritability of 0.4 (Baudouin and Durand-Gasselín, 1991) and a coefficient of variation of 20%.

Following the clone comparative trial, only 20% of the clones tested will be distributed (5% of the palms from the initial cross), ensuring a mean progress of 25%.

The genetic progress achieved will therefore be:

- 18 years after planting the trial: + 30%.
- 25 years after planting the trial: + 40%.

### Investment Required

There are several areas of investment: firstly the buildings for exploitation and land for planting the palms. To illustrate the situation, we give an example of producing one million units (seeds or ramets) under average soil and climatic conditions (tertiary sands, water deficit: 300 mm).

#### Conventional seeds

##### a. Buildings

Buildings are required for artificial pollination (pollen preparation, packaging and storage, packaging the talc-pollen mixture used for pollination), seed preparation (seed preparation and packaging), seed storage and germination (release from dormancy and germination).

The needs are 175 m<sup>2</sup> per million seeds produced.

##### b. Planting

The palms required for sexual seed production are *dura* and *tenera* selfs - the *pisiferas* are chosen from the latter (25% of the palms planted).

The limiting factors are the *dura* selfing capacity (on average three bunches/palm of 600 seeds each are produced), *pisifera* pollen production (often only one palm or less in five produces pollen) and the number of pollen units obtained (33 units per bearing palm/year).

Unavailability of the palms for various reasons should be taken into account (Jacquemard *et al.*, 1981), particularly from vertical growth (*dura* and *pisifera*) and mesocarp:fruit rate (*dura*) of around 30%.

For a million seeds, 5.6 hectares of *dura* selfs and 4.7 hectares of *tenera* selfs or between-group crosses have to be planted, with some of the areas at double density.

#### Clonal seeds

##### a. Buildings

Clonal seed production needs a small *in vitro* culturing unit for vegetative propagation from 10 - 20 parents. The laboratory floor area is 80 to 100 m<sup>2</sup>. This is a costly building to be equipped with complex equipment.

The seed production unit would need a similar building to that for conventional seed production (175 m<sup>2</sup> for 1 million seeds).

b. Planting

Cloning the best *dura* and *pisifera* parents to produce clonal seed makes it possible to optimize the areas planted with pedigree trials. 4.1 hectares of *dura* clones and 0.75 hectare of *pisifera* clones planted at double density are needed to produce one million biclonal seeds annually.

The advantage of clonal seeds is less if the group B parent is *tenera*. Selfing has to be carried out and semiclonal seeds are produced.

### Cross reproduction by cloning

a. Buildings

Cloning needs extensive, highly complex buildings and is usually done by commercial production units using costly equipment (air filtration system, laminar flow hoods, temperature-controlled rooms, precision air-conditioning, etc.). It is estimated that one square metre of light shelf is enough to produce 3,000 ready-to-wean ramets a year.

For clone production and field trials, a 600 m<sup>2</sup> *in vitro* culture unit is required to culture around 200 zygotic embryos or young plants. The commercial unit should have a floor area of 650 m<sup>2</sup>.

b. Planting

It was seen above that the exploitation of clones requires field tests of potential planting material before certification. This makes it possible to detect abnormalities and pick the best clones.

104 palms are required to test a clone, that is, about 0.73 hectare. The planting area required is not proportional to the number of palms to be produced per year, but to the number of clones to be tested. In this case, it is necessary to test about 100 clones with potentials similar to that of the initial cross.

### Cloning elite individuals

a. Buildings

To produce clones and test them in the field, a 200 m<sup>2</sup> *in vitro* culturing unit is required, making it possible to culture around 50 ortets per year.

As in the above case, the commercial unit should have a floor area of 650 m<sup>2</sup>.

b. Plantings

The excellent productivity of the palms is at least equal to the mean productivity of the cross from which they were derived.

Land requirements to develop 20 clones are 14.6 hectares. Their potentials - at least equal to that of the initial cross - guarantee commercial profitability.

c. The risks involved

By the risks involved, we mean the legitimacy of reproduction, the absence of abnormalities and security of reproduction.

In reproduction from seed, a complex and cumbersome system of checks on artificial pollination was implemented very early on (Bernard and Malingraux, 1965). The system (Fig. 6) manages to prevent most mishaps - faulty bagging, inadequate pollen, mix-up of crosses, mistaken identity - to mention only the most serious.

The production of abnormal seedlings or palms is limited by systematic elimination of low to mediocre quality pollen and seeds.

Most abnormal palms are culled in the nursery, and only 2% of the palms eventually planted are unproductive or "foreign".

Germination hazards (heating or storage problems, etc.) can sometimes threaten the success of the operation.

As regards clone production, equally strict procedures throughout the process (Fig. 7) make it possible to check planting material identity and compositions of the media used for the various stages.

Lastly, ramets leaving the production unit have to comply to a strict norm; misfits are culled in the nursery. The possible existence of mantleness is screened for at the stations. Clones so affected that they are not economical to plant are not distributed commercially. This means that commercial plantations should have only 1% - 2% unproductive, mantled palms.

Production security is ensured by the large number of clones used - if one clone is unsatisfactory, production of the others are stepped up to satisfy demand.

Clonal seeds pass through all the controls for both of the above types of materials, and the risks involved are the same as those for normal seeds. In principle, the grower does not assume the risk of mantleness.

d. Operational flexibility

Excluding the initial investment, reproduction of the best palms from parents tested by cloning is the most flexible method. In effect, cloning is a continuing process that is possible to integrate the success of the procedure in meeting production targets. Replacing one programme by another is done naturally by replacing one culture with another.

Pedigree field interchangeability is not immediate, as it means either a loss of time (felling - replanting) or a loss of land (immobilization of new areas).

However, the operating and amortization costs for a commercial clone production unit make it much more sensitive to significant changes in production volume.

In cross reproduction from seed, marketing problems can, to a certain extent, be buffered by building up stocks over a maximum period of two years.

Clonal seeds seem to have the drawbacks of both methods, and call for the absolute success of cloning both parents. Given that the success rate for somatic embryogenesis is 0.8 for palms aged 20 years, it will only be possible to reproduce two-thirds of the progenies.

## CONCLUSION

It is clear that the genetic progress shown by tested clones is much greater than that from seeds. Cloning also increases the number of genotypes available that satisfy the criteria stipulated (e.g. productivity, oil quality, growth and disease tolerance). The heritability estimates derived show that a cross or its reproduction is at least 1.5 times as variable as a clone obtained from the same cross.

It is also worth remembering that, on average, the cost of seed supplied to growers increases by around 1% per year (Fig. 8) and that cloning gives a 19% improvement compared to the seeds currently available.

It would also be interesting, in view of the increasing reliability of planting material production strategies and techniques, to set international production norms to provide growers with additional guarantees of the legitimacy and fidelity of planting materials.

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**Table 1: Improvement obtained for a cross depending on the method**

	Seed	Clonal Seeds	Repro. By Cloning	Elite Clones	Time
Control	100	100	100	100	10
Cross chosen	115	115	115	115	10
Value of Planting	108	108	-	-	11
Material Produced	115	115	-	-	15
	115	115	115	130	18
	115	115	140	140	25

**Table 2: Time management in the different methods**

Years	Conventional Seeds	Clonal Seeds	Reproduction by Cloning	Cloning Elite Trees
1	Trial planted crossing plan Selfing + recomb.	Trial planted Parents cloned	Trial planted	Trial planted
5 and 6	Selfs and recomb.planted	Parents clones planted	Crosses repeated	
10 and 11	End of trial start of seed production (Part. repro.)	End of trial Start of seed reproduction (Part. Repro.)	End of trial Cloning of zygotic embryos or young plants	End of trial Cloning of elite individuals
11 - 15	Use of <i>pisifera</i> (further repro.)	Use of <i>pisifera</i> (further repro.)	Start producing first clones Field trials	Start producing first clones Field trials
18			Start marketing clones representative of cross	Start marketing clones from best 5% or ortets
25			Start marketing best 5% of clones	

Figure 1: Population Breeding

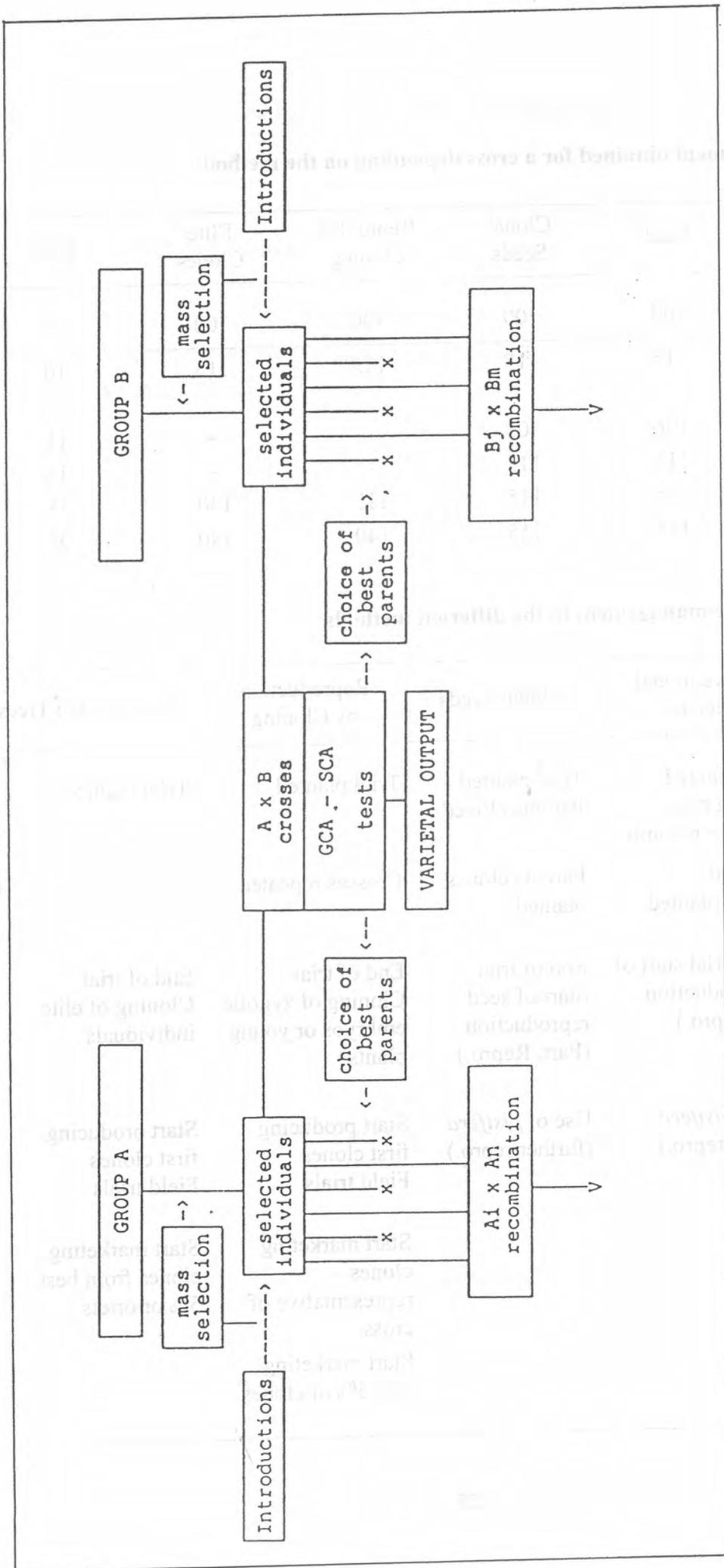


Figure 2: The Different Varietal Outputs

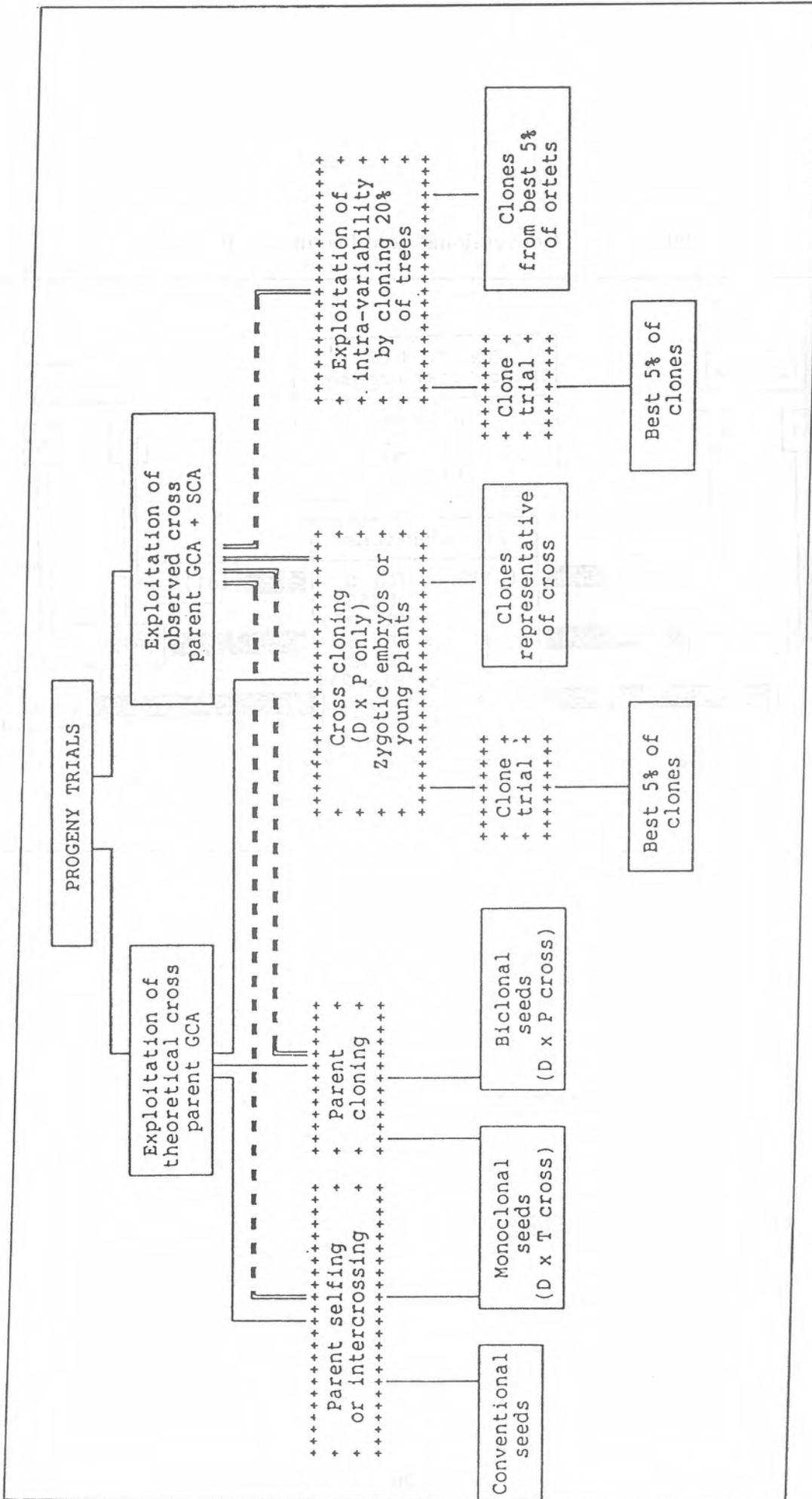


Figure 3: Conventional Seed Production Procedure

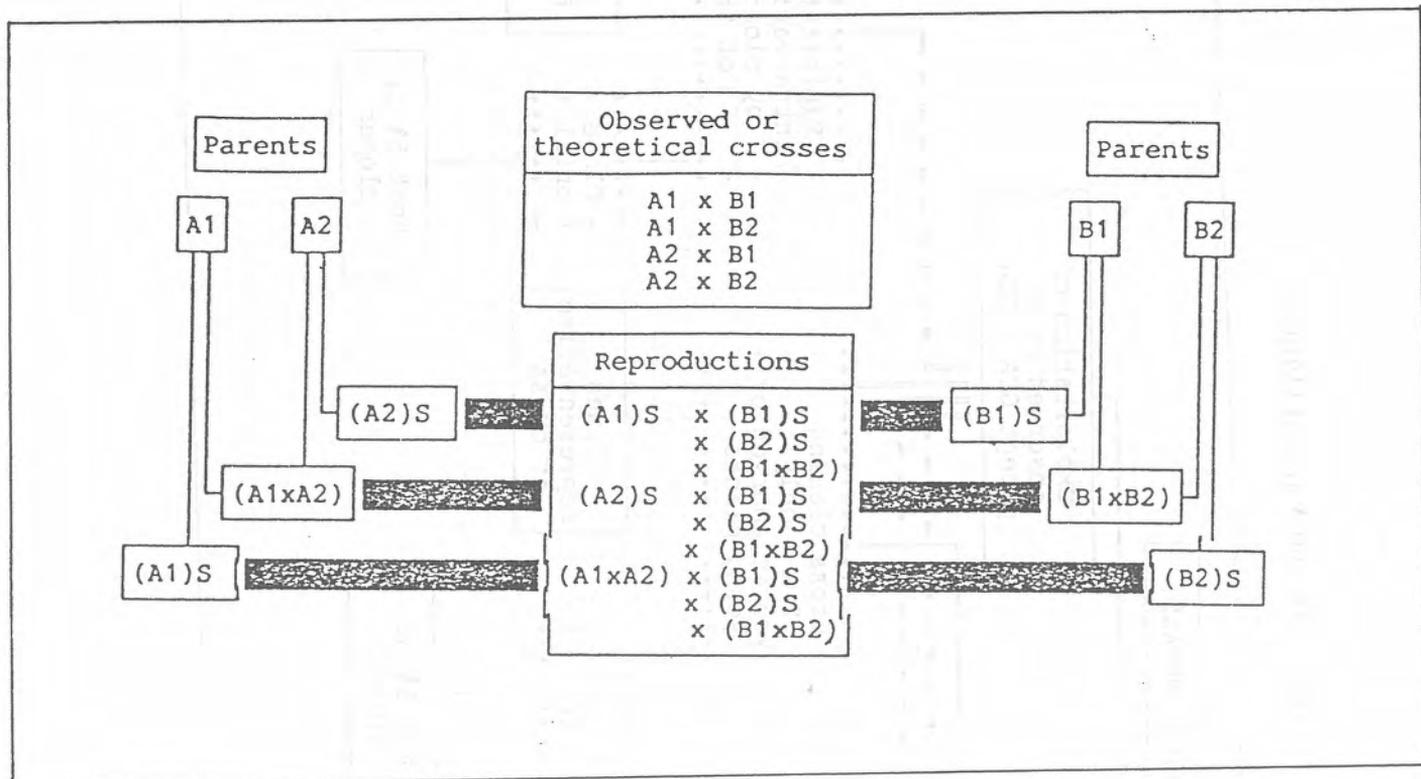


Figure 4: Clonal Seed Production Procedure

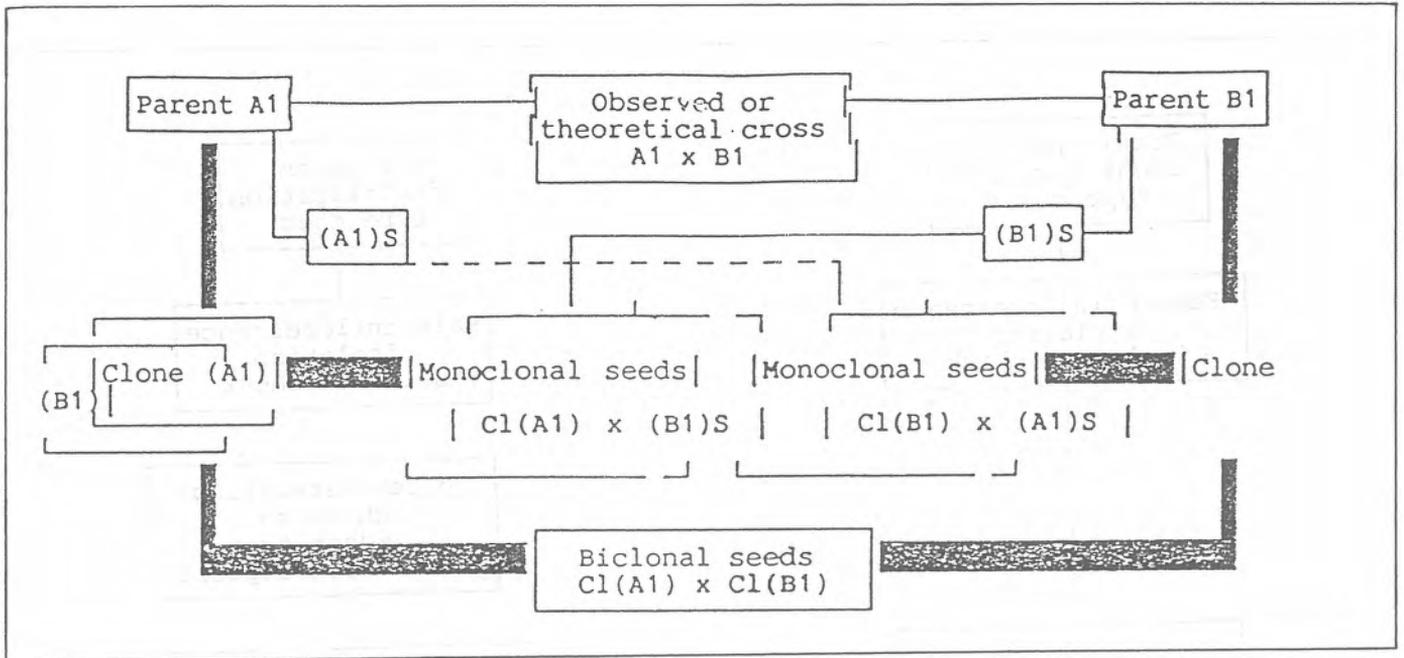


Figure 5: Clone Production Procedure

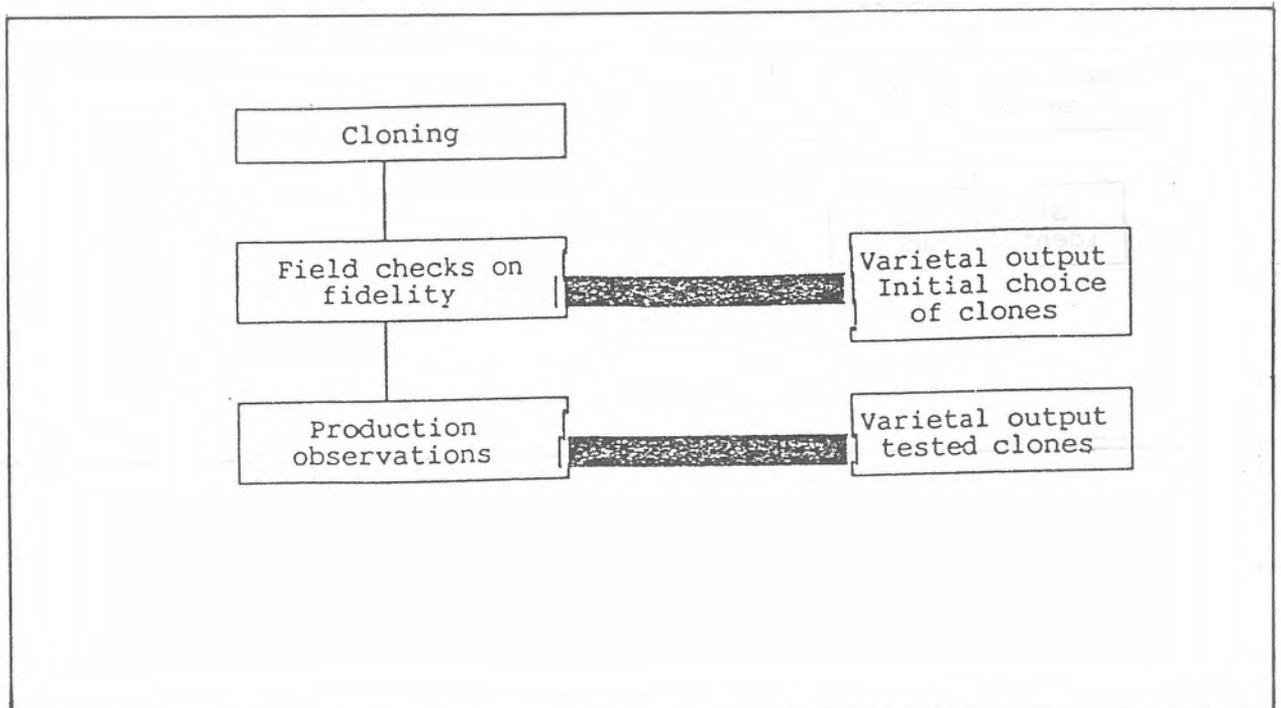


Figure 6: Seed Production Quality Controls

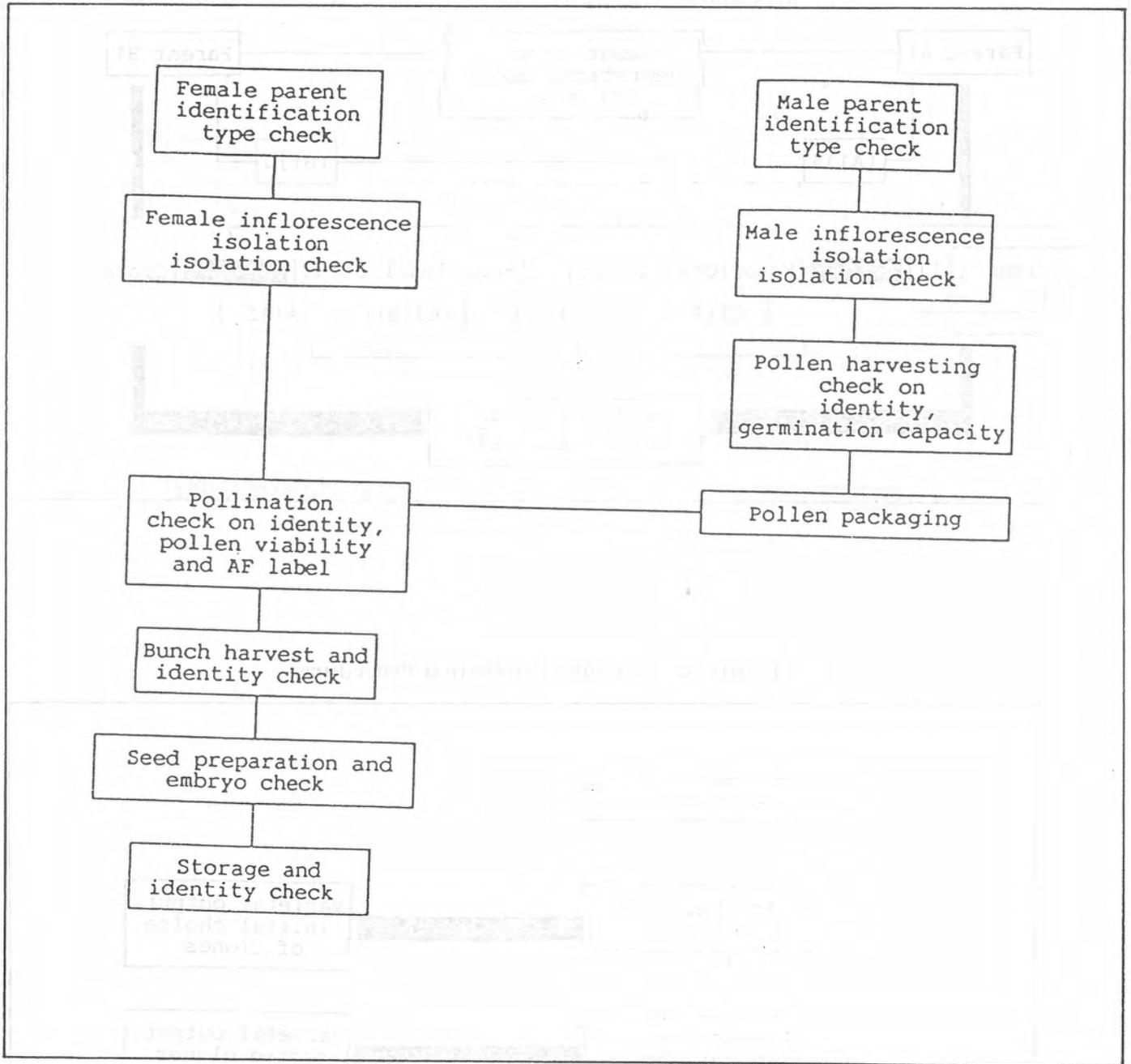


Figure 7: Quality Control in the Clone Operation

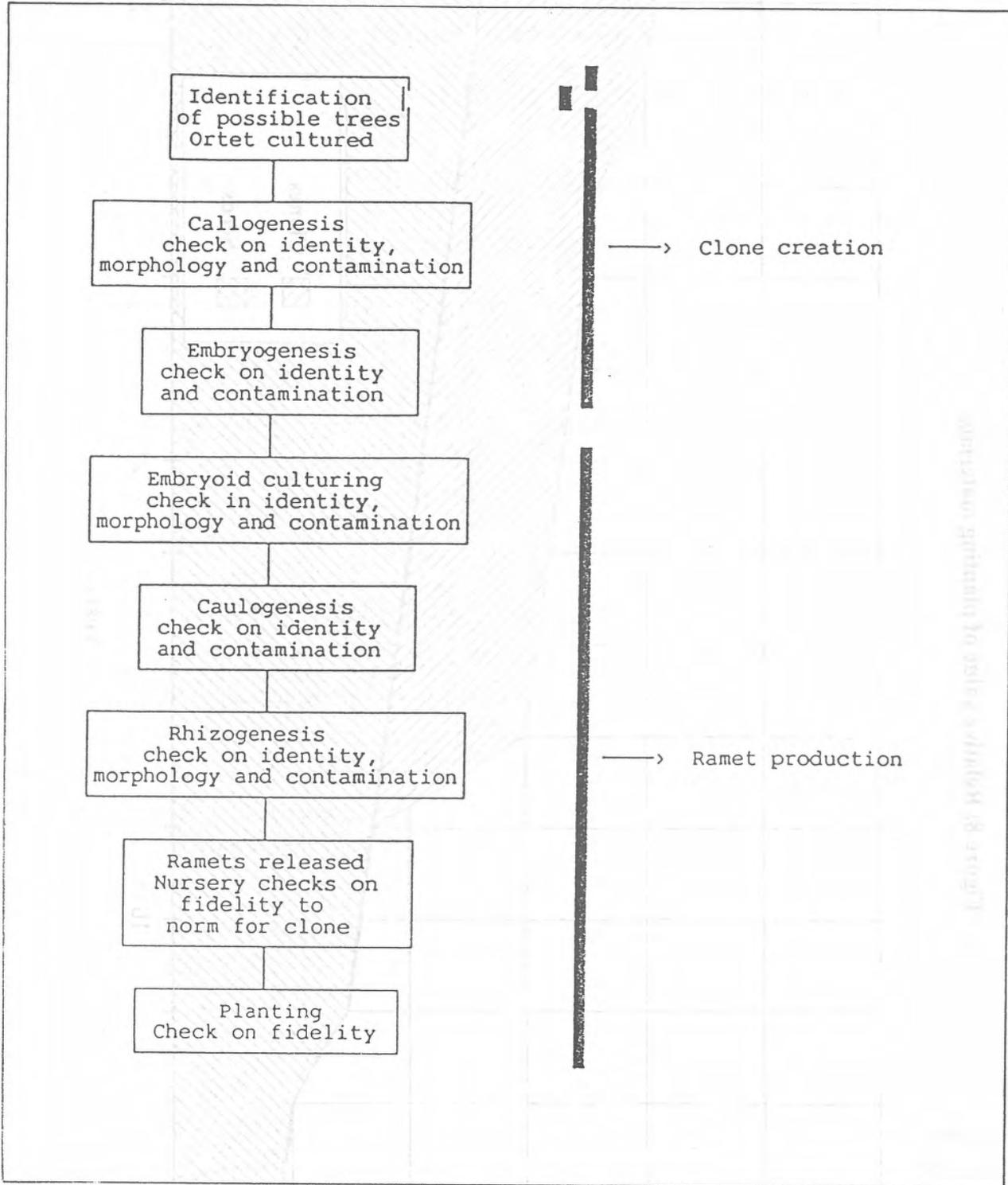
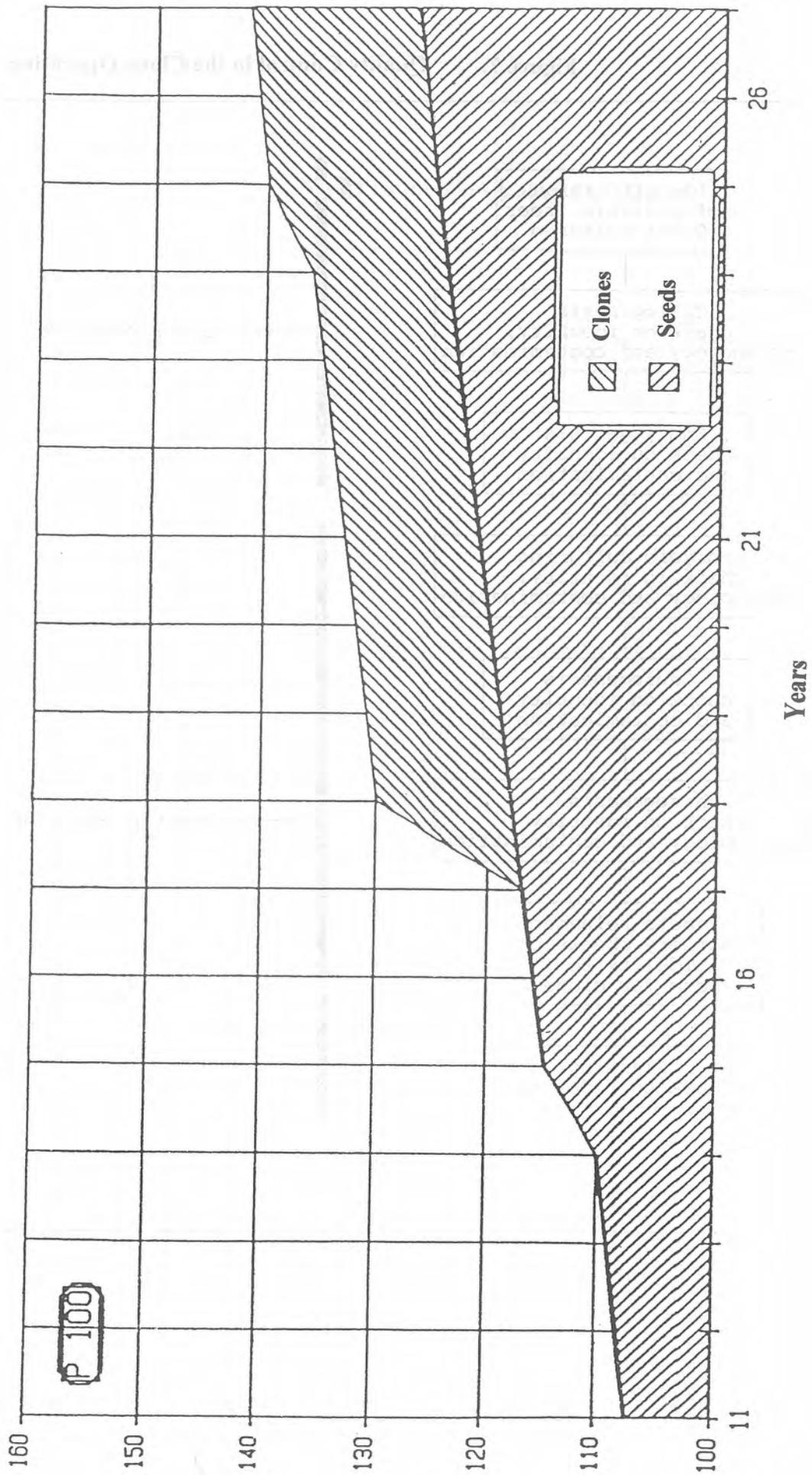


Figure 8: Relative value of planting materials



## OIL PALM BREEDING PROGRAMME IN NIGERIA

Okwuagwu C.O.<sup>1</sup> and Ataga C.D.<sup>1</sup>

### INTRODUCTION

The African Oil palm *Elaeis guineensis* Jacq. is endemic to the entire Guinean zone of West Africa (Hartley, 1967). It occurs in a fairly narrow coastal belt within the forest zone from Guinea, including The Cape Verde Islands, to Angola and further to the east in Central Africa including Zaire, the Congo, Uganda, Tanzania and Madagascar. Zeven (1964), Van der Vossen (1974) postulated that the oil palm natural habitat is likely to be within the West African oil palm belt principally in the south-eastern part of Nigerian and south-western part of the Cameroons.

Although the oil palm has been used by man in West Africa since ancient times, its commercial exploitation is of recent origin. With the realization of the economic potential of the crop, concerted effort towards its improvement started as early as the turn of this century in both West and Central Africa. An account of the history of the development of the various breeding programmes in Africa has been given by Hartley, 1967. Notable in these early efforts are the works of the "Institut National pour des Etude, Agronomiques an Congo" (INEAC), and two major West African programmes, viz: The West African Institute for Oil Palm Research (WAIFOR) in Nigeria, Ghana, and Siera-Leone and the Institut de Recherche pour les Huiles et Oleagineux" (IRHO) in Cote d'Ivoire and Benin.

The objective of this paper is to present the status of the major oil palm breeding programmes in Nigeria.

### OIL PALM BREEDING IN NIGERIA

The history of oil palm breeding and selection in Nigeria dates back to the turn of this century (Hartley, 1967). As early as 1912, the genetic exploitation of the Calabar, Aba, Nkwele (Umuahia) and later Ufuma natural groves of the then Eastern Nigeria had begun. With the establishment of the Oil Palm Research Station (OPRS) in 1939 (now the Nigerian Institute for Oil Palm Research (NIFOR), breeding and selection became established. With the recognition of the advantages of the Deli *dura* of South East Asia over the African *dura*, an aggressive

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exchange policy was pursued to ensure that NIFOR acquires and incorporates Delis from many divergent sources into its breeding programme. The Deli parents that combine their characteristic high fruit quality with an above average mean annual bunch number, were to produce the best yielding *tenera* progenies in either DxP or DxT trials. Through the several extensive prospectings of the oil palm belt and the marginal regions of Nigeria and also through exchange programmes with other African programmes, NIFOR was able to acquire a very diverse oil palm germplasm base which in 1962 was used to formulate a comprehensive oil palm breeding programme based on the modification of the Reciprocal Recurrent Selection (RRS) (Sparnaaij *et al.* 1963). The programme is divided into the following sections (see Fig. 1).

- (i) The comparative trials: These are *dura* x *tenera* test crosses laid out in statistically designed trials. From these trials the best crosses are identified and the *tenera* progenies reproduced for seed production using selfings of their corresponding *dura* and *tenera* parents.
- (ii) Selfings: All *dura* and *tenera* palms are selfed and planted in unreplicated progeny rows. These selfed progenies are used primarily as source of *dura* (from *dura* selfs) and *pisifera* (from *tenera* selfs) parents to reproduce elite teneras of the comparative trial.
- (iii) Reassortment and recombination: The *dura* x *dura* and *tenera* x *tenera* crosses between parent palms are designed to produce improved materials by reassortment and recombination. Selections from this population are used to repeat the crossing pattern adopted in the previous generation.
- (iv) Continued inbreeding: This is a phase of continued inbreeding of selfed progenies of those parents which have shown outstanding overall performance in the comparative trial. The aim is to develop more or less inbred lines of *dura* and *tenera* parents. The combining ability of these lines would be tested at each stage of inbreeding by parallel comparative DxP trials of parents from *dura* and *tenera* inbred lines.

The improvement which have been attained in NIFOR planting material over the years is presented below:

Period	Type of Material	Yield, ton/ha/year	
		FFB	Oil
1930s - 1950s	Open and controlled pollinated DxP, DxT and DxP crosses of selected groves palms.	2.5 - 5.0	0.5 - 1.0
1960s - 1970s	DxP controlled pollinated EWS materials from the 1st stage oil palm breeding programme	5.0 - 10.0	1.0 - 1.5
Late 1970s - the present	DxP controlled reproduction of elite <i>tenera</i> progenies from the first cycle of the RRS programme	15 - 18	2.0 - 3.0

Outcome of the NIFOR First Cycle RRS Breeding Programme:

### **Genetic Control of the major Components of Yield**

The inheritance of the components of oil yield in the NIFOR breeding population has been reported upon by Blaak *et al*, 1965; West *et al*, 1976 and West (1976). The inheritance of bunch yield was found to be very low with significant non-additive genetic control. The inheritance of the two components of bunch yield - mean annual bunch number (bn) and single bunch weight (sbw) were found to be moderate for (sbw) and higher for (bn). However, the significant negative genetic correlation between these two bunch yield components negates the exploitation of their additive genetic control.

Generally, fruit quality components mesocarp to fruit ratio (m/f); shell to fruit ratio (s/f) and kernel to fruit ratio (k/f) have high heritability. However, there is clearly a low correlation for these fruit components between *dura* parents and their *tenera* offspring and between *dura* and *tenera* full-sibs (Sparnaaij, 1969; van der Vossen 1974; Okwuagwu and Ataga, 1984). The implications are that the *tenera* genotypic values for these fruits components of a *dura* parent can most reliably estimated from their *tenera* offspring in DxT or DxP trials. Secondly, the selection of *dura* parents for the production of *tenera* hybrids based on the *dura* parent performance or DxD progeny performance will be ineffective. Most recently, the evaluation of the inheritance of kernel to fruit ratio in all the possible intra and inter-fruit form crosses implicated a maternal inheritance conditioned by the presence of the *dura* genotype (Okwuagwu and Okolo, 1992).

### **Improvement of the Deli Breeding Population**

Because of the comprehensive nature of the NIFOR first cycle breeding programme, it was possible to evaluate the future of breeding within and among different populations of the Deli *dura* selections from different sources for which fixation for different group of genes has occurred, is as effective as mating among unrelated African *dura* parents, in generating heritable genetic variation essential for selection progress (Okwuagwu, 1992). The exploitation of intra Deli crosses in oil palm breeding was found to be unfruitful. Immediate genetic improvement of the Deli breeding populations can rely on the build-up of heritable genetic variation for economic yield traits through intercrossing divergent Deli sub-populations. The introgression with the African *dura* may have the limitations of the decline in fruit quality, but will on the long term be the most effective strategy of exploiting both the Deli and African *dura*.

### **Standardization of the Fruit and Bunch Analysis Procedures**

As a result of the research carried out in NIFOR on the selection criteria for the major oil yield trait of the fresh fruit bunch (FFB) and the fruit components, Blaak (1963), developed a procedure of fruit and bunch analysis by weight percentages which has since become the standard method of fruit and bunch evaluation. By this method the yield of oil is derived by the following:

Palm oil yield = yield of ffb x % fruit/bunch x % mesocarp/ fruit x % oil/wet mesocarp.

Palm kernel yield = yield of ffb x % fruit/bunch x % kernel/fruit.

### **Large Scale Commercial Seed Germination**

Through concerted early research efforts at NIFOR, the appropriate conditions necessary to break the dormancy of oil palm seeds was developed. The heat and moisture requirements to overcome the problem of oil palm seed which is normally characterised by prolonged dormancy of up to one year became well defined. The construction requirements of a large scale germination which could hold up to 1 million seeds at a time as well as maintain the exacting heat requirement of  $39^{\circ}\text{C} \pm 1^{\circ}\text{C}$  and the seed relative humidity of 18% was first described by Rees (1959). NIFOR has the capability of producing 7.4 million seeds annually, however our mean annual production has remained 5 million, based on the national demand.

### **Progeny Response to Differential Density Plantings**

The four-row planting trials (with every fifth row unplanted) incorporated in the NIFOR main breeding programme progeny trials was aimed at evaluating progeny response to 2 density plantings viz: the standard density of about 150 palms per hectare and a lower density of 100 palms per hectare. No differences were observed in the first 4 years of bunch yield under the 2 different densities. On individual palm basis, the ffb yield of the low density plantings was higher for the 5th - 8th years mean. However when yield was expressed on per hectare basis, the higher individual palm yield of the low density plantings was not enough to compensate for the fewer palms per hectare. Earlier density studies carried out in NIFOR (Sly and Chapas 1963; Maliphant, 1968), indicate that the optimum planting density varies from one progeny to another. From the 4-row planting trials, when comparison was made among the very productive progenies for the mature yield, no individual yield difference were observed between the 2 density plantings. Thus for such highly productive progenies which did not show response to wide density differences (150 palms/ha. and 100 palms/ha.). Higher density plantings over the current practice may result in higher bunch yield/ha. New density trials are now being formulated to further explore, exploit this possibility.

## SECOND CYCLE BREEDING PROGRAMME

The results obtained from the first cycle of the modified RRS, the second cycle breeding programme was initiated in 1984. Basically, the breeding scheme followed the modified RRS scheme. The grouping of parents for complementary traits as was the case in the first cycle was found unnecessary. Parents were selected from the first cycle recombinant populations and also new introductions were made from the gene pools. The second cycle parents include 5 Deli *dura*, 9 African *dura* and 13 *tenera* selections. These parents have been recombined as follows: (Figure 2).

1. One set of 5 x 5 (Deli x Deli)  $\frac{1}{2}$  diallel of 10 crosses
2. Two sets of 5 x 5 (*dura* x *dura*)  $\frac{1}{2}$  diallel of 10 crosses
3. Three sets of 5 x 5 (*tenera* x *tenera*)  $\frac{1}{2}$  diallel of 10 crosses each
4. Three sets of 5 x 5 (Deli x *tenera*) factorial of 25 crosses each
5. Three sets of 5 x 5 (*dura* x *tenera*) factorial of 25 crosses each,

giving a total of 210 crosses. Each of the parents was selfed for possible use in future seed production. 20% yield improvement is expected from selections from the second cycle over the first cycle population. The yield evaluation of the early plantings of the second cycle test cross trials commenced in 1991. A significant observation from the early results from these trials is high precocity and slower height increment of some progenies compared to the first cycle EWS standards.

### Breeding for Slow Height Increment (Short-Stemmed) Oil Palm

The rate of height increment determines the economic life span of the palm. The reduction in the rate of height increment not only reduces harvesting cost but also increases the economic life span of the palm. In view of its importance, NIFOR within the past 20 years embarked on different approaches to the development of short-stemmed oil palm variety.

- (i) The development of short stem oil palm variety using indigenous short-stemmed oil palms. Efforts in this direction have consistently shown a negative relationship between slow height increment and the yield of fresh fruit bunch. Thus making variety development by the conventional method rather slow and unencouraging. However, variants have been identified which combine slow growth with economic yield. With the availability of the tissue culture technique, clonal multiplication of these variants could be productive.
- (ii) The development of short-stemmed oil palm variety through interspecific hybridization of the indigenous oil palm (*E. guineensis*) and the South American oil palm (*E. oleifera*).

Through this method, a slower height increment was attained but the interspecific  $F_1$  hybrids were characterized by various forms of flowering abnormalities which has limited their direct use. To improve the flowering characteristics and ensure better bunch development, the hybrids were back-crossed to *E. guineensis* as the recurrent parent. The evaluation of these  $BC_1$  population showed that the flowering problem still persisted to a large extent while the population segregated for height and other vegetative growth characteristics. With the present knowledge of the flowering abnormality in the interspecific hybrids, efforts are being made by NIFOR to increase its sources of *E. oleifera*.

- (iii) The use of mutagens: In our effort to develop short stemmed oil palm variety without the sacrifice of good bunch yield, a mutation programme was initiated using chemical mutagens and ionizing irradiation on pollen and young embryos. The result from the first mutant population ( $M_1$ ) showed that indeed ionizing irradiation affects oil palm height among other traits. Palms from the  $M_1$  population have shown such useful traits such as earliness (bunch yield starts 18 months from field planting), compact canopy and very high sex ratio. The  $M_1$  palms with these useful traits have been used to plant  $M_2$  population with the aim of determining those useful mutations that breed true.

### BREEDING FOR DROUGHT RESISTANCE

This involves breeding for economic yield levels in environment dominated by water deficit. Most of the areas within the oil palm belt and the marginal regions of Nigeria usually have 5-8 months dry season. The water deficit experienced by the oil palm during this period could be as high as 890mm. This single factor accounts for the high yield gap between Nigeria and South East Asia where the ffb yield could be as high as 30-40 tonnes/ha/year compared to the maximum yield of 15-20 tonnes/ha/year attainable in Nigeria. From the past results of the breeding programme, there is every indication that there are significant differences in the extent to which different progenies respond to water stress. A programme aimed at developing drought tolerant oil palm varieties has been initiated.

### TISSUE CULTURE

The value of clonal propagation in a perennial tree crop like the oil palm has long been recognized. NIFOR realizes the importance of meeting up with the changing demand of the oil palm industry and the role of clonal palms in the provision of uniformly high yielding disease resistant planting materials to the growers. From the NIFOR breeding programmes excellent

materials have been identified which through clonal multiplication of either parents or the individual hybrids, would result in considerable yield increases.

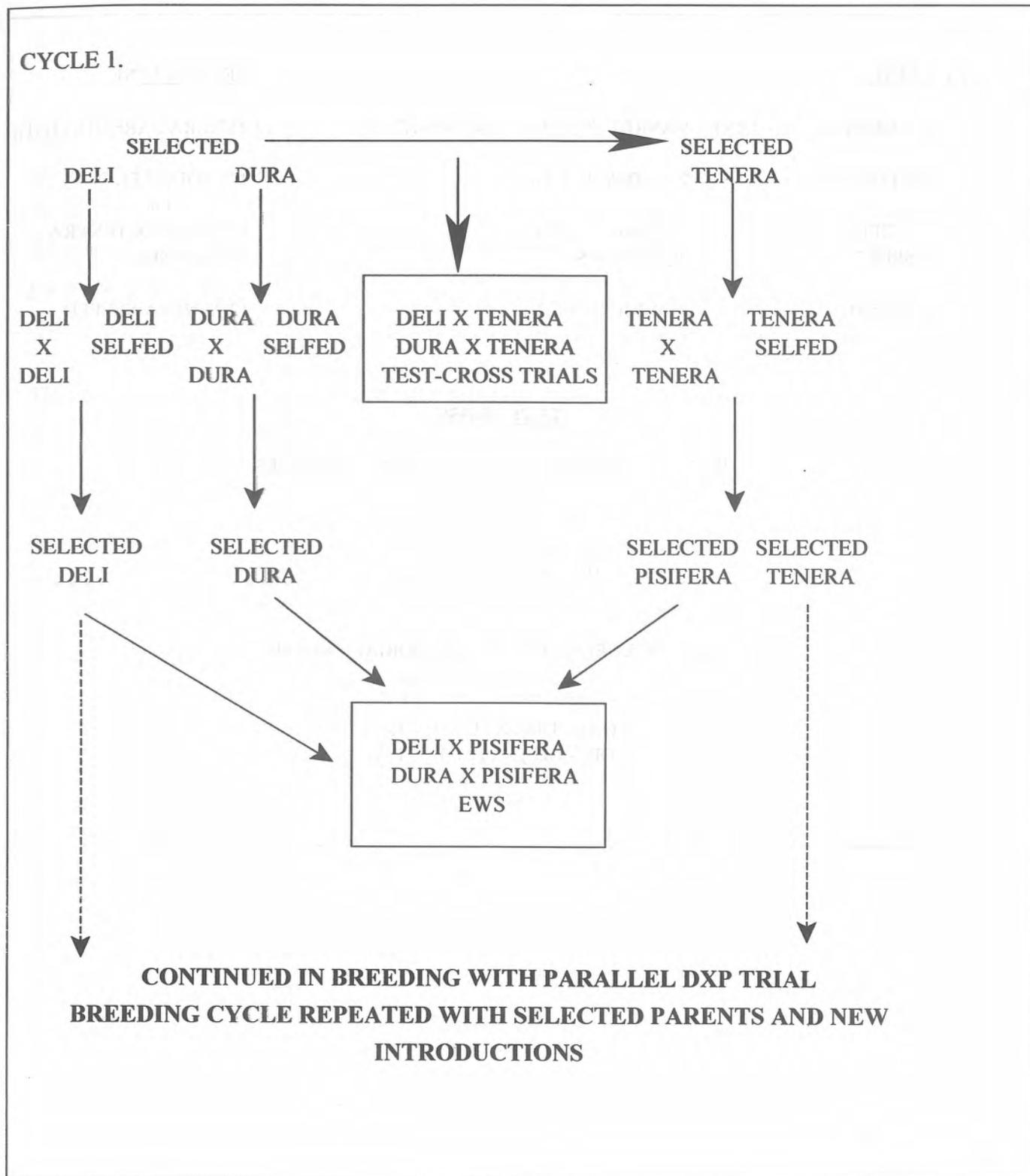
The execution of the tissue culture programme, research and technical personnel have been trained and are still being trained in the area of clonal propagation and biotechnology. Using the very limited facility available, it has been possible to reduce considerably the duration for callus induction from explant. With the recent assistance from EEC, a well equipped tissue culture laboratory is being built on the NIFOR Main Station. This, when completed will facilitate the realization of the objective of the NIFOR tissue culture programme.

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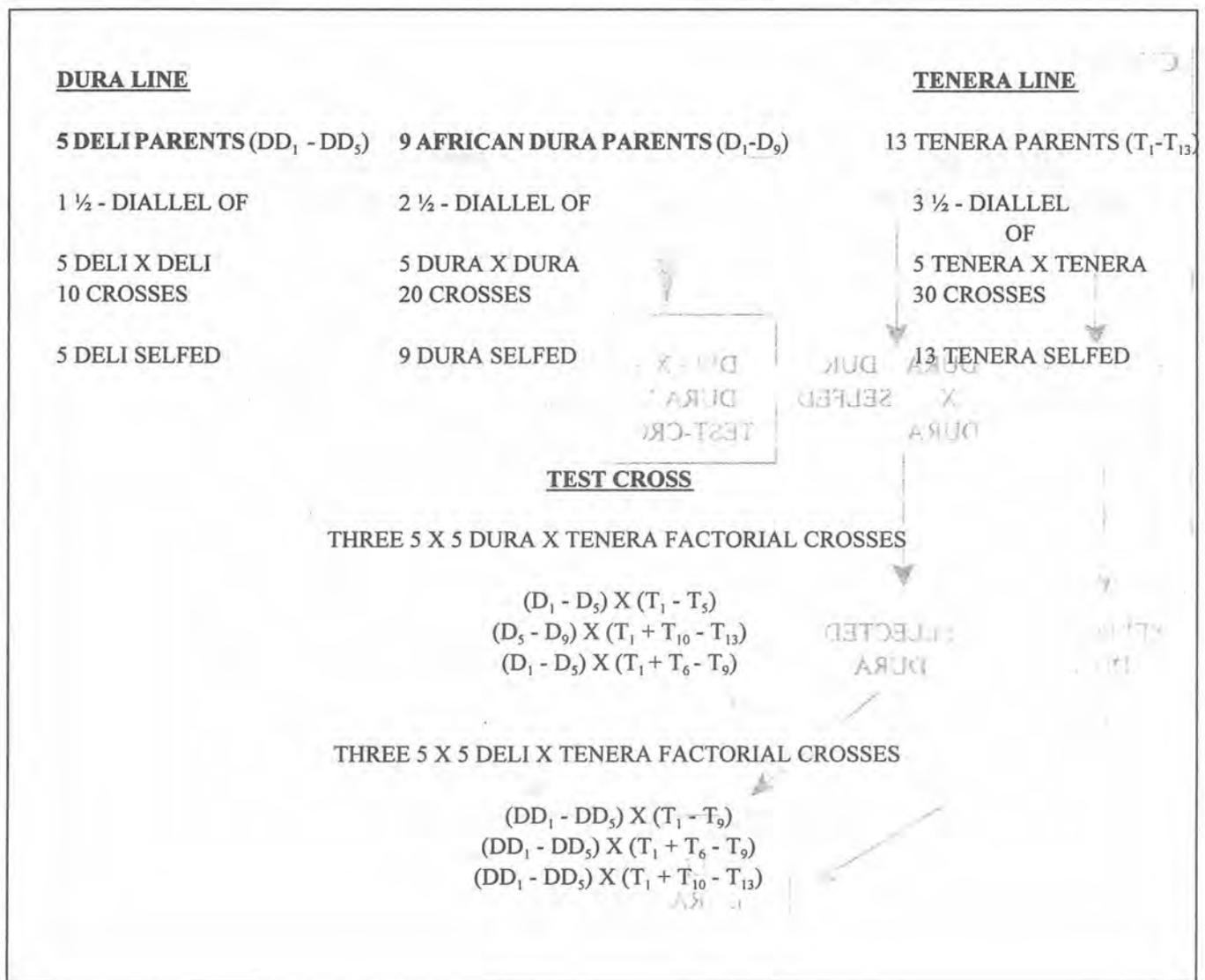
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**FIGURE 1. SCHEME OF THE NIFOR RRS BREEDING PROGRAMME**



**FIGURE 2. NIFOR BREEDING PROGRAMME CROSSING SCHEME - SECOND CYCLE**



## THE OIL PALM BREEDING PROGRAMME IN MALAYSIA

Yong Y.Y.<sup>1</sup>

### ABSTRACT

*The annual DxP seed requirement in Malaysia is about 30-35 million. Thirteen research organisations including PORIM, Guthrie, Socfin, United Plantations, Highland Research Unit, Golden Hope, Felda, Pamol, I.O.I., Sime Darby, Applied Agricultural Research and Eastern Plantation Agency have embarked on oil palm breeding programmes. These conventional oil palm breeding programmes are supported by the tissue culture laboratories of each organisation.*

*The genetic resources for these breeding programmes come from the Deli dura populations and breeding populations developed by INEAC, IRHO and Nifor. These populations have been widely distributed through cooperative and exchange schemes, such as the International Experiment (1946), the Cooperative Breeding Scheme (1956) and the Oil Palm Genetics Laboratory (1963). The recent prospections undertaken also by PORIM in Africa provide a valuable reservoir of germplasm for the oil palm breeding programmes in Malaysia.*

*The Malaysian breeding scheme follows that of a modified recurrent selection which stresses on the exploitation of the additive genetic variation. Further yield improvement would probably come through the exploitation of the non additive genetic variation in the advanced breeding populations. Elite DxP hybrids showing specific combining ability can be reproduced through selfs or clones of parents.*

*The breeding programmes should be geared to meet the present and future needs of the industry. The vegetative propagation of oil palms through tissue culture will enable the rapid exploitation of recombinants existing in the breeding programmes.*

### INTRODUCTION

The oil palm was first introduced into Malaysia in 1902 and 1905 by the Department of Agriculture as experimental palms (Jagoe, 1952). Independently a private company. The Rantau Panjang Rubber Estate, Kuala Selangor, also imported oil palm seeds into Malaysia in 1911.

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<sup>1</sup>Kumpulan Guthrie Berhad

These importations were believed to be the descendants of the four palms which were introduced from Mauritius via Amsterdam and planted in Bogor Botanic gardens, Java in 1848. Descendants of these palms are also known as the *Deli dura* and they provided seeds for the first commercial planting in Malaysia which was established at the Tennamaran Estate, Kuala Selangor in 1917. 1930 to 1969 saw the rapid and large scale cultivation of oil palm in Malaysia. The demand for superior oil palm seeds rose. The oil palm improvement programme through breeding and selection was initiated by the Department of Agriculture in the early 1920's and this was soon complemented by oil palm breeding programmes of the private plantation companies in Malaysia. They currently supply around 30-35 million DxP seeds annually to the industry.

### **THE OIL PALM BREEDING INSTITUTIONS IN MALAYSIA**

The oil palm breeding programmes in Malaysia are undertaken by thirteen research institutions belonging to the government as well as the private sector and are listed below:

- (1) Palm Oil Research Institute Malaysia (PORIM) 1979 (continuation of the programme of Department of Agriculture, 1922)
- (2) Guthrie Research Chemara (1933)
- (3) Socfin (1940's)
- (4) United Plantations (1950's)
- (5) Highland Research Unit (1951)
- (6) Golden Hope (1957)
- (7) Federal Land Development Authority (FELDA) (1961)
- (8) Department of Agriculture, Sabah (1966)
- (9) Pamol (1974)
- (10) Industrial Oxygen Incorporated (1974) - formerly Dunlop Research Station
- (11) Sime Darby (1983)
- (12) Applied Agricultural Research (1985)
- (13) Eastern Plantation Agency (1989)

PORIM, established by the Malaysian government in 1979, inherited the oil palm breeding programme which was initially carried out by the Department of Agriculture at the Federal Experimental Station, Serdang in 1922. This was subsequently taken over by the Malaysian Agricultural Research and Development Institute (MARDI) in 1970. Currently, PORIM is playing an important role in the distribution of advanced and prospected oil palm genetic materials to the various Malaysian oil palm breeding programmes.

Socfin in Malaysia terminated its oil palm breeding programme in 1983 and is mentioned here since it was one of the earlier oil palm breeding stations in Malaysia and had been instrumental in the introduction of a wide range of genetic materials especially those developed by Institute de Recherches pour les Huiles et Oleagineux (IRHO). These genetic materials were generously made available to the industry when Socfin terminated its breeding programme.

The Guthrie Research Chemara, United Plantations, Highland Research Unit and Golden Hope (Oil Palm Research Station, Banting) were the early oil palm breeding stations set up by the private companies. They provided the bulk of planting materials to the oil palm industry. The oil palm breeding programme of FELDA was aimed at the production of seeds to meet the large requirement of seeds for the FELDA land schemes.

The breeding programmes of Pamol and I.O.I. were established through their association with Oil Palm Genetics Laboratory (OPGL), Sime Darby, Applied Agricultural Research (AAR) and Eastern Plantation Agency initiated their breeding programmes in the eighties to produce seeds for their own requirements and obtain additional revenue from the lucrative seed market. The breeding programme of Applied Agricultural Research is in fact a continuation of the HRU programme which is now under a different management. The HRU and AAR programmes diverged as each proceeded their separate ways.

Oil palm is a perennial crop and oil palm breeding is difficult because of the long generation time and requirement for large areas for experimentation. It must be mentioned here that the year of establishment of the oil palm breeding programme of the various research stations is no indication of the superiority or advancement of the breeding populations of the various programmes, for the Malaysian oil palm breeders have been fortunate in that the advanced genetic materials were widely distributed among the breeding stations through PORIM and various other cooperative and exchange schemes which will be discussed later.

### **THE BREEDING OBJECTIVES**

The primary objective of all the oil palm breeding programmes is the production of commercial oil palm planting material with high oil yield potential. Improvements of the secondary traits such as oil quality, carotene, compact palms (short and/or smaller crown) and disease tolerance are gaining the attention of the oil palm breeders.

### **High Oil Yield Per Hectare**

High oil yield could be achieved through increasing the fresh fruit bunch (FFB) yield and/or the oil to bunch (O/B). In the past, oil palm breeders have tried to increase the oil yield without worrying too much whether it was achieved through improvement in FFB or O/B. The critical labour shortage for the harvesting of FFB in Malaysia should see oil palm breeders aiming for higher average bunch weight rather than bunch number for the same amount of FFB and higher O/B rather than FFB for the same amount of oil yield per hectare. The O/B, being the product of fruit to bunch (F/B), mesocarp to fruit (M/F) and oil in mesocarp (O/M), can be improved through any of these components.

### **Oil Quality**

Palm oil has a well balanced ratio of 50 percent of saturated to 50 percent unsaturated fatty acids in its fatty acid composition. Improvement in the unsaturation level of palm oil will ensure that it can make further inroads the vegetable oils market. This was recognised very early in the oil palm breeding programme in Malaysia. This was attempted through the hybridisation of the *E. guineensis* with the *E. oleifera*, which has about 80% unsaturated fatty acids composition. Commercially, this programme has not achieved its desired impact in the industry because of the low oil yields of the interspecific *E.o.* x *E.g.* hybrids to *E. guineensis* or selection and breeding within the *E. guineensis* continues in the breeding programmes. Selection within the Nigerian materials will be important in providing the genetic variation for oil quality.

### **Shorter Palms**

The advantages of slow vertical growth in oil palm are well known. Reduced rate of height increment would ensure a longer replanting cycle and ease harvesting of matured palms. The Dumpy palm (E206) or its derivatives have featured prominently in some of the oil palm breeding programmes in Malaysia. The Dumpy genes have been introgressed in the *pisifera* lines of HRU, AAR and Golden Hope materials. Breeding for shorter palms will continue to receive the attention of oil palm breeders.

### **Kernel Oil**

The demand for the kernel oil, mainly lauric oil, will increase in the future with the expansion of the oleochemical industry. The oil palm breeders will therefore have to produce palms with high kernel content, probably at the expense of the mesocarp oil, for this specific market.

### **Disease Tolerance/Resistance**

Oil palm growers in Malaysia are fortunate as there are no serious diseases of oil palm in Malaysia. Most of these diseases can be controlled by prudent agricultural practices. The only notable disease worth mentioning is the basal stem rot caused by species of *Ganoderma* which may be prevalent in mature palms grown in the coastal areas of Malaysia. Not much effort has been directed towards breeding for tolerance to *Ganoderma* due to the lack of understanding of the process of infection by *Ganoderma* and the lack of a reliable inoculation technique (Ariffin *et al.* 1989). This disease will become more prevalent with successive replanting cycles. Breeding for tolerance or resistance to this disease may be important in future. The resistance could probably come from *E. oleifera* as Tan (1987) had found significantly lower incidence of basal stem rot in *E.o.* x *E.g.* hybrids.

### **Improving the Physiological Traits**

The Oil Palm Genetics Laboratory (OPGL) has contributed significantly to crop physiological studies in oil palm in Malaysia. Breeding for increased physiological efficiency through selection for leaf-area ratio (LAR), photosynthesis efficiency and harvest index had been advocated at various times (Hardon *et al.*, 1972; Breure & Corley, 1983; Rosenquist *et al.*, 1990). However, the selection based on physiological traits with the exception of harvest index, is not very popular among the oil palm breeders in Malaysia at this moment.

### **Yield Stability**

The performance of the oil palm will vary under different environments. Significant genotype x environment interactions have been found in oil palm breeding trials in Malaysia (Ong *et al.*, 1986; Lee *et al.*, 1987; Yong and Chan, 1990 a or b; Rajanaidu *et al.*, 1990; Yong *et al.*, 1991; Lee *et al.*, 1990). It is therefore desirable to select for yield stability. Yield stability here refers to varieties that respond positively to a favourable change in environment and should not be confused with a stable variety that does not respond to changing environments. Breeding for yield stability will be of greater importance in the future when biclonal seeds or clonal palms are commercialised. Such materials are of a narrower genetic make-up and will be more sensitive to the environment.

## BREEDING POPULATIONS AND GENETIC RESOURCES

The success in achieving the objectives of the oil palm breeding programmes depends on the availability of the genetic variability that exists in oil palm populations. The breeding and selection efforts in oil palm have resulted in distinct oil palm breeding populations which can be traced to a few palms of different origins. These breeding populations, often referred to as breeding populations of restricted origin (BPRO), have been well documented by Rosenquist (1986). The importations and liberal exchange of germplasm among research stations in the early years have resulted in the wide distribution of the BPROs. The important BPROs in the Malaysian breeding programmes will be briefly discussed. No attempt will be made to list the breeding populations in the various breeding programmes of Malaysia as it is expected that most breeding stations would have similar populations either in their pure or introgressed forms.

### The Deli *dura*

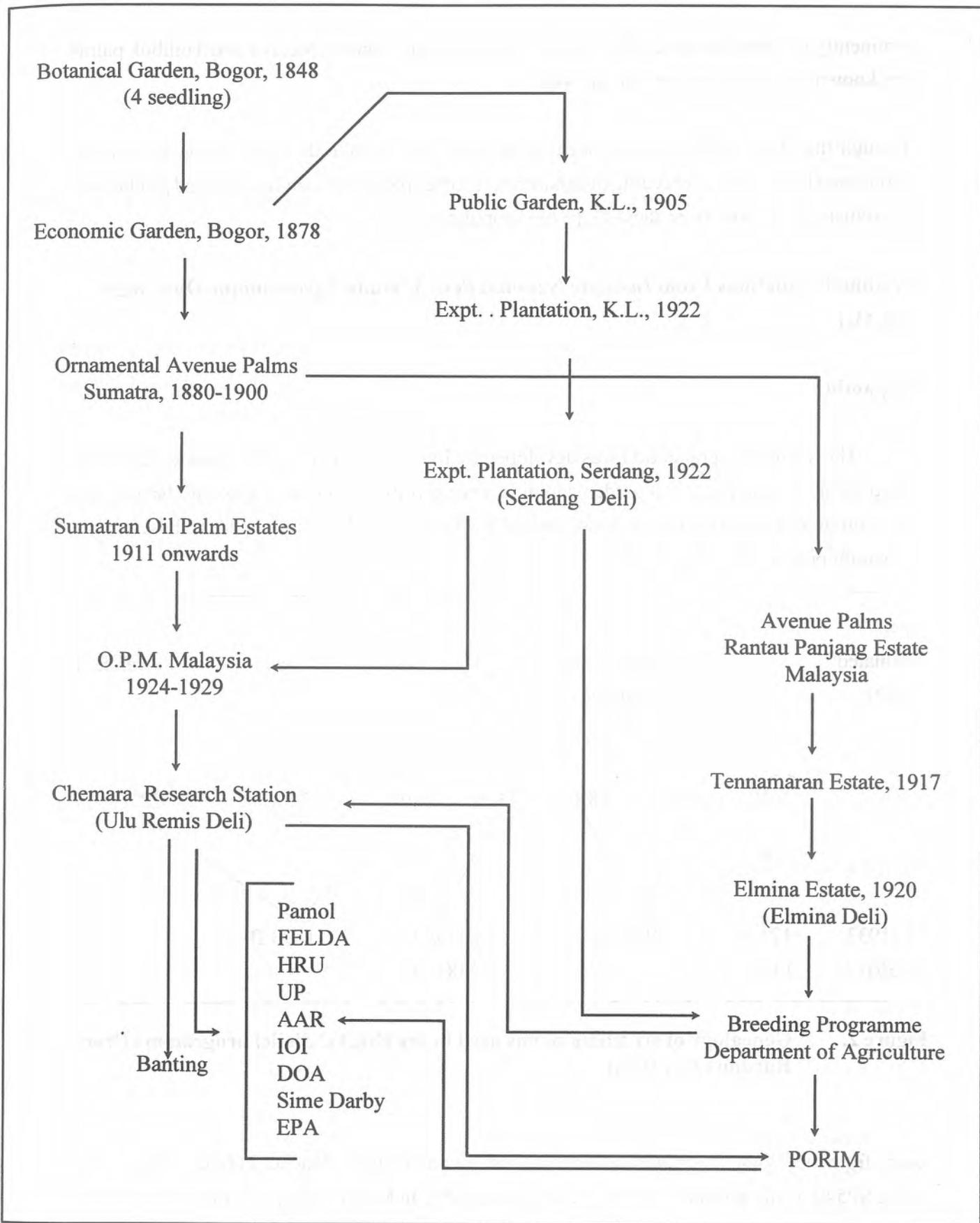
The Deli *dura* population, descended from the four palms, was introduced into Indonesia in 1848. The early oil palm breeding and selection efforts were mainly concentrated on this population. The Deli BPROs available in the Malaysia oil palm breeding programmes are shown below:

- Serdang Deli
- Elmina Deli
- Ulu Remis Deli (URD)
- Dumpy Deli
- Socfin Deli (JL)
- Dabou Deli
- Gunung Melayu Deli
- Tumbuk Deli

The Serdang, Elmina and Ulu Remis Deli are very closely related and have been widely distributed among research stations (Figure 1). The history and development of these BPROs have been well documented by Hardon and Thomas (1968).

The Socfin deli *dura* was developed independently from Deli *dura* introduced from Medang Arang, Indonesia. The Dabou deli is probably developed from Deli palms of the same source.

The Dumpy BPRO, developed from a single palm (Jagoe, 1952) through selfing and sibmating is an important source of genes for the slow vertical growth. Dumpy genes have featured



**Figure 1: The Origin and Distribution of *Deli dura* in Malaysia**  
(After Hardon and Thomas, 1968)

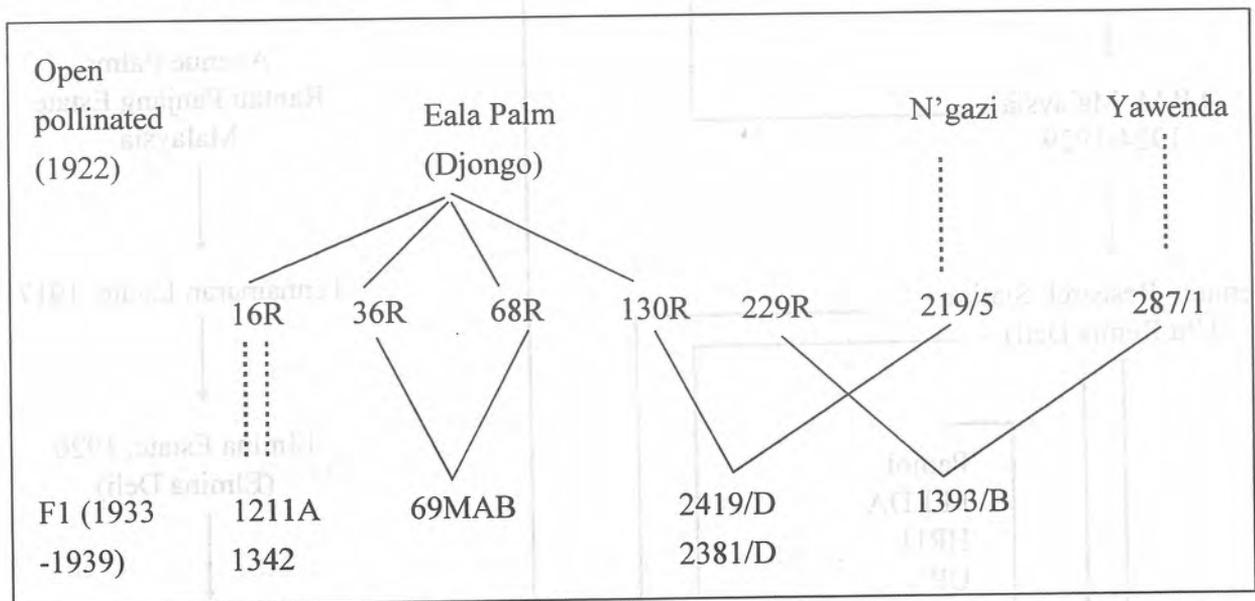
prominently in some Malaysian DxP seeds. Similarly, the Gunung Melayu and Tumbuk palms were known for their slow vertical growth.

Although the above BPRO's have been recognised, these materials would have been freely intercrossed in the various breeding programmes. Mother palms for commercial seed production are exclusively derived from these Deli *dura* populations.

### Breeding Populations From Institute National Pour L'etude Agronomique Du Congo (INEAC)

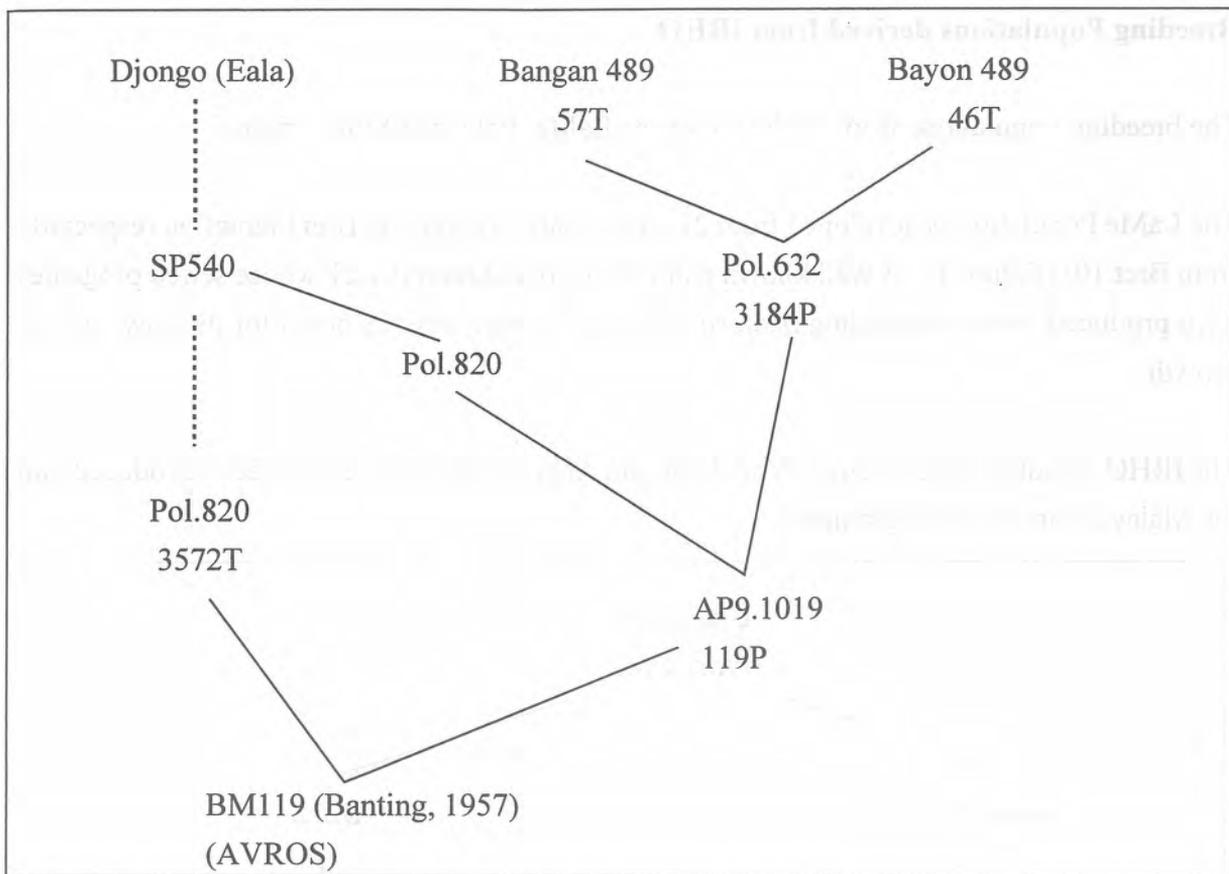
#### Yangambi

The Yangambi population was developed by INEAC from open pollinated seeds of one *tenera* palms in Eala Botanical garden and nine *teneras* from Yawenda, N'gazi and Isangi. Six *tenera* progenies developed from crosses and selfs of the selected palms formed the based of the Yangambi populations (Figure 2).



**Figure 2. Genealogy of six *tenera* palms used in the INEAC diallel programme (from Hardon *et al*, 1976)**

Seeds from the Djongo palm at Eala were also sent direct to Sungei Panchor in Indonesia and one palm, SP540, is the ancestor of BM119, often referred to in Malaysia as the AVROS (Figure 3).



**Figure 3. Origin of AVROS *pisifera* (After Rosenquist, 1986)**

The AVROS is well known for its precocity, high yields and vigorous growth.

The AVROS population in its pure or introgressed form has provided many *pisiferas* for commercial DxP seeds.

The Yangambi populations from INEAC has also been used in the development of Ulu Remis *Tenera* (URT) from which Guthrie's first *pisiferas* were derived (Rosenquist, 1989).

The Binga breeding population developed by INEAC is closely related to the Yangambi population and is also available in the breeding programmes.

### **Breeding Populations from Nigerian Institute for Oil Palm Research (NIFOR)**

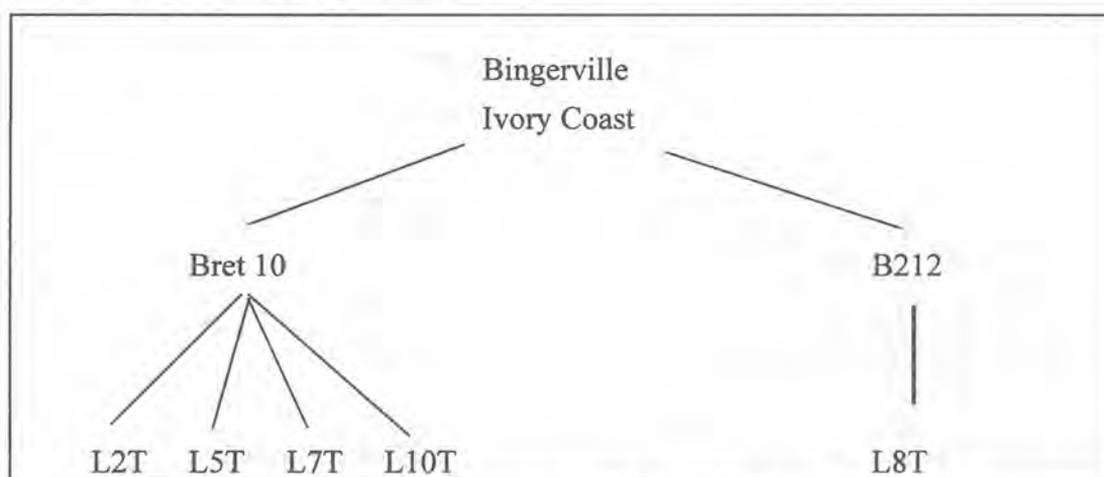
The Nigerian populations in the Malaysian breeding programmes are also important in the breeding of *teneras* from which commercial *pisiferas* are derived; e.g. Sabah Breeding Programme and Guthrie programme. A number of origins have been recognised including Aba, Calabar, Ufuma and Benin (Rosenquist, 1986). The NIFOR population will continue to play an important role in the oil palm breeding programmes in Malaysia.

## Breeding Populations derived from IRHO

The breeding populations from IRHO are of the LaMe, Pobe and Sibiti origins.

The LaMe Population is developed from 21 *tenera* palms mainly on Bret Plantation (especially from Bret 10) (Figure 4). A well known palm in this population is L2T whose selfed progenies have produced some outstanding *pisifera*. The LaMe population is noted for its slow vertical growth.

The IRHO breeding populations of Yocoboue and Angola origins have also been introduced into the Malaysia breeding programmes.



**Figure 4. The Origin of LaMe *Pisiferas* (After Rosenquist, 1986)**

The genetic resources from the oil palm breeding programmes in Malaysia will be further supplemented by the introduction of *Elaeis guineensis* prospections from Nigeria, Cameroon, Zaire, Madagascar, Tanzania, Angola and of *E. oleifera* germplasm from South America (Rajanaidu, 1985). This reservoir of germplasm will ensure the continued improvement of the oil palm breeding populations in Malaysia.

The oil palm breeding populations were made available to the Malaysian oil palm breeding programmes through importations, cooperative and exchange schemes. Some of the more important cooperative schemes are listed below:

- (1) The International Experiment (1946)
- (2) Cooperative Breeding Scheme (1956)
- (3) Oil Palm Genetic Laboratory (1963), now known as the Technical Liaison Committee
- (4) The Sabah Breeding Programme (1966)

### **The International Experiment**

In 1946, five breeding stations, namely IRHO stations at LaMe, Sibiti and Pobe, INEAC (Zaire) and SOCFIN (Malaysia) participated in an exchange of planting material on a large scale. This operation was subsequently known as the International Experiment (Hardon *et al*, 1976). This exchange programme introduced the Yangambi (Zaire), LaMe (Ivory Coast), Pobe (Dahomey) and Sibiti (Congo) materials into the Socfin breeding programme in Malaysia. These materials were subsequently distributed to the other breeding stations.

### **The Cooperative Breeding Scheme**

This scheme was initiated by the Department of Agriculture in 1956 (Hardon and Tong, 1959). The participants were Golden Hope, Guthrie, Socfin and United Plantations. This scheme resulted in the distribution of improved breeding populations especially the Deli *dura* (Dumpy, Elmina and Serdang), Serdang *pisiferas* and the *E. oleifera* among participating companies.

### **The Oil Palm Genetic Laboratory (1963-73)**

In 1963, Pamol, I.O.I., Guthrie and Golden Hope set up a joint research unit known as the Oil Palm Genetics Laboratory (OPGL) in Malaysia to improve oil palm breeding and undertake physiological studies on oil palm. Major efforts were directed towards the introduction of new breeding material, notably from Nigeria (NIFOR), the Cameroons, the Republic of Zaire and the Ivory Coast.

The limited oil palm breeding programmes of Pamol and I.O.I. were the result of their association with OPGL. The improved Ulu Remis Deli *dura* populations from Guthrie and Golden Hope and *tenera* populations from Nigeria, Cameroon and Zaire were made available to Pamol and I.O.I. in 1974.

### **The Sabah Breeding Programme (1966)**

This breeding programme for Sabah was initiated by C.W.S. Hartley for the production of high yielding oil palm planting materials for the Sabah agroclimatic conditions (Rajanaidu *et al*, 1990). Breeding materials for the Sabah Breeding Programme were obtained through an exchange scheme, organised among Malaysian (Guthrie, Golden Hope, Socfin and PORIM) and three African (NIFOR, Unilever Nigeria and Unilever Cameroons) groups.

Many other exchange schemes among breeding stations, both local and overseas, have taken place. These exchange schemes have made advanced genetic materials widely distributed among the Malaysian breeding programmes.

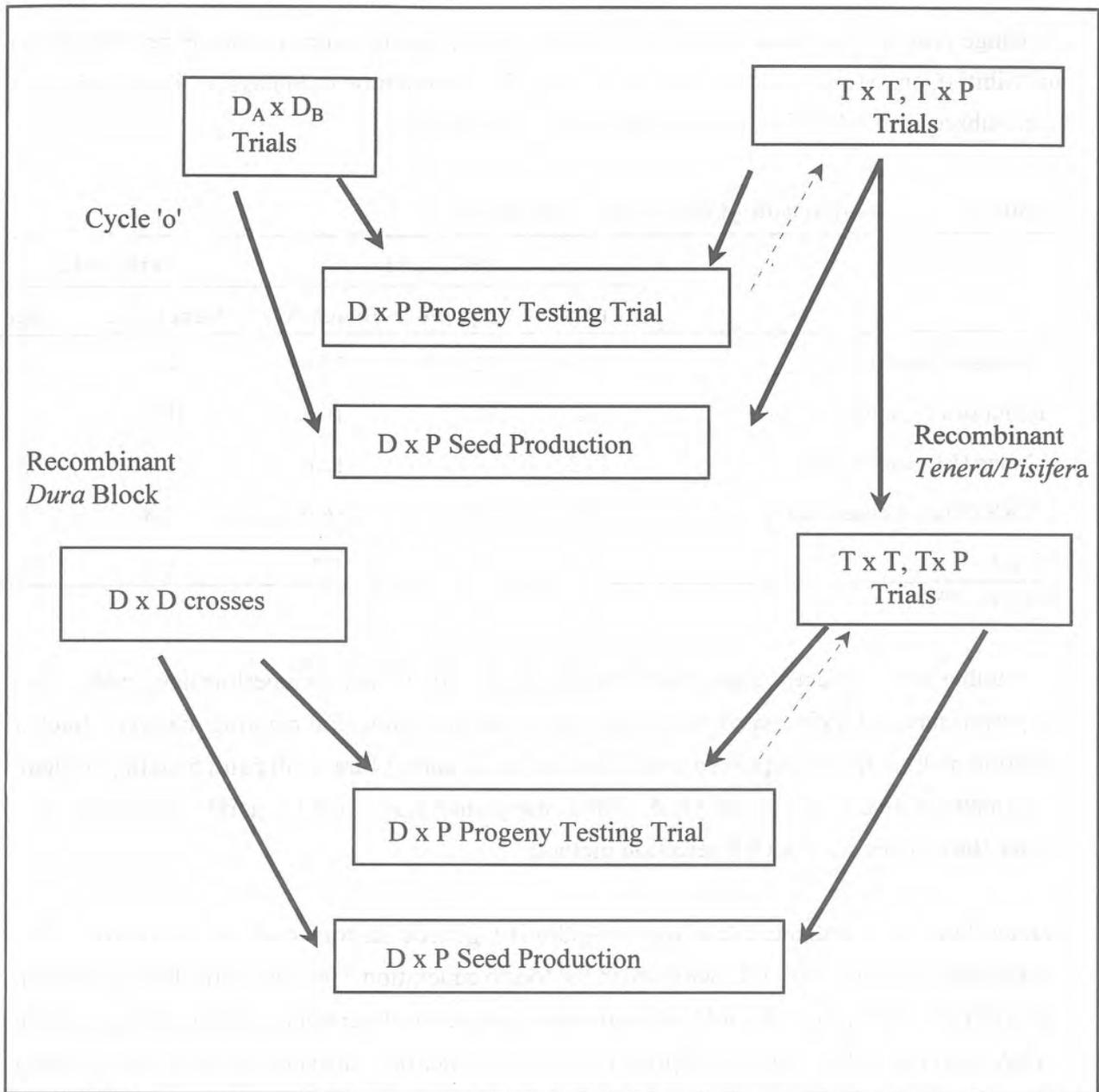
## BREEDING STRATEGIES

The early planting material before the 50's was the thick shell Deli *dura* and the improvement of Deli *dura* was done initially through mass selection. Family and individual palm (FIP) selection (Rosenquist, 1989) was subsequently followed as the initial studies had indicated low heritability of FFB yield and the bunch and fruit characters.

The discovery of the single gene inheritance by Beirnaert and Vanderweyen (1941) led to the exclusive commercial exploitation of the *tenera* the early sixties. The *tenera*, the thin shell oil palm variety, is derived through the hybridisation of the thick shell *dura* and the shell-less *pisifera*. The present day oil palm breeding programme is thus based on the breeding and selection of *dura* as the female palms and the *pisifera* as the male palms which, when hybridised, give high yielding DxP *tenera* progenies. A more sophisticated breeding method was therefore required.

The Malaysian oil palm breeding programmes follow basically the same breeding scheme which has often been referred to as Hardon's scheme or Modified Recurrent Selection and is shown in Figure 5. Generally, the *dura* and the *tenera* populations are kept separate to prevent inbreeding in the eventually DxP hybrids. Heterosis could also be exploited in the DxP inter-origin DxP crosses. Crosses within the respective populations are planted in replicated trials. *Dura* mother palms are selected following family and individual (FIP) strategy. *Pisifera* pollen parents are selected on their *tenera* sib performance and on DxP progeny test results.

Yield improvements achieved in the *dura* populations have been demonstrated by Yong and Chan (1988) and Lee *et al* (1988). The former indicated FFB yield improvements of 38% and 54% over that of the F1 generation for the F2 and F3 generations respectively. However, these increases were attributed to both breeding as well as agronomic efforts. The later authors indicated that the first generation of selection in Deli *dura* populations 12.4% and 19.4% improvements in FFB and oil yield respectively. Subsequent selection showed these in Deli populations achieved a selection progress of 8.8% and 11.7% per generation for FFB and oil yield respectively (Table 1). It will be difficult to achieve such rates of improvements in the advanced Deli population as there would be little additive genetic variation for these traits. This



**Figure 5: Malaysian Oil Palm Breeding Scheme**

exchange programme introduced the Yangambi (Zaire), LaMe (Ivory Coast), Pobe (Dahomey) and Sibiti (Congo) materials into the Socfin breeding programme in Malaysia. These materials were subsequently distributed to the other breeding stations.

**Table 1. Comparison of Deli *dura* Populations**

	FFB Yield			Oil Yield	
	t/ha	kg/palm	Bunch No.	Bunch Wt.	t/ha
F1 Bogor (unselected)	17.9	121.3	12.9	9.4	3.1
TjMorawa (unselected)	16.6	112.0	10.8	10.4	3.1
Elmina (F1 generation)	21.3	144.2	12.6	11.5	3.7
OPRS (F3&F4 generation)	26.9	181.5	16.0	11.4	5.0
F-test	**	**	**	**	**

(Lee *et al.*, 1990)

The mother palms selected using the FIP strategy depend on their own performance rather than the performance of their *tenera* progenies - the eventual commercial planting material. Such a selection method has been proven to be effective in the early years of oil palm breeding as there was much additive variation for yield. The consequent reduction in the additive variation will reduce the efficiency of such a selection method.

Studies have also indicated that the non-additive genetic factors may be important. The improvements in the FFB yield achieved in the fourth generation over that of the third generation were not reflected in the FFB yields of their *tenera* progenies of a common male parentage (Table 2, from Lee *et al.*, 1990). Parent offspring regression studies have also indicated that low yielding *dura* palms could also produce higher yielding *tenera* progenies than higher yielding *dura* palms, and vice versa, suggesting that the non-additive factors may be important (Figure 6, from Yong and Chan, 1990).

**Table 2. Mean yield on coastal soils of DxP progenies derived from different generations of Deli *duras***

Deli <i>dura</i>	<i>Pisifera</i>	No. of Progenies	Mean Yield (t/ha)	
			FFB	Oil
UR (3rd generation)	BM 119	52	30.9	7.80
UR (4th generation)	BM 119	8	31.0	8.30

(Lee *et al.*, 1990)

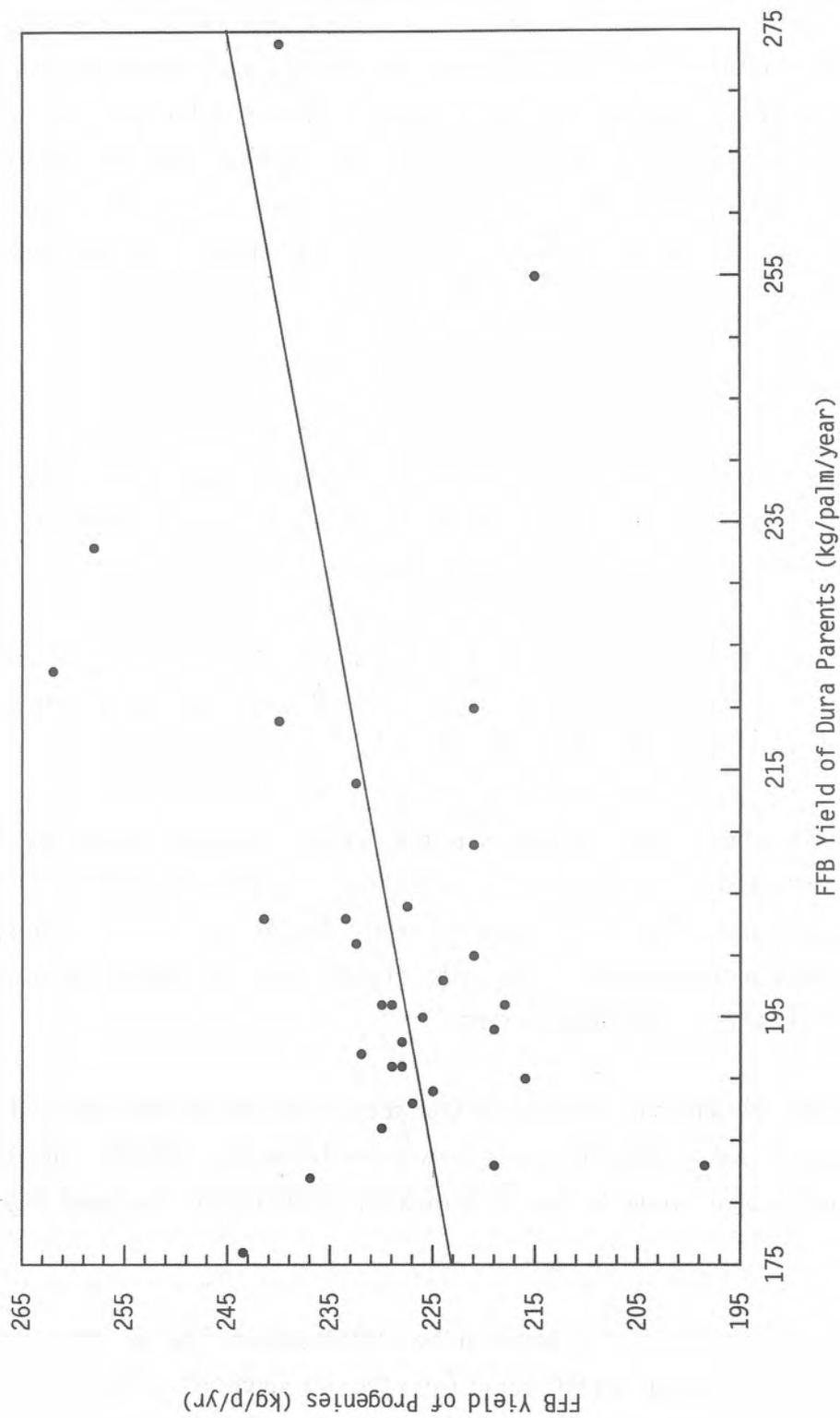


Fig 6: The linear regression of progenies on *Dura* parents.  
for FFB yield ( $a=193+20$ ,  $b=0.175+0.1$ ,  $P < 0.09$ )

It would be appropriate at this juncture for the Malaysian oil palm breeding programmes to exploit the non-additive genetic variation in the production of high yielding DxP oil palm planting material. A breeding strategy more akin to that of the Reciprocal Recurrent Selection (RRS) scheme as practised by IRHO could be followed. In fact many Malaysian oil palm breeding stations have of late introduced the RRS scheme, albeit in a limited way, in their breeding programmes. The Sabah Breeding programme is one example. The utilisation of the Nigerian propection material in the Malaysian breeding programmes has also proceeded in this manner. The creation of commercial *dura* mother palm blocks developed from intercrosses of progeny tested mother palms have also been attempted.

### **Commercial DxP Seeds**

Guthrie, Felda, Golden Hope, HRU, U.P. and DOA, Sabah are the main producers of the 30-35 million commercial DxP seeds which are required annually. Pamol, I.O.I. Sime Darby, AAR and EPA produce limited quantity of seeds for their own estates.

All the commercial seed producers use the Deli *dura* populations (Serdang, Elmina and Ulu Remis) as the source of their mother palms. The commercial DxP seeds in Malaysia are therefore recognised by the origin of their *pisifera* populations.

The Guthrie *pisiferas* were developed from the Ulu Remis *Tenera*, a population developed from the cross of URD with bulked Yangambi pollen from 52 palms imported from INEAC in 1947. The URT were crossed among URT, Serdang *pisifera* and INEAC *pisifera* to produce the *pisiferas* for the production of commercial DxP seeds. Guthrie have also introduced *pisiferas* developed from IRHO (LaMe + Sibiti) populations.

Felda produces two different kinds of commercial DxP seeds, one with *pisiferas* derived from IRHO (LaMe) population and the other from *pisiferas* derived from the AVROS. The former possess slower vertical growth while the later is known for its high yields but more vigorous growth.

The Golden Hope materials are based on *pisiferas* of the AVROS origin. The next improvement in the Golden Hope DxP materials would come from the introduction of Dumpy AVROS (AVROS introgressed with the Dumpy genes). This would reduce the rapid vertical growth of these material.

The HRU DxP material is based on the Dumpy Avros *pisiferas* which are noted for their slow vertical growth. The AAR DxP materials are similarly produced from the *pisiferas* derived from the same Dumpy Avros progenies.

United Plantations produces commercial DxP seeds with *pisiferas* derived from Yangambi populations developed by IRHO.

The DOA, Sabah *pisifera* palms are derived from *tenera* populations of Cameroon and Nigerian origins.

Pamol, I.O.I., EPA and Sime Darby produces only limited number of seeds for their own estates. Being relatively new in oil palm breeding programmes, they would prefer to use the proven *pisiferas* of AVROS origin developed by PORIM.

### **The Inter-Origin Trial**

The inter-origin trial evaluating the commercial DxP progenies of various origins was conducted at selected sites in Malaysia, Indonesia, South America, West Africa and Zaire in the mid-seventies. The results of the FFB yield extracted from published data of four of these trials - one in Indonesia (Nouy *et al.*, 1991) and three in Malaysia (Rajanaidu, *et al.*, 1986) are shown in Table 3. The mean FFB yield of four DxPs common to four locations was computed to indicate the environmental effect. Yields recorded in Indonesia was higher than that recorded in Malaysia and the coastal areas of Malaysia yielded higher than the inland area.

The FFB yields were also expressed as a percentage of the Deli x LaMe, a high yielding material and very popular in Indonesia. The Malaysia DxP (Deli x AVROS, Deli x Deli + Congo + Nigeria + Serdang) were comparable to the Deli x LaMe in the inland trial at Guthrie and they were higher yielding than the Deli LaMe in the coastal areas.

The oil yield per palm also indicated that the Malaysian DxP of Deli x Avros and Deli x Deli + Congo + Nigeria + Serdang performed favourably compared to that of Deli LaMe (Table 4). These results have indicated the effectiveness of the oil palm breeding programme in Malaysia.

Whilst it is true that current planting material may be different from that used in the comparative trial. Such trial are nevertheless very useful in indicating the effectiveness of the oil palm breeding programmes and should be conducted regularly through a joint international effort.

Table 3: The comparative performance of commercial DxP from various at origin four locations in Indonesia and Malaysia.

	Source	*Indonesia		**Guthrie (inland)		**Golden Hope (coastal)		** United Plantation (coastal)	
		FFB Yield (5th-9th year)		FFB Yield (4 <sup>th</sup> -8 <sup>th</sup> year)		FFB Yield (4 <sup>th</sup> -7 <sup>th</sup> year)		FFB Yield (3 <sup>th</sup> -7 <sup>th</sup> year)	
		(kg/pa/yr)	(% of A)	(kg/pa/yr)	(% of A)	(kg/pa/yr)	(% of A)	(kg/pa/yr)	(% of A)
A	Deli x La Me	214.0	100.0	141.4	100.0	192.0	100.0	200.4	100.0
B	Deli x Yangambi	204.0	95.3	132.4	93.6	190.3	99.1	203.2	101.4
C	African <i>Duras</i> x Cowan	212.0	99.1	127.0	89.8	176.3	91.8	193.2	96.4
D	African <i>Duras</i> x Yaligimba	212.0	99.1	128.0	90.5	165.3	86.1	195.0	97.3
E	African <i>Duras</i> x Binga	216.0	100.9	-	-	-	-	195.8	97.7
G	Deli x AVROS	-	-	126.2	89.3	204.5	106.5	201.6	100.6
H	Deli x (Deli + Congo + Nigeria + Serdang)	-	-	145.4	102.8	218.0	113.5	223.8	111.7
I	Marihat	206.0	96.3	-	-	-	-	-	-
	Mean (A.B.C.D.)		210.5		132.2		180.9		198.0

\* Extracted from Nouy et al 1991, \*\* Extracted from Rajanaidu et al, 1985

Table 4. The Comparative Performance of commercial DxP from various at origin four locations in Malaysia and Indonesia.

	Source	** Malaysia (Mean of 3 locations)			* Indonesia		
		(O/B)	(O/P) (kg/pa)	(% of A)	(O/B)	(O/P) (kg/pa/yr)	(% of A)
A	Deli x La Me	23.5	41.62	100	24.1	51.36	100
B	Deli x Yangambi	25.4	44.50	107	24.3	48.96	95
C	African Duras x Cowan	22.9	37.97	91	23.0	48.76	95
D	African Duras x Yaligimba	21.5	36.24	87	23.0	48.76	95
E	African Duras x Binga	-	-	-	22.0	47.52	93
F	Deli x (27B+233B+Local Un.)	21.9	38.56	93	-	-	-
G	Deli x AVROS	23.4	46.13	111	-	-	-
H	Deli x (Deli + Congo + Nigeria + Serdang)	23.9	46.58	112	-	-	-
I	Marihat	-	-	-	24.5	49.44	96
	Mean (A, B, C, D)	23.3	-	-	23.6	-	-

\* Extracted from Nouy et al, 1991

\*\* Extracted from Rajanaidu et al, 1985

## Clonal Palms

The potential of oil palm clones in raising the yields and fixing other desirable traits in oil palm has been recognised by the research stations. Exploited yield improvements of 30% and 15% had been quoted by Hardon *et al.* (1987) and Soh (1986) respectively. Many of these research organisations have established their oil palm tissue culture laboratories since the early eighties. Currently they are nine tissue culture laboratories and they are listed below:

- (1) Pamol (1975)
- (2) Golden Hope (1979)
- (3) PORIM (1980)
- (4) HRU (1981/82)
- (5) Sime Darby (1981/82)
- (6) FELDA (1983)
- (7) GUTHRIE (1984)
- (8) I.O.I. (1985)
- (9) Eastern Plantations Agency (1989)

Most laboratories have reported abnormalities in their clonal palms though the frequency differed (Corley *et al.*, 1981; Corley *et al.*, 1986; Ho & Tan, 1989). The factors leading to abnormalities are yet to be ascertained and much of the research work in the laboratories are veiled in secrecy.

Each year more and more clones are being evaluated in the field and the successful commercialisation of oil palm clones will depend on their field performance.

Elite DxP hybrids identified from progeny trials can also be reproduced in large numbers through cloning of the parents and producing seeds from these clonal parents. This approach has been followed by some of the laboratories. Though the abnormality problem may be overcome, the full potential of clonal palms will not be realised.

## CONCLUSION

The importance of planting high yielding oil palm planting material has been recognised from the very onset of the oil palm industry in Malaysia. The oil palm breeding programmes in Malaysia are being carried out by thirteen research institutions. The ease of accessibility of breeding populations through cooperative and exchange programmes among international and local research stations has contributed towards the success of the breeding programme.

PORIM's accessions of germplasms from Nigeria, Cameroon, Zaire, Madagascar, Tanzania and Angola would ensure the availability of a reservoir of genetic variability which is necessary for the continued improvement of the oil palm.

The Malaysian oil palm breeding programmes generally follow the modified form of recurrent selection method and this has been successful in the exploitation of the additive genetic variation in the production of high yielding commercial DxP seeds. The oil palm breeders are now turning their attention into exploiting the non-additive variation in their breeding populations by introducing the reciprocal recurrent selection strategy for the reproduction of elite hybrids using selfs or clones of parents.

The existence of different commercial seed producers have provided the industry with the choice of planting material to suit their needs.

The vegetative propagation of oil palm through tissue culture will play an important role in the exploitation of the genetic variability existing in the current oil palm breeding programmes. Desirable trials can be fixed more rapidly than through the conventional breeding methods. This will enable the breeding programmes to respond more quickly to the needs of the industry.

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## OIL PALM BREEDING IN GHANA

Wonkyi-Appiah, J.B.<sup>1</sup>

### INTRODUCTION

Oil palm breeding in Ghana has mainly involved research in oil yield and characters directly or indirectly influencing oil yield i.e. bunch yield (bunch number and average single bunch weight), fruit-to-bunch ratio, shell-to-fruit ratio, kernel-to-fruit ratio and oil-to-bunch ratio. Other characters which indirectly affect yield eg. disease resistance and height are also investigated. Genetic investigations have also been conducted on fertile *pisifera* palms.

The improvement plan of oil yield and its components is the adaptation of the reciprocal recurrent selection (RRS). The aim is to improve the oil production of planting material intended for commercial plantations. This requires the manipulation of quantitative characters which, for the most part, have low heritability. The best progress is therefore obtained by combining phenotypic choices for characters with good heritability in progeny tests. The superior combination of materials with large number of small bunches, on the one hand, and small number of large bunches, on the other, is greatly exploited.

### Breeding Methods

The main methods or approaches employed are:

- introduction of genetic material
- prospection
- selection
- hybridisation with *Elaeis oleifera* (South American oil palm)
- tissue culture or *in vitro* propagation
- mutation breeding
- exploitation of the fertile *pisifera*

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## **Introduction of genetic materials**

Parent breeding materials were introduced mainly from NIFOR (Nigerian Institute for Oil Palm Research). Materials were of the following origin: Nigeria, Cote d'Ivoire, Angola, Zaire, Malaysia and Indonesia. Later additions were made through special 'Exchange Programme' with Chemara Research Station of Malaysia.

The materials consisted of seeds and pollen (Deli *dura*, African *dura* and *tenera* crosses and selfings). The introduced materials were planted in 1961, 1964, 1966, 1967, 1968, 1973, 1979 and 1980 at Okumaning and Kusi near Kade. They were either planted as progeny trials or unreplicated blocks depending on the type of material.

## **Prospection**

A greater part of the OPRI genetic material is from NIFOR. It is a remarkable fact that most of the oil palms at present being cultivated originated from a few selections or from palms chosen during prospecting in certain very small areas, the only quality of such areas being their easy accessibility.

Several generations of breeding and selection have raised the productivity of the local material to much higher levels. It is, however, likely that these levels could have been reached much earlier if the original parent palms used for the breeding work had not been selected until more extensive prospecting had been carried out in the main grove areas. The average quality in some parts of these groves is remarkably good and, even now, the introduction of systematic prospecting would be most rewarding.

Under the auspices of the Scientific Committee of the African Oil Palm Development Association (AFOPDA) a joint prospection for oil palm germplasm is being envisaged. The prospection will cover the following areas:

- i) Angola
- ii) Zaire
- iii) Cameroun
- iv) Congo and Gabon
- v) Cote d'Ivoire
- vi) Tanzania and Burundi (along the banks of Lake Tanganyika)
- vii) Gambia, Ghana, Guinea, Liberia, Senegal and Sierra Leone
- viii) Madagascar.

## **Selection**

The principal elements of the selection plan are as follows:-

- the planting material is divided into two groups, Group A consisting of material with a small number of large bunches and Group B containing those with a large number of smaller bunches;
- trees are selected from A and B for characters with good heritability;
- the A x B crosses produced between selected trees are planted in comparative trials to assess their characteristics and combining ability of the trees chosen;
- combinations within A and B are performed to concentrate favourable genes. At this stage, it is also possible to introduce new types of material coming from prospectations. Trees are selected from the re-combinations to initiate a new breeding cycle;
- seed production is from A x A and B x B progenies of the trees which have given the best crosses.

### **Selection in the Nursery**

The long pre-nursery and nursery period (11-16 months) provides ample opportunity for selection before field planting. At an even earlier stage during the germination, selection on time of germination is possible, but experiments to date (Sparnaaij, 1955; Menendez and Blaak, 1964) have not shown that this procedure has any effect on the performance in the field.

Selection in the pre-nursery is not promising either as at this stage observed difference reflect only the date of germination. Chlorotic and malformed seedlings are of course removed before transplanting to the nursery.

Nursery selection can be either negative, consisting of the removal of abnormal or unhealthy plants, or positive, aimed at selecting plants or progenies which are likely to be more precocious and perhaps, more productive.

The advantages of the positive selection of individual plants are more questionable. The most vigorous plants can easily be detected by comparative leaf measurement and, because of their advanced development, these palms are, in general, the first to start fruiting. Their early yields are for the same reasons, usually somewhat higher than those of unselected palms, but nothing can be said about their mature yields.

The results of a statistical experiment at NIFOR in Nigeria show that the 50% best palms selected in the nursery have a small (4%) but non-significant advantage over the unselected ones in the first 5 years of production. Even if this advantage is maintained in later years, it is still too small to justify the doubling of seed production and, consequently, the lowering of selection standards for seed trees.

There is a correlation between the number of leaflets in the leaves of very young nursery seedlings and yields in the mature palms. Palms with a higher leaflet number retain this characteristic in later years and show a significantly higher average bunch weight, although the yields do not differ noticeably (WAIFOR, 1961 - 1964; de Berchoux and Gascon, 1965).

There is a significant correlation between the *average height of a progeny* in the nursery and *early yields* (WAIFOR, 1965). A selection for (genetic) *precocity* thus appears practicable.

### **Selection on Yield**

The oil yield is a composite figure, the major elements being the bunch number, the average bunch weight, the fruit-to-bunch ratio, the mesocarp-to-fruit ratio and the oil-to-mesocarp ratio. In defining selection criteria a choice must be made as to priority to be given to any of these elements. Such a choice must be based on the *variation* and on the *heritability* of the various factors.

The variation is greatest in the bunch yield factors (bunch number and average bunch weight). The highest observed individual bunch yield in a selection field is usually several times higher than the field average. The maximum oil-to-bunch value that is theoretically possible is only 1½ to 2 times the present day commercial figures. The scope of yield selection is therefore greater than that of bunch quality selection. In most breeding centres, however, priority has been given to the improvement of fruit and bunch quality because this is simpler to effect. Reliable data become available at a much earlier date (one year is usually sufficient) and heritabilities are higher. Since the average fruit and bunch quality has risen to such a high level that further spectacular increases are unlikely, the bunch yield is at present the most important selection criterion.

In a breeding programme as outlined above yield selection has to be carried out both at the progeny level (comparative progeny trials) and at the individual level. In order to be effective, selection must be based on reliable yield records and on some knowledge of the inheritance of yield and its components.

Progeny trials are designed to determine the relative yields of a number of progenies under a given set of external conditions. Replication and randomisation eliminates to some extent the confusing effect on relative progeny yields of soil variations within the trial area, but it cannot eliminate the effect of soil variations on the comparison of individual palms. Progeny selection should therefore, whenever possible, precede individual selection and any high yielding individuals found in poor progenies should be regarded with suspicion.

Yield recording over a period corresponding to the economic life span of a plantation undoubtedly provides the best basis for selection. In practice, however, selection must usually be based on much more limited data if a rapid succession of generations is to be achieved.

Before the relative performance in a progeny trial can be translated into the relative value of a progeny (or individual) for further breeding or seed production, the following factors affecting selection efficiency must be considered:

- Age and mutual competition for light;
- Soil conditions;
- Climatic conditions;
- Inheritance of yield and its components

### INHERITANCE OF YIELD AND ITS COMPONENTS

It is assumed by all oil palm breeders that bunch number and bunch weight are independently inherited as additive quantitative factors, and most breeding schemes (including that of OPRI) are based on this assumption. Pronk and Westenberg (1955) were the first to formulate this hypothesis on the basis of the results of crosses between Deli *dura* palms and imported *dura* and *tenera* palms from Africa. Other results confirm that *all yields and quality factors (excepting for the time being, the oil-to-mesocarp ratio) are additively inherited* (Sparnaaij, 1969).

Consequently, it seems advisable to amend the present breeding procedure and to endeavour to obtain a reliable determination of the genetic values for as many parents as possible rather than to test a large number of promising combinations between a limited number of parents. This is more so for the economy of space.

Theoretically one estimate per parent would be adequate, but to achieve reasonable reliability, it is preferable to do at least 2 separate estimates of the genetic values.

## GENOTYPIC VALUES AND HERITABILITY

The improvement of oil production requires the manipulation of a number of quantitative characters. The progress will be achieved by concentrating on characters with good heritability.

A genetic study was conducted on the results of progeny trials at NIFOR and OPRI to assess genotypic values and heritability. Phenotypic and genotypic values of the bunch yield and its components and fruit characteristics were compared. These values will indicate the extent of progress to expect in the selection programme. The method of calculating individual genotypic (A) values for components of oil yield in the oil palm from the means of full-sib families was first proposed by Sparnaaij (1969).

The components of oil yield are inherited independently as additive quantitative factors (Pronk and Westenberg, 1955; Noiret *et al.*, 1966). The average relative performance of the progeny from two parents will therefore, be given by the equation

$$P = \frac{x + y}{2}$$

where P is the progeny mean value of the character concerned and x and y the respective parental (genotypic values). A solution of 3 algebraic equations of this type will give the genotypic value (a, b and c) of a character for the three palms A, B & C.

The correlation between phenotypic and genotypic values indicates the extent of response to be obtained if selection is based on phenotype. Values for number of bunches are generally much better than for single bunch weight, while the lowest coefficients were found for total bunch yield. Correlation coefficients are generally not substantially different when the genotypic value is estimated over year 1 + 2 or year 2 + 3. Results of these estimations are summarised in Tables 1 - 6 (van der Vossen, 1974).

The ultimate goal of an oil palm breeding programme is the production of good *tenera* palms. *Dura* parent palms should therefore be assessed by the performance of their *tenera* progeny for their bunch and fruit composition and consequently genotypic value (A) for components of bunch and fruit quality were estimated from the means of the *tenera* full-sib families and denoted as  $A_t$ . Nevertheless, for the *dura* and Deli *dura* palms A values were also estimated from means of *dura* offspring ( $A_d$ ) in order to be able to compare  $A_t$  and  $A_d$  values (Table 1).

## Prediction of Yield and Fruit Qualities in the Oil Palm

Studies conducted on the performance of individual palm trees involved in different crosses showed that yield and fruit quality factors of progenies can be predicted if the genotypic values (additive genetic variation) of the parents are assessed. Genotypic values of palms for different characters were calculated from which were derived correlation values showing the relationship between relative mean progeny performance and calculated values (Wonkyi-Appiah, 1974).

## Mesocarp Oil Determination

Results of mesocarp oil determinations by the direct Soxhlet extraction method and two other indirect methods were compared. In the indirect methods only the percentage of water is determined. A percentage fibre-to-mesocarp (constant) is then used to calculate the percentage oil-to-mesocarp. In one of the indirect methods a progeny mean fibre-to-mesocarp is used in the calculation, while in the other a general fibre-to-mesocarp mean of 16% is used. It was observed that in all the progenies studied, there was no significant difference between the results of the direct Soxhlet extraction and those of the indirect methods using the progeny mean fibre percentage for calculation (Tables 7 & 8).

It is suggested that the latter may be adopted for routine laboratory mesocarp oil determination in place of the Soxhlet in order to save time and reduce cost. (Wonkyi-Appiah, 1974).

## Exploitation of the Fertile *Pisifera*

For commercial oil palm plantations *tenera* is preferred to *dura* because of its superior fruit characteristics - thin shell and higher percentage of mesocarp, hence a higher yield per hectare. *Pisifera*, which is generally female sterile, is used only as the pollen parent for the production of the hybrid *tenera* commercial seed. However, of special interest is the fertile *pisifera* palm. Some *pisifera* palm trees have a good or even better bunch production than the *tenera* palms and give higher oil yield because of their higher oil-to-bunch ratio (extraction rate) see Table 9. Wonkyi-Appiah, 1980). Genetic factors controlling fertility in the oil palm have been investigated (Wonkyi-Appiah, 1987, 1989).

It is hypothesised that the gene controlling fertility, F (or f) is linked to the gene controlling shell D (or d). Some examples are given below showing the segregation pattern of crosses in the OPRI breeding material:

i)	<b>Parents</b>	<b>Progeny (Segregation)</b>			
	(851.53T x 4/3029FP)				
	<i>Tenera</i> x fertile <i>pisifera</i>	→	<i>Tenera</i>	<i>Pisifera</i>	
	Genotype D-F // d-f x d - F // d - f		D-F // d-F, D-F // d-f, d - F // d - f, d - f // d - f	Fertile	Fertile
			Fertile	Fertile	Partially Female Sterile
			2	:	1 : 1

ii)	851.194T x 4/3029F				
	<i>Tenera</i> x fertile <i>pisifera</i>	---->	<i>Tenera</i>	<i>Pisifera</i>	
	Genotype D - F // d - F d-F // d-f		D-F // d-F, D-F // d-f	d-F // d-f, d-F // d-f	
			Fertile	Fertile	Fertile
			Fertile	Fertile	Fertile
			1	:	1

To get a pure stand of fertile *pisifera* the following crosses can be made:-

<b>Parents</b>	<b>Progeny (Segregation)</b>
Fertile <i>pisifera</i> x fertile <i>pisifera</i>	→ Fertile <i>pisifera</i>
d.F // d - f x d-F // d-F	d-F // d-F
Genotype or	
d-F // d-f x d-F // d-F	→ d-F // d-F, d-F // dF, d-f // d-F
	d-f // d-F
	all fertile

The *pisifera* has the highest sex ratio. The fertile *pisifera* has the best fruit composition of all the 3 fruit forms because of the absence of shell and high percentage of mesocarp. It should therefore be theoretically ideal for commercial exploitation.

One of the problems to be encountered in commercial exploitation of the *pisifera* will be germination. The germination of the *pisifera* seed under laboratory conditions has been investigated by many workers (Thomas & Hardon, 1968; Arasu, 1970; Wonkyi-Appiah, 1975, Nwankwo, 1981).

*Pisifera* seeds can also be germinated under plantation conditions (Wonkyi-Appiah, 1973). The problem of germinating *pisifera* seeds may be avoided by making *tenera* x fertile *pisifera* crosses

with *tenera* as the female parent to get *tenera* seeds for germination. This will lead to the establishment of a population of 1:1 mixture of *tenera* and fertile *pisifera* palms.

## MUTATION BREEDING

Mutation breeding is aimed at increasing genetic variation by inducing mutation through irradiation. The work done so far on oil palm has been preliminary and exploratory (Wonkyi-Appiah, 1990).

### Effect of Gama Irradiation of Seeds and Pollen Grains of Oil Palm (*Elaeis guineensis* Jacq.)

It was observed that gamma irradiation (0-400Gy) from a cobalt 60 source induced germination in oil palm *dura* seeds within 2-5 weeks (Wonkyi-Appiah and Amu, 1976). There was no germination in the non-irradiated material during the experimental period of 8 weeks (Table 10).

The standard method of germinating oil palm *dura* seeds takes up to 3 months. Even though higher doses of irradiation gave higher percentage germination, it was observed that further growth after the emergence of the embryos, was greatly retarded leading to eventual death of the seedlings.

Work at the OPRI/GAEC has revealed that pollen tube formation of oil palm pollen grains was not inhibited up to a radiation dose of 50Gy (Wonkyi-Appiah, 1982). The irradiated pollen was used to artificially fertilize female flowers on *dura* palms. The effect of the different dosages on the fruits formed and the viability of the seeds are summarized in Table 11. There were significant differences between treated and untreated bunches in some of the fruit characters. The production of infertile fruits was very high in the treated material regardless of dosage. Consequently there was a depressive effect on seed germination and the correlation between irradiation dose and percentage germination was negative and significant (0.74 at 1% level). The seeds obtained above were germinated and planted for subsequent study.

Plants developed from the irradiated oil palm *dura* seeds were planted in 1977 (Field K12). Because of the depressive effect on germination a few or no plants survived from some of the doses for further study. The seedlings which were produced by artificially fertilizing female flowers with irradiated pollen were planted in 1979 (Field K14) and 1980 (Field K15). Here also some of the dosages did not produce any materials at all due to the high incidence of infertile fruits and poor germination (Table 10).

### **Seed Irradiation (Field K12)**

The plants derived from the non-irradiated material produced more bunches per tree than those derived from the irradiated material. However, some of the treated material performed equally well when total bunch production is considered (Fig. 1). Some individual trees in the treated material performed better.

### **Pollen Irradiation (Fields K14 and K15)**

In Field K14 pollen irradiation of up to 50Gy depressed bunch yields in the resulting plants (Fig. 2). However, in Field K15, the plants derived from the irradiated pollen grains performed equally well irrespective of dosage (Fig. 3).

So far only the effect of gamma irradiation on bunch production (B.Wt.) has been studied. The oil palm, like other perennial tree crops, remain in the field for several years. Data on the other production characteristics are available for study to find out if any useful mutants have been produced by irradiation in the area of sex ratio, percentage of shell, height and resistance to pests and diseases.

### **Hybridisation with *Elaeis Oleifera***

This project, aimed at the exploitation of the desirable features of the South American oil palm, *Elaeis oleifera*, is at an initial stage (though far advanced elsewhere, eg. IRHO Station in Cote d'Ivoire). Work done so far is introduction of pure *oleifera* material from Costa Rica. Materials were planted in 1979 and 1980 and are under observation. Some of the desirable features of the South American palm are oil fluidity (less saturated hydrocarbons), low growth, resistance to certain diseases and pests.

### **Tissue Culture/*In-Vitro***

The OPRI has started a preliminary work on tissue culture/*in vitro* propagation in collaboration with the Ghana Atomic Energy Commission (GAEC), Kwabenya. Training of specialists and installation of a laboratory could be achieved under contract or collaboration with one of the organisations having the necessary facilities and techniques. The technique of regenerating plantlets from young leaf tissue have been developed by the IRHO and Unilever.

## SEED PRODUCTION

Results of extensive progeny trials at NIFOR, Benin City Nigeria and at The Oil Palm Research Institute, Kade, Ghana were exploited to derive a crossing plan for DxP commercial seed production. The OPRI has issued about 20.5 million germinated seeds from 1969 to 1991. So far about 720,000 transplantable seedlings have been distributed. If properly utilised, 92,343 ha (1991) should have been planted using these materials. This is about 70% of the total estimated planted area of 129,607 ha (1991) in the country.

In Ghana the present yield potential for improved DxP is 17-20t FFB/ha (12-14t in commercial plantations). Allowing an extraction rate of 22%, this will give palm oil yield of 3.7-4t/ha (2.6-3.0t) in commercial plantations.

## CONCLUSION

In conclusion the following observations are being made:

i) The grouping of materials into A and B:

The basis for the superiority in yield for this combination has been demonstrated mathematically (Sparnaaij, 1969) and shown to be not due to the different origins but rather to the contrasting production characteristics of the two groups. However some breeding centres seem to identify one group as Deli *dura* and the other as African material and commercial seed production is on this basis. If this tendency continues and *duras* are selected from Delis only there is the danger of losing considerable African *dura* material of great potential.

ii) **Use of Genotypic Values**

Estimation of genotypic values for different characters of palms from a limited number of crosses which could be used to assess the potential of other combinations has been demonstrated. This will not only provide values for combinations which will be impossible to make and plant, but also partially solve the problem of lack of adequate space and time.

(Sparnaaij, 1969; Van der Vossen, 1974; Wonkyi-Appiah, 1974).

iii) **Estimation of oil in the mesocarp.**

A new modified indirect method of mesocarp oil estimation which is a modification of the Desassis method could be adopted to reduce time and cost since it has been demonstrated to be accurate.

(Van der Vossen, 1974; Wonkyi-Appiah, 1982).

iv) **Exploitation of fertile *pisifera* palms for bunch production**

Some breeding centres have rich source of fertile *pisifera* palms of good bunch production. These are ideal to commercial exploitation to increase palm oil yield.

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Table 1.

## Components of bunch yield

Phenotypic (P) and genotypic (A) mean annual values per palm in some groups of *tenera* and *dura* palms

Group size/fruit form	P1			A2		
	nb	w(kg)	yield (kg)	nb	w(kg)	yield (kg)
21 <i>tenera</i>	11.5	5.5	62.3	10.0	4.8	48.0
coeff. of variation (%)	28	23	34	26	29	28
15 <i>dura</i>	9.0	7.0	63.0	9.6	4.0	38.6
coeff. of variation (%)	44	54	32	39	49	48
9 Deli <i>dura</i>	7.5	8.4	62.2	6.9	7.4	51.5
coeff. of variation (%)	16	19	19	30	31	43
45 <i>tenera</i> + <i>dura</i>	9.8	6.6	59.2	9.2	5.1	46.0
coeff. of variation (%)	37	41	32	34	43	39

1. P: mean values over year 1+2+3+4
  2. A: mean values over year 1+2+3
- nb - number of bunches per palm per year  
w - average single bunch weight

Table 2. Coefficients of correlation between phenotypic (P) and genotypic (A) values for bunch yield and its two components, calculated over different sets of years.

	P(1+2+3+4) A (1+2+3)	P(1+2+3+4) A (1+2)	P(1+2+3+4) A (2+3)
<i>n<sub>b</sub></i>			
<i>tenera</i>	0.487* (n=21)	0.456* (n=24)	
<i>dura</i>	0.691*** (n=24)	0.654*** (n=26)	
<i>tenera</i> + <i>dura</i>	0.632*** (n=45)	0.568*** (N=50)	0.698*** (n=30)
<i>w</i>			
<i>tenera</i>	0.491* (n=21)	0.258* (n=24)	
<i>dura</i>	0.478* (n=24)	0.460* (n=26)	
<i>tenera</i> + <i>dura</i>	0.441** (n=45)	0.450*** (n=50)	0.382* (n=30)
Total yield			
<i>tenera</i>	0.328 (n=21)		
<i>dura</i>	0.281 (n=24)		
<i>tenera</i> + <i>dura</i>	0.297* (n=45)		
* P<0.05	<i>n<sub>b</sub></i> -	number of bunches per palm per year	
** P<0.01	<i>w</i> -	average single bunch weight	

**Table 3: Components of bunch and fruit quality. Phenotypic (P) and genotypic (A) mean values of some groups of *tenera* and *dura* palms. In brackets coefficients of variation(%)**

Group size/fruit form	fr (%)	m(%)	s(%)	k(%)	s.fr.w.(g)
31 <i>tenera</i>					
P	63.8 (9)	83.1 (7)	9.0 (45)	7.9 (34)	8.0 (35)
A <sub>t</sub>	65.6 (7)	79.0 (7)	12.5 (35)	8.5 (21)	6.5 (42)
18 <i>dura</i>					
P	66.7 (6)	55.9 (8)	33.0 (11)	11.1 (14)	
A <sub>t</sub>	66.6 (6)	77.2 (10)	12.9 (40)	9.9 (31)	
A <sub>d</sub>	67.7 (8)	50.7 (12)	37.5 (15)	11.8 (16)	
19 Deli <i>dura</i>					
P	68.2 (7)	59.7 (8)	30.9 (12)	9.4 (18)	
A <sub>t</sub>	72.0 (5)	77.8 (5)	13.8 (20)	8.4 (19)	
A <sub>d</sub>	72.5 (5)	35.9 (10)	34.7 (14)	9.5 (14)	

fr = fruit to bunch %

s(%) = shell to fruit

m(%) = mesocarp to fruit

k(%) = kernel to fruit

s.fr.w.(g) = single fruit wt.

**Table 4: Coefficients of correlations between phenotypic (P) and genotypic (A) values of components of bunch fruit quality.**

Component	P <sub>t</sub> -A <sub>t</sub>	P <sub>d</sub> -A <sub>d</sub>	P <sub>d</sub> -A <sub>t</sub>
single fruit weight	0.56** (n=22)	0.179 (n=30)	0.045 (n=37)
fruit to bunch ratio	0.577*** (n=31)	0.872*** (n=30)	0.249 (n=37)
mesocarp to fruit ratio	0.912*** (n=31)	0.744*** (n=30)	0.176 (n=37)
kernel to fruit ratio	0.178*** (n=31)	0.584*** (n=30)	0.541*** (n=37)

\* P<0.05

\*\* P<0.01

\*\*\* P<0.001

**Table 5: Estimates of narrow-sense heritability from offspring-mid-parent regressions for *tenera* components of oil yield, 95% confidence limits.**

Component	<i>tenera</i> x <i>tenera</i> families			<i>dura</i> x <i>tenera</i> families		
	h <sup>2</sup> /n = b	t.test b/sb	n	h <sup>2</sup> /n = 2b	t.test b/sb	n
number of bunches per palm per year	0.512 ± 0.212	4.83***	84			
average single bunch weight	0.206 ± 0.132	3.12**	84			
Total Yield	0.091	n.s.	84			
fruit to bunch ratio	0.550 ± 0.273	4.04***	50	0.179 ± 0.163	2.15	154
single fruit weight	0.686 ± 0.218	8.37***	48	0.604 ± 0.208	5.70***	145
mesocarp to fruit ratio	0.956 ± 0.177	10.99	50	0.799 ± 0.231	6.77*	154
shell to fruit ratio	1.064 ± 0.195	11.07***	50	0.793 ± 0.163	4.84***	154
kernel to fruit ratio	0.658 ± 0.252	5.78***	50	0.596 ± 0.188	6.21***	154
oil to mesocarp ratio	0.248	n.s.	34	0.230	n.s.	116

\* P<0.05      \*\* P<0.01      \*\*\* P<0.001      n.s. not significant

**Table 6. Estimates of narrow-sense heritability (h<sup>2</sup>) for components of oil yield and quality from the regression of genotypic (A) on phenotypic (P) values based on *tenera*, *dura* or *tenera* + *dura* progeny; 95% confidence limits.**

Component	Regression	n	h <sup>2</sup> /n = b	t test b/s <sub>b</sub>
number of bunches per palm per year	A <sub>t+d</sub> - P <sub>t+d</sub>	45	0.557 ± 0.210	5.34***
average single bunch weight	A <sub>t+d</sub> - P <sub>t+d</sub>	45	0.352 ± 0.220	3.23***
fruit to bunch	A <sub>t</sub> - P <sub>t</sub>	31	0.447 ± 0.241	3.79***
	A <sub>d</sub> - P <sub>d</sub>	30	0.235	n.s.
	A <sub>t</sub> - P <sub>t</sub>	37	0.05	n.s.
single fruit weight	A <sub>t</sub> - P <sub>t</sub>	22	0.559 ± 0.378	3.08***
mesocarp to fruit	A <sub>t</sub> - P <sub>t</sub>	31	0.881 ± 0.333	5.41***
	A <sub>d</sub> - P <sub>d</sub>	30	1.129 ± 0.438	5.28***
	A <sub>t</sub> - P <sub>d</sub>	37	0.323	n.s.
shell to fruit	A <sub>t</sub> - P <sub>t</sub>	31	0.993 ± 0.376	5.40***
	A <sub>d</sub> - P <sub>d</sub>	30	0.976 ± 0.340	5.88***
	A <sub>t</sub> - P <sub>d</sub>	37	0.193	n.s.
kernel to fruit	A <sub>t</sub> - P <sub>t</sub>	31	0.653 ± 0.241	5.33***
	A <sub>d</sub> - P <sub>d</sub>	30	0.603 ± 0.324	3.82***
	A <sub>t</sub> - P <sub>d</sub>	37	0.711 ± 0.396	3.80***

\* P<0.05      \*\* P<0.01      \*\*\* P<0.001      n.s. not significant  
t - *tenera*      d - *dura*

Table 7. Progeny Means of % fibre-to-mesocarp and % oil-to-mesocarp determined by different methods

Progeny	% fibre-to-mesocarp (Progeny mean) (*)	% oil-to-mesocarp			Difference	L.S.D
		0/M1	0/M2	0/m3		
Experiment 852-1						
5.12D x 4.493T	17.19 ± 1.76	42.52 ± 4.70	43.52 ± 4.96	42.26 ± 4.98	NS	
38/0401T x 26.0932D	17.58 ± 1.67	43.00 ± 4.01	44.52 ± 4.28	42.96 ± 4.31	NS	
1.2209D x G.147T	17.58 ± 1.89	46.07 ± 2.17	47.58 ± 1.62	46.30 ± 1.37	NS	
3.3164D x 4.1624T	17.57 ± 1.76	48.13 ± 7.06	50.20 ± 8.13	48.63 ± 8.11	NS	
1.53D x G.147T	18.26 ± 1.38	40.47 ± 4.40	42.74 ± 4.30	40.45 ± 4.31	NS	
1.2209D x 32.3005T	16.67 ± 1.38	44.83 ± 4.18	45.79 ± 4.02	45.18 ± 4.08	NS	
Experiment K1-1						
1.2229T x 5.1295D	16.40 ± 0.81	45.38 ± 2.90	46.71 ± 2.44	46.44 ± 2.39	NS	
4.17T selfed	18.96 ± 2.53	43.78 ± 3.51	46.47 ± 3.82	43.46 ± 3.79	S*	2.48
3.415T x 1.2209D	17.09 ± 1.26	41.83 ± 7.01	44.21 ± 5.87	42.97 ± 5.85	NS	
14.892T shelved	18.26 ± 1.89	37.14 ± 6.43	39.50 ± 5.13	37.24 ± 5.11	NS	
1.2229T shelved	16.11 ± 1.45	43.44 ± 4.84	44.79 ± 4.49	44.68 ± 4.49	NS	
Mean	17.42 ± 0.86	43.33 ± 2.94	54.09 ± 2.79	43.69 ± 3.12		

0/M1 = % oil-to-mesocarp determined by the 'direct method' (Soxhlet)

0/M2 = % oil-to-mesocarp determined by the 'indirect method'

0/M3 = % oil-to-mesocarp determined by the 'modified indirect method' (modifiee)

NS = Differences not significant

S\* = Difference significant at

(\*) Mean of 40 samples (10 palms; 4 bunches per palm)

Table 8. Palm means of % oil-to-mesocarp determined by different methods

Palm No.	% fm*	% oil-to-mesocarp			Difference	L.S.D.
		0/M1	0/M2	0/M3		
852.654	17.19 ± 1.76	38.45 ± 6.19	39.04 ± 7.09	37.70 ± 7.00	NS	
852.110	17.58 ± 1.67	42.66 ± 2.88	45.03 ± 2.53	43.43 ± 2.53	NS	
852.5	17.58 ± 1.89	45.68 ± 4.24	45.91 ± 4.52	44.29 ± 4.85	NS	
852.157	17.57 ± 1.76	46.16 ± 3.55	46.52 ± 3.49	45.03 ± 3.47	NS	
852.286	18.26 ± 1.38	45.30 ± 2.77	48.06 ± 2.44	45.78 ± 2.44	S*	2.13
852.82	16.67 ± 1.38	48.87 ± 1.77	50.84 ± 2.35	50.14 ± 2.35	NS	
K1 2964	16.40 ± 0.81	43.84 ± 4.52	45.23 ± 3.59	44.82 ± 3.58	NS	
K1 3281	18.96 ± 2.52	43.74 ± 4.35	44.92 ± 5.38	41.92 ± 5.38	NS	
K1 3047	17.09 ± 1.26	51.20 ± 3.54	51.67 ± 2.27	50.57 ± 2.27	NS	
K1 3692	18.26 ± 1.89	43.26 ± 3.26	47.13 ± 2.53	44.84 ± 2.50	S**	2.86
K1 2188	16.11 ± 1.45	44.30 ± 4.17	46.20 ± 4.04	46.10 ± 4.04	NS	
Mean	17.42 ± 0.86	44.86 ± 0.86	44.41 ± 3.33	44.97 ± 3.54		

\* fm = progeny mean fibre %  
 0/M1 = % oil-to-mesocarp determined by the 'direct method' (soxhlet)  
 0/M2 = % oil-to-mesocarp determined by the 'indirect method'  
 0/M3 = % oil-to-mesocarp determined by the 'modified indirect method'  
 NS = Difference not significant  
 S\* = Difference significant at  
 S\*\* = Difference significant at

**Table 9. Bunch fruit characteristics and yield of 21 best palms out of 60 palms in the cross 851, 194T x 4/3029FP (planted in 1969)**

	Bunch and Fruit Characteristics						Yield per year (kg) (1972-1978)	
	% F/B	%/M	%/S	%/K	%/OM	Extraction rate	Bunch wt	Palm oil
K3: 2621FP	62.3	96.1	-	3.9	57.2	0.342	125.6	43.0
K3: 3193T	65.7	64.5	19.2	16.4	52.3	0.222	116.0	25.8
K3: 2980FP	63.7	90.7	-	3.3	54.9	0.338	112.8	38.1
K3: 3124FP	63.3	95.0	-	5.1	57.2	0.344	112.7	38.8
K3: 3190FP	72.6	95.6	-	4.4	61.5	0.427	111.4	47.6
K3: 2745FP	68.7	97.8	-	2.2	62.9	0.423	107.4	45.4
K3: 2867T	65.9	76.4	12.1	11.5	65.8	0.331	105.3	34.9
K3: 2622FP	63.3	98.3	-	1.8	57.0	0.355	102.8	36.5
K3: 2981T	65.4	69.4	15.6	15.0	54.6	0.248	102.2	25.3
K3: 3158FP	70.2	97.7	-	2.3	57.1	0.392	98.8	38.7
K3: 3324FP*							98.6	
K3: 3988T*							94.9	
K3: 2746FP	65.4	96.4	-	3.7	56.1	0.354	94.9	33.6
K3: 3226FP	62.3	95.2	-	4.8	62.0	0.368	94.8	34.9
K3: 2904FP	63.8	94.8	-	5.3	56.3	0.341	94.7	32.3
K3: 3225T*							91.8	
K3: 3125FP	63.5	94.8	-	5.2	51.2	0.308	91.4	28.2
K3: 3015FP	63.6	97.7	-	2.3	56.1	0.349	91.0	31.8
K3: 2705FP	66.6	97.0	-	3.0	52.6	0.340	90.2	30.7
K3: 3052FP	70.0	97.5	-	2.5	57.4	0.392	90.1	35.3
K3: 2703FP	74.0	96.7	-	3.3	54.5	0.390	88.5	34.5

FP = Fertile *pisifera*

T = *Tenera*

% F/B = Fruit-to-bunch ratio

%M = Mesocarp-to-fruit ratio

%S = Shell-to-fruit ratio

%K = Kernel-to-fruit ratio

% O/M = Oil-to-mesocarp ratio

\* = Fruit characteristics not determined due to absence of bunches at the time of sampling.

**Table 10. Weekly cumulative percentage germination of dura oil palm seeds irradiated after soaking in water**

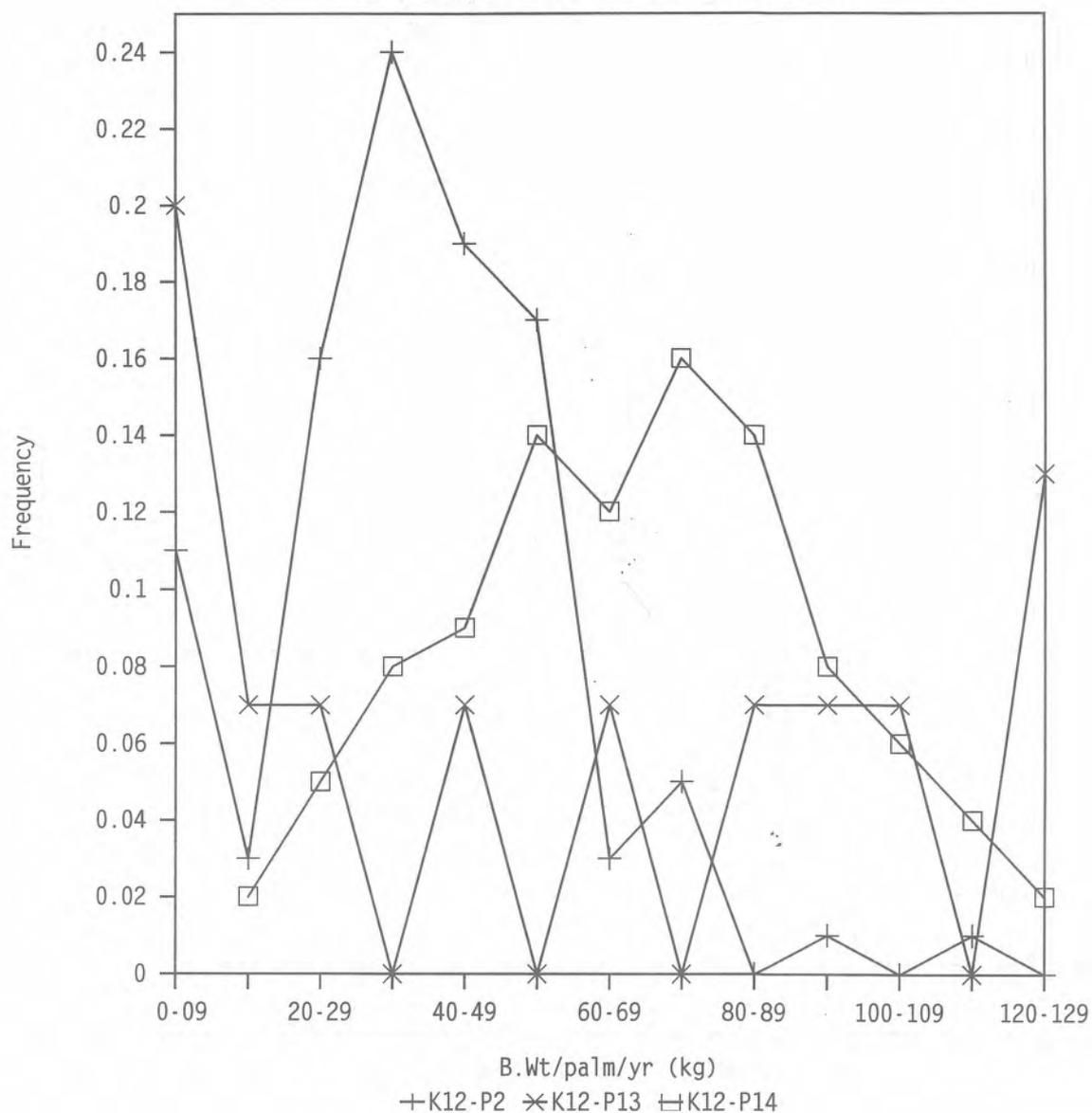
Dose (Krad)	Cumulative percentage germination						
	2 <sup>nd</sup>	3 <sup>rd</sup>	4 <sup>th</sup>	5 <sup>th</sup>	6 <sup>th</sup>	7 <sup>th</sup>	8 <sup>th</sup>
0 (control)	0	0	0	0	0	0	0
1	0	0	0	1	1	1	1
5	1	1	1	1	1	1	1
10	0	0	2	4	4	4	4
20	0	9	16	22	24	24	24
40	0	3	20	28	29	30	31
60	0	0	5	17	31	38	39
80	0	0	3	25	55	59	61
100	0	0	18	35	47	51	56
120	0	0	1	15	40	58	63
140	0	0	1	10	36	48	55

**Table 12 Fruit and bunch characteristics and viability of seeds of treated and untreated bunches**

Palm Number	Doses of irradiation (Gy)	Fruit-to-bunch ratio (F/B%)		Mesocarp-to-fruit ratio (M%)		Shell-to-fruit ratio (S%)		Kernel-to-fruit ratio (K%)		% of Infertile fruit		% of kernels without embryos	
		Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
K1. 3981	10	62.6	67.2	58.2	55.5	33.6	36.0	8.2	8.5	*	*	*	*
K1. 1988	10	70.3	69.4	54.4	59.2	35.5	32.2	10.1	8.6	23.0	0	8.4	1.6
K1. 2520	15	66.7	71.0	55.6	57.3	37.0	33.2	7.4	9.5	15.0	3.7	50.0	0
K1. 3967	20	63.8	68.1	70.4	62.5	25.0	26.7	4.6	7.8	11.8	0	*	3.5
K1. 2823	20	43.4	62.1	57.7	53.1	36.6	34.2	6.3	12.8	68.8	4.2	84.4	0
K1.3964	25	56.8	68.3	70.3	61.4	25.4	29.7	4.2	8.9	17.9	0	29.4	0
K1. 2234	30	50.0	61.4	51.7	53.8	42.0	34.4	4.6	11.9	52.6	7.3	*	0
K1. 2065	35	64.0	72.2	70.7	63.4	24.7	28.5	1.4	8.2	19.9	0	69.8	4.0
K1. 2316	40	51.2	65.5	74.8	58.2	23.8	31.9	3.2	10.0	73.9	4.6	93.8	3.5
K1. 3751	40	59.7	65.3	69.6	60.9	27.3	28.6	6.9	10.6	38.8	1.6	39.5	3.8.
K1. 2333	45	51.1	72.2	65.2	54.8	27.9	35.0	7.8	10.2	35.9	40.6	19.2	5.3
K1. 3843	50	66.5	66.9	62.5	62.2	29.7	28.3	6.2	9.4	*	*	*	*
K1. 2339	50	44.4	69.4	64.7	63.1	29.1	29.3	5.9	7.6	51.0	2.2	6.3	2.7
Mean		57.7	67.6	63.5	58.9	30.6	31.4	5.9	9.5	38.1	5.8	44.5	2.2
Significant Level	0.1%	not significant		not significant		not significant		significant		significant		significant	
	1%	significant		not significant		not significant		significant		significant		significant	

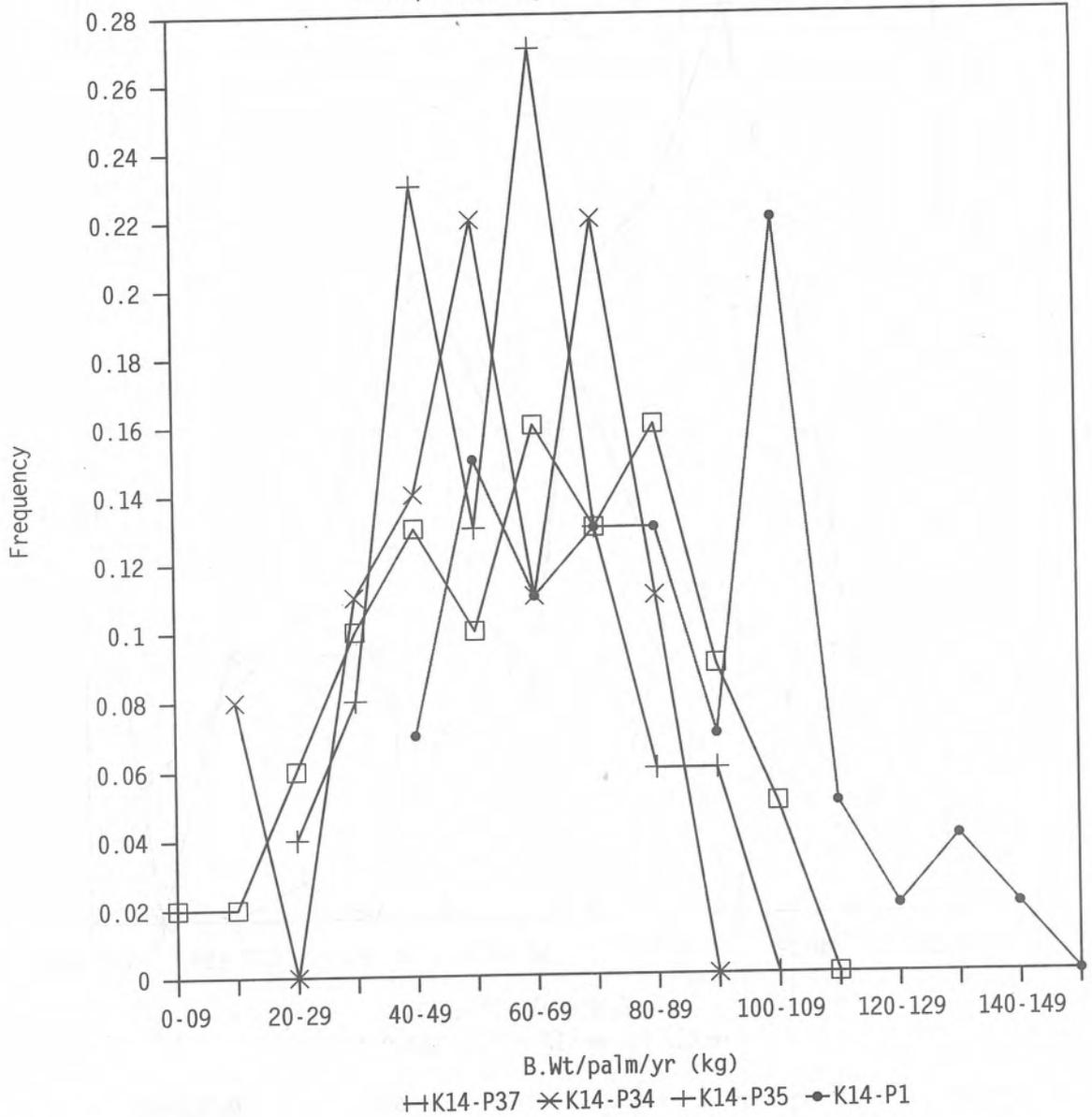
\* not determined

Fig. 1: Relative Frequency Distance of  
B.Wt/palm/yr (1981-1988) K12 planted 1977



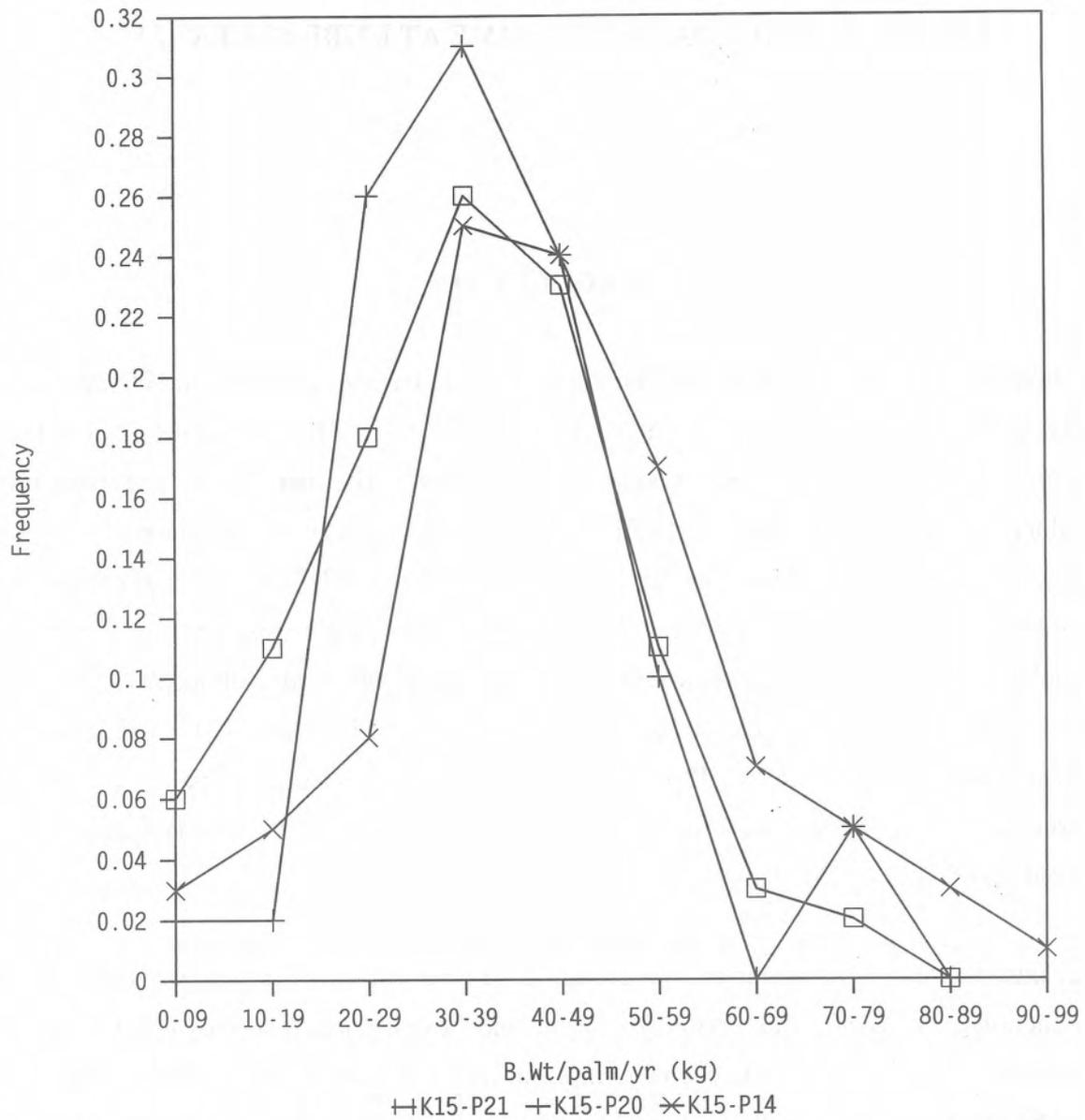
IDENTIFICATION	MEAN	STD. ERROR	STD. DEVIATION	VALID CASES
K12-P2: CONTROL (852.802T x 852.121D)	68.739	2.211	25.015	128
K12-P13: (10 Gy)	38.725	1.812	19.770	119
K12-P14: (20 Gy)	57.395	10.881	42.141	15
K12-P15: (30 Gy) Palm No. K12.2469	49.04			(Single Observation)
K12-P16: (50 Gy) " K12.1082	104.75			"
" K12.1083	99.24			"
" K12.1084	119.50			"
" K12.1085	87.68			"
" K12.1086	106.34			"
" K12.1087	106.40			"
" K12.1088	59.81			"
" K12.1089	36.20			"
" K12.1090	141.65			"

Fig. 2: Relative Frequency Distance of  
B.Wt/palm/yr (1983-1988) K14 planted 1979



IDENTIFICATION	MEAN	STD. ERROR	STD. DEVIATION	VALID CASES
K14-P1: CONTROL (K1.3967D x 851.805P)	84.179	3.445	25.548	55
K14-P34: (K1.3843D x 851.805P/50 Gy)	56.063	3.351	20.109	36
K14-P35: (K1.3981D x 851.805P/10 Gy)	58.808	2.353	16.969	52
K14-P37: (K1.1988D x 851.805P/10 Gy)	62.766	3.031	24.056	63
K14-P36: (K1.2520D x 851.805P/15 Gy):-				
Palm No. K14.544	65.82			(Single Observation)
K14.545	56.37			"
K14.546	60.00			"

Fig. 3: Relative Frequency Distance of  
B.Wt/palm/yr (1985-1988) K15 planted 1980



IDENTIFICATION	MEAN	STD. ERROR	STD. DEVIATION	VALID CASES
K15-P14: (K1.1988D x 851.805P/10 Gy)	43.622	1.285	18.220	201
K15-P20: (K1.3981D x 851.805P/10 Gy)	36.974	2.081	13.489	42
K15-P21: (K1.3843D x 851.805P/50 Gy)	34.315	2.018	15.892	62

## OIL PALM BREEDING PROGRAMME AT EMBRAPA/BRAZIL

Barcelos E.<sup>1</sup> and Amblard P.<sup>2</sup>

### INTRODUCTION

The story of the introduction of oil palm breeding material in Brazil goes back to 1963, when two similar collections of crosses selected by IRHO were sent to Brazil in order to develop a programme aimed at producing seeds under local conditions. The first IRHO set of crosses was introduced in the State of Bahia by CEPLAC ("Comissao Executiva do Plano da Lavoura Cacaueira"), and planted at the Una Research Station in 1966. The second set of crosses was established between 1964 and 1965 at CPATU ("Centro de Pesquisa Agropecuaria do Tropico Umido"), State of Para. These genetic resources consisted in Deli *Duras* Dabou, plus L2T selfed, L2T x L5T and L5T x L7T as La Me source of *pisiferas*, and S7T selfed, S10T x S7T, and S17T x S9T as Yangambi *pisiferas*. CEPLAC has been employing the best *dura* palm progenies for the production of seeds for smallholders. The material introduced to CPATU was abandoned and suffered severe disease problems.

Other introductions of advanced breeding material in Brazil occurred in the seventies, with the introductions of MARDI (Malaysian Agricultural and Development Institute of Malaysia) lines in Bahia State (in exchange with cocoa germplasm from CEPLAC) and the bulk introductions made by IRHO in Manaus, through a cooperative programme with EMBRAPA ("Empresa Brasileira de Pesquisa Agropecuaria"). Details of these programmes are as follows:

#### 1. CEPLAC, STATE OF BAHIA

In 1976, MARDI sent 10 crosses (5T x T and 5D x D) to CEPLAC. Only 80 plants of the five D xD crosses survived. This material was planted mixed in a single observation

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<sup>1</sup>Oil palm breeder - EMBRAPA/CPAA/Manaus/Brazil

<sup>2</sup>Oil palm breeder - CIRAD/IRHO - Based at EMBRAPA/CPAA/Manaus/Brazil

plot at the Lemos Maia Experimental Station in Una, Bahia. The ancestry of the 5 crosses mixed are as follows:

- 1) 0.102/8428 x 0.102/8539
- 2) 0.82/2258 x 0.34/73
- 3) 0.82/2159 x 0.34/55
- 4) 0.82/2029 x 0.102/2054
- 5) 0.82/2029 x 0.102/2054

A second introduction of MARDI material occurred in 1977. Of the 8 DxD crosses introduced, 5 crosses survived and were planted in single observation plots. These DxD crosses were:

- 1) 0.85/4251 x 47/114
- 2) 0.105/8914 x JL 18024
- 3) 0.105/8949 x JL 18152
- 4) 0.107/1681 x 47/94
- 5) 0.105/8914 x 47/146

Finally, in 1978 a set of 26 crosses, 11 of which were TxT's SP540 derivatives and 15 DxD's Deli and Deli Dumpy were received.

## 2. **EMBRAPA**

EMBRAPA is a national organization with research activities all over Brazil. EMBRAPA's oil palm breeding programme is conducted by the Agro-Forestry Research Center (Centro de Pesquisa Agroflorestral da Amazonia Ocidental, CPAA), located at Manaus, Amazonas State. This centre has 5,000 ha of land at its Urubu River Oil Palm Research Station (Estacao Experimental do Rio Urubu-EERU), located 140 km from Manaus, of which 400 ha were planted with breeding and agronomy experiments (Table 1). In this area the rain is relatively well distributed throughout the year, with an average total rain ranging from 2,040 to 2,749 mm per year with mild annual water deficit of 4 to 215 mm (Table 2). EERU is located on clayey textured weathered soil with a low organic matter content, strongly acid and low fertility (low phosphorus & exchangeable bases contents).

EERU's *guineensis* breeding programme is composed mainly by IRHO materials introduced since 1983. Table 3 presents a summary of the Progenies Test by parental line planted at EERU during 1983-88.

The progenies test presented in Table 3 originated from Deli *dura* parental lines mated to classical IRHO sources of *pisifera*, namely La Me and Yangambi (Table 4). The ascendance of these male parents which were introduced to Brazil as selfings is described in Figure 1. The Deli *dura* lines (Table 5), include parents from the well known Sumatra-Dabou and Medan Ara (Indonesia)-Johore Labis (Malaysia, Socfindo) populations as shown in Figure 2.

The parental lines shown in Table 3 correspond to the first selection cycle of the IRHO programme, and were extensively tested in various locations in Africa and Asia (Gascon, *et al.*, 1981; and Nouy, *et al.*, 1991). Several related DxP and DxT crosses of the second cycle were introduced for testing at Brazil-EERU. However, no conclusive results have been recorded yet.

In addition, 3 clonal evaluation trials were planted at EERU, since 1988, involving a total of 23 IRHO's clones. These trials are part of an international network for evaluation of the genetic x environment interaction. Vegetative observations showed a good conformity in the first planted trials.

A trial of commercial seeds with different origins, was also planted in 1985, involving material from 6 origins:

- IRHO (2)
- OPALMA (2)
- PALMOL (2)
- ASD (1)
- H&C (1)
- UNILEVER/ZAIRE (7)

No conclusive results are available as yet.

Additional germplasm has been introduced from IRHO to enrich the pool of EERU since 1984 (Table 6).

In addition to the oil palm advanced breeding material and germplasm introduced by IRHO, Embrapa also obtained 246 introductions of identified open-pollinated seeds sampled from sub-spontaneous oil palm groves of the Brazilian State of Bahia, which were planted at EERU during 1984-86. These sub-spontaneous populations developed from the seeds brought by the African slaves, and dispersed along the coast, from the State of Rio de Janeiro in the south towards the State of Ceara in the North. However, the greatest concentration of groves occurs in the southern coast of Bahia State, close to Valenca, Taperoa and Nazare districts. Ooi, *et al.* (1982), collected 31 open-pollinated bunches at different processing plants in Bahia, and planted the material in a replicated trial at EERU. In 1984, Melo (1985) carried out a collection in 6 districts of Bahia State. From these collections, EMBRAPA introduced 215 entries, identifying the different materials by geographic origin and planting the accessions in a bunch-to-row trial for evaluation. According to Melo (1985), most of the bunches collected were *dura*, only a frequency of 4.9% of *pisiferas* were found in the collection. The same author, indicates that the low presence of *pisiferas* can be explained by the fact that the first African slaves could not have brought other, than *dura* seeds. Similar low *pisifera* frequencies were also found in collections in Africa by Rajanaidu and Meunier cited by Melo (1985). A summary of the characteristics of the germplasm collected by Melo (1985) is presented in Table 7. The importance of this material can be seen as a new source of genetic material for breeding better locally adapted oil palm types, especially seeking resistance to major oil palm diseases present in South America.

In the *E. guineensis* breeding programme is planned the utilization of the material prospected at Bahia and also the utilization of the material available at the germplasm collection. Once evaluated, these material will go into the reciprocal recurrent selection design.

With the trial results of Rio Urubu and other IRHO's stations, where the same breeding material has been tested, the third cycle of RRS will be conducted. The utilization of tissue culture in collaboration with IRHO will allow exploitation of the outstanding individuals in the trials. Besides the breeding programme, the EERU is producing commercial seeds. A total of 4 million seeds is the annual production potential of the station.

### **Germplasm of *Elaeis oleifera* and its hybrids**

The *E. oleifera* germplasm collection, collected in the Brazilian Amazon, includes 226 open pollinated lines, collected from 15 localities in the region, representing 26 ha of planted area and 3.726 individuals (Table 8). The collected material shows interesting genetic variability (Ghesquiere, 1987), very promising for the hybridization programme with *E. guineensis*. The interspecific hybrids programme is also being conducted, with some progenies already in the field (Table 9).

Due to the great importance of "spear rot" in the Latin American oil palm cultivation, this programme must be enforced by the production of a considerable amount of hybrids and back crosses, in view to selecting outstanding individuals to be multiplied by tissue culture and be evaluated in high "spear rot" incidence areas (Le Guen, *et al.*, 1991).

### **CONCLUSION**

In spite of the national and regional importance and scientific value of the Rio Urubu Station, lack of financial and human resources are major constraints to its immediate survival. In this context EMBRAPA is looking for partners and other collaborators.

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**Table 1. Research conducted at Rio Urubu Experimental Station - EERU - EMBRAPA/Manaus**

Research	Lines	Number of Trials	Planted Area - ha
Breeding	259	22	212
Agronomy	-	4	48
Germplasm <sup>1</sup>	521	8	48
Seed Production <sup>2</sup>	215	9	92
<b>TOTAL</b>	<b>995</b>	<b>43</b>	<b>400</b>

1. 245 accessions collected in sub-spontaneous oil palm in Bahia/Brazil.

50 accessions introduced from Africa/IRHO

226 accessions *E. oleifera* collected in the Amazonian Region

2. 156 *dura* lines

55 *tenera/pisifera* lines

**Table 3. Number of progenies test between parental lines at EMBRAPA's Rio Urubu research station in Manaus.**

Deli <i>dura</i> Female Lines	Male Lines				Total
	L2T Self	L10T self	L10T x L312P	L431TxL319P	
D5D x D3D	11	9	11	13	44
L404 x D#D	6	9	13	13	41
L404D x D10D	10	11	10	10	41
D115D x L269D	9	11	6	10	36
D8D x D115D	5	7	9	16	37
D8D x D118D	6	7	8	8	29
<b>Total</b>	<b>47</b>	<b>54</b>	<b>57</b>	<b>70</b>	<b>228</b>

5 crosses (D5D x D3D) x (L5T x L10T)

5 crosses (D5D x D3D) x (L5T x L2T)

4 crosses (D5D x D3D) x (L2T x L10T)

3 crosses ASD

7 crosses MARDI-CEPLAC x L2T AF

6 crosses IRHO-CEPLAC x MARDI-CEPLAC

1 cross Control L2T x D10D

Table 2: Rainfall, water deficit and temperature at EERU, EMBRAPA-CPAA, Brazil

Month	1984	1985	1986	1987	1988	1989	1990	1991	Average
Jan	-	268.8	177.4	314.0	242.8	324.4	133.8	311.8	221.6
Feb	-	196.7	240.8	266.0	307.2	254.4	192.4	146.2	200.5
Mar	-	244.4	226.8	375.4	174.8	310.6	467.1	327.3	265.8
Apr	-	162.4	332.6	353.6	328.8	307.0	308.6	318.8	264.0
May	-	226.2	183.0	236.0	442.4	405.0	303.4	345.0	267.6
Jun	64.6	212.9	160.8	68.1	260.6	217.8	76.0	192.0	156.6
Jul	150.4	136.8	151.6	60.6	179.8	204.0	117.6	116.8	139.7
Aug	59.8	123.4	29.8	141.0	52.2	64.4	49.0	91.0	76.3
Sept	218.2	129.2	49.8	79.8	150.4	42.0	85.4	85.4	105.0
Oct	179.0	280.8	171.2	47.2	166.8	115.8	49.8	78.2	136.1
Nov	87.7	94.2	236.8	76.0	220.6	213.2	124.4	29.2	135.3
Dec	229.8	298.0	158.7	221.7	222.6	173.4	133.0	68.2	188.2
Total	-	2373.8	2119.3	2239.4	2749.0	2632.0	2040.5	2109.9	2323.4
Water Deficit (mm)	-	4.0	87.0	164.0	21.0	67.0	192.0	215.0	107.0
Light Hours	-	2032.0	1952.0	2030.0	1787.0	1824.0	2019.0	2016.0	1925.0
Days with rain	-	177.0	173.0	146.0	177.0	177.0	-	185.0	-
Temperature Max	32.2	32.9	33.0	32.2	32.0	32.7	31.8	34.0	32.0
Min	20.4	21.1	21.2	21.4	21.4	21.7	21.8	19.7	21.1

Source: EMBRAPA-CPAA, Manaus (1992)

Table 4: *Tenera/pisifera* lines in EERU

Original Cross	Number of Lines
L2T SELF	7
L2T SELF x (L10T x L312P)	6
L10T SELF	5
L2T SELF x (431T x L319P)	6
L2T SELF x L10T SELF	2
L2T SELF x L2T SELF	3
(L5T x L2T) x L10T SELF	1
(L431P x L319P) SELF	11
(L10T x L312P) SELF	6
(L2T x L10T) SELF	2
(L5T x L2T) SELF	1
L2T SELF x (L5T x L2T)	3
L10T SELF x (L2T x L10T)	1
CHE 131 SIB	1
L5T SELF x (L11T x L2T)	1
HC 129 SIB	2
CAM 236 x CAM 244	1
17	59

**Table 5: *Dura* lines in EERU**

Original Cross	Number of Lines
(D5D x D3D) SELF	17
(D115D x L269D) SELF	14
(L404D x D3D) SELF	15
L404 x D10D) SELF	18
(D8D x D118D) SELF	10
(D8D x D118D) SELF	10
(D5D x D3D) SIB	5
(L404D x D10D) SIB	4
(D8D x D118D) SIB	3
(D5D x D3D) x (L404D x D3D)	2
(D8D x D118D) x (D115D x L269D)	3
(L404D x D3D) x (L404D x D10D)	2
(D8D x D115D) x (L404D x D10D)	3
(D5D x D3D) x (D8D x D118D)	3
(D8D x D118D) x (L404D x D10D)	4
(D8D x D115D) SIB	4
(D8D x D118D) x (D8D x D115D)	4
(L404D x D3D) SIB	5
(D115D x L269D) SIB	5
(L404D x D3D) x (D115D x L269D)	4
(D5D x D3D) x (D115D x L269D)	3
(D5D x D3D) x (L404D x D10D)	2
(L404D x D3D) x (D8D x D118D)	2
(L404D x D10D) x (D115D x L269D)	1
(D8D x D115D) x (L404D x D3D)	2
(D5D x D3D) x (D8D x D115D)	2
(D8D x D115D) x (D115D x L269D)	2
CHE 135 x HC 132	1
HC 133 x HC 132	1
HC 132 x HC 136	1
CHE 135 x HC 136	1
(D118D x D10D) x (D22 x D5D) *	1
(D22D x D5D) x (D102D) x D3D) *	1
(P519D x P511D) x (D118D x D10D)*	1
34	156

\*CPATU

**Table 6.: Germplasm accessions introduced in Brazil by IRHO since 1984.**

ACCESSION	Number of lines per year of introduction					Total
	1984	1985	1986	1987	1988	
<i>DURA</i>						
Dabou Deli	4	4				8
Johore Labis Deli	1	3		3		7
Layang Layang Deli		1			1	2
Deli "Dumpy" Serdang		3				3
<i>TENERA/PISIFERA</i>						
Bingerville	1	4				5
Yocoboue		1				1
"Porto Novo" Pobe		1		1		2
Yangambi INEAC	1	1	3			5
"Sibiti" La Rive					2	2
Lobe		1			1	2
Widikoum, Cameroon		2				2
Aba, Calabar (Nigeria)			1	2		3
Salazar, Novo Redondo	3					3
Short Stem (Pobe)		5				5

**Table 7. Bunch characteristics of the sub-spontaneous material prospected by Melo (1985) in Bahia, Brazil.**

Characteristics	Number of Observations	Mean	Range	C.V. (%)
Bunch weight (kg)	329	17.3	3.3 - 65.4	52.6
Fruit to Bunch (%)	329	72.3	27.1 - 87.4	11.9
Average Fruit Weight (g)	329	5.5	4.9 - 49.9	33.0
Mesocarp to Fruit (%)	328	51.1	34.6 - 76.5	10.7
Shell to Fruit (%)	327	38.9	13.9 - 49.2	11.7
Oil to Fresh Mesocarp (%)	255	45.9	11.0 - 60.6	16.7

**Table 8. Germplasm of *E. oleifera* collected in Brazil.**

Location	Number of acces/year of planting			
	1984	1985	1987	Total
Careiro	11	28	-	39
Manicore	29	42	-	71
Novo Aripuana	-	12	-	12
Amatari	-	13	-	13
Autazes	-	12	-	12
Maues	-	11	4	15
BR 174	2	14	-	16
Perimetral Norte	-	8	-	8
Acajatuba	-	9	1	10
Tefe	-	6	-	6
Anori	-	5	-	5
Moura	-	12	-	12
Tonantins	3	1	-	4
Benjamin Constant	1	-	-	1
Barcelos	2	-	-	2
15	48	173	5	226
Number of palms	645	3.015	66	3.726

**Table 9. Interspecific oil palm breeding programme**

Hybrids	1985	1986	1991	1992	1993*	Total
<i>E. oleifera</i> x	17	12	62	5		96
<i>E. guineensis</i>						
Back cross BC 1		3	6	10	11	30
Back cross BC2				4	14	18
Bac cros F2						1

\* in the nursery

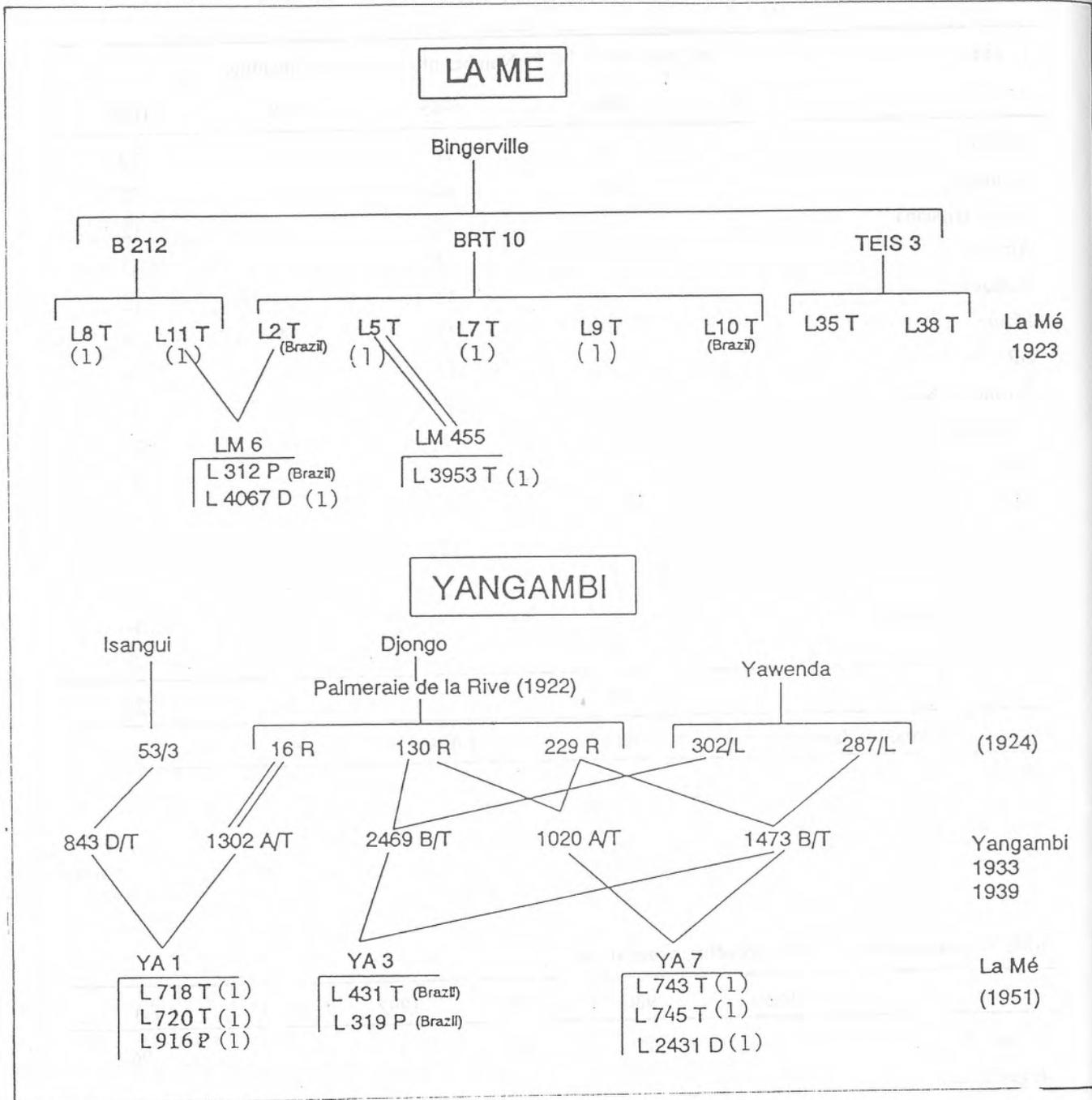


Fig 1. Ancestry of Lame and Yangambi male parents used at EERU program in Brazil.

(1) Also introduced to EERU



## **OIL PALM BREEDING PROGRAMMES IN INDONESIA**

### **A case study of the Marihat - RCEC**

Ridwan A. Lubis<sup>1</sup>, A. Razak Purba<sup>1</sup> and Adlin U. Lubis<sup>1</sup>

#### **ABSTRACT**

*The current status of the oil palm improvement strategies at Marihat-RCEC is presented. Different kinds of second cycle crosses trials have been set up and some double recombinations for the third cycle have already been carried out. Based on the elimination of bad genitors and on the recombinations set up in dura or tenera selection in 1975-1980, the average potential of RRS 2 should be 10-15% higher than RRS 1, and much more progress will be obtained with the RRS 3.*

*The current cloning programme involves around 36 progenies of 41 very good DxT or DxP families. The progenies of selected ortets are estimated produce 0.7 tonnes more oil per hectare per year, than the ordinary Marihat seed production. More progress is expected to be realized through specific programmes of creation of ortets.*

*There are several programmes at Marihat for improving hybrid materials and ensuring their utilization, taking into account the new possibilities offered by in vitro cloning. The programme is not only based on materials introduced at Marihat, but also use some crosses not represented at Marihat-RCEC. They are planted by Socfindo and IRHO at other places in Indonesia.*

#### **INTRODUCTION**

Since 1974, oil palm improvement strategies implemented by Marihat RCEC has been based on a adaptation of Reciprocal Recurrent Selection (RRS) scheme (Meunier and Gascon, 1972). The main characteristic of these programmes is to make the best possible use of the local population created previously, whilst testing numerous introduction from Africa. Implementation of the second selection cycle is now well established.

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Another priority of this oil palm improvement is given to the *guineensis* x *oleifera* hybrid programme. Some crosses were made in 1956 and others in 1975 with the *oleifera* from Colombia, Brazil and Suriname. The first general study of these hybrids was made in 1987 (Lubis *et al*, 1987). Actually, these hybrids were in the F2 and the back-cross programmes. The strategies for improving these hybrid materials are based on carrying out backcrosses or F2 crosses which, in the case of genic sterility, should restore the fertility, whilst enabling selection based on positive agronomic characters.

In an effort to produce high quality oil palm planting material in sufficient quantity, the Marihat RCEC launched the clonal programme in 1975. The present cloning programme involves utilization of the best individuals from the best crosses in trials that have been analyzed (first cycle of the RRS trial). In order to distribute only those that perform best, the other programme is to reclone the result of the first clones.

## RECIPROCAL RECURRENT SELECTION

### CYCLE 1

#### Population Being Studied

Between 1974 and 1980 Marihat-RCEC set up 27 genetic trials which were 460 crosses carried out between 180 *dura* genitors and 142 *tenera/pisifera* genitors. The origin of the various sources of the genitors was quite well known (Gascon, 1973; Lubis, 1988).

The *dura* origins were coded according to the estate from where the first palms used as genitors were originated, i.e. Marihat, Tinjowan and Dolok Sinumbah estates. The genitors of the other two origins were originated both from "Marihat population" and from another source, which was unknown as "DS x ?" origins, i.e. "Gunung Bayu", "Dabou", "Socfin" and "Dumpy" were not related to "Marihat population". The codes of the very few sub-origins depended on the name of the ancestral genitors. So, the majority of the *dura* genitors of the "Marihat population" were related to the same genitor "533". Table 1 gives the list of different *dura* origins and sub-origins studied by Marihat-RCEC.

The majority of *tenera* genitors came from Zaire. Based on the estates or the research stations which conducted selection of the original genitors, several origins could be distinguished as : the

“Bangun” origin; the “Dolok Sinumbah” origin which were the origin of the well known *pisifera* DS 76P or EX. 5; the “Bah Jambi” origin was made up of the offspring of the *pisifera* DS 76P and DS 66P. The “Sungei Pancur” origin, was made up of the famous *tenera* SP 540T and the “Sungei Pancur x Bangun” origin was made up of the Sungei Pancur genitor and Bangun genitor. The “Yangambi” origin, was selected by IRHO. The other origins came from Cameroon (the “Marihat” origin), from Ivory Coast (the “La Me” and “Yocoboue” origins) and from Nigeria (the “Nifor” origin). Table 2 shows the different *tenera* origins studied.

### Experimental Design and Parameters Analysed

The trials were set up using randomized block (sometimes 5x5 or 4x4 balanced lattice), containing from 6 to 25 treatments and generally 6 replicates. The elementary plots contain 4 lines of 3 or 4 trees. The planting density is 143 trees/ha, except for 2 trials where it is 130 trees/ha. These trials had no standard cross, but a significant proportion had common crosses and progenies from different origins which were compared inside the same trials. The cross design between the *dura* and *tenera* groups was made in such a way that allowed crossing of every genitor with several genitors of other group.

The FFB harvesting and recording were made on each tree from the third year after planting to at least the ninth year, and sometimes to the twelfth year. The bunch analysis was carried out before the introduction of *Elaeodobius* from a sample of 40 trees 5 to 6 years old per cross analysed twice. The percentage of oil to bunch was estimated from this analysis. The yearly vertical growth was measured generally between 6 and 10 years old trees.

### Results

An estimation of the general combining abilities (GCA) of the different *dura* and *tenera* origins had been studied by Marihat RCEC (Lubis *et al.*, 1989). This study showed that the *tenera* inter-origin variability was quite important, much higher than the *dura* inter-origin variability. Later study carried out by Purba and Nouy (1992) confirmed this difference between the *tenera* origins, but they also state that the *dura* inter-origin variability appeared more important than assumed. The *dura* inter-sub origin variability was much wider and the differences were always (and generally highly) significant. Table 3 shows the importance of the *tenera* inter-origin variability.

On the other hand, greater differences was noted between the *tenera* origins, in the number of bunches. Lubis *et al.* (1989) showed that the GCA observed for oil production only reflected GCA for the number of bunches. In other words, the variation in oil production mostly depended on the number of bunches.

An estimation of the GCA for the FFB (fresh fruit bunch), oil to bunch ratio and vertical growth rate for each *dura* and *tenera* origins were schematized in Figures 1, 2 and 3. These figures did not indicate the standard error, but Purba and Nouy (1992) showed that standard error of the *dura* genitors were much higher than the *tenera* genitors. This estimation of the GCA could be summed up as follow:

- Average combining abilities for FFB in the *dura* origins of the Marihat populations, except for the M-RISPA origin. Even so, certain sub-genitors were found having good GCA : A-501 had good GCA for bunch production, oil to bunch ratio and rather good for vertical growth. Genitors originating from "34C" had good GCA for bunch production; "M-533" and "M-533 x (24x240)" gave slow growing crosses. The genitors from Ivory Coast or Cameroon had very high GCA as well as for FFB or oil to bunch ratio, but with a fast vertical growth. The others origins different from the Marihat population gave good GCA for bunch production except the Dumpy origins which had slow vertical growth.
- There was much greater variability in the *tenera* group. The Dolok Sinumbah origin had low GCA for most parameters, but one of the sub origins, DS-1102 x 1117, of which DS 76P was a part, was characterized by high GCA for bunch production and for bunch quality. The GCA of Sungei Pancur origin was quite high for FFB and for oil to bunch ratio. This origin had average GCA for vertical growth compared to the whole materials tested. The La Me origin was of particular interest because its slow vertical growth and excellent combining abilities for bunch number. The characteristics of the Yocoboue origin appeared to be close to those of the La Me origin, but generally inferior. The Marihat origin had very low GCA for bunch production. The only promising quality of this material was its slow vertical growth.

This study of combining abilities was valid for the whole material selected by Marihat-RCEC for its breeding programme, but could not be generalized to materials tested by other research stations. The above analysis of combining abilities for the bunch production were based on the

fact that the period of 6 - 9 years old gave a good indication of the long term yield (12 years) (Lubis *et al*, 1991).

The application of the combining abilities studies was an useful guide to define the crossing plans:

- to seed production : the Marihat seed production had been completely revised. The genitors with bad GCA had been discarded.
- to next RRS cycles : only the offsprings of the elite genitors were selected in the next cycles.

## CYCLE 2

The second cycle trials could be distinguished in two steps:

1. A preliminary interpretation of the progeny trials planted between 1982 and 1990 (306 crosses of 19 trials). Despite the fact that these trials began before exploitation of the first cycle was completed, some of the trial analyses might provide interesting information.
2. A complementary interpretation of exploitation of the first cycle and after checking all the data, lead to the setting up of 300 - 350 new progenies.

Different kinds of second cycle crosses trials were set up:

- a. Those concentrating on exploitation of specific combining abilities, with:
  - the reproduction of single good DxT crosses already tested; the selfing of the genitors *dura* and *tenera*.
  - the joint reproductions of 2 or 4 good crosses already tested, i.e. D1 x T1 and D2 x T1 and to D1 x D2 and T1 selfed; D1 x T1 and D1 x T2 and to D1 selfed and T1 x T2; D1 x T1, D1 x T2, D2 x T1, D2 x T2 and to D1 x D2 and T1 x T2.
- b. Those seeking to exploit good general combining abilities after carrying out crosses between second cycle genitors originating from selfings or crosses of *dura* and *tenera* first cycle genitors. These genitors never tested in the same combination but with good GCA.

Compared to RRS 1, the average potential of RRS 2 should be higher of 10 - 15%. This progress is not exceptional, because it is only based on the elimination of bad genitors and on the recombinations set up in *dura* or *tenera* selection in 1975 - 1980, which are generally made between genitors of the same origin e.g. "La Me x La Me", "Yangambi x Yangambi", etc. much more progress will be obtained in RRS 3.

### CYCLE 3

Although the third cycle should normally be set up after the interpretation of the second cycle, but to save time it is useful and interesting to carry out already some double recombinations, (between second cycle genitors not yet tested but is originating from selected first cycle).

There are two policies in the Marihat RRS 3 programmes :

- Classic exploitation of the variability within each *tenera* or *dura* origin.
- General exploitation of all the variability observed in Marihat and introduced materials. In this way, the LM 2T x LM 9T and RS 27T x MA 860T *tenera*, which are never tested individually with the Deli in the second cycle, are recombined without knowing their crossing value. Selection is less effective, but the test with the Deli *dura* quickly exploits greater variability.

### GENERAL COMMENT

The exploitation of the variability should produce very important progress in RRS 3. However, there is an important problem which hinders the improvement and the utilisation of the best genitors i.e. the female characteristics of the best *pisifera* genitors. It would be necessary to know more the floral biology in order to get pollen from the best *pisiferas*, or to have special location to produce pollen, or to adapt the definition of the groups of RRS. Concerning the last point, it is interesting to observe that in RRS 1 trials very good yield was obtained with crosses between the *dura* from "Deli x SP 540T" and the *pisifera* from La Me or Cameroon. Therefore, why not to have a *dura* group with La Me or Cameroon origin and a *tenera* group with Deli x Yangambi?

## CLONAL PROGRAMME

The selection of the ortets for *in vitro* multiplication requires two steps : The choice of the best progenies (high yielding and good secondary characteristics) and the choice of the best trees within those best progenies. The other programme is to multiply some exceptional trees of the RRS 1 cycle. This choice has been done on 36 progenies of 41 very good families and 174 ortets of the selected materials are already cloned. The progenies where are selected ortets produce 11% higher oil/ha than the average of the Marihat seed (7, 2 vs 6, 5 ton/ha).

However, these ortets are selected in trials set up for progeny tests. More progress will be obtained through specific programmes of creation of ortets, like F2 recombination started in 1992 between trees of two different DxT (or DxP) high yielding crosses with complementary characteristics, for example: *dura* of "BJ 7D x L 2T" by *tenera* of "BJ 348D x DS 76P". The "BJ 7D x L 2T" cross gives high GCA for FFB and vertical growth while the "BJ 348D x DS 76P" cross has an excellent GCA for oil to bunch ratio.

Due to the production of clones, Marihat has also started the setting up of an important "Genotype x Environment" study. The aim is to get a good estimate of the potential between different ecologies of oil palm plantations in Indonesia. Twenty (20) clones will be studied in different location, and 7 of them in each location.

## HYBRID PROGRAMME

The objective of the hybrid programme is to transfer some of the *Oleifera*'s positive characters to the *guineensis*. In the first step, 79 hybrids have been set up in 11 trials from 1975 to 1980.

It is now time to exploit these interesting materials by:

- Backcrossing to *guineensis* :
- Short term approach (cloning after one backcross)
- Long term approach (DxP seed after several generation)

F2 production (utilization of maximum recombinations and exploitation by cloning)

Backcross to *oleifera* :

- long term programme (produce pure *oleifera* as productive as *guineensis*).

The variability of *Elaeis oleifera* tested in Sumatra is very large : Brazil, Costa Rica, Colombia and Surinam origins. The observation made by Lubis *et al.* (1987) showed that the variability between the hybrids of those *oleifera* was still large as well as for yield, bunch compositions, fatty acid compositions and height increment/year.

To complete the hybrid programme, Marihat has therefore planned to use a few crosses not represented at Marihat-RCEC but planted by Socfindo at Bangun Bandar (Brazil x La Me) and those set up by IRHO/Socfindo at Aek Kwasan (Monteria and Costa Rica x La Me and Nifor).

### CONCLUSION

At the beginning the Marihat-RCEC oil palm breeding programme was hindered by:

- Setting up of a selection scheme was late.
- The available genetic materials were slightly selected and studied.

Since than improvement has been made in :

- the selection of an efficient selection scheme,
- the study of the wide variability of its materials and the policy of introduction of new genitors,
- the volume information brought by the important network of trials set up by Marihat,
- the introduction of new technology, e.g. *in vitro* propagation.

Today, important progress has been obtained. The breeding programme Marihat-RCEC can contribute significantly to the bright future and more efficient improvement of oil palm.

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**Table 1**      **Origins of the *dura* genitors used in the Marihat RRS first cycle**

Origin	Sub-Origin	Nb. of genitors
DS	M-5333	23
	M-533x (24x240)	15
	M-533x (6x23)	12
	M-533x (9x41)	6
MA	M-34C	7
	M-34Cx434B	5
	M-434Bx213B	9
	M-434Bx435B	9
	M-435B	1
	M-435Bx145B	3
T	M-501	2
	M-511x520	1
	M-511x571	2
	M-554x571	2
DSxMA	M-(1x44)x34C	2
DSxT	M-533x501	1
	M-533x(531x501)	1
M-RISPA	M-RISPA	5
DSx?	MxD56	24
M-SP540	M-SP540	7
GB	GB	4
DA	DA	5
SOC	SOC	5
RS-DUMPY	RS-DUMPY	5

“Marihat Population” = Groups MA + DS + T + M-RISPA

MA = Marihat

DS = Dolok Sinumbah

T = Tinjowan

Populations different from Marihat Population:

SP540 = SP540T

DA = Dabou

SOC = SOCFIN

GB = Gunung Bayu

533, 24, 240 ... = Genitor number

**Table 2. Origin of the *tenera* genitors used in the Marihat RRS first cycle**

Population	Origin	Sub-Origin	NB of genitors	
Zaire	B	B	1	
	BJ	1103x(1102x117)	25	
	DS		1102	5
			1102x1117	2
			1103	10
			1117	3
	SP	SP	15	
	SP/B	SP/B	10	
YA	YA	9		
Ivory Coast	LM	LM	9	
	YO	YO	3	
Cameroon	MA	103x329	24	
		103x333	2	
		103x582	4	
		30	2	
		30x333	1	
		30x374	2	
		333x329	4	
Nigeria	NI	NI	2	

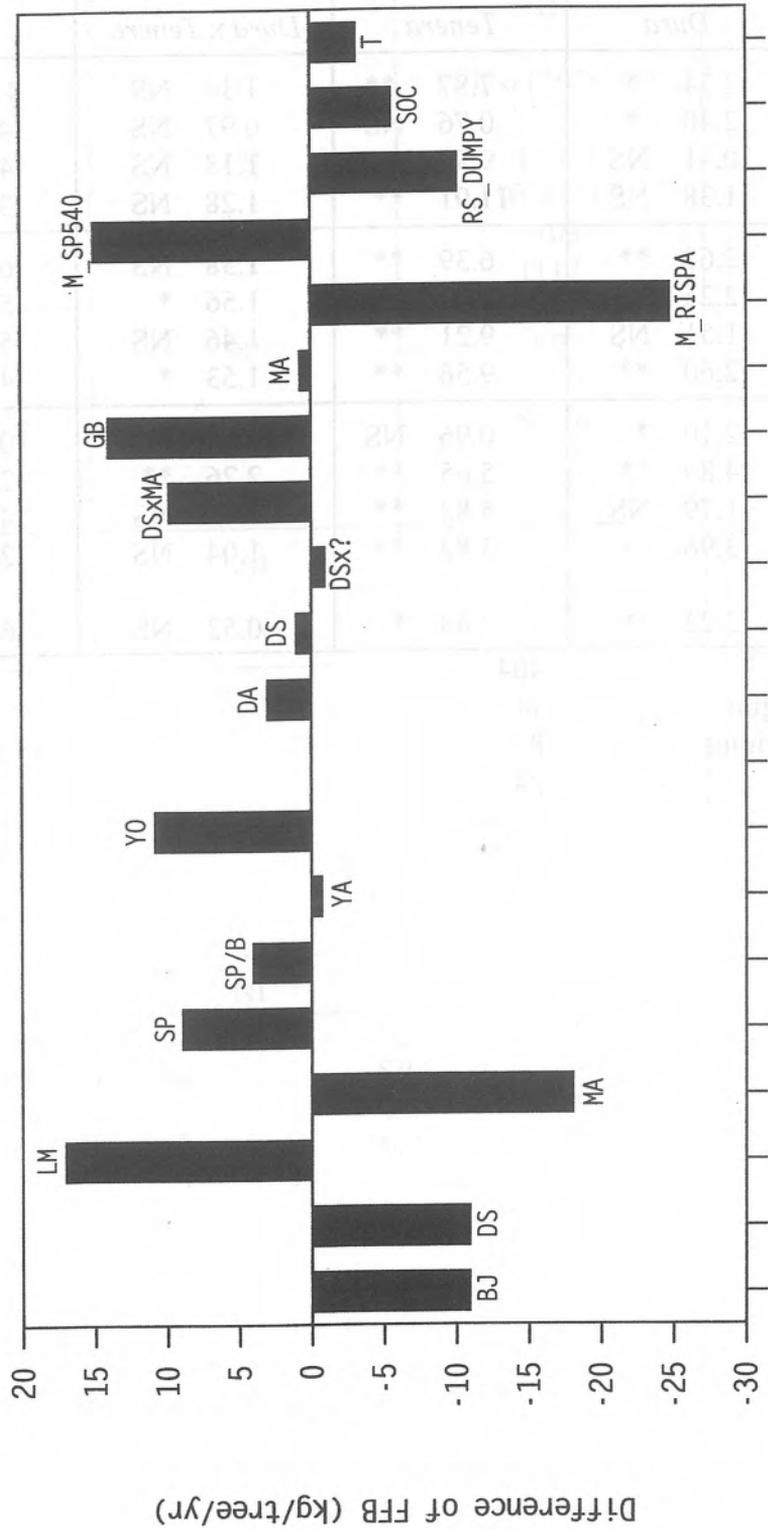
B	=	Bangun	BJ	=	Bah Jambi
DS	=	Dolok Sinumbah	SP	=	Sungai Pancur
YA	=	Yangambi	YO	=	Yocoboue
LM	=	La Me	MA	=	Marihat
NI	=	Nifor			

**Table 3: Analysis of the mains effects Origins, Trial and of inter-origin interaction (after Purba and Nouy, 1992)**

Parameters	F		F		F		F	
	<i>Dura</i>		<i>Tenera</i>		<i>Dura x Tenera</i>		Trial	
BN 4-6 yrs	2.34	*	7.87	**	1.34	NS	4.17	**
ABW 4-6 yrs	2.40	*	0.76	NS	0.97	NS	4.36	**
FFB 4-6 yrs	0.41	NS	9.90	**	1.18	NS	4.48	**
Oil 4-6 yrs	1.38	NS	11.91	**	1.28	NS	3.73	**
BN 7-9 yrs	2.68	**	6.39	**	1.38	NS	6.05	**
ABW 7-9 yrs	2.25	*	0.97	NS	1.56	*	5.72	**
FFB 7-9 yrs	1.51	NS	9.21	**	1.46	NS	5.71	**
Oil 7-9 yrs	2.60	**	9.56	**	1.53	*	4.72	**
% Fruit/B	2.10	*	0.96	NS	0.81	NS	10.07	**
% Mesoc/F	4.89	**	5.65	**	2.26	**	2.76	**
% Oil/M	1.79	NS	5.82	**	1.06	NS	4.85	**
% Oil/B	3.96	**	3.82	**	1.04	NS	2.35	**
Vert. Growth	3.22	**	2.49	*	0.52	NS	6.26	**

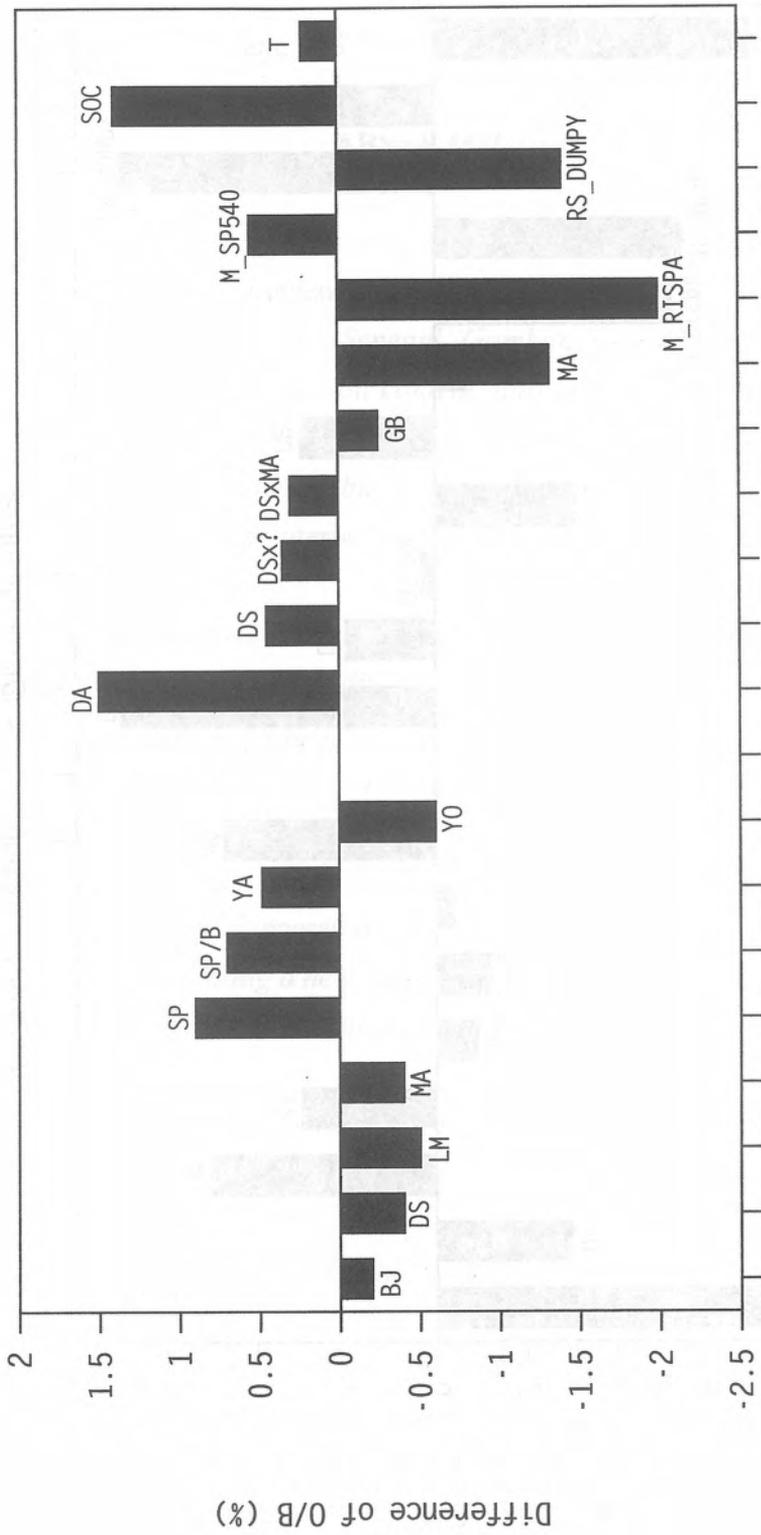
Number of crosses : 404  
 Number of *dura* origins : 11  
 Number of *tenera* origins : 8  
 Number of trials : 24

Fig. 1: FFB PERIOD 7-9 YEARS OIL differences due to the origins.



< Tenera group >  
< Dura group >  
Tenera and Dura groups studied.

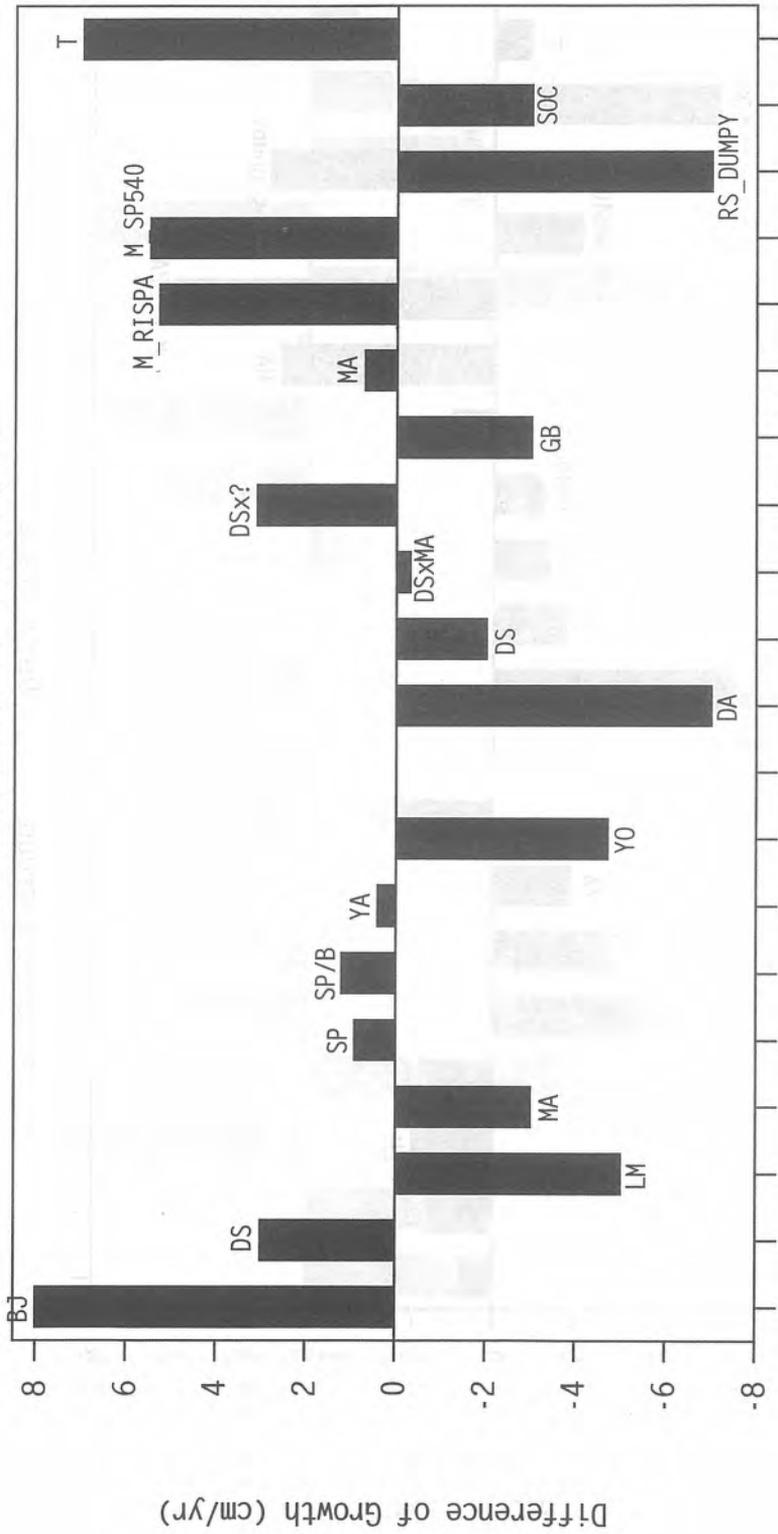
Fig. 2: PERCENTAGE OF OIL TO BUNCH differences due to the origins.



< Tenera group > < Dura group >

Tenera and Dura groups studied.

Fig. 3: GROWTH RATE differences due to the origins.



< Tenera group > < Dura group >  
 Tenera and Dura groups studied.

**OIL PALM GENETIC RESOURCES:  
Collection, evaluation, utilization and conservation**

Rajanaidu, N.<sup>1</sup>, Jalani, B.S.<sup>1</sup>, Ahmad Kushairi Din <sup>1</sup> and Rao, V.<sup>2</sup>

**ABSTRACT**

*PORIM has assembled the largest oil palm *Elaeis guineensis* and *Elaeis oleifera* germplasm collection in the world. *Elaeis guineensis* collections were sampled in Nigeria, Camerouns, Zaire, Tanzania, Madagascar, Angola, Senegal, Gambia, Sierra Leone and Guinea. These materials are being evaluated for yield, oil content, fatty acid composition, physiological and vegetative traits (e.g. height). The *Elaeis oleifera* genetic materials were collected in Honduras, Nicaragua, Costa Rica, Panama, Colombia and Suriname. In addition, exotic palms such as *Jessenia*, *Oenocarpus*, *Bactris* and *Euterpe* were also gathered in Peru and Colombia.*

*The palms collected in Nigeria were evaluated in the field and some of the palms could yield up to 10-12 t oil per hectare per year. In addition, these palms are short, with a height increment of 20-25cm per year as compared to 45-75cm per year with the present planting material. Dwarf palm would be extremely useful to the industry because they would reduce the cost of harvesting and lengthen replanting cycle.*

*The selected Nigerian duras and teneras are being utilized for broadening the variability of current duras, teneras and initiating a new foundation breeding programme. The high yielding dwarf teneras are being multiplied by tissue culture techniques.*

*At present the oil palm germplasm is conserved as field genebanks. Experiments are being conducted to preserve oil palm germplasm using in vitro methods such as cryopreservation of seed and somatic embryos. Molecular techniques such as RFLP and RAPD are being used to study the genetic variation of natural oil palm populations.*

*PORIM has developed two types of oil palm planting materials i.e. PS1 and PS2 from the elite Nigerian germplasm material. The PS1 is known for its high yields and dwarfness while PS2 was bred for high iodine value.*

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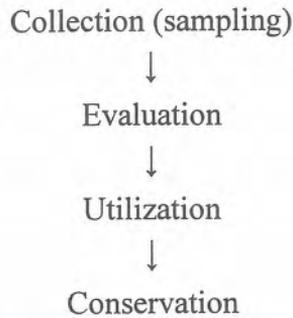
## INTRODUCTION

The history has shown us the importance of widening the genetic base of crops. The Irish potato famine is one of the most dramatic examples of the fragility of crop yields due to a narrow genetic base. In 1846, the late blight fungus (*Phytophthora infestans*) reduced production dramatically. At that time Irish farmers were growing a limited number of potato clones derived from only two samples brought from South America (Crist, 1971).

A similar situation occurred in the early 1970's. Nearly 50% of the corn in the USA was wiped out by *Helminthosporium maydis*. During that period most maize hybrids were developed from one source of cytoplasmic male sterile line (NAS, 1972).

It has been generally recognized that the narrowness of effective gene pools has been a major obstacle to rapid selection progress in oil palm (Arasu and Rajanaidu, 1975, 1976; Hardon *et al.*, 1985; Hardon and Thomas, 1968; Hartley, 1988; Jagoe, 1952; Ooi *et al.*, 1973; Rajanaidu and Abdul Halim Hassan, 1986; Thomas *et al.*, 1969). It was the concern generated by this situation that provided the initial impetus for the prospection of oil palm genetic materials in its natural environment. A number of expeditions were mounted to collect oil palm genetic materials by early oil palm workers. After the second world war workers in Belgian Congo sampled oil palm germplasm at a number of sites (Vanderweyen, 1952; Pichel, 1956). Between 1961-1965, plant breeders from the Nigerian Institute for Oil Palm Research (NIFOR) collected genetic material at local markets and through village chiefs. This material was established at NIFOR. Evaluation of prospected 72 open-pollinated progenies had been concluded and outstanding palms were selected for introduction into the current cycle of the breeding programme (Okwuagwu, 1985). Blaak (1967) sampled materials in Bamenda Hills of Cameroons and some of those materials were planted at Lobe, Cameroons and a part of it was distributed elsewhere. In Ivory Coast, the French oil palm workers systematically evaluated palms in the wild and the selected palms were progeny-tested for their breeding value (Meunier, 1969; Meunier and Baudouin, 1985). Earlier, Institut de Recherches pour les Huiles et Oleagineux (IRHO) selected 38 palms at Pobe in Dahomey and 4 palms at Bingerville in Ivory Coast. These formed their basic *tenera* stock.

Genetic resources programme involved four major steps. They are:-



Each of these activities has been described in detail in this document.

The major objectives of the oil palm genetic resources programme are:-

- ▶ To find new source of genes to broaden the genetic base of current oil palm breeding programme which is based on an extremely narrow genetic base;
- ▶ To conserve a cross-section of oil palm germplasm for future use because natural palm groves in Africa are disappearing at a rapid rate due to development and human population pressure;
- ▶ To introduce novel genes for fatty acid composition e.g. high iodine value (more unsaturated oil), dwarfness, high bunch index etc. Current breeding materials possess limited variation for these traits;
- ▶ To create an entirely new oil palm breeding population based on the new introductions;
- ▶ To understand the level of natural variation for various traits in the oil palm species;
- ▶ To study the organisation of variation between and within natural oil palm groves. This information is extremely useful to devise a sampling strategy for germplasm collection in the wild and conservation.

This paper outlines PORIM's oil palm genetic resources programme and its implication to the oil palm industry in Malaysia.

## COLLECTION AND SAMPLING

The objective of plant exploration is to collect material with the maximum amount of useful genetic variability within a strictly limited number of samples (Marshall and Brown, 1975). The basic sampling strategy which is optimal in attaining this objective is to (i) collect 50-100 individuals per site, (ii) to sample as many sites as possible within the time available and (iii) to ensure that sample sites represent as broad a range of environments as possible.

In the case of oil palm, 2-32 palms per site were collected and the number of sites visited in a country depended on the distribution and density of natural palm groves.

The details on the various oil palm germplasm collections carried out by PORIM are given below:-

### Nigeria

In 1973, the Malaysian Agricultural Research and Development Institute (MARDI) and NIFOR collected oil palm genetic materials at 45 sites with an average of 20 palms per site and 200 seeds per palm. A total of 919 (595 *duras* and 324 *teneras*) bunches were harvested during this prospection. One bunch was harvested from each of the sampled palms and the fruits from each bunch were kept separate until field planting. For the sampled palms, data on bunch weight, bunch length, bunch breadth, bunch depth, fruit diameter, nut diameter, kernel diameter, mesocarp to fruit (%), shell thickness, fruit weight and nut weight were recorded *in situ* (Obasola *et al.*, 1983; Rajanaidu *et al.*, 1979, Ooi *et al.*, 1979).

The overall variation of the different characters of *dura* and *tenera* palms is given separately in Table 1. Their mean, standard deviation, sample size, maximum and minimum values and coefficient of variation (C.V.) are given. For *duras* the fruit weight varied from 2 to 23g and the CV was 34%. For *teneras* it was 1.2 to 15.4g and the CV was 30%. The mesocarp to fruit (%) ranged from 15 to 79% for *duras* and 16 to 97% for *teneras*.

### Cameroons

In the past, as indicated earlier, some material had been collected in the Bamenda Highlands (Blaak, 1967). During 1974-1975, IRHO prospected *guineensis* material at Muyuka, Mamfe, Kendem, Numba, Dinkom and Widikum of Western Cameroons.

In 1984 PORIM with the cooperation of Unilever, collected material both in the Western and Eastern part of Cameroons.

Samples were collected at 32 different sites distributed throughout Cameroons. One to 15 palms per site were chosen at random; the objective was to cover the whole country as far as possible. A total of 95 (58 *duras* and 37 *teneras*) palms were sampled during the prospection. The method of collection was similar to that adopted in Nigeria.

The characteristics of the material collected in Cameroons are given in Table 2. For *duras* the mean bunch weight was 16.8kg, mesocarp to fruit 40.0% and single fruit wt 10.3g. In the case of *teneras* the bunch wt was 17.3kg, single fruit wt was 8.6kg and mesocarp to fruit was 62.4%.

### **Zaire**

PORIM also collected oil palm genetic materials in Zaire from April-July 1984 with the cooperation of Unilever. In the past Belgian workers had prospected for oil palm germplasm in early twenties and fifties in Congo on an *ad hoc* basis. During this expedition, palms were sampled at 56 different sites distributed throughout Zaire i.e. Equator, Kivu, Kikwit - Kwango and Bas Zaire. In most sites 5 - 10 palms were sampled. A total of 369 (283 *duras* and 86 *teneras*) bunches were collected.

The characteristics of Zaire *duras* and *teneras* are given in Table 3. The Zaire *duras* had a mean bunch wt of 17.6kg, mean fruit wt of 14.2g and mesocarp to fruit of 43.9% and the corresponding figures for *teneras* were 17.4kg, 12.6g and 64.1% respectively.

### **Tanzania and Madagascar**

In the past collections were made in the main oil palm belt in West Africa i.e. from Sierra Leone to Angola. The populations in Tanzania and Madagascar were considered basically fringe populations. The collections in Tanzania and Madagascar were carried out in 1986 with the cooperation of the Ministry of Agriculture in Tanzania and Madagascar (Rajanaidu, 1986) and with a partial financial grant from the International Board for Plant Genetic Resources (IBPGR).

In Tanzania, samples were collected at 13 sites located near Kigoma along the Lake Tanganyika. At each site 1-7 palms were sampled and a total of 60 (42 *duras* and 18 *teneras*) bunches were collected. In 1979, Blaak collected some Tanzanian material and it is being studied in Costa Rica (Richardson *et al.* 1986).

During the expedition the team found dense palm groves at Ujiji, Mwandiga, Kiganza and Simbo in Tanzania. However, these palm groves were not as dense as those found in Nigeria, Camerouns and Zaire. The frequency of *duras* was about 90%; *teneras*, 10%; *virescens*, 10% and *nigrescens* 90%.

The characteristics of materials collected in Tanzania are given in Table 4. For *duras* the mean bunch wt was 18.4 kg, mean fruit wt 16.9g, mean nut wt 8.9g, mesocarp to fruit 46.7%, fruit length 4.3cm, fruit diameter 2.7cm, nut diameter 2.0cm, and kernel diameter 1.3cm. The corresponding figures for *teneras* were bunch wt 13.7kg, mean fruit wt 15.5g, mean nut wt 8.5g, mesocarp to fruit 70.6%, fruit length 4.4cm, fruit diameter 2.7cm, nut diameter 1.7cm, and kernel diameter 1.4cm.

In Madagascar, palms were sampled at 4 sites and 17 samples were collected. At each site 1-6 palms were sampled. The method of collection of data was similar to that of Nigeria.

In Madagascar, the distribution of oil palm was very sparse. Most of the palm groves were noticed along the road from Miandrivazo to Malaimbandy. The palms were confined to sandy river valleys intermingled with forest trees.

In the case of Madagascar, limited data were scored in the field because of lack of harvestors to obtain intact bunches for measurements. It was not possible to identify the fruit forms because the fruits were extremely small and the mesocarp of the fruits was generally damaged by birds. The mean nut weight of 17 samples was 1.8g as compared to 8.5g and 8.9g for *teneras* and *duras* collected in Tanzania. In general, the palm growth, bunch and fruit traits were poor when compared to the materials collected elsewhere in Africa. This could be due to the poor environment and low rainfall in the western part of Madagascar.

### **Angola**

Oil palm germplasm was sampled at 8 sites. At each site 2-14 samples were collected. A total of 54 (42 *duras* and 12 *teneras*) bunches were collected in Angola.

In the field, data on bunch weight, bunch length, width, depth, spine characters, fruit weight, nut weight, mesocarp/fruit (%), fruit length, fruit diameter, nut diameter and shell thickness were collected.

The palm groves at Cabinda were dense and actively exploited by the farmers for the oil. The natural palm groves at Sumbe and Benguela were sparse. The palms at Caxito and Funda were moderate in density (Rajanaidu *et al.* 1991).

### **Senegal**

Collection was carried out in July/August 1993 with the cooperation of the Ministry of Agriculture, Senegal. The palms were sampled at 13 sites. At each site 5-10 palms were sampled. A total of 104 samples were collected in Senegal. Only *dura* palms were encountered. This could be due to the low rainfall and the differential survival of *duras* and *teneras* in this harsh environment.

The mean and coefficient of variation (C.V.) for the traits scored in the field are given. The mean bunch wt is only 5.9 kg and the mesocarp/fruit (%) (M/F) is 35.1 (Rajanaidu and Jalani, 1994).

### **Gambia**

Gambia is a narrow strip of land which is wedged within Senegal. During the course of oil palm germplasm collection in Senegal, a limited number of accessions were sampled in Gambia. The oil palm germplasm collection in Gambia was carried out with the cooperation of the Ministry of Agriculture and Forestry. Collections were made at six sites. At each site 5-10 palms were sampled. As in Senegal, only *dura* palms were encountered in Gambia. A total of 45 palms (bunches) were sampled. Isolated palm groves were noticed and the natives regularly harvested the bunches and extracted oil using crude village methods (Rajanaidu and Jalani, 1994).

The field data scored in Gambia were analysed. The mean bunch wt was 5.7 kg and mesocarp/fruit (%) was 33.47. The mean and C.V. values for the characters scored in Gambia and Senegal are rather similar in magnitude.

### **Sierra Leone**

In April/May 1994, oil palm genetic material was collected in Sierra Leone with the cooperation of the Ministry of Agriculture.

The team visited 14 sites covering York, Waterloo, Mckorowo, Mayira, Bonyeya, Kabaiama, Matopie, Rotifunk, Rogbene, Mayonkoli, Mamanka, Kambia, Masenie and Rokupr. Extensive natural palm groves were encountered around Kambia and Rokupr. At each site 2-6 palms were sampled. A total of 56 samples (52 *duras*, 3 *teneras*, 1

*pisifera*) were collected. In terms of fruit colour, 54 samples were *nigrescens*, 1 *virescens* and 1 *albescens*. The collected seeds were divided equally between the Ministry of Agriculture, Sierra Leone and PORIM.

The average *dura* bunch wt was 9.47kg, single fruit wt 5.6g and mesocarp to fruit was 34.8%.

### **Guinea**

Oil palm germplasm was collected in Guinea in May 1994. The plant exploration was carried out jointly with the Ministry of Agriculture, Guinea and PORIM.

The collections were made at 14 sites involving Kissidougou, Guekedou, Macenta, Sereidou, Somota, Golowe, Bomati, Kindia, Boffa, Boke, Kamsar and Coyah. Dense palm groves were noticed around Boffa and Boke areas.

At each site 3-5 palms were sampled. A total of 61 samples (58 *duras*, 3 *teneras*) were collected. Only *nigrescens* fruits were encountered in Guinea.

The mean *dura* bunch wt was 11.4kg, mean fruit wt 6.4g, and the mesocarp/fruit(%) was 35.01%.

In Table 5, a general comparison is made between the populations studied *in situ* in Senegal, Gambia, Ivory Coast (Meunier, 1969), Nigeria, Cameroons, Zaire, Tanzania and Angola. The data show that the *dura* mean bunch wt seemed to increase from Senegal to Angola. The Tanzanian collection, a fringe population, had bunch and fruit qualities which were comparable to those collected in Ivory Coast, Nigeria, Cameroons, Zaire and Angola. Richardson (1986) has also noticed outstanding bunch and fruit qualities of the Tanzanian materials planted and evaluated at Coto, Costa Rica. These palms were collected by Blaak in 1979.

A number of workers have studied the inheritance of some of the qualitative traits in oil palm and their frequencies in the wild (Ooi and Rajanaidu, 1979). The genotype *nigrescens* is recessive but occurs in high frequency in the wild but not the genotypes *pisifera*, *albescens* and *idolaritica*.

The dominant genotype, the *dura* occurs at a high frequency in the wild but not in the case of *virescens* and *mantled*. Portères (1947) and Meunier (1969) pointed out the

exceptional high frequency of *virescens* at Man, Ivory Coast.

It is interesting to note that the high frequency of *teneras* and *virescens* was observed by Rajanaidu (1986) at sites 12, 13, 14 near Ufuma. Toovey (1947), before the introduction of modern D x P (*teneras*), had observed such a very high frequency of *teneras* at Ufuma indicating unconscious selection by the locals.

Later, we will notice that the area near Ufuma in the East Central state of Nigeria is very important in terms of finding high yielding dwarf palms.

### **Genetic Structure of Populations and Sampling Strategy**

The oil palm populations (p) sampled at random in Nigeria were studied to understand the genetic structure of natural oil palm population. At each site (population) 5 palms (families - f) were sampled and the seeds from each palm were kept separate and 12 seedlings (s) per family were field planted in a completely randomised design. Data collected were used to understand the distribution of variation between and within natural oil palm populations. The information is useful to devise effective sampling strategies for collection and conservation. ANOVA showed significant differences between populations (p), families (f) for most traits except for some fatty acid at the population level.

The variation due to populations contributed 0-28% , families 3-35% and seedlings 74-94% towards the total variation (Rajanaidu *et al.*, 1988). The distribution of the variation between and within populations shows that sufficient emphasis must be given at the time of sampling of the population i.e. the number of sites visited and at the family level i.e. the number of palms sampled at each site.

### ***Elaeis oleifera***

*Elaeis oleifera* oil palm was collected in Colombia, Panama, Costa Rica, Honduras, Brazil and Suriname in 1981-1982 (Rajanaidu, 1986). The palms were screened for fatty acid compositions (FAC). The collections from Colombia, Panama and Costa Rica had an iodine value (I.V.) of more than 90. The C18:1 level ranged from 52-66% and C18:2 level varied between 15 and 23%. The I.V. in the Brazilian *Elaeis oleifera* ranges from 76-81 and the level of C18:1 was lower than the accessions from Colombia, Panama, Costa Rica and Honduras. The FAC of Suriname *Elaeis oleifera* is rather unique. It has the highest level of C18:1 and the lowest C18:2

when compared to other populations. The mean I.V. in Suriname population is the lowest i.e. 67.5. The lowest level of C16:0 in the *Elaeis oleifera* collections is 13% (Rajanaidu *et al.* 1994).

The iodine value of *Elaeis oleifera* is much higher than *Elaeis guineensis* oil. However, the oil yield of pure *Elaeis oleifera* is much lower with the oil to bunch ratio of 5% as compared to *Elaeis guineensis* (*tenera*) > 25%. One way to exploit the *Elaeis oleifera* oil is through the medicinal/pharmaceutical industry rather than catering for culinary purposes.

### **Exotic Palms**

These palms are found in tropical lowland areas of the Amazon region of Peru, Colombia, Venezuela and Brazil. The palms are exploited for oil, beverage and palm heart.

### **Jessenia-Oenocarpus**

In 1989, a large quantity of *Jessenia-Oenocarpus* germplasm was collected in five major areas Colombia. It has been estimated that wild adult palm could produce about 2.2kg of oil per palm per year (Balick, 1989). The *Jessenia* oil characteristics resemble olive oil.

### ***Bactris gasipaes* (Pejibaye)**

This oil palm species is distributed from Honduras to Bolivia. *Pejibaye* has a main trunk and a number of shoots arising from the base of the palm. The *pejibaye* is cultivated mainly for fruits and palmito or palm heart.

The *pejibaye* fruit consists of protein (6.3%), fats (5.8%), carbohydrates (35.7%) and high level of vitamin A (U.I. 867.7) (Mora - Urpi *et al.*, 1984).

It is estimated that 3 tonne/ha/yr of palmito could be harvested from the third year of production. However, based on Clement's findings (1988) fresh fruit yields could reach 30-50 tonnes per hectare per year at the fifth year after field planting.

The oil quality of *Bactris* is compared with high I.V. *Elaeis guineensis*. The I.V. of *Bactris* oil is 73 compared to 64 of *guineensis*.

## **Introduction of Exotic Germplasm and Quarantine Procedure.**

The oil palm industry aims at preventing the introduction and spread of harmful organisms elsewhere in Malaysia. So far, Malaysia is fairly free of major diseases such as *Fusarium* infection in Africa and Marchitez, South America. Obviously, Malaysia had adopted very strict quarantine measures to prevent these diseases.

The Department of Agriculture (DOA), Malaysia has drawn up a number of quarantine measures. They are:-

**i) Phytosanitary measures in the country of origin.**

The seeds or pollens are collected from healthy palms and the genetic material is inspected by the local quarantine officer and certified free from any harmful diseases.

The seeds must be free from all mesocarp before despatch to intermediate quarantine stations, normally a temperate country. At present, the intermediate quarantine station is the International Mycological Institute, U.K.

**ii) Intermediate quarantine station.**

A representative sample of the seeds is examined for *Fusarium oxysporum* and *Cercospora elaeidis*. If any serious pathogen is detected, the consignment is not transmitted to the importing country. The seeds and pollens are packed before despatching to the recipient country.

**iii) Phytosanitary measures in Malaysia.**

In Malaysia, samples of the seeds are examined for *Fusarium oxysporum*, *Cercospora elaeidis* and other pathogens. The seeds are also fumigated with methyl bromide.

The seeds are germinated and planted in a post-entry quarantine nursery for one year and are observed closely by pathologists.

After the nursery stage, the seedlings will be released by DOA for field planting. The pathologists and breeders are required to report to DOA if any exotic disease is detected in the germplasm materials.

## EVALUATION AND PERFORMANCE OF OIL PALM GENETIC MATERIALS

The oil palm genetic materials collected in the wild were planted in the form of 'open-pollinated' families at PORIM Research Station, Kluang, Johore. Experimental designs such as cubic lattice, randomised complete block (RCBD) and completely randomised (CRD) were used to study the performance of the material.

The various stages and the time frame involved in the collection, establishment, evaluation and release of elite planting materials is given in Figure 1. It takes more than twenty years from the collection of germplasm in the wild to the release of oil palm planting materials.

Detailed data were collected on an individual palm basis. They are:-

- 1) Yield (FFB)
- 2) Bunch number (BNO)
- 3) Average Bunch wt (ABwt)
- 4) Mean nut wt (MNW) (g)
- 5) Fruit/bunch (%) (F/B)
- 6) Mesocarp/fruit (%) (M/F)
- 7) Oil/dry mesocarp (%) (O/DM)
- 8) Oil/Wet mesocarp (%) (O/WM)
- 9) Oil/bunch (%) (O/B)
- 10) Kernel/fruit (%) (K/F)
- 11) Shell/fruit (%) (S/F)
- 12) Mean fruit wt (MFW)
- 13) Frond production (FP)
- 14) Rachis length (RL)
- 15) Leaflet number (LN)
- 16) Height/yr (HT) : Measured from ground to frond 42 and divided by the age of palm from field planting.
- 17) Leaf Area (LA) i.e. = 
$$\frac{\sum_6(L \times W)}{6} \times \frac{\text{No. of leaflets} \times 2 \times 0.57}{10,000}$$
- 18) Trunk Dry wt (Tr. Dia) in kg per palm
- 19) Total dry matter (TDM) = Vegetative dry matter (VDM) + bunch dry matter (BDM)
- 20) VDM (t per hectare per yr) = 
$$\frac{(148 \times \text{frond dry wt} + \text{trunk dry wt})}{1000}$$

- 21) BDM (t per hectare per yr) i.e. =  $0.53 \times \text{FFB} \times [(1-\text{O/B}) + (2.1 \times \text{O/B})] \times 148$
- 22) Harvest index i.e. =  $\frac{\text{BDM}}{\text{VDM} + \text{BDM}}$
- 23) Fractional interception (f) =  $e^{-0.47}$  (leaf area index - 0.3) where leaf area index (LAI) =  $(40 \times \text{LA} \times 148) \div 10000$
- 24) Conversion efficiency (e) =  $\frac{\text{BDM} + \text{VDM}}{3.1 \times f}$
- 25) Frond index (FI) =  $\frac{\text{Leaf area}}{\text{Leaf dry wt}}$
- 26) Lauric acid (12:0)
- 27) Myristic acid (14:0)
- 28) Palmitic acid (16:0)
- 29) Stearic acid (18:0)
- 30) Oleic acid (18:1)
- 31) Linoleic acid (18:2)
- 32) Iodine value (IV) =  $(16:1 \times 0.9504) + (18:1 \times 0.8601) + (18:2 \times 1.732) + (18:3 \times 2.616)$
- 33) Oil/palm/yr (kg) =  $\text{FFB} \times \text{O/B}$
- 34) Kernel/palm/yr (kg) =  $\text{FFB} \times \text{K/F}$
- 35) Total Economic Product = Total oil + (k/p/yr) 0.6

Most of the vegetative and physiological traits were computed as given in papers by Corley (1981) and Squire (1986).

Rosenquist (1990) tabulated the Nigerian summary data supplied by PORIM. Population summary for yield, oil/bunch and oil yield (kg/palm/yr) and the ranking of the populations was computed. A similar information on height, bunch index, harvest index, rachis length and frond index was calculated. Rosenquist has also estimated the population yield index. Based on the overall ranking, the top 5 populations are 12, 14, 19, 13 and 16. These populations are found in the Uda, Akwa, Uli Ihiala areas of East Central state. The information on the performance of the populations for FFB, oil yield, height and harvest index shows that East Central State of Nigeria is the most productive area. However, the populations from the drier areas (39-40) near Bida had higher of kernel content (Figure 2).

Based on the performance of populations and families within populations, the elite palms for the following traits were selected. They are:-

i) High yield and dwarfness

*Tenera* palms yielding more than 10t oil per hectare per year have been discovered in the Nigerian collection. In addition, they are also short; their annual height increment is only 15-25 cm compared to 45-75 cm of current commercial D x P planting material. The selected elite *dura* and *tenera* for exploitation are given in Table 6. A number of these palms sampled at sites 12, 13 and 14 in East Central State possessed these traits.

ii) Palms producing high iodine values (high unsaturation) (more liquid oil)

A number of palms have been selected for high iodine value (I.V.) They have been multiplied to produce high I.V. planting material. The characteristics of high I.V. palms are shown in Table 7.

iii) Palms with high kernel content

The prospects of breeding for high kernel to bunch is bright. Some Nigerian families had mean kernel to bunch of more than 12% compared to 6% in current breeding progenies. It is possible to double the kernel content of future oil palm planting material. An economic analysis shows that breeding for the highest possible content of kernel in oil palm fruits yields the highest rate of return from the oil palm (Rajanaidu and Jalani, 1994).

## **Biochemical and Molecular Screening**

### **Biochemical screening**

Ten populations from the Nigerian oil palm germplasm collection were analysed with polyacrylamide gel electrophoresis to examine their isoenzyme variation. They are glutamate - oxaloacelate transaminase (GOT), acid phosphatase (AcP) and esterase (Est). The population 39 is the most polymorphic and population 44 the least. The overall polymorphic index (PI) was 0.130 in the Nigerian population and a similar study in the Zaire population showed that the PI was 0.086.

The results indicate that the Nigerian population is genetically more diverse than Zaire population. The quantitave data analysis of these two countries further reinforces the above results (Rajanaidu *et al.*, 1993).

## **Molecular screening**

The natural palms sampled in Nigeria were screened thoroughly for quantitative traits and isoenzyme variation. Since these traits are influenced by the genetic and environmental factors, variation at the molecular level was studied using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD).

Nigerian genetic materials were originally collected at 45 sites (populations). For this study 11 populations from three ecotypes were screened.

### **RFLP**

DNA was extracted from young leaves and the DNA was digested with 5 restriction enzymes, Taq I, Hind III, Bgl II, Bcl I and Bst NI. Hybridization was carried out with a cDNA probe pOP-G142.

The level of genetic variation was estimated by using polymorphic index (PI).

Using the RFLP technique, population 44 had the highest PI value (0.108) while population 43 the lowest (0.067). It was found that the four restriction enzymes used in this study revealed polymorphism when hybridized with pOP - G142 (Maizura, 1998).

### **Random Amplified Polymorphic DNA (RAPD)**

Fifty DNA samples from 10 Nigerian populations were amplified using twenty primers. A considerable variation in the RAPD banding pattern was noted.

### **Cameroon**

Three years (1990-1993) yield data have been collected and analysed. ANOVA shows that there are significant differences between populations and families. The population mean is given in Table 8 for yield (FFB), bunch number (BNO) and average bunch weight (ABWT). The FFB yield for the population means varies from 55-98 kg per palm per year. These early yields are lower than the early Nigerian populations.

The yield (FFB) in Cameroon populations varies from 12-164 kg per palm per year and the bunch number from 2.5-32.0 and average bunch weight from 2.2-11.6 kg.

Means and coefficients of variation (C.V) for individual populations sampled in Cameroon show that the high yielding populations are found in Eastern Cameroon e.g. populations 22 and 28. Populations with high bunch number are found in the Western Cameroon e.g. population (2).

### **Zaire**

ANOVA has shown significant differences between populations and families. Populations means for (1990 -1993) for FFB, BNO and ABWT are given in Table 9. The yields are slightly better than that of Cameroons. However, these yields are lower than Nigerian populations.

The variation of the yield parameters (FFB, BNO and ABWT) was studied and the results showed that the FFB values varied from 11.5-183.4 kg, BNO varied from 2.3-28.8 and ABWT from 2.8 to 15.4 kg per palm per year. The range for FFB and BNO was similar in magnitude for Cameroon and Zaire populations.

Population means and their corresponding coefficient of variation (C.V.) showed that the populations (30,31) sampled along Lake Tanganyika had highest FFB and BNO. Population 3 sampled near Binga had highest ABWT.

### **Tanzania**

The Tanzania collection has been field planted in trial 0.256 at Bukit Lawiang, Kluang (August 1990). Early screening of the palms had shown the presence of extremely thin-shell *teneras* in this collection.

### **Madagascar collection**

This collection was field planted in trial 0.240 at Bukit Lawiang. The palms have started bearing bunches. The palms are extremely small and this is comparable to *Elaeis oleifera* palms found in Suriname.

### **Angola collection**

The Angolan seedlings have been arranged into various experiments and field planted at Kluang in 1994.

## GENETIC RESOURCES INFORMATION MANAGEMENT SYSTEM

The effective use of germplasm depends largely on the computer system related to data recording and retrieval. Systematic description of various morphological, agronomic traits, results of evaluation, maintenance and distribution records is vital.

The descriptors for oil palm including the collection, characterization and evaluation data are electronically stored.

Following data are gathered:

### Accession Data

Accession number

Scientific name

### Collection Data

Collector's name

Collecting institute

Date of collection

Country of collection

Location of collection site

Collection source

Local name

Number of seeds sampled

Fruit form

Fruit color

Height

Population size

Ecology

Bunch

Bunch analysis

Vegetative measurement

Photograph/video

Pests/pathogen

Other notes from collector

### Characterization and preliminary evaluation

Further characterization and evaluation

## UTILIZATION

Sizeable oil palm germplasm collections have been made by PORIM. It is important that germplasm collections are made and maintained for the present and future. It is even more important, however, that the collections be used effectively (Sprague, 1986). The oil palm germplasm have been evaluated and are being utilized in a number of ways for crop improvement. They are:-

- **Direct selection of individuals from the Nigerian collection**

Oil palm is a cross-pollinated, perennial tree crop with a generation interval of more than 10 years. Hence its breeding and selection is time-consuming and difficult. Normally, the newly introduced gene pool has no potential for direct exploitation as commercial material. It has to be introgressed into another advanced material first. The new gene pool will only be considered for immediate exploitation if it possesses interesting traits and acceptable yields. In the case of Nigerian collection about 3% of the *teneras* had oil yields comparable to current planting materials. Approximately a third of these had an annual height increment significantly less than current commercial D x P materials. Attempts are being made to clone these elite palms by tissue culture techniques.

The performance of the selected *teneras* are given in Table 10. Data show that the outstanding families and individual *teneras* are normally found in East Central State of Nigeria. In selecting the *teneras*, the relatively high O/B standard of 28% was found to be the main limiting factor. FFB yields were generally excellent and most palms were significantly shorter than current D x P palms of similar age.

- **Progeny-testing of Nigerian elite palms**

Some of the extremely outstanding Nigerian *teneras* listed in Table 9 were progeny-tested with a range of *Deli duras* available in the industry and PORIM. The T x D or D x T hybrids and their *dura* and *tenera* parents are being selfed simultaneously. The aim of the crossing programme is to progeny test the Nigerian *teneras* to study their combining ability. The selfs will be used for seed production following the procedure of reciprocal recurrent selection (Jacquemard *et al.*, 1981). The *pisiferas* in the Nigerian collection too are progeny tested to study their combining ability with *Deli duras*. Meunier and Baudouin (1985) have evaluated the breeding value of Yacoboue population sampled in Ivory Coast by crossing with a sample of palms of known breeding value.

- **Broadening the genetic base of *Deli duras* and *teneras***

The overall genetic variability of current *Deli duras* and *teneras* could be broadened by crossing *Deli duras* with Nigerian *duras* and *teneras* such as AVROS, La Me, Yangambi, URT, and 27B could be mated to Nigerian *teneras* or *pisiferas*. These introgressed populations, with increased amounts of heritable variation for desired traits, will be the basis for further selection and breeding. Careful choice of the germplasm at this stage will increase selection efficiency and the probability of obtaining desirable segregants.

- **Foundation Breeding Programme**

The outstanding *dura* and *pisifera* palms from the Nigerian collection are being used to initiate an entirely new breeding programme; with the objective of producing superior alternatives to the current *Deli duras* and modern *pisiferas*.

- **Development of elite D x P planting materials**

PORIM has developed 2 types of planting material using the elite Nigerian germplasm.

- i) **PORIM Series 1 (PS1)**

- Features of parental palms**

- The experiment was laid down in the field in 1976 and data were collected from 1982-1986. High yielding dwarf palms were identified for seed production.

- Progeny Test Results**

- The progeny test trials were planted in 1989 and yield records were collected from December till May 1994. The oil/bunch and oil yield per palm per year are 25% higher than the D x P control.

- ii) **PORIM Series 2 (PS2)**

- The trial was laid down in 1976 and the yield records were collected from 1982-1986. The mother palms for high yields and iodine value were selected (Table 7).

- Characteristics of progenies**

- The high yielding palms with iodine value were selfed and planted in 0.282 at Ulu Paka, Terengganu. Table 10 shows the transmission of the high I.V. trait from parents to their progenies.

### **Availability of PORIM PS1 and PS2 Elite Planting Materials.**

Initially PORIM will be supplying a limited quantity from 1994-1988 for experimental planting. The production will be scaled up from 2000 onwards. In addition to PORIM, the industry seed producers will be producing a larger quantity of seeds based on elite Nigerian material.

## **CONSERVATION**

Oil palm germplasm collected in different parts of the world are maintained in the form of field genebank or living collections. At present, there is no long-term method to store oil palm seeds at very low temperature and humidity as in cereals such as rice and wheat. The oil palm germplasm can be conserved in the following ways:-

### **Field Genebank (living collection)**

The preservation of germplasm in the form of field genebank makes maintenance efforts very expensive. The field genebanks require large amounts of land and regular maintenance. In the case of oil palm only 148 palms per hectare are planted.

At present, PORIM field genebank maintains 59625 palms covering 406ha (1000 ac) (Table 11). One great advantage of field genebank is that the material is readily available for evaluation and breeding purposes.

### ***In-vitro***

Experiments are being conducted to study the long-term conservation of oil palm tissues e.g. embryos using cryopreservation technique where the temperature of the tissues is reduced to that of liquid nitrogen (-196°C) (Paranjothy *et al.*, 1986).

### ***In-situ* Conservation**

The natural palm groves in Africa possess genetic diversity which is essential to plant breeders to develop high yielding planting material which could meet the future challenges of the oil palm industry. However, these resources are disappearing at a rapid rate in Africa due to development and population pressure. Every effort must be made to preserve these resources for posterity. PORIM is playing a major role in the conservation and utilization oil palm genetic resources to safeguard the long-term interest of Malaysian oil palm industry.

## CONCLUSIONS

The oil palm genetic resources research programme is long-term in nature. It started way back in 1973 and the benefits are seen only after 20 years.

In the case of Nigerian palms the following results have been achieved:-

### **High yield**

Palms yielding more than 10t oil per ha per yr have been selected. These palms could double the current oil palm yields.

### **Dwarfness**

The height increment of high yielding dwarf palms is only 15-25 cm per year compared to 45-75 cm per year of current planting materials. These palms will facilitate easier harvesting and length the replanting cycle which is beneficial economically and to the environment.

### **High iodine value (I.V.) (unsaturation) (more liquid oil)**

High yielding palms producing crude palm oil (CPO) with high I.V. have been selected. These palms will yield higher olein fraction in CPO and it is possible to penetrate the lucrative liquid oil market.

### **High kernel content**

Families with high kernel content i.e. more than 12% kernels in bunches have been selected. The kernel content of current planting materials is only 6%. These materials will cater for the oleochemical industry which is expanding at a rapid rate.

Based on these Nigerian elite materials, three types of planting materials are being developed by PORIM. They are:-

- High yielding dwarf D x P planting material, PS1;
- High yielding planting material with high iodine value, PS2;
- High yielding planting material with high kernel content, PS3.

In future, collections from other countries have the potential to provide genes for high level of Vitamin E, carotene and further enhance the prospects for palm oil and oil palm by products.

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TABLE 1: VARIATION OF THE CHARACTERS OF NIGERIAN PALMS  
(FIELD DATA)

Fruit from	Statistics	Bunch weight	Bunch length	Bunch breadth	Bunch depth	No. of spikelets	Fruit length	Fruit diameter	Nut diameter	Kernel diameter	Shell thickness	Fruit spk.	Single fruit weight	Single nut weight	Mesocarp (%)	
Dura	$\bar{X}$	11.82	41.25	32.18	23.90	186.53	3.44	2.11	1.60	1.10	0.25	8.02	7.98	4.17	47.31	
	S.D.	6.13	6.35	5.19	4.18	53.52	0.53	0.36	0.31	0.22	0.09	2.38	2.74	1.52	7.75	
	n	595	595	594	449	595	595	595	595	595	595	436	591	591	591	591
	Max	41.36	60	51	390	360	5.7	3.7	3.1	2	0.7	20.4	23	11.7	79	
	Min	2.27	19	17	13	27	2.3	1.1	0.9	0.6	0.05	2.8	2	1	15	
	CV	51.86	15.39	16.13	17.49	28.69	15.41	17.06	19.38	20	36	29.68	34.34	36.45	16.38	
Tenera	$\bar{X}$	10.91	40.31	31.85	23.90	189.51	3.43	2.06	1.26	1.01	0.12	8.43	6.50	1.87	70.96	
	S.D.	5.14	6.58	5.20	3.65	57.37	0.46	0.35	0.22	0.19	0.06	2.55	1.95	0.95	9.78	
	n	322	319	319	319	258	324	324	324	324	324	243	320	320	320	320
	Max	28.64	65.0	47.0	36.0	340	4.6	5.8	2.1	1.7	0.4	19.4	15.4	11.9	97	
	Min	1.82	21.0	19.0	12.0	42.0	2	1.2	0.6	0.4	0.04	3.3	1.2	0.5	16	
	CV	47.09	16.32	16.34	15.27	30.27	13.41	16.99	17.46	18.81	50.0	30.25	30.00	50.90	13.78	

Key:  $\bar{X}$  = Mean  
 S.D. = Standard deviation  
 n = Sample size  
 Max = Maximum  
 Min = Minimum  
 CV = Percentage coefficient of variation

TABLE 2: CHARACTERISTICS OF CAMEROONS GENETIC MATERIALS  
(FIELD DATA)

	Cameroon Dura				Tenera			
	N	$\bar{X}$	SD	CV	n	$\bar{X}$	SD	CV
Bunch wt (kg)	52	16.83	9.086	53.99	38	17.28	7.84	45.37
Bunch length (cm)	52	36.50	16.26	44.55	-	-	-	-
Bunch breadth (mm)	52	32.00	11.31	35.34	-	-	-	-
Bunch depth (cm)	52	27.00	7.07	26.18	-	-	-	-
Stalk wt (kg)	51	2.73	1.50	54.94	40	2.66	0.95	35.71
Fruit length (cm)	52	3.90	0.55	14.10	41	3.68	0.47	12.77
Fruit diameter (cm)	53	2.23	0.41	18.38	42	2.08	0.35	16.83
Nut diameter (cm)	53	1.80	0.38	21.11	42	1.35	0.19	14.07
Kernel diameter (cm)	53	1.16	0.32	27.86	42	1.13	0.29	25.66
Single fruit wt (g)	53	10.33	3.31	32.04	42	8.56	2.84	33.17
Single nut wt (g)	53	6.24	2.25	36.06	42	3.15	1.13	35.87
Stalk/Bunch (%)	51	16.54	6.01	36.34	38	16.55	5.10	30.82
Mesocarp/Fruit (%)	53	39.96	7.67	19.32	42	62.37	7.61	12.20
Shell thickness (mm)	53	3.29	1.07	32.52	42	1.37	0.78	56.93

TABLE 3: CHARACTERISTICS OF ZAIRE MATERIAL (FIELD DATA)

Variable	Dura				Tenera			
	n	Mean	Std. Dev.	CV	n	Mean	Std. Dev.	CV
Bunch wt (kg)	232	17.60	7.24	41.44	79	17.38	8.47	48.73
Bunch length (cm)	223	42.53	6.95	16.34	76	44.13	7.29	16.52
Bunch breadth (cm)	223	35.45	5.05	14.25	76	36.08	5.53	15.34
Bunch depth (cm)	223	27.06	3.52	13.00	76	27.71	4.29	15.48
Stalk wt (kg)	232	2.15	0.94	43.72	77	2.20	1.02	46.36
Fruit length (cm)	233	4.02	0.49	12.19	82	4.08	0.53	12.99
Fruit diameter (cm)	233	2.55	0.36	14.12	82	2.44	0.37	15.16
Nut diameter (cm)	233	1.92	0.31	16.15	82	1.55	0.26	16.77
Kernel diameter (cm)	233	1.18	0.25	21.19	82	1.22	0.24	19.67
Single fruit wt (g)	215	14.22	4.26	29.95	80	12.62	4.80	38.03
Single nut wt (g)	214	7.89	2.51	31.81	80	4.34	1.91	44.00
Stalk (%)	230	12.22	2.77	22.67	76	12.63	3.83	30.32
Mesocarp/Fruit (%)	213	43.92	7.20	16.39	79	64.14	13.09	20.41
Shell thickness (mm)	233	3.68	0.93	25.27	81	1.68	0.79	47.02

TABLE 4: VARIATION OF THE CHARACTERS SCORED IN THE FIELD  
(Tanzania and Madagascar Genetic Material)

Fruit From	Statistics	B.Wt	MFW	MNW	M/F (%)	LF	DF	DN	DK	ST
Dura Tanzania	$\bar{X}$	18.4	16.9	8.9	46.7	4.3	2.7	2	1.3	0.35
	SD	13.54	4.1	2.7	10.08	0.46	0.47	0.31	0.29	0.09
	n	42	42	42	42	42	42	42	42	42
	Max	90	25.5	16.6	64.9	5.2	3.8	2.8	2.1	0.55
	Min	6.4	9.1	4.28	18.5	3.3	1.9	1.3	0.8	0.1
	CV	73.6	24.4	30.13	21.55	10.87	17.57	15.34	22.3	26
Tenera Tanzania	$\bar{X}$	13.69	15.5	8.5	70.63	4.41	2.69	1.71	1.38	0.16
	SD	6.51	5.24	1.94	8.84	0.63	0.44	0.12	0.1	0.04
	n	17.0	17	17	17	16	16	16	16	16
	Max	27.0	30.39	12.32	82.84	5.8	3.9	2	1.7	0.3
	Min	5	6.74	5.71	50	3.1	2.1	1.4	1.1	0.05
	CV	47.5	33.87	22.82	12.52	14.46	16.6	7.1	7.36	25.58
Madagascar	$\bar{X}$			1.76						
	SD			0.98						
	n			17						
	Max			5.11						
	Min			0.99						
	CV			56						

Key:

- $\bar{X}$  = Mean
- SD = Standard deviation
- n = Sample size
- Max = Maximum
- Min = Minimum
- CV = Coefficient of variation
- B. Wt = Bunch wt (kg)
- MFW = Mean fruit wt (g)
- M/F (%) = Mesocarp/fruit
- MNW = Mean nut wt (g)
- LF = Length of fruit
- DF = Diameter of fruit
- DN = Diameter of nut
- DK = Diameter of kernel
- ST = Stalk weight (kg)

**Table 5: A comparison of bunch characters of oil palm genetic material collected in West African countries.**

Country	Bunch wt (kg)	<i>Dura</i>			<i>Tenera</i>		
		Single wt(g)	Mesocarp fruit (%)	Bunch wt (kg)	Single wt(kg)	Mesocarp fruit (%)	
Senegal	5.94 (36.90)	2.65 (26.63)	35.18 (18.75)	-	-	-	
Gambia	5.74 (39.52)	2.54 (22.07)	33.47 (16.35)	-	-	-	
Guinea	11.4	6.4	35.0	-	-	-	
Sierra Leone	9.5	5.6	34.8	-	-	-	
Ivory Coast	10.9 (37.0)	6.9 (28.87)	41.8 (12.92)	9.8 (40.72)	5.8 (27.99)	61.2 (10.46)	
Nigeria	11.8 (51.86)	7.98 (34.34)	47.3 (16.38)	10.9 (47.09)	8.5 (30.00)	70.99 (13.78)	
Cameroon	16.8 (53.9)	10.3 (32.04)	39.7 (19.32)	17.3 (45.37)	8.6 (33.17)	62.4 (12.20)	
Zaire	17.6 (41.14)	14.2 (29.95)	43.9 (16.39)	17.4 (48.73)	12.6 (33.03)	64.1 (20.41)	
Tanzania	18.4 (73.60)	16.9 (24.40)	46.7 (21.55)	13.7 (47.50)	15.5 (33.87)	70.8 (12.52)	
Angola	21.4	14.24	8.9	16.0	11.7	70.9	

Note: Figures in parenthesis are coefficient of variation

**Table 6: Performance of Selected Nigerian Teneras**

No.	Palm No.	Prog.	O/B	K/B	n*	Average yield (6-8 yr)	F.L. (m)	Ht. Increase (cm/an) (8 yr)
1	2657	8 - 16	28.9	3.4	3	163.30	4.40	16.30
2	6380	4 - 01	27.5	7.1	3	185.30	4.91	25.40
3	2793	9 - 3	29.5	6.5	4	228.70	5.45	19.40
4	5180	9 - 28	28.7	6.7	3	185.50	4.35	30.20
5	2422	10 - 2	28.7	4.5	4	171.00	5.27	21.00
6	5385	16 - 07	28.8	5.1	6	188.00	5.14	14.20
7	289	10 - 12	28.4	6.4	6	174.80	5.12	13.80
8	1269	10 - 15	29.6	6.4	4	174.70	3.75	17.00
9	4814	16 - 10	27.4	6.4	4	140.80	5.14	23.90
10	5843	13 - 3	28.9	6.7	3	167.20	4.97	23.40
11	3933	17 - 06	31.2	2.4	3	206.70	5.02	27.50
12	8480	15 - 12	28.2	6.0	3	149.30	5.04	23.90
13	7285	18 - 23	28.6	4.7	3	172.00	4.98	18.30
14	7748	15 - 23	28.8	5.6	3	176.20	5.05	25.40
15	7403	16 - 5	28.1	4.4	2	217.50	5.61	22.80
16	3207	19 - 06	29.1	2.9	3	198.00	4.92	17.60
17	6763	16 - 21	29.8	5.4	5	168.50	5.75	15.90
18	1791	18 - 8	30.5	3.9	4	201.00	5.12	15.60
19	4575	18 - 8	31.7	4.5	4	179.60	4.91	24.10
20	4576	18 - 8	28.6	2.8	3	161.70	4.91	16.00
21	2190	18 - 23	27.7	5.4	4	182.70	5.11	24.10
22	1751	19 - 2	28.4	4.5	3	217.20	5.25	23.80
23	7628	19 - 6	27.8	3.1	6	185.80	5.50	15.50
24	692	19 - 8	30.2	5.4	4	168.50	5.03	23.00
25	9037	29 - 22	29.3	3.2	3	149.30	4.65	23.10
26	4385	19 - 14	30.3	5.2	3	219.30	5.16	23.50
27	3752	27 - 02	28.5	4.5	4	212.50	4.94	25.40
28	7717	19 - 14	27.6	6.5	2	217.80	5.47	25.00
29	1931	19 - 15	29.4	3.9	5	200.20	4.45	16.00
30	6959	27 - 2	29.8	2.8	4	170.30	4.80	21.50
31	6566	28 - 4	30.5	6.5	3	158.70	5.08	23.00
32	5159	28 - 12	27.3	4.5	3	194.20	4.15	21.30

**Table 7: Fatty acid composition of high iodine value Nigerian germplasm palms**

Palm No.	C14:0	C16:0	C18:0	C18:1	C18:2	I.V.
22	0.6	32.8	6.5	43.7	15.3	63.9
38	0.5	32.5	7.9	44.3	13.5	61.3
48	0.7	35.4	5.5	43	14.2	61.4
128	0.6	35.3	5.3	42.1	15.8	63.4
146	0.5	32.4	5.6	45	15.5	65.4
151	1.1	36.1	5.4	41.6	14.8	61.2
618	0.6	33.7	6.7	44.2	13.5	61.2
814	0.5	32.6	6.6	47.4	11.8	61.1
903	0.4	30.8	7.3	46.5	13.9	63.9
971	0.3	30.2	7.0	49.1	12.9	64.4
1861	1.4	37.0	6.4	36.2	17.6	61.4

**Table 8: Trial 0.219: Yield *dura*; population mean of year 1990-1993 (Cameroons)**

Pop	FFB	BNO	ABW
1	69.88	14.65	4.80
2	84.79	18.64	4.65
3	67.47	10.75	6.57
4	74.28	16.85	4.46
5	77.74	14.24	5.85
6	90.35	16.64	5.53
7	82.11	15.33	5.14
8	80.62	14.01	5.93
9	81.28	15.60	5.21
10	81.03	15.24	5.52
11	86.50	15.45	5.75
12	55.58	13.89	3.91
13	62.35	15.06	4.42
14	80.58	14.62	5.61
15	81.40	16.25	5.09
16	72.98	12.29	6.08
17	75.70	13.85	5.65
18	86.94	16.56	5.41
19	88.92	15.19	6.15
20	72.67	13.69	5.28
21	97.70	14.63	6.83
22	72.34	12.45	6.02
23	88.12	15.85	5.58
24	82.15	13.06	6.59
25	57.65	11.04	5.09
26	80.58	14.37	5.81
27	96.70	16.40	6.01
28	79.95	14.90	5.46
29	85.67	14.94	5.81
30	56.07	12.20	4.74
31	64.27	11.08	5.69
Mean	79.77	14.88	5.51

Table 9: Zaire; Pop Mean for FFB, BNO, ABWT (1990-1993)

Pop	FFB	BNO	ABWT
1	102.77	13.47	7.74
2	77.96	12.16	6.60
3	84.75	10.57	7.97
4	74.58	10.43	7.45
5	85.46	12.02	7.29
6	80.39	12.61	6.56
7	90.47	12.28	7.43
8	83.61	13.17	6.47
9	79.73	12.99	6.46
10	81.68	11.62	7.14
11	89.59	13.75	6.81
12	82.85	11.51	7.56
13	79.10	12.11	6.44
14	85.05	12.66	7.05
15	84.63	12.40	6.95
16	78.42	11.03	7.28
17	83.42	11.62	7.58
18	90.65	12.42	7.42
19	83.59	13.33	6.56
20	79.84	11.61	7.05
21	92.75	14.50	6.36
22	83.37	12.07	7.13
23	102.53	18.11	5.71
24	85.81	12.69	7.01
25	89.26	11.87	7.72
26	82.51	11.72	7.65
27	89.89	12.35	7.42
28	83.82	10.97	7.88
29	89.16	14.55	6.25
30	100.26	16.77	6.06
31	94.37	17.22	5.71
32	83.05	13.37	6.52
33	79.27	12.08	6.89
34	78.28	12.48	6.64
35	80.15	12.79	6.43
36	78.88	12.80	6.51
37	79.07	11.70	6.91
38	79.76	11.83	6.74
39	75.25	12.15	6.42
40	82.82	12.39	6.85
41	84.03	11.53	7.06
42	86.83	12.08	7.32
43	71.90	11.57	6.38
44	77.40	10.63	7.72
45	101.30	17.61	5.89
46	73.09	12.13	6.25
47	82.77	15.06	5.59
48	84.74	13.91	6.20
49	80.48	13.08	6.27
50	91.93	15.33	6.42
51	83.09	15.09	5.66
52	89.44	16.07	5.82
53	85.32	13.73	6.28
54	86.83	15.03	5.93
55	85.92	15.50	5.63
Mean	83.72	12.76	6.84

**Table 10. Performance of selected Nigerian teneras**

No.	Palm No.	Prog.	O/B	K/B	n	Average yield (6-8 yr)	F.L. (m)	Ht. Increase (cm/an) (8 yr)
1	6380	4-01	27.5	7.1	3	185.3	4.91	25.4
2	2793	9-3	29.5	6.5	4	228.7	5.45	19.4
3	5180	9-28	28.7	6.7	3	185.5	4.35	30.2
4	2422	10-2	28.7	4.5	4	171.0	5.27	21.0
5	5385	16-07	28.8	5.1	6	188.0	5.14	14.2
6	289	10-12	28.4	6.4	6	174.8	5.12	13.8
7	1269	10-15	29.6	6.4	4	174.7	3.75	17.0
8	3933	17-06	31.2	2.4	3	206.7	5.02	27.5
9	7285	18-23	28.6	4.7	3	172.0	4.98	18.3
10	7748	15-23	28.8	5.6	3	176.2	5.05	25.4
11	7403	16-5	28.1	4.4	2	217.5	5.61	22.8
12	3207	19-06	29.1	2.9	3	198.0	4.92	17.6
13	1791	18-8	30.5	3.9	4	201.0	5.12	15.6
14	4575	18-8	31.7	4.5	4	176.9	4.91	24.1
15	2190	18-23	27.7	5.4	4	182.7	4.10	24.1
16	1751	19-2	28.4	4.5	3	217.2	5.11	23.8
17	7628	19-6	27.8	3.1	6	185.8	5.25	15.5
18	4385	19-14	30.3	5.2	3	219.3	5.16	23.5
19	3752	27-02	28.5	4.5	4	212.5	4.94	25.4
20	7717	19-14	27.6	6.5	2	217.8	5.47	25.0
21	1931	19-15	29.4	3.9	5	200.2	4.45	16.0
22	6959	27-2	29.8	2.8	4	170.3	4.80	21.5
23	5159	28-12	27.3	4.5	3	194.2	4.15	21.3

**Table 11: Number of Palms and Area Occupied by Field Genebank**

Collection	Species	No. of palms planted in the field	Area (ha)
Nigerian	<i>E. guineensis</i>	31434	212
Zaire	<i>E. guineensis</i>	13750	93
Cameroons	<i>E. guineensis</i>	3590	24
Tanzania	<i>E. guineensis</i>	3104	21
Madagascar	<i>E. guineensis</i>	38	1
Angola	<i>E. guineensis</i>	2507	17
Senegal	<i>E. guineensis</i>	To be planted	
Gambia	<i>E. guineensis</i>	To be planted	
Sierra Leone	<i>E. guineensis</i>	To be planted	
Guinea	<i>E. guineensis</i>	To be planted	
Honduras	<i>E. oleifera</i> )	4248	29
Nicaragua	<i>E. oleifera</i> )		
Costa Rica	<i>E. oleifera</i> )		
Panama	<i>E. oleifera</i> )		
Colombia	<i>E. oleifera</i> )		
Suriname	<i>E. oleifera</i> )		
Brazil	<i>E. oleifera</i> )		
Colombia, Peru	<i>Bactris</i>	30	1
Colombia, Peru	<i>Oenocarpus</i>	265	2
Colombia, Peru	<i>Jessenia</i>	639	5
Colombia, Peru	<i>Euterpe</i>	20	1

Figure 1 : **STAGES AND TIME FRAME**  
 (collection, establishment, evaluation and release of elite planting materials)

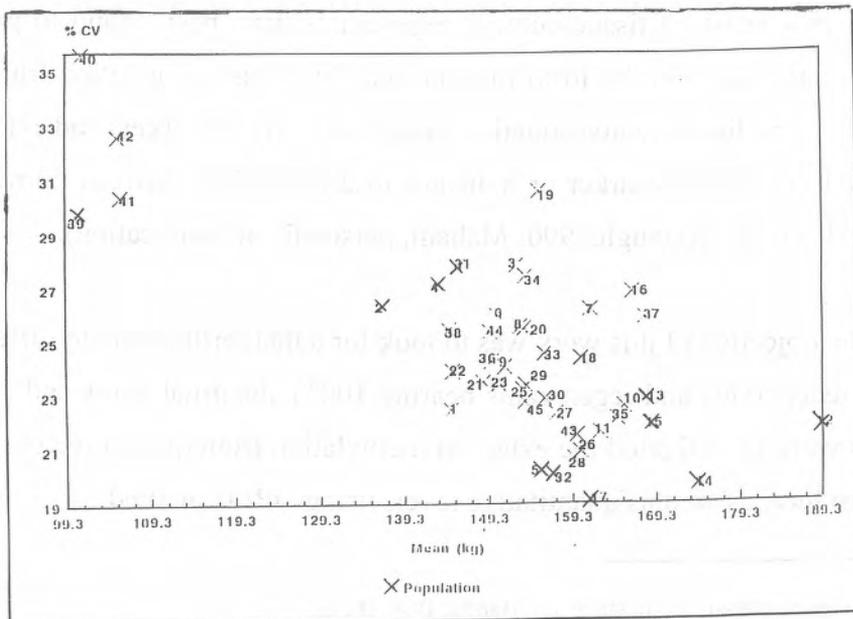
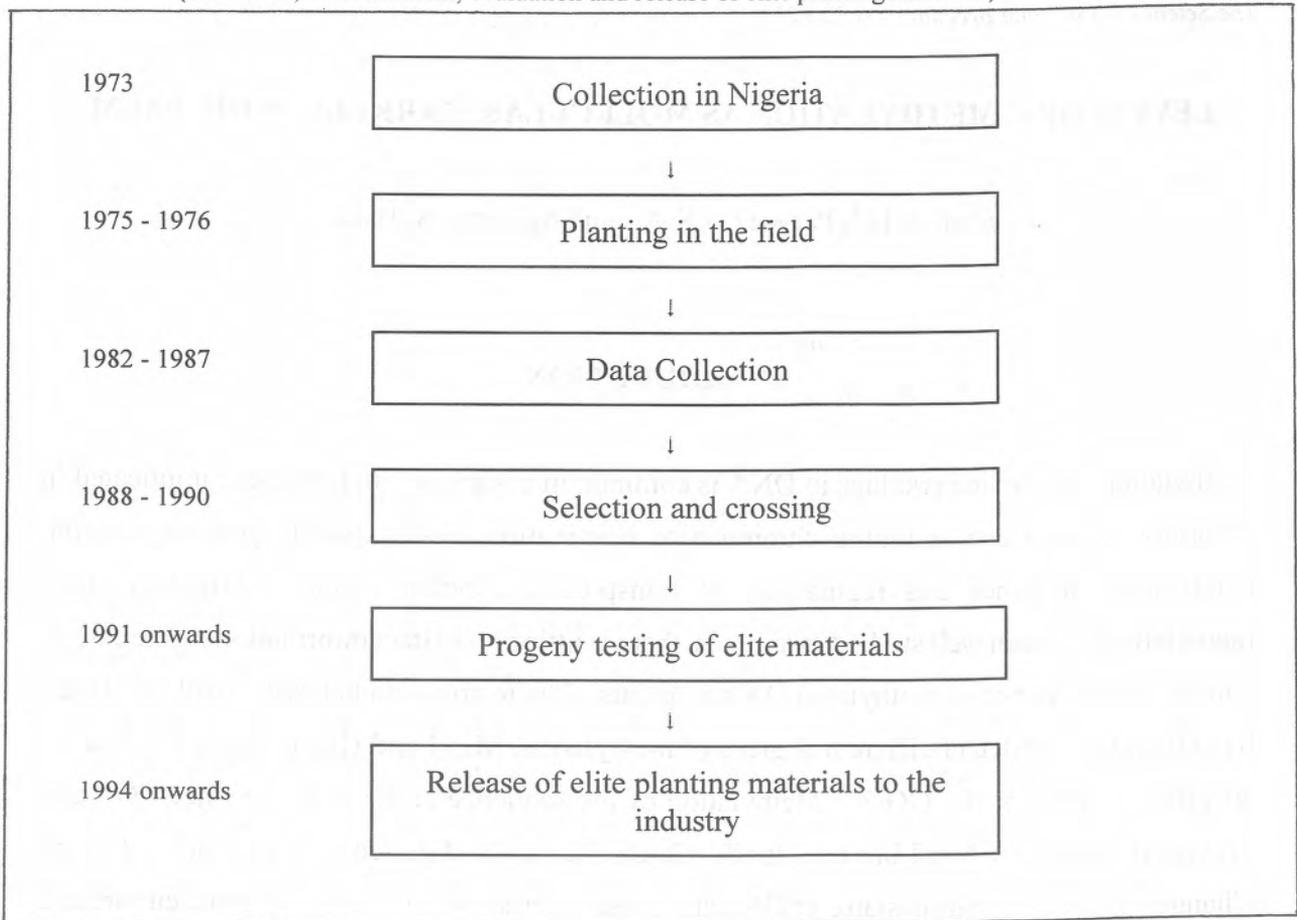


Figure 2. Trial 0.148 (Dura) Fresh Fruit Bunch (82-86).

## LEVELS OF C-METHYLATION AS MOLECULAR MARKERS IN OIL PALM

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### INTRODUCTION

Methylation of cytosine residues in DNA is common in eukaryotes and has been implicated in a variety of functions including chromosome inactivation, tissue specific gene expression, inactivation of genes and regulations of transposons (Doefler, 1983). Although DNA methylation has been well studied in animals, there is little or no direct information on the extent, pattern or inheritance of methylated DNA in plants. The restriction nucleases MspI and HpaII have been used to detect different degrees of methylation. Msp I and Hpa II digest DNA at the specific sequence 5' -CCGG-3'. Methylation of the sequence at the 5' and, 5'-meCCGG can prevent digestion by MspI but not HpaII. CmeCCGG can be digested by HpaII but not MspI. Changes in the methylation status of DNA have also been shown as a result of tissue culture and linked to phenotypic changes (Phillips *et al.*, 1990).

In oil palm, as a result of tissue culture, regenerants have been found to produce abnormal phenotypes, particularly in the form of abnormal fruits called 'mantled fruits' (Corley *et al.*, 1986). Previous preliminary investigation using biochemical markers and cytogenetic methods were unable to provide a marker or indicator to differentiate between normal and abnormal regenerants (Rao and Donough, 1990; Mahani, personal communication).

Therefore the objective of this work was to look for a marker/markers to differentiate between the normal regenerants and regenerants bearing 100% abnormal fruits and 50-70% abnormal fruits. This work investigated the extent of methylation from qualitative observation of DNA digestion products as well as quantitative levels using HPLC method.

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At the same time, in a previous experiment (Shah and Parveez, 1992) it was noted that the oil palm *E. guineensis* var *Tenera* (which is a cross between *E. guineensis*, *dura* and *pisifera*) showed a higher degree of methylation compared to its parental types when digested with PstI, a methylation-sensitive enzyme. So we were interested to determine whether differences in these levels do indeed exist. Therefore, similarly the degree of methylation and HPLC analyses were carried out on these different varieties. It is hoped that the results may provide a better understanding of the methylation status in oil palm as well as providing a better understanding towards changes in the methylation pattern as a result of tissue culture.

## MATERIALS AND METHODS

### Materials

Lead samples of *Elaeis guineensis*, *Dura* (246/5), *Pisifera* (281/26) and their progeny *Tenera* (543/40) were obtained from the Golden Hope plantation. *Elaeis oleifera* leaves were obtained from the PORIM plantation. Also plants used in the first part of the study are the regenerated plants from 3 different clones (parental plants) of different genotypes Fc028, Fc071 and Fc080. For each of the 3 parental types, normal and abnormal plants producing 100% 'mantled' fruits and plants producing 50-70% mantled fruits were analysed and their numbers listed below (Table 1). The leaves from the plants were supplied by the Federal Land Development Agency (FELDA) plantation.

### Methods

#### Extraction of DNA

Total DNA was isolated using the modified method of Dellaporta *et al.* (1983).

#### Digestion of DNA

DNA was digested with EcoRI, HpaII and MspI as recommended by the manufacturers (BRL), separated on 1% gel and blotted on to Nylon membranes (Hoffer). The hybridisation was performed as described by Maniatis (1982) with a final wash of 4xSSC, and 0.1% SDS at 55°C.

### HPLC determination of base composition

For HPLC analysis, 40  $\mu\text{g}$  of DNA was redissolved in deionised water at 1  $\mu\text{g}$  per ml and 4 vol of formic acid was added. DNA was hydrolysed by heating in an oven for 1 hr at 170°C, after which the hydrolysed DNA was cooled to room temperature and then freeze-dried. The freeze dried hydrolysate was dissolved in 0.1M Hcl and left at 4°C until used.

Control solution (containing adenine cytosine, 5-methylcytosine, guanine and thymine and uracil) was prepared by dissolving at a concentration of 6 mg per ml in 0.1M Hcl. HPLC was carried out using waters (501 HPLC) pump; waters 991 photodiode array detector) and analyzed by computerisation. The bases were eluted with a buffer containing 20mM  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 5mM SDS and 33% methanol (pH 5.4) over a period of 20 minutes. Bases were detected at 260 nm and percentage were calculated using waters software package and calibration curves. Percentage of 5-methylcytosine was calculated as a ratio of 5-methylcytosine to 5-methylcytosine + cytosine (Citti *et al.*, 1983).

## RESULTS AND DISCUSSION

### Methylation levels in *E. guineensis* and *E. oleifera*

In this research, the degree of methylation was at first observed qualitatively using two methylation sensitive enzymes such as Msp I and Hpa II. In the various digestions, enough enzyme was put to ensure that there was complete digestion. Therefore the susceptibility or resistance to digestions would be a reflection of the degree of methylation rather than incomplete digestion due to insufficient enzyme.

In a previous experiment, when DNA from *E. guineensis*, *dura*, *pisifera* and *tenera* were digested with a methylation sensitive enzyme such as Pst I, it was observed that the *tenera* displayed a higher level of methylation due to its insusceptibility to digestion by the enzyme Pst I. A non-methylation sensitive enzyme Hind III was also used as a control for complete digestion. Repetition of the experiment to ensure that was sufficient enzyme and quantitative analysis of result of digestion showed that there was indeed higher degree of methylation in the *tenera* when compared to its parental types, *dura* and *pisifera* (Figure 1).

HPLC analysis of the oil palm samples is presented in Table 2. Calculation of percentage methylation is based on the ratio of methylcytosine (methylcytosine + cytosine) x 100. HPLC analysis confirmed our observation that the intraspecific hybrid, *tenera* showed a much higher degree of C-methylation than its parental plants and also when compared to the other oil palm species that is, *Elaeis oleifera*. Although the intraspecific hybrid showed a higher degree of methylation, the interspecific cross between (O x P) displayed an almost similar level of methylation of 25% as its male parent, that is the *Pisifera* of 22%. Results obtained from HPLC confirmed the qualitative observation of the extent of digestions from agarose gel electrophoresis (Figures 1 and 2) using restriction enzymes Pst I, Hpa II and Msp I which are sensitive to C-methylation. Some of the HPLC profiles including the calibration are shown in Figure 3A, B, C and D).

Although the reason for the higher level of methylation in *tenera* is not apparent, it is suggested that the levels of methylation could be used as a marker in selection for *Tenera* in an  $F_1 \times F_1$  population or a population of *E. guineensis* accessions from Africa. Work is being carried out to determine the levels in the  $F_2$  segregating population. Therefore higher levels of methylcytosine could provide a means for culling out *dura* and *pisifera* types without waiting for the plants to bear fruits to be classified for selection procedures.

HPLC results showed a difference in the level of both species of oil palm *E. guineensis* and *E. oleifera*. In tomato, *Lycopersium esculentum* and *L. peruvianum*, no difference was detected between the two species (Messeguer *et al.*, (1991)). Our results in *E. guineensis*, *tenera* showed a higher level of methylated cytosines for more than those reported, which is up to 30% methylation (Gruenbaum *et al.*, 1981). However, all the documented levels have been for annual crops. This is the first documented data on the methylation status in perennial a crop. It has been shown in plants that 5-methylcytosine levels can be high, as plant DNA have an additional methylation site at sequence CNG as well as the doublet CG.

DNA methylation has been correlated with chromatin structure with the highly heterochromatic region in X-chromosome is highly methylated. Satellite DNA is heavily methylated and that, highly repeated sequences which are in the centromeric region and interstitial heterochromatin are also heavily methylated (Deumling, 1981). Therefore, it would be interesting to determine if there is a difference in the heterochromatin in the genomes of *E. guineensis dura*, *pisifera* and *tenera* as well as in *E. oleifera*. Unfortunately however, not much is known about the genome

in the oil palm. It has also been shown that the bigger the genome size, the higher the level of methylated cytosine (Wagner & Capesuis, 1981).

Enzymes Msp I and Hpa II have different sensitivity towards the sequence CCGG. Hpa II will not cut CmeCGG and Msp I will not cut the sequence meCCGG. Using these two enzymes, it was observed that there were much extensive digestion when Msp I was used. Results show that there were more cytosine methylation at the 5' end than in the internal cytosine. This result is in agreement with that of Gruenbuan *et. al.*, (1981) which show that in plants there is higher degree of methylation at the CG doublet than at the CNG triplet.

### **C-methylation levels in tissue culture regenerants**

The quantitative levels were determined by HPLC. Figures 4, 5, 6 show the electrophoresis of digestion products of DNA from the parent plants and their various regenerants, after digestion with Hpa II and Msp I. Figure 7 shows the digestibility of DNA from palm 115M (abnormal) and normal (115N) regenerants obtained from a different plantation (Golden Hope). The parent plant for palm 115M was not investigated due to nonavailability of tissue material. Figure 4 shows the digestion of DNA from parent plant Fc 028 and its abnormal regnerant bearing 100% mantled fruits and 70% mantled fruits and normal regenerants, after digestion with enzymes Hpa II and Msp I. Results show that there is less digestion with palms number 106 and 109 (producing 100% mantled fruits and 70% mantled fruits respectively) than the normal regnerant palm or their parent plant. Similarly Figures 5 and 6 showed that there appeared to be less digestion when the DNA from the abnormal regenerants was digested with Hpa II and Msp I when compared to their parental DNA or their respective normal regnerant. Therefore from the extent of digestion using both these enzymes, there appeared to be an increase in the degree of methylation in abnormal regenerants when compared to normal regenerants. Normal regenerants exhibited a similar extent of methylation when compared to the parent plants from which the explants were derived.

There was no consistency in the extent of methylation between regenerants showing 100% abnormality and 40-70% abnormality. Two regenerants (100% abnormality) exhibited a higher degree of methylation when compared to their sibling plants showing 40-70% abnormality (Figures 5 and 6). Regnerant Fc 028/109 (40-70% abnormality) however showed a higher extent of methylation when compared to the regnerant bearing 100% abnormality (Figure 4).

This observation differs from those observed for regenerants from clones Fc 071 and Fc 080. All the observations from electrophoresis of digestion products were confirmed by HPLC. Results from HPLC which detected the levels of methylcytosine in the various clones are shown in Table 3 and some HPLC profiles (Fig. 8A, B, C) are shown. HPLC results also confirmed the higher level of methylcytosine in regenerant Fc 028/109 (40-70% abnormality) when compared to Fc 028/106 (100% abnormality). In general therefore, all parent plants exhibited levels or extent of C-methylation similar to that observed for other *tenera* types (as shown in previous section). All mantled regenerants show a higher level of methylation from 48%-56%. Normal regenerant and parental plants showed a methylation level no exceeding 45%. This level could perhaps be used as a marker to determine which regenerants are abnormal and which are normal. Analyses on more regenerants within each pedigree was not carried out due to nonavailability of materials at the time of the project.

Each of the normal and abnormal regenerants were compared for their digestibility with Hpa II and Msp I. Regenerants from Fc 028 showed almost similar extent of digestion with Hpa II and Msp I. Similarly there were obvious differences between extent of digestibility with Hpa II and Msp I in the other regenerants which may reflect a similar level of methylation at both cytosine residues.

A substantial amount of work has shown the effects of tissue culture on the genome and efforts has turned towards a study of the mechanisms involved. One of the more recent work has emphasised on the role of methylation in generating somaclonal variation (Phillips, 1989). Studies on maize (Brown and Lorz, 1986; Brown, 1989) and on rice (Muller *et al.*, 1990) of regenerated plants by examining the extent of digestion by Hpa II and Msp I showed changes in the methylation status in 16% of the regenerated plants.

Using Southern blots and probing with cloned functional genes have revealed altered methylation (Brown, 1989) although none of the probes used related to abnormal phenotypes (Phillips *et al.*, 1990).

Some work reported on hypomethylation or hypermethylation occurring during tissue culture. Our results showed evidence of hypermethylation. It has also been reported that use of hormones such as 2, 4D and NAA at different concentration could induce methylation, particularly 2,4D. In carrots there was a positive correlation between exogenously added auxin and methylation of

cytosine residues, leading to an increase of methylated cytosine of up to 70% (Lo Schiavo *et al.*, 1989).

Despite the accumulating evidence that tissue culture can cause significant changes in DNA methylation, the basis of these alterations is still unknown. It has been suggested that cell cycle perturbation could cause methylation changes (Phillips *et al.*, 1990). Detection of such changes would require analysis of small groups of cells within the callus. Detection or assessment of genetic stability or instability at an early stage during tissue culture would prove to be important and valuable during tissue culture.

In oil palm, tissue culture of oil palm was previously carried using exogenous hormones. These could lead to the increase in methylation level. However, why the hypermethylation leads to production of abnormal palms remains to be investigated.

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**Table 1. Leaf samples of parent plants and regenerants**

Fc 028 ortet (parent plant)  
 Fc 028/026/106 100% mantled regenerant  
 Fc 028/026/109 50-70% mantled regenerant  
 Fc 028/026/122 normal

-----  
 Fc 071 ortet (parent plant)  
 Fc 071/04/2 100% mantled regenerant  
 Fc 071/04/3 50-70% mantled regenerant  
 Fc 071/04/8 normal

-----  
 Fc 080 ortet (parent plant)  
 Fc 080/037/156 100% mantled regenerant  
 Fc 080/037/164 50-70% mantled regenerant  
 Fc 080/037/155 normal

-----  
 115E 100% mantled regenerant  
 115E normal

**Table 2. Percentage cytosine and methylcytosine in oil palm species**

DNA samples	% cytosine	% methylcytosine
<i>Dura</i> 85/4338 (D)	88	12
<i>Pisifera</i> 98/6901 (P)	77	23
<i>Tenera</i> 1	53	47
<i>Tenera</i> 2 DxP	60	40
<i>Tenera</i> 3	62	38
<i>Dura</i> 246/5 D	85	15
<i>Pisifera</i> 281/26 D	75	25
<i>Tenera</i> 543/40 (DxP)	50	50
<i>Oleifera</i> (O)	67	33
<i>Pisifera</i> (P)	78	22
OxP (F <sub>1</sub> )	75	25

**Table 3. Percentage cytosine and methylcytosine in various oil palm parent plants and their normal and abnormal regenerants.**

		% cytosine	% methylcytosine
Fc 028 ortet		57	43
Fc 028/26/106	100% mantled regenerant	50	50
Fc 028/026/109	50-70% mantled regenerant	46	54
Fc 028/026/122	normal	58	42
Fc 071 ortet		60	40
Ce 071/04/2	100% mantled regenerant	49	51
Fc 071/04/3	50-70% mantled regenerant	52	48
Fc 071/04/8	normal	57	43
Fc 080 ortet		55	45
Fc 080/037/156	100% mantled regenerant	44	56
Fc 080/037/164	50-70% mantled regenerant	50	50
Fc 080/037/155	normal	56	44
115E	100% mantled regenerant	57	43
115E	normal	66	34

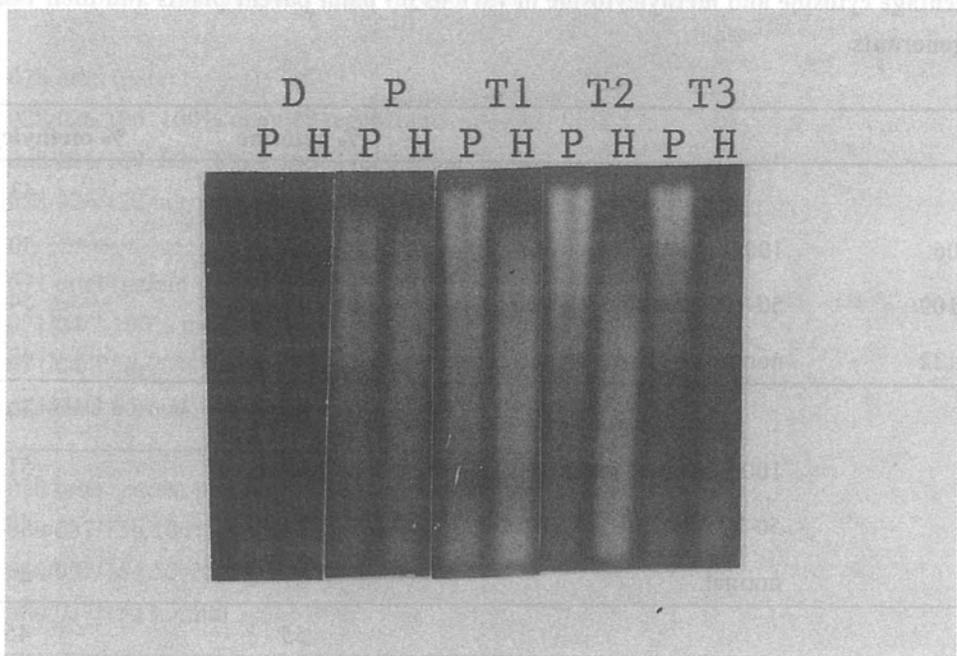


Figure 1. DNA digestion products after digestion with Pst I(P) and Hind III(H). D, T and P represent *dura*, *tenera* and *pisifera* respectively.

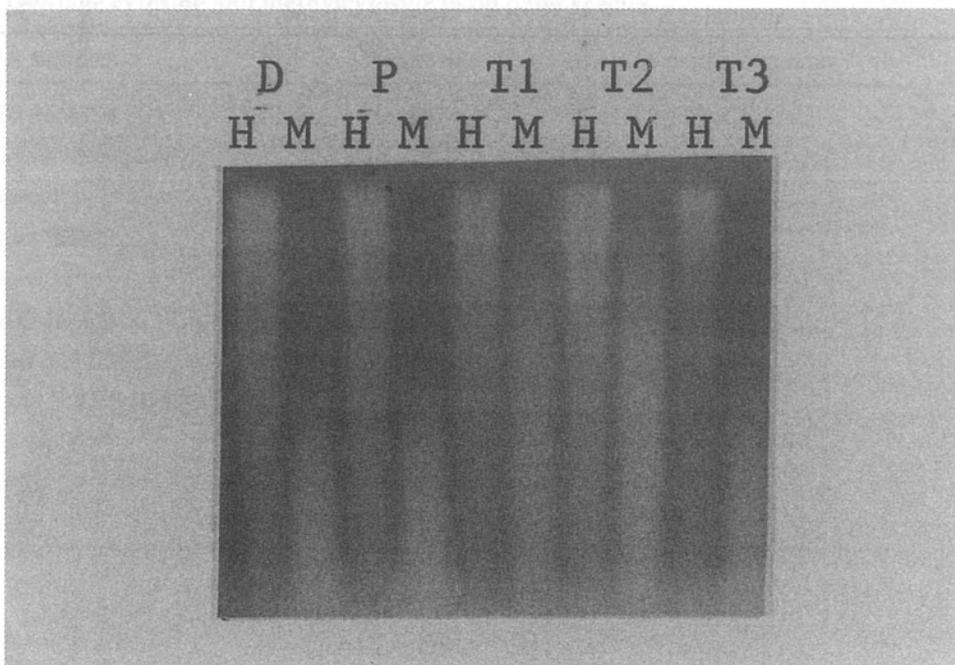


Figure 2. DNA digestion products after digestion with Hpa II (H) and Msp I(M). D, P and T represent *dura*, *pisifera* and *tenera* respectively.

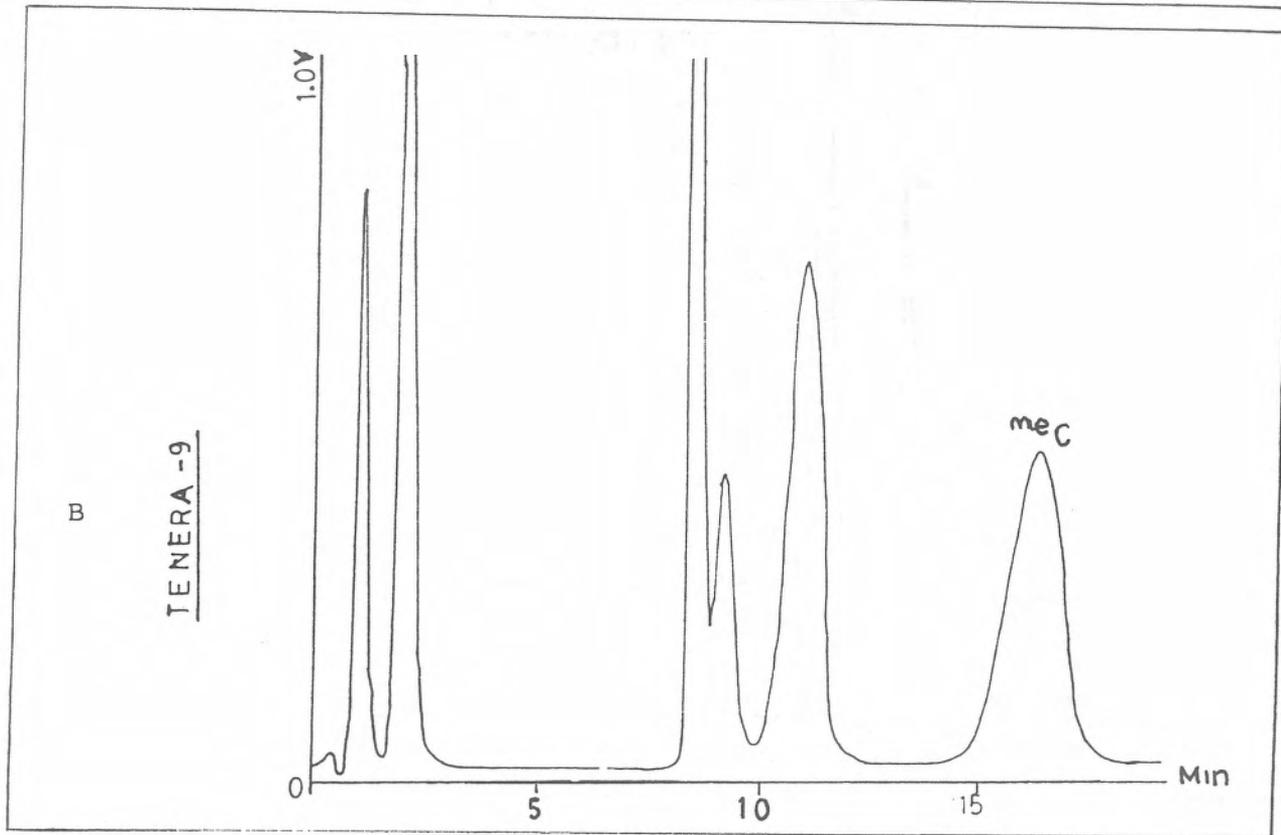
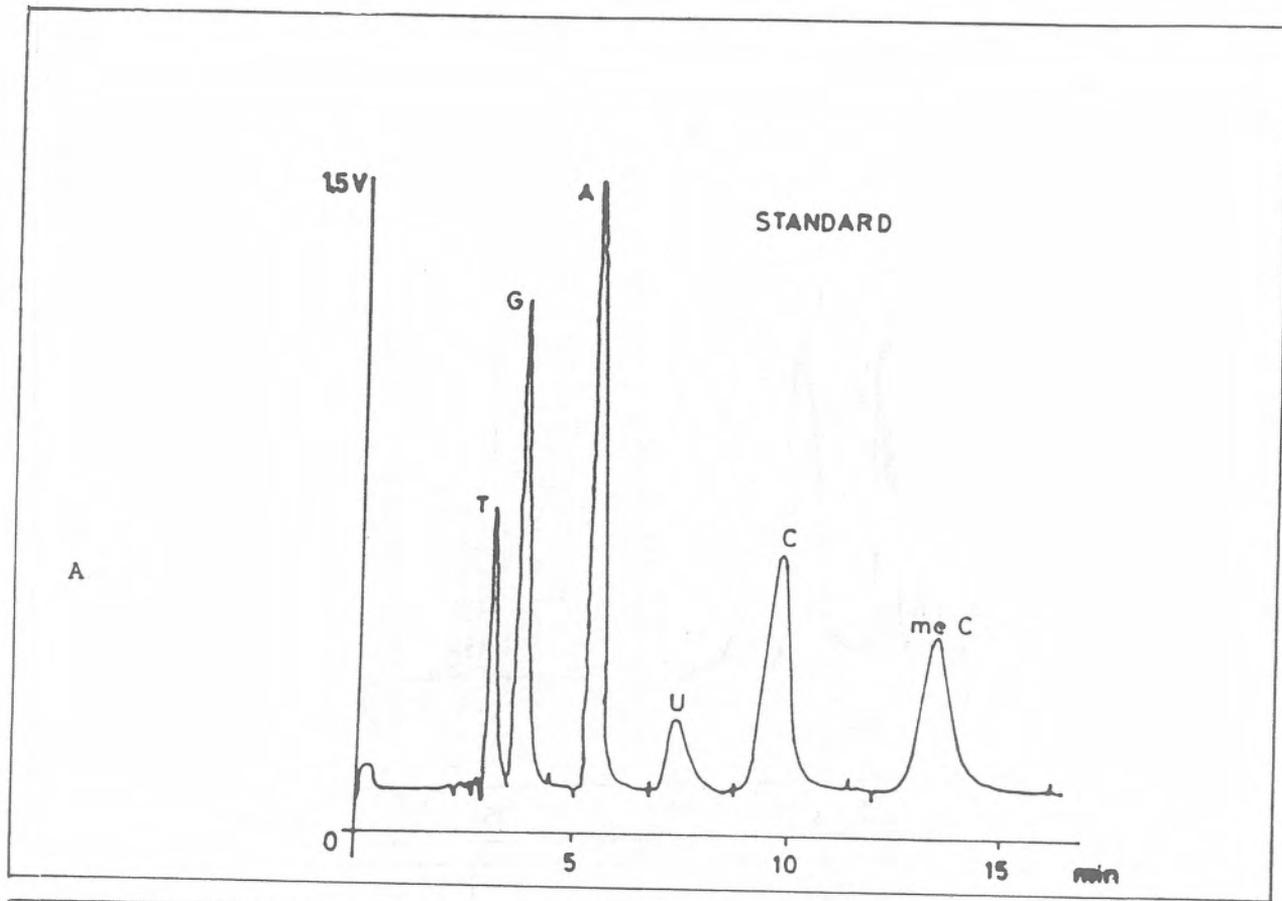
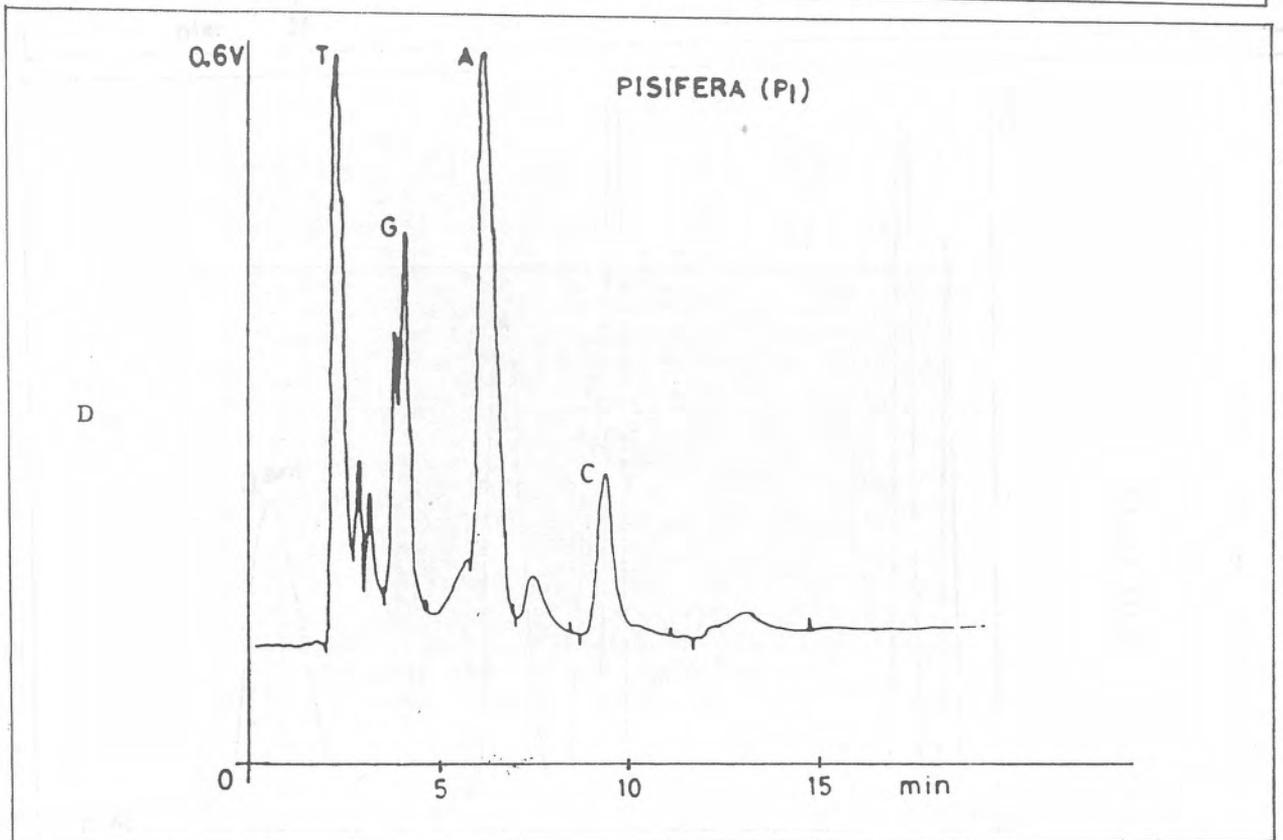
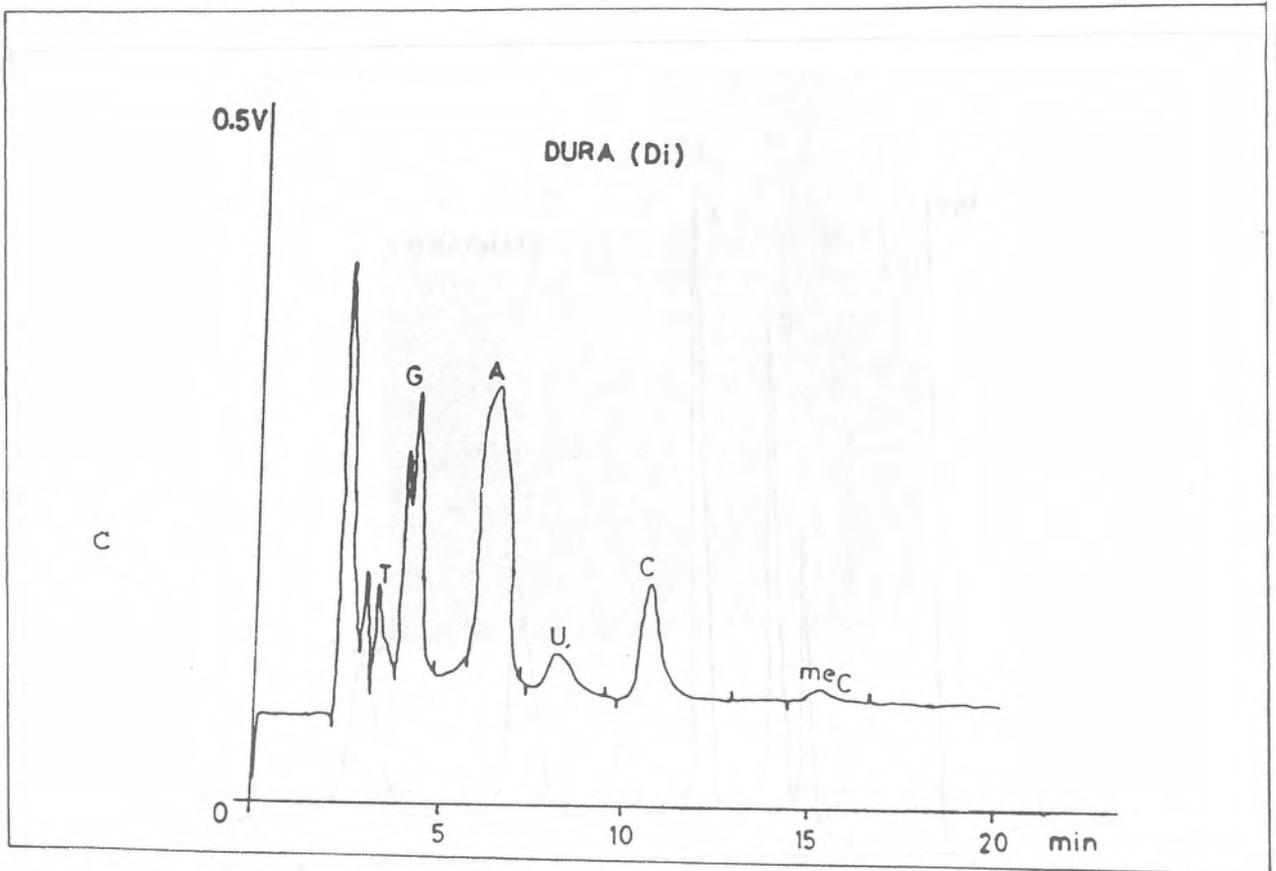
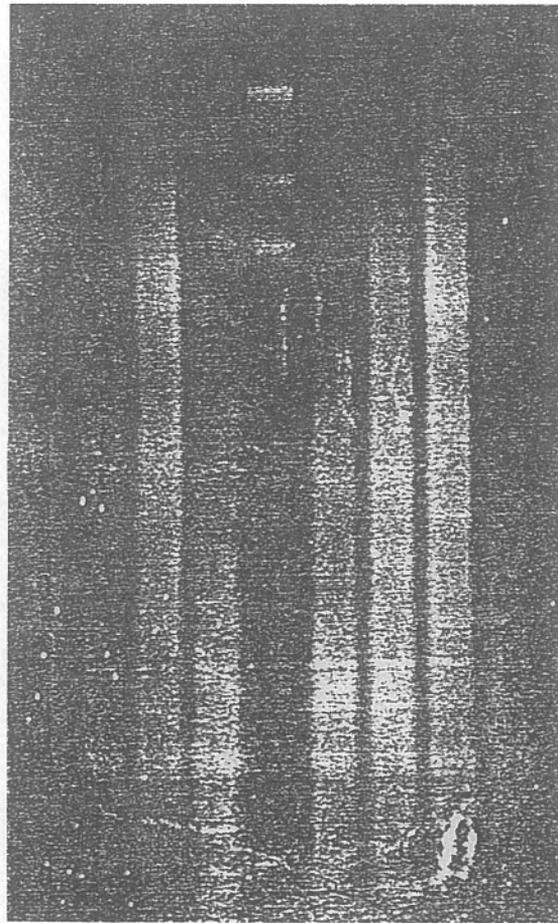


Figure 3A, B, C, D. Elution profiles of HPLC of DNA from *dura*, *pisifera*, *tenera* and the standard





1 2 3 4 λ 5 6 7 8  
MspI HpaII

Figure 4. Lanes 1 and 5, 2 and 6, 3 and 7, 4 and 8 represent DNA from samples 028, 106, 109 and 122 respectively after digestion with Msp I and Hpa II.

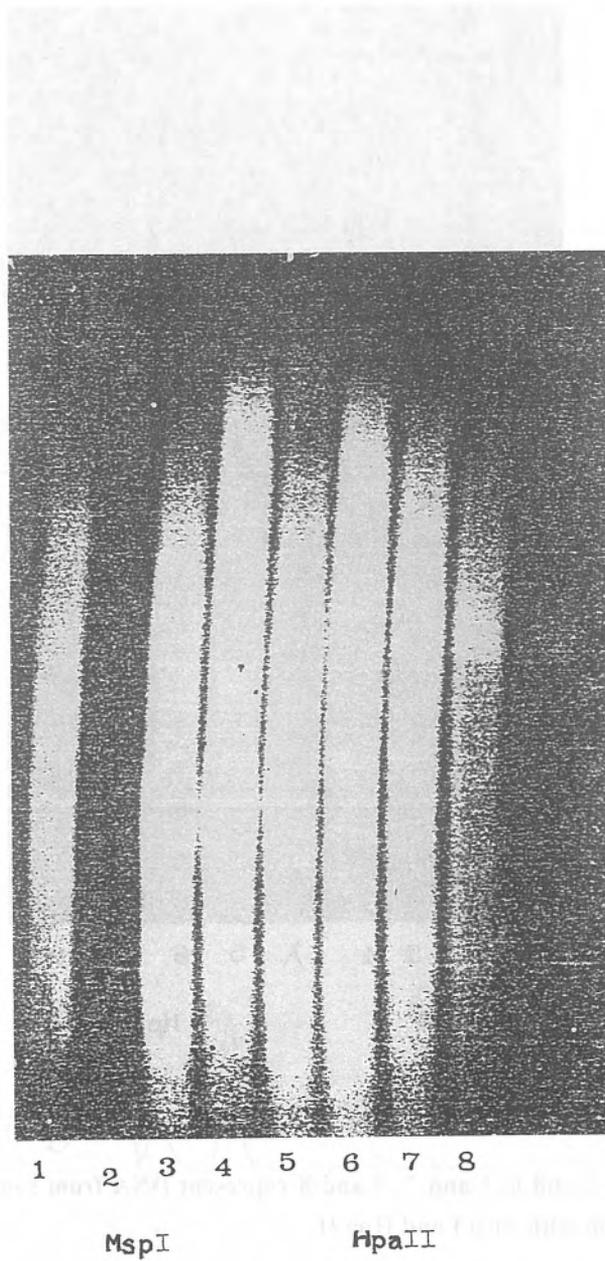


Figure 5. Lanes 1 and 5, 2 and 6, 3 and 7, 4 and 8 represent samples 071, 2, 3 8 respectively digested with Msp I(M) and Hpa II(H).

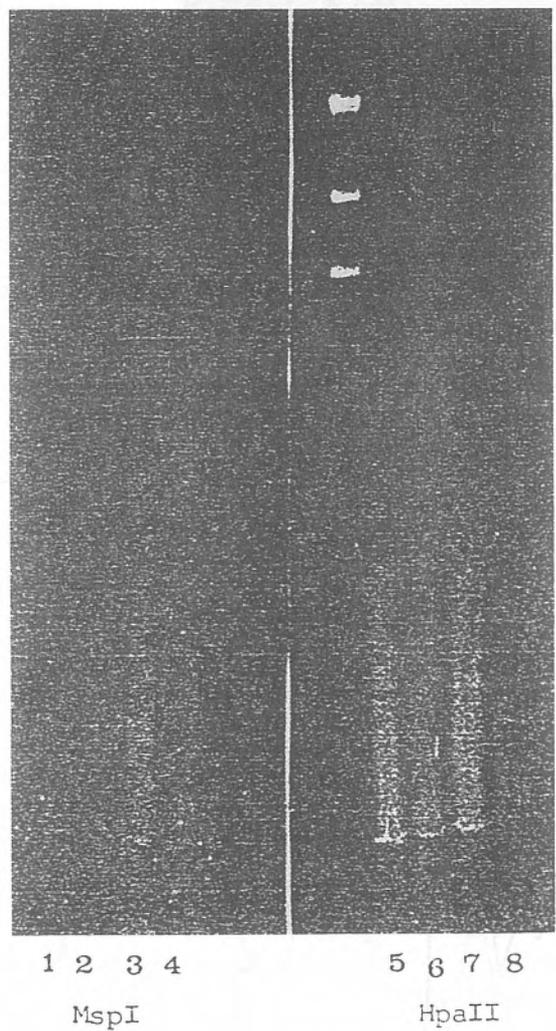


Figure 6: Lanes 1 and 5, 2 and 6, 3 and 7, 4 and 8 represent 080, 155, 164 respectively after digestion with Msp I and Hpa II.

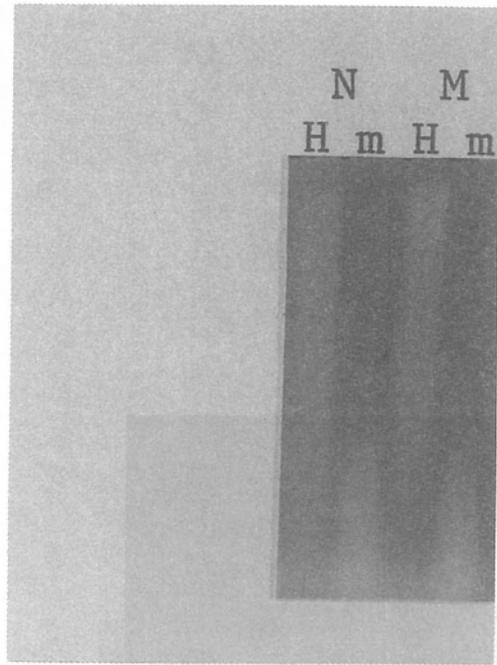


Figure 7. M and N represent mantled and normal leaf samples of clone 115.  
H: Hpa II and m: Msp I.

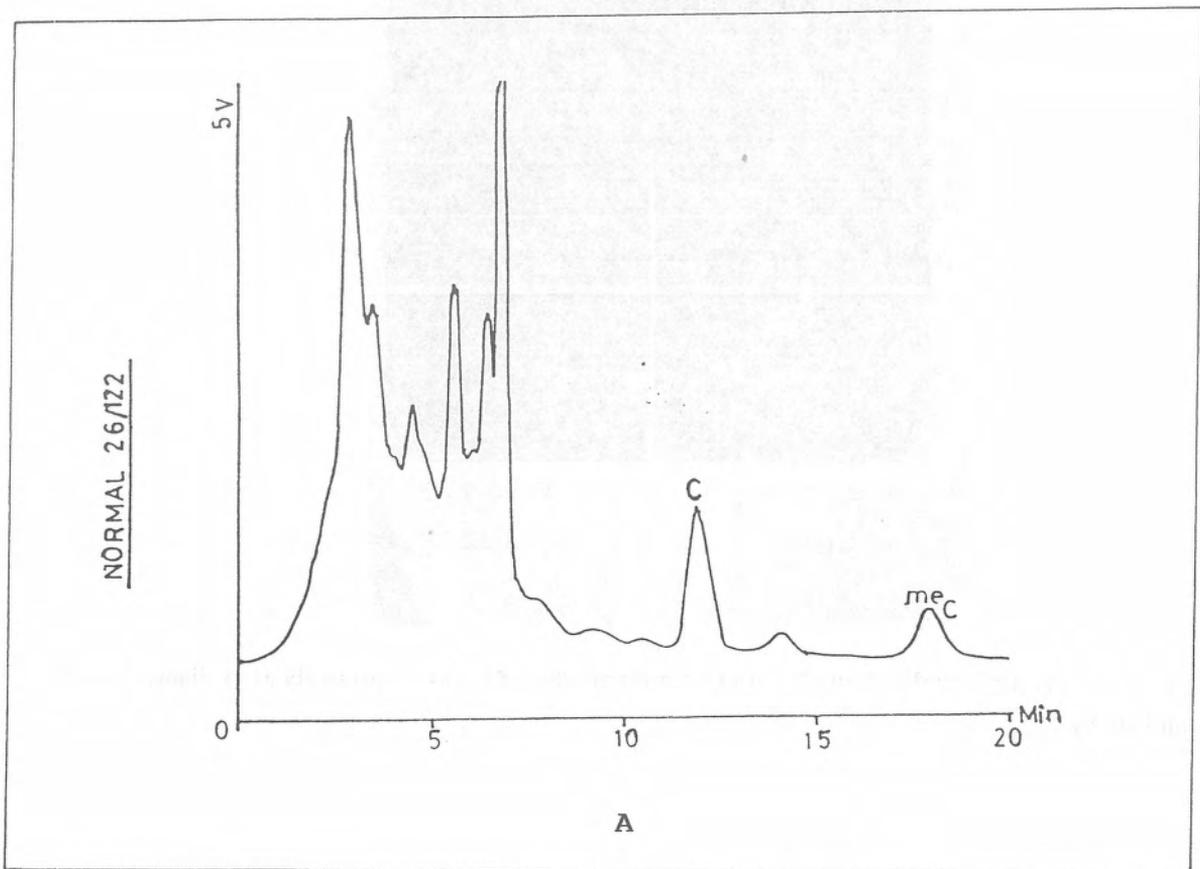


Figure 8A. HPLC elution profiles of DNA samples from 028/106, 028/109 and 028/122

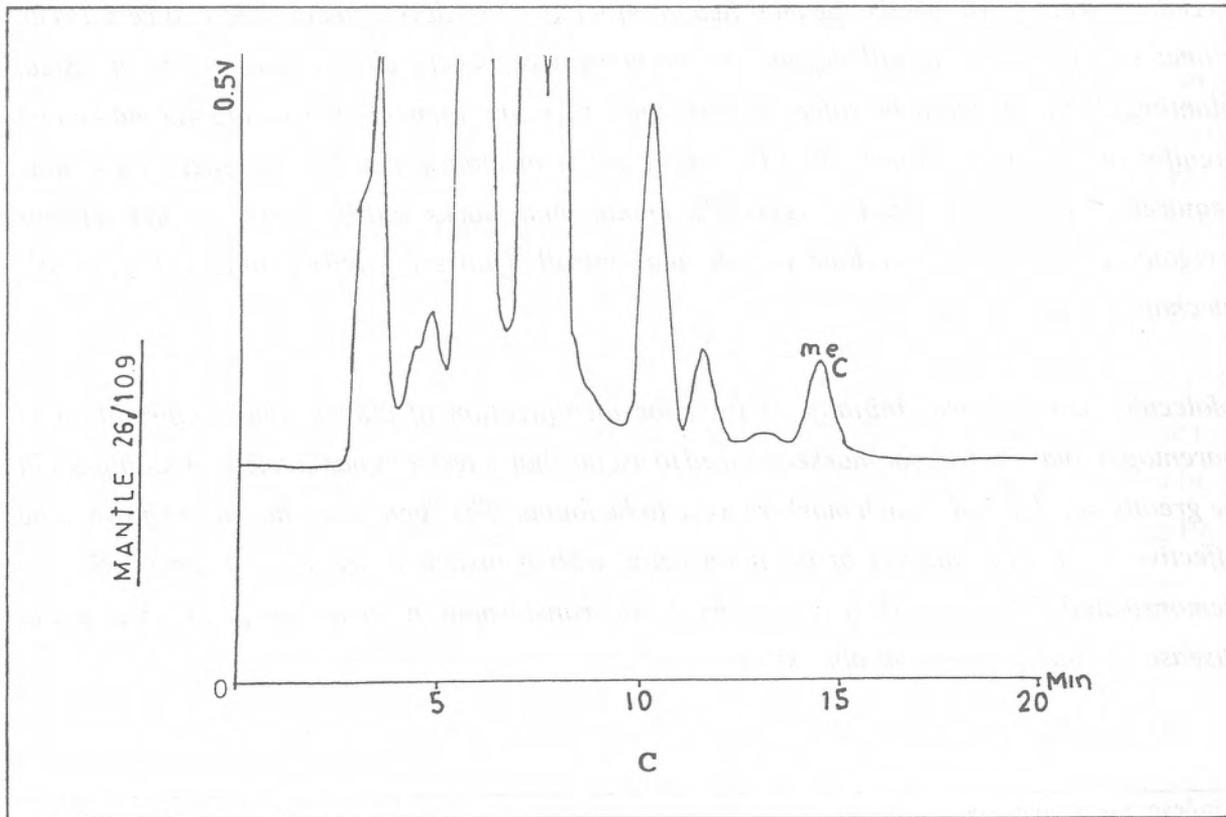
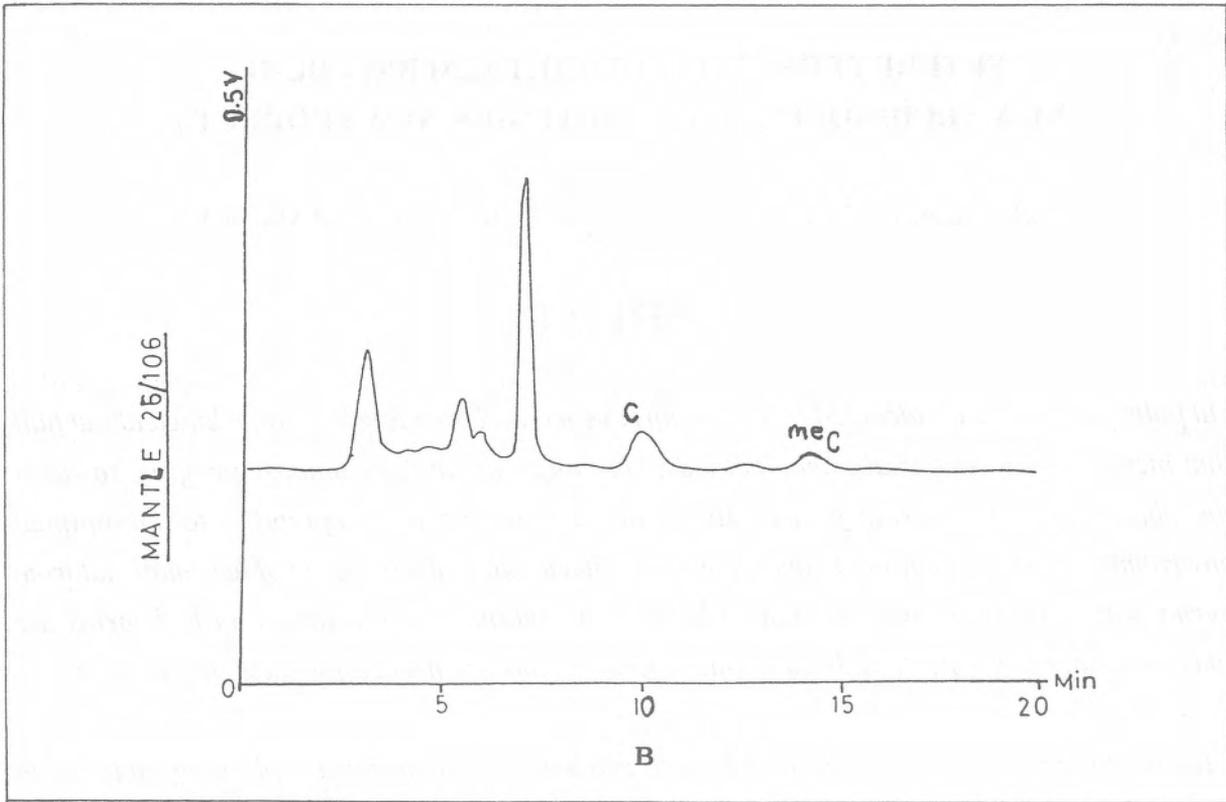


Figure 8B, C. HPLC elution profiles of DNA samples from 028/106, 028/109 and 028/122

## **FUTURE PROSPECTS FOR OIL PALM BREEDING: NEW TECHNIQUES, NEW STRATEGIES, NEW PRODUCTS**

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### **ABSTRACT**

*Oil palm yields have quadrupled in the last forty years, and breeding has contributed about half that increase. Through traditional methods, breeding will continue to make progress towards the objectives of increasing yields still further and improving adaptability to sub-optimal environments. The adoption of novel selection criteria, and introgression of new material from germplasm collections, will make significant contributions. Inbred lines and F<sub>1</sub> hybrids are likely to remain unimportant, because many generations are needed for their development.*

*Clonal propagation will be essential for the production of commercial planting material in future, with either clones or clonal seed being planted in preference to conventional seed. Breeding strategy will need to be modified for efficient clone development. The choice between clones and clonal seed will depend on circumstances; where clones have not been tested, planting clonal seed may be safer, because genotype x environment interactions should be less significant than with clones. For full exploitation of clones, research on their agronomic requirements will be needed. Eventually, monoclonal blocks will be fertilised, and perhaps irrigated, according to each clone's needs, and controlled flowering and ripening will allow fully mechanised harvesting.*

*Molecular markers will initially be used for identification of clones, and confirmation of parentages, but a search for markers linked to useful characteristics has started; breeding could be greatly accelerated if such markers were to be found. The "gene gun" has been shown to be effective for transformation of oil palm cells, with transient expression of the GUS gene demonstrated. The most likely initial targets for transformation are oil composition, pest and disease resistance, and fruit abscission.*

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*The potential yield of oil palm has been estimated at 17 t/ha/yr of vegetable oil. To achieve this, increases in both bunch index and oil/bunch will have to be combined with more efficient photosynthetic conversion of radiation. The latter is most likely to be achieved by reducing maintenance respiration rates.*

## INTRODUCTION

In this paper we shall consider the scope for future progress by conventional breeding in the oil palm, and the need for the introduction of new techniques. First, we will review breeding progress up to the present day. We will then discuss some of the objectives of oil palm breeding; current objectives are constrained by the limits of conventional breeding methods, so it will therefore be useful to review some of the new techniques now becoming available, before considering possible future breeding objectives. Finally we shall speculate as to the most likely developments over the next 20 years.

### Past Breeding Progress

Yields of oil from oil palms in Malaysia have quadrupled since 1950 (Davidson 1991), and approximately half this improvement has come through breeding. There has been steady progress in the yield of the breeding populations, with a 60% yield increase from Deli *dura* breeding between 1920 and 1970 (Lee *et al.*, 1990). This steady progress has been combined with a major improvement through the exploitation of a single gene, that controlling shell thickness.

The yield increase in the Deli *dura* population appears to have come from improvement in the photosynthetic conversion of intercepted radiation (Table 1; Corley and Lee, 1992). The main effect of the shell thickness gene is to improve the partition of assimilates in the *tenera* bunch compared to the *dura*, with oil being produced instead of shell (Table 2; Corley and Lee, 1992).

### Objectives of Oil Palm Breeding

**Yield** The primary objective of oil palm research is to increase the profit per hectare from the plantation; this applies as much to the oil palm breeder as to the agronomist or anyone else. The main way in which agricultural research contributes towards this objective is by increasing yields per hectare; increase yields lead to lower production costs, and improved margins. When grown in favourable environments, such as those of Malaysia and North Sumatra, the oil palm is already

much the highest yielding oil crop. Yields of over 5 tons/ha/yr are commonplace, and the highest recorded yields are over 9 tons/ha. (see, for example, Lee and Toh, 1990). When kernel oil is included, total vegetable oil yields are even higher. The potential yield of the crop may be as high as 17 tons oil/ha., though, so there is considerable scope for further improvement (Corley, 1983).

In many situations yields can be increased by reducing losses to pests or disease. Breeding for resistance can make a useful contribution here; the best example with oil palm, so far, is resistance to *Fusarium* wilt in Africa.

**Adaptability** The high yields recorded from oil palm in favourable environments often lead to its being planted in less favourable conditions, where the yields realised may be much lower. The breeder can contribute here by developing drought tolerant material for environments with a prolonged dry season, and perhaps cold tolerant material for planting at higher altitudes and higher latitudes than at present. Improving adaptation to unfavourable environments will not necessarily be very useful, though; unless production costs in such environments can be brought down to the same level as those in Malaysia and North Sumatra, the producer may be unable to compete on the world market. At present, many palm oil producing countries have protected internal markets, but such protection is gradually diminishing.

**Oil composition** The range of oil composition within the genus *Elaeis* is not very great, and major changes in oil composition are unlikely to be achieved by conventional breeding. It is often stated that the market for palm oil would be increased, if a more liquid oil could be produced. The argument is that over half of world trade in oils and fats is in liquid oils, but palm oil is not classified as a liquid oil. However, much of the liquid oil is actually hardened in manufacture to produce solid fats for margarine, and palm oil can be used in such products with little or no hardening. It should also be remembered that a liquid oil will only command a premium if it is sold as a special product. Palm oil which is more liquid than the standard, but is sold simply as palm oil, will possibly not conform to specifications for the manufacturing process, and may be rejected. Small increases in iodine value may be of little practical value, therefore.

At present, there is considerable demand for polyunsaturated oils, and, again, palm oil is not classified as polyunsaturated. However, the oil palm yields as much linoleic acid per hectare as do the major polyunsaturated oil crops, sunflower, maize and soya bean. In the case of palm oil, the linoleic acid is mixed with larger volumes of monounsaturated and saturated fatty acids, but

it may be easier to produce a polyunsaturated oil palm oil by transesterification and fractionation than by breeding.

Oils with high oleate or stearate levels are of interest, but could probably not be produced from oil palm without transgenic technology (see below).

## BREEDING TECHNIQUES

Conventional breeding concentrates on the plant population. Where cloning is possible, we can start to study individual plant genotypes. Molecular markers allow us to identify individual genes within plants, and by transgenic technology we can manipulate the genes within individual cells.

### Conventional Breeding

There is undoubtedly considerable scope for further improvement in yield, and in the other subsidiary objectives discussed above, through conventional breeding in existing breeding populations. For example, it has been suggested that there is relatively little useful variation left in the Deli *dura* population, but Okwuagwu (1991) showed that crossing of two Deli *dura* populations which had been developed independently generated significant amounts of variability. Other populations have a broader genetic base than the Deli *dura*, and the scope for further selection and improvement must be greater.

New or improved selection criteria and selection methods have a lot to contribute. For example, in most cereal crops harvest index has been increased by reducing the amount of dry matter going into vegetative growth, but Corley and Lee (1992) show that, at least up to 1970, breeders had not reduced the amount of dry matter going into vegetative growth in the Deli *dura* population. Numerous studies have shown significant genetic variation in bunch index (eg. Hardon *et al.*, 1972; Tan, 1978; Breure and Corley, 1983), so there must be scope for improvement, though some progress has probably been made since 1970, as many breeders now include bunch index among their selection criteria.

Selection for photosynthetic characteristics should be useful (Smith, 1991), though breeders have apparently already improved photosynthetic conversion coefficient (Corley and Lee, 1992). Modification of the leaf area trend with palm age could significantly improve the yield profile (Breure, 1985). Further selection criteria are discussed in other papers at this conference.

Collections of wild material (Rajanaidu and Rao, 1988) can be used to broaden the genetic base of breeding programmes, but almost certainly at the expense of some of the progress already achieved. Several generations of breeding may be needed to get back to the present level, but the scope for further improvement should then be increased. Molecular markers might help to accelerate the introgression process.

Interspecific hybridisation offers another approach to broadening the genetic base of breeding populations. *Elaeis oleifera* has a more liquid oil than *E. guineensis*, with hybrids between the two species being intermediate (Hardon, 1969). *E. oleifera* also has a shorter trunk and smaller leaves (Hardon, 1969), but vegetative dry matter production by the hybrids is not lower than in *E. guineensis* (Meunier and Hardon, 1976). Interspecific hybrids are reputed to be resistant to two major diseases in Latin America, but there is a lack of hard data on this resistance, and also on the aetiology of at least one of the diseases.

With maize, a similarly cross-pollinated species, the main breeding strategy has been the production of homozygous inbred lines, which are then crossed to produce genetically uniform but heterozygous  $F_1$  hybrids. This has not been attempted with the oil palm (though the reproduction of individual crosses by selfing the parents and then crossing between the two selfings (Gason *et al.*, 1981) is a step in this direction). Presumably the time involved has been a deterrent; at least eight generations of inbreeding are generally considered necessary to achieve more or less complete homozygosity.  $F_1$  hybrids are genetically equivalent to clones, and the development of cloning techniques obviates the need for an  $F_1$  hybrid programme. Conversely, though, if  $F_1$  hybrids were to be produced, some of the problems inherent in cloning (high cost, risk of somaclonal variation) could be avoided.

An alternative approach has also been suggested: according to Pooni *et al.* (1989), inbred lines could exhibit heterosis to the same extent as  $F_1$  hybrids. In the oil palm, this approach has the disadvantage that the preferred *tenera* genotype is heterozygous at the shell thickness locus. In theory, one might develop a genotype which was homozygous at all loci except that for shell thickness, but that would probably require more generations of inbreeding than achieving complete homozygosity. Alternatively, one could develop fertile *pisifera* lines, but most fertile *pisiferas* are less than fully fertile, and yield rather poorly.

## Clonal Propagation

Commercial oil palm plantings consist of genetically heterogeneous seedling populations, and the scope for yield improvement by cloning the best individuals is undoubtedly large. The development of abnormal flowers on cloned palms (Corley *et al.*, 1986) has set back the introduction of clones by nearly ten years, but this problem now appears to have been overcome (Corley, 1991), and clones should soon start to play a major part in commercial plantings. The recent description of a liquid suspension culture technique (de Touchet *et al.*, 1991) may allow the introduction of automated culture systems, thus reducing the cost of clonal plants, but even with the high cost of the present laborious techniques, there is no doubt that planting of clones will be profitable (Corley *et al.*, 1988; Corley, 1991). Extensive field testing will be necessary to identify the best clones, and clone testing techniques still need improvement. Cryopreservation (Engelmann and Duval, 1986) may be a useful way of storing cultures of new clones until they have been proven. The cost and problems of cryopreservation need to be compared with the alternative of recycling cultures from field grown palms of proven clones.

Through tissue culture, homozygous diploid plants might be developed from haploid pollen mother cells, offering a rapid way of developing homozygous lines, for production of  $F_1$  hybrids. While this would remove the need for an extended inbreeding programme, the scale of testing necessary to identify the best combinations for production of high yielding  $F_1$  hybrids might still be prohibitive, and needs to be examined.

## Molecular Marker-assisted Selection

The use of recently developed DNA marker systems, notably restriction fragment length polymorphisms (RFLPs), is dealt with in another paper at this conference (Jack and Mayes, 1992) and only a brief overview will be given here.

Over the last 6 years or so, DNA probes have become widely used in a number of important crop species, initially within those major temperate crops with extensive breeding histories such as maize, barley, oilseed rape and potato (for reviews see Chang and Meyerowitz, 1991, and Tanksley *et al.*, 1989) but more recently within less well characterised crops such as bamboo (Friar and Kochert, 1991), cocoa (Wilde *et al.*, 1992) and yam (Terauchi *et al.*, 1992). Broadly speaking such technology has two distinct though overlapping applications, the first being genotype characterisation (e.g. genetic fingerprinting and germplasm evaluation) whilst the second is identification of specific genes through linkage analysis. The major difference between

these two applications is that for the first, no knowledge of probe location within the genome is required; any DNA marker which shows differences between genotypes can be used. Gene tagging, on the other hand, requires an ordered linkage set of DNA markers covering the whole genome, as well as a mapping population segregating for the traits of interest. Clearly such mapping requires a substantial degree of effort, though equally it represents an important resource.

Within Unilever we have substantial experience in the development and exploitation of DNA probes for genotyping, for example in confirming clone (ramet) : palm (ortet) identities following tissue culture, and for assessing progenies of selected crosses for spurious out-crossing. More recently we have embarked upon the construction of an oil palm linkage map. Details of this work are given in the accompanying paper.

### **Transgenic Technology**

Despite considerable effort, monocotyledons have proven intransigent to genetic transformation. They lack the necessary plasticity in tissue culture to be regenerated from protoplasts following direct uptake of DNA (with the recent exception of rice and maize: Shimamoto *et al.*, 1989; Gordon-Kamm *et al.*, 1991) and appear to have defence mechanisms that inhibit the growth and virulence of *Agrobacterium* (Sahi *et al.*, 1990). However, a recent development in gene transfer technology offers the prospect of a near universal transformation system, the gene gun.

At present, we consider microprojectile bombardment ("biolistics") to be the technique most likely to transform oil palm. The gene gun employs a 0.22 blank charge to propel a polyethylene projectile at around  $350 \text{ m.s}^{-1}$  into a stopper plate. The face of the macroprojectile carries a 2.5  $\mu\text{l}$  drop containing a suspension of DNA-coated tungsten particles, each approximately  $1 \mu\text{m}$  in diameter. These pass through a hole in the stopper plate and penetrate the tissue below, ultimately lodging within cells. The damage to target tissues and frequency of gene delivery depend upon a number of factors e.g. the force of the tissue and its distance from the stopper plate. Experience gained in ballistic transformation of cereals should prove to be generally applicable to monocotyledonous crops including oil palm.

In order to successfully and efficiently apply the gene gun to a new tissue, it is important to know how many cells have received DNA and where they are located. The method currently favoured is to shoot plasmids that contain the *E. coli*  $\beta$ -glucuronidase gene (GUS), driven by the constitutive CaMV 35S promoter. Plant cells (including a wide range of monocots) whose nuclei

receive the plasmid are able to express GUS without its incorporation into the host chromosomal DNA, a phenomenon known as transient expression. This form of expression results in a pulse of enzyme production, peaking around 48 h after shooting, and diminishing as the free DNA is degraded and with the half life of the enzyme. Cells that are expressing GUS are able to cleave the clear substrate 5-bromo-4-chloro-3-indolyl glucuronide (X-GLUC) to yield a stable, blue crystalline solid (Jefferson, 1987). These cells appear as blue spots against an unstained background. The number of transiently expressing (i.e. blue) cells gives an indication of the efficiency with which the gun is delivering DNA into cell nuclei, and where those recipient cells are located. It is estimated that for every thousand cells that transiently express a gene, one stably incorporates it into the chromosomal DNA (Gordon-Kamm *et al.*, 1990).

One of the first priorities of the project has been to establish that the GUS marker gene system works in regenerable oil palm tissue and to begin optimization experiments. Immature spear leaf tissue was bombarded with a plasmid vector containing 35S-GUS and, after X-GLUC staining, up to 400 GUS positive (blue) cells were observed per shot (mean = 132), which compares very favourably with similar work with cereals. These results indicate that bombardment is an efficient method for delivering foreign genes into regenerable oil palm cells and that those cells are capable of expressing foreign genes once delivered into the nucleus. These are two fundamental requirements for development of a transformation protocol.

Successful application of ballistic transformation to oil palm must take into account the limitations of both gene gun technology and oil palm tissue culture; large numbers of regenerable cells must be made accessible to accelerated microprojectiles without compromising their viability. Sectioning of stained material from early shooting experiments with oil palm and other species revealed that the gun was only delivering DNA into the surface layers of multicellular structures. In the light of this evidence, three regenerable target oil palm tissues are being evaluated. First, there is some evidence that proembryos and meristematic nodules can originate from the surface layers of some types of callus (Schwendiman *et al.*, 1990). It is likely that chimeric transformants would result from treating callus, and recloning from transformed sectors of any regenerants would be required to produce solidly transformed lines. Secondly, regenerable primary explants such as immature spear leaf could be bombarded and callus induced under selection before regeneration. The advantage of this route would be that solid transformants could be produced in the first cycle of tissue culture. Finally, bombardment of small cell clusters such as those found in embryogenic cell suspension cultures has proved to be a successful route to transformation of maize (Gordon-Kamm *et al.*, 1991) and we are also exploring the feasibility of this option for oil palm.

## NEW BREEDING STRATEGIES

### Conventional Breeding

Breeding will increasingly be aimed at the production of clones, either *teneras* for commercial planting or *dura* and *pisifera* clones for the production of clonal seed, and breeding strategies will have to be modified accordingly.

Where the objective is clonal seed, the strategy may not be too different from that for conventional seed. Progeny testing will be of vital importance. In the past, D x P trials were usually regarded as *pisifera* progeny tests, and often employed an NCM1 design, where each *pisifera* was crossed with perhaps four randomly chosen *duras*, using different *duras* for each *pisifera*. This design was appropriate because each *pisifera* can be used to pollinate many *duras*, whereas the number of seed which could be produced from any one *dura* was very limited. If the *dura* parent is to be cloned, though, then progeny testing becomes equally important for both *dura* and *pisifera*, and a crossing design which allows breeding values for both parents to be estimated with equal reliability (NCM2, partial diallel) is preferable. For most characters, it appears that general combining ability predominates in oil palm populations (Breure *et al.*, 1992), but if specific combining ability plays any part, it should be exploited. So far we are aware, no methods of predicting specific combining effects have been developed, but Breure *et al.* (1992) propose a two stage progeny test programme, with SCA effects being tested for at the second stage, between those parents with the best GCA values.

Where *tenera* clones are intended to be the commercial planting material, breeding strategies are likely to change. Whereas for seed, phenotypically uniform D x P crosses were the objective, for clones we need to create relatively variable families from which to select individual ortets. Hardon *et al.* (1987) showed that, when Deli *duras* were outcrossed to African material, the average yield was lower than for pure Deli *dura*, but the range of variation was greater, and the best individuals outyielded the best pure Deli *dura* (Table 3). The greater the genetic distance between the parents, the more variable will be their offspring; genetic distance can be guessed at from the limited ancestral information within breeding programmes, but molecular markers may have an important part to play here (see below).

*Teneras* can be created in a number of ways. D x T crosses have an advantage over D x P crosses, in that both parents can be selected on phenotype, whereas sterile or partially fertile *pisiferas* cannot be so reliably selected. TxT crosses have advantages where selection for fruit characters

is concerned, because the heritability of such characters from *duras* to their *tenera* offspring is poor (van der Vossen, 1974). However, the occurrence of 25% of vegetatively very vigorous, sterile *pisiferas* biases individual palm selection within such crosses. In some programmes, there is a move towards fertile *pisiferas*, which are generally less vigorous, so this problem may diminish in future.

Important questions to be answered are how best to screen new germplasm, either from wild collections or from interspecific hybridisation for useful characters, and how to incorporate such material into existing breeding programmes. Unless molecular markers linked to useful characteristics can be identified (see below), introgression of new material must remain laborious and time consuming.

### Use of Molecular Markers

**Random markers** RFLPs are already being used for identifying, and confirming the ancestry of, individual palms and clones (Mayes and Jack, 1991). Confirmation of the legitimacy of individual palms will be important in planning programmes, particularly to avoid inbreeding; there is no doubt that, at least until the shell thickness gene was recognised and could be used as a marker, the level of contamination in individual crosses, and the corresponding frequency of illegitimacy, was sometimes high.

Isoenzymes have been used to study the genetic distance between breeding populations (Ghesquiere, 1985) and RFLPs could be used for the same purpose. Estimates of genetic distance have been used to predict the level of heterosis in maize crosses (Smith *et al.*, 1990), and a similar approach may be useful in breeding oil palm ortets.

Random polymorphic markers could also be used to accelerate the production of homozygous lines by inbreeding, by identifying the most homozygous individuals in each generation.

**Molecular markers linked to useful genes** Once markers linked to useful characteristics have been identified, the scope for their use will expand considerably. At a simple example, a marker linked to the shell thickness gene might permit identification of *pisiferas* in the nursery, so that they could be planted separately, thus eliminating an important source of bias in selection in TxT crosses. Markers linked to other characters which may be based on one or a few genes could also be screened for at the nursery stage. For example, one might screen for disease resistance under conditions where the disease did not occur. The suggestion that resistance to *Fusarium* wilt may

be based on only two loci (de Franqueville and de Greef, 1988) is important in this context, and needs to be tested.

In other crops, RFLP markers have been identified which are associated with (linked to genes controlling) a significant proportion of the variation in quantitative or polygenic characters. A search for such linkages in oil palm could be very rewarding.

Linked markers may be particularly important in the exploitation of new germplasm. In a backcrossing programme, a marker linked to the desired character from the nonrecurrent parent could be used to ensure that the character was retained at each generation. Alternatively, linked or random markers from the recurrent parent could be used to accelerate recovery of the original parental genotype, while screening at each generation for the added character by other means.

## NEW PRODUCTS

### Clones

There can be no doubt that the highest yields will be obtained by cloning outstanding *tenera* ortets. While  $F_1$  hybrids are genetically identical to clones, both the time required to develop inbred lines and the scale of testing necessary to identify good hybrids are against their use. At present, the best individual palms typically yield at least 25% more than the family mean, and sometimes as much as 50%. While some of this superiority is undoubtedly environmental, the broad sense heritability for oil yield may be quite high (Baudouin and Durand-Gasselín, 1991), and thorough clone x environment testing may allow much of the superiority to be realised in practice.

Genotype x environment interactions are likely to be much more marked with clones than with seedling progenies, which are genetically heterogeneous (Lee and Donough, 1991). For an environment where clones have not previously been tested, planting clonal seed, which should give less G x E interaction, may be the safest approach. Planting a wide range of good clones might be as good, though, and would allow the best clones to be identified in due course. Where strong interactions do occur, advantage may be taken by planting only clones shown to be well adapted to the environment, thus obtaining the maximum possible yield.

In the long term, we need to understand the physiology of clone x environment interactions, so that we can start to predict at least the direction of the interactions, even if not their magnitude.

As commercial planting of clones expands, we must start to study the agronomic requirements of clones. Clones differ in optimal planting density (Corley and Donough, 1990) and in fertiliser requirements (Donough, personal communication, 1992). Crop management, controlling flowering, fruiting and ripening patterns to simplify harvesting, will probably only be possible by combining growth regulator treatments with the inherent uniformity of flowering and fruiting patterns within clones (Corley *et al.*, 1982). Eventually, we may expect to see monoclonal blocks, of clones carefully for the environment, and each fertilised and perhaps irrigated according to its needs. Crop management will ensure a predictable supply of fruit, allowing fully mechanised harvesting.

### Clonal Seed

Clonal seed will not really be a new product, since it involves only the scaling up of production of crosses which could be produced conventionally. It is often assumed that somaclonal variation would not be a problem with clonal seed, but if the same recessive mutation were to occur in both parent clones, some of the offspring would have the mutant phenotype. This may seem improbable, but it now appears that the mantled fruit character in clones involves a heritable change (Rao and Donough, 1990), and the same mutation appears in many different clones. The possibility that other mutation "hot spots" exist cannot be ruled out until some clone x clone crosses have been produced, and compared with crosses between the corresponding ortets.

How close the performance of clonal seed may approach to that of clones depends in part on the amount of variation between palms within the clone seed crosses. As noted above, the best individuals usually significantly outyield their families, and unless inbred parents are used (in which case they may perhaps be multiplied by selfing as easily as by cloning) the within-family variation will remain significant, and exploitable through *tenera* clones.

A misconception which should be dispelled is that it is technically easier to produce clonal seed than clones. Other things being equal, the timescale should be shorter for clonal seed: once an outstanding D x P cross has been identified, it can be reproduced as soon as clones of the parents start to flower. Conversely, the best individuals within the outstanding cross will have to be field tested as clones before they are planted commercially. In practice, though, clonal seed has the important disadvantage that two specific palms, the *dura* and *pisifera* parents, must be cloned; tissue culture techniques are not yet sufficiently reliable that any specified palm can be cloned with certainty. In contrast, for *tenera* clones, it does not much matter which among a population of outstanding *teneras* are successfully cloned, provided that sufficient clones are developed to

give a high probability of identifying good ones after field testing. If the success rate is low, one can simply increase the number of ortets sampled.

### **Clones are Research Tools**

In principle, clones might be used as standards in trials, to detect soil fertility gradients and other environmental effects. In one trial, early yield of a clone was significantly correlated with position on a slope (Corley *et al.*, 1981). There are likely to be interactions of clones with microenvironment, though and the value of this approach has yet to be proven. Clones were used as standards in single palm plot breeding trials planted by Pamol Plantations in the early eighties. The coefficient of variation between palms of a clone was often nearly as large as that between seedlings within a progeny - in four trials, the average CV between palms within progenies for FFB yield over 3 years was 26.5%, compared to 22.8% within clones (Donough, personal communication, 1988) - but it is not the CV between clonal palms which determines whether a clone will be a useful standard. If covariance adjustment of progeny yields using the clonal standards reduces the CV between palms within progenies, then something has been achieved. This needs to be studied.

Clones are also being used as standards for comparison between locations; for example, Unifield T.C. Ltd has used the same standard clones for trials in Asia, Africa and South America. If clone x environment interactions prove as important as expected, though, such standards may not be very useful except for comparison of trials within a single location.

Uniformity for many characters makes clones useful subjects for physiological and other studies (in contrast to the yield data mentioned above, in four trials the CV between seedlings within families for petiole cross section was 18%, while that within clones was only 11% - Donough, personal communication, 1988). The number of leaf and inflorescence primordia in the apical bud, and the stages at which sex differentiation and other events occur, show little variation between palms within a clone, but large differences between clones (Corley, unpublished); this will allow more precise studies of flowering responses than have been possible with seedlings. Different clones show different responses to planting density (Corley and Donough, 1990); understanding the reasons for these differences should allow improved selection criteria for yield per hectare to be developed.

## **Molecular Markers**

Molecular markers are available now for such things as determining genetic distance between palms (for predicting heterotic performance) and characterising germplasm collections (e.g. to ensure the maintenance of individuals with the greatest levels of heterozygosity). A first map should emerge within the next 2 years and we expect that markers to shell thickness genes would be identified shortly afterwards. It is also possible that we will have markers to *Fusarium* wilt resistance genes within 3 - 4 years. However markers to components of polygenic characters such as yield (quantitative trait loci - QTL) may take considerably longer.

## **Possible Products of Transgenic Technology**

**Background Considerations** In common with conventional breeding, objectives for the application of transgenic technologies fall into two classes, those related to agronomic criteria (adaptability, yield potential), and those related to the quality of the commercial product. However, while we expect small but progressive improvements to come from conventional breeding, in contrast our expectations of transgenic technologies are for "step changes", or for introgression of novel traits not accessible to the conventional breeder. New agronomic traits could be introduced gradually, without major transition in plantation practice, but when we consider step changes in quality traits, these must be introduced as large blocks or whole plantations, in order to segregate the new product from regular palm oil.

Furthermore, the long replacement cycle of oil palm plantations implies that we must start thinking beyond the technical barriers to application of transgenic technologies, and consider the strategies we must adopt to reach commercial objectives. This aspect will be crucial in evaluating the feasibility of the breeding objectives we can address via transgenic technologies.

A further consideration is the breeding system of the oil palm. Do we need to concern ourselves about the consequences of the escape of transgenes, as we would with other outcrossing species such as grasses? In principal the answer is yes, but, if we can limit cultivation to regions of the world where there is no indigenous wild palm population capable of cross fertilisation by oil palm, then chance escape of the transgenes can effectively be ruled out. We therefore argue that it is reasonable to go ahead with the transgenic approach without the need for additional technology to control male fertility, outside those regions where oil palm is indigenous.

The agronomic targets towards which we have seen greatest progress in other crops are: insect resistance (Bt toxin), herbicide resistance and virus resistance (see Flavel, 1989). These are not of great relevance to oil palm. The quality trait for which most progress has been made is the control of tomato fruit softening (pectin degradation) via manipulation of polygalacturonase (Kramer *et al.*, 1990). This may be of relevance to controlling the abscission of oil palm fruit (see below).

### **Potential Targets - quality traits**

*Oil composition* is a particularly interesting target, for several reasons. It is a technically feasible target. A significant number of genes with potential to modify oil composition have been isolated and are currently being evaluated in annual oil crops. For instance it has been announced by Calgene Inc. that they can produce a high stearic oil in rape by modifying the activity of the delta-9 desaturase (stearoyl-ACP desaturase) (Knutzon *et al.*, 1992).

Palm oil is milled at the site of production, which means that fruit with special oil compositions can be milled separately from the bulk material and thus reach specific end uses with an added value relative to standard palm oil. This position contrasts with other oil crops such as, for example, low erucic acid rape where a modified oil rapidly displaced the existing commodity and lost the opportunity of selling at a premium.

More specific targets for the manipulation of mesocarp oil composition would be: a high stearate oil, achieved by enhancing the activity of the palmitoyl elongase; high oleate oil by enhancing the activity of palmitoyl elongase and delta-12 desaturase. Two of the necessary genes are available and are being tried out in oil seed rape, and these genes, or their palm homologues, could be used in sense or anti-sense\* configurations to control the activity of the relevant enzymes.

*Endogenous lipase activity* within the mesocarp results in the accumulation of free fatty acids, and reduces the value of the extracted oil (Henderson and Osborne, 1991). Reduction of the activity of this enzyme would be beneficial. When the lipase responsible has been fully characterised and we have sufficient knowledge to find the corresponding gene, we could then use the antisense configuration of this gene in the oil palm to control lipase activity in the mesocarp.

*Carotenoid content* of the mesocarp oil could be modified. The biochemical pathway for carotenoid pigment synthesis is well understood and it should not prove difficult to isolate the genes responsible for the rate-controlling steps. Manipulation of the genes in either the sense or antisense configurations could then be exploited to adjust the levels of carotenoids. An example of this has been demonstrated in tomato (Bird *et al.*, 1991) where the manipulation of the prephytoene pyrophosphate synthase gene can result in the production of yellow fruit.

A central requirement for the manipulation of mesocarp quality is the availability of promoter genes which are specific in their expression to the developmental phase of the mesocarp. These will be a necessary appendage to any foreign gene which is introduced with the intention of modifying the biochemical pathways of this tissue, but such genes should not be difficult to isolate.

### **Potential Targets - agronomic traits**

*Fusarium oxysporum* Vascular wilt is a major problem for oil palm, although at the moment it is almost restricted to West Africa. Fungal disease resistance is receiving intense attention via the most advanced molecular techniques. We can expect that within a few years the so called non-host resistance factors will be well understood and that gene cassettes (packages of structural genes, with the necessary regulatory genes, and perhaps markers for selection after transformation) for fungal resistance will be available. It is not far fetched to expect that these will be effective against *Fusarium oxysporum*. Should we be concerned about the introgression of wilt resistance into the wild population? This could also happen with a conventional breeding programme (though some of the strongest sources of resistance have come from wild or semi-wild populations).

*Non-abscinding fruit* is often mentioned as a valuable characteristic since it could improve harvesting efficiency. We are starting to gain some information on the process of fruit abscission in oil palm (Henderson and Osborne, 1990) which should allow us to draw parallels with other species for which the relevant genes are known, and allow us to develop strategies for manipulation these genes. Polygalacturonase has been implicated as one of the key enzymes (Osborne *et al.*, 1992) so the inhibition of synthesis of this enzyme in the abscission zone by antisense technology could be contemplated.

*Parthenocarpy*, without the normally attendant problem of restricted fruit development and bunch failure could be a valuable trait. This will probably ultimately require the manipulation of a specific set of hormone receptors and is a long way off.

Going one step further, some form of *facultative apomixis* (agamospermy - the production of seed without meiosis), could allow breeders to produce the genetic equivalent of F1 hybrid seed, without the need for inbreeding, or for clonal propagation.

These targets have been listed in order of decreasing feasibility as seen at this moment, but relative to the normal breeding cycle of the oil palm, this position may change rapidly. Take for example, facultative apomixis. At the moment we understand little of this phenomenon, and still less of its molecular or genetic basis. Attempts to transfer the potential for apomictic reproduction via normal wide crossing procedures (e.g. from *Trypsacum* to wheat) have been unsuccessful, but given the pace at which plant science is now moving with the help of molecular techniques, 10 years, or one oil palm breeding cycle, will probably see detailed understanding plus the possibility of manipulation of breeding systems to permit facultative apomixis. One breeding cycle is a short time in which to modify breeding strategies to take account of this, but for oil palm the strategy would be the same as that for the support of clonal propagation via tissue culture, as the objective would be to identify outstanding individuals, which could then be reproduced apomictically.

## CONCLUSIONS

Clones should have a major commercial impact within the next four or five years. Early trial results have already indicated a few promising clones (Corley *et al.*, 1988; Le Guen *et al.*, 1991), and commercial planting of these should start soon. For safety, though, a range of clones should be planted in any commercial development, and a sufficiently large portfolio of adequately tested clones may not be available for some years yet. We may speculate that by 2005, or even earlier, perhaps 80% of annual planting programmes on the large estates will be done with clones, but because of the long economic life of the palm, it will be up to another 25 years before complete conversion to clones has occurred, even where a pure clone strategy is adopted. In practice, most companies will probably plant some areas of clonal seed as well.

Molecular markers are already starting to be useful in breeding programmes, with random markers being used for confirming identify, and perhaps soon for estimating genetic distance. Later, and less certain, developments will be the use of linked markers to accelerate selection.

The likely contribution of collections of wild germplasm and of interspecific hybrids is difficult to evaluate. In the absence of linked markers, the contribution of either may remain small, simply because of the long timescale involved in introgressing new material into existing advanced populations. The most useful characteristic of *E. oleifera* is probably its liquid oil, but this may not be sufficiently liquid to lead to a major expansion of palm oil into new markets. Major changes in oil composition are more likely to come from transgenic techniques. Developments on a commercially significant scale are probably at least 15 years away.

If commercially important single genes can be identified, either in the collections of wild germplasm, or in other species altogether, they could be transferred to high yielding clones. Given this possibility, the conservation of collections of wild germplasm is clearly very important.

What can we expect from the oil palm of the future? The potential yield has been estimated at 17 tons of oil/ha/yr (Corley, 1983). This estimate was based on the assumption of a continuously efficient photosynthetic canopy, 75% of gross assimilation being lost to respiration, and a harvest index of 57% (in energy terms).

It appears that the harvest index should be relatively easily achievable: with oil/bunch of 30%, we need a bunch index of 67%. That is high, but if oil/bunch could be increased to 40% (not an impossible level; at least one *tenera*, palm 2/5622 in Cameroun, yielded such bunches), then bunch index need only be 50%.

Continuous high photosynthetic rates may be more difficult. With total photosynthetically active radiation of 31 GJ/ha/yr, and light interception of 95%, a photosynthetic conversion coefficient,  $e^*$ , of 2.1 g/MJ would be needed. The highest recorded value of  $e^*$  for oil palm is about 1.6 g/MJ (Squire, 1985), but annual crops give values up to 3 g/MJ. Increasing the rate of photosynthesis may be possible (Smith, 1991), but decreasing respiration losses may prove easier.

Maintenance respiration probably consumes about 50% of gross photosynthesis production (Breure, 1988; van Kraalingen *et al.*, 1989), and growth respiration a further 25%. A reduction in maintenance respiration should increase net photosynthetic production. According to the now standard model of oil palm assimilate partitioning (Corley *et al.*, 1971; Squire and Corley, 1987; van Kraalingen *et al.*, 1989), any extra photosynthetic production should all go into fruit. With 75% respiratory losses, and a bunch index of 50%, 12.5% of gross photosynthesis goes into fruit bunches. A 10% reduction in maintenance respiration from 50 to 45% should increase the

assimilates available for bunch production by 5% of gross photosynthesis; half of this would be consumed in growth respiration, giving a 20% increase in bunch yield (2.5% extra on a total of 12.5%). Significant changes in maintenance respiration rate have been achieved in rye grass (Wilson, 1982), and have resulted in useful increases in total dry matter production. How easy it will be to achieve such changes in oil palm, and whether they will come through conventional breeding or genetic engineering, remains to be seen.

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**Table 1**      **Dry matter production of three groups of *Deli dura* oil palms**  
 (From Corley and Lee, 1992)

<b>Generations of selection</b>	<b>0</b>	<b>1</b>	<b>4</b>
Total dry matter production (kg/palm/yr)	172.3	189.8*	201.3**
Vegetative dry matter (V - kg/palm/yr)	108.3	110.5	99.8
Yield (dry matter - kg/palm/yr)	64.0	79.3***	101.4***
Bunch index (yield/total dry matter - %)	37.3	41.9*	50.7***
Harvest index (energy in oil/total - %)	22.8	24.0	30.0***
Leaf area index (L)	4.52	4.78	3.87
Light interception (f - estimated - %)	84.8	87.0	80.2
Net assimilation rate (kg/m <sup>2</sup> /yr)	0.56	0.58	0.76**
Energy conversion (e - g dry matter/MJ)	1.05	1.14	1.36***

\* Different from mean of unselected population, P > 95%

\*\* P > 99%

\*\*\* P > 99.9%

**Table 2** Yield and composition of fruit bunches for *dura* and *tenera* sibs  
(Yields in kg fresh fruit bunches per palm per year; from Corley and Lee, 1992)

Expt	No. of crosses	<i>Duras</i> -----					<i>Teneras</i> -----				
		Yield kg	Oil ----- as % fresh bunch	Shell ----- as % fresh bunch	Kernel ----- as % fresh bunch	Fibre ----- as % fresh bunch	Yield kg	Oil ----- as % fresh bunch	Shell ----- as % fresh bunch	Kernel ----- as % fresh bunch	Fibre ----- as % fresh bunch
1	10	130	18.7	19.7	4.6	16.7	128	24.8	4.3	4.9	19.5
2	10	132	18.1	19.0	4.4	17.1	138	23.6	4.2	4.7	19.5
3	8	155	17.6	21.1	6.0	16.3	163	23.4	5.8	6.1	19.2
4	5	132	16.0	24.9	6.5	15.8	131	22.5	5.2	6.3	19.5
5	22	156	15.7	20.6	5.4	16.8	162	21.1	6.5	5.8	19.5
6	10	153	18.4	22.5	6.2	15.2	155	24.1	6.3	6.4	18.1
Mean		146	17.2	20.9	5.4	16.4	150	22.9	5.6	5.7	19.2
Energy (GJ)		2.42	0.98	0.76	0.22	0.46	2.34	1.34	0.21	0.24	0.55

**Table 3** Variation in yield of fruit of individual *dura* palms from two different types of cross (From Hardon *et al.*, 1987)

	<i>Deli dura</i> x <i>Deli dura</i>	<i>Deli dura</i> x <i>African dura</i>
Number of crosses	5	3
Number of palms	270	75
Mean yield per palm (kg/year)	173	164
Standard deviation (kg)	40	59
Coefficient of variation (%)	23	36
Yield of best 5% of palms:		
a) Observed	251	271
b) Expected from normal distribution	255	286