Newsletter BERITA ISOPB

THE INTERNATIONAL SOCIETY FOR OIL PALM BREEDERS
PERSATUAN AHLI-AHLI PEMBIAK BAIK KELAPA SAWIT ANTARA BANGSA

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EDITORIAL

Breeding for short palms or slower height increment is a quiet pursuit of most oil palm breeders while oil yields obviously remain the principal preoccupation. Slow vertical growth was after all the main reason for interest in the Dumpy E206 palm in the 1950's, a principal reason for three decades of research in *E. oleifera* and, more recently, interest in backcrosses and populations 12, 13 and 14, among others, of the Nigerian Germplasm Collection with PORIM and NIFOR.

Among various small collections of shorter palms, rare records refer to the "Tumbuk" palms, which or whose descendants occur in some of the larger breeding programmes. The feature article in this issue brings these palms to light.

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FEATURE ARTICLE

ORIGIN AND BACKGROUND OF THE TUMBUK PALMS

The Dumpy E206, Gunung Melayu and Tumbuk palms are the few sources of dwarf genes in oil palm breeding. All these palms are of the Deli *dura* ecotype. The Tumbuk palms were laid by the Department of Agriculture (DoA) in trial 0.10 at Field C, Tumbuk Estate - thus the name "Tumbuk palms". The trial comprised of six non-experimental collection of Dumpy progenies *(Table 1)*, planted in November 1952 at 30 x 30 ft square covering an area of 38.89 ac. The dwarf habit of the Tumbuk palms were not surprising as these palms were derivatives of the Dumpy E206.

Table 1 : Pedigree of the Tumbuk palms in Trial 0.10.

Progeny Number	Type of Cross	Parents		Grand Parents			
P.99	D x D	0.3/1.9	0.3/3.5	E206	E206	E206	E206
P.97	D x D	0.3/3.9	0.3/3.7	E206	E206	E206	E206
P.84	D x D	0.3/3.9	0.3/2.4 E206	E206	E206	E206	
P.91	D x D	0.3/1.9	0.32.4	E206	E206	E206	E206
P.86	D x D	0.3/2.4	0.3/2.4	E206	E206	E206	E206
P.83	D x D	0.3/3.6	0.2/55.8	E206	E206	E206	E206

The Tumbuk palms were featured in the early Cooperative Breeding Scheme (CBS) trial 0.28 planted at Klanang Baru in 1957. The following excerpts from the Annual Report of the Department of Agriculture (DoA) for the year 1957 described the origin of these materials and its basis of selection.

"The short dura palms on Tumbuk Estate were grown from seed collected at Tennamaram Estate, which also supplied the seed of the Dumpy palm of the Serdang Dumpies. No yield records are available for the Tumbuk palms, but the field mean yield is extremely good. The palms used were selected primarily for their habit and progenies."

It was reasonable that the Tumbuk palms were not yield recorded as it was a collection of non-experimental Dumpy palms. However, basic information of its parents (Serdang Field 4 and 3a) were gathered as early as 1946 and extended to 1961 *(Table 2)*. It was reported that the Dumpy palms in Field 4 (trial 0.2) were yielding much less than those in Field 3a (trial 0.3). Soil fertility might account for this in part but the difference was attributed mainly to the over shading of the Dumpy palms in Field 4 by neighbouring tall palms.

Table 2: Background information on parents of the Tumbuk palms.

Year planted	Palm number	F/B %	M/F %	(Mean 1946-1961) FFB Kg/p/yr	BNO /p/yr
1939	0.2/55.8	64.4	60.4	46.4	3.4
1940	0.3/1.9	70.7	61.8	113.2	5.5
1940	0.3/2.3	58.6	67.8	127.7	5.2
1940	0.3/2.4	54.3	61.7	132.7	6.0
1940	0.3/3.5	53.8	67.9	107.7	6.1
1940	0.3/3.6	52.3	64.9	112.3	6.5
1940	0.3/3.7	39.7	71.7	95.5	6.2
1940	0.3/3.9	57.3	61.0	103.2	5.8

It is thus concluded that the Tumbuk palms are derivatives of the famous Dumpy E206 originating from Elmina Estate.

Contributed by: A. Kushairi and N. Rajanaidu

SOCIETY NEWS

Proceedings of the 1991 ISOPB

INTERNATIONAL WORKSHOP ON GxE STUDIES IN PERENNIAL TREE CROPS

Publication of this proccedings had been somewhat delayed but is now at the printersd and should now be ready by October.

Contents of this Proccedings are as follows :

International symposium on

RECENT DEVELOPMENTS IN OIL PALM TISSUE CULTURE AND BIOTECHNOLOGY

This 1½ days symposium is being organised by the ISOPB on 24th and 25th September during the week of the PORIM International Palm Oil Congress, at the Istana Hotel, Kuala Lumpur.

While most of ISOPB members would be at the congress we have given below some selected abstracts of papers that will be presented at the Symposium.

THE APPLICATION of BIOTECHNOLOGY to OIL PALM - Potentials and Progress

by

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The potential which recent developments in biotechnology offer for the improvement of oil palm is enormous. In the immediate future, the long awaited potential of clonal propagation is likely to be realised and more recent developments, such as the application of molecular markers have been utilised and even the development of transformation systems have begun to be investigated.

There are potentially two strategies to the genetic improvement of oil palm - marker assisted breeding and genetic manipulation (so-called Reverse Genetics). Both methods hold out the prospect of substantial gains for oil palm breeders, the former, in the immediate future and the latter in a decade or two.

The majority of our work to date has been focussed around the application of molecular markers to oil palm, but recently we have also begun a pilot project into the feasibility of oil palm transformation.

We report our progress in the use of molecular markers in a number of ways, including; unequivocal identification of palm identity; identification of genetic relationships and confirmation of pedigree; investigation of origins and genetic relationships with respect to heterotic effects.

In addition we present the current state of the Genetic Linkage Map which we have been constructing in a population of *E. guineensis* derived from a self.

We also present the results of our initial investigation into methods and targets for oil palm transformation, including our progress in cloning of promoters which we might wish to use to direct transgene expression.

DNA and PROTEIN CHANGES in RELATION to CLONAL ABNORMALITIES

by

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With the advent of molecular techniques, DNA and protein based procedures are being used for clonal

and somaclonal characterisation. Among the DNA techniques being utilised, a novel technique (RAPD) based on the amplification of random DNA sequences by the polymerase chain reaction (PCR) with arbitrary primers is the simplest and most rapid. The amplified products segregate in Mendelian fashion and can therefore be used as genetic markers. Restriction enzyme analysis of organelle DNA is another simple DNA based method to characterise differences. Amongst the protein based techniques, SDS-PAGE, IEF and 2-D separation have all been used to characterise clonal and somaclonal variation.

RAPD studies were carried out with polyembryogenic cultures with the aim of detecting differences at the *in vitro* stage in relation to abnormalities. DNA from polyembryogenic cultures, established from a normal clonal palm and from an abnormal mantled clonal palm, were screened for polymorphisms with arbitrary primers obtained commercially. Of about 100 primers screened, at least 30 primers showed clear differences in the profiles of amplified DNA bands on agarose gels. In some instances differences in intensity of bands were also evident. Further comparisons between normal and abnormal tissues are being evaluated to characterise polymorphisms that are specific to clones and abnormalities. It is evident that the technique has applications in characterising clonal and somaclonal variations in oil palm.

It has been postulated that changes in organelle DNA might be causal to the clonal abnormalities. Tissue culture induced changes in mitochondrial DNA are known in several crops. Mitochondrial DNA preparations from six pairs of clonal palms, one normal and another abnormal, have been compared by restriction enzyme analysis. No differences were detected using EcoR1, HINDIII and other enzymes in the profiles of the digested DNA. It is unlikely on the evidence available that the abnormalities are due to changes in the restriction sites that have been examined or to gross changes in the mitochondrial DNA.

Changes in protein profiles were examined by SDS-PAGE and IEF. No differences were evident in protein extracts from cultures of normal and abnormal palm origin. However, differences were evident in SDS-PAGE separations of protein extracts from normal and abnormal palms.

These studies demonstrate that RAPD techniques have potential application in monitoring changes in *in vitro* cultures in relation to abnormalities. The advantages of this technique stem from its ability to detect extensive polymorphisms, simplicity, rapidity and the need for small amounts of DNA, which allows examination of very small tissue explants.

APPLICATION of MOLECULAR MARKER TECHNIQUES IN OIL PALM TISSUE CULTURE

by

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The appearance of abnormalities in palms obtained from *in vitro* micropropagation suggests that genetic changes could have been induced during the culture process. This study was initiated to determine if such changes had indeed occurred. Palms derived from tissue culture were analysed using molecular marker techniques. The genetic stability of plants derived from cultures grown in media with various concentrations of cytokinin, namely, kinetin and benzylaminopurine (BAP), was compared with respect to transfer regime and time in culture. In the course of the study, it was observed that the clones could be distinguished by differences in their probe hybridisation patterns. Eight clones have been identified using twenty probe/restriction enzyme combinations. Two of these clones were monitored for clonal fidelity from subculture to subculture during propagation of the embryoids. To date, DNA change has not been observed any earlier than in the eleventh subculture showing that the oil palm is relatively stable in culture.

ISOENZYME VARIATION in NIGERIAN OIL PALM (*Elaeis guineensis*) GERMPLASM

by

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Ten populations from the PORIM Nigerian oil palm germplasm collection were analysed with polyacrylamide gel electrophoresis to examine isoenzyme variation. Leaf tissue was used to study the variation in three isoenzyme systems, viz., glutamate-oxaloacetate transaminase (Got), acid phosphatase (Acp) and esterase (Est).

It was found that 81% of the bands obtained were polymorphic. Population 39 is the most polymorphic and population 14 the least. The rare alleles found in some populations may have an important role as genetic markers in oil palm breeding. No correlation was found between the pattern of variation for morphological characters and that shown by the three isoenzymes.

OIL PALM TISSUE CULTURE - CURRENT PRACTICE and CONSTRAINTS

BY

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Vegetative propagation of oil palm (*Elaeis guineensis* Jacq.) was initiated in the early 1970's. Despite two decades of research and development, little is yet known of the biochemical processes and physiology of oil palm tissues in culture. Much of the progress achieved towards large scale production of oil palm ramets has been from contributions of empirical experimentation. Several large private organisations with interests towards commercializing the technology are involved in tissue culture of oil palm, hence many important details in the protocols developed are seldom or more likely never published. Generally, it would appear that apart from having similar basic techniques in producing oil palm clones, most laboratories also share similar constraints in scaling up of the process into an efficient commercial micropropagation operation.

COMMERCIAL POTENTIAL OF OIL PALM CLONES : EARLY RESULTS OF THEIR PERFORMANCE IN SEVERAL LOCATIONS

by

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The early performance of oil palm clones in terms of bunch yield (FFB), oil to bunch (O/B) and oil yield (OY), from various sources: IRHO (Institut de Recherche pour les Huiles et Oleagineux), PORIM (Palm Oil Research Institute of Malaysia) and HRU/AAR (Highlands Research Unit/ Applied Agricultural Research) were studied on trials in different environments.

Clones apparently performed differently in different environments. There were no particularly outstanding clones in terms of OY, across trials although some clones showed good promise within trials.

Inefficient ortet selection and /or clone x environment (GxE) interaction might account for the lack of any superior clones across environments. Significant GxE effect implied the need for extensive clonal testings to pick out the best clones. Improved selection methods will help shortlist candidate clones for GxE tests and when coupled with bias on O/B selection, will ensure capturing superior clones.

INCIDENCE OF ABNORMALITIES IN RELATION TO *IN VITRO* PROTOCOLS

by

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Tissue culture methods for large-scale clonal propagation are well established. The commercial exploitation of these methods is constrained by floral and fruit abnormalities in the clonal palms. These abnormalities essentially represent a feminisation of the male parts of the flower resulting in mantled fruits in the bunches and 'androgynous' flowers in the male inflorescence.

Field evaluation of several clones reveals that the first batches of palms are almost always normal with respect to these abnormalities. The abnormalities appear after subculture, often increasing with continued subculture. There is some evidence for higher incidence of abnormalities in palms derived from cultures maintained on media containing cytokinins. Dendrogram analysis indicates that the abnormalities develop independently in different cell lineages i.e. abnormalities are not confined to a particular lineage. The abnormalities are transmissible meiotically, but the F1 abnormal palms have been found to revert to normal. Strategies to minimise abnormalities in clonal palms are discussed in the light of these observations.

YIELD OF OIL PALM CLONES IN DIFFERENT ENVIRONMENTS

by

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Results of seven oil palm clone trials, in 5 different environments, are briefly summarised. There is some evidence for clone x environment interactions of FFB yield, but less for oil/bunch. Two clone x density trials, one in Malaysia and one in Sumatra, are discussed in more detail. Yields in Sumatra were higher, apparently as a result of faster growth, and also high photosynthetic efficiency. Clone x site interactions for yield were significant in the second year of production, but not in the third year.

ASSAYING ENDOGENOUS CYTOKININ LEVELS IN INFLORESCENCES OF OIL PALM RAMETS

by

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In attempting to understand the possible causes of mantled fruit abnormalities in oil palm ramets, the endogenous cytokinin levels in inflorescences from normal and abnormal oil palm ramets were characterised. ELISA coupled with HPLC separation of extracts was used to quantitate cytokinins in the plant extracts. Antibodies to zeatin riboside, isopentenyl adenosine and dihydrozeatin riboside were produced by immunizing rabbits with cytokinin-bovine serum albumin conjugates. The titre and cross-reactivity of these antibodies were determined by ELISA.

Inflorescences from various fronds of normal and abnormal palms of Clone LMC 088 were sampled. Cytokinin extracts were prepared from sections of spikelets and isolated ovaries and then separated by HPLC. These analysis showed that all cytokinins increased with development of inflorescences. Our studies to-date indicate that reliance on frond number is a poor criterion for comparison, as inflorescence development in individual palms varies differently with frond number. It is evidently necessary to develop a proper sampling procedure before useful comparisons of cytokinin levels are made.

OTHER NEWS

In Vol. 7 (1), January - June 1990, of this newsletter readers were informed of some pioneering work published in the journal NATURE on sex determination in mammals. Transgenic male mice were produced when the Sry gene was inserted into xx (female) mice, but other factors are also involved as not all transformants expressed the gene.

In a recent issue of the same journal (Vol. 364, 19 August 1993) two articles, one on mice and the other on primates, independently describe finding rapid evolution of the gene, in particular the flanking sequences of a well conserved HMG-box domain. Readers are offered two suggestions as to what this could well mean. We reproduce here abstracts of the two articles.

Rapid evolution of the sex determining locus in Old World mice and rats

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THE Y chromosome-linked sex determining locus (*Sry*) responsible for testis determination in mammals[1-5] contains a DNA-binding motif (HMG box) that is conserved across species of marsupial and placental mammals (infraclasses Metatheria and Eutheria, respectively)[1,4,6,7]. But little to no sequence similarity is observed in flanking sequences between these two infraclasses, or among orders within each infraclass. We investigated the rate and pattern of evolution for the coding sequence of *Sry* in Old World mice and rats (subfamily Murinae). We found typical rates of synonymous substitution throughout the gene, but high rates of non-synonymous substitution, especially in the C-terminal (non-HMG box) region, when compared to other genes[8]. This region is also characterized by a frame-shift mutation and variation in a trinucleotide repeat motif. These data suggest that the non-box region is either functionally unconstrained or has undergone species-specific adaptive divergence.

Rapid sequence evolution of the mammalian sex-determining gene *SRY*

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IN mammals, induction of male sex determination requires the Y-chromosome gene *SRY*[1]. *SRY* encodes a protein with a central 'high mobility group' domain (HMG box) of about 78 amino acids[1-3]. HMG boxes are found in a wide variety of proteins that bind to DNA with high affinity but differing degrees of sequence specificity[4]. The human SRY protein binds to linear DNA with sequence specificity[5] and to cruciform DNA structures without sequence specificity[6]. The DNA-binding activity of the SRY protein resides in the HMG box and mutations in this region are associated with sex reversal in XY females[7-9]. No function has been ascribed to the portions of the SRY protein outside the HMG box. *SRY* belongs to a family of genes that are related by sequence homology within the DNA-binding domain: the genes most similar to *SRY* (>60%) have been named *SOX* genes (SRY box genes). None of the known *SOX* genes is homologous to *SRY* outside the HMG-box region. Although *SRY* is an important developmental regulator, its sequence is poorly conserved between species apart from the HMG-box domain. Here we investigate the coding sequence of *SRY* in primates and find that evolution has been rapid in the regions flanking the conserved domain. The high degree of sequence divergence and the frequency of non-synonymous mutations suggest either that the majority of the coding sequence has no functional significance or that directional selection has occurred.