



Newsletter BERITA ISOPB

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

P.O. BOX 10620 KUALA LUMPUR

Vol. 2 No. 3

SEPTEMBER 1985

ISSN 0127-6581 P.P. 80/1/85

EDITORIAL

It has been now four years since the last International Oil Palm Conference in Kuala Lumpur where much excitement has been created IRHO's announcement of the field planting of oil palm clones from their tissue culture research, the second group to do so and by the revelation of the promising early yield results of the first oil palm clones by Bakasawit/Unilever, the first group to succeed in regenerating oil palm in vitro. Since then many more groups, mainly plantation based groups and also commercial tissue culture laboratories, have ventured into this area, and many tissue cultured oil palms have been field planted. 1985 is the year predicted earlier when commercial availability of clones will make its scene and thus it appears to be an appropriate time to feature oil palm tissue culture as the main theme in this issue of the Newsletter.

In this issue are included reports volunteered by various tissue culture laboratories on their respective progress and development to date and their future plans. The reports do vary in their contents, most tend to talk in terms of generalities, some more so than others, some strike a somewhat optimistic note while others a subdued note. It is a bit unfortunate due to commercial competitiveness most organisations tend to veil their oil palm tissue culture work in a shroud of secrecy from public knowledge, although at the scientist level, most of them do have some idea of the progress in other laboratories. Also included in this issue is an abstract of a paper by yours truly estimating the expected yield increase with the first oil palm clones using the broad sense heritability approach and its implications to breeding and ortet selection.

Before we dig into the meat of this Newsletter it would be interesting to list down the milestones in the development of oil palm tissue culture in relation to the milestones in development of plant tissue culture in general.

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<u>Year</u>	<u>Tissue Culture Development</u>
1902	First unsuccessful attempt at in vitro culture of plant tissue's by Haberlandt.
1934	White's successful continuous growth of tomato roots in a liquid medium, and the discovery of the importance of adding Vitamin B, especially thiamin in the medium.
1937	Identification of IAA as one of the hormones controlling cell enlargement in the shoots of plants. Identification of other auxins followed.
1939	Nobecourt and Gautheret and White reported the indefinite culture of plant callus tissue in a synthetic medium.
1950	Ball's reported success in tissue culture of Sequoia, a forest tree.
1956	Miller <u>et al</u> identified, purified and characterized the first cytokinin, kinetin. Cytokinins were found to stimulate mature cells to subdivide and proliferate in the presence of auxin.
1957	Skoog and Miller found that by modifying the concentration of cytokinin in the presence of a fixed concentration of IAA they could control the morphogenetic expression of tobacco tissue in culture.
1958	Development of single-cell culture by nurse culture technique by Muir <u>et al</u> and development of the concept of cellular totipotency.
1960	Development of protoplast culture technique by Cocking.
	Demonstration of the concept of cellular totipotency by Bergman and subsequently by Steward <u>et al</u> and Earle and Torrey.

Morel pioneered the commercial vegetative propagation of orchids and other plants using the meristem culture technique.

- 1962 Murashige and Shoog devised the widely used MS medium for callus tissue and laid the foundation for commercial propagation of plants by tissue culture.
- 1964 Guha and Maheshwaris' discovery of the anther culture technique.
- 1968 First reports of embryoid and plantlet formation in tissue cultures of monocotyledonous crops e.g. asparagus, rice, wheat and sugar cane.
- 1970 Early reports on attempts of tissue culturing oil palm and success in obtaining callus cultures and shoot and rootlike structures from the cultures by Rabechault, Smith & Jones and Staritsky.
- 1974 Unilevers' announcement of their success in obtaining plantlets from tissue culture of oil palm using root explants.
- 1976 Rabechault and Martins' success in regenerating oil palm plantlets from callus cultures derived from leaf explants. This formed the basis of IRHO oil palm tissue culture process.
- 1980/81 PORIM/Wye obtained their first oil palm plantlets.

Formation of Unifield tissue culture laboratory in U.K. and subsequently Bakasawit tissue culture laboratory in Malaysia to produce oil palm clones commercially.

Preliminary results of field trials of the first oil palm clones of Unilever revealed the fidelity of the cloning technique. 1983 Reported successes in obtaining oil palm plantlets by other groups e.g. HRU, Sime Darby, Indian scientists followed.

A. Reports from Oil Palm Tissue Culture Laboratories

Bakasawit Sdn. Bhd.

Bakasawit's roots go back to 1968 when Unilever Research in the U.K. began the first experiments with tissue culture of the oil palm. In 1975 the techniques of clonal propagation were transferred to Malaysia with the setting up of Unipamol's Clonal Oil Palm Research Unit at Layang-Layang and 4 years later a partnership between Unipamol and Harrisons Malaysian Plantations (then Harrisons Malaysian Estates) was formed for the purpose of commercial scale production of clonal oil palms. In 1983 this partnership became Bakasawit Sdn. Bhd. Situated on a 5 acre site on the Dusun Durian Estate at Banting, the purpose built laboratories presently employ a workforce of 42 including 5 executives. With large culture room, medium preparation and nursery facilities, the unit currently has capacity for the production of around 250,000 clonal plants per year using existing techniques, and is designed for expansion. In addition to its function as producer of clonal planting material (currently for the Partners but shortly for sale to third parties), Bakasawit has a strong research and development effort aimed at a more efficient production of new clones, improvement of the production process and field testing of clonal material. The latter involves not only the identification of high yielding clones but also investigations into the best use of clonal oil palms on a commercial scale. The trials programme began in 1977 and to date some 280 hectares of trials and test plantings, involving some 39 clones, have been planted.

Bakasawit, Malaysia

BPPM (RISPA)

BPPM (Balai Penelitian Pekebunan Medan) is also working on tissue culture on the oil palm. Due to the organization in the Government Research bodies the tissue culture programme is carried out by Bogor Research Institute for Estate Crops at Bogor. Its programme covers the tissue culture for oil palm, coconut, cocoa, rubber and stevia. For the oil palm BPPM supplies the materials. At the present time work is carried out on the oil palm seedling and so far the callus stage has been reached. Several techniques have been used to induce root and shoot formation. If everything goes well then by mid-1986, hopefully the first plantlet will be coming out. At that time BPP Medan will supply them with the materials which we think are appropriate for tissue culture.

For the future the tissue culture programme will be aimed to:

- a. Duras and pisiferas which combine very well in the progeny testing. These materials will be used to produce planting materials for the smallholders which cannot afford to buy the clonal plantlets.
- b. DxP hybrids which give the best performance in the progeny testing. These materials will be supplied to large private Companies or Government Estates which can afford to buy them.
- c. Out-standing palms for the next breeding cycle.

BPPM, Medan Indonesia

Unifield T.C. Ltd.

Unifield T.C. Ltd. of Bedford, England, a company jointly owned by Unilever and Harrisons and Crosfield and sister company to Bakasawit Sdn. Bhd. in Malaysia, began producing clonal oil palms by tissue culture in 1981. Covering the oil palm growing world outside Malaysia, it draws its clones from the partner's breeding programmes in Africa and Papua New Guinea. By mid-1985, its 20,000 square foot laboratory had exported some 400,000 plantlets to 14 countries and its workforce of over 80 persons currently produces a range of 44 clones for use at selected sites in both large and small scale field trials.

Unifield U.K.

Guthrie Tissue Culture Laboratory

Guthrie Tissue Culture Laboratory is located at Chemara Research Complex in Seremban. It is a temporary lab established by the conversion of a bungalow during mid-1984. The main building consists of a media preparation room, an inoculation room with twelve laminar air flow hoods, three culture rooms, a staff room and a washing and autoclaving area. This lab has a capacity to produce 200,000 plantlets per year.

Tissue culture work commenced in September 1984 with a present work force of one executive, 3 staffs and 13 workers.

Currently, this lab is actively engaged in oil palm tissue culture research, aiming for commercial production of elite oil palm planting material as well as the propagation of selected parent palms.

Young leaves carefully excised near the 'palm heart' form the main source of explant material. Specially induced tertiary roots derived directly from the base of the trunk also serve as explants. To-date, callus formation has been successfully induced from explants of seedlings and selected ortet palms. Callus are being subcultured for further multiplication and induction for embryogenesis.

In addition to tissue culture, other techniques such as embryo culture technique etc. are also being used as a tool in germplasm conservation and for other purposes.

In future, it is envisaged that the lab will be expanded and further equipped for research and development of other aspects of biotechnology such as automation for large scale plantlet production by hydroculture, somaclonal variation, protoplast culture, and other novel areas.

Guthrie Research Chemara, Malaysia

HRU Tissue Culture Laboratory

HRU ventured into oil palm tissue culture in December 1981 on a very preliminary basis with one laboratory assistant using an old plant pathology inoculation hood. Actual substantial work began when the tissue culturist joined the unit in November 1982.

The first phase of the HRU oil palm tissue culture project to produce the first clonal plantlet and transplanted into soil was achieved in 28 months in March 1981. The second and third phases of the project are proceeding simultaneously i.e. improvement of the culturing techniques to increase success rates and production of adequate clonal materials for field evaluation and pilot commercial plantings.

Success in the culturing techniques has been highly variable and usually apparently related to the original explant materials and cultures used i.e. the control over development processes of the respective cultures is usually poor. However this limitation has been overcome to some extent by very high work input and large throughput of cultures and overall success to-date appears not unsatisfactory. More than 400 mature palms and 200 seedlings have been put into culture. Callus lines have been obtained from about 300 mature palms and 160 seedlings; while embryogenic lines have been achieved for about 40 mature palms and 25 seedlings. For effective commercialization of the process however it is imperative that the culturing success rate should be greatly improved.

The first field trials of HRU clonal materials will be plantlet in 1986 with 7 clones to be followed by 14 clones in 1987. Anticipated preliminary commercial production will begin in 1986 with about 100,000 plants being for testing.

HRU, Malaysia

IRHO (France/Ivory Coast)

IRHO has 12 clones derived from selected mature palms planted into the field in 1983 and are thus coming into bearing. They have to-date put 150-170 selected ortet palms into culture, of which about 50 have become embryogenic. IRHO is at the moment seeking collaboration with various oil palm research stations all over the world to evaluate their clones. They reckoned that commercial sales will begin in 1990.

IRHO is very cautious about genetic variation arising from plants propagated from tissue culture. They prefer to field test the plants everytime there is a modification or change in the culturing process especially in the phytohormone treatments. They have also planted a large scale trial to check for differences between plantlets derived from different types of embryoids or calluses from the same clone. From nursery observations no differences were apparent.

Editor (From discussions with
Noiret, IRHO)

Tissue Culture of the Oil Palm in FELDA
Agricultural Services Corporation

Following the signing of an agreement between the IRHO and FELDA in 1981, Dr. Maheran Abu Bakar was sent for training in the IRHO's laboratories in France and Ivory Coast. Upon her return, a temporary tissue culture laboratory was set up in Kuala Lumpur in 1983. Ortets were selected from the various progeny trials and cultured using the technology of the IRHO. Another officer, Mr. Aw Khoo Teng, was recruited to assist Dr. Maheran in running the laboratory. In late 1984, all operations were shifted to the newly completed tissue culture building, located within the FELDA Headquarters Complex. Clonal material will be field tested first before being released for planting purposes.

P.K.P Felda, Malaysia

Oil Palm Tissue Culture in PORIM

Objectives

PORIM embarked on tissue culture studies in 1980. The primary objectives have been to:

1. Develop and optimise methods for rapid micropropagation of oil palm.
2. Field evaluation of clones.

Ancillary objectives now include:

1. Cryopreservation of seeds, embryos, embryoids and callus.
2. Haplogensis by culture of anthers.
3. Embryo culture as an alternative to conventional seed germination for recalcitrant seeds e.g. seeds from pisifera and tenera palms.

Staff and facilities

A team of four research officers is involved in these studies. A modern well equipped laboratory exists at its headquarters in Bangi which is adequate for research and development purposes. Additional laboratory facilities in Serdang are also being utilised. A laboratory has also been established at PORIM's Research Station in Kluang for initiation of callus from ortets selected at the Research Station and neighbouring collaborating agencies.

Progress and Future Plans

PORIM carries out research in collaboration with various agencies, the latter providing ortets from their breeding trials. Established embryogenic lines are distributed to collaborating agencies.

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Field trials commenced in 1983. Newer clones are continuously being produced for field evaluation.

A laboratory for large-scale production of plantlets has been planned for construction at Bangi.

Embryo culture is routinely used for regeneration of seedlings from selected seeds for breeders. Seeds have been successfully cryopreserved, providing a means for long-term germplasm storage.

PORIM, Malaysia

Sime Darby (Ebor Research) Tissue Culture Project

Sime Darby's tissue culture laboratory was set up in 1981/82 to undertake research and development and commercial production of the oil palm. Work began from 1981 and liaisons with overseas organisations aided in developing the technique. The first callus was produced from roots of mature palms in 1982. The first embryogenesis was observed from callus from seedling material in mid-1984.

At present Sime Darby has embryogenic lines from more than 200 sources; mostly seedling sources but including mature palms. About 750 plantlets are being transplanted into soil each week for large scale clonal test. Another 200 selected mature palms are expected to be tissue cultured in the near future.

Anticipated plantlet reproduction is 100,000 by end 1986 increasing to $\frac{1}{2}$ million annually depending on the circumstances. Sime Darby has a team of 2 tissue culturists and 16 laboratory workers on this project.

Ebor Research, Malaysia

B. Paper Abstract (Oliaguieux, Feb 1986)

Anticipated Yield Increase with Selected Oil Palm Clones from Current DxP Seedling Materials and its Implications on Clonal Propagation, Breeding and Ortet Selection.

Soh, AC, HRU.

Abstract

An increase in oil yield of at least 30% has been projected with commercial plantings of the first oil palm (Elaeis guineensis Jacq) clones obtained from current DxP seedling derived planting materials. This widely accepted figure presumes a very high broad sense heritability (h_B^2). Heritability (h_B^2) estimates obtained from four representative DxP trials at HRU average 0.19 with expected selection responses averaging 12-16%. The low genetic variation between palms and the large confidence intervals associated with individual palm oil yield measurements make ortet selection unreliable and necessitate extensive clonal testings. This together with the uncertainty in the tissue culture technique to clone every palm make feasible other alternative cloning strategies i.e.:

- i) Cloning the parents of a superior DxP cross to produce biclonal F_1 seeds
- ii) Cloning the recreated seedlings of a superior DxP cross
- iii) Cloning all the ortets of a superior DxP cross in a trial

All of which would be expected to give yield improvements of about 13%.

Clonal propagation will play a significant if not dominant role in oil palm. Future breeding programmes should have provisions for exploitation by these alternative forms of clonal propagation besides seed production. Greater emphasis should be placed on TxT/P and DxT/T outcrosses between different genetic origins in the

parental populations to generate wider genetic variability than on further improvement in a particular hybrid by recurrent selection to exploit SCA, which can be shortcircuited by cloning. More 3-4 way cross TxD progeny testings should be made to screen their combining abilities for future breeding and to generate superior combinations and individuals for cloning. Ortet selection within such crosses should be more effective because of greater genetic variability. Reliability in ortet selection can be further assured by improvements in accuracy of oil yield measurements which minimize seasonal, yield cycle and interpalm competition effects. The greatest advantage of having clones now is the feasibility to study and quantify these environmental effects thus enabling more effective selection techniques to be developed.

c. NEWS

Conferences

The second Malaysian National Plant Tissue Culture Symposium and the International Rubber Tissue Culture Workshop was held on 15-17 October in Serdang, Malaysia. It was jointly organised by Rubber Research Institute of Malaysia (RRIM), Agricultural University of Malaysia (UPM), Malaysian Agricultural Research and Development Institute and PORIM. Guest speakers present included Kenneth Giles of Twyford Lab and Koh, Chong Lek of University of Malaya who spoke on plant genetic engineering. Ivor Symkin of Hatfield Polytechnic, U.K who spoke on physiological aspects of field conditioning and acclimatization of tissue culture plantlets and Barba of University of Philippines who reported on their tissue culture work on Calamus sp. (rattan). The oil palm crowd made a significant contribution to this conference with Rohani (PORIM) presenting her work on embryo culture of oil palm, Ali Sekak (PORIM) and Ismail Hamzah (UPM) describing their respective preliminary studies on oil palm anther and protoplast cultures and Rajanaidu espousing his strategies in breeding and selection for vegetative propagation in oil palm. Laurie Jones of Unilevers, Colworth Lab as well all the local oil palm tissue culturists also attended the conference.

Following the National Tissue Culture Conference and International Rubber Tissue Culture Workshop was the International Rubber Conference hosted by RRIM. In this Conference, Professor Simmonds outlined his views on new breeding strategies for rubber in the light of the crop being pushed to less optimal areas from good areas which have been supplanted by oil palm, and that large rubber acreages are now in the hands of smallholders, who are unlikely to provide the necessary inputs to achieve high yields from the present clones.

Paranjothy, PORIM's tissue culturist, presented a paper on Recent Developments in Cell and Tissue Culture of Oil Bearing Palms at the Workshop on Biotechnology in Agriculture organised by the International Centre of Genetic Engineering and Biotechnology at New Delhi, September 17-22.

Cheah, Keng Tuan, and Maheran, tissue culturists at Sime Darby and Felda respectively, attended the Workshop on In Vitro Problems in Brussels, September 15-21.

Hot Air

In view of the fact that most of the Malaysian plantation companies are controlled by the government owned National Investment Board (PNB) there is speculation of an imminent establishment of a consortium involving these companies to undertake research and development into oil palm tissue culture and other aspects of plant biotechnology. There is also talk that Bakasawit may also be involved in this set-up. There has been an earlier proposal to set up a national biotechnology group involving all governmental research institutes.

Owing to the rather tight seed supply situation PORIM has been requested by the industry to expand its oil palm clonal propagation efforts quickly to hopefully achieve 200,000 plantlets for sale by next year.

Words in Fashion

a. Biotechnology

The most fashionable and perhaps the most abused word used by nonscientists and scientists alike these days. But what is actually biotechnology? To the cynical, making 'tau-ewe' (soysauce) or 'samsu' (rice-wine) is also biotechnology i.e. it is just a new name given to the fermentation and microbial degradation derived industries. At other times, biotechnology has been equated to tissue culture and genetic engineering. As one university lecturer pointed out, biotechnology is but the industrial application of such research techniques. A definition of plant biotechnology is given by Torrey (American Scientist, July-August 1985):

"The application of existing techniques of plant organ, tissue and cell culture, plant molecular biology, and genetic engineering to the improvement of organs and of plant productivity for the benefit of man". Anybody care to disagree?

b. High technology

The term high tech or technology was at one time synonymous with the computer industry. Now, it is also applied to biotechnological industries using techniques of molecular biology. High tech industries are the main thrust of venture capitalism. In this part of the world venture capitalists have singled out tissue culture propagation of tropical crops and particularly oil palm, as a high tech area. As a consequence of this and in line with the Singapore Government's economic policy to move into high tech industries, a biotechnology company, Plantek International (Pte) Ltd. was formed by a consortium of companies involving Native Plants Inc. of USA, Tata Groups of India, Sumitomo Corp and Kyowa Hakko of Japan and Intraco of Singapore.

Recently Plantek made a press announcement highlighting their interest and expertise in the tissue culture propagation of ornamentals, fruit trees, coffee, oil palm and other economic palms.