December 2008

AAR NEWS

AAR started its oil palm tissue culture research in 1982 as an R&D effort and has since expanded its operations, featuring an advanced tissue culture cloning laboratory which was officiated on 17 January 2008 by YB Datuk Peter Chin Fah Kui, Minister of Plantation Industries and Commodities.

Situated within Tuan Mee estate, the laboratory has a production capacity that exceeds one million clonal palms and to-date is the largest oil palm tissue culture lab in the world in terms of area and production capacity. The success of which is attributed to the years of research and development that has gone into establishing the tissue culture cloning process, primarily with the Liquid Suspension System (LSS) which has enabled a higher turn-around capacity as compared to the industrial standard, termed Gel Culture System (GCS). However, the success of the laboratory could only be achieved by a strong and dedicated workforce led by Mr. Tan Cheng Chua (AAR's Crop Improvement Section Head), Mdm. Ho Yuk Wah (AAR's Senior Tissue Culturist) and her tissue culturist team, Mdm. Choo Chin Nee and Mdm. Norazura Anuar, Dr. Soh Aik Chin (AAR's plant-breeding consultant), in addition to the strong support and encouragement by the company's Senior Management led by Dr. Kee Khan Kiang and Mr. Goh Kah Joo.

In this issue, Mdm. Ho Yuk Wah has written a paper entitled "Biotechnological approaches in oil palm planting material - a success story", which was presented at the 2008 National Oil Palm Conference held in India.

HAPPY READING!

Tasren Nazir Mahamooth

INSIDE THIS ISSUE :

Biotechnological Approaches in Producing Oil Palm Planting Material – A Success Story

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Abstract

Success in cloning oil palms was first reported by Jones (1974) and Rabechault and Martin, (1976). Results from successful field trials followed in the late seventies. Realising the huge yield potential of cloning and planting of elite clones, Malaysian plantation companies including Applied Agricultural Resources Sdn. Bhd. (AAR) commenced their tissue culture programmes in the early eighties. By the mid 1980s there were 10 clonal oil palm laboratories in Malaysia (Wooi, 1990) and others elsewhere (Le Guen *et al*., 1991). The euphoria was dampened by the first report by Corley *et al* (1986), on mantling phenomenon that affected the reproductive structures and yield of clonal oil palms after which all laboratories reverted back to research and development. In a span of more than 20 years of research at AAR we concentrated on fine tuning the tissue culture processes in conjunction with experimental clonal plantings in 1990 and large scale commercial plantings in 1997. With the successful performances of our commercial plantings we

are now forging ahead with the recent commissioning of our new state of the art tissue culture laboratory of 4800m^2 and commercial nursery of 0.5 ha.. These have a capacity for 1.5 million ramet production annually. Mantled somaclonal variants still occur in the tissue culture process however the problem can be circumvented with better management of cultures. Thus it is possible to produce oil palm clones commercially by keeping the mantling level to less than 5% (Corley *et al*., 1998 and Wong *et al*, 1999, Ho *et al*, 2006). With the success in recloning, the issue of insufficient ortets has been overcome. Our commercial production utilizes both ortets and reclones and a combination of gelled culture system and liquid suspension system.

Introduction

Commercial oil palm planting materials are mixtures of hybrid families. Since genetic variability exists within these DxP planting materials it is possible to select high yielding individuals as ortets for clonal propagation. Generally, the selection of elite ortets is based on high fresh fruit bunch (FFB) yield and oil to bunch ratio of 28% and above from breeding trials. Other characteristics such as cloning of inter-specific hybrids for more liquid oil and slower height increment (Ho 1987, 2005), compact palm architecture for high density planting (Escobar *et al*, 2006), had also been reported. However, the greatest benefit of planting clones will be the high expected increase in oil yield.

The Tissue Culture Process

This involves the culturing of sterile tissue samples (explants) such as tips of tertiary roots, immature leaf or inflorescences on a gelled culture media. A modified Murashige and Skoog (1962) culture medium supplemented with sugar, vitamins and growth regulators to stimulate growth of callus is commonly used. Callus can be observed from as early as one month after culture initiation. Callusing rate of 20% was obtained for Yangambi Avros, Dumpy Avros and Cameroon materials compared to 16% for Avros & La Me & 11% for Nifor. Table 1. The influence of genotypes on rate of callus induction was also reported by Ginting et al (1995).

Table1 – Callusing Rate of Genotypes Cultured at AAR Sdn Bhd

Origin	No. of Ortets	Callusing (%)	
		Mean	Range
Yangambi Avros	24	20	3 – 55
Dumpy Avros	46	20	2 – 53
Cameroon	28	20	5 – 46
Avros	15	16	2 – 42
La Me	10	16	5 - 31
Nifor	7	11	3 - 24

The callus is then induced to form embryoids which then develop into shoots. Embryoid multiplication is carried out by subculturing onto fresh media every 4 to 6 weeks.

Success with liquid suspension systems have been reported (Malaurie, 1987; Teixeira et al. 1990 and Wong et al. 1999) In the liquid suspension system, embryogenic callus from gelled cultures are transferred to liquid media and agitated on orbital shakers at 80 $\pm$ 10 rpm. This system allows for automation of the tissue culture process thus eliminating manual labour required for transfers onto gelled media. Embryogenic cell suspensions are then plated out onto gelled media for germination, shoot development and rooting.

Cloning and Recloning Efficiencies

In the gelled-culture system, callogenesis can be induced in all ortets and reclones. On palm basis, the reclones are more amenable to culture with embryogenesis rate of 88% and shoot regeneration 85% compared to ortets with 72% and 56% respectively (Table 2). Only 7% of callogenic explants of reclones could differentiate into embryoids, this is however, more than doubled that of callogenic explants of ortets. Shoot regeneration from embryogenic lines of both ortets and reclones appear similar at 95% to 96%.

Table 2 – Oil palm cloning and recloning efficiencies of AAR's "Gelled-Culture System"

Culture Stage	Success rate (%)			
	Based on Palms		Based on Explants	
	Ortets[a] (n=216)	Reclones[b] (n=110)	Ortets[a] (n=400,000)	Reclones[b] (n=200,000)
Callogenesis	100	100	19 (1 to 67)	14 (1 to 41)
Embryogenesis	72	88	3 (1 to 6)	7 (6 to 20)
Shoot Regeneration	56	85	96[c]	95[c]

Ref: a = Cloning of palms derived from seeds,
b= Recloning of palms derived from tissue cultured ramets
c= Based on embryogenic lines
( )=Range

The frequency of ortet clones and reclones that can proliferate on liquid suspension system is less than that in gel system, 59% and 86% palm wise respectively. The frequency of embryoids converting to shoots is also lower (Table 3).

Palm Type	Basis	Embryogenic callus proliferation (%)	Shoot conversion from embryoids (%)
Ortet (n = 37)	Embryogenic palms (n = 22)	59*	57*
	Embryogenic callus (n = 377)	58*	50*
Reclones (n = 35)	Embryogenic palms (n = 30)	86*	78*
	Embryogenic callus (n = 575)	48*	49*

Table 3 - Oil palm cloning and recloning efficiencies of AAR's "Liquid-Suspension System" (Compared to Gelled-Culture System expressed as 100%)

Ref: * = compared to gelled culture system which is expressed as 100%

Rooting

Shoots which had attained a height of 5 cm and above are excised for rooting on a root induction medium containing an auxin such as NAA or IBA. The advantages of using a double layer technique have also been reported by Zamzuri (1999). Root initials are normally observed after 3 to 4 weeks.

Acclimatisation in Tissue Culture Nursery

Rooted plantlets of height 10 cm and above are acclimatised in high humidity chambers before transplanting in the pre-nursery. A total of 1.6 million ramets were transplanted in our acclimatization nursery in year 2006 and 2007. Mean survival rates after transplanting in acclimatization chambers was 98.1% and 99.4% for year 2006 and 2007 respectively. (Table 4). These extremely high survival rates contribute to the success in our commercial oil palm cloning program.

Table 4 – Survival of ramets in acclimatization chamber at 1 month after transplanting

Planted		At 1 month after planting		% Survival
Year	Total	No. Dead	No. Alive	Mean
2006	794,663	15234	779,429	98.1
2007	818,149	5252	812,897	99.4
Total	1,612,812	20,486	1,592,326	-
Mean	-	-	-	98.7

Table 5 showed the substantial increase in ramets being acclimatized between Year 2001 to 2007, from 0.24 million in 2001, reaching a peak of 1.1 million in 2005 and 0.8 million ramets in 2006 and 2007. Correspondingly, ramet despatched to clients rose from 0.17 million in 2001 and stabilized at 0.7 million between 2005 and 2007. Despatches are expected to reach 1 million by year 2009/2010

Table 5 – Ramets Acclimatised & Despatched

Ref: M = million; * = Projected production

Year	Ramets Planted in Acclimatisation Chamber	Ramets Despatched to Clients
2001	242,000	170,000
2002	254,000	180,000
2003	435,000	300,000
2004	563,800	400,000
2005	1,100,000	700,000
2006	800,000	700,000
2007	800,000	700,000
2008	800,000*	700,000*
2009-2010	1,200,000*	1,000,000*

Hardened ramets are despatched to estate pre-nurseries for transplanting into soil in 150 mm x 225 mm (6" x 9", flat measurements) polybags for 3 months after which they are then transferred into large polybags in the main nursery for a further 9 months before field planting.

Field Trials and Results

Between 1997 and 2006, we have planted oil palm clones in over 11,197 hectares throughout our estates in Malaysia. Of these ramets, 80% were propagated from primary ortets, 7% from re-clones and 13% from Liquid-suspension system.

Table 6 – Trial Performance of AAR Clones

Trial	Year of record-ing	FFB Production (Tonnes/ha)			% FFB increase of Clones over DxP	Oil Yield (Tonnes oil/ha)			% oil increase of Clones over DxP
		Clone		AA DxP		Clone		AA DxP	
		Range to date	Mean			Range to date	Mean		
BCT13-97	00-06	22.6-28.0	25.3	21.7	117	6.0-7.9	6.9	5.4	128
BCT14-97	01-06	21.2-29.5	26.1	24.3	107	6.4-9.1	7.7	6.6	117
BCT15-98	00-06	22.3-30.4	27.6	26.9	103	6.6-8.5	7.9	6.6	120
BCT16-98	01-06	15.7-24.8	20.8	18.9	110	4.7-7.5	6.1	5.2	117
BCT18-00	02-06	21.7-33.4	26.5	25.1	106	6.2-9.1	7.8	7.0	111

Yield production of 5 separate clonal trials in different estates is summarized in Table 6. Trial mean for clones ranged from 20.8 to 27.6 tonnes ffb/ha/yr whilst the DxP control ranged from 18.9 to 26.9 tonnes ffb/ha/yr.. The increase in ffb yield over the DxP control ranged from 103% to 117%. In terms of oil yield, the clones produced 6.1 to 7.9 tonnes oil/ha/yr and DxP control ranged from 5.2 to7.0 tonnes oil/ha/yr over the 5 locations. The increase in oil yield over the DxP control ranged from 111% to 128%. The best clones produced more than 9 tonnes oil/ha/yr. Results showed the significant gain in oil yield with clonal plantings and concur with Baudouin *et al.*(1991), Maheran *et al.* (1995) that oil to bunch trait is highly heritable and transmitted from ortets to clones.

Table 7a – Commercial Performance of AAR Clones (same age plantings)

Material	Area Planted (Ha)	Year of Production (Tonnes ffb/ha/yr)			
		1st	2nd	3rd	3 Year Mean
Clones	**251**	**20.8 (101%)**	**25.7 (118%)**	**26.7 (102%)**	**26.7 (107%)**
DxP	**69**	**20.5**	**21.8**	**26.1**	**22.9**

Ref: ( ) = % increase over DxP

Table 7b – Commercial Performance of AAR Clones

Planting material	Planted Area (Ha)	FFB Yield Production (tonnes/ha/yr)		
		Lowest	Highest	Mean
AA Clones	249	31.3	39.8	33.7
AA DxP	1622	20.0	34.6	27.3
% increase of clones over DxP	-	157	115	123

Clones planted in commercial fields have also proven to yield between 7% (Table 7a) to 23% (Table 7b) more ffb than the DxP controls. Similarly commercial oil extraction data (Table 8) also showed that clones have 8% to 22% more oil than the control DxP. Of the 4 commercial OER test runs carried out , the clonal OER ranged from 24.1% to 26.7% whist OER of DxP ranged from 19.7% to 22.4%

Table 8 – Test Runs on Oil Extraction Rates (OER) at Principals' Mills

Test Date	Total Estates	Mill Identity	CLONES			DxP OER (%)	% OER increase of clones over DxP
			Area Harvested (ha)	Total ffb Processed (tonnes)	OER (%)		
Sept 04	1	I	464	390	24.3	22.4	108
Oct 04	3	II	423	343	26.7	21.9	122
June 05	3	II	251	389	25.6	22.1	116
Jul 06	4	III	1341	914	24.1	19.7	122

Limitations to Large Scale Propagation

Low embryogenic rates contributed by genotype variation necessitate the setting up of a larger pool of ortets. The lack of elite ortets can be addressed by setting up ortet gardens with elite crosses of known performance. The success in recloning will alleviate the demand for primary ortets.

The bottleneck in large scale micropropagation of oil palms is the unavailability of reliable molecular markers for detecting somaclonal variants, particularly for the mantling phenotypes.

Commercial Planting Issues

Perhaps the most important issue undermining confidence to cloning of oil palms has been the mantled fruit abnormality phenomenon. This can only be detected when the ramets produce the first few inflorescences at 1.5 to 2 years after field planting. The abnormality is typified by the vestigial stamens in the female flowers developing into fleshy carpels to form a mantle surrounding the fruit. Such mantled fruits can be parthenocarpic or fertile. Parthenocarpic fruits normally abort before ripening thus affecting yield production.

The mantling rate in AAR's clonal plantings is summed up in Table 9. The initial high rates between 1986 and 1995 were due to experimentation in the early years. However with improved protocols and diligent culture management we are able to consistently keep the mantling rates to less than 3% in clonal plantings since 1996. Mantling rates of ramets produced from gelled culture system and liquid suspension system are similarly low at less than 3% (Table 10).

The risk in production of clones producing mantled fruit bunches is currently kept at a low level by culture management such as reduced use of phytohormones in the culture media, reduced time in culture and limiting production to 5000 ramets per embryogenic line. Such limitation would not be necessary when molecular marker tools become available. Current strategy is to adopt the planting of a package of different clones rather than single clone planting to lessen the risk of high abnormality rates i.e. minimize the impact of planting any "risk" clone in large numbers at any planting.

Table 9 – Mantling Rates in AAR Clones

Year Planted	Explant Source	Clones		Ramets	
		Number Planted	Mantled (%)	Number Planted	% Mantled
1986-1991	Ortet	12	67	7427	6.2
1989-1993	Embryos (Reprod. cross)	17	12	6840	1.1
1992-1995	Seedlings (Reprod. Crosses)	63	75	80642	7.6
1996	Ortets	3	67	451	2.0
1996-1997	Ortets	20	80	9378	2.7
1997-1998	Ortets	18	50	11865	1.4
1998-2001	Ortets	45	34	29171	1.1
2001-2003	Ortets	16	88	26243	2.7
2004	Ortets	3	0	412	0

Table 10 – Mantling Rates of AAR Clones and Reclones Produced by Gelled-Culture and Liquid Suspension Systems

Culture Type	Mantling (%)	
	Clones	Reclones
Gel	2.9	2.0
Liquid	1.6	1.5

Poor pollination is being addressed with the incorporation of 20% DxP as pollinators in clonal plantings. Genotype and environmental (GxE) interaction is being attended to with multi location trials.

Field and estate planting strategy such as tall clones in poorer areas, high bunch number to buffer low yielding period can be investigated. The selection of vigorous materials with more compact architecture as ortets and ramets for cloning or recloning should not be overlooked.

Concluding remarks

Our success in commercializing the production of clonal planting materials depends on the following factors:

1. availability of a constant supply of primary ortets and reclones as explant materials for uninterrupted production
2. a combination of gel and liquid culture system to build up ramet numbers
3. reduced contamination rates in the laboratory and high survival rates in the nursery for maximum ramet output
4. accountability and traceability in every step of the tissue culture process for quality management

5. much improved protocols with minimum somaclonal variants (mantling) supported and verified by field performance
6. The mantling rate (sterile type) can be reduced to less than 5% with the adoption of a clonal package of minimum 5 to10 clones per planting and restricted ramet production to less than 5,000 ramets per embryogenic line.

Our timely collaboration with centres of excellence around the world and setting up a joint molecular laboratory with the University of Nottingham Malaysia Campus (AAR-UNMC Research Centre) would facilitate the development of molecular markers for screening clonal oil palms for abnormality.

Our success is a story of perseverance, dedication, teamwork, disciplined workforce and last but not least, the belief and trust of our principals namely Boustead Holdings Bhd. and Kuala Lumpur Kepong Bhd in our ability to deliver quality planting materials for the oil palm industry.

Acknowledgements

The author wishes to acknowledge Advanced Agriecological Research Sdn. Bhd. and its Principals, Boustead Holdings Bhd. and Kuala Lumpur Kepong Bhd., for permission to present this paper at the National Conference "Oil palm for farmers prosperity and vegetable oil security" organized by The Soc. for Promotion of Oil Palm Research and Development (SOPOPRAD)in collaboration with National Research Centre for Oil Palm (ICAR) India at Vijayawada, Andhra Pradesh, India on 2-4 February 2008.

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Official opening of
AAR TISSUE CULTURE LABORATORY

The official opening of AAR Tissue Culture Laboratory was successfully held on 17 January 2008. It was officiated by YB Datuk Peter Chin Fah Kui, Minister of Plantation Industries and Commodities. About 250 guests attended the opening ceremony. The following are some photos taken during the event.

Launching of AA Vitroa
From left: Dato' Seri Lee Oi Hian, Datuk Azzat Kamaluddin,YB Datuk Peter Chin, Dr. Kee Khan Kiang and Mr. Chow Kok Choy,

Ribbon cutting ceremony
From left: Mr. Chow Kok Choy, YB Datuk Peter Chin and Datuk Azzat Kamaluddin

Souvenir presentation
From left: Mr. Chow Kok Choy presenting a momento to YB Datuk Peter Chin.

Tour of the Tissue Culture Laboratory
From left: Dato' Seri Lee Oi Hian, Dr. Kee Khan Kiang and YB Datuk Peter Chin accompanied by Mdm. Ho Yuk Wah (Senior Tissue Culturist).

Social & Personal

Congratulations

Newly confirmed recruits (January – August 2008):

Dr. Wong Wei Chee graduated with B.Sc. in Industrial Biotechnology (Hons.) from the University of Malaya in 2002 and received her Ph.D. in Plant Biotechnology from the same university in 2008, working on the "Genetic transformation of embryogenic cell suspension of *Musa acuminata* cv. Mas (AA group) with a transcription factor associated with early flowering." Before she joined The Advanced Agriecological Research Sdn. Bhd. in August 2007 as the biotechnologist, she worked as a research officer at the Centre for Research in Biotechnology for Agriculture (CEBAR) in the University of Malaya. She joined AAR in August 2007 and is currently stationed at the AAR-UNMC Biotechnology Research Centre in Semenyih, Selangor.

Miss Hor Mei Ling was born in Seremban, Negeri Sembilan. She is now pursuing her studies in Chartered Institute of Management Accountants (CIMA), Kuala Lumpur. She has been working since 1994. In 1996, she worked independently as an Accounts Supervisor in a trading company. She handled the full set of accounts, fund management, cash control, and payroll in that company. Later, she also worked in several companies in different industries. She joined AAR in March 2008 as the Accounts Executive.

Miss Sita Astari graduated with a B.Sc. in Biology (Hons.) from Institut Teknologi Bandung (ITB) in 2007 and pursued her Master of Science degree in Biology, specialising in Applied Entomology from the same institute. She joined PT. AAR Indonesia in November 2007 as an Assistant Research Officer. She is currently involved in oil palm agronomy in addition to being an entomologist for the company.

Miss Elza Sisilia graduated with a Bachelor of Economy degree in Accounting (Hons.) from the University of Riau in 2001. Prior to her employment under PT. AAR Indonesia as the Accounts and Administrative Officer, she had previously worked at PT. Asuransi Dayin Mitra (2006-2008) as an Accounts Officer and at PT. Altra Excis Investima (2002-2006) as a Finance & Accounting Officer.

Social & Personal

AAR Sport Club Family Day

This year the Organizers did something different for the Sports Club family day held on the 2nd of August 2008. We played host to more than 50 children and their caretakers from PDK Selayang orphanage at our office field. There were many interesting programs both for the young and old. It was all smiles and screams as each cheered on their team. Red team emerged the overall winner.

ready, get-set GO!!!!!

Kids enthralled with the gimmicks of the clown

children enjoying their nasi lemak

One for the road….

Its fun time…. kids and oops, our CHEERLEADERS ?

AAR Durian Fest – July, 4 2008

The thorny or "King of the fruits" or the "smelly" fruit (according to the mat salleh) season is back. You can smell them miles away, and if you are on the road, you will be tailing behind lorries and cars returning home from their kampungs or holidays, fully laden with these thorny fruits. A durian fest was held in the office compound and members had a great time sitting under shady trees sampling different varieties of the durians.

Contributed by Mdm. Julie Yee