Production, Performance and Advances in Oil Palm Tissue Culture[1]

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Abstract

*The Malaysian national average palm oil yield had been stagnating at 3.5 to 3.9 t/ha/yr for more than two decades. With increasing global demands for vegetable oils coupled with challenges in production efforts, there is an urgent need to improve productivity. Current oil palm (*Elaeis guineensis*) planting materials are produced through the hybridization of* dura *x* pisifera *(DxP). To accelerate and capture the maximum yield potential of a selected genotype, oil palm needs to be vegetatively propagated. Oil palm tissue culture is not without problems, such as the floral abnormality. A prerequisite for clonal production is the availability of a large number of high quality ortets, selected from breeding programmes. Another option for improved planting materials is through the production of clonal seeds, using clonal parents with good specific combining ability (SCA). The low rate of embryogenesis for large-scale propagation is being addressed via the liquid culture, innovative shake flask and bioreactor systems. The biomarker approach, leading to the development of a diagnostic tool for screening clonal amenity and conformity is being investigated. in 2009, production of clonal oil palm in Malaysia was 2.53 million ramets, thus the need to increase the production capacity. Agronomic inputs such as in fertile land and tailored fertilizer regimes are necessary for clones to express its genetic potential.*

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INTRODUCTION

Yield improvements were achieved through breeding supported by agronomic principles. Oil palm (*Elaeis guineensis*) is an exceptional example where a quantum leap in yield improvement is obtained through a single gene. The "first wave" in yield improvement of up to 30% was by switching from the *dura* to the *tenera* (DxP) planting materials. However, the oil yield has been stagnating between 3.5 and 3.9 for more than two decades. In 2009, the Malaysian palm oil industry achieved an average oil yield of 3.93 t/ha, fresh fruit bunches (FFB) of 19.20 t/ha/yr and palm kernel yield of 1.01 t/ha/yr (MPOB, 2009). The approach of enhancing competitiveness through expansion of land for cultivation has limited option. A viable alternative is to increase productivity per unit land area, leading to the national aspiration of increasing FFB yield of 35 t/ha/yr and oil extraction rate (OER) of 25% by the year 2020, referred as Vision 35:25.

High yielding genetic materials are available in the industry. It had been reported that the best experimental plot produced oil yield of 8.6 t/ha/yr (Corley *et al*., 1976), selected progeny producing 12.2 t/ha/yr (Rajanaidu *et al*., 1990), individual palm yielding 13.6 t/ha/yr (Sharma and Tan, 1999) and the maximum theoretical oil yield of 18.2 t/ha/yr (Corley, 1998). Cloning such palms would provide high-yielding planting materials for the industry, which would be the "second wave" (Kushairi *et al.*, 2006) in yield improvement in the oil palm, in tandem with Vision 35:25.

Oil palm tissue culture technique was rigorously attempted in the 1960s through the 1970s. The early success of plantlet production in the 1970s (Jones, 1974; Rabéchault and Martin, 1976) inspired many oil palm organizations to exploit the *in vitro* propagation technique. In 1986, the problem of clonal mantled fruits, floral and vegetative abnormalities of clonal palms planted at United Plantations Berhad, Malaysia was brought to the attention of the scientific community during the *Colloquium on Breeding and Selection for Clonal Oil Palm* in 1985 organized by the International Society for Oil Palm Breeders (ISOPB). This caused a major furore among oil palm tissue culturists. Later, Corley *et al*. (1986) published the first report on abnormality in clonal oil palm. In the following two decades, as more information and understanding on the tissue culture process and the problems arising from it accumulated, there was renewed interest to proceed with large-scale propagation of oil palm clones to increase productivity.

In Malaysia, there are 12 tissue culture laboratories, including MPOB, producing clonal oil palm ramets. In 2009, oil palm clone producers in Malaysia produced 2.53 million ramets. However, this production of ramets is far below the amount needed by the industry. Clonal production for sale is limited, even to smallholders. As such, the industry is expanding their existing tissue culture facilities, while more new ones are being established.

WORLD PRODUCTION OF OIL PALM PLANTING MATERIALS

World production of oil palm planting materials was reported by Kushairi and Rajanaidu (2009). Some of the updated production figures are reported here.

A) *Dura* x *Pisifera* (DxP)

Oil palm *dura* x *pisifera* (DxP) hybrid seeds are largely based on Deli *dura* selections at various research centres such as Malaysian Palm Oil Board (MPOB), Chemara, Banting, Dami, Socfindo and Dabou. The main sources of *pisiferas* are AVROS, NIFOR (Calabar), Ekona, Yangambi and La Me.

Indonesia

In 1995, there were only three major oil palm seed producers in Indonesia that produced 61 million seeds. Then, the Indonesian Oil Palm Research Institute (IOPRI) was the dominant seed producer at 50 million seeds. However, the situation has changed in 2008 (*Table 1*). The number of seed producers in Indonesia has increased from three to nine and the quantity of seed production increased substantially from 61 million in 1995 to 125 million in 2008 and 250 million in 2009. The price of DxP seeds in Indonesia as in *Table 2*.

Table 1: Oil palm DxP seed production in Indonesia, 2009

Company	**Million seeds/year**
Marihat, IOPRI	50
PT Socfindo	45
PT London Sumantera Ind. Tbk.	20
PT Dami Mas Sejahtera	24
PT Bina Sawit Makmur	24
PT Tunggal Yunus Estate	20
PT Tania Selatan	6
PT Bakti Tani Nusantara	6
PT Bakrie Sumantera Plant. Tbk.	20
PT Sarana Inti Pratama	18
PT Sasaran Ehsan Mekarsari	17
Total	**250**

Table 2: Oil palm DxP seed prices in Indonesia, 2008/2009

Company	**Price (USD/seed)**
Marihat, IOPRI	0.60/0.70
PT Socfindo	0.95
PT London Sumantera Ind. Tbk.	1.20
PT Dami Mas Sejahtera	0.90
PT Bina Sawit Makmur	0.90
PT Tunggal Yunus Estate	0.80
PT Tania Selatan	0.90
PT Bakti Tani Nusantara	0.65

Malaysia

Oil palm DxP seed production in Malaysia increased marginally from 50 million in 1995 to 65 million in 2007 and 88 million in 2008. The number of seed producers in Malaysia remained constant over the years (*Table 3*).

Table 3: Average oil palm DxP seed production capacity in Malaysia, 1995-2008

Company	**Million seeds/year**
Federal Land Development Authority (Felda)	17
Sime Darby (including Guthrie, Golden Hope)	30
United Plantations Berhad	10
Industrial Oxygen Incorporated (IOI)	6
Highlands Research Unit (HRU)	8
Borneo Samudera	5
Sasaran Ehsan Utama (SEU)	2
Rubber Industry Smallholders Development Authority (RISDA)	1
IJMP, Sabah	1
SPAD, Sarawak	1
Malaysian Palm Oil Board (MPOB)	0.5
Total	**81.5**

Other Asian Countries

Other major oil palm DxP seed producing countries in Asia are Papua New Guinea, Thailand and India (*Table 4*). Thailand has become a significant seed producer in the past five years.

Table 4: Other major oil palm seed producing countries in Asia

Country	**Million seeds/year**
Papua New Guinea	30
Thailand – Univanich	8
Thailand – Department of Agriculture	5
India	2
Total	**45**

Central-South America

Costa Rica is a major oil palm seed producer, producing 30 million DxP seeds annually. CIRAD, a French based organization, has developed a network in Central-South America in seed production. It is expected that a significant amount of CIRAD seed production in Latin America (*Table 5*).

Table 5: Oil palm DxP seed production in Central-South America

Company	**Million seeds/year**
ASD Costa Rica	30
Gene Palm Honduras	2
Murgas & Lowe, Colombia	2
La Cabána (CIRAD resale)	1.5
INIAP, Ecuador	2
Embrapa, Brazil	1
Total	**38.5**

Africa

The major oil palm seed producers in Africa are CIRAD and its partners in Ivory Coast (CNRA), Cameroon (IRAD, SOCFINCO) and INRAB in Benin, OPRI in Ghana, NIFOR in Nigeria and Unipalma in the Democratic Republic of Congo and Ghana. An estimated 25 million oil palm seeds per annum are produced in Africa (*Table 6*).

Table 6: Oil palm DxP seed production in Africa

Company	**Million seeds/year**
Benin	6
Nigeria	2
Cameroon, La Dibamba	1
Cameroon, Pamol	1
Ghana	2
Democratic Republic of Congo, Unipalma	3
Ivory Coast, CNRA	10
Total	**25**

B) Oil Palm Clones

Some three million oil palm tissue culture plantlets are produced annually worldwide (*Table 7*). In Malaysia, the current production of clonal oil palm planting materials is 2.5 million ramets annually (*Table 8*) and is expected to grow to five million by 2010 from 11 commercial tissue culture laboratories. This is still a remote number to supply 40 million ramets by 2017. In addition, most tissue culture laboratories cater only to the needs of their own plantations, limiting the quantity available for the industry, including smallholders. Most tissue culture labs, such as Advanced Agriecological Research (AAR) and Felda are equipped with advanced facilities. AAR and Felda are poised to produce about one million ramets per year. Productions by these companies are expected to increase to at least two million each over the next 3-5 years. The mean oil yield of AAR clones is 7.5 t/ha/yr compared to 6.5 t/ha/yr that of its DxP hybrid seeds.

Table 7: Estimated world production of oil palm tissue culture plantlets

Country	**Million ramets/year**
Malaysia	2.5
Costa Rica	0.5
Indonesia	0.5
Total	**3.5**

Table 8: Oil Palm Ramet Production in Malaysia (million)

Agency	2009	2010 (expected)
Clonal Palm	0.13	0.25
FELDA	0.35	1
AAR, Selangor	1	0.6
Sime Darby	0.3	0.75
Kulim-TopPlant	0.01	0.02
SEU	0.003	0.01
UPB	0.06	0.06
IOI	0.3	1
TSH	0.2	0.8
AgroCom	0.1	0.12
Borneo Samudra, Tawau	0.08	0.2
Total Production	2.53	4.81

C) Clonal Seeds

Another alternative to developing high yielding planting materials is through clonal seed production (Veerappan *et al.*, 2000; Soh, 2005). United Plantations Berhad (UPB) in Malaysia is the pioneer company in the world to produce bi-clonal seeds. The estimated annual production by UPB is one million DxP bi-clonal seeds.

In this method of seed production, the *dura* and/or *pisifera* palms are cloned as parents. The clonal parents are crossed to produce seeds the same way as that in conventional DxP seed production. The selection of parents for cloning is largely based on specific combining ability (SCA). Bi-clonal seeds are produced when both the parents are cloned, while semi-clonal seeds are those with either of the parent is a clone. It is preferable to clone the maternal parent (*dura*) and use normal (non-clonal) progeny-tested *pisiferas* to produce the DxP clonal seeds. This will ensure a large number of female *dura* parents are available for use in seed production. It is sufficient to have a small number of *pisifera* parents as the pollen source.

The advantages of semi-clonal and bi-clonal seeds over the conventional DxP seeds include:

- Bi-clonal and semi-clonal seeds will have greater degree of uniformity because the crossings are confined to a limited number of parental combinations;
- Cost of seed production is very much lower than tissue culture plantlets;
- Low risk of clonal abnormality because of limited plantlets production from each parent;

- Require a small tissue culture set up to clone the parents and the number of plantlets production per ortet are limited;
- An oil yield gain of 15% is expected compared to conventional DxP hybrid seeds.

Sharma (2006) reported that oil yields of UPB semi- and bi-clonal DxP seeds ranged from 7.95 to 9.52 t/ha/yr.

D) Interspecific Hybrid Seeds

It is estimated that 2.5 million *E. oleifera* x *E. guineensis* (OG) interspecific hybrid seeds are produced worldwide. The production is localized in South America (*Table 9*). Interspecific hybrids are somewhat tolerant to spear rot, the palms are short and compact with a more liquid oil compared to the DxP intraspecific hybrids. The OG interspecific hybrids based on Taisha (Ecuador) *E. oleifera* is expected to produce oil palm yields close to that of the DxP.

Table 9: World oil palm interspecific hybrids seed production

Country	**Million seeds/year**
Ecuador (CIRAD partner)	1
Colombia (La Cabaña)	0.3
Colombia (Indupalma)	0.2
Brazil (Embrapa)	1
Total	**2.5**

Summary of the world production of oil palm planting materials is given in *Table 10.*

Table 10: World production of oil palm planting materials

Type of planting materials	**Million planting materials/year**
DxP	315
Clones	3.0
Bi-clones/Semi-clones	1.0
Interspecific hybrids	2.5
Grand Total	**321.5**

TISSUE CULTURE PROTOCOLS

Oil palm is a monocotyledonous plant, and unlike most other species, vegetative propagation is made possible only *via* tissue culture. Oil palm tissue culture is unique, undergoing callusing and embryogenesis processes, which had been rigorously attempted in the 1960s through the 1970s. The early successes of plantlet production was seen in the 1970s (Jones, 1974; Rabéchault and Martin, 1976) inspiring various organizations to delve into *in vitro* propagation.

Oil palm tissue culture though successful, but was not without difficulties. The first case of abnormality in clonal oil palm was brought to the attention of participants of a colloquim organised by the International Society for Oil Palm Breeders (ISOPB) in 1985 and later reported by Corley *et al*. (1986). Fruit (mantled) and vegetative abnormalities caused a major

furore among oil palm tissue culturists. Many laboratories reduced production, but maintained enough for field evaluation. After two decades, as more information and understanding on the tissue culture process and the problems arising from it accumulated, there was renewed interest to go ahead with large-scale propagation of oil palm clones (Kushairi *et al*., 2006). The Malaysian oil palm industry, in a survey in 2004 produced ca. two million ramets annually (Kushairi et al., 2006). Most laboratories have established their own improved culture media and protocols for cloning and conducted field trials. Clonal plantlets derived from selected ortets were reportedly more uniformed and in many cases yielded at least 20% more than the seed-derived DxP standards.

Although clonal abnormalities and amenability still persist but at manageable levels, clonal production capacities in existing and emerging labs are accelerating in accordance to the escalating demand for improved planting materials. Land expansion for new plantings has nearly come to a standstill but the replanting programme continues as 22% of the total area under oil palm is constituted of old plantings, aging 19 years and above (Kushairi *et al*., 2006). At 148 palms/ha planting density, the industry would require an astounding 140 million ramets (seedlings) for replanting, staggered over several years.

Due to the slow process in oil palm *in vitro* propagation, which takes two to five years from explants to nursery seedlings, oil palm clones cannot meet the entire demand for improved planting materials in the near future. While forging ahead with the production of clones, high-performing *dura* and *pisifera* with good combining abilities are cloned to mass produce the sexual parents (especially the *dura*). Using either one (semi-clonal) or both (bi-clonal) parents, DxP "clonal seeds" are produced. Meanwhile, with the large requirements for clonal planting materials, establishment of ortet gardens was proposed to overcome shortages in explant sources (Kushairi *et al.*, 2006). With the enthusiasm for large scale clonal production, a Malaysian Standard in ortet selection was developed (MS 2099:2008) to ensure only high quality ramets are produced from high quality ortets.

Modifications of the culture media and protocols with reduced abnormality have somehow limited large-scale propagation. This is further aggravated by the incidence of low rate of embryogenesis ranging from 3% to 6% (Rajanaidu *et al*., 1997). Of that, about 50% of the embryoids failed to establish (Wooi, 1995). However, most laboratories have now established improved media and protocols for cloning of oil palm and conducted field trials.

Ho *et al.* (2009) of AAR Malaysia reported that a callusing rate of 20% was obtained for Yangambi-AVROS, Dumpy-AVROS and Cameroon materials compared to 16% for AVROS and La Me and 11% for NIFOR (Table 11). The influence of genotypes on rate of callus induction was also reported by Ginting and Fatmawati (1995).

Table 11: Callusing rate of genotypes cultured at AAR, Malaysia

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

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 12*). Only 7% of callogenic explants of reclones could differentiate into embryoids, this is however, more than doubled than that of callogenic explants of ortets. Shoot regeneration from embryogenic lines of both ortets and reclones appear similar at 95% to 96% (Ho *et al*, 2009).

Table 12: Oil palm cloning and recloning efficiencies of AAR "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

Reliable tissue culture procedures and stringent culling at various cloning stages (Maheran *et al*., 1995; Simon *et al*., 1998) and using a wider range of ortets (Tan *et al*., 2003) help reduce the incidence of abnormality. Current abnormality rate is less than 5% (MPOB, 2006b). Since late 1980s, MPOB has evaluated close to 70 clones of which seven are very high yielding, producing more than 7 t/ha/yr oil yield (*Table 13*).

Table 13: Performance of MPOB clones (4 – 7 yrs)

No	Clone	Oil-to-bunch (%)	Oil Yield (t/ha/yr)	Soil type*
1a	P164	30.6	8.71	Inland
1b	P164	33.8	10.81	Coastal
2	P162	29.3	7.80	Inland
3	P135	28.4	7.56	Inland
4	P194	29.1	7.75	Inland
5	P149	30.8	7.25	Inland
6	P200	29.1	7.74	Inland
7	P203	30.8	8.01	Inland

* Inland = fertile soil, Coastal = less fertile soil

ISSUES AND CHALLENGES

Availability of ortets

One of the most important requirements for a successful venture in the production of oil palm clonal materials is the availability of high-quality ortets, arising from breeding and selection programmes. A breeding programme demands large areas for ortets selection. Furthermore, a large-scale propagation of oil palm clones requires a large number of elite ortets, which are currently limiting. Based on an ortet selection in progeny trials, some 2% to 11% of palms are selected as ortets.

Currently, palms selected as ortets are coincidental, i.e. selected from trials with other objectives, such as progeny trials in breeding and selection programmes, and was not initially meant for ortet selection. As such the number of ortets selected would be few compared to total palms planted. Thus, it would be beneficial if 'ortet gardens' were to be developed for ortet selection (Kushairi *et al.*, 2006). This could be carried out by selecting the cream of high yielding *dura* and *pisifera* palms to create high yielding progenies. Unlike DxP seed production, where majority of the parents are selected based on general combining ability (GCA), parents in crosses to create progenies for ortet gardens should be based on specific combining ability (SCA). The same parents are repeatedly used to produce the desired number of DxP (*tenera*) progenies for ortet gardens. The other source for palms to be planted in ortet gardens is the reclones of the proven clonal palms.

Efficiency in tissue culture process

Low embryogenesis rates (3% to 6%) remain the stumbling block to large-scale ramet production. The tissue culture process is costly and labour intensive. The entire process requires very specialized infrastructure to ensure a clean and controlled environment, ample laboratory space to house the cultures and most importantly skilled workers, the lifeline of any tissue culture laboratory. Therefore, any cost and labour reduction strategies are welcomed. Since the new millennium, MPOB has been actively developing innovations that can help improve the efficiency of the tissue culture process. Examples of technologies or methods to simplify parts of the tissue culture process include the "double-layer rooting" technique (Zamzuri, 1998; 2001) and "flameless sterilizer" (Zamzuri, 2002).

In addition to a high cost of production, tissue culture laboratories are faced with shortage of operators with high turnover rates. Further improvement in tissue culture process can be expected from the liquid media system, which opens up automation possibilities. Propagation via liquid media enables the increase in embryogenic cultures by several folds. However, as the conventional solid culture system does not permit the regeneration of cultures/embryoids directly in liquid, the best option is to synergize the use of solid cultures with liquid systems (shake flask and bioreactor). In order to mass produce or for bulking up the production of ramets, the suspension culture system was developed (de Touchet *et al*., 1991; Teixera *et al*., 1995; Wong *et al*., 1999; Tarmizi, 2002). The bioreactor is the preferred alternative if rapid large scale proliferation is needed (Tarmizi *et al*, 2003). In relation to this, the MPOB developed the Fast Transfer Technique (MoFaTT) in liquid culture system (Tarmizi and Zaiton, 2005), the 2-in-1 MoSlim (MPOB Simple Impeller for liquid culture) with later upgraded to SLIM-FaTT (simple impeller with fast transfer technique) (Tarmizi and Zaiton, 2006a; 2006b) and the MPOB Modified Vessel (MoVess) (Tarmizi *et al*. 2007). Besides

regeneration of embryogenic aggregates from liquid culture system, these cultures could also be encapsulated as artificial seeds.

There is a need for proper management of information flow generated from the laboratory to the field as well as integration with information collected from other disciplines e.g. breeding and molecular biology. MPOB has developed a tissue culture database system using a relational database management software for computerized audit trail (Zamzuri, 2001b) and further enhanced with bar-coding for monitoring and recording purposes in OPTRACKS (Tarmizi *et al*., 2003). OPTRACKS has been licensed to three oil palm agencies in Malaysia.

Pollination problem

The high sex-ratio observed in oil palm clones poses pollination problem and fruit set. Some plantations have resorted to assisted pollination to improve fruit set and oil to bunch (O/B) in clonal planting material. It is practical to plant DxP material in every four rows of clonal material to supply the pollen needed by clonal materials.

OTHER RELATED STUDIES ON TISSUE CULTURE

Histological Study of Oil Palm Embryogenesis

Histological study of somatic embryogenesis from oil palm leaf explants was conducted by Schwendiman *et al.* (1988). Histological analysis carried out on somatic embryo stages cultivated in Malaysian laboratories showed similarities compared with Schwendiman *et al.* (1988). This was a follow up from the work reported by Ong (2001). Embryogenic calli growing from primary nodular calli were observed to comprise of proembryos, usually with thickened cell walls (*Figure 1a*) (Ong, 2001; Ong-Abdullah and Ooi, 2006). In the oil palm, it would appear that the formation of proembryos are surrounded by several layers of highly vacuolated cells with shrunken cytoplasm, supporting previous reports on the isolation of these proembryo units as it becomes multicellular (Ong, 2001, Halperin, 1970, Konar *et al*., 1972). On the other hand, the non-embryogenic calli normally do not have these structures. Moreover, the non-embryogenic calli also do not appear to contain meristematic centers that were characteristic of embryogenic calli as well as embryogenic suspension calli (*Figure 1 a,b,c*). Histologically stained sections of embryogenic suspension cultures of various stages showed that the embryogenic calli in the suspensions were at various developmental stages (Ooi, 2003). Some of them looked like the initial embryogenic calli with meristematic centers that was used as the inoculum while others showed defined vasculature formation or procambial strands, indicative of somatic embryogenesis progression. As the suspensions were all maintained in the same type of liquid medium and culturing was initiated at the same time, indicating that the development of calli from different clones progressed at different rates. When the embryogenic calli, either from solid or liquid cultures, were sub-cultured onto the next media with reduced auxin levels allowing embryogenesis progression, the callus turned into opaque white structures as observed by Schwendiman *et al.* (1988). Within the opaque embryoid, a shoot apex could be observed, sometimes accompanied by two first leaves. Vascular strands were more defined in these opaque embryo structures. As the embryoids proceeded to turn green, signifying photosynthetic capabilities, stained sections of the cells showed less meristematic cells and starch accumulation, observed by the highly vacuolated cells stained pinkish with the periodic acid-Schiff reagent.

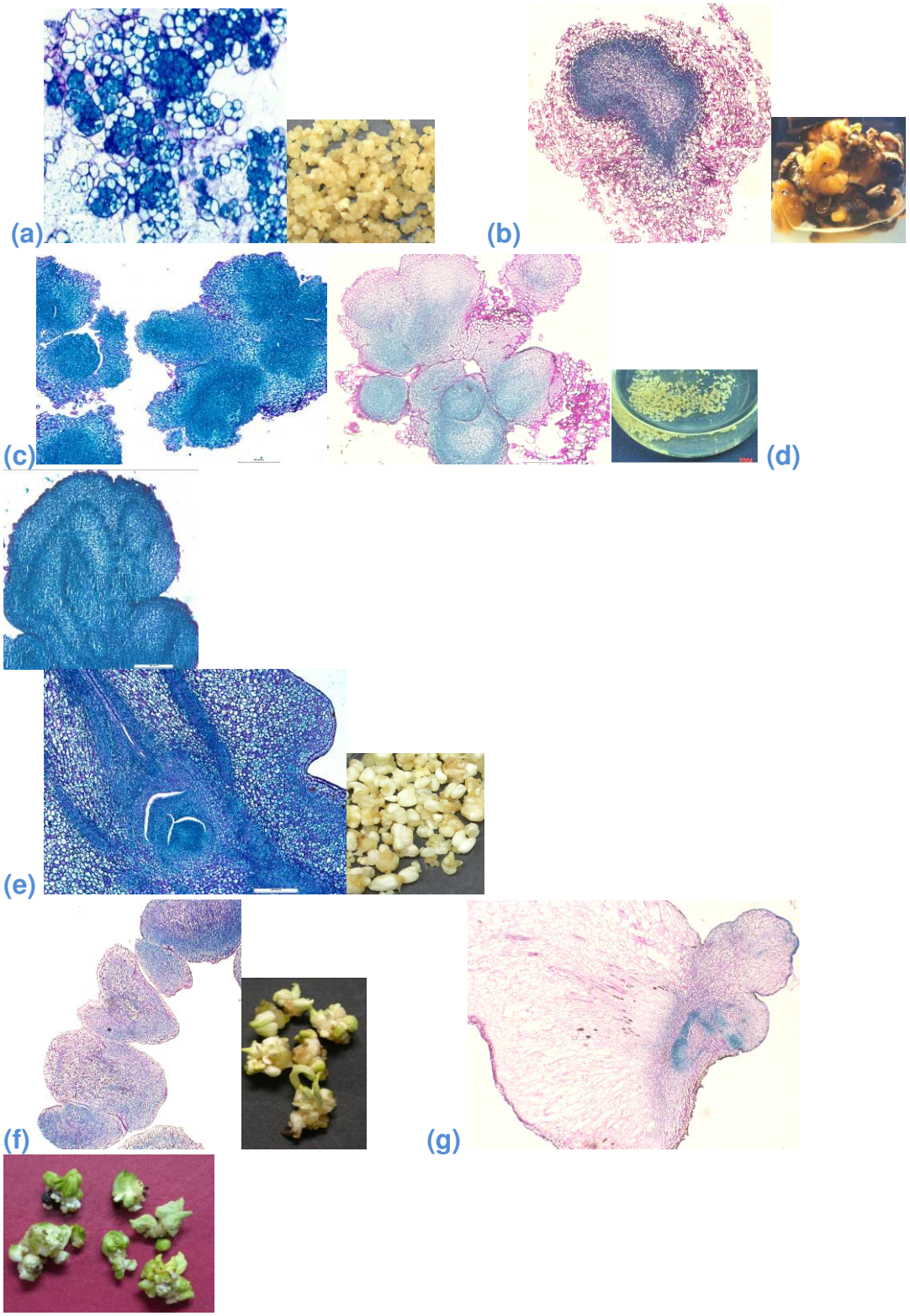

Figure 1. Histological analysis of different somatic embryogenic stages. (a) embryogenic callus; (b) non-embryogenic callus; (c) embryogenic suspension cultures of two clones ; (d) embryogenic suspension cultures cultivated in MS-basal medium ; (e) bipolar embryos; (f) white embryoids; (g) green embryoids.

Molecular markers for quality control

Molecular research for the tissue culture area has been geared towards developing biomarkers to address the issues of tissue culture amenity and abnormality. Part of it entails gene expression studies for both embryogenesis and abnormality. In the medical field, biomarkers were developed to predicting disease susceptibility, progression and drug response; hence the term "personalized medicine" was coined (Hood *et al*., 2004; Jain, 2004; Nevins *et al*., 2003). However, in oil palm, the predictive tool for embryogenesis and abnormality will not be to remedy the condition but primarily for screening purposes, for now.

A rough estimation has indicated that tissue culture laboratories would be operating at a loss of approximately RM80,000 (USD1 = RM3.3) per year just for cloning oil palm materials without any prior screening to gauge the embryogenic potential or the tissue culturability of the material (Ong-Abdullah, 2005). The obvious reason for this is that callogenesis and embryogenesis are random processes and are unable to be externally controlled. Although the figure may seem small, but when it is extrapolated to abnormality in the field, if it happens, the cost would escalate! This is the reason why tissue culture laboratories continue to practice 'basket sampling' [Wallenius (1973) cited by Kelly and Cumberland, 1985] to ensure that demand for elite oil palm planting materials can be met.

Biomarkers that are being developed will eventually lead to production of a diagnostic tool for clonal amenity and conformity. To date, some potential biomarkers (gene expression-based) have been identified and have undergone some validation. Biomarkers for embryogenesis may be used for screening stages as early as the explants. However, MPOB studies indicated that the biomarkers may be specific to different tissue culture laboratories due to differences in genotypes and culturing protocol/media used (*Table 14*). The discovery of biomarkers for the mantling abnormality has been somewhat challenging, postulated to be an epigenetic phenomenon. Research in this area suggested that methylation status during the development of the palm is a dynamic process. Hence, screening of adult palms exhibiting the normal or abnormal fruiting phenotype may not be translatable to the earlier development stage, such as the nursery stage.

However, the cause of clonal abnormality still remains unknown. Based on the phenotype of the mantled flowers, with conversion of the stamen whorl into a carpel whorl, it is not unlikely that changes in the expression of the MADS box genes specifying the affected flower whorls underlie the mantled phenotype. The MADS box directed differential display has proven to be an excellent method of isolating a broad set of members from the MADS box gene family expressed in a variety of tissues (Van der Linden *et al*., 2002; 2005). Through this technique eighteen MADS box genes from the oil palm were isolated and characterized (Syed Alwee *et al*., 2006; Auyong, 2006).

Table 14: Suggested timepoints for explant screening using different candidate markers to predict embryogenesis

	Agencies		
Candidate Markers	A	B	C
EgHOX1	1d	1m/5m	1d/3m
EgPER1	1m	5m	1d
EgSERK	1d	1m	nc
EgPK1	1m	1d	nc
OPKN1	1m	7d	2m
EgNAC1	nc	4m	nc
OPSC10	1d/1m	7d/5m	1d
EgSAPK	1m	7d	nc
EgHAD	1m	nc	1d

Explant cultures – 1d: 1-day explant culture; 7d: 7-day; 1m: 1-month; 2m: 2-month; 3m: 3-month; 4m: 4-month; 5m: 5-month.

nc: no correlation to embryogenesis observed

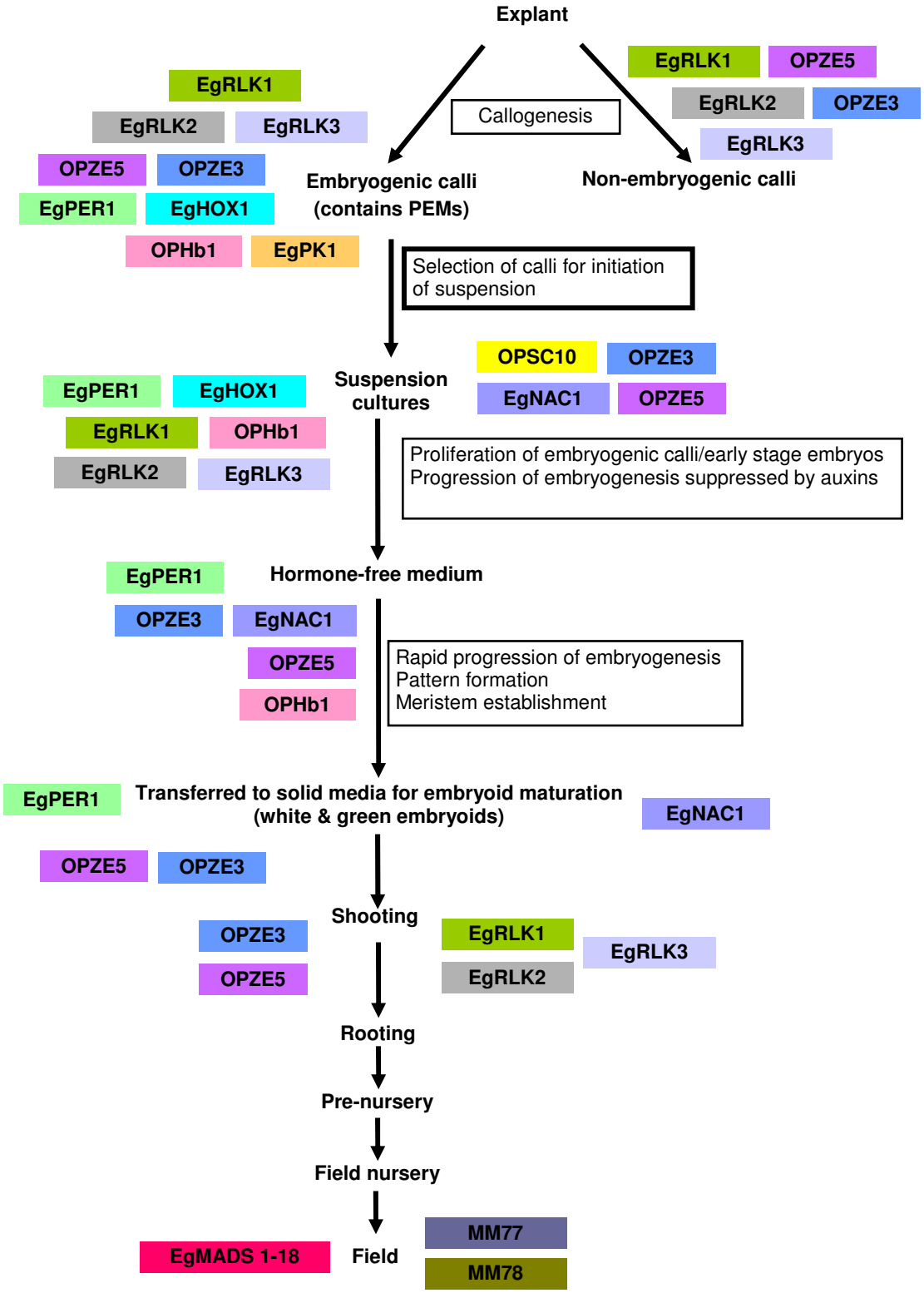

Figure 2. Schematic representation of the expression profile of potential markers for somatic embryogenesis (PEMs, proembryogenic masses) and flowering, including potential markers for clonal conformity.

YIELD PERFORMANCE

Yield performance of clones was reportedly superior to those of commercial DxP seedlings (Khaw and Ng, 1997). The clones yielded at least 25% higher than seedlings (Rohani *et al*., 2000; Tan *et al*., 2003). In Sarawak, Malaysia the cumulative oil yields of 6-7 year-old clones exceeded that of the DxP by 10% to 37% (Simon and Koh, 2005). Similarly, in Sabah, Malaysia for 9-10 year-old palms, the clones maintained an advantage over DxP cumulative oil yield by 28% to 55%. Felda (Felda brochure) and UPB (Mukesh, 2006) reported that the oil yield increment of clones was more than 20%. Generally, the average cumulative oil yield produced in clones gave an advantage over DxP by 44% or 48 t/ha (Simon and Koh, 2005).

MPOB Clonal Performance

Planting clonal palms is one of the best options to improve productivity per unit area. Several laboratories have reported that clonal palms showed an increase in oil yield over the DxP at least by 20%. *Table 15* and *Figure 3* show the performance of MPOB clones planted by a private plantation company in Sabah, Malaysia. The oil to bunch (O/B) ratio of the clonal materials was 28.5% as compared to 21.2% of Standard DxP material.

Table 15: FFB yields (ton/ha/yr) of an estate in Sabah

Age (years)	3	4	5	6	7	8	9	10	11	12
Year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
MPOB clones	20.67	23.30	32.01	27.35	31.06	32.46	30.00	36.26	34.55	32.60
Standard DxP	19.89	24.93	25.84	24.22	26.86	26.17	26.54	30.93	29.23	26.09
Increment (%)	3.92	-6.54	23.88	12.92	15.64	24.04	13.04	17.23	18.20	24.95

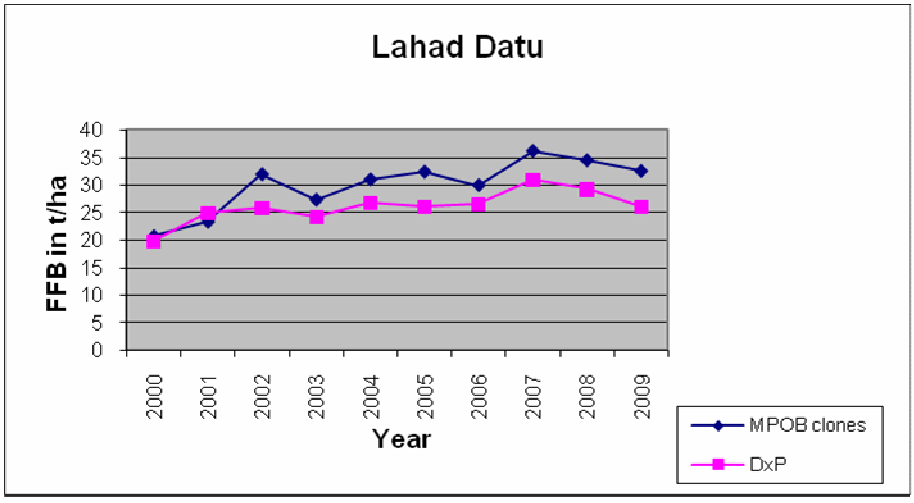

Figure 3. MPOB clones vs DxP

AGRONOMIC INPUTS

An important aspect to elevate oil palm yield is the plant clonal materials only on prime land. Planting materials, such as clones, with high yielding potential should only be planted in fertile soil having good site-yield potential. Potential of the genotype, environment and their interaction can be realized under such situation.

In addition, good agricultural practice (GAP) is a necessity for good harvests. With high yielding potential, the clones might need a tailored fertilizer regime, different from those commonly applied to DxP seedlings. It is hypothesised that additional nutrients are required for clones to express their potentials. It was reported that various genotypes performed differently under varying fertilizer rates (Kushairi *et al*., 1998).

STANDARDS AND LICENSING POLICIES

Malaysian Standards in Ortet Selection

Yield stagnation for nearly three decades, land scarcity for cultivation and record-high price of palm oil mooted the industry to increase efficiency, hence productivity. One of the ways is using clonal planting materials. The Malaysian Standard on "Oil Palm Ortet Selection for Cloning – Specification" was developed to authenticate production of high quality clonal planting materials. Production centres in Malaysia, are by law, licensed by MPOB after accreditation by the Standards and Industrial Research Institute of Malaysia (SIRIM).

The objectives of the Malaysian Standards for ortet selection are:

- To develop a standard for oil palm clonal materials,
- To ensure that only *bona fide* producers of clonal materials are in the market,
- To ensure that only high quality clonal materials are produced in the country,
- To improve productivity of oil palm plantations by using improved and high quality planting materials.

Specification for oil palm ortet selection for cloning is outlined according to Introduction, Scope, Definitions, Requirements for ortet selection, Guidelines for production practices and facilities, Packaging and transportation of plantlets/ramets, Legal requirement and Certification mark.

Specifications for ortet selection as sources of explants for cloning towards commercial planting were grouped into three:

1) Materials of known pedigree and known performance of family and individual palm,
2) Materials of unknown pedigree and known performance from known seed producer[2],
3) Materials from field tested clones for recloning.

The followings are minimum standards required for the selection of an ortet:

1) Materials of known pedigree and known performance of family and individual palm.
2) Materials of unknown pedigree and known performance from known seed producer.

   a) Oil yield (OY), minimum : 50 kg/palm/year
      Oil to bunch (O/B), minimum : 27%
   b) The ortet shall be derived from a family size with a minimum of 30 palms, where
      i). No. of palms per plot, minimum : 10 palms
      ii) No. of replications, minimum : 3
   c) The Oil Yield values shall be derived from at least four consecutive years of FFB yield recording.
   d) The O/B values shall be derived from at least five analyses per ortet.

3) Materials from field tested clones for recloning.
   The specifications are the same as (a – d) above.
   Additional requirements are as follows:
   e) Select only clonal palm/ ramet with no history of fruit or vegetative abnormalities.
   f) Average percentage of abnormality should not be more than 5% based on a minimum of 100 palms evaluated, for that specific clone in a particular year of field planting.

Conclusion

In striving to achieve an overall elevated productivity, one must first look at improvements that tackle core issues such as ortet selection. Members of industry are urged to intensify their breeding programmes for ortet selection. Policies to enable cloning and recloning of elite palms should be implemented to encourage large scale production. The industry can also exploit the *in vitro* technology by cloning the parental palms to produce clonal seeds. This is a source of more affordable quality planting materials. With the reasonably priced planting materials may encourage more participation from all sectors including smallholders leading to a more aggressive replanting programme. The high demand for clonal materials would require labs to expand their production and encourages setting-up of new labs. To ensure successful implementation of the strategic plan towards increasing oil yield, full support from various sectors and the government is needed. Clonal planting material is expected to create the “second wave” in yield improvement. With tissue culture labs imposing higher than minimum selection standards, coupled with Good Agricultural Practices (GAP) in plantations, oil yield is expected to increase substantially from existing national average of 3.5 to 3.9 t/ha/yr, towards achieving the national aspiration of Vision 35:25.

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