Oil Palm Tissue Culture: Fast Tracking Elite Commercial Lines

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Abstract

Enhancing oil palm productivity through land expansion is no longer a viable option, and thus, improving yield per hectare is the utmost priority for the Malaysian Oil Palm Industry. In order to produce improved and high-yielding planting materials, the Malaysian Palm Oil Board (MPOB) has leveraged its rich genetic resource established since 50 years ago through extensive bioprospection as well as augmenting its cloning facilities to fast-track the breeding programmes. Cloning via the tissue culture system is by far the most viable approach to vegetatively propagate elite palms. After more than 33 years of research and development, reliable protocols for both solid and liquid culture systems coupled with various innovative technologies were established. The SureSawit^{TM} Karma biomarker, which is related to clonal abnormality, was developed as a tool for quality assurance of clonal production. To date, clonal trials have been extensively conducted, and the results have been encouraging. Clones such as CPS1, CPS2 and a few others recorded superior performance in comparison with D×P standard crosses. Tissue culture has also enabled selection of clones with special characteristics such as virescens, compactness, high carotene content and other traits for future recloning.

5.1 Introduction

Oil palm (*Elaeis guineensis* Jacq.) planting materials are being sexually produced by crossing *dura* and *pisifera* (D×P), resulting in the *tenera* offspring. While the D×P seed materials have contributed to quantum leaps in yield improvements, there still exists considerable variations for exploitations through vegetative propagation via tissue culture. This method enables true-to-type reproduction of the best genotypes which is very much desired by the plantation community.

Vegetative propagation of oil palm via tissue culture technique took place as early as 1960s through 1970s. The early success of ramets (tissue culture derived planting materials) production was seen in the 1970s (Jones 1974; Rabéchault and Martin 1976). This success inspired Malaysian Palm Oil Board, MPOB (then Palm Oil Research Institute Malaysia, PORIM), and many other oil palm organizations to proceed with large-scale production of ramets. In the mid-1980s, the first report on floral abnormality in clonal oil palm (Corley et al. 1986), known as mantling, had caused a commotion among oil

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Fig. 5.1 Normal and mantled fruit form of the oil palm. Floral abnormality found in tissue culture derived planting materials will lead to the formation of mantled fruits which have significantly reduced oil content, or at worse prevents the fruit formation

palm tissue culturists (Fig. 5.1). Many laboratories reduced their production and produced only enough plantlets for field evaluation.

After a couple of decades, as more data and deeper understanding obtained on the tissue culture process and its associated problems, ramets' producers regained their confidence to bulk-produce clonal materials for field plantings. Despite recording significantly reduced abnormality occurrence, the large-scale production of ramets has been impeded as a repercussion on the modified approach applied to the formulation of tissue culture media and its protocols. The low rate of embryogenesis, ranging from an average of 3–6% (Rajanaidu et al. 1997; Corley and Tinker 2003), had been adding insult to injury. Of that, about 50% of the embryoids failed to establish (Wooi 1995). Against all odds, most laboratories have now established: (i) enhanced tissue culture media and protocols and (ii) field evaluation of their clonal materials. The floral abnormality observed in the clonal trials of the later clonal production gave an abnormality rate of less than 5% compared to the earlier production, whereby the rate exceeded 5% (Tan et al. 2003). In order to keep this rate low, reliable tissue culture procedures and stringent culling at various cloning stages (Maheran et al. 1995; Simon et al. 1998) as well as the use of a wider range of ortets (Tan et al. 2003) need to be practiced. To date, the abnormality rate still remains less than 5% as reported by tissue culture laboratories (Hashim et al. 2018). Performance wise, clonal plantlets derived from selected ortets have been reported with higher yield compared to commercial D×P seedlings (Khaw and Ng 1997). In general, clones yielded approximately 20% more than seedling standards (Rohani et al. 2000; Corley and Tinker 2003; Tan et al. 2003). However, there are still issues and challenges to be addressed as covered in this review.

5.2 Tissue Culture as a Fast Track Breeding Programme

According to a survey conducted by MPOB, yields from areas planted with oil palm in Malaysia have been stagnating, hovering at 18–20 tonnes Fresh Fruit Bunches (FFB) per hectare per year (t/ha/yr) and Oil Extraction Rate (OER) of 19–20% since 1994 with the national average oil yield (OY) of less than 3.7 t/ha/yr (Basiron 2007). Furthermore, in recent years since 2013–2016, the Oil Yield (OY) has further dropped from 3.7 to 3.51 t/ha/yr (MPOB Pocketbook 2017). The stagnating and declining yield is not new as the issue has been brought up in several forums for many years.

To address this issue, the oil palm breeders continue to establish improved planting materials through introgression of existing and germplasm materials (Rajanaidu and Jalani 1999). Using this technique, high-yielding materials have been propagated; however, despite rigorous selection, the problem of segregation in the seed-derived progenies still persists. Another bottleneck of this conventional method of oil palm breeding is the fact that it is tedious and time consuming. In addition, expansion of land as a means of increasing productivity is not feasible and sustainable, and hence, increasing yield and profitability on the existing plantation area is the only viable alternative. According to Corley and

Tinker (2003), the genetic potential of palm oil yield can reach up to 18.5 t/ha/yr, and at the moment, this figure is far from achievable with the national average never recorded above 4 t/ha/yr OY per year. Therefore, to fast-track the progress and to capture the maximum potential of a selected genotype, vegetative propagation through tissue culture is the best option available to the industry.

Tissue culture or micropropagation is a technique used to propagate and maintain plant cells, tissues or organs on a culture medium of known and controllable nutrient composition under sterile conditions. Plant tissue culture is widely used to produce clones of economically important crops, including the oil palm. In oil palm breeding programmes, tissue culture is incorporated to shorten the time taken to develop commercial planting materials. Via cloning, it is expected that multiplication of individuals with desired economic traits can be expedited. The process of oil palm tissue culture is illustrated in Fig. 5.2. Tissue culture offers an extremely valuable strategy which is not only limited for multiplying high-yielding individuals but also to produce elite clones with economically and agronomically desirable traits such as high bunch index, low height increment, more compact palm, disease resistance, among others. However, the main focus in this review is on cloning of high-yielding planting materials.

5.3 Performance of High-Yielding Clonal Palms

Clonal palms have been field tested by MPOB and few other agencies in various plantation areas. As mentioned previously, clones generally show superior performance compared to commercial D×P planting materials. For instance, the cumulative FFB of 6–7-year-old clones planted in Sarawak was reported 10–37% higher compared to the standard D×P (Simon and Koh 2005). Similar trend was observed in a plantation in Sabah, Malaysia, the cumulative FFB for 9–10-year-old clones exceeded those of D×P by 28–55%. Agencies such as FELDA (Roowi 2010) and UPB (Sharma 2006) also reported increased in FFB by 20% or more. In general, clones produced on average 48 t/ha of FFB, which is 44% increase over D×P (Simon and Koh 2005).

5.3.1 MPOB Clonal Performance

Performance of MPOB clones in few field trials from different locations in Malaysia is as follows.

5.3.1.1 Field Trials of MPOB Clones at MPOB Station

Table 5.1 shows seven years data of mean FFB yield per hectare from third to ninth year after planting at MPOB Station in Keratong. In this respect, all clones were superior to the D×P control ranging from 3 to 38%. Clone P90 outperformed the D×P by 38% while both P56 and P143 by 32%. The average FFB increment of the ten clones over D×P was 21%. In comparing individual clones at the ninth year, P75, P90 and P56 produced 34.8, 34.3 and 31.2 t/ha FFB, respectively. Based on the FFB means and the % oil to bunch (O/B) obtained, OY for all clones were higher than the D×P from 7 to 48% with an average of 20% (Zamzuri 2004).

In terms of FFB yield per hectare, both clones and D×P started to increase their yields at the fifth year after planting. The clones exceeded D×P steadily throughout the years except at the seventh year (in 2001) where the D×P showed a dramatic increase in yield to more than 30 t/ha/yr but then dropped to 20 t/ha/yr in the following two years. However, the clones remained stable between 20 and 25 t/ha/yr.

5.3.1.2 Field Trials of MPOB Clones at Private Estates

(a) *JC Chang Plantations in Sabah*

This trial was planted on March 1997 near Lahad Datu in Sabah. Two clones (P149 and P164) were compared with D×P commercial palms. The total number of clonal materials was 720 palms. Only 0.3% palms were found mantled initially but later mostly reverted to normal fruiting. Table 5.2 shows the performance of

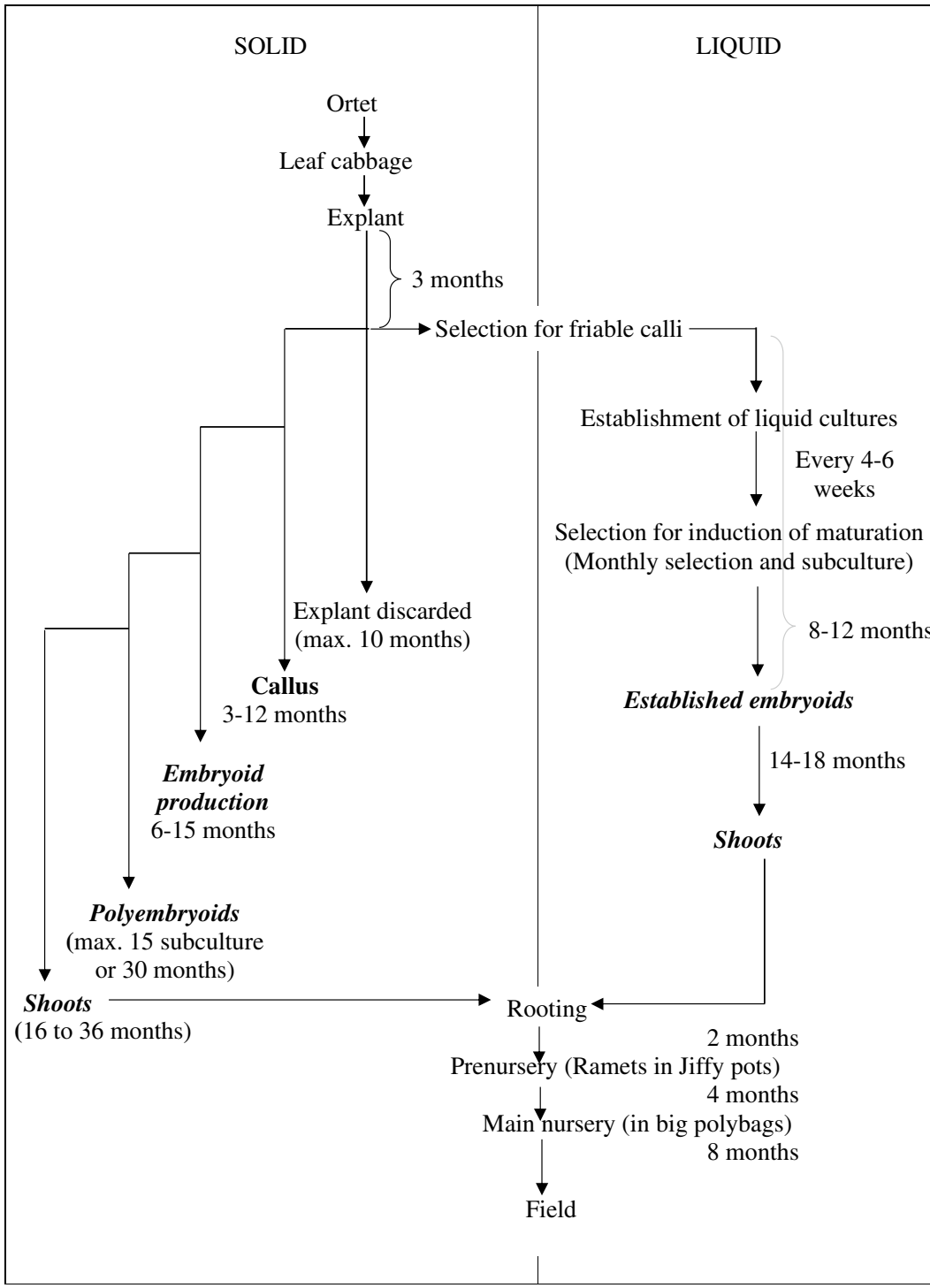

Fig. 5.2 MPOB oil palm tissue culture protocol with approximate time scale. The figure is adapted from Tarmizi et al. (2018)

Table 5.1 FFB, O/B and OY from third to ninth year after field planting

Clone no.	FFB (t/ha/yr)							Mean FFB	Incr[b] (%)	O/B (%)	OY t/ha	Incr[a] (%)
	Years after planting											
	3	4	5	6	7	8	9					
P56	4.88	10.41	11.51	15.16	28.43	25.74	31.19	18.19	32	24.07	4.38	26
P57	7.31	7.08	11.67	14.27	26.37	20.91	23.85	15.92	16	23.84	3.80	9
P75	6.90	8.01	16.67	8.80	14.84	15.16	34.79	15.02	9	25.61	3.85	11
P79	8.40	6.47	17.60	14.06	26.03	20.84	18.71	16.01	17	26.39	4.23	21
P90	8.72	9.49	15.33	16.80	24.29	23.32	34.26	18.89	38	27.28	5.15	48
P123	7.69	6.31	20.15	11.53	20.39	15.13	22.18	14.77	8	27.15	4.01	15
P126	6.55	9.65	22.35	21.22	22.95	24.65	18.35	17.96	31	23.91	4.29	23
P127	9.50	7.42	6.04	19.00	38.99	28.56	14.30	17.69	29	21.04	3.72	7
P135	6.43	9.27	13.26	17.52	21.82	15.63	14.82	14.11	3	26.97	3.80	9
P143	8.29	11.84	22.59	17.06	24.36	20.84	22.06	18.15	32	24.71	4.48	9
Clone mean	7.47	8.60	15.72	15.54	24.85	21.08	23.45	16.67	21	25.10	4.17	18
SC	5.89	5.13	9.16	12.72	32.61	17.44	13.19	13.73	–	25.35	3.48	–
Incr (%)[c]	27	68	72	22	−24	21	78	21				

SC Standard cross D×P
[a]OY increment of clones against SC, [b]FFB increment of clones against SC, [c]FFB increment of multiple clones against SC

Table 5.2 FFB yields (t/ha/yr) of clones against SC from JC Chang Plantations, Sabah, Malaysia

	Age of clones/palms (years)										
	5	6	7	8	9	10	11	12	13	14	15
MPOB Clones	32.01	27.35	31.06	32.46	30.00	36.26	34.55	32.60	32.68	37.03	29.65
SC[a]	25.84	24.22	26.86	26.17	26.54	30.93	29.23	26.09	30.93	30.37	23.93
Incr (%)[b]	23.88	12.92	15.64	24.04	13.04	17.23	18.20	24.95	24.95	21.93	23.90

[a]Standard cross D×P, [b]FFB increment of clones against SC

MPOB clones with an average FFB increment of 20.06%. The oil to bunch (O/B) ratio of the clonal materials in this plantation was 28.5% as compared to 21.2% of standard D×P materials (Details of O/B and OY data were not provided by the estate)

(b) *Serijaya Industri Sdn Bhd in Sabah*

TSH has planted over 900 hectares of MPOB clone P456 and the standard cross D×P at Gunung Rara Estate, Kalabakan, Sabah. In this trial, clone P456 showed an outstanding FFB yield performance over D×P material even at early age of production and at high altitude area of Gunung Rara estate in Kalabakan, Sabah as summarized in Table 5.3. O/B and OY data were not provided by the estate.

(c) *United Plantations Berhad, Bagan Datuk, Perak*

United Plantations Berhad has conducted a trial with D×P standard planting materials obtained from various plantation agencies to be compared with two MPOB clones. A trial plot was planted

Table 5.3 FFB Yield (t/ha/yr) of P456 against D×P standard cross at Gunung Rara Estate, Serijaya Industries Sdn Bhd, Sabah, Malaysia

	Age of clones/palms (years)		
	4	5	6
Clone P456	24.53	29.75	30.21
SC[a]	15.86	20.43	21.05
Incr (%)[b]	54.7	45.6	43.5

[a]Standard cross D×P, [b]FFB increment of clones against SC

Table 5.4 Performance of MPOB clones against agencies' D×P at the fifth year after planting in Bagan Datuk, Perak, Malaysia

Agency	FFB (t/ha/yr)	O/B (%)	OY (t/h/yr)
MPOB P126	24.23	27.09	6.56
MPOB P456	33.03	31.80	10.50
Agency A	27.44	29.41	8.07
Agency B	28.43	27.19	7.73
Agency C	30.86	28.34	8.74
Agency D	29.57	28.54	8.44
Agency E	29.20	24.73	7.22
Agency F	29.11	22.97	6.69
Agency G	26.25	25.47	6.69
Agency H	30.42	26.47	8.05
Agency I	28.29	26.69	7.55
Agency J	29.41	23.70	6.97

with D×P progenies from ten agencies (Agency A-J) and two MPOB clones in 1999. Table 5.4 shows the yield record at fifth year after planting. It is obvious from the table that MPOB clone P456 outperformed D×P of all agencies in almost every parameters measured. The second MPOB clone, P126, although seemed not to be performing well against D×P, has a potential to be used in high-density (HD) planting programme (200 palm/ha) compared to 148 palm/ha (normal-density planting), owing to its short rachis length. If the trial had implemented HD for P126, the hypothetical data would have been like these, FFB 32.7 t/ha/yr and OY 8.86 t/ha/yr.

(d) *FELDA Sungai Tekam, Pahang*

FELDA has conducted a trial with D×P standard planting materials obtained from various plantation agencies to be compared with two MPOB clones. A trial plot was planted with D×P from nine agencies (Agency A-I) and two MPOB clones in 1999. Table 5.5 shows the yield record at fifth year after planting. Clone P456 showed the highest FFB of 34.48 t/ha/yr, while P126 showed the highest O/B of 30.18%. If the trial had implemented HD for P126, the hypothetical FFB and OY would be 35.7 t/ha/yr and 10.8 t/ha/yr, respectively.

Table 5.5 Performance of MPOB clones against agencies' D×P at the fifth year after planting in Sungai Tekam, Pahang, Malaysia

Agency	FFB t/ha/yr	O/B (%)	OY (t/ha/yr)
MPOB P126	26.43	30.18	7.28
MPOB P456	34.48	29.05	9.14
Agency A	28.16	29.68	7.62
Agency B	27.27	28.38	7.06
Agency C	26.85	28.42	6.96
Agency D	28.26	27.41	7.06
Agency E	25.66	26.49	6.20
Agency F	24.96	25.70	5.85
Agency G	28.71	25.86	6.77
Agency H	27.81	27.61	7.01
Agency I	25.58	27.19	6.34

5.4 MPOB Superior Clones for High-Yield

5.4.1 Clone P456

P456 is an outstanding MPOB standard clone because of its high OY, very low mantling rate and ease in tissue culturing. Recent planting of 20,000 P456 ramets in Sabah by a private company showed that the mantling rate is about 0.013% (Zamzuri 2011). Figure 5.3 shows mature fruit bunches and cross-section of fruits of clone P456. This clone was officially commercialized as Clonal Palm Series 1 (CPS1) through Malaysian Commercialization Year 2017.

5.4.2 Clone P126

Clone P126 features a distinctive characteristic, which is short rachis length (RL) of approximately 4.5 m as compared to 5–6 m of D×P and other clones. The short RL of this clone makes it suitable for HD planting (198 palms/ha) as compared to 148 palms/ha for normal density. P126 also produces smaller bunches, which is favourable for ease of harvesting. Despite smaller bunches, the expected annual FFB yield for 1 ha of P126 is 32.6 tonnes, which is still far more productive compared to D×P (28 t/ha/yr as stated by Azman and Mohd Noor 2002). In addition, clone P126 showed no incidence of mantling when field tested at various locations

Fig. 5.3 Palm, bunches and cross-section of P456 fruits

Table 5.6 Performance of P126 in high density planting design

Age (years)	Age of clones (years)					
	4	5	6	7	8	9
FFB/palm (kg)	95.72	133.21	167.52	175.86	160.30	164.42
Bunch no.	28.8	29.84	26.92	24.23	22.11	17.81
Bunch weight (kg)	3.36	4.44	6.22	7.25	7.19	9.20

Fig. 5.4 Palms, bunches and cross-section of clone P126 fruits

(Zamzuri 2011). The yield data are presented in Table 5.6. Figure 5.4 shows photos of P126 clone.

5.4.3 Clone P379

A high-yielding palm is usually related to high production of female bunches, i.e. having a high sex ratio of female to male inflorescences. From preliminary observations on some trial plots, clone P379 exhibited the characteristic of having frequent male inflorescences (MI) along with fruit bunches especially during dry spell (Zamzuri 2011). P379 (Fig. 5.5) also has potential to be incorporated with other clones as pollen source palms, substituting the use of D×P palms. The incorporation of D×P palms in clonal plots, planted in certain ratios such as 1:4, is to ensure sufficient pollen supply for pollinating clonal female inflorescences. Despite their usefulness, planters are concerned that this may cause a mix-up in the harvested fruit bunches that will create inconsistency in yield performance. Thus, planting clone P379 which potentially acts as source of pollen for pollination could produce consistent

Fig. 5.5 Palm, bunches and cross-section of P379 fruits

fruit bunches. The performance of P379 in a clonal trial at Tawau, Sabah, is as follows: FFB 182.69 kg (2010–2016), O/B of 32.87%, BNO 13.66 and BWT 13.3 kg.

5.4.4 Clone P325

Another potential clone is P325 (Fig. 5.6), which exhibited zero mantling was planted in a trial plot in Johor (Zamzuri 2011). Latest result showed that the mean FFB production was 195.7 kg/p/yr from clones ages between six and nine years with a very high O/B of 37.5%, giving an estimated OY of 10.86 t/ha/yr.

5.5 Issues and Challenges

5.5.1 Availability of High-Quality Ortets

The availability of high-quality ortets which originates from a good and proper breeding programme is prerequisite for a successful cloning process. This programme enables the identification of superior mother palms. A breeding programme also demands large areas of good land for the purpose of producing elite ortets. For a large-scale propagation of oil palm clones, a large number of elite ortets would be required. The Malaysian Standard (MS) on "Oil Palm Ortet Selection for Cloning–Specification" was developed to check production authenticity for desired productivity and sustenance of the oil palm industry, whereby only high-quality ramets, derived from high-quality ortets, are produced. Details of the MS specification are as mentioned by Zamzuri (2011).

However, the number of quality ortets that met the MS criteria is very limited. Based on selection in progeny trials, only ~2–11% of the palms are suitable as ortets (Kushairi et al. 2006). One of the alternatives that could ensure the availability of elite ortets is through recloning of the proven clonal palms as clonal ortets. Besides that, production of clonal seeds can also be considered as an alternative. This could be exploited by creating clonal parents with good combining ability.

5.5.2 Low Somatic Embryogenesis Rate

Somatic embryogenesis is a progressive development of a plant somatic cell dedifferentiated to a totipotent embryonic stem cell possessing the ability to develop to an embryo under optimal conditions. This new embryo can further evolve into a complete plant (Guan et al. 2016). To date, the low rate of embryogenesis of 3–6% (Rajanaidu et al. 1997; Corley and Tinker 2003) remains the stumbling block to large-scale ramet production. One of our efforts at MPOB is to improve embryogenesis rate. Recently, improved rates of 8.2 and 29.3% from several elite *tenera* and clonal *tenera* ortets were reported (Siti Rahmah et al. 2017).

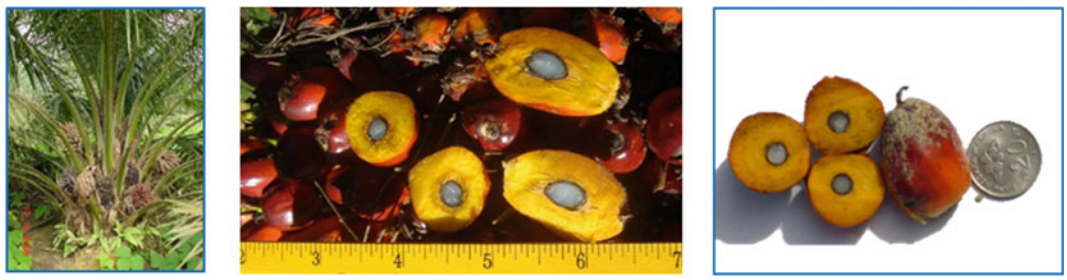

Fig. 5.6 Palm, bunches and cross-section of P325 fruits

5.5.3 Inefficiency in Current Tissue Culture Process

The solid culture system is regarded as the "first generation" technique, which is characterized by high input of manual labour and low level of automation. This process is laborious and a high cost of production especially when scaling up. It was for this reason that led to a concentration of efforts to further improve the tissue culture process by developing plant propagation methods in liquid media. For mass propagation, the liquid culture system is one option to address the high input. At MPOB, the liquid culture system was developed to overcome the proliferation rate of friable embryogenic calli. The system basically manipulates shaking or stirring activity so that the cultures could be exposed efficiently to oxygen and nutrients as compared to the gelled system with only partial exposure.

5.5.3.1 Oil Palm Liquid Culture–MPOB Protocol

MPOB has developed the basic protocol for a liquid culture system (Tarmizi 2002) using shake flasks. It includes selection of suitable callus (friable type), media formulation, sieving of specific aggregate sizes, maturation induction, embryoid regeneration and production of rooted plantlets which involve a dual phase system (solid and liquid media). About 1000 normal-looking shoots could be obtained from one gram of mature aggregates from prolific embryogenic clones after 9–12 months in solid medium. Several innovations were developed to enhance the liquid culture system.

5.5.3.2 MPOB Fast Transfer Technique (MoFaTT) in Liquid Culture

In a shake flask culture system, the operators, in general, have to move the flasks out of the culture rooms to the laminar flow chambers to replenish the nutrient medium for culture growth sustenance. A system (MoFaTT) was developed as a rapid and convenient means for liquid medium replenishment without having to move the cultures to and fro from the culture rooms to the sterile cabinets (Tarmizi and Zaiton 2005). The benefits of the MoFaTT system (Fig. 5.7) are that the conventional ten-step protocol could be reduced to two steps for medium replenishment. In fact, the medium replenishment could be undertaken on the shaker itself at any time. The risk of contamination would thus be reduced.

5.5.3.3 Two in One MPOB Simple Impeller (2-in-1 MoSLIM) in Liquid Culture System

In a conventional process, the establishment, maintenance and maturation of liquid cultures were limited to flask and shaker sizes (Tarmizi 2002). For scaling up of cultures, bioreactors or special commercial flasks were used, but these were expensive (Tarmizi et al. 2003). Furthermore, most systems use different devices for agitation and aeration. To address this issue, the two-

Fig. 5.7 MoFaTT system

Fig. 5.8 MoSLIM system

in-one MoSLIM (Fig. 5.8) was developed as a new innovation process to provide simultaneous aeration and agitation (two in one) for the establishment, maintenance and maturation of liquid the cultures (Tarmizi and Zaiton 2006a). This approach is economical and practical for culture maintenance in liquid culture medium. The two-in-one MoSLIM is able to increase fresh weight of cell aggregates by two–six-fold from five oil palm clones after multiplication for 30–40 days.

5.5.3.4 Simple Impeller with Fast Transfer Technique (SLIM–FaTT) in Liquid Culture System

In the two-in-one MoSLIM, there is still a need to conduct replenishment of media in a laminar flow cabinet. Thus, there is still a risk of contamination. To overcome this problem, the system was further modified so that no movement of cultures is required, and medium replenishment could be carried out at the culture site itself. The new system is a combination of the two earlier innovations (MoFaTT and MoSLIM) and is called SLIM–FaTT (Fig. 5.9). The added benefit of the SLIM–FaTT system is that the conventional eight-step protocol is reduced to one step for media replenishment. The benefits from both of the earlier systems also apply in this new system as well (Tarmizi and Zaiton 2006b).

5.5.3.5 Multiplication of Oil Palm Liquid Cultures in Bioreactors

A bioreactor technology was primarily developed for biomass production. Hence, the culture vessels needed adaptations to the specific requirements of embryogenic suspension. The purpose of using a bioreactor is for large-scale propagation. From the previous studies at MPOB, the fresh weight of the cultures from selected clones increased 4–20-fold after 50–80 days in a B Braun Bench-top two litre Bioreactor and five-fold after about 60 days in a Biotron bioreactor (Fig. 5.10). Figure 5.11 shows the distinctive growth increase of cultures in a bioreactor as compared to the shake flask system.

The present findings on the bioreactor system show the possibility of large-scale production of embryogenic suspension cultures in a single run. Once the bioreactor method has become more efficient, time and space could be saved.

Fig. 5.9 SLIM-FaTT system

Fig. 5.10 Proliferation in a bioreactor

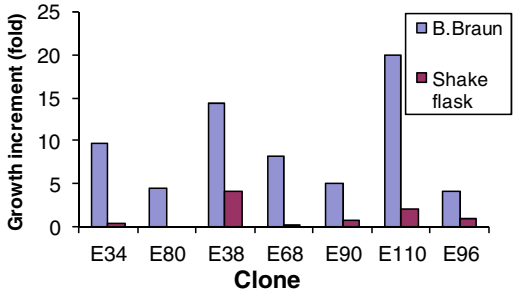

Fig. 5.11 Growth comparison of oil palm cultures maintained in a B.Braun bioreactor or shake flask after 50 days incubation

5.5.3.6 MPOB Modified Vessel (MoVess) for Liquid Tissue Culture System

This innovation is a vessel modified with adaptors for controlling growth conditions such as medium replenishment. This vessel, called MoVess (Fig. 5.12), was designed to overcome the high costs of a bioreactor and at the same time provides a simpler culture inoculation operation (Tarmizi et al. 2007). Fresh weight of cell aggregates increments of up to 35-fold were obtained for cultures of selected oil palm clones after 30–60 days growth in MoVess. This new system is simple and more economical for scaling up oil palm liquid cultures. Again, the medium replenishment can be carried out on site without the need to move the vessel to a sterile cabinet, which also reduces the risk of contamination.

Fig. 5.12 MoVess system

5.5.3.7 MPOB Motorized Vessel (MPOB-Motovess) for Liquid Tissue Culture

Motovess is an improved version of MoVess (Tarmizi et al. 2007). This system omits the use of the magnetic stirrer used in MoVess. The magnetic stirrer limits the culture volume between 1 and 2 L, and omission of the stirrer will allow handling of a larger culture volume of between 2 and 9 L (Fig. 5.13). MotoVess consists of a motor with a stand and a shaft with impeller, made of perforated stainless steel for agitation and aeration of the medium. It was recorded that two–six-fold increment in fresh weight of cultures could be obtained after 40 days using MotoVess.

5.5.3.8 Motorized Vessel with Fast Media Transfer (MoVeFast)

MPOB motorized vessel (5–10 L capacity) was developed to propagate oil palm cultures for increased production (Tarmizi et al. 2009). The device offers economical and practical means of culturing cell aggregates in larger volumes of liquid media compared to traditional shake flasks. The system is not fully automated, whereby the vessel needs to be brought to a laminar flow cabinet for media replenishment in

Fig. 5.13 MotoVess system

a sterile manner. However, handling of the system is inconvenient and impractical owing to bulkiness and weightiness of the vessel. The risk of contamination also increases by having to substitute used media with fresh media. To overcome these problems, a mechanism was developed to integrate the media replenishment process. This new system is called MoVeFast (Fig. 5.14).

In a MoVeFast system, a stainless steel scaffold with a raised platform holding a bottle containing at least five litres of fresh media is connected to a motorized vessel. To ensure efficient media replenishment, the top of the bottles is equipped with top plates attached to vent devices. Clamps attached to tubings are used for controlling the flow of media during media replenishment. On the other end of the motorized vessel, an empty pre-sterilized modified bottle of preferred size, placed on the lower level of the scaffold, is connected for the purpose of discarding spent media. This new system allows media replenishment on site. This system generated a fresh weight increment of about five-fold for cultures of selected oil palm clones after about 40 days in the MoVeFast system.

Fig. 5.14 MoVeFast system

5.5.3.9 MultiVessel (MV) Bioreactor for Liquid Tissue Culture System

Improvements to the oil palm liquid culture system are a continuous process. This led to the development of an innovative technology, namely the MultiVessel (MV) bioreactor (Fig. 5.15), for the simultaneous multiplication of cell aggregates of various clones and/or application of various treatments (Tarmizi et al. 2016). MV provides convenient alternative to the conventional shake flask system. Multiplication of cultures in the MV bioreactor does not require any shaker or a large area. With a working volume of 300–700 ml, this system uses a simple impeller and a pump for agitation and aeration purposes. Basically, the MV bioreactor is an improvement of the MPOB Simple Impeller (two-in-one MoSLIM) system, previously developed using commonly available Schott bottles (Tarmizi and Zaiton 2006a). However, the two-in-one MoSLIM system can only multiply cultures of a single clone or for the application of a single treatment. To overcome this problem, improvements were made to the system to enable more vessels to be connected to single pump(s). Moreover, this system is more cost effective than a commercial lab scale multi-

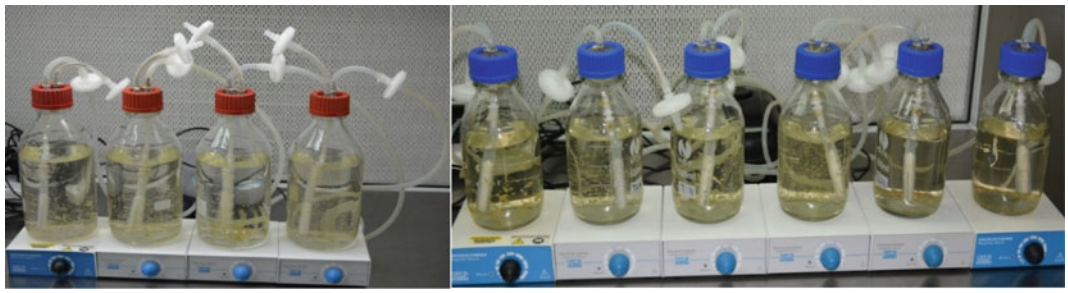

Fig. 5.15 MultiVessel (MV) 4 and 6 systems

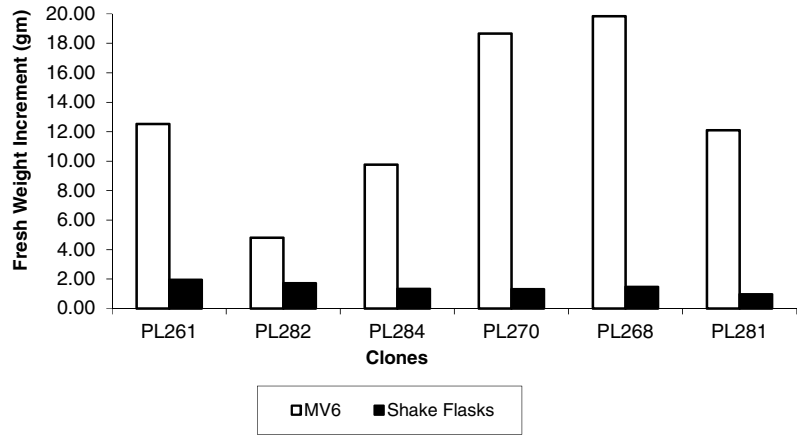

Fig. 5.16 Fresh weight increment of cultures for clones after approximately 30 days of multiplication in MV6, compared to the shake flask system (conventional method)

fermenter, whereby the preparation for culture inoculation is also tedious. This new system can be applied to liquid culture systems of any crop with further potential for automation. Two to 14-fold increments in fresh weight of cultures were obtained after about 30 days (Fig. 5.16) for oil palm clones when multiplied in MV4 (four vessels) and MV6 (six vessels) systems. Normal regeneration of cell aggregates was observed (Fig. 5.17).

The application of liquid media technique has opened up possibilities for automation of the system. Propagation via liquid media has also proven to increase the number of embryogenic cultures by several folds. This is due to the nature of the system in which the cell cultures are fully surrounded by media hence allowing efficient nutrient transfer into cells and removal of metabolic waste out of these cells. The rapid development of suspension cultures showed that a reliable alternative method is available for multiplication of friable embryogenic calli in the oil palm (Soh et al. 2011; Tan et al. 2003; Tarmizi and Zaiton 2005). However, as the conventional culture system does not permit the regeneration of these cultures or embryoids directly in liquid system, the best option is to synergize the use of both, solid and liquid cultures (shake flask and bioreactor).

5.6 Land Scarcity

In a report by Khoo and Chandramohan (2002), the authors indicated that prime agriculture land is currently facing serious shortage. Further expansion of land would risk coming under fire by the environmental non-governmental organizations. Moving to or using marginal areas with poorer soils, terrain and rainfall as an alternative would inevitably lead to escalating problems. Planting in these areas would cost more to

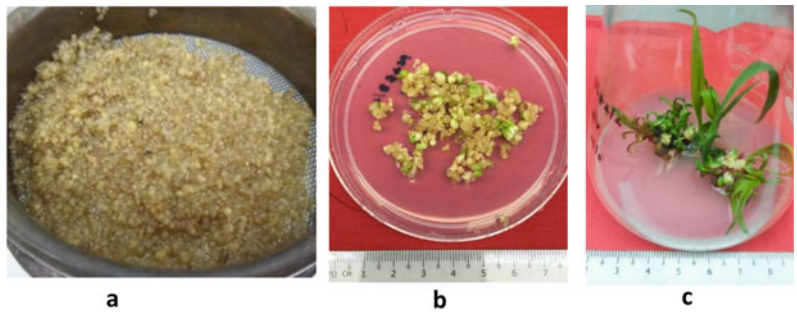

Fig. 5.17 **a** Cell aggregates of clone PL261 from the MV6 system, **b** regeneration of cell aggregates and **c** development into embryoids and shoots

develop, is less productive which would also lead to higher production costs. In addition to that, when production figures are tabulated, it would eventually affect the overall national oil yield!

Taking all these into consideration, it is obvious that to achieve the increase in production output by expanding land areas devoted to oil palm is not an option. Therefore, that leaves the fact that yield production will have to be increased substantially within the same amount of land planted with the crop. This is where quality planting materials such as high-yielding clones play a pivotal role in improving the national productivity.

5.7 Supply and Demand of Clones

The current production of clonal oil palm is still low when compared to the demand for this material. The total production of ramets by major tissue culture laboratories in the country is approximately 5 million (MPOB, unpublished data). To improve this situation, each company that is involved in clonal production should be encouraged to increase its production capacity to more than half million ramets annually.

For this purpose, MPOB strongly encourages big and medium-size plantations to set up their own tissue culture laboratories. To help them in this endeavour, MPOB is providing consultations and training. Up till now, MPOB had provided consultations and training to seven commercial tissue culture laboratories. However, it should be noted that 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, selected from a comprehensive breeding programme. Such programme is essential for the identification of superior mother palms. Thus, tissue culture method is not a standalone field. The current listing of tissue culture laboratories worldwide is as follows:

Malaysia:

1. FELDA Agricultural Services Sdn Bhd
2. Applied Agricultural Resources Sdn Bhd (AAR)
3. IOI Corporation Bhd
4. Sime Darby Plantation Sdn Bhd
5. United Plantations Bhd
6. Clonal Palms Sdn Bhd
7. Agrocom Enterprise Sdn Bhd
8. TSH Biotech Sdn Bhd.
9. Sawit Kinabalu Bio-Tech Sdn Bhd
10. Sabah Softwoods Berhad
11. KULIM-Top Plant Sdn Bhd
12. RISDA
13. FELCRA Bhd
14. Tradewinds Plantation Bhd
15. MPOB.

Indonesia

1. Indonesian Oil Palm Research Institute (IOPRI)
2. PT. Socfin Indonesia
3. SUMBIO (PT. London Sumatera/IndoAgri)
4. PT. Bina Sawit Makmur (Sampoerna Agro)

5. PT. Tunggal Yunus Estate (Asian Agri)
6. PT. Dami Mas Sejahtera (Sinar Mas)
7. PT. Bakti Tani Nusantara
8. Sarana Inti Pratama (IndoAgri)
9. Bakrie-ASD (Bakrie)
10. Sasaran Ehsan Mekarsari
11. *Dura* Inti Lestari (Darmex Agro).

Costa Rica
1. Agricultural Services & Development (ASD).

Papua New Guinea
1. Dami Oil Palm Research Station Biotechnology Laboratory, New Britain Palm Oil Ltd.

Thailand
1. Univanich Palm Oil Public Company Ltd.

Higher production of clonal palms from both existing and new laboratories will definitely reduce the cost per ramet. The current price for a bare-rooted ramet ranges from USD 5–10 per ramet (MPOB 2006). At a reduced price, more growers would plant tissue culture materials in their plantations. It is envisaged that this will increase Malaysia's crude palm oil (CPO) production annually.

Currently, the estimated land planted with clonal materials in Malaysia is about 200,000 hectares (MPOB, unpublished data). Based on the age profile of the Malaysian oil palm, approximately 6.4% (365,581 ha) of the total oil palm planted area is represented by old palms, which are more than 25-year old in 2016 (Shahari and Ismail 2019). Based on this figure, the national requirement of new planting materials is approximately 54.1 million palms. With the current production capacity of 5 million ramets per year, oil palm companies and smallholders can incorporate 10% of clonal materials into their replanting programmes.

Independent smallholders contribute about 16.3% (934,000 ha) of the total hectarage planted with oil palm. To realize the national target of high-quality planting materials, MPOB will initiate a programme called "Contract farming", specifically targeted for independent smallholders. In this concept, an identified company would purchase FFB from the smallholders at a premium price, based on higher expected OER of clones compared to the D×P. The clones will be planted in areas near the palm oil mills. The company, jointly with MPOB, will also provide advisory services on good agricultural practices (GAP) to smallholders.

5.8 Standard for Licensing Policies

Commercialization of clonal materials requires specific standards and license in order to protect buyers from counterfeit materials. Companies are required to meet specific criteria prior to license issuance. In Malaysia, licenses are issued for three activities, namely producing plants from oil palm tissues, selling and moving plants from oil palm tissues and storing plants from oil palm tissues. The fee for each activity is RM100.00 per year.

5.9 Recent Advances in Oil Palm Tissue Culture

5.9.1 Molecular Markers for Quality Control

Research groups working on the oil palm have reported numerous gene expression studies for both embryogenesis and abnormalities arising from tissue culture (Low et al. 2008; Ho et al. 2007; Adam et al. 2007a; Lin et al. 2009; Roowi et al. 2010; Beule et al. 2011; Habib et al. 2014). These studies mainly strive to discover possible biomarkers to address the issues of tissue culture amenity and abnormalities. In the medical field, biomarkers were developed for the purpose of 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 the case of the oil palm, the availability of a predictive tool for embryogenesis and abnormality would

be prioritized for screening and perhaps leading to improvements in the process to prevent the undesirable conditions.

Two main issues affecting oil palm clonal propagation are the poor embryogenesis rate and the risk of obtaining the mantled fruits. The latter is discussed in the next section. The average embryogenesis rate has not improved from the previously reported 6% (Wooi 1995). As oil palm tissue culture goes through somatic embryogenesis, callusing, embryogenesis and shoot regeneration are the main factors crucial to the success of clonal propagation. Callusing is considered not an issue for palms with rates ranging from 11 to 20% (Soh et al. 2011; Ho et al. 2009). The formation of embryogenic callus which generally leads to somatic embryo formation is a sporadic and unpredictable process. As phytohormones, primarily auxin, are used in early tissue culture, studies to investigate targeted molecular pathways involving auxin have been reported. Several gene families including the Aux/IAA gene family are rapidly induced by auxin (Abel and Theologis 1996; Hagen and Guilfoyle 2002; Nemhauser et al. 2006; Paponov et al. 2008). EgIAA9, a putative Aux/IAA gene, was moderately associated with somatic embryogenesis potential (Ooi et al. 2012). Several genes including EgHOX1, Eg707 and EgPK1 have been reported to be highly expressed in embryogenic callus compared to non-embryogenic callus (Ooi et al. 2008, 2016; Thuc et al. 2011). Ectopic expression of EgAP2-1 could enhance regeneration capacity in transgenic Arabidopsis (Morcillo et al. 2007). Other molecular approaches have also been taken to identify molecular markers for embryogenesis such as genomics (Ting et al. 2013; Tranbarger et al. 2012), epigenetics (Ho et al. 2013) and proteomics (Tan et al. 2016; De Carvalho et al. 2014).

5.9.2 Discovery of an Epigenetic Marker for the Mantled Somaclonal Variant

In the 80s, a somaclonal variant arising from oil palm clonal propagation was reported which resulted in fruit abortion and yield losses (Corley et al. 1986). This phenotype, called mantled, involves feminization of the male staminodes in female inflorescences and occasionally the stamens in the male inflorescences as well. Due to the range of severity and reversible occurrences of mildly mantled phenotypes, an epigenetics origin had been widely hypothesized (Jaligot et al. 2000). Numerous studies had been conducted to investigate epigenetic changes particularly DNA methylation, in relations to mantling (Jaligot et al. 2000; Tregear et al. 2002; Jaligot et al. 2004; Lei et al. 2006; Jaligot et al. 2014; Ong-Abdullah et al. 2015).

Floral organ identity is known to be regulated mainly by the MADS-box family of genes (Coen and Meyerowitz 1991). As the mantling abnormality involves changes in floral organs, expression studies on the oil palm B-type MADS-box genes showed that EgDEF1 and EgGLO2 genes were downregulated in developing mantled inflorescences (Adam et al. 2007a, b). Further investigations revealed no DNA methylation differences between normal and mantled clones in two retrotransposons located within the introns of EgDEF1 (Jaligot et al. 2014), but demethylation of the Karma retroelement, also located within an intron of EgDEF1, was found to be involved in defining the mantled phenotype (Ong-Abdullah et al. 2015). Subsequently, a KARMA screening assay was developed commercially that can be used to screen nursery plantlets (Ong-Abdullah et al. 2016). This would allow assessment of the mantling risk and early culling of plants to circumvent economic and time losses involved in the event of planting mantled palms.

5.10 Cloning of Oil Palms with Special Traits

Although establishing high-yielding planting materials is of priority, palms with other important economic traits have also been identified. Selected oil palm possessing agronomic and secondary traits (as below) has also been cloned:

i. High bunch index
ii. Low height
iii. High vitamin E
iv. High carotene
v. Virescens.

5.10.1 High Bunch Index

The bunch index (BI) of the present planting materials is about 0.4. The benchmark to be achieved is a bunch index of >0.6. A Tanzanian virescens *dura* (with a BI of 0.68) and a *tenera* (with BI = 0.58) were cloned. About 50 ramets of both clones are already planted in the field.

5.10.2 Low Height Increment (Compact Palm)

The height increment of the present planting materials ranged from 45 to 75 cm/year. The benchmark to be achieved is palm with a height increment of <30 cm/year. More than low height increment, ortets were cloned, and the cultures are in various forms of growth, from callus to nursery stages. The first batch of 21 ramets cloned from selected low height increment palms was field planting for further evaluation.

5.10.3 High Vitamin E

The current vitamin E content of the present planting materials is about 600 ppm. The benchmark to be achieved is vitamin E content ranging from 1000 to 1500 ppm (Mohd Din et al. 2005). A *dura* palm (with 1551 ppm of vitamin E) and a *tenera* (containing 1392 ppm vitamin E) were cloned. A limited number of ramets are already planted for further observation.

5.10.4 High Carotene

The current carotene content of *E. guineensis* is 500 ppm, whereas for *E. oleifera*, it is 1500 ppm. The benchmark to be achieved for the fast track programme is 2000 ppm for *E. guineensis* and 3000 ppm for *E. oleifera*. The oil from *E. oleifera* is advocated as potentially useful for the neutraceutical industry (Choo and Yusof 1996). Out of 22 *E. oleifera* ortets sampled for cloning using various types of culture media, only one palm with 3146 ppm carotene content was successfully cloned (Zamzuri and Siti Rahmah 2007). A total of 53 ramets were produced and planted in Hulu Paka, Terengganu, Malaysia.

5.10.5 Virescens (Clone PS1, P505)

The origin of this clone is from cross-breeding of Nigerian *dura* and AVROS *pisifera*. This clone produced *virescens* fruit. *Virescens* fruits are green when unripe and change to orange when the bunch matures (Fig. 5.18) (Singh et al. 2014). Since *virescens* fruits undergo a more profound colour change upon ripening, it is easier to identify ripe bunches, particularly in tall palms where they can be obscured by fronds, thus minimizing yield loss due to fallen fruits or harvesting of unripe bunches. In terms of yield, the ortet produces an average of 28.9 t/ha/yr of FFB, 30.14% O/B and OY of 8.71 t/ha/yr.

Fig. 5.18 Virescens palms are easily monitored, immature bunches are green, and matured bunches are orange in colors

5.11 Conclusion

Oil palm improvement via conventional breeding is a slow process. Cloning is an alternative method to propagate true-to-type genotypes with desired traits. Cloning also provides means to exploit exotic genetic materials such as palms with high carotene and high-unsaturated oil contents. Planting clones can be the way forward to increase the oil palm productivity, and thus, the industry should actively include clonal materials for replanting programme. Initially, in vitro production of oil palm clones on a large-scale was hampered by the abnormality problem. However, the in vitro cloning process of oil palm has tremendously improved since its early days, and now, the chances of planting mantled palms can be minimized with the discovery of KARMA gene. Based on this discovery, a diagnostic assay, *SureSawit*™ KARMA, has been developed for early screening of clonal plantlets and prediction of mantled fruit.

Field results of clonal materials have been very encouraging. The superior performance of clones is due to the inheritance of good traits. Although environmental factors such as the soil type can affect yield, the performance of clones thus far was better than the standard D×P. With reliable ortet selection, it is also possible to forecast the clonal potential based on the heritable traits applied in ortet selection. As clonal materials are generated from elite high-yielding palms, these materials would help to boost the overall oil yield and create the "second wave" in yield improvement.

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