MPOB Clonal Propagation Programme[1]

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Abstract

Besides oil yield as the main cloning target in increasing productivity per unit land area, nine other traits of the oil palm are being pursued to expedite the breeding programme in MPOB. This could shorten the time taken to develop commercial planting materials with desired traits. Production of high value added products might be strategic for sustaining the oil palm industry. A standard of specifications designated for ortet selection, MS 2099:2008, should be followed by all the oil palm tissue culture laboratories in Malaysia. In doing so, ramets produced for commercial planting are certified to be derived from high yielding ortets. Collaboration with private agencies in planting of clones is necessary for testing on wider locations, different agronomic and cultural practices. So far, eight out of 70 clones contributed mainly to the mantling problem at MPOB clonal field trials. Planting more clones per plot and the use of more reclones in commercial plantings could reduce the mantling rate. For recloning, it is important to select normal ramets from 'non-sensitive' clones with no history of mantling. A few outstanding MPOB clones highlighted in this paper are P456, P126, P379 and P325.

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1. INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) planting materials had, until recently, been produced by sexual means through *dura* x *pisifera* (DxP), resulting in the *tenera* offspring. While the DxP seed materials had contributed to quantum leaps in yield improvements, there still exist considerable variations for exploitations through vegetative propagation via tissue culture (TC). This method enables true-to-type reproduction of the best genotypes which is very much desired by the plantation community. Cloning of the oil palm – a monocotyledonous plant – via tissue culture is unique and though successful, it is not without its difficulties. Clonal amenability and abnormalities are among such problems.

Tissue culture techniques had been rigorously attempted in the 1960s through 1970s for vegetative propagation of the oil palm. The early success of plantlet production was seen in the 1970s (Jones, 1974; Rabéchault and Martin, 1976). This success inspired the Malaysian Palm Oil Board, MPOB (then PORIM) and many other oil palm organizations to exploit the *in vitro* propagation technique. In the mid 1980s, the first report on abnormality in clonal oil palm (Corley *et al*., 1986) caused a major furore among oil palm tissue culturists. Many laboratories reduced their production and produced only enough plantlets for field evaluation. After two decades, as more information and understanding on the tissue culture process and the problems arising from it has accumulated, there is renewed interest to go ahead with the large-scale propagation of oil palm clones.

The floral abnormality rate observed in the trials of the later clonal production is less than 5% compared to the earlier production, whereby rates exceeded 5% (Tan *et al*., 2003). Reliable tissue culture procedures and stringent culling at various cloning stages (Maheran *et al*., 1995; Simon *et al*., 1998) and the use of a wider range of ortets (Tan *et al*., 2003), can help to reduce the incidence of abnormality in the field effectively. Currently, the abnormality rate remains less than 5% as reported by tissue culture laboratories from a recent survey conducted by MPOB. In terms of clonal performance, Khaw and Ng (1997) reported that clonal plantlets derived from selected ortets were significantly superior to the commercial DxP seedlings. The clones yielded at least 25% more than seedling standards (Rohani *et al*., 2000; Tan *et al*., 2003, Zamzuri *et al.*, 2005).

2. Current Status

The approach of enhancing competitiveness by the expansion of land for oil palm to raise production by economies of scale is no longer a viable option. Thus, the alternative is to increase productivity per unit land area, *e.g*. by increasing yield and profitability per hectare on the same land. Attaining superior oil yield per unit area of the oil palm is the most important avenue for the future competitiveness and sustainability of the oil palm (Simon, 2005). Most of the high yielding materials are currently propagated through cross-pollination of selected seed materials with improved genetic potential. Despite rigorous selection, the problem of segregation in the seed derived progenies still persists. While the industry is currently achieving a national average oil yield of about 4 tonnes per hectare per year, the genetic potential of the palm has been reported to be 18.5 tonnes of oil per hectare per year (Corley, 1998). Therefore, to capture the maximum potential of a selected genotype, oil palm has to be propagated vegetatively. Cloning of the oil palm seems to be the best approach to achieve high yields.

The current production of clonal oil palm is still low compared to the demand for this material. In 2011, the total production of ramets by 13 tissue culture labs in the country was approximately 4.2 million. To improve this situation, each existing lab should be encouraged to increase its production capacity to more than one million ramets annually. A comparative study showed that higher production levels substantially reduced the cost of production (Zamzuri *et al*., 1998). MPOB strongly encourages the big and medium-size plantations to set up their own tissue culture labs. To help them in doing so, MPOB provides consultations and training. Up till now, with the support of these consultations and training, four agencies have established their own oil palm tissue culture labs. However, it should be noted that one of the most important requirements for a successful venture into the production of oil palm clonal materials is the availability of high quality ortets, thus a good and proper breeding programme is necessary. A proper breeding programme is essential for the identification of superior mother palms/ ortets, and thus tissue culture is not a standalone field.

The current listing of laboratories in Malaysia is as follows:

- Sime Darby
- IOI Corporation

- Applied Agriecological Resources (AAR)
- FELDA Agricultural Services
- United Plantations
- Agrocom Enterprise
- Clonal Palms
- Borneo Samudera
- Sasaran Ehsan Utama (SEU)
- TSH
- EPA Kulim -Top Plant
- RISDA
- MPOB

The current price for a bare-rooted ramet quoted by commercial laboratories ranges from RM25 to RM40. Higher production of clonal palms from both existing and new labs will definitely reduce the cost per ramet and this will encourage more growers to plant these new planting materials in their plantations. It is envisaged that this will increase Malaysia’s CPO annual production.

3. Tissue Culture on Fast Track Breeding Programme

Conventional oil palm breeding programmes takes many years to complete, especially in generating new or improved planting materials. This is in view of the perennial nature of the crop. It is therefore, appropriate that another strategy be adopted to get results on a fast track basis especially when individual palms with traits of economic interest have been identified. Tissue culture or cloning is one strategy that can be incorporated into oil palm breeding programmes. Via cloning, it is expected that multiplication of these interesting individuals can be expedited. It is an extremely valuable strategy for multiplying oil palm elite lines. This technique may shorten the time taken to develop a commercial planting material.

Ten (agronomic and secondary) traits of the oil palm that are being pursued based on a brainstorming session held between MPOB and members of the industry are as follows;

- High oil yield
- Ganoderma tolerance
- High bunch index
- Low height
- Long stalk
- Low lipase

- High oleic
- Large kernel
- High vitamin E
- High carotene

<u>Cloning Achievements;</u>

1) Oil Yield trait:

   High oil yield (OY) is undoubtedly the main target for cloning by most laboratories because of its commercial value which is much desired by the plantation community. MPOB's high oil yielding clones will be highlighted in the later part of this paper.

2) 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 was 2000 ppm for *E.guineensis* and 3000 ppm for *E.oleifera.* The oil from *E. oleifera* was advocated as potentially useful for the neutraceutical industry (Choo and Yusof, 1996; Mohd. Din *et al*., 2002).

   Out of 22 *Elaeis oleifera* ortets sampled using various types of culture media, only one palm with 3146 ppm carotene content was successfully cloned (Zamzuri & Rahmah, 2007), and designated as P500. Only 53 ramets of P500 were produced and planted in MPOB Hulu Paka in 2010.

3) High vitamin E:

   The current vitamin E content of the present planting materials is about 600 ppm. The benchmark to be achieved was vitamin E ranging from 1000 to 1500 ppm (Mohd Din, 2005).

   A dura palm (designated as P534D with 1551 ppm vitamin E) and a tenera (P532 containing 1392 ppm) were cloned. A limited number of ramets are currently at the rooting and nursery stages respectively.

4) High bunch index:

The bunch index (BI) of present planting materials is about 0.4. The benchmark to be achieved was a bunch index of > 0.6.

A Tanzanian virescent dura (assigned as P531D with a BI of 0.68) and a tenera (assigned as P530 with BI = 0.58) were cloned. About 50 ramets of both clones are presently growing at the nursery stage**.**

5) Low height increment:

The height increment of present planting materials ranged from 45-75cm/year. The benchmark to be achieved was palm with a height increment of < 30cm/year.

More than 20 PS1 ortets from MPOB/UKM station were cloned and the cultures are in various forms of growth, from callus to nursery stages. The first batch of 21 ramets cloned from a PS1 palm (assigned as P471) was sent for planting to Kluang station in 2009.

6) Long stalk:

A tenera palm with this trait was cloned but limited embryoids were obtained which unfortunately failed to produce shoots. More samplings will be carried out in future.

4**. Ortet selection standard**

The demand for high OY clonal planting materials is expected to increase whereby many existing laboratories are expanding and new ones are emerging. With such a large volume of ramets being produced and planted, it is important that the ramets are truly derived from high OY ortets. To ensure this, a standard of specifications was developed for selecting good mother palms (or ortets) as the source of explant.

The Malaysian Standard (MS 2099:2008) on “Oil Palm clones for commercial planting – Specification for ortet selection” 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. In developing this standard, the Working Group on Oil Palm Clonal Planting Material which was managed by MPOB-SIRIM and comprised of representatives from eight other organisations, started the programme since 2006 through a series of meetings and brainstorming sessions.

This specification is outlined according to: i) Introduction, Scope, Definitions, Requirements for ortet selection, ii) Guidelines for production practices and facilities, iii) Packaging and transportation of plantlets/ ramets, iv) Legal requirement and v) Certification mark. Specifications for the ortet selection as sources of explants for cloning towards commercial planting were categorised into three groups:

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

The minimum standards required to be adhered to for the selection of an ortet for group 1) materials of known pedigree and known performance of family and individual palm, are as follows:

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 comprising a minimum of 30 palms, whereby;
   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.

For group 2) materials of unknown pedigree and known performance from known seed producer, and group 3) materials from field tested clones for recloning, the specifications are as stated below;

a) Oil yield (OY), minimum : 55 kg/palm/year
   Oil to bunch (O/B), minimum : 28%
b) similar specifications as for group 1: b) – d)

but with additional requirements for recloning as follows:

e) Selection of 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.

5. Cloning Achievement of high yielding clones

Clonal trials were mostly carried out at several MPOB stations. However with shortage of land, collaborative clonal trials with other oil palm agencies were also carried out as listed below. In total, about 70 clones were planted over an area of approximately 160 ha. of field trials and 220 ha. of commercial test plantings. The collaborative trials were divided into two groups, called the bilateral clonal trials and standardized clonal trials.

I. Bilateral Clonal Trials (BCT):

In these trials, MPOB clones that were available at that time were planted at the agencies' plantations. The BCT was initiated in the late 90's. The agencies/ plantations involved were:

- JC Chang (Sabah & Pahang)
- Foong Lee (Perak)
- Lembing Plantations (Pahang)
- Borneo Samudera (Sabah)
- EPA-KULIM (Johor)
- Ladang ESPEK (Perak & Pahang)
- TSH (Sabah)
- SPAD (Sarawak)

II. Standardized Clonal Trials (SCT):

In these trials, three private plantations were involved. Two MPOB clones (P368 & P379) were field tested at 5 locations along with each agency's clones under approximately similar planting design, number of palms and planting time (initiated in year 2007). The agencies/plantations involved in the SCT were:

- AAR (Johor)
- Perlis Plantation Bhd (Sabah & Sarawak)
- United Plantations Bhd (Perak)
- MPOB Keratong (Pahang)

Results

Generally, the clonal field trials could be summarised as below;

1. Oil yield

   Most clones showed normal fruit formation, uniform and high yielders. Higher oil yield than the DxP by 7 to 34%. 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 as in Table 1 (Kushairi, 2010).

   Table 1: Performance of MPOB clones (4 – 7 yrs)

No	Clone	Oil-to-bunch (%)	Oil Yield (t/ha/yr)	Soil type*
1a 1b	P164 P164	30.6 33.8	8.71 10.81	Inland 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

2. Mantling rate

   Overall mantling rate is less than 5%. From more than 70 clones tested, most of the mantled palms were contributed by 8 clones. These 8 ‘sensitive’ clones were P209, P210, P236, P273, P291, P297, P300 and P330 as in Table 2. MPOB is using these materials for molecular studies to understand the abnormality and for biomarker discovery. For liquid culture system of MPOB, mantling was reported to occur less than 5% (Tarmizi, 2005).

   Table 2. Mantled clones of MPOB

No	Clone	No planted	% mantled	No of locations
1	*P297	120	82.8	2
2	P330	30	46.7	1
3	P209	231	45.8	3
4	P236	658	31.1	3

5	P210	315	26.1	2
6	*P291	34	20.6	1
7	P300	73	20.6	1
8	**P273	78	16.7	1

*Average and **Poor yielding palms under the Ortet selection efficiency study

3. Clone P456

   P456, which is a reclone of P164, has been 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%.

4. Clone P126

   Ramets from reclone of P126 showed almost zero mantling. The clone showed high O/B although small bunches was produced (FFB 152kg/p/yr and O/B 28%). This might ease harvesting procedures in future. P126 palms also exhibit short rachis lengths of about 4.4 m and has been deemed possible for use in high density (HD) plantings. The palms were field tested at a distance of 25' x 25' at MPOB Kluang (inland soil) in 2007 and MPOB Teluk Intan (peat soil) in 2008. It was also planted at 27'x27' and 29'x29' planting distances in 2008 by Ladang RISDA at Ulu Keratong. Comparative yield according to palm age would be notified later.

5. Sex-ratio of 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 bunches. From preliminary observations on some trial plots in SCT, P368 showed a high sex ratio with numerous fruit bunches as usually exhibited by most clones. On the contrary, the clone P379 exhibited the characteristic of having frequent male inflorescences (MI) along with fruit bunches. Further observations on the MI and yield recordings (including pollen viability) should be done continuously for several years to ascertain whether this phenomenon is genetically or physiologically/ environmentally related. If it is genetically linked, P379 could be incorporated with other clones in any future clonal planting as the pollen source palms, substituting the use of DxP palms. Moreover, this pollinating clone could also produce high quality fruit bunches. The incorporation of DxP palms in clonal plots, usually planted in alternate number of rows, may cause a mix-up in the harvested fruit bunches that will create inconsistency in the OY output. This matter could be avoided by using clonal high OY-cum-pollinating palms. Ortet performance of the these clones in terms of FFB and O/B% are as follows;

- P368 = 181.5 kg/p/yr & 28.8%
- P379 = 195.1 kg/p/yr & 31.8%

6. Extraordinary and future clone - P325

Another clone with good potential is P325, which was planted in MPOB stations and in a BCT plot at EPA-KULIM. P325 exhibited zero mantling and its ortet performance was superior with an FFB production of 198.8 kg/p/yr and O/B of 35.2%. Early results from the EPA-KULIM plots showed that O/B was very high and quite consistent, ranged from 34.5 – 39.4, with an average of 37.3%, while the FFB production ranged from 60.8 – 228.3, with an average of 158.7 kg/p/yr giving an estimated OY of 8.04 t/ha/yr. These data was obtained from a total of 39 palms at 6 years of age. Further observations and recordings will be continued as the palms grow older.

Discussion

The occurrence of mantled or ‘sensitive’ clones cannot be predicted at present, but detection using biomarkers in future holds promise. Mantling can occur to any ortet whereby its genetic or physiological state during time of culturing may be sensitive to the media protocol, environment, etc. For the time being, before molecular tools are perfected, housekeeping or cultural practices on clonal planting could be carried out in avoiding or minimizing this abnormality problem. Ramets of new clones should first be tested on clonal field trials or in a semi-commercial scale, while ramets from recloning can go forth to commercial scale.

From the performances of P456 and P126 in MPOB, recloning is deemed possible for production of ramets for commercial plantings. Cloning of clones that originates from the ‘sensitive’ type should be avoided, even though the ortets have no history of mantling. It was reported that Initial experiences with recloning were discouraging because of the higher susceptibility to mantling encountered (Wong et al 1999), even though reclones appeared to be more amenable and gave higher embryogenesis rates. Subsequent reclonings by AAR produced lower mantling rates (Soh et al, 2010). Konan et al (2005) have also reported similar experiences as well and indicated the importance of selecting normal ramets from clones with no history of mantling for recloning.

Conclusion

Production of palms with desired agronomic characters and with high value added traits is a strategy for sustaining the oil palm industry. The yield potential of oil palm is largely determined by the quality of oil palm planting material. 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 the existing national average of 4 t/ha/yr to more than 8 t/ha/yr. Agronomic inputs such as using fertile land and tailored fertilizer regimes are necessary for clones to express its genetic potential.

Cloning superior palms provides uniform high yielding planting materials, sparking the ‘tsunami’ in yield improvement, in tandem with the national aspiration of increased productivity to 35 t/ha/yr FFB and 25% OER or Vision 35:25 by the year 2020

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