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COMMERCIAL-SCALE PROPAGATION AND PLANTING OF ELITE OIL PALM CLONES: RESEARCH AND DEVELOPMENT TOWARDS REALIZATION

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ABSTRACT

The announcements of breakthroughs in plant regeneration from tissue cultures of oil palm in the 1970s ushered in a new chapter in oil palm genetic improvement with projected yield increase of clones exceeding 30% over hybrid seeds. However, the subsequent ubiquitous appearance of the mantled fruit somaclonal variant in regenerated palms resulted in the early commercial oil palm tissue culture laboratories reverting to further research and development (R&D). Applied Agricultural Resources Sdn Bhd persisted and, through its R&D, circumvented the impeding issues of unacceptably high fruit mantling, and low efficiencies of tissue culture amenability and selection of elite palms. In the process, it has established viable large-scale commercial propagation of oil palm clones by gel and liquid culture methods by the late 1990s. This achievement has since been emulated by more than a dozen commercial laboratories in Malaysia and elsewhere, producing about 3.5 million ramets per year. This apparent success has led industry to believe that oil palm cloning is an established and efficient technology, and that more tissue culture laboratories to produce more high-yielding clones to replace hybrid seeds in planting and replanting will resolve the stagnating national yields.

Much of the increased ramet production comes from more laboratories culturing more palms than in improved cloning efficiencies. Most of the clones currently produced are also derived either from advanced dura *(D) x* pisifera *(P) hybrids or from commercial DxP fields with low heritability for yield. Consequently, the expected yields of the clones would not be much different from those of near true F1 and clonal hybrids which are currently available at a much reduced cost and without the attendant mantling risk. The agro-management needs of clonal plantings to maximize their fruit bunch yield potential have yet to be systematically addressed through scientific experimentation. Cloning ortets from the early or recombinant phases of hybrid breeding programmes would be more efficient with the wider genetic variability and higher heritability for yield and other desirable traits. Perhaps the biggest advantage of cloning would be in the early commercial exploitation of new genetic materials from introgression programmes of wide intra- or inter-specific crosses which would also broaden the genetic base of the commercial plantings to reduce the risk of genetic vulnerability to pests, diseases and environmental stress.*

Clones are unlikely to supersede hybrid seeds as the dominant oil palm planting material until the amenability and fidelity deficiencies in tissue culture have been further resolved or circumvented, and their field performance advantage over concurrent improved hybrids clearly demonstrated.

Keywords: oil palm, clonal propagation, R&D, commercialization and field planting, existing issues.

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INTRODUCTION

Palm oil is Malaysia's second largest revenue earner, worth about RM 50.7 billion in 2009 (Department of Statistics, Malaysia). Industry provides direct employment to more than half a million people, or more than two million people overall if those indirectly employed are included. Demand for palm oil will continue to rise particularly in emerging economies, *e.g*. China and India, due to increased per capita consumption and demand as biofuel, a renewable resource. Oil palm is essentially a commodity crop where high yield and consequent reduction in production costs are crucial to its long-term competitiveness and sustainability, particularly in the light of labour and land scarcity in Malaysia resulting in higher costs. The national average annual oil palm yield has stagnated for the past three decades, and planting improved oil palm varieties will be critical to long-term high production efficiency, productivity and sustainability of the crop. Current commercial oil palm planting materials, being a mixture of *dura* (D) × *pisifera* (P) or *tenera* (T) hybrids from non-fully inbred parents, have considerable between palm genetic variability arising from between and within family genetic variability depending on the relatedness and inbreeding status among their D and P parents. Individuals could vary by more than 30% of the mean yield of the hybrids (Jones,1974; Hardon *et al.*, 1987). Although the differences are not entirely genetic, nevertheless, they provided the rationale and impetus in the 1960s to develop clonal propagation of the oil palm by tissue culture because the species has no natural means of vegetative propagation. Conventional hybrid breeding methodology would require at least three generations or over 20 years to achieve the superior yields of these individuals (Soh *et al*., 2005). Success in cloning would short-circuit the process. The oil palm, being a monocotyledon and a perennial tree, was considered a recalcitrant species for *in vitro* propagation. However, with concerted research efforts, the first *in vitro* clones were obtained by two overseas laboratories, *i.e*. Unilever (UK) and CIRAD or Centre de Co-operation Internationale en Recherche Agronomique pour le Developpement (France), in the mid-1970s (Jones, 1974; Rabechault and Martin, 1976). Following this, despite more than two decades of research and development (R&D), involving the setting up of more than 20 laboratories worldwide with expenditures running into tens of millions of Malaysian ringgit in clonal propagation for trials and field tests, large-scale commercial propagation and planting of proven T clones were not successful until recently. The commercial laboratories set up by the British and French groups and their Malaysian and foreign associates had to close when excessive numbers of abnormal palms (in the form of mantled parthenocarpic fruits which result in sterility) were regenerated from tissue cultures in their laboratories and subsequently also in others. The Applied Agricultural Resources Sdn Bhd (AAR) laboratory, without any direct external or foreign assistance, persisted and succeeded in circumventing this principal problem, and has achieved commercial-scale clonal palm production (*Figures 1* and *2*). This article reviews the R&D advances made by AAR to achieve this position. AAR's achievement has also encouraged other laboratories to pursue this effort, resulting in the current annual production capacity of about 2.5 million ramets or clonal plantlets from 11 commercial oil palm tissue culture (OPTC)

Figure 1. AAR's commercial oil palm tissue culture laboratory (capacity 1.5 million plantlets per year).

Source: Soh *et al.* (2006).

Figure 2. Field plantings of AAR clones.

TABLE 1. ESTIMATED WORLD PRODUCTION OF OIL PALM TISSUE CULTURE PLANTLETS

Country	Number of plantlets per year (million)
Malaysia	2.5
Costa Rica	0.5
Indonesia	0.5
Total	3.5

Source: Kushairi *et al*. (2010).

TABLE 2. OIL PALM TISSUE CULTURE PLANTLET PRODUCTION (million) IN MALAYSIA

Agency	2009	2010 (expected)
Clonal Palm	0.13	0.15
FELDA	0.35	1.0
AAR	1.0	0.6
Sime Darby	0.3	0.75
Kulim-Top Plant	0.01	0.02
SEU	0.003	0.01
UPB	0.06	0.06
IOI	0.3	1.0
Agrocom	0.01	0.12
Borneo Samudra	0.08	0.2
Total	2.53	4.81

Source: Kushairi *et al*. (2010).

laboratories in Malaysia, and about 3.5 million ramets worldwide (*Tables 1* and *2*; Kushairi *et al*., 2010). This article also highlights the future R&D advances needed for clones to become a significant, if not dominant, oil palm planting material.

HISTORICAL DEVELOPMENT

The historical development of OPTC clonal propagation in industry and at AAR is summarized in *Table 3*.

As indicated, commercial clonal propagation of oil palm, which was a logical progression from the first reports of breakthroughs in plantlet regeneration from *in vitro* culture of oil palm, took a step backwards when the pioneering Bakasawit/Unifield and Tropiclone commercial laboratories reverted to further R&D soon after experiencing the fruit mantling somaclonal variation which resulted in varying degrees of sterility (*Figure 3*). Other OPTCLs (OPTC laboratories), *e.g*. Agrocom, FELDA, United Plantations including AAR, which were established subsequent to the pioneering laboratories experienced the same problem. Nevertheless, they persisted in clonal propagation in a modest manner.

It was soon realized at AAR (Soh *et al*., 2001) that for successful large-scale commercial plantings of proven T clones, a number of critical issues needed to be resolved besides somaclonal variation, *i.e*. cloning efficiency, ortet (parent palm of clone) selection efficiency, feasibility of recloning (using tissues of clonal palms), the liquid suspension system, conditioning or acclimatization of plants produced *in vitro*, and field testing requirements.

TABLE 3. HISTORICAL DEVELOPMENT IN OIL PALM TISSUE CULTURE CLONAL PROPAGATION

Date	Event
1974	First reports on successful plant regeneration from tissue culture of oil palm by the British (Unilever) and the French (CIRAD) groups.
1982	AAR team began R&D on tissue culture clonal propagation of oil palm at HRU Sdn Bhd, its previous company.
1984	AAR's first regenerated plantlet from a seedling clone was planted.
1986	AAR's first clone trials of mainly seedling clones and one ortet clone were planted.
	First announcement of flowering and fruiting (mantling) abnormality in oil palm clones by the Unilever group (Corley *et al.*, 1986). Subsequent similar reports by CIRAD and other groups, including AAR. However, AAR's first clone trials were free from mantling. Publication by Soh (1986) on the expected yield increase with oil palm clones and proposed breeding/selection strategies in the light of ortet selection inefficiency and the mantling abnormality risk. The team from HRU moved over to AAR. AAR inherited half of the cultures from HRU in 1987.
1987	Trial to test CIRAD's clones resulted in severe mantling abnormality in some clones.
1989	AAR's first formal trials on ortet clones were planted.
	Severe mantling was observed in one clone which was free of mantling in the earlier trial. Other clones had negligible to mild mantling. Pilot field testing of these averaged 6% mantling, with a range from 0 to 36%. Commercial production laboratories set up by Unilever and CIRAD were discontinued because of the mantling problem, and the groups reverted to further R&D.
1989-2005	Papers by AAR in local and international journals on ortet selection efficiency and breeding strategy, *e.g.* Soh (1990; 1998; 1999; 2004; 2005), Soh and Chow, (1989; 1993), Soh *et al.* (1994; 1995; 2001; 2003b, c).
1990-1995	Trial and pilot commercial field tests of embryo- and seedling-derived clones of reproduced superior crosses or progenies besides the early ortet clones were conducted. The embryo- and seedling-derived clones resulted from the alternative cloning strategies proposed by Soh (1986). Mantling was negligible in the embryo-derived clones; the high yield of their superior family (achieving 40 t FFB) was reproduced and the top clones have been recloned. Except for one clone, mantling in the other ortet clones was minimal. The seedling-derived clones still averaged about 8% mantling, presumably from the limited number of seedlings sampled.
1996	The beginning of a large-scale clone trial and commercial field tests of ortets from improved cloning protocol and ortet selection strategies adopted at AAR. Confidence was built from the results of the pilot commercial field tests. On average, about two trials were planted per year totaling >24 trials to date. Commercial clonal or ramet field test plantings rose to 100 000 – 300 000 plants per year.
1997	AAR's paper (Wong *et al.*, 1997) to an international audience and in an international journal announced the feasibility of large-scale clonal propagation of oil palm using the gel culture protocol.
1999-2003	The announcement and publication to an international audience of the feasibilities of recloning and the liquid suspension culture techniques for efficient mass propagation of superior proven oil palm clones (Wong *et al.*, 1999b; Soh *et al.*, 2001; 2003a; Tan *et al.*, 2003).
2004-2005	The building and commissioning of AAR's new commercial tissue culture laboratory with the capacity to produce 1.5 million proven clonal palms for commercial planting.
2006-2008	Commercial clonal or ramet field plantings rose to 500 000 – 750 000 plants per year for the last few years, totalling about 30 000 ha of commercial areas to date from 1990s.

Source: Soh *et al.* (2006).

Figure 3. Fruit mantling somaclonal variation.

TABLE 4. MANTLING INCIDENCE RATES IN CLONES

Year cloned	Year planted	Explant source	No. of clones	No. clones mantled[z] (%)	No. ramets[y] planted	Mantled ramets in clones	
						Range (%)	Mean (%)
1983/4	1986-91	Ortet[x]	12	67	7 427	0-35.7	6.2
1987	1989-92	Embryos (RC)[w]	17	12	6 840	0-72.0	1.1
1987/88	1992-95	Seedlings (RC)	63	75	80 632	0-35.4	7.6
1992	1996	Ortet	3	67	451	0-3.5	2.0
1993	1996-97	Ortet	20	80	9 378	0-9.1	2.7
1994	1997-98	Ortet	18	50	11 865	0-3.5	1.4
1995	1998	Ortet	22	32	3 412	0-6.0	1.0

Note: [z]A clone with any of its ramets expressing the mantling somaclonal variation is considered mantled.
[y]Ramets or plantlets = clonal plants or members of a clone.
[x]Ortet = parent tree of clone.
[w]Repeated cross of a superior family.
Source: Soh *et al.* (2003a).

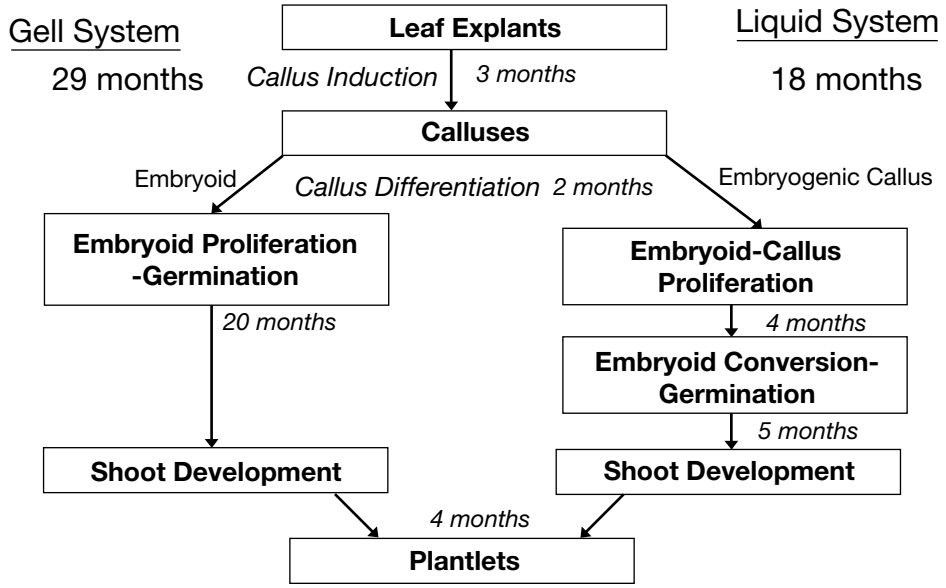
The following summarizes the R&D approaches and advances made by AAR in these areas:

Somaclonal Variation

This has been managed to an acceptably low level (<5%) via the following strategies resulting from the R&D trials:

- cloning a large pool of selected palms with different genetic backgrounds (genotypes) as there are genetic/clonal differences in susceptibility/tolerance to the mantling abnormality.
- optimizing the tissue culture protocol (hormone treatments; nutrient and medium requirements; physical conditions, such as light, temperature and type of vessel; culture selection and transfer for multiplication, such as transfer frequency; and production life/level of culture).
- practising strict process quality control through stringent laboratory 'house-keeping' to ensure negligible contamination and optimum growing conditions for the cultures, coupled with stringent culture selection at each transfer. Well-trained personnel are crucial.

Table 4 documents the progressive reduction in mantling rates that has been achieved.



Source: Soh *et al.* (2006).

Figure 4. Culture stages and duration in gel vs. *liquid systems to produce 5000 shoots.*

TABLE 5. OIL PALM CLONING AND RECLONING EFFICIENCIES IN THE GEL CULTURE SYSTEM

Explant source[z]	Basis	Callogenesis % (range)	Embryogenesis % (range)
Ortet (cloning)	Palm	100	80 (43-100)
	Explant	17 (12-27)	4 (0-36)
Ramet (recloning)	Palm	100	89 (77-100)
	Explant	16 (11-21)	12 (0-52)

Note: [z]Ortets = parent palms of clones; ramets or plantlets = clonal plants.
Source: Soh *et al.* (2003a).

Cloning Efficiency

Tissue culture cloning of oil palm is an inefficient process (*Figure 4*). The efficiency of callogenesis or callus formation is not an issue for all palms, as about 15% of the leaf explants that are cultured yield calli. Although 80% of the palms cultured are embryogenic (*i.e.* they produce embryoids or somatic embryos), less than 25% of these embryogenic palms and less than half of the 5% of the embryoids that differentiate from the calli can actively proliferate and regenerate into plants in sufficiently large numbers for commercial production. Again, there are genetic/clonal differences in amenability.

The strategies involved to circumvent this limitation are similar to those adopted for the abnormality issue.

Table 5 illustrates the achievements made in improving cloning efficiency in gel culture by AAR (Wong *et al.*, 1999a; Soh *et al.*, 2003a).

Recloning and Liquid Culture

Owing to inefficient ortet palm selection, *i.e.* the yield of the ortet is not directly translated to clone yield due to environmental effects, actual clonal trial results are mandatory to select for the better clones (Soh, 1986; Soh *et al.*, 2003b). Unfortunately, by the time the field trials are completed, the original palm is usually no longer existing or is less amenable to tissue culture. Recloning, *i.e.* cloning of the selected clones, is therefore necessary. The technical issues in recloning are similar to those for cloning, *i.e.* phenotype abnormality and efficiency, and thus can be resolved in a similar manner. Recloning efficiency appears to be better, in terms of embryogenesis on both palm and explant basis, than that of cloning (*Table 5*). This is somewhat to be expected based on genetic or habituation arguments. Low mantling rates in reclones, comparable to those of clones, have also been achieved (*Tables 6* and *7*).

The original gel culture system (*Figure 5*) that was established was inefficient in terms of proliferation rate. Besides, its non-synchronous culture development required tedious manual culture selection and transfer, and was less amenable to automation. The issues of abnormality/efficiency and the solutions to overcome these would also apply.

TABLE 6. MANTLING INCIDENCE RATES IN RECLONES

Year recloned	Year planted	No. of primary clones recloned	No. of primary ramets[z] recloned	Reclones[y] of primary clones mantled[x] (%)	Reclones of primary ramets mantled[w] (%)	No. of secondary ramets planted	Mantled ramets in reclones	
							Range (%)	Mean (%)
1989	1993-95	3	6	100	100	35 330	2.9-14.1	11.3
1991	1994-97	3	8	100	100	7 853	2.3-9.2	4.9
1992	1995-97	5	8	100	75	1 016	0-5.2	3.2
1993	1997	4	4	50	50	478	0-3.6	1.2
1994	1997-98	10	24	80	67	2 779	0-12.7	2.1

Note: [z]Ramets or plantlets = clonal plants.
[y]Reclones = clones obtained from cloning ramets.
[x]Clones with any of its secondary ramets (from recloning) mantled.
[w]Ramets with any of its secondary ramets (from recloning) mantled.
Source: Soh *et al.* (2003a).

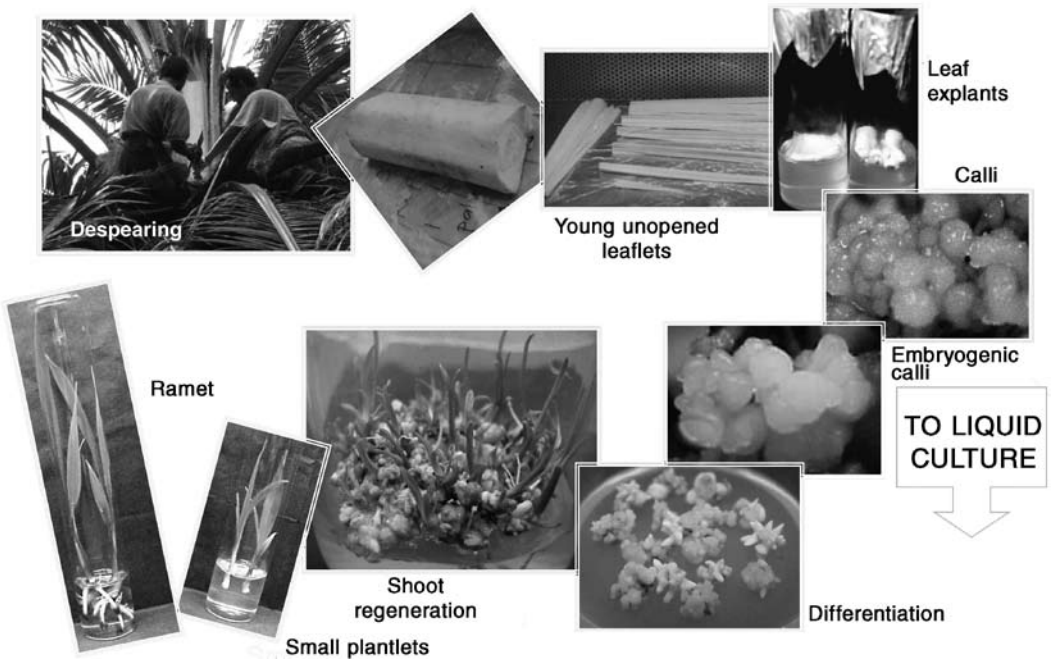
TABLE 7. MANTLING RATES OF AAR CLONES AND RECLONES FROM GEL AND LIQUID CULTURES

Culture type	Clone type	Mantling (%)
Gel	Clones	2.6
	Reclones	2.0
Liquid	Clones	1.9
	Reclones	1.5

Source: Soh *et al.* (2003a).

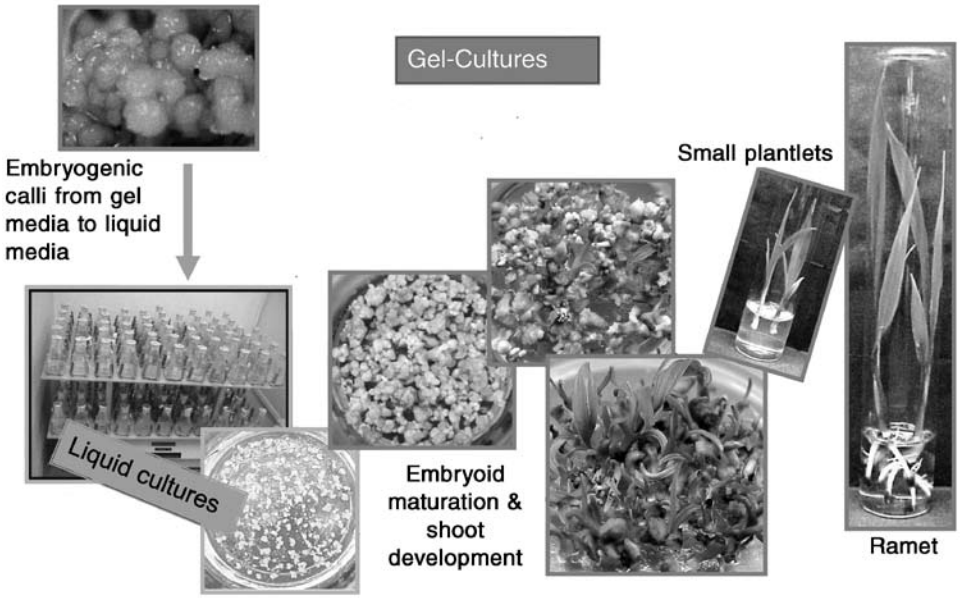
Breakthroughs in the feasibility of recloning and liquid culture were essential for the large-scale commercial clonal propagation of elite palms, and these have been achieved by the AAR team (Wong, 1999b).

The efficiencies of the liquid suspension system in terms of amenability and proliferation rate as well as mantling risks are given in *Figure 6* and *Tables 8* and *9* (Soh *et al.*, 2003a) for both clones and reclones. It is evident that a very high percentage of palms and cultures are amenable to



Source: Soh *et al.* (2006).

Figure 5. AAR's oil palm tissue culture: gel system.



Source: Soh *et al.* (2006).

Figure 6. AAR's oil palm tissue culture process: liquid system.

TABLE 8. OIL PALM CLONING EFFICIENCIES IN LIQUID SUSPENSION SYSTEM

Explant source[z]	Basis	Embryogenic callus proliferation (%)	Shoot conversion from embryo (%)
Ortet (cloning)	Embryogenic palms	83	94
	Embryogenic callus	63	95
Ramet (recloning)	Embryogenic palms	93	100
	Embryogenic callus	95	100

Note: Ortets = parent palms of clones; ramets or plantlets = clonal plants.
Cultures were developed on gel media and proliferated in liquid media. Shoots were converted on gel media.
Source: Soh *et al.* (2003a).

TABLE 9. PLANTLET PRODUCTION AND MANTLING RATES OF CLONES IN GEL *vs.* LIQUID CULTURE SYSTEMS AT VARIOUS SUBCULTURE LEVELS

Clone No.	Gel-culture system			Liquid suspension system		
	Subculture No.[z]	No. of shoots obtained ('000)	Mantled ramets (%)	Subculture No.	No. of shoots obtainable[y] ('000)	Mantled ramets (%)
41	11	1.120	73	11	3 000	93
178-46	7, 11, 13	0.375	7	7, 8, 9	90	0
100-24	13, 14	0.314	4	3, 15, 16	3	0
154-44	9	0.290	0	9, 13	20	9
94-195	7	0.147	0	9	2 000	2
124-24	6	0.636	0	1, 3	1	0
180-2	6, 8, 9	0.169	0	6, 7	10	0
79-24A	14, 15	0.201	0	13, 17	400	0
121-29A	6, 9	0.342	0	3, 8	600	0
176-86	9, 14	1.108	0	4, 8, 9, 14	1 000 000	0

Note: [z]About 40 ramets or plantlets (clonal plants) were sample-tested per subculture.
[y]Extrapolated figures based on proliferation and germination rates of cultures.
Source: Soh *et al.* (2003a).

proliferation in the liquid system. Again, reclones are more amenable. Proliferation rates are very much higher in liquid culture although there is wide variation among clones. Proliferation rates in gel and liquid systems appear to be correlated. In fact, repeatability is apparent between clones and reclones, and between gel and liquid cultures for both amenability and mantling risk.

Conditioning/Acclimatization of Plants Produced *in vitro*

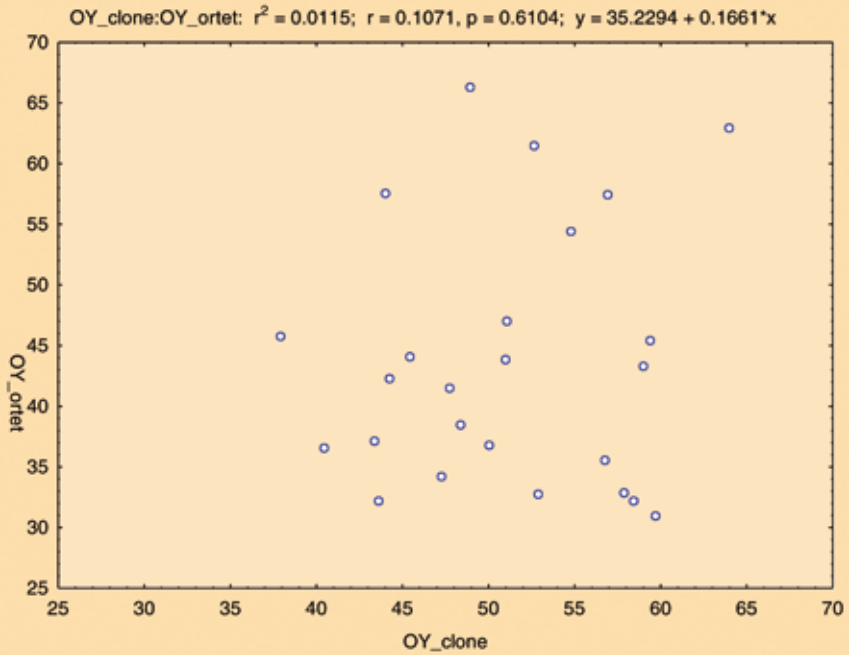
Poor recovery (less than 60%) of hardened plants, for subsequent nursery and field planting, from the acclimatization process of the plants produced *in vitro* was problematic in many laboratories (particularly in the earlier years), despite attempts at more sophisticated treatments. These include the use of sterile potting media, mycorrhiza soil amendment, application of leaf anti-transpirant, and acclimatization in climate control chambers (Wooi *et al*., 1981; Corley, 1993; Wuidart and Konan, 1989; Blal and Gianninazzi-Pearson,1990; Tan *et al*., 1999). Through R&D, AAR has achieved over 95% recovery of hardened plants of uniform size and good vigour for large-scale production with a simple system using plastic shelters, light organic potting media, mist irrigation and a simple rigorous protocol of plant conditioning, selection, and handling (*Figures 1* and *7*; Tan *et al*., 2003).

Conditioned AAR ramets have been dispatched with relative ease in consignments of up to 15 000 ramets in paper carton boxes as bare-root plantlets by road, rail or air, meeting the strict phytosanitary requirements of the various authorities, both in Malaysia as well as in Indonesia. As a result of the vigour of the conditioned ramets and the strict culling done at AAR's laboratory and conditioning nursery, combined casualty and culling rates in the estates have averaged 7%, significantly lower than those of seedlings.

Ortet/Clone Selection

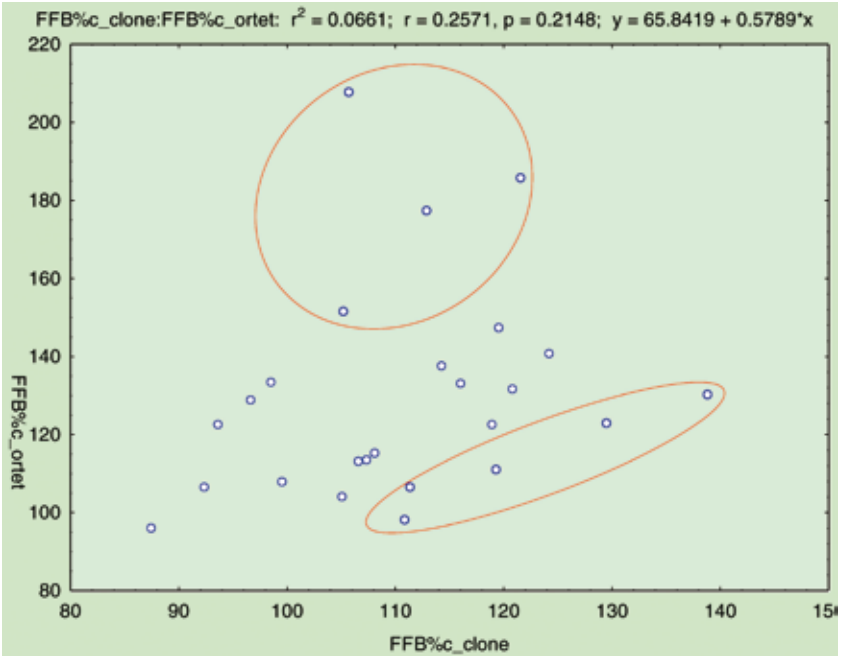
Cloning is only a propagation tool. Improved clonal varieties arise from the mass propagation of superior genotypes. The breeders in the AAR team have demonstrated that ortet selection or selection of palms for cloning from commercial fields is inefficient due to poor ortet-clone correlation for oil yield (*Figures 8*, *9* and *10*). They have thus devised selection and breeding strategies from field experiments to support the laboratory team in the continuous provision of sufficient elite ortets in view of the inefficient cloning process (Soh, 1986; Soh *et al*., 2003b; 2006a,b). Ortets are selected from the best (but not limited to) families within the established progeny test trials. Particular emphasis is given to oil-to-bunch and related component traits, *e.g.* mesocarp-to-fruit, oil-to-wet/dry mesocarp and palm stature (short, light petioles and canopy), with selection standards exceeding those specified by SIRIM (Standards and Industrial Research Institute of Malaysia) certification. Selected ortets generally constitute less than 5% of the palms tested. Only the top clones (making up less than 20% of the clones tested) possessing the best total merit from

Figure 7. Despatch of AA Vitroa I oil palm acclimatized ramets.



Source: Soh *et al.* (2006).

Figure 8. Ortet – clone correlation: oil yield.



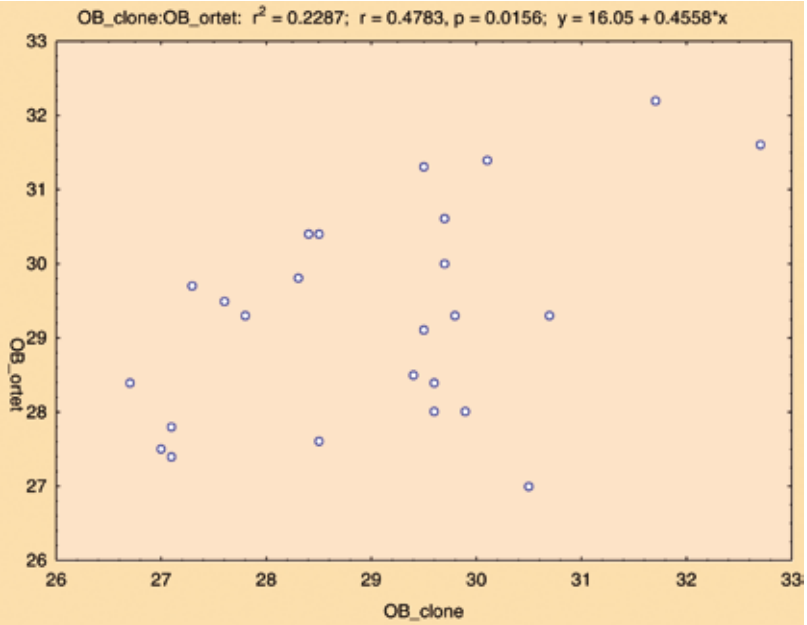
Source: Soh *et al.* (2006).

Figure 9. Ortet – clone correlation: FFB yield (% of DxP control).

a combination of desirable traits are selected for recloning. Ramets that are sampled must reflect the clone phenotype in all aspects, with no suspected somaclonal variation or clone mix-up or misidentification. Genotypic fidelity confirmation through DNA-fingerprinting follows.

Field Evaluation and Commercial Plantings

Field evaluation of clones is mandatory for commercial clone production as 'the proof of the pudding is in the eating'. Field trials are carried out to prove the fidelity of the clones in terms of



Source: Soh *et al.* (2006).

Figure 10. Ortet – clone correlation: oil to bunch %.

abnormality risk and expected yield increase to:

- help validate the selection and cloning procotols, and for their further optimization;
- select clones for recloning; and
- demonstrate the superiority of the clones over hybrid seed varieties.

Besides formal clonal evaluation trials which are laid down for every new clone or reclone produced, the initial ramets that are produced are field-nursery screened for early detection of somaclonal variation risk. Similarly, ramet samples are taken from different production level batches of each clone to field-screen for clone fidelity/stability. Pilot commercial-scale plantings, as well as trial oil mill extraction runs, are done to further support these data.

The clones are tested at different estate locations to assess the importance of clone × environment interactions. Three types of treatment controls – a good clone tested previously, a DxP control representing the previous generation commercial material (from which the clones are derived), and a DxP control representing the current generation commercial material) – are included in all the clonal trials planted although the achievement of the full set of controls has not been always successful. The controls are included to enable valid comparisons of the advantage of clones or reclones over their concurrent improved DxP hybrid materials. This will be further discussed later.

Pilot commercial-scale test plantings are also made at different estate locations. The planting arrangement adopted is: 4 rows per clone × 4 clones + 4 rows of DxP. This is to hedge against somaclonal variation (mantling, others), unpredictable yield performance and inadequate pollination risks.

From the evaluation trials to date, on average, the clones exceeded the DxP controls by 5% for bunch yield (FFB) and 14% for oil yield (OY), although there have been clones that yielded more, indicating the prospective yield improvements achievable with recloning (*Table 10*). There were also clones yielding less than the DxP controls, an indication of the inefficiency of ortet selection.

From pilot commercial test plantings, clones have outyielded DxP by 7%-34% in FFB (*Tables 11, 12* and *13*). A similar and higher FFB yield advantage of clones has also been claimed by others (Khaw and Ng, 1997; Simon and Koh, 2005; Sharma, 2006; Kushairi *et al.*, 2010). Reports derived from commercial field results have to be tempered by the fact that the clones had usually been initially planted in smaller and better areas, and might have been given better management inputs than the commercial DxP, while for reports on trials, the appropriateness of the DxP controls used for valid comparison would need to be scrutinized. For oil extraction rates (OER) from mill test runs by AAR, ramet crops have achieved 24%-27% as compared to 19%-22% with DxP (*Tables 14* and *15*).

CONCLUSION

Latest Results, Experiences and Issues

Following the lead by AAR in the commercial mass production of ramets, there are now at least 11 OPTCL in Malaysia, and a few more in Indonesia,

TABLE 10. SUMMARY OF AAR CLONE TRIAL RESULTS: FRESH FRUIT BUNCH (FFB) AND OIL YIELDS (OY) OF CLONES COMPARED AGAINST COMMERCIAL DxP HYBRIDS

Trial	Year of yield data	Clone FFB yield range (t ha^{-1})	Mean clone FFB yield (t ha^{-1})	AAR DxP FFB yield (t ha^{-1})	No. of clones > 10% > DxP	Clone OY range (t ha^{-1})	Mean clone OY (t ha^{-1})	AAR DxP OY (t ha^{-1})	No. of clones > 10% > DxP
BCT4-89	1997-2001	26.3-35.6	30.7	31.8	2/12	6.9-10.3	8.3	8.4	1/12
BCT5-89	1998-2000	24.6-35.8	31.1	30.8	2/14	7.2-10.3	8.6	8.2	3/14
BCT7-90	1994-2001	26.4-35.1	29.8	28.5	1/5	8.2-9.2	8.7	7.8	2/5
BCT9-91	1994-2000	19.4-29.4	26.0	19.9	11/14	7.4-8.4	7.0	5.0	13/14
BCT13-97	2000-2007	20.2-28.4	26.0	23.0	9/12	5.9-78	7.0	5.8	11/12
BCT14-97	2001-2007	22.0-29.3	26.0	24.6	5/11	6.8-8.9	7.5	6.4	8/11
BCT15-98	2000-2009	25.0-32.4	29.4	29.0	1/8	7.2-9.3	8.4	7.0	7/8
BCT16-98	2001-2009	18.5-26.0	23.1	21.7	8/24	5.3-7.9	6.6	5.6	20/24
BCT17-99	2002-2010	12.0-23.3	19.2	18.4	6/14	3.3-6.3	5.2	4.3	11/14
BCT18-00(Loc1)	2002-2010	19.9-36.2	29.5	29.2	5/19	5.3-9.3	8.2	7.1	14/19
BCT18-00(Loc2)	2003-2010	17.6-26.0	21.6	21.6	2/8	5.3-6.8	6.2	5.6	4/8
BCT19-01	2003-2010	10.0-20.0	17.1	15.6	8/10	2.5-5.4	4.5	3.8	8/10
BCT20-03(Loc3)	2005-2010	17.8-25.0	22.4	22.2	3/8	4.5-7.3	6.2	5.8	4/8
BCT20-03(Loc4)	2006-2010	17.7-25.3	21.6	20.3	2/8	4.8/7.5	6.0	5.3	5/8
Overall mean (wtd. av.)%	-	-	253 105%	240 100%	65/167 (39%)	-	70 114%	6.2 100%	111/167 (67%)

TABLE 11. COMPARISON OF FRESH FRUIT BUNCH YIELDS (t ha^{-1}) OF AAR COMMERCIAL CLONES AGAINST DxP PLANTINGS IN SIGALONG ESTATE, SABAH (1999 planting)

Material	Area (ha)	2004	2005	2006	Mean
AAR clones	251.4	20.8 (101%)	25.7 (118%)	26.7 (102%)	24.4 (107%)
AAR DxP	68.7	20.5	21.8	26.0	22.9

Source: Ho *et al*. (2009).

TABLE 12. COMPARISON OF FRESH FRUIT BUNCH YIELDS (t ha^{-1}) OF AAR COMMERCIAL CLONES AGAINST DxP PLANTINGS IN SEGARIA ESTATE, SABAH

Material	Planting	Area (ha)	2004	2005	Mean
AAR clones	1998	212.9	25.9 (132.7%)	27.8 (134.3%)	26.9 (133.2%)
AAR DxP		30.6	19.7	20.7	20.2
AAR clones	1999	240.2	20.6 (99.7%)	25.4 (112.9%)	23.0 (106.5%)
AAR DxP	-	96.5	20.7	22.5	21.6

Source: Soh *et al.* (2006a, b).

TABLE 13. COMPARISON OF FRESH FRUIT BUNCH YIELDS (t ha^{-1}) OF AAR COMMERCIAL CLONES AGAINST DxP PLANTINGS IN KAMPAR ESTATE, PERAK

Location	Material	Area (ha)	Year										Average	%
			1	2	3	4	5	6	7	8	9	10		
Kampar (AAR) 1992 planting	AAR clones	169	13	19	26	28	32	39	36	36	34	34	29.7	121
	DxP	21	6	26	31	29	30	32	28	27	32	32	24.6	100

Source: Soh *et al.* (2006a, b).

TABLE 14. OIL EXTRACTION RATE (OER) FROM MILL TEST RUNS OF AAR COMMERCIAL CLONES *vs.* DxP PLANTINGS

Mill	Crop type	Area (ha)	FFB processed (t)	OER (%)
Segaria	Clone	464.3	390	24.3
	DxP			22.4
KDC	Clone	423	343	26.7
	DxP			21.9

Source: Soh *et al.* (2006a, b).

TABLE 15. OIL EXTRACTION RATE (OER) FROM MILL TEST RUNS OF AAR CLONES *vs.* DxP CROPS AT KDC MILL

Item	Unit	AAR clones (1999 planting)	AAR DxP (1999 planting)	Mixed DxP (1978/79 planting)
FFB crop processed	T	388.7	339.6	502.9
OER	%	25.6	22.1	19.7

Source: Soh *et al.* (2006a, b).

Costa Rica, Colombia and Papua New Guinea have ventured into commercial propagation together with FELDA, Sime Darby, United Plantations and IOI, besides AAR leading the pack. These OPTCL are backed by historical breeding programmes. The ramets produced so far have been mainly used for proprietary plantings. There are also other OPTCL without breeding programmes which are franchising the technology and ortets from others, *e.g*. from MPOB (Malaysian Palm Oil Board). The national annual ramet production was about 2.5 million in 2009, and is projected to reach 5 million by 2010 (Kushairi *et al*., 2010). To reverse the stagnating national average yields to a rising trend to achieve the stipulated National Key Result Area (NKRA) of 35 (t ha^{-1} FFB): 25 (% OER) by the year 2020, the industry has been pressured to expand the number of OPTCL and their capacities in order to be able to supply smallholdings currently faced with laggard yields. This will be a daunting task and the targeted results are unlikely to be achieved within the stipulated period. The planting of clones by smallholders is also inadvisable based on the existing issues:

Culture amenability. Although national ramet production has increased, this came from the establishment of more OPTCL than from improved efficiency in amenability (Syed Alwee *et al*., 2010). In fact, the efficiencies reported are lower than those achieved earlier by AAR, especially for liquid culture (Wong *et al*., 1999b; Soh *et al*., 2003b). MPOB has reported on their R&D progress in automated and prospective high throughput processes, *e.g*. bioreactor, immersion culture (Tarmizi *et al*., 2003; 2007; Tarmizi and Zaiton, 2006. While these are encouraging developments, their general applicability and somaclonal variation risks are still unclear.

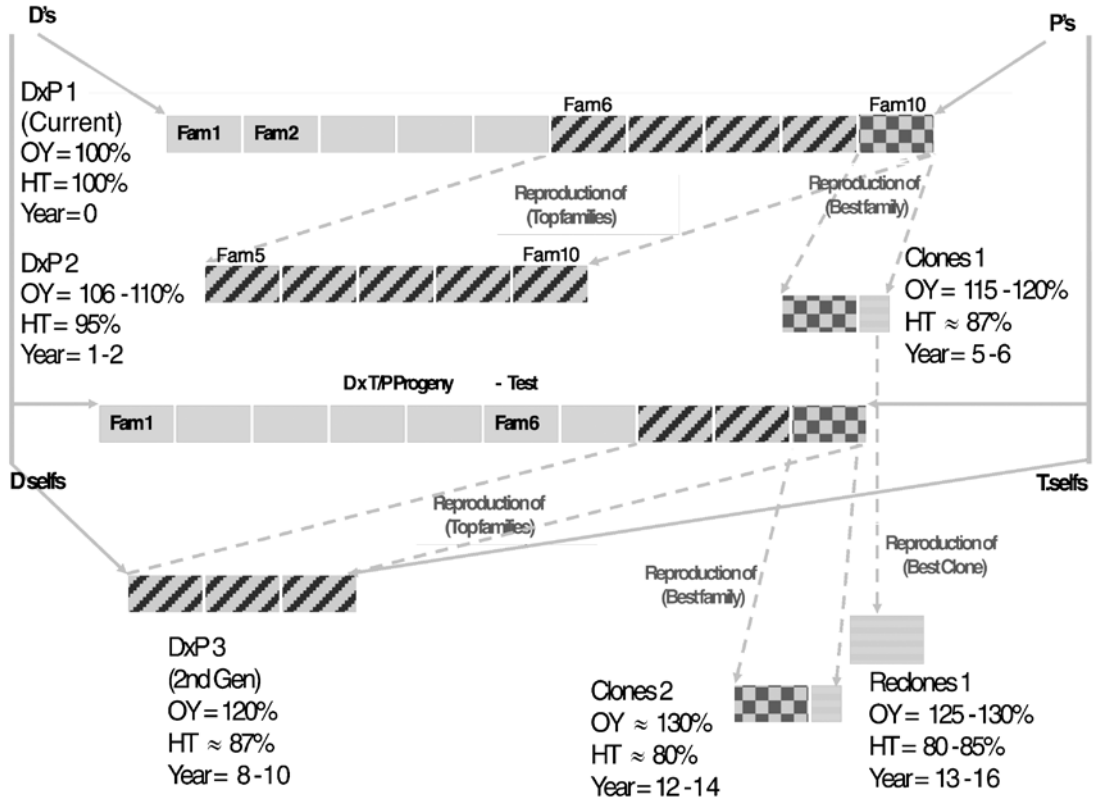
Putative expression biomarkers for OPTC amenability/embryogenesis have been obtained in MPOB (Ong-Abdullah and Ooi, 2006; Kushairi *et al*., 2010) but these need to be validated in clones of other OPTCL for genotype/protocol dependencies.

Somaclonal variation risk. The mantling risk of <5% reported by laboratories has been averaged over a large number of clones produced over time. There could be wide variations in susceptibility in any one batch of clones produced. If the clones constituting a large field planting are few, the economic consequence of a renegade clone is likely to be very great. The risks will be exacerbated with ramets from reclones and liquid culture where proliferation levels and concomitant somaclonal variation risks are higher. Reports on somaclonal variation risks with reclones and liquid culture are still not forthcoming besides those reported by AAR earlier. Apparently only a few (ca. 6) laboratories have ventured into R&D in liquid culture although most would have done some recloning. Although it may be considered preliminary, some further evidence on the relative efficiency and fidelity of these two techniques would be helpful in confirming their utility for large-scale propagation. A diagnostic molecular marker for mantling, an epigenetic change, has so far been elusive (Mathes *et al*., 2001; Morcillo *et al*., 2006; Syed Alwee *et al*., 2006; Rival *et al*., 2008).

An interesting development in OPTC is the recent report by Smith *et al.* (2010) from Dami Oil Palm Research Station, Papua New Guinea, on successful plantlet regeneration via 'true or direct' somatic embryogenesis (simultaneous shoot and root development from an embryoid) from oil palm inflorescence explants cum proliferation in liquid culture. Apparently the system is amenable to mass propagation and as the plantlets are derived from direct embryogenesis without a callus phase, somaclonal variation risk is expected to be reduced. Field results are as yet unavailable. The system was adapted from that of *Pinus radiata* pine (Smith, 1997).

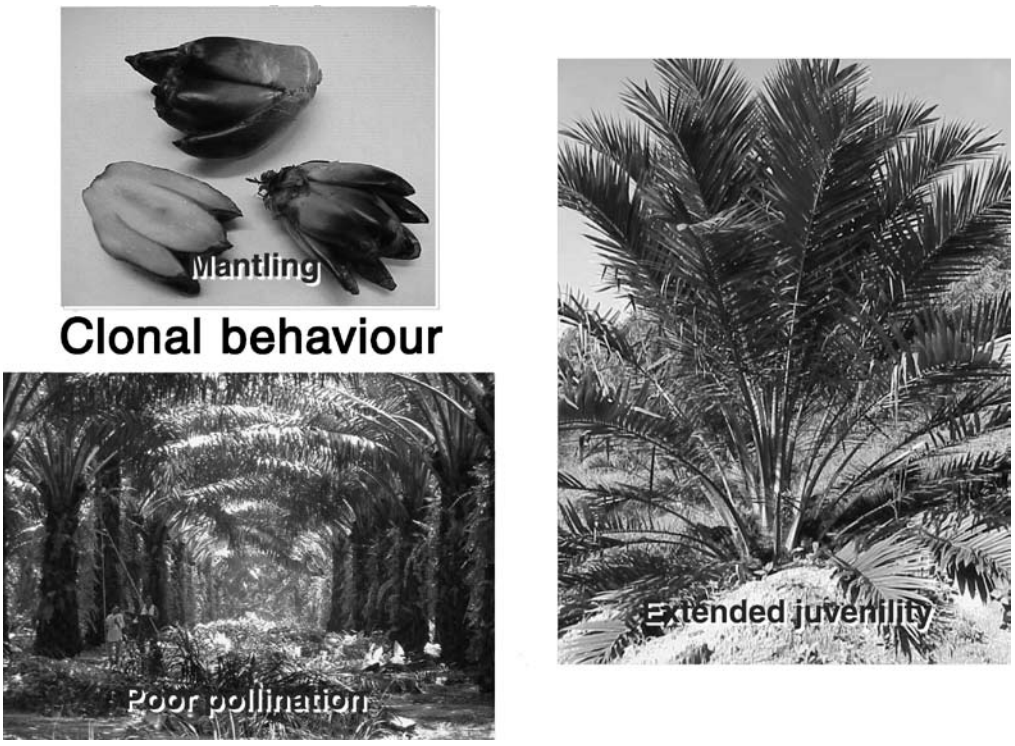
Ortet selection efficiency and clonal performance. CIRAD compared 42 clone-ortet sets and 17 parent crosses from their second cycle reciprocal recurrent selection programmes (Potier *et al.*, 2006) and found that although the mean OY of the clones was 7% better than the control cross mean (representing their previous generation DxP mean), it was lower than the current generation cross mean by 9%. The best clones were only as good in OY as the best crosses indicating that reclones would be only as good as the reproduced best crosses. Similar observations have been made by AAR. This would imply that with a good breeding programme, cloning ortets from advanced DxP populations is unlikely to result in substantial yield improvement as Soh (1986) (*Figure 11*) had predicted earlier. These observations also concur with those from AAR (Soh, 1986; Soh *et al*., 2003b) on the low heritability for OY, which would imply the low likelihood of success in ortet selection from commercial fields as is currently practised by some OPTCL. This would raise questions on the advantage and relevance of commercial clonal plantings with their higher cost and attendant risks of somaclonal variation and pest, disease and environmental stress vulnerabilities.

Cloning in the earlier or recombinant phases would be more efficient with wider genetic variabilities and higher heritabilities for yield and other desirable traits. This would also apply to the alternative clonal seeds (hybrid seeds derived from clonal parents: biclonal with both clonal parents, semiclonal with only one clonal parent, usually the D parent) and approaches in cloning embryos/



Source: Soh *et al.* (2003).

Figure 11. Expected cloning vs. *breeding improvement.*



Source: Soh *et al.* (2006).

Figure 12. Field planting issues.

seedlings, especially the former which has now become a proven commercial technology (Soh, 1986; 2010; Soh *et al*., 2003c; Wong, 2010).

Perhaps the biggest advantage of clones is the early commercial exploitation of new genetic materials from introgression breeding progenies derived from wide (intra- and interspecific) crosses, thus broadening the genetic base of the commercial crop to reduce its genetic vulnerability risk to biotic and abiotic stresses and ensuring its sustainability.

Lastly, adaptability trials are an integral part of a breeding and cultivar development programme. The issues of commercial field plantings of clones in terms of their composition, planting configuration and agro-management practices, including GxE interactions to be considered, to fully exploit the advantage of clones and the field trials needed, have been highlighted earlier (Soh *et al*., 2003a; 2006a, b) (*Figure 12*). If these issues are not addressed, the advantage of clones *vis-á-vis* hybrid seeds would be further undermined.

OPTCL which are not backed by good breeding programmes are less likely to be sustainable due to the inability to generate the necessary elite ortets to support the lower success rates expected, the larger field evaluation programmes required, and the high investment and operational costs. Industry needs to consider greater efforts in building up very comprehensive breeding programmes (existing or new), an approach which has been pursued in Indonesia, simultaneously with developing improved tissue culture techniques and commercial laboratories.

Finally, clones will only capture a major share of the oil palm planting material market when the issues of low culture amenability and unpredictability of fidelity can be resolved (via protocol manipulations) or circumvented (presumably through molecular marker technologies), and their field performance advantage over concurrent improved hybrids has been clearly demonstrated.

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REFERENCES

BLAL, B and GIANINAZZI-PEARSON, V (1990). Interest in endomycorrhizae for the production of micropropagated oil palm clones. *Agric. Ecosyst. Environ*., *29*: 39-43.

CORLEY, R H V (1993). Fifteen years of experience with oil palm clones – a review of progress. *Proc. of the International Oil Palm Conference - Agriculture Conference* (Basiron, Y *et al*., eds.). PORIM, Bangi. p. 69-81.

CORLEY, R H V; LEE, C H; LAW, I H and WONG, C Y (1986). Abnormal flower development in oil palm clones. *The Planter*, *62*: 233-240.

DEPARMENT OF STATISTICS, MALAYSIA. Monthly external trade statistics. Department of Statistics, Malaysia.

HARDON, J J; CORLEY, R H V and LEE, C H (1987). Breeding and selecting the oil palm. *Improving Vegetatively Propagated Crops* (Abbot, A J and Atkin, R K eds.). Academic Press, London. p. 63-81.

HO, Y W; TAN, C C; SOH, A C; WONG, G; CHONG, S P; CHOO, C N and NORAZURA, A (2009). Biotechnological approaches in producing oil palm planting materials – a success story. Oil palm for farmers' prosperity and edible oil security. *Proc. of the National Conference on Oil Palm* (Rethinam, P; Reddy, V M; Mandal, P K; Suresh, K and Prasad, M V eds.). SOPOPRAD, Pedavegi-534450, A.P., India. p. 86-93.

JONES, L H (1974). Propagation of clonal oil palm by tissue culture. *Oil Palm News*, *17*: 1-8.

KHAW, C H and NG, S K (1997). Performance of commercial scale clonal oil palm (*Elaeis guineensis* Jacq.) plantings in Malaysia. Paper presented at the International Society of Horticultural Science Symposium. Brisbane, Australia.

KUSHAIRI, A; TARMIZI, A H; SAMSURI, I; ONG-ABDULLAH, M; SAMSUL KAMAL, R; OOI, S E and RAJANAIDU, N (2010). Production, performance and advances in oil palm tissue culture. Paper presented at the ISOPB Seminar on Advances in Oil Palm Tissue Culture. Yogyakarta, Indonesia. 29 May 2010.

MATHES, M; SINGH, R; CHEAH, S C and KARP, A (2001). Variation in oil palm (*Elaeis guineensis* Jacq,) tissue culture-derived regenerants revealed by AFLPs with methylation-sensitive enzymes. *Theoretical and Applied Genetics*, *102*: 971-979.

MORCILLO, F; GAGNEUR, C; ADAM, H; RICHAUD, F; SINGH, R; CHEAH, S C; RIVAL, A; DUVAL, Y and TREGEAR, J W (2006). Somaclonal variation in micropropagated oil palm: characterization of two novel genes with enhanced expression in epigentically abnormal cell lines and in response to auxin. *Tree Physiology*, *26*: 585-594.

ONG-ABDULLAH, M and OOI, S E (2006). Biomarkers: finding a niche in oil palm tissue culture. Part 1 - laying the foundation. *Oil Palm Bulletin No. 53*: 30-35.

POTIER, F; NOUY, B; FLORI, A; JACQUARMARD, J C; EDYANA SURYNA, H and DURAND GASSELIN, T (2006). Yield potential of oil palm (*Elaeis gueneensis* Jacq.) clones: preliminary results observed in the Aek loba genetic block in Indonesia. Paper presented at the ISOPB Seminar on Yield Potential in Oil Palm. Phuket, Thailand, December 2006.

RABECHAULT, H and MARTIN, J P (1976). Multiplication vegetative du palmier a huile (*Elaeis guineensis* Jacq.) a l'aide de culures de tissues foliares. *C.R. Acad. Sci. Paris, Ser. D 283*: 1735-1737.

RIVAL, A; JAGLIGOT, E; BEULE, T and JEAN FINNEGAN, E (2008). Isolation and expression analysis of genes encoding MET, CMT, and DRM methyltransferases in oil palm (*Elaeis guineensis* Jacq.) in relation to the 'mantled' somaclonal variant. *J. Exp. Bot.* doi:10.1093/jxb/ern178. p. 1-11.

ROOWI, S H (2010). Progress of oil pal tissue culture in Felda anits challenges. Paper presented at the ISOPB Seminar on Advances in Oil Palm Tissue Culture. Yogyakarta, Indonesia. 29 May 2010.

SHARMA, M (2006). Challenges facing the Malaysian palm oil industry: multi-pronged strategy for raising the oil yield, productivity and profitability. *Proc. of the Clonal and Qty. Rep. Material* (Kushairi, A; Sambanthamurthi, R; Ong-Abdullah, M and Chan, K W eds.). MPOB, Bangi.

SIMON, S and KOH, H L (2005). An update of oil palm tissue culture clones in PPB Oil Palm Bhd - East Malaysia. Advances in breeding and clonal technologies for super yielding planting materials. 2005 National Seminar. Kuala Lumpur. 7-8 March 2005.

SMITH, D R (1997). The role of in-vitro methods in pine plantation establishment: the lesson from New Zealand. *Plant Tissue Culture and Biotechnology, 3*: 63-73.

SMITH, D R; YIMBANG, R; LEE, C and LORD, S (2010). High efficiency vegetative amplification – A new oil palm improvement system. Paper presented at the ISOPB Seminar on Advances in Oil Palm Tissue Culture.Yogyakarta, Indonesia. 29 May 2010.

SOH, A C (1986). Expected yield increase with selected oil palm clones from current DxP seedling materials and its implications on clonal propagation, breeding and ortet selection. *Oleagineux*, *41*: 51-56.

SOH, A C (1990). Oil palm breeding – breeding into the 21st century. *Plant Breeding Abstr.*, *60(12)*: 1437-1444.

SOH, A C (1998). Review of ortet selection in oil palm. *The Planter, 74*: 217-226.

SOH, A C (1999). Breeding plans and selection methods in oil palm. *Proc. Symp.: The Science of Oil Palm Breeding* (Rajanaidu, N and Jalani, B S eds.). PORIM, Bangi. p. 65-95.

SOH, A C (2004). Selecting the ideal oil palm. What you see is not necessarily what you get! *J. Oil Palm Research Vol. 16*: 121-128.

SOH, A C (2005). Super yielding oil palm - strategic breeding plan. Advances in breeding and clonal technologies for super yielding planting materials. 2005 National Seminar. Kuala Lumpur. 7-8 March 2005.

SOH, A C (2010). Review of strategies in oil palm clonal propagation. Paper presented at the ISOPB Seminar on Advances in Oil Palm Tissue Culture. Yogyakarta, Indonesia, 29 May 2010.

SOH, A C and CHOW, C S (1989). Index selection in oil palm for cloning. *Proc. of the 6th International Congress SABRAO* (Iyama, S and Takeda, G eds.). Tsukuba, Japan. p. 713-716.

SOH, A C and CHOW, C S (1993). Index selection utilizing plot and family information in oil palm. *Elaeis*, *5*: 27-37.

SOH, A C; CHOW, S C; IYAMA, S and YAMADA,Y (1994). Candidate traits for index selection in choice of oil palm ortets for clonal propagation. *Euphytica, 76*: 23-32.

SOH, A C; YONG, Y Y; HO, Y W and RAJANAIDU N (1995). Commercial potential of oil palm clones: early results of their performance in several locations. *Recent Developments in Oil Palm Tissue Culture and Biotechnology* (Rao, V; Henson, I E

and Rajanaidu, N eds.). Int. Society for Oil Palm Breeders, Selangor. p. 134-144.

SOH, A C; WONG, G; TAN, C C; CHEW, P S; HOR, T Y; CHONG, S P and GOPAL, K (2001). Recent advances towards commercial production of elite oil palm clones. *Proc. of the 2001 PIPOC International Palm Oil Congress*. MPOB, Bangi. p. 33-44.

SOH, A C; WONG, G; HOR, T Y; TAN, C C and CHEW, P S (2003a). Oil palm genetic improvement. *Plant Breeding Reviews, 22* (Janick, J ed.). p. 165-219.

SOH, A C; GAN, H H; WONG, G; HOR, T Y and TAN, C C (2003b). Estimates of within family genetic variability for clonal selection in oil palm. *Euphytica*, *133*: 147-163.

SOH, A C; WONG, G; TAN, C C; HOR, T Y and WONG, C K (2003). Revisited: cloning seedlings of reproduced best DxP cross strategy. Paper presented at ISOPB Seminar, Medan. 6-7 October 2003.

SOH, A C; WONG, G; TAN, C C; CHONG, S P; CHOO, C N; NOR AZURA, A and HO, Y W (2006a). Progress and challenges in commercial mass propagation of clonal oil palm. Paper presented at the IOPC 2006 International Oil Palm Conference. 19-23 June 2006.

SOH, A C; WONG, G; TAN, C C; CHONG, S P; CHOO, C N; NOR AZURA, A and HO, Y W (2006b). Advances and issues in commercial propagation of oil palm clones. Paper presented at the Ministry of Plantation Industry and Commodities Workshop.

SYED ALWEE, S; VAN DER LINDEN, C G; VAN DE SCHOOT, J; DE FOLTER, S; ANGENENT, G C; CHEAH, S C and SMULDERS, M J M (2006). Characterization of oil palm MADS box genes in relation to the mantled flower abnormality. *Plant Cell, Tissue and Organ Culture, 85*: 331-344.

SYED ALWEE, S; ROOWI, S H; AW, K T and OTHMAN, A Z (2010). Progress of oil palm tissue culture in Felda and its challenges. Paper presented at the ISOPB Seminar on Advances in Oil Palm Tissue Culture.Yogyakarta, Indonesia. 29 May 2010.

TAN, C C; WONG, G and SOH, A C (1999). Acclimatization and handling of oil palm tissue cultured plantlets. *Proc. of the 1999 PORIM International Palm Oil Congress*. PORIM, Bangi.

TAN, C C; WONG, G; SOH, A C; HOR, T Y; CHONG, S P and GOPAL, K (2003). Experiences from clonal evaluation trials and commercial test plantings. *Proc. of the 2003 PIPOC International Palm Oil Congress.* MPOB, Bangi.

TARMIZI, A H and ZAITON, R (2006). Two-in-One MPOB Simple Impeller (2-in-1 MOSLIM in Liquid System. *MPOB Information Series No. 303*.

TARMIZI, A H; NORIHAN, M A and ZAITON, R (2003). Multiplication of oil palm suspension cultures in bench-top two litre bioreactor. *J. Oil Palm Research Vol. 16*: 44-49.

TARMIZI, A H; ZAITON, R and ROSLY, M Y (2007). MPOB modified vessel (MoVess) for liquid system. *MPOB Information Series No. 355*.

WONG, C K (2010). Bench-marking best performing semi-clonal seeds against *tenera* clones: avenue for superior clonal ortet identification. Paper presented at the ISOPB Seminar on Advances in Oil Palm Tissue Culture.Yogyakarta, Indonesia. 29 May 2010.

WONG, G; TAN, C C and SOH, A C (1997). Large scale propagation of oil palm clones: experiences to date. *Acta Hort*., *447*: 649-658.

WONG, G; TAN, C C; SOH, A C and CHONG, S P (1999a). Clonal propagation of oil palm through tissue culture. *The Planter, 75*: 221-230.

WONG, G; CHONG, S P; TAN, C C and SOH, A C (1999b). Liquid suspension culture: a potential technique for mass production of oil palm clones. *Proc. of the 1999 PORIM International Palm Oil Congres - Emerging Technologies and Opportunities in the Next Millennium*. PORIM, Bangi. p. 3-11.

WOOI, K C; WONG, C Y and CORLEY, R H V (1981). Tissue culture of palms – a review. *Proc. of the COSTED Symposium: Tissue Culture of Economically Important Plants* (Rao, A N ed.). p. 138-144.

WUIDART, W and KONAN, K (1989). Weaning oil palm ramets. *Oleagineux*, *44*: 573-582.