International Seminar on
Status of Oil Palm Tissue Culture Technology

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Malaysian Palm Oil Board
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The International Society for Oil Palm Breeders
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Indonesian Oil Palm Research Institute
www.iopri.org
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INTERNATIONAL SEMINAR ON
STATUS OF OIL PALM TISSUE CULTURE TECHNOLOGY
ISOPB
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2017-2021

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   Vice President of the International Society for Oil Palm Breeders
ORGANISERS

Malaysian Palm Oil Board (MPOB)

Director-General: Datuk Dr Ahmad Kushairi Din
www.mpob.gov.my

MPOB is the premier government agency entrusted to serve the country’s oil palm industry. Its main role is to promote and develop national objectives, policies and priorities for the well-being of the Malaysian oil palm industries. It was established on 1st May 2000, taking over, through merger, the functions of the Palm Oil Research Institute of Malaysia (PORIM) and the Palm Oil Registration and Licensing Authority (PORLA). Each of these respective organisations has been involved in the oil palm industry for more than 20 years and it is to render more effective services as well as to give greater national and international focus to the industry that MPOB was instituted. The organisation’s activities include research, publication, development and implementation of regulations and the promotion of the palm oil industry in Malaysia. The MPOB oversees all stages of palm oil production in Malaysia, from planting to exporting to enhance the well-being of the Malaysian oil palm industry through research, development and excellent services.

The International Society for Oil Palm Breeders (ISOPB)

President: Dr Ahmad Parveez Ghulam Kadir
isopb.mpob.gov.my

The society is known as ‘Persatuan Ahli-ahli Pembiak Baka Kelapa Sawit Antarabangsa’or ‘The International Society for Oil Palm Breeders’. The general aim of the Society is to advance the knowledge of oil palm breeding through international co-operation. In order to achieve this aims, the Society has in the past and will continue to engage itself in the activities such as organising seminars and workshops both locally and internationally to facilitate the exchange and sharing of new knowledge in oil palm breeding and related aspects of oil palm cultivation. The Society also actively involved in establishment of various committees/working groups to deal with specific aspects or problems of oil palm for the purposes of exchange of views, international collaboration and dissemination of information. In addition, the Society also promotes and assists in international exchange of genetic material for breeding and selection.

Indonesian Oil Palm Research Institute (IOPRI)

Director: Dr Hasril Hasan Siregar
www.iopri.org

IOPRI is major oil palm research institute in Indonesia. Various technological packages have been produced by IOPRI and widely used in Indonesian oil palm industry such as superior oil palm planting materials, biological agents for pest and disease control, fertiliser formulations, integration of palm oil and cattle, and others. Additionally, IOPRI also provides laboratory analysis services (leaves, soil, water, oil and waste), technical assistance, and training. IOPRI has primary responsibilities to do research and development in all aspects of oil palm and distribute the results to the oil palm industry. The scope of the research done by IOPRI includes; breeding and plant biotechnology, soil science and agronomy, plant protection, technology engineering and environmental management yield processing and quality, and socio-techno economic.
WELCOME REMARKS

by

Dr Ahmad Parveez Ghulam Kadir
Deputy Director-General (R&D) of Malaysian Palm Oil Board
President of the International Society for Oil Palm Breeders (ISOPB)

Y Bhg Dr Hasril Hasan Siregar
Director, Indonesian Oil Palm Research Institute (IOPRI)

Distinguished guests, speakers and participants,

Ladies and gentlemen,

Good morning, on behalf of International Society For Oil Palm Breeders (ISOPB), I wish a warm welcome to speakers, members of the society, colleagues and participants to the international seminar on “Status of Oil Palm Tissue Culture Technology”. Recalling this topic was discussed 8 years ago in Yogyakarta, it is worth to attend to the progress of oil palm tissue culture since. I am certain, exciting progress in this discipline will be observed from various oil palm agencies worldwide, through the presentations delivered by speakers, who are oil palm tissue culturist themselves.

Ladies and gentlemen,

The first report on the success of oil palm tissue culture was in year 1975. Since then, this tool has given much room of imagination to crop improvement personnel, to exploit it for research and commercial. However, this discipline faced a major hurdle from a type of somaclonal variation, known as mantling, which was an abnormality at the oil palm female reproductive organ, first reported by Mr Tan Yap Pau (the oil palm breeder of United Plantation Bhd then), and in one of the seminars organised by ISOPB in the 1980s. Nevertheless, oil palm tissue culture had continued. Of those who persevered, fruit of success was observed; this fits well to a key character for scientific research and discovery.

Ladies and gentlemen,

Today, we heard of commercial sales of tenera clones. We heard of commercial clonal seeds; semi- and bi-. We also heard of high OER in mills attributing to clonal planting materials. In research, we heard of the discovery of scientific explanation to mantling and diagnostics kits for early detection is entailed; oil palm liquid suspension culture to improve tissue culture efficiencies; and possibly somatic artificial seeds. These exciting information of oil palm tissue culture development, warrant a detail look, and hence, this seminar is justified.
Ladies and gentlemen,

As the society has a mandate to encourage scientific information sharing, apart from presentations by eminent speakers, this seminar has also scheduled a forum session. Through forum discussion, we hope the participants would gather insights to oil palm tissue culture and hence, shaping the direction for future in this discipline, both research and commercial.

Ladies and gentlemen,

Last but not least, I would like to thank MPOB and IOPRI, being the co-organizer to this event, the speakers for sparing their time to share their knowledge with us, the organising committees and the secretariats, for ensuring all aspects of the seminar is in orderly manner, and most importantly, to all participants who had come near or far, making this seminar possible.

Thank you.
OPENING
REMARKS

by

Dr Hasril Hasan Siregar
Director of Indonesian Oil Palm Research Institute (IOPRI)

Dr Ahmad Parveez Ghulam Kadir,
President, International Society for Oil Palm Breeders (ISOPB),

Dr N Rajanaidu, the Chairperson of the Organising Committee of this International Seminar,

Distinguished Guests, Speakers, Participants

Ladies and Gentlemen,

It gives me a great pleasure to address this distinguished gathering of scientists in “International Seminar on Status of Oil Palm Tissue Culture Technology”. This event is held in conjunction with International Oil Palm Conference (IOPC) 2018, 17-19 July 2018. This Seminar is the seventh ISOPB and IOPRI collaboration after 1988 in Pematang Siantar, 2003 in Medan, 2010 in Yogyakarta, 2014 in Bali and 2016 in Kisaran.

Ladies and Gentlemen,

Oil palm tissue culture is interesting topics to elaborate, and to my understanding this is the third ISOPB gathering with theme on tissue culture after 1986 and 2010. Tissue culture technology promises up to 30% increase of oil productivity compared to seed planting material, yet until now few planters adopt the technology due to somaclonal variation. I hope with your expertise and knowledge will overcome this obstacle, hence the adoption of tissue culture in oil palm will thrive.

Ladies and Gentlemen,

In this International Seminar, 10 papers will be presented, and hopefully it will stimulate productive discussion among all of the participant. I hope our foreign guest will have an enjoyable stay in Medan and I look forward to meet you in IOPC in the next three days. I would like to wish thanks to chairpersons, speakers, participants, organising committee and others on organising this seminar. Finally, I now have the pleasure to declare open the ISOPB-IOPRI International Seminar on Status of Oil Palm Tissue Culture Technology.

Thank you.
SESSION 1
ABSTRACT

Commercial oil palm tissue culture clonal propagation took-off (with many production labs set-up) at the start of the new millennium since the first report of plantlet regeneration in the mid-1970s and its subsequent set-back due to the ubiquitous mantled fruit syndrome reported since 1986. However, to date commercial ramet production and plantings have yet to make a significant impact in the Industry, constituting much less than 10 percent of the 400 to 500 million seeds produced worldwide annually. The risk of mantling and poor cloning efficiency resulting in high cost of ramets and the inability to demonstrate convincingly the superior yield advantage of clones over concurrently improved DxP hybrids have been the dominant issues. With the discovery of the epigenetic cause of mantling and the imminent development of a more efficient molecular marker and that ramets derived from recloning and liquid proliferation of non-mantled clones appear to be repeatable, in terms of non-mantling and amenability, further opens up the feasibility of larger scale production of proven clones. However, OPTC is still an inefficient process owing to the low and very low frequency of ortets amenable to cloning and liquid culture resulting in <0.2% success on explant and <10% and <1% of the superior ortet genotypes captured respectively. In simplistic plant breeding genetic terms, these genotype (G) and genotype x protocol/medium (GxE) effects need to be researched and addressed with a wider range of genotypes and protocols to broaden the genetic base of clones for sustainability besides profitability from increased ramet production. The development of OPTC amenability (embryogenesis, proliferation) biomarkers especially for the liquid suspension system together with the mantling marker would facilitate these efforts. There have been few reports that demonstrate the superior advantage of clones objectively with statistically replicated trials and with concurrently improved DxPs as controls. The possibility of using genomic selection to screen superior seedlings from DxP hybrids for commercial planting poses another challenge to the basic premise and rationale of cloning. Continuing R&D is essential to ensure the sustainability of commercial oil palm clone production.

* University of Nottingham Malaysia Campus, Kuala Lumpur
INTRODUCTION

The first attempts at commercial oil palm tissue culture (OPTC) propagation started in the early 1990s following the first reports on oil palm plantlet regeneration in the mid-1970s (Jones 1974, Rabechault and Martin, 1976) initially by Unilever (Unifield/Bakasawit) and Cirad (Tropiclone), the companies which achieved the first plantlets, and soon followed by others e.g AAR, Agrocom, UP etc (Soh et al. 2017a). When the spectre of the sterile mantled fruit syndrome reared its ugly head in clonal palms produced by all the commercial labs in the late 1980s and early 1990s, all of them reverted to R&D. Confidence in commercial clonal propagation resurrected at the turn of the new millennium with the first reports of successful large scale commercial production and planting of clonal palms or ramets by gel (GC) and liquid suspension (LSC) cultures with minimal mantling risk by e.g. AAR, Agrocom, Felda (Wong et al 1997, 1999; Soh et al. 2011). The “mushrooming” of commercial OPTC labs soon followed in Malaysia and elsewhere even by labs without supporting oil palm breeding programs.

COMMERCIAL CLONE PROPAGATION: THEN, NOW AND FUTURE

Then & Now

Commercial clones have yet to make significant impact in terms of production and field plantings on the Industry for the past 15 years or so. In fact, ramet production could have stagnated or fallen with labs limiting production for in-house plantation use or even dropped out of the market due to non-competitiveness for one reason or another (Soh et al 2017b). The prevailing impeding issues have been:

Risk of Mantling. The unpredictability of mantling and its severity clone and ramet-wise which are only expressed when the ramets are fruiting. Recloning (RC) from ramets and LSC have traditionally been deemed to have higher risk of somaclonal variation.

Inefficient OPTC Process. Callus production is not an issue: ortet-wise (100%) and explant-wise (ca.30%) because of the large number of explants cultured (ca.1000). Embryoid formation (embryogenesis) and proliferation are the key issues: ortet-wise (up to 70%) and explant-wise (<5%). However, only <10% and <1% of the ortets can produce actively proliferating embryos for commercial ramet production for the GC and LSC cultures respectively (Soh et al, 2011, Choo C.N. per comm.). In practice, to hedge on risk of mantling yet achieving commercial scale production, commercial labs are obliged to clone a basket of ortets (primary ortets and ramets) and from GC and LSC cultures.

It has been estimated that to achieve an annual ramet production rate of 500,000 from 5 years onwards in the future, about 100 ortets need to be put into culture annually, which poses a great challenge to most programs. Stringent culture selection with frequent subculture onto fresh media (2-3 months’ interval) and limiting production cycles to e.g. 18 subcultures or 5000 shoots per line are commonly practised to hedge on somaclonal variation risk.
**Inefficient Ortet Selection.** Fresh fruit bunch (FFB) yield per palm is poorly heritable ($h^2 = 0.01-0.14$); oil yield (OY, $h^2 = 0.22-0.30$); oil to bunch, (OB, $h^2 = 0.37-0.56$) are better (Soh et. al. 2003), the higher values were from progenies of more outbred parents. Preferably, primary ortets should be selected from the best palms in the best families from progeny-test trials, based on OY based biasedly towards the more heritable oil yield components OB, mesocarp to fruit (MF) and oil to mesocarp (OM) and the palms should not be competitive in vegetative growth or enjoy undue advantage (space, soil fertility) over its neighbours (Soh 1998). Selection from commercial DxP is even more inefficient due the very low individual palm heritability arising from variable environment and palm genotype x environment interaction. Selection of ramets from the best clones from clonal evaluation trials for recloning (RC) should be very efficient as each ramet has been replicated in the plot and block, so long it is true-to-type (genotype) and has no apparent signs of somaclonal variation besides mantling.

**Yield Advantage of Clones**

**Trials.** Superior yield advantage >20-30% from clones has commonly been claimed from trials with the prevailing generation of DxP (commercial or standard cross) as controls. The general experience from a number of well-run clonal trials revealed that on average the mean OYs of the clones were about 10-15% at best better than the DxP control and could be even lower if the clones were derived from DxP of highly selected (inbred) parents (Soh et. al. 2003a, 2011; Poitier et al., 2006 ). Undoubtedly there would be clones yielding >30% which could be recloned. But by the time the reclones are available they have to be judged against the concurrently available improved DxP (generally < 10-15% OY improvement). Nevertheless, a 5-10% OY advantage of the RC over the concurrently improved DxP (if realized) would be still substantial and justifiable for the higher cost of the ramet. An experiment is under way to concurrently clone the seedlings of new DxP crosses and plant them together with the cross seedlings undergoing progeny-testing (Soh et al. 2003b). When results become available the best clones superior to the best crosses (which will form the next generation of improved DxP) could be identified for recloning. In so doing the commercial clones are always ahead of the new improved DxP (Soh et al, 2011).

**Commercial fields.** Often-times it is difficult to objectively demonstrate the advantage of clones for the following reasons:

Ramets tended to be planted on better areas and sometimes given better agro-management inputs.

Very high FFB yields reported for ramet plantings have also been achieved by DxP plantings

Commercial ramets comprise a basket (1 to many) clones with varying ramet numbers per clone depending on the ‘flavor of the day” predisposing the plantings to somaclonal variation, poor pollination, poor adaptability and P&D risks.

FFB yields are greatly influenced by environment and management. Different clones have different agro-management requirements e.g. fertilizer; a common management practice may not exploit the potential of individual clones. Current clones selected from high yielding palms usually from good growing areas are usually not adaptable to poor environments.

Improved oil extraction rates (OER) from clonal plantings are easier to demonstrate by charting the rising OER trend from a mill corresponding with increasing areas of ramet plantings or dedicated OER runs with ramet crop against commercial DxP crop and better still compared to DxP crop of similar age as the ramet planting (Soh et al, 2011).
Now & Future

With the discovery of the epigenetic cause of mantling followed by the imminent development of a more efficient molecular marker and that ramets derived from RC and LSC proliferation of non-mantled clones appear to be repeatable or heritable, in terms of non-mantling and amenability, further opens up the feasibility of larger scale production of proven clones (Low et al. 2008, Chan et al. 2010, Choo et al. 2013, Ooi et al. 2012, 2013, Ting et al. 2011, Ong-Abdullah et al. 2015). However, OPTC is still a very inefficient process owing to the low and very low frequency of ortets amenable to cloning (GC and LSC) resulting in <0.2% success on explant and <10% and <1% of the superior ortet genotypes captured respectively. In simplistic plant breeding genetic terms, these genotype (G) and genotype x protocol/medium (GxE) effects need to be researched and addressed with a wider range of genotypes and protocols to broaden the genetic base of clones for sustainability besides profitability from increased ramet production. Previously, tissue culturists shied from such R&D attempts which would incur protracted and expanded efforts to raise cycles of cultures from the lab through to the field till fruiting to ascertain the fidelity of the clones. The development of OPTC amenability (embryogenesis, proliferation) biomarkers especially for the LSC together with the mantling marker would facilitate these efforts especially if the screening could be effected early, ideally at the lab level. These breakthroughs with the RC of best clones and the automated large scale LSC production of embryoids that could be packaged into artificial or synthetic seeds, the technology of which has been established (Mariani et al. 2014, Ong-Abdullah 2017) would bring down the cost of ramets with high yielding potential substantially. These breakthroughs would also allow early commercial cloning exploitation of ortets from wide crosses e.g. Oleifera (O) x Guineensis (G) F1 and backcrossed hybrids, GxG hybrids involving less advanced breeding parents which would otherwise entail a protracted breeding program, to improve genetic diversity in commercial plantings (Alvarado et al. 2017, Sritharan et al. 2017). The breeding for clonal propagation approach as practised in traditional clonally propagated crops e.g. cassava, rubber, could also be adopted (Soh et al. 2017).

The current developments in Genomic Selection (GS) in oil palm may prove to be a boon or bane to OPTC (Cros 2017, Cros et al. 2015a,b, Jacob et al. 2017, Kwong et al. 2016) The former would facilitate the identification of superior combining parents for cloning for clonal seed production and their hybrid progenies for selection of ortets for ramet production. This coupled with screening markers that would identify cultures amenable to cloning, RC and proliferation in LSC would enable very large production and planting of superior planting materials. The latter would be like the “new elephant in the room” or “spanner in these OPTC works” case whereby GS is used to select the best DxP seedlings for commercial field planting and obviate the need for cloning. Such an experiment has been planted to validate this (Teh et al. 2017, et al, Kulaveerasingam et al. 2017). Some currently perceived caveats: high cost of genotyping individual seedlings which could possibility be reduced in time with high throughput technological advancement; larger than usual breeding population sizes needed; limited to single trait, inclusion of other desirable and may be related traits would require a multiple selection index type or multi-trait BLUP GS which may be more complicated analytically but would improve accuracy of prediction (Marchal et al. 2016); may be less efficient with wide-crosses especially with Guineensis (G) and Oleifera (O) hybrids and backcrosses due to negative linkages and genomic instability (Soh et al. 2017c).
CONCLUSION

OPTC is entering interesting and challenging times. With the imminent development of efficient biomarkers for mantling, tissue culture amenability (embryogenesis, prolificacy) GC and LSC-wise, which are heritable traits, cultures could be screened early and enable larger scale propagation. However, genotype and genotype x media/protocol differences restrict considerably the range of genetic materials planted in the field predisposing to vulnerability to biotic and abiotic stresses and hence reduced sustainability. With the early screening biomarkers now available R&D to test a wider genetic range of ortets against different media/protocols is now feasible and should be pursued rigorously. Genomic selection has the potential to assist in selection of the parents of superior hybrid families to clone for clonal seed production and the superior families as source of ortets for cloning. Conversely, GS on individual palms within superior families/populations if proven feasible has the potential to undermine the rationale for cloning. The winning technology i.e. OPGS versus OPTC would be the one with highest yield achievable with comparatively lower cost and ease of field management.

The other applications or technology arising from OPTC are already in place: cryopreservation (genetic conservation, immortal reference lines (Texiera et al 2013), protoplast culture for transgenic lines (Masani et al 2013), ploidy lines (Iswandar et al. 2010).

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A Holistic Perspective of Current Progress in Oil Palm Tissue Culture

Ong-Abdullah M; Tarmizi, A H; Zamzuri, I; Samsul Kamal, R; Ooi, S E; Naqiuddin, M H; Dalilah, A B; Norashikin, S; Nuraziyan, A; Pang, J T Y; Siti Rahmah, A R; Mohd Isa, Z A; Singh, R; Low, E T L; Nookiah, R; Ordway, J M; Jiang, N; Smith, S W; Lakey, N; Mohamad Arif, A M; Ahmad Parveez, G K; Martienssen, R A and Sambanthamurthi, R

ABSTRACT

Plant tissue culture is one of the earliest and most widely used agriculture technology available. Since its introduction in the 1970’s, it brought along a promise of increased productivity of at least 10-15% against the most improved current DxP and was touted as the ‘second wave’ in yield improvement for the oil palm. The main advantage of clones is in the fact that they are carbon copies of the selected individual (ortet) generally sourced from the best crosses. And when these are planted in a designated plots, their uniformity or true-to-typeness contribute to increased yields per planted area. This is becoming more important as agricultural products are expected to increase by 70% and net exports of oilseeds and vegetable oils are likely to triple by 2050 to meet the projected population growth of ~9 billion. Thus, there is a need to intensify productivity amidst the pressure of a shrinking land resource whilst balancing the environmental and social goals. However, after close to 40 years since its first introduction, the oil palm clonal industry has yet to really make a significant impact to uplift the national productivity. The two main challenges faced with the cloning technology are the inefficiency of the tissue culture process and the possible emergence of somaclonal variants. Despite the fact that most oil palms selected for cloning and recloning have 72 to 88% success rates, their respective callogenesis and embryogenesis rates are at a mere 14-19% and 3-7%. This has certainly impacted the cost of producing ramets as compared to seed-derived materials. In addition to that, the scaling up of ramet production in the early 1980s unearthed yet another hurdle for tissue culture, that is an increased frequency of abnormal clones following field transplanting.

* Malaysian Palm Oil Board, 6, Persiarian Institusi, Bandar Baru Bangi, 43000, Kajang, Selangor, Malaysia.
The most common somaclonal variant observed was the mantled fruit phenomenon. These challenges have certainly affected the growth of the clonal industry as a whole and contributed to the lack of confidence in pushing forward plans of increasing the clonal hectarage in oil palm plantations. On the contrary, with conventional hybrid breeding requiring inbred parents which would take at least 20 years to achieve superior yields of individual palms, cloning technology would allow the process to be fast-tracked. Furthermore, over the years, tissue culture processes have undergone improvements and new innovations have been developed to expedite production. Clonal trials have been extensively conducted and the results have been encouraging. This has led to the selection of clones with special characteristics for future cloning and recloning. Leveraging cutting-edge molecular tools as well as platform technologies have also helped in regaining confidence in the utilization of clones. Biomarkers associated to embryogenesis and KARMA have been developed into predictive tools that can easily be adopted as part of a quality assurance procedure in clonal production. Generally, for clonal planting materials to have an impact on the nation’s productivity its utilization needs to be more extensive. Currently, only about 2-3% of the planted oil palm area is represented by tissue culture-derived materials. In order to encourage investment in clonal plantings amongst smallholders, contract farming was suggested. It will come a time when yield improvements would one day reach its plateau and genetic manipulation may be the only approach able to break these yield barriers. In this situation, cloning technology would be crucial as it is the prerequisite for its success. Despite the current inadequacies in clonal technology, some industry players have reported positive benefits from utilizing clones in their respective plantations and we have also witnessed more laboratories being established. We believe the situation will improve as we continue to leverage new science and technologies to further enhance the cloning process.
The Current Status of SE Technology Developed by IRIBB to Reduce the Abnormality in Oil Palm

Sumaryono*; Imron Riyadi and Priyono

ABSTRACT

Abnormal floral and fruit development of tissue culture derived oil palm (*Elaeis guineensis* Jacq.) has hindered the progress of tissue culture of oil palm in Indonesia. Tissue culture of oil palm is usually carried out on agar-solidified media for all stages. Alternative approach involving the use of cell suspension culture for oil palm somatic embryogenesis (SE) has been attempted in order to explore its potential for automation and scaling-up of somatic embryos production and to reduce floral abnormality of oil palm. Suspension cultures of oil palm at IRIBB were started in shake flasks and then scaled up in stirred tank and temporary immersion system (TIS) bioreactors. The growth rate of embryogenic calli of oil palm in TIS immersed for 3 min with 6 h interval was significantly higher than that of on solid media. Through better synchronization of embryo development, maturation and germination using TIS, production of uniform somatic embryos and recovery of normal plantlets of oil palm become more feasible. In addition, the brief exposure of propagules to the medium is expected to decrease the abnormality of ramets. Field tests of the ramets showed that the level of floral and fruit abnormalities (androgynous, hermaphrodite, and mantled) was less than 1%. The use of TIS for oil palm SE in conjunction with stringent practice of tissue culture protocol, proper selection of embryogenic calli, and limitation of culture period have demonstrated to reduce floral abnormality in oil palm.

Keywords: oil palm, somatic embryogenesis, suspension culture, TIS, floral abnormality

INTRODUCTION

Tissue culture of oil palm (*Elaeis guineensis* Jacq.) has been developed since early 1970s and world production of oil palm plantlets was estimated around 3.5 million per year in 2009 (Kushairi *et al*., 2010). This number is only a small fraction of the total oil palm planting material, considering its superior characteristics such as higher yield than hybrid seeds. Two of the possible reasons, among others for the minor use of clonal plants are floral abnormality found in the field and inefficiency protocol of oil palm tissue culture (Soh *et al*., 2011).

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Oil palm tissue culture (OPTC) is usually carried out on agar-solidified media for all developmental stages. However, solid cultures have several disadvantages such as low callus multiplication rate, unsynchronized somatic embryo development, low maturation and germination rates of somatic embryos, and time consuming in cleaning, filling and handling of a large number of containers. These features make the protocols on agar-solidified medium unsuitable for large-scale production and very costly (Duval et al., 1993).

The use of suspension culture system has a potential for enhancement of somatic embryo multiplication and synchronization of embryo development for scaling-up production of somatic embryos under bioreactor conditions. In addition, the liquid media can be easily renewed without changing the vessels. Thus, production cost can be reduced because of the relative ease of handling bulk cultures in bioreactors. The use of bioreactor vessels also requires smaller space in a culture room than the use of many containers.

The suspension cultures of oil palm have been developed by de Touchet et al. (1991), Duval et al. (1993), Sumaryono et al. (1994; 2008; 2010), Teixeira et al. (1995), Ginting & Fatmawati (1997), Tahardi (1998ab; 1999), Tarmizi et al. (2003; 2004), Syed Alwee et al. (2010), and others in order to scale up the production of oil palm SE. The latest suspension cultures of oil palm that IRIBB developed is temporary immersion system (TIS) to scale up SE production but possibly has other benefit which is to reduce the floral abnormality because of a brief exposure of the inoculum to the medium. Syed Alwee et al. (2010) reported that mantling rate of clonal oil palms derived from gelled medium on average was 2.5% whereas from liquid medium was 1.5%.

**LIQUID CULTURE IN SHAKE FLASKS**

The Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) started to carry out OPTC in liquid cultures since early 1990s (Sumaryono et al., 1994; Tahardi, 1998a) using Erlenmeyer flasks. Friable embryogenic calli initiated from immature leaves were cultured in DF and modified MS media in 100 mL Erlenmeyer flasks. Treatments were applied such as basal media, different macro and micro nutrients, vitamin, auxins, cytokinins, and activated charcoal in order to obtain higher calli growth rate and more synchronous development. The flasks were placed on a rotary shaker at 100 rpm. Homogenous embryogenic calli of oil palm in suspension culture have been obtained where the callus was white, small, and started to form proembryos (Sumaryono et al., 1994).

Tahardi (1998a) developed further suspension cultures of oil palm in shake flasks by employing medium composition of B5 containing very low concentrations of 2,4-D and kinetin. Callus growth followed sigmoid curve where the first two weeks was a lag phase then increased drastically after two weeks and after which it began to level off. It indicates that the appropriate time for subculture should be 4 weeks. The embryogenic cell growth rate was 1.5-2 fold in 4 weeks, less than that obtained by de Touchet et al. (1991) at 4 fold in a month. The embryogenic suspension cultures were homogenous, consisting of small, white and opaque callus clumps of less than 1 mm in size (Figure 1a), and no browning of tissue was observed.

Induction of somatic embryogenesis was triggered by transferring the embryogenic aggregates to agar-gelled medium without plant growth regulators. The aggregates started to
differentiate, forming numerous small nodules connected to one another. As the nodules began to develop, they were dissociated and formed individual lobed structures representing proembryos indicating the early formation of somatic embryos (Figure 1b). Proliferation and further differentiation of these proembryos led to the formation of many clumps of globular and elongated embryos (Figure 1c). However, asynchronous germination of somatic embryos occurred after several passages on the solid medium (Figure 1d).

![Figure 1](image)

Figure 1. Embryonic suspension culture of oil palm in shake flasks and induction of somatic embryos in the solid media (Tahardi, 1998a).

**LIQUID CULTURE IN STIRRED TANK BIOREACTORS**

For commercial purposes, the use of shake flasks for somatic embryogenesis mass production is considered not feasible. Therefore, the suspension culture procedure of oil palm SE that has been developed in shake flasks must be scaled up in bioreactors. The scaled up of oil palm SE in bioreactors including TIS after all conditions, especially soluble components like nutrients and plant growth regulators, have been optimized in small scale shake flasks. Stirred tank bioreactors with volume capacity of 1 L (Bioindustrie Mantovane) and 5 L (Applikon) (Figure 2) have been used (Tahardi, 1999). Different types of bioreactors have been used for the production of SE in many crops (for a comprehensive review see Fei & Weathers, 2016).

Stock suspension culture was prepared in 100 mL Erlenmeyer flasks. Embryogenic cell aggregates were filtered through nylon clothe sieves of 1000 and 200 µm pore size at the time of subculture to obtain more homogeneous cell suspensions. Fifty mL of the stock suspension culture were inoculated into 1-L bioreactor, and 200 mL into 5-L bioreactor. The operation of the bioreactor was under the command of the Control Module (Figure 2 left). The module controlled the agitated speed at 60 rpm, medium pH at 5.3-5.7, dissolved oxygen at 60-100%, and temperature at 27 °C.
Tahardi (1999) reported that oil palm embryogenic cells can be successfully cultured in a bioreactor environment with reasonably high growth rates. With the lowest initial cell density of 5% packed cell volume (PCV), the cells grew as much as 5-6 fold in 4 weeks. High growth rate was also reported by Tarmizi et al. (2004) using a 2-litre bioreactor that the proliferation rate of embryogenic cells was 9-14 fold after 50 days in culture, and by Gorret et al. (2004) reported an increase in biomass of embryogenic cells of 3.5 fold per month. Tahardi (1999) showed that cell viability was maintained at nearly 90% level throughout the culture period of 12 weeks. However, the embryogenic cells were not able to be induced to somatic embryos. In addition, some problems were encountered in bioreactor suspension cultures such as foaming, cells attached to the vessel wall, and clumping of cell aggregates. Some problems such as most cell aggregates were lodged in between the baffle cage and inner wall of the vessel and severe shear damage during stirring (Tarmizi et al., 2004) before they modified the bioreactor.

**LIQUID CULTURE IN TIS**

The use of cell suspension culture for oil palm propagation via somatic embryogenesis is very promising but suspension cultures still pose a major weakness, leading to tissue hyperhydricity which may be due to continuous immersion of propagules in liquid culture and lack of aeration (Tahardi, 1998b). These disadvantages of liquid cultures can be overcome by periodic immersion system or known as temporary immersion system (TIS). This system allows for periodic wetting of the inoculum with nutrient medium for a specified frequency and duration.

Nutrient medium is placed in the bottom chamber, while the plant materials (calli, somatic embryos, or germinants) are in the upper chamber. The medium is pressure fed via tubing by an air pump from the bottom chamber into the top growth chamber to the level of the inoculum placed on a polyurethane foam disc. The medium is held in the top chamber for a short period of time and then drained back to the bottom chamber until the next filling. The plant materials are immersed in the bubbling medium, so providing gentle mixing and headspace gas renewal.

TIS was used successfully in SE cultivation of coffee (Ducos et al., 2007), sugarcane (Mordocco et al., 2009), rubber (Etienne et al., 1997), cacao (Niemenak et al., 2008), tea (Tahardi et al., 2003), peach palm (Heringer et al., 2014), sago palm (Riyadi & Sumaryono,
2009), date palm (Othmani et al., 2011), and many other crops (Etienne & Berthouly (2002). TIS has also been used in SE of oil palm (Tahardi, 1998b; Tarmizi et al., 2003; Sumaryono et al., 2008; Syed Alwee et al., 2010; Marbun et al., 2015).

At IRIBB somatic embryogenesis of oil palm in TIS was first developed using Nalgene flasks with 250 mL volume capacity (Tahardi, 1998b). This apparatus needs a double timer to push the air to lift the medium into the upper chamber, and to pull air to make the medium back into the bottom chamber. The nutrients and plant growth regulators used were the same as for shake flask cultures. Tahardi (1998b) compared the growth and the expression of somatic embryos of oil palm for 8 weeks of culture in TIS and on solid medium. Approximately 0.5 g nodular calli were placed on a screen disc in the upper chamber and 250 mL medium was placed in the lower chamber. Immersion of the calli was programmed for a duration of 3 minutes at a frequency of every 6 hours.

After two culture periods (4 and 8 weeks) in TIS the calli texture that initially large nodular callus clumps has changed gradually into smaller granular callus aggregates. This change was not evident on the solid medium. In the first culture there was no significant increase in somatic embryo numbers on both culture systems, but after the second period of 8 weeks the number of somatic embryos had increased by 35 folds in TIS, whereas insignificant on solid medium (Table 1). After 8 weeks of culture, somatic embryos regenerated under the TIS conditions, 71.9% of the total 523 embryos were globular embryos, 21.8% developing embryos, and 6.3% mature embryos, whereas on solid medium all 27 embryos were at globular stage. It was concluded also that TIS allows better synchronization of somatic embryo development thus enabling production of more uniform somatic embryos of oil palm.

| TABLE 1. EFFECT OF CULTURE SYSTEM AND PGR ON THE EXPRESSION OF OIL PALM SOMATIC EMBRYOS FROM NODULAR CALLI AFTER 4 AND 8 WEEKS OF CULTURE (TAHARDI, 1998B) |
|---|---|---|---|
| Culture system | Plant growth regulator | No. of somatic embryo | Stages of embryo development |
| | 2,4-D Kin | Culture period (mg/L) | Pro-embryo | Developing embryo | Mature embryo |
| | | (w) | (%) |
| TIS | 0.01 0.1 | 4 8 | 71.9 | 21.8 | 6.3 |
| | 0.10 0.1 | | 75.3 | 15.9 | 8.8 |
| Solid medium | 0.01 0.1 | 17 27 | 100 | 0 | 0 |
| | 0.10 0.1 | 8 11 | 100 | 0 | 0 |

IRIBB also employed TIS with Nalgene flasks using embryogenic calli, instead of nodular calli as the starters for suspension cultures (Sumaryono et al., 2008) to determine callus
proliferation as well as induction and maturation of somatic embryos. Fresh weight of callus biomass increased steadily on recycled cultures up to 24 weeks. The best transfer interval was 4 weeks with the growth rate at 0.38 g/g/week, but to decrease the numbers of subculture, transfer interval of 6 weeks should be chosen. At 6-week transfer interval, somatic embryos started to emerge after one culture cycle, and at 18 weeks culture period (3 culture cycles), almost a half of the biomass consisted of somatic embryos. At the fourth of culture period, the culture was composed of 80% somatic embryos. Maturation of embryos was carried out in DF medium containing 0.5 mg/L kinetin + 0.05 mg/L ABA and added with GA₃ for germination. The number of germinants was 56 per flask after 6 weeks (Sumaryono et al., 2008).

Since 2007 RITA flasks (Vitropic) (Figure 3) have been used for TIS of somatic embryogenesis of oil palm and other crops. This system is still used two chambers (compartments), the bottom one is for the liquid medium while the upper one for the inoculum, but it is easier in handling than Nalgene flasks because the screen disc on the upper chamber together with the inoculum can be subcultured directly to the bottom chamber containing new medium of a new flask. In addition, the capacity is also bigger at 1 L volume. This apparatus needs a single timer only to push the air to lift the medium into the upper chamber. When the air flow is stopped, the pressure in the two chambers adjusts and the medium returns to the bottom of the vessel by gravity force.

Collaborating with PT Sampoerna Agro Tbk., IRIBB has developed SE-TIS of six varieties of oil palm using RITA flasks. All conditions such as nutrients, plant growth regulators, and environmental factors were the same as for the Nalgene flasks, except the medium volume was 170 mL per flask. The immersion duration was adjusted to 3 min with interval every 6 hours. Other study by Marbun et al. (2015) found that the best immersion was 3 min with 3 h interval. The growth of embryogenic callus in TIS was significantly faster than that of solid medium. After 8 weeks of culture, biomass fresh weight in TIS increased by 3-fold, twice as that of on solid medium (Sumaryono et al., 2010). Initiation, maturation and germination of somatic embryos of oil palm can be conducted in TIS.

![Figure 3. Temporary immersion system (TIS) RITA® Vitropic.](image)
Culture stages of SE-TIS of oil palm (Figure 4) is started with calli initiation from young leaf explant on solid medium. Calli formation (callogenesis) in oil palm is very slow, the first calli emerge after 3 months of culture. After several times of proliferation on solid medium, primary calli can be cultured in TIS for proliferation of calli, and initiation, maturation and germination of somatic embryos. Small shoots development is conducted on solid medium and then rooting of the plantlets is conducted in liquid medium in the culture tubes. Plantlets with height of at least 10 cm and have a good root system are ready to be acclimatized to *ex vitro* conditions.

![Figure 4. Culture stages of SE-TIS of oil palm.](image)

**FIELD TESTS**

Tissue culture derived plants of oil palm (so called ramets) from TIS and solid medium cultures were field planted in Mesuji district, South Sumatra and in Bogor, West Java, Indonesia. In Mesuji, the first ramet batch was planted in 2010, followed by others in the next 3 years, with total of almost 2000 ramets in 14.7 ha area. In the first year of productive period, the growth of ramets (Figure 5a) were taller than the hybrid trees (Figure 5b). It means that the growth of ramets was better than that of hybrid seedling trees. Observations on generative parameters (Figure 5b-d) showed that the floral sex ratio was more than 90%, and the percentage of abnormal fruits (androgynous, hermaphrodite, and mantled) was less than 1%. In the fifth year of productive period (8 years of age) the abnormal fruits were not found anymore. On the younger ages, only two trees were found to have mantled fruits and both trees were derived from plantlets cultured on solid media.

A field test in Bogor, West Java was conducted in 2014 consists of 77 ramets and 29 hybrid seedling trees (Figure 6). The floral sex ratio was 95% on ramets and 72% on seedling
trees. Hermaphrodite fruits were found 0.07% on ramets and 0.18% on seedling trees. Abnormal fruits in form of androgynous and mantled fruits were not found on all oil palm trees.

**Figure 5. A Field-test of ramets in Mesuji, South Sumatra. Growth of (a) ramets and (b) hybrid oil palms, (c & d) Normal fruits, and (e) Hermaphrodite fruits.**

**CONSTRAINTS AND PROSPECT**

In spite of many promising results, culturing in bioreactors proves to be trickier than culturing on agar-solidified media in terms of contamination risks and hyperhydricity (vitrification). Contamination is one of the major problems in cell and tissue cultures (Leifert & Cassells, 2001). In TIS there is an air flow from outside into the liquid medium that increases the chance of contamination compared with a stationary solid medium. In our laboratory, in the past the level of contamination, mostly due to bacteria was high at 25-30% in SE of oil palm, sago palm and sugarcane (Sinta et al., 2014). Experiments have been carried out to solve this problem and the results revealed that from all components of TIS compartment, washer (a small ring seal connecting screen disc and basket) was the main source of TIS contaminant (41.2%). Four contaminants found were bacteria identified as *Bacillus macerans, B. megaterium, B. sphaericus* and *B. firmus*. Two times sterilization of the washer in an autoclave at temperature of 121 °C and air pressure of 1 kg/cm² for 20 minutes before and after being installed reduced the contamination.
level on TIS culture to 10% (Sinta et al., 2014). Efforts on reducing further the level of contamination are still being conducted.

Hyperhydricity is mainly found in suspension cultures with continuous immersion of plant materials in the liquid medium. Albarran et al. (2005) observed that hyperhydricity on coffee somatic embryos increased with the immersion frequency from two times to six times per day. In TIS the duration and frequency of immersion can be adjusted accordingly to decrease somatic embryo and shoot hyperhydricity.

Other concern is light transmittance into the inoculum especially when the somatic embryos become mature in the cotyledonary stage that already have chlorophyll. Light is important for germination of SEs and their following plantlet development (Afreen et al., 2002). However, the cylindrically shaped and less transparent vessels in TIS restrict light penetration into the center of the compartment, therefore restricting SE development (Ducos et al., 2007).

The prospect to apply oil palm suspension cultures using temporary immersion system is very promising to scale up the culture production, to increase embryo production, to synchronize somatic embryo development, to shorten culture period, to decrease the use of labors and culture space, as well as to reduce the floral abnormality of oil palm. The use of TIS for oil palm SE in conjunction with stringent practice of tissue culture protocol, appropriate selection of embryogenic calli, and limitation of culture period to less than 20 subcultures have shown to reduce floral abnormality of clonal oil palms in the field. Early this year IRIBB has started to produce more than 300,000 ramets for the next 2-3 years using this SE-TIS technology.

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Bactris gasipaes
Saccharum
Elaeis guineensis
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Menara Perkebunan Sumaryono research and development towards realization. Azura Soh, (Indonesian).

liquid culture of temporary immersion system. Sinta maturation of somatic embryos of sago palm Riyadi Netherlands date palm micropropag

free amino acids in different tissues. of somatic embryos in Niemenak hybrids). immersion system (RITA Mordocco A

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SESSION 2
Experience and Challenges in Commercial Production of Elite Oil Palm Clones in Applied Agricultural Resources Sdn Bhd

Choo, C N; Wong, C K and Melody, M P

ABSTRACT

The tissue culture laboratory of Applied Agricultural Resources Sdn Bhd (AAR) was set up in 1986 initially as a research unit to micropropagate oil palm and other crops. Early cloning results indicated that the clones performed well in the field with minimal somaclonal variation. With the confidence in our cloning system, the expansion of the laboratory took place in 2005 with an annual capacity of one million ramets annually. Many challenges need to be overcome in order to ensure the success of large scale ramet production. One of the challenges is effectively selecting genetically superior ortets for cloning and also to improve the efficiency of current oil palm tissue culture techniques/process. Sourcing and training sufficient competent workforce also plays a major role in ensuring the successful establishment of a commercial tissue culture laboratory. The availability of labour saving devices will overcome the labour shortage problem. Even though the mantling rates could be kept at minimal levels, the availability of reliable and robust oil palm mantling marker for early culture screening still remains as a major requirement for large scale ramet production. The performances of clonal materials in trials and commercial field are discussed in this paper.

INTRODUCTION

Clonal propagation has become a popular tool for the production of commercial panting materials as it has several advantages over the conventional propagation method such as rapid multiplication of uniform planting materials with the desired traits, expeditious released of improved varieties and production of disease-free plants.

Success in oil palm cloning was first reported in the mid-seventies (Jones, 1974; Rabechault and Martin, 1976), and in Malaysia in the early eighties (Wooi et al, 1981; Paranjothy

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and Othman, 1982). Encouraged by the early successes of clonal oil palm plantings, approximately ten tissue culture laboratories had been in operation in Malaysia by the mid-eighties (Wooi, 1990) and currently, there are about 12 oil palm tissue culture laboratories in Malaysia.

AAR is a subsidiary company of Boustead Plantations Bhd. and Kuala Lumpur Kepong Bhd. The main objective of AAR is to formulate best agro-management practices and provision of high yielding planting materials for the two principals to realize maximum profits and achieve sustainable production through scientific research and development.

Since the formation of AAR in 1986, the first batch of ramets had been successfully planted in trial plots as well as commercial fields between 1990 and 1992 for evaluation. Over a span of more than 30 years of research, AAR has focused on fine tuning the tissue culture processes and evaluates the performance of the clonal planting materials in trial plots as well as large scale commercial fields. Based on the satisfactory performances of the commercial planting in 1997, AAR has forged ahead with the commissioning of the commercial tissue culture laboratory in 2005 with a capacity of one million ramets production annually.

PRINCIPLES AND PROCEDURES APPLIED IN ORTET SELECTION

The success of oil palm cloning begins with the selection of the ‘best’ ortet source. The best approach would be selecting the best palms from the best families in the progeny-test plots. A comprehensive breeding programme incorporating various desirable traits is essential in order to create good sources of ortets for cloning. The criteria for selecting a promising ortet has been described by many authors (Soh, 1986; and Rajanaidu, 1986); selection is based on a combination of yield components (Soh and Chow, 1989) and high bunch index for high yield productivity (Breure and Corley, 1983). Other characteristics such as cloning of inter-specific hybrids for more liquid oil and slower height increment and compact palm architecture for high density planting (Escobar et al, 2006) had also been reported.

AAR’s ortet selection criteria were drawn up based on the SIRIM MS 2099 standard. The ortets are initially selected based on data and are further scrutinised, verified and confirmed by breeders, tissue culturists and agronomists in the field. AAR’s main criteria in the selection of ortets for cloning is based on a minimum four consecutive years of records on yield and oil to bunch values derived from at least five analyses per ortet. For a primary ortet, the fresh fruit bunch (FFB) yield must be at least 10% more than that of the progeny DxP control in the trial and its fruit bunches must have a minimum oil to bunch ratio of 28%. The height of the ortet should ideally be less than 90% of the DxP control. All ortets are selected from our in-house breeding trials. Whilst for recloning exercise, the criteria for clonal ortets are similar to that of the primary ortets but the minimum oil to bunch ratio is raised to 28.5%. Other minor components and factors such as the sizes of kernels, fruits and bunches, vegetative characteristics such as compactness, vigour, nutrient status, parthenocarpy level and free from abnormalities and diseases are also considered during the selection.
OIL PALM TISSUE CULTURE PROCESS

The oil palm tissue culture process starts with the selection of suitable explants such as immature leaves, young inflorescences, seed embryos or tips of tertiary roots. Adopting the somatic embryogenesis pathway, the oil palm tissue culture process consists of six stages namely callus induction, callus differentiation, embryoid proliferation and germination, shoot development, root induction and finally plantlet conditioning. Generally, the entire process may take 12 months to over two years to generate the first batch of conditioned ramets (Wong et al., 1997). To date, the oil palm cloning process is still inefficient with average callusing rates of around 25% and embryogenesis rates around 3% - 6%. At AAR, although in general all primary ortets are able to produce callus, however, only 76% of the primary ortets are embryogenic. Callusing rates for primary ortets vary from 1% - 74% with a mean of 23%, whilst, embryogenesis rates vary from 0.1% - 24.9% with a mean of 3.0% (Table 1).

TABLE 1. CALLUSING AND EMBRYOGENESIS RATES OF VARIOUS GENOTYPES (PRIMARY ORTET)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Ortets</th>
<th>Callusing Rate (%)</th>
<th>Embryogenesis Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>Yangambi AVROS</td>
<td>57</td>
<td>24</td>
<td>1 – 67</td>
</tr>
<tr>
<td>Dumpy AVROS</td>
<td>225</td>
<td>24</td>
<td>1 – 74</td>
</tr>
<tr>
<td>AVROS</td>
<td>33</td>
<td>22</td>
<td>2 – 54</td>
</tr>
<tr>
<td>Dumpy Yangambi AVROS</td>
<td>177</td>
<td>26</td>
<td>1 – 59</td>
</tr>
<tr>
<td>Cameroon</td>
<td>43</td>
<td>18</td>
<td>1 – 46</td>
</tr>
</tbody>
</table>

Similar to primary ortets, all clonal ortets are able to produce callus, but in terms of embryoid production, clonal ortets are more responsive with 96% of them able to produce embryoid. Callusing rates for clonal ortets range from 1% - 60% with a mean of 24%, whilst, embryogenesis rates range from 0.1% - 71.4% with a mean of 5.0% (Table 2).
Table 2. Callusing and Embryogenesis Rates of Various Genotypes (Clonal Ortet)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>No. of Ortets</th>
<th>Callusing Rate (%)</th>
<th>Embryogenesis Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Yangambi</td>
<td>16</td>
<td>25</td>
<td>11–39</td>
</tr>
<tr>
<td>Cameroon</td>
<td>76</td>
<td>26</td>
<td>6–43</td>
</tr>
<tr>
<td>Yangambi AVROS</td>
<td>98</td>
<td>26</td>
<td>1–60</td>
</tr>
<tr>
<td>NIFOR</td>
<td>18</td>
<td>10</td>
<td>2–39</td>
</tr>
<tr>
<td>AVROS</td>
<td>101</td>
<td>23</td>
<td>2–56</td>
</tr>
</tbody>
</table>

Table 1 and 2 also indicated that callusing and embryogenesis rates for both primary and clonal ortets were found to differ widely within and between the genotypes. The influence of genotypes on the rates of callus induction had also been reported by Ginting et al. (1995) and Choo et al. (2015).

In general, callus can be induced from both primary and clonal ortets. On palm basis, clonal ortets are more amenable to culture with an embryogenesis rate of 96% and a shoot regeneration rate of 85% compared to primary ortets at only 76% and 56% respectively (Table 3). About 5% of callogen explants of clonal ortets differentiated into embryoids, which is one and a half times more than the primary ortets. Shoot regeneration from embryogenic lines for both the primary and clonal ortets appears similar at 88%.

Table 3. Cloning and Recloning Efficiencies of AAR Ortets (Primary & Clonal)

<table>
<thead>
<tr>
<th>Culture Stage</th>
<th>Success Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Palm Basis</td>
</tr>
<tr>
<td></td>
<td>Primary Ortet</td>
</tr>
<tr>
<td>Callusing</td>
<td>100</td>
</tr>
<tr>
<td>Embryogenesis</td>
<td>76</td>
</tr>
<tr>
<td>Shoot Regeneration</td>
<td>56</td>
</tr>
</tbody>
</table>
CLONAL FIELD PLANTING RESULTS

Trial performance of AAR clones in different locations and soil types are summarized in Table 4. The increase in FFB yield over the control ranged from 99% to 116%. In terms of oil yield (OY), the increase over the control ranged from 103% to 122%. Results showed a significant gain in OY with clonal planting materials and well corresponded with findings of Baudouin et al. (1991) and Maheran et al. (1995) that the oil to bunch trait is highly heritable and transmitted from ortets to clones.

TABLE 4. TRIAL PERFORMANCE FOR AAR CLONES

<table>
<thead>
<tr>
<th>No.</th>
<th>Trial</th>
<th>Year of Recording</th>
<th>No. of clones</th>
<th>% FFB of clones over control</th>
<th>% Oil Yield of clones over control</th>
<th>Control type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>BCT13-97</td>
<td>2000-07</td>
<td>12</td>
<td>113</td>
<td>121</td>
<td>AA DXP</td>
</tr>
<tr>
<td>2.</td>
<td>BCT14-97</td>
<td>2001-07</td>
<td>11</td>
<td>106</td>
<td>117</td>
<td>AA DXP</td>
</tr>
<tr>
<td>3.</td>
<td>BCT15-98</td>
<td>2000-09</td>
<td>8</td>
<td>101</td>
<td>120</td>
<td>AA DXP</td>
</tr>
<tr>
<td>4.</td>
<td>BCT16-98</td>
<td>2001-09</td>
<td>24</td>
<td>106</td>
<td>118</td>
<td>AA DXP</td>
</tr>
<tr>
<td>5.</td>
<td>BCT17-99</td>
<td>2002-10</td>
<td>14</td>
<td>104</td>
<td>121</td>
<td>AA DXP</td>
</tr>
<tr>
<td>6.</td>
<td>BCT18-00 (1)</td>
<td>2002-10</td>
<td>19</td>
<td>101</td>
<td>115</td>
<td>AA DXP</td>
</tr>
<tr>
<td>7.</td>
<td>BCT18-00 (2)</td>
<td>2003-10</td>
<td>7</td>
<td>100</td>
<td>111</td>
<td>AA DXP</td>
</tr>
<tr>
<td>8.</td>
<td>BCT19-00</td>
<td>2003-10</td>
<td>10</td>
<td>112</td>
<td>118</td>
<td>AA Hybrida1</td>
</tr>
<tr>
<td>9.</td>
<td>BCT20-00 (3)</td>
<td>2005-10</td>
<td>8</td>
<td>101</td>
<td>107</td>
<td>AA DXP</td>
</tr>
<tr>
<td>10.</td>
<td>BCT20-00 (4)</td>
<td>2006-10</td>
<td>8</td>
<td>106</td>
<td>113</td>
<td>AA DXP</td>
</tr>
<tr>
<td>11.</td>
<td>BCT21B-04</td>
<td>2007-15</td>
<td>13</td>
<td>107</td>
<td>109</td>
<td>AA Hybrida1</td>
</tr>
<tr>
<td>12.</td>
<td>BCT22-07</td>
<td>2010-17</td>
<td>16</td>
<td>116</td>
<td>122</td>
<td>AA Hybrida1</td>
</tr>
<tr>
<td>13.</td>
<td>BCT 23-10</td>
<td>2012-17</td>
<td>19</td>
<td>99</td>
<td>105</td>
<td>AA Hybrida1</td>
</tr>
<tr>
<td>14.</td>
<td>BCT 24-10</td>
<td>2013-17</td>
<td>12</td>
<td>99</td>
<td>103</td>
<td>AA Hybrida1</td>
</tr>
</tbody>
</table>

Range: 7-24

Note: AA Hybrida1 control was 22% higher than AA DXP control in oil yield.
Commercial yield data of five of our principal’s estates in Sabah showed that mean FFB yield for our clones ranged from 21.5 t/ha to 29.2 t/ha between 2013 and 2016 (Table 5). The highest yield recorded was 42.6 t/ha in 2013 whilst the lowest was 17.7 t/ha in 2016. The clones managed to maintain above 6.0 t/ha/yr of oil yield from 2013-2015, before dropping to 5.3 t/ha in 2016. The mean OY ranged from 5.3 to 7.0 t/ha/yr. The highest annual OY recorded was 10.1 t/ha in both 2013 and 2014 whilst the lowest was 4.3 t/ha in 2016. The reduced of both FFB and OY between 2015 and 2016 most probably was due to El Nino effect.

**TABLE 5. COMMERCIAL PERFORMANCES OF AAR CLONES**

<table>
<thead>
<tr>
<th>Area</th>
<th>Palm Age</th>
<th>Year</th>
<th>2013</th>
<th>2014</th>
<th>2015</th>
<th>2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Estate: 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of Field: 28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Area: 2,609 ha</td>
<td>13-20 years old</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean: 94.4 ha/field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range: 14-161 ha/field</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>FFB (t/ha)</th>
<th>Mean</th>
<th>29.2</th>
<th>28.3</th>
<th>26.8</th>
<th>21.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>24.4 to 42.6</td>
<td>24.2 to 39.9</td>
<td>23.3 to 35.2</td>
<td>17.7 to 29.7</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>OY (t/ha)</th>
<th>Mean</th>
<th>6.8</th>
<th>7.0</th>
<th>6.7</th>
<th>5.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>5.5 to 10.1</td>
<td>5.7 to 10.1</td>
<td>5.8 to 9.1</td>
<td>4.3 to 7.5</td>
<td></td>
</tr>
</tbody>
</table>

Seven oil extraction rate (OER) test runs in different palm oil mills at our principal’s estates were carried out to have a better comparison between the clonal and normal DxP planting materials. Figure 1 shows that the clones’ OER ranged from 24.1% to 26.7% while the mill OER ranged from 19.7% to 23%. The increase in the OER of clones over DxP ranged from 108% to 122%. These results show that clones definitely produce more oil compared to DxP.
COMMERCIAL PLANTING ISSUES

The most important issue undermining confidence in the mass propagation of oil palm clonal material is the mantled fruit phenomenon. This abnormality can only be detected when the ramets produce the first few inflorescences at one and a half to two years after field planting. It is characterized by the development of vestigial stamens in female flowers and later turning into fleshy carpels that form a mantle surrounding the fruit. These mantled fruits can be parthenocarpic or fertile. Severe parthenocarpic fruit set resulting in bunch failure which negatively affects yield production.

Mantling rates in AAR’s clonal plantings are summarised in Table 6. The initial higher rates between 1986 and 1995 were due to protocol tests being evaluated in the early years. However with improved protocols and stringent culture management, AAR managed to consistently keep the average mantling rates to less than 3% in the clonal plantings since 1996.

Figure 1. Test Runs on Oil Extraction Rates (OER) at Principals’ Mills
Mantling incidence is currently kept at a low level by good culture management such as minimising the amount of plant growth hormone used in the culture media, reducing time in culture and limiting production per embryogenic line or clone. The risk of mantling rate in the field is mostly limited to less than 5% with the adoption of a clonal package of 5 to 10 clones per planting. Ong-Abdullah et al. (2015) reported that the mantling phenomenon was due to loss of *karma transposon* methylation in oil palm tissue during the tissue culture process. The availability of a diagnostic kit/marker for screening of mantled clones at the nursery stage (if not earlier) would lead to greater confidence in clonal plantings.

### CHALLENGES IN COMMERCIALISING ELITE OIL PALM CLONES

Due to the inefficiency of the oil palm tissue culture process, especially in embryogenesis, a large number of ortets is constantly required in order to scale up production. About 100 ortets need to be inoculated each year in order to produce more than 500,000 ramets annually. However, the requirement is unlikely to be fulfilled by most existing breeding programs as only about 20% to 30% of the palms per trial are selected as ortets. A series of progeny testing trials must be available to meet this continuous ortet demand. One alternative approach is to reproduce members of the best progeny-tested family and plant them as large ortet gardens (Soh 1986, 1987, 1998). The other alternative is to reproduce the best tested clones, which usually also exist in larger numbers or can be planted as clonal ortet gardens.

The inefficiency in oil palm tissue culture process is also a great hindrance to large-scale ramet production. The whole tissue culture process requires specialized infrastructure to ensure a clean and controlled environment with sufficient laboratory space for incubating the cultures and most importantly, adequate supply of skilled workers. In addition to the high cost of production, commercial tissue culture laboratories are also facing shortage of laboratory operators with high turnover rates. The tissue culture process is costly and labour intensive,

### TABLE 6. MANTLING RATES IN AAR CLONES

<table>
<thead>
<tr>
<th>Year Planted</th>
<th>Explant Source</th>
<th>Mantling (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986-1991</td>
<td>Ortets (Immature Leaves)</td>
<td>6.2</td>
</tr>
<tr>
<td>1989-1993</td>
<td>Ortet (Embryos)</td>
<td>1.1</td>
</tr>
<tr>
<td>1992-1995</td>
<td>Seedlings (Immature Leaves)</td>
<td>7.6</td>
</tr>
<tr>
<td>1996-2000</td>
<td>Ortets (Immature Leaves)</td>
<td>0.3 - 2.0</td>
</tr>
<tr>
<td>2001-2007</td>
<td>Ortets (Immature Leaves)</td>
<td>0 – 2.7</td>
</tr>
<tr>
<td>2007-2015</td>
<td>Ortets (Immature Leaves)</td>
<td>0 – 3.0</td>
</tr>
</tbody>
</table>
hence, it is essential to adopt measures to improve efficiency and better utilisation of resources leading to reduction in cost of production. One such approach is the usage of liquid suspension system (Wong et al. 1999a) which can increase the speed of multiplication and growth of cultures. However, the inability to induce embryogenic callus which is amenable to the liquid suspension system remains a bottleneck. Labour saving devices are good options for future investments. For example, automated operations/devices can lower the cost of production and overcome problems with labour shortage, although the initial cost of investment could be very high. Generally, the investment will be paid off after some defined period if the integration of machinery into the oil palm tissue culture process is successful. Regardless of the successful innovations to improve efficiency, the ultimate test remains in the fidelity of clones in the field.

Another challenge in mass production of elite clones is to overcome field planting issues associated with clonal materials. Besides the risk of mantling as discussed earlier, clonal materials are required to be planted interspersed with DxP materials to minimize issues with fruit set. A 4-palms row of a lower sex ratio DxP to be interspersed with every 4x4 clones strip is normally practiced to ensure adequate pollen supply and to sustain weevil populations; both being prerequisites for effective pollination (Tan et al. 2003). The availability of a mantling marker for nursery screening would greatly reduce the risk of mantled palms being planted in the field which would then need to be cumbersomely replaced. Despite the low mantling risks, planting of large monoclonal blocks are not advisable due to the risk of vulnerability to pest and disease outbreaks and certainly ineffective pollination.

**CONCLUSION**

Our success in commercial production of elite oil palm clones depends on several factors including the availability of constant supply of primary and clonal ortets for uninterrupted production, a more efficient culture system for ramets’ mass production, improved tissue culture protocols with minimum somaclonal variants supported and verified by field performance.

**ACKNOWLEDGEMENT**

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**REFERENCES**


Ong-Abdullah, M *et al.* (2015). Loss of *karma transposon* methylation underlies the mantled somaclonal variant of oil palm. Nature do: 10.1038/nature15365


Status of Oil Palm Cloning Program at Asian Agri

Ida Febriantine*; Ang Boon Beng; Izharul Ihsan; Manjit Sidhu; Yohannes Samosir and Mukesh Sharma

ABSTRACT

Asian Agri commenced planting material improvement in 1996 with the introduction of a wide range of genetic materials for its breeding program and released four varieties called Topaz in 2004. A complementary program was then launched in 2005 by establishing oil palm tissue culture laboratory. In 2012, a cutting edge new facility, Clonal Oil Palm Propagation Unit (COPPU) was built to support the multiple objectives of tissue culture program i.e to develop efficient, reliable and cost effective propagation techniques for the culturing of elite parental palms for semiclonal and biclonal seed production, and elite tenera palms with specific traits for commercial planting. COPPU has also continuously improved tissue culture protocols by working on different type of explant tissues, sampling techniques, reducing the risk of abnormality of ramets produced, shortening the tedious tissue culture process, and improving multiplication rate.

Elite ortet palms were rigidly selected from our breeding trials based on CPO, O/B, FFB, together with other important specific traits of interest such as Ganoderma tolerance, slow height increment, compact canopy, and specific oil quality. A total of 613 ortets have been sampled for explants since 2005 comprising of 41 Dura, 7 Pisifera and 565 Tenera palms.

Efficiency and consistency of propagation process in the laboratory still remain challenging. However, our work on process development has shown promising results. Contamination in the laboratory was low at less than 1% per month. Callus induction and callus differentiation rates based on ortet were 96% and 84%, respectively for Tenera ortets. Similar rates applied to Dura while Pisifera was much less responsive. It is interesting to note that Nigeria and Ekona genotype Teneras were relatively more responsive compared to Ghana. Equiped with a modern acclimatization chamber, the survival rate of 91% was achieved despite the extreme hot temperature that may occur in Riau.

______________________________

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*Corresponding author, email: ida_febriantine@asianagri.com
Promising results have also been seen in the field. More than 1,000 ha have been planted with clones in different soil and climatic conditions. Floral and fruit mantling rate was less than 0.3% indicating the reliability of tissue culture protocol in regards to including media formulation, culling, discipline of workers, and close supervision. The top 5 best clones produced oil yield > 30% over control DxP seedlings. In one clonal trial on volcanic soil in North Sumatra, 5 best clones gave 9.3 ton CPO/ha/yr (average of the first 2 years harvesting), while DxP control seedlings only produced 6.0 ton CPO/ha/yr. However, consistency of clone performance planted in different locations still varies indicating strong interaction of clones with environment conditions. Selection of ortet palms also requires a better approach to ensure that clones would perform better than their respective ortet progenies and current elite progenies.

In addition to Tenera cloning, progress in parental palm cloning has also been satisfactory. A total of more than 1,000 Dura ramets and 40 Pisifera ramets have been planted, to produce semiclonal and biclonal seeds. Ramets regenerated from inflorescence tissue and ramets produced through suspension culture have been also planted in the field. Fidelity of these clones are yet to be observed.

In the future, tissue culture in oil palm is not only tasked with provision of high yielding clones to meet the growing problems of land scarcity for plantation expansion, but also to support breeding and biotechnology programs. These include propagation of new planting materials which may need long cycle of selection and backcrossing like interspecific hybrids.

**Keywords:** clone, mantling, oil palm, tissue culture, Topaz

**INTRODUCTION**

Oil palm (*Elaeis guineensis* Jacq) is the highest yielding oil crop per hectar at present. The high and increasing yield of oil palm led to a rapidly expanding world industry. Indonesia and Malaysia are among the countries which provide the most of the oil entering international trade.

Improvement of oil palm materials have also been carried out by embarking program of intensive breeding. This program has contributed to quantum leap in yield improvement (Zamzuri, 2011). However, since the conventional oil palm breeding had been facilitated by sexual means through dura x pisifera (DxP), it involves random segregation which will add to highly heterozygous offspring. Furthermore, breeding program requires progeny testing to be carried out in the large area, over long period and high cost of trials (Anonim, 2017).

Full potential of oil palm can only be realized through vegetative propagation. Tissue culture is only way due to the nature of oil palm which is single stem monocot. Tissue culture could expedite breeding program of desired traits by shorten the time, producing clones with high value added. Indonesia is one of the leading palm oil producers which is now entering the world of clonal oil palm plantations.

At Asian Agri, tissue culture program commenced in 2005 by the bold initiative of Mr. Ang Boon Beng. Three shop lots at Riau Andalan Pulp and Paper Complex were renovated to accommodate the requirement of a tissue culture laboratory. By the year 2012, the lab moved to the new state of art tissue culture laboratory, Clonal Oil Palm Production Unit (COPPU) in
Buatan Estate. The facility was built to support the objectives of clonal oil palm program i.e to multiply elite parental palms for the production of semi-clonal and bi-clonal seed in order to further improve yields, to explore the commercial potential of planting Tenera clones with specific traits of interest e.g. high oil yield, specific oil quality, slow height increment, compact canopy and resistance/tolerant to disease. The current capacity of COPPU is 0.5 M ramet per year, and the lab is expandable to 1 M ramet per year.

The process in oil palm tissue culture involves the reversing the cell differentiation from organized tissue (leaf and inflorescence tissue) to form a mass of undifferentiated cells called “calli”, then to form embryoid which are then coaxed to produce shoots followed by root to complete the process of restoration to be whole plantlets that would continue to develop into normal plants that are genetically identical to the ortet pams (Sharma, 2008). This process does not occur in nature, and has become the ‘black sheep’ of oil palm tissue culture work, potentially could result in high abnormality for some prone genotypes. Clonal amenability and abnormalities are among such problems (Zamzuri, 2011). Floral abnormality rate was observed in the trials as well, although the rate now is less than 5% compared to the earlier clonal production (Tan et al., 2003).

This paper highlights the experience and progress made by COPPU to improve clonal propagation technique and its utilization.

**PRODUCTION PROCESS**

Tissue culture process starts with the selection of ortets as the source of explants. Ortets were selected from the best individual palms within the best progenies with detailed yield records based on minimum three bunch analysis. Tender spear-leaf tissue (cabbage) and young inflorescences were sampled using minimum destructive sampling techniques to reduce damage to the palm and increase recovery time (8 – 18 months).

![Figure 1. Tissue culture process](image-url)
In the field, the leaf-cabbage sample ca. 60 cm long was carefully lowered down to the ground. The sample was surface sterilized using alcohol then transported to the lab where further work was undertaken in laminar air flow cabinet; open the spear by cutting the sheath to expose the leaves within.

Old leaves (L-1 to L-3) were discarded as well as the very young leaves (L-9 to L-12) for their pinnae were short and narrow. Selected leaves were then surface sterilized using alcohol and carefully cut to get explants of 2 mm width and placed into petridish containing callus induction media. A total of 750 to 1000 petridishes of explants could be obtained from a cabbage.

The callus and embryoid could be seen 3-12 months after inoculation. Embryoids were harvested and plant in a development media for shoot growth and development. Once the shoots reached 8 cm height, they were transferred into root induction media for 1 to 2 months to develop roots (become plantlets) before transferred to acclimatization chamber with controlled humidity and temperature. This acclimatization phase would gradually expose the plantlets to ambient condition. Direct transfer of the plantlets from in vitro condition to nursery which was exposed to natural environment would certainly kill them due to excessive water loss through transpiration. The extreme weather at Riau, Indonesia, had been somewhat quite difficult to handle. Nevertheless, we were able to achieve high survival rate and should be possible to improve further (Graph.1).

Graph 1. Survival Rate of Plantlet at Acclimatization Chamber from 2013-2017

From 2005 to date, a total of 613 ortets have been sampled comprising of 41 *dura* palms, 7 *pisifera* palms and 565 *tenera* palms. Their response to tissue culture varied depending on the ortet (*Table 1, Table 2*).
TABLE 1. SUMMARY OF TENERA, DURA AND PISIFERA CLONING, 2005-2015

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Ortet inoculated</td>
<td>512</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>Ortet produced callus</td>
<td>490</td>
<td>96</td>
<td>26</td>
</tr>
<tr>
<td>Ortet produced embryoid</td>
<td>410</td>
<td>84</td>
<td>20</td>
</tr>
<tr>
<td>Explant inoculated</td>
<td>1,782,531</td>
<td>3,481/p</td>
<td>90,870</td>
</tr>
<tr>
<td>Clean explant</td>
<td>1,441,872</td>
<td>81</td>
<td>81,594</td>
</tr>
<tr>
<td>Explant produced callus</td>
<td>171,020</td>
<td>12</td>
<td>3369</td>
</tr>
<tr>
<td>Explant produced embryoid</td>
<td>17,881</td>
<td>10</td>
<td>611</td>
</tr>
</tbody>
</table>

For tenera, the callus induction and callus differentiation rate based on ortet were 96% and 84% respectively, dura palms were more responsive with 100% ortet produce callus, and 77% produce embryoid. On the other hand, cloning pisifera was rather difficult as indicated by only 86% of the ortet produced callus and 50% of them produced embryoid.

TABLE 2. RESPONSE OF PARENTAL GENOTYPE TO TISSUE CULTURE

<table>
<thead>
<tr>
<th>Fruit Type</th>
<th>Parental Background</th>
<th>Number of Ortet</th>
<th>Callus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Induction (%)</td>
</tr>
<tr>
<td>DURA</td>
<td>Cemara</td>
<td>6</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>Dura Deli</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>HC</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>PISIFERA</td>
<td>Ekona</td>
<td>3</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Ghana</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Nigeria</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>TENERA (male parent)</td>
<td>Avros</td>
<td>53</td>
<td>98</td>
</tr>
<tr>
<td></td>
<td>Dami</td>
<td>32</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>Ekona</td>
<td>143</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Ghana</td>
<td>79</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Lame</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Nigeria</td>
<td>115</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Yangambi</td>
<td>13</td>
<td>100</td>
</tr>
</tbody>
</table>

Further observation on group of parental genotypic background showed interesting results (Table 2). The results however are subject to cautious interpretation as the number of ortet used varies greatly and some of them are still fewer than 10 palms. For dura, Harrison & Crossfield genotype is likely to respond good with 100% of the ortet produced callus and embryoid, and the same results occurred for tenera or pisifera Yangambi and Lame. Tenera with Nigeria and Ekona
*pisifera* genotype were relatively more responsive than that of Ghana. Sampling of *pisifera* ortet is still few and therefore the difference in response to tissue culture is yet to be seen (*Table 2*).

Production process described above uses solid based culture which is hampered with low multiplication rate and less uniform regeneration of shoots. Working on Liquid Suspension System (LSS) is in progress aiming at uniform and synchronized development of desired quantity could be made and therefore may suit commercial mass ramet production. Ramets from LSS are now available for field testing in comparison with their respective lines from solid culture system.

*Note:* PE – Polyembryoid (stage where embryoid starts developing shoots)

**Figure 2. Liquid Suspension Cultures**
Stringent culling in all stages of tissue culture process is done to eliminate off types such as inferior embryoid/hyperhidricity type, browning culture, rosette and terminal inflorescence. This practice is thought to result in a low abnormality of ramets in the field. More than 100,000 ramets of 45 clones have been planted in the field since 2010 and abnormal rate (mantle flowering/fruit) is very low at 0.3%.

In addition to selection of ortet and culling off types, controlling contamination is also pivotal in the success of tissue culture laboratory. Bacteria or fungi contamination at our lab has been strictly controlled and achieved less than 1% a month. This requires good team work and tight supervision and regular check on equipment and cleanliness of room and air. Limitation of guests visiting the lab particularly to clean room is part of the rules.

**FIELD EVALUATION**

Abnormal flowering and fruit formation of clonal palms in the field have frequently been reported (Soh, A C, et all. 2006) and this has set back the commercial application of clonal propagation. In addition, yield performance of clones has also been debated for long in comparison to D x P seedlings palms. Therefore field evaluation of clones is critical prior decision-making on upscaling of clonal propagation program particularly of *tenera* types. More than 1,000 ha of land in seven estates (mineral, volcanic and deep organic soils) have been planted with different clones for field evaluation purposes (Table 3).

**TABLE 3. CLONAL PLANTING PROGRESS AT ASIAN AGRI**

<table>
<thead>
<tr>
<th>YOP</th>
<th>Location (Hectar)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KGD</td>
</tr>
<tr>
<td>2010</td>
<td>78</td>
</tr>
<tr>
<td>2011</td>
<td>45</td>
</tr>
<tr>
<td>2012</td>
<td>66</td>
</tr>
<tr>
<td>2013</td>
<td>-</td>
</tr>
<tr>
<td>2014</td>
<td>-</td>
</tr>
<tr>
<td>2015</td>
<td>-</td>
</tr>
<tr>
<td>2016</td>
<td>-</td>
</tr>
<tr>
<td>2017</td>
<td>-</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>189</strong></td>
</tr>
</tbody>
</table>

*Notes:*

<table>
<thead>
<tr>
<th>GEO LOCATION</th>
<th>SOIL TYPE</th>
<th>ESTATE LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Sumatera</td>
<td>Volcanic Soil</td>
<td>KBA, KSN</td>
</tr>
<tr>
<td></td>
<td>Alluvial Soil</td>
<td>KTD</td>
</tr>
<tr>
<td></td>
<td>Organic soil (&gt;3m)</td>
<td>KNS, KTS, KNU</td>
</tr>
<tr>
<td>Riau</td>
<td>Inland Soil</td>
<td>KGD, KUK</td>
</tr>
</tbody>
</table>
The results presented in the following discussion were based on performance of clones planted from 2010 to 2013 which used ortets of Gen-1 progeny trials. D x P Topaz seedlings palms used as the control were not necessarily the progenies of the respective ortet palms. In fact, some of the control palms were D x P seedlings from commercial seeds other than Topaz. Therefore, the comparisons have not been fairly made in relation to genotype make-up of the clones and D x P palms. Nevertheless, the results indicated that the top 5 clones planted in any type of soils (inland, volcanic and organic) outperform in yield both FFB and CPO compared to the D x P control (Table 4, 5, 6, 7, and 8). The clones produced more FFB by 12 up to 44% compared to that of D x P control (Table 7). Similar trend was also found in CPO (Table 8).

It is noted, however, that a number of clones are not consistent in their ranking indicating the strong G x E effects apply in clones when planted in different agro-climate locations. Clonal trials in inland soil (Table 4), shown a decline of yield (FFB and/or CPO) in the fourth year of harvesting but then they recovered in the following year. The decline occurred in both clone and D x P control and it was thought due to severe drought and haze in the previous year in Riau Province.

**TABLE 4. PERFORMANCE OF GEN-1 CLONAL TRIALS AT INLAND SOIL (LSC 1)**

<table>
<thead>
<tr>
<th>Trial Number</th>
<th>YOP</th>
<th>Range of yield data</th>
<th>Mean</th>
<th>Yield Parameter</th>
<th>Yield year (ton/ha/y)</th>
<th>O/B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1 2 3 4 5</td>
<td></td>
</tr>
<tr>
<td>CT01_KGD</td>
<td>2010</td>
<td>01/13-12/17</td>
<td>Top 5</td>
<td>FFB</td>
<td>14.6 27.7 38.4 31.9 41.3</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.6 6.7 9.4 7.8 10.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12 clones FFB</td>
<td>15.4 27.4 36.6 30.2 35.5</td>
<td>26.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.5 6.3 8.4 6.9 8.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>- - - - - -</td>
<td></td>
</tr>
<tr>
<td>CT02_KGD</td>
<td>2010</td>
<td>03/13-02/17</td>
<td>Top 5</td>
<td>FFB</td>
<td>17.7 30.9 36.0 34.3 33.8</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>4.6 8.1 9.4 9.0 8.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>14 clones FFB</td>
<td>15.2 27.2 32.9 32.0 33.7</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.7 6.6 8.0 7.8 8.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>7.0 20.2 27.7 27.2 32.6</td>
<td>30.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>FFB</td>
<td>1.8 5.3 7.2 7.1 8.5</td>
<td></td>
</tr>
<tr>
<td>CT07_KGD</td>
<td>2012</td>
<td>06/15-05/17</td>
<td>Top 5</td>
<td>FFB</td>
<td>25.7 32.3</td>
<td>32.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>7.0 8.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31 clones FFB</td>
<td>23.5 30.0</td>
<td>29.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>5.8 7.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>24.5 30.8</td>
<td>27.5</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.8 7.3</td>
<td></td>
</tr>
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</table>
### TABLE 5. PERFORMANCE OF GEN-1 CLONAL TRIALS AT ORGANIC SOIL > 3 M DEPTH (LSC 4)

<table>
<thead>
<tr>
<th>Trial Number</th>
<th>YOP</th>
<th>Range of yield data</th>
<th>Mean</th>
<th>Yield Parameter</th>
<th>yield year (ton/ha/y)</th>
<th>O/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT04_KNS</td>
<td>2011</td>
<td>06/14-05/17</td>
<td>Top 5</td>
<td>FFB</td>
<td>19.8 34.0 36.0</td>
<td>29.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>5.1 8.7 9.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15 clones</td>
<td>FFB</td>
<td>17.9 29.6 31.9</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>4.3 7.1 7.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>FFB</td>
<td>14.5 28.0 29.0</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.3 6.3 6.5</td>
<td></td>
</tr>
<tr>
<td>CT06_KNS</td>
<td>2012</td>
<td>05/15-04/17</td>
<td>Top 5</td>
<td>FFB</td>
<td>17.4 29.5</td>
<td>30.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>4.5 7.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>29 clones</td>
<td>FFB</td>
<td>15.3 26.3</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.6 6.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>FFB</td>
<td>13.6 26.4</td>
<td>33.2</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.9 7.5</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 6. PERFORMANCE OF GEN-1 CLONAL TRIALS AT VOLCANIC-ALLUVIAL SOIL (LSC 1)

<table>
<thead>
<tr>
<th>Trial Number</th>
<th>YOP</th>
<th>Range of yield data</th>
<th>Mean</th>
<th>Yield Parameter</th>
<th>yield year (ton/ha/y)</th>
<th>O/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT03_KBA</td>
<td>2011</td>
<td>04/14-03/17</td>
<td>Top 5</td>
<td>FFB</td>
<td>21.1 32.8 35.9</td>
<td>32.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>5.9 9.2 10.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31 clones</td>
<td>FFB</td>
<td>21.5 31.2 30.9</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>5.9 8.5 8.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>FFB</td>
<td>24.4 30.3 30.7</td>
<td>30.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>6.3 7.8 7.9</td>
<td></td>
</tr>
<tr>
<td>CT05_KSN</td>
<td>2012</td>
<td>08/15-07/17</td>
<td>Top 5</td>
<td>FFB</td>
<td>30.9 34.8</td>
<td>32.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>8.7 9.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25 clones</td>
<td>FFB</td>
<td>28.7 31.1</td>
<td>31.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>7.7 8.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>FFB</td>
<td>23.1 27.2</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>5.5 6.5</td>
<td></td>
</tr>
<tr>
<td>CT08_KTD</td>
<td>2013</td>
<td>01/16-12/17</td>
<td>Top 5</td>
<td>FFB</td>
<td>19.0 30.0</td>
<td>31.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>5.1 8.0</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>45 clones</td>
<td>FFB</td>
<td>15.2 25.9</td>
<td>29.8</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.9 6.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control 1</td>
<td>FFB</td>
<td>10.1 23.6</td>
<td>29.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>2.5 5.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control 2</td>
<td>FFB</td>
<td>14.0 26.1</td>
<td>30.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.7 6.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Control 3</td>
<td>FFB</td>
<td>16.5 26.2</td>
<td>26.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CPO</td>
<td>3.7 6.0</td>
<td></td>
</tr>
</tbody>
</table>

**Notes:** Control 1 is D x P material from internal seed source (Topaz), whereas control 2 and 3 are seed from outside seed suppliers.
### TABLE 7. SUMMARY OF AVERAGE FFB (KG/PALM/YR) OF CLONES AND THEIR PERCENT SUPERIORITY OVER D X P SEED IN CLONAL TRIALS.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Year of Yield Data</th>
<th>Range Data</th>
<th>Range of Clonal FFB Yield (kg/palm/yr)</th>
<th>Ave. Top 5 Clonal Yield</th>
<th>DxP Yield</th>
<th>% increase of clones &gt; DxP</th>
<th>No. Clones &gt;10% DxP</th>
<th>Soil Type</th>
<th>Previous Crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT01_KGD</td>
<td>01/13-12/17</td>
<td>1-5 year</td>
<td>158-224</td>
<td>215</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Inland</td>
<td>Rubber</td>
</tr>
<tr>
<td>CT02_KGD</td>
<td>03/13-02/17</td>
<td>1-4 year</td>
<td>147-246</td>
<td>208</td>
<td>144</td>
<td>44.4</td>
<td>12/14</td>
<td>Inland</td>
<td>Rubber</td>
</tr>
<tr>
<td>CT03_KBA</td>
<td>04/14-03/17</td>
<td>1-3 year</td>
<td>123-251</td>
<td>234</td>
<td>209</td>
<td>12.0</td>
<td>4/31</td>
<td>Volcanic</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>CT04_KNS</td>
<td>06/14-05/17</td>
<td>1-3 year</td>
<td>84-210</td>
<td>199</td>
<td>159</td>
<td>25.2</td>
<td>9/15</td>
<td>Organic</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>CT05_KSN</td>
<td>08/15-07/17</td>
<td>1-2 year</td>
<td>167-265</td>
<td>242</td>
<td>185</td>
<td>30.8</td>
<td>18/25</td>
<td>Volcanic</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>CT06_KNS</td>
<td>05/15-04/17</td>
<td>1-2 year</td>
<td>97-162</td>
<td>146</td>
<td>125</td>
<td>16.8</td>
<td>14/29</td>
<td>Organic</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>CT07_KGD</td>
<td>06/15-05/17</td>
<td>1-2 year</td>
<td>98-237</td>
<td>207</td>
<td>182</td>
<td>13.7</td>
<td>7/22</td>
<td>Inland</td>
<td>Rubber</td>
</tr>
</tbody>
</table>

### TABLE 8. SUMMARY OF AVERAGE CPO (TON/HA/YR) OF CLONES AND THEIR PERCENT SUPERIORITY OVER D X P SEED IN CLONAL TRIALS

<table>
<thead>
<tr>
<th>Trial</th>
<th>Year of Yield Data</th>
<th>Range Data</th>
<th>Range of Clonal CPO Yield (ton/ha/yr)</th>
<th>Ave. Top 5 Clonal Yield</th>
<th>DxP Yield</th>
<th>% increase of clones &gt; DxP</th>
<th>No. Clones &gt;10% DxP</th>
<th>Soil Type</th>
<th>Previous Crop</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT01_KGD</td>
<td>01/13-12/17</td>
<td>1-5 year</td>
<td>4.4-7.7</td>
<td>7.3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Inland</td>
<td>Rubber</td>
</tr>
<tr>
<td>CT02_KGD</td>
<td>03/13-02/17</td>
<td>1-4 year</td>
<td>4.7-7.2</td>
<td>7.0</td>
<td>5.3</td>
<td>32.1</td>
<td>6/14</td>
<td>Inland</td>
<td>Rubber</td>
</tr>
<tr>
<td>CT03_KBA</td>
<td>04/14-03/17</td>
<td>1-3 year</td>
<td>4.0-9.5</td>
<td>8.8</td>
<td>7.3</td>
<td>20.5</td>
<td>13/31</td>
<td>Volcanic</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>CT04_KNS</td>
<td>06/14-05/17</td>
<td>1-3 year</td>
<td>3.2-7.9</td>
<td>7.6</td>
<td>5.4</td>
<td>40.7</td>
<td>9/15</td>
<td>Organic</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>CT05_KSN</td>
<td>08/15-07/17</td>
<td>1-2 year</td>
<td>6.3-9.4</td>
<td>9.2</td>
<td>6</td>
<td>53.3</td>
<td>21/25</td>
<td>Volcanic</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>CT06_KNS</td>
<td>05/15-04/17</td>
<td>1-2 year</td>
<td>3.4-6.2</td>
<td>6.0</td>
<td>5.2</td>
<td>15.4</td>
<td>4/29</td>
<td>Deep Peat</td>
<td>Oil Palm</td>
</tr>
<tr>
<td>CT07_KGD</td>
<td>06/15-05/17</td>
<td>1-2 year</td>
<td>3.0-8.2</td>
<td>7.8</td>
<td>6</td>
<td>30.0</td>
<td>11/22</td>
<td>Inland</td>
<td>Rubber</td>
</tr>
</tbody>
</table>
Continuous supply of ortet of high yielding palm is very important in clonal propagation program. Our current strategy is ‘shooting the stars’ meaning giving priority to the highest yielding palms from progeny trials to establish field collection of ramet which would serve as ortet source. Ortet selection in the Gen-2 program has been raised to >13 ton CPO/ha/yr. Soh et al (2006) emphasized on the importance of carrying of good clonal trials to identify outstanding
and stable clones for large scale propagation. In our case, this approach has been adopted by establishing multi-location clonal trials. It was found that three clones have shown consistency in their ranking with high yields across sites. They could then be used as clone controls for future program.

Field evaluation of ramets of *dura* and *pisifera* is also ongoing. In 2014, more than 1,000 *dura* ramets and 40 ramets of *pisifera* have been planted in Buatan Estate, Riau to support semiclonal and biclonal seed production program.

**FUTURE DIRECTION**

The challenge faced by oil palm industry has been growing in relation to scarcity of suitable land for expansion and also the increase of labor cost as well as environmental issues. In the future, development of efficiency and reliability of clonal propagation techniques are still needed in light of commercial scale production. In addition, tissue culture is also important to support breeding and biotechnology programs. These include propagation of new planting materials which may need long cycle of selection and backcrossing like interspecific hybrids. Application of tissue culture to study and improve adaptation of oil palm to its environment such as drought may also of interest.

**CONCLUSION**

Tissue culture techniques have been consistently developed at Asian Agri since 2005 resulting confidence on our protocol being low risk of abnormality (mantling). However, its efficiency is yet to be improved in the light to upscale the production to commercial production, hence combination of solid and liquid culture systems deserved further studies. Stringent selection of ortet palm is very important to increase yields higher than 30% of current DxF progenies, but this could be also overtaken by new progenies come by conventional breeding. A strong GxE effect in clonal trials has been noticed, implying the need to careful planning in planting clones at different locations. Tissue culture technique is used to propagate elite parental palms both *dura* and *pisifera* for the production of semiclonal and biclonal seeds, which are thought to be more uniform and therefore higher yielding.

**REFERENCES**


Overview on Clonal Propagation of Oil Palm at Dami OPRS, Papua New Guinea

Baskaran Ponnusamy; Elizabeth Muruna; Marie-Louise Imaroto; Marnie Light and Luc Bonneau

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ABSTRACT

Dami Oil Palm Research Station (OPRS) houses the research section of New Britain Palm Oil Ltd (NBPOL) in West New Britain Province, Papua New Guinea. NBPOL has the largest sustainable palm oil production in Papua New Guinea, and Dami OPRS has had ongoing plant breeding programmes for the development of improved oil palm since 1968. The breeding research is associated with an ISO9001 certified seed production unit (Dami PNG) that supplies seed for local plantations, as well as overseas seed sales. The development of a commercial tissue culture facility in the late 1990s further expanded the capability of Dami OPRS to deliver on the production of high quality oil palm planting material. Currently, the Dami OPRS tissue culture laboratory has a main focus on cloning proven elite parental palms for production of high-yielding semi-clonal seeds, sold under the registered trademark name of “SUPERFAMILY®”. In addition, the laboratory supports the breeding programmes and agronomic research through conservation of elite germplasm and production of clonal material for experimental purposes. This presentation will provide an overview of the clonal propagation of oil palm at Dami OPRS via somatic embryogenesis from immature inflorescence of elite parental palms, as well as the successful acclimatization of clonal plantlets and establishment in the rametry and field for seed production. Future challenges will also be discussed, with the present aim of optimizing the current protocol to improve efficiencies for more rapid clonal production of selected material, and for the production of synthetic seeds for deployment of clonal tenera.

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New Britain Palm Oil Limited (NBPOL) currently has 86,542 ha of oil palm plantations in Papua New Guinea and the Solomon Islands, with production in 2016/17 in excess of 6 MT/ha of total palm products. In 1967, the Dami Oil Palm Research Station (OPRS) was established with the objectives of ensuring the continued improvement of oil palm planting material, securing the availability of oil palm seed for NBPOL, and supporting the expansion of the oil palm industry in Papua New Guinea. These objectives have been successfully achieved, and are ongoing, with three key areas of focus, namely: plant breeding, tissue culture, and seed production. Thus, the breeding research links to the ISO9001 certified seed production unit (Dami PNG) which produces and supplies seed for local plantation development (plantations and smallholders), as well as overseas seed sales. The Dami tissue culture laboratory, which was set up in December 1996, also plays an important role in research and seed production, particularly in the deployment of clones from elite parental palms.

Since its establishment, Dami OPRS has had a strong reputation for successful plant breeding programmes for the development of improved oil palm. The first oil palm breeding trials were implemented in 1968, and a few of the original palms from those early trials are still standing. Notable persons involved with the early plant breeding research are Eric Rosenquist (HFAS consultant) and Kees Breure, who was Head of Agronomy from 1971 to 1977. In the 1990s, Frédéric Dumortier played an important role in the advancement of the plant breeding research, and now acts in the capacity of Breeding Consultant for Dami OPRS.

In 1996, an on-site tissue culture laboratory was set up at Dami OPRS by Dr Simon Lord in order to produce clonal oil palm plants. Prior to that, from the late 1980s until the mid-1990s, ramets were obtained from Unifield (UK) and planted in Dami OPRS trials. However, given the difficulties of shipping material to and from other countries it was deemed necessary to develop the facilities and capacity at Dami OPRS to produce clonal material, and by 2004, more than 1000 palms from 38 clones had been planted in seed gardens at Dami (Vovola and Smith, 2004). In the early 2000s, research into double haploid production was led by Bill Anderson, and Dr Dale Smith introduced the use of liquid suspension cultures for somatic embryo development (Smith et al., 2010). This was a successful endeavour, and has allowed for the production of semi-clonal seeds (i.e. cloned elite dura mother palms used for ‘vegetative amplification’) and some pisifera clonal palms as pollen source for bi-clonal seed production. More recently, Dr Baskaran Ponnusamy has joined the Biotechnology Department at Dami OPRS, and some current and future aims for the further development of clonal technology are discussed below.

CURRENT STATUS OF PLANT BREEDING AT DAMI OPRS

Dami OPRS has a firm commitment towards short- and long-term breeding programmes in order to continuously improve on their production of quality, high-yielding planting material. This is especially important since the availability of high-grade planting material is a key factor in supporting sustainable oil palm production. The original germplasm collections at Dami OPRS were obtained from Banting in Malaysia in 1967, and efforts to improve on this material have been ongoing (i.e. Dami Deli and AVROS base populations). The Dami germplasm collection
also includes material from the Binga and Lobe breeding programmes (from the Unilever Combined Breeding Programme), and some Ghana material (ex-Nifor). The continuous improvements of the base populations at Dami OPRS is based on the method of Reciprocal Recurrent Selection (RRS), wherein a three-step selection process is followed: (1) Intensive phenotypic selection for testing in progeny trials; (2) Progeny testing with intensive yield recording, bunch analysis and vegetative characteristics to determine general combining abilities (GCA); and (3) Multiplication of the best parents for commercial exploitation. Thus, all parental material currently utilised for seed production and for clonal production have been selected based on data from extensive DxF progeny trials, which has been gathered over the last 50 years from around 60 trials covering more than 1,200 ha of trial plantings. Currently, Dami OPRS has 32 active progeny testing trials, 1 immature, 2 in nursery stage, and >20 new DxF trials in seed collection. These new trials aim to evaluate ~150 dura mothers in combination with ~130 pisifera fathers. Analysis of early yield data should allow for preliminary selections of the parental material by 2025.

**CURRENT STATUS OF PLANT TISSUE CULTURE AT DAMI OPRS**

Smith et al. (2010) demonstrated the successful use of inflorescence explants for production of clonal plantlets from somatic embryos initiated via suspension culture at Dami OPRS. Immature inflorescences are preferred as explant material, since less damage is done to the selected palm, which are often palm of interest in the Dami OPRS breeding programmes. The inflorescence enclosed within the spathe is carefully excised from the leaf axil of frond 8, 9 or 10 and then transferred to the laboratory for sterilisation and excision, as described by Vovola and Lord (2004). Individual spikelets are placed into tubes of induction medium and subdivided some weeks later onto proliferation medium (Smith et al., 2010). Within 12 weeks, there may be formation of some directly formed somatic embryos, or compact pro-embryogenic tissues which is then transferred to liquid culture to allow for the development of somatic embryos. Individual clumps of somatic embryos are placed on germination medium and following the growth and development of these, they are hardened before planting *ex vitro*.

This methodology has allowed for current production of up to 6,000 ramets per year (*dura*, *pisifera* or *tenera*). Many of these are for SUPERFAMILY® semi-clonal seed production, and there are currently ~1,000 clonal dura mother palms under production for the SUPERFAMILY cultivars. Seeds are produced from eight dura mother lines that are control-pollinated with selected pollen from nine commercial *pisifera*, allowing for 18 SUPERFAMILY® cultivars. Since 2013, almost 13,000,000 SUPERFAMILY® seeds have been produced from clonal mother palms, equating to a sufficient amount of seeds to supply roughly 65,000 ha of planting area. To date, we have observed no mantling in our parent palms, and seed production from such semi-clonal seed gardens has proven to be far more efficient and cost-effective than obtained from traditional seed gardens. This is an important consideration, besides the more obvious advantage of being able to maximise on the potentially highest-yielding genetic material. In the last 4 years, NBPOL has exclusively planted SUPERFAMILY® material across 13,000 ha of replant areas in our plantations and associated smallholder blocks. On average, for this material, the expected start of bunch production is 21-24 months (after field planting), 30.3-30.8 MT/ha of FFB/ha/year (average 4-9 yrs, 120 palm/ha) and 9 ton/ha/year of CPO, oil/ha/year (average 4-9 yrs, 120 palm/ha).
Finally, the Dami OPRS tissue culture laboratory also supports the breeding programmes and agronomic research through conservation of elite germplasm, and production of clonal material for experimental purposes. The opportunity to use clonal palms as experimental material allows for implementation of nutrient trials or physiological experiments where it is particularly useful to have material of a uniform genotype. Thus, the tissue culture laboratory plays a key role in enhancing the research capacity of Dami OPRS.

**FUTURE CHALLENGES**

With maturation of our current breeding trials, and the continued advancement of our breeding programmes, new selections of elite material is ongoing and work is in progress to expand our SUPERFAMILY® clone lines (both *dura* and *pisifera*). However, the time from selection of an elite palm to seed production from clonal parent palms may take up to 6 years. Considering that there is not much time-saving that can be gained through improvements at the nursery and immature planting stages, one of the major challenges is to optimize the current tissue culture protocol to more rapidly produce clones of the selected material. Thus, this is an important area of focus, and we have several experiments currently in progress to improve efficiencies in our current protocols. Furthermore, forthcoming validation from our trials evaluating bi-clonal seed material is in progress, and an additional area of interest and research is on the production of synthetic seeds for the deployment of clonal *tenera*.

**REFERENCES**


Is There a Future in Tissue Culture for the Oil Palm Industry?

Siti Habsah Roowi, Rafidah Mohammed Kassim, Raja Bahiyah Nur Raja Hirdan, Abdul Fatah Daud, Muhammad Nazmi Burhan, Noorsusilawati Mandangan, Muhammad Farid Abdul Rahim, Mohd Latif B. Kamarudin, Mohd Mahfuz Roslan, Naderman B Samin, Nur Adibah Ishak, Mohd Nasruddin Hj Mohamad, Tan Joon Sheong and Sharifah Shahrul Rabiah Syed Alwee

ABSTRACT

Oil palm tissue culture in Malaysia started since early 1980’s. Since then, the transition of oil palm tissue culture from a mere research activity to a full scale commercial operation has been slow and full of challenges. To date, FGV has successfully produced 10 million oil palm ramets since 1983 and these has been planted in over 41000 ha semi-commercial areas. Production of oil palm ramets in FGV has always been coupled with continuous improvements of the cloning system due to limited starting materials (ortets) and to further reduce the cost. Through these efforts, we have managed to reduce abnormality to about 3% or less since 2010. Although cloning only pushes the FFB yield increase upwards by an average of about 4%, the oil yield gain can be significant at an average of about 17%. In general, we can confidently say that clones out-perform DxP in most if not all plantings. Although oil palm cloning technology for commercial production maybe slow in adoption, it’s importance as a tool for advance crop improvement technology such as gene editing, genomic selection, genetic transformation cannot be denied.

Keywords: cloning, crop improvement, oil palm, yield increase

REFLECTION OF PAST 30 YEARS OF TISSUE CULTURE

Oil palm cloning technologies has begun since 1970s. Using tissue culture technologies, the increased in oil yield production has been estimated to reach 25%-30% (Soh et al., 1986) and this forecast was confirmed by large scale field trials (Cochard et al., 2000). Although there has been gradual increased in large scale production of oil palm clones, the total production of about 4.0 million oil palm clones still constitutes a very small proportion compared to 300 million oil palm seeds (Khusairi et al., 2010).

Oil palm tissue culture begins by culturing immature leaves (Duval et al 1995), immature inflorescence (Wooi et al., 1981, Teixeira et al., 1994), zygotic embryos (Teixera et al.,1993, Rajesh et al., 2003); or through the development of cell suspension (Wong et al., 1999a, 1999b) and protoplast (Masani et al.,2013 ). FGV embark in oil palm tissue culture technology through cloning of young leaves since 1983 and successfully produced more than 10 million ramets to date that has been planted in over 41000 ha semi-commercial areas. Over the years, we have continuously made improvements in the technology to enhance the efficiency of the cloning system and at the same time reduce the cost. In early years (1998-2008), the cloning technologies used a gelled system which is less efficient for the production of large number of ramets. Later, we moved into suspension technology for large scale production. Figure 1 clearly indicates that with the introduction of suspension culture since 2009, the production numbers can be increased very fast while at the same time, number of ortets cultured are markedly reduced. A total amount of more than 1 million ramets per year are successfully produced since 2009 to 2017.

![Figure 1: Ortet cultured and total ramets production ex-lab in FGV](image)

Abnormality in FGV was recorded from trials and semi-commercial area. The abnormality rates in trial areas fluctuates from year to year (Figure 2). However, the fluctuation clearly shows a downward trend with the abnormality rate hovering at around 5% since the last few years. Clearly abnormal flowering varies greatly between clones with some clones have higher abnormalities than others but with improvement in technology, this can be controlled significantly. Data gathered from these trials also provide information for the lab to make decision on the cultures within the lab whether they will proceed into large scale production or terminated. Using this information, FGV have managed to minimize the risk of abnormality in semi-commercial area. The current average abnormality rates at semi-commercial area are being control at an average of 3% (Figure 3).

Figure 2: Abnormality level in trials area
Among the first FGV clonal trial took place in 1987 at Pusat Penyelidikan Pertanian Tun Razak (PPPTR) where La Mé materials were planted. In this trial (C3), 4 clones with La Me origin were planted against commercial Tenera (DxP) material. As summarised in Figure 4, the mean fresh fruit bunch (FFB) yield for 20 years for all the clones is relatively higher than the commercial DxP. It was noticed that there is a sudden drop in FFB on the 12th year of planting and this was mainly due to lower rainfall recorded (1,772 mm) as compared to the year before (2,368 mm). This observation correlates to the previous findings where water deficit of 100 mm per year can have a detrimental effect on FFB production of up to 10% to 20% depending on soil quality (Ochs and Daniel, 1976; Caliman and Southworth, 1998).
Figure 4: FFB yield profile for clonal trial (C3) planted La Mé origin at Phase 9/19 in 1987

As FGV begins its stringent ortet selection criteria in the 1990s, we observed a much improved FFB yield when the Yangambi-clonal materials were evaluated in the field. Notably, the yield observation period had been reduced to 10 years as compared to the previous trial as the latter is a standard practise by majority of the plantations for field evaluation. Cutting short the trial period allows for more trials to be evaluated. 3 clonal trials with Yangambi-origin where the first 2 trials (C26 and C27) planted in Kota Gelanggi 5 in 1995 (Figure 5 and Figure 6) and trial C29 was planted at PPPTR in 1996 (Figure 7). It is notable that the mean FFB yield for all Yangambi origin clonal materials across the 3 trials are relatively higher than the La Mé origin.

Figure 5: FFB yield profile for clonal trial (C26) planted with Yangambi origin at Phase 1B1 Kota Gelanggi in 1995
Another important aspect that is given great emphasis when it comes to clonal material is their bunch component particularly oil to bunch (O/B) that is directly translated into oil yield potential (OYP). Figure 8 summarizes the comparison between the 4 clonal trials (C3, C26, C27 & C29).
It is clear that the O/B in Yangambi clonal material has a greater potential with at least 1% gain over La Mé material. Although the gain is minute, the translation into oil extraction rate (OER) is significant (0.855%). On top of that, the oil yield potential coming from the Yangambi clonal material is on average at 0.7 tonne more than La Mé material on the same hectare basis.

**HECTARAGE OF PLANTING**

Since 1987 to 2015, more than 500 ha have been planted with clonal materials as trials (*Figure 9*). Meanwhile for commercial planting, since 2006 to 2017, a total of more than 41,000 ha semi-commercial area has been planted with clones (*Figure 10*).
Figure 10: Total hectare planted with clonal materials as Semi-Commercial in 2006 to 2016

COMPANIES INVOLVED IN TC

There are about 13 laboratories involved in oil palm cloning in Malaysia (Table 1).

**TABLE 1: RAMETS PRODUCTION (EX-LAB) FROM 2006 TO 2015 OF TISSUE CULTURE LABORATORIES IN MALAYSIA ('000)***

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Source: MPOB

A total amount of 42.6 million ramets ex-lab has been produced in Malaysia of 13 laboratories from year 2006 to 2015 (Table 1 & Figure 11). The main player in oil palm clonal ramets production are AAR, TSH, IOI and FGV.
Since 1998 to 2017, FGV produced a total amount of 17 million ramets (Figure 12). However, within this 17 million ramets produced, only 60% are sold, and the remaining is for internal usage.

Figure 11: Ramets production (ex-lab) from 2006 to 2015 of Tissue Culture Laboratories in Malaysia ('000)

Figure 12: Ramets production numbers in FGV, Ex-lab and the saleable ramets from 1998 to 2017.
Oil palm cell suspension culture has been conducted in FGV for large scale production since 2006 and sales of ramets from liquid cells suspension begun in 2009. However, not all ramets can be produced through the suspension system. Out of 80% palms producing embryoid, only 20% of palms cultured can give the right embryogenic structures that would be able to proliferate in liquid suspension system. Throughout the cloning years of 2009 to 2017, continuous improvement on embryogenesis rate has further increased the percentage of amenable palms producing the right embryogenic cell structure from 20% to 40% (Figure 13).

The ramets produced for large scale production since 2009 to 2017 is obtained from a combination of gelled (70%) and liquid system (30%). With the combined use of both systems, the overall average abnormality of semi-commercial area remain below 3%.

**SCALING UP FURTHER**

One of the challenges in FGV oil palm cloning is the availability of high quality ortets arising from breeding and selection programme. The current ortets obtained for clonal production in FGV mostly are selected from clonal trials, which are the reclones of proven clonal palms.

Although FGV has manage to get higher embryogenesis rate than before (Figure 13), the tissue culture process still remains costly and labor intensive (Figure 14). Further fine tuning of the tissue culture process allow for a reduction of 20% of manpower involve in oil palm cloning from year 2013 to 2018. To further reduce dependency on skilled labour, a shift towards use of automation for rapid large scale propagation needs to happen.
There are other technologies that are yet to be explored within the cloning sphere such as cloning using inflorescence as starting material, single plantlet germination in liquid system (in a simple fermenter) and the development of cloning platform through protoplast and anther culture. Regeneration of oil palm protoplast with limited success has been done by Masani et al. (2013). These should be further explored as protoplast can become indispensable tools in genetic engineering and gene editing for crop improvement. Gene editing technology has been touted as a game changer in crop improvement. Implementation of such technology in oil palm would be reliant on the use of protoplast technology for gene transformation and subsequently regeneration of oil palm clones carrying the edited genes.

Anther culture is another cloning technology that should be revisited as it can be an alternative method to produce homogeneous planting materials through generation of haploid plantlets. The haploid plantlets are self-sterile due to presence of single set of chromosome which are not able to participate in meiotic segregation. By colchicine treatment, haploids are made homozygous diploid, or isogenic diploid which are fertile. Some preliminary work on culture of oil palm microspores has been published (Odewale, 1989; Latif, 1991; Tirtoboma, 1998), but as yet there are no reports of homozygous diploid oil palm being produced by this method.

The use of tissue culture technique as a tool for crop plant improvement has long been demonstrated for many crops. Tissue-culture protocols are available for most crop species, although continued optimization is still required for many crops, especially in oil palm. Tissue culture techniques, in combination with molecular techniques, have been successfully used to incorporate specific traits through gene transfer in many plant species. \textit{In vitro} techniques for the culture of protoplasts, anthers, microspores, ovules and embryos have been used to create new genetic variation in the breeding lines, often via haploid production in many other plant species.
CONCLUSION

Tissue culture in FGV from young leaves has been routinely used for clonal oil palm production. FGV has been producing more than one million ramets ex-lab annually since 2009. The rates of abnormality are currently being controlled at an average of 3% for plantings in 2010 to 2014. In clone vs DxP comparative trials, clones always outperform DxP in both FFB and oil yield. New developments in protoplast and another culture will need to be made to ensure the continuity of this technology in the post-genomic era. In conclusion, clones have proved themselves as a superior planting material for the oil palm industry and will continue to be relevant in the post-genomic era as a tool for crop improvement. Refusal to accept this is akin to the refusal of adopting advance technology for the prosperity of the industry as we move into the future.

REFERENCES


SESSION 3
A New Perspective to the Oil Palm Cloning Program in Sime Darby Plantation

Suzaini Yahya, Nita Azlin, Hasnoor Laili Mat Hassan, Musa Bilal, Mohd Hafizul Azlan Omar, Teh Chee Keng and David Ross Appleton

ABSTRACT

The objective of the oil palm cloning program in Sime Darby is to commercially produce high yielding tenera clonal planting material, potential to produce 30% higher oil yield compared to Dxp seedlings. Adopting a two-stage ortet selection by assessing the field performance of clones produced in terms of the yield and clonal abnormality in a trial prior to commercial production is necessary to ensure that the best clones are reproduced or recloned for larger-scale planting and have minimal abnormalities. It takes 7 to 8 years to select ortets from a Breeding trial, followed by another 7 years to complete the evaluation of a clonal trial. By then, the next generation of high yielding Dxp materials from conventional Breeding is already available, which can reduce the yield benefit of the clones compared to current Dxp. Recently, Sime Darby Plantation developed GenomeSelect™ (GS) technology that enables early yield prediction of individuals of the latest generation progeny based on genotyping. By incorporating GenomeSelect™ (GS) into tissue culture, together with usage of zygotic embryos, selection of highest yielding materials can be applied to the latest generation of breeding materials through a 2-stage system along with significant increases in efficiency saving at least 7 years in the selection cycle.

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*Sime Darby Biotech Laboratories Sdn Bhd
Progress of Tissue Culture Research and Monitoring of Clones in IOPRI

Hernawan Yuli Rahmadi*, Erwin Nazri.*, Ernayunita*, Arfan N. Simamora*, A. Razak Purba, Retno D. Setiowati*, Sri Wening* and Edy Suprianto*

ABSTRACT

Indonesian Oil Palm Research Institute (IOPRI) in collaboration with CIRAD had started the tissue culture research since 1985. However due to soma clonal variation, the tissue culture commercialization in IOPRI has not been established up until now. IOPRI had several techniques to reduce the soma clonal variation, such as: minimizing subculture in all culture phase under 23 times, plant hormones related to soma clonal variation usage reduction, plantlet morphological selection before acclimatization, traceable clones using computerized database, and monitoring of clones planted in Riau and Jambi in 2013 and 2015/2016.

From the monitoring of clones in Riau and Jambi we observed that the flower abnormality were at 2.11% and 2.71%, respectively. This suggests that maintaining subcultures under 23 times of all phases could reduce flower abnormality. Before subcultures restriction, the abnormality rate could reach up to 53.35% in one of our field trial planted in 2009. Even though our clones monitoring in Jambi was not completed yet since several palms were planted in 2016 and were not bearing any fruit yet (immature).

Other than the Elaeis guineensis tissue culture, IOPRI in collaboration with Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) has conducted cloning Elaeis oleifera x Elaeis guineensis F1 hybrids and backcrosses (BC) since 2016/2017. From the study we found that the average of callogenesis rate was 1.71% in BC and 1.14% in F1 hybrid explants. The highest callogenesis rate was 5.93%, occurred in BC from Brazil. After 3 subcultures, the calli have become embryogenic and will be transferred to Temporary Immersion System (TIS) for somatic embryo induction. Somatic embryo induction, maturation, and germination will be conducted in TIS.

Keywords: oil palm, tissue culture, soma clonal variation, OG hybrids, TIS

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INTRODUCTION

Oil palm tissue culture in Indonesian Oil Palm Research Institute (IOPRI) started in 1985 when Pusat Penelitian Marihat (PPM) in collaboration with IRHO/CIRAD-CP, France build a tissue culture laboratory in Marihat (Ginting and Ginting, 2007). The first oil palm clones produced from this collaboration were planted in Cot Girek, PT Perkebunan Nusatara I, Aceh, in 1987. More clones had been planted in numerous location in Sumatera, Borneo, West Java and Celebes. Recently IOPRI had developed several procedures in the clone shipments to accommodate these vast trials.

At the beginning of the clone trials, the performance was promising and the clone yields were 20-30% higher compared to palms from seedling (Latif, 2004). This result was mainly determined by how the individual ortet was selected from their progeny trials. However, clonal variations occur in the clones’ flower that gave mantled fruits, abortion, androgynous and gynandromorph flowers. This finding hinder IOPRI clones to be commercialized, since the abnormalities observed in the field were inconsistent, with abnormality rates ranging from 0% up to 100% (Setiowati, et al., 2011). Therefore, IOPRI had develop several techniques to reduce these abnormalities.

Other than that, since 2016 IOPRI in collaboration with Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB), has conducted cloning on Elaeis oleifera x E. guineensis F1 hybrids and backcrosses (BC).

DIFFICULTIES IN SEARCH OF THE BEST PALM

To gain 20-30% higher yield compared to palms from seedling, ortets must be selected from the best-of-the-best palms available in a progeny trial. Nevertheless, this is not an easy task since the oil palm phenotype was subject to environmental and observation factors. Therefore, to select the best palms is not based on its phenotype observed but from its genotype value. Most of IOPRI progeny trials were set in Randomized Complete Block Design (RCBD) (Figure 1), hence the statistical model is:

\[ y_{ijk} = \mu + F_i + B_j + H_{ijk} + E_{ijk} \]

- \( F_i \) = i – treatment effect (genotype)
- \( B_j \) = j – plot effect
- \( H_{ijk} \) = observation error
- \( E_{ijk} \) = experimental error
Figure 1. Randomized Completely Block Design of IOPRI progeny trial.

If \( \hat{H}_{ijk} \) is known, then \( \hat{G}_{ijk} = \hat{F}_i + \hat{H}_{ijk} \) can be calculated, which is the estimated genotype value. However, the \( H_{ijk} \) is not separable from the \( E_{ijk} \). Because of this inability to separate the observation error from the experimental error, it is valuable to try to analyse based on the palm kinship structure within the progeny trials by Best Linear Unbiased Prediction (BLUP). Other approach to select the ortet is using molecular markers from the association mapping.

CHALLENGES IN ACCLIMATIZATION AND CLONES SHIPMENTS

After the ortet was selected, the next challenges occur when the plantlet acclimatization took place. Before current procedure the acclimatization success rate was low, while current procedure on average had 83.35% of success rate (Hidayat, et al., 2011). The procedure includes: (1) Washing the media from the roots, then ± 2 minutes dipping in fungicide. (2) The plantlets were planted in mixed of sand, soil and compos in 10:3:1 ratio. (3) The plantlets were individually covered by transparent plastics bag for 1 month (Figure 2) (4) The individual covers were replaced with “global cover” for 2 weeks (Ernayunita et al., 2017).
Figure 2. Individual covers in plantlet acclimatization.

Beside acclimatization, shipment is a challenge on its own, if the monitoring trials are far away from the laboratory and in different island. Plantlet shipment in testube is packed using styrofoam box filled with styrofoam grains (Figure 3) (Ernayunita, et al., 2014).

Figure 3 Plantlet testube shipment in styrofoam box.
Shipment of clones to pre-nursery (PN) is done by bare-root treatment without soil, wrap in paper and sealed in plastic bag (Figure 4) (Ernayunita, et al., 2013). While bare-root procedure in main-nursery (MN) stage for clone was not recommended since the clone’s vigor was rapidly depleted in bare-root procedure (Ernayunita, et al., 2014).

**Figure 4 Bare-root procedure of shipment of clone to PN: a) gently clean all the soil from the roots, b) wrap the plantlets’ roots in paper, d) do not allow the plantlets’ leaves to be in contact with water, hence stack the wrapped plantlets in an opposite direction, and d) sealed the plantlets in plastic bag.**

**TECHNIQUES TO MINIMIZE ABNORMALITIES**

Different techniques to minimize soma clonal variation in oil palm clones produced by IOPRI are (Ernayunita, et al., 2017):

1) Minimizing subculture in all culture phase under 23 times.
2) Plant hormone related to soma clonal variation usage reduction.
3) Plantlet morphological selection before acclimatization (Figure 5).
4) Clones’ traceability through computerized database (Figure 6).
5) Monitor clones planted in Riau and Bah Jambi (Table 1).
These measures have led to the drop of abnormalities from 53.5% in clone trial planted in 2009, to only 2.11% and 2.17% (Table 1) respectively on clones planted in Riau (2009) and Jambi (2015 and 2016). Even though our monitoring of clones in Jambi was not completed yet since several palms were planted in 2016 and were not bearing any fruit yet (immature).
**TABLE 1. ABNORMALITIES RATE OF IOPRI CLONES IN RIAU AND JAMBI**

<table>
<thead>
<tr>
<th>Planting Year</th>
<th>Location</th>
<th>Clones Planted</th>
<th>Clones Observed</th>
<th>Abnormal Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>Riau</td>
<td>95</td>
<td>94</td>
<td>2.11%</td>
</tr>
<tr>
<td>2015/2016</td>
<td>Jambi</td>
<td>1255</td>
<td>422</td>
<td>2.71%</td>
</tr>
</tbody>
</table>

**E. OLEIFERA x E. GUINEENSIS F1 HYBRIDS AND BACKCROSSES CLONING**

Cloning attempt of F1 hybrids started in late 1980s, yet only few clones were produced and planted in the vicinity of tissue culture laboratory in Marihat. The BC1 cloning started when ortet of Suriname BC1 at West Kalimantan was collected in 2006. The clones were planted in 2013 in Riau and North Sumatera and the abnormality rate was 100%, even though techniques to minimize abnormalities were implemented. At the same field trials IOPRI also planted BC2 of Suriname origins. From the BC2 trials we found out that the BC2 also produces similar abnormal fruits and flowers found in the BC1 clones (Fig. 7). This finding suggest that the abnormalities was not solely due to tissue culture procedure, but also from its genetic background.

![Abnormal fruits of Suriname BC2](image)

Since 2016, funded by Badan Pengelola Dana Kelapa Sawit (BPDPKS), IOPRI in collaboration with IRIBB try to clones F1 hybrids and BC1 both from *E. oleifera* origin Brazil and Suriname. From the study we found that the average of callogenesis rate was 1.71% in BC and 1.14% in F1 hybrid explants. The highest callogenesis rate was 5.93%, occurred in BC from Brazil. After 3 subcultures, the calli have become embryogenic and will be transferred to Temporary Immersion System (TIS) for somatic embryo induction. Somatic embryo induction, maturation, and germination will be conducted in TIS (Sumaryono, *et al.*, 2017).
REFERENCES


IOI’s Experience in Oil Palm Tissue Culture

Lim Loon Lui*¹, Chew Yee Chern¹ & Ng Shee Kiat²

ABSTRACT

IOI’s involvement in oil palm tissue culture started after it acquired, in September 1990, Dunlop Estate Berhad (DEB), which besides the 12 estates, 2 palm oil mills and 2 rubber factories, also included the Research Centre and Tissue Culture Laboratory, all located in Peninsular Malaysia. Research on tissue culture propagation of oil palm, which had already started in 1987 in DEB’s Tissue Culture Laboratory, was continued. With the successful development of the tissue culture protocol for the production of clonal ramets, IOI’s Tissue Culture Laboratory had undergone two expansions in production capacity in 2002 and 2008, to allow for the scaling up in productions of clonal palms for clonal trials and test plantings, semi commercial and eventually large scale commercial planting of clonal palms in IOI’s estates. As of to-date, millions of clonal palms had been produced and field planted in tens of thousands of hectares of IOI’s plantations in Peninsular, Sabah and Indonesia.

Successful large scale production of clonal ramets starts with stringent selection of high yielding ortets, followed by a series of processes from ortet sampling, explant preparation and culturing, culture incubation, stages of tissue culture process, hardening and establishment of ramets in pre- and field nursery and finally field planting. Experiences obtained over the years, and views on some pertinent success factors for large scale commercial production of clonal palms will be shared.

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CLOSING REMARKS
by
Dr N Rajanaidu
Vice President of the International Society for Oil Palm Breeders (ISOPB)

Dear Colleagues,

The theme of this International Seminar is “Status of Oil Palm Tissue Culture Technology” and it is jointly organised by IOPRI, MPOB and ISOPB. The Welcome Remarks for the Seminar was delivered by current President of ISOPB, Dr Ahmad Parveez and Opening Remarks by Dr Hasril Hasan Siregar, Director of Indonesian Oil Palm Research Institute (IOPRI). Dr Parveez traced the historical development oil palm tissue culture technology from 1975. He mentioned topics related to somaclonal variation/mantling, tenera clones, semi-/bi-clonal seeds, high OER, liquid suspension culture, somatic artificial seeds will be reviewed during the Seminar.

Dr Hasril Hasan Siregar indicated that this Seminar is held in conjunction with International Oil Palm Conference (IPOC) 2018. So far, ISOPB and IOPRI organised six seminars; 1988 in Pematangsiantar, 2003 in Medan, 2010 in Yogyakarta, 2014 in Bali and 2016 in Kisaran. In this International Seminar 10 papers will be presented and hopefully it will stimulate productive discussion among all of the participants.

Prof Soh Aik Chin delivered the Keynote Paper: Quo Vadis (Wither) Oil Palm Tissue Culture Clonal Propagation? He traced oil palm tissue culture technology development from mid-1970s and its subsequent set back due to mantle fruit abnormality reported since 1986. The ramet production and plantings have yet to make a significant impact in the industry. The oil palm ramets contribute less than 5% of the total oil palm planting materials. Generally, the clones tend to yield 10%-15% more than seedlings. The risk of mantling and poor cloning efficiency contributed to the high cost of ramets. With the finding of epigenetic mechanism for mantling, markers being identified to weed out abnormal ramets in the nursery before field planting.

Dr Ong-Abdullah,M et al. paper “ A Holistic Perspective of Current Progress in Oil Palm Tissue Culture” highlighted the need to improve the productivity of oil seed crops including oil palm to feed the world population of 9 billion by 2050. Most oil palm selected for cloning and recloning have 72% to 88% success rates. However, their respective callogenesis and embryogenesis rates are at a mere 14%-19% and 3%-7%. This has resulted in high cost of ramets compared to DxP seeds. Biomarkers associated to embryogenesis and KARMA have been developed into predictive tools and for quality assurance.
The next paper “The Status of Somatic Embryogenesis (SE) Technology Developed by IRIBB to Reduce the Abnormality in Oil Palm” by Sumaryono et al. describe the use of temporary immersion system (TIS) bioreactors. The growth rate of embryogenic calli of oil palm in TIS immersed was significantly higher than that of on solid media. Field observation of ramets showed that the level of floral and fruit abnormalities was less than 1%.

The presentation by AAR on “Experience and Challenges in Commercial Production of Elite Oil Palm Clones in Applied Agricultural Resources Sdn Bhd” highlighted the need to selecting genetically superior ortets for cloning and improve tissue culture protocols. Sourcing and training sufficient competent workforce plays a critical role in determining success of a commercial tissue culture laboratory. The paper provides information on callusing and embryogenesis rates of various genotypes. The data shows that AAR clones oil yield is 3-22% higher than DxP seedling control. AAR was able to minimise the mantling (<5%) risk by planting a package of 5-10 clones. Clonal materials are required to be planted interspersed with DxP materials to overcome pollination problem.

Asian Agri paper on “Status of Oil Palm Cloning Program at Asian Agri” covers the selection criteria for the ortet selection. Traits such as CPO, O/B, FFB, *Ganoderma* tolerance, slow height increment, compact canopy and oil quality were considered. Callus induction and callus differentiation rates based on *tenera* ortets were 96% and 84% respectively. Similar rates applied to Dura while *pisifera* was less responsive. Nigerian and Ekona Tenera genotypes were more responsive compared to Ghana. More than 1000 ha have been planted with clones in different environments. Floral and fruit mantling rate was less than 0.3%. A strong interaction of clones with environment was suspected as the consistency of clone performance in different locations varies. Asia Agri has field planted 1000 *dura* ramets and 40 *pisifera* ramets to produce semi- and bi-clonal seeds.

The paper “Overview on clonal propagation of oil palm at Dami OPRS, Papua New Guinea” by Baskaran Ponnusamy et al. highlights the main focus on cloning proven elite parental palms for production of high- yielding semi- clonal seeds, sold as “SUPERFAMILY”. The clonal propagation at DAMI is via somatic embryogenesis from immature inflorescence of elite parental palms. Since 2013, nearly 13 m SUPERFAMILY seeds have been produced from clonal mother palms. To-date, there was no mantling in the clonal parental palms and seed production from semi-clonal seed gardens has proven to be more efficient and cost-effective compared traditional seed gardens. SUPERFAMILY planting material planted at 120 palms/ha has FFB yield potential of 30t/ha and 9 t oil per ha.

The paper “Is there a future in tissue culture for oil palm industry” by FGV outlined to-date FGV produced 10 m oil palm ramets since 1983 and these have been planted in 41 000 ha. With the continuous improvement of cloning system, FGV was able to keep level of abnormality below 3% since 2010. FGV is confident that generally clones out- perform DxP; the FFB yield increase by 4% and oil yield on average by 17%. One of the challenges in FGV oil palm cloning is the availability of high quality ortets arising from its breeding programme. Hence, FGV has to depend on clonal trials as a source of ortets; which are reclones of proven clonal palms. FGV is
one of the major oil palm tissue culture laboratories in Malaysia; producing more than one million ramets annually since 2009.

Sime Darby paper “A New Perspective to the to the Oil Palm Cloning Program Sime Darby Plantation” adopted two-stage ortet selection by assessing the field performance of clones for yield and clonal abnormality in trial and best clones are recloned for large-scale planting. Recently, Sime Darby Plantation developed GenomeSelect™ (GS) technology that enables early yield prediction of individuals of latest generation progeny based on genotyping.

IOPRI paper “ Progress of Tissue Culture Research and Clones Monitoring in IOPRI” described the tissue culture protocol. By limiting subculture below 23 times, reduction of plant hormone related to somaclonal variation, plantlet morphological selection before acclimatization, traceability of clones using database and field observation, IOPRI was to reduce the somaclonal variation. IOPRI in collaboration with Indonesian Research Institute for Biotechnology and Bioindustry (IRIBB) initiated a programme to clone Elaeis oleifera x E. guineensis F1 hybrids and backcrosses.

IOI has one of the largest oil palm tissue culture laboratories in Malaysia; producing more than one million ramets per annum. IOI planted thousands of hectares of clonal palms in its plantations in Peninsular Malaysia, Sabah and Indonesia.

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See You at
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19-21 November 2019