Progress of Oil Palm Tissue Culture in Felda and Its Challenges

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Abstract

FASSB has been increasing its clonal production from 250,000 annually in 2007 until 1 million in 2010. With this increase, many challenges have to be overcome to ensure the success of scale up. One of the main issues is ortet selection and availability. Significant effort on recloning may help to alleviate this issue. However, questions on abnormality and age of palm for recloning need to be addressed. Another aspect is to look at increase the cloning efficiency to reduce the number of ortets required for large scale production. Suspension culture technology is touted to improve oil palm tissue culture efficiency significantly. Beyond the laboratory, managing the delivery of 1 million ramets pose several challenges. This includes method of delivery, tight participation of buyers and also setting up of servicing teams to ensure smooth progression of ramets from weaning, to field planting.

Introduction

Success in cloning oil palms was first reported by Jones (1974) and Rabechault and Martin, (1976). Generally, oil palm tissue culture in Malaysia started since early 1980's. Since then, the developments in the cloning techniques, albeit slow, have raised expectations in the industry regarding the possibility of commercial scale propagation. However, issues regarding the economics and technical feasibility of scaling up the cloning techniques have been raised. For oil palm, this means handling hundreds of plant multiplication at various stages manually on a daily basis. As the technique is still very much labour intensive, it is a daunting task to manage the production of 1 million oil palm ramets from field (ortet) to field (planting). It is a delicate marriage between science, art and management.

FELDA's effort towards producing 1 million oil palm ramets annually has started approximately 30 years ago. Although many challenges were faced, FELDA continues its research and clonal trial programme to improve the processes in terms of reducing the floral abnormality, improving the amenability of ortets, increasing the efficiency of the process and reducing the cost of cloning. Based on these experiences, we can conclude that scaling up oil palm cloning, although with much trials and tribulations, is technically possible, economically feasible and can be considered as key towards planting material diversification.

Ortet availability

Oil palm cloning begins with the 'right' ortet selection methodologies. Selecting the 'true' high yielding palms as ortet have always been difficult due to large environmental variance, low heritability and even possibly inaccurate measurements. This could lead to unreliable ortet selection or poor correlation between ortet and its ramets. In Felda, the selection criteria includes:

- 9.0 ton oil / ha / year
- >30% oil /bunch
- Height increment < 0.5 m / year

Comprehensive breeding scheme incorporating various materials is necessary in order to create source of ortets. It has been shown that selection of individual palms will not contribute towards oil yield improvement due to its low heritability (Soh et al., 2006). Instead, selection of high oil yielding ortets from superior progenies will increase the efficiency of cloning. Although selection is made based on oil yield, more emphasis is made towards selecting for palms with higher bunch number and oil to bunch as these parameters are more heritable. Ortets with higher bunch number with medium size bunches are preferred over those having bigger bunches with lower bunch number.
FELDA's oil palm breeding program which started in late 1960s have proven successful in producing good materials to be selected as ortet for cloning. FELDA has also started a programme towards recreating crosses that will give good progenies that are amenable to cloning and also recloning. For recloning, it was observed that the amenability rate is higher (Table 1). These efforts are expected to increase the availability of good ortet.

Table 1: Cloning and recloning efficiencies

Stages	Success rate	
	Cloning	Recloning
Callus induction rate for leaf explants	17%	18.20%
Callus induction rate for palms	100%	100%
Callus differentiation rate for leaf explants	3%	5%
Callus differentiation rate for palms	70%	100%
No. embryoid lines able to proliferate	60%	79%
Average no of shoots obtainable per line	300	~2600
No.palms able to produce > 50,000 shoots	21%	na
Mantling rate of ramets	< 5%	na

Note:
na : Not available yet

The tissue culture process

Oil palm cloning technology is generally inefficient with embryogenesis rate in callus cultures averaging around 5%. Although callusing rate can be as high as 100% (palm wise) with about 60% of palms being embryogenic, the proliferation rates are still not optimal. In general, 30% of ortets provided the bulk of clonal planting produced. As such, in commercial propagation, large number of ortets has to be cultured to make up for the inefficiency of the cloning process. A variable, but generally small number of plantlets (100 to 10,000) can be produced from a single ortet, and each ortet can only be harvested for leaf explants once in 3 to 5 years. This means that a steady supply of superior ortets is crucial for maintaining the quality of oil palm clones produced.

On top of being inefficient, oil palm cloning is often genotype dependent and in general, about 60% of ortets available in FELDA is amenable to the current tissue culture process (Table 2). The remaining 40% is non-amenable. As such a program dedicated to continually developing cloning protocol for newer varieties or crosses is being carried out. This allows the cloning programme to be at par with the breeding progress rather than maintaining the cloning programme for a few selected background.

Table 2: Success in cloning (% of ortets able to produce ramets according to genotype)

Stages	Yangambi	NIFOR	La Me	Others
% Ortet	20%	37%	41%	18%

Improvement in the efficiency of cloning can be achieved through the use of plant growth regulators. We have evaluated the improvements that can be made in terms of cloning efficiency with the usage of growth regulators in the culture media (Table 3). However, the use of growth regulators must be made with caution as previously it has been implicated with the rise of abnormality. Quality ‘checkpoints’ need to be made along the tissue culture process to ensure that stringent culling and selection be made to reduce occurrence of abnormality.

Table 3: Success rate at each stages in oil palm tissue culture.

Stages	Success rate	
	Without growth regulator	With growth regulator
Callus induction rate for leaf explants	17%	23.17%
Callus induction rate for palms	100%	100%
Callus differentiation rate for leaf explants	3%	5%
Callus differentiation rate for palms	70%	70%
No. embryoid lines able to proliferate	60%	79%
Average no of shoots obtainable per line	300	2600
No. palms able to produce > 50,000 shoots	10%	21%
Mantling rate of ramets	< 5%	< 5%

To further increase the efficiency of cloning, Felda has embarked on the research and development of suspension culture technology. Through this technology, larger numbers of ramets per ortet can be achieved (Table 4). Tarmizi et al. (2004) has shown that using the bioreactor, proliferation rates of about 10 to 14 fold weight increment of embryogenic cells can be achieved after 50 to 80 days in culture. The suspension culture system offers advantages in reproducibility, versatility and efficiency with high potential for scaling up propagule production. Clonal trials conducted on limited numbers liquid culture clones suggested that the technology is viable to be exploited for oil palm clone production (Table 5).

Table 4: Gel versus Suspension culture.

Stages	Success rate	
	Gelled	Suspension
Callus induction rate for leaf explants	23.17%	23.17%
Callus induction rate for palms	100%	100%
Callus differentiation rate for leaf explants	5%	5%
Callus differentiation rate for palms	70%	70%
No. embryoid lines able to proliferate	79%	20%
Average no of shoots obtainable per line	2600	10000
No. palms able to produce > 50,000 shoots	21%	50%
Mantling rate of ramets	< 5%	< 5%

Table 5: Clonal Trial results of clones from suspension culture

Treatments	Total number of clone tested	Mantling rate	Average
T9 (Gelled)	5	0.67%	2.5%
T10 (Gelled)	3	2.45%	
T13 (Gelled)	3	4.62%	
T14 (Gelled)	9	2.19%	
S6 (Liquid)	7	1.56%	1.5%
S9 (Liquid)	9	1.80%	
S12 (Liquid)	7	4.91%	
S13 (Liquid)	7	1.52%	
S29 (Liquid)	3	1.53%	
S30 (Liquid)	3	0.00%	
S38 (liquid)	4	0.0%	
S39 (liquid)	2	0.5%	

Overcoming Floral Abnormality through Culture and Field Management

As the search for floral abnormality marker continues, oil palm tissue culture labs must find ways to circumvent this problem. We cannot fully eliminate floral abnormality in oil palm clones but with better management of cultures, the occurrence can be maintained at below 5% level. This includes establishing checkpoints at various stages of tissue culture, stringent selection throughout the tissue culture processes and strict culling in the nursery. To assist the buyers, we have also established a clonal quality unit where all processes in the nurseries and field are being carried out or vetted by this team to avoid delays or errors in determinations of floral abnormality occurrence. However, ultimately there must be a prediction system that is fast enough and efficient enough for us to 'catch' abnormality at its' earliest onset.

Field Planting

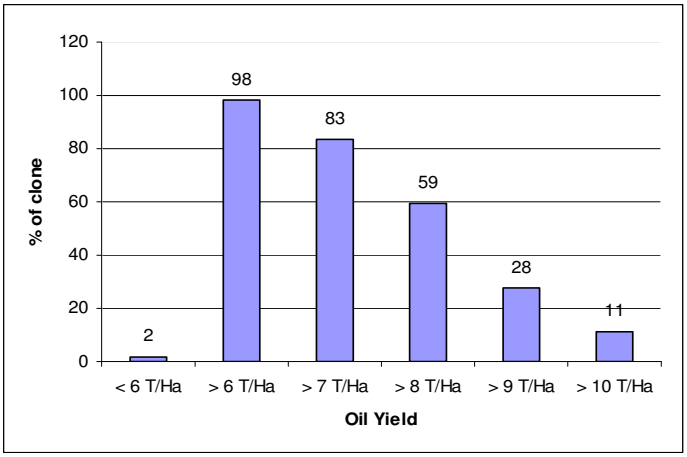
An important aspect of clonal planting for high yield is that only prime land should be utilized. High yielding clonal planting materials should only be planted on fertile soil with good site-yield potential in order to realize the potential of the genotype, the environment and its combination (G X E). Numerous publications on strategies and requirements for yield enhancement have been made (Chan, 1998; Chew and Goh, 2003; Goh and Chew, 2000; Goh et al., 1994, 2000). In addition, good agricultural practices are crucial to ensure good yield are sustained. This includes establishment of a good leguminous cover, proper soil and

moisture conservation practices, integrated pest management and discriminatory manuring practices.

The fertilizer regimes for clones must be tailored specifically for the planter to reap its maximum potential. Preliminary studies showed that the quantitative nutrient demand of clones does not differ much from D x P, but strong indication suggest that more attention should be paid to maintain correct nutrient balances and ratios and the use of suitable fertilizer sources (Ng et al., 2003).

Based on 6 trials of over 50 clones comparing performance of clones, 48% of FELDA clones were capable of giving more than 8 tonnes oil per ha of which 50% of them gave more than 8.8 tonnes oil per ha (Figure 1).

Figure 1: Clonal Performance of FELDA clones over D x P



When looking at yield performance based on origin, Yangambi was found to give the highest oil yield increment at an average of 22 percent more oil compared to DxP, followed by NIFOR (20%), La Me (17%) and others (14%) (Table 6).

Table 6: Clone Yield According to Origin

% over D xP (mean)	Yangambi	NIFOR	La Me	Others
FFB	105	107	106	102
Oil	122	120	117	114

Clonal planting material yielding 40 to 50 tonnes FFB is not new. Such yields represent the combination of genotype, agro-management and environmental condition. However, such combinations are infrequent or they may occur in small locations. Yields of 30-35 tonnes are closer to the site yield potential for most fields planted on reasonably suitable areas and should be the more realistic targets. Coupled with extraction rates of about 24%-26%

reported for clonal plantings, oil yields of 7.2 – 9.1 t/ha are achievable, thus enabling the industry to break out from its stagnating yield syndrome.

Problems and Limitations

Scaling up of oil palm cloning poses several limiting factors. One of it is the high production cost due to the labour intensive manner of the process and the slow proliferation rates. To date, in all oil palm tissue culture labs, skilled operators select and manipulate culture cells in sterile laminar flow hoods. This poses a limitation on the number of cultures that can be handled on a daily basis by an operator. On top of this, the current method of oil palm tissue culture is difficult to automate. If automation is to become the thing of the future, the current tissue culture techniques need to be reviewed. This include the way the media is prepared to cater for the various genotypic backgrounds that enters the lab to the way the cultures can be selected and manipulated during subculturing processes.

Another major limiting factor is the unavailability of a system to detect floral abnormality earlier. The cloning process does produce clones or lines that are very prolific that it can produce a million ramets with no problems. However, due to the uncertainty of clonal abnormality, such lines or clones have to be limited in their productions to avoid possible increase in the rates of clonal abnormality. This results in the removal of potentially high quality ramets from the lab purely due to speculations of clonal abnormality. This practice results in very high cost of the production of ramets.

Availability of a screening system that can be used even at the culturing stage would also allow research into media formulations for increasing tissue culture amenability to move faster as each step can be tested rather than having to wait till field planting results are observed.

Conclusion

The success in commercial production of oil palm clonal planting materials depends on several factors including availability of ortets for uninterrupted production, the efficiency of the system used to allow for production of high number of ramets per ortet, low contamination in the lab to avoid culture loss and high survival rates in the nursery. To maintain the abnormality level below 5%, a good traceability system must be put in place to ensure that each and every ramet has gone through stringent selection system.

Breeding programme has made significant progress in terms of producing high yield planting material. The improvements that can be made through clonal production are only as progressive as the breeding programme. With the high cost of clonal material, seeds are still the planting material of choice. However, the ability of the cloning technology to multiply palms of specific traits apart from yield traits must be capitalize to ensure the production of niche planting materials.

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