Strategies to develop oil palm clones for Latin American and Africa.

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1 Introduction

Palm Oil will remain at the first rank of vegetable oil for long despite some unfair NGO's attack, of course not all. This leads the world of oil palm to improve the sustainability of the industry. The breeders are part of the stakeholders as they provide the planting material for the plantation. They have to design a strategy that will enable continuous improvement of "sustainable intensification" of oil palm cultivation.
Breeders may help right now in many ways. Yields per unit of soil, per hour of man, per hour of mill can still be improved dramatically. In addition, as oil palm plantations can be devastated by difficult to control diseases, breeders can, or have to, propose planting material which is resistant to the most worrisome diseases. In the future, new improvements will come from planting material easy to harvest, or with low requirement in fertilizers, or event more resistant to drought etc... These are huge challenging aims!
Breeders work will be part of the future success of oil palm plantation and will need excellent work from agronomist and manager in order to show the extraordinary potential of this crop.
Up to now classical breeding have aloud to achieve great genetic progress and will probably be the core method which will generate continuous improvements. Nevertheless, in addition breeders need new tools to improve their efficiency in order to make all the promise happen as soon as possible. Of course molecular markers is one of them, genetic transformation of oil palm will very likely be a useful tool and tissue culture can help a lot in order to take advantage of exceptional genotypes.
This paper will focus on tissue culture. This technique as first been developed in the 70's (Smith and Jones, 1970; Rabéchault *et al*., 1970; Corley *et al*., 1977) and we still have a lot of challenges to achieve. Among them we will focus on:

- Management of trueness to type trough statistic or other tools.
- Tissue culture methodology and its technical and economic limits.
- Clone yield: selection of ortet and field evaluation.
- Disease resistances to fusarium wilt, blast, Ganoderma, Bud rot…

Of course most of these points should be considered whatever the continent is: Africa, Latin America and Asia. But we have to consider that for Africa and Latin America resistance to disease is probably a first ranking characteristic to be considered, as compared to Asia where only Ganoderma will be consider in a limiting number of plantation entering in the 3rd or the 4th generation.

2 Trueness to type

Clonal propagation of palms through somatic embryogenesis may lead to abnormalities. Many of them have been observed bud one cannot be detected in the early stage of the ramets development and it is observed only when the palms are two to three years in plantation. It is commonly called the ‘‘mantled’’ floral abnormality, which can make ramets unproductive (Corley et al. 1986).

Another question remains: do we face “silent” abnormalities? In other words: does it exist abnormalities that cannot be detected by normal observations but that affect performances of the clones?

2.1 ‘‘Mantled’’ floral abnormality

This abnormality, which is seen only once palms start flowering in the field, i.e. 2 to 3 years after planting, appear as a defect of flower architecture (Durand-Gasselin et al. 1993). In the more severe cases there is no fruit development, whilst in less severe cases only some of the female flowers are affected. Such abnormalities have been observed by all the research teams with various intensity depending on the in vitro culture protocol adopted.

Most of the protocols resulted in a small percentage of variant palms (Corley et al. 1986; Duval et al. 1988; Durand-Gasselin et al. 1993; Konan et al. 1995). We will not discuss here the various possible causes that have been suggested for the ‘‘mantled’’ floral abnormality as the most probable causes are link with DNA regulation through methylation (Jaligot *et al.*, 2000; Jaligot *et al.*, 2002; Jaligot *et al.*, 2004).

The fact is that we do not have in hand a tool that enables us to screen ramets for their trueness to type. Therefore, we have to manage the abnormality through a statistical scheme that will take into account different factors.

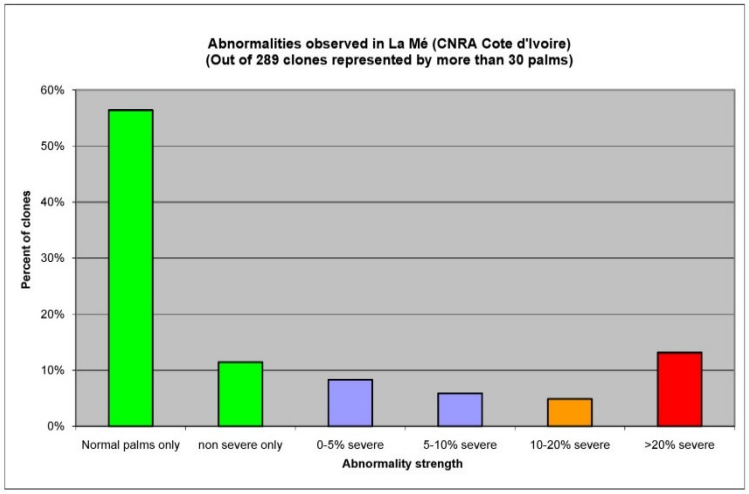

Figure 1: Unbalance proportion of mantledness between clones

2.1.1 The unbalance % of mantled palms

The first one is the unbalance % of mantled palms between clones : it varies from 0% to more of 20% (Figure 1). In order to observe and average abnormality rate close to the mean rate of abnormality (3%) a minimum number of clones should be planted. If possible from different families as it is not yet clear if a family effect can be found. 8 to 10 should be a minimum.

2.1.2 New diseases

A second risk is linked to new diseases: it is well-known that a single genotype can suddenly be affected by a new disease. One way to evaluate this risk is to consider the probability of bankrupt if such an event arises.

Let us imagine a population where, if such an event occurred, 10% of the plants would be destroyed and the others would remain untouched. In this binomial hypothesis, and if selection for this character is neutral, 10% of the clones drawn from this population will be susceptible and 90% resistant. In addition, the farmer defines a threshold β beyond which mortality is judged to be excessive. Let us use $\beta = 25\%$.

- Monoclonal plantations.

It is clear that 10% of the plantations will be a failure and 90% a success.

- Bi-clonal plantations

1% of plantations will have two susceptible clones and 18% a single susceptible clone; in both cases, the 25% mortality threshold will be exceeded. The risk of failure is therefore greater than for a monoclonal plantation (19%).

Calculations for a few additional cases are shown in figure 2. When n becomes large, the loss rate tends towards 0.10, the probability of drawing a susceptible clone and the risk of exceeding β becomes nil. A plantation set up with a small number of clones can therefore be more dangerous than a monoclonal plantation!

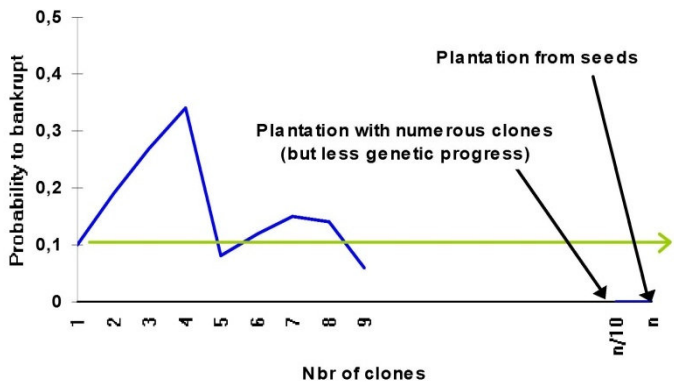

Figure 2: Probability of exceeding or equalling "unacceptable" loss threshold β depending on the number of clones planted. $\beta = 25\ \%$ and Proba (drawing a susceptible clone) = 10 %

2.1.3 Duration of in vitro culture.

Most of the teams working on tissue culture have observed an increase of abnormality linked to the duration of in vitro culture. For oil palm, a splendid work has been recently presented by Konan (Konan *et al.,* 2010). It shows that part of the clones is quite stable and is not affected by mantled abnormalities even 10 years after the first plantation (Figure 3). Unfortunately and other part is clearly affected by the "Mantled

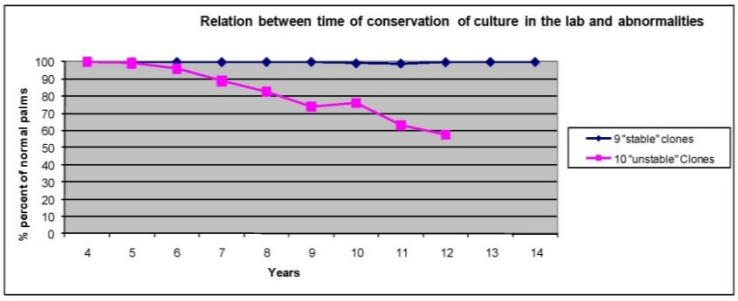

Figure 3: relation between duration of in vitro culture and percentage of normal palms in the field.

abnormality" right after the second year of plantation. The main problem seems to be that, for the time being, it is not possible to predict which clone is or will be abnormal.

Therefore most of the tissue culture labs have decided to limit the time of in vitro propagation. Many options have been proposed, they are not well documented and it is not always clear whether the decision is taken base on experiences or a mix of economic constrains and carefulness. Limitations results from a compromise between duration of the culture, number of palms per ortet sample, number of palm per embryogenic events.

2.2 Re-cloning.

Recloning strategy is of course a key point in building the strategy. At the time of re-cloning you should know the exact agronomic value of the clone as one had take time to evaluate it within a proper trial. In addition, one can concentrate its effort on clones which does not show any abnormalities (see point 2.1.3). The resulting strategy will therefore be really simplified.

But another aspect has to be taken into account: the trans mission of "Mantled" abnormality through vegetative reproduction; We know that Mantled abnormality can be transmitted through sexual reproduction, even if not totally. Crosses between two abnormal palms results in a variable proportion of abnormality (table 1).
Through vegetative reproduction our experience is that the resulting abnormality status of the palms is worse than the status of the iriginal ramet which is re-cloned :

- Recloning of a severely abnormal mantled palm results in a 100% very severely mantled palms.
- Recloning of a non-severely abnormal mantled palms results in a mixture of severe and non severe abnormal plams.
- Recloning of young normal palms (nursery, or 1 to 3-4 years in the field) results generally in an increase of non-severely mantled palms.
- Recloning of "adult" palms (8 years or more) does not, so far, lead to an increase of abnormality.

Table 1 : Transmission of mantled abnormality through meioses. % of abnormal palms.

Genitors/palms within a single clone	Mâle	Palm 1	Palm 2	Palm 3	Palm 4	Palm 5	Palm 6
Female	Flowers	Normal	Normal	Normal	Abnormal	Abnormal	Abnormal
Palm 1	Normal				0% (0/33)	0% (0/4)	0% (0/35)
Palm 2	Normal				0% (0/28)	0% (0/15)	0% (0/34)
Palm 3	Normal				**2% (1/50)**	0% (0/14)	0% (0/40)
Palm 4	Abnormal	0% (0/22)		0% (0/31)	**69%(20/29)**	**21% (8/37)**	
Palm 5	Abnormal		0% (0/2)			**28% (7/25)**	**32% (6/19)**
Palm 6	Abnormal	0% (0/11)	?% (0/0)		**23% (9/39)**		**29% (12/41)**

2.3 General conformity.

Results of recloning may let us think that there exists a sort of continuum of abnormality. This is very clear for "Mantled" abnormality, but might also happened for other characters of palms that are not easily observed. For example we have observed that bunch number have surprisingly low broad sense heritability: is that character affected by "silent" abnormalities?

For years we did try to plant a trial which will help us to demonstrate this hypothesis. Fortunately we will be able to plant in 2010 and 2011 two trials (table 2) which will compare:

- One progeny (A x B)
- 10 to 20 clones derivated from this progeny
- A top cross between palm of the self of A cross by the original pisifera B.

The mean of clones for the most important agronomic characteristics will be compare to the mean of the original cross and to the mean of the top cross. Of course additional interesting results are expected as the assessment of the genetic variance within the cross and therefore the expected genetic progress that is expected from clones.

Table 2: schematic protocol of trials which aim at assessing the general trueness to type of clones.

Material	**Trial 1 (Ecuador)**	**Trial 2 (Guatemala)**
Original AxB cross	144 palms	144 palms
Top cross: A (self) x B	288 palms	288 palms
Different clones from AxB	20 clones (x 72 palms each)	11 clones (x 72 palms each)

3 Tissue culture methodology.

The first tissue culture protocols which were developed were based on somatic embryogenesis followed by adventitious embryogenesis which allowed increasing the multiplication rate of the different processes. Most of the field trials have been obtain using this protocol.

Following de Touchet work of embryogenic suspension (Touchet (de) *et al.*, 1991) many teams have developed a new protocol based on embryogenic suspension using liquid media. AAR group have been a pioneer in that field and have for the time being the greatest experience in the field. (Wong *et al.* 1996).

The suspension protocol helps a lot to build a tissue culture strategy and have two main advantages. The multiplication rate is fantastic and limitation in numbers is linked to the risk management of abnormalities. The second advantage is that it is much more predictable than the original "solid" protocols. Within a reasonable time of around three years it is possible to predict and therefore organize a commercial production.
Field results have not been widely published, but information gathered from different sources indicates that, as long as multiplication during the suspension liquid phase is reasonable (what is reasonable ?), one has not observed a significan increase in abnormality level.
In Latin America, ASD and Cirad add a collaborative research project that lead to the development of a new protocol. Cirad/PalmElit uses exclusively this protocol in its laboratory in Colombia. Cenipalma has developed its own project based on

4 Expected clone yield.

In the 80's very few data were available on clones yield potential. Most of the groups were using theoretical to predict the expected value of clones (Sow, 1986; Meunier *et al.,* 1988; Baudouin and Durand-Gasselin, 1991) which was linked to different methods used to select the ortets (Baudouin et al 1994; Soh et al., 2003). The predictions shows that as a mean clones yield will not exceed that of the progenies (in which they have been selected) by more than 15 %, furthermore some authors predict very limited progress. This does not mean that some individual clones will not over yield progenies by 25 or 30 % but they will be quite rare (Cochard *et al.*, 1999).

An interesting work has been presented by B. Nouy and F. Potier a few years ago (Nouy *et al.,*2006 ; Potier *et al.,* 2006) which show from field clone comparative trials that broad sense heritability was high only for a small number of parameters. (Table xx)

Table 3: Correlation between the ortets at Aek Kwasan and the clones at Aek Loba
Broad sense heritability of the traits (i: immature; m: mature) (From Potier *et al.,* 2006)

	Ortet/clone correlation	h^2
FB	0.472**	0.506
MF	0.792**	0.788
OM	0.724**	0.572
OER	0.476**	0.432
BNi	0.482**	0.364
FFBi	0.484**	0.396
ABWi	0.592**	0.587
Oili	0.309*	0.244
BNm	NS	0.076
FFBm	0.322*	0.239
ABWm	0.424**	0.358
Oilm	NS	0.152

Broad sense heritability for FFB or OIL are weak also heritability for OER is workable (0.43) and very good for mesocarp to fruit (0.79). More surprisingly bunch number broad sense heritability is low while narrow sense heritability is generally high. It might happen that this character is affected by tissue culture.

Our breeding programme allows us to have continuous improvement of the seeds (Figure 4) (Durand-Gasselin *et al.*, 2002) and because of poor heritability most of the first cycle clones are not better than the current commercial seeds (Figure 5)

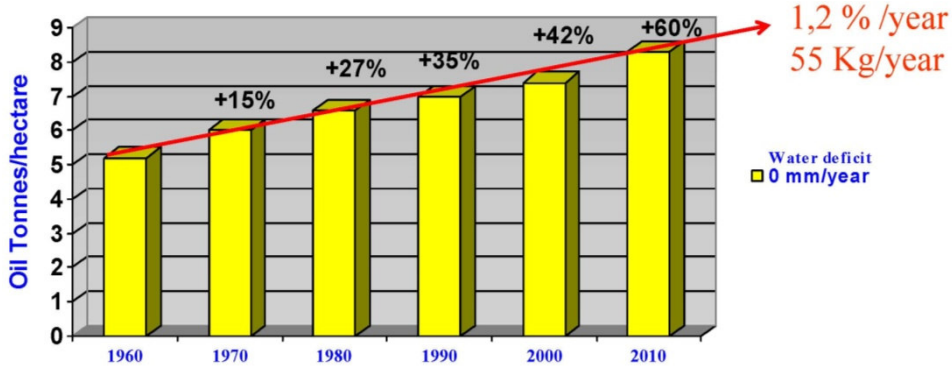

Figure 4: Illustration of the continuous pure genetic progress that can be achieve through a "normal" breeding programme.

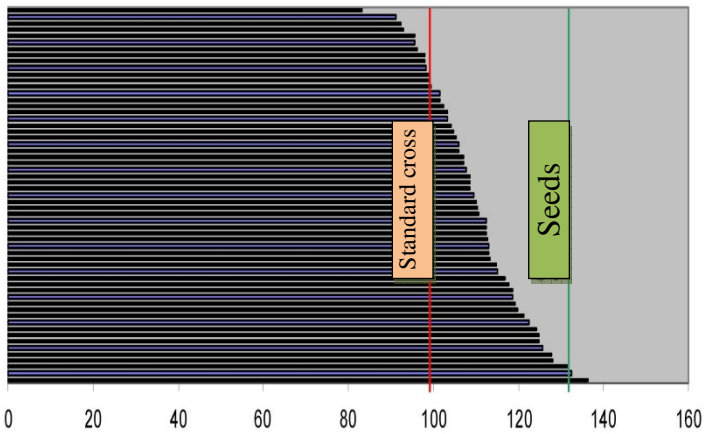

Figure 5: 1st cycle clones vs 2nd cycle seeds

Some breeding programmes are stick with a narrow genetic base and the seeds value does not increase rapidly. In that case clones can appear better than the in house seeds but not as good as seeds from other suppliers.

Therefore, facing a good genetic programme, the strategy for clonal propagation should anticipate seed genetic progress. One has to take into account the poor heritability of oil production and to create clones only from the very top ranking progenies. If the progenies are available in field, selection will focus on heritable traits i. e. OER only (and reasonable "normal" FFB yield).

5 Resistance of clones to diseases

Resistance to diseases is probably one field where clones can help a lot sustainable culture of palm oil. To develop disease resistant planting material is especially imperative for Latin America regarding bud rot development. In Africa it might appears not to be so important as excellent wilt resistant seeds already exist (Franqueville and Renard, 1990) but *Ganoderma* is also present and quite active in some area (Congo basin and Cameroun) and resistance to both diseases have to be combined, clonal propagation of such genotype will obviously help. In

Asia only *Ganoderma* has an economic impact on a limited number of plantations but resistance to that disease will very likely be consider by the planters as an additional security factor.

5.1 Resistance to Bud Rot

Both in South America and Central America, the existence of a disease, bud rot, is slowing down the development of the area under oil palm. This is particularly detrimental given the potential in the continent.
"Bud rot" (BR) is a disease with a complex symptomatology. Thus, many have perceived it as several diseases. It has been described in many forms (Louise *et al.*, 2006; G. Martinez *et al.*, 2008). To date the only solution to the disease is to plant interspecific E. o. x E. g. hybrids which are, in most of the case, resistant to the disease (Durand-Gasselin *et al.*,2009). But Philippe Amblard (Amblard *et al.*, 2009) has recently presented new progress in research toward selection of resistance to bud rot in E. guineensis. From this work, it is clear that some progenies are quite resistant to bud rot (figure 6)

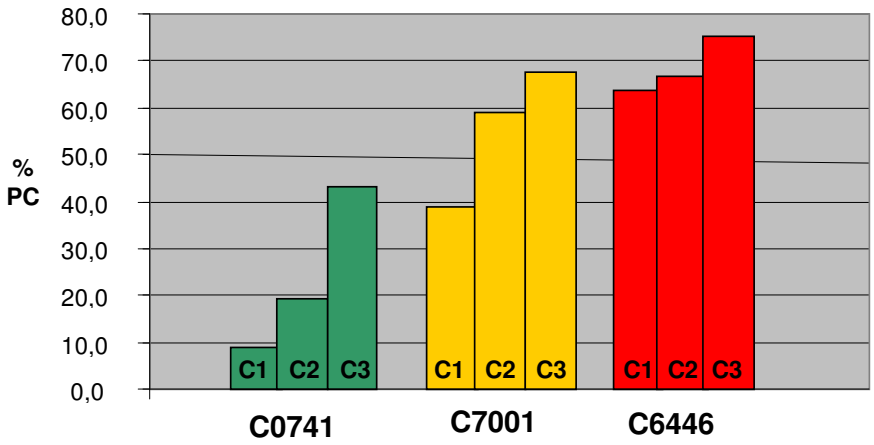

Figure 6: % of Bud Rot infection at 9 years in three families of three different materials

Clones are currently being developed from the family C1 of the C0741 material. As variability for quantitative traits is in oil palm generally huge, and we think that we face a quantitative resistance, we expect to find some strongly resistant material in such cross.

To illustrate that expectation, we can see what has happen with one clone (BTC 40) derivated from C1001 material: this material has been badly affected by bud rot also the infection of the BTC 40 clone remains much lower.
Obviously, is is possible to find resistant clones from families which are not that resistant. Of course in order to maximise our chances to find such material it is safer to start from families which are already resistant.

Figure 7: Comparison of the performance of clone TRL 70 and the cultivar from which it is derived (C1001) in relation to bud rot. (Shushufindi estate, Ecuador, Danec group) ;

5.2 Resistance to Fusarium wilt.

As cirad clonal programme has been first developped in Cote d'Ivoire, we have a wide experience in resistance of clones to fusarium wilt. The main results have been synthetized by Hubert de Franqueville (Franqueville et al., 1993). The resistance to fusarium wilt of 89 differents clones have been assessed (252 tests of 160 ramets each).
The ranking of resistance is wider than for progenenies : it exist extremely resistant clones as well as quite susceptible ones. Again it is shown that resistant material can be found in non resistant progenies and vice versa. An interesting example is given with the family LM10T x DA28D. This family is excellent for yield but is not resistant to this disease. Not surprisingly most of the clones derivated from this family are susceptible (Index >> 100) but one clone is average (index ~ 100) and one (LMC 111) is highly resistant (Index <<100) (table 4)

Table 4: results of fusarium wilt resistance of clones from the family LM10T x DA28D in early screening tests. LM10T x DA28D is a susceptible family.

Clone	Family	Nbr of tests	Mean Index
LMC 087	L10T x D28D	3	115
LMC 088	L10T x D28D	4	149
LMC 111	**L10T x D28D**	**3**	**67**
LMC 165	L10T x D28D	3	139
LMC 166	L10T x D28D	2	96

Table 5: results of fusarium wilt resistance of clones from the family LM10T x DA28D in early screening tests. LM2T x LM269D is a resistant family

Clone	Family	Nbr of tests	Mean Index
LMC 057	L2T x L269D	2	53
LMC 063	L2T x L269D	10	79
LMC 065	L2T x L269D	4	73
LMC 152	L2T x L269D	4	86
LMC 167	L2T x L269D	3	73

In a resistant family most of the clones are resistant to the disease (Table 5).

If we have in hand a very interresting family, for example very high yielding, it will be possible to invest some effort in looking for reistance to the disease, even if the family is not resistant. The remark remains probably valid for bud rot as well as for *Ganoderma* resistance.

To illustrate that point we can have a look to what has happen in a clonal trial in Cote d'Ivoire. Clone LMC 111 effectively shows a perfect resistance to fusarium wilt despite some of its neighborough have been destroyed (FEC 166). (Figure 8)

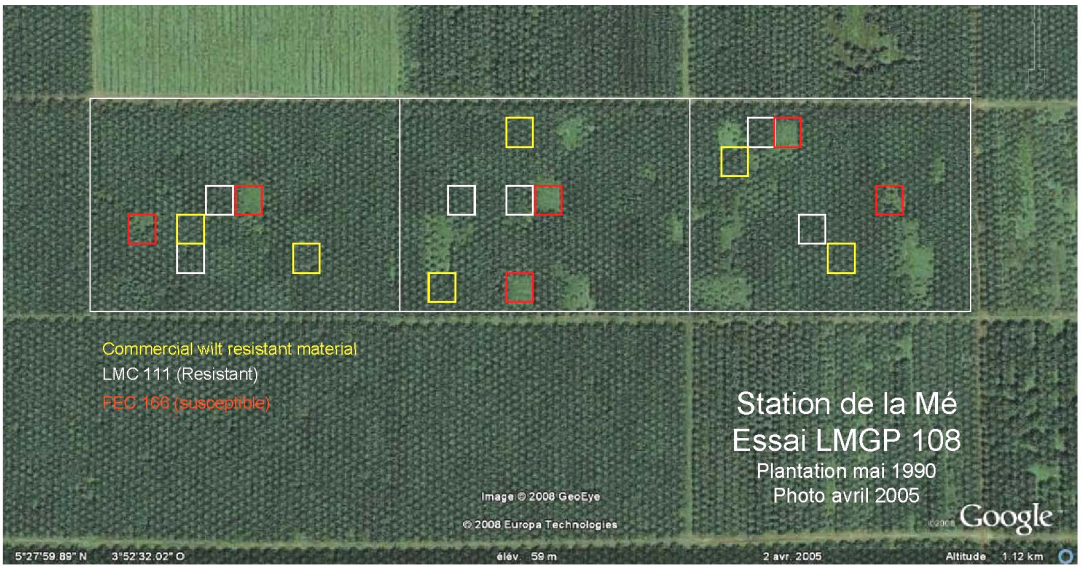

Figure 8 : Field observation of fusarium wilt in a clonal trial; Commercial resistant material (Yellow), LMC 111, resistant clone (White) and FEC 166, susceptible clone.

Experience gain from this work could be very useful to define strategies for resistance to the other disease. Up to now, it seems that it is possible to select clones resistant to Bud rot (see 5.1) as well as to Ganoderm (5.3)

5.3 Resistance to Ganoderma.

By chance (?) some clonal trials have been planted in Ganoderma infected field. The results were presented a few years ago (Durand-Gasselin *et al.*, 2005).
Once again it is clear that it seems possible to find very resistant material (Soc 1503), in this case in a resistant progeny.

Unfortunately, it has not yet been possible to assess the resistance of this clonal material in an early screening test.

Figure 9 illustrate what can happen if susceptible clone are planted : they can be devastated by the disease.

Table 6 – Performance of some crosses in relation to BSR, and of clones derived from those crosses.

Cross	Comments on the cross	Clones derived from the cross	Indexes obtained by the clones
LM10T x DA8D	One of the clearly highly susceptible crosses (Indexes obtained: 265 and 193)	LMC 009 SOC 0901 SOC 0902 SOC 0903	170 51, 153 183, 210, 267, 175
BB91D x LM311P	LM311P transmits a good resistance	SOC 1502 SOC 1503	67 39, 23
LM404D x LM718T	LM404D (?) and LM718T display a satisfactory performance	SOC0403	100, 87, 91

Figure 9 : Field observation of Ganoderm in a clonal commercial plot (Mata Pao, Socfindo, Indonesia); Clone Soc 001 is susceptible to ganoderma, the other clone show an average resistance.

5.4 Combining resistance to several diseases.

Early indications indicate that there is not obvious correlation between resistance to *Ganoderma* and resistance to *Fusarium* wilt (Franqueville, personal communication). It is clear that the defenses mechanism that are activated by the oil palm to provide some partial resistance to the different diseases are not necessary the same. Therefore it might be difficult du combine high yielding, and resistance to 2 or more diseases.

Clonal strategies can be developed to overcome this difficulty. From one or two excellent crosses, resistant to fusarium wilt (as an example), it might be possible to select clones which are also resistant to Ganoderma. This type of material will be specialy devoted to Congo Bassin and some part of Cameroun where the two disease are present in the same fields.

6 Conclusion

To develop a strategy for clonal propagation of oil palm one has to take into account a set of parameters which are sometime difficult to combine. The situation to day will probably (and hopefully) evolve

We can classified the parameter in two groups

1. Parameter link to tissue culture :
    a. Trueness to type: to manage the risk of abnormalities we have to produce 10 to 15 clones.
    b. Number of ramets per sampling must be limited.
2. Parameter link to yield of clone.
    a. Because of continuous genetic progress of seeds one must work only with very high yielding progenies.
    b. Resistance to disease can benefit from a clonal programme.

Obviously from the first set of parameters the trend is to increase the number of clones to be produce commercially, on the other hand, the second set tend to reduce the number of clones in order to maximize the benefit. Is it possible to compromise ?

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