A retrotransposon-based- strategy for the assessment of genetic and epigenetic stability of oil palm embryogenic suspensions

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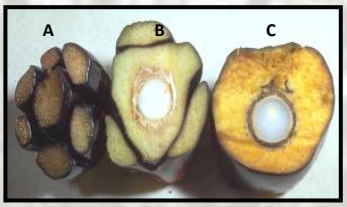
Background

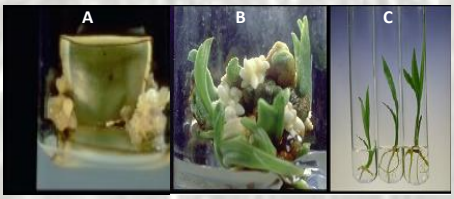
The oil palm (Elaeis guineensis Jacq.) is the highest yielding crop for edible vegetable oil. Due to a high level of heterozygosity, clonal propagation of oil palm by tissue culture has become an essential tool for breeding. However a significant proportion of off-type individuals display a variant phenotype called *mantled* [1] which affects both floral morphology and fruit development. Consequently this undesirable trait directly affects oil yields. As somaclonal variation is only visible tree years after planting, developing an early screening method is now a major challenge for the certification of clonal material to growers. To date and despite several studies, it has not been possible to isolate early detection markers of the abnormality, partly due to the high level of epigenetic alterations associated with the *mantled* phenotype (see [2] for a review).

Objectives

We are aiming at investigating the genetic and epigenetic stability of oil palm embryogenic suspensions during long–term *in vitro* proliferation. This will be achieved through the assessment of the part played by retro-elements. Evidence has been found for the reactivation of retrotransposons through tissue culture and a role in the emergence of somaclonal variations has been suggested [3-4-5]. Moreover, transposable element activity is controlled by DNA methylation which was demonstrated to vary both during oil palm tissue culture and in relationship with the occurrence of the *mantled* variation [2].



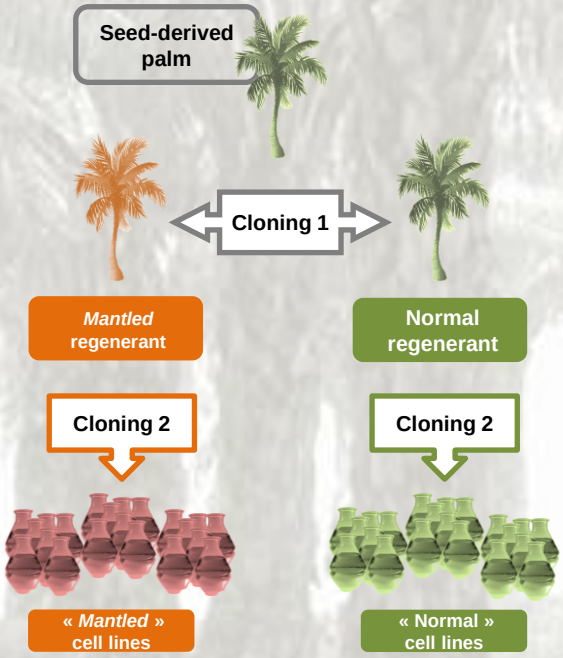
Oil palm fruit
(A) severely *mantled*
(B) slightly *mantled*
(C) normal



Micropropagation of oil palm though somatic embryogenesis
(A) foliar explant-derived callus
(B) polyembryonic culture
(C) rooted plantlets

Experimental design

Two seed-derived palms from distinct genetic origins were used to generate normal and *mantled* cell lines though two successive cloning events. Embryogenic suspensions were subcultured for one year and sampled every month for nucleic acids extraction.



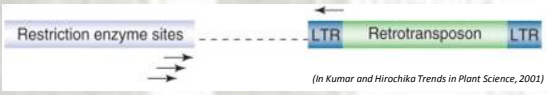
Identification of Transposable Elements in the oil palm nuclear genome

1) Partial sequencing of oil palm genome by 454 GSFLX titanium technology :
410Mb of high-quality data were generated with a sequence average size of 454bp covering about 0.23 X of oil palm genome.

2) *In silico* data mining with the AAARF software [6] from 454-generated sequences in order to 1) identify the different classes of transposable elements in the oil palm genome according to Wicker [7] and to 2) evaluate their relative abundance.

Investigation of dynamic changes in LTR-Retrotransposon families during *in vitro* culture

1) LTR specific primers are designed using sequence information from each class of retrotransposons.



2) S-SAP approach
We are developping a retrotransposon-based molecular marker system using a S-SAP approach [8]. Insertion polymorphims for each retrotransposon will be studied i) between time points during *in vitro* proliferation and ii) between cell lines of different genetic origin and phenotype.

Expected Outputs

Our present project will provide a mean to evaluate the impact of *in vitro* culture duration on oil palm genome stability and its incidence on *mantled* flowering. Through the development of a fingerprinting system, our study will help understanding the role of transposable elements in genetic and epigenetic instability. These data will enable the assessment of *in vitro* protocols and will provide the bases for the development of a clonal conformity test.

[1] Corley *et al.*, 1986, Planter, Kuala Lumpur 62: 233-240.
[2] Jaligot *et al.*, 2011, Annals of Botany, 108(8): 1453-1462.
[3]Granbastien, 1998, Trends in Plant Science,3 (5): 181-187
[4] Kaeppler *et al.*, 2000, Plant. Mol. Biol., 43: 179-188
[5] Bairu *et al.*, 2011, Plant Growth Regul., 63: 147-173
[6] DeBarry *et al.*, 2008, BMC Bioinformatics., 9: 235
[7] Wicker *et al.*, 2007, Nat Rev Genet., 8(12):973-82
[8] Waugh *et al.*, 1996, Mol Gen Genet., 253:687- 694