DNA METHYLATION IS INVOLVED IN SOMACLONAL VARIATION IN OIL PALM

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Introduction

Populations of somatic embryo-derived oil palms (*Elaeis guineensis* Jacq.) display on average 5% somaclonal variant individuals of the "mantled" type, involving feminisation of male floral organs [1] (Fig. 1). The incidence of this phenotype among regenerants varies according to the type of embryogenic callus used. Nodular Compact Calli (NCC) and Fast Growing Calli (FGC) yield respectively about 5 and 100% abnormal plantlets. In order to investigate the relationship between the "mantled" somaclonal variant phenotype and possible alterations in genomic DNA methylation rate, two complementary approaches have been used [2].

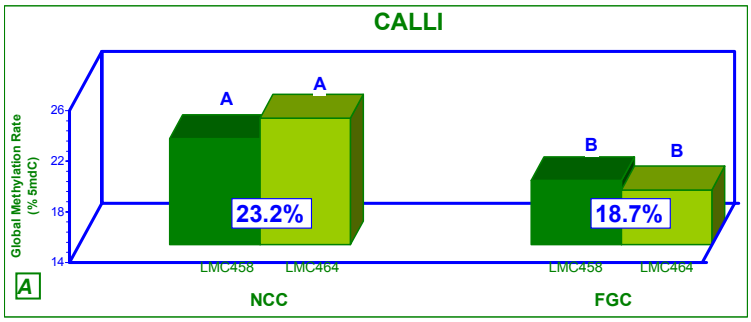
Fig. 1. From left to right: Oil palm fruits from severely mantled. slightly mantled, and normal regenerants.

Material and methods

Plant material : Two different clonal lines of embryogenic calli (NCC and FGC types were used for each) were obtained through *in vitro* culture of leaf explants [1]. Leaves were harvested from adult palms of four different clonal lines.

Estimation of global methylation rates :

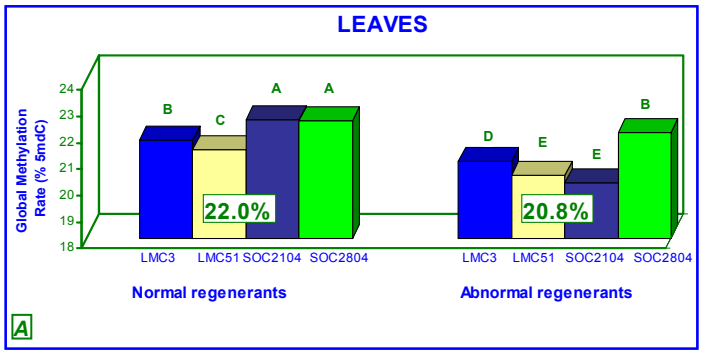
- ***HPLC analysis*** : enzymatic (P1 nuclease/alkaline phosphatase) hydrolysis of genomic DNA into nucleosides, subsequently separated and quantified [2]. The Global Methylation rate (GMR) is calculated as [5mdC]/([dC]+[5mdC]).
- ***SssI-Methylase accepting assay*** : enzymatic saturation of CG sites with tritiated methyl groups [3]. The Normalised Methylation Index (NMI) reflects the relative DNA methylation in the sample compared to that in the standard (DNA from leaves of seed-derived oil palm).



B

Clonal line	Type	NMI ± SD
LMC458	NCC	1.74 ± 0.43^{a}
	FGC	1.01 ± 0.04^{b}
LMC464	NCC	1.39 ± 0.15^{a}
	FGC	0.93 ± 0.29^{b}

Fig. 2. Global methylation rates (***A***) and normalised methylation index (***B***) calculated respectively from HPLC and SssI-MAA analysis performed on calli DNA. Values followed by different letters are significantly different at the 1‰ level (GMR) or at the 5% level (NMI).



B

Clonal line	Type	NMI ± SD
LMC3	NORMAL	1.51 ± 0.26^{cd}
	MANTLED	0.90 ± 0.04^{bc}
LMC51	NORMAL	0.68 ± 0.13^{ab}
	MANTLED	0.86 ± 0.19^{bc}
SOC2104	NORMAL	**1.05 ± 0.01^{cd}**
	MANTLED	**0.59 ± 0.06^{a}**
	NORMAL	

Fig. 3. Global methylation rates (***A***) and normalised methylation index (***B***) calculated respectively from HPLC and SssI-MAA analysis performed on leaf DNA. Values followed by different letters are significantly different at the 1‰ level (GMR) or at the 1% level (NMI).

Results & Discussion

A significative relationship between genomic hypomethylation and abnormal phenotype has been demonstrated for two different types of plant material of various different genotypes using two complementary approaches.

The overall global hypomethylation in DNA of abnormal individuals was more marked in calli (Fig. 2A) than in leaves (Fig. 3A), but the extent of the decrease in CG methylation was similar in both plant material (Fig. 2B & 3B). In neither case was it possible to determine a « threshold level » below which the methylation rate could be defined as « abnormal ». Nevertheless, these results constitute the first demonstration of an epigenetic defect correlated with the « mantled » abnormality in oil palm.

We are currently focusing our investigations of DNA methylation on sequence-specific phenomena. We are identifying an oil palm homolog of the *METI* DNA-methyltransferase gene of *Arabidopsis,* as decreases in the expression of the latter have been proved to result in genome-wide hypomethylation and alterations in floral morphology [4]. Moreover, MSAP analysis (*ie* AFLP using isoschizomeric restriction enzymes of differential methylation sensitivity [5]) has been undertaken to detect phenotype-specific changes in methylation patterns. The methylation status of individual cytosines in candidate markers is also being addressed using the bisulfite sequencing method [3], with the eventual aim of formulating a discriminant test based on methylation-sensitive PCR.

References

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