

An epigenetic approach for the determinism of the “mantled” somaclonal variation in oil palm

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The oil palm



- ◆ A giant perennial grass:
Monocotyledoneous,
Arecaceae (Palmaceae)
Coconut palm, date palm, rattan, edible palms,..
- ◆ Two cultivated species :
 - *Elaeis guineensis*
 - *Elaeis oleifera*
(enriched in unsaturated FAs)
 - Interspecific hybrid



Facing the global context ...

✓ The **past 10 years** have been marked by:

- a significant **increase in demand** for fat: + 50 %
- a **twofold increase in the production** of oil palm and palm kernel, which now account for one third of total vegetable fat production.



✓ This trend is likely to continue over the next few years:

In addition to **traditional uses** for vegetable fat, there is a increase interest and forecasted **demand for bio-fuels**.



A 2025 perspective ...

✓ Meeting these demands will be extremely difficult, if not impossible, unless there is a considerable **increase in oil palm production**.



✓ The necessary increase in oil palm production will involve **extending plantations** but also **improving yields**.

✓ This will only be possible if planters can rely on **quality planting material**

✓ Today, clonal planting material accounts for **less than 2%** in the global supply of improved planting material (seeds)

✓ The part played by oil palm vitroplants is **expected to rapidly increase** significantly in a 2025 perspective



Oil palm micropropagation



A collaborative IRD-CIRAD
research programme



Scaling up micropropagation



- Feasibility of SE-based process
 - 2 millions vitroplants
 - Sizeable genetic progress
- Transferred in producing countries: Indonesia, Malaysia, Côte d'Ivoire, Costa Rica, Colombia
- Bottlenecks from scaling-up:
 - Production costs: 2 to 4 US\$ per vp (5 to 7 x seeds)
 - Genetic fidelity



Bottlenecks in commercial development

1. Production costs

To set up an improved production process for oil palm

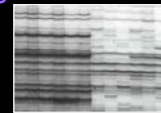
- ❖ large scale production ($\times 10^5$ vitroplants / year / clonal line)
- ❖ significant reduction in manpower costs



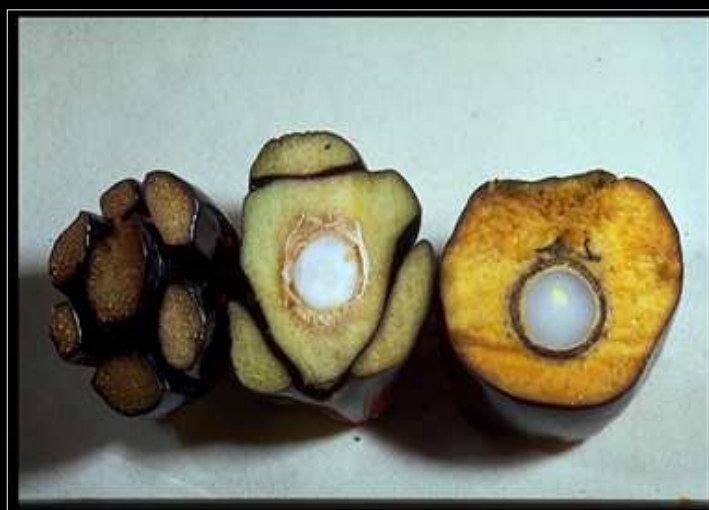
2. Clonal fidelity

To set up a set of DNA/RNA/serological markers

- ❖ monitoring of SE process
- ❖ discard off-type lines as early as possible



The "mantled" somaclonal variant phenotype



Characteristics of the "mantled" somaclonal variation

- ✓ Inter clonal variability: 0 to 85%
- ✓ Intra clonal variability: between production batches
- ✓ Variable expression on a given palm :
 - from : one fruit on one single bunch
 - to : all the fruits from all the bunches
- ✓ Expression varying with time
 - 100% of the slightly mantled palms reverted to the normal phenotype after 10 years in the field
 - 50% of severely mantled reverted to normal
- ✓ Non-Mendelian sexual transmission

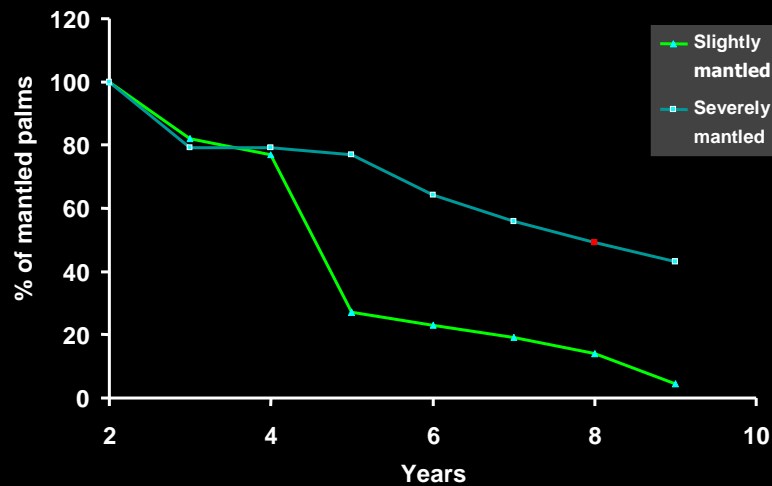


Impact of the "mantled" somaclonal variation

	Observed palms	Normal palms	Slightly abnormal	Severely abnormal
IDEFOR Côte d'Ivoire	29,415	90.3%	3.7 %	6.0 %
FELDA Malaysia	18,935	92.0%	5.6 %	2.4 %
IOPRI Indonesia	6,771	87.3%	5.3 %	7.4 %



Reversion of somaclonal variation in the field



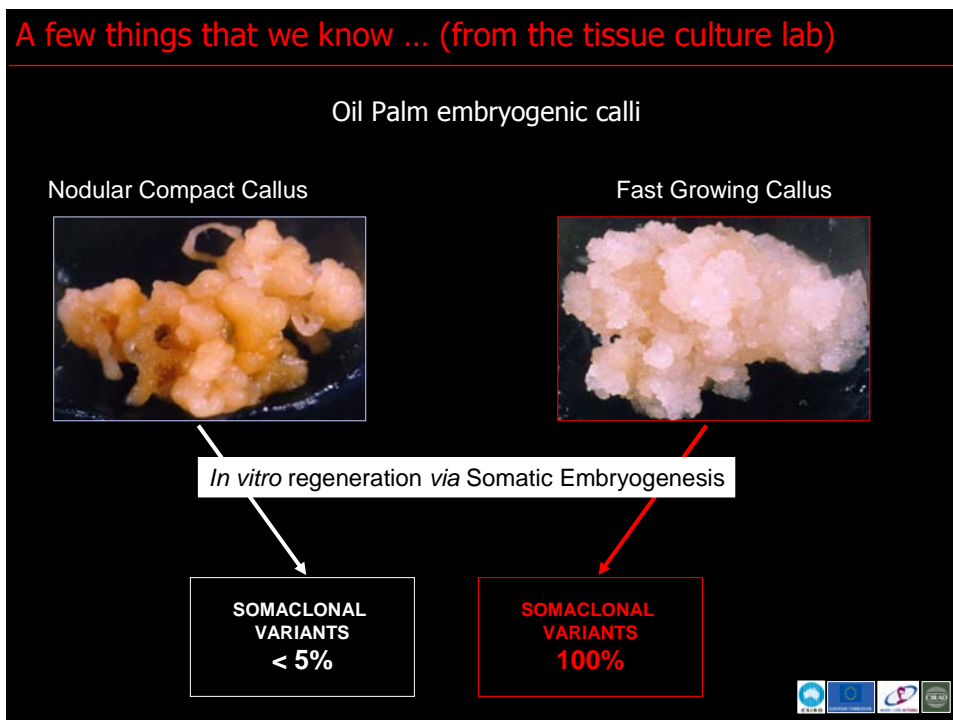
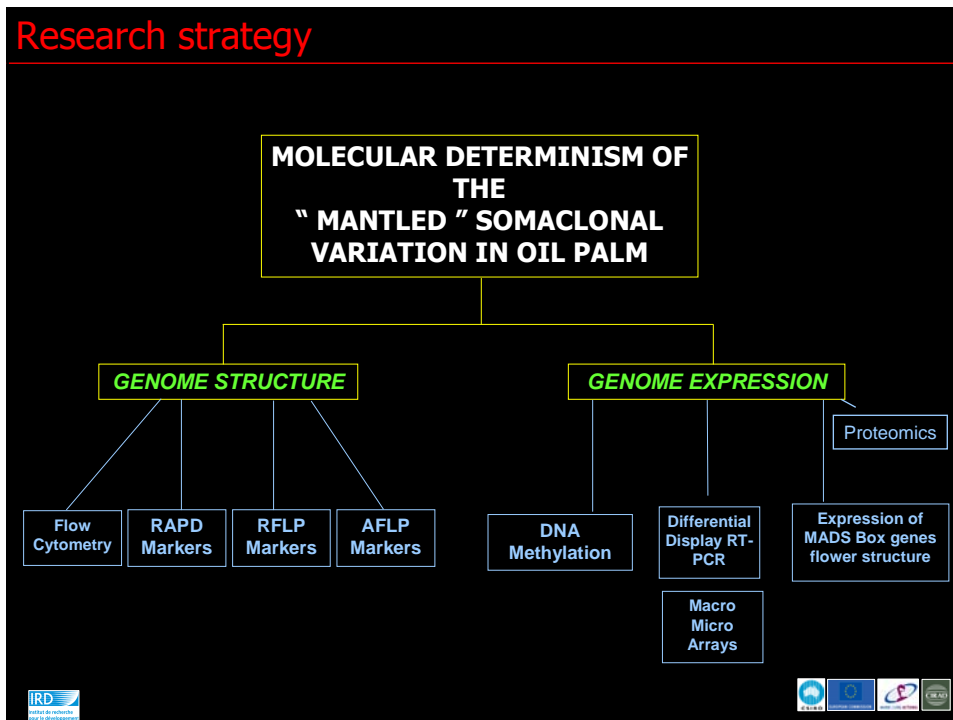
Data from CNRA La Mé Research Station (Côte d'Ivoire)



A few things that we know ... (from the field)

- ✓ The "mantled phenotype occurs very rarely in progenies originating from sexual reproduction (a handful of individuals in 500 millions commercial seeds sold yearly...)
- ✓ One spontaneous ecotype of *Elaeis guineensis* showing a stable "mantled" phenotype has been described and named "poissonii"
- ✓ Several different SE protocols gave rise to the same variant phenotype
- ✓ Recloning from leaf explant sampled on variant somaclones always gives rise to variant somaclones
- ✓ Somaclonal variants in Date Palm (*Phoenix dactylifera*) originating from SE are reported to show supernumerary carpels





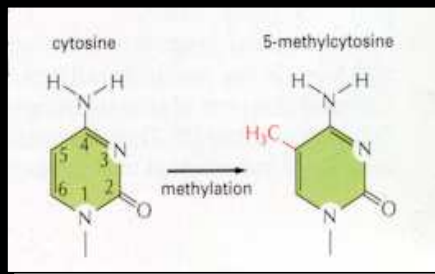
The DNA methylation hypothesis

- ✓ Epigenetic nature of the *mantled* abnormality
 - Field results
 - Standard DNA markers (RAPDs, AFLPs)
- ✓ DNA methylation involves the addition of a methyl group to the 5' position of a cytosine base.
- ✓ This modification is associated with gene silencing
- ✓ DNA methylation rates changes with developmental stages
- ✓ Tissue culture induced instability
- ✓ Growth regulators (2,4-D) affect DNA methylation rates
- ✓ Defects in DNA methylation (anti MET1) generated abnormal flower phenotypes in *Arabidopsis*

Plant Breeding, 117(1), 73-76.



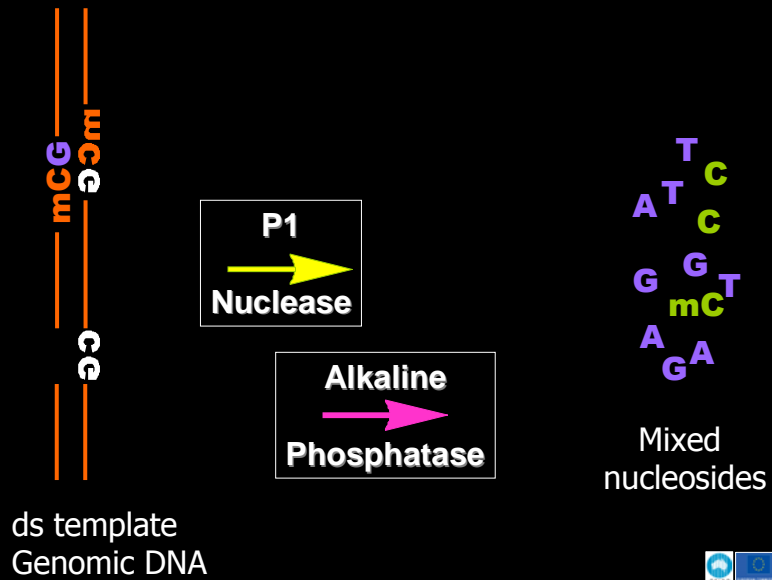
Estimation of Global DNA Methylation Rates



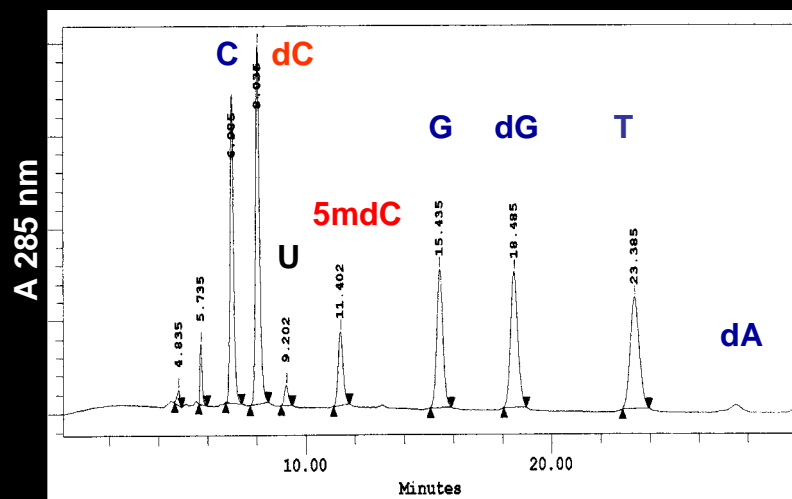
$$\frac{5\text{mdC}}{\text{dC} + 5\text{mdC}}$$



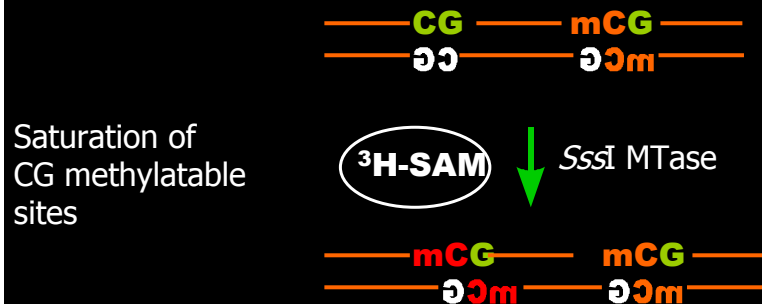
Enzymatic hydrolysis of genomic DNA



HPLC separation of nucleosides



SssI-Methylase Accepting Assay



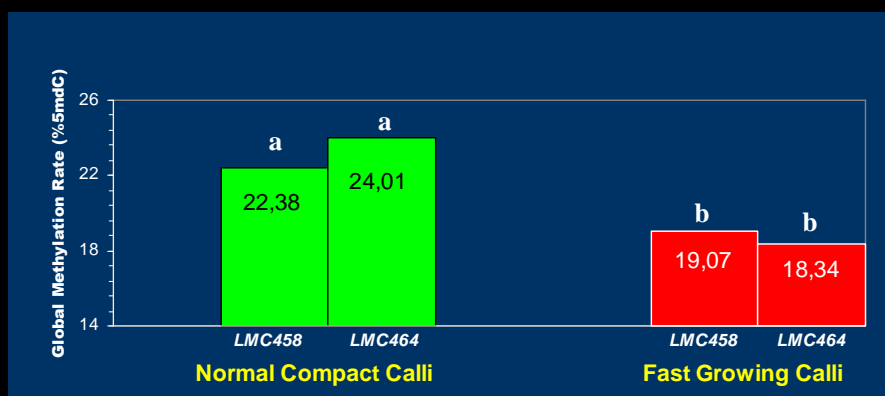
$$\text{Radioactivity } \alpha = \frac{1}{\% \text{CpG Meth}}$$



European Molecular Biology Laboratory
E. Oakeley & J.P. Jost



Global Methylation Rates in embryogenic calli



No Clone effect; Type effect : $F(1, 11) = 58.19$; $p < 0.0000$

Plant Cell Rep. 19 (7): 684-690.



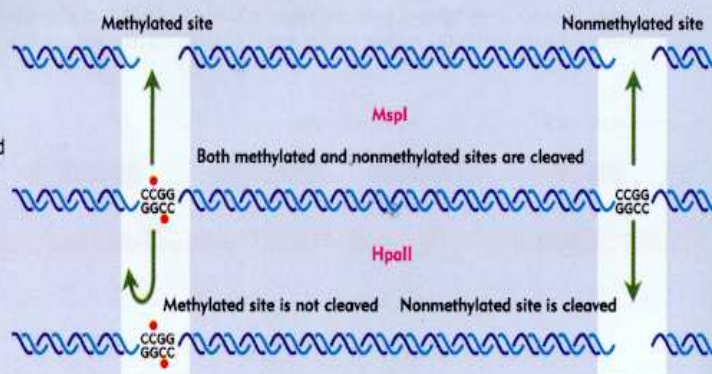
Search for Methylation Sensitive DNA Markers

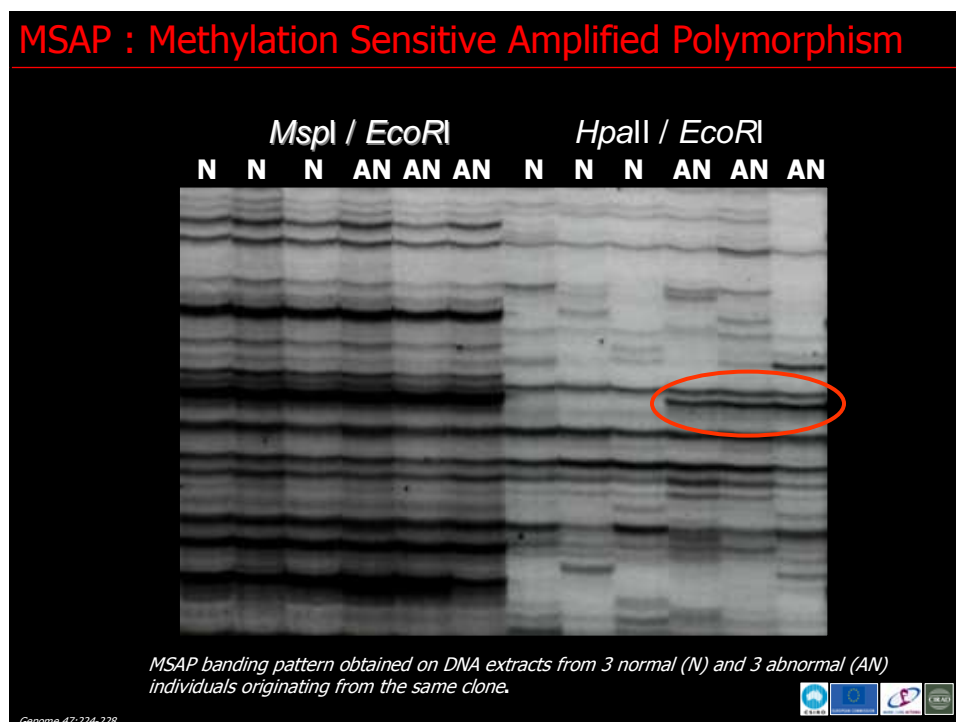
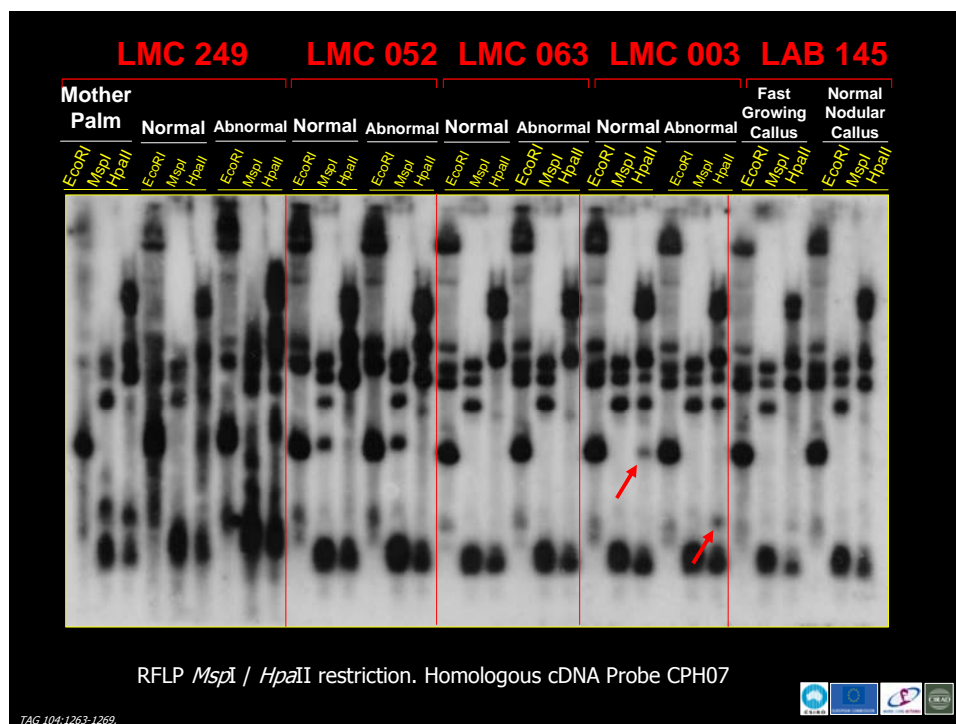
- Oil palm cDNA probes from immature inflorescences and/or calli
- Isoschizomeric restriction enzymes (*Msp*I/*Hpa*II)
- Search for differential genomic DNA Methylation patterns



Isoschizomeric restriction enzymes

The restriction enzyme *Msp*I cleaves all CCGG sequences whether or not they are methylated at the second C, but *Hpa*II cleaves only nonmethylated CCGG tetramers.





VARIOMETH

EXPLORING THE ROLE OF DNA METHYLATION IN EPIGENETIC VARIATION IN HIGHER PLANTS

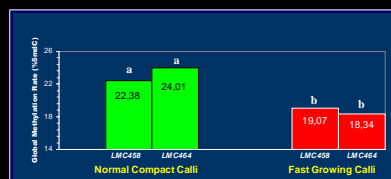
- The VARIOMETH fellowship will focus on the role of DNA methyltransferases on the determinism of somaclonal variation and on the exploration of the relationship between DNA methylation and chromatin remodelling.
- Both approaches will be developed in parallel with the aim of describing specific molecular events which could be used for the development of markers of epigenetic instability in plants.
- These markers will be integrated in a strategy aimed at the identification of *in vitro* treatments which are prone to generate epigenetic variability in somatic embryogenesis-based micropropagation processes.



European Commission
Human Resources and Mobility
Marie Curie Outgoing International Fellowship
2004-2007

A few things that we would like to know ...

- ✓ Could expression profiles of METases in calli / inflorescences/leaves explain the previously observed differences in global methylation rates ?



- ✓ Which part is played by each studied family of METase?
 - MET1: Maintenance MTase (CG motifs)
 - CMT3 : Methylation of heterochromatic DNA (CNG motifs)
 - DRM : Methylation of isolated Cs



DNA-methyltransferases oil palm genes MET/CMT/DRM

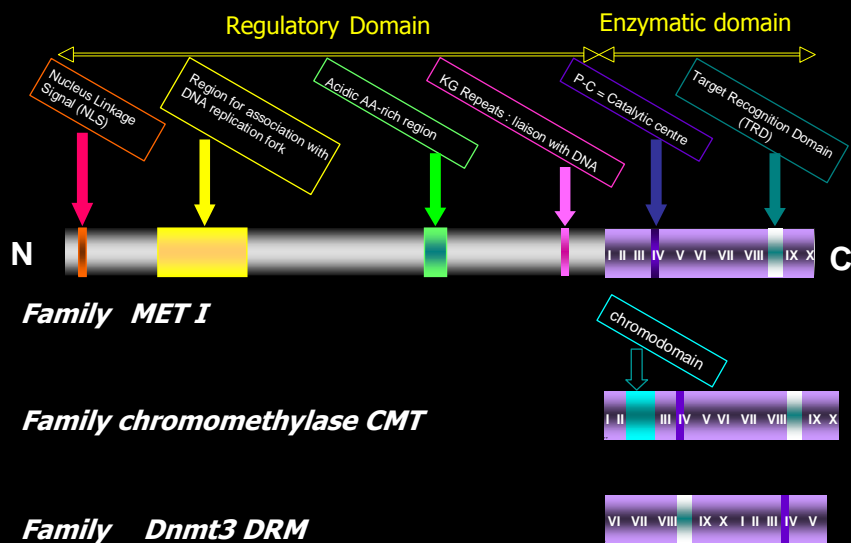
- ✓ Isolate full length cDNAs
 - from cDNA librairies
 - from EST libraries (2411 sequences)
 - RACE experiments
 - CODEHOP experiments

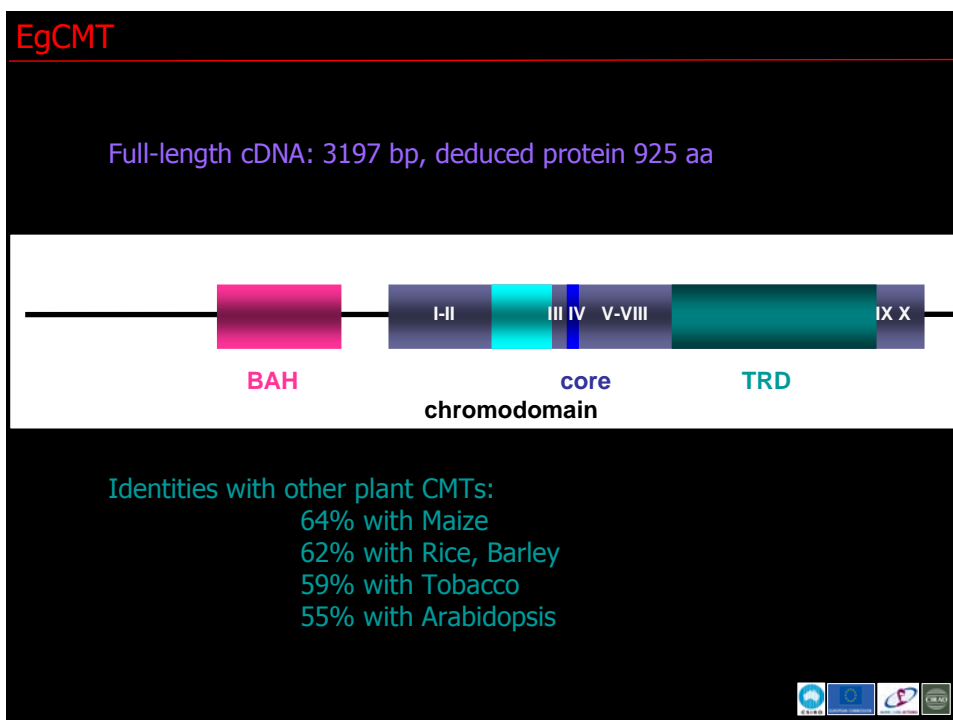
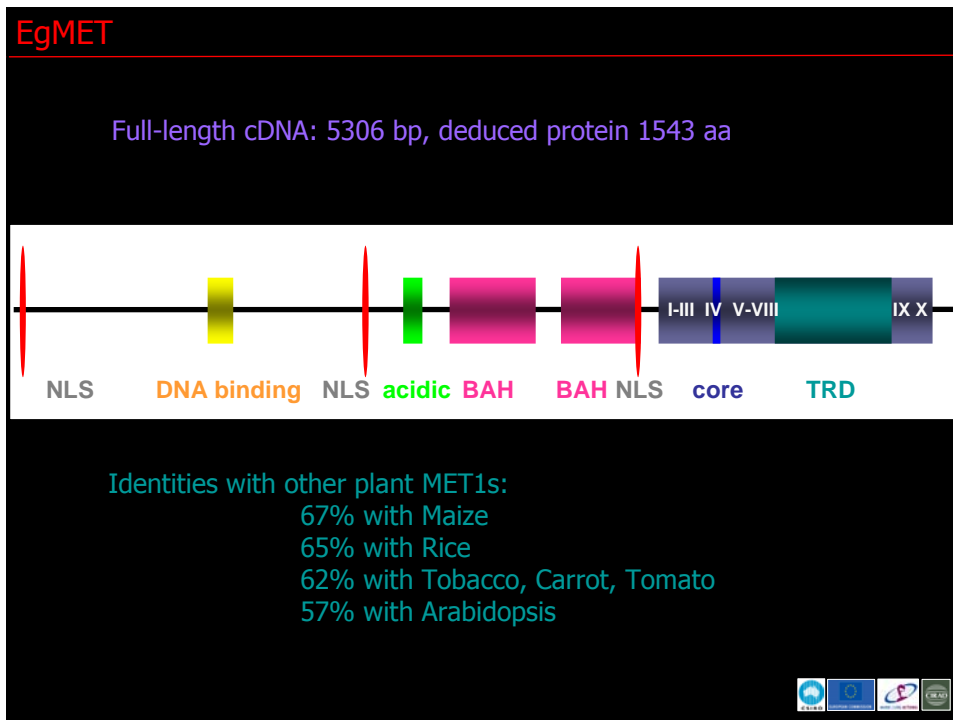
- ✓ Expression studies in embryogenic calluses, somatic embryos and leaves
 - Semi quantitative RT-PCR
 - Real Time qPCR

FEBS Letters 579 (2005) 2709–2714



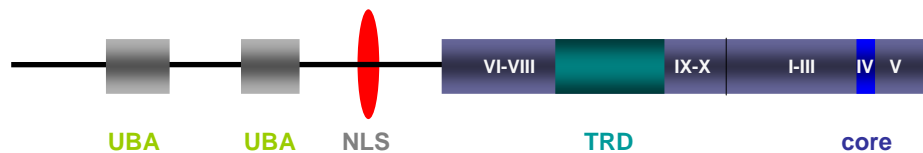
DNA-Methyltransferases in plants





EgDRM

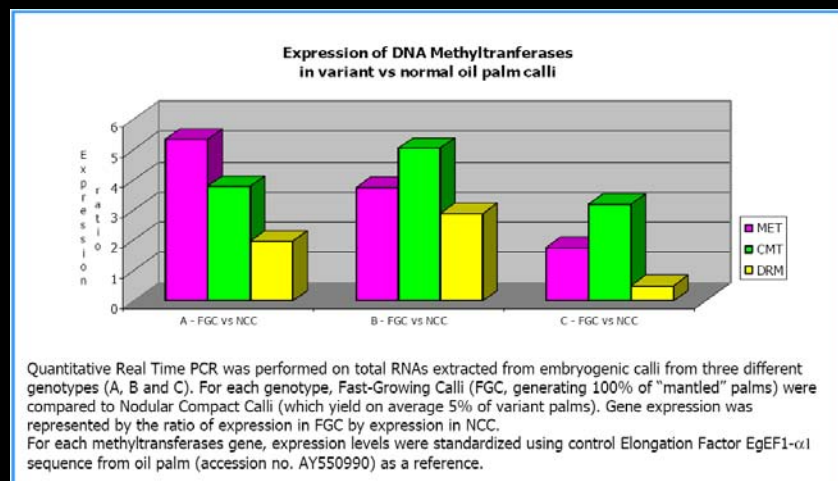
Full-length cDNA: 2477 bp, protein 591 aa



Identities with other plant DRMs:
 68% with Barley
 59% with Tobacco
 55% with Rice
 54% with Maize
 53% with Arabidopsis



Real Time qPCR analysis on embryogenic calli



Conclusions

- **Full lengths cDNAs** coding for three different DNA (cytosine-5)-methyltransferases families (namely *EgMET*, *EgCMT* and *EgDRM*) were isolated from oil palm (*Elaeis guineensis* L) and the corresponding *EgMET*, *EgCMT* and *EgDRM* products were characterised.
- **Expression of oil palm DNMTs** was compared between normal and variant calli and inflorescence tissues using quantitative reverse-transcription PCR. A consistent increase in transcript levels of *EgMET1* and *EgCMT1* was found in variant fast-growing calli relative to nodular compact calli.
- The genome-wide hypomethylation previously described in 'mantled' material cannot be explained by a decrease in expression levels of the de novo or maintenance DNMTs, a **paradox** which has been previously reported in tumour cells, where there is evidence for global hypomethylation of DNA.

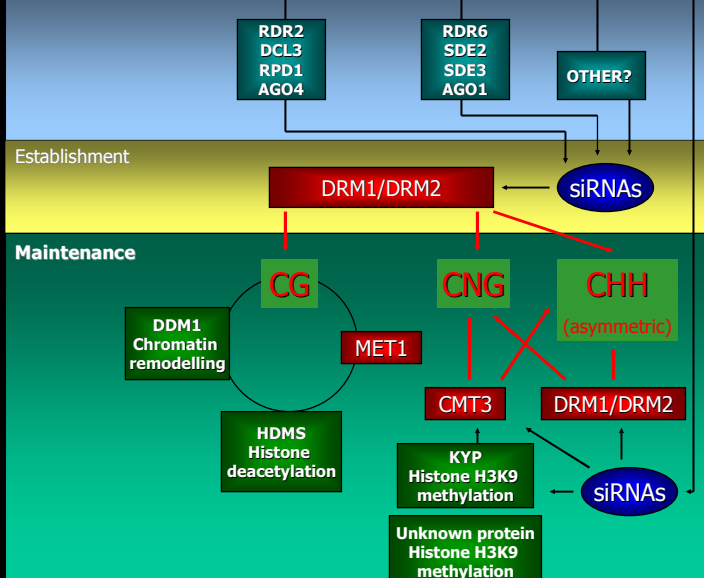


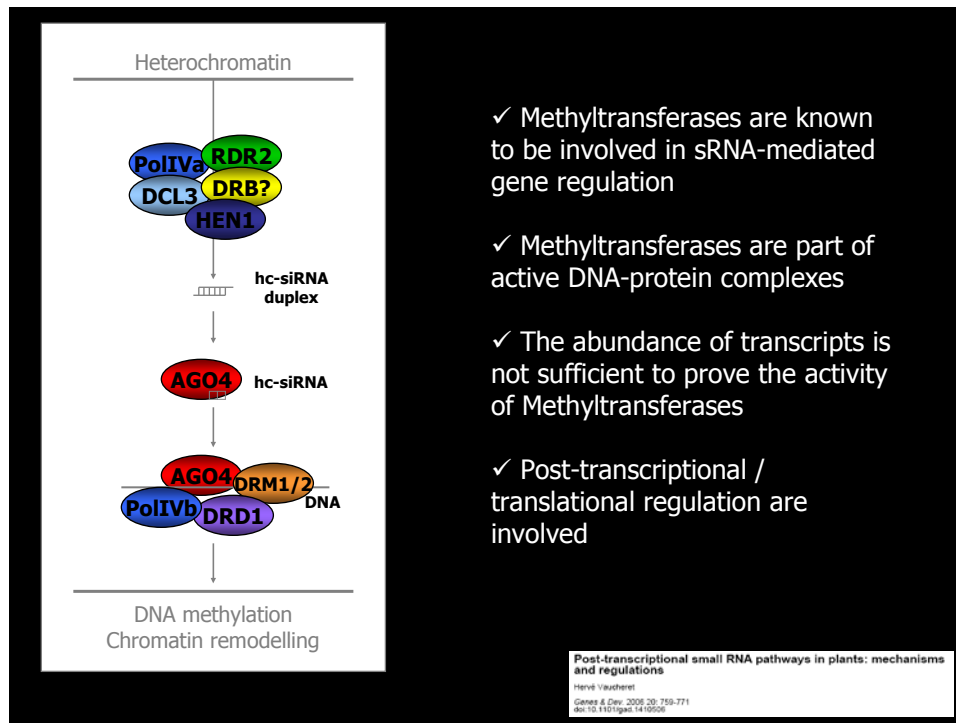
Establishment and Maintenance of DNA methylation

GARDENING THE GENOME
DNA METHYLATION IN
ARABIDOPSIS THALIANA

Simon W. L. Chan¹, Ben H. Henderson² and James R. Jacobsen¹

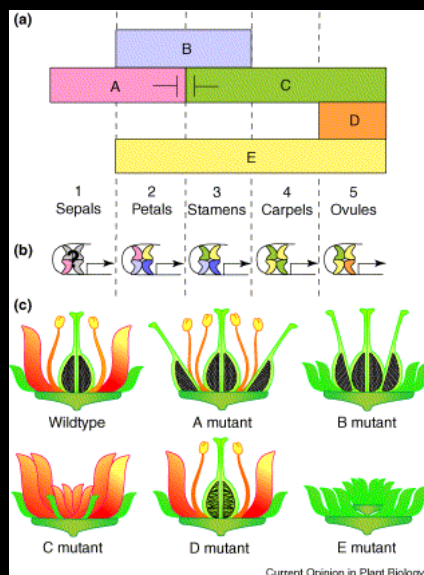
siRNA-generating pathway





- ✓ Methyltransferases are known to be involved in sRNA-mediated gene regulation
- ✓ Methyltransferases are part of active DNA-protein complexes
- ✓ The abundance of transcripts is not sufficient to prove the activity of Methyltransferases
- ✓ Post-transcriptional / translational regulation are involved

Methylation around MADS Box candidate genes

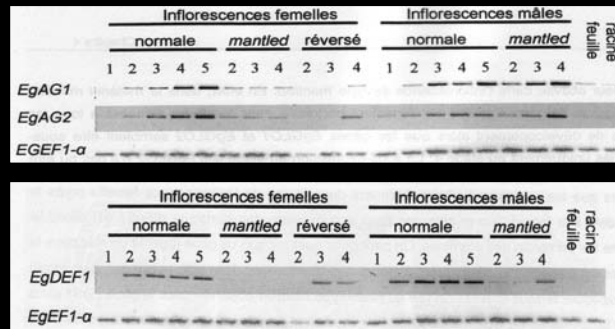


Several oil palm MADS box genes have shown differential expression patterns according to the presence of *mantled* abnormality

Alterations in expression affect not only B-type, but also C, D and E-type genes

Whorls 2, 3 and 5 are affected by homeotic changes

Methylation around MADS Box candidate genes



Reduced expression of genes Eg DEF1 and EgGLO2 (B type), EgAG2 (C and D type) and EgS1 (type E) in abnormal oil palm flowers

Am. J. Bot. 92(11):1836–1852, 2005

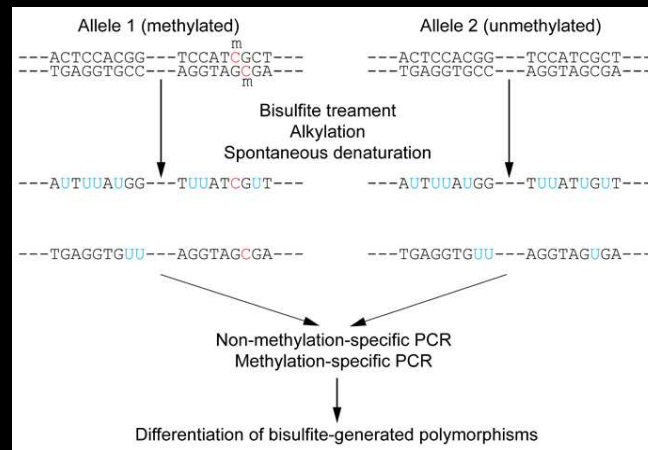


Methylation around candidate genes

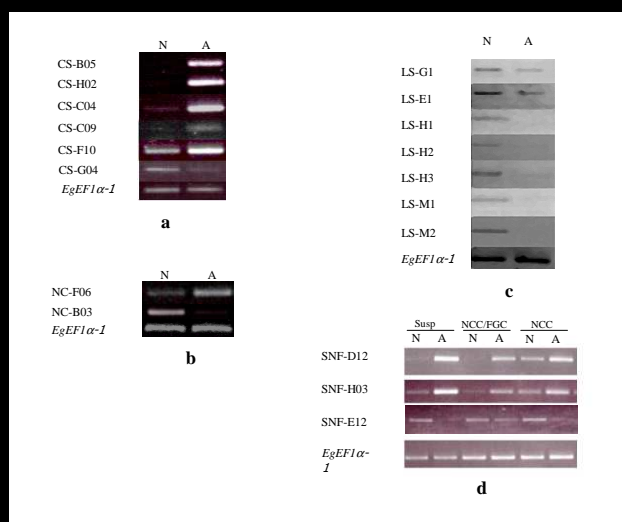
- ✓ This study is being undertaken by Southern analysis of DNA from normal/abnormal inflorescences, somaplants and calluses, which are differentially cleaved with methylation-sensitive restriction enzymes (*MspI/HpaII*; *McrBC*) and hybridized with probes specific to the coding and/or promoter region of the target genes.
- ✓ We have used *McrBC* for a preliminary survey aimed at identifying putative promoters of oil palm MADS box genes which may be the target for control by DNA methylation. If we find any evidence for methylation in normal or *mantled* material, then we will follow up with Bisulfite Sequencing for a more detailed study.
- ✓ Specific probe DEF1 and AG2 coding region



Methylation-specific PCR: towards MS markers...



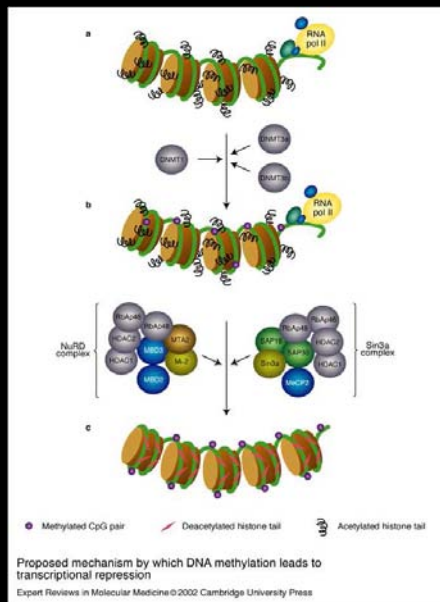
Other candidate genes...



Semi-quantitative RT-PCR profiles of the macroarray markers producing differential signals between normal (N) and abnormal material (A) from suspensions [a]; NCC [b]; leafy shoots [c] and three materials in common: suspension, NCC, FCC [d]. The oil palm elongation factor *EgEF1α-1* gene was used as a reference.



Exploring chromatin conformation and DNA methylation

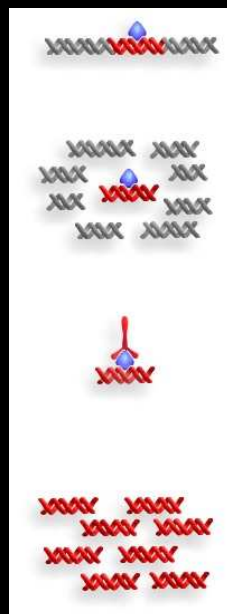


Evidence suggests that deacetylation of histones is linked to the methylation status of the surrounding DNA.

In particular, deacetylation of H3 lysine 9 seems to be strongly linked to methylation of the DNA in the chromatin region in which it occurs.



Chromatin immunoprecipitation (ChIP) protocol



DNA-binding proteins are crosslinked to DNA with formaldehyde in vivo.

Isolate the chromatin.
Shear DNA along with bound proteins into small fragments.

Bind antibodies specific to the DNA-binding protein to isolate the complex by precipitation

Reverse the cross-linking to release the DNA and digest the proteins.
Use PCR to amplify specific DNA sequences



Relevant literature

- JALIGOT E., RIVAL A., BEULÉ T., DUSSERT S. & VERDEIL J.-L. (2000) Somaclonal variation in Oil Palm (*Elaeis guineensis* Jacq.): The DNA methylation hypothesis. *Plant Cell Reports* 19 (7): 684-690.
- TREGAR J., MORCILLO F., RICHAUD F., BERGER A., SINGH R., CHEAH S.C., HARTMANN C., RIVAL A. & DUVAL Y. (2001) Characterisation of a defensin gene expressed in oil palm inflorescence: induction during tissue culture and possible association with epigenetic somaclonal variation events. *Journal of Experimental Botany*, 53 : 1387-1396.
- JALIGOT E., BEULÉ T. & RIVAL A. (2002) Methylation-sensitive RFLPs reveal a differential banding pattern associated with somaclonal variation in oil palm (*Elaeis guineensis* Jacq.). *Theoretical and Applied Genetics*. 104:1263-1269.
- JALIGOT E., BEULÉ T., BAURENS F.C., BILLOTE N. & RIVAL A. (2004). MSAP screening for differentially methylated markers associated with the « mantled » somaclonal variation in oil palm (*Elaeis guineensis* Jacq.). *Genome* 47:224-228.
- MORCILLO F., GAGNEUR C., ADAM H., JOUANNIC S., RICHAUD F., RAJINDER S., CHEAH S.C., RIVAL A., DUVAL Y. & TREGAR J.W. (2005) Somaclonal variation in micropropagated oil palms: Characterization of two novel genes displaying enhanced expression in epigenetically abnormal cell lines and investigation of the influence of auxin on their activity. *Tree Physiology* : 26, 585-594.
- ADAM H, JOUANNIC S, ESCOUTE J., DUVAL Y, VERDEIL J-L & J.W. TREGAR (2005) Reproductive developmental complexity in the african oil palm (*Elaeis guineensis*, Arecaceae). *American Journal of Botany* 92(11): 1836-1852.
- RIVAL A. , E. JALIGOT, T. BEULÉ & J. FINNEGAN (2008) Isolation and differential expression of MET, CMT and DRM methyltransferase genes from oil palm (*Elaeis guineensis* Jacq.) in relation with the "mantled" somaclonal variation. *Journal of Experimental Botany* (doi:10.1093/jxb/ern178).



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