

Epigenetic analysis of somaclonal variation in higher plants: Oil palm as a case study

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Background

Epigenetics is the study of heritable changes in gene expression that occur without a change in DNA sequence. In higher plants, these phenomena have economic relevance in the case of somaclonal variation: a genetic and phenotypic variation among clonally propagated plants from a single donor genotype.

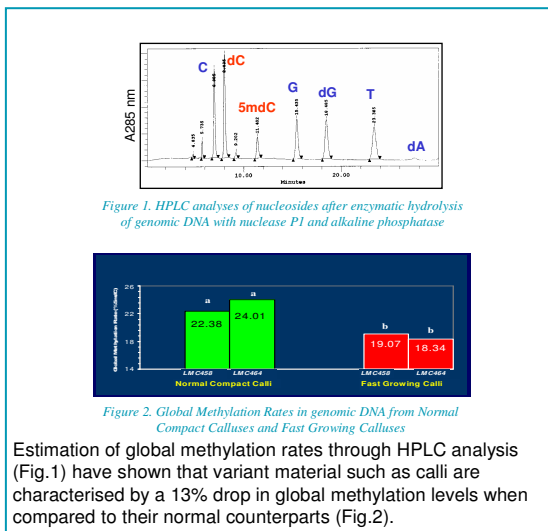
In oil palm (*Elaeis guineensis* Jacq), approximately 5% of somatic embryo-derived palms show abnormalities in their floral development. This variant phenotype offers a very interesting model, because somaclonal variation alters the expression of genes coding, directly or indirectly for the flower structure.

Studies on genomic DNA methylation changes induced by tissue culture together with results from long term field studies have suggested that epigenetic mechanisms play an important role in the determination of somaclonal variation in oil palm.

Aims

1. To use complementary approaches for the development of methylation-sensitive markers of epigenetic instability in regenerant palms,
2. To integrate these markers in a strategy aimed at the identification of *in vitro* treatments which are prone to generate epigenetic variability in somatic embryogenesis-based micropropagation processes

Results



Using MSAP (Methylation Sensitive Amplified Polymorphism) the methylation status of CCGG sites was studied in four distinct genotypes. Overall, 64 selective primer combinations were used and they have amplified 23 markers exhibiting a differential methylation pattern. Each of the 23 markers isolated in the present study could discriminate between the two phenotypes only when they were from the same genetic origin. Valuable information on putative target sequences will be obtained from the further characterization of these markers.

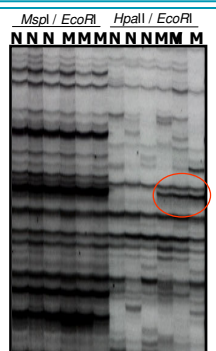


Figure 4. MSAP analysis of leaf DNA extracted from 3 Normal (N) and 3 Mantled (M) regenerants from the same clonal line

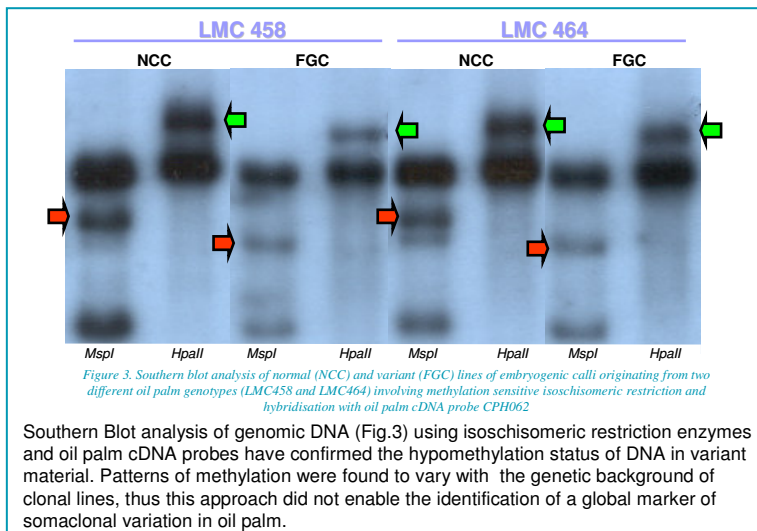


Figure 3. Southern blot analysis of normal (NCC) and variant (FGC) lines of embryogenic calli originating from two different oil palm genotypes (LMC458 and LMC464) involving methylation sensitive isoschisomic restriction and hybridisation with oil palm cDNA probe CPH062

Southern Blot analysis of genomic DNA (Fig.3) using isoschisomic restriction enzymes and oil palm cDNA probes have confirmed the hypomethylation status of DNA in variant material. Patterns of methylation were found to vary with the genetic background of clonal lines, thus this approach did not enable the identification of a global marker of somaclonal variation in oil palm.

We are presently studying expression profiles of DNA Methyltransferases in normal and mantled material, in order to explore their part in the previously observed differences in global methylation rates. Three different families of METases are being analyzed, namely: MET (maintenance of methylation at CG sites), CMT (methylation of heterochromatic DNA at CNG motifs) and DRM (methylation of isolated Cytosine). Differences in global DNA methylation rates between normal and variant callus material could not be attributed to changes in MET or CMT expression. The isolation of full-length cDNAs of oil palm Methyltransferases from the three families is under way together with the study of DRM expression.

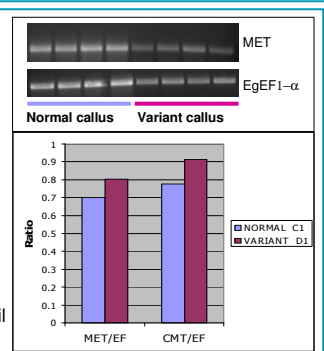


Figure 5. Semi-quantitative RT-PCR analysis of MET1 DNA Methyltransferase expression in embryogenic calli

Conclusions

- ✓ The mantled somaclonal variation in oil palm is characterised by a sizeable hypomethylation of genomic DNA
- ✓ Methylation-sensitive DNA markers such as MS-RFLPs and MSAPs were found to provide genotype-dependant profiles which hampered their use in the early detection of somaclonal variation.
- ✓ Analysis of DNA-MTase expression suggest the presence of a post-transcriptional regulation of MET and CMT activities.