Annals of Botany **108**: 1453–1462, 2011
doi:10.1093/aob/mcq266, available online at www.aob.oxfordjournals.org

ANNALS OF BOTANY
Founded 1887

REVIEW: PART OF A SPECIAL ISSUE ON PALM BIOLOGY

Epigenetic imbalance and the floral developmental abnormality of the *in vitro*-regenerated oil palm *Elaeis guineensis*

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Received: 1 October 2010 Returned for revision: 1 November 2010 Accepted: 19 November 2010 Published electronically: 10 January 2011

• *Background* The large-scale clonal propagation of oil palm (*Elaeis guineensis*) is being stalled by the occurrence of the *mantled* somaclonal variation. Indeed, this abnormality which presents a homeotic-like conversion of male floral organs into carpelloid structures, hampers oil production since the supernumerary female organs are either sterile or produce fruits with poor oil yields.
• *Scope* In the last 15 years, the prevailing point of view on the origin of the *mantled* floral phenotype has evolved from a random mutation event triggered by *in vitro* culture to a hormone-dependent dysfunction of gene regulation processes. In this review, we retrace the history of the research on the *mantled* variation in the light of the parallel advances made in the understanding of plant development regulation in model systems and more specifically in the role of epigenetic mechanisms. An overview of the current state of oil palm genomic and transcriptomic resources, which are key to any comparison with model organisms, is given. We show that, while displaying original characteristics, the *mantled* phenotype of oil palm is morphologically, and possibly molecularly, related to MADS-box genes mutants described in model plants. We also discuss the occurrence of comparable floral phenotypes in other palm species.
• *Conclusions* Beyond its primary interest in the search for discriminating markers against an economically crippling phenotype, the study of the *mantled* abnormality also provides a unique opportunity to investigate the regulation of reproductive development in a perennial tropical palm. On the basis of recent results, we propose that future efforts should concentrate on the epigenetic regulation targeting MADS-box genes and transposable elements of oil palm, since both types of sequences are most likely to be involved in the *mantled* variant phenotype.

Key words: Epigenetics, flower development, clonal fidelity, MADS-box, *mantled* phenotype, somaclonal variation, transposable elements, *Elaeis guineensis*.

THE *MANTLED* PHENOTYPE IN OIL PALM: EMERGENCE AND MORPHOLOGICAL DESCRIPTION

The African oil palm (*Elaeis guineensis* Jacq.) has become the first world source of vegetable oil thanks to both its oil yield (up to 6·5 t ha^{-1}), which is the highest of all oleaginous plants and the large areas dedicated to its culture (Corley, 2009; Koh *et al.*, 2009). Indonesia and Malaysia alone account for nearly 9 Mha of oil palm plantation producing >80 % of the world's palm oil (FAO statistics 2008, http://faostat.fao.org/site/339/default.aspx). From the late 1970s onwards, the increasing commercial interest in oil palm culture has prompted the optimization of oil production through the genetic improvement of planting material. Because of its long life cycle (25 years) which makes conventional breeding programmes expensive and time-consuming and its recalcitrance to natural vegetative propagation, *in vitro* cloning methods based on somatic embryogenesis have been implemented (Pannetier *et al.*, 1981).

The subsequent up-scaling of clonal production from the laboratory to the pilot production plant allowed the detection of a proportion of several somaclonal phenotypes among the clonal progenies. The term *mantled* was coined by R. H. V. Corley (Corley *et al.*, 1986) to describe the singular aspect of the abnormal fruit displayed by some of those variants. On their flowers, fleshy structures resembling carpels replace stamens and give its typical 'wrapped' aspect to the fruit. The effect on oil production can be dramatic since the mesocarp of the resulting fruits accumulate very low levels of oil and, in the most severe cases, the flowers are sterile (Rival, 2000; Rival and Parveez, 2005).

Although the occurrence of this variant phenotype is rather modest since it affects approx. 5 % of the regenerant palms on average, it varies widely and unpredictably depending on the genotype of the mother palm and on *in vitro* culture conditions (Jones *et al.*, 1995; Eeuwens *et al.*, 2002). The severity of this abnormality is spatially heterogeneous as it shows different degrees of organ transformation between palms originating from the same clonal progeny, between inflorescences borne on the same palm, or between flowers composing the same inflorescence. A spontaneous and gradual reversion to the normal phenotype is observed in the field and its duration

depends directly on the original severity of the abnormality (Rival and Parveez, 2005). However, the reversion takes years to complete and this period of non-productivity must be added to the 2–3 years of *in vitro* somatic embryogenesis and micropropagation and the 5 years during which the oil palms are sexually immature. As a consequence, the *mantled* abnormality results not only in a considerable deficit in oil production but also in a waste of investment in state-of-the-art biotechnological infrastructures, skilled labour time and plantation area. Since it is impossible to detect the *mantled* material before flowering and as it is not correlated to any vegetative abnormality, the early detection of variants became a priority for breeders and biotechnologists. Since the early 1990s, research aimed at identifying early markers of the *mantled* phenotype has tackled simultaneously the twin problems of the 'original trauma' causing the abnormality and the means of its early detection.

THE SEARCH FOR EARLY MARKERS, AND HOW IT CHANGED THE SHAPE OF THE QUESTION

The term 'somaclonal variation' designates new phenotypes arising randomly in a somatic cell line, notably after *in vitro* clonal propagation. Indeed, tissue culture protocols generally include the abrupt termination of tissue correlations between the explant and the donor plant and a subsequent radical change in environmental and trophic conditions. Also, plant growth regulators such as auxins and cytokinins can be used at high levels to induce the de-differentiation and the re-differentiation of tissues and the rooting of the somaplant (Rival, 2000; Eeuwens *et al.*, 2002). Therefore, the generic phenomenon of somaclonal variation encompasses very different situations since variant phenotypes are likely to originate from a wide range of distinct alterations arising after tissue culture (Larkin and Scowcroft, 1981; Kaeppler and Phillips, 1993; Kaeppler *et al.*, 2000).

Amongst the perturbations that are associated with somaclonal variation, changes in ploidy level are frequent, through either the loss of chromosome or chromosome fragments (aneuploidy) or the duplication of the whole set of chromosomes without cell separation (polyploidy). Indeed, Stelly *et al.* (1989) showed that high frequencies of aneuploidy and tertiary monosomy in cotton (*Gossyptum hirsutum*) regenerants indicate that cytogenetic anomalies were a major source of somaclonal variation in this species. More recently, flow cytometry unambiguously identified euploid (diploid, mixoploid, triploid and tetraploid) and aneuploid papaya micropropagated plantlets (Clarindo *et al.*, 2007). In oil palm, flow cytometry analyses performed on embryogenic calli and regenerated shoots showed no alteration of the ploidy level in these materials when compared with the mother palm (Rival *et al.*, 1997).

In several cases, DNA markers proved useful in the detection of somaclonal variants in plants; indeed, Palombi and Damiano (2002) showed that SSR markers but not RAPD could detect genetic variation induced in micropropagated kiwifruit plants. In oil palm, extensive RAPD and AFLP analyses revealed no DNA polymorphisms that could be linked to the *mantled* variant phenotype (Rival *et al.*, 1998), making it unlikely that a gross genetic defect could be the source of the somaclonal variation of oil palm.

These results, as well as the observed instability of the variation in time, led to the hypothesis of an epigenetic origin of the *mantled* phenotype. In the late 1990s the prominent role played by both the extent and the distribution of DNA methylation on the time and space of gene expression was spreading fast from the field of cancer research to that of plant developmental biology (Laird and Jaenisch, 1994; Jones, 1996; Finnegan *et al.*, 1998). It had been shown that a given gene undergoes extensive changes in its methylation profile in the course of cell differentiation and organ specialization and that its transcription is modulated throughout plant life in response to various environmental cues (Finnegan *et al.*, 1998, 2000; Matzke and Matzke, 2004; Jullien *et al.*, 2006; Xiao *et al.*, 2006; Chinnusamy and Zhu, 2009; Law and Jacobsen, 2010). More specifically, the documented correlations between perturbations of DNA methylation patterns and both somaclonal variations (Peraza-Echeverria *et al.*, 2001; Joyce and Cassells, 2002; Joyce *et al.*, 2003; see also more recent examples in Guo *et al.*, 2007; Pietsch and Anderson, 2007; Park *et al.*, 2008; Schellenbaum *et al.*, 2008; Chang *et al.*, 2009; Baránek *et al.*, 2010) and abnormalities in floral induction and development (Finnegan *et al.*, 1996; Ronemus *et al.*, 1996; Jacobsen and Meyerowitz, 1997; Cubas *et al.*, 1999; Jacobsen *et al.*, 2000; Soppe *et al.*, 2000; Kankel *et al.*, 2003) were a strong incentive to look in that direction.

Nevertheless it must be noted that this idea was by no means exclusive of other probable influences, such as those of plant growth regulators. Indeed, the work of Besse *et al.* (1992) demonstrated that a type of embryogenic calli (fast-growing calli or FGC) generating nearly 100 % of *mantled* palms displayed a decreased level of endogenous cytokinins compared with the nodular compact calli type (NCC), which yields on average 5 % of variant regenerants. A complementary study on calli obtained by the re-cloning of normal and abnormal regenerants showed that these differences could be attributed to a defect in cytokinin uptake (Jones, 1998). Furthermore, Morcillo *et al.* (2006) later observed in *mantled* palm-derived calli a relative overexpression of the *EgIAA1* gene which could be involved in promoting auxin-induced changes in gene expression. Taken together, these studies support the idea that the abnormal phenotype is probably the result of altered sensitivity and/or response to growth regulators in the *in vitro* culture material. Interestingly, it has been proposed that the action of plant growth regulators (PGRs) is mediated through modifications of the epigenetic marks in the corresponding hormone-responsive genes (Stokes *et al.*, 2002; Xiao *et al.*, 2006; Alexandre *et al.*, 2009; Krogan and Long, 2009). So far evidence has been found, both *in vitro* and *in planta*, for the implication of chromatin-remodelling factors and transcription factors in PGR response pathways, especially in relationship to flower development (Murray *et al.*, 2003; Kaufmann *et al.*, 2009; Kapazoglou *et al.*, 2010; Kim *et al.*, 2010). The direct involvement of DNA methylation in PGR-induced gene regulation is still to be demonstrated but nevertheless the hypothesis of its impact in the case of the *mantled* phenotype is appealing.

An epigenetic misregulation can be characterized either directly, through its effectors or the immediate modifications

that they control or, indirectly, through its consequences on gene expression. With the aim of screening the largest amount of polymorphisms possible, the first searches for epigenetic markers of the *mantled* somaclonal variation tried therefore to investigate changes in DNA methylation or in gene expression in the whole oil palm genome or in a subset thereof (Jaligot *et al.*, 2000; Tregear *et al.*, 2002). Global DNA methylation rates were investigated by HPLC quantitation of nucleosides in embryogenic calli (NCC vs. FGC), leaves and inflorescences from normal vs. *mantled* adult clonal oil palms (Jaligot *et al.*, 2000). The quantitation of 5-methylcytosine in a large number of DNA samples from different genotypes and tissues showed that a major epigenetic imbalance is at work in the *mantled* material. Indeed, a significant deficit in DNA methylation was demonstrated in all the abnormal tissues compared with their normal counterparts: the average decrease in methylation level was −19·3 % in FGC, −7·4 % in *mantled* inflorescences and −5·5 % in mature leaves from variant palms (Jaligot *et al.*, 2000; E. Jaligot *et al.*, unpubl. res.). Similar results were obtained when CG methylation rates were examined in the same study through an enzymatic saturation assay. In spite of the strong and statistically significant correlation that was demonstrated between the *mantled* phenotype and genome hypomethylation, it was impossible to define a unique 'methylation threshold' for clonal conformity because of the combination of high individual- and genotype-dependent variations in global DNA methylation levels.

Because of this background noise, several subsequent papers targeted single-sequence methylation polymorphisms in the hope of narrowing down the scope of the study to those of the differentially methylated cytosines that bore relevance to the regulation of genes (Matthes *et al.*, 2001; Jaligot *et al.*, 2002, 2004; Lei *et al.*, 2006). Depending on the pre-existence or absence of candidate markers, either selected or anonymous sequences, respectively, were examined for the occurrence of methylation polymorphisms with isoschizomeric methylation-sensitive enzyme pairs. Overall, at the single-sequence level both increases and decreases in DNA methylation were observed in relationship to the *mantled* somaclonal variation. Similar to the whole-genome analysis, most of the differences that were found were attributed to genotype-dependent variation in DNA methylation, independent of the phenotype. As a consequence, the markers that were isolated because of their ability to discriminate between normal and variant palm material on a limited range of genotypes later proved to be inefficient for the screening of material with different clonal origins.

Likewise, potential transcriptional markers for either 'true-to-type' or 'mantled' phenotypes were scored by comparing tissues from either normal or abnormal palms or tissue cultures. The differential display technique was first implemented but was found to be time-consuming and yielded a comparatively low number of candidate markers (Tregear *et al.*, 2002; Morcillo *et al.*, 2007). The search for expression markers was taken to a higher level with the popularization of systematic sequencing campaigns aimed at building multi-purpose molecular resources for non-model organisms. Numerous wxpressed sequence tags (ESTs) libraries from various adult or *in vitro* tissues of oil palm were then constructed by research teams in France and in Malaysia (Jouannic *et al.*, 2005; Ho *et al.*, 2007; Low *et al.*, 2008; Mayes *et al.*, 2008; Singh *et al.*, 2008; Roowi *et al.*, 2010).

When considering the ensemble of the results, the majority of the expression polymorphisms that were detected were dependent on the genotype. Among the abundant phenotype-dependent expression polymorphisms that were indentified, most were of a quantitative (more vs. less) rather than of a qualitative (presence vs. absence) nature and proved elusive for the non- or semi-quantitative detection techniques that were available at the time, such as northern blotting and RT-PCR. Moreover, as happened in the case of sequence-specific methylation studies it proved difficult to assign the candidate markers to definite biological pathways, since the structure of these sequences pointed to a wide variety of functions. And it was even more difficult to connect these markers to a plausible and unique scenario explaining the emergence of the *mantled* phenotype from independent somatic embryogenesis events and unrelated oil palm genotypes.

In the light of these conclusions, it appeared crucial to consider a change in the strategies used to achieve the proposed objectives. This was performed both (*a*) by looking at the problem through a complementary perspective, focused on likely sources rather than on likely effects of the oil palm somaclonal variation through a candidate gene approach, and (*b*) by taking the search for anonymous discriminant markers to a higher level using the novel high-throughput techniques.

THE 'CANDIDATE GENE' APPROACH: CONFRONTING THE USUAL SUSPECTS

The candidate gene approach proposes that a gene with known function is linked to a given complex trait (Pflieger *et al.*, 2001; Glazier *et al.*, 2002). Originally used as a complement to mapping studies, this approach has now derived a new meaning from the field of functional biology where it consists of checking the hypothesis that a known gene function is involved in the mechanism of interest (Somerville and Somerville, 1999; Andersen and Lübberstedt, 2003; Remington and Purugganan, 2003).

In the context of the *mantled* abnormality, two gene families were studied following the candidate-gene approach. The occurrence of abnormal flower phenotypes involving organ conversions in *Arabidopsis thaliana* plants expressing low levels of the DNA-methyltransferase 1 (*MET1*) gene has been described in pioneering studies in plant epigenetics (Finnegan *et al.*, 1996; Ronemus *et al.*, 1996). As for the *mantled* phenotype, its correlation with a significant genomic hypomethylation prompts the question of an initial impairment of DNA-methyltransferase activity. This issue has been addressed by identifying, in the oil palm, members of the three DNA-methyltransferase gene families that have been characterized in plants: the Dnmt1/MET1 family, the chromomethylase (CMT) family and the domain-rearranged methyltransferase (DRM) family (for a review, see Finnegan and Kovac, 2000). The former two classes of enzymes are responsible for the maintenance of methylation at sites that are symmetrical across the two DNA strands (i.e. CG and CHG sites, respectively, where H is A, T or C) (Cao and Jacobsen, 2002*b*;

Cao *et al.*, 2003; Aufsatz *et al.*, 2004). Moreover, DNA-methyltransferases of the CMT family are crucial for the stable silencing of high copy number sequences such as transposable elements which make up a large portion of plant genomes (Bennetzen, 2000; Papa *et al.*, 2001; Slotkin and Martienssen, 2007; Feschotte, 2008). The third category of DNA-methyltransferases ensures both the imperfect maintenance of methylation at isolated Cs ('asymmetrical' CHH sites) and the *de novo* transfer of methyl groups on previously unmethylated cytosines in any sequence context (Cao *et al.*, 2000, 2003; Cao and Jacobsen, 2002*a*; Cao *et al.*). The enzymes belonging to the CMT and DRM classes are assisted in their functions by small non-coding RNAs which direct them to their target sequences, a process called RNA-directed DNA methylation (RdDM) that is central to the phenomenon of gene silencing (Herr *et al.*, 2005; Kanno *et al.*, 2005; Onodera *et al.*, 2005; Pontier, 2005). Therefore, a dysfunction of any of these three enzyme families would be likely to cause a genomic hypomethylation such as the one occurring in *mantled* oil palm tissues. The prominent member of each of the three gene families was identified in the oil palm genome and its transcription level was assessed through real-time PCR quantitation (Rival *et al.*, 2008). Surprisingly, a slight but significant increase in transcript accumulation was observed for both maintenance DNA-methyltransferases at the embryogenic callus stage (FGC vs. NCC). This apparent inconsistency between the variations in DNA-methyltransferase gene expression and the changes in DNA methylation rates is comparable to what has been found in tumour tissues, in which genome-wide hypomethylation and local hypermethylation of tumor-suppressor genes can be paradoxically coupled to the overexpression of DNA-methyltransferases (for recent reviews on the subject, see Jones and Baylin, 2007; Esteller, 2008; McCabe *et al.*, 2009). However, in the case of oil palm this situation is partially erased through the regeneration and maturation of the clonal plant, since no differences in DNA-methyltransferase RNA levels were found between normal and *mantled* inflorescences. Although a post-transcriptional regulation of the DNA methyltransferase genes or an alteration of the enzymatic activities cannot be ruled out, it was concluded that the reduced DNA methylation was most likely a side effect of the *mantled* somaclonal variation rather than being causal.

The other gene family under investigation is formed by the genes contributing to what was originally named the ABC model (Coen and Meyerowitz, 1991). This model is based upon the hypothesis that the formation of the concentric whorls of floral organs in arabidopsis can be explained by the combined expression of a limited set of genes in partially overlapping cell territories. This model was later validated and completed in different plant models with occasional amendments due to differences in floral architecture between species, and has become the ABCDE model (Kang *et al.*, 1998; Davies *et al.*, 1999; Ambrose *et al.*, 2000; Brunner *et al.*, 2000; Honma and Goto, 2001; Münster *et al.*, 2001; Johansen *et al.*, 2002; Nagasawa *et al.*, 2003; Whipple *et al.*, 2004; Kaufmann *et al.*, 2005; Leseberg *et al.*, 2008; Zhao *et al.*, 2010). As a matter of fact, the inactivation of either one of the genes corresponding to the A, B or C functions results in the 'overflowing' of the neighbouring organ types beyond the whorl boundaries. Of particular interest for the study of the *mantled* phenotype of oil palm are the plants that are defective in B-type functions: their petals and stamens are replaced by supernumerary sepals and carpels, respectively (Coen and Meyerowitz, 1991; Zahn *et al.*, 2005). Considering that the oil palm flowers have tepals instead of both sepals and petals, the B-type mutant phenotype is very similar to the sexual organ conversion seen in the *mantled* flowers. Actually, Adam *et al.* (2005*a*) demonstrated at the histological level that both the residual stamens or staminodes of the female flowers and the stamens of the male flowers were transformed into carpelloids and carpels, respectively, in the *mantled* oil palm regenerants. Abnormal patterns of floral organogenesis have recently been observed in somaclonal variants of grapevine (*Vitis vinifera*), so the occurrence of such a phenomenon in clonal regenerants of oil palm is plausible (Chatelet *et al.*, 2007).

The functions predicted through the ABCDE model are mostly encoded by genes belonging to the MADS-box superfamily of transcription factors. Since this superfamily is much wider than the relatively small number of organ identity genes, many distinct MADS-box genes have been identified in the oil palm genome by different teams (Adam *et al.*, 2005*b*; Syed Alwee *et al.*, 2006) but only a small fraction has been assigned to functions in flower development. To date, putative representatives of the B, C and E gene groups have been identified and their status of true orthologues of the genes cloned in the model plants arabidopsis and snapdragon (*Antirrhinum majus*) has been asserted through both the study of their respective expression patterns in the different floral whorls by *in situ* hybridization and their ectopic expression in transgenic arabidopsis plants (Adam *et al.*, 2007*a*, *b*). Among them, it has been found that the *EgDEF1* and *EgGLO2* genes are structurally and functionally equivalent to the *APETALA3/DEFICIENS* and *PISTILLATA/GLOBOSA* B-group genes of arabidopsis and snapdragon, respectively. From what has been demonstrated in studies based on model species it can be assumed that the protein products of the oil palm genes act through the formation of a tetramer made of the B-group DEF/GLO heterodimer and one protein from each of the A (or C) and E groups, according to the 'quartet' theory of floral organ specification (Egea-Cortines *et al.*, 1999; Pelaz *et al.*, 2000; Honma and Goto, 2001; Yang *et al.*, 2003; Melzer *et al.*, 2008; Melzer and Theissen, 2009). If this hypothesis is correct then a misregulation of either one of the B-type genes of oil palm could lead to the *mantled* phenotype if the relative amount of at least one of the quartet members deviates from the normal 1 : 1 : 2 ratio, possibly affecting the DNA-binding properties of the complex or its role in transcriptional activation. As a matter of fact, a decrease in the mRNA levels of both the *EgDEF1* and *EgGLO2* genes has been demonstrated in male and female inflorescences displaying the *mantled* phenotype compared with those from true-to-type palms (Adam *et al.*, 2007*b*). However, this decrease has not been detected consistently throughout the development of the inflorescences so this preliminary work clearly needs to be continued. Interestingly, Kaufmann *et al.* (2009) have suggested a possible interplay between the promotion of floral organogenesis and auxin response pathways in arabidopsis. Indeed, these authors

demonstrated through the identification of the targets of the E-type SEPALLATA3 protein that the complexes involving this protein mediate auxin-dependent flower development. This hypothesis raises the exciting possibility of a link between an auxin-induced variation emerging during the *in vitro* propagation of oil palm and the homeotic conversion of floral organs observed in the *mantled* regenerants, and needs to be explored further.

EPIGENETIC LANDSCAPING AND THE OIL PALM

The concept of 'epigenetic landscape' invented by Waddington (1957) has recently been revived in the wake of high-throughput technologies. Originally it designated an ensemble of stimuli and constraints influencing the differentiation of a cell. Today this idea has been revamped and the landscape is now made of a rich stratification of epigenetic mechanisms and of the gene expression changes that they promote. Taking advantage of the NGS (next generation sequencing) methods for the transcriptional analysis of whole genomes, several studies published in recent years have proposed a snapshot of the so-called 'epigenome' in model plants such as arabidopsis and rice (*Oryza sativa*) (Zhang *et al.*, 2007; Cokus *et al.*, 2008; Li *et al.*, 2008; Zhang *et al.*, 2008). This image is the reflection of the transcriptional state within a given tissue at a given developmental stage and in a precise physiological context and therefore it is very well suited for comparative purposes.

Several research groups and consortia have recently announced the complete sequencing of the oil palm genome. Indeed, Zieler *et al* (2010) very recently reported on the generation of whole-genome shotgun sequences of the oil palm genome, using primarily Sanger reads to enable high-quality assemblies. This breakthrough was achieved by a consortium joining Synthetic Genomics Inc. (USA), ACGT Sdn. Bhd. (Malaysia) and the J. Craig Venter Institute (USA). According to these authors, the genome sequences have been supplemented with a high volume of EST and transcriptome sequencing and the genome has been fully annotated.

In November 2009, The Malaysian Palm Oil Board announced that a consortium co-led by the Advanced Biotechnology and Breeding Centre had sequenced three oil palm genomes from the two related species *Elaies oleifera* and *Elaeis guineensis*, including the *pisifera* and *dura* varieties of oil palm, which display thin-shelled and thick-shelled fruits, respectively. The consortium included Orion Genomics and MOgene LC based in St Louis, MO and The Genome Center at Washington University, Macrogen Inc. based in South Korea, and GeneWorks Pty Ltd based in Adelaide, Australia. In addition to sequencing and assembling the genomes of the three oil palm varieties, the consortium sequenced the expressed genes (or transcriptome) from multiple tissue types for all three types of oil palm (Wahid, 2009).

In May 2009, a Malaysian consortium created by Sime Darby Bhd, one of the world's largest oil palm company and Synamatix Sdn Bhd, a bio-informatics company had announced the successful sequencing, assembly and annotation of the oil palm genome with 93·8 % completeness.

The diversity and the size of the investments in these joint ventures raise concerns in the oil palm researchers' community about the availability of these genome sequences to the public since issues on intellectual property rights are predictable. For this reason it is likely that the epigenetic landscaping of the oil palm genome will not become feasible for several years. However, in the meantime, it is possible to get a taste of the epigenome on a fraction of the oil palm genome or transcriptome. To obtain a reduction in sequence complexity prior to sequencing, a preliminary filtration or fractionation step can be applied to remove the sequences that are highly redundant and/or methylated as these are assumed to be irrelevant for the study of the differential expression of low-copy genes. Such an enrichment strategy, already suggested for the targeting of oil palm genes by Mayes *et al.* (2008), has been found to be suitable for the sequencing of the gene-containing fraction from large and repeat-rich genomes such as those of maize (*Zea mays*) (Palmer *et al.*, 2003; Whitelaw *et al.*, 2003), sorghum (*Sorghum bicolor*) (Bedell *et al.*, 2005) and onion (*Allium cepa*) (Jakše *et al.*, 2008). So these 'reduced-representation approaches' (as they have been called by Paterson, 2006) could have two beneficial effects for oil palm research, by accelerating the sequencing process of the gene-rich part of the genome and by providing the basis for its epigenetic mapping.

Recently Beulé *et al.* (2010) used subtractive hybridization to eliminate expressed sequences that were common to both normal and *mantled* male inflorescence tissues. This resulted in the enrichment in either normal- or variant-type-associated sequences, depending on the orientation of the subtraction process. The two corresponding libraries were then constructed and the differential accumulation of their cDNA contents was verified by macroarray hybridization. Eventually, the most differentially expressed phenotype-related transcripts were assessed on a larger range of palm tissues and genotypes using semi-quantitative RT-PCR. This approach led to the identification of six candidate markers, among which one was most strongly expressed in abnormal inflorescences, whereas the remaining five accumulated preferentially in true-to-type inflorescences. However, the analysis of the respective expression patterns of these transcripts in different oil palm tissues offered no clues as to their role in the onset of the somaclonal variation of oil palm, nor did the putative function of two of the corresponding gene products as components of the ubiquitin-proteasome pathway. In any case, it will be interesting to establish whether the genes corresponding to these candidate markers undergo methylation changes in conjunction with their phenotype-dependent variations in transcriptional levels.

TREADING ON THE *MANTLED* TRAIL: PERSPECTIVES

Throughout the years, a large amount of data has been gathered about the *mantled* somaclonal variation of oil palm. Although the many complexities of this abnormality still make it a puzzle, several exciting trails have been uncovered and need to be followed further.

A misregulation of one or both B-type floral organ genes *EgDEF1* and *EgGLO2* could help explain the morphology of

the *mantled* flowers. For this reason, the magnitude and the time-course of the decrease in the *EgDEF1* and *EgGLO2* gene expression needs to be quantified accurately using quantitative PCR. Whatever the results of these new experiments, the impact of the decrease in gene expression on the actual protein levels and on the availability of functional tetrameric complexes will have to be assessed. This point is made even more relevant because of the isolation in the oil palm genome of a different sequence with similarities to the *GLOBOSA* gene, named *EgGLO1*. Its protein product shares 87·1 % of identical residues with EgGLO2 while the expression patterns of the corresponding genes differ slightly from each other (Adam *et al.*, 2005*b*, 2007*b*). As several authors have pointed out in different monocotyledonous and dicotyledonous species (Münster *et al.*, 2001; Zahn *et al.*, 2005; Geuten and Irish, 2010), the occurrence of paralogous genes ensuring the formation of B-type floral organs could reflect a diversification in the roles performed by the protein complex to which their products contribute.

It is of paramount scientific interest to study the epigenetic mechanisms regulating the expression of the floral MADS-box genes, in the course of the normal oil palm flower development as well as in the *mantled* context. Indeed, several clues point to a general hypersensitivity towards flower malformations residing in the palm family. The occurrence in seed-derived populations of the rare (and supposedly mutant) *diwakkawakka* variety of oil palm, whose flowers are similar to those of the *mantled* somaclones (Hartley, 1988), mirrors the observation of *mantled* phenotype in seed-derived palms from the parent species *E. oleifera.* Very intriguing also is the plasticity and reversibility of sex differentiation in palms: Corley *et al.* (1976) demonstrated that gibberellic acid could favour the formation of male inflorescences in the monoecious oil palm, whereas hermaphrodism can be induced *in vitro* in female flowers of the dioecious date palm (*Phoenix dactylifera*) after treatment with growth regulators (Masmoudi-Allouche *et al.*, 2008). Moreover, in the latter species, somaclonal variants displaying supernumerary carpels have been observed (Cohen *et al.*, 2004, 2007) The possibility of a 'hotspot' within the oil palm genome being the starting point of the *mantled* somaclonal variation would fit with the observation that variant palms can originate, albeit at varying frequencies, from any of the micropropagation protocols known to date (Eeuwens *et al.*, 2002). Future work aimed at tackling this question will have to include the localization of the floral MADS-box genes in palm genomes, since the identification of a cluster formed by some of these genes could both support the 'hotspot' hypothesis in oil palm and contribute to explain the recurrence of abnormal floral phenotypes in different palm species. These reflections imply that the study of the epigenetic mechanisms governing the expression of the floral identity genes in oil palm must take into account their respective genomic environments in order to be informative. The considerable role played by chromatin remodelling (with or without concomitant changes in DNA methylation) in long-range, co-ordinated regulation of gene expression has been well described, especially in the case of floral induction and development (Schubert *et al.*, 2005; Baroux *et al.*, 2007; Adrian *et al.*, 2009; Cazzonelli *et al.*, 2009; Charron *et al.*, 2009). Thus it would be interesting to investigate the possible changes in chromatin condensation associated with the *mantled* somaclonal phenotype in the vicinity of the floral MADS-box genes. Small non-coding RNA populations of oil palm should be studied in parallel as these molecules can promote changes in both DNA methylation and chromatin conformation (Lippman *et al.*, 2003; Vaucheret, 2006; Poethig, 2009) and as an increasing number of papers illustrate their participation to the regulation of flower development (Park *et al.*, 2002; Baker *et al.*, 2005; Swiezewski *et al.*, 2007; Hultquist and Dorweiler, 2008; Nag *et al.*, 2009; Liu *et al.*, 2010; Nair *et al.*, 2010; Yant *et al.*, 2010).

In addition, the influence of transposable elements in the *mantled* phenotype should be examined further. It is well known that both the immobilization and the silencing of transposable elements are mediated by a combination of DNA hypermethylation and heterochromatinization and that this repression is frequently alleviated after tissue culture and/or as a consequence of defects in the gene silencing machinery (Grandbastien, 1998; Miura *et al.*, 2001; Slotkin and Martienssen, 2007; Tanurdzic *et al.*, 2008; Reinders *et al.*, 2009; Slotkin *et al.*, 2009). In spite of the fact that transposable elements constitute a large portion of the oil palm genome and are widely distributed among its chromosomes (Castilho *et al.*, 2000; Price *et al.*, 2002), no large-scale phenotype-dependent alterations in transposable elements repartition or methylation were detected by Kubis *et al.* (2003) in regenerant oil palms. Still, the occurrence of such phenomena in relation to the *mantled* abnormality cannot be completely excluded since the recent study of Mirouze *et al.* (2009) demonstrated that different families of transposable elements can be regulated through distinct pathways. In addition, recent experiments undertaken in our group have revealed the insertion of a retrotransposon within one of the candidate marker genes for the abnormality and its coincidence with an altered production of transcripts from this gene (Jaligot *et al.*, unpubl. res.). Provided that a causal relationship is demonstrated between this specific insertion and the *mantled* somaclonal variation, it is tempting to hypothesize that the epigenetic mechanisms involved could resemble those underlying the retroelement-induced *Hose in Hose* floral phenotype of primrose (*Primula vulgaris*) (Li *et al.*, 2010).

ACKNOWLEDGEMENTS

The research discussed in this paper was part of the EPIDEV (Epigenetic Control of Developmental Gene Expression), a FAST (French-Australian Science and Technology) collaborative research project between the International Science Linkages programme established under the Australian Government's innovation statement Backing Australia's ability – Project FR070100 and French Partenariat Hubert Curien, Project 16079RJ. A.R. was supported through a Marie Curie Outgoing International Fellowship from the 6th Framework Program of the European Commission (Contract MOIF-CT-2004-509893 VARIOMETH). E.J. was sponsored by a Lavoisier Post Doctoral Grant awarded by the French Ministry of Foreign Affairs (Contract BFE2002/340634B). The Directors and dedicated staff of PalmElit SAS (France), CNRA-LaMé (Côte d'Ivoire), Hacienda La Cabana (Colombia), Malaysian Palm Oil Board, FELDA Agricultural

Services, and United Plantations Bhd (Malaysia) are gratefully thanked for their co-operation.

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