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INVITED REVIEW

Determination of Flower Structure in *Elaeis guineensis*: Do Palms use the Same Homeotic Genes as Other Species?

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• *Aims* In this article a review is made of data recently obtained on the structural diversity and possible functions of MADS box genes in the determination of flower structure in the African oil palm (*Elaeis guineensis*). MADS box genes play a dominant role in the ABC model established to explain how floral organ identity is determined in model dicotyledon species such as *Arabidopsis thaliana* and *Antirrhinum majus*. In the monocotyledons, although there appears to be a broad general conservation of ABC gene functions, the model itself needs to be adapted in some cases, notably for certain species which produce flowers with sepals and petals of similar appearance. For the moment, ABC genes remain unstudied in a number of key monocot clades, so only a partial picture is available for the Liliopsida as a whole. The aim of this article is to summarize data recently obtained for the African oil palm *Elaeis guineensis*, a member of the family Arecaceae (Arecales), and to discuss their significance with respect to knowledge gained from other Angiosperm groups, particularly within the monocotyledons.
• *Scope* The essential details of reproductive development in oil palm are discussed and an overview is provided of the structural and functional characterization of MADS box genes likely to play a homeotic role in flower development in this species.
• *Conclusions* The structural and functional data provide evidence for a general conservation of the generic 'ABC' model in oil palm, rather than the 'modified ABC model' proposed for some other monocot species which produce homochlamydeous flowers (i.e. with morphologically similar organs in both perianth whorls), such as members of the Liliales. Our oil palm data therefore follow a similar pattern to those obtained for other Commelinid species in the orders Commelinales and Poales. The significance of these findings is discussed.

Key words: Palm, MADS box, flower, *Elaeis*, monoecious, homeotic.

INTRODUCTION: WHY STUDY FLOWER FORM?

Given the remarkable diversity of form displayed by flowers and their great utility as a morphological character in taxonomic studies, it is not surprising that they have been a focus of attention in recent years in evolutionary developmental biology. Innovations in flower structure were probably a key factor which contributed to the success of the angiosperms and the great diversification of lineages which occurred early in the evolution of this group is reflected in abundance of floral forms observed today in extant species. During the last decade, the development of molecular phylogenies has allowed the elucidation of phylogenetic relationships between most major angiosperm clades (Savolainen and Chase, 2003; Davies *et al.*, 2004). Moreover, for an increasing number of angiosperm species, whole genome sequences are available, along with associated functional tools for molecular genetic studies. This has made it possible for researchers to carry out in-depth analyses of the molecular determination of flower structure in model plants such as thale cress (*Arabidopsis thaliana*), snapdragon (*Antirrhinum majus*) and rice (*Oryza sativa*) (Theissen *et al.*, 2000). The large body of data thus obtained provides a useful starting point for studies in other higher plant taxa for which fewer molecular resources and functional tools are available.

In this article, we summarize recent work carried out on the structure and function of floral MADS box genes in the African oil palm, *Elaeis guineensis* (Arecoideae, Cocoseae; Dransfield *et al.*, 2005), an economically important member of the palm family (Arecaceae), which constitutes the order Arecales within the monocotyledons. We compare and contrast our results with those obtained from other angiosperm lineages, especially within the monocotyledons. Gene structure/function studies on palms pose a number of technical difficulties, but are vital in order to understand how flower structure determination in the Arecaceae fits in with that of other groups.

FLOWERING IN OIL PALM

Flowering in Arecaceae

Palms are probably one of the most easily recognizable plant families, despite the relatively large size of the group – around 2400 species, according to a recent

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checklist (Govaerts and Dransfield, 2005). As noted by Tomlinson (1990), palms are characterized by generally highly branched inflorescences with a basal prophyll, a tendency towards monoecy and dioecy and the association of flowers within small groups (usually condensed cincinni) which are often characteristic of specific clades. A wide range of studies of inflorescence and flower development has been reported for palms (Tomlinson and Moore, 1968; Uhl, 1976; Uhl and Moore, 1977, 1978, 1980; De Mason *et al.*, 1982; Uhl and Dransfield, 1984; Uhl, 1988; Barfod and Uhl, 2001; Stauffer *et al.*, 2002; Rudall *et al.*, 2003). As is typical of monocotyledonous species, palm species usually produce trimerous flowers. Some features have been noted as characteristic of specific groups, including the apocarpous character typical of (but not universal to) Coryphoid palms and the presence of distinct overlapping scales around the ovules of Calamoid species. Probably the most striking variation in flower structure is exhibited by the tribe Phytelepheae. This group is characterized by flowers with more than three organs per whorl and stamens which develop centrifugally in large numbers (from 120 to over 900 per flower; Uhl and Moore, 1977). With regard to their perianth, palm flowers often display distinguishable sepals and petals (Dransfield and Uhl, 1998). However, it is also common to observe a perigon-type perianth composed of two whorls of organs of similar appearance referred to as tepals. Floral bauplan forms part of the wide body of morphological and anatomical data which facilitate the classification of palm species into specific clades, complemented more recently by in-depth studies of molecular phylogeny (Dransfield *et al.*, 2005). In this article, we focus our attention mostly on oil palm flower development as compared with other angiosperm families, particularly within the Liliopsida, for which an increasing body of molecular data is becoming available.

Reproductive development in oil palm

An in-depth microscopic analysis of oil palm inflorescence and floral development was carried out recently (Adam *et al.*, 2005), complementing partial studies reported previously (Beinaert, 1935; Corley and Gray, 1976; Van Heel *et al.*, 1987). The key developmental stages of inflorescence and flower development in *E. guineensis* are illustrated in Fig. 1. Oil palm is a long-lived single stemmed palm which bears, like the majority of palm species, a single vegetative shoot apical meristem maintained throughout the lifetime of the plant. Under favourable climatic conditions, this meristem is continuously active, producing a new leaf primordium approximately every 2 weeks in mature palms (Corley and Gray, 1976). The leaf takes 2–3 years to develop from initiation to the time when leaflets unfold in the centre of the palm crown. Inflorescences are formed throughout the year in the axils of their subtending leaves. *Elaeis guineensis* is monoecious, producing separate male and female inflorescences on the same palm in alternation, although mixed sex inflorescences are occasionally observed. Whereas the male inflorescence bears individual staminate flowers, the female inflorescence produces floral triads consisting of a pistillate flower flanked by two accompanying staminate flowers. The latter develop up to, and including, the appearance of microsporocytes in the pollen sac, after which no further development occurs and abscission takes place before the pistillate flower reaches maturity. In the perianth of oil palm flowers, sepals and petals are of a similar petaloid appearance, particularly in the pistillate flower. The reproductive organs of staminate flowers are composed of six stamens with connate filaments surrounding a pistillode, whereas pistillate flowers display rudimentary stamens (staminodes) and a gynoecium of three carpels.

A homeotic floral variant in oil palm: the mantled abnormality

For the purpose of understanding the molecular processes which determine flower structure in oil palm, a previously described homeotic epimutant, known as *mantled* (Corley *et al.*, 1986) is of particular interest. *Mantled* palms exhibit a transformation of stamens and staminodes into carpel-like structures in staminate and pistillate flowers, respectively (Adam *et al.*, 2005). In the *mantled* staminate flower, the transformation of the stamens into pseudocarpels results in sterility, whereas in the *mantled* pistillate flower, fertilization may occur in less severe cases to produce characteristic fertile fruits. In more severe cases, parthenocarpy or arrested development occurs. The *mantled* phenotype is observed in oil palms regenerated from tissue culture (Corley *et al.*, 1986) and may be transmitted through meiosis (Rao and Donough, 1990). However, reversion to wild type is observed in the field in some but not all individuals (Durand-Gasselin *et al.*, 1990), indicating an epigenetic origin. In the female inflorescence of *mantled* palms, all stages up to and including reproductive organ initiation appear the same as in normal palms. Developmental divergence occurs shortly afterwards, when organs resembling carpels are seen to develop in the androecium in place of staminodes. These carpeloid structures lack ovules and are thus sterile. In the case of the *mantled* staminate flower, the divergent developmental pattern is witnessed at the same stage, i.e. during the elongation of the organs of the third whorl, which are seen to display a central vascularization characteristic of carpels, whereas stamens normally have a peripheral vascularization (Fig. 1). The homeotic transformation of stamens to sterile carpel-like structures may also be observed in the accompanying staminate flowers of floral triads on the female inflorescence. As previously demonstrated with model flowering plants, the study of floral variants in oil palm is likely to provide a useful means to understand the molecular mechanisms which regulate floral morphology in this species.

THE MOLECULAR BASIS OF FLOWER STRUCTURE: WHAT DO WE KNOW FROM OTHER SPECIES?

The ABC model

Genetic studies performed on model species, including *Arabidopsis thaliana* and *Antirrhinum majus*, led in the

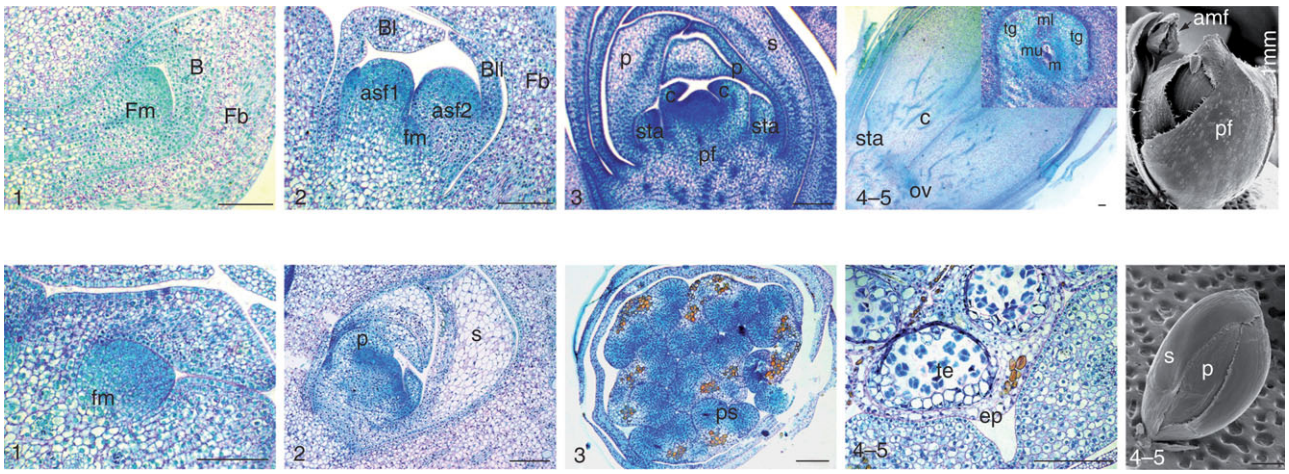

FIG. 1. Key stages of pistillate (upper panel) and staminate (lower panel) flower development in oil palm. Developmental stages (indicated at bottom left of each photo) were assigned on the basis of the differentiation of the floral whorls. Stage 1 corresponds to a floral meristem. Stage 2 corresponds to the initiation of perianth organs. Stage 3 corresponds to the development of perianth organs and the initiation of reproductive organs. Stage 4 corresponds to the development of reproductive organs and stage 5 to a mature flower. Photographs are of either PAS/NBR-stained transverse and longitudinal sections or scanning electron micrographs (right-hand photographs, upper and lower panels). Abbreviations: asf1/asf2, accompanying staminate flowers 1 and 2; B, bracteole; BI/BII, bracteoles I and II; c, carpel; ff, pistillate flower; fm, floral triad meristem; Fb, floral triad bract; o, ovule; p, petal; s, sepal; sta, staminodes; c, carpel; m, megaspore mother cell; ps, pollen sac; sta, staminodes; te, tetrads; tg, integuments.

early 1990s to the identification of regulatory pathways and genes which control various aspects of flowering in a wide range of plants (Coen and Meyerowitz, 1991; Levy and Dean, 1998; Meyerowitz, 1998). At the outset, molecular genetic studies of higher plant flowering revealed the existence of a generalized floral signalling hierarchy within which individual genes were found to act at specific levels. Several classes of genes were identified in this way, including those determining flowering transition, inflorescence meristem identity and floral organ identity (Okada and Shimura, 1994; Weigel, 1995). An important advance was achieved in the formulation of the ABC model to explain floral organ identity determination based on studies performed on *A. thaliana* and *Antirrhinum* (Coen and Meyerowitz, 1991). According to this model, the identity of each whorl of the flower is governed by the expression of one or more homeotic genes of function A, B or C. Expression of the A-class function alone specifies sepal formation. The combination of A- and B-class functions specifies the development of petals, and the combination of B- and C-class functions results in the formation of stamens. The expression of the C function alone determines the development of carpels. Since its initial conception, this model has been modified to take account of newer data, revealing a D-type activity involved in the specification of ovules (Angenent and Colombo, 1996) and an E function necessary for the determination of the corolla, androecium and gynoecium (Pelaz *et al.*, 2000). The essential details of the current generic ABC model are shown in Fig. 2 (top right).

Despite this overall conservation of gene function between species, several limitations to the generic organ identity model have been identified, including the apparently poor conservation of A-type function, which is likely to be a relatively recent development in higher plant evolution (Egea Gutierrez-Cortines and Davies, 2000). Another aspect of flower structure determination which appears increasingly more complex in reality than in the ABC model is the distinction of roles between C and D genes, which in *A. thaliana* are closely related and overlap in their functions (Favaro *et al.*, 2003; Pinyopich *et al.*, 2003). The frequent occurrence of paralogues in lineages involved in flower structure determination makes gene structure/function studies complicated; however, the duplications from which they have arisen will in some cases have been important evolutionary events.

The key role of MADS box genes in flower development

Nearly all floral homeotic genes, including those mentioned above, code for MADS box transcription factors. These proteins are common to all eukaryotic groups; however those found in higher plants are distinguishable by their characteristic MIKC structure, referring to the four different domains which they possess (Theissen *et al.*, 2000). Phylogeny reconstructions reveal that the MADS box gene family is composed of a number of defined gene clades (Becker and Theissen, 2003). In eudicotyledons, 14 different paralogous MIKC-type MADS box gene subfamilies, have been defined (Alvarez-Buylla *et al.*, 2000; Becker *et al.*, 2000; Theissen *et al.*, 2000, Becker and Theissen, 2003). Thanks to studies performed on a number of different species, strong structure/function relationships have been established for a number of these groups. Thus A function has been inferred for certain members of the *SQUAMOSA* (*SQUA*) subfamily, B function for genes of the *GLOBOSA* (*GLO*) and *DEFICIENS* (*DEF*) classes, C and D functions for members of the *AGAMOUS* (*AG*) group and E function for various *AGAMOUS-like2* (*AGL2*)

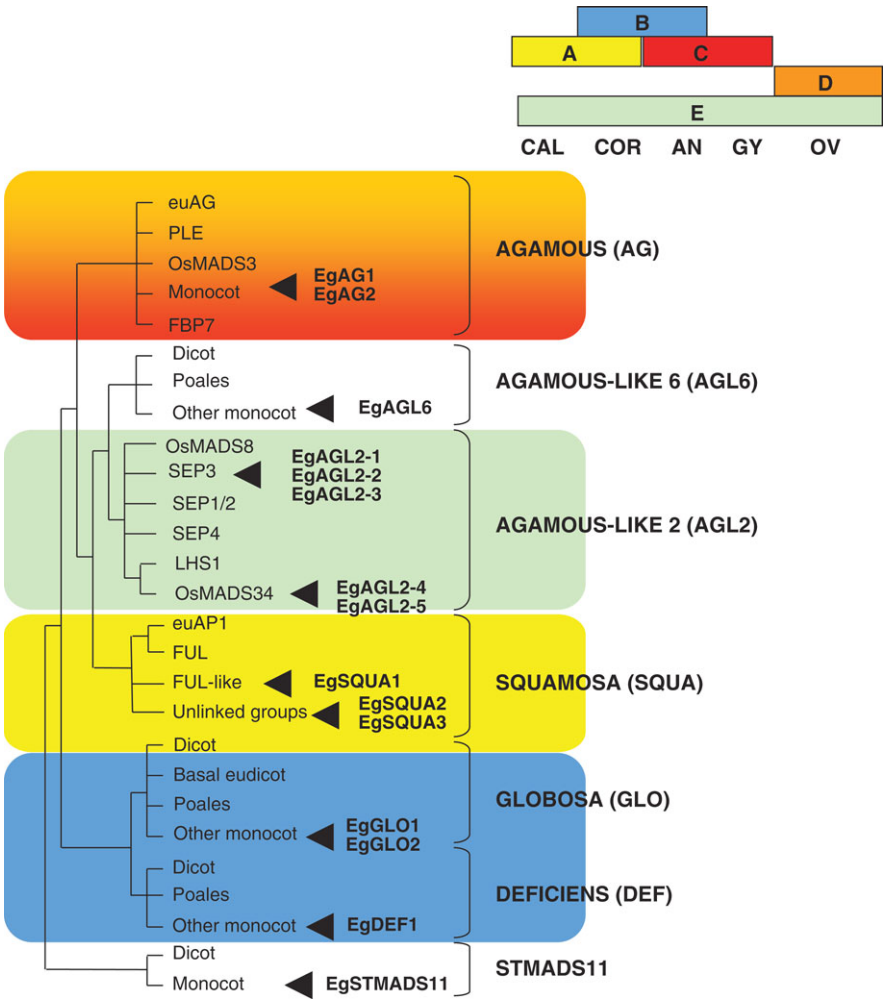

FIG. 2. Dendrogram illustrating sequence affinities between oil palm MADS box proteins and selected relatives from other angiosperm groups. Subfamilies are designated according to the system of Becker and Theissen (2003). The tree shown is a schematic representation of topologies obtained using the maximum parsimony (MP) method with full-length MADS box amino acid sequences. Abbreviations: euAG, euAGAMOUS clade; PLE, PLENA clade; FBP7, FLORAL BINDING PROTEIN 7 clade; SEP3, SEPALLATA3; SEP1/2, SEPALLATA1/2; LHS1, LEAFY HULL STERILE1; euAP1, euAPETALA1 clade; FUL, FRUITFULL; CAL, calyx; COR, corolla; AN, androecium; GY, gynoecium; OV, ovule.

genes. We took advantage of these conserved structure/function relationships in order to study genes likely to regulate flower structure in oil palm.

MADS BOX GENES IN OIL PALM

In our studies, MADS box genes of oil palm were identified and characterized *via* the isolation of full-length cDNAs. Most sequences were isolated by PCR amplification with degenerate MADS box-specific primers using cDNA derived from male or female inflorescences. The resulting PCR fragments were then used to screen cDNA libraries prepared from the same type of plant material (Adam *et al.*, 2006). An additional MADS box gene was identified by systematic sequencing of cDNA clones as part of an EST (expressed sequence tag) collection (Jouannic *et al.*, 2005). As a result, 15 different oil palm MADS box genes were identified and named according to their sequence affinities as follows: *EgSQUA1*, *EgSQUA2* and *EgSQUA3* (*SQUAMOSA* or *SQUA* group); *EgDEF1* (*DEFICIENS* or *DEF* group); *EgGLO1* and *EgGLO2* (*GLOBOSA* or *GLO* group); *EgAG1* and *EgAG2* (*AGAMOUS* or *AG* group); *EgAGL2-1*, *EgAGL2-2*, *EgAGL2-3*, *EgAGL2-4* and *EgAGL2-5* (*AGAMOUS-like2* or *AGL2* group); *EgAGL6-1* (*AGAMOUS-like6* or *AGL6* group); and *EgSTMADS11-1* (*STMADS11* group). Genes were assigned initially to MADS box subfamilies on the basis of sequence similarities. The overall sequence relationships of the proteins encoded by the oil palm MADS box genes are shown pictorially in the dendrogram in Fig. 2, which also reveals the different clades identified within each subfamily. These data were described in detail previously (Adam *et al.*, 2006) except for the AGL6 and STMADS11 groups. For this study, entire amino acid sequences were used in conjunction with the maximum parsimony (MP) method. A summary of essential details of the oil palm MADS box genes and the proteins which they encode is given in Table 1. This table includes information on the possible

TABLE 1. *Summary of data obtained on the complexity of the different oil palm MADS box groups, on the spatio-temporal expression patterns of five selected oil palm MADS box genes and on the phenotypic effects induced when they were overexpressed in transgenic* Arabidopsis thaliana *plants*

Gene	Size of cross-hybridizing gene group	Expression pattern			Phenotype when ectopically expressed in *A. thaliana*	Possible role(s)
		Staminate flowers	Pistillate flowers	Mantled palms		
EgSQUA1	2	Inflorescence and floral meristems	Inflorescence and floral meristems	Unaffected	Tall phenotype (larger number of nodes)	Inflorescence/ floral meristem identity (A function?)
EgDEF1	1	Stamens and petals	Staminodes and petals	Decreased expression (both sexes)	No alterations observed	B function
EgGLO2	2	Sepals, petals and stamens	Sepals and petals	Decreased expression (both sexes)	Transformation of sepals to petals	B function
EgAG2	2	All whorls (at immaturity)	Carpel primordia/ ovules	Decreased expression (mainly in pistillate flower)	No alterations observed	C and/or D function
EgAGL2-1	2–5	Petals and stamens	Petals and ovule primordia	Decreased expression (both sexes)	Leaf-Like sepals and petals; 'flower within a flower'	E function

existence of closely related genes as evaluated by Southern hybridization and their specificity of expression as revealed by RT-PCR analysis on several different organs/developmental stages of the plant.

In subsequent work, we focused our attention on those groups for which a role in floral organ identity determination has been demonstrated, namely the SQUA (A function), GLO (B function), DEF (B function), AG (C/D functions) and AGL2 (E function) subfamilies. As can be observed from the dendrogram in Fig. 2, most subfamilies can be further resolved into smaller clades, some specific to monocots or dicots. Some sequences were observed to be rooted in an unresolved fashion at the base of their subfamily group. This included in the case of oil palm the SQUA homologues EgSQUA2 and EgSQUA3. All other oil palm MADS box sequences were found to branch with related sequences from other species within their subfamily, providing some initial clues as to their possible functions.

POSSIBLE FUNCTIONS INFERRED FROM EXPRESSION ANALYSIS AND TRANSGENIC STUDIES

The phylogenetic reconstructions carried out for each MADS box subfamily provide a useful insight into evolutionary relationships both within the group of oil palm genes studied and also with respect to other plant taxa. Although this may provide clues as to the possible roles of the oil palm genes, studies of a functional nature are essential in order to validate any hypotheses made. The investigation of floral gene function in oil palm is complicated by the large size and long life cycle of the plant, flowering occurring only about 3 years after germination. Thus, although genetic transformation of oil palm has been achieved (Parveez *et al.*, 2000), transgenic studies of floral gene function in this species would require many years to yield results. In order to circumvent this problem, we employed ectopic expression in *A. thaliana* (under the control of the CaMV 35S promoter) as a means of assessing the conservation of MADS box protein function between oil palm and dicots. The data obtained by genetic transformation were coupled with information on the spatial and temporal expression patterns of each gene in normal and *mantled* palms, obtained by *in situ* hybridization and RT-PCR, respectively, so as to propose putative functions. Five of the oil palm MADS box genes were selected for detailed investigations of their possible function in oil palm reproductive development (Adam *et al.*, 2007). On the basis of their DNA sequences, the genes selected showed similarities with various different homeotic genes implicated in the ABC model: *EgSQUA1* (A class and/or meristem identity); *EgGLO2* and *EgDEF1* (B class); *EgAG2* (C or D class); and *EgAGL2-1* (E class). The isolation of MADS box cDNAs from oil palm was also recently described by Alwee *et al.* (2006), who identified genes similar or identical to each of the above, with the exception of *EgDEF1*.

Petal and sepal identities are clearly distinguished by MADS box gene expression in Elaeis guineensis

Within the general objective of identifying possible functions for floral MADS box genes in oil palm, we wished to address the specific question of whether petals and sepals have distinct identities in this species, and whether the *mantled* flower abnormality involves a change of organ identity in the perianth, as seen in the B-class mutants of model species. It is important to deal with this question at the outset when interpreting the data obtained, in order to establish the overall framework within which ABC functions can be attributed to specific genes. *Elaeis guineensis*

produces, like many monocot species, flowers containing an inner and outer perianth of similar appearance, the petals and sepals sometimes being referred to collectively as tepals. In the case of the pistillate flower, as noted by Beinaert (1935), the only major difference between the inner and outer perianth whorls is in the size of the organs produced. Beinaert therefore considered the pistillate flower to be homochlamydeous (i.e. with morphologically similar organs in both perianth whorls). In the case of the staminate flower, some colour distinction exists between the inner and outer perianth organs, the former being more translucent in appearance. In the palm family as a whole, the morphological distinction of petals and sepals is possible for many species (Dransfield and Uhl, 1998). Our results indicate that the identities of petals and sepals can be distinguished by MADS box gene expression patterns in the case of oil palm. This is well illustrated by the *EgDEF1* gene, for which expression was detected in the petals but not in the sepals of pistillate flowers (Fig. 3 and Table 1). *EgDEF1* was also observed to be expressed in the androecium of flowers of both sexes. This spatial expression pattern is typical of classical B-class genes in model species such as *A. thaliana* in which the calyx and corolla are structurally different, as will be discussed below. Differences between sepals and petals of oil palm were also revealed by the spatial expression pattern of *EgAGL2-1*, a *SEPALLATA3* (*SEP3*)-like gene of putative E function.

Utility of the mantled epimutant for the elucidation of floral homeotic functions in oil palm

Floral mutants are an invaluable tool for studies aimed at elucidating gene function. In the case of oil palm, for which genetic approaches would require dauntingly long periods of time, we capitalized on the availability of the *mantled* epimutant induced by tissue culture. The *mantled* phenotype resembles that of B-class mutants, such as *apetala3* (*ap3*) and *pistillata* (*pi*) of *Arabidopsis thaliana* (*AP3* is a *DEF*-type gene and *PI* a *GLO*-type one) in which stamens are homeotically transformed to carpel-like structures. The *ap3* and *pi* mutants also exhibit a conversion of petals into sepals. We therefore investigated whether a petal to sepal transformation could be revealed in *mantled* palms by MADS box gene expression changes. Our results corroborated this hypothesis; for example, no *EgDEF1* transcripts were detected in the inner perianth of *mantled* flowers, implying that a homeotic conversion had occurred.

More generally, our RT-PCR results (summarized in Table 1) revealed that the expression of four of the five MADS box genes investigated was lower in *mantled*

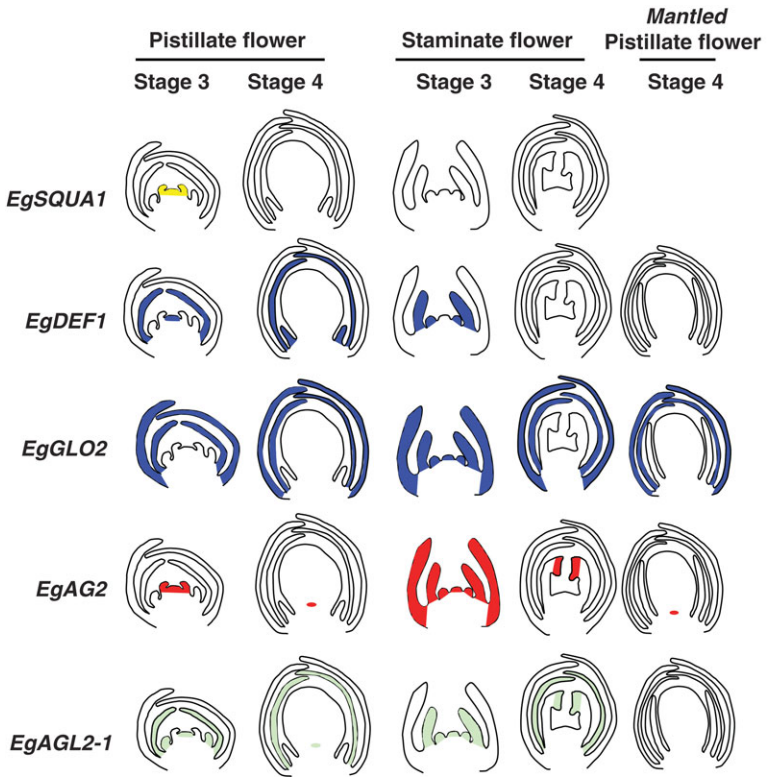

FIG. 3. Spatial expression patterns of oil palm MADS box genes in staminate and pistillate flowers: schematic representation of *in situ* hybridization results. Two different developmental stages are depicted: firstly, the developing androecium and gynoecium stage (Stage 3); and secondly, the stage where gynoecium and androecium have reached full size but not maturity (Stage 4). Observations with *mantled* pistillate flowers are shown for Stage 4 only. Floral zones in which an *in situ* hybridization signal was observed are shown in colour, depending on the gene involved (yellow for *EgSQUA1*, blue for the B class genes *EgDEF1* and *EgGLO2*, red for *EgAG2* and green for *EgAGL2-1*).

palms. This raises interesting questions as to the identity of the gene or genes initially perturbed in their activity in epigenetically altered plants. It seems unlikely that several different genes would all be directly perturbed; thus one hypothesis which can be made is that the initial gene target is an upstream regulator of the MADS box genes. This hypothesis suffers from the drawback that mutations in the genes which regulate organ identity genes generally produce much wider phenotypic effects than those seen in *mantled* palms. For example, mutations in the *UNUSUAL FLORAL ORGANS* (*UFO*) gene result in changes not only to floral organ identity, but also to inflorescence structure (Hepworth *et al.*, 2006). To account for the specific nature of the *mantled* abnormality, it would therefore be necessary to invoke the existence of a target gene possessing either a novel function or exercising a much narrower role than its orthologues in previously described systems. An alternative and probably more plausible way to explain why several different MADS box genes show reduced expression in *mantled* plants is based on the fact that MADS box genes can regulate each other. Indeed many MADS box genes contain in their promoter regions regulatory sequences known as CArG boxes which are themselves binding sites for MADS box transcription factors. Binding may often occur to heteromultimeric complexes containing two or more different MADS proteins, thus the interactions taking place are complex. In the context of the *mantled* abnormality, it is interesting to note that in *A. thaliana*, mutations in the *APETALA3* gene result in reduced transcript levels of the *PISTILLATA* gene and vice versa (Goto and Meyerowitz, 1994). Thus it is possible that one of the MADS box genes described here might be the initial genomic target of the epigenetic somaclonal variation event with secondary consequences on the expression of other MADS box genes. Further studies will be required to reveal the identity of the gene(s) involved and the exact nature of the epigenetic deregulation which has occurred.

Summary of functional data obtained: a model to explain the possible functions of five different floral MADS box genes of oil palm

Table 1 and Fig. 3 summarize the data obtained from gene expression and transgenic studies. For each of the genes investigated, a possible function is proposed on the basis of available results.

In the case of *EgSQUA1*, a role in the determination of inflorescence and floral organ identity is suggested. *EgSQUA1* expression is concentrated in meristematic zones of the inflorescence and flower and a tall phenotype results when the gene is overexpressed in transgenic *A. thaliana* plants due to a larger number of nodes produced during the reproductive phase of the plant.

In the case of *EgDEF1*, its specific expression pattern in petals and sepals, coupled with the observed reduction in transcripts associated with the *mantled* abnormality, argue strongly in favour of a B function, as suggested by its sequence affinities.

In the case of *EgGLO2*, a functional similarity with the *A. thaliana PISTILLATA* gene was observed, since both produce the same phenotypic changes when ectopically expressed in transgenic plants of the latter species, namely the transformation of sepals into petals. Using the same approach, an identical phenotype was observed by Alwee *et al.* (2006) for the oil palm gene *EgMADS16*, a paralogue of *EgGLO2* also isolated in our laboratory under the name of *EgGLO1*. In their study, Alwee *et al.* demonstrated that *EgMADS16* was able to complement an *A. thaliana pistillata* mutant.

The homeotic modifications seen in 35S:*EgGLO2* transgenic plants, taken together with the observed reduction of *EgGLO2* transcripts in *mantled* palms, suggest a B function for this gene. Nevertheless, *EgGLO2* appears to diverge compared with its *A. thaliana* relative inasmuch as transcripts of this gene are detected in sepals. This observation should be interpreted with caution, given that it does not necessarily signify the accumulation of the EgGLO2 protein. Indeed, in *Lilium longiflorum*, it has been shown that transcripts of the *DEF* gene *LMADS1* accumulate in all whorls of the flower, but that the corresponding protein is present only in petals and stamens (Tzeng and Yang, 2001). An additional factor to bear in mind is that according to the generic ABC model, the GLO protein cannot exercise a B function on its own, but requires the presence of DEF, with which it forms a heterodimer and probably other types of heteromultimeric complexes (Theissen and Saedler, 2001). Nevertheless, it appears that in the Liliales at least, GLO-type proteins possess the capability of binding *in vitro* in a sequence-specific manner to DNA as a homodimer (Winter *et al.*, 2002; Kanno *et al.*, 2003).

On the basis of *in situ* hybridization studies, a C and/or D function can be proposed for *EgAG2*, since transcripts were detected both in ovule primordia and more generally in the carpel, as well as in other parts of the flower at earlier stages. The existence of genes possessing a mixed C/D function has already been demonstrated in *A. thaliana* (Favaro *et al.*, 2003; Pinyopich *et al.*, 2003).

In the case of *EgAGL2*, ectopic expression in transgenic *A. thaliana* produced an altered flower phenotype similar to the quadruple *sepallata* (*sep1/sep2/sep3/sep4*) mutant (Ditta *et al.*, 2004). It was noted that sepals and petals acquired leaf-like characteristics; also a 'flower within a flower' was observed in place of the ovule in the gynoecium. This phenotype is hypothesized to be due to a dominant negative effect whereby the oil palm EgAGL2-1 protein is able to bind to the SEP3 protein partners in *A. thaliana* without possessing all necessary specificities for biological activity. Collectively, these data argue for an E function for *EgAGL2*. The *AGL2-like* subfamily appears to have expanded and functionally diversified in the monocots compared with dicots; nevertheless it is interesting to note that E function was recently demonstrated to be conserved in rice through studies of loss of function mutants of the *OsMADS1* gene (Kumar *et al.*, 2005).

By combining the functional data, we were able to propose a tentative model to explain the action of the five oil palm MADS box genes studied in detail (Fig. 4). This model represents in essence the generic ABC model and

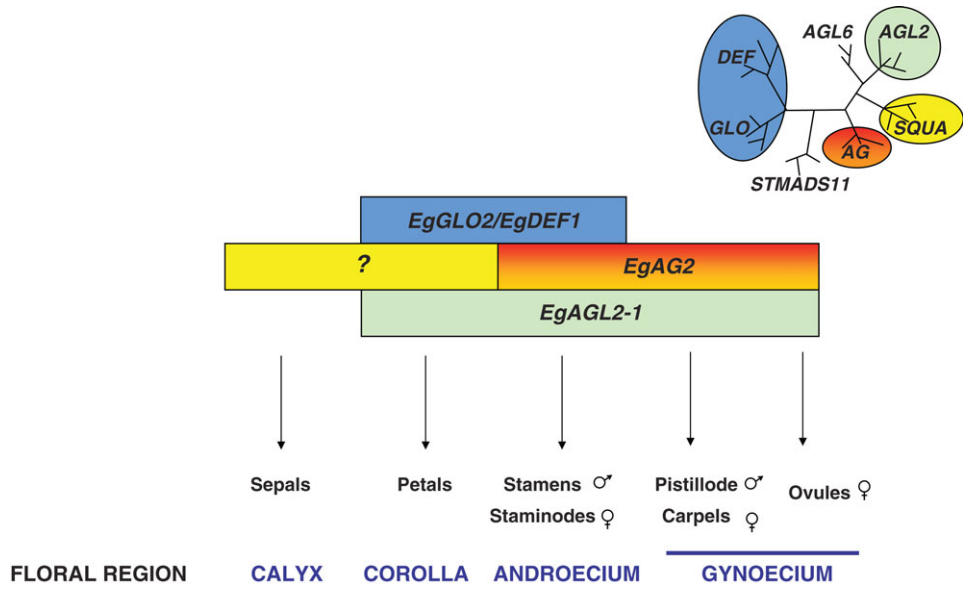

FIG. 4. A model to explain the possible roles of various oil palm genes in the determination of flower structure in oil palm, as based upon the generic eudicot ABC model (Coen and Meyerowitz, 1991; Angenent and Colombo, 1996; Pelaz *et al.*, 2000). In the top left-hand corner is shown a schematic representation of sequence relationships between the oil palm MADS box genes studied. Boxes are colour shaded according to homeotic functions as follows: yellow, A function; blue, B function; orange, C and D functions; green, E functions.

distinguishes oil palm from some other monocot species, as is discussed below.

ABC model variation amongst the monocotyledons

Since the ABC model was first conceived for eudicots, a number of studies have been undertaken to investigate whether floral organ identity is determined in a similar way in monocots. Figure 5 provides a summary of current knowledge of floral organ identity regulation within the Liliopsida, with putative ABC models shown where sufficient data exists to allow hypotheses to be made.

With regard to the determination of floral reproductive organs, studies on a wide range of species have revealed that there is a structural conservation of C and D MADS box gene lineages in monocots (Skipper *et al.*, 2006). A general, if not universal conservation of C- and D-type gene expression patterns in monocots with respect to dicots suggests a corresponding functional conservation, and has been corroborated by transgenic studies in some cases (e.g. Kyozuka and Shimamoto, 2002; Tzeng *et al.*, 2002; Benedito *et al.*, 2004). More generally, global conservation of the generic ABC model has been demonstrated for some species. This was found to be the case for the Poales, the first monocot group to be studied in detail (Kramer and Jaramillo, 2005). B-class genes have been a matter of particular interest in monocot studies, since their activities appear to be important in the distinction of petals and sepals, which are sometimes similar in appearance in this group. Studies on members of the Poales such as maize and rice (Ambrose *et al.*, 2000; Nagasawa *et al.*, 2003) revealed that B-class gene expression followed a similar pattern to that seen in model eudicots, B-type activity being detected in lodicules but not in paleae/lemmae. Another monocot order in which the generic ABC model appears to be conserved is the Commelinales (Ochiai *et al.*, 2004). In this case, two species were studied, namely *Tradescantia reflexa* and *Commelina communis*, both of which display distinct petal and sepal morphologies. In both plants, the expression of *DEF* and *GLO* genes was found to clearly mark petal identity compared with that of the sepals, although in the case of *T. reflexa*, a second *GLO* paralogue was identified and found to be expressed in sepals.

In contrast to the Poales and Commelinales, species from some other monocot clades were found not to conform to the generic ABC model. In the Liliales, studies of two species have been reported, namely tulip (*Tulipa gesneriana*; Kanno *et al.*, 2003) and lily (*Lilium longiflorum*; Tzeng and Yang, 2001). In these two cases, a perianth containing similar petaloid organs in both whorls is produced. In order to explain how this floral configuration might be determined, an altered model was proposed whereby B-class gene expression is expanded to include the outer perianth. This was referred to as the 'sliding boundary model' (Kramer *et al.*, 2003) or 'modified ABC model' (Van Tunen *et al.*, 1993). In the former case, the model was proposed to take account of data obtained with species of the Ranunculaceae. The studies performed by Tzeng *et al.* (2001) and Kanno *et al.* (2003) provided support for the modified ABC model in *Lilium longiflorum* and *Tulipa hybrida*, respectively.

In contrast with the other monocot orders mentioned, data obtained on species of the Asparagales revealed divergences within the group, with *Agapanthus praecox* apparently conforming to the modified ABC model (Nakamura *et al.*, 2005) and *Asparagus officinalis* not (Park *et al.*, 2003, 2004). This is particularly surprising given that the flowers of *A. officinalis* display two whorls of almost

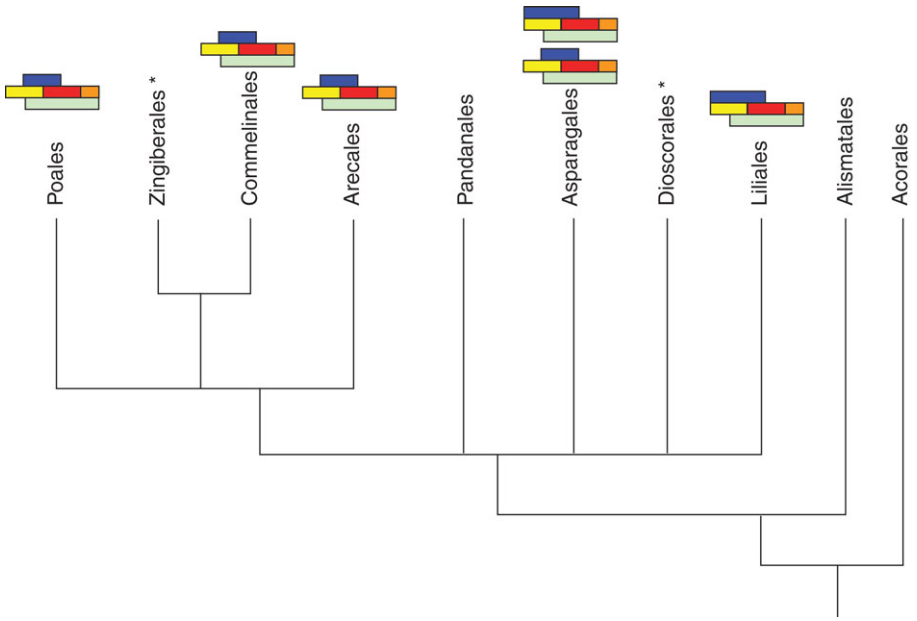

FIG. 5. Summary of MADS box structural and functional data obtained for monocot orders. The characterization of species putatively following the generic ABCDE model or 'modified ABC model' (involving B function in the outer perianth) is indicated where data are available. Orders for which sequences are available but not functional, data are indicated by an asterisk. Note that the tree (not to scale) is for illustrative purposes only and is based on the topology indicated by Savolainen and Chase (2003), which is undergoing revision.

identical petaloid organs in their perianth; thus it could be hypothesized that a divergent signalling pathway for petaloid tepal specification exists in this species. Nevertheless, as pointed out by Kramer and Jaramillo (2005), some caution should be exercised when comparing data between the different species, since the developmental stages studied were not always the same and in the case of *L. longiflorum*, only a single late stage was investigated.

Although the data available on ABC gene function in monocots are as yet fragmentary, one general trend which can be observed in Fig. 5 is the conservation of a molecular distinction (in the form of B gene expression) between the two perianth whorls within the Commelinid group, which includes palms. In the specific case of oil palm, in which this molecular distinction is not accompanied by clear morphological differences, it can be hypothesized that additional signalling mechanisms have evolved in organ identity determination, as suggested also for *A. officinalis* (Park *et al.*, 2003, 2004). Outside the Commelinid group, the modified ABC model might well be a characteristic feature of the Liliales, but this appears not to be the case for the Asparagales, in which both types of ABC scenario can be found.

When comparing B gene data for the different monocot clades, it is interesting to note that the presence of multiple *GLO* (but not *DEF*) genes in many cases. In addition to the cases of *E. guineensis* (Arecales) and *Tradescantia reflexa* (Commelinales) mentioned previously, other examples include: the Burmese fishtail palm *Caryota mitis* (Arecales; Genbank accessions AAY56602 and 56603); rice, maize and wheat (Poales; Chung *et al.*, 1995; Münster *et al.*, 2001; Hama *et al.*, 2004), *Asparagus officinalis* (Asparagales; Park *et al.*, 2004), the Martagon lily *Lilium martagon* (Liliales; Genbank accessions AAY56593 and AAY56594) and the ornamental banana *Musa ornata* (Zingiberales; Genbank accessions AAY56605 and AAY56606). This contrasts with dicots, in which often only a single *GLO* gene is observed and suggests that a *GLO* gene duplication occurred early in the evolution of the monocots (Zahn *et al.*, 2005). Such an event is likely to have been followed by functional diversification, which might account for the unexpected expression patterns of certain *GLO* genes in vegetative tissues of both monocots and dicots (Southerton *et al.*, 1998; Münster *et al.*, 2001; Skipper, 2002; Kanno *et al.*, 2003). In the case of oil palm, *EgGLO2* (but not *EgGLO1*) transcripts were detected in roots (Adam *et al.*, 2006).

In summary, the very limited data available provide evidence for an overall conservation of the generic ABC model within the Commelinid clade. In contrast, the modified ABC model can be applied to species in the more phylogenetically distant Liliales, while in the case of the Asparagales, both types of scenario can be found. It would clearly be of great interest to know which of the two models described (if either) represents the plesiomorphic character of the monocots and indeed of the angiosperms. The resolution of this question is complicated by the great diversity of flower structure which exists amongst basal angiosperms and the fact that the calyx (and possibly even the corolla) is generally considered to have arisen independently several different times during evolution (Zanis *et al.*, 2003). This question and others need to be addressed by wider and deeper sampling of molecular data amongst monocots, so as to obtain a better evolutionary perspective and to identify new gene orthologues and paralogues. It should be borne in mind that at least some floral bauplan

diversity in monocots may not be attributable to the functioning of ABC MADS box genes. An interesting illustration of this point is the lodicule of the grass flower, which is considered to be a floral morphological innovation and which expresses B-class MADS box genes despite its non-petaloid characteristics (Kramer and Jaramillo, 2005). No doubt novel floral gene functions will also have arisen during the evolution of the palm family.

On a more general level, the sequence and functional data described here for oil palm should help to shed further light on the occurrence of duplication, neofunctionalization and subfunctionalization amongst MADS box genes during the evolution of the monocots. In the absence of a complete sequence of the oil palm genome, the current picture is inevitably fragmented; nevertheless, our data tend to confirm trends observed in other species. Apart from the example of *GLO* gene duplication mentioned above, another subfamily in which gene duplication and functional diversification is likely to be prevalent is the *AGL2* group. Indeed, five different oil palm genes of this clade have been identified and found to display divergent expression patterns (Adam *et al.*, 2006, 2007), a situation similar to that occurring in other monocots such as rice (Malcomber and Kellogg, 2005). Given that the expression of all five oil palm *AGL2* genes characterized is specific to the inflorescence, their functions are likely to be restricted to the development of the reproductive structures of the plant, as is typical but not universal in this MADS box subfamily.

One aspect of reproductive development in oil palm upon which the current data do not shed light is sex determination, none of the oil palm MADS box genes described having been found to display a sex-dependent expression pattern. Given that there appears to be no common mechanism for sex determination in higher plants (Ainsworth, 2000), it is impossible to speculate on the nature of the genes involved in this process in oil palm, although it is interesting to note that sex-dependent expression of MADS box genes has been observed in some dioecious plants such as *Rumex acetosa* (Ainsworth *et al.*, 1995).

CONCLUSIONS

MADS box genes probably lie at the heart of many key evolutionary events in plants through the fundamental role which they play in the regulation of reproductive development in general and floral structure in particular. Our data on the structure, expression and functional analysis of oil palm MADS box genes help to fill in a gap in existing knowledge and will allow the palm family to be compared and contrasted with other groups which have traditionally received more attention in this field. It is hoped that in future years, new information will be gathered from other members of the Arecaceae. This should help in the long term to provide an insight into the regulatory processes which underlie the rich structural diversity seen within the family.

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