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RESEARCH PAPER

Functional characterization of MADS box genes involved in the determination of oil palm flower structure

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Abstract

In order to study the molecular regulation of flower development in the monoecious species oil palm (*Elaeis guineensis*), cDNAs of 12 MADS box genes from this plant belonging to seven distinct subfamilies were previously isolated and characterized. Here studies carried out on five of these genes, each likely to be involved in floral morphogenesis: *EgSQUA1* (*SQUAMOSA* subfamily); *EgAGL2-1* (*AGL2* subfamily); *EgGLO2* (*GLOBOSA* subfamily); *EgDEF1* (*DEFICIENS* subfamily); and *EgAG2* (*AGAMOUS* subfamily), are described. In order to determine where and when in the plant these genes are likely to function, their spatial and temporal patterns of expression were studied during the development of male and female inflorescences, either of normal phenotype or displaying a homeotic flowering abnormality known as *mantled*. In parallel, the phenotypic effects of ectopically expressing these genes in transgenic *Arabidopsis thaliana* plants were analysed. The data suggest a broad conservation of floral homeotic gene functions between oil palm and previously described model species, although a few minor variations in the zones of activity of certain genes cannot be excluded. The data also indicate distinct molecular identities for the morphologically similar floral organs of whorls 1 and 2. They also reveal reduced expression of putative B, C/D, and E class genes in *mantled* flowers, which undergo a homeotic transformation comparable to B class mutants of model species.

Key words: ABCDE model, flower, homeotic, MADS box, *mantled*, oil palm.

Introduction

The mechanisms which govern the morphology of flowers in higher plants have been extensively studied in recent years. In the generic hermaphrodite angiosperm flower, floral organs are arranged in a series of concentric whorls containing sepals, petals, stamens, and carpels. Many variations to this basic structure exist; however, a large body of data has revealed that the regulation of flower morphology works along similar lines in a wide range of higher plant species. Floral organ identity determination can be explained by the ABCDE model (Coen and Meyerowitz, 1991; Colombo *et al*., 1995; Pelaz *et al*., 2000; Honma and Goto, 2001) with variations to the basic scheme applying to specific plant groups (Theissen *et al*., 2000). According to the generic ABCDE model, the expression of class A genes specifies sepal formation in the calyx. The combination of class A, B, and E gene activities specifies the formation of petals in the corolla. In a similar way, class B, C, and E gene expression specifies stamen formation in the androecium, while class C and E gene activities determine the formation of carpels in the gynoecium. Additionally, class C, D, and E genes determine ovule identity. With the exception of some class A genes such as *APETALA2* from *Arabidopsis thaliana*, all floral organ identity genes characterized to date code for proteins of the MADS box transcription factor family displaying a typical MIKC modular structure. Molecular studies have revealed interesting parallels between the phylogeny of the MADS box gene family and the evolution of land plants (Purugganan *et al*., 1995; Theissen *et al*., 1996, 2000; Alvarez-Buylla *et al*., 2000; Lamb and Irish, 2003). The known A, B, C, D, and E class genes fall into separate clades on the basis of their sequence similarities, namely those containing the *SQUAMOSA* (class

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Abbreviations: PBS, phosphate-buffered saline; RT-PCR, reverse transcription-polymerase chain reaction.

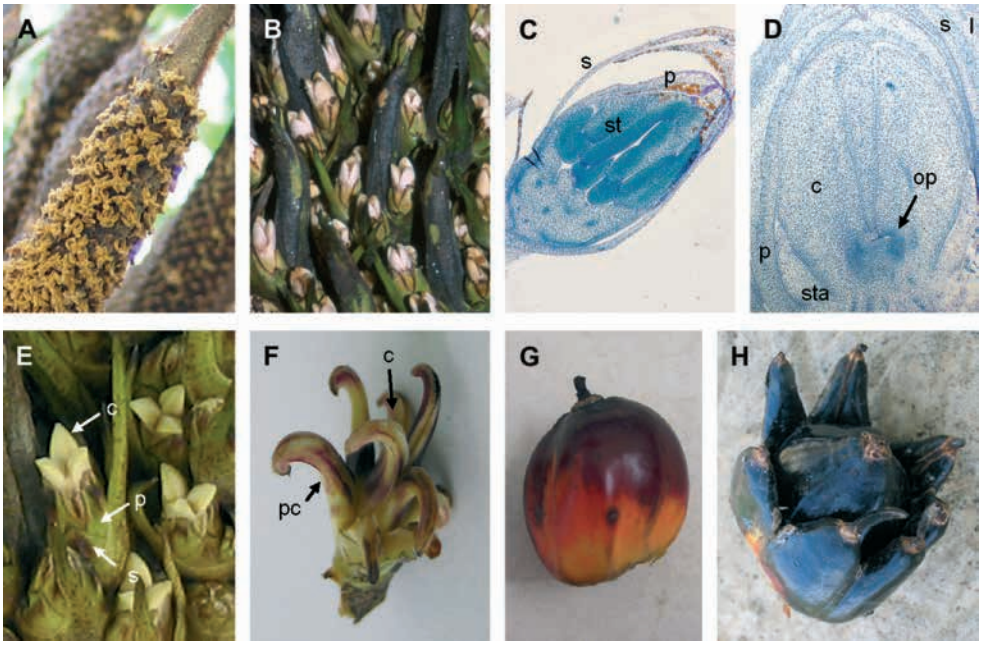

Fig. 1. Pistillate and staminate flowers of wild-type and *mantled* oil palm. Macroscopic view of staminate (A) and pistillate (B) flowers on male and female rachillae, respectively, at maturity. Microscopic longitudinal sections of wild-type staminate (C) and pistillate (D) flowers. Macroscopic view of pistillate flowers from wild-type (E) and *mantled* (F) rachillae. The *mantled* pistillate flower has been dissected to show only the gynoecium. Macroscopic view of wild-type (G) and *mantled* (H) fruits. c, Carpel; op, ovule primordia; p, petals; pc, pseudo-carpels, sepals; st, stamens; sta, staminode.

A), *DEFICIENS* (class B), *GLOBOSA* (class B), *AGAMOUS* (classes C and D), and *AGL2*-like (class E) genes, as defined by Becker and Theissen (2003).

Since its conception, the ABCDE model has been shown to be widely applicable amongst dicot species (Pnueli *et al*., 1994; Kater *et al*., 1998, 2001; Berbel *et al*., 2001; Immink *et al*., 2003). In more recent years, homologues of the dicot ABCDE MADS box genes have also been identified in a range of monocot species (Mena *et al*., 1995; Ambrose *et al*., 2000; Jeon *et al*., 2000; Fornara *et al*., 2003; Xiao *et al*., 2003). Overall, less is known about the function of floral MADS box genes in monocots. It should also be noted that the majority of functional MADS box gene studies in monocots to date concern the grass family (Poaceae). Whilst some MADS box gene studies have been described in the literature for species of the Asparagales, Liliales, and Commelinales groups, none has as yet been reported for the other monocot orders. One of the most notable gaps concerns the palm family (Arecaceae), which forms the order Arecales, a group of great economic significance, particularly in tropical countries, consisting of 187 recognized genera (Dransfield *et al*., 2005). One of the most agriculturally important species of this group is the African oil palm (*Elaeis guineensis*), a plant cultivated extensively in inter-tropical regions of Asia, Africa, and Latin America. Here the first reported study of floral MADS box gene function in this species is presented, which should serve as a useful basis for understanding how the flower structure determination of palms fits in with those of other monocot groups.

The inflorescences of oil palm are compound panicles consisting of a central rachis which gives rise to branches known as rachillae. The oil palm normally produces in alternation male and female inflorescences carrying unisexual flowers (Fig. 1A, B). The staminate flower consists of a perianth surrounding an androecium of six stamens (in two concentric whorls of three) which themselves enclose a rudimentary gynoecium (Fig. 1C). The perianth consists of a calyx and a corolla of three sepals and three petals, respectively, sometimes collectively referred to as tepals. The female inflorescence produces floral triads each consisting of two accompanying male flowers flanking a single pistillate flower. Unlike functional staminate flowers, the accompanying male flowers do not develop sufficiently to produce pollen. The structure of pistillate flowers is similar to that of staminate flowers except that the perianth encloses a rudimentary androecium of usually six staminodes, themselves surrounding a tricarpellate gynoecium (Fig. 1D). In the staminate flower, petals and sepals display some subtle differences in their appearance whereas in pistillate flowers, which are considered to be homochlamydeous, they show a close resemblance.

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 (Fig. 1E, F; 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 characteristic fertile or parthenocarpic fruits may be produced in less severe cases (Fig. 1G, H). The *mantled* phenotype is observed in oil palms regenerated from tissue culture (Corley *et al*., 1986) and may be transmitted by genetic crossing (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. It has been proposed that the mechanism involved might involve differential genomic DNA methylation (Jaligot *et al*., 2000).

A structural characterization of oil palm MADS box cDNAs and their encoded proteins was carried out previously (Adam *et al*., 2006). Twelve oil palm cDNAs identified in this way were revealed by phylogenetic reconstructions to belong to five different MADS box gene subfamilies of known importance in flower structure determination, namely the *SQUAMOSA* (*SQUA*), *DEFICIENS* (*DEF*), *GLOBOSA* (*GLO*), *AGAMOUS* (*AG*), and *AGL2* groups described by Becker and Theissen (2003). The broad aim of the work described herein was to identify putative functions for the genes in question. The investigation of floral gene function in oil palm is complicated by its large size and long life cycle, the production of functional flowers starting only 3 years after germination. On the basis of their sequence similarities and expression profiles, five different oil palm MADS box genes likely to be involved in floral morphogenesis were selected for detailed analyses. A study of the temporal and spatial expression of these five MADS box genes in oil palm and an investigation of their phenotypic effects when ectopically expressed in transgenic *A. thaliana* plants are described here. In parallel with the transgenic approach, a comparative study of *mantled* and wild-type material was performed, which helped provide an additional insight into the molecular regulation of petal and stamen/staminode development. Taken together, the results allow a model of floral organ identity control for oil palm to be proposed, which resembles the generic ABCDE dicot model, with the possibility of some minor divergences.

Materials and methods

Plant material

Inflorescence material of seed-derived (Deli×La Mé origin) and regenerant (FC166 clonal lines) oil palms used for the reverse transcription-polymerase chain reaction (RT-PCR) study was harvested at the Tun Razak plantation, Malaysia, with the kind permission and help of FELDA Agricultural Services Sdn Bhd. Root and leaf material were harvested from seed-derived greenhouse plants grown in Montpellier.

Plant material used for *in situ* hybridization (Deli×Avros origin) was harvested at the Coto plantation, Costa Rica, with the kind permission and help of the ASD company. Additional material was harvested in Malaysia as described above.

RT-PCR

Total RNA was isolated from different tissues of oil palm according to Corre *et al*. (1996). First-strand cDNA was prepared using an oligo(dT) primer and 2 μg of RNA in conjunction with a SuperScript first strand kit (Invitrogen). The primer pairs used and conditions for RT-PCR amplification are shown in Supplementary Table S1 at *JXB* online. The primers of the control elongation factor gene *EgEF1-α1* (accession no. AY550990) were designed around an intron sequence to test for the presence of genomic DNA contamination in the reverse transcription reaction. Amplified cDNAs were cloned and sequenced to check the specificity of the amplification products.

Total RNA of *A. thaliana* flowers was extracted using the RNeasy kit (Qiagen). First-strand cDNA was prepared using oligo(dT) as primer and 0.7 μg of RNA in conjunction with a SuperScript first strand kit (Invitrogen). The primer pairs used and conditions for RT-PCR amplification are shown in Supplementary Table S2 at *JXB* online.

RNA in situ *hybridization*

DNA substrates for riboprobe synthesis were PCR products obtained with a 5′ primer and a 3′ primer, containing sequences detailed in Supplementary Table S1 at *JXB* online, plus the T7 RNA polymerase initiation site. Primers used are shown in Supplementary Table S3 at *JXB* online. The resulting fragments were used as templates for synthesizing antisense digoxigenin-labelled riboprobes employing UTP-DIG (Roche) and a T7 Maxi Script kit (Ambion). Each amplification product was tested by Southern blotting to check the specificity of the probe (data not shown).

Flower material was fixed for 8 h at room temperature in fixation buffer (4% paraformaldehyde, 0.1 M phosphate buffer pH 7), dehydrated through a graded series of ethanol and butanol dilutions, embedded in Paraplast plus (Paraplast X-Tra, Oxford Labware) and sectioned to 7 μm with a microtome. Tissue sections were deparaffinized with Safesol (LaboNord, France), rehydrated through an ethanol series, and then pre-treated with proteinase K (0.1 U ml^{-1}) in TRIS-HCl pH 7.5 at 37 °C for 15 min. Digestion was stopped by washing with 0.2× phosphate-buffered saline (PBS)/glycine and then twice with PBS for 2 min each. After dehydrating in ethanol baths, hybridization was performed at 42 °C overnight with 0.2 μg ml^{-1} of the digoxigenin-labelled RNA probe in hybridization solution (50% formamide, 2× SSC, 4× Denhardt, 20% dextran, 2 mg ml^{-1} tRNA). After hybridization, slides were washed in 2× SSC at 50 °C for 45 min and twice in 1× NTE (TRIS-HCl 1 mM, NaCl 0.5 M, EDTA 1 mM, pH 7.5) at 37 °C for 2 min each. An RNase A digestion (20 μg ml^{-1} in 1× NTE) was carried out for 30 min at 37 °C and stopped by washing with 1× NTE at 37 °C. Final washes were conducted in 2× SSC, 1× SSC, and 0.5× SSC for 15 min each at 50 °C prior to rinsing in PBS. For signal detection, samples were incubated in blocking reagent [Roche; 10% (w/v) in PBS] for 1 h and afterwards for 30 min in blocking reagent containing anti-digoxigenin alkaline phosphatase-conjugated Fab fragment antibody (Roche) diluted at 1:500. After three washes for 10 min in PBS, tissues were equilibrated in detection buffer (100 mM TRIS-HCl pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$) for 15 min prior to incubating in the same buffer supplemented with 0.2 mM nitroblue tetrazolium (NBT) and 0.2 mM bromochloroindolyl phosphate (BCIP) substrates.

Plant transformation and transgenic plant analysis

The complete open reading frames of *EgSQUA1*, *EgDEF1*, *EgGLO2*, *EgAGL2-1*, and *EgAG2* were each generated by PCR using a 5′ primer containing a restriction enzyme site and a reverse primer designed after the stop codon with a restriction site. The

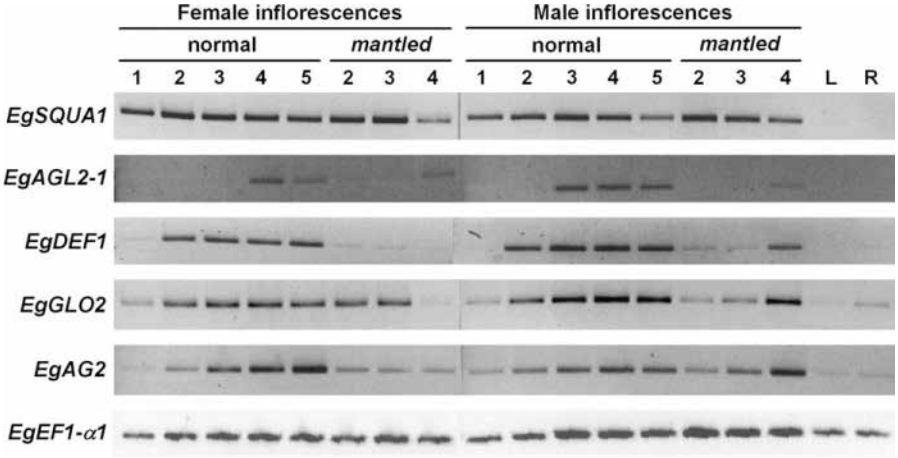

Fig. 2. Analysis of the expression patterns of oil palm MADS box genes. RT–PCR analysis of MADS box gene transcript accumulation during the development of oil palm normal and *mantled* female and male inflorescences, and in leaves (L) and roots (R). Numbers correspond to different stages of inflorescence development. Stage 1 corresponds to the floral meristem stage. 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 the mature flower. The names of genes are indicated on the left.

PCR fragment was placed downstream of the cauliflower mosaic virus 35S promoter in the 35S-2 plasmid (see http://www.pGreen.ac.uk) and the expression cassette was subsequently cloned into the pGreen binary vectors (Hellens *et al*., 2000).

Arabidopsis thaliana plants of the ecotype Columbia were transformed using the floral dip method (Clough and Bent, 1998), and the *Agrobacterium tumefaciens* C58C1 strain was used for the transformation. Transformants that survived in the medium containing phosphinotricine were checked for transgene insertions by PCR. The plants were grown at 22 °C under short day conditions.

Results

Temporal and spatial expression patterns of oil palm MADS box genes during wild-type flower development

A structural characterization of 12 oil palm MADS box cDNAs belonging to the *SQUAMOSA* (*SQUA*), *DEFICIENS* (*DEF*), *GLOBOSA* (*GLO*), *AGAMOUS* (*AG*), and *AGL2* subfamilies was described previously (Adam *et al*., 2006). In the study presented here, a detailed analysis of the expression of five of these genes, chosen on the basis of their sequences and expression patterns, was carried out. The five genes were: *EgSQUA1* (*SQUA* subfamily); *EgGLO2* (*GLO* subfamily); *EgDEF1* (*DEF* subfamily); *EgAG2* (*AG* subfamily); and *EgAGL2-1* (*AGL2* subfamily) (see Supplementary Fig. S1 at *JXB* online). The expression pattern of each MADS box gene was characterized by gene-specific semi-quantitative RT–PCR using rachillae of normal and *mantled* oil palm inflorescences at five different developmental stages plus leaves and roots (Fig. 2). The key features of each developmental stage studied are described in detail elsewhere (Adam *et al*., 2005) and reference microscopic sections are shown in Supplementary Fig. S2 at *JXB* online. The spatial expression pattern of each of the five chosen genes was studied by *in situ* hybridization on longitudinal sections of pistillate and staminate flowers (Fig. 3). *In situ* hybridization was also performed in the same way on floral triads of *mantled* female inflorescences and on the rachilla meristem for some of the genes characterized (Fig. 3).

SQUA subfamily: The *EgSQUA1*, *EgSQUA2*, and *EgSQUA3* genes belong to the *SQUAMOSA* subfamily and display similar expression patterns (see Supplementary Fig. S3 at *JXB* online). The focus in the present study was on *EgSQUA1* which is more related to *FRUITFULL*-like genes of the *SQUA* subfamily (Adam *et al*., 2006; see Supplementary Fig. S1 at *JXB* online). RT-PCR analysis revealed the presence of *EgSQUA1* transcripts in the inflorescence, rachilla, and floral meristems, but not, in contrast to *EgSQUA2* and *EgSQUA3*, in the vegetative shoot meristem (see Supplementary Fig. S3 at *JXB* online). Thus, its function appears to be restricted to reproductive development. *EgSQUA1* transcript abundance is relatively constant during the development of pistillate and staminate flowers; however, no signal was detected in either leaves or roots (Fig. 2).

The histo-cytological localization of *EgSQUA1* transcripts was studied on several different types of material by *in situ* hybridization (Fig. 3). In longitudinal sections of the developing rachis, a signal was detected in cells of the outer layers of the rachilla meristem (Fig. 3A). *In situ* hybridization performed on pistillate and staminate flowers at different stages of development revealed a localization of *EgSQUA1* transcripts concentrated in the central dividing zone of floral primordia in the case of the pistillate flower (Fig. 3B). At stage 3 of development in the staminate flower, *EgSQUA1* was observed to be expressed in the cells of stamen primordia and weakly in sepals and

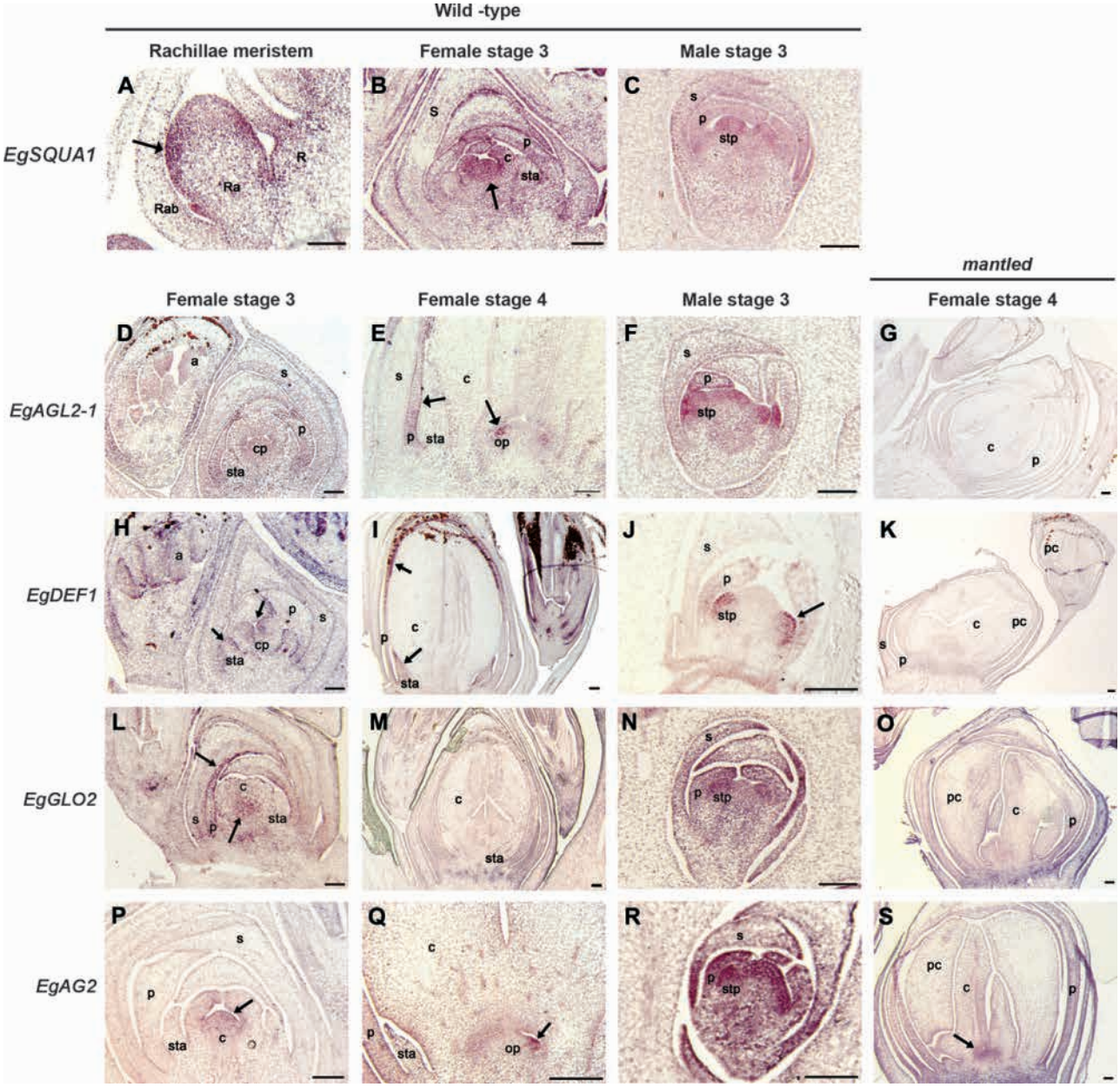

Fig. 3. *In situ* localization of *EgSQUA1*, *EgAGL2-1*, *EgDEF1*, *EgGLO2*, and *EgAG2* transcripts in wild-type staminate and pistillate flowers, and in *mantled* pistillate flowers of oil palm. Longitudinal sections were hybridized with digoxigenin-labelled antisense RNA fragments of *EgSQUA1* (A, B, C), *EgAGL2-1* (D, E, F, G), *EgDEF1* (H, I, J, K), *EgGLO2* (L, M, N, O), and *EgAG2* (P, Q, R, S). (A) *EgSQUA1* expression in rachilla meristem. (B, D, H, L, P) *EgSQUA1*, *EgAGL2-1*, *EgDEF1*, *EgGLO2*, and *EgAG2* expression in pistillate flowers in which reproductive organs are initiated. (E, I, M, Q) *EgAGL2-1*, *EgDEF1*, *EgGLO2*, and *EgAG2* expression in mature pistillate flower. (C, F, J, N, R) *EgSQUA1*, *EgAGL2-1*, *EgDEF1*, *EgGLO2*, and *EgAG2* expression in staminate flowers in which stamen primodia are initiated. (G, K, O, S) *EgAGL2-1*, *EgDEF1*, *EgGLO2*, and *EgAG2* expression in *mantled* pistillate flowers. a, Anther; c, carpel; cp, carpel primodium; op, ovary primordium; p, petals; pc, pseudocarpel; R, rachis; Ra, rachillae; Rab, rachilla bracts; s, sepals; sta, staminode; stp, stamen primordium. Bar lengths: 0.1 mm.

petals (Fig. 3C). Overall, it appears that the oil palm *EgSQUA1* gene is expressed at a generally constant level during inflorescence development, with no noticeable changes occurring at the stages associated with floral organ initiation and differentiation. The RT-PCR and *in situ* hybridization data obtained suggest that this gene might play a role in determining the meristematic character of cells during inflorescence and flower development, with a possible additional function in the later phases of development of sepal and petals.

AGL2 subfamily: For the detailed studies reported here, it was decided to focus attention on *EgAGL2-1*, which belongs to the gene clade represented by the *A. thaliana SEPALLATA3* (*SEP3*) E function gene (Pelaz *et al.*, 2000; Honma and Goto, 2001; see Supplementary Fig. S1 at

JXB online). RT-PCR analysis revealed that *EgAGL2-1* is expressed during the late stages of floral development, transcripts being detected from stage 4 onwards in the female inflorescence and from stage 3 onwards in the male inflorescence (Fig. 2).

In situ hybridization studies performed on pistillate flowers revealed a weak accumulation of *EgAGL2-1* transcripts principally at the base of petals at stage 3 (Fig. 3D). Once all organs of pistillate flowers were in place, expression was observed to be concentrated in the cells of petals and in ovule primordia (Fig. 3E). In stage 3 staminate flowers, *EgAGL2-1* transcripts were seen to be localized in petals and in the primordia of stamens (Fig. 3F).

In conclusion, the spatial pattern of expression revealed for *EgAGL2-1* suggests that this gene might be involved in the development of petals and ovule primordia in pistillate flowers, and in stamen and perianth development in the staminate flower.

DEF subfamily: One gene belonging to the DEFICIENS subfamily, named *EgDEF1*, was identified and characterized in oil palm. *EgDEF1* transcripts were not detected at the floral meristem stage in female and male inflorescences (Fig. 2), but were found to be present in similar quantities at all subsequent stages studied. *EgDEF1* transcripts were not detected in leaves or roots.

In situ hybridization revealed a weak signal in staminodal cells and at the centre of the carpel (Fig. 3H). In the pistillate flower displaying developed reproductive organs, *EgDEF1* transcripts were detected mostly in staminodes and in the distal regions of petals (Fig. 3I). Accompanying male flowers also displayed an accumulation of *EgDEF1* transcripts in petal cells and in stamens. In stage 3 staminate flowers, an intense signal was observed in the primordia of stamens (Fig. 3J). Overall, the transcript accumulation pattern of *EgDEF1* in pistillate and staminate flowers suggests a role for this gene in the determination of the identity of the inner perianth whorl and stamens as seen for genes belonging to the *DEF* subfamily in other species.

GLO subfamily: Two closely related genes belonging to the *GLOBOSA* subfamily, named *EgGLO1* and *EgGLO2*, were identified in oil palm. Their closely related sequences (Adam *et al*., 2006) and qualitatively identical expression patterns (see Supplementary Fig. S3 at *JXB* online) suggest that they are young paralogues. In the present study attention was focused on the expression and function of *EgGLO2*. A progressive accumulation of *EgGLO2* transcripts was observed during the development of the female and male inflorescences. Transcripts were only barely detected at stage 1 (Fig. 2), a weak signal also being observed in roots.

In situ hybridization studies revealed that *EgGLO2* transcripts were mostly localized in sepals, petals, and in the floral meristem in stage 3 pistillate flowers (Fig. 3L). In stage 4 pistillate flowers, *EgGLO2* transcripts were only weakly detected, principally in whorls 1 and 2 (Fig. 3M). In staminate flowers, *EgGLO2* transcripts were found to be localized in the organs of all whorls at stage 3 (Fig. 3N).

Overall, the expression of *EgGLO2* seems to be mainly localized in sepals, petals, and staminodes or stamens of pistillate and staminate flowers, respectively, at early stages of development, whereas at later stages, transcripts are detected mostly in the perianth. It is doubtful that the presence of *EgGLO2* transcripts in sepals signifies a homeotic activity for this gene in these organs, since this would require the production of not only a functional EgGLO2 protein, but also its putative heterodimeric partner EgDEF1. Given the apparent lack of expression of *EgDEF1* in sepals, this scenario seems unlikely. On the basis of currently available data, the most plausible role for *EgGLO2* appears to be, like many of its homologues, in the determination of the identity of the inner perianth and androecium. Nevertheless, it is interesting to note that *GLO* gene transcript accumulation in whorl 1 has already been reported for other monocot species such as tulip (Liliales; Kanno *et al*., 2003) and *Tradescantia reflexa* (Commelinales; Ochiai *et al*., 2004), although in the former case this was to be expected since in tulip flowers the outer perianth whorl is petaloid, with *DEF* gene expression also being detected.

AG subfamily: The *EgAG1* and *EgAG2* genes belong to the *AGAMOUS* subfamily and are similar in both their sequence and expression pattern (see Supplementary Figs S1 and S3 at *JXB* online), suggesting that, like *EgGLO1* and *EgGLO2*, they are young paralogues. Attention was focused on *EgAG2*, for which transcripts were weakly detected in roots and leaves, progressively increasing amounts being observed during female and male inflorescence development (Fig. 2).

In situ hybridization experiments showed that *EgAG2* transcripts were localized mostly in the cells of carpel primordia at stage 3 of pistillate flower development (Fig. 3P). Subsequently at stage 4, *EgAG2* transcripts were most strongly detected in cells of ovule primordia (Fig. 3Q). In the staminate flower at stage 3, *EgAG2* transcripts were observed to accumulate in each of the four whorls (Fig. 3R). Overall, the spatial expression pattern of *EgAG2* suggests a function comparable with that of C or D class genes as observed for genes of this subfamily in other species.

Analysis of gene expression patterns associated with the mantled *phenotype*

RT-PCR analysis revealed that the expression of several oil palm MADS box genes was perturbed in *mantled* palms (Fig. 2). The *mantled* phenotype may be identified morphologically from stage 4 (initiation of androecium and

gynoecium organs) onwards in pistillate and staminate flowers. No difference in the accumulation of *EgSQUA1* transcripts was observed between normal and *mantled* material. In contrast, transcripts of *EgAGL2-1* were found to be accumulated in smaller quantities in male and female inflorescences displaying the *mantled* phenotype. The most marked case of perturbed expression in *mantled* palms was that of *EgDEF1*, transcripts of this gene accumulating in smaller amounts at all stages studied for the female inflorescence. However, in the *mantled* male inflorescence, *EgDEF1* transcripts were less abundant only at stages 2 and 3 compared with the same stage of development in the wild type. For *EgGLO2*, a pattern of reduced expression in *mantled* palms was observed, at stage 4 for the female inflorescence and at stages 2 and 3 for the *mantled* male inflorescence. In the case of *EgAG2*, a *mantled*-dependent profile of expression was observed only in the female inflorescence, for which reduced transcript abundance was seen at stages 3 and 4.

In order to characterize the spatial pattern of *EgAGL2-1*, *EgDEF1*, *EgGLO2*, and *EgAG2* expression in *mantled* plants, *in situ* hybridization was performed on longitudinal sections of *mantled* floral triads at stage 4, which display the formation of pseudocarpels in the androecium of both pistillate and accompanying male flowers (Fig. 3; see Supplementary Fig. S4 at *JXB* online). The question of whether the *mantled* phenotype also involves a transformation of petals into sepals as seen in class B mutants was unresolved at this point, owing to the close physical resemblance of petals and sepals in oil palm. In experiments performed with *EgAGL2-1* and *EgDEF1* probes, no hybridization signal was observed either in petals or in the androecium, in contrast to the situation seen in normal staminate and pistillate flowers (Fig. 3G, K; see Supplementary Fig. S4 at *JXB* online). Similar experiments performed with an *EgGLO2* probe enabled the identification of a hybridization signal localized mostly in whorls 1 and 2 of *mantled* pistillate flowers (Fig. 3O; see Supplementary Fig. S3 at *JXB* online). Finally, *in situ* hybridization analysis of *EgAG2* expression revealed a concentration of transcripts of this gene, as for wild-type pistillate flowers, in the ovule primordia of whorl 4. No significant signal was seen in the pseudocarpels, which do not form ovules (see Supplementary Fig. S3 at *JXB* online).

Taken together, these data indicate that in *mantled* plants, the accumulation of transcripts of several genes belonging to the *DEF*, *GLO*, *AGL2*, and *AG* subfamilies is reduced. This suggests that these genes might be involved in the determination of organ identity in the androecium and also possibly in the inner perianth. Indeed the *in situ* hybridization data obtained revealed differences in gene expression between normal and *mantled* flowers, not only for the androecium but also for whorl 2 organs, suggesting a homeotic transformation of both as seen in the class B mutants of model plants. For the genes belonging to *DEF* and *GLO* subfamilies, transcript accumulation is perturbed in distinctly different developmental patterns in male and female inflorescences of the *mantled* phenotype, suggesting that the temporal regulation of these genes is different in pistillate and staminate flowers.

Analysis of oil palm MADS box gene function by ectopic expression in A. thaliana

To investigate the biological functions of the proteins encoded by the oil palm MADS box genes, transgenic plants ectopically expressing *EgSQUA1*, *EgDEF1*, *EgAG2*, *EgGLO2*, and *EgAGL2-1* were generated. For the genes *EgAG2* and *EgDEF1*, a total of 44 and 61 independent transgenic lines, respectively, were obtained. However, none of the transformants obtained in these two cases was observed to display phenotypic alterations compared with wild-type plants, either in flowers or in any other organs or tissues of the plant. In contrast, the results obtained with the transgenic lines overexpressing *EgSQUA1*, *EgGLO2*, and *EgAGL2-1* revealed changes in phenotype.

Heterologous expression of EgSQUA1 *in* A. thaliana *results in a tall phenotype*: Previous functional studies of *SQUAMOSA*-like genes showed that when overexpressed, they cause an early flowering phenotype (Mandel and Yanofsky, 1995; Berbel *et al*., 2001; Shchennikova *et al*., 2004). To investigate whether *EgSQUA1* has a similar biological effect, 25 independent transgenic lines overexpressing this gene were obtained. No significant effect on flowering time was observed; however, 17 plants were found to display a greater height than wild-type plants. Four independent transgenic lines were studied, and for each line, five T_2 plants were analysed (Table 1). All plants displayed the same tall phenotype. Various measurements were performed on the transgenic plants at the same defined time points. Measurements of the transgenic plants showed that they were on average 17 cm taller than the wild type (Table 1; Fig. 4A). This elongation was found to be due to a greater number of internodes

Table 1. *Analysis of height, number of internodes, pedicel length, and silique length in wild-type plants and T_2 35S*::EgSQUA1 *transgenic lines*

Average pedicel length corresponds to an average of average values obtained for each individual plant. Measurements were performed on four individual plants per line.

T_2 lines	Height (cm)	Number of internodes in reproductive phase	Length of pedicels in inflorescence (cm)	Length of silique (cm)
Wild type	46.2±4.8	25.0±6.1	0.8±0.6	0.97±0.14
T_2 no. 8-3	65.5±1.3	51.2±3.9	2.4±0.2	0.39±0.05
T_2 no. 8-1	65.0±3.1	44.0±5.2	2.3±0.2	0.39±0.07
T_2 no. 7-3	60.5±5.2	44.6±4.3	1.8±0.5	0.34±0.04
T_2 no. 7-1	67.7±3.3	47.8±6.4	2.3±0.3	ND

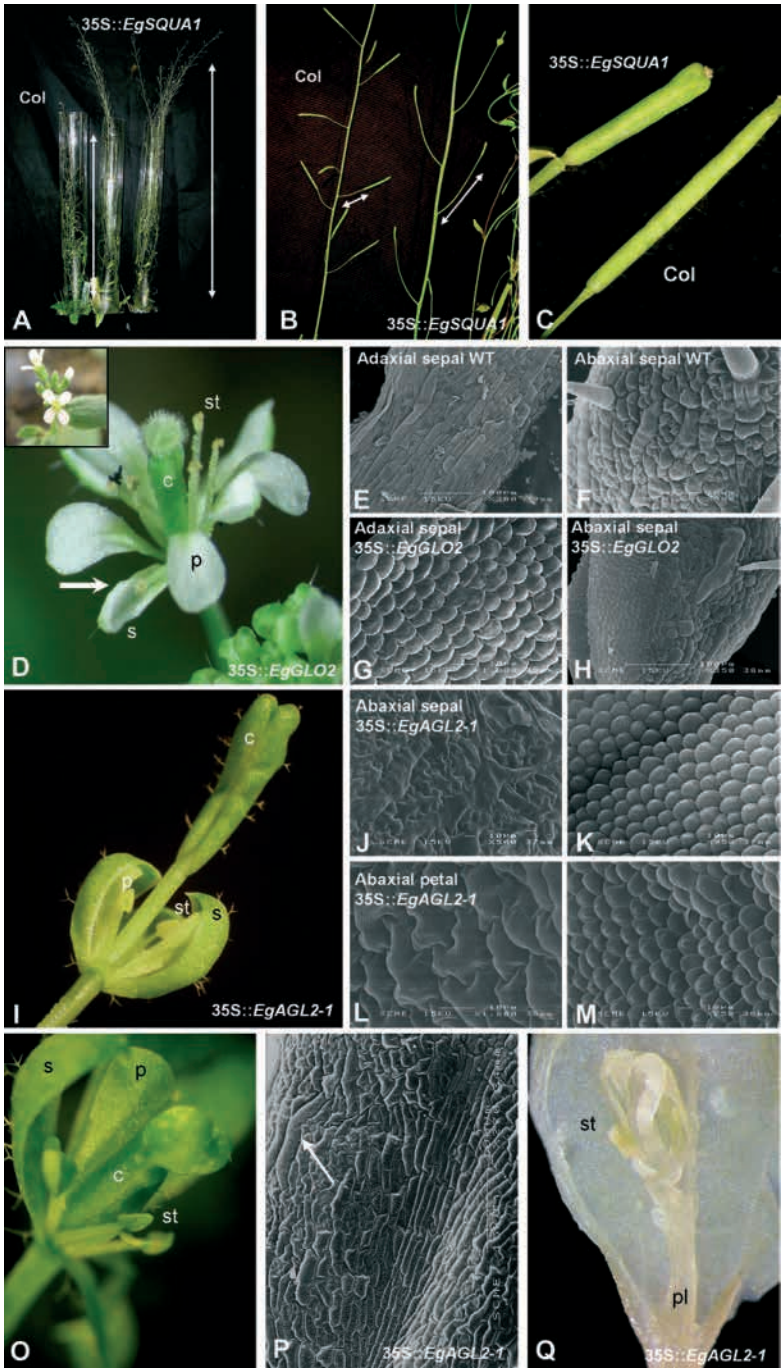

Fig. 4. Phenotypes of 35S::*EgSQUA1*, 35S::*EgGLO2*, and 35S::*EgAGL2-1 A. thaliana* transgenic lines. (A) A wild-type plant (left) and a 35S*::EgSQUA1* plant (right) of the same age. Note the difference in size of the transgenic plants (arrows). (B) Inflorescence branching in wild-type (left) and 35S::*EgSQUA1* (right) plants. Note the difference in size of the pedicel of flowers. (C) Silique of a wild-type (left) and a 35S::*EgSQUA1* (right) plant. (D) Flower of a 35S::*EgGLO2* plant. Sepals are partially transformed in petals. A wild-type flower is shown at the top left. (E) Scanning electron micrograph (SEM) of the adaxial surface of a wild-type sepal. The cells on the epidermis consist primarily of elongated rectangular cells. (F) SEM of the abaxial surface of a wild-type sepal. (G) SEM of the adaxial surface of a 35S::*EgGLO2* sepal. (H) SEM of the abaxial surface of a 35S::*EgGLO2* sepal. (I) Flower of a 35S::*EgAGL2-1* line. The flower has green petals, stamens are short, carpels are malformed and have branched stellate trichomes. (J) SEM of the abaxial surface of a 35S::*EgAGL2-1* sepal. (K) SEM of the adaxial surface of a wild-type petal. (L) SEM of the abaxial surface of a 35S::*EgAGL2-1* petal. (M) SEM of the adaxial surface of a 35S::*EgAGL2-1* petal. (O) Flower of a 35S::*EgAGL2-1* line showing deformed carpels without stigmas. (P) SEM of the gynoecium surface of a 35S::*EgAGL2-1* flower. The arrow shows a cell resembling those seen in sepals. (Q) Carpel of a 35S::*EgAGL2-1* line opened to reveal an additional flower with stamens and stigma contained alongside developing ovules. c, Carpel; p, petals; pl, placenta; s, sepals; st, stamen.

produced during the reproductive phase of the plant. Internode length was found to be unaffected. Transgenic plants carrying the 35S::*EgSQUA1* construction also displayed longer pedicels and shorter siliques in comparison with those of the wild type (Fig. 4B, C).

Overall, it can be seen that the heterologous expression of *EgSQUA1* in transgenic *A. thaliana* did not affect floral structure but rather the architecture of the plants, probably due to a modification of the meristematic activity of the plants during their reproductive phase.

Ectopic expression of EgGLO2 *in* A. thaliana *results in the same phenotype as 35S::*PI *plant lines*: Twenty-six independent transgenic lines that overexpressed *EgGLO2* were obtained. Six of these lines (31%) displayed homeotic transformation of floral organs, of the same type in each case. This involved a partial conversion of sepals to petals in whorl 1 (Fig. 4D). In the wild-type plant, the adaxial (inner) and abaxial (outer) epidermis of sepals were seen to contain elongated cells (Fig. 4E, F) whereas petals displayed rounded cells with prominent cuticular thickenings on their abaxial surface and ovoid cells on their adaxial surface (Fig. 4K). Examination of the flowers of 35S::*EgGLO2* lines revealed that whorl 1 contained mosaic organs with both sepal-like and petal-like tissues (Fig. 4G, H). The peripheral regions of the first whorl organs were found to be characteristic of petals, while cells in their central region appeared to be characteristic of sepals. The same type of homeotic conversion was observed in the case of ectopic expression of *PI* in *A. thaliana* (Krizek and Meyerowitz, 1996), suggesting that the EgGLO2 and PI proteins have similar biochemical specificities.

Ectopic expression of EgAGL2-1 *in* A. thaliana *results in modifications to the epidermis of sepals and petals and the production of a flower in the place of the ovule*: Twenty-nine independent transgenic lines that overexpressed *EgAGL2-1* were obtained. Six of these lines (20%) displayed the same floral alterations, namely homeotic transformation of sepals and petals, shorter stamens, and gynoecium malformation (Fig. 4I). The gynoecium was often seen to be supported by an elongated gynophore, to display malformed carpels, to lack a style, and to possess only a short stigma. Upon microscopic examination, floral whorls 1 and 2 of 35S::*EgAGL2-1* plants were found to be transformed into leaf-like organs. The abaxial epidermal cells of the sepals of transgenic flowers appeared to have a mixture of both leaf- and sepal-like characteristics (Fig. 4J). Whereas wild-type petals displayed an abundance of rounded cells with prominent cellular thickenings (Fig. 4K), the abaxial surface of petals of 35S::*EgAGL2-1* transgenic lines were found to consist almost entirely of irregular puzzle-shaped cells typical of those normally found on leaves (Fig. 4L). The adaxial surface of petals did not show homeotic transformation, although the cells displayed less cuticular thickening (Fig. 4M). Whereas the sepals from flowers of wild-type plants had simple unbranched trichomes on the abaxial surface and no trichomes were formed on the pistil, flowers of 35S::*EgAGL2-1* transgenic lines possessed sepals and carpels which displayed some branched stellate trichomes (Fig. 4I, 4N). In severe cases, the carpels of 35S::*EgAGL2-1* transgenic lines were completely malformed (Fig. 4N). Moreover, the epidermis of carpels displayed some elongated rectangular cells instead of smaller epidermal cells (Fig. 4O), although the puzzle-shaped cells characteristic of leaves were not seen. The development of the ovules of 35S::*EgAGL2-1* transgenic lines was studied. It was observed that some ovules were malformed with an undeveloped funiculus (data not shown). In several cases, flowers of 35S::*EgAGL2-1* plants displayed a loss of determinacy in their innermost whorl, witnessed by the production of a flower within a flower (Fig. 4P). The additional flower was found to be formed in the place of an ovule. The new flower was found to be composed of perianth organs, stamens, and organs similar to the stigma (Fig. 4P). In order to assess whether *A. thaliana* floral organ identity genes were affected by the ectopic expression of the oil palm *EgAGL2-1* gene, RT-PCR was performed on wild-type and transgenic flowers at two stages of development. The expression of the endogenous *A. thaliana* genes *AP1*, *SEP1*, *SEP2*, *SEP3*, *SEP4*, *AP3*, *PI*, and *AG* was found to be unaffected, in terms of transcript accumulation, in transgenic flowers (see Supplementary Fig. S5 at *JXB* online). It is interesting to note that the modified floral phenotype observed in 35S::*EgAGL2-1* plants has common features with that of the *sep1sep2sep3sep4* quadruple mutant of *A. thaliana* (Ditta *et al*., 2004), including the production of a flower within the carpel.

Overall, these results suggest that the EgAGL2-1 protein may act in a dominant negative manner to perturb the SEP regulatory pathway in transgenic *A. thaliana* plants.

Discussion

An in-depth characterization is described of five florally expressed MADS box genes in oil palm belonging to five different MADS box subfamilies, namely the *SQUA*, *DEF*, *GLO*, *AGL2*, and *AG* groups (Adam *et al*., 2006). The study included a detailed analysis of their expression during flower development in relation to sex and floral phenotype, and a functional analysis by means of genetic transformation of *A. thaliana*. Genetic transformation has been achieved for oil palm (Parveez *et al*., 2000); however, studies of floral gene function in transgenic oil palm would require many years and a large amount of space in order to yield exploitable results. On the other hand, by using ectopic expression in *A. thaliana* as a functional tool, it was possible to obtain information on the conservation of MADS box protein function between oil palm

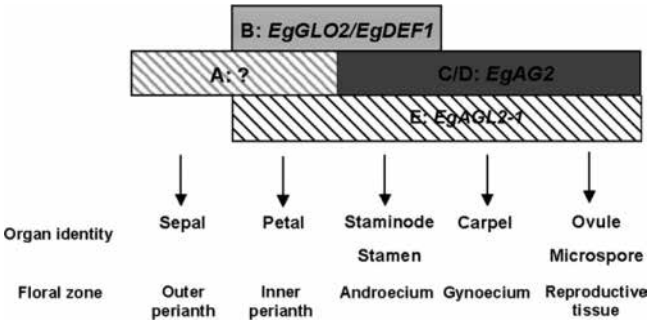

Fig. 5. Model to explain MADS box gene functions governing oil palm floral structure. Boxes represent the different functions defined according to the modified ABC model. Genes putatively associated with each function are indicated inside the corresponding boxes.

and its dicot counterpart. On the basis of the available data, a working model of homeotic gene activities regulating oil palm flower structure is proposed here (Fig. 5).

Meristem identity and A function

The main function of *SQUA*-like genes in higher plants appears to be the determination of the identity of the floral meristem. In *A. thaliana*, three genes belonging to the *SQUA* subfamily act in redundancy in the determination of inflorescence architecture (*AP1*, *FUL*, and *CAL*; Ferrandiz *et al*., 2000). The *AP1* gene also acts to specify the identity of sepals and petals, thus performing an A function (Mandel *et al*., 1992). Despite evidence that a rice *SQUA* gene might exert a dominant negative effect on floral organ identity when heterologously expressed in *Arabidopsis* (Fornara *et al*., 2004), there has been no clear demonstration to date of an A function for *SQUA* genes in monocots (Kater *et al*., 2006). Indeed, as illustrated by the wheat *VRN1* gene involved in vernalization, *SQUA*-like gene function may have diverged in various species (Yan *et al*., 2003).

EgSQUA1 transcripts accumulate in cells that retain meristematic characteristics in the rachilla and floral meristems and also to a lesser extent throughout most regions of the staminate and pistillate flower. The specific expression of *EgSQUA1* in inflorescence and floral meristems is a pattern commonly observed amongst members of the *SQUA* subfamily of MADS box genes (Huijser *et al*., 1992; Mandel *et al*., 1992; Mena *et al*., 1995; Moon *et al*., 1999*a*; Kyozuka *et al*., 2000). The functional studies of *EgSQUA1* in *A. thaliana* revealed that its ectopic expression increases the number of internodes during the reproductive phase of the plant. Floral morphogenesis and floral induction timing are not affected, in contrast to *Arabidopsis* plants which overexpress *AP1* (Mandel and Yanofsky, 1995). When interpreting the phenotype of 35S::*EgSQUA1* plants, it is of interest to note that like the *Arabidopsis ful* mutant, reduced fertility is observed (Gu *et al*., 1998). Thus the hypothesis of a dominant negative effect cannot be ruled out. Whatever the original cause of the reduced fertility of 35S::*EgSQUA1* plants, it is a possibility that this phenotypic effect might lead to changes in resource allocation which could, in turn, affect inflorescence growth.

B function

The archetypal B function observed in the model dicot *A. thaliana* involves the activity of *APETALA3* and *PISTILLATA*, genes belonging, respectively, to the *DEF* and *GLO* subfamilies. The corresponding proteins act in a multimeric complex. In monocots, the situation appears to be similar, but often more complex, multiple *GLO* and *DEF* genes have been observed in a number of cases. In the case of maize, a *DEF*-like gene (*SILKY1*) and three *GLO*-like genes (*ZMM16*, *ZMM18*, and *ZMM29*) have been identified. Expression patterns and functional data support the idea that these genes do indeed exert a B function (Ambrose *et al*., 2000; Münster *et al*., 2001). In the case of rice, similarly based on expression patterns in flowers and functional analyses, two *GLO* orthologues (*OsMADS2* and *OsMADS4*), and a *DEF* orthologue (*OsMADS16* or *SUPERWOMAN1*, *SPW1*) with B class activity have been identified (Kang *et al*., 1998; Moon *et al*., 1999*b*; Kyozuka *et al*., 2000; Nagasawa *et al*., 2003). In oil palm, three genes of putative B function have been identified: *EgGLO1* and *EgGLO2* belonging to the *GLO* subfamily; and *EgDEF1* belonging to the *DEF* subfamily. *In situ* hybridization analysis revealed that unlike their model dicot counterparts, *EgDEF1* and *EgGLO2* do not display the same spatial pattern of expression in the flower. *EgDEF1* expression is essentially restricted to the inner perianth and stamens/staminodes in staminate/pistillate flowers, respectively. This result indicates a distinct molecular identity for whorl 2 organs compared with those of whorl 1, despite their similar appearance in oil palm. It is relevant to note in this context that most palm species produce distinguishable petals and sepals (Dransfield and Uhl, 1998). In contrast to *EgDEF1*, *EgGLO2* expression is mainly detected in sepals and petals in nearly mature oil palm pistillate flowers, whereas in staminate flowers a strong signal is also seen in the stamens. The question of homeotic identity in perianth organs has been addressed for a number of other monocot species, including some which produce morphologically similar tepals rather than distinct petals and sepals. In the case of tulip, which produces tepals of a petaloid nature, *DEF* and *GLO* gene expression is observed in both whorls of the perianth (Kanno *et al*., 2003). In contrast, *Asparagus*, which also has tepal organs resembling petals, exhibits expression of *DEF* and *GLO* genes in the inner tepals and in stamens, but not in the outer tepals (Park *et al*., 2003, 2004). It is also interesting to compare the oil palm data with results obtained for *Tradescantia reflexa* and *Commelina communis* (Ochiai *et al*., 2004), which produce

distinctly different sepals and petals. The expression of the *DEF* gene homologues was found to be restricted to the inner perianth and androecium. In contrast, one of the *GLO* genes isolated was found to be expressed in whorl 1, as observed for *EgGLO2* in oil palm.

Consistent with their similarities to known B class genes in respect of sequence and spatial expression in the flower, *EgDEF1* and *EgGLO2* both display reduced expression in *mantled* palms, which resemble B class mutants in that they show transformation of stamens or staminodes into carpeloid structures. The additional question remains as to whether the *mantled* abnormality involves, like the *def* and *glo* mutants of *Antirrhinum*, a transformation of petals into sepals. This appears to be the case, on the basis of the perturbation of expression of *EgDEF1* in the inner perianth of *mantled* palm flowers. Thus the phenotype of *mantled* palms may be considered to be similar in nature to previously characterized B mutants. In the case of *EgGLO2*, although a general quantitative reduction in transcript amounts is observed in *mantled* flowers at specific stages, spatial changes observed by *in situ* hybridization are less marked.

Additional indications concerning the conservation of B function in oil palm can be drawn from the *A. thaliana* ectopic expression data. Ectopic expression of *EgGLO2* in transgenic *A. thaliana* results in conversion of sepals into petals as observed with *PI* (Krizek and Meyerowitz, 1996), suggesting that *EgGLO2* has common biochemical specificities with its *A. thaliana* homologue which is necessary for petal and stamen formation. In the case of *EgDEF1*, it might be considered surprising that the ectopic expression of this gene does not result in homeotic transformation in *A. thaliana* flowers. However, a similar result was obtained by Tzeng and Yang (2001) for a lily *AP3* homologue *LMADS1*. If the *A. thaliana AP3* gene is ectopically expressed in its native species, a homeotic conversion of carpels to stamens in whorl 4 is observed to be induced. This involves the induction of the expression of *PI* (Jack *et al*., 1994), which is, in turn, maintained through activation of the *PI* promoter by the PI–AP3 complex (Honma and Goto, 2000). One possible explanation for the absence of homeotic transformation in *A. thaliana* flowers ectopically expressing *EgDEF1* (or *LMADS1*) is that the two monocot DEF proteins mentioned might not have the specificities necessary for binding to their protein partner homologues in *A. thaliana* and/or interaction with the *PI* promoter.

Overall, the spatial expression data indicate that the two perianth organs of oil palm have a different homeotic identity despite being morphologically similar and that the oil palm *DEF* and *GLO* genes described here might have a B function. On the basis of the available data, *EgDEF1* is likely to be involved in petal determination, and in stamen/staminode initiation and functional stamen development. Similarly, *EgGLO2* might play a role in stamen and petal determination. The additional detection of *EgGLO2* expression in sepals might be taken to suggest a role for this gene in whorl 1; however, it should be stressed that no studies to date have as yet demonstrated a DEF-independent biological function for GLO proteins.

C and D functions

Two oil palm genes belong to the *AG* subfamily, which is known to be involved, in model plants, in the determination of stamens and carpels in the case of C class genes and in ovule development in the case of C and D class genes. Phylogenetic studies indicate that the two oil palm *AG* genes do not belong to the monophyletic D class group (Kramer *et al*., 2004; Adam *et al*., 2006). *EgAG2* expression was found to be localized principally in stamens and carpel primordia in staminate and pistillate flowers, respectively. In mature flowers, transcripts are present in the precursor cells of ovules and in the microspores of stamens. RT-PCR analysis of *EgAG2* transcript accumulation shows that the expression of this gene is perturbed in *mantled* plants, suggesting a role in the determination of androecium identity as for other C class genes. In model species, C class genes are expressed in stamens and carpels while D class genes show specific expression in ovules. However, it has been shown that, in the *A. thaliana* mature flower, the expression of *AG* occurs in restrictive cellular regions of stamens and carpels (Bowman *et al*., 1991). Moreover, it was shown that *AG* controls the microsporogenesis process leading to pollen formation via the regulation of *SPOROCYTELESS* (Ito *et al*., 2004). It has been shown that ovule identity in *A. thaliana* is promoted by three redundant genes closely related to *AG*, namely *SHATTERPROOF1* (*SHP1*), *SHATTERPROOF2* (*SHP2*), and *SEEDSTICK* (*STK*), which act in concert with *AG* (Favaro *et al*., 2003; Pinyopich *et al*., 2003). Functional analyses indicate that in *A. thaliana* a C class gene may be involved in the development of male and female gametes and that some genes may have a specific D function and others a mixed C/D function.

Based on these observations and on the expression pattern of *EgAG2*, it is possible to hypothesize that this oil palm gene exhibits a mixed C/D function involving the control of organ identity in the androecium and the gynoecium and that it might also play a role in male and female gamete development. This situation is similar to that proposed for the *SHP1* and *SHP2* genes of *A. thaliana*. Nevertheless, ectopic expression of *EgAG2* in *A. thaliana* does not result in either a homeotic floral phenotype, or in any of the other developmental alterations associated with *AG* gene heterologous expression, such as altered leaf morphology or early flowering (Mizukami and Ma, 1992; Kater *et al*., 1998; Rutledge *et al*., 1998). These observations suggest that the specificities of the EgAG2 protein are divergent from those of the *A. thaliana* homologues and/or that *EgAG2* is not

sufficient on its own to provide a C or C/D function in *A. thaliana*. A number of studies in various different species have revealed that the C/D function is often shared by two or more closely related genes as a result of gene duplication, and that partial redundancy in the control of reproductive organ identity is common amongst angiosperms (Ferrario *et al*., 2004). Interestingly, in rice, the functions regulated by *AG* have been partially partitioned between two paralogous genes, *OsMADS3* and *OsMADS58*, which display subfunctionalization in terms of their activity in different whorls of the flower (Yamaguchi *et al*., 2006).

On the basis of the present data, the possibility that a specific D function controlling ovule identity and development exists in oil palm cannot be excluded. Ovules are initiated from the remaining meristematic region located inside the three carpels of the gynoecium (Adam *et al*., 2005). The hypothesis of a specific D function in oil palm is compatible with the *mantled* phenotype in which carpels are produced instead of stamens or staminodes in the androecium. These carpels do not form ovules, possibly implying that in oil palm the development of the ovule requires additional homeotic gene functions compared with those necessary for carpel development.

E function

EgAGL2-1, belonging to the AGL2/SEP subfamily, shows late expression in pistillate and staminate flowers from the stage of reproductive organ initiation, and this expression is affected in *mantled* plants. *In situ* hybridization shows that *EgAGL2-1* is expressed in petals and ovules in pistillate flowers and in petals and stamens in staminate flowers. *EgAGL2-1* might therefore play a role in petal development and in ovule and stamen development in pistillate and staminate flowers, respectively, like previously described E class genes.

The ectopic expression of *EgAGL2-1* in *A. thaliana* results in modifications in different whorls of the flower. The overall phenotype has similarities to that of the *sep1sep2sep3sep4* mutant (Ditta *et al*., 2004), although, in the latter case, the additional flower produced within the carpel was found to originate from the floral meristem and not from the conversion of an ovule as seen in 35S::*EgAGL2-1* plants. Interestingly, no *A. thaliana* floral organ identity genes were found to be affected in their transcript accumulation in 35S::*EgAGL2-1* plants. A possible explanation of these data is that the protein encoded by *EgAGL2-1* might interact with native *A. thaliana* proteins to form a biologically inactive complex in a dominant negative manner. All floral whorls display modifications when *EgAGL2-1* is introduced, suggesting that *EgAGL2-1* is able to interact within each of the different native protein complexes necessary for correct whorl identity in *A. thaliana*. On the other hand, the fact that 35S::*EgAGL2-1* plants display a *sep* mutant phenotype suggests that the functional specificities of EgAGL2-1 and *Arabidopsis* SEP proteins have diverged significantly during evolution. Clearly it would be of interest to assess whether *EgAGL2-1* is able to complement the *Arabidopsis sep* mutants genetically. Overall, the data support the idea that EgAGL2-1 acts as an E class protein involved in petal, androecium, and gynoecium development in oil palm flowers and that it may be involved in protein complexes which are slightly different from those of *A. thaliana*. The complement of E class genes functioning in monocots appears to be different since *SEP1/2* and *SEP4*-like genes do not seem to be present, in contrast to the *SEP3*-like genes. Moreover *SEP*-related genes specific to monocots have been described, notably the *LHS1* and the *OsMADS34* clades (Kramer *et al*., 2004; Adam *et al*., 2006). *LHS1*-like genes play a role in determining the identity of the paleas and lemmas, in inflorescence meristem identity, and in specifying the upper floret in certain species (Malcomber and Kellogg, 2004). In rice, five *SEP*-related genes are present: two from the *SEP3* clade, two from the *LHS1* clade, and one from the *OsMADS34* clade (Adam *et al*., 2006). All of them show specific expression patterns during flower development (Kang and An, 1997; Prasad *et al*., 2001; Pelucchi *et al*., 2002). Within the second group, an E-type mutant phenotype was recently demonstrated for the *OsMADS1* gene (Kumar Agrawal *et al*., 2005).

Conclusion

The working model of flower structure determination in oil palm implicates different combinations of gene activities necessary for correct organ identity in each whorl (Fig. 5). The scheme in Fig. 5 shows a strong overall resemblance to the generic dicot ABCDE model and contrasts with the 'modified ABC model' proposed for members of the Liliales (Tzeng *et al*., 2001; Kanno *et al*., 2003) and Asparagales (Nakamura *et al*., 2005) displaying petal identity in floral whorl 1. Overall, the oil palm data indicate a closer similarity to the situation observed in species of the Commelinales (Ochiai *et al*., 2004), which show a clear distinction of sepals and petals and which are more closely related to palms (with which they form the Commelinid group) than to the Liliales or Asparagales. Further studies will now be required in order to obtain a deeper understanding of the diverse roles played by MADS box genes in the determination and evolution of flower form both within the palm family and throughout angiosperms as a whole.

Supplementary data

The following supplementary data are available at *JXB* online.

Fig. S1. Relationships between MADS box proteins from *Elaeis guineensis* and their relatives from other angiosperms.

Fig. S2. Histological definition of developmental stages in staminate and pistillate flowers of oil palm.

Fig. S3. Expression pattern analyses of oil palm MADS box genes.

Fig. S4. *In situ* hybridization studies to investigate the accumulation of transcripts of the oil palm genes *EgAGL2-1*, *EgDEF1*, *EgGLO2* and *EgAG2* in *mantled* flowers (stage 4) of the male oil palm inflorescence.

Fig. S5. Endogenous gene expression levels in flowers of wild type and transgenic 35S::*EgAGL2-1 Arabidopsis* plants.

Table S1. List of primers and RT-PCR conditions used for the expression analysis of oil palm MADS box genes.

Table S2. List of primers and RT-PCR conditions used for the expression analysis of *A. thaliana* MADS box genes.

Table S3. List of primers used for the synthesis of *in situ* hybridization probes.

Acknowledgements

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Note added in Proof

Syed Alwee *et al*. (*Plant Cell Tissue and Organ Culture* **85,** 331–344; 2006) recently described the identification of oil palm MADS box genes including EgMADS16, a paralogue of EgGL02 (identical to EgGL01; Adam *et al*., *Journal of Molecular Evolution* **62,** 15–31; 2006) which complements the *pistillate* mutant.

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