INSIGHTS FROM ANA-GRADE ANGIOSPERMS INTO THE EARLY EVOLUTION OF CUP-SHAPED COTYLEDON GENES

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Background and Aims The closely related NAC family genes NO APICAL MERISTEM (NAM) and CUP-SHAPED COTYLEDON3 (CUC3) regulate the formation of boundaries within and between plant organs. NAM is post-transcriptionally regulated by miR164, whereas CUC3 is not. To gain insight into the evolution of NAM and CUC3 in the angiosperms, we analysed orthologous genes in early-diverging ANA-grade angiosperms and gymnosperms.

Methods We obtained NAM- and CUC3-like sequences from diverse angiosperms and gymnosperms by a combination of reverse transcriptase PCR, cDNA library screening and database searching, and then investigated their phylogenetic relationships by performing maximum-likelihood reconstructions. We also studied the spatial expression patterns of NAM, CUC3 and MIR164 orthologues in female reproductive tissues of Amborella trichopoda, the probable sister to all other flowering plants.

Key Results Separate NAM and CUC3 orthologues were found in early-diverging angiosperms, but not in gymnosperms, which contained putative orthologues of the entire NAM + CUC3 clade that possessed sites of regulation by miR164. Multiple paralogues of NAM or CUC3 genes were noted in certain taxa, including Brassicaceae. Expression of NAM, CUC3 and MIR164 orthologues from Am. trichopoda was found to co-localize in ovules at the developmental boundary between the chalaza and nucellus.

Conclusions The NAM and CUC3 lineages were generated by duplication, and CUC3 was subsequently lost regulation by miR164, prior to the last common ancestor of the extant angiosperms. However, the paralogous NAM clade genes CUC1 and CUC2 were generated by a more recent duplication, near the base of Brassicaceae. The function of NAM and CUC3 in defining a developmental boundary in the ovule appears to have been conserved since the last common ancestor of the flowering plants, as does the post-transcriptional regulation in ovule tissues of NAM by miR164.

Key words: CUP-SHAPED COTYLEDON, CUC, NO APICAL MERISTEM, NAM, MIR164, Amborella trichopoda, Cabomba aquatica, Ginkgo biloba, angiosperm, gymnosperm.

INTRODUCTION

The genes NO APICAL MERISTEM (NAM) in Petunia hybrida and CUP-SHAPED COTYLEDON3 (CUC3) in Arabidopsis thaliana encode distinct members of the large NAC family of plant-specific transcription factors (Ooka et al., 2003). NAC proteins contain a highly conserved, DNA-binding ‘NAC’ domain at their N terminus, and a more variable C-terminal region (Ernst et al., 2004). NAM, CUC3 and their respective orthologues in diverse angiosperms, which are here referred to generically as CUC genes, are expressed at, and control the formation of, tissue boundaries both within and between plant organs. Accordingly, the inactivation of CUC genes reveals a range of unique, redundant and partially overlapping phenotypes in processes including: shoot apical meristem (SAM) establishment, lateral meristem formation, lateral organ separation, leaf lobing and ovule development (Souver et al., 1996; Aida et al., 1997; Ishida et al., 2000; Vroemen et al., 2003; Hibara et al., 2006; Nikovics et al., 2006). In ovule development, CUC genes are expressed at and define the boundary between the nucellus and chalaza in several distantly related eudicots (Souver et al., 1996; Ishida et al., 2000; Weir et al., 2004; Hibara et al., 2006). The degree of genetic redundancy among CUC genes varies between species. For example, the inactivation of the single genes NAM in P. hybrida (Souver et al., 1996) and CUPULIFORMIS (CUP) in Antirrhinum majus (Weir et al., 2004) causes cotyledon fusion and prevents SAM formation, whereas in Ar. thaliana any two of the genes CUC1, CUC2 and CUC3 must be mutated to produce the equivalent phenotype (Aida et al., 1997; Hibara et al., 2006).
Arabidopsis thaliana CUC1 and CUC2 are post-transcriptionally regulated by miR164 (Laufer et al., 2004; Mallory et al., 2004), and both P. hybrid a NAM and An. majus CUP also possess target sites for this microRNA. This regulatory mechanism appears to control the balance between tissue separation and fusion in various situations in the plant. For example, the post-transcriptional regulation of CUC2 in Ar. thaliana is necessary for fusion to take place between the two carpels of the syncarpic gynoecium, while the balance of CUC2 and miR164 expression also controls the depth of leaf lobes (Nikovics et al., 2006; Sieber et al., 2007; Larue et al., 2009). Interestingly, a similar mechanism controls leaf dissection and compound leaf formation in several distantly related eudicots in which these morphological traits were clearly shown to be of independent origin (Blein et al., 2008; Berger et al., 2009). The regulation of CUC genes by miR164 thus seems to form a conserved genetic module that has been repeatedly recruited during angiosperm evolution to both leaf dissection and floral organ fusion.

To provide insights into the early evolution of CUC genes in the angiosperms, we studied their phylogenetic relationships, in both angiosperms and their sister group, the gymnosperms. To make conclusions on the CUC genes that were present in the last common ancestor of the extant angiosperms, we included in our analyses representatives of the ANA grade (ANA for Amborellales, Nymphaeales and Austrobaileyales), which, according to molecular phylogenetic analyses (Bremer et al., 2009), comprises the first three extant lineages to have diverged from a common remaining lineage from which all other living angiosperms are descended. In particular, we focused on Amborella trichopoda, a shrub endemic to New Caledonia, which, as the only representative of Amborellales, is the probable sister to all other extant angiosperms. This work is complementary to an earlier study of miR164 evolution, which identified the presence of at least two MIR164 genes in the last common ancestor of the extant angiosperms (Jasinski et al., 2010).

The present study investigated the origin, through duplication, of the angiosperm NAM and CUC3 lineages, and the presence or absence of post-transcriptional regulation in these lineages in the last common ancestor of the extant angiosperms. Studies in Am. trichopoda have demonstrated NAM and CUC3 orthologues to be expressed between the nucellus and chalaza in the ovule, as is also the case in eudicots, suggesting the conservation of CUC gene function in defining this tissue boundary throughout angiosperm evolution. As the NAM orthologue from Am. trichopoda appears to be regulated by miR164, we also investigated the expression of this microRNA in ovule tissues. The results suggest that the NAM/miR164 genetic module has operated in ovule tissues since the last common ancestor of the extant angiosperms.

MATERIALS AND METHODS

Plant material

Material of Amborella trichopoda Baill. was field-collected at Col d’Amieu, New Caledonia, and that of Ginkgo biloba L. was obtained from the City of Lyon Botanic Garden. Plants of Cabomba aquatica Aublet were obtained from Anthias SA (Les Chêres, Rhône, France) and grown to maturity in a small aquarium.

NAC gene identification

RNA was extracted (Chang et al., 1993) from plant reproductive tissues and reverse transcribed into cDNA using M-MuLV reverse transcriptase (Fermentas, Hanover, MD, USA). Conserved regions of NAM and CUC3 homologues, within the 5′ NAC domain, were amplified by PCR using the partially degenerate primers: 5′-GGTTYCAYCCNACTGAYGARGAGCT and 5′-CTGCANATNACCCATTCCYTCTTT. DNA fragments thus obtained were sequenced and used in initial phylogenetic analyses (data not shown) to determine their possible orthology to P. hybrid a NAM and/or Ar. thaliana CUC3. Amplified fragments of possible NAM and/or CUC3 orthologues were then used to obtain longer or full-length cDNAs by screening cDNA libraries, prepared as previously described (Fourquin et al., 2005; Finet et al., 2010). The sequence of GbiNACa from G. biloba was completed by RACE (rapid amplification of cDNA ends) PCR using a Marathon (Clontech, Mountain View, CA, USA) kit, while CaqNACa was initially identified from a C. aquatica flower expressed sequence tag database (C. Scott and C. Finet, unpubl. data). Further possible NAM and CUC3 orthologues, and closely related NAC genes from diverse angiosperms and gymnosperms, were identified by BLAST searching of publicly available databases.

Phylogenetic analyses

Amino acid sequences from NAC genes were aligned using MUSCLE in the SEAVIEW program (Gouy et al., 2010) and homologous sites for phylogenetic reconstructions were chosen manually. Maximum-likelihood phylogenies were then generated from these alignments in PhyML (Guindon et al., 2009) using an LG evolutionary model. Amino acid alignments were also back-translated using TranAlign (http://emboss.sourceforge.net/), thus conserving homologous sites determined from amino acid alignments, and used to generate maximum-likelihood phylogenies in PhyML using a GTR evolutionary model. Statistical support for all phylogenetic analyses was provided by performing 1000 bootstrap replicates.

In situ hybridizations using polynucleotide probes

Expression of AttrNAM and AtrCUC3 in female reproductive tissues was detected using digoxigenin-labelled antisense riboprobes synthesized using T7 RNA polymerase (Promega, Madison, WI, USA) and DIG-RNA labelling mix (Roche, Basel, Switzerland) from full-length cDNAs amplified as PCR products to incorporate bacteriophage T7 promoter sequences. Negative and positive control hybridizations were also performed, using sense strand riboprobes and an antisense strand riboprobe of a HISTONE4 gene from Am. trichopoda, respectively. In situ hybridizations were carried out as described by Nikovics et al. (2006), except that hybridizations were performed in solutions containing 50 % (v/v) formamide, 10 % (w/v) dextran sulphate, 3.5 mg mL⁻¹ tRNA, 2.5 × Denhardt’s reagent, 0.3 μM NaCl, 10 mM Tris/HCl, 10 mM sodium phosphate
and 5 mM EDTA (pH 6.8). Photographs were taken using an AxioCam MRC cooled camera (Zeiss, Jena, Germany) in conjunction with a Zeiss AxioImager M2 microscope, and images were processed with Photoshop software (Adobe, San Jose, CA, USA).

In situ hybridizations using oligonucleotide probes

Expression of miR164 in female reproductive tissues was detected using a 5′-digoxigenin-labelled miRCURY LNA probe (Exiqon, Vedbaek, Denmark) complementary to the predicted mature Atr-miR164a sequence from Am. trichopoda (Jasinski et al., 2010). An LNA oligonucleotide complementary to murine miR124 (Lagos-Quintana et al., 2002), which has no predicted target sequences in known plant genomes, was used as a negative control. In situ hybridizations were carried out as described by Adam et al. (2007) at probe concentrations of 0.02 mM. Detection was performed using the Vector Blue Alkaline Phosphatase Substrate Kit III (Vector Laboratories, Burlingame, CA, USA). Photographs were taken using an Evolution MP 5.0 cooled camera (MediaCybernetics, Bethesda, MD, USA) in conjunction with a DMRB microscope (Leica, Solms, Germany), and images were processed with Photoshop software (Adobe).
RESULTS

The NAM and CUC3 lineages separated prior to the radiation of the extant angiosperms

To gain insight into the evolution of CUC genes in the angiosperms, we performed phylogenetic analyses using sequences from both angiosperms and gymnosperms, including *Am. trichopoda* (*Amborellales, Amborellaceae*) and *C. aquatica* (*Nymphaeales, Cabombaceae*), as representatives of the two earliest diverging flowering plant lineages (see Supplementary Data Table S1). We first performed analyses on the entire data set obtained (Fig. S1) to identify the relationship of major clades containing CUC genes.

Phylogenies obtained using both amino acid and nucleotide alignments (Fig. S2) yielded trees of similar overall topology in which a grade containing the genes *PNAC111*, *PNAC109*, *VvLOC100254997* and *ANAC074* grouped externally to all remaining sequences, followed by a clade containing the *Ar. thaliana* gene *NAC1*, which mediates auxin signalling in lateral root development (Xie et al., 2000). Within the remaining clade of sequences analysed, sub-clades respectively containing *P. hybrida* NAM (*PhyNAM*) and *Ar. thaliana* CUC3 (*AthCUC3*) occupied sister positions in both of the analyses performed. However, in the phylogeny produced using a nucleic acid alignment (Fig. S2B), a group of Poaceae monocot sequences grouped externally to the clade containing *PhyNAM*, as compared with its position in the phylogeny performed using an amino acid alignment (Fig. S2A). This effect may be due to highly biased codon usage in Poaceae. Another unstable feature of our analyses occurred in the position of *CaqNACA*, a possible NAM orthologue from *C. aquatica* (*Nymphaeales*). This sequence occupied a basal position within the NAM clade in a nucleic acid-based phylogeny (Fig. S2B), but appeared basal to the entire NAM + CUC clade in an amino acid-based phylogeny (Fig. S2A). Following our initial studies, we performed a phylogenetic analysis using a sub-set of sequences centred on the sister clades containing *PhyNAM* and *AthCUC3* to better resolve the nodes within these, but omitting the Poaceae and *CahNACA* sequences which had previously shown variable positions. This analysis (Fig. 1) indicated the internal structure of the NAM and CUC3 clades to broadly recapitulate angiosperm phylogeny, with sequences from the ANA-grade angiosperm *Am. trichopoda* occupying basal positions in both clades. Thus, despite the apparent instability of certain sequences in our phylogenies, the presence of separate NAM and CUC3 orthologues in diverse flowering plant groups, including the earliest diverging order Amborellales, strongly suggests these lineages to have separated through a gene duplication event prior to the last common ancestor of the extant angiosperms.

In phylogenetic analyses performed using a nucleic acid alignment from an extensive NAC family data set (Fig. S2B), a clade of four gymnosperm genes occupied a sister position to the combined angiosperm NAM + CUC3 clade. Like NAM orthologues, these gymnosperm genes possess sites of regulation by *miR164* (Fig. S3). However, bootstrap support for the phylogenetic placement of these genes was not strong, and only one of them, *PtaEST1163314*, occupied the same position in phylogenetic analyses performed using an amino acid alignment (Fig. S2A). Although questions thus remain concerning the precise orthology of the *NAM/CUC3*-like genes identified here from gymnosperms, it appears significant that none of these genes grouped within the angiosperm NAM and CUC3 clades in any of our phylogenetic analyses. These findings are consistent with the possibility that the NAM and CUC3 lineages separated specifically in the angiosperm lineage, after its divergence from that of the extant gymnosperms. However, other interpretations of these data are possible. For example, separate gymnosperm orthologues of NAM and CUC3 might have been missed in cloning procedures, or may have existed but have been lost from the gymnosperm clade subsequent to the separation of the angiosperm and gymnosperm lineages.

The CUC1 and CUC2 lineages separated by gene duplication near the base of Brassicaceae

The largely redundant *Ar. thaliana* paralogues CUC1 and CUC2 are positioned in our phylogenetic analysis in each of
two sub-clades occupying sister positions within the main NAM clade of angiosperm NAC genes. Each of these sub-clades exclusively contains genes from Brassicaceae (Fig. 1), which groups within the eurosids II clade (Bremer et al., 2009). The closest relatives of Brassicaceae sampled in this study are *Pisum sativum* and *Populus trichocarpa* from the eurosids I clade, which, like the other species external to Brassicaceae included in our analyses, do not appear to contain separate orthologues of CUC1 and CUC2. We therefore conclude that the duplication that generated CUC1 and CUC2 occurred after the separation of eurosids I and II, and before the relatively early speciation event within Brassicaceae (Bailey et al., 2006) that separated the common lineage of *Brassica* and *Raphanus* from that of *Arabidopsis* and *Cardamine*. These conclusions are summarized in Fig. 2. Further sampling within eurosids II could be used to localize this gene duplication event more precisely. At least one further gene duplication event within the CUC1 clade appears to have occurred to generate two CUC1 paralogues per diploid genome in *Brassica*, thus explaining the presence of four CUC1 paralogues in the tetraploid genome of *Brassica napus* (Fig. 1). Our phylogenetic analyses also reveal the presence of recent gene duplications outside Brassicaceae in both the NAM and the CUC3 clades, leading for example to the paralogues *PsaNAM1* and *PsaNAM2* in *Pisum sativum* and *PtrCUC3a* and *PtrCUC3b* in *Populus trichocarpa* (Fig. 1). Zimmermann and Werr (2005) noted the presence of a conserved motif, termed Motif 3, within the C-terminal region of certain NAM-like genes. Given the phylogeny of the NAM clade, as shown in Fig. 1, the absence of this motif in Brassicaceae CUC1 proteins (Fig. S4) suggests that sequences encoding it might have been lost from the CUC1 lineage in a common ancestor of Brassicaceae.

CUC3 lost its regulation by miR164 before the radiation of the extant angiosperms

All angiosperm NAM genes for which sequence data from the 3’-end of the coding region were available to this study were found to contain sites of potential regulation by miR164 (Fig. 1), as were possible gymnosperm orthologues of the combined NAM/CUC3 clade (Fig. S3). However, all CUC3 orthologues analysed to date, including AtrCUC3 from the ANA-grade angiosperm *Am. trichopoda*, lack sites of regulation by miR164. As all sites of regulation by miR164 occur in the same position in their respective NAC genes, these sites appear to be homologous, and as CUC3 groups within a clade of NAC genes that contain sites of regulation by miR164, we conclude the CUC3 lineage to have previously possessed a site of regulation by miR164, but to have lost this site before the radiation of the extant angiosperms.
Expression patterns of NAM, CUC3 and miR164 orthologues suggest the conservation of function in ovule development since the last common ancestor of the extant flowering plants

An interest in female reproductive development led us to study the expression patterns of the NAM and CUC3 orthologues, AtrNAM and AtrCUC3, in female flowers of Am. trichopoda. Expression of these genes was found to co-localize in the peripheral region of the ovule, corresponding to the boundary of the nucellus and chalaza (Fig. 4). This expression pattern strongly resembles those of the orthologues of AtrNAM and AtrCUC3 in Ar. thaliana (Ishida et al., 2000; Hibara et al., 2006) and those of AtrNAM in P. hybrida (Souver et al., 1996) and An. majus (Weir et al., 2004). These data suggest that NAM and CUC3 define a developmental boundary in the Am. trichopoda ovule, as do the paralogous NAM clade genes CUC1 and CUC2 in Ar. thaliana (Ishida et al., 2000). Such conservation of expression in a very precise tissue boundary strongly suggests the conservation of function in the studied plant lineages since the last common ancestor of the living flowering plants.

As AtrNAM possesses a site of regulation by miR164, we also performed in situ hybridizations using an LNA oligonucleotide probe corresponding to the known mature miR164 sequence from Am. trichopoda (Jasinski et al., 2010). These hybridizations (Fig. 5) demonstrated co-localization of miR164 expression with that of its putative NAM target AtrNAM in ovule tissues, closely resembling the expression pattern of miR164 in the eudicot Nicotiana benthamiana (Valoczi et al., 2006). These results strongly suggest that the post-transcriptional regulation of NAM by miR164 has been conserved in the ovule since the radiation of the extant angiosperms.

**DISCUSSION**

**A partial reconstruction of NAM and CUC3 evolution in the angiosperms**

We have analysed NAC family sequences from angiosperms and gymnosperms to clarify a number of points in the evolution of CUC genes in the flowering plants. We incorporated sequences from early-diverging angiosperms and gymnosperms in these analyses to infer the structure of the NAC family in the last common ancestor of the extant angiosperms. We also included all available NAM clade sequences from Brassicaceae to resolve questions relating to the evolutionary origin of the much studied paralogues CUC1 and CUC2 in Ar. thaliana.

The results of our analyses, which are summarized in Fig. 6, clearly indicate that both the NAM and CUC3 lineages were present in the last common ancestor of the extant flowering plants. The apparent absence of separate orthologues of NAM and CUC3 in gymnosperms suggests the duplication that generated these paralogous lineages occurred after the separation of the extant angiosperms and gymnosperms. Gymnosperm genes that group closely to the combined angiosperm NAM + CUC3 clades in phylogenetic analyses have been identified in our analyses, although further work will be needed to adequately support the possible orthology of some or all of these to genes of the angiosperm NAM + CUC3 clade.

Our analyses have also resolved a question relating to the evolutionary origin of CUC1 and CUC2 in Ar. thaliana. Earlier phylogenies of NAM genes, in which only Ar. thaliana was included to represent Brassicaceae, showed CUC1 to group externally to a clade in which Ar. thaliana CUC2 was present, together with NAM genes from distantly related eudicots such as An. majus and P. hybrida (Ooka et al., 2003; Vroemen et al., 2003; Weir et al., 2004; Zimmermann and Werr, 2005; Blein et al., 2008).
However, our phylogenetic analysis (Fig. 1), which incorporates four genera that form two clades within Brassicaceae, better resolves the NAM clade, with the result that CUC1 and CUC2 appear as paralogous lineages that are unique to Brassicaceae, at least among the taxa included in our analysis (as summarized in Figs 2 and 6).

The basal positioning of CUC1 observed in the phylogenies cited above suggests these analyses were distorted by long-branch attraction, possibly due to rapid evolution in the CUC1 lineage. Such rapid evolution might suggest CUC1 to have evolved specific functions through the positive selection of non-silent changes to its coding sequence. Specific functions of CUC1 in cotyledon development, which might support this hypothesis, are revealed in plants in which PIN-FORMED1 (PIN1) and PINOID (PID), which act to limit CUC expression in cotyledon tissues, are mutated. Accordingly, cuc1 pin1 double mutants show enhanced defects in cotyledon separation compared with cuc2 pin1 double mutants (Aida et al., 2002), while cuc1 pin1 pid triple mutants show a greater recovery of the cotyledon development that is lost in pin1 pid double mutants compared with cuc2 pin1 pid triple mutants (Furutani et al., 2004). However, these effects may reflect differential regulation of CUC1 and CUC2 in response to auxin, rather than differences in the biochemical properties of the proteins encoded by these genes. Thus, an alternative to positive selection operating on CUC1 is relaxed selection, which may have allowed the coding sequence of this gene to change more rapidly than that of its parologue CUC2. Indeed, both positive and relaxed selection may have operated on distinct domains of CUC1.
In addition to gene duplications in the NAC family, we have also addressed the question of the post-transcriptional regulation of NAC genes by miR164. We conclude that a loss of regulation by this microRNA has occurred in at least five NAC family lineages in the angiosperms, including that of CUC3, and further show that loss of post-transcriptional regulation in this lineage preceded the radiation of the extant angiosperms. By contrast, we have found no loss of regulation by miR164 in any gene of the angiosperm NAM clade. It is interesting to note that the conservation of regulation by miR164 in NAM genes since the last common ancestor of the extant angiosperms is paralleled by conservation in the structure of the miR164 gene family (Fig. 6). Accordingly, at least two miR164 genes appear to have been present in the last common ancestor of the extant flowering plants and these gene clades have been conserved to the present day in several distantly related angiosperm lineages, including those of the ANA grade (Jasinski et al., 2010).

The function of NAM and CUC3 in early angiosperms

We have focused here on the earliest diverging of all angiosperm lineages, uniquely represented by Am. trichopoda, to gain insight into the CUC genes present in the last common ancestor of the extant angiosperms. We show that expression of NAM and CUC3 orthologues co-localizes with a developmental boundary that forms between the nucellus and chalaza of the Am. trichopoda ovule. This highly specific expression pattern appears to be entirely conserved between Am. trichopoda and diverse eudicots (Souer et al., 1996; Ishida et al., 2000; Weir et al., 2004; Hibara et al., 2006), suggesting its conservation since the last common ancestor of the extant angiosperms. We argue that conservation of such a highly specific expression pattern, in a developmental boundary that is also conserved between the species under comparison, implies the conservation of gene function. A full test of this hypothesis will, however, have to await the development of functional genetics approaches in Am. trichopoda or other ANA-grade angiosperms.

Our study has also demonstrated co-localization of miR164 expression with that of its putative target AtNAM, in Am. trichopoda ovule tissues, closely resembling the situation in eudicots (Valoczi et al., 2006). No specific phenotype has yet been associated with the expression of miR164 in ovules. However, expression of miR164 in Ar. thaliana leaf and other tissues is known to modulate, rather than eliminate, the expression of its CUC gene targets (Laufs et al., 2004; Nikovics et al., 2006). Accordingly, the co-localization of miR164 and NAM expression reported here suggests this fine-tuning mechanism also to operate in ovule tissues, and to have been conserved at this location since the last common ancestor of the extant flowering plants.

NAM genes and their miR164 regulator are known to form a genetic module that has been recruited many times independently to processes such as compound leaf development and leaf lobe formation (Nikovics et al., 2006; Blein et al., 2008; Berger et al., 2009). In addition, this module is known to be involved in the fusion of carpels into a syncarpic gynoecium, a process which has also evolved many times independently in the angiosperms (Armbruster et al., 2002). For a genetic module to remain in a state of readiness to be recruited to such evolutionarily labile processes, it may also play some more constant developmental role that acts to maintain it over long evolutionary periods. Thus, the functions of NAM, CUC3 and miR164 in ovule development, which the results presented here suggest to have persisted throughout flowering plant evolution, in addition to basic functions in the SAM, may represent conserved roles from which these genes have been recruited to other more variable functions, such as leaf dissection, during angiosperm evolution.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Fig. S1: Sequence alignments used in phylogenetic analyses. Fig. S2: Maximum-likelihood phylogenetic analyses of CUC genes and related sequences in angiosperms and gymnosperms. Fig. S3: Conservation of miR164 target sites in CUC-like genes from angiosperms and gymnosperms. Fig. S4: Conserved domains in selected CUC proteins. Table S1: Accession numbers of sequences analysed.

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LITERATURE CITED


