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Short communication

Nucleotide sequence of a flower-specific MADS box cDNA clone from orchid

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Abstract

An orchid (*Aranda* deborah) mature flower cDNA library was screened with an *agamous* cDNA probe from *Arabidopsis*. One positive clone for *agamous* gene was isolated, cloned and sequenced. This cDNA clone (*om1*) has a full length open reading frame of 750 bp corresponding to 250 amino acid residues. Comparison of *om1* MADS box with that of its counterparts in tomato and *Arabidopisis* reveals significantly high homology (> 95%). Northern analysis indicated this gene is expressed in mature flowers and not in young developing inflorescences or young floral buds. In the mature flowers, it is only expressed in petals and weakly in sepals but not in the column (gynostemium).

Floral development in orchid is a complicated process regulated by both environmental and genetic factors [2, 3]. Although much information has been obtained through horticultural and physiological studies [3, 4], little is known about the molecular mechanism underlying its flower formation. Recently, a number of floral homeotic genes have been isolated and characterized in species including *Arabidopsis*, *Antirrhinum*, *Petunia* and tomato [5, 6, 11, 1, 9]. Among these development genes, *agamous* and *agamous*-like genes have received much attention. Coordinately, these genes act on floral morphogenesis as a large gene family. In addition, their N-terminal amino acids share a DNA binding domain (MADS box) which may play pivotal roles in transcription regulation during flower development. So far all the MADS box genes were isolated from dicotyledons. In this communication, we report a cDNA clone which encodes an orchid MADS box containing transcription factor with homology to the *agamous* gene product.

The nucleotide sequence and derived amino acid sequence of a full-length MADS box cDNA from *Aranda* deborah, designated as *om1* (orchid MADS box gene 1) is shown in Fig. 1. The clone was isolated by screening a million plaques of a λ-ZAP cDNA library from mature flowers under non-stringent conditions, utilizing a 1.0 kb *agamous* cDNA fragment from *Arabidopsis* (*Eco* RI

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X69107.

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                                   GGCACGAGAGCAAAGGAGATTAAGAG      26

ATGGGAAGAGGGAGAGTGGAGCTGAAGATGATTGAGAACAAGATCAATCG      76
M  G  R  G  R  V  E  L  K  M  I  E  N  K  I  N  R       17

TCAAGTAACCTTTGCCAAGCGCCGGAAACGCCTTCTCAAGAAGGCCTATG     126
Q  V  T  F  A  K  R  R  K  R  L  L  K  K  A  Y  E       34

AGCTCTCCGTCCTCTGCGATGCGGAGGTTGCCCTCATCATTTTCTCCAAT     176
  L  S  V  L  C  D  A  E  V  A  L  I  I  F  S  N       50

CGTGGAAAGCTCTACGAGTTCTGCAGCAGCACAAGTATGTTAAAAACGTT     226
R  G  K  L  Y  E  F  C  S  S  T  S  M  L  K  T  L       67

AGAGAAGTACCAAAAATGCAATTTTGGAAGCCCAGAATCAACTATCATAT     276
 E  K  Y  Q  K  C  N  F  G  S  P  E  S  T  I  I  S      84

CAAGAGAGACCCAGAGTAGTCAGCAGGAGTACTTGAAACTTAAAAACCGA     326
  R  E  T  Q  S  S  Q  Q  E  Y  L  K  L  K  N  R      100

GTGGAAGCCTTACAAAGATCGCAGAGAAATTTGCTTGGCGAGGATCTCGG     376
V  E  A  L  Q  R  S  Q  R  N  L  L  G  E  D  L  G      117

TCCACTTGGCAGCAAGGAGCTCGAGCAATTAGAGCGGCAACTGGACTCCT     426
 P  L  G  S  K  E  L  E  Q  L  E  R  Q  L  D  S  S     134

CTCTTAGACAGATTCGATCAACACGGACGCAGTTCATGCTTGATCAGCTA     476
  L  R  Q  I  R  S  T  R  T  Q  F  M  L  D  Q  L      150

GCCGACCTCCAGCGAAGGGAGCAAATGCTTTGCGAGGCGAATAAGACCCT     526
A  D  L  Q  R  R  E  Q  M  L  C  E  A  N  K  T  L      167

AAAGAGAAGGTTTGAGGAAAGCAGCCAGGCGAATCAGCAGCAGGTGTGGG     576
 K  R  R  F  E  E  S  S  Q  A  N  Q  Q  Q  V  W  D     184

ATCCCAGCAATACGCACGCCGTAGGATACGGGAGGCAGCCTGCTCAGCAC     626
  P  S  N  T  H  A  V  G  Y  G  R  Q  P  A  Q  H      200

CATGGAGAGGCTTTCTATCATCCCTTGGAGTGCGAGCCGACATTGCAGAT     676
H  G  E  A  F  Y  H  P  L  E  C  E  P  T  L  Q  I      217

CGGGTATCATTCTGATATAACGATGGCAACGGCGACTGCTTCAACTGTTA     726
 G  Y  H  S  D  I  T  M  A  T  A  T  A  S  T  V  N     234

ATAATTACATGCCACCTGGTTGGCTTGGACAAATTTCAGGCTCCTACGAA     776
  N  Y  M  P  P  G  W  L  G  Q  I  S  G  S  Y  E      250

TAGCTACAAATTTCTAGCTTTCCCCCCTATTACATCATGTATCCTAAAAC     826
>

TTCTTTCTTATTATTATTTTGGTTTTTTTGTGTCAATTTCTGGCTTTTGT     876

ATTCTATATGATTTCCTCAGGGTCATAATTATTTGAAATCTATAAAATGC     926

CGTAAACAAGTGGGTGTGTAATAGAAGATACGTTTTGTTTTCAAAAAAAA     976

AAAAAAAAAA      987
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Fig. 1. Nucleotide and deduced amino acid sequence of *om1* gene. The potential phosphorylation site (RXXT/S) and glycosylation site (NXT/S) are underlined. Four leucine and one isoleucine residues proposed to be on the hydrophobic face of the helical wheel are double-underlined.

Orchid	OM	1	MGRGRVELKMIENKINRQVTFAKRRKRLLKKAYELSVLCDAEVALIIFSNRGKLYE	100%
Petunia	FBP1	1	----KI-I-R---SS-----YS---NGI----K-I------R-SV---ASS--MH-	66%
Petunia	FBP2	1	---------R---------------NG----------------------------	95%
Tomato	TM5	1	---------R--G-----------NG----------------------------	93%
Yeast	MCM1	16	KE-RKI-I-F----TR-H---S--KHGIM---F-----TGTQ-L-LVV-ET-LV-T	46%
Arabidopsis	AgL4	1	---------R---------------N----------------------------	96%
Arabidopsis	Ag	50	S---KI-I-R---TT------C---NG-----------------V--S--R---	77%
Antirrhinum	DEFA	1	-A--KIQI-R---QT-----YS---NG-F---H--------K-SI-MI-STQ--H-	59%
Human	SRF	142	R--VKIKMEF-D--LR-YT--S--KTGIM-------T-TGTQ-L-LVA-ET-HV-T	41%

Fig. 2. An alignment between various MADS box regions: *Aranda* deborah (orchid), *Petunia hybrida* (petunia), *Lycopersicon esculentum* (tomato), *Saccharomyces cerevisiae* (yeast), *Arabidopsis thaliana*, *Antirrhinum majus* (snapdragon), *Homo sapiens* (man) [1, 9, 8, 6, 12, 11, 7]. The number refers to the position in a given ORF of the first residue of the domain. A dash indicates amino acids identity. The percentage represents the degree of resemblance with the OM amino acid sequence as a reference.

fragment from the cDNA clone pGEM7Z(+)) as probe.

The polypeptide (250 amino acids) encoded by *om1* has a calculated molecular mass of 28774 Da and exhibits 80.8%, 78.6% and 57.3% amino acid identity to FBP2, TM and AgL4 respectively [1, 9, 6]. A more significant homology could be observed in the N-terminal region when the first 56 amino acid residues of the O-MADS box were aligned to all the other presumptive DNA binding domains listed in Fig. 2. The OM central domain (57–150), when compared with the equivalent regions of its homologues, shows only moderate homology whereas their C-terminal regions (151–250) vary distinctively (data not shown). The sole potential calmodulin-dependent phosphorylation site (RQVT) is perfectly conserved in the *om1* gene whereas the proposed glycosylation site (NKT) is found at amino acid position 164–166. The K box domain (position 109 to 147) is rich in leucine residues and can potentially form an α-helical conformation similar to the leucine zipper motif [1]. It is also interesting to note that, unlike *agl-2* and *agl-4* which have an unusually long 5′-untranslated region containing a short ORF, the *om1* gene has only a short untranslated region similar to *tm* genes [6, 9].

The cDNA clone originating from the O-MADS gene has been obtained from a cDNA library raised against mature floral poly(A)$^+$ RNA. Therefore we can conclude that this gene is expressed in flowers. The comparison of expression of the *om1* gene in different organs as well as in different floral development stages is further characterized by RNA gel blot analysis

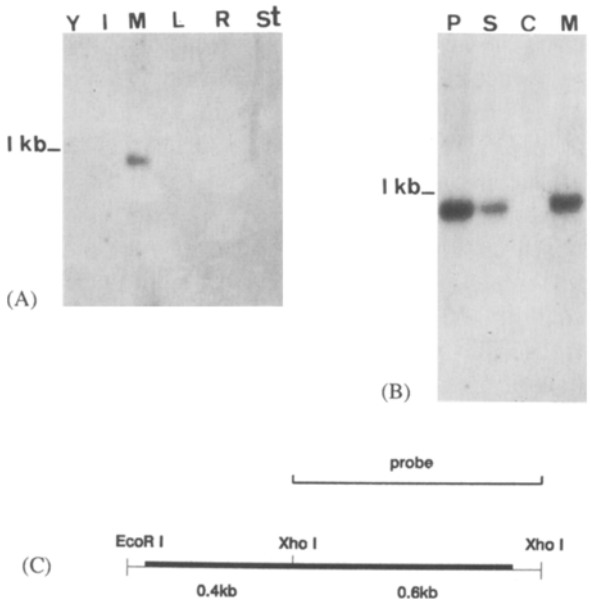

Fig. 3. RNA gel blot analysis of *om1* gene expression in orchid organs. A. Analysis of *om1* gene expression in different organs of orchid. Methods for northern blot analysis were performed as described in Fig. 1. Y, young inflorescence bud; I, intermediate flower; M, mature flower; L, leaf; R, root; St, stem. B. Localization of *om1* gene expression pattern within different organs of orchid mature flowers. 8 μg of poly(A)$^+$ RNA prepared from different floral organs were used in each lane. RNA was fractionated by 1.5% denaturing agarose gel, transferred onto hybond filter and probed with a gel-purified *Xho* I fragment (0.6 kb) which does not contain the MADS box DNA-binding domain. P, petals; S, sepals; C, column; M, mature flowers. C. Diagram of the *om1* cDNA clone and its restriction map. Probe used for northern analysis is indicated.

and the result is presented in Fig. 3. Poly(A)$^+$-enriched RNA (8 μg) prepared individually from young inflorescence bud (5 mm), young floral bud (1 cm), mature flowers, leaf, stem and root were fractionated on 1.5% agarose gel containing formamide as denaturant. The RNA was subsequently blotted onto nylon Hybond and challenged with a *oml* cDNA probe lacking the 5′ MADS box to avoid cross-hybridization. A transcript with an estimated size of 1.0 kb was only found in mature flower indicating that the *oml* gene is exclusively expressed in flowers. The failure of detecting the *oml* transcripts in inflorescence bud and in young floral bud (intermediate stage of flower development) implies that the *oml* gene is probably not an early or immediate early gene as we previously expected. Schultz *et al.* have pinpointed that *agamous* gene in *Arabidopsis* functions downstream of the *Flo10* in the flower developmental pathway [8]. To further localize the expression patterns of *oml* gene within different floral organs, poly(A)$^+$ RNA isolated from petals, sepals and columns (fused structure of stamens and style) were analysed by northern blot hybridization. The *oml* transcripts were only detectable in petals and sepals, but not in columns. In petunia flowers, however, the MADS box *fbp2* transcripts were barely detected in sepals [1]. Sepals of orchid flower are morphologically similar to the petals. The high level expression of *oml* transcripts in orchid petals and sepals may imply involvement of *oml* gene in petal development.

MADS box genes isolated from dicot species are shown to constitute a large gene family. In the present study, only one MADS box clone (*oml*) was isolated from the cDNA library of mature flowers. Additional clones of MADS box genes could be obtained if cDNA libraries of other stages of flowers were screened. Whether the monocotyledons also exhibit multiple-gene family organization still remains to be studied.

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