計畫類別：個別型計畫
計畫編號：NSC93-2311-B-002-024-
執行期間：93年08月01日至94年07月31日
執行單位：國立臺灣大學生態學與演化生物學研究所
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報告類型：精簡報告
處理方式：本計畫可公開查詢

中華民國94年 0月 0日
Abstract

*Mussaenda pubescens* is a plant with enlarged and petaloid calyx lobe, named calycophylls, they are phenotypically similar to true petals in having bright color and papillate epidermal cells. We proposed the petaloid structure were formed by the ectopic expression of genes that specifying petal organ identity, but our preliminary data suggested that there might be unknown factors involved other than floral ABE genes. In the first year of the three-year project, we have successfully identify the full-length sequences of one A, three B, and one E class genes. We have also successfully constructed clones for yeast-2-hybrids. The RNAs were extracted and ready for cDNA library construction and expression analysis.

**Keywords:** *Mussaenda pubescens*, floral organ identity genes, protein-protein interactions
Whitlock, 1999). The project's rationale is to apply this model to the petaloidy regulation in *Mussaenda pubescens*.

In our previous survey, we have successfully identified A, B, and E class genes from *M. pubescens*, namely *MupAP1* (A class homologue); *MupDEF, MupGLO, MupTM6* (all B class homologues); and *MuSEP* (E class homologue). The expression patterns based on RT-PCR results suggest that B and E class genes are expressed in the normal sepals as well as calyphylls. The only exception is *MupGLO*, its transcript is not detected in calyphylls and very weak in normal sepals. The complex expression pattern is intriguing and indicates that the formation of calyphylls might correlate with *MupDEF* and *MupTM6* expression, both these two genes are homologues to AP3 of *Arabidopsis*. These floral gene products needed to form a multimer complex in order to function properly, and different functions are likely carried out by combinations of different proteins (Theißen and Saedler, 2001, Theißen, 2001). The preliminary results prompted us to propose a thorough screening of candidate proteins that interact with *MupDEF, MupTM6* and/or *MupSEP*, in order to identify the unknown factors that are responsible for the petaloid structure.

三、研究方法

(1) Sample collections and RNA isolation

Plant materials of *Mussaenda pubescens* have been collected from the populations at Shu-Mei-Pin (Taipei Co.) and Kenting (Pingtung Co.). Total RNAs were isolated from different organs of *Mussaenda pubescens* by Pine Tree method (Chang et al. 1993).

(2) Full-length open reading frame of cDNAs

Since previously identified ABE genes were obtained through 3’ RACE (Rapid Amplification of cDNA Ends, Invitrogen, Life technologies, Carlsbad, CA, USA), specific primers were designed at K domain region of previously identified ABE genes for 5’ RACE (Rapid Amplification of cDNA Ends, Invitrogen, Life technologies, Carlsbad, CA, USA) reaction in order to obtain 5’ region of the genes. The 5’ RACE products were then cloned into pGEM®-T vector (Promega, Madison, WI, USA), and determined the sequences. New specific primers at the 5’ end of the genes were then designed to amplify full-length cDNAs in conjugated with 3’ specific primers for PCR. The products were then cloned again into pGEM®-T vector (Promega, Madison, WI, USA), and determined the sequences.

(3) Construction of clones for yeast-2-hybrid

The cDNAs were constructed into the lambda vector using the HybriZAP 2.1® two-hybrid system, into pAD-GAL4 and pBD-GAL4 following the manufactury's suggestions (Stratagene, La Jolla, CA, USA). New primers were designed to include a *Eco*RI or *Sal*I overhead at their 5’ (Table 1 and Fig. 1) for amplifying open reading frame of cDNAs, and then cloned into pAD or pBD vectors and transformed into *E. coli*. Colony PCR were used to confirm the identity of these clones.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequences (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MuAP1_Eco</td>
<td>ggAATTCATggggAgAggAAAg</td>
</tr>
<tr>
<td>MuAP1_Sal</td>
<td>ACgCgTCgACgggCTTTATCAggC</td>
</tr>
<tr>
<td>MuTM6_Eco</td>
<td>ggAATTCATgggTCgTgggAAAg</td>
</tr>
</tbody>
</table>

Table 1. Primer design for yeast-2-hybrid constructs
MuTM6_Sal  ACgCgTCgACCATgCAATAggATg
MuGLO_Eco  ggAATTCATTgggAgAggTAAg
MuGLO_Sal  ACgCgTCgACgTTCTTgggTAgAgC
MuDEF_Eco  ggAATTCATggCTCggTgggAAg
MuDEF_Sal  ACgCgTCgACgTAAATAAATTTGCTAC
MuSEP_Eco  ggAATTCATggAAgAggTAAg
MuSEP_Sal  ACgCgTCgACgTATCATggTAACC

Fig. 1. Cloning strategy for yeast-2-hybrid constructs.

1. Full length cDNA construction
We have obtained full-length cDNA sequences of all five genes (1 A, 3 B, and 1 C class genes), and have them cloned into pGEM-T vectors. No new homologues were found during 5' RACE or full-length cDNA PCR amplification after examining the sequence alignments.

2. Clone constructs for yeast-2-hybrid
We have obtained full-length cDNA clones of all five genes in either pAD-GAL4 or
pBD-GAL4 vectors, except for MuDEF (Table 2). The sequence of the MuDEF clone contained a stop codon, and was different from cDNA sequences we had, therefore, needed to be re-amplified. The sequences of all of the clones were examined to prevent any errors at every cloning step. We are designing another sets of clones that only include IKC domains of these genes, i.e. excluding MADS-box domain. This is to eliminate a possible auto-regulation effect of these constructs (Elena Kramer, personal communication).

Table 2. A check-list for the obtained constructs. Checked marks () are correct clones we had. Triangles are not finished cloning, whereas the cross marks mean a wrong sequence was obtained as explained in the text.

<table>
<thead>
<tr>
<th>Insert</th>
<th>Ligation &amp; Transformation</th>
<th>Colony PCR</th>
<th>Plasmid extraction</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD-MuAP1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>BD-MuAP1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>AD-MuDEF</td>
<td>Δ</td>
<td>Δ</td>
<td>Δ</td>
<td>x</td>
</tr>
<tr>
<td>BD-MuDEF</td>
<td>Δ</td>
<td>Δ</td>
<td>Δ</td>
<td>x</td>
</tr>
<tr>
<td>AD-MuGLO</td>
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<td>✓</td>
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<td>✓</td>
</tr>
<tr>
<td>BD-MuGLO</td>
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<td>✓</td>
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<td>✓</td>
</tr>
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<td>AD-MuTM6</td>
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<td>✓</td>
</tr>
<tr>
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</tr>
<tr>
<td>BD-MuSEP</td>
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</table>

五、参考文献


