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玉葉金花花部器官決定基因之功能與蛋白質交互作用研究

(1/3)

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一、中英文摘要

毛玉葉金花（*Mussaenda pubescens*）是台灣低海拔中常見的植物之一。玉葉金花屬植物常可以見到花萼其中的一個裂片尖端膨大成一個外形類似葉子、但顏色為白色或紅色的構造，稱為葉狀萼片（calycophylls, 或 floral semaphylls）。而玉葉金花名稱的由來，即源於它的花序中黃色的花瓣和白色的葉狀萼片而來。這些葉狀萼片不管在細胞形狀或細胞排列上，都與正常的花瓣類似，加上有著顯著的顏色，一般皆認為這些葉狀萼片在功能上與花瓣無異，我們稱之為類花瓣(petaloid)的構造。我們在這個三年期的研究計畫中，期望藉由花部基因的選殖與功能性的研究，希望瞭解有關花瓣與類花瓣調控的各類因子。在第一年的研究中，我們順利的取得了毛玉葉金花中一個 A 群，三個 B 群，以及一個 E 群基因的全長序列，並構建了 Yeast-2-hybrid 所需的各個 clones。另外也完成毛玉葉金花的各部器官的 RNA 萃取，俾後續的 cDNA library 建置，以及 RNA 表現分析。

關鍵詞：玉葉金花，花部器官決定基因，蛋白質交互作用

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 papilliate 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

二、前言與研究目的

The majority of flowering plants uses petals as the attractant agent for luring pollinators. However, there are many examples that use non-petal organs to mimic showy petals, and thus called "petaloid structures". In order to elucidate the possible genetic mechanism for the formation of petaloid structures, we selected *Mussaenda pubescens* as our working system. *M. pubescens* has an enlarged and petaloid sepals named "calycophylls" on the peripheral flowers of a inflorescens. The calycophylls have vivid white color and loose mesophylls, and have papilliate epidermis, all typical features of a true petal. Based on the current floral genetic model, petals are determined by combination of three classes of genes, A (e.g. *AP1* and *AP2*), B (e.g. *PI* and *AP3*), and E (*SEP*s). In *Arabidopsis* studies, overexpression a combination of *AP1*, *AP3*, *PI*, and *SEP* showed an interesting phenotype that converts all its leaves into petals (Honma and Goto, 2001). Theses results suggest that, petaloid structures could be regulated by ectopic expression of petal identity genes, as proposed by several authors (Bowman, 1997; Albert et al., 1998; Baum, 1998; Baum and

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 calycophylls. The only exception is *MupGLO*, its transcript is not detected in calycophylls and very weak in normal sepals. The complex expression pattern is intriguing and indicates that the formation of calycophylls 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 (Theiβen and Saedler, 2001, Theiβen, 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 manufactory'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 1. Primer design for yeast-2-hybrid constructs

Primer name	Sequences（5'→ 3'）
MuAP1_Eco	ggAATTCATggggAgAggAAAg
MuAP1_Sal	ACgCgTCgACgggCTTTATCAggC
MuTM6_Eco	ggAATTCATgggTCgTgggAAg

MuTM6_Sal	ACgCgTCgACCATgCAATAggATg
MuGLO_Eco	ggAATTCATggggAgAggTAAg
MuGLO_Sal	ACgCgTCgACgTTCTTgggTAgAgC
MuDEF_Eco	ggAATTCATggCTCgTgggAAg
MuDEF_Sal	ACgCgTCgACgTAAATAAATTTGCTAC
MuSEP_Eco	ggAATTCATgggAAgAggTAgg
MuSEP_Sal	ACgCgTCgACgTATCATggTAACC

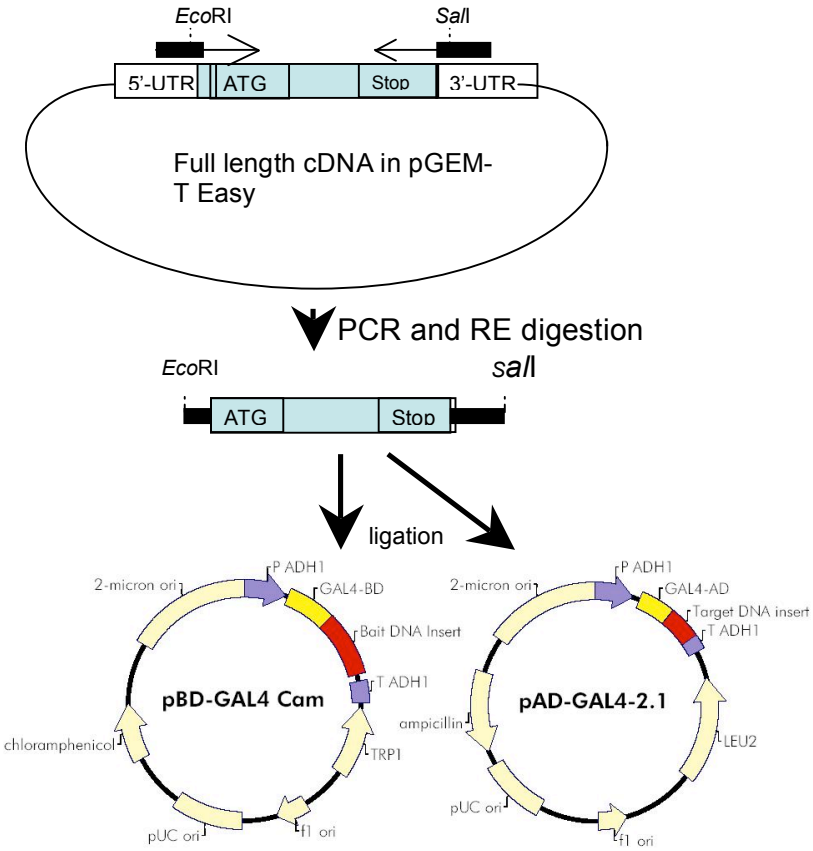

Fig. 1. Cloning strategy for yeast-2-hybrid constructs.

四、研究結果與討論

(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.

	Insert	Ligation & Transformation	Colony PCR	Plasmid extraction	Sequence
AD-MuAP1	✓	✓	✓	✓	✓
BD-MuAP1	✓	✓	✓	✓	✓
AD-MuDEF	Δ	Δ	Δ	Δ	×
BD-MuDEF	Δ	Δ	Δ	Δ	×
AD-MuGLO	✓	✓	✓	✓	✓
BD-MuGLO	✓	✓	✓	✓	✓
AD-MuTM6	✓	✓	✓	✓	✓
BD-MuTM6	✓	✓	✓	✓	✓
AD-MuSEP	✓	✓	✓	✓	✓
BD-MuSEP	✓	✓	✓	✓	✓

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