APPENDIX A

MADS Box Genes

Most plant homeotic genes belong to a class of related genes known as MADS box genes. The MADS box is a highly conserved sequence motif found in a family of transcription factors. The acronym **'MADS'** is derived from the first letter of the first discovered members of this class of genes

1) **M**CM1 – a yeast gene which encodes a transcription factor necessary for mating type determination

2) **A**GAMOUS/**D**EFICIENS – floral organ identity genes

3) Mammalian serum response factor (**S**RF) is found to induce the transcription of proto-oncogenes.

Many genes known to determine floral organ identity are MADS box genes such as DEFICIENS gene of *Antirrhinum* (Snapdragon) and AGAMOUS, APETALA1 and APETALA3 of *Arabidopsis*. MADS box genes encode proteins that function as transcription factors but they differ among themselves in structure. The MADS box genes share a characteristic conserved nucleotide sequence known as the MADS box, which encodes a protein structure known as MADS domain. The MADS domain enables these transcription factors to bind to a region of DNA with a specific nucleotide sequence. Such binding causes the transcription of the gene in the immediate vicinity. Proteins that bind to specific DNA sequences and act as 'switches' to 'turn on' or induce the expression of genes are called transcription factors.

(Sources: Datta and Das, 2002b)

APPENDIX B

The ABC model of organ identity determination

The basic structure of a complete flower consists of four concentric whorls (Figure1). A simple model has been proposed to predict organ formation in flowers, where three classes of homeotic genes, so-called ABC-class genes, act alone or together to give rise to sepals (A), petals (A+B), stamens (B+C), and carpels (C).

Figure 1

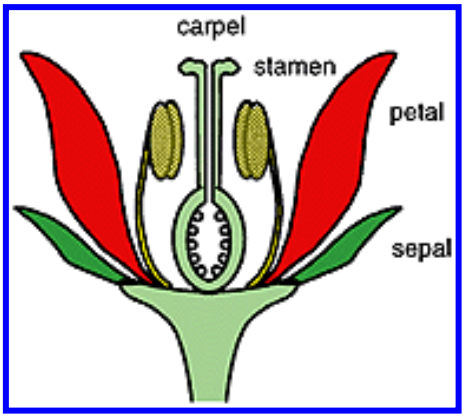
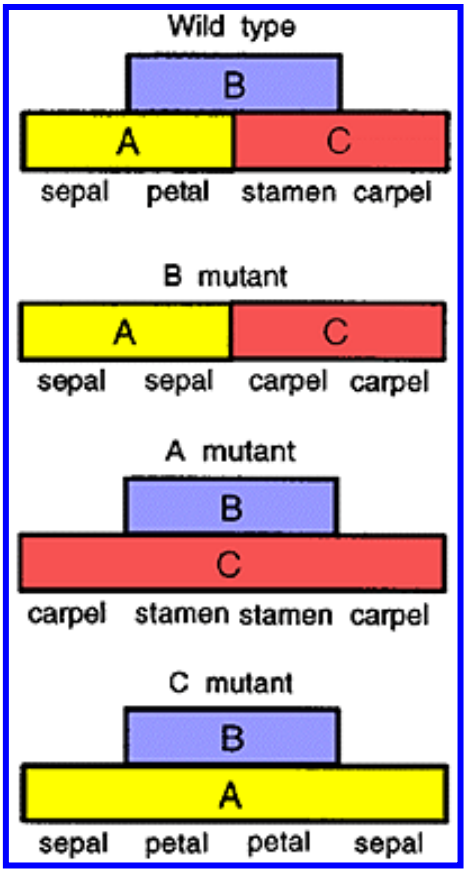


Figure 2

According to the ABC-model (Figure 2), organ determination in the whorls depends on the combinatorial action of three regulatory functions. A mutation disrupting one of the functions causes a homeotic change in organ identity. Note that the A and C functions are negatively regulating each other: Mutation in one causes expansion in the expression domain of the other.



APPENDIX C

Vacin and Went Medium (VW; Vacin and Went, 1949)

Item number	Component	Amount per liter of culture medium (final concentration in culture medium)
	Macroelements	
1.	Tricalcium phosphate, $Ca_{3(}PO_4)_2$	200 mg
2.	Potassium nitrate, KNO_3	525 mg
3.	Potassium phosphate, KH_2PO_4	250 mg
4.	Magnesium sulfate, $MgSO_4.7H_2O$	250 mg
5.	Ammonium sulfate, $(NH4)_2SO_4$	500 mg
	Microelement	
6.	Manganese sulfate, $MnSO_4.H_2O$	6.8 mg
	Iron	
7.	Ferrous sulfate, $FeSO_4.7H_2O$	27.8 mg
8.	Disodium ethylene diaminetetraacetate, Na_2EDTA	37.3 mg
	Sugar	
9.	Sucrose	20 g
	Complex additive	
10.	Coconut water	150 ml
11.	Water, distilled	to 1000 ml

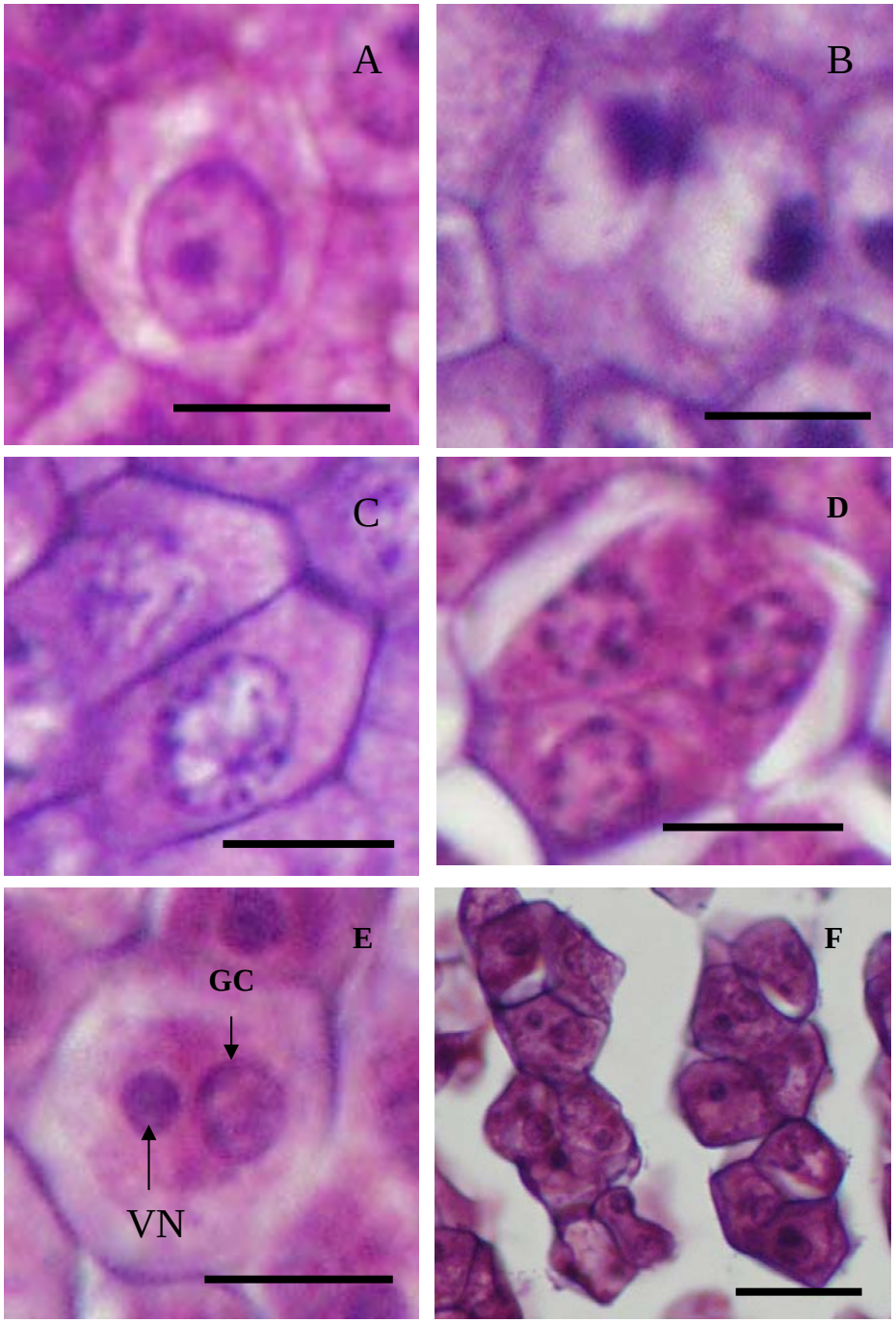
APPENDIX D

Knudson C medium (KC; Knudson, 1946)

Item number	Component	Amount per liter of culture medium (final concentration in culture medium)
	Macroelements	
1.	Calcium nitrate, $Ca(NO_3)_2.4H_2O$	1 g
2.	Ammonium sulfate, $(NH_4)_2SO_4$	500 mg
3.	Magnesium sulfate, $MgSO_4.7H_2O$	250 mg
4.	Potassium phosphate, KH_2PO_4	250 mg
5.	Ferrous sulfate, $FeSO_4.7H_2O$	250 mg
	Microelement	
6.	Manganese sulfate, $MnSO_4.4H_2O$	5.6 mg
	Sugar	
7.	Sucrose	20 g
	Complex additive	
8.	Coconut water	150 ml
9.	Water, distilled	to 1000 ml

APPENDIX E (FIGURE)

Pollen grain development of *D. crumenatum* from the induction day to anthesis



APPENDIX E (CONTINUED)

Pollen grain development of *D. crumenatum* was observed in nature. It was examined from the induction day (d0) to the day of flower blooming (d9). Cell division occurred synchronously. Uninucleate microsporocyte or pollen mother cell at a premeiotic stage was observed from d0 to d2 (A). The first-meiotic division of telophase I was discerned on d3 (B). Dyad, resulting from the end of meiosis I, and the beginning of the second meiotic division at the early prophase II appeared on d4 (C). Tetrad was completely formed following the mitotic division of nucleus on d5 (D). The two-celled stage pollen grain (PG) indicating PG maturity was first recorded on d6 (E). It showed the vegetative nucleus (VN) and the generative cell (GC) lying in the cytoplasm of the vegetative cell. A group of PGs, known as pollinia, adhered together and remained together until anthesis. Mass of bi-nucleate pollen grains was shed in early morning of d9 (F). A-E; Bar =10 μm, F; Bar = 20 μm

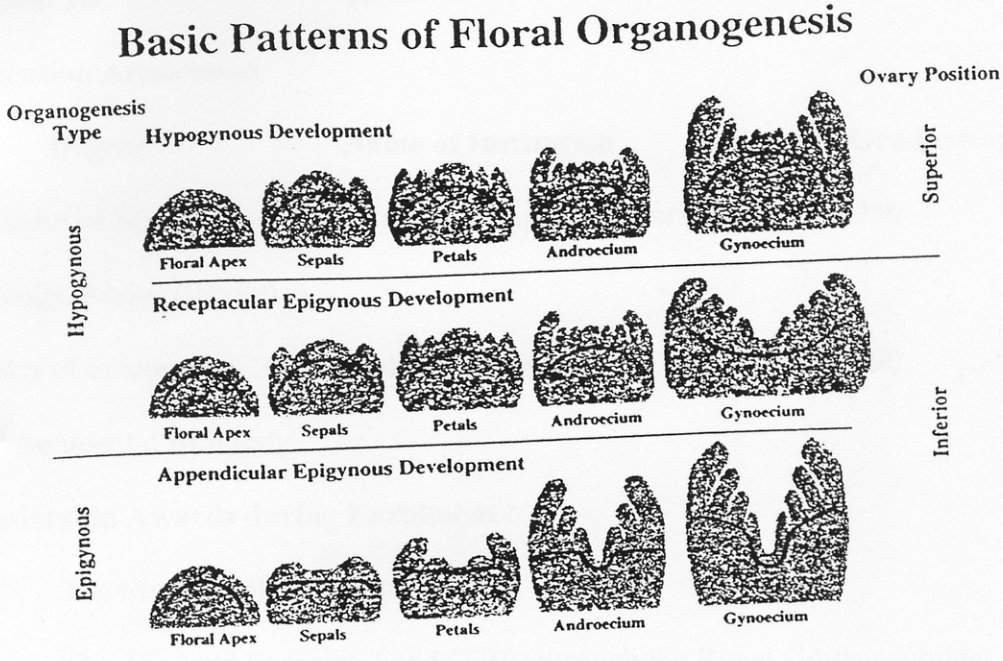
APPENDIX F

Table 5 Details of a typical pattern of temperature variation throughout the day on induction days and non-induction days. Data of temperature changes was automatically recorded every 1 hr using a data logger (Hobo Proseries).

	Temperature (°C) on a day				Temperature drop after peak (mean)		Decreasing of	Flowering
	Minimum.	Maximum	Maximum-Minimum	Mean	1 hr. after	2 hr. after	temperature	on the 9th day
Temperatures on induction days (day 0) , 9 days before flower blooming								
The 1st flowering flush	24.79	34.01	9.22	27.43	6.44	7.28	markedly	YES
The 2nd flowering flush	25.60	37.40	11.80	27.82	7.40	9.90	markedly	YES
The 3rd flowering flush	24.40	37.00	12.60	27.30	7.90	9.88	markedly	YES
The 4th flowering flush	26.00	38.80	12.80	28.13	8.90	11.70	markedly	YES
					(7. 66)	(9. 69)		
Representative temperatures on non-induction days throughout the year (2001)								
January	26.63	30.31	6.68	26.36	1.21	1.61	gradually	NO
April	25.17	39.67	14.50	30.85	0.45	2.67	gradually	NO
July	25.56	34.01	8.45	28.95	1.67	0.42	gradually	NO
October	23.63	34.85	11.22	26.69	4.09	6.07	gradually	NO
					(1. 86)	**(2. 69)**		

APPENDIX G

Basic pattern of floral organogenesis



Hypogynous flowers generally maintain a convex floral apex through floral organogenesis. **Receptacular-epigynous** flowers have a similar pattern of growth through gynoecial initiation. However, after gynoecial initiation, the periphery of the floral apex expands and rises, generating a basin in the center of the floral apex. **Appendicular-epigynous** flowers also begin floral organogenesis with a convex floral apex, however, during perianth initiation, the floral apex flattens and before or during androecial initiation a concavity is created in the center of the floral apex, due to floral up growth of the floral cup. Subsequently, the gynoecial primordial are initiated on the flanks of this concavity. (source: Kuzoff *et al.*, 2001)

List of Publications and Proceeding

1. Meesawat, U. and Kanchanapoom, K. 2001. Morphological changes during floral development of pigeon orchid (*Dendrobium crumenatum* Sw.). Proceeding of the XVIII Annual conference on electron microscopy, p72-73. January, 17-19, 2001, Khonkean, Thailand. *J.E.M.S.T.* 15(1): 72-73.
2. Meesawat, U. and Kanchanapoom, K. 2002. *In vitro* plant regeneration through embryogenesis and organogenesis from callus culture of pigeon orchid (*Dendrobium crumenatum* Sw.). *Thammasart Int. J. Sc. Tech.* 7(2): 9-17.
3. Meesawat, U. 2003. The shoot apical meristem (SAM). *Suranaree J. Sci. Technol.* 10: 244-250.
4. Meesawat, U. and Kanchanapoom, K. 2006. *In vitro* floral bud formation in the pigeon orchid (*Dendrobium crumenatum* Swartz). *Thai J. Agri. Sc.* (accepted)