Plant Cell Physiol. 43(1): 52–57 (2002)

Ectopic Expression of *SUPERMAN* Suppresses Development of Petals and Stamens

Jae-Young Yun [1], Detlef Weigel [3, 4] and Ilha Lee [1, 2, 3, 5]

[1] *School of Biological Sciences, Seoul National University, Seoul, 151-742 Korea*
[2] *Plant Metabolism Research Center, Kyung Hee University, Suwon, 449-701 Korea*
[3] *Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, CA 92037, U.S.A.*
[4] *Department of Molecular Biology, Max Planck Institute for Developmental Biology, D-72076 Tübingen, Germany*

The floral regulatory gene *SUPERMAN* (*SUP*) encodes a C2H2 type zinc finger protein that is required for maintaining boundaries between floral organs in Arabidopsis. It has been proposed that the main function of *SUP* is to balance cell proliferation in the third and fourth whorl of developing flowers, thereby maintaining the boundaries between the two whorls. To gain further insight into the function of *SUP*, we have ectopically expressed *SUP* using the promoter of *APETALA1* (*AP1*), a gene that is initially expressed throughout floral meristems and later becomes restricted to the first and second whorls. Flowers of *AP1::SUP* plants have fewer floral organs, consistent with an effect of *SUP* on cell proliferation. In addition, the *AP1::SUP* transgene caused the conversion of petals to sepals and suppressed the development of stamens. The expression of the B function homeotic gene *APETALA3* (*AP3*) and its regulator *UNUSUAL FLORAL ORGANS* (*UFO*) were delayed and reduced in *AP1::SUP* flowers. However, *SUP* does not act merely through *UFO*, as constitutive expression of *UFO* did not rescue the defects in petal and stamen development in *AP1::SUP* flowers. Together, these results suggest that *SUP* has both indirect and direct effects on the expression of B function homeotic genes.

Key words: B function — Cadastral gene — Cell proliferation — Flower development — SUPERMAN.

Introduction

The Arabidopsis flower consists of four organ types, sepals, petals, stamens, and carpels, which are arranged in a series of concentric rings or whorls. The specification of floral organ identity in the different whorls is explained by the ABC model, according to which three classes of homeotic genes, A, B, C each act in two adjacent whorls to control organ identity (Bowman et al. 1991, Coen and Meyerowitz 1991, Weigel and Meyerowitz 1994). Class A genes, which include *APETALA1* (*AP1*) and *APETALA2* (*AP2*), are required for the development of sepals and petals; class B genes, which include *APETALA3* (*AP3*) and *PISTILLATA* (*PI*), are required for petals and stamens; and the class C gene *AGAMOUS* (*AG*) is required for stamens and carpels (Bowman et al. 1989, Bowman et al. 1991, Bowman et al. 1993, Kunst et al. 1989, Irish and Sussex 1990). More recently, additional factors required for A, B, and C functions have been discovered (Alvarez and Smyth 1999, Byzova et al. 1999, Conner and Liu 2000, Pelaz et al. 2000).

How expression of ABC genes, which is largely controlled at the transcriptional level, is regulated has been subject to several studies. A crucial factor in activating ABC genes is the floral identity gene *LEAFY* (*LFY*), which regulates the three classes of homeotic genes through three genetically distinct mechanisms (Parcy et al. 1998). *LFY* encodes a DNA-binding transcription factor that directly regulates expression of the A function gene *AP1* and the C function gene *AG* (Parcy et al. 1998, Busch et al. 1999, Wagner et al. 1999, Lohmann et al. 2001). It is not known whether LFY directly regulates B function genes as well, but it has been shown that LFY acts together with the F-box protein UNUSUAL FLORAL ORGANS (UFO) to activate B function genes (Levin and Meyerowitz 1995, Wilkinson and Haughn 1995, Lee et al. 1997, Parcy et al. 1998, Samach et al. 1999, Honma and Goto 2000). In contrast to *LFY*, which is expressed throughout the young flower (Parcy et al. 1998), *UFO* is expressed in a discrete pattern in both shoot and floral meristems, and region-specific expression is required for normal expression of *AP3* and *PI* (Lee et al. 1997, Samach et al. 1999).

Another regulator of B function genes is *SUPERMAN* (*SUP*), which was originally thought to be simply a repressor of *AP3* and *PI*, because their expression domains are expanded in *sup* mutants (Schultz et al. 1991, Bowman et al. 1992). *SUP* has therefore been termed a cadastral gene (Bowman et al. 1992). Molecular analysis of *SUP* revealed, however, that *SUP* is expressed in the third whorl, and that *SUP* expression is largely dependent on B function genes as early *SUP* expression is decreased and late *SUP* expression is not detected in *ap3* and *pi* mutants (Sakai et al. 1995, Sakai et al. 2000). These findings led to the conclusion that the main function of *SUP*, which encodes a C2H2 type transcription factor, is to maintain the boundary between whorls three and four, by regulating the balance of cell proliferation in these two whorls.

To further study the interaction between B function genes and their regulators *SUP* and *UFO*, we have generated trans-

[5] Corresponding author: E-mail, ilhalee@plaza.snu.ac.kr; Fax, +82-2-872-1993.

Table 1 Numbers of floral organs in *AP1::SUP* transgenic plants

		Wild type	Strong	Intermediate	Weak
First whorl	Sepal	4.0±0.0	1.5±0.3	3.7±0.5	4.0±0.0
	Filament		0.2±0.1	0	0
	Ca/Se mosaic		1.5±0.2	0	0
Second whorl	Petal	4.0±0.0	0	0	2.5±0.5
	Sepal		0	2.0±0.3	0
	Pe/Se mosaic		0.0±0.1	0.1±0.2	0.3±0.2
Third whorl	Stamen	5.9±0.2	0	0	1.0±0.3
	Pe/St mosaic		0	0	0.3±0.2
	Filament		0	0.1±0.1	0.1±0.2
	St/Ca mosaic		0	0	0.4±0.2
Fourth whorl	Carpel	2.0±0.0	0	1.6±0.2	1.8±0.2
	Ca/Se mosaic		0	0.7±0.1	0.2±0.1
	Carpel-like		0	0.5±0.2	0.3±0.1
Total organ number		15.9	3.2	8.7	10.9

Total of five representative plants from transgenics that showed strong, intermediate, weak phenotype were chosen respectively. Twenty basal most flowers from individual plants were used for organ number counting. Carpel-like structure has residual amount of stigmatic tissues on top of filamentous structure. Abbreviations: Se, sepal; Pe, petal; St, stamen; Ca, carpel.

genic Arabidopsis plants that express *SUP* ectopically from the *AP1* promoter. Although two previous studies have examined the consequences of ectopic *SUP* expression (Kater et al. 2000, Nandi et al. 2000), both studies used heterologous species. Our results in Arabidopsis differ from those previous studies, and reveal for the first time that SUP can repress B function both directly and indirectly most likely through its effect on cell proliferation.

Results

Ectopic expression of SUP from the AP1 promoter causes floral defects

The *AP1* gene is expressed from stage 1 throughout the entire floral meristem. *AP1* RNA expression is maintained in the outer two whorls, but subsides in the inner two whorls three and four (Mandel et al. 1992). In contrast to the endogenous gene, activity of the *AP1* promoter persists in the central whorls after stage 3, suggesting that elements repressing *AP1* are located downstream of the initiation codon (Hempel et al. 1997, M. Yanofsky, personal communication). We therefore chose the *AP1* promoter to express *SUP* ectopically throughout the young flower. We produced 47 transgenic *AP1::SUP* lines; none of them showed any defect during vegetative development.

AP1::SUP plants had several floral defects, especially in the number of floral organs (Table 1). According to the severity of the phenotype, *AP1::SUP* plants were classified into three groups. Twelve *AP1::SUP* lines had a strong phenotype, and produced flowers that lacked most organs. They typically produced fewer than 2 sepals, and fewer than 2 carpels or carpel-like structures. Seventeen *AP1::SUP* lines had an intermediate phenotype. Organ number was decreased in whorls two and three, with almost all third-whorl organs missing. Organ number in whorl one was more variable than in wild type, and organ number in the fourth whorl was increased. The latter was likely due to a transformation of third-whorl stamens into carpels, which then fused with the fourth-whorl carpels, as frequently seen in *ap3*, *pi*, or *ufo* mutant flowers (Bowman et al. 1989, Bowman et al. 1991, Levin and Meyerowitz 1995, Wilkinson and Haughn 1995). Second-whorl petals were converted into sepals. Infrequently, filamentous organs were found in the third whorl. Eighteen *AP1::SUP* lines had a weak phenotype. The first whorl was normal, but second and third whorl organs were reduced in number. Some third-whorl stamens were converted into stamen/carpel mosaic organs. As with intermediate lines, the fourth whorl gynoecium was often abnormal, and included some carpel-sepal mosaic organs. The morphology of mature flowers in representative transgenic plants is shown in Fig. 1.

AP3 and UFO expressions are delayed and reduced in AP1::SUP

The lack of petals and stamens, along with the homeotic conversion of petals into sepals and stamens into carpels, suggested a reduction in B function, which specifies petals and stamens. To investigate the molecular basis of these phenotypes, we analyzed one intermediate transgenic line, IL25.213, in more detail. Because *AP3* and *UFO* are necessary for the development of petals and stamens and it has been proposed that they have a role in cell proliferation (Samach et al. 1999,

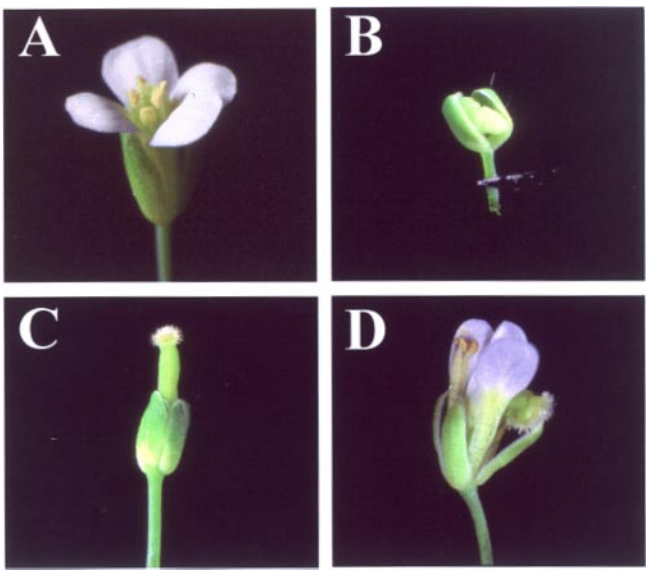

Fig. 1 Flower morphology of *AP1::SUP* in Arabidopsis. (A) Wild type. (B–D) Representative flowers from transgenic plants with strong (B), intermediate (C), and weak (D) phenotype.

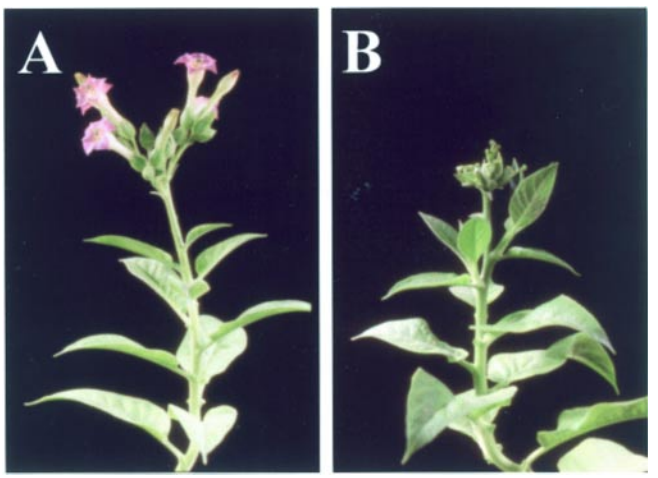

Fig. 3 Inflorescences of *AP1::SUP* in tobacco. (A) Wild type. (B) *AP1::SUP*. *AP1::SUP* flowers show relatively normal sepals but are lack of petals and stamens. The number of carpels is increased and the length of style is reduced in *AP1::SUP* flowers.

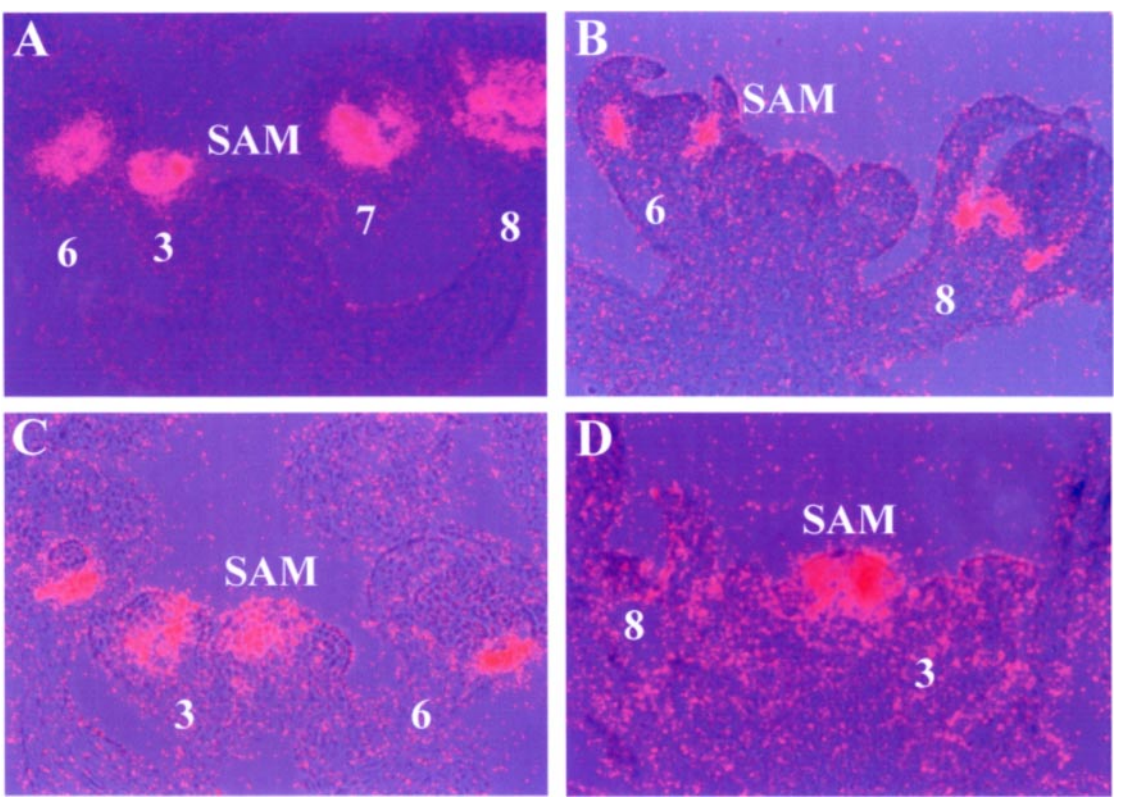

Fig. 2 RNA expression of *AP3* and *UFO* in wild-type and *AP1::SUP* inflorescences. (A) and (C) wild type. (B) and (D) *AP1::SUP*. *AP3* expression is shown in (A) and (B) and *UFO* expression in (C) and (D). SAM, shoot apical meristem. Numbers indicate floral stages (Smyth et al. 1990).

Table 2 Effect of *35S::UFO* on floral organ numbers in *AP1::SUP*

		AP1::SUP	*AP1::SUP 35S::UFO*
First whorl	Sepal	4.0±0.2	3.8±0.3
Second whorl	Petal	2.5±1.0	2.4±0.6
	Sepal	0	0
	Pe/Se mosaic	0.6±0.3	0.2±0.1
Third whorl	Stamen	0	0
	Filament	0.1±0.1	0
Fourth whorl	Carpel	3.1±0.7	2.5±0.6
	Carpel-like	0.1±0.1	0.7±0.4

AP1::SUP line IL25.213 and *35S::UFO* line DW229.5.3 were used. Five T2 plants from IL25.213, and five doubly transgenic plants were examined. Twenty basal-most flowers from individual plants were used for organ number counting. Carpel-like structure has residual amount of stigmatic tissues on top of filamentous structure. Abbreviations: Se, sepal; Pe, petal; St, stamen; Ca, carpel.

Sakai et al. 2000), we analyzed the expression of *AP3* and *UFO* in IL25.213 by in situ hybridization (Fig. 2). In wild type, *AP3* is detected from floral stage 3 in second- and third-whorl primordia, where it persists until late stages of development (Jack et al. 1992) (Fig. 2A). In contrast, *AP3* expression is not detected in IL25.213 flowers until about floral stage 6 (Fig. 2B). *AP3* RNA is detected after floral stage 6, but it remains confined to the margins of second-whorl primordia. Occasionally, *AP3* is detected at the base of third-whorl primordia in IL25.213. Therefore, *AP3* expression is delayed and the expression domain is reduced in IL25.213. The delayed and reduced expression of *AP3* in *AP1::SUP* are consistent with the phenotype of the mature flowers.

Because *UFO* is an upstream regulator of *AP3* (Lee et al. 1997, Samach et al. 1999), we also analyzed *UFO* expression in IL25.213. In wild type, *UFO* is strongly expressed in the shoot apical meristem and in floral meristems (Fig. 2C). In flowers, *UFO* expression is dynamic, first being detected in a central domain and then resolving into a cup-shaped domain. After stage 5, it becomes confined to the base of second-whorl primordia (Lee et al. 1997, Samach et al. 1999). As expected, *UFO* expression was unaffected in the shoot apical meristem of IL25.213 (Fig. 2D). In contrast, no *UFO* expression was detected in floral meristems of IL25.213 until at least floral stage 6 (Fig. 2D). After floral stage 6, *UFO* expression was occasionally detected at the base of second and third whorl primordia (data not shown).

35S::UFO does not rescue the B function defects in AP1::SUP

Because both expression of the B function gene *AP3* and its regulator *UFO* is reduced in *AP1::SUP* flowers, we wanted to determine whether constitutive expression of *UFO* could rescue some of the floral defects in *AP1::SUP*. We therefore crossed IL25.213 plants with transgenic plants that express *UFO* from the constitutive *35S* promoter (Lee et al. 1997). *35S::UFO* flowers have an expanded *AP3* expression domain and show an increase in petal and stamen number, both of which are opposite to the effects seen in *AP1::SUP* plants. Doubly transgenic *AP1::SUP 35S::UFO* plants were identified by PCR genotyping. The phenotype of *AP1::SUP 35S::UFO* flowers was very similar to that of the *AP1::SUP* parental line IL25.213. The flowers had reduced organ number, and petals and stamens were converted into sepals and carpels, respectively (Table 2). Thus, overexpression of *UFO* cannot rescue the defect of B function in *AP1::SUP* plants.

AP1::SUP ablates petals and stamens in tobacco

Genetic engineering for male sterility is a major target of biotechnology. *AP1::SUP* in Arabidopsis produces flowers that lack stamens but have relatively normal carpels. To determine whether targeted misexpression of *SUP* could be used to engineer male sterility in plants other than Arabidopsis, we generated *AP1::SUP* transgenic tobacco plants (*N. tabacum*). Of 11 transgenic tobacco plants, all showed normal vegetative development. Four plants, however, had flowers that showed defects in floral organ development. The first whorl organ, sepals were relatively normal, although the basal part of tube was unfused occasionally. The second and third whorl organs, petals and stamens were absent in the transgenic plants. The identity of fourth whorl organ, carpels were not changed but the number of carpels were increased to four or five instead of two as in wild type. In addition, the length of style was decreased to make a stout gynoecium. The representative transgenic tobacco plant is shown in Fig. 3.

Discussion

In *sup* loss-of-function mutants, the number of third-whorl stamens is increased, while the number of fourth-whorl carpels is decreased (Schultz et al. 1991, Bowman et al. 1992). This phenotype is accompanied by an expansion of the expression domain of the *AP3* (Bowman et al. 1992, Sakai et al. 2000). It has been suggested that the expansion of the *AP3* domain is merely an indirect consequence of the effect of *SUP* on cell

proliferation in the third and fourth whorls (Sakai et al. 1995, Sakai et al. 2000). Consistent with effects of *SUP* on cell proliferation that are independent of B function gene activities, null mutations in the B function genes *AP3* and *PI* are not epistatic to *sup* mutants (Sakai et al. 2000).

We have shown that overexpression of *SUP* in the flower reduces organ number, indicating an instructive role of *SUP* in controlling cell proliferation. However, if *SUP* affected B function only through its effects on cell proliferation, one would expect that the *SUP* gain-of-function phenotype is at most the opposite of the *sup* loss-of-function phenotype, namely increase in carpel number at the expense of other floral organs. In contrast to this prediction, we have found that ectopic expression of *SUP* can also convert petals into sepals, which is accompanied by a delay and reduction in *AP3* expression.

Further evidence for a rather direct interaction between *SUP* and B function genes comes from the observation that *AP1::SUP* affected the second and third whorls, in which B function genes are normally expressed, more strongly than the first and fourth whorls. A third argument for a relatively direct effect of *SUP* on B function genes comes from the observation that overexpression of *UFO* did not rescue the *AP1::SUP* defects, even though *UFO* expression is reduced in *AP1::SUP* flowers.

Two previous studies have assessed the effects of ectopic *SUP* expression. One of them reported the effects of constitutive expression of Arabidopsis *SUP* in transgenic rice (Nandi et al. 2000). The other study reported the effects of expressing Arabidopsis *SUP* in petunia and tobacco under the control of the promoter from the petunia B function gene *FBP1* (Kater et al. 2000). In rice, an increase of carpel number at the expense of stamens was reported, along with an increase in the number of lodicules, the monocot equivalents of petals. In petunia, no effects on organ identity were reported, but cell expansion was affected. Consistent with the study by Nandi et al. (2000), we found that the most pronounced defect of weak *AP1::SUP* lines was a reduction in organ number. That organ identity effects were only obvious in our intermediate and strong lines suggests that higher activity levels of *SUP* are required for the regulation of B function genes. The differences between our and the study by Nandi et al. (2000) may be due to the fact that Nandi et al. (2000) used a heterologous system, which may reduce the effective levels of *SUP* activity. It is more difficult to reconcile our results in tobacco with those by Kater et al. (2000), but an important difference is that we used the *AP1* promoter, which is active before homeotic genes are activated, while Kater et al. (2000) used the promoter of a B function homeotic gene, *FBP1*. Thus, it is possible that driving *SUP* from the *AP1* promoter is more effective than from the promoter of a B function gene, which is negatively regulated by *SUP*.

Genetic engineering of male sterility is an important target of agrobiotechnology. We tested the feasibility of using *SUP* to eliminate petals and stamens in a heterologous system, tobacco, and found that the expected phenotype, lack of petals and stamens but relatively normal carpels, was found in about a third of *AP1::SUP* tobacco plants. Thus, *AP1::SUP* may be another tool for generating male-sterile plants.

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana, ecotype Columbia, was used in this study. *35S::UFO* transgenic plants have been described (Lee et al. 1997). Plants were grown at 23°C in long days (16 h light) under Cool White fluorescent lights.

Generation of transgenic plants

To generate the *AP1::SUP* vector pIL25, the *SUP* coding sequence (Sakai et al. 1995) was PCR-amplified from Arabidopsis Columbia genomic DNA with oligonucleotide primers 5′-TTG GTA CCA TTG TCA TAC ATA AAA CGG-3′ and 5′-ATA TGG ATC CGG AGA GAT CAA ACA GCA TAG-3′, and linked to the 1.7 kb *AP1* promoter (Hempel et al. 1997). The final construct was in the pCGN1547 binary vector (McBride and Summerfelt 1990). The construct was introduced into Columbia by vacuum infiltration (Bechtold et al. 1993). Transgenic plants were selected on plates with 0.5× MS medium supplemented with 25 μg ml^{-1} kanamycin for 10–12 d before transplanting them to soil. Transgenic tobacco plants were generated using leaf discs of tobacco (*Nicotiana tabacum*) by *Agrobacterium*-mediated transformation as described previously (Horsch et al. 1985).

In situ hybridization

In situ hybridization and synthesis of probes were performed as described (Drews et al. 1991, Lee et al. 1997).

Acknowledgments

We thank M. Yanofsky for communicating unpublished results. Supported by Parson and Aron Foundation fellowships (I.L.), and grant IBN 97–23818 from the National Science Foundation (D.W.). This research was also supported by a grant (code PF003201–01) from Plant Diversity Research Center of 21st Century Frontier Research Program funded by Ministry of Science and Technology of Korean government. J.-Y. Yun was supported by the Brain Korea 21 program.

References

Alvarez, J. and Smyth, D.R. (1999) *CRABS CLAW* and *SPATULA*, two *Arabidopsis* genes that control carpel development in parallel with *AGAMOUS*. *Development* 126: 2377–2386.

Bechtold, N., Ellis, J. and Pelletier, G. (1993) *In planta Agrobacterium* mediated gene transfer by infiltration of adult *Arabidopsis thaliana* plants. *C. R. Acad. Sci.* 316: 1194–1199.

Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M. and Smyth, D.R. (1993) Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* 119: 721–743.

Bowman, J.L., Sakai, H., Jack, T., Weigel, D., Mayer, U. and Meyerowitz, E.M. (1992) *SUPERMAN*, a regulator of floral homeotic genes in *Arabidopsis*. *Development* 114: 599–615.

Bowman, J.L., Smyth, D.R. and Meyerowitz, E.M. (1989) Genes directing flower development in *Arabidopsis*. *Plant Cell* 1: 37–52.

Bowman, J.L., Smyth, D.R. and Meyerowitz, E.M. (1991) Genetic interactions among floral homeotic genes of *Arabidopsis*. *Development* 112: 1–20.

Busch, M.A., Bomblies, K. and Weigel, D. (1999) Activation of a floral homeotic gene in *Arabidopsis*. *Science* 285: 585–587.

Byzova, M.V., Franken, J., Aarts, M.G., de Almeida-Engler, J., Engler, G., Mari-

ani, C., Campagne, M.M. and Angenent, G.C. (1999) *Arabidopsis STERILE APETALA*, a multifunctional gene regulating inflorescence, flower, and ovule development. *Genes Dev.* 13: 1002–1014.

Coen, E.S. and Meyerowitz, E.M. (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* 353: 31–37.

Conner, J. and Liu, Z. (2000). LEUNIG, a putative transcriptional corepressor that regulates *AGAMOUS* expression during flower development. *Proc. Natl. Acad. Sci. USA* 97: 12902–12907.

Drews, G.N., Bowman, J.L. and Meyerowitz, E.M. (1991) Negative regulation of the Arabidopsis homeotic gene *AGAMOUS* by the *APETALA2* product. *Cell* 65: 991–1002.

Hempel, F.D., Weigel, D., Mandel, M.A., Ditta, G., Zambryski, P., Feldman, L.J. and Yanofsky, M.F. (1997) Floral determination and expression of floral regulatory genes in *Arabidopsis. Development* 124: 3845–3853.

Honma, T. and Goto, K. (2000). The *Arabidopsis* floral homeotic gene *PISTILLATA* is regulated by discrete cis-elements responsive to induction and maintenance signals. *Development* 127: 2021–2030.

Horsch, R.B., Fry, J.E., Hoffman, N.L., Eichholtz, D., Rogers, S.G. and Fraley, R.T. (1985) A simple and general method for transferring genes into plants. *Science* 227: 1229–1231.

Irish, V.F. and Sussex, I.M. (1990) Function of the *apetala-1* gene during *Arabidopsis* floral development. *Plant Cell* 2: 741–751.

Jack, T., Brockman, L.L. and Meyerowitz, E.M. (1992) The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS-box and is expressed in petals and stamens. *Cell* 68: 683–697.

Kater, M.M., Franken, J., Van Aelst, A. and Angenent, G.C. (2000) Suppression of cell expansion by ectopic expression of the *Arabidopsis SUPERMAN* gene in transgenic petunia and tobacco. *Plant J.* 23: 407–413.

Kunst, L., Lenz, J.E., Martinez-Zapater, J. and Haughn, G.W. (1989) *AP2* gene determines the identity of perianth organs in flowers of *Arabidopsis thaliana*. *Plant Cell* 1: 1195–1208.

Lee, I., Wolfe, D.S., Nilsson, O. and Weigel, D. (1997) A *LEAFY* co-regulator encoded by *UNUSUAL FLORAL ORGANS*. *Curr. Biol.* 7: 95–104.

Levin, J.Z. and Meyerowitz, E.M. (1995) *UFO*: an Arabidopsis gene involved in both floral meristem and floral organ development. *Plant Cell* 7: 529–548.

Lohmann, J.U., Hong, R., Hobe, M., Busch, M.A., Parcy, F., Simon, R. and Weigel, D. (2001) A molecular link between stem cell regulation and floral patterning in *Arabidopsis*. *Cell* 105: 793–803.

Mandel, M.A., Gustafson-Brown, C., Savidge, B. and Yanofsky, M.F. (1992) Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. *Nature* 360: 273–277.

McBride, K.E. and Summerfelt, K.R. (1990) Improved binary vectors for *Agrobacterium*-mediated plant transformation. *Plant Mol. Biol.* 14: 269–276.

Nandi, A.K., Kushalappa, K., Prasad, K. and Vijayraghavan, U. (2000) A conserved function for *Arabidopsis SUPERMAN* in regulating floral-whorl cell proliferation in rice, a monocotyledonous plant. *Curr. Biol.* 10: 215–218.

Parcy, F., Nilsson, O., Busch, M.A., Lee, I. and Weigel, D. (1998) A genetic framework for floral patterning. *Nature* 395: 561–566.

Pelaz, S., Ditta, G.S., Baumann, E., Wisman, E. and Yanofsky, M.F. (2000) B and C floral organ identity functions require *SEPALLATA* MADS-box genes. *Nature* 405: 200–203.

Sakai, H., Krizek, B.A., Jacobsen, S.E. and Meyerowitz, E.M. (2000) Regulation of *SUP* expression identifies multiple regulators involved in *Arabidopsis* floral meristem development. *Plant Cell* 12: 1607–1618.

Sakai, H., Medrano, L.J. and Meyerowitz, E.M. (1995) Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. *Nature* 378: 199–203.

Samach, A., Klenz, J.E., Kohalmi, S.E., Risseeuw, E., Haughn, G.W. and Crosby, W.L. (1999) The *UNUSUAL FLORAL ORGANS* gene of *Arabidopsis thaliana* is an F-box protein required for normal patterning and growth in the floral meristem. *Plant J.* 20: 433–445.

Schultz, E.A., Pickett, F.B. and Haughn, G.W. (1991) The *FLO10* gene product regulates the expression domain of homeotic genes *AP3* and *PI* in *Arabidopsis* flowers. *Plant Cell* 3: 1221–1237.

Smyth, D.R., Bowman, J.L. and Meyerowitz, E.M. (1990) Early flower development in *Arabidopsis*. *Plant Cell* 2: 755–767.

Wagner, D., Sablowski, R.W.M. and Meyerowitz, E.M. (1999) Transcriptional activation of APETALA1 by LEAFY. *Science* 285: 582–584.

Weigel, D. and Meyerowitz, E.M. (1994) The ABCs of floral homeotic genes. *Cell* 78: 203–209.

Wilkinson, M.D. and Haughn, G.W. (1995) *UNUSUAL FLORAL ORGANS* controls meristem identity and organ primordia fate in *Arabidopsis*. *Plant Cell* 7: 1485–1499.

(Received July 2, 2001; Accepted October 17, 2001)