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Activation of the *WUS* gene induces ectopic initiation of floral meristems on mature stem surface in *Arabidopsis thaliana*

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Abstract

A gain-of-function *Arabidopsis* mutant was identified *via* activation tagging genetic screening. The mutant exhibited clustered ectopic floral buds on the surface of inflorescence stems. The mutant was designated as *sef* for stem ectopic flowers. Our detailed studies indicate that the ectopic flower meristems are initiated from the differentiated cortex cells. Inverse PCR and sequence analysis indicated that the enhancer-containing T-DNA from the activation tagging construct, SKI015, was inserted upstream of the previously cloned *WUS* gene encoding a homeodomain protein. Studies from RT-PCR, RNA *in situ* hybridization and transgenic plant analysis further confirmed that the phenotypes of *sef* are caused by the overexpression of *WUS*. Our results suggest that overexpression of *WUS* could trigger the cell pluripotence and reestablish a new meristem in cortex. The type of new meristems caused by *WUS* overexpression was dependent upon the developmental and physiological stages of a plant. With the help of some undefined factors in the reproductive organs the new meristems differentiated into floral buds. In a vegetative growth plant, however, only the new vegetative buds can be initiated upon the overexpression of *WUS*. These studies provide new insights of *WUS* on flower development.

Introduction

The shoot apical meristem (SAM) is the ultimate source of aerial parts of a plant. At the transition from vegetative growth to reproductive development, the shoot meristem turns into an inflorescence meristem. The floral meristem is then initiated from the periphery of inflorescence meristem. Expression of the meristem-identity gene *LEAFY* (*LFY*) in the peripheral zone activates the expression of a series of floral organ-identity genes and promotes the transition from the inflorescence meristems to floral meristems (Parcy *et al*., 1998). In *Arabidopsis*, the floral meristem produces four whorls, including four sepals, four petals, six stamens and two carpels. According to the ABC model, sepals are determined by A (*APETALA1, AP1; APETALA2, AP2*) genes, petals by A and B (*APETALA13, AP3; PISTILLATA, PI*) genes, stamens by B and C (*AGAMOUS, AG*) genes, carpels by C-function genes, respectively (Bowman *et al*., 1991; Coen and Meyerowitz, 1991). The spatio-temporal distribution of AG not only controls the development of floral organs, but also plays an important role in the determination of floral meristem identity through a regulatory loop between *WUSCHEL* (*WUS*) and *AG*. *WUS* is expressed in shoot meristems and its expression can

be reestablished in floral primordia (Fletcher, 2002; Weigel and Jurgens, 2002). WUS and LFY can then activate the expression of *AG* at stage 3 of floral development. *WUS* expression, on the other hand, is inhibited by AG at stage 6, when the carpel primordia have formed. The repression of *WUS* terminates the stem cell identity in developing flowers and allows gynoecium differentiation (Lenhard *et al*., 2001; Lohmann *et al*., 2001).

The formation of different types of meristems is dependent on their morphological positions and developmental stages (Kerstetter and Hake, 1997). For example, tobacco floral buds can only be initiated on the explants collected from the flowering stage plants but not from the explants isolated from the vegetative stage plants (Rajeevan and Lang, 1993). It was reported that the cell fates of ectopic meristems in roots are influenced by auxin (Gallois *et al*., 2004). Flowers or floral organs can be regenerated from cultured explants and the fate of floral buds was determined by medium composition (Li *et al*., 2002b), especially auxin and cytokinin (Rajeevan and Lang, 1993). For instance, under high concentrations of cytokinin and auxin only tepals were initiated from the explants, at reduced levels of these two hormones the stamens and carpels were initiated (Lu, 2002; Li *et al*., 2002b). *WUS* ectopic expression combined with *STM* can activate ectopic organ formation on cotyledon and hypocotyl (Gallois *et al*., 2002). An inducible overexpression of *WUS* promotes somatic embryogenesis in roots (Zuo *et al*., 2002). Ectopic expression of *ANT::WUS* transgene is sufficient to induce integument formation (Groβ-Hardt *et al.,* 2002). *35S::WUS* constitutive expression induces *CLV3* activity near the vein (Lenhard *et al*., 2002). The floral meristem occurred in root in the co-existence of WUS and LFY (Gallois *et al*., 2004). So far, however, there are only few reports to show that the completely differentiated tissue can redifferentiate into floral organs *in planta*.

Here we identified a gain-of-function mutant by activation tagging screen. Molecular analyses indicated that four copies of a CaMV 35S enhancer were inserted upstream of the *WUS* gene. As a result, numerous ectopic flowers were formed on the mature stem surface of the mutant plants. RT-PCR and *in situ* hybridization data demonstrated that the expression of endogenous *WUS* was dramatically elevated in the inflorescence stems. Tissue section and *LFY* RNA distribution analysis indicated that the ectopic floral buds were initiated from the cortex. Our results suggest that overexpression of *WUS* could trigger cell pluripotence and reestablish new meristems from differentiated tissues.

Materials and methods

Plant growth conditions and transformation

Transgenic plants were generated in a *bri1-5* (Ws-2 ecotype) background with the activation tagging vector pSKI015 (Weigel *et al*., 2000). The gain-of-function mutant was identified in a screen of more than 2500 transgenic plants as described previously (Li *et al*., 2001). Plants were grown at 22 °C under long-day condition (16 h day/8 h night).

Seeds of mutant *pga6-1* were kindly provided by Dr Jian-Ru Zuo and Prof Nam-Hai Chua (The Rockeffeller University, USA). To induce the expression of *WUS* in mutant *pga6-1*, plants were grown on solid MS medium (Murashige and Skoog, 1962) supplemented with 50 mg l^{-1} kanamycin according to Zuo *et al*. (2002). When the inflorescence stem appeared, the plants were transferred onto the new medium with 5 μmol l^{-1} 17-β-estradiol for two weeks under long-day condition.

Histological analysis

Tissues were fixed in FAA (50% ethanol, 5% acetic acid and 3.7% formaldehyde) at room temperature for 16 h. The fixed tissues were dehydrated in a series of ethanol, cleared with xylene and embedded in Paraplast (Sigma). Serial 10 μm thick sections were cut and transferred to polylysine-coated slides (Sigma) and stained with 0.02% toludine-blue for 5–10 min at room temperature after dewaxing. Photographs were taken on an Olympus VANOX microscope.

In situ *hybridization*

Shoot and stem sections were prepared as above. After dewaxing and rehydrating, sections were digested in 5 μg ml^{-1} proteinase K in the solution containing 100 mmol l^{-1} Tris·Cl pH 7.5 and 50 mmol l^{-1} EDTA for 30 min at 37 °C. The digestion reaction was stopped by washing off the

proteinase K with DEPC-treated water thoroughly. Acetylation was performed with anhydride 0.25% in triethanolamine of 100 mmol l^{-1} pH 8.0 for 5 min after that the section was dehydrated in a graded ethanol series to 100% and dried at room temperature.

The products of PCR with primer KPN (5′ TTC TGG TAC CAT GGA GCC GCC ACA GCA TCA GC) and primer SAC (5′ TCT TGA GCT CCT AGT TCA GAC GTA GCT CAA) were subcloned into a pGEM-T vector (Promega) and used as a template for RNA probe. Probe preparation, hybridization and detection were carried out as described previously (Xu *et al*., 2001; Yong *et al*., 2003).

Scanning microscopy

Materials were fixed overnight at 4 °C in FAA, dehydrated for 12 h in 90% ethanol followed by 20 min treatments each with 95%, 100% ethanol, 75% ethanol–25% isoamyl acetate, 50% ethanol–50% isoamyl acetate, 25% ethanol–75% isoamyl acetate and 100% isoamyl acetate. Plant material was critical-point dried in liquid CO_2, coated with gold and observed at 30 kV scanning electron microscope (type S-800, HITACHI).

Identification of T-DNA insertion site

The T-DNA insertion site was identified *via* an inverse PCR analysis as reported previously (Li *et al*., 2001). To confirm the four enhancers in transgenic plant, about 2.4-kb PCR product was sequenced with primer UPSAL (5′ ATG GTC GAC AGT TAA AAA ATG AGT AAT C) and primer T7 (from the T-DNA of pSKI015; 5′ GTA ATA CGA CTC ACT ATA GGG CGA ATT G).

PCR genotyping

The locus *bri1-5* was identified by dCAP (Neff *et al*., 1998) using the primer Bri-F (5′ TAC TTT CGA TGG CGT TAC CT) and primer Bri-R (5′ AAG CCA GAA ACG GAG CC). After digestion with *Pst*I, PCR products give 168 bp fragments in homozygotes, 23, 145 and 168 bp in heterozygotes and 23 and 145 bp in the wild-type.

The genotype of the *sef* locus was identified with the upstream primer UPSAL (see above, an upstream sequence of T-DNA insertion site) and downstream primer DPXHO (5′ GGC TCG AGG TGT GTT TGA TTC GAC TTT TG, a downstream sequence of T-DNA insertion site) and primer T7 (see above). The *sef* heterozygotes should give two bands at 1.65 kb (from UPSAL and DPXHO) and 2.4 kb (from UPSAL and T7), homozygotes should give 2.4 kb band only and the wild-type gives 1.65 kb PCR products.

RT-PCR

Total RNA was isolated from the stem segments with Trizol reagent, according to the protocol provided by the manufacturer (Gibco BRL, USA). First-strand cDNA generated from the total RNA sample using the RT-PCR Kit (TaKaRa, Japan). PCR products were amplified using the first strand cDNA as a template with the primer KPN and the primer SAC (see above) for *WUS*, 5′ GGG GAT CCA CTA GTA TGT TCC AAA CCC AAT TTT CC3′ and 5′ GGG GAT CCG AGC TCG GTA ACG AGC ATG CAG 3′ for *At2g17960*. PCR reaction was carried out as the following conditions: denaturation 30 s at 94 °C, annealing at 60 °C for 30 s and extension 90 sec at 72 °C for 30 cycles. The products were fractionated on an agarose gel and stained with ethidium bromide.

Results

sef was identified by an activation tagging genetic screen

In an attempt to identify *bri1-5* genetic suppressors *via* activation tagging (Li *et al*., 2001, 2002a), we isolated a gain-of-function mutant in the *bri1-5* background. To exclude possible genetic contribution from *bri1-5*, the double mutant was backcrossed with wild-type plants (ecotype Ws-2). The resulting F1 plants were allowed to self-pollinate to generate a segregating F2 population. F2 plants were genotyped with PCR-based dCAPs (described in Materials and methods). In the wild-type, 168 bp PCR products can be digested by *Pst*I and produced two fragments of 23 and 145 bp. The mutation in *bri1-5*, however, renders the 168 bp fragments is resistant to *Pst*I digestion. For heterozygous plants, PCR products contain a mixture of 23, 145 and 168 bp fragments after *Pst*I digestion. Individuals possessing the gain-of-function

T-DNA insertion, but lacking the *bri1-5* mutation, were selected for further analysis. The mutant exhibited multiple morphological defects including twisted primary inflorescence stems, elongated pistils, and fasciated siliques (Figure 1B–D). One of the most striking phenotypes, however, is that ectopic floral buds were formed on the surface of the inflorescence stems (Figure 2B and D). There were numerous floral buds observed on the surface of the inflorescence stems. The epidermal cells in the ectopic meristematic area were smaller and rounder than the adjacent cells (Figure 2E), suggesting that these epidermal cells possess a meristematic cell identity. Cross section analysis indicated that the ectopic floral buds are initiated from cortex (Figure 2G). The gain-of-function mutant was designated as the *sef* for Stem Ectopic Flowers.

Expression of WUS is enhanced in the ectopic floral meristems

We cloned the flanking sequence of the T-DNA by inverse PCR (Li *et al*., 2001, 2002a; Zhou et al., 2004). Sequence analysis showed that the T-DNA was inserted in a region between *At2g17960* and *WUS* on chromosome II, with the right border of the T-DNA pointing to *At2g17960*. The number of enhancers was confirmed by sequencing the PCR products with primers UPSAL and T7 (see Materials and methods). The four enhancers are about 7.5 kb upstream of the *WUS* start codon (Figure 3A).

To examine whether the insertion of the T-DNA led to the overexpression of *At2g17960* or *WUS*, semi-quantitative RT-PCR analysis were carried out. Our results indicated the *WUS* transcripts are increased significantly in the stem internodes with floral outgrowth structures on their surfaces, while the expression of *At2g17960* is not altered (Figure 3B).

It was reported that *WUS* is expressed under the third cell layer in SAM and under the second cell layer in floral meristem of wild-type *Arabidopsis* plants (Schoof *et al*., 2000). RNA *in situ* hybridization analysis indicated that the *WUS* expression zone in ectopic floral meristems has been considerably expanded compared to normal floral meristems of wild-type plants. In *sef*, the *WUS* mRNA can be detected in all of the floral meristematic cells (Figure 3C and D). These results suggest that the four CaMV 35S enhancers from activation tagging vector caused a significant enhancement of endogenous *WUS* gene expression in the SAM. In addition, there are distinct hybridization signals in cortical tissues in *sef* plants (Figure 3E).

Ectopic floral phenotype can be recapitulated in pga6-1 plants upon the induction with 17-β-estradiol

To further substantiate that the *sef* phenotype was caused solely by the overexpression of *WUS*, transgenic plants harboring *35S* (with dual enhancers)::*WUS* cDNA construct were generated. Most of the transgenic plants do not develop beyond the vegetative growth stage and the ectopic flowers seen in *sef* cannot be observed in these transgenic plants. Therefore, the mutant *pga6-1* plants were carefully examined. This mutant was previously identified by a functional screen using chemical-inducible activation tagging system for genes involved in the promotion of somatic embryogenesis (Zuo *et al*., 2002). Molecular analysis indicated that a single copy of the T-DNA was inserted about 1 kb upstream of the start codon of the *WUS* gene in *pga6-1* mutant, i.e., *PGA6* is identical to *WUS*. Upon the supplementation of the chemical inducer in the plant culture medium, expression of *WUS*/*PGA6* can be induced and the somatic embryo formation was promoted even without the addition of external plant hormones (Zuo *et al*., 2002). It is not reported, however, whether the ectopic floral bud can be initiated on the inflorescence stem surface of the *pga6-1* plants. Using the method described by Zuo *et al*. (2002), *WUS* overexpression in *pga6-1* was induced on the medium containing 17-β-estradiol following the transition from vegetative to reproductive growth. A cluster of adventitious shoots was observed on the stem surface of *pga6-1*. Like the *sef* mutant, a flower-like outgrowth appeared on the stem surface of the *pga6-1* plants (Figure 4). These results supported our hypothesis that overexpression of *WUS* in *sef* mutant plants induced the formation of ectopic floral buds on the mutant inflorescence stems.

LFY is induced in the same tissues where WUS is overexpressed

There were numerous floral meristems on the stem surface of *sef* mutant plants. Meristematic out-

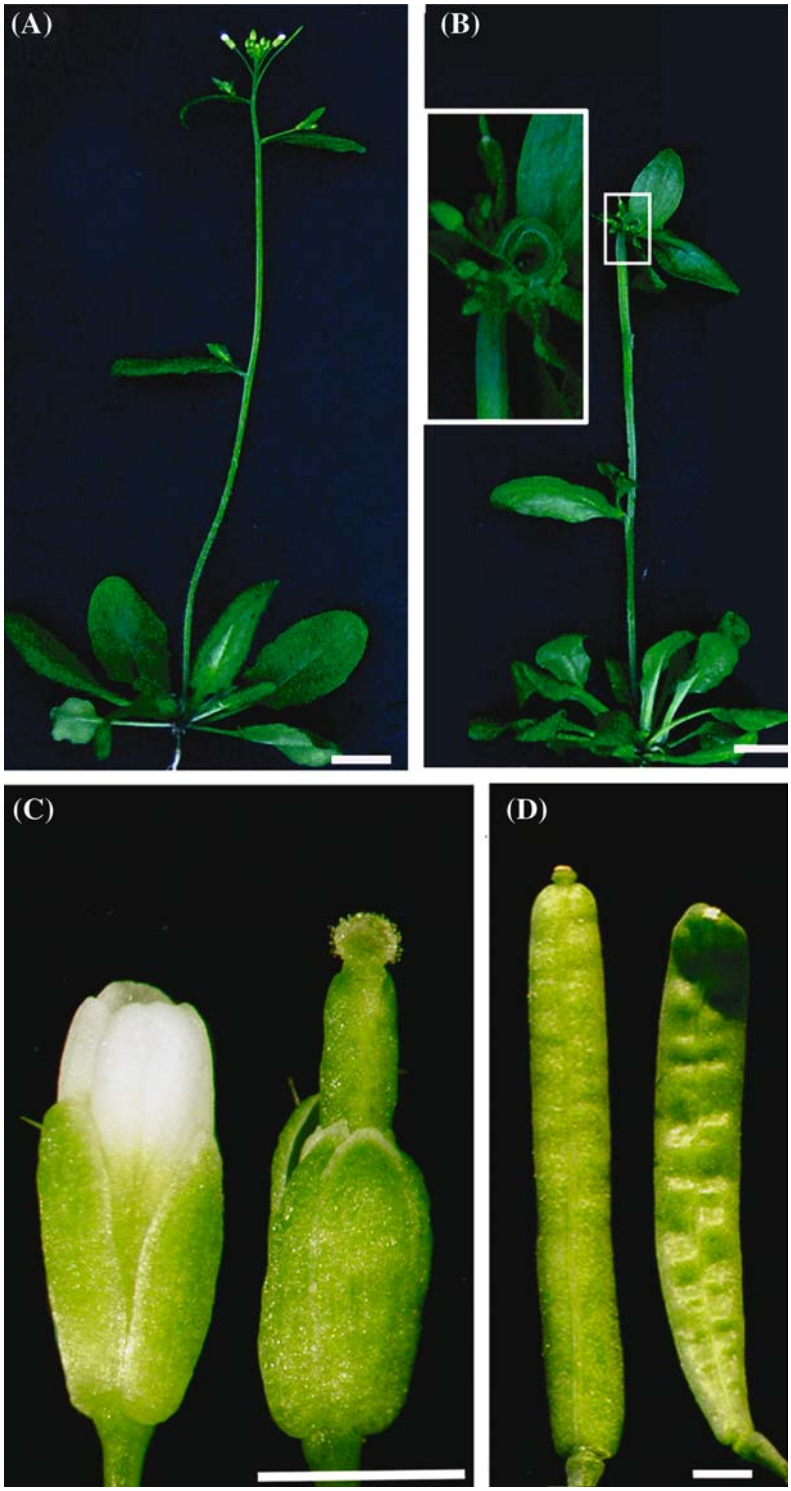


Figure 1. Phenotypes of mutant *sef*. (A) A 4-week-old wild-type plant. (B) The inflorescence stem of the mutant is curled at the anthesis stage and the boxed part is enlarged. (C) The gynoecia cannot be seen before anthesis in a wild-type plant (left), however, the stigma of mutant flower is already higher than the perianth (right). (D) The corrugated carpel (right) of mutant *sef* compared with that of wild-type (left). The scale bar is 1 cm in A and B, and 1 mm in C and D.

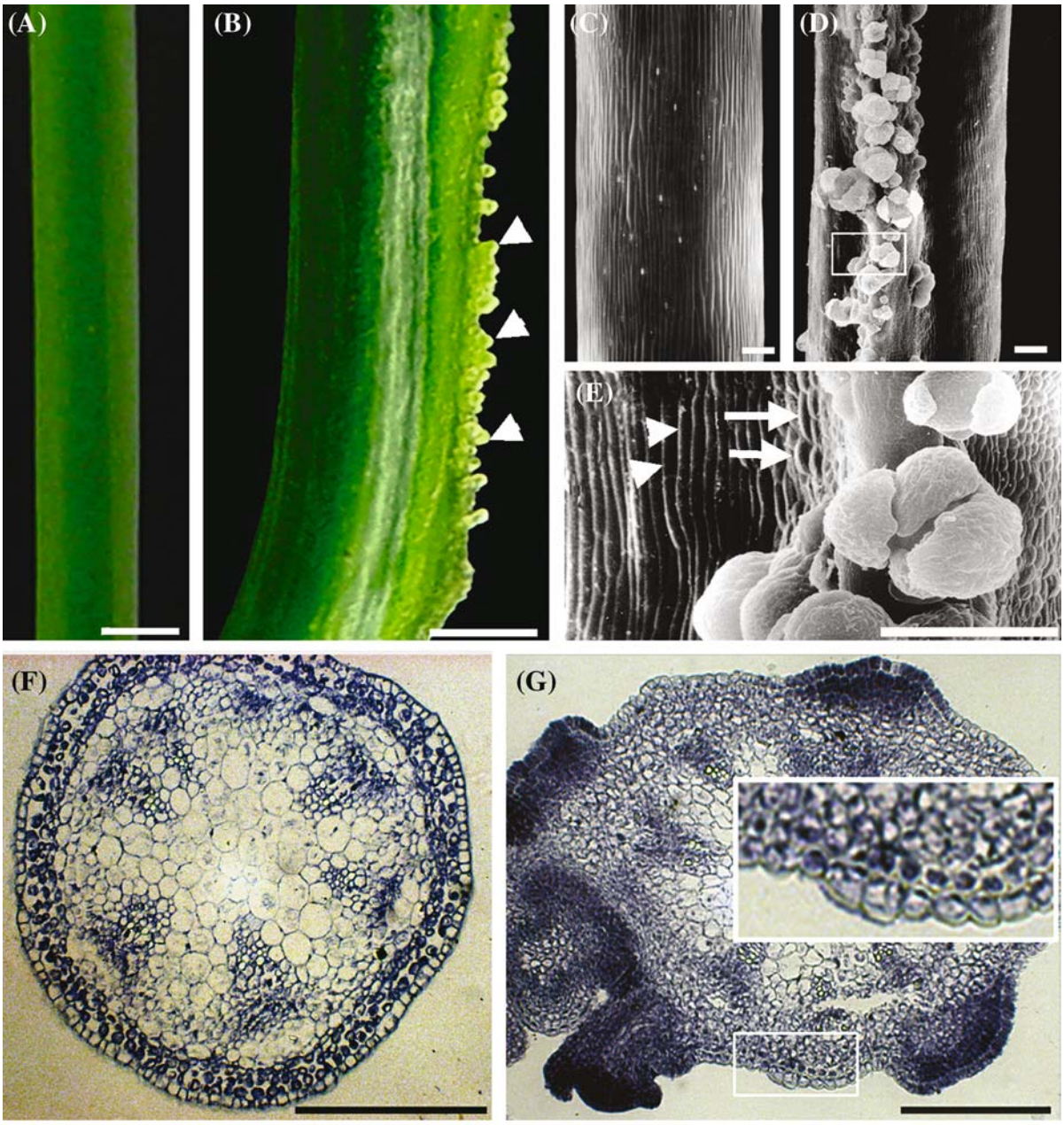
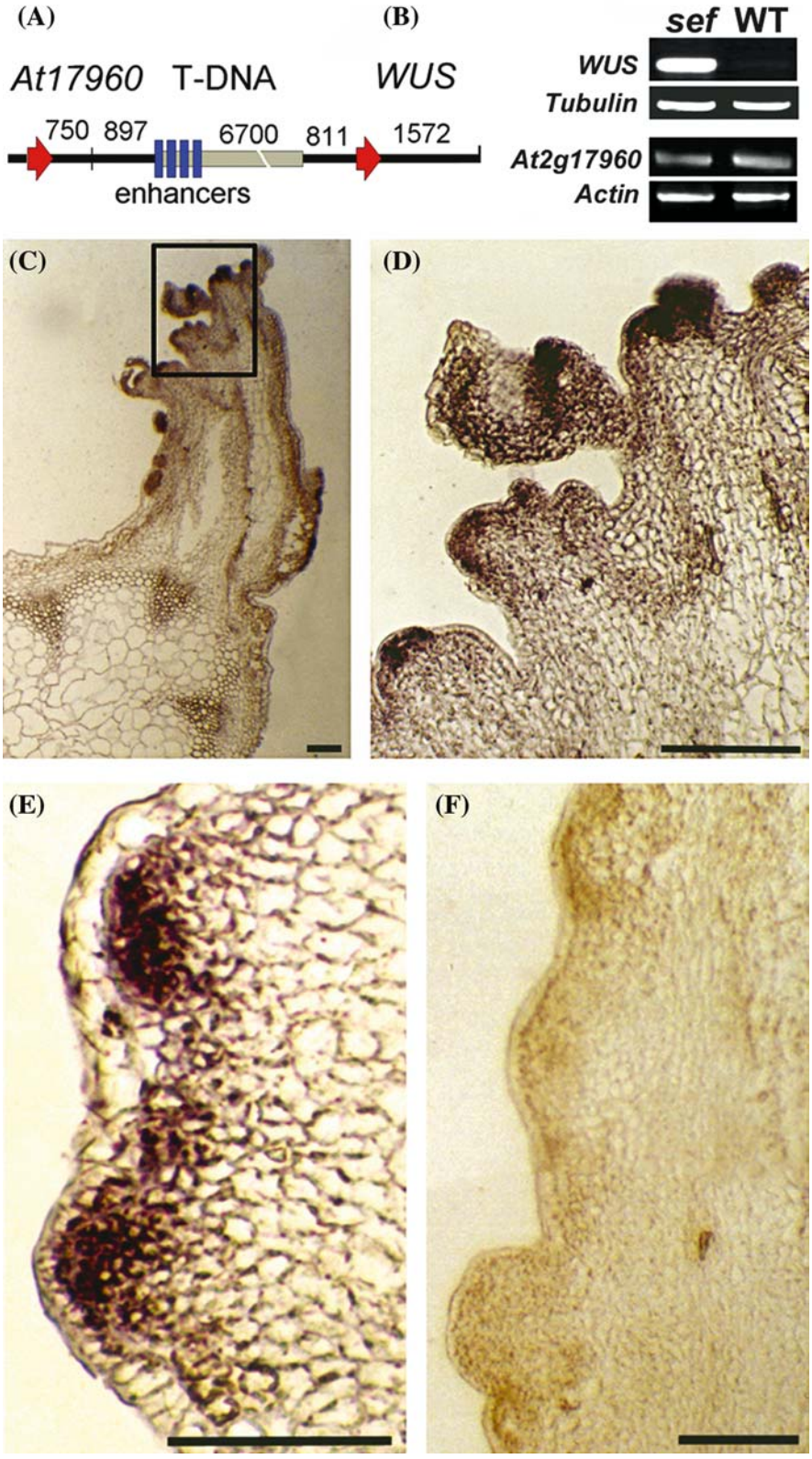


Figure 2. Ectopic floral buds formed on the stem surface of *sef*. (A) The stem of a wild-type plant. (B) Many floral buds initiated on the inflorescence stem surface of the mutant and no obvious developing order. (C) Scanning electronic microscopy image of the wild-type plant. There are not any outgrowths found on it. (D) Floral buds distributed along mutant inflorescence axis. (E) Enlargement of the marked part in D, ectopic meristematic cells in epiderm were small and round (arrows) while epidermic cell is larger and longer (arrow heads). (F, G) Transversal section of wild-type (F) and *sef* mutant (G) inflorescence axis stained by toludine-blue. The ectopic floral buds initiated from the cortex of mutant. Scale bar is 1 mm in A and B; 150 μm in C–G.

growths appeared on inflorescence stems and arranged longitudinally along the axis of inflorescence stems (Figure 5). Some outgrowths developed into single flowers as development proceeded. These meristems, which were formed on the inflorescence axis, do not show apical dominance, and the floral buds initiated randomly from the outgrowth on the stem surface. Analysis


(A)
At17960 T-DNA
750 897 6700 811 1572
enhancers
(B)
WUS
(B)
sef WT
WUS
Tubulin
At2g17960
Actin
(C)
(D)
(E)
(F)

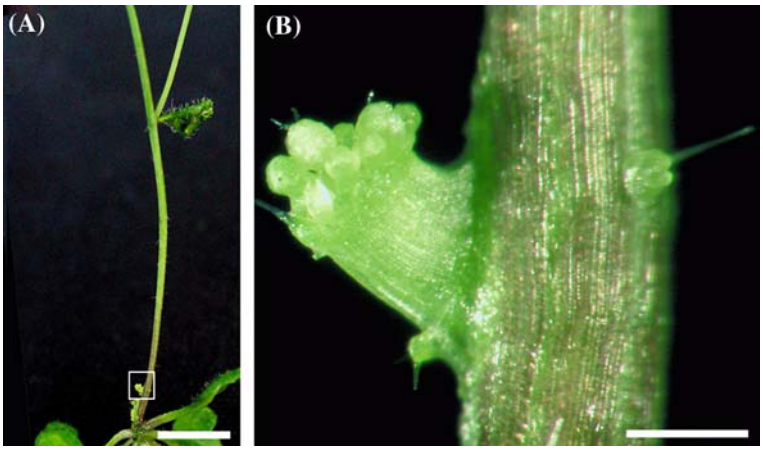


Figure 4. *WUS* inducible overexpression in *pga6-1* recapitulated the *sef* phenotype. (A) The Inflorescence buds were originated ectopically on the stem surface of the mutant *pga*6-1 after induction on the medium supplemented with 5 μM 17-β-estradiol for 2 weeks. (B) Enlargement of the area marked in A indicating the ectopic floral buds on stem surface. Scale bar is 1 cm in A and 1 mm in B.

of tissue sections clearly indicated that the meristems were derived from the cortical cells (Figure 5E–I).

LFY is a floral meristem identity gene and is expressed in floral and leaf primordium and the increase of its expression could promote the meristem transition from the vegetative to the reproductive state (Weigel and Nilsson, 1995; Blazque *et al*., 1997). To further understand the origin of the *sef* ectopic floral meristems, the *LFY* gene was used as a marker and its expression patterns during ectopic flower development were detected by RNA *in situ* hybridization. The hybridization results showed that some stained domains were found underneath the epidermal cell layer (Figure 5A) similar to that of *WUS* (compared with Figures 5B and 3E). *LFY* transcripts were detected in a few cortical cells even before an outgrowth is apparent (Figure 5E). The expression domain of *LFY* is significantly expanded due to *WUS* activation in these cortical tissues (Figure 5F). The epidermal cells in the same area as these outgrowth structures eventually become meristematic cells (Figure 5G). Finally the overlying epidermal cells become part of the ectopic meristem cells (Figure 5H). *LFY* expression can be detected at a high level in an entire ectopic flower bud (Figure 5I). At flower stage 6, overexpressed *LFY* can still be detected (Figure 5D). These results suggested that overexpression of *WUS* can alter the expression pattern of *LFY*.

Ectopic leaf-like structures were seen in vegetative tissues

The ectopic floral buds were only observed on the surface of reproductive tissues including inflorescence (Figure 2), pedicels (Figure 7A), and siliques (Figure 7B). Bud-like structures can be seen sometimes on the surface of rosette leaves. These bud-like structures usually developed into leaves, rather than ectopic flowers (Figure 6).

Figure 3. Localization of the T-DNA insertion and the *WUS* expression pattern in *sef*. (A) The T-DNA from the activation tagging construct, pSKI015, was inserted in chromosome II. The stop codon of the putative protein *At2g17960* is 897 bp from 4 × 35S enhancers. Arrows indicating the transcription direction, number is the length of the fragment. (B) RT-PCR products of *WUS* and *At2g17960* were fractionated on agarose gel and stained with ethidium bromide. Total 30 cycles of reaction were carried out. Total RNA was extracted from the stem segment containing outgrowths of *sef* or the internodes of wild-type. The actin and tubulin gene fragments were amplified as a control. (C–F) Detection of *WUS* mRNA with *in situ* hybridization. (C) Transversal section of the inflorenscence axis of mutant across an ectopic inflorescence hybridized with *WUS* antisense RNA. (D) Enlargement of the area marked in C indicating the enlarged domain in floral primordia. (E) Longitudinal section of inflorescence axis, indicating the *WUS*-expressing cell underneath epiderm, which is similar to that of *LFY* expression pattern in mutant cortex (compared with Figure 5B). (F) Negative control, *WUS* RNA detected with sense probe. Scale bar is 100 μm in C–F.

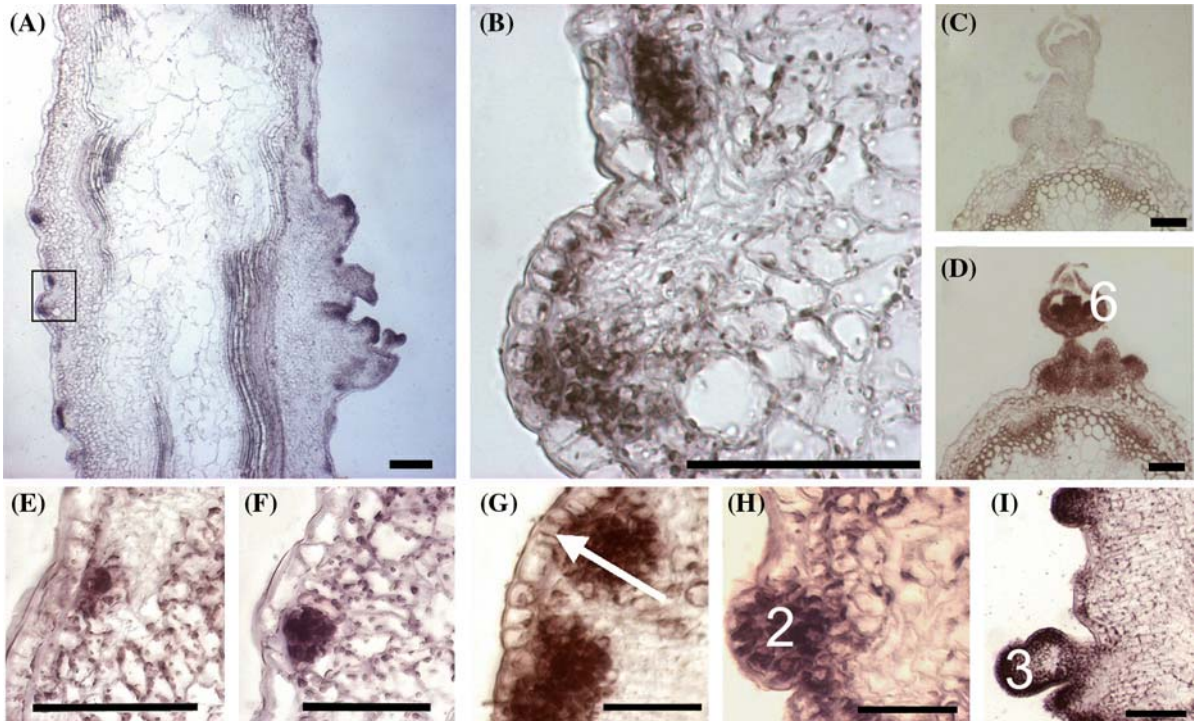


Figure 5. Distribution of *LFY* mRNA detected by *in situ* hybridization. (A) Longitudinal section of the inflorenscence axis of mutant, detected with *LFY* antisense probe. It can be found that many *LFY*-expressing domains in the cortex. (B) Enlargement of the boxed part in A. No *LFY* RNA was detected in epiderm cell but *LFY* expressed underneath epiderm. (C) Transversal section of the inflorescence axis of mutant across an ectopic flower bud, the RNA was detected with sense probe (negative control). (D) The adjacent section to C, *LFY* expressed strongly in flower on stage 6. (E–I) The origination and fate of *LFY*-expressing cells in the cortex. Longitudinal sections of mutant inflorescence axis hybridized with *LFY* antisense probe. *LFY* RNA was detected firstly in a few of small cells beneath the epiderm (E). As the increase in number of these small cells, the epiderm bulged slightly (F). The epidermic cells overlying *LFY*-expressing domain began to divide (arrow) in (G). Subsequently, these epidermic cells expressed *LFY* also and became a part of the ectopic floral meristem (H). Finally the flower buds formed (I). Number indicating the developmental stage of floral buds; Scale bar is 100 μm in A, C and D; 50 μm in B and E–I.

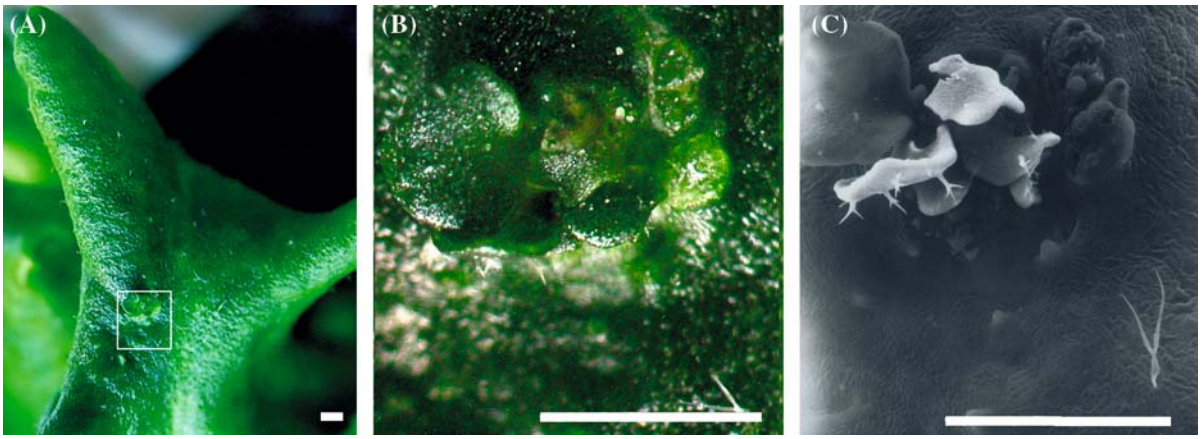


Figure 6. Ectopic shoots formed on the rosette leave. (A) The vegetative shoots occurred on the adaxial side of rosette leave. (B) Higher magnified image of the boxed part in A. (C) Scanning electronic microscopy image of the shoot in B. Bar 1.0 mm.

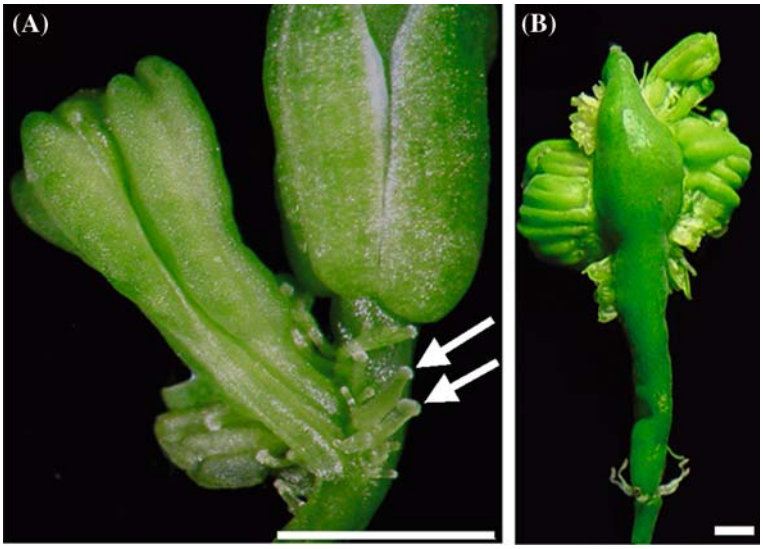


Figure 7. Ectopic floral organs in *sef*. (A) A cluster of carpels initiated on the pedicel and carpelloid organs tipped with stigmatic tissue (arrows). (B) Supernumber primordia of carpels formed in the medial furrow between carpels. Scale bar is 1 mm.

Discussion

The *WUS* gene expression is limited to a domain underneath the outermost three and two cell layers in shoot and floral meristems, respectively. The homeostasis of the stem cell population in these meristems is controlled by two regulatory loops between *WUS* and *CLV3* (Brand *et al*., 2000; Schoof *et al*., 2000) and between *WUS* and *AG* (Lenhard *et al*., 2001; Lohmann *et al*., 2001). Here we report our identification and characterization of a gain-of-function mutant, *sef*. The *sef* mutant was isolated *via* an activation tagging genetic screen. Our detailed analysis suggests that the overexpression of endogenous *WUS* can result in the formation of ectopic meristems in the cortex. With the assistance of some unknown factors, the floral meristem identity gene *LFY* was activated. The gain-of-function mutant *sef* is the first example of floral buds initiated from differentiated tissue *in planta* although many researchers have established *in vitro* flower or floral organ regeneration systems (Lu *et al*., 1988, 2002; Pang *et al*., 1993; Li *et al*., 2002b).

In wild-type plants, *WUS* RNA can be detected only in the organizing center of the meristematic cells (Mayer *et al*., 1998) and in the nucleus of the ovule (Groβ-Hardt *et al*., 2002). In *sef* mutants, however, *WUS* RNA was detected underneath the epidermal cell layer (Figure 3D). This raised a question: why the *WUS*-expressing cells were distributed in the cortex of the *sef* mutant? One explanation is that *CLV3*-expressing cells were left behind in the cortex zone during stem elongation because the stem cell domain is expanded in *sef* plants. Endogenous *WUS* was expressed *de novo* underneath the ectopic stem cells in the cortex according to the same mechanism employed in normal floral meristem (Weigel and Jurgens, 2002), furthermore, its expression is enhanced by the four 35S enhancers.

In the gain-of-function mutant *sef*, multiple meristems and the ectopic *AG*-dependent organs can be found. The multiple meristems suggest that stem cells are initiated at different tissues in the *sef* mutant due to the overexpression of *WUS,* which specifies the identity of the stem cells (Laux *et al*., 1996; Mayer *et al*., 1998). The ectopic meristem is regulated by *CLV3*/*WUS* feedback loop (Brand *et al*., 2000; Schoof *et al*., 2000). The second feature can be explained by the regulation loop between *AG* and (*WUS* + *LFY*) (Lenhard *et al*., 2001; Lohmann *et al*., 2001). *AG* specifies the stamen and carpel identity (Coen and Meyerowitz, 1991; Mandel *et al*., 1992; Mizukami and Ma, 1992; Jack *et al*., 1997). In the pedicel and carpel of the mutant, *WUS* overexpression induced AG at a high level, the latter made the fourth whorl floral organ formed preferably.

The ectopic floral buds (Figure 2) and the distinct distribution of *LFY* transcripts (Figure 5) suggested *LFY* expressed ectopically in *sef*. Why

was *LFY* expressed in the cortex of *sef* inflorescence stem? A reasonable explanation is that *LFY* expression was induced by WUS and other factors in the stem. At the reproductive stage, some unknown cues may interact with WUS and trigger the expression of *LFY*. The co-existence of LFY and WUS cause the ectopic floral meristem (Gallois *et al*., 2004). Lacking these undefined factors, stem cells induced by *WUS* overexpression can only remain at vegetative growth stage (Figure 6).

In *sef* mutants, the vegetative shoots produced on the rosette leave (Figure 6), flower buds initiated from stem surface (Figure 2D), and cluster of organs formed on the surface of the pedicel (Figure 7A) and on the medial furrow of the carpels (Figure 7B). The difference among ectopic organs in *sef* implies that the expression pattern of *WUS* downstream genes is tissue specific. *CLV3*, one of the target genes of *WUS* (Brand *et al*., 2000; Schoof *et al*., 2000), can be activated by *WUS* in cells within meristem apex and in cells near the vascular tissues in the transgenic *35S::WUS* seedlings (Brand *et al*., 2002; Lenhard *et al*., 2002). In transgenic *AP3::WUS* plants, however, no *CLV3* mRNA was detected in the second and the third whorl of the floral organ primordium (Lenhard *et al*., 2001). Similarly, no *CLV3* mRNA was detected in developing ovules where endogenous *WUS* was expressed (Groβ-Hardt *et al*., 2002). These results suggested the cell competence is necessary for response of *CLV3* to WUS signal. The tissue-specific expression of *LFY* in *sef* might employ similar mechanism used by *CLV3*.

As described above, the ectopic meristems in *sef* displayed different differentiation fates. Somatic embryos formed in roots of *WUS* inducible plants (Zuo *et al*., 2002; Gallois *et al*., 2004). Leaf primordia were observed on rosette leaves of *35S::KNAT1* transgenic plants (Chuck *et al*., 1996). The inflorescence meristems were reported on cauline leave of *35S::KNAT1* transgenic plants (Chuck *et al*., 1996), on rosette leaves and cotyledons of double-mutant *fil-8 yab3-2* (Kumaran *et al*., 2002). Organogenesis can be found on cotyledons and hypocotyls at co-expression of *WUS* and *STM* (Gallois *et al.,* 2002; Lenhard *et al*., 2002). Floral meristems were induced in the stems of our mutant *sef* and in the roots with co-expression of *WUS* and *LFY* (Gallois *et al*., 2004). These observations indicated that the differentiation direction of reestablished meristems are affected by near environmental and/or physiological status, which was confirmed by *in vitro* experiments that single flower differentiated from floral organ (Li *et al*., 2002b).

In conclusion, our results suggest that endogenous *WUS* expression was enhanced in the gain-of-function mutant *sef*. Accumulation of WUS alone can trigger cell pluripotence, which is a novel genetic phenotype of gene regulation in differentiation of organs. WUS is sufficient for the reestablishment of the meristem *de novo* in the inflorescence stem, and the cell fate of the reestablished meristem depended on its microclimate.

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