Whorl-Specific Expression of the *SUPERMAN* Gene of *Arabidopsis* Is Mediated by *cis* Elements in the Transcribed Region

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Supplemental Experimental Procedures

Plasmid Construction

All reporter genes were constructed by standard molecular biology techniques.

All constructs, except for #3, were based on pSUP-GUS (BsaBI), which was made by inserting a *uidA*-coding region in the BsaBI site (8243094 of chromosome 3; blunt end, position 1848 of TAC clone K14B15 [GenBank accession AB025608]) of a 6.7 kb *SUP* genomic fragment (8237177–8243842 of chromosome 3; position 77784 of MXC7 [GenBank accession AB026655] to 2601 of K14B15 [GenBank accession AB025608] [S1]) in pBluescript II SK+ (pSUP). The blunt end *uidA*-coding region was cut out from pBI101 (Clontech) by using SmaI for the 5′ end and SacI (blunt ended by T4 DNA polymerase) for the 3′ end for adjustment with the *SUP* gene open reading frame. pSUP-GUS (BsaBI) was digested with NcoI (8242572–8242812 of chromosome 3; position 1326–1566 of K14B15), filled in, and self ligated to produce pSUP-GUS (BsaBI) Δ, construct #2. To produce construct #3, pSUP was digested with BsaBI and was ligated with a BS linker, BS-F (5′-GGATCTAGAACTCCGTTTGGGTTTCGCTGGC GCC-3′) and BS-R (5′-GGCGCCAGCGAAACCCAAACGGAGTTCTA GATCC-3′), to produce pSUP-BS. The plasmid pSUP-BS was digested with NarI and was ligated with a PCR-amplified GUS-coding region fragment with a NarI end. Oligonucleotides GUSKA1 (5′-CCG GCGCCGGTGGTCAGTCCCTTATG-3′) and GUSKA2 (5′-CCGGCGC CAGGCTGTAGCCGACGATG-3′) were used as primers for PCR using pBI101 as a template. To produce constructs #4–#7, pSUP-GUS (BsaBI) was digested with NcoI (1325 and 1566 of K14B15) and was ligated with a series of deleted NcoI fragments. The deleted NcoI fragments were amplified by PCR using pSUP as a template and primers NC2 and BS1 for #4, NC1 and BS1 for #5, NC4 and BS1 for #6, and NC5 and BSI for #7 and were cloned into the pBluescript SK+ TA-vector and sequenced to avoid PCR error. To produce constructs #8–#10, pSUP-GUS (BsaBI) was digested with NcoI (1325 of K14B15) and Bsu36I (1780 of K14B15) and was ligated with a series of truncated fragments with NcoI (5′) and Bsu36I (3′) ends. The truncated fragments were amplified by PCR using pSUP as a template with primers NC3 and BS1 for #8 and Q203 and BS4 for #10, or they were synthesized as an NCBS linker by annealing NCBS-F (5′-CATGGGTTAACC-3′) and NCBS-R (5′-TCAGGTTA ACC-3′) for #9. After recloning, the direction of the insert DNA was checked by PCR or sequencing. The following oligonucleotides were used as primers for PCR: NC1 (5′-CCATGGGAATTCAGAT CGGCTC-3′), NC2 (5′-CCATGGTCTCCATCATCATCTTCAAC-3′), NC3 (5′-CCATGGAGATATAGGGCAGGTTTGATC-3′), NC4 (5′-GGCCAT GGTTTTCATGGCCACCA-3′), NC5 (5′-GGCCATGGCAACAGGATCA TGATTATCT-3′), BS1 (5′-CCTCAGGATCTTGCAAGC-3′), BS4 (5′-GCC CTCAGGGCCTGTTGTAATCTGAGTCTTGC-3′), Q203 (5′-CTCTCTAA GAGACAGACAGAC-3′), and Q201 (5′-CCTTAGAGAAGCATCATA TCTTCT-3′). To produce constructs #11 and #12, pSnaBI-SUP was digested with BanII (with a site at −15 relative to the longest cDNA, 8242265 of chromosome 3; 1019 of K14B15 [AB025608]) and BglII (+113 for the longest cDNA, 8242393 of chromosome 3; 1147 of K14B15) for #11 or with BglII (+113 for the longest cDNA, 8242393 of chromosome 3; 1147 of K14B15) and SpeI (8242442 of chromosome 3; 1196 of K14B15) for #12 and was blunted with T4 DNA polymerase to make it blunt ended and self ligated. These plasmids were cut out by AccB7I and Bsu36I, and the fragment was cloned into pSUP-GUS (BsaBI) Δ digested with AccB7I and Bsu36I. To produce a series of promoter deletions, pSUP-GUS (BsaBI) Δ was digested with ClaI (8239000 of chromosome 3; position 603 of TAC clone K13C10 [GenBank accession AP000734]) for construct #13, with PvuII (8239987 of chromosome 3; 1590 of K13C10) for construct #14, and with AvaII (8240783 of chromosome 3; 2386 of K13C10) for construct #15. For #16, AflII (8238951–8242020 of chromosome 3; 554 of K13C10 to 774 of K14B15 [AB025608]) sites were used to delete the internal fragment. All constructs used the pCGN1547 plant transformation vector [S2].

Plant Transformation and Growth Conditions

Arabidopsis thaliana plants of the ecotype Landsberg *erecta* were transformed by using the vacuum infiltration method after placement of the plasmid into the *Agrobacterium tumefaciens* strain ASE. We recovered transgenic plants by selecting for kanamycin resistance. All plants were grown under continuous illumination at 22°C.

GUS Staining and Microscopy

Two inflorescences from each T1 transformant were stained for GUS activity as described previously [S3]. The patterns of whole-mount floral staining from at least six independent stable transformants for each construct were characterized. The number of transgenic lines examined for each construct is given in Figures 1 and 5. Whole-mount tissues were mounted in 100% ethanol or a clearing solution (72% chloral hydrate and 11% glycerol).

In Situ Hybridization

Flowers were fixed, embedded, sectioned, hybridized with the ^{35}S-labeled *uidA* antisense probe, and exposed for 8–10 weeks as described previously [S1]. To generate a *uidA* antisense probe, the *uidA*-coding region of pBI101 (Clontech) was cloned into pBluescript SK+ (Stratagene) and was used as an in vitro transcription template.

Supplemental References

S1. Sakai, H., Medrano, L.J., and Meyerowitz, E.M. (1995). Role of SUPERMAN in maintaining *Arabidopsis* floral whorl boundaries. Nature *378*, 199–203.

S2. McBride, K.E., and Summerfelt, K.R. (1990). Improved binary vectors for Agrobacterium-mediated plant transformation. Plant Mol. Biol. *14*, 269–276.

S3. Sieburth, L.E., and Meyerowitz, E.M. (1997). Molecular dissection of the AGAMOUS control region shows that cis elements for spatial regulation are located intragenically. Plant Cell *9*, 355–365.

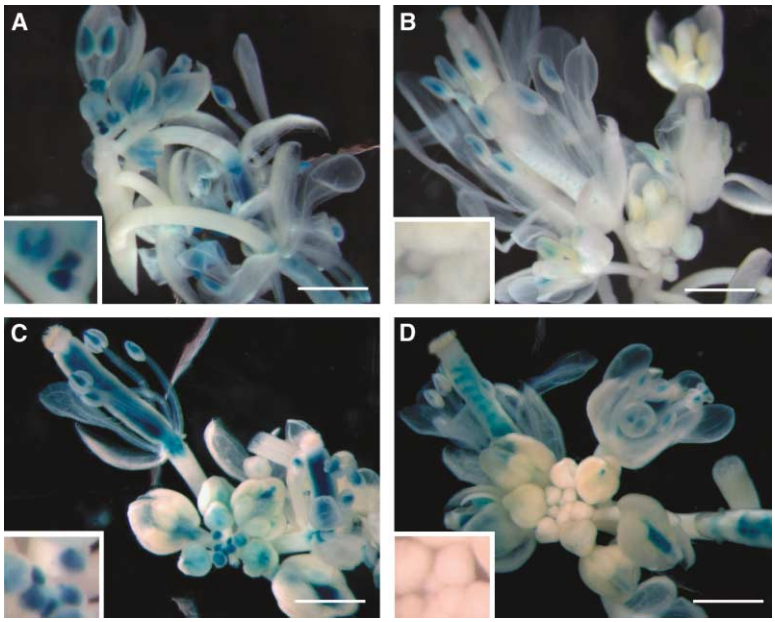

Figure S1. Expression Patterns of SUP-GUS Reporter Genes with Various Deletions in the *SUP* Gene-Coding Region

(A and B) Inflorescence transgenic for (A) construct #6 and (B) construct #7.

(C and D) (C) Construct #9 and (D) construct #10.

The inset in each case is a close-up view of the early-stage floral buds. The scale bars in (A)–(D) represent 1 mm.