Synergistic disruptions in *seuss* cyp85A2 double mutants reveal a role for brassinolide synthesis during gynoecium and ovule development

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Abstract

**Background:** The Arabidopsis *SEUSS* (*SEU*) gene encodes a transcriptional adaptor protein that is required for a diverse set of developmental events, including floral organ identity specification, as well as gynoecium, ovule and embryo development. In order to better understand the molecular mechanisms of *SEUSS* action we undertook a genetic modifier screen to identify *seuss*-modifier (*sum*) mutations.

**Results:** Screening of M2 lines representing approximately 5,000 M1 individuals identified mutations that enhance the *seuss* mutant phenotypic disruptions in ovules and gynoecia; here we describe the phenotype of the *sum63* mutant and enhanced disruptions of ovule and gynoecial development in the *seu sum63* double mutant. Mapping and genetic complementation tests indicate that *sum63* is allelic to *CYP85A2* (AT3G30180) a cytochrome p450 enzyme that catalyzes the final steps in the synthesis of the phytohormone brassinolide.

**Conclusions:** Our identification of mutations in *CYP85A2* as enhancers of the *seuss* mutant phenotype suggests a previously unrecognized role for brassinolide synthesis in gynoecial and ovule outer integument development. The work also suggests that *seuss* mutants may be more sensitive to the loss or reduction of brassinolide synthesis than are wild type plants.

**Background**

*SEUSS* (*SEU*) is a member of a family of transcriptional co-regulators that controls a diversity of developmental events in *Arabidopsis thaliana* [1,2]. *SEU* is required for repression of *AGAMOUS* during floral organ identity specification. The *SEU* protein has been shown to physically interact with members of the MADS domain homeobox transcription factor family as well as other transcriptional co-regulators (*LEUNIG* (*LUG*) and *LEUNIG_HOMOLOGUE* [3-6]). These protein interactions mediate repression of *AG* transcription through the recruitment of a histone deacetylase protein, as well as components of the mediator complex [4,5,7]. These data taken together support a model in which *SEU* functions as a bridging protein that enables the recruitment of *LUG* and associated histone deacetylase activities by DNA binding proteins of the MADS domain family. In this model *SEU* is required for repression of *AG* in floral whorls that will give rise to perianth organs where these protein complexes are most active [5].

*SEU* and *LUG* are also required for development of the medial domain of the gynoecium [8,9]. The medial domain of the *Arabidopsis* gynoecium contains the carpel margin meristem, a vital meristem that gives rise to the ovules and other tissues required for female reproductive competence. The effect of *seu* or *lug* single mutants on medial domain development is relatively mild, however both *seu* and *lug* single mutants display a dramatic synergistic interaction with *aintegumenta* (*ant*) mutants. In *seu ant* or *lug ant* double mutants development of the gynoecial medial domain is greatly disrupted resulting in the loss of ovule primordia. These results suggest that *SEU* and *LUG* participate with *ANT* in gene regulation events that are required for the development of the medial gynoecial domain.

*ANT* encodes a DNA binding transcription factor of the *AP2* gene family that functions during organogenesis [10,11]. *ANT* potentiates organ growth by engendering a...
competence for cellular divisions during organ development [12,13]. The ant single mutants display fewer and smaller lateral organs in both vegetative and reproductive parts of the plant as well as alterations in the development of the ovule integuments [10,11]. The integuments are layers of cells that later form into the seed coat. In the ant single mutant both the inner and outer integuments fail to develop properly. SEU and LUG also play a role in the development of the ovule integuments [1,14]. However, the seu and lug single mutants display a relatively mild disruption of ovule integument development that is incompletely penetrant.

Brassinosteroid hormones are a class of plant hormones that play a role in a wide variety of developmental processes [15,16]. The two main active brassinosteroid hormones in Arabidopsis are castasterone (CS) and brassinolide (BL). The synthesis of these two hormones in Arabidopsis requires a cytochrome p450 (cyp450)-type enzyme, CYP85A2 (At3G30180) that is rate-limiting for the conversion of 6-deoxyCS to CS and CS to BL [17,18]. However, the phenotype of the cyp85A2 mutant is much less severe than that of the brassinosteroid insensitive bri1 mutant [19]. This is due in part to the partially redundant activity of a paralogous cytochrome p450 enzyme, CYP85A1 (At5G38970) and by the presence of CYP85A-independent pathways for the production of CS [17,18].

Here we report synergistic genetic interactions between mutations in the CYP85A2 gene and seu mutants that affect the development of the gynoecial and ovule defects of the seu-1 mutant (Methods). We uncovered eleven seuss-modifier (sum) mutations that enhanced the seu-1 phenotype. We focused our initial efforts on sum63 as it showed a synergistic genetic interaction with seu with respect to gynoecial development and female fertility.

**Molecular identification and phenotypic analysis of sum63 single mutants**

In order to better characterize the sum63 single mutant phenotype in a wild type background, the seu sum 63 double mutant identified in the screen was backcrossed to the Ler parental ecotype three times and sum63 single mutant lines were isolated. The sum63 single mutants conditioned a moderate reduction in plant height as well as alterations in rosette leaf development (Figure 1M; Table 1). Rosette leaves of the sum63 single mutant were darker green and rounder in shape when compared to wild type leaves. The morphology of the sum63 flower was similar to wild type except that the stamens were shorter than wild type (Figure 1D). This resulted in a slightly reduced ability of the sum63 flower to self fertilize.

We also generated a F2 mapping population by crossing the seu sum63 double mutant (Ler ecotype) to wild type Col-0 plants. The sum63 mutation was rough mapped to an interval of chromosome III between ciw11 and T32N15.42 that included the CYP85A2 (AT3G30180) gene. Comparison of the sum63 single mutant phenotype to the published phenotype for loss-of-function alleles of CYP85A2 suggested that sum63 might be allelic with CYP85A2 [17,18]. We compared the sequence of the CYP85A2 genomic DNA from Ler and sum63 individuals in an effort to identify the sequence alteration underlying the sum63 allele. We successfully amplified and sequenced the first 1274 nucleotides of the CYP85A2 gene sequence (transcriptional start site as +1) as well as a 3’ portion of the CYP85A2 gene from position +2427 into the 3’untranslated region (See Table 2 for primer sequences). We found no changes in the sequence of these regions in the sum63 mutant relative to the sequences we derived from Ler DNA. However, we were unable to amplify the intervening portions of the CYP85A2 gene from the sum63 mutants, while these same regions were successfully amplified from Ler individuals. Furthermore, oligonucleotide primers that spanned the intermediate region (i.e. between +1274 and +2427) also failed to generate amplicons from the sum63 genomic DNA. These results suggested the presence of a genomic rearrangement in sum63 individuals that disrupts the CYP85A2 gene sequence. Complementation tests between the sum63
allele and the previously characterized cyp\textsubscript{85A2-1} and cyp\textsubscript{85A2-2} alleles [18,20] revealed that sum\textsubscript{63} was allelic with these cyp\textsubscript{85A2} alleles (data not shown). Thus we have renamed the sum\textsubscript{63} allele cyp\textsubscript{85A2-4} (Table 3). Characterization of the T-DNA insertion sites in cyp\textsubscript{85A2-1} and cyp\textsubscript{85A2-2} individuals confirmed disruption of this gene in these lines (Methods). The cyp\textsubscript{85A2-1} allele has been previously reported as a null allele based on a failure to detect transcript in RT PCR assays [17]. The phenotypes of the cyp\textsubscript{85A2-1}, cyp\textsubscript{85A2-2} and cyp\textsubscript{85A2-4} alleles are similar suggesting that they are all strong loss-of-function alleles (Figure 1).

The seu cyp\textsubscript{85A2} double mutant conditions enhanced disruptions of gynoecial and ovule development

To further characterize the seu cyp\textsubscript{85A2} double mutant phenotype we created and analyzed the following double mutants: seu\textsubscript{3} cyp\textsubscript{85A2-1}, seu\textsubscript{3} cyp\textsubscript{85A2-2} (both in the Col-0 background) and seu\textsubscript{1} cyp\textsubscript{85A2-4} (Ler background). We did not detect an enhancement of the homeotic transformations previously reported for the seu\textsubscript{1} allele [1] in the seu cyp\textsubscript{85A2} double mutants. However, all three seu cyp\textsubscript{85A2} double mutant combinations displayed enhanced defects in ovule and gynoecial development. Double mutants conditioned enhanced splitting of the apex of the gynoecium relative to the single mutants (Figure 1). The cyp\textsubscript{85A2} single mutants did not display splitting of the gynoecial apex and splitting in the seu single mutant was mild and rarely observed in the early arising flowers. In contrast, the gynoecial apex in the seu cyp\textsubscript{85A2} double mutants was nearly always split and extended horn like protrusions of the valves were observed. The splitting of the apex and the horn-like protrusions may be the result of a reduction in the growth of the medial domain of the gynoecium. Analysis of earlier stage gynoecia indicate that even as early as floral stage 7 or 8 [21] the medial domain of the gynoecium appears retarded in its growth relative to the lateral or valve domains (Figure 1N-P). The seu cyp\textsubscript{85A2} double mutants also display a significant reduction in the number of ovule primordia initiated relative to wild type and either single mutant (Table 1). Kim et al. have previously reported that over-expression of CYP85A2 conditions an increased number of seeds per siliqua, further suggesting a role for CYP85A2 in the development of ovules from the gynoecial medial domain [17].

The seu cyp\textsubscript{85A2} double mutants also conditioned an extreme loss of fertility as these double mutants did not generate viable seeds upon self-fertilization. Our analysis of ovule defects indicated the ovule developmental defects of the double mutant were enhanced relative to either single mutant. The double mutant ovules displayed a reduced growth of the outer integument relative to either single mutant (Figs. 2 and 3). In severe cases the outer integument failed to develop, resulting

![Figure 1](image_url)

Figure 1 The seu cyp\textsubscript{85A2} double mutants condition enhanced gynoecial defects. Photomicrographs of indicated genotypes: panels A-L, flowers where some sepals and petals have been removed to allow viewing of gynoecia; panel M, rosette morphology. A) Col-0 wild type flower. B) seu\textsubscript{1} mutant flower. C) seu\textsubscript{3} mutant flower. Note slight split at gynoecial apex (arrowhead). D) sum\textsubscript{63} single mutant flower. E) cyp\textsubscript{85A2-2} mutant flower. F) cyp\textsubscript{85A2-1}. G) seu\textsubscript{1} sum\textsubscript{63} double mutant. H) seu\textsubscript{3} cyp\textsubscript{85A2-2} double mutant. I) seu\textsubscript{3} cyp\textsubscript{85A2-1} double mutant. Enhanced splitting at the gynoecial apex is detected in the seu cyp\textsubscript{85A2} double mutants relative to the respective single mutants. J-L) higher magnification of gynoecial apices shown in A, C and H, respectively. M) Rosette phenotypes. N-P) Nomarski optical images of chloral hydrate cleared stage 7 or early stage 8 gynoecia. N) In seu\textsubscript{3} the medial domain (md) extends to apex of gynoecium. At this stage the seu\textsubscript{3} mutant gynoecium shown is indistinguishable from wild type (not shown). O) In the seu\textsubscript{3} cyp\textsubscript{85A2-1} double mutant the extent of the medial domain is reduced. P) Severely effected seu\textsubscript{3} cyp\textsubscript{85A2-1} gynoecium. Adaxial portions of the medial domain are very reduced resulting in a "hollowed out" gynoecium. Scale bars in A is 1 mm for panels A-L; scale bar in J is 1 mm for images J-L; scale bar in M is 5 cm; scale bar in N is 0.1 mm for N-P.
in a somewhat orthotropic ovule morphology (Figure 2G). Although brassinolide has not previously been reported to play a role in outer integument development, the double mutant phenotype suggests a requirement for the cyp85A2 gene for outer integument development in the seu mutant background. It is likely that the loss of SEU sensitizes the ovule to disruptions in the levels of brassinolide.

We also noted defects in the development of the female gametophytes within the seu cyp85A2 ovules (Figure 3). Often the female gametophyte was missing or failed to reach a mature morphology with a recognizable set of gametophyte cells. As we did not observe segregation distortion with these alleles, it is most likely that the defects in female gametophyte development are an indirect result of the development of the sporophytic tissue of the ovule and not due to a requirement for SEU and CYP85A2 activity in the female gametophyte directly.

Levels of CYP85A2 transcript are reduced in seuss mutant inflorescence samples

We examined the steady state levels of transcript accumulation for the CYP85A2 and SEU transcripts in wt (Col-0), seu-3 and cyp85A2-1 mutant inflorescences. In the seu-3 mutant tissue the level of the CYP85A2 transcript was significantly less (41% of wild type) than that detected in wild type inflorescence samples (Table 4). These data suggest that SEU activity may be required for wild type levels of CYP85A2 transcript accumulation in the inflorescence. No statistically significant difference in the expression of SEU transcript was detected between the wild type and the cyp85A2 inflorescences tested. Thus CYP85A2 does not appear to be required for expression of the SEU transcript. The cyp85A2-1 allele has been previously reported as a null allele, yet we detected a low level of expression of the CYP85A2 transcript in the cyp85A2-1 inflorescences. However, the cyp85A2-1 allele is still likely a null or near null allele

**Table 1 Quantitative phenotypic analysis of cyp85A2 seu double mutants**

|                  | Ler  | seu-1 | sum63 | seu-1; sum63 | Col-0 | seu-3 | cyp85A2-1 | cyp85A2-2 | seu-3; cyp85A2-1 | seu-3; cyp85A2-2 |
|------------------|------|-------|-------|--------------|-------|-------|-----------|-----------|------------------|------------------|
| plant height (cm)| 12.8 | 8.7<sup>a</sup> | 9.1<sup>b</sup> | 4.4<sup>b</sup> | 14    | 9.4<sup>c</sup> | 11<sup>a</sup> | 10<sup>d</sup> | 6.8<sup>c</sup> | N.D.             |
| +/-              | +/- 0.71 | +/- 0.54 | +/- 0.52 | +/- 0.24 | +/- 0.62 | +/- 0.68 | +/- 0.57 | +/- 0.54 | +/- 0.38        |                  |
| ovule number per siliqe | N.D. | N.D | N.D | N.D | 53 | 49 | 45<sup>b</sup> | 45<sup>d</sup> | 31<sup>c</sup> | 32<sup>c</sup> |
| +/-              | +/- 20 | +/- 1.4 | +/- 1.3 | +/- 1.6 | +/- 1.1 | +/- 1.3 |                  |                  |

<sup>a</sup> indicates statistically different from Ler (ANOVA and pair wise Tukey-Kramer HSD; alpha less than 0.05)
<sup>b</sup> indicates statistically different from Ler, seu-1 and sum63
<sup>c</sup> indicates statistically different from Col-0
<sup>d</sup> indicates statistically different from Col-0, seu-3, cyp85A2-1 and cyp85A2-2
<sup>+</sup> indicates standard error of the mean
N.D. - not determined
based on the site of the T-DNA insertion that is expected to truncate the CYP85A2 protein product after just 45 amino acids. Thus it seems unlikely that the synergistic enhancement of the seu cyp85A2 double mutants is entirely conditioned by a seu-mutant dependent reduction of CYP85A2 transcript.

We propose that additional genes are misregulated in the seu mutant background and that these disrupted gene regulation events contribute to the phenotypic enhancement. We speculate that these genes might lie in the brassinosteroid synthesis pathway or might lie in parallel pathways that support common cellular responses (e.g. cell division or cell expansion) in the ovule or the gynoecial medial domain. SEU is required for proper response to the phytohormone auxin and altered auxin signaling may in part condition the disruption of gynoecial medial domain development seen in seu ant double mutants [2,22]. Given the extensive overlap of brassinosteroid-responsive and auxin-responsive genes and the documented requirement of brassinosteroids for transcriptional responses to auxin [23-28], it is possible that the enhanced seu cyp85A2 double mutant phenotypes result from the combined weakening of brassinosteroid- and auxin-dependant signaling pathways.

Conclusions

Our screen for second site genetic modifiers of the seu mutant gynoecial and ovule phenotypes has identified cyp85A2 as a genetic enhancer of the seu mutant. These results suggest that brassinolide hormones play a previously unappreciated role in the development of the outer integument of the ovule and the gynoecial medial domain. They also suggest that loss of SEU activity may sensitize ovule and gynoecial development to the loss of brassinosteroid hormones. The seu mutant background thus may represent a sensitized genetic background to identify additional regulators of gynoecial and ovule function.

Methods

Microscopic and morphometric analysis

For chloral hydrate clearing inflorescences were fixed in ethanol:acetic acid (9:1) for two hours at room temperature, washed in 90% ethanol (two times) and then cleared overnight at room temperature in either chloral hydrate (Sigma) (2.5 g dissolved in 1 ml of 30% glycerol) or Hoyer’s solution (70% chloral hydrate, 4% glycerol, 5% gum arabic (Sigma)) [29]. Gynoecia were then dissected and mounted on slides in Hoyer’s solution, and examined on Axioscop2 microscope (Zeiss) with Nomarski optics. Ovule counts were made in chloral hydrate cleared stage 9-12 gynoecia. Estimations of outer integument defects and female gametophyte development were made from mature ovules or young seeds observed in stage 12 -14 flowers. Images were captured with a micropublisher 5.0 RTV digital camera and Q capture software (Q Imaging, Surrey, BC, Canada). Cropping and contrast adjustment of images was done in Adobe Photoshop CS2 (Adobe Software). Plant heights were measured manually when plants were at an equivalent developmental age as determined by the number of post-abscission siliques observed on the primary shoot. Statistical analysis was carried out in JMP7 (SAS Institute Incorporated) using ANOVA followed by pair wise comparisons with a Tukey-Kramer HSD test and an alpha value cutoff of 0.05.

Genotyping and T-DNA insertion site mapping

The T-DNA insertion site was mapped for salk_056270 (cyp85A2-1) in At3g30180 within the end of exon 1 in the codon encoding glycine 45. The asterisk (*) indicates the insertion site within the genomic DNA sequence: 5’ GCCAATATTTGTGA*AACGACTGAGTTTCT3’.

The T-DNA insertion site was mapped for salk_129352 (cyp85A2-2) within the end of exon 4 in the codon encoding glutamic acid 302. The asterisk (*) indicates the insertion site within the genomic DNA sequence: 5’AGCTCT TGAAGAACT*CAGAGTATGTACTGTTCT3’.

Table 2 Sequences of primers used for amplification and sequencing of the CYP85A2 gene

| primer name                     | sequence                          |
|---------------------------------|-----------------------------------|
| AT3G30180-F1                    | TAAACAACGGCAACACACACCC            |
| AT3G30180-613B                  | CACCGAGCTCTCCATTAGGCC             |
| AT3G30180-445F                  | TGTTTTGCCCAACAAAGGTCTC            |
| AT3G30180-1274R                 | TCCACACACAGCTGCTGAAAA             |
| AT3G30180-2427F                 | TTTGGTCCTTCTGTGTTCGGTGG           |
| AT3G30180-3UTRR                 | CATTGCGAATAGGCCCAGGATTC           |
| AT3G30180-2938R                 | TTCCATTTCCTTCTCTCTCTC             |

Table 3 CYP85A2 mutant alleles described in this study

| Allele   | Other Designation | Allele Disruption                      | Reference |
|----------|-------------------|----------------------------------------|-----------|
| cyp85A2-1 | salk_056270       | T-DNA insertion in CDS at amino acid 45 | [17,18]   |
| cyp85A2-2 | salk_129352       | T-DNA insertion in CDS at amino acid 302| [17,18]   |
| cyp85A2-4 | salk_129352       | T-DNA insertion in CDS at amino acid 302| [17,18]   |
|          | sum63             | uncharacterized rearrangement          | This study|

§ this allele (salk_129352) has been designated cyp85A2-2 by Nomura et al., 2005. The allele designated cyp85A2-2 in Kim et al., 2005 is a different allele (salk_068754) that was designated cyp85A2-3 by Nomura et al.
The oligonucleotide pairs: SALK_056270 LP (5′ GAATTTCTGTGCTGAAATTGC3′), SALK_056270 RP (5′ACCCGAGATTCAGATTCAATG3′); and SALK_129352 LP (5′CGTAAATTCTCCAACCTTT TGG3′), SALK_129352 RP (5′TTGTGTGGGAACCTCTATC G3′) were used with oligo LBB1 (5′GGAGGTGGAGGTTTAGGGT TCG3′) for genotyping and insertion site mapping.

qRTPCR analysis
For analysis of transcript abundance, inflorescences (inflorescence meristem through floral stage 12) were collected, frozen in liquid nitrogen and ground in microcentrifuge tubes. RNA extraction, cDNA synthesis, and qRT-PCR were performed as previously described [8]. A single qRT-PCR run contained four biological replicates, and each biological replicate was assayed in triplicate. Results shown in Table 4 are the mean expression of the indicated gene normalized to ADENOSINE PHOSPHORIBOSYL TRANSFERASE (At1g27450). Results are averages and standard error of the mean for four biological replicates. Statistical analysis of differences of means was carried out in JMP7 (SAS Institute Incorporated) using a Tukey-Kramer HSD test and a p value cutoff of 0.05. Sequences of the oligonucleotides used for qRT-PCR analysis: At3G30180-1440/1441F (5′GGAGGTGGAGGTTTAGGGT TCG3′) and At3G30180-1440/1441R - (5′TCTTCTCCATTTCTCT TCCATCTAT3′)

Mutagenesis
Approximately 5,000 homozygous seu-1 seeds were imbibed overnight in 4 mM ethyl methane sulfonate in a 50 ml conical tube. Seeds were then rinsed several times and planted to soil. Pools of M2 seeds were collected from approximately fifteen M1 plants. M2 pools were screened for enhanced gynoecial defects or female sterility.

Mapping of sum63 mutation and sequencing of CYP85A2 genomic DNA from the sum63 mutant
Pollen from sum63 seu-1 double mutants (in the Ler background) was used to pollinate Col-0 plants. Resulting F2 plants were visually screened and genomic DNA was prepared from leaves of 53 plants that displayed the sum63 phenotype. The sum63 mutation was rough mapped between ciw11 and T32N15.42 on the third chromosome. We estimated the percentage recombination between the ciw11 marker and the sum63 mutation was 9.8% and between T32N15.42 and sum63 was 10.2%. The oligonucleotide pairs AT3G30180-F1 and AT3G30180-613R as well as AT3G30180-1274R were used to amplify and sequence the first 1274 nucleotides (relative to transcriptional start site as position +1) of the CYP85A2 gene, as well as primer pairs expected to hybridize to the intervening portions of the CYP85A2 gene, as well as primer pairs that were expected to span the intervening region (i.e. AT3G30180-445F and AT3G30180-2938R) did not generate amplicons from the sum63 DNA while they did yield amplicons from the Ler DNA samples.

List of abbreviations used
SEUSS (SEU); seu: modifier (sum); (CYP): cytochrome p450; (CS): castasterone; (BL): brassinolide; (Ler): Landsberg erecta; (Col): Columbia; (LUG): LEUNIG; (ANT): ANTEGUMENTA;

Table 4 qRT-PCR quantification of mean expression levels of CYP85A2 and SEU transcripts in wt, seu, and cyp85A2 mutants

|         | wt (Col-0) | seu-3  | cyp85A2-1 |
|---------|------------|--------|-----------|
| CYP85A2 | 0.29       | 0.12±  | 0.12±     |
| +/-     | 0.03       | +/- 0.01 | +/- 0.02 |
| SEU     | 0.43       | 0.12±  | 0.39      |
| +/-     | 0.02       | +/- 0.01 | +/- 0.05 |

* indicates statistically different from Col-0
(ANOVA and pair wise Tukey-Kramer HSD; alpha less than 0.05)
+/- indicates standard error of the mean

 normalized to ADENOSINE PHOSPHORIBOSYL TRANSFERASE (AT1G27450)
Authors' contributions
SNW, EER, HB, and RGF all contributed to data collection and analysis. RGF devised the screen and wrote the paper. All authors have read and approved the final manuscript.

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References
1. Franks RG, Wang C, Levin JZ, Liu Z: SEUSS, a member of a novel family of plant regulatory proteins, represses floral homeotic gene expression with LEUNIG. Development 2002, 129(1):253-263.
2. Bao F, Ashahanandam S, Franks RG: SEUSS and SEUSS-LIKE transcriptional adaptors regulate floral and embryonic development in Arabidopsis. Plant Physiol 2010, 152(2):821-836.
3. Sitaraman J, Bui M, Liu Z: LEUNIG_HOMOLOG and LEUNIG perform partially redundant functions during Arabidopsis embryogenesis and floral development. Plant Physiol 2008, 147(2):672-681.
4. Sridhar VJ, Suendreddara A, Gonzalez D, Conlan RS, Liu Z: Transcriptional repression of target genes by LEUNIG and SEUSS, two interacting regulatory proteins for Arabidopsis flower development. Plant Physiol 2004, 131(3):1194-1199.
5. Sridhar VJ, Suendreddara A, Liu Z: APETALA1 and SEUSS-LIKE interact with SEUSS to mediate transcription repression during flower development. Development 2006, 133(16):3159-3166.
6. Grego V, Sessa A, Colombo L, Kater MM: AGL24, SHORT VEGETATIVE PHASE, and APETALA1 redundantly control AGAMOUS during early stages of flower development in Arabidopsis. Plant Cell 2006, 18(6):1373-1382.
7. Gonzalez D, Bowen AJ, Carroll TS, Conlan RS: The transcription coexpressor LEUNIG interacts with the histone deacetylase HDA19 and mediator components MED14 (SWP) and CDK8 (HEN3) to repress transcription. Mol Cell Biol 2007, 27(15):5306-5315.
8. Ashahanandam S, Nole-Wilson S, Bao F, Franks RG: SEUSS and AINTEGUMENTA Mediate Patterning and Ovule Initiation during Gynoecium Medial Domain Development. Plant Physiol 2008, 148(3):1165-1181.
9. Liu Z, Franks RG, Klink VP: Regulation of gynoecium marginal tissue formation by LEUNIG and AINTEGUMENTA. Plant Cell 2000, 12(10):1879-1892.
10. Elliott RC, Betzner AS, Huttner E, Oakes MP, Tucker WQ, Gerentes D, Perez P, Smyth DR: AINTEGUMENTA, an APETALA2-like gene of Arabidopsis with pleiotropic roles in ovule development and floral organ growth. Plant Cell 1996, 8(2):155-168.
11. Klucher KM, Chow H, Reiser L, Fischer RL: The AINTEGUMENTA gene of Arabidopsis required for ovule and female gametophyte development is related to the floral homeotic gene APETALA2. Plant Cell 1996, 8(2):137-153.
12. Krizek BA: Ectopic expression of AINTEGUMENTA in Arabidopsis plants results in increased growth of floral organs. Dev Genet 1999, 25(3):224-236.
13. Mizukami Y, Fischer RL: Plant organ size control: AINTEGUMENTA regulates growth and cell numbers during organogenesis. Proc Natl Acad Sci USA 2000, 97(2):942-947.
14. Liu Z, Meyerowitz EM: LEUNIG regulates AGAMOUS expression in Arabidopsis flowers. Development 1995, 121(4):915-921.
15. Fujita S, Yokota T: Biosynthesis and metabolism of brassinosteroids. Annu Rev Plant Biol 2003, 54:137-164.
16. Clouse SD, Sasse JM: Brassinosteroids: Essential regulators of plant growth and development. Annu Rev Plant Phys 1998, 49:227-251.
17. Kim TW, Hwang JY, Kim YS, Joo SH, Chang SC, Lee JS, Takatsuto S, Kim SK: Arabidopsis CYP85A2, a cytochrome P450, mediates the Baeyer-Villiger oxidation of castasterone to brassinolide in brassinosteroid biosynthesis. Plant Cell 2005, 17(8):2397-2412.
18. Nomura T, Kusitano T, Yokota T, Kariya Y, Bishop GJ, Yamaguchi S: The last reaction producing brassinolide is catalyzed by cytochrome P-450, CYP85A3 in tomato and CYP85A2 in Arabidopsis. J Biol Chem 2005, 280(18):17873-17879.
19. Clouse SD, Langford M, McMorris TC: A brassinosteroid-insensitive mutant in Arabidopsis thaliana exhibits multiple defects in growth and development. Plant Physiol 1996, 111(3):671-678.
20. Alonso JM, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shen P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, et al: Genome-wide insertional mutagenesis of Arabidopsis thaliana. Science 2003, 301(5633):653-657.
21. Smyth DR, Bowman JL, Meyerowitz EM: Early flower development in Arabidopsis. Plant Cell 1999, 20(7):755-767.
22. Pflug J, Zambrsky P: The role of SEUSS in auxin response and floral organ patterning. Development 2004, 131(19):4697-4707.
23. Nemhauser JL, Mockler TC, Chory J: Interdependency of brassinosteroid and auxin signaling in Arabidopsis. PLoS Biol 2004, 2(9):e258.
24. Hardtke CS: Transcriptional auxin-brassinosteroid crosstalk: who’s talking? Bioessays 2007, 29(11):1115-1123.
25. Goda H, Sawa S, Asami T, Fujitaka S, Shimada Y, Yoshida S: Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in Arabidopsis. Plant Physiol 2004, 134(4):1553-1573.
26. Moucheif CF, Osmond KS, Hardtke CS: BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth. Nature 2006, 443(7110):458-461.
27. Bao F, Shen J, Brady SR, Mudgett GK, Asami T, Yang Z: Brassinosteroids interact with auxin to promote lateral root development in Arabidopsis. Plant Physiol 2004, 134(4):1624-1631.
28. Nakamura A, Higuchi K, Goda H, Fujitaka M, Sawa S, Kobayashi T, Shimada Y, Yoshida S: Brassinolide induces IAA5, IAA19, and DR5, a synthetic auxin response element in Arabidopsis, implying a cross talk point of brassinosteroid and auxin signalling. Plant Physiol 2003, 133(4):1843-1853.
29. Berleth T, Burgens G: The role of the monopteris gene in organising the basal body region of the Arabidopsis embryo. Development 1993, 118(2):575-587.

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