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Research Article

Altered expression of *SELF-PRUNING* disrupts homeostasis and facilitates signal delivery to meristems

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Abstract

Meristem maintenance, achieved through the highly conserved CLAVATA-WUSCHEL (CLV-WUS) regulatory circuit, is fundamental in balancing stem cell proliferation with cellular differentiation. Disruptions to meristem homeostasis can alter meristem size, leading to enlarged organs. Cotton (*Gossypium* spp.), the world's most important fiber crop, shows inherent variation in fruit size, presenting opportunities to explore the networks regulating meristem homeostasis and to impact fruit size and crop value. We identified and characterized the cotton orthologs of genes functioning in the *CLV-WUS* circuit. Using virus-based gene manipulation in cotton, we altered the expression of each gene to perturb meristem regulation and increase fruit size. Targeted alteration of individual components of the *CLV-WUS* circuit modestly fasciated flowers and fruits. Unexpectedly, controlled expression of meristem regulator *SELF-PRUNING* (*SP*) increased the impacts of altered *CLV-WUS* expression on flower and fruit fasciation. Meristem transcriptomics showed *SP* and genes of the *CLV-WUS* circuit are expressed independently from each other, suggesting these gene products are not acting in the same path. Virus-induced silencing of *GhSP* facilitated the delivery of other signals to the meristem to alter organ specification. SP has a role in cotton meristem homeostasis, and changes in *GhSP* expression increased access of virus-derived signals to the meristem.

Introduction

Meristems depend on intercellular signals to balance stem cell proliferation with cellular differentiation. In the shoot apical meristem, this regulation is achieved through the highly conserved CLAVATA (CLV)-WUSCHEL (WUS) circuit (Brand et al. 2000; Schoof et al. 2000). *WUS*, expressed in the organizing center of the Arabidopsis (*Arabidopsis thaliana*) shoot apical meristem, encodes a homeodomain transcription factor essential for stem cell activity (Laux et al. 1996; Mayer et al. 1998). WUS moves through plasmodesmata to the stem cells where it directly binds to and activates the expression of *CLV3* (Schoof et al. 2000; Yadav et al. 2011; Daum et al. 2014). The CLV3 ligand, processed to a 13 amino acid glycopeptide, is secreted from the stem cells and diffuses to the underlying organizing center (Fletcher et al. 1999; Rojo et al. 2002; Kondo et al. 2006; Ohyama et al. 2009). By binding to leucine-rich repeat receptor kinase CLV1 and related receptors, CLV3 initiates signaling cascades to promote cell differentiation and restrict *WUS* expression (Clark et al. 1997; Fletcher et al. 1999; Jeong et al. 1999; Schoof et al. 2000; Müller et al. 2008; Ogawa et al. 2008). As a result of this negative feedback, WUS promotes stem cell proliferation while CLV signaling proteins prevent the over-proliferation of stem cells (Clark et al. 1995, 1997; Laux et al. 1996; Mayer

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et al. 1998; Fletcher et al. 1999; Schoof et al. 2000). Disruptions in CLV-WUS feedback signaling alter meristem size and lead to changes in inflorescence size and floral organ number. Arabidopsis *clv1*, *clv2*, and *clv3* mutants have enlarged meristems with supernumerary floral organs, including carpels that give rise to fruit and seed, whereas the floral meristems from *wus* loss-of-function mutants terminate prematurely, with flowers lacking most stamens and carpels (Laux et al. 1996; Clark et al. 1997; Brand et al. 2000).

The domestication of many crops resulted in larger inflorescences and fruits. In tomato (*Solanum lycopersicum*) and maize (*Zea mays*), larger fruits resulted from disrupted meristem homeostasis. In tomato, mutations in the *CLV1* homolog, *FASCIATED AND BRANCHED* (*FAB*), and *SlCLV2*, and mutations that disrupt *SlCLV3* expression (*fasciated*) or protein activity (*fasciated inflorescence*, *fin*, encoding a necessary arabinosyltransferase), and those that increase SlWUS activity (*locule number*), all result in meristem fasciation, extra floral organs, and large fruits with 12 or more locules for strong *fin* alleles (Cong et al. 2008; Munos et al. 2011; Rodriguez et al. 2011; Xu et al. 2015). Editing the *SlCLV3* cis-regulatory regions yields tomatoes with a continuum of locule numbers (Wang et al. 2021). In maize, mutations in the *CLV1* and *CLV2* putative orthologs *thick tassel dwarf* and *fasciated ear2* (*fea2*), respectively, and in a leucine-rich receptor-like kinase encoded by *fasciated ear3* (*fea3*) produce more rows of kernels per inflorescence (Taguchi-Shiobara et al. 2001; Bommert et al. 2005, 2013; Je et al. 2016). Quantitative trait locus mapping and functional assays indicate that weak alleles of *fea2* and *fea3* contributed to maize domestication by increasing rows of kernels (Bommert et al. 2013; Je et al. 2016). This is further supported as generating weak alleles by editing *ZmCLAVATA3/EMBRYO SURROUNDING REGION-RELATED* (*CLE*) promoters increases several yield-related traits in maize (Liu et al. 2021).

Cotton (*Gossypium* spp.) is our most important fiber crop and a valuable source of seed oil and protein. Although naturally a photoperiodic (short-day) perennial tree, cultivated cotton, mostly Upland (*Gossypium hirsutum*; ~95% of worldwide production) and Pima (*Gossypium barbadense*; ~5% of worldwide production), is grown as a day-neutral, annual crop. The earliest bolls generate seed fiber with the highest favorable attributes of length and strength, while those forming later in the season can have reduced quality, and consequently, value. Large-scale, mechanical harvesting strategies favor single-pass harvesting late in the season, resulting in a less-than-desirable blend of high- and low-quality fibers for downstream processing. More determinate cotton architectures that synchronize flowering times and reduce branching can enhance crop yield and quality (Chen et al. 2015, 2019; Si et al. 2018; McGarry and Ayre 2021). We previously demonstrated that *SINGLE FLOWER TRUSS* (*GhSFT*) and *SELF-PRUNING* (*GhSP*) control cotton growth habit by regulating meristem phase change: high levels of *GhSFT*, encoding the long-distance flowering signal florigen, accelerated flowering, even in "early" day-neutral varieties; silencing *GhSP* expression terminated growth and vegetative branching (McGarry et al. 2016). Additional strategies to enhance yield, such as increasing the size of the earliest and highest-quality bolls, are needed and could benefit newer technologies for more frequent robot harvesting as well as mitigate environmental impacts.

Cotton bolls on a single plant show inherent variation in locule number: Pima cotton usually produce bolls with three or four locules whereas Upland cotton has four or five locules per boll; bolls with six locules are rare (Dunlavy 1921). A higher proportion of five-locule bolls per plant, preferred by producers, is associated with general crop vigor, meaning optimal water, nutrients, light, and temperature. Understanding the genetic regulation of cotton meristem homeostasis offers untapped potential to impact boll size and crop value. While numerous *CLE* genes are recently identified in cotton (Wan et al. 2021), the molecular mechanisms underlying meristem homeostasis are not addressed. We hypothesized that the genetic networks regulating meristem size could be functionally assessed in cotton, and the activities of specific cotton genes could be manipulated to increase boll size. To test these hypotheses, we identified the cotton orthologs of the *CLV-WUS* meristem maintenance circuit, and transiently perturbed these regulatory networks to investigate their roles in meristem fasciation. In these undertakings, we identified a separate and expanded role for SP in meristem homeostasis and showed that regulated expression of *GhSP* facilitated access of other virus-derived signals to cotton meristems.

Results

Identifying the cotton orthologs of the *CLV-WUS* meristem maintenance circuit

CLV3 is the founding member of the *CLE* genes which encode a large family of small extracellular signaling ligands. CLE propeptides, harboring an amino-terminal signal sequence and conserved CLE domain near the carboxyl terminus, are proteolytically processed to the 12 to 14 amino acid CLE protein and post-translationally modified (Fletcher 2020). To identify the cotton CLV3 orthologs, we queried the AtCLV3 (At2g27250.3) and SlCLV3 (Solyc11g071380) propeptide sequences against the *G. hirsutum* v2.1, *G. barbadense* v1.1, and *G. raimondii* v2.1 databases using tBLASTn and BLASTp searches (Supplemental Fig. S1). These searches identified two homologs in *G. hirsutum* (Gohir.A09G098550 and Gohir.D09G097950) and *G. barbadense* (Gobar.A09G103400 and Gobar.D09G110700), and a single protein in *G. raimondii* (Gorai.006G114900); each cotton protein shared 70% and 77% amino acid identity with the Arabidopsis and tomato proteins, respectively. As there is typically low sequence conservation outside of the CLE domain (Sharma et al. 2003; Strabala et al. 2006), we queried the processed AtCLV3 and SlCLV3 peptides, consisting of only the 13 amino acid CLE box (LRT/GVPS/AGPDPLHH), against the *G. hirsutum* database in BLASTp searches. This approach identified two additional homologs, Gohir.A08G077600 and

Gohir.D08G087400, sharing 83% identity with the Arabidopsis and tomato queries (Supplemental Fig. S2A). Comprehensive clustering analysis of CLE peptides from 57 plant genomes identified a distinct CLE domain conserved in each of 12 phenetic groups, suggesting shared function among peptides of each cluster (Goad et al. 2017). Consistent with this clustering analysis, the four identified *G. hirsutum* proteins harbored the CLE domain characteristic of those regulating meristem homeostasis (Goad et al. 2017) and this was not observed in other cotton CLE peptides (Supplemental Fig. S2B). Together, these analyses support homoeologs Gohir.A08G077600 and Gohir.D08G087400, and Gohir.A09G098550 and Gohir.D09G097950, as the strongest candidates to encode the GhCLV3 ligands. Each homoeologous pair was tested in initial functional assays (Supplemental Appendix S1), and Gohir.A08G077600 and Gohir.D08G087400 were analyzed further.

Multiple receptors, typically leucine-rich repeat receptor-like kinases, can perceive the secreted CLV3 ligand (Fletcher 2020). To identify the cotton orthologs of receptor proteins CLV1 and CLV2, we queried the Arabidopsis and tomato protein sequences against the *G. hirsutum* genome in tBLASTn searches: AtCLV1 (At1G75820) and SlCLV1 (FAB; Solyc04G081590) each identified 96 hits; AtCLV2 (At1G65380) identified 34 hits in cotton. Phylogenetic analysis of the strongest cotton CLV1 hits, having more than 50% identity over the full protein sequence, identified two homoeologous pairs of proteins, Gohir.A02G082800, Gohir.D02G098300, Gohir.A05G185500, and Gohir.D05G188300, as orthologous to AtCLV1 and SlCLV1 (Supplemental Figs. S3 and S4A). The corresponding Gohir.A02G082800 and Gohir.D02G098300 genes shared similar exon/intron organizations with *AtCLV1*, supporting these as candidate *GhCLV1* genes for functional testing (Supplemental Fig. S4B). Among the CLV2 hits, only two cotton homologs, Gohir.A05G390700 and Gohir.D04G026500, shared more than 50% amino acid identity over the full AtCLV2 protein (Supplemental Fig. S5). The corresponding genes were used in subsequent functional analyses.

The arabinosyltransferase FASCIATED INFLORESCENCE (SlFIN), and transcription factor PERIANTHIA (AtPAN) and the maize ortholog FASCIATED EAR4 (ZmFEA4) affect the CLV-WUS circuit to regulate meristem size (Running and Meyerowitz 1996; Chuang et al. 1999; Pautler et al. 2015; Xu et al. 2015). To identify the cotton orthologs, we queried the SlFIN (Solyc11g064850) and AtPAN (At1g68640) peptide sequences against the cotton proteome using BLASTp. SlFIN identified 13 hits in cotton: nine of these had more than 70% identity over the full query sequence and were encoded by six genes (Supplemental Fig. S6). AtPAN identified 100 hits in cotton: the 13 strongest, having more than 50% identity over the full protein sequence, were encoded by four genes (Supplemental Fig. S7). The genes encoding the strongest FIN, Gohir.A06G174700 and Gohir.D06G184700, and PAN, Gohir.A12G169200 and Gohir.D12G172500, homologs, sharing the greatest identity over the longest protein sequence, were used for functional analyses.

Cotton *CLV-WUS* orthologs are expressed in meristems

The components of the CLV-WUS feedback circuit are predicted to regulate meristem size, and in other systems, their expression is exquisitely restricted to specific domains of the meristem. Therefore, we sought to investigate the expression of the genes in cotton. We mined publicly available cotton meristem transcriptomes to quantify expression levels (NCBI GEO GSE144546). Meristems and immature flanking leaves were isolated from seedlings of day-neutral and photoperiodic cotton, and from the apices of the main stem and branches of mature photoperiodic plants grown under inductive short-day conditions or noninductive long days (Prewitt et al. 2018). The *CLV-WUS* homologs were expressed in meristems at all stages tested (Fig. 1; Supplemental Table S1). *GhCLV3*, *GhCLV1*, and some of the *GhWUS* genes showed increased expression in the sympodial branch meristems of mature plants grown under inductive conditions compared to the monopodial main stem meristems, meristems from plants grown in the noninductive environment, or meristems from early development (Fig. 1). Only *GhCLV1* showed a significant increase in expression in branch meristems from mature, short-day grown plants compared to seedlings (Supplemental Table S1). As previously reported, *GhSP* was significantly enhanced in meristems isolated from the branches and main stems of plants grown under short days (Fig. 1; Supplemental Table S1; Prewitt et al. 2018). The expression patterns of the cotton *CLV-WUS* homologs were generally consistent with predicted roles in meristem regulation.

We demonstrated that *GhSFT* and *GhSP* acted through diverse genetic networks to impact different metabolic pathways and developmental patterns (McGarry et al. 2020). To test if the *CLV-WUS* meristem maintenance genes interacted with *GhSFT* or *GhSP*, we used weighted gene co-expression network analysis. Fifty-four cotton libraries, comprised of meristems harvested at different developmental stages and from different photoperiods, and from the apices of TX701 and DP61 plants with altered expression of *GhSFT* and *GhSP*, were used for co-expression analysis (McGarry et al. 2020). Among the 16 modules of co-expressed genes, one *WUS* homolog, *Gohir.A10G098300*, was significantly co-regulated with *GhSP* (Supplemental Table S2). None of the other cotton *CLV-WUS* genes was co-expressed with *GhSFT* or *GhSP*. We then questioned how accelerating meristem determinacy, by over-expressing *GhSFT* or silencing *GhSP* (McGarry et al. 2016; Si et al. 2018; Chen et al. 2019), affected the *CLV-WUS* circuit. Apices from day-neutral DP61 or photoperiodic TX701 with altered *GhSFT* or *GhSP* expression levels were isolated and transcriptomes were prepared as described (NCBI GEO GSE144546) (McGarry et al. 2016). As shown in Table 1, changes in *GhSFT* or *GhSP* did not affect the expression of genes involved in meristem maintenance. The *GhCLV3* and *GhWUS* genes were barely detected, likely reflecting the dilution of their low expression

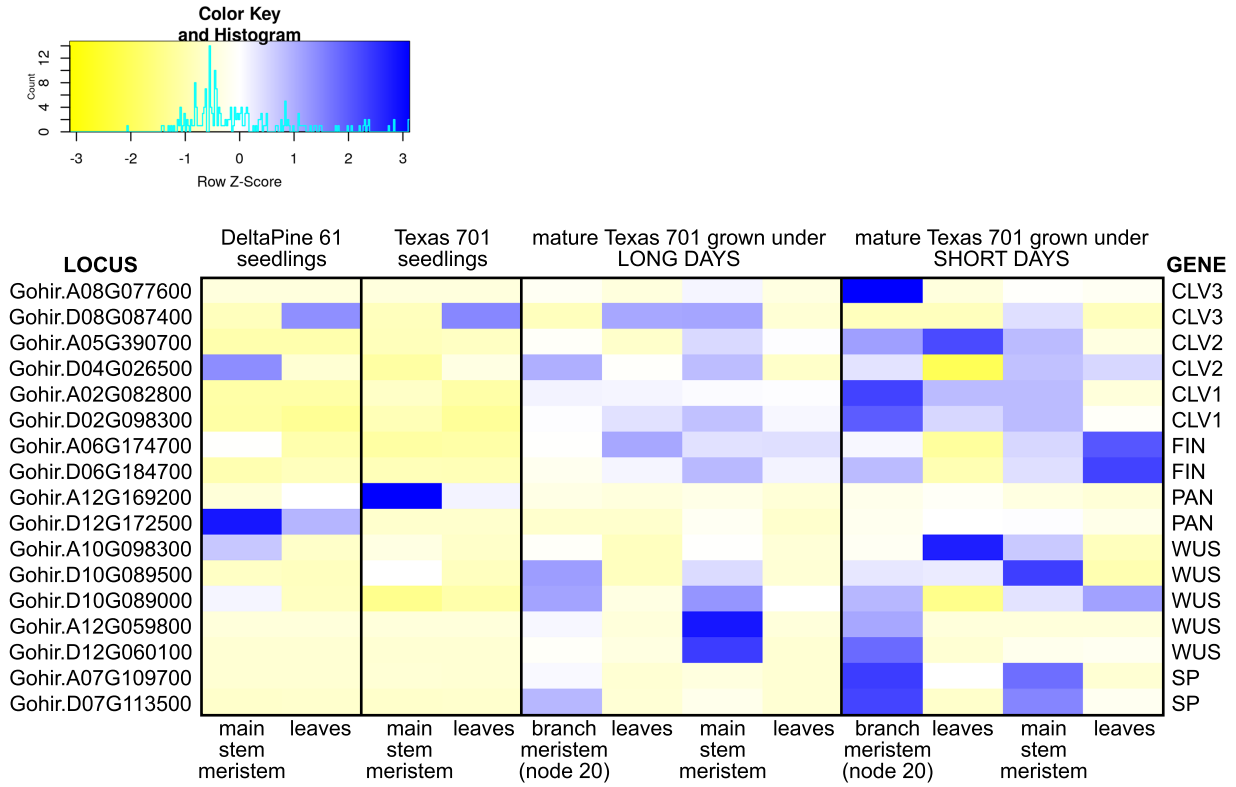

Figure 1. The *CLV-WUS* genes are expressed in cotton meristems. The expression of each gene in meristems and flanking leaves was determined. Meristems were isolated from the monopodial main stems of day-neutral DeltaPine 61 and photoperiodic Texas 701 seedlings; from the monopodial main stem and monopodial lateral branch at node 20 of mature Texas 701 grown under noninductive long days (plants were not flowering); and from the monopodial main stem and sympodial fruiting branch at node 20 of mature Texas 701 grown under inductive short days (plants had flowering sympodial branches). The loci and corresponding gene names are indicated along the *y*-axis. The expression values, reported as FPKM and indicated in the color key (see also Supplemental Table S1), were normalized by *Z*-score across rows and visualized by heatmap using web tool shinyheatmap (Khomtchouk et al. 2017).

Table 1. Changes in *GhSFT* or *GhSP* do not affect the expression of *CLV-WUS* genes in cotton apices

Gene name	Locus	DP61 uninoc	DP61 CLCrV-GhSFT	DP61 TRV-GhSP	TX701 uninoc	TX701 CLCrV-GhSFT	TX701 TRV-GhSP
CLV3	Gohir.A08G077600	0.00	0.00	0.00	0.00	0.00	0.00
CLV3	Gohir.D08G087400	0.00	0.00	0.00	0.00	0.00	0.00
CLV2	Gohir.A05G390700	2.63	3.19	3.34	2.36	4.10	3.99
CLV2	Gohir.D04G026500	2.28	3.90	3.01	2.49	2.61	2.80
CLV1	Gohir.A02G082800	0.67	0.19	0.27	0.34	0.52	0.53
CLV1	Gohir.D02G098300	0.33	0.41	0.76	0.28	0.84	0.97
FIN	Gohir.A06G174700	11.51	8.52	8.65	8.56	9.27	8.34
FIN	Gohir.D06G184700	6.97	6.06	4.01	6.33	5.16	5.52
PAN	Gohir.A12G169200	1.82	2.51	3.73	2.43	1.75	0.00
PAN	Gohir.D12G172500	0.78	2.65	3.16	0.38	0.69	0.89
WUS	Gohir.A10G098300	0.10	0.00	0.00	0.00	0.00	0.00
WUS	Gohir.D10G089500	0.00	0.18	0.15	0.00	0.00	0.00
WUS	Gohir.D10G089000	0.72	1.38	1.10	1.87	1.36	1.12
WUS	Gohir.A12G059800	0.00	0.00	0.00	0.00	0.00	0.00
WUS	Gohir.D12G060100	0.00	0.00	0.00	0.00	0.00	0.00
SP	Gohir.A07G109700	0.00	0.00	0.00	0.00	0.76	0.00
SP	Gohir.D07G113500	1.94	2.03	0.00	0.00	0.00	0.00
SFT	Gohir.A08G227700	0.00	0.62	0.00	0.00	3.5	0.00
SFT	Gohir.D08G248000	0.40	34.22[a]	0.33	0.00	103.45[a]	0.00

The expression of each transcript from apices of plants with different *GhSFT* and *GhSP* expression is reported in FPKM values.
[a]Significantly different pair-wise comparisons at $P < 0.05$; reported *P*-values are corrected using the Benjamini–Hochberg method.

in apices, whereas *GhCLV1*, *GhCLV2*, *GhFIN*, and *GhPAN* showed relatively consistent expression across treatments within each genotype. These findings suggested that, while most of the genes of the *CLV-WUS* circuit functioned independently from *GhSFT* and *GhSP*, a *GhWUS* homolog was significantly co-regulated with *GhSP*.

Transiently disrupting gene expression alters flower and fruit morphology

Tobacco rattle virus (TRV) and the disarmed *Cotton leaf crumple virus* (dCLCrV) are highly effective tools for manipulating gene expression in cotton (Tuttle et al. 2008; Gao et al. 2011; Qu et al. 2012). TRV is a potent vehicle for virus-induced gene silencing (VIGS) and dCLCrV can deliver gene activity when target sequences are expressed in the sense orientation or mild VIGS when targets are oriented in the antisense direction (McGarry and Ayre 2012; McGarry et al. 2016).

We predicted that if the identified cotton orthologs have roles in balancing stem cell maintenance with cellular differentiation, altering their expression using virus-based gene manipulation may impact growth patterns and, most noticeably, affect flower and fruit development. To test this, we silenced each gene from TRV and over-expressed *GhCLV3* from dCLCrV in day-neutral Coker 312. Transient manipulation of meristem homeostasis genes affected the transition to flowering and floral organization. Silencing *GhCLV2* from TRV significantly delayed the onset of reproductive growth compared to controls, as quantified by the node of the first fruiting branch (Fig. 2). Remarkably, silencing *GhCLV2* delayed flowering nearly as much as silencing the florigenic signal *GhSFT* (McGarry et al. 2016), and suggested that *GhCLV2* is required for the normal transition to flowering in cotton. This finding is consistent with reports in Arabidopsis where the *clv2*-null mutant shows late and disrupted flowering, emphasizing that CLV2 signaling affects meristem fate change (Wang et al. 2008; Jones et al. 2021). Altering *GhCLV3* expression affected floral meristems. Consistent with the predicted imbalance reducing meristem size, cotton plants over-expressing *GhCLV3* from dCLCrV produced clusters of small floral buds at apices yet had fewer fruits compared with uninoculated and dCLCrV-infected plants (Supplemental Fig. S8, A–C). Conversely, silencing *GhCLV3* from TRV produced floral buds with multiple unfused carpels (Supplemental Fig. S8, D and E). Taken together, these findings supported that the identified cotton *CLV* orthologs had roles in meristematic activities and that transiently disrupting the expression of these genes altered meristem regulation.

To determine whether virus-mediated changes to target gene expression affected fruit size, we quantified the number of locules present per flower or fruit from three replicate plants of each treatment. As shown in Fig. 3A and E, developing fruits from Coker 312 typically had four or five evenly distributed locules, with septa separating each and joining in the center (columella). Similar distributions and numbers of locules were observed among TRV- and dCLCrV-infected Coker 312 plants (Fig. 3E). VIGS plants showed fewer bolls with four locules per fruit and/or a small proportion of fruit with additional locules (Fig. 3E). Notably, these extra locules were not part of the outer fruit wall. As examples, TRV:GhCLV3-infected plants produced fruits with an extra locule squeezed in between more prominent locules and/or an enlarged central columella with ovules (Fig. 3, B–D). These increases in fruit complexity were not evident from intact bolls as the pericarps showed four or five septa. Over-expressing *GhCLV3* rarely produced fruits with three locules (Fig. 3E). Collectively, these results supported that the cotton gene products functioned in the CLV-WUS meristem circuit and transiently downregulating their expression modestly fasciated floral meristems in a manner suggesting late timing or low penetrance of the silencing signals.

Co-silencing meristem homeostasis genes with *GhSP* exacerbates meristem defects

We questioned if low incidences of floral meristem fasciation were due to the instability of TRV at elevated temperatures and prolonged time to flowering and fruiting (Senthil-Kumar and Mysore 2011). To test this, we included the *GhSP* silencing signal on each TRV virus. We predicted that silencing *GhSP* would accelerate termination, with a distinctive pattern marking flowers where the second meristem silencing signal would be active. As anticipated, plants co-silencing *GhSP* with a target gene of the *CLV-WUS* circuit consistently yielded the *GhSP*-silenced phenotype: growth terminated by the fifth node with a terminal flower and all axillary buds terminated with flowers. Flowers from co-silenced plants showed pronounced disruptions to floral organization not observed in TRV:GhSP-silenced, TRV-infected, nor uninoculated plants (Fig. 4). TRV:GhSP-GhCLV2 plants consistently produced terminal flowers with expanded whorls of carpels and extended and unfused styles (Fig. 4, F–H). TRV:GhSP-GhFIN flowers displayed supernumerary organization of floral organs including extra petals, branched and lobed anthers, and multiple fused stigmas and styles with ovaries showing increased numbers of locules. TRV:GhSP-GhPAN-inoculated plants showed supernumerary petals, and increased complexity of the gynoecium (compare Fig. 4, E and H with Fig. 3A). Supernumerary petals were observed in flowers from TRV:GhSP-GhCLV3-inoculated plants (Fig. 4I). To test if co-silencing *GhSP* with a meristem maintenance gene affected fruit size, we quantified the number of locules per fruit from replicate plants of each treatment from two pooled greenhouse trials. Uninoculated and TRV-infected plants generally yielded bolls with four or five locules, as previously shown (compare Fig. 4J with Fig. 3E). Consistent with their premature termination, TRV:GhSP-infected plants produced bolls containing fewer locules per fruit compared to uninoculated and TRV-infected controls (Fig. 4J). As we did not observe differences in the

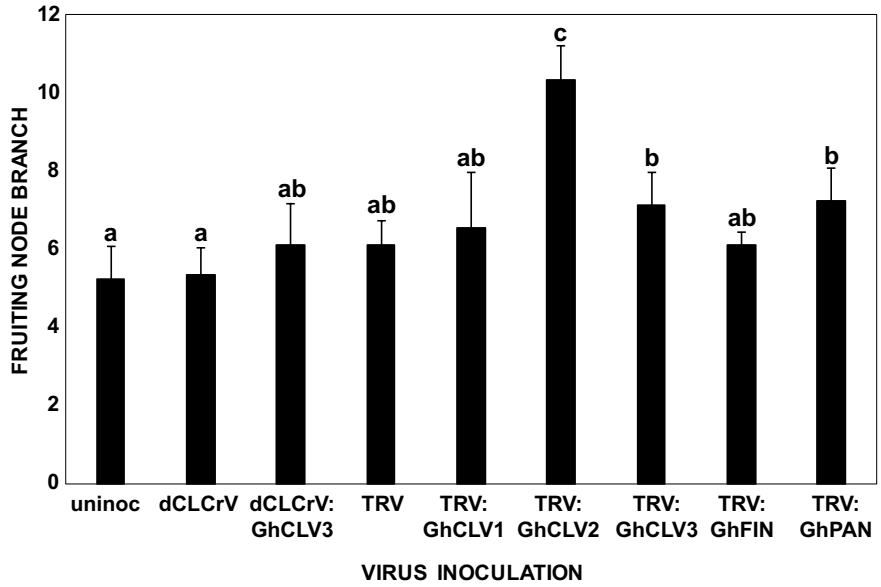

Figure 2. Silencing *GhCLAVATA2* delays the transition to reproductive growth. Meristem homeostasis genes were over-expressed from dCLCrV or silenced from TRV, and the effect of these treatments on flowering time in day-neutral Coker 312 cotton was quantified by the node of the first fruiting branch. Uninoculated (uninoc) plants were included as controls. Error bars reflect the standard deviation among replicate plants per treatment ($n = 6$). The Tukey HSD mean separation test at $P < 0.05$ was used to determine significant differences among treatments, and these are indicated with lower-case letters.

numbers of sepals or petals among flowers from TRV:GhSP-silenced plants, the effect on fruit size suggested that GhSP was needed in the innermost floral whorl, and, in this regard, was reminiscent of the roles for *WUS* in the inner whorls of Arabidopsis flowers (Laux et al. 1996). Co-silencing *GhSP* with *GhCLV1*, *GhCLV2*, *GhCLV3*, *GhFIN*, or *GhPAN* increased the number of locules per fruit and the yield of larger-sized fruits (Fig. 4J). At most, 11 locules per fruit were observed, and bolls with six or more locules comprised over 16% of the yield from TRV:GhSP-GhCLV2-treated plants (Fig. 4J).

We sought to confirm these findings using a different genetic background. Wild, photoperiodic Texas 701 are tall, lanky plants, with pronounced apical dominance (McGarry and Ayre 2012). While these short-day photoperiodic cotton remained vegetative under noninductive long days (McGarry and Ayre 2012), TRV:GhSP-infected Texas 701 terminated by the fifth node irrespective of day-length, and the induced terminal and axillary flowers were productive (McGarry et al. 2016). When Texas 701 plants were infected with TRV viruses targeting *GhSP* alone or with a target of the *CLV-WUS* circuit and grown under noninductive long days, growth terminated. Co-silenced plants produced flowers with six to ten petals whereas flowers from TRV:GhSP-infected plants consistently had five petals (Supplemental Fig. S9, A–C). Notably, one of the TRV:GhSP-GhFIN-infected plants produced a fasciated boll with six septa evident on the pericarp while TRV:GhSP-infected Texas 701 yielded bolls with three or four locules (Supplemental Fig. S9D, E). The uninoculated and TRV-infected controls remained vegetative and did not flower. The findings from this experiment were consistent with the results in Coker 312 (Fig. 4) and emphasized that altered *GhSP* levels magnified defects in meristem homeostasis.

To test if the observed phenotypes were consistent with expected changes in target gene expression, we measured transcript levels in isolated meristems from co-silenced Coker 312 plants. At 15 d post-inoculation (dpi), meristems were harvested from three replicate plants, RNA was isolated, and libraries were prepared for Illumina sequencing. As shown in Table 2, silencing *GhSP* with TRV consistently reduced *GhSP* expression in meristems. This finding supports the observed terminated phenotype in all TRV:GhSP-treated plants, and is consistent with previous reports (McGarry et al. 2016). Similarly, *GhCLV1*, *GhCLV2*, *GhCLV3*, *GhPAN*, and *GhFIN* transcripts were reduced with their respective treatments compared with TRV or TRV:GhSP control treatments. Consistent with the early flowering resulting from *GhSP* silencing, *GhPAN* transcript levels increased in TRV:GhSP and co-silencing treatments compared with TRV alone. Notably, silencing *GhSP* did not significantly affect the expression of *GhCLV1*, *GhCLV2*, *GhCLV3*, *GhPAN*, *GhFIN*, or *GhWUS* in isolated meristems (Table 2) or apices (Table 1). We questioned if changes to *GhSP*, *GhCLV1*, *GhCLV2*, *GhCLV3*, *GhPAN*, and *GhFIN* perturbed the same genetic networks. We identified transcripts significantly differentially expressed by each treatment and visualized their intersections. Few transcripts overlapped all treatments (Supplemental Fig. S10A). Each meristem maintenance gene influenced the expression of distinct groups of genes, however, *GhSP* affected the largest number of unique

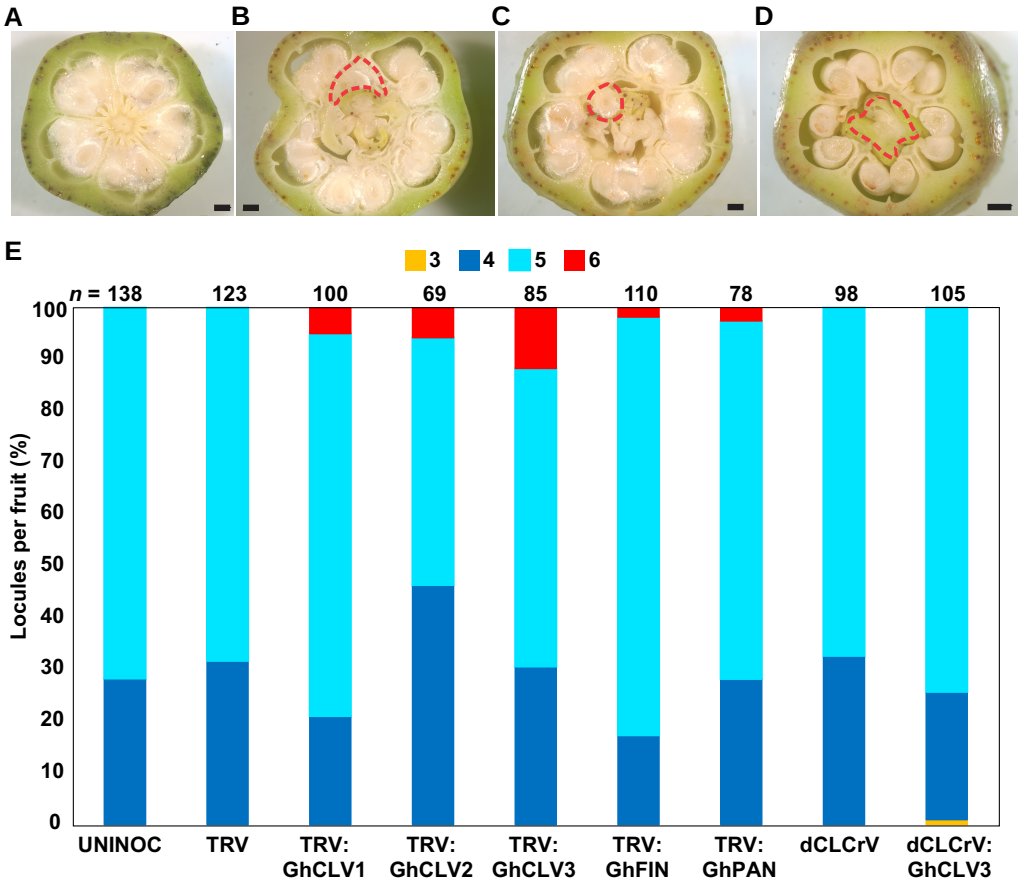

Figure 3. Altering expression of genes from the *CLV-WUS* circuit modestly fasciates flowers and fruits. **A)** A transverse section of a maturing boll from an uninoculated (uninoc) control plant reveals five evenly distributed locules with clear septa extending from the tightly organized central columella. **B**–**D**) Meristem homeostasis genes were over-expressed from dCLCrV or silenced from TRV. In TRV:GhCLV3-silenced plants, some bolls had an extra locule positioned closer to the center of the fruit (B); ectopic ovules forming outside the septum (C); or a loosely organized and expanded columella with ovules (D). **E)** The distribution of locule number per fruit is quantified across treatments. All flowers and fruits were harvested from virus-infected plants and controls. The total number of flowers and fruits analyzed from three replicate plants per treatment is indicated by *n*. The percentage of fruits with three, four, five, or six locules is shown. The designation of six locules was used for fruits like those shown in (B)–(D), with an extra locule in the fruit wall and/or an expanded columella harboring ovules. Scale bars are 1 mm.

transcripts (Supplemental Fig. S10A). To characterize these, we used gene ontology (GO) enrichment analysis. GO terms related to photosynthesis, transcription, and transporter activity were significantly enriched among the 829 unique transcripts impacted by *GhSP* (Supplemental Fig. S10B), consistent with previous reports (McGarry et al. 2020). Additionally, we noted significant enrichment of transcripts related to the RNA exosome (Supplemental Fig. S10B). Taken together, these experiments demonstrate that co-silencing genes of the *CLV-WUS* circuit with *GhSP* enhanced the severity of meristem perturbations in different cotton accessions, and these phenotypes were consistent with quantitative changes in target transcript levels.

Manipulating expression of meristem regulator *GhSP* enhances access to cotton meristems

We questioned if silencing *GhSP* enhanced the penetrance of other silencing signals in the meristem. To test this hypothesis, we silenced *AGAMOUS* (*AG*), encoding a MADS-box transcription factor. AG is required to repress *WUS* expression to terminate the floral meristem and to specify stamen and carpel floral organ identities (Lenhard et al. 2001). When 193 nucleotides of sequence targeting *GhAG* for co-silencing with *GhSP* in TRV:GhSP-GhAG(MADS) was delivered to day-neutral Coker 312 plants, we consistently observed plants with multiple flowers having extra whorls of petals in lieu of stamens and carpels (Fig. 5, E and F). These phenotypes were not observed when *GhAG* was silenced alone, nor in TRV:GhSP silenced plants, TRV-infected, or uninoculated controls (Fig. 5, A–D). In replicate experiments, TRV:GhSP-GhAG(MADS)-infected plants yielded flowers with smaller, petal-like structures surrounding reduced stamens and carpels, and these phenotypes were not observed in other treatments (Supplemental Fig. S11). Taken together, these findings suggest that, in cotton, SP has a role in meristem homeostasis and in controlling signals reaching the stem cell niche.

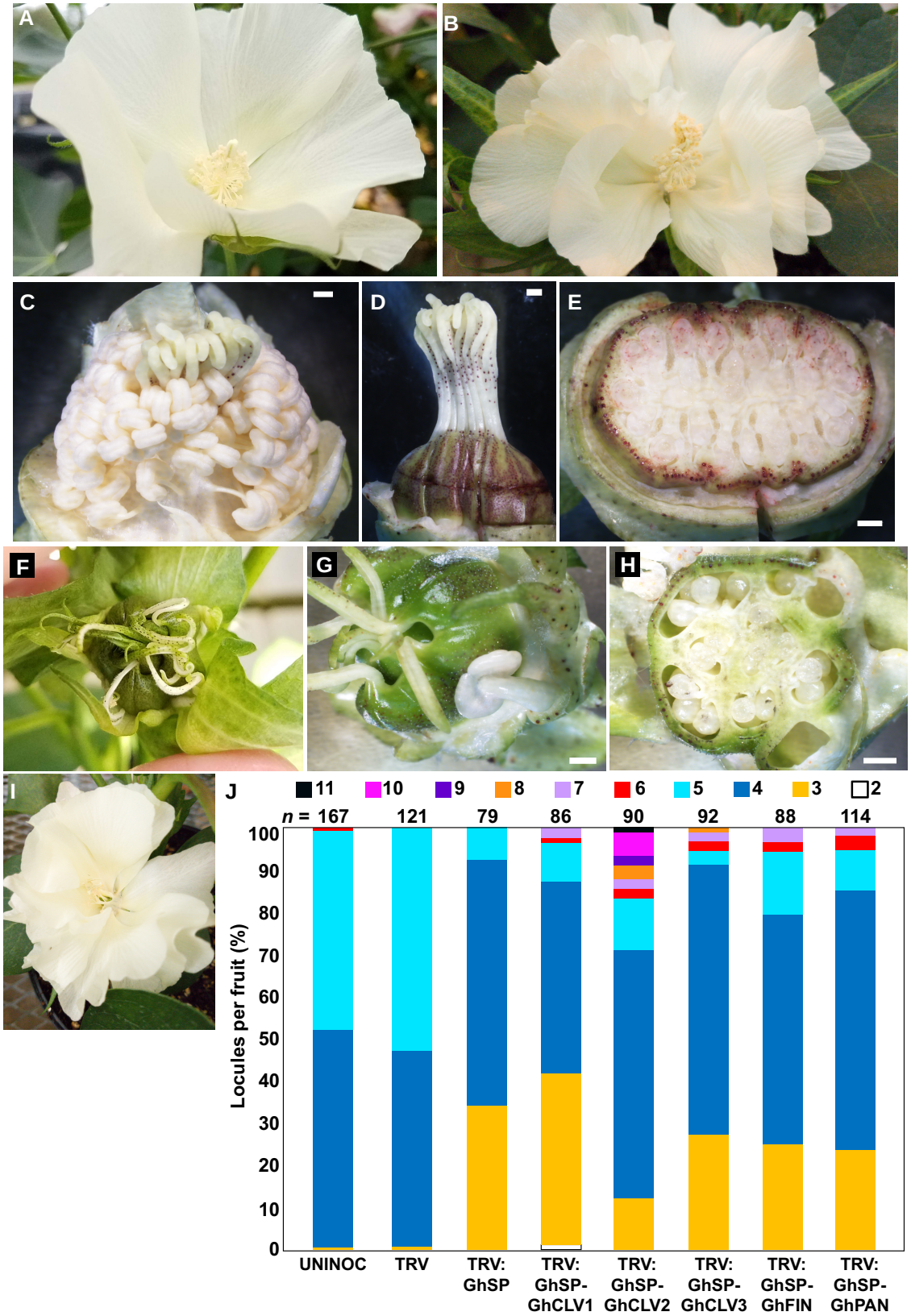

Figure 4. Co-silencing meristem homeostasis genes with *GhSP* enhances fasciation. **A)** A cotton flower typically has five petals, as shown in this flower from an uninoculated Coker 312 plant. The stamens surround the styles, and four or five fused stigmas are evident. **B)** Meristem genes were co-silenced with GhSP from TRV. A flower from a TRV:GhSP-GhPAN-infected plant has more than 11 petals. **C–E)** A flower from a TRV: GhSP-GhFIN-infected plant shows supernumerary organs: more than 10 fused stigmas emerge above the sheath of stamens (C); removing the

(Continued)

Discussion

Meristem maintenance—SP and the CLV-WUS circuit

Shoot architectures are controlled by proliferation, dormancy, and termination of meristems. The highly conserved CLV-WUS meristem maintenance circuit is critical for balancing the stem cell population with the differentiation of stem daughter cells. Disruptions to this circuit are demonstrated to profoundly impact meristem size (Fletcher et al. 1999). In tetraploid rapeseed (*Brassica napus*), mutating both *BnCLV3* homoeologs via CRISPR/Cas9 mutagenesis produced multilocular siliques with significantly more seeds and greater seed weight than wild-type or single mutants (Yang et al. 2018). Impressively, mutations in the tomato and maize homologs show substantial and quantitative changes in fruit size, and these mutations were drivers in crop domestication (Xu et al. 2015; Rodríguez-Leal et al. 2017; Chu et al. 2019; Liu et al. 2021; Wang et al. 2021). Leveraging this genetic circuit to increase yields in diverse crops is a highly desirable and sought-after goal, but not widely realized. In part, these limitations reflect the redundancy among the large gene families encoding receptors and secreted ligands, and diverse compensatory mechanisms used in different plants to safeguard meristem maintenance (Rodriguez-Leal et al. 2019; Liu et al. 2021; Wang et al. 2021). In addition, intertwining circuits regulate meristem homeostasis. The CLE40 secreted peptide controls the size of Arabidopsis shoot apical meristems in a negative feedback loop opposite to that of CLV3 (Schlegel et al. 2021). CLE40, which can compensate for CLV3 mutations, is secreted from the peripheral zone of the meristem and binds the BARELY ANY MERISTEM 1 (AtBAM1) receptor to activate *WUS* expression; *CLE40* expression is negatively regulated by WUS. It is suggested that, together, the CLV3-CLV1 and CLE40-BAM1 circuits modulate WUS activity to balance the stem cell population with cellular differentiation.

In addition to its increasingly complex roles in regulating meristem phase change, SP, and its ortholog CENTRORADIALIS (CEN), may aid in maintaining and protecting the stem cell niche independently from the CLV-WUS circuit. As plants transition from vegetative growth to flowering, the shoot apical meristem enlarges and domes. In tomato, SP and components of the CLV-WUS circuit promote this developmental process (Xu et al. 2015; Tal et al. 2017). The *late termination* (*ltm*) mutant shows precocious meristem doming, delayed flowering, and upregulation of *SP* expression; similarly, *35S:SlSP* plants and *fab* mutants have more domed meristems. These findings suggest that LTM regulates *SP* expression to promote and coordinate growth of the primary shoot apical meristem with the floral transition (Tal et al. 2017). In doing so, SP protects meristems from strong flowering signals that can terminate the meristem (Tal et al. 2017). In citrus (*Citrus sinensis* "Washington" sweet orange X *Poncirus trifoliata*), CsCEN safeguards meristem homeostasis to ensure continued vegetative growth from indeterminate meristems (Zhang et al. 2021). The leaf axils of most citrus species produce a determinate thorn and an indeterminate branch, and these structures result from the differential expression of *CsCEN*. In the indeterminate axillary meristem, CsCEN represses expression of thorn-specifying transcription factor *THORN IDENTITY 1* (*TI1*); in the determinate vegetative meristem, where *CsCEN* is not expressed, TI1 represses *WUS*. Loss-of-function *Cscen* mutants have two axillary thorns and no branches whereas *CsCEN* gain-of-function produces bushy and thornless citrus plants. These findings suggest that CsCEN acts in indeterminate meristems to protect the *WUS* domain for continued vegetative growth.

We propose that by restricting meristem access, GhSP shields the cotton stem cell niche, possibly protecting *GhWUS* expression, as suggested by co-expression network analysis (Supplemental Table S2). Transiently disrupting expression of components of the *CLV-WUS* circuit modestly fasciated cotton bolls (Fig. 3) but modifying *GhSP* expression enhanced the impact of other signals regulating meristem size (Fig. 4; Supplemental Fig. S9). *SP* and *CLV-WUS* do not likely act in the same pathway since their altered expressions did not have reciprocal effects (Table 2). Rather, our experiments showed that disruptions to *SP* expression manifested earlier and synergistically with disruptions to genes of the *CLV-WUS* circuit, suggesting overlapping functions in apical and axillary meristems. Altering *GhSP* levels enhanced the penetrance of signals affecting the late-acting floral organ identity gene, *AG*. Co-silencing *GhSP* and *GhAG* impacted nearly every flower on infected plants (Fig. 5), suggesting GhSP is needed to buffer against meristem-terminating signals. Silencing *GhSP* broadly affected meristematic gene expression, with significant enrichment of transcripts related to the RNA exosome (Supplemental Fig. S10B). A subunit of the RNA exosome, ribosomal RNA-processing protein 44A (RRP44A), interacts with plasmodesmata to move *KNOTTED1* and *SHOOT MERISTEMLESS* mRNA, and this mobility is required for meristem maintenance (Kitagawa et al. 2022). It is intriguing to consider that *GhSP* may use a similar mechanism to control meristem access.

Figure 4. (Continued)
stamens reveals the fused styles and stigmas (D); and a cross-section through the gynoecium of this flower shows an increase in the number of locules (E; compare with Fig. 3A). **F–H)** TRV:GhSP-GhCLV2-infected Coker 312 plants produced terminal flowers with extended and unfused styles (F); fasciated stamen and nested ovaries can be observed (G); and a cross-section through this fruit reveals a whorled arrangement of multiple locules (H). **I)** Supernumerary petals are observed in TRV:GhSP-GhCLV3-infected plants. **J)** Locule number per fruit is quantified by treatment. Results from two greenhouse trials were pooled, with flowers and fruits harvested from nine replicate plants per treatment. The total number of flowers and fruits quantified is reported as *n*. The number of locules per fruit, expressed as a percent, ranged from two to eleven.

Table 2. TRV co-silences *GhSP* and components of the *CLV-WUS* circuit in meristems isolated from VIGS plants

Gene name	Locus	TRV	TRV-GhSP	TRV-GhSP-GhCLV1	TRV-GhSP-GhCLV2	TRV-GhSP-GhCLV3	TRV-GhSP-GhPAN	TRV-GhSP-GhFIN
CLV3	Gohir.A08G077600	0.82	0.07	0.00	0.02	0.00	0.42	0.00
CLV3	Gohir.D08G087400	0.86	1.39	0.88	0.08	0.31	1.40	0.00
CLV2	Gohir.A05G390700	3.13	1.78	1.16	0.22	1.66	2.50	1.89
CLV2	Gohir.D04G026500	3.55	2.97	2.36	0.47	2.18	3.57	2.34
CLV1	Gohir.A02G082800	7.26[a]	2.46	1.51[a]	7.20	3.76	2.83	1.46
CLV1	Gohir.D02G098300	3.14	1.59	0.58	4.17	1.61	1.84	3.31
FIN	Gohir.A06G174700	12.49	14.12[b]	9.63	9.53	6.20	5.17	5.79[b]
FIN	Gohir.D06G184700	5.41	7.77[c]	6.46	4.91	5.20	3.76	1.46[c]
PAN	Gohir.A12G169200	2.43	17.67	25.24	30.56	26.69	9.75	37.64
PAN	Gohir.D12G172500	5.37	11.66[d]	19.92	17.99	16.99	5.20[d]	22.21
WUS	Gohir.A10G098300	1.50	0.60	0.93	0.96	1.28	1.29	0.36
WUS	Gohir.D10G089500	1.02	1.09	2.48	1.55	1.13	2.82	1.03
WUS	Gohir.D10G089000	2.73	4.14	4.45	3.85	5.87	3.42	3.29
WUS	Gohir.A12G059800	0.19	0.02	0.05	0.00	0.00	0.00	0.00
WUS	Gohir.D12G060100	0.37	0.05	0.00	0.00	0.00	0.00	0.00
SP	Gohir.A07G109700	7.50	0.19	0.25	0.00	0.24	0.24	0.00
SP	Gohir.D07G113500	10.39	0.82	0.53	0.76	0.24	0.10	0.20

Expression data are reported as FPKM values meristems isolated at 15 dpi from Coker 312 plants. Significantly different pair-wise comparisons in target gene expression are indicated relative to TRV or TRV:GhSP controls with [a]$P < 0.01$, and [b,c,d]$P < 0.05$; *P*-values were corrected for false discovery using the Benjamini–Hochberg method.

Larger cotton bolls—Applications for crop biotechnology

Fruit size and quality are fundamental to harvest value. Cotton is mechanically harvested, using heavy equipment, once at the end of a 150 to 180 d season. However, cotton flowers and fruits asynchronously, with the first and highest quality bolls opening ~85 d after planting. Fiber quality then degrades as it is exposed to wind, rain, dust, and insects before harvest. In addition, mechanical once-over harvesting is aggressive: parts of the plant are removed along with the desired seed fiber, and extraneous plant materials and field debris contaminate yields. Distributed, autonomous robots are the next transformative technology for field crops (Gebbers and Adamchuk 2010; Tang et al. 2020). Such technologies in cotton fields could reduce soil compaction and maximize fiber quality while reducing harvesting and baling costs. Consequently, larger bolls with more fiber, even with fewer bolls per plant, are prominent goals to increase crop yields and make robot harvesting more feasible. Here, we identified and functionally characterized the cotton orthologs of the highly conserved CLV-WUS meristem maintenance circuit. We demonstrated that targeted disruptions to the *CLV-WUS* circuit in cotton resulted in fasciated floral meristems, emphasizing the value of these genes as strong candidates for crop improvement via cotton biotechnology.

Viruses and meristems

Shoot apical meristems are generally considered to be immune from virus infection. In Arabidopsis, *WUS* suppresses the accumulation of several viruses, including TRV, in meristems by repressing the expression of methyltransferases and impairing protein synthesis to restrict viral replication and spread (Wu et al. 2020). Thus, in stem cells, *WUS* triggers antiviral immunity. The work presented here shows we can use VIGS to manipulate meristem events: co-silencing target sequences with *GhSP* increased access and penetrance of virus-delivered signals to meristems. These findings offer insights into meristem dynamics in general and consideration for targeting meristematic events in cotton.

Materials and methods

Phylogenetics analyses

The cotton (*G. hirsutum*) orthologs of Arabidopsis (*A. thaliana*) and tomato (*S. lycopersicum*) genes active in the *CLV-WUS* stem cell maintenance circuit were identified using the *G. hirsutum* v2.1 genome (Chen et al. 2020) available at Phytozome v13 (Goodstein et al. 2011) and CottonGen (Yu et al. 2021) (Fig. 1; Supplemental S1). The Arabidopsis and tomato proteins were aligned with cotton homologs using the CLUSTAL multiple sequence alignment tool MUSCLE with default parameters (Edgar 2004) (Supplemental Figs. S2, S3, and S5–S7). The CLV1 alignment was manually trimmed at the amino and carboxy termini to construct a phylogenetic

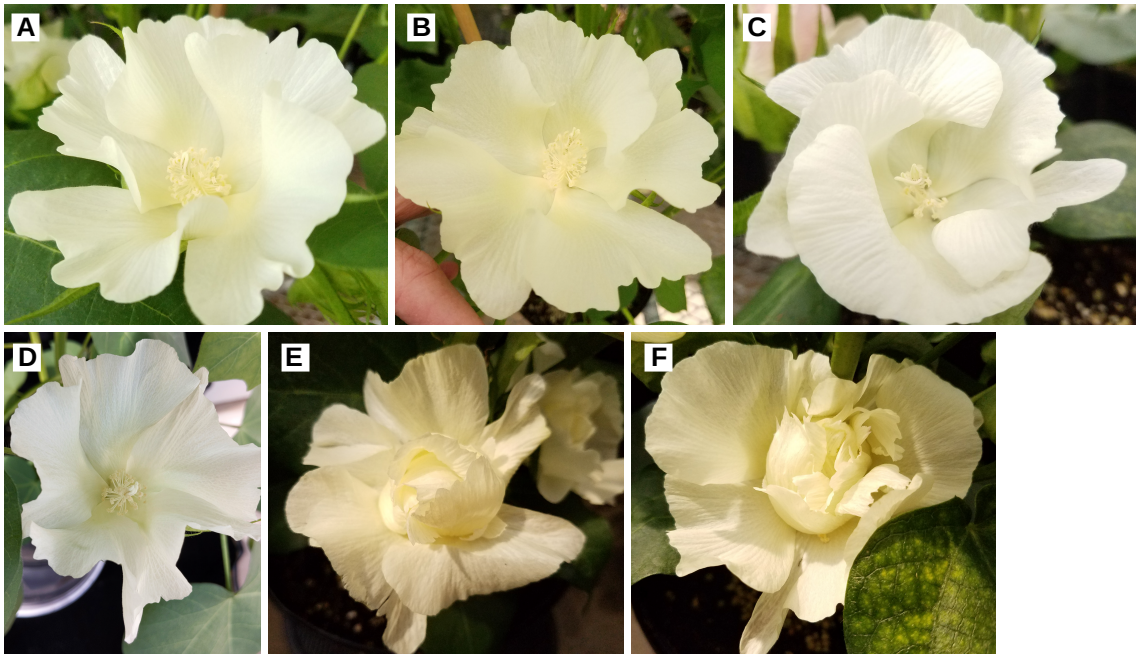

Figure 5. Co-silencing AG with *GhSP* enhances floral phenotypes. **A)** Flowers from uninoculated cotton plants show the characteristic arrangement, consisting of five petals, and a sheath of stamens surrounding the fused styles with four to five fused stigmas emerging from the center. Similar flowers are observed among **B)** TRV-, **C)** TRV:GhSP-, and **D)** TRV:GhAG-infected plants. **E, F)** TRV:GhSP-GhAG-infected plants show flowers with an outer whorl of five petals surrounding an inner whorl of petals. The androecium and gynoecium are not evident (E, F).

tree by neighbor-joining. The phylogeny was tested by the bootstrap method with 1,000 iterations in Mega X (Kumar et al. 2018). The Poisson substitution model and pairwise deletion of gaps/missing data were the default parameters used (Supplemental Fig. S4A).

Virus constructs for transient gene manipulation

The VIGS target sequences were designed within the coding sequences of each gene, and the specificity of each was predicted using the SolGen VIGS tool (Fernandez-Pozo et al. 2015). The VIGS target sequences were expected to silence the A and D homoeologs, and included 494 nts of *GhCLV1* (spanning nts 1,649 to 2,142 of coding sequence Gohir.D02G098300); 500 nts of *GhCLV2* (nts 1 to 500 of coding sequence Gohir.D04G026500); 255 nts of *GhCLV3* (full Gohir.D08G087400 coding sequence); 432 nts of *GhFIN* (nts 1 to 432 of coding sequence Gohir.D06G184700); and 300 nts of *GhPAN* (nts 1,081 to 1,380 from the coding sequence of Gohir.A12G169200). For each VIGS target sequence, the A and D sub-genome homoeologs differed by, at most, 11 noncontiguous nucleotides. Target sequences were synthesized by Twist Bioscience (San Francisco, CA, USA) and incorporated unique XbaI and SacI restriction sites at the 5′ and 3′ ends, respectively; the *GhCLV3* coding sequence incorporated 5′ XbaI SphI sites and 3′ NheI SacI sites. Sequences for VIGS were digested with XbaI and SacI (New England Biolabs, Ipswich, MA, USA), and ligated into the same sites of pYL156 (TRV RNA2) (Burch-Smith et al. 2006) to generate pYL156:GhCLV1, pYL156:GhCLV2, pYL156:GhCLV3, pYL156:GhFIN, and pYL156:GhPAN. The *GhCLV3* coding sequence was released by SphI and NheI digests and ligated into the same sites of pJRTCLCrVA.008 (Tuttle et al. 2008) to construct gain-of-function vector pJRTCLCrV:GhCLV3. This vector was digested with XbaI and SacI and cloned into the same sites of binary vector pAgroJRTCLCrVA (Tuttle et al. 2012), producing dCLCrV:GhCLV3.

To construct viruses co-silencing *GhSP* with a second target gene, the Twist Bioscience cloning vectors described above were digested with XbaI and SacI to release each insert, and pART7-GhSP (Prewitt et al. 2018) was digested with EcoRI and XbaI to release the *GhSP* fragment. These fragments were ligated into pYL156 digested with EcoRI and SacI to produce pYL156:GhSP-GhCLV1, pYL156:GhSP-GhCLV2, pYL156:GhSP-GhCLV3, pYL156:GhSP-GhFIN, and pYL156:GhSP-GhPAN.

The cotton orthologs of the Arabidopsis *AGAMOUS* (At4g18960) were identified as tandem repeats on the A and D sub-genomes (Gohir.A10G033000, Gohir.A10G033100, Gohir.D10G033700, and Gohir.D10G033800). The VIGS targeting sequences were designed against the K-box and spanned nts 599 to 865 of Gohir.A10G033000, or the MADS-box and included nts 125 to 320 of Gohir.A10G033000. Each VIGS targeting sequence was PCR-amplified from cotton meristem or flower cDNA using oligonucleotides Kbox-fwd (5′: tccaaaTCTAGAGAGCTGTTGTTTGCTGAAATAGAG) and Kbox-rev (5′: ctcgtgCTCGAGGAGCTCGACTAGTTGAAGAACTATCTGGTCTTGC), or MADS-fwd (5′: ctcgtgTCTAGAATGGTGTAC

CCCAACGAATCCCTTG) and MADS-rev (5′: ctcgtgCTCG AGGAGCTCTGGAGAAGACAATCAAAGCAACCTC). PCR products were digested with XbaI and XhoI, and cloned into the same sites of pYL156 to create pYL156: GhAG(Kbox) or pYL156:GhAG(MADS), respectively. To co-silence each with *GhSP*, pYL156:GhSP-GhCLV2 was digested with XbaI and XhoI, releasing the *GhCLV2* fragment, and gel-purified; the digested PCR products were ligated into the same sites to yield pYL156:GhSP-GhAG(Kbox) or pYL156: GhSP-GhAG(MADS).

Plant growth conditions

G. hirsutum accessions Coker 312 and short-day photoperiodic accession Texas 701 were used in inoculation experiments. Seeds were germinated in 3.5 inch square pots with Metro-Mix 852 growth mix (Sungro Horticulture, Agawam, MA, USA) and placed in a growth chamber at 25 °C (16 h light/8 h dark) with balanced warm and cool white T5 fluorescent lights.

Virus inoculations

Each viral vector was introduced to *Agrobacterium tumefaciens* GV3101 pMP90 by electroporation. Single colonies were cultured for *Agro*-inoculation as described (McGarry et al. 2016). TRV and dCLCrV are bipartite viruses: *A. tumefaciens* strains harboring each genome were cultured and prepared separately; equal volumes of inoculum were mixed immediately before infiltration. *Agro*-inoculation of viruses was delivered to saturation into the cotyledons of seedlings at 4 d post-germination using a 1 mL syringe. At minimum, six plants were infiltrated with each virus. Inoculated plants were covered with plastic domes overnight at room temperature, and then transferred to a growth chamber at 25 °C (16 h light/8 h dark) for 3 wk. Plants were transplanted to 4.5 L pots with Metro-Mix 852 growth mix and grown to maturity in a greenhouse (16 h light/8 h dark, 30 °C/25 °C) with supplemental lighting provided by metal halide and mercury lamps (light intensity at leaf level was 1,300 µmol photons m^{-2} s^{-1}). Each virus was tested in at least two independent greenhouse experiments.

RNA-seq library construction, sequencing, and analysis pipeline

At 15 dpi, apices from three replicate co-silenced plants and controls were harvested into acetone. Acetone was changed twice, and samples were stored at 4 °C before processing. With the aid of an SMZ1500 stereomicroscope (Nikon, Melville, NY, USA), meristems were dissected from apices and returned to acetone. Acetone was removed and meristems were frozen in liquid nitrogen and homogenized using a Retsch mill (MM400; Retsch USA, Newtown, PA, USA). RNA was isolated by hot borate (Wan and Wilkins 1994) followed by column clean-up (Zymo Research, Irvine, CA, USA). Total RNA concentration was determined by nanodrop, and 20 ng was used to prepare libraries with NEBNext Single Cell/ Low Input RNA Library Prep Kit for Illumina. Library quality was assessed at the University of North Texas Health Science Center Genomics Core Facility (Fort Worth, TX, USA) by Agilent High Sensitivity D5000 Screen Tape assay (Agilent Technologies, Santa Clara, CA, USA). Libraries with concentrations ≥2 nM were sequenced for 75 cycles on an Illumina NextSeq 550 Sequencer (University of North Texas Health Science Center Genomics Core Facility). In total, libraries constructed from three plants each of TRV:GhSP, TRV:GhSP-GhCLV1, TRV:GhSP-GhCLV2, and TRV: GhSP-GhCLV3 treatments, and two plants each of TRV, TRV:GhSP-GhPAN, and TRV:GhSP-GhFIN treatments were of suitable quality for sequencing.

Clustering differed between flowcells, with the first generating ~387×10^6 clusters and the second producing ~56×10^6 clusters. Samples from the lower-yielding flowcell were repeated, and the sequences generated from this third flowcell, with ~46×10^6 clusters, were incorporated. Read quality was determined using FASTQC in the CyVerse Discovery Environment (Goff et al. 2011), with median quality scores ≥ 32 for 75 bp reads. The reads generated from four lanes for each sequenced library were concatenated. The sequencing yields per sample are included in Supplemental Table S3. Reads were mapped to the *G. hirsutum* v2.1 genome (Chen et al. 2020) and transcripts were assembled using RMTA_v2.6.3 in the CyVerse Discovery Environment. Differential expression was determined using Cuffdiff2 (Trapnell et al. 2010). Normalized expression data was reported as fragments per kilobase of transcript per million mapped reads (FPKM).

Accession numbers

Transcriptome data are deposited in the NCBI Gene Expression Omnibus (accessions GSE206189 and GSE144546). Cotton DNA and proteins sequences may be accessed at Phytozome v13 (Goodstein et al. 2011) and CottonGen (Yu et al. 2021) using identifiers Gohir.A08G077600 and Gohir.D08G087400 (GhCLV3); Gohir.A05G390700 and Gohir.D04G026500 (GhCLV2); Gohir.A02G082800 and Gohir.D02G098300 (GhC LV1); Gohir.A06G174700 and Gohir.D06G184700 (GhFIN); Gohir.A12G169200 and Gohir.D12G172500 (GhPAN); Gohir.A10G098300, Gohir.D10G089500, Gohir.D10G089000, Gohir.A12G059800, and Gohir.D12G060100 (GhWUS); and Gohir.A07G109700 and Gohir.D07G113500 (GhSP).

Disclosure

Based in part on this work, authors R.C.M. and B.G.A., with the University of North Texas, filed U.S. Utility Patent Application No. 17/749,560 with the United States Patent and Trademark Office.

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Author contributions

B.G.A., L.E.W., E.v.d.K., and R.C.M. designed the experiments; R.C.M. conducted the experiments with B.G.A., H.K., Y.T.L., and G.L.P.; R.C.M. and B.G.A. analyzed the data; R.C.M. wrote the paper with input from all the authors.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. Identifying cotton orthologs of the CLV-WUS circuit.

Supplemental Figure S2. Identifying cotton CLV3 proteins.

Supplemental Figure S3. Multiple sequence alignment of CLV1 by MUSCLE.

Supplemental Figure S4. Identifying cotton CLV1 orthologs.

Supplemental Figure S5. Multiple sequence alignment of CLV2 proteins.

Supplemental Figure S6. Multiple sequence alignment of FIN by MUSCLE.

Supplemental Figure S7. Multiple sequence alignment of PAN by MUSCLE.

Supplemental Figure S8. *GhCLV3* affects floral development.

Supplemental Figure S9. Co-silencing *GhSP* with components of the *CLV-WUS* circuit causes fasciation in photoperiodic cotton Texas 701.

Supplemental Figure S10. Visualizing the overlaps and ontology of differentially expressed genes identified in meristems from VIGS plants.

Supplemental Figure S11. Co-silencing *GhSP* with *GhAGAMOUS* affects floral development.

Supplemental Table S1. *CLV-WUS* genes are expressed in different cotton meristems.

Supplemental Table S2. Weighted gene co-expression network analysis identifies *GhWUS* as co-regulated with *GhSP*.

Supplemental Table S3. Illumina sequencing yields.

Supplemental Appendix S1. Silencing other candidate *GhCLV3* genes.

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