

SHORT COMMUNICATION



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SUPERMAN genes: uncovering a new function in the development of complex inflorescences

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Abstract

The Arabidopsis *SUPERMAN* (*SUP*) gene and its orthologs in eudicots are crucial in regulating the number of reproductive floral organs. In *Medicago truncatula*, in addition to this function, a novel role in controlling meristem activity during compound inflorescence development was assigned to the *SUP*-ortholog (*MtSUP*). These findings led us to investigate whether the role of *SUP* genes in inflorescence development was legume-specific or could be extended to other eudicots. To assess that, we used *Solanum lycopersicum* as a model system with a cymose complex inflorescence and *Arabidopsis thaliana* as the best-known example of simple inflorescence. We conducted a detailed comparative expression analysis of *SISUP* and *SUP* from vegetative stages to flower transition. In addition, we performed an exhaustive phenotypic characterisation of two different *slsup* and *sup* mutants during the plant life cycle. Our findings reveal that *SISUP* is required for precise regulation of the meristems that control shoot and inflorescence architecture in tomato. In contrast, in Arabidopsis, *SUP* performs no meristematic function, but we found a role of *SUP* in floral transition. Our findings suggest that the functional divergence of *SUP*-like genes contributed to the modification of inflorescence architecture during angiosperm evolution.

1 | INTRODUCTION

SUPERMAN (*SUP*) is a member of the C₂H₂ zinc-finger family of transcription factors in *Arabidopsis* and has been classically considered a floral-specific gene (Sakai et al., 1995; Englbrecht et al., 2004; Xu et al., 2018). The specific expression of *SUP* in the boundaries between primordia of reproductive organs (Sakai et al., 1995) is consistent with increased stamens and carpels in *sup* mutant flowers (Breuil-Broyer et al., 2016). *SUP* controls floral organogenesis by repressing auxin biosynthesis of floral stem cells in the flower meristem (Xu et al., 2018).

Notwithstanding certain particularities between species, *SUP* orthologs in *Petunia hybrida*, *Medicago truncatula*, and *Solanum lycopersicum* have a common expression pattern and function during flower development, as

they are involved in the control of the number of stamens and carpels (Nakagawa et al., 2004; Rodas et al., 2021, 2023; Zhang et al., 2023). However, in addition to this conserved function, the *Medicago SUP* ortholog also modulates meristematic determinacy of the secondary inflorescence meristem (I2), controlling the number of flowers in the compound inflorescence (Rodas et al., 2021). *Medicago* and *Arabidopsis* have a monopodial growth in which the shoot apical meristem (SAM) transitions from vegetative to reproductive meristem. In *Arabidopsis*, the inflorescence meristem (IM) generates flowers in its flanks. On the contrary, in *Medicago*, the specification of an additional secondary inflorescence meristem (I2) is associated with compound inflorescence development Berbel et al., 2012; Benlloch et al., 2015; (Cheng et al., 2018).

In contrast to *Arabidopsis* and *Medicago*, a sympodial growth pattern describes the tomato plant architecture (Pnueli et al., 1998). Although the main stem follows a monopodial growth during vegetative

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stages, a shift to sympodial growth takes place from the last vegetative axillary meristem after the transition to flower, producing leaves and flowers (Pnueli et al., 1998; Lippman et al., 2008). Inflorescence development in tomato requires sequential and coordinated behaviour of the different types of involved meristems, fluctuating between meristem determinacy and resumption. A new meristem does not emerge until the prior has undergone differentiation, resulting in sympodial growth and multiple-flower arrangement in a zigzag pattern. Such complexity involves many genes with essential roles in meristem specification and determinacy, which are ultimately responsible for tomato shoot and inflorescence architecture (Park et al., 2012; Périlleux et al., 2014; Gaarslev et al., 2021; Périlleux x & Huerga-Fernández, 2022).

This work analysed the role of *SUP* and its tomato ortholog (*SISUP*) in plant architecture. We performed an exhaustive phenotypic analysis of *slsup* and *sup* mutants during the plant life cycle. Our findings reveal undescribed functions for *SISUP* in tomato plant architecture by controlling the activity of axillary and reproductive shoot meristems. No function in plant architecture was observed for the Arabidopsis *SUP* gene. However, a new *SUP* function was shown in the control of flowering time and axillary bud outgrowth. Our data suggests that the evolution of *SUPERMAN*-like genes is linked to the evolutionary variation of inflorescence architecture among angiosperms.

2 | MATERIALS AND METHODS

2.1 | Plant material and growth conditions

Solanum lycopersicum cultivar Moneymaker was the reference as a wild type for tomato experiments. The wild type, CRISPR-Cas mutants *slsup-4* and *slsup-5* (obtained thereafter) were grown in a greenhouse as described by Salazar Sarasua et al. (2023). For *Arabidopsis thaliana* experiments, Landsberg *erecta* (*Ler*) and Columbia (*Col-0*) ecotypes were used as wild-type plants, and the mutant lines *sup-2* (*flo-10*) (Schultz et al., 1991), and *sup-5* (Gaiser et al., 1995) were obtained from the Nottingham Arabidopsis Stock Centre. Seedling samples for *in situ* hybridisation grew in sterile plates. Arabidopsis plants were grown in controlled-environment chambers at 22°C under a long-day photoperiod (16 h light/8 h dark).

2.2 | In situ hybridisation

The RNA sense and antisense probes were generated with the T7 and SP6 RNA polymerases using specific fragments of the genes *AtSUP* and *Solyc09g08959*, the putative ortholog of *SUP* in tomato (*SISUP*) (Zhang et al., 2023) (Table S1). These fragments were cloned into the pGEM-T Easy vector (Promega). In tomato, we used tomato seedlings containing different stages of the primary shoot meristem (PSM) maturation from the vegetative phase to flower meristem termination and shoot meristems. We used Arabidopsis seedling samples 4, 7, and 10 days after stratification. These samples were fixed and embedded into wax in the automated processor (Leica TP1020). *In situ* hybridisation with digoxigenin-labelled probes was performed according to Rodas et al. (2021). *In*

situ experiments for the antisense probes were performed more than twice, obtaining the same results.

2.3 | Design CRISPR-Cas9 construct for *Solyc09g089590* gene editing and molecular characterisation of *slsup* mutants

Breaking-Cas (<https://bioinfogp.cnb.csic.es/tools/breakingcas/index.php?gset=SARS2>) was used to select a guide (*sgRNA-1*) targeting 48 bp after the start codon of *Solyc09g089590* (Zhang et al., 2023) (Figure S2B). GoldenBraid 2.0 assembly system (Sarrion-Perdigones et al., 2011) was used to generate the CRISPR-Cas9 construct (*SISUP*-*sgRNA*). First, a single gRNA sequence was obtained by annealing of complementary primers SgSISUP-F and SgSISUP-R (Table S1) and then assembled with GB1001 (U626 promoter) and GB0645 (scaffold RNA) parts into the destination vector pDGB3α1. In successive multipartite GB reactions, this first module was assembled with the GB2578 (containing *hCas9*, *nptII*, and *dsRED* transcriptional units) into a destination vector pDGB_Ω2 (Figure S2 C). *SISUP*-*sgRNA-1* construct was used to transform the *Agrobacterium tumefaciens* strain LBA4404.

Tomato transformants were obtained as described by Salazar-Sarasua et al. (2022). The genomic DNA of diploid transgenic plants was isolated from leaves or flowers and PCR-analysed using the primers 35SDIR and *hCAS9*-REV primers (Table S1). Direct DNA sequencing (Sarrion-Perdigones et al., 2011) of PCR products (496-bp) of the genomic region spanning target sites (CRISPR seq-F and CRISPR seq-R primers; Table S1) was used to select the Cas9-free T0 edited lines. The type of edition in the targeted sequence was identified by using the online tools TiDE (<http://shinyapps.datacurators.nl/tide/>) and software ICE 2 CRISPR analysis (<https://ice.synthego.com>) (Table S1). Null segregants for the Cas9 among C1.7 T1 plants were identified using fluorescent DsRED seeds (Figure S2H,I). Direct DNA sequencing of PCR products of the genomic region surrounding the CRISPR target site identified *slsup-4* and *slsup-5* mutant lines. The F2 population was generated by crossing *slsup-4* and *slsup-5* alleles (pollen donor) and the wild-type.

A CAPS marker was designed to identify mutant lines. Genetic changes in the recognition site of the *SspI* restriction enzyme of PCR products (CRISPR seq-F/CRISPR seq-R primers, Table S1) allowed us to identify mutants. Three *slsup* mutant sets were grown during the present work at different times for phenotypic analysis and sample collection, always observing comparable phenotypes between all *slsup* mutant plants. To determine the number and time of activation of the lateral branches, we used five plants from each genotype: wild-type, *slsup-4* and *slsup-5* (Figure S3 D). Also, we analyzed five plants of a second set of wild-type and *slsup-4* plants for bud activation (Figure S3E). We classified the lateral branches from L1 to L7 as active if their size was longer than 1 cm and inactive if they were visible but smaller than 1 cm. The third classification was as absent (O) (Figure S3). We analysed the first to the fourth inflorescence from 8 plants for each genotype (WT, *slsup-4* and *slsup-5*) to determine the number of flowers per inflorescence. The number of leaves in the primary shoot meristem was measured using eight plants from each phenotype. We counted the stamen and carpel numbers using thirty

flowers of each phenotype. Data showing plant height and size came from five plants from each genotype.

2.4 | Phenotypical characterisation of the *Arabidopsis sup* mutants

For flowering time experiments, seeds of the wild type and *sup* mutants were grown on soil in LD at 22°C after stratification at 4°C for three days. Flowering time (bolting) was defined as the day when the floral stem was longer than 1 cm. At least 30 plants of each genotype were used for each experiment (*Ler* = 35, *sup-5* = 38; *Col-0* = 36; *sup-2* = 32). This experiment was repeated more than three times, obtaining the same results.

2.5 | Evolutionary analysis, graph construction and statistical analysis

The *Arabidopsis SUPERMAN* (*At3g23130*) gene is located at chromosome 3: 8242256–8243372 (TAIR10), and the putative *SUP* ortholog in tomato (*Solyc09g089590*) in the chromosome 9: 65424385–65424978 (SL4.0) of the tomato genome. We compared both genomes using the Orthology prediction methods Synima (<https://pubmed.ncbi.nlm.nih.gov/29162056/>) and OrthoFinder (<https://genomebiology.biomedcentral.com/articles/10.1186/s13059-019-1832-y>), finding 463 orthogroups containing at least one gene from chromosome 3 of *Arabidopsis* and one from chromosome 9 of tomato (Additional File 1). We extracted 100 upstream and 100 downstream genes surrounding *At3g23130* and *Solyc09g089590* (Additional File 1) and found which of those *Arabidopsis* genes have orthologs in these tomato genes. Using the genome browsers of the surrounding regions of *At3g23130* (<https://jbrowse.arabidopsis.org/>) and *Solyc09g089590* (https://solgenomics.net/jbrowse_solgenomics), we generated Figure S2A and Additional file 2. Statistical tests were performed using the web statistical calculators (<https://astatsa.com/>). PLOT of DATA web tool (<https://huygens.science.uva.nl/PlotsOfData/>) and Excel program were used to construct the graphics.

3 | RESULTS

3.1 | *SISUP* mutations affect shoot and inflorescence architecture

The *Solyc09g089590* gene was reported as the putative *SUP* orthologue in tomato, with a conserved function during flower development (Zhang et al., 2023). We performed microsynteny analysis and found that *SUP* (*At3g23130*) and *SISUP* (*Solyc09g089590*) derived from a common ancestral genomic region, supporting that *Solyc09g089590* is the *SUP* ortholog in *S. lycopersicum* (*SISUP*) (Figure 1A; Additional Files 1 and 2).

We studied the expression pattern of *SISUP* in the meristems involved in tomato plant architecture (Périlleux et al., 2014; Gaarslev et al., 2021; Périlleux and Huerfano-Fernández, 2022). During the early

vegetative growth, *SISUP* was first detected in the axillary meristems (AM) (Figure 1B). AM expression was maintained at the late vegetative stage (Figure 1C) and even after floral transition (Figure S1A). A weak expression was detected in the primary shoot meristem (PSM) before the floral transition (Figure 1C). Following this, *SISUP* was expressed at the late transitional meristem (TM) in the emerging first flower meristem (FM) and the inflorescence meristem (IM) (Figure 1D). *SISUP* transcript was also detected in the vegetative sympodial meristem (SYM), FM, IM, and the leaf primordia (Figure 1E). In agreement with Zhang et al. (2023), *SISUP* was detected in the primordia of all floral organs except for the sepals in the early stages of flower development (Figure 1F). Later, *SISUP* was expressed in stamens and carpel primordia (Figure 1G).

We studied *SISUP* function by phenotypically characterising two *slsup* mutant alleles (*slsup-4* and *slsup-5*) generated by CRISPR-Cas9 technology (Figures 1H–J and S2A–E). The *slsup-4* and *slsup-5* mutations introduced premature stop codons and would encode putative smaller N-peptides with no function (Figure S2C). Compared to the wild type, *slsup* mutants showed delayed axillary branching (Figure S3), the formation of extra vegetative phytomers on the primary stem, reduced plant height (Figures S4 and S5) and defects in the flowers with extra organs (Figure S6).

During sympodial growth, the wild-type plants generate sympodial units (SUs) with three leaves and an inflorescence with 6 to 8 flowers sequentially developed in the typical zigzag pattern (Figure 2A, H). *Slsup* mutants develop two main shorter axes with similar thickness and size, resembling two SUs (Figure 2B,C). These alterations occur throughout development, resulting in smaller plants with bushier phenotypes (Figures S4 and S5). The simultaneous maturation of the AM and SYM could explain such variations (Figure 2E, F). *Slsup* mutant phenotype is quite variable: not all SUs and lateral branches looked equal or formed the same number of leaves, and individual SUs form inflorescences arranged irregularly with a variable number of flowers (Figure 2G, J). These oscillations indicate variable expressivity of *slsup* phenotypes.

In the wild type, the PSM matures until a TM, which gives rise to the first FM and an IM. Each new IM develops perpendicular to the one previously formed, maturing sequentially into FMs (Figure 2E, I). However, *slsup* mutants showed multi-flowered asynchronous inflorescences (Figure 2I, J). SEM analysis showed duplicated inflorescences emerging on opposite sides (Figure 2L). In agreement with Zhang et al. (2023), *slsup* plants developed flowers with increased stamens and carpels and a few seedless fruits with additional locules (Figure S6). Overall, these results suggest that *SISUP* controls the outgrowth of the axillary meristems, the sympodial branching, and the number of flowers per inflorescence, sharing this last function with *MtSUP* (Rodas et al., 2021).

3.2 | *SUP* mutations affect flowering time and the outgrowth of the axillary meristems

The results obtained in tomato plants motivated us to study the expression and function of *SUP* in *Arabidopsis* from the vegetative to the floral stage. In contrast to the *Medicago* and tomato *SUP* orthologs, *SUP* transcript was absent of the SAM at vegetative stages and

in the inflorescence (IM) or floral meristems (FM). However, *SUP* expression was detected in vegetative tissues close to the SAM, such as stipules, leaves, and the vascular tissue of cotyledons (Figures 3A-D and S1). After the floral transition, *SUP* exhibited its

well-known expression in the boundary between the reproductive organs of flowers (Figure 3C). Thus, we examined the vegetative development of *sup-2* and *sup-5* plants and found that *sup-2* and *sup-5* flowered earlier than wild-type plants (Figure 3E). Also, the primary

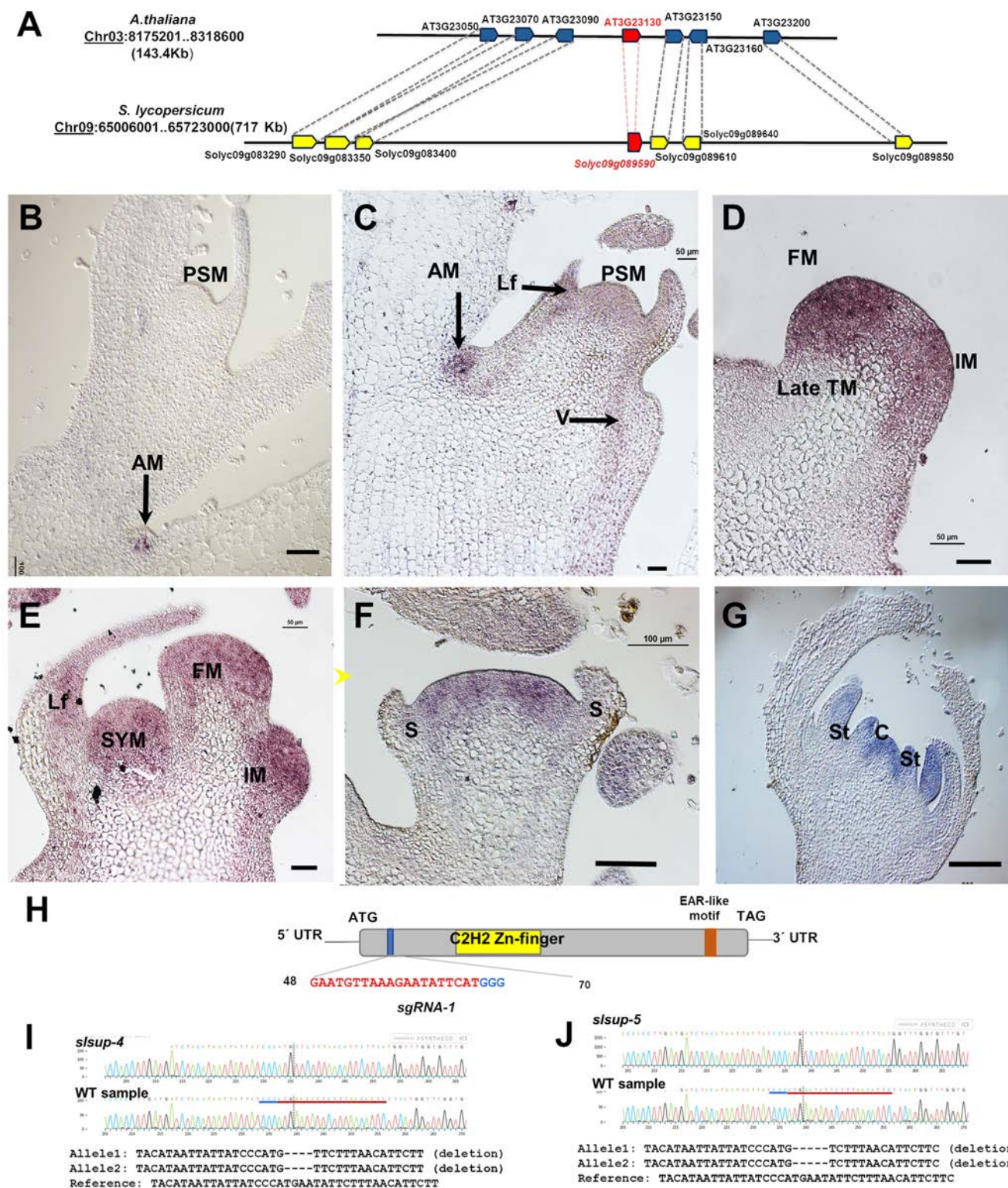


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rosette branches (PRB) showed moderately earlier activation in *sup* mutants (Figure S7A). The mutants produced more PRB over time, being more pronounced in *sup-2* than in *sup-5* compared with their respective wild types (Figure S7B). These results showed a novel expression and function for *SUP* in *Arabidopsis thaliana*.

4 | DISCUSSION

Our results showed that the tomato *SUPERMAN* ortholog is a new meristem regulatory gene underlying tomato shoot and inflorescence architecture. These findings confirm that the novel function of a *SUP*-like gene in eudicots found in *Medicago truncatula* during inflorescence development might be extended to other phylogenetically distant species, such as tomato.

The phenotype of *slsup* mutants exhibits variable expressivity. *Slsup* plants showed a moderately delayed floral transition, delayed axillary bud initiation, and a significant impact on the development of reproductive shoots. Mutant phenotypes are consistent with the simultaneous expression of *SISUP* in all the meristems that shape the tomato plant. Similarly, variable expressivity was also observed in the phenotype of the inflorescences of *Medicago mtsup* mutants ranging from one to four flowers (Rodas et al., 2021) as well as in the number of reproductive organs within a single plant in all *sup* *Arabidopsis* alleles (Breuil-Broyer et al., 2016). However, contrasting with the meristem-specific expression of *MtSUP* (Rodas et al., 2012) and *SISUP*, the *Arabidopsis SUP* was absent in the SAM, IM, or FM. Nevertheless, we discovered novel expressions for *SUP* during vegetative development, especially in the stipules. A possible connection between *SUP* and the stipules may be behind the molecular mechanism that explains the *SUP* function and the function of stipules in plant development. Stipules are the first site of high free-auxin production with a protective function of the apical meristem (Aloni et al., 2003). The molecular function of *SUP* in the floral meristems is to repress auxin biosynthesis genes *YUC1* and *YUC4* to control cell differentiation near the FM central region (Xu et al., 2018). Interestingly, *YUC1* and *YUC4* genes overlap their expression with *SUP* in the

stipules (Cheng et al., 2007), and the presence of stipules influences flowering time in *Pisum sativum* (Sharma and Kumar, 2012). Despite not being expressed in the apical meristems, we demonstrated that *SUP* has a role in the flowering initiation and axillary bud outgrowth in *Arabidopsis*. This is a novel function for *SUP* in *Arabidopsis thaliana*.

In *Arabidopsis*, floral phenotypes of *sup* mutants were classified according to the quantitative effect on FM indeterminacy (Breuil-Broyer et al., 2016). Similarly, the high degree of I2 indeterminacy in *Medicago mtsup* mutants produces more flowers per inflorescence than in the wild type (Rodas et al., 2021). *SUP* regulates FM determinacy by controlling cell proliferation and differentiation by repressing auxin biosynthesis. This hormone plays a critical role in the activity of meristems and apical dominance (Gallavotti, 2013). While future studies could unravel how this C2H2 zinc finger protein (C2H2 ZFP) influences the tomato plant architecture, it could be hypothesised that higher levels of indeterminacy at the *slsup* shoot apex result in stronger apical dominance, thus inhibiting the outgrowth of axillary branches (Shimizu-Sato & Mori, 2001; Bennett and Leyser, 2006). However, we cannot ignore that C2H2 ZFPs can interact with other proteins, DNA or RNA, meaning that *SUP*-like genes can potentially regulate many different genes involved in the tomato meristem maturation program (Park et al., 2014; Meir et al., 2021; Zhang et al., 2022).

Different models have explained how the meristem regulation program is an essential process that defines the distinct inflorescence architectures (Lippman et al., 2008; Prusinkiewicz et al., 2007; Park et al., 2012; Périlleux et al., 2014; Gaarslev et al., 2021). Mutations in *SISUP* lead to changes in both shoot and inflorescence architecture. These alterations could result from simultaneous rather than sequential initiation and maturation rate of the meristem involved in the tomato sympodial growth (Périlleux and Huerga-Fernández, 2022). The disruption of the maturation rate of these meristems could negatively affect cell proliferation in elongating internodes. However, the evaluation of this hypothesis will need further research. In contrast, inflorescence phenotypes agree with higher levels of IM indeterminacy, defining the number of flowers this meristem produces.

FIGURE 1 *SISUP* is the putative ortholog of the *Arabidopsis SUP*. **A**. The scheme shows the collinearity of neighbouring matching gene pairs between the *Arabidopsis* AT3G23130 gene and the *Solyc09g089590* gene in tomato. The blue boxes joined by dotted grey lines represent the putative orthologs near *SUP* and the putative *SISUP*. Non-orthologous genes were not represented. **B–G**. *In situ* hybridization of *SISUP* during the primary shoot meristem (PSM) maturation. **B**. Longitudinal section of 4 DAG seedlings. *SISUP* mRNA was detected in the axillary meristem (AM). **C**. Longitudinal section of 10 DAG seedlings. *SISUP* transcripts accumulated in the axillary meristem (AM) and a weak expression was observed in the PSM, leaf primordia (Lf) and vasculature (V). **D**. *SISUP* was detected in the late transitional meristem (TM) toward a first flower meristem and the lateral inflorescence meristem (IM). **E**. Longitudinal section of 13 DAG seedlings. After the floral transition, *SISUP* was detected in the floral meristem (FM), inflorescence meristem (IM) and sympodial meristem (SYM). **F–G**. Longitudinal sections of developing flowers. *SISUP* expression was first detected in all the floral organ primordia except the sepals (S), and at late floral stages (F) expression was restricted to stamens (St) and carpel primordia (C). **H**. Schematic representation of the guide sgRNA-1 position and sequence in the target gene *Solyc09g089590* (*SISUP*) indicated in red. The PAM site (GGG) is indicated in blue. **I**. Sequencing chromatograms and *SISUP* deletion in *slsup-4* homozygous mutant aligned with the WT sequence. *slsup-4* has a deletion mutation of 4 (59–62 del AATA) nucleotides. The red and blue lines indicate the sgRNA and PAM sequence, respectively. **J**. Sequencing chromatograms and *SISUP* deletion in *slsup-5* homozygous mutant aligned with the WT sequence. *slsup-5* has a deletion mutation of 5 nucleotides (59_63 del AATAT). The red and blue lines indicate the sgRNA and PAM sequence, respectively. Bars in B and F indicate 100 μ m. Bars in C to E indicate 50 μ m. Bars in G indicates 200 μ m. DAG = days after germination.

The petunia *SUP*-ortholog (*PhSUP*) has only been assigned a function during floral development (Nakagawa et al., 2004). However, it would be interesting to explore whether this gene is also involved in the genetic control of plant architecture. Diversification of the Solanaceae *SUP*-like genes could have been involved in the variation among sympodial shoot structures with solitary flowers in *Petunia hybrida* and *Capsicum annuum* and multiple flowers in *Solanum lycopersicum* (Lippman et al., 2008; Castel et al., 2010; Cohen et al., 2014). In

monocots, the *SUP*-like gene *RAMOSA1* (*RA1*) regulates the determinacy of spikelet pair meristem, controlling the branching degree in maize (Vollbrecht et al., 2005). Excitingly, minimal differences in the temporal expression of the *RA1* orthologs in *Zea mays*, *Sorghum bicolor* and *Setaria viridis* lead to variation in branch architecture in these grasses (Strable et al., 2023). Despite the considerable evolutionary distance between species with complex inflorescences such as Medicago, tomato, and maize, *MtSUP*, *SISUP*, and *RAMOSA1* are conserved

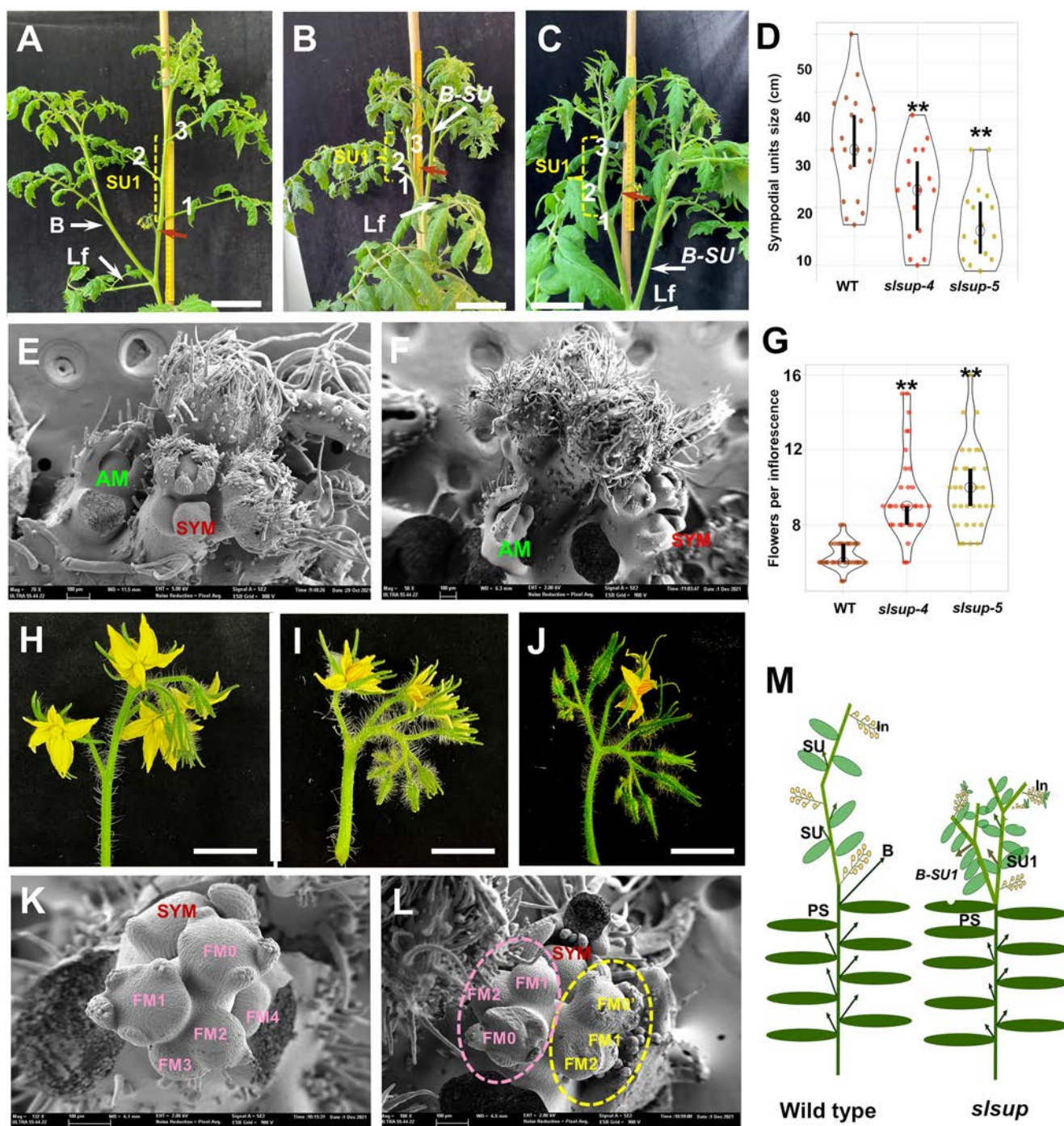


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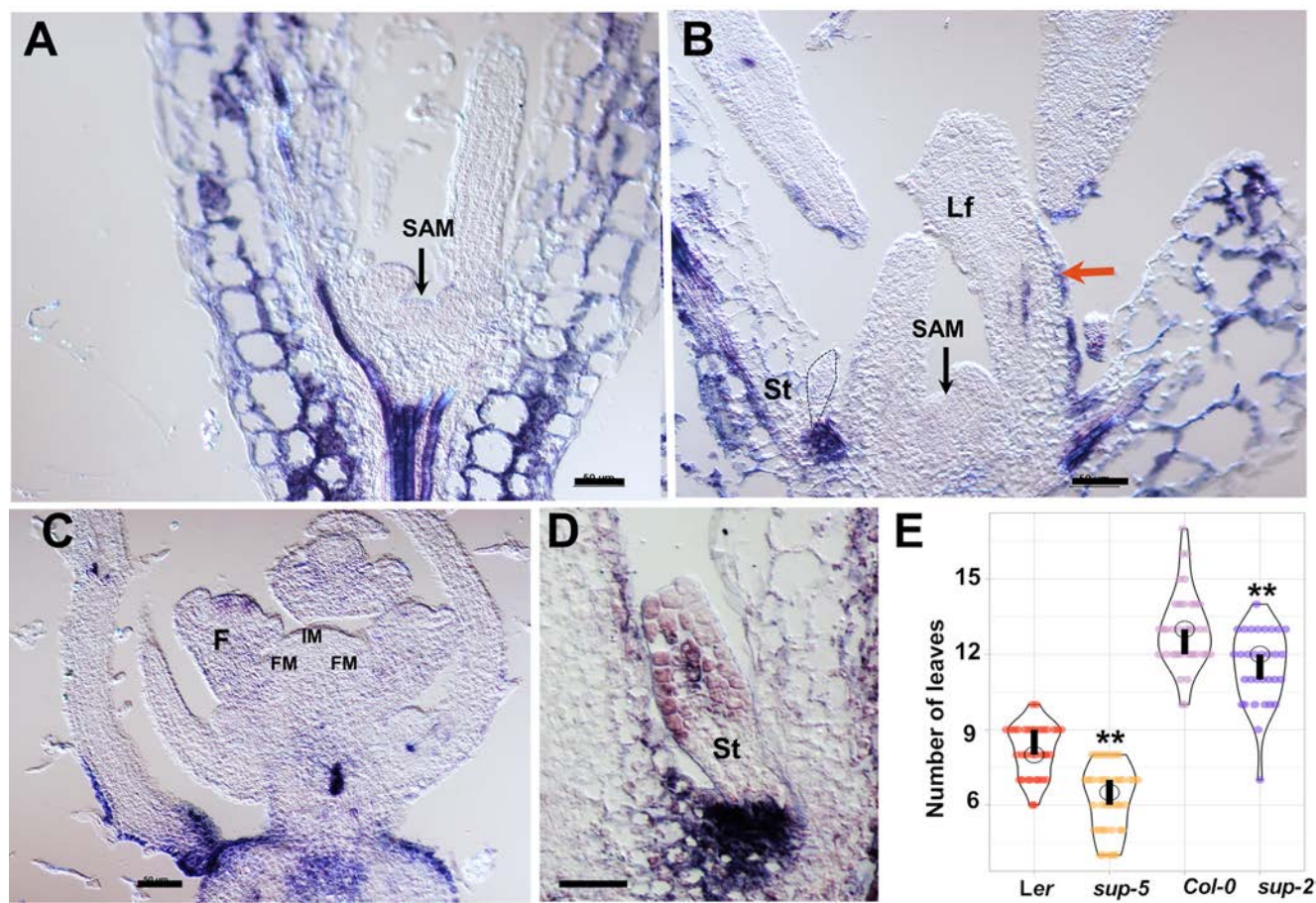


FIGURE 3 Functional characterization of *SUP* during vegetative to flower transition in *Arabidopsis thaliana*. A–D. *In situ* hybridization with *SUP* antisense probe. A. Longitudinal section of four-day-old seedling apices. *SUP* transcript was detected in the vasculature of the vegetative plant and was absent in the shoot apical meristem (SAM). B. Longitudinal section of seven-day-old seedling apices. *SUP* showed a strong expression at the base of stipules (St). Also, *SUP* mRNA was detected in the vasculature of cotyledons and the abaxial side of leaf (Lf) (orange arrow). C. Longitudinal section of ten-day-old seedling apices. *SUP* expression was maintained in the vascular tissue (yellow arrow). *SUP* mRNA accumulates between stamens and carpels primordia of a developmental flower (F). D. Longitudinal section of five ten-day-old seedling apices. Strong *SUP* expression was detected at the base and tip of stipules (St). E. Flowering time of *sup-5* (Ler) and *sup-2* (Col-0) mutants measured as the total leaf number at flowering grown in long-day conditions (LD). Statistically significant difference was determined by one-way ANOVA ($p < 0.05$) followed by Bonferroni post-hoc test multiple comparison $^{**}p < 0.01$. Bars in A, B and D indicate 50 μ m. Bar in C indicates 20 μ m.

FIGURE 2 Phenotypic characterization of *slsup* mutants during plant growth. A. Representative wild-type sympodial shoots at six weeks old. After the first inflorescence produced by the primary shoot (orange arrow), a sympodial unit (SU) developed 3 leaves before the emergence of the next inflorescence. A big branch (B) emerges on the axils of the last leaf (Lf). B–C. Representative *slsup-4* and *slsup-5* sympodial units at six weeks old. Two central axes with similar thickness and size can be observed. One axis corresponds to the first SU. The lateral branch simulates another sympodial unit (B-SU). Both SU and B-SU developed shorter internodes than the wild type. D. Violin plot of the sympodial unit size in wild type and *slsup* mutants. An open circle indicates the median of the data. A vertical bar indicates for each median the 95% confidence interval determined by bootstrapping. Statistically significant differences were determined by one-way ANOVA ($p < 0.05$) followed by Bonferroni post-hoc test multiple comparison $^{**}p < 0.01$. E. SEM analysis of wild-type shoots shows a premature sympodial meristem (SYM) close to the next inflorescence and a matured axillary meristem (AM) on the opposite side. F. SEM analysis of representative *slsup-5* shoot shows the SYM and AM meristems at the comparable maturation stage. G. Violin plot of the number of flowers per inflorescences in wild type and *slsup* mutants. The open circles indicate the median of the data, and the vertical bar indicates the 95% confidence interval determined by bootstrapping. Statistically significant difference was determined by one-way ANOVA ($p < 0.05$) followed by Bonferroni post-hoc test multiple comparison $^{**}p < 0.01$. H. Wild-type inflorescence with six flowers and the typical zigzag arrangement. I. Representative inflorescence of *slsup-4* mutant showing ten flowers. J. Representative inflorescence of *slsup-5* mutant showing sixteen flowers resulting from abnormal inflorescence duplication. K. SEM analysis of shoots in the wild type. The inflorescence contains flowers in sequential developmental stages arranged in the typical zigzag pattern of tomato inflorescences. L. SEM analysis of representative *sup-5* shoot showing duplicated inflorescences (pink and yellow ovals). Two flowers at similar developmental stages and two almost identical floral meristems (FM) emerged on opposite sides. M. Schematic representation of the tomato plant architecture of the wild type and *slsup* mutants. PS. Primary stem. SU. Sympodial unit. B-SU. Branch resembling a sympodial unit (B-SU). In. Inflorescence. Bars in A to C indicate 10 cm. Bars in E, F, K and L indicate 100 μ m. Bars in H to J indicate 2 cm.

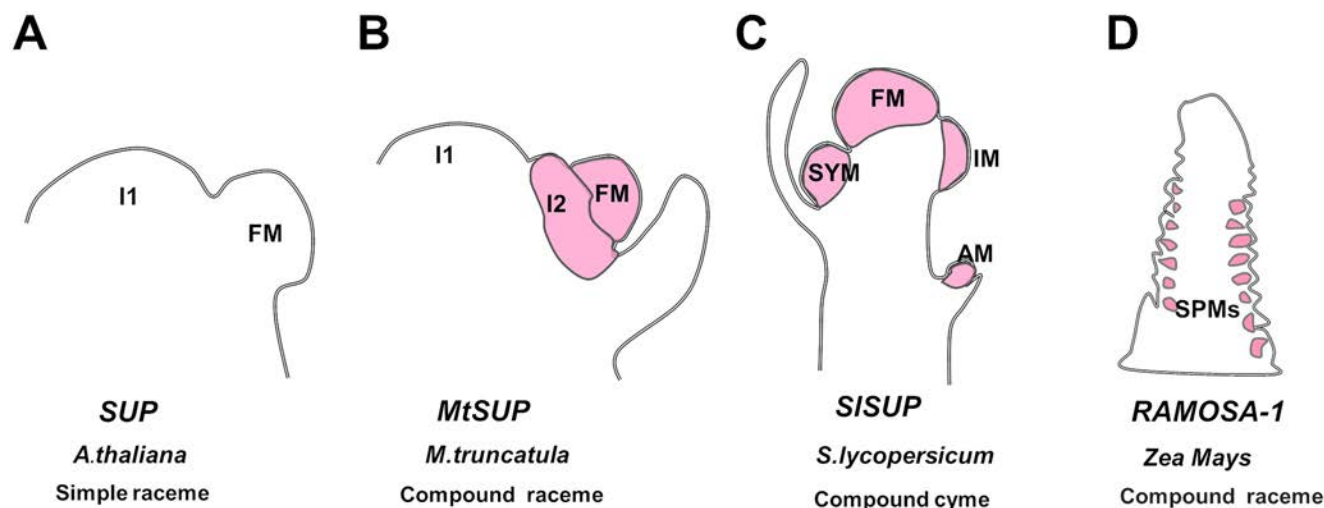


FIGURE 4 Schematic representation of *SUP*, *MtSUP*, *SISUP* and *RAMOSA1* expression patterns in the meristems that build the inflorescences in their respective species. **A.** In *Arabidopsis*, with a simple inflorescence, *SUP* is not expressed in the IM (This study). **B.** In *M. truncatula*, *MtSUP* is expressed in the secondary inflorescence meristem (I2), whose activity decides the number of flowers of the Medicago compound inflorescence (Rodas et al., 2021). **C.** *SISUP* controls the determinacy of the meristems in the tomato reproductive shoot apices (This study). **D.** *RAMOSA1* regulates the determinacy of spikelet pair meristem (SPMs), controlling the branching degree in maize (Vollbrecht et al., 2005).

meristem regulatory genes that share functions in the activity of inflorescence meristems in their respective species (Figure 4) (Rodas et al., 2021, 2023; Vollbrecht et al., 2005). However, in *Arabidopsis*, which develops simple inflorescences, *SUP* is not expressed in any meristem (Figure 4). Our results suggest that compound inflorescences, which independently appeared during angiosperm evolution, could be related to changes in the spatial and temporal expression patterns of *SUP*-like genes (Figure 4). Many unanswered questions remain, but our work has contributed to taking a crucial step forward.

AUTHOR CONTRIBUTIONS

Conceptualization: E.R, A.L.R.; C.G.M. L.A.C, J.P.B; **Methodology:** E.R, A.L.R, C.G.M **Data curation.** E.R, A.L.R; **Validation:** E.R, A.L.R; **Formal analysis:** E.R, A.L.R, C.G.M; **Investigation:** E.R, ALR, CGM **Writing-original draft:** E.R; C.G.M **Supervision:** A.L.R, L.A.C, J.P.B.

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DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting this article's findings are included in its Supplementary materials and Additional Files.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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