Arabidopsis thaliana SHOOT MERISTEMLESS Substitutes for Medicago truncatula SINGLE LEAFLET1 to Form Complex Leaves and Petals

Véronique Pautot, Ana Berbel, Thibaud Cayla, Alexis Eschstruth, Bernard Adroher, Pascal Ratet, Francisco Madueño, and Patrick Laufs

Abstract: LEAFY plant-specific transcription factors, which are key regulators of flower meristem identity and floral patterning, also contribute to meristem activity. Notably, in some legumes, LFY orthologs such as Medicago truncatula SINGLE LEAFLET (SGL1) are essential in maintaining an undifferentiated and proliferating fate required for leaflet formation. This function contrasts with most other species, in which leaf dissection depends on the reactivation of KNOTTED-like class I homeobox genes (KNOXI). KNOXI and SGL1 genes appear to induce leaf complexity through conserved downstream genes such as the meristematic and boundary CUP-SHAPED COTYLEDON genes. Here, we compare in M. truncatula the function of SGL1 with that of the Arabidopsis thaliana KNOXI gene, SHOOT MERISTEMLESS (AtSTM). Our data show that AtSTM can substitute for SGL1 to form complex leaves when ectopically expressed in M. truncatula. The shared function between AtSTM and SGL1 extended to the major contribution of SGL1 during floral development as ectopic AtSTM expression could promote floral organ identity gene expression in sgl1 flowers and restore sepal shape and petal formation. Together, our work reveals a function for AtSTM in floral organ identity and a higher level of interchangeability between meristematic and floral identity functions for the AtSTM and SGL1 transcription factors than previously thought.

Keywords: Medicago truncatula; meristematic activity; flower development; SINGLE LEAFLET/LEAFY; SHOOT MERISTEMLESS transcription factors

1. Introduction

Meristems are essential for plant development, as they are required for the continuous growth and development that are distinguishing features of plants. Amongst all the different types of meristems, the shoot apical meristem (SAM) and the floral meristem (FM) share many features and have been well characterized. The class I KNOTTED-like homebox (KNOXI) SHOOT MERISTEMLESS (STM) and CUP-SHAPED COTYLEDON (CUC1), CUC2 and CUC3 genes are essential regulators of meristem and boundary activities in Arabidopsis thaliana (A. thaliana) [1,2]. Boundaries are domains of restricted growth located between the meristem and initiating organ primordia or between two organs. These domains control organ separation, inflorescence architecture, organ abscission, fruit opening and leaf shape. Boundaries share overlapping features with meristems, and the regulation of both involves common factors [3]. CUC genes are required for SAM initiation and establish boundaries together with STM, which is in turn required for SAM maintenance [4–11]. The three A. thaliana CUC genes, CUC1, CUC2 and CUC3, share partially
LEAFY (LFY) is a key regulator of flower meristem identity and floral patterning [14–17]. LFY acts as a pioneer transcription factor and promotes chromatin accessibility to its target genes APETALA1 (API) and AGAMOUS (AG) [18,19]. LFY also contributes to meristem function, particularly to the formation of floral meristems in A. thaliana [20–23]. LFY acts together with UNUSUAL FLOWER (UFO), an F-box protein, which is a substrate adaptor of CULLIN1–RING ubiquitin ligase complexes (CRL1) [24,25] to control meristem function and identity [17,26]. In flowers, both LFY and auxin transport contribute to proper positioning of sepal primordia through the regulation of CUC2 expression [27]. Besides determining floral identity and patterning, LFY also contributes to the meristematic identity of floral or axillary meristems with several regulators [21,28–31]. Among them are PENNYWISE (PNY) and POUNDFOOLISH (PNF), two BEL1-like (BELL) homeodomain partners forming heterodimers with STM [32,33]. The LFY implication in axillary meristem emergence is mediated through REGULATOR OF AXILLARY MERISTEMS1 (RAX1), a MYB transcription factor [34,35], and through the repression of ARABIDOPSIS RESPONSE REGULATOR (ARR7), encoding a cytokinin signaling component [21,36].

Meristematic features can also be found outside bone fide meristems, such as in the leaves. This attribute is particularly obvious in compound leaves, in which leaflet formation requires a transient maintenance of a meristematic-like stage. Indeed, in most species with compound leaves, KNOXI gene down-regulation at leaf initiation is only transient and these genes are reactivated following leaf initiation, leading to leaflet formation [37–40]. In the inverted repeat-lacking (IRLC) clade of legume species, the formation of compound leaves requires the activity of the LFY orthologs called UNIFOLIATA (UNI) in pea (P. sativum) and SINGLE LEAFLET1 (SGL1) in Medicago truncatula (M. truncatula) [41,42]. These LFY orthologs substitute for KNOXI expression, which is permanently excluded from the initiating leaf primordia [43,44]. SGL1 is expressed in the entire SAM and highly expressed in developing leaves, where its prolonged expression is required for the formation of compound leaves [42,45]. However, ectopic expression of KNOXI genes in M. sativa and M. truncatula leaves can further increase leaf dissection [44,46], suggesting that these two Medicago species retain the capacity to respond to both LFY and KNOXI pathways. Consistent with a central role of LFY orthologs in IRLC legume leaf morphology, loss of function of the pea UFO ortholog, STAMINA PISTILLOIDA (STP), leads to leaf complexity reduction [47]. In contrast, in non-IRLC legumes such Lotus japonicus and soybean, LFY orthologs only play a minor role, and KNOX1 proteins accumulate in leaves and are likely associated with compound leaf development [44,48].

In simple leaves, such as in A. thaliana, repression of KNOXI genes is permanent, limiting their meristematic features [49,50]. However, these leaves are still able to develop an increased complexity in response to ectopic expression of KNOXI genes [40,51–53] and to UFO [54].

The observation that depending on the species, LFY and KNOXI genes can similarly increase leaf complexity (through the formation of leaflets or serrations) and that some species are able to respond to both factors, suggests that both pathways may at least partially converge to control leaf development. CUC genes could be such a convergence point as both KNOXI and LFY pathways require the activity of CUC2 to make compound leaves [55,56]. Similar to CUC1/2 in A. thaliana, the expression of the M. truncatula NO APICAL MERISTEM (MtNAM) ortholog is regulated by mir164 [57], and MtNAM is required to maintain boundaries both for cotyledon and leaflet separation besides its role in apical meristem initiation [58]. SGL1 function in leaflet primordium initiation is epistatic to MtNAM activity and MtNAM RNAs levels are reduced in sgl1 mutant [58], suggesting that SGL1 acts upstream of MtNAM in this species.

Besides its function in leaves, SGL1 also plays a role in floral meristem identity. M. truncatula is a legume species developing compound inflorescences. Upon floral transition, the shoot apical meristem transforms into a primary inflorescence meristem (II) and gives

redundant roles, while also having specific functions. CUC1 and CUC2 but not CUC3 transcripts are negatively regulated by microRNA164 (miR164) [12,13].
rise to a lateral secondary inflorescence meristem (I2), which produces a bract, one to three flowers and a spike [59–61]. In contrast to other flowering species which show a sequential floral ontogeny with successive formation of sepals, petals, stamens and carpels, each floral organ derives from a specific primordium; petals and sepals differentiate from common primordia in *M. truncatula* [59]. Thus, each floral meristem gives rise sequentially to five sepals and four common primordia, which further differentiate into five petals and ten stamens, and one carpel. The *Arabidopsis* floral organ identity genes are conserved in legumes [62]. Loss of function of *SGL1* leads to the reversion of common primordia into incomplete floral meristems, giving rise to sepals and carpels without petals and stamens [42]. This phenotype is related to a B function loss. Similar to *LFY* in *Arabidopsis* [63], *SGL1* acts synergistically with *MiPROLIFERATING INFLORESCENCE MERistem (MiPIM)*, the *A. thaliana* ortholog of *APETALA1 (API)* in *M. truncatula* to determine floral meristem identity [61,64]. *MtNMH7* and *MtTM6* are the *A. thaliana* *AP3-like* paralogs. *MNMH7* determines petal identity whereas *MtTL6* controls stamen identity [65]. *MiPISTILLATA (MiPI)* and *MtNGL9* are the two *A. thaliana* PI-like paralogs, with *MiPI* functioning as the master regulator of B function [66,67]. The *M. truncatula* genome harbors two redundant *MiAG* members, *MtAgA* and *MtAgB*, which specify stamen and carpel identity and floral meristem determinacy [68,69]. Recently, a novel regulator of inflorescence development and floral organ identity was identified in *M. truncatula*: the AGAMOUS-like FLOWERS (AGLF) gene, which encodes a MYB domain protein that promotes the C floral identity function besides repressing A and B functions [69,70].

Here, we further compared the meristematic activity of *SGL1* (*LFY*) and *AtSTM* (*KNOX1*) using *M. truncatula* compound leaf as a model system. We first showed that *AtSTM* could substitute for *SGL1* to form complex leaves. We next tested whether *AtSTM* could also substitute for *SGL1*’s role during floral development. Indeed, *AtSTM* expression could restore petal formation in *sgl1* flowers, revealing that *AtSTM* could substitute for *SGL1* function to specify petal identity and promote floral organ identity gene expression. Therefore, our data reveal a high level of interchangeability between *SGL1* and KNOX1 activities in *M. truncatula* that extends beyond the generally accepted meristematic function to the determination of the identity and growth of the flower perianth.

2. Results

2.1. *AtSTM* Substitutes for *SGL1* in *M. truncatula* to Form Compound Leaves

The *M. truncatula* genome harbors two MtSTM-like genes, *MtKNOXI* and *MtKNOX6*, and a previous report describes in vitro plantlets overexpressing the MtSTM-like genes, *MtKNOXI* and *MtKNOX6*, in *M. truncatula* [46]. However, only the vegetative phenotype was described, as the phenotype of *MtKNOXI* and *MtKNOX6* overexpressors was extremely severe. Therefore, to overcome such strong phenotypes, we thought to use a KNOX gene from a heterologous system. *AtSTM* shares 62.8% amino acid identity with *MtKNOXI* and 64.86% with *MtKNOX6*, and in addition to modifying leaf shape when ectopically expressed, *AtSTM* also has an established role in *Arabidopsis* floral identity [5,6,32,52,71,72]. Thus, we selected *AtSTM* to be expressed in *M. truncatula* and to explore its potential more widely; we expressed it under two different promoters by generating the *p35S:AtSTM* and *pSGL1:AtSTM* constructs that we first introduced in wild-type plants (see Section 4 and Supplemental Figure S1).

Transgenic lines expressing high levels of *AtSTM* presented a severe phenotype and were not viable in the greenhouse, similar to in vitro plantlets overexpressing MtKNOXI-like genes [46] (Figure S2). Only transgenic plants with low levels of *AtSTM* expression could be investigated (Figures 1 and S3). The overall development of these lines was quite normal, although their fertility was reduced. In wild-type *M. truncatula*, the juvenile first leaf is simple, while adult later leaves are trifoliate and composed of a terminal leaflet with two lateral leaflets (Figure 1A–D). Ectopic *AtSTM* under the *p35S* or the *pSGL1* promoters occasionally led to the formation of an additional leaflet fused to the terminal leaflet of adult leaves (Figures 1E,H and S3). Quantitative analyses were performed using the *p35S:AtSTM*
The wild-type first leaves (rank 1) were simple, while the majority of adult leaves (ranks 2 to 5) were trifoliate (only 4 out of 72 leaves had more than three leaflets). The p35S:AtSTM sequences seldom led to complex leaves, as only 8 out of 72 adult leaves (ranks 2 to 5) were more complex (Figure 1Q).

Figure 1. Ectopic expression of AtSTM rescues the sgl1 leaf phenotype. Phenotype of juvenile (L1) and adult leaves (rank L2, L3, L4) of 5-week-old plants. (A–D) R108 control line. Juvenile leaves are simple, while adult leaves are trifoliate and composed of a terminal leaflet plus two lateral leaflets. The petiole (p) and the rachis (r) are indicated. (E–H) p35S:AtSTM, a transgenic line expressing AtSTM under the p35S promoter producing a L2 and a L4 heart-shaped adult leaves with an ectopic leaflet fused to the terminal leaflet (arrowheads). This phenotype was occasionally observed. Leaflet margins are serrated. (I–L) sgl1 line, showing simple juvenile (L1) and adult leaves (L2–L4). (M–P) p35S:AtSTM sgl1 line, showing trifoliate L3 and L4 leaves similar to wild-type. (Q) Quantification of the leaflet number. Four-week-old plants were analyzed (n = 18 plants per genotype). Average ± SD are shown. Lowercase letters indicate significant differences between genotypes at each leaf rank (one-way ANOVA with Tukey’s post hoc test; p ≤ 0.001). Bars = 5 mm.
We then tested whether *AtSTM* expression is sufficient to rescue the *sgl1* leaf phenotype (see Section 4). In the *sgl1* mutant, the majority of leaves are simple (Figure 1I–L). All rank 5 leaves were simple, but 12 out of 54 leaves (ranks 2 to 4) were bi- or trifoliate in the *sgl1* mutant (Figure 1Q). In contrast, in *p35S:AtSTM sgl1* plants, the majority of adult leaves were trifoliate as in wild-type (Figure 1B–D,O,P). The *p35S:AtSTM* construct restored almost systematically the capacity to form trifoliate leaves, with 52 out of 54 leaves (ranks 3 to 5) producing at least three leaflets (Figure 1Q). Therefore, we concluded that *AtSTM* can replace *SGL1* to promote leaflet formation.

To explore the developmental origin of the extra or rescued leaflets in the different backgrounds, we imaged by SEM young developing leaf primordia (Figure 2). As observed in wild-type apices, a pair of lateral leaflets and a terminal leaflet initiated in *AtSTM* transgenic lines during early leaf primordium development (Figure 2A,C). At stage S8, additional leaflets could form at the base of the terminal leaflet in *AtSTM* (arrows Figure 2D), which were not observed in the wild-type (Figure 2B) and therefore resulted from secondary morphogenesis. This indicates that the morphogenetic window during which leaflets can be initiated is extended following *AtSTM* expression. In *p35S:AtSTM sgl1* plants, the terminal primordium was surrounded by two lateral primordia (Figure 2G,H), already visible at early stages (S4), as seen in the wild-type (Figure 2A,G). Thus, leaflet restoration in *p35S:AtSTM sgl1* does not appear to rely on a late production of leaflets but a rescue of the normal developmental process with a restoration of early lateral leaflet initiation, as occurs in the wild-type.

**Figure 2.** SEM analysis of early stages of leaf development. (A,B) R108 wild-type control line. (A) SAM and a leaf primordia at S6 stage showing a terminal leaflet (TL) developing trichomes with one of the lateral leaflets (LL) and one stipule (ST). (B) S8 stage leaf primordia with one terminal leaflet folded on itself between the two lateral leaflets. (C,D) *p35S:AtSTM* line. (C) SAM with a typical S4 stage leaf primordia. (D) At S8, the *p35S:AtSTM* line has formed a new leaflet (NL) at the base of the terminal leaflet (arrow). Leaflet margins are dissected. (E,F) *sgl1* mutant. (E) SAM with a simple S5 leaf primordia (SL). (F) At S8, the leaflet is folded on itself. (G,H) *p35S:AtSTM sgl1* line. (G) SAM with S4 leaf primordia that has formed lateral leaflets similar to wild-type. (H) At S8, the *p35S:AtSTM sgl1* leaf primordia was similar to wild-type with the terminal leaflet surrounded by two lateral leaflets. Bars = 100 μm.

2.2. *AtSTM* Substitutes for *SGL1* in *M. truncatula* in Specifying Petal Formation

*M. truncatula* is a legume species developing compound inflorescences. The wild-type *M. truncatula* mature flower (Figure 3A–I) comprises four whorls consisting of a calyx formed by five sepals fused at their base (Figure 3B), a corolla containing three types of yellow petals, the standard or the vexillum at the adaxial position (Figure 3C), the keel...
formed by two fused petals at the abaxial position surrounded by two lateral petals and the alae or wings (Figure 3D–G). The third whorl consists of an independent stamen filament at the adaxial position, the vexillary stamen filament and nine stamen filaments fused into a staminal tube that surrounds a monocarpous gynoecium [59] (Figure 3H,I). The sgl1 mutants produce inflorescences with cauliflower-like floral structures, containing incomplete floral meristems (FMs), elongated sepals and occasionally carpels [42] (Figure 3U). These cauliflowers do not produce petals nor stamens, similar to lfy mutants in Arabidopsis [73].

Figure 3. Ectopic expression of AtSTM in M. truncatula promotes petal identity. (A–J) R108 wild-type control line. (A) The wild-type flower showing the calyx (C) and the corolla containing 5 petals: the standard or vexillium (Vx), the keel (K) and two alae (A) or wings. (B–I) A wild-type dissected flower:
(B) the calyx, formed by 5 fused sepals at their base. (C) The standard or vexillum, abaxial side. 
(D,E) The keel formed by two fused petals (arrows) surrounded by two lateral petals, the alae or 
 wings, adaxial (D) and abaxial (E) sides, (F) a dissected wing. (G) A dissected keel petal. (H,L) A 
single carpel enclosed by a staminal tube comprising nine fused stamens plus one independent
 “vexillary” stamen at the adaxial position (I). (J) After fertilization, the carpel grows out to form 
a coiled fruit with spines. (K–T) p35S:AtSTM line. (K–Q) Phenotypes of p35S:AtSTM flowers. 
(L,M) Petals were abnormal and can show dissected margins. (N) Flower showing petaloid sepal.
 (M–O.Q) Flowers forming two or three carpels (arrowheads), with some showing an unfused carpel
 (arrows). (O,P) Flowers showing petaloid stamens. (Q) A dissected flower (the corolla was removed)
 showing 3 carpels with one developing petaloid sectors. (R) Dissected petals, some of them showing
 sepal sectors. (S) A dissected calyx showing petaloid sepal. (T) Fruits were smaller with unbent
 spines. (U) sg/l inflorescence containing three flowers with a cauliflower-like morphology. sg/l
 flowers contain sepal and carpeloid structures and lack petals and stamens. (V–Y) A p35S:AtSTM
 sg/l flower showing petals. This flower contains inside incomplete FMs that produce mainly petals
 or petaloid sepal and a few sepals. (V) FMs are visible (*). (W) Bottom view showing the calyx
 (arrow), the sepals form is restored (see also Figure 4M), some other sepals are visible (arrowhead).
 Dissected petals: a vexillum-like petal (X), a keel-like (arrow Y) and wing-like petals (arrowhead Y). 
Bars = 2 mm, except for F, G, I, N and U, for which bars = 1 mm.

Figure 4. SEM analysis of flower development. (A–D) Flower development in wild-type R108 line.
 (A) An inflorescence showing floral meristems (FMs) at different stages, including a late S5 stage
 primordia and a spike (spk) at the base of the floral meristem. (B) S4 stage FM showing the abaxial sepal
We then tested the effects of p35S:AtSTM sgl1 with five sepals fused at their base (Figure 3B,W). Inside the calyx, the petaloid stamens (Figure 3O,P) and petaloid carpels (Figure 3Q). The fertility was severely reduced, with some plants infertile. The fruits were small, with fewer discs and unbent spines compared with wild-type fruits (Figure 3T,J). These fruits contained a few seeds. The same phenotypes were occasionally observed in pSGL1:AtSTM flowers (Figure S3F,G). We then tested the effects of p35S:AtSTM on sgl1 flower development. Surprisingly, the ectopic expression of AtSTM rescued sepal shape and petal formation in the sgl1 mutant (Figure 3V–Y). Similar to wild-type flowers, p35S:AtSTM sgl1 flowers formed a calyx with five sepals fused at their base (Figure 3B,W). Inside the calyx, the p35S:AtSTM sgl1 flowers showed a cauliflower phenotype with incomplete FMs, producing a few sepals and a majority of petals or petals with sepallike shape or serrated margins (Figure 3N,S). These flowers did not form carpels, in contrast to sgl1 flowers, suggesting a deficiency in C function (Figure 3). The majority of organs formed were petals, as one cauliflower flower from a line could produce up to 65 petals (Figure S4).

Wild-type plants for SGL1 overexpressing AtSTM occasionally produced abnormal flowers showing fused organs and are characterized by an increase in petal identity with petaloid sepals and petaloid stamens (Figure 3K–S). Some petals showed alterations in shape or serrated margins (Figure 3L,M). These flowers occasionally produced two to three unfused carpels (Figure 3M–O). Flowers can show petaloid sepals (Figure 3N,S), petaloid stamens (Figure 3O,P) and petaloid carpels (Figure 3Q). The fertility was severely reduced, with some plants infertile. The fruits were small, with fewer discs and unbent spines compared with wild-type fruits (Figure 3T,J). These fruits contained a few seeds. The same phenotypes were occasionally observed in pSGL1:AtSTM flowers (Figure S3F,G). We then tested the effects of p35S:AtSTM on sgl1 flower development. Surprisingly, the ectopic expression of AtSTM rescued sepal shape and petal formation in the sgl1 mutant (Figure 3V–Y). Similar to wild-type flowers, p35S:AtSTM sgl1 flowers formed a calyx with five sepals fused at their base (Figure 3B,W). Inside the calyx, the p35S:AtSTM sgl1 flowers showed a cauliflower phenotype with incomplete FMs, producing a few sepals and a majority of petals or petals with sepallike shape. Petals were partially restored as some of them had a vexillium-like, wing-like or keel-like shape (Figure 3X,Y). Thus, when ectopically expressed, AtSTM restores petal formation in sgl1. These flowers did not form carpels, in contrast to sgl1 flowers, suggesting a deficiency in C function (Figure 3). The majority of organs formed were petals, as one cauliflower flower from a 35S:AtSTM sgl1 line could produce up to 65 petals (Figure S4).

SEM analyses were performed to further characterize these flowers at early developmental stages. Figure 4A–D shows wild-type floral development. At stage 4, the wild-type floral meristem had formed five sepal primordia, four common primordia and a carpel primordium (Figure 4B). At late stage 5, the wild-type floral meristem displayed the complete set of floral organ primordia, with petal and stamen primordia deriving from the differentiation of common primordia (Figure 4D). Figure 4E–H shows the floral development of a p35S:AtSTM plant wild-type for SGL1. Figure 4F shows a late stage 5 p35S:AtSTM floral meristem. Based on sepal development, a delay in the formation of the inner floral organ primordia could be observed compared with the wild-type (Figure 4F,D). In contrast, Figure 4G shows a stage 5 floral meristem containing differentiated petals and stamen primordia and two carpel primordia, indicating that the delay in internal organ primordia differentiation is variable between flowers. Figure 4H shows a p35S:AtSTM flower developing two carpels. Similar to previous data [42,61], sgl1 inflorescences showed multiple incomplete FMs, elongated sepals, defective common primordia and carpel primordia (Figure 4I–L). Sepal primordia further develop into elongated sepals and carpel primordia into a carpel-like structure. The cauliflower phenotype is caused by the iterative
conversion of common primordia into incomplete floral meristems (Figure 4I,L). In sgl1 mutants overexpressing AtSTM (Figure 4M-O), the sepal form was restored, suggesting that AtSTM could take over SGL1 function for the control of sepal shape. The late stage 5 floral meristems showed a delay in the differentiation of other floral organ primordia, as observed in p35S:AtSTM SGL1 plants (Figure 4N,F). Later, petals and sepals differentiated from these primordia (Figure 4O). Together, these observations show that expression of AtSTM partly restored normal early morphogenesis of sgl1 flowers.

2.3. AtSTM Substitutes for SGL1 to Promote Floral Organ Identity Gene Expression

To determine if AtSTM activates A and B functions to promote petal formation in sgl1 flowers, we used in situ hybridization to analyze the expression pattern of floral organ identity genes in p35S:AtSTM sgl1 flowers. We first investigated the expression of the A class geneMtPIM, the A. thaliana ortholog of AP1 in M. truncatula. MtAPI has a conserved role with orthologous genes and is required to specify floral meristem and floral organ identity [61,64]. In wild-type inflorescences, MtAPI transcripts localize to the floral meristem and bract (Figure 5A,B). In a stage 4 flower meristem, MtAPI expression was observed in sepal primordia and was restricted to the outer domain of the common primordia that further gives rise to sepals and petals and was absent from the inner part, which differentiates into stamens and carpel (Figure 5C) [61,64]. At later stages, MtAPI expression was maintained in sepals and petals (Figure 5D). Similar to the pattern described in [61], in sgl1 flowers, MtAPI was expressed in the floral meristem and in the bract (Figure 5E). MtAPI was expressed uniformly in defective common primordia and in reiterated floral meristems (Figure 5F–H). At later stages, MtAPI expression localized to the outer incomplete floral meristem and disappeared from the central domain that further differentiates into carpels (Figure 5E,G). In p35S:AtSTM sgl1 flowers, MtAPI was more widely expressed than in sgl1 flowers, with MtAPI detected in reiterated floral meristems and in developing petals (I–K). Thus, in p35S:AtSTM sgl1 flowers, AtSTM acts as a positive regulator of A function, contributing to enhanced petal identity.

Figure 5. MtAPI expression in wild-type, sgl1 and p35S:AtSTM sgl1 flowers. (A–D) R108 wild-type flowers. (A, B) MtAPI was expressed in the floral meristem (FM) and in the bract primordia (Br) and absent in secondary inflorescence meristem (I2). (C) At stage 4, MtAPI was expressed in sepals (S). MtAPI was restricted to the outer part of the developing common primordia (CP), which will give rise to petal (P) and was absent in the inner part that will give rise to stamen (St). (D) At stage 6, MtAPI...
expression was maintained in sepals and petals. MtAPI1 was absent in carpels (C) and stamens. (E–H) sglI flowers. (E) MtAPI1 was expressed in bract (Br) and floral meristem (FM). (F,G) MtAPI1 was uniformly expressed in defective common primordia (CP’), unlike in wild-type common primordia, MtAPI1 was absent in the inner part of the floral meristem where carpel will develop (C). (H) MtAPI1 was expressed in reiterated floral meristems deriving from floral primordia. (I–K) p35S:AtSTM sglI flowers. MtAPI1 expression was detected in reiterated floral meristems (FM), in defective common primordia (CP’) and in developing petals (P). Bars: (A–I) = 50 µm, (J,K) = 100 µm.

We then investigated the expression of the B class gene MtPI. In wild-type, MtPI transcripts were localized to common primordia cells and later restricted to petal and stamens (Figure 6A,B) and [66,67]. In the sglI mutant, no MtPI expression was detected in defective common primordia, consistent with the phenotype of sglI flowers, which lack petals and stamens (Figure 6C). In sglI flowers overexpressing AtSTM, MtPI expression was detected in defective common primordia (Figure 6D,E inset-a). At a later stage, MtPI localized to the outer domain of the defective common primordia that further gives rise to petal-like organs (Figure 6E and inset-b). Later, MtPI is expressed in petal-like organs (Figure 6D,E). Thus, AtSTM acts as a positive regulator of MtPI expression consistent with the restoration of petal identity.

Figure 6. MtPI expression in wild-type, sglI and p35S:AtSTM sglI flowers. (A,B) MtPI expression in R108 wild-type flowers. (A) Mt PI expression was detected at stage 3 in cells of the common primordia (CP). (B) At later stages, MtPI expression was restricted to stamens (St) and petals (P). (C) MtPI was not detected in sglI flowers. (D,E) MtPI expression in p35S:AtSTM sglI flowers. (D) A flower developing sepals (S) and petals (P). MtPI expression was detected in defective common primordia (CP’) and in petals (P). (E) A cauliflower showing several floral meristems and developing sepals (S) and petals (P). MtPI was detected in defective common primordia (CP’) and in petals (P). Unlike in wild-type, in which the expression of MtPI is observed in the whole common primordia, the expression of MtPI was restricted to the periphery of the defective common primordia, which will give rise to petals (see details in (a,b)). (a,b) Close-ups of the areas marked in (E). Bars: (A,C,a,b) = 50 µm, (B–E) = 100 µM.

We further determined the expression of the M. truncatula ortholog of the A. thaliana C-class gene AG. MtAGb was used as a probe as its signal is stronger and it is more restricted than that of MtAGa [68]. In wild-type flowers, MtAGb expression was first detected at stage 2 in the central part of the floral meristem where the carpel will develop (Figure 7A). At stage 4, MtAGb expression was mainly localized to the inner domain of the common primordia that will further give rise to stamens and to carpel primordia (Figure 7B). At later stages, its expression was restricted to stamens, carpel and ovules (Figure 7C,D). In sglI flowers, a weak signal was detected in floral meristems and defective common
primordia and was absent in the L1 layer (Figure 7E–G). Later, its expression was detected in carpel-like structures and ovules (Figure 7H). In sgl1 plants overexpressing AtSTM, MtAGb expression was detectable in only a few flowers (3 of 13). In these flowers, the signal was weak and restricted to a few cells in FM beneath the two outer most layers (Figure 7I).

Figure 7. MtAGb expression in wild-type, sgl1 and p35S:AtSTM sgl1 flowers. (A–D) MtAGb expression in wild-type flowers. (A) MtAGb expression was located in the center cells of the floral meristem (FM) at stage 2. (B) At stage 4, MtAGb expression was detected in the carpel primordia (C) and the half of the common primordia (CP) that will give rise to the stamens. (C) At stage 5, MtAGb expression was detected in stamen (St) and carpel (C) primordia. (D) In later stages, MtAGb expression was located in stamen (St), carpel (C) and developing ovules (Ov). (E–H) MtAGb expression in sgl1 flowers. MtAGb was detected in FM (E, arrowhead) and in defective common primordia (CP’, F arrowhead, G) and absent in the L1 layer. (H) In later stages, MtAGb expression was detected in carpel (C) and ovules (Ov). (I,J) MtAGb expression in p35S:AtSTM sgl1 flowers. Expression was detected in 3 flowers out of 13. (I) An apex showing expression underneath the outermost cell layers in floral meristem (FM). (J) An apex showing no expression. Bars = 50 µm.

3. Discussion

Here, we compared the activity of two transcription factors, AtSTM and SGL1, in M. truncatula. Our analysis is based on transgenic plants that were able to grow in a greenhouse and therefore expressed AtSTM at low levels. This allowed us to investigate the activity of AtSTM during flower development.

An increase in the leaflet number was only occasionally observed following AtSTM ectopic expression in wild-type M. truncatula. This limited effect of AtSTM could be linked to AtSTM expression levels in these lines, which were low. The additional leaflets were formed at the base of the terminal leaflet and resulted from a secondary morphogenesis. This suggests that AtSTM leads to additional leaflets through the extension of the meristematic activity, allowing more leaflets to emerge, and not from the division of the lateral leaflets into two structures. In M. truncatula, the terminal leaflet derives from the terminal zone where auxin maxima are located through the activity of SMOOTH LEAF MARGINI (SLM11), the PIN1 ortholog in M. truncatula [74]. Lateral leaflets result from the marginal blastozone activity and the formation of local auxin maxima that depend on SGL1 activity [74]. The tetrafoliate pattern seen in AtSTM transgenic lines likely results from a defect in auxin distribution in the terminal zone. This leaf patterning is also found in M. truncatula plants inactivated for HEADLESS (HDL) or MtREVOLUTA1 (MtREV1), the putative orthologs of A. thaliana WUSCHEL and REVOLUTA, of which mutants are altered
in auxin homeostasis [75,76]. The ectopic expression of AtSTM could rescue the formation of lateral leaflets in the sgl1 mutant. These data show that AtSTM could substitute for SGL1 via an independent pathway to form complex leaves. This suggests that AtSTM could bypass the requirement for SGL1 during the formation of compound leaves in M. truncatula, indicating shared functions between these proteins, a conclusion further reinforced by the study of the floral phenotype of p35S:AtSTM sgl1 plants.

Our data revealed an unexpected effect of AtSTM on floral development, as AtSTM could induce petal identity. The effect of AtSTM on petal identity was moderately visible in an SGL1 wild-type background, as only few chimeric petaloid floral organs were formed, but was dramatic in an sgl1 mutant background. Indeed, all p35S:AtSTM sgl1 flowers produced petals or petals with sepal sectors, while such organs were missing in sgl1. Although the increase in petal number could be in part due to the indeterminate state conferred by the sgl1 mutation, it nevertheless indicates that AtSTM can restore petal formation in an sgl1 mutant. The shape of sgl1 sepal was also restored following AtSTM expression, showing that AtSTM could substitute for other functions of SGL1 during flower development. The formation of petals in p35S:AtSTM sgl1 was correlated with an activation of MtAPI and more notably of MiPI expression, suggesting that AtSTM could promote the expression of these floral organ identity genes to restore petals, and not through an indirect effect on floral meristem growth, for instance. Such a role for KNOXI genes in the promotion of B function was not yet reported in either M. truncatula nor in A. thaliana [44,46,72].

On the contrary, p35S:AtSTM sgl1 flowers did not form stamens, and in contrast to sgl1 cauliflowers, which developed carpels, AtSTM sgl1 cauliflowers lacked carpels. MtAGb expression was only rarely detected in p35S:AtSTM sgl1 cauliflowers, in agreement with the lack of carpel identity. Interestingly, the expression of MtAGb was systematically detected in floral meristems beneath the outermost layers in sgl1 background. The localization and the low intensity of the MtAGb signal in sgl1 cauliflowers suggest that SGL1 influences MtAGb expression.

In Arabidopsis, a link for AtSTM with carpel identity was revealed with the analysis of plants compromised for AtSTM activity in line with AtSTM expression in flowers [5,6,71,73]. A more direct contribution to carpel identity was illustrated with the phenotype of A. thaliana KNOXI overexpressors showing homeotic conversion of ovules into pistils. However, KNOXI ectopic expression does not complement the ag mutant [52,72]. In line with these conclusions made in Arabidopsis, we observed that in M. truncatula, ectopic expression of AtSTM could not induce the C function in the absence of SGL1 activity. It is possible that in p35S:AtSTM sgl1 flowers, AG is playing a role related to floral meristem termination more than a function related to the specification of carpel identity.

The impact of AtSTM was more obvious both in leaves and flowers of the sgl1 mutant compared with wild-type SGL1 plants. This distinct impact could suggest that the STM pathway is more effective in the absence of SGL1 activity. It is likely that SGL1 acts in part through the M. truncatula UFO ortholog, as it does in Arabidopsis and other legumes. Indeed, in pea and in Lotus japonicus defective in STAMINA PISTILLOIDA (STP) or in PROLIFERATING FLOWER ORGAN (PFO), the A. thaliana UFO orthologs lack petals and stamens and show a reduced carpel formation similar to sgl1 flowers [47,77]. On the other hand, AtSTM was shown recently to function together in A. thaliana with AP1 to specify floral meristem identity in part via UFO [78]. This suggests that SGL1 and STM pathways may converge on MtUFO and that a competition for UFO interaction or for targets shared between SGL1 and AtSTM could be the basis for the higher effect of AtSTM in the absence of SGL1.

Our work shows that AtSTM substitutes for SGL1 function in M. truncatula during both vegetative and reproductive development. A parallel has been proposed between compound leaflet primordia and common primordia formation. Both of these processes seem to require the maintenance of an indeterminate phase controlled by SGL1 [61]. While in leaves, SGL1 maintains the indeterminate state, in flowers, SGL1 acts in opposite by
promoting the formation of common primordia. The capacity for AtSTM to substitute for SGL1 in both leaves and flowers underlines this parallel and the control of meristematic activity shared by these two transcription factors.

4. Materials and Methods

4.1. Plant Growth and Plant Material

*M. truncatula* plants were grown in a greenhouse or in growth chambers under long-day conditions (16 h light at 23 °C and 8 h dark at 15 °C). The wild-type (R108) and the sgl1-1 mutant *M. truncatula* lines have been described [42].

The pSGL1:GUS reporter construct was generated as follows. A 2.7 kb fragment corresponding to the SGL1 (Medtr3g098560) promoter sequence used in [42] (wild-type *M. truncatula* cv Jemalong) was amplified from the *M. truncatula* R108 ecotype using primers pSGL1-for, incorporating a BgIII site, and pSGL1-rev, incorporating a BamHI site. The promoter was cloned into pCR Blunt II-TOPO vector to create pTOPO-pSGL1 and sequenced. The pSGL1 promoter was moved into the binary vector pCAMBIA 3301 in front of the β-glucuronidase (GUS) gene. For this, a BgIII-BamHI fragment containing the SGL1 promoter was ligated into pCAMBIA3301 cut with BamHI and BgIII to replace the 35S terminator.

The pSGL1:HA-AtSTM construct was generated as follows (AtSTM, AT1G62360). pTOPO-pSGL1 was cut with EcoRI-BamHI to release the pSGL1 promoter, which was cloned into the pCAMBIA 3300 binary vector cut with EcoRI and BamHI to create pCAMBIA 3300 pSGL1. The alli2AtSTM plasmid harboring the triple hemagglutinin (HA) tag-AtSTM fusion under the double enhanced cauliflower Mosaic Virus 35S promoter was used as a template to amplify the HA-AtSTM fusion using primers AtSTM-for and AtSTM-rev incorporating BamHI and EcoRI sites, respectively. This fragment was ligated into the pALC vector (Syngenta Ltd., Jeolotts Hill, UK) cut with BamHI and EcoRI. The BamHI-XbaI fragment containing the HA-AtSTM fusion and the 35S terminator was cloned into pCAMBIA 3300 pSGL1 to create pCAMBIA pSGL1:HA-AtSTM 35S term.

The p35S:HA-AtSTM construct was generated as follows. The pSGL1 promoter sequence of the pCAMBIA pSGL1:HA-AtSTM 35S term was replaced with the 35S promoter sequence from pCAMBIA 3301 using the BgIII and BamHI sites. The pCAMBIA 3301 was cut with BamHI and BgIII to release the 35S terminator, and the pCAMBIA 3300 containing the pSGL1:HA-AtSTM construct was cut with BgIII and BamHI to replace the pSGL1 promoter with the 35S promoter to create pCAMBIA p35S:HA-AtSTM 35S term. pSGL1-GUS, p35S:Astem and pSGL1:AtSTM constructs were introduced into A. tumefaciens GV3101. The pSGL1-GUS construct was used to transform *M. truncatula* R108 wild-type plant, while p35S:Astem and pSGL1:AtSTM constructs were used to transform *M. truncatula* R108 plants heterozygous for the sgl1-1 mutation. *M. truncatula* transgenic lines were created using a leaf disc protocol [79]. Transgenic calli were selected on media containing 3 mgL⁻¹ Basta (glufosinate-ammonium). Primers are listed in Table S1.

Four independent pSGL1:GUS transgenic lines were analyzed for SGL1:GUS activity. The SGL1:GUS activity was detected in meristem, vascular tissue and young leaves in R108 *M. truncatula* (Figure S1), which was similar to the activity of the SGL1 promoter isolated from the JemalongA17 ecotype [42], and in axillary meristem, young floral buds and carpels (Figure S1).

Most of the transgenic plantlets expressing AtSTM were not viable when transferred to soil. RT-PCR were realized to compare the level of expression of AtSTM in transgenic lines. Total RNA was extracted from AtSTM transgenic lines expressing p35S:AtSTM (in vitro seedlings and transgenic plants grown in the greenhouse) using Tri reagent (Sigma-Aldrich, Saint-Quentin-Fallavier, France) and treated with DNase I (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions. AtSTM levels were monitored using qAtSTM-F and qAtSTM-R primers. Primers specific for the *M. truncatula* UBQUITIN gene (Medtr3g091400) were used as an internal control [80]. Only transgenic plantlets expressing AtSTM at low levels were viable in the greenhouse. Four p35S:AtSTM independent lines
and three $pSGL1:AtSTM$ lines were obtained. Of these, two independent $p35S:AtSTM$ lines and one $pSGL1:AtSTM$ based on their phenotype were chosen for further characterization. These plants showed reduced fertility. Plants homozygous for the $p35S:AtSTM$ construct and heterozygous for $sgl1$ were obtained and confirmed by PCR genotyping [42].

4.2. Phenotypic Observations

Leaves and flowers were observed under a binocular microscope (Nikon, SMZ1000) and imaged with a digital camera (ProgRes C10[plus]). $M. truncatula$ meristems showing GUS activity were dissected and photographed using a LeicaMZ12 dissecting microscope fitted with an AxioCam ICc5 digital camera.

4.3. Quantitative Analyses of Leaf Development

Progenies of $SGL1+/sgl1$ (R108) and $p35S:AtSTM$ $SGL1+/sgl1$ lines were grown in a greenhouse. Four-week-old plants were used. The leaflet number was determined on R108 wild-type, $sgl1/sgl1$, $p35S:AtSTM$ $SGL1+$ and $p35S:AtSTM$ $sgl1/sgl1$ plants. Eighteen plants per genotype were analyzed.

4.4. Scanning Electron Microscopy (SEM)

Three to eight-week-old plants were dissected to observe leaf and flower primordia. The samples were imaged using SEC DESKTOP SEM (Scanning Electron Microscope, (SNE-1500M), SEC, Suwon, Korea) at an accelerating voltage of 15 kV.

4.5. In Situ Localization of GUS Activity and In Situ Hybridization

GUS staining and tissue embedding have been described in [81]. RNA in situ hybridization with digoxigenin-labeled probes was performed as previously described [82]. The RNA antisense and sense probes of $MtAPI1$ (Medtr8g066260) $MtPIM$, $MtPI$ (Medtr3g088615) and $MtAGb$ (Medtr8g087860) were generated using as cDNA templates a 426 bp fragment of $MtPIM$ (282–707 from ATG), a 298 bp fragment of $MtPI$ (504–801 from ATG) or a 215 bp fragment of $MtAGb$ (558–773 from ATG), respectively, cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) and using the corresponding SP6 and T7 RNA polymerases in the vector for transcription. SP6 was used for transcription of RNA antisense probes and T7 for the sense. The in situ hybridization with control sense probes is presented in Figure S5.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms232214114/s1, Figure S1: $pSGL1:GUS$ expression in $M. truncatula$, Figure S2: In vitro transgenic $M. truncatula$ plantlets overexpressing $AtSTM$, Figure S3: Phenotype of a $pSGL1:AtSTM$ transgenic line expressing $AtSTM$ under the $pSGL1$ promoter, Figure S4: Petal production following $AtSTM$ expression in $sgl1$ flowers, Figure S5: In situ hybridization with control sense probes, Table S1: List of primers.

Author Contributions: All authors made essential contributions to the project. V.P. performed most of the experiments; A.E. made the $Medicago truncatula$ transgenics; T.C. performed the GUS assays, some leaf phenotypic characterization and some SEMs; B.A. provided technical assistance to V.P.; A.B. and F.M. performed the in situ analyses; P.L., P.R. and V.P. designed the research; V.P. and P.L. wrote the article. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by IJPB’s Plant Observatory technological platforms. The IJPB benefits from the support of Saclay Plant Sciences-SPS (ANR-17-EUR-0007). Work at F.M.’s lab was supported by the Spanish Ministerio de Ciencia Innovacion y Universidades and FEDER (grants BIO2015-64307-R and PGC2018-099232-B-I100). Thibaud Cayla and Alexis Eschstruth were supported by the ANR-11-BSV2-0005 Charmful.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.
Acknowledgments: We thank Bruno Letarnec for help in the greenhouse. We are grateful to Shelley Hepworth and Nicolas Arnaud for critical reading.

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Hepworth, S.R.; Pautot, V.A. Beyond the divide: Boundaries for patterning and stem cell regulation in Plants. Front. Plant Sci. 2015, 6, 1052. [CrossRef] [PubMed]
2. Maugarny-Cales, A.; Gontcharova, B.; Jouannic, S.; Melkonian, M.; Ka-Shu Wong, G.; Laufs, P. Apparition of the NAC transcription factors predate the emergence of land plants. Mol. Plant 2016, 9, 1345–1348. [CrossRef] [PubMed]
3. Žádníková, P.; Simon, R. How boundaries control plant development.Curr. Opin. Plant Biol. 2014, 17, 116–125. [CrossRef] [PubMed]
4. Aida, M.; Ishida, T.; Tasaka, M. Shoot apical meristem and cotyledon formation during Arabidopsis embryogenesis: Interaction among the CUP-SHAPED COTYLEDON and SHOOT MERISTEMLESS Genes. Development 1999, 126, 1563–1570. [CrossRef]
5. Endrizzi, K.; Moussian, B.; Haecker, A.; Levin, J.Z.; Laux, T. The SHOOT MERISTEMLESS gene is required for maintenance of undifferentiated cells in Arabidopsis shoot and floral meristems and acts at a different regulatory level than the meristem genes WUSCHEL and ZWILLE. Plant J. 1996, 10, 967–979. [CrossRef] [PubMed]
6. Long, J.A.; Moan, E.I.; Medford, J.I.; Barton, M.K. A Member of the KNOTTED class of homeodomain proteins encoded by the STM gene of Arabidopsis. Nature 1996, 383, 66–69. [CrossRef] [PubMed]
7. Takada, S.; Hibara, K.; Ishida, T.; Tasaka, M. The CUP-SHAPEDCOTYLEDON1 gene of Arabidopsis regulates shoot apical meristem formation. Development 2001, 128, 1127–1135. [CrossRef] [PubMed]
8. Belles-Boix, E.; Hamant, O.; Wittaik, S.M.; Morin, H.; Traas, J.; Pautot, V. KNAT6: An Arabidopsis homeobox gene involved in meristem activity and organ separation. Plant Cell 2006, 18, 1900–1907. [CrossRef]
9. Spinelli, S.V.; Martin, A.P.; Viola, I.L.; Gonzalez, D.H.; Palatinik, J.F. A Mechanistic link between STM and CLC1 during Arabidopsis development. Plant Physiol. 2011, 156, 1894–1904. [CrossRef]
10. Scofield, S.; Murison, A.; Jones, A.; Fozard, J.; Aida, M.; Band, L.R.; Bennett, M.; Murray, J.A.H. Coordination of meristem and boundary functions by transcription factors in the SHOOT MERISTEMLESS Regulatory Network. Development 2018, 145, 157081. [CrossRef] [PubMed]
11. Aida, M.; Tsubakimoto, Y.; Shimizu, S.; Ogisu, H.; Kamiya, M.; Iwamoto, R.; Takeda, S.; Karim, M.; Mizutani, M.; Lenhard, M.; et al. Establishment of the embryonic shoot meristem involves activation of two classes of genes with opposing functions for meristem activities. Int. J. Mol. Sci. 2020, 21, 5864. [CrossRef] [PubMed]
12. Laufs, P.; Peaucelle, A.; Morin, H.; Traas, J. MicroRNA Regulation of the CUC genes is required for boundary size control in Arabidopsis meristems. Development 2004, 131, 4311–4322. [CrossRef] [PubMed]
13. Mallory, A.C.; Reinhart, B.J.; Jones-Rhoades, M.W.; Tang, G.; Zamore, P.D.; Barton, M.K.; Bartel, D.P. MicroRNA Control of PHABULOSA in leaf development: Importance of pairing to the MicroRNA 5′ Region. EMBO J. 2004, 23, 3356–3364. [CrossRef] [PubMed]
14. Parcy, F.; Nilsson, O.; Busch, M.A.; Lee, I.; Weigel, D. A Genetic framework for floral patterning. Nature 1998, 395, 561–566. [CrossRef] [PubMed]
15. Wagner, D.; Sablowski, R.W.M.; Meyerowitz, E.M. Transcriptional activation of APETALA1 by LEAFY. Science 1999, 285, 582–584. [CrossRef] [PubMed]
16. Lohmann, J.U.; Hong, R.L.; Hobe, M.; Busch, M.A.; Parcy, F.; Simon, R.; Weigel, D. A Molecular link between stem cell regulation and floral patterning in Arabidopsis. Cell 2001, 105, 793–803. [CrossRef] [PubMed]
17. Chae, E.; Tan, Q.K.-G.; Hill, T.A.; Irish, V.F. An Arabidopsis F-Box Protein Acts as a transcriptional co-factor to regulate floral development. Development 2008, 135, 1235–1245. [CrossRef] [PubMed]
18. Jin, R.; Klasfeld, S.; Zhu, Y.; Fernandez Garcia, M.; Xiao, J.; Han, S.-K.; Konkol, A.; Wagner, D. LEAFY is a pioneer transcription factor and licenses cell reprogramming to floral fate. Nat. Commun. 2021, 12, 626. [CrossRef] [PubMed]
19. Lai, X.; Blanc-Mathieu, R.; GrandVuillemin, L.; Huang, Y.; Stigliani, A.; Lucas, J.; Thévenon, E.; Loue-Manifel, J.; Turchi, L.; Daher, H.; et al. The LEAFY floral regulator displays pioneer transcription factor properties. Mol. Plant 2021, 14, 829–837. [CrossRef] [PubMed]
20. Moyroud, E.; Kusters, E.; Moniaux, M.; Koes, R.; Parcy, F. LEAFY blossoms. Trends Plant Sci. 2010, 15, 346–352. [CrossRef] [PubMed]
21. Chahtane, H.; Vachon, G.; Le Masson, M.; Thévenon, E.; Périgon, S.; Milhajlovic, N.; Kalinina, A.; Michard, R.; Moyroud, E.; Moniaux, M.; et al. A Variant of LEAFY reveals its capacity to stimulate meristem development by inducing RAX1. Plant J. 2013, 74, 678–689. [CrossRef] [PubMed]
22. Li, W.; Zhou, Y.; Liu, X.; Yu, P.; Cohen, J.D.; Meyerowitz, E.M. LEAFY controls auxin response pathways in floral primordium formation. Sci. Signal. 2013, 6, ra23. [CrossRef] [PubMed]
23. Yamaguchi, N.; Wu, M.-F.; Winter, C.M.; Berns, M.C.; Nole-Wilson, S.; Yamaguchi, A.; Coupland, G.; Krizek, B.A.; Wagner, D. A molecular framework for auxin-mediated initiation of flower primordia. Dev. Cell 2013, 24, 271–282. [CrossRef] [PubMed]
24. Zhao, D. The ASK1 gene regulates B function gene expression in cooperation with UFO and LEAFY in Arabidopsis. Development 2001, 128, 2735–2748. [CrossRef]
25. Gagne, J.M.; Downes, B.P.; Shiu, S.-H.; Durski, A.M.; Vierstra, R.D. The F-Box subunit of the SCF E3 complex is encoded by a diverse superfamily of genes in Arabidopsis. Proc. Natl. Acad. Sci. USA 2002, 99, 11519–11524. [CrossRef] [PubMed]

26. Risseeuw, E.; Venglat, P.; Xiang, D.; Komendant, K.; Daskalchuk, T.; Babic, V.; Crosby, W.; Datla, R. An activated form of UFO alters leaf development and produces ectopic floral and inflorescence meristems. PLoS ONE 2013, 8, e83807. [CrossRef] [PubMed]

27. Yamaguchi, N.; Wu, M.-F.; Winter, C.; Wagner, D. LEAFY and polar auxin transport coordinately regulate Arabidopsis flower development. Plants 2014, 3, 251–265. [CrossRef]

28. Running, M.P.; Fletcher, J.C.; Meyerowitz, E.M. The WIGGLUM gene is required for proper regulation of floral meristem size in Arabidopsis. Development 1998, 125, 2545–2553. [CrossRef]

29. Chen, Q.; Atkinson, A.; Otsuga, D.; Christensen, T.; Reynolds, L.; Drews, G.N. The modifier of auxin and flower development (MOAF) gene is required for flower development. Development 1999, 126, 2715–2726. [CrossRef]

30. Sawa, S.; Watanabe, K.; Goto, K.; Kanaya, E.; Morita, E.H.; Okada, K. FILAMENTOUS FLOWER, a meristem and organ identity gene of Arabidopsis, encodes a protein with a zinc finger and hmgl-related domains. Genes Dev. 1999, 13, 1079–1088. [CrossRef]

31. Norberg, M.; Holmlund, M.; Nilsson, O. The PLATE OF PETIOLE acts redundantly to control the growth and development of lateral organs. Development 2005, 132, 2203–2213. [CrossRef] [PubMed]

32. Kanrar, S.; Onguka, O.; Smith, H.M.S. Arabidopsis inflorescence architecture requires the activities of KNOX-BELL homeodomain heterodimers. Planta 2006, 224, 1163–1173. [CrossRef] [PubMed]

33. Kanrar, S.; Bhattacharya, M.; Arthur, B.; Courtier, J.; Smith, H.M.S. Regulatory networks that function to specify flower meristems require the function of homeobox genes PENNYWISE and POUND-FOOLISH in Arabidopsis. Plant J. 2008, 54, 924–937. [CrossRef] [PubMed]

34. Keller, T.; Abbott, J.; Moritz, T.; Doerner, P. Arabidopsis REGULATOR OF AXILLARY MERISTEMS1 controls a leaf axil stem cell niche and modulates vegetative development. Plant Cell 2006, 18, 989–991. [CrossRef] [PubMed]

35. Müller, D.; Schmitz, G.; Theres, K. Blind homologous R2R3 myb genes control the pattern of lateral meristem initiation in Arabidopsis. Plant Cell 2006, 18, 586–597. [CrossRef] [PubMed]

36. Lee, D.J.; Park, J.Y.; Ku, S.-J.; Ha, Y.-M.; Kim, S.; Kim, M.D.; Oh, M.-H.; Kim, J. Genome-wide expression profiling of ARABIDOPSIS RESPONSE REGULATOR 7(ARR7) overexpression in cytokinin response. Mol. Genet. Genomics. 2007, 277, 115–137. [CrossRef]

37. Hareven, D.; Gutfinger, T.; Parnis, A.; Eshed, Y.; Lifschitz, E. The making of a compound leaf: Genetic manipulation of leaf architecture in tomato. Cell 1996, 84, 735–744. [CrossRef]

38. Bharathan, G.; Goliber, T.E.; Moore, C.; Kessler, S.; Pham, T.; Sinha, N.R. Homologies in leaf form inferred from KNOXI gene expression during development. Science 2002, 296, 1858–1860. [CrossRef]

39. Hay, A.; Tsiantis, M. The genetic basis for differences in leaf form between Arabidopsis thaliana and its wild relative Cardamine hirsuta. Nat. Genet. 2006, 38, 942–947. [CrossRef]

40. Shani, E.; Burko, Y.; Ben-Yaakov, L.; Berger, Y.; Amsellem, Z.; Goldshmidt, A.; Sharon, E.; Ori, N. Stage-specific regulation of Solanum lycopersicum leaf maturation by class 1 KNOSSEI LIKE HOMEBOX proteins. Plant Cell 2009, 21, 3078–3092. [CrossRef]

41. Hofer, J.; Turner, L.; Hellens, R.; Ambrose, M.; Matthews, P.; Michael, A.; Ellis, N. UNIFOLIATA regulates leaf and flower morphogenesis in Pea. Curr. Biol. 1997, 7, 581–587. [CrossRef]

42. Wang, H.; Chen, J.; Wen, J.; Tadege, M.; Li, G.; Liu, Y.; Mysore, K.S.; Ratet, P.; Chen, R. Control of compound leaf development by FLORICAULA/LEAFY ortholog SINGLE LEAFLET1 in Medicago truncatula. Plant Physiol. 2008, 146, 1759–1772. [CrossRef] [PubMed]

43. Hofer, J.; Gourlay, C.; Michael, A.; Ellis, T.H.N. Expression of a Class 1 KNOSSEI-like homeobox gene is down-regulated in pea compound leaf primordia. Plant Mol. Biol. 2001, 45, 387–398. [CrossRef]

44. Champagne, C.E.M.; Goliber, T.E.; Wojciechowski, M.F.; Mei, R.W.; Townsley, B.T.; Wang, K.; Paz, M.M.; Geeta, R.; Sinha, N.R. Compound leaf development and evolution in the legumes. Plant Cell 2007, 19, 3369–3378. [CrossRef] [PubMed]

45. Busch, A.; Gleissberg, S. EcFLO, a FLORICAULA-like gene from Eschscholzia californica is expressed during organogenesis at the vegetative shoot apex. Plant Cell 2003, 23, 841–848. [CrossRef] [PubMed]

46. Zhou, C.; Han, L.; Li, G.; Chai, M.; Fu, C.; Cheng, X.; Wen, J.; Tang, Y.; Wang, Z.-Y. STM/BLP-like KNOXI is uncoupled from ARP in the regulation of compound leaf development in Medicago truncatula. Plant Cell 2014, 26, 1464–1479. [CrossRef]

47. Taylor, S.; Hofer, J.; Murfet, I. Stamina pistilloida, the pea ortholog of Fim and UFO, is required for normal development of flowers, inflorescences, and leaves. Plant Cell 2001, 13, 31–46. [CrossRef] [PubMed]

48. Dong, Z.; Zhao, Z.; Liu, C.; Luo, J.; Yang, J.; Huang, W.; Hu, X.; Wang, T.L.; Luo, D. Floral patterning in Lotus japonicus. Plant Physiol. 2005, 137, 1272–1282. [CrossRef]

49. Nikolov, L.A.; Runions, A.; Das Gupta, M.; Tsiantis, M. Leaf development and evolution. Curr. Top. Dev. Biol. 2019, 131, 109–139.

50. Challa, K.R.; Rath, M.; Sharma, A.N.; Bajpai, A.K.; Davuluri, S.; Acharya, K.K.; Nath, U. Active suppression of leaflet emergence as a mechanism of simple leaf development. Nat. Plants 2021, 7, 1264–1275. [CrossRef]

51. Lincoln, C.; Long, J. A KNOSSEI-like homeobox gene in Arabidopsis is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants. Plant Cell 1994, 6, 1859–1876. [PubMed]

52. Pautot, V.; Docks, J.; Hamant, O.; Kronenberger, J.; Grandjean, O.; Jublot, D.; Traas, J. KNAT2: Evidence for a link between KNOSSEI-like genes and carpel development. Plant Cell 2001, 13, 1719–1734. [CrossRef] [PubMed]
Piazza, P.; Bailey, C.D.; Cartolano, M.; Krieger, J.; Cao, J.; Osowski, S.; Schneeberger, K.; He, F.; de Meaux, J.; Hall, N.; et al. *Arabidopsis thaliana* leaf form evolved via loss of KNOX expression in leaves in association with a selective sweep. *Curr. Biol.* 2010, 20, 2223–2228. [CrossRef] [PubMed]

Lee, I.; Wolfe, D.S.; Nilsson, O.; Weigel, D. A LEAFY co-regulator encoded by UNUSUAL FLORAL ORGANS. *Curr. Biol.* 1997, 7, 95–104. [CrossRef]

Blein, T.; Pulido, A.; Viallette-Guiraud, A.; Nikovics, K.; Morin, H.; Hay, A.; Johansen, I.E.; Tsiantis, M.; Laufs, P. A conserved molecular framework for compound leaf development. *Science* 2008, 322, 1835–1839. [CrossRef]

Bilsborough, G.D.; Runions, A.; Barkoulas, M.; Jenkins, H.W.; Hasson, A.; Galinha, C.; Laufs, P.; Hay, A.; Prusinkiewicz, P.; Tsiantis, M. Model for the regulation of *Arabidopsis thaliana* leaf margin development. *Proc. Natl. Acad. Sci. USA* 2011, 108, 3424–3429. [CrossRef]

Viallette-Guiraud, A.C.M.; Chauvet, A.; Gutierrez-Mazariegos, J.; Eschstruth, A.; Ratet, P.; Scutt, C.P. A conserved role for the NAM/MiR164 developmental module reveals a common mechanism underlining carpel margin fusion in monocarpous and syncarpous eurosids. *Front. Plant Sci.* 2016, 6, 1239. [CrossRef]

Cheng, X.; Peng, J.; Ma, J.; Tang, Y.; Chen, R.; Mysore, K.S.; Wen, J. NO APICAL MERISTEM (MtNAM) regulates floral organ identity and lateral organ separation in *Medicago truncatula*. *New Phytol.* 2012, 195, 71–84. [CrossRef]

Benlloch, R.; Navarro, C.; Beltrán, J.; Cañas, L.A. Floral development of the model legume *Medicago truncatula*: Ontogeny studies as a tool to better characterize homeotic mutations. *Sex. Plant Reprod.* 2003, 15, 231–241. [CrossRef]

Zhou, C.; Han, L.; Hou, C.; Metelli, A.; Qi, L.; Tadege, M.; Mysore, K.S.; Wang, Z.-Y.; Long, R.; et al. HEADLESS regulates auxin response and compound leaf morphology in *Medicago truncatula*. *Front. Plant Sci.* 2019, 10, 1024. [CrossRef]
77. Zhang, S.; Sandal, N.; Polowick, P.L.; Stiller, J.; Stougaard, J.; Fobert, P.R. Proliferating Floral Organs (Pfo), a Lotus japonicus gene required for specifying floral meristem determinacy and organ identity, encodes an F-Box Protein. *Plant J.* 2003, 33, 607–619. [CrossRef]

78. Roth, O.; Alvarez, J.P.; Levy, M.; Bowman, J.L.; Ori, N.; Shani, E. The KNOXI transcription factor SHOOT MERISTEMLESS regulates floral fate in *Arabidopsis*. *Plant Cell* 2018, 30, 1309–1321. [CrossRef]

79. Cosson, V.; Eschstruth, A.; Ratet, P. *Medicago truncatula* transformation using leaf explants. *Methods Mol. Biol.* 2015, 1223, 43–56. [CrossRef]

80. Kakar, K.; Wandrey, M.; Czechowski, T.; Gaertner, T.; Scheible, W.-R.; Stitt, M.; Torres-Jerez, I.; Xiao, Y.; Redman, J.C.; Wu, H.C.; et al. A community resource for high-throughput quantitative RT-PCR analysis of transcription factor gene expression in *Medicago truncatula*. *Plant Methods* 2008, 4, 18. [CrossRef]

81. Nikovics, K.; Blein, T.; Peaucelle, A.; Ishida, T.; Morin, H.; Aida, M.; Laufs, P. The balance between the MIR164A and CUC2 genes controls leaf margin serration in *Arabidopsis*. *Plant Cell* 2006, 18, 2929–2945. [CrossRef] [PubMed]

82. Ferrándiz, C.; Liljegren, S.J.; Yanofsky, M.F. Negative Regulation of the SHATTERPROOF genes by FRUITFULL during *Arabidopsis* fruit development. *Science* 2000, 289, 436–438. [CrossRef] [PubMed]