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RESEARCH PAPER

Co-ordinated regulation of flowering time, plant architecture and growth by *FASCICULATE*: the pepper orthologue of *SELF PRUNING*

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Abstract

Wild peppers (*Capsicum* spp.) are either annual or perennial in their native habitat and their shoot architecture is dictated by their sympodial growth habit. To study shoot architecture in pepper, sympodial development is described in wild type and in the classical recessive *fasciculate* (*fa*) mutation. The basic sympodial unit in wild-type pepper comprises two leaves and a single terminal flower. *fasciculate* plants are characterized by the formation of floral clusters separated by short internodes and miniature leaves and by early flowering. Developmental analysis of these clusters revealed shorter sympodial units and, often, precocious termination prior to sympodial leaf formation. *fa* was mapped to pepper chromosome 6, in a region corresponding to the tomato *SELF-PRUNING* (*SP*) locus, the homologue of *TFL1* of *Arabidopsis*. Sequence comparison between wild-type and *fa* plants revealed a duplication of the second exon in the mutants' orthologue of *SP*, leading to the formation of a premature stop codon. Ectopic expression of *FASCICULATE* complemented the *Arabidopsis tfl1* mutant plants and as expected, stimulated late flowering. In agreement with the major effect of *FASCICULATE* imposed on sympodial development, the gene transcripts were localized to the centre of sympodial shoots but could not be detected in the primary shoot. The wide range of pleiotropic effects on plant architecture mediated by a single 'flowering' gene, suggests that it is used to co-ordinate many developmental events, and thus may underlie some of the widespread variation in the Solanaceae shoot architecture.

Key words: *FASCICULATE*, flowering time, pepper, plant architecture, *SELF PRUNING*, sympodial development.

Introduction

The overall plant architecture is the sum of many physiological and genetic pathways giving rise to a unique morphological appearance of each and every species (Sussex and Kerk, 2001). In the vegetative phase, plant architecture can be divided into primary components such as phylotactic patterns, leaf shape, length of internodes, and by the relative strength of apical dominance. In the reproductive phase, architecture is determined by inflorescence position, composition, timing of release of apical dominance, growth habit (sympodial versus monopodial growth), and the number of internodes in the sympodial units. Architecture regulation represents an important component of plant development and has a major impact on the agronomic performance of agricultural plants. A notable change in plant architecture, i.e. the use of semi-dwarf wheat and rice varieties allowed a dramatic increase in yields known as the green revolution (Peng *et al.*, 1999). In recent years, the genetic and molecular bases of plant architecture components began to unravel by the identification of genes regulating internode length, apical dominance and branching, floral transition and growth habit (reviewed by Reinhardt and Kuhlemeier, 2002; Wang and Li, 2006).

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The growth habit of Solanaceae plants is characterized by sympodial development in which the shoot apical meristem terminates by a flower or an inflorescence and further development continues from the upper most axillary meristems. This growth pattern is referred to as sympodial or 'determinate', in contrast with the monopodial or ''indeterminate'' growth in plants such as *Arabidopsis* and *Antirrhinum*, where the development of the shoot apical meristem is maintained throughout the entire life span of the plant. In tomato, a gradual reduction in the length of the sympodial units is caused by a mutation in a single gene, *SELF PRUNING* (*SP*). This mutation causes a dramatic change in plant architecture giving rise to a small bushy plant relative to the normal vine habit tomatoes. While in wild-type tomato, three leaves separate adjacent inflorescences, in *sp* plants the number of leaves per successive sympodial units is gradually reduced until no leaf is produced and growth 'terminates' (Pnueli *et al.*, 1998). This growth habit facilitates mechanical harvest on which the entire processing tomato industry is based.

SP was identified as a homologue of *TERMINAL FLOWER1* (*TFL1*) and *CENTRORADIALIS* (*CEN*), which control inflorescence architecture in *Arabidopsis* and *Antirrhinum*, respectively (Pnueli *et al.*, 1998). For both *TFL1* and *CEN*, recessive mutations result in the conversion of the indeterminate shoot into a determinate flower (Bradley *et al.*, 1996, 1997). In addition to controlling regulation of meristem function, *TFL1* also has a role in the repression of initial flowering (Ratcliffe *et al.*, 1998). In pea, another plant with a sympodial habit, two different *TFL1* homologues were isolated and mapped to distinct mutations that affect apical meristem development (*DETERMINATE*) and flowering time (*LATE FLOWERING*) (Foucher *et al.*, 2003). Therefore, the dual functionality of *TFL1* in *Arabidopsis* may be separated into distinct functions in different members of the gene family of pea. *TFL1* shares homology with mammalian phosphatidylethanolamine binding proteins. These proteins are likely to have a role in signal transduction; however, their precise biological function is still unknown. Yeast-two hybrid screens identified several proteins that interact with *SP* (Pnueli *et al.*, 2001). One of these proteins, a bZIP G-Box was found later to be the homologue of *Arabidopsis* flowering time regulator *FD* (Abe *et al.*, 2005; Wigge *et al.*, 2005).

TFL1 is a member of a small gene family in *Arabidopsis* that includes *FLOWERING LOCUS T* (*FT*) whose function is antagonistic to *TFL1* as it promotes flowering (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999; Hanzawa *et al.*, 2005). *FT* is a major integrator of several flowering-promoting pathways as it is activated by the long-day photoperiod, vernalization, and autonomous pathways (reviewed by Jack, 2004). The rice *FT* orthologue corresponds to the heading date QTL *Hd3a* that promotes flowering in short-day conditions (Kojima *et al.*, 2002). In tomato, *SP* is also a member of a small gene family that includes at least five other members (Carmel-Goren *et al.*, 2003). *SP3D* from this family, the orthologue of *FT*, was shown to encode a florigen precursor and the gene is mutated in the late flowering and shoot architecture *single flower truss* (*sft*) plants (Lifschitz *et al.*, 2006). On the basis of their phenotypic interaction, it was hypothesized that the ratio of *SFT*/*SP* regulates vegetative to reproductive transitions in tomato (Lifschitz and Eshed, 2006).

The present model for the *TFL1* role in the inhibition of flowering is via negative regulation of the floral meristem identity gene *LFY* (Liljegren *et al.*, 1999). *LFY*, in turn, represses *TFL1* activity in flower meristems (Parcy *et al.*, 2002). Similarly, *TFL1* negatively regulates *AP1* and the two genes are expressed in non-overlapping patterns; *AP1*, in turn, mutually represses *TFL1* (Liljegren *et al.*, 1999). The direct regulation of *TFL1* is poorly understood: it is not known whether *LFY* directly binds to its promoter or acts through an intermediate factor. However, the fact that both genes are expressed in the same cells in tomato, suggests that direct transcriptional repression is not the only mode of action (Pnueli *et al.*, 1998). Recently it was suggested that TFL1 protein is mobile within the shoot meristem and that this movement is indirectly regulated by LFY (Conti and Bradley, 2007).

Pepper (*Capsicum* spp.) is a member of the Solanaceae family and is a close relative to tomato. However, unlike tomato, for which ample information exists on the development of the shoot and architectural mutants are available, pepper architecture is poorly documented. The general branching pattern in the reproductive phase of *Capsicum* was described by Child (1979). However, to our knowledge, no detailed characterization of the sympodial development in *Capsicum* has been reported. Still, several branching mutants were described, such as one that controls branching of lateral axillaries prior to the first bifurcation (Bergh and Lippert, 1975). Even earlier, inheritance of the fruit clustering syndrome was determined to be controlled by a single recessive gene (Barrios and Mosokar, 1972; Deshpande, 1944).

In this paper, the shoot architecture and sympodial development in wild-type pepper as well as in the *fasciculate* (*fa*) mutation, which is characterized by the formation of clusters of flowers and fruits and compact 'determinate' plant architecture (Daskalov and Poulos, 1994) are described. The *fa* mutation is utilized for ornamental peppers but can also be utilized in breeding fresh-market and processing peppers by creating an ideotype with concentrated fruit setting suitable for mechanical harvest (Poulos, 1994). It is next shown, by mapping and allele sequencing, that *FA* is encoded by the pepper orthologue of *SP*. Furthermore, by means of ectopic expression of *FASCICULATE* in *Arabidopsis*, it is shown that it is functionally similar to *TFL1*. Recently, the pepper homologue of *SP* was isolated by Kim *et al.* (2006) and was shown to be mutated in a determinate line, most likely a *fasciculate* mutant. The present paper describes new data on the phenotypic and molecular characterization of *fasciculate* that implicate the importance of *FASCICULATE* on determining pepper shoot architecture. The study of plants such as pepper that have distinct architecture from most other model plant

species allows further understanding of the mechanisms by which the diversification of plant architecture occurs.

Materials and methods

Plant material and traits measurements

A *C. annuum* accession, 5219 that carries the *fa* mutation was obtained from Dr C Shifriss, The Volcani Institute, Israel. An F_2 mapping population consisting of 244 plants was constructed by crossing 5219 with the *C. frutescens* wild-type accession BG 2816. The population was grown in the greenhouse in the Volcani Center during the winter of 2003 and used to harvest leaves for DNA extraction and for scoring of the *fasciculate* phenotype. F_2 plants were recorded as having the wild-type or mutant phenotype by having a single flower per node or a cluster of flowers, respectively. Additional phenotypic measurements taken at the red mature fruit stage included the number of leaves on the main stem to first flower, height of the main stem to first bifurcation, total height of the plant from its most basal point to the top, length of the internodes in first three sympodial units, and weight and total soluble solids of five fruits (Ben Chaim *et al.*, 2001).

In order to quantify the relation between internode length and leaf size, both parameters were measured in the two shoots that branch in one sympodium, in five randomly selected pairs (starting from the third unit), taken from five independent wild-type plants (a total of 100 sympodial measurements). Internode length (cm) was measured by a ruler and leaf area (in cm^2) was determined from scanned leaf images by the Image Gauge v3.3 software (Fuji). Correlation coefficients between internode and leaf growth using data across all plants were calculated using the ratios of the two internodes (short/long) and the two leaves (small/large) measurements in each sympodial unit.

Mapping and data analyses

The tomato *SP* gene (obtained from Professor Dani Zamir, The Hebrew University of Jerusalem) was mapped in pepper as an RFLP probe using *Bcl*I polymorphism between the parents of the mapping population. Procedures for RFLP analysis and genetic mapping were described by Ben Chaim *et al.* (2001). To determine the effect of the allelic state at the *FA* locus on the measured traits in the F_2 population, one-way analysis of variance ($P \leqslant 0.05$) was used to contrast the means of the three genotypic classes based on the genotype of *SP* for each trait by JMP v.3 software (SAS Institute, 1994).

Scanning electron microscopy

Tissue was fixed, osmium-treated, and critically point dried as previously described by Alvarez *et al.* (1992). Scanning electron microscopy was performed on a Hitachi S-3500N SEM. Digital images were captured at 5 kV and assembled in Adobe Photoshop.

Isolation of the FASCICULATE *gene*

To isolate *FASCICULATE*, primers were used from its tomato homologue *SP* (GenBank Accession no. U84140); SP-F: 5′-GTGAACCCCTTGTGATTGGT-3′ located in the first exon and SP-R: 5′-GTTTCCCTCTGGCAATTGAA-3′ located in the fourth exon and used them to amplify the corresponding partial gene from pepper genomic DNA of *C. frutescens* BG 2816 by PCR. A fragment of 2312 bp was cloned into the pDrive vector (Qiagen) and sequenced. All sequences were determined in The Center for Genomic Technologies, The Hebrew University of Jerusalem. The partial *FASCICULATE* gene was used to screen a bacterial artificial chromosome (BAC) library of pepper constructed from *C. frutescens* BG 2816 (J Vrebalov and J Giovannoni, unpublished data) available from the Arizona Genomics Institute (http://www.genome.arizona.edu/orders/). Four positive clones were identified, of which, clone 121 B1 was used as a template for extending the sequence of *FASCICULATE* to the 5′ and 3′ regions. Based on the genomic sequence, the open reading frame (ORF) of *FASCICULATE* was amplified by RT-PCR using RNA extracted from the apical meristem of the primary shoot of the wild-type parent BG 2816 at the stage of six leaves using the primers FAORF-F: 5′-ATGGCTTCGAAAATGTGTGAACC-3′ and FAORF-R: 5′-ACTAAACCCGAAAAACAACAG-5′. For first-strand cDNA synthesis, total RNA extracted with the RNeasy plant mini kit (Qiagen) was used. The RNA was reverse-transcribed with AMV reverse transcriptase (CHIMERx) using random primers. Primers SP-F/R were also used to amplify the recessive allele of *FASCICULATE* using genomic DNA and RNA from the mutant parent 5219. The *FASCICULATE* cDNA from BG 2816 as well as cDNA and genomic DNA from 5219 were cloned and sequenced.

In situ *hybridization*

Samples were fixed and sectioned according to standard protocols (Szymkowiak and Irish, 2005). Antisense RNA probes, labelled by digoxigenin, were generated from the 5′ ends of cDNA clones of *FASCICULATE* and the pepper orthologue of *LFY* using T7 RNA Polymerase. Hybridization, washes, and detection were performed according to standard *in situ* hybridization techniques (Szymkowiak and Irish, 2005).

Ectopic expression of FASCICULATE *in* Arabidopsis

The ORF of *FASCICULATE* was subcloned into the *Bam*HI and *Xba*I sites of pART7 downstream of the CaMV 35S promoter. Subsequently, the *35S:FA* fragment was cloned into the *Not*I site of the binary vector BART. The resultant clone was transformed into the *Agrobacterium tumefaciens* strain Agro ASE by electroporation. Transformation of wild-type *Arabidopsis* (Landsberg *erecta*) and the *tfl1-2* mutant was done by the floral dip method. Transformed seeds were planted in flats and selected by spraying with BASTA. Detection of *35S:FA* transgenes was

done by PCR with genomic DNA using 35S forward and *FA* reverse primers.

GenBank Accession numbers for *FASCICULATE* are FJ042775 (wild type) and FJ042776 (mutant).

Results

Shoot development in wild type and fasciculate

Wild-type pepper shoots typically produce 8–15 leaves on the main stem before termination with a single flower. Branching of the main shoot results from release of two to three sympodial shoots from the axils of the leaves preceding the flower. Each sympodial shoot consists of two leaves and a single terminal flower. The leaves subtending the sympodium are 'carried up' by the dramatic elongation of the stem internode below the sympodial unit and are placed above the preceding flower (Fig. 1A, B). Within the sympodial unit, the two opposite leaves appear to emerge almost simultaneously and new sympodial shoots emerge from their axils shortly after their initiation. Here too, elongation of the internode between the leaf and the shoot 'push' the leaf above the terminal flower of the same sympodium. This cycle of development repeats itself and can theoretically continue for an indefinite period of time. However, in many large-fruited plants, flowers develop in a few nodes only, and further growth is repressed.

fasciculate plants form a cluster of flowers after the termination of the main stem instead of the solitary flower found in a wild-type plant. Further sympodial development is suppressed and the typical continuous dichotomous branching is considerably reduced (Fig. 1C, D). After termination of the main stem, lower lateral shoots are released from apical dominance, and these too produce clusters of flowers as in the main stem. The growth suppression of *fasciculate* plants results in compact 'determinate' plants.

Examination of initiating sympodial shoots of wild-type plants using scanning electron microscopy could not differentiate the initiation of its components. The two leaves and the apical flower, all seem to initiate simultaneously (Fig. 2A-C). In *fa* plants, the same basic structure of the sympodial unit remained unchanged compared with the wild type, however, the leaf primordia were considerably smaller than in the wild type (Fig. 2D). Occasionally, undeveloped leaf primordia were observed, and no new sympodial shoots were produced (Fig. 2D, E). The progressive shortening of internodes within sympodial shoots, and the failure to initiate some of them, resulted in the clustering of small leaves and flowers and the 'determinate' structure of the mutant architecture. Occasionally, a lateral shoot initiated from one

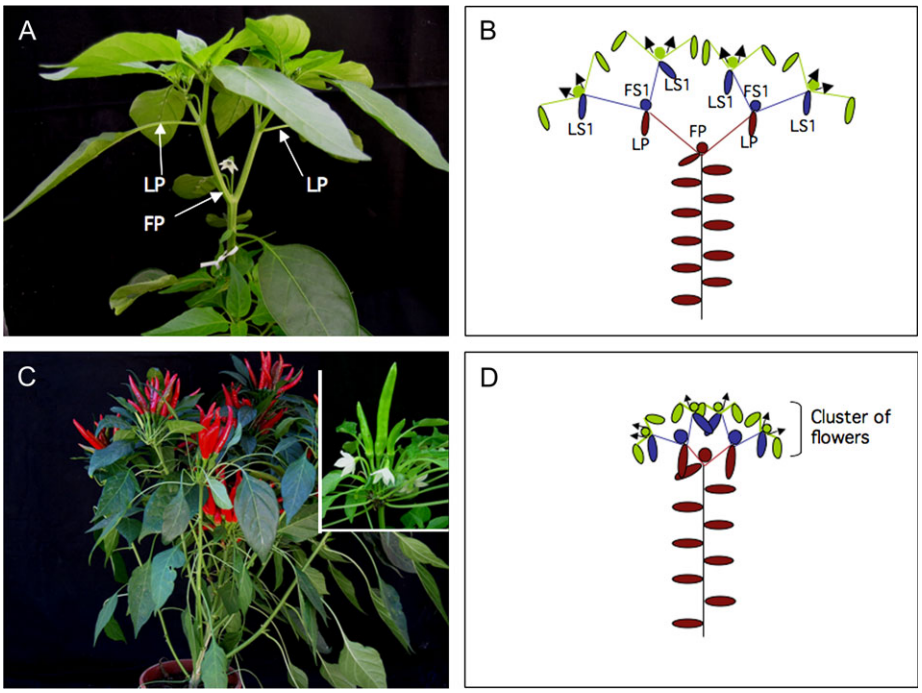

Fig. 1. Phenotypes of wild-type and *fasciculate* plants. (A) Wild-type plant forming a single flower after the termination of the main stem. At the first bifurcation, the flower (FP, flower of primary shoot) and the first two leaves (LP, leaf of primary shoot) are originated from the primary shoot. (B) Schematic drawing of a wild-type plant. Each sympodial unit composed of two leaves (oval shape) and a flower (circle) is presented in a different colour. Subsequent to the first bifurcation, the next flower (FS1, flower of sympodial shoot1) and the two leaves (LS1, leaf of sympodial shoot1) form the first sympodial unit. (C) Mature *fasciculate* plant. *fasciculate* cluster consists of flowers and fruits at different developmental stages is inserted in the upper right corner. (D) Schematic drawing of a single *fasciculate* shoot. The basic structure of the sympodial unit remains the same as in the wild type. Clusters of flowers result from reduced internodes within the sympodium. Small black arrows indicate sympodial meristems.

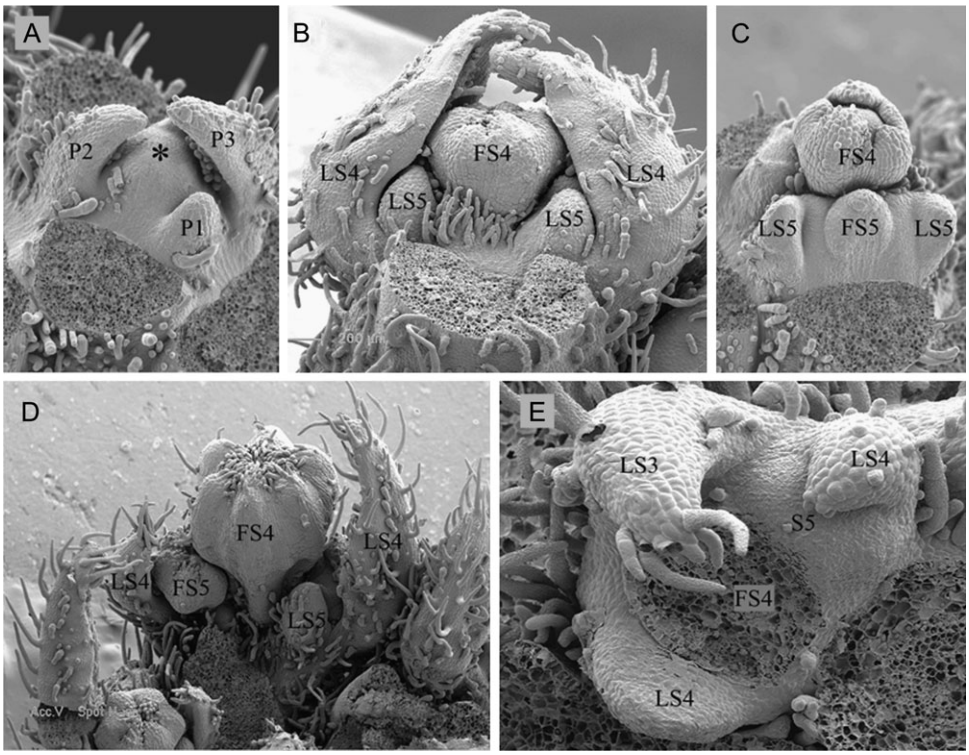

Fig. 2. Scanning electron micrographs of apical meristems and sympodial units of wild type (A–C) and *fasciculate* (D, E) plants. (A) Vegetative shoot apical meristem (SAM). The SAM is indicated by an asterisk. Leaf primordia are marked by P. (B) The wild-type meristem produces a terminal flower (FS4) and two equally developing leaves (LS4). Leaf primordia of the next sympodial units (LS5) are developed in the axils of the preceding leaves. (C) The younger sympodial unit is composed of a single terminal flower (FS5) and two leaf primordia (LS5). (D) The *fasciculate* meristem consists of a single terminal flower (FS4) and two leaves (LS4), however, the left leaf is much smaller than the right one. The younger sympodial unit developed in the axil of the left leaf (LS4) consists of a flower (FS5) without leaf. (E) The next sympodial unit (S5) is observed in the axil of the right leaf (LS4) but not in the axil of the undeveloped left leaf.

of the compact sympodial cluster, and generated 7–10 leaves topped again by clustered flowers.

In *fasciculate*, a concomitant reduction in leaf size and internode length occurs during sympodial development. While the average leaf size in line 5219 prior to sympodial development (at the base of the cluster) is 14.7 ± 1 cm^2, there is a considerable reduction in leaf size to an average of 6.5 ± 1.2 cm^2 within the compact cluster of fruits. This relationship between internode length and leaf size is not restricted to *fasciculate* plants, but also exists in wild-type ones. Except for the first one or two branching points, for which the two shoots of the sympodial unit develop approximately equally, at each further branching point, the two shoots develop asymmetrically, i.e. the growth of one shoot is greater than the other (Fig. 3A). The mean internode length in the small and large shoot pairs at each sympodial unit across genotypes was 4.6 ± 0.2 cm and 8.5 ± 0.2 cm, respectively. Similarly, the mean leaf area in the small and large shoot at each sympodial pair across genotypes was 12.4 ± 1.1 cm^2 and 16.9 ± 1.1 cm^2, respectively. There was a high positive correlation between internode growth and leaf growth (r=0.77; Fig. 3B).

The 'determinate' growth habit and altered plant architecture of *fa* mutants, promoted examination of a possible association with the *SP* gene that controls similar traits in tomato (Pnueli *et al.*, 1998). An F_2 population from a cross of the mutant line 5219 (*fa*) and the wild-type accession BG 2816 (*FA*) was scored for the *fa* mutation and compared with the segregation of *SP*. The *fa* mutation segregated as a single recessive gene as expected (Chi square=0.1, P=0.75 for an expected ratio of 3:1). Southern blot under high stringency conditions and RFLP analyses indicated that *SP* recognizes a single copy gene that completely co-segregated with the *FA* locus. The *FA* locus was mapped in chromosome 6 of pepper in the syntenic region of tomato containing *SP*.

In order to compare growth characteristics of *fa* to wild type and in the absence of isogenic material for *fasciculate*, F_2 progenies of the above mapping population were measured and evaluated. All plants were subjected to RFLP analysis with *SP* and the phenotypic means of the three genotypic classes were contrasted. While homozygous recessive mutant plants produced the first flower after 15.7 ± 0.6 leaves, heterozygous and homozygous wild-type plants flowered after 17.7 ± 0.6 and 20.3 ± 0.6 leaves, respectively (Fig. 4A). As a result, the height of mutant plants was significantly shorter than wild-type plants giving rise to a more compact growth of the mutant. The height of the main stem until first flower was 25.1 ± 1 cm for homozygous mutants, 26.8 ± 0.9 cm for heterozygotes, and 31.1 ± 1 cm for

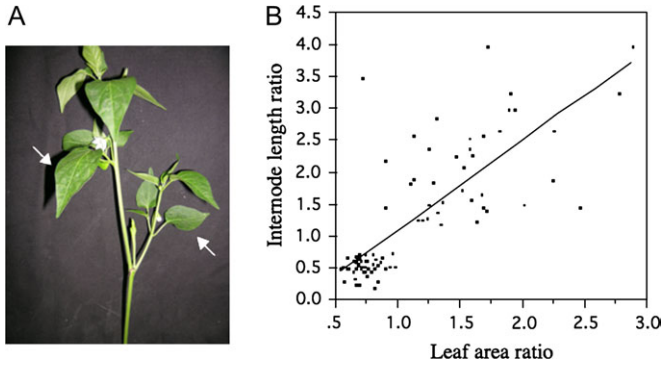

Fig. 3. Asymetric sympodial development in wild-type pepper. (A) Sympodial shoot showing short and long internodes and the corresponding small and large leaves (indicated by arrows). (B) The correlation plot between the ratios of internode length and leaf area measured in pair of shoots developed at the same sympodial unit.

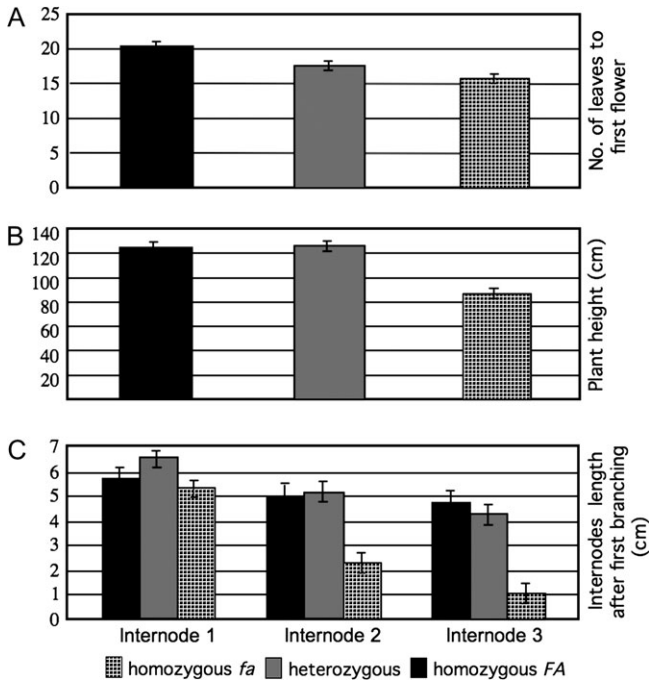

Fig. 4. Phenotypic characterization of *fasciculate* plants. Phenotypic measurements for each genotype were taken in F_2 plants based on the RFLP pattern at the *FA* locus. (A) Number of leaves on the main stem before flowering. (B) Total plant height. (C) Internodes length in the first three sympodial units after first branching. Standard errors are presented as bars.

homozygous wild type. Therefore, *FA* exerted its effect on both flowering time and stem height in a co-dominant manner as the three genotypic classes were significantly different from each other.

The average length of the internodes on the main stem was similar in both mutant and wild-type plants. Therefore, the reduced stem length was attributed to a reduced number of internodes and not to reduction in their size. The total plant height of homozygous *fa* plants was 84.7±4.2 cm, compared to 126.8±3.7 cm and 125.3±4.2 cm for heterozygotes and homozygous *FA* plants, respectively (Fig. 4B). The length of the internodes in the first sympodial unit was similar in both mutant and wild-type plants (approximately 5 cm). However, starting from the second sympodial unit, the length of the internodes in mutant plants (2.2±0.4 cm and 1.0±0.4 cm for the second and third sympodial nodes, respectively) progressively decreased compared to wild-type ones (4.9±0.5 cm and 4.7±0.5 cm for the second and third sympodial nodes of homozygous wild-type plants) (Fig. 4C). No significant differences in the length of the internodes were detected between the heterozygous and homozygous wild-type plants. No significant differences among the three genotypic classes at *SP* were observed for either fruit size or for total soluble solids (data not shown).

Isolation of FASCICULATE *and examination of the molecular basis of the* fa *mutation*

The pepper orthologue of *SP* was isolated from the wild-type *C. frutescens* accession BG 2816 as described in the Materials and methods. The ORF of *FA* is composed of 528 bp and it shares the highest homology to tomato *SP* (93% amino acid identity). Phylogenetic analysis of GenBank FA-related proteins indicated that FA is more closely related to SP and to the homologous non-Solanaceae proteins TFL1 and CEN than to other members of the tomato SP family whose function except for SFT, is not presently known (data not shown). Comparison of the ORF and the genomic sequence of *FA* revealed the presence of four exons in the gene, identical in size to the four exons of the tomato *SP* gene (Carmel-Goren *et al.*, 2003). PCR amplification of *FA* using genomic DNA from several wild-type lines representing three *Capsicum* species and from 12 *C. annuum fa* mutants whose origin is not known (kindly provided by C Shifriss, The Volcani Center, Israel) revealed a similar size difference of the amplified fragment that differentiates all the wild-type and mutant lines (Fig. 5A), indicating the common occurrence of an insertion in the *fa* mutants.

RT-PCR amplification using RNA extracted from apical meristems of plants 4 weeks after germination from BG 2816 and 5219 revealed an insertion of approximately 60 bp in the cDNA of the *fa* mutant (Fig. 5B). Sequence comparison of genomic DNA from BG 2816 and 5219 identified a duplication of 858 bp in the *fa* mutant that contains part of intron 1, exon 2, and part of intron 2. Comparing the sequences of the cDNA clones from both parents identified a duplication of exon 2 in 5219 that created a premature stop codon in the junction of the two exons (Fig. 5C). The mutation results in the formation of a truncated protein of 88 amino acids compared to 174 amino acids in the intact one.

Expression of FA *and* CaLFY

Using *in situ* hybridization of *FA*, no hybridization signal was detected at the vegetative apical meristem of young seedlings with two leaves. However, expression was detected

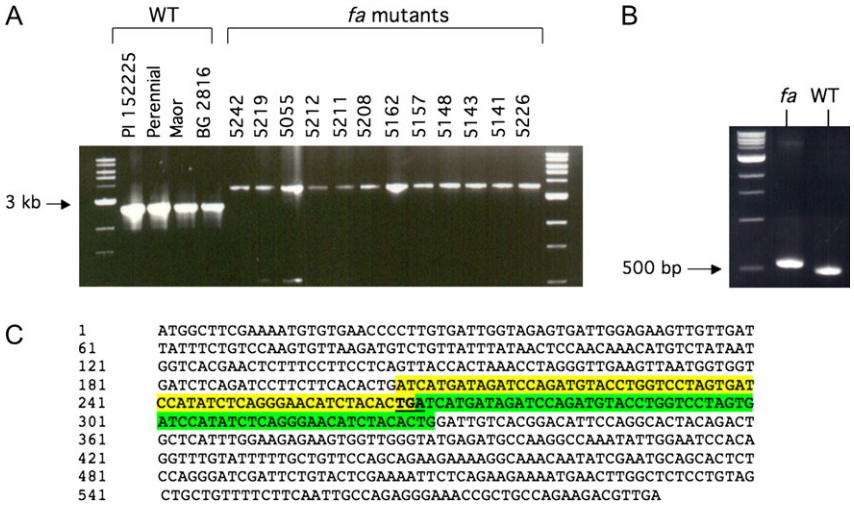

Fig. 5. The molecular nature of *fasciculate*. (A) PCR amplification of *FASCICULATE* using genomic DNA from wild-type (WT) and mutant (*fa*) plants. (B) RT-PCR using cDNA from wild-type (WT) and mutant (*fa*) plants. (C) Nucelotide sequence of *FA* cDNA from *fasciculate* mutant. The duplicated exon#2 is colored by yellow (first repeat) and green (second repeat). The stop codon created at the junction of the duplication is marked by bold and is underlined.

at this stage using RT-PCR, indicating that the gene is expressed albeit at a low level, or in a dispersed manner (data not shown). After flower initiation, clear expression foci were detected in subapical cells of the sympodial meristem and in axillary meristems of the primary shoot (Fig. 6A, B). No signal was detected in the flower. Because of the role of *LFY* in determining inflorescence architecture and promoting flowering in *Arabidopsis* (Liljegren *et al.*, 1999), the spatial expression of its homologue in pepper (*CaLFY*) was determined, as compared with *FA*. A partial sequence of *CaLFY* was isolated by PCR amplification of cDNA from the shoot apex using primers from the tomato *LFY* homologue (GenBank accession AF197934). *In situ* hybridization with *CaLFY* indicated that the gene is expressed at the apical dome of the vegetative meristem of young seedlings as well as in the provascular bundle of the leaf primordia (Fig. 6C). After induction of flowering, *CaLFY* is expressed in the flower as well as in the sympodial meristems (Fig. 6D).

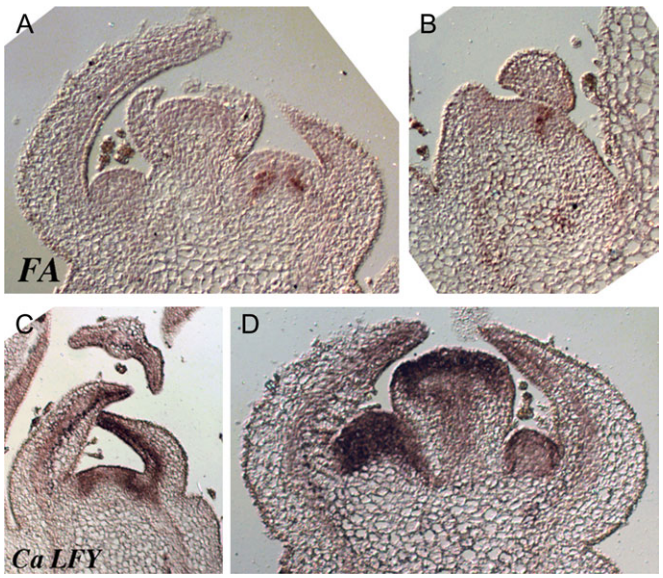

Fig. 6. Expression of *FA* and *CaLFY*. (A, B) *In situ* localization of *FA* transcripts in wild-type plants. (A) Longitudinal section of the apex in seedlings with six true leaves. Expression is detected in the sympodial meristem but not in the flower. (B) Longitudinal section of the axillary meristem in seedlings with six true leaves. (C, D) *In situ* localization of *CaLFY* transcripts in wild-type plants. (C) Longitudinal section of the apex in seedlings with two true leaves. Expression is detected in the vegetative apical meristem and in the leaf primordia. (D) Longitudinal section of the apex in seedlings with six true leaves. Expression is detected in the sympodial meristems and in the flower.

Complementation of Arabidopsis tfl1-2 *by ectopic expression of* FA

In order to test whether the cloned *FA* encodes for functional protein, it was introduced into wild-type and *tfl1 Arabidopsis* under the control of the 35S promoter. A total of 17 *35S*:*FA* Landsberg *erecta* plants in the wild-type background (six primary transformants and 11 T_2 plants resulted from three primary transformants) were examined. An increased vegetative phase and delayed flowering was observed in all transformed plants compared to wild type, indicating a role for *FA* in the repression of flowering. While untransformed wild-type plants had, on average, eight rosette leaves and three cauline leaves before flowering, *35S*:*FA* plants in the wild-type background had, on average, 12 rosette leaves and eight cauline leaves before flowering. The flowers of 35S:*FA* transgenic plants had a proliferation of additional flower buds within them. Similarly, plants ectopically expressing *35S*:*FA* in a *tfl1* background (three primary transformants and 13 T_2 plants) had, on average, 12 rosette leaves and eight cauline leaves

before flowering, compared with five rosette leaves and two cauline leaves of the untransformed *tfl1* mutants (Fig. 7A–C). Ectopic expression of *FA* in *tfl1* complemented the mutant phenotype, as the determinate inflorescence of *tfl1* was converted to an indeterminate one as in wild-type *Arabidopsis* (Fig. 7D–F).

Discussion

Higher plants display large variation in their growth pattern which is manifested among others by patterns of branching, and leaf and inflorescence structure. The genetic control of plant architecture has been studied in detail in a limited number of model species such as *Arabidopsis*, *Antirrhinum*, tomato, and petunia (Angenent *et al.*, 2005; Wang and Li, 2006; Prusinkiewicz *et al.*, 2007; Quinet and Kinet, 2007). An emerging conclusion from these studies is that, although many of the genes dictating architecture are common to different plant species, their function is commonly modified in each plant species. Therefore, while the model species are imperative for describing basic models of architecture and for studying developmental pathways, in order to understand plant diversity further it is necessary to compare these models and gene functions in less explored species with divergent architectures.

FASCICULATE *is a major determinant of pepper sympodial development*

In the present study, a description of sympodial development in wild-type and *fasciculate* peppers is provided and there is evidence that *FASCICULATE* has a major impact on pepper architecture. Although the *fasciculate* mutation has been known for a long time in pepper and numerous *fasciculate* varieties, mostly ornamentals, exist in *Capsicum*, reports on its phenotypic characterization are not available. Three main characteristics differentiate *fasciculate* from wild-type peppers: reduction of flowering time, reduction of the length of the internodes in the sympodial units, and inhibition of leaf growth during sympodial development. The combined effect of these characteristics is the appearance of a compact plant architecture and concentrated flower and fruit setting. Although *FASCICULATE* functions during the vegetative phase as a repressor of flowering, its prime function is in the regulation of flowering of the sympodial shoot.

FASCICULATE *is the orthologue of* CEN *and* SP

Gene mapping, allele sequencing, phylogenetic relationships, and phenotypic complementation of *Arabidopsis* homologous mutation, all collectively indicate that the pepper *FA* gene is the orthologue of *CEN* and *SP*. The mapping of *FA* to chromosome 6 in the same genomic region containing *SP* in tomato agrees with the overall good syntenic relationships of pepper and tomato chromosomes (Livingstone *et al.*, 1999). Compared with *SP* that belongs to a small gene family, *FA* was detected as a single copy gene in the pepper genome based on Southern blot analysis. Reduction in the stringency of the hybridization will possibly reveal additional members of the *FA* family. Recently, the pepper homologue of *SP* was isolated and its sequence was shown to differ in an insertion of one nucleotide in a determinate line compared to wild type, resulting in a putative truncated protein in the determinate line (Kim *et al.*, 2006). Based on the description of the determinate plants (flowered earlier than wild type and had clusters of flowers and fruits), it can be assumed with confidence that the determinate line is a *fasciculate* mutant. The availability of two independent *fasciculate* mutations in *FA* that result in putative truncated protein excludes the possibility that the truncated protein may still be functional. Furthermore, the availability of two independent *fasciculate*

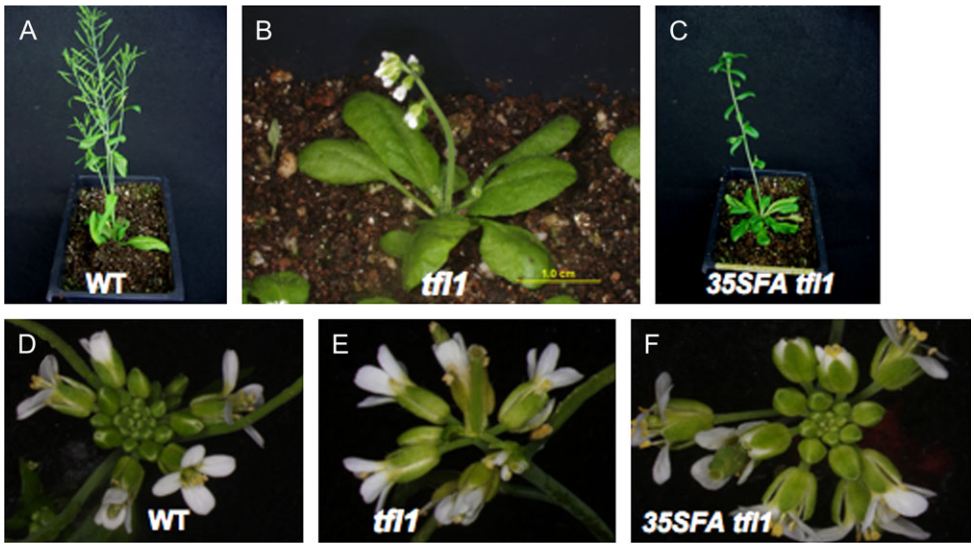

Fig. 7. Ectopic expression of *FA* in *tfl1* of *Arabidopsis*. (A) Wild type. (B) *tfl1* mutant. (C) *tfl1* plants expressing *35S:FA*. (D) Inflorescence of wild type. (E) Inflorescence of *tfl1*. (F) Inflorescence of *tfl1* expressing *35S:FA*.

mutations also excludes the possibility that the effect on flowering time and determinate growth habit may be controlled by two independent closely linked loci.

Ectopic expression of *FA* in *Arabidopsis* resulted in similar phenotypes to those reported in other studies in which *TFL1* or its homologues were over-expressed in *Arabidopsis* (Ratcliffe *et al.*, 1998; Nakagawa *et al.*, 2003; Pillitteri *et al.*, 2004). The emergence of new inflorescences within flowers in transgenic *Arabidopsis* overexpressing *FA* was also reported by Boss *et al.* (2006) who overexpressed the *TFL1*-homologue from grapevine in *Arabidopsis*. As expected from its role in the suppression of flowering, over-expression of *FA* resulted in increased vegetative growth. However, the increased branching reported in *35S:TFL1* plants by Ratcliffe *et al.* (1988) was not observed in the present study. The phenotypic rescue of the *tfl1* mutation indicated that the function of *FA* is conserved to *Arabidopsis TFL1*. These results agree with the broad conservation of *TFL1* homologues in determining shoot architecture in sympodial and monopodial plant systems in both dicot and monocot plants.

Expression of genes that determine the transition of the shoot from the vegetative to the reproductive phase

Functional analyses of *fa* mutant plants uncovered pleiotropic functions for the gene product in primary and sympodial flowering, internode length and leaf growth. However, the expression pattern of *FA* as detected by RNA *in situ* hybridization resembles that of *CET2* and *CET4*, the tobacco *TFL1* homologues (Amaya *et al.*, 1999): expression of both genes is detected in vegetative axillary meristems. In addition, expression of *FA* is detected in subapical cells of the sympodial meristem similar to the spatial expression pattern of *TFL1* (Bradley *et al.*, 1997). The absence of a detectable expression signal in the apical meristem is in contrast to *Arabidopsis* and *Antirrhinum* in which expression of *TFL1* and *CEN* was detected in this tissue (Bradley *et al.*, 1996, 1997). The amplification of *FA* by RT-PCR using RNA from vegetative apices implies that the gene is expressed at this stage but at a level that is undetectable by the *in situ* technique used. Regardless, the expression of *FA* before flowering is consistent with its role in determining flowering time. It should be noted that analysis of *SP* expression in tomato apices by a different procedure, using S^{35} labelling, also failed to uncover expression in primary tomato apices (Thouet *et al.*, 2008). At the same time, a wide range of pleiotropic effects of *sp* mutants were documented in tomato (Pnueli *et al.*, 1998). Thus, even if *FA* and *SP* are expressed at different levels during different stages of development, expression levels may not indicate the significance of expression in each domain (Lifschitz, 2008).

The expression of the pepper *LFY* homologue in the apical meristem was similar to the expression pattern of *NFL*, the tobacco *LFY* homologue, although in pepper, expression was detected throughout the dome of the apical region, while in tobacco expression was detected in a ring outside the central dome (Amaya *et al.*, 1999). The expression of *FA* and the pepper *LFY* homologue in separate domains in the apex conforms to the non-overlapping expression pattern of *TFL1* and *LFY* in *Arabidopsis* (Bradley *et al.*, 1997). This pattern of expression differs from that of *SP* and the tomato *LFY* homologue that show overlapping expression pattern in all apices (Pnueli *et al.*, 1998). Therefore, although tomato, pepper, and tobacco are all related Solanaceous species, each has a unique pattern of expression of the genes that determine the transition of the shoot from the vegetative to the reproductive phases. But since RNA *in situ* may not be sensitive enough, these differences may be primarily quantitative rather than qualitative, and may account for the unique balance between vegetative growth and flowering and the different types of inflorescence architectures in each of the Solanaceae species (Prusinkiewicz *et al.*, 2007).

Comparison of sympodial development in pepper and tomato

Pepper and tomato are close relatives in the Solanaceae family. However, these two species differ in several aspects of their architecture resulting in a unique growth pattern of each one. Tomato is characterized by a vine growth habit compared with a bush habit of pepper. Tomato flowers earlier than pepper (after 5–12 leaves compared to 10–20 leaves for pepper, depending on the genotype). Tomato has a compound leaf and inflorescence compared to a simple leaf and single flower in pepper. Upon flowering and the release of apical dominance, a single shoot is developed in tomato compared to two shoots that are developed in pepper. Finally, while each sympodial unit of wild-type tomato consists of three leaves and an inflorescence (an exception to this is the wild tomato species *Solanum pennellii* that has two leaves per sympodial unit), in pepper (*C. annuum*), each sympodial unit consists of two leaves and a single flower.

These differences in wild-type architecture of tomato and pepper are also manifested by the different characteristics of the homologous *self pruning* and *fasciculate* mutations and the function of the corresponding genes. While no change in flowering time of the primary shoot is observed in tomato *self pruning*, pepper *fasciculate* plants flower earlier than in the wild type. *self pruning* may have an effect on flowering time in combination with other mutants (Lifschitz and Eshed, 2006), therefore, it did not completely lose its capacity to affect this trait. The reduction in the size of the sympodial unit in *self pruning* occurs via a reduction in the number of leaves which can be regarded as early flowering of the sympodium. By contrast, in *fasciculate*, the reduction in the size of the sympodial unit occurs via shortening the internodes and inhibition of leaf development, however, the basic structure of the sympodial unit remains unchanged. Therefore, while the function of *SELF PRUNING* is to repress flowering in the sympodial shoot, *FASCICULATE* functions as a flowering repressor in the primary shoot as well as a promoter of stem and leaf growth in the sympodial shoot. The structure of the inflorescence and the solitary

flower in tomato and pepper, respectively, remain unchanged in the *self pruning* and *fasciculate* mutants. This is in contrast to the monopodial plant systems such as in *Arabidopsis* and *Antirrhinum* in which the inflorescence structure has terminal differentiation.

Compared to *fasciculate* in which the mutation results from the formation of a truncated protein which presumably abolishes its function, the two known *self pruning* mutations result from a change in a single amino acid (leucine instead of proline) whose consequences on the function of the protein are not known (Pnueli *et al.*, 1998). Because most mutagenesis studies in tomato have been done in a *self pruning* background (Emmanuel and Levy, 2002; Menda *et al.*, 2004), knockout mutations at the *SELF PRUNING* locus have not been identified. Therefore, it is possible that once such mutations are available, some phenotypic consequences may differ from the current existing *self pruning* mutations.

How can common genes control diversity of plant architecture?

The phenotypic differences in the homologous *self pruning* and *fasciculate* mutations point to one of the most fundamental questions regarding plant development: how do similar genes confer diverse phenotypes in different plant species (Doebley and Lukens, 1998)? Phenotypic diversity can be caused by diverse mechanisms such as changes in gene function due to sequence divergence (a less likely cause for *SP/FA* because of the high sequence similarity of the two genes), changes in regulation pattern, or differential association with upstream and downstream components. Attempts to understand diversification in gene function in plants were carried out by comparisons of sequence, expression patterns or examination of gene function by transgenic means (Yoon and Baum, 2004). Recently, it was demonstrated that diversity of inflorescence architectures in *Arabidopsis* and petunia can be explained by differential patterns of expression of floral meristem identity genes (Souer *et al.*, 2008).

In order to gain a more comprehensive understanding of the mechanisms by which diversification of tomato and pepper architectures occur, we plan to isolate the genes controlling plant architecture in both plants, identify homologous mutations in these genes, and examine their interactions. In tomato, numerous mutant stocks are available (Emmanuel and Levy, 2002; Menda *et al.*, 2004; www.tgrc.ucdavis.edu). Mutations affected in plant architecture were isolated and characterized and, in some cases, the genes controlling the mutations were identified. These include *falsiflora* (Molinero-Rosales *et al.*, 1999), *blind* (Schmitz *et al.*, 2002) *lateral suppressor* (Schumacher *et al.*, 1999), *dwarf* (Bishop *et al.*, 1996), *jointless* (Mao *et al.*, 2000), and *sft* (Lifschitz *et al.*, 2006). We have recently initiated an EMS mutagenesis project in pepper with the goal of identifying and characterizing mutations and isolating the genes that control pepper growth architecture. Several mutants with altered sympodial development, plant size, and flowering time were identified and are currently being studied (Paran *et al.*, 2007). This will allow the comparison of the phenotypic effects and genetic regulatory networks of homologous genes controlling similar developmental processes in the two related Solanaceae species.

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