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

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Received 21 September 2008; Revised 24 November 2008; Accepted 27 November 2008

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).
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 phosphatidylinositol-4,5-bisphosphate and ethanolamine 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 Ha3a 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 F2 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. F2 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 cm2) was determined from scanned leaf images by the Image Gauge v3.3 software (Fujifilm). 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 F2 population, one-way analysis of variance (*P* ≤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’-GTGAACCCCTTGTGATTTG-GT-3’ located in the first exon and SP-R: 5’-GGTTCCCTGTGCAATT-GAA-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’-ATGGCCTCGAAAATGTGT-GAACC-3’ and FAORF-R: 5’-ACTAAACCCGAAA-CAACAG-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 *BamH*I and *Xba*I sites of pART7 downstream of the CaMV 35S promoter. Subsequently, the 35S:*FA* fragment was cloned into the NorI 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

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**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.
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 cm², there is a considerable reduction in leaf size to an average of 6.5±1.2 cm² 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 cm² and 16.9±1.1 cm², 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 F₂ 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*, F₂ 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
homzygous 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).

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...
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).

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 tfl 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 T2 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 tfl background (three primary transformants and 13 T2 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 in 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 S35 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. annum), 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 un-
changed 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 pre-
sumably 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 (Doebly 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 SPI1A 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 avail-
able (Emmanuel and Levy, 2002; Menda et al., 2004;
www.tgrc.ucdavis.edu). Mutations affected in plant archi-
tecture were isolated and characterized and, in some cases,
the genes controlling the mutations were identified. These
include falsiflora (Moliner-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 iso-
lating 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 develop-
mental processes in the two related Solanaceae species.

Acknowledgements

We thank G Kaluzki for her excellent technical assistance.
This research was supported by The Israel Science Founda-
tion (Grant No. 687/05).

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