

## Complementation of wild strawberry (*Fragaria vesca* L.) *SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR*) genes in *Arabidopsis thaliana*

Norbert HIDVÉGI<sup>1,2</sup> – Andrea GULYÁS<sup>1,2</sup> – Jaime A. TEIXEIRA DA SILVA<sup>2,3</sup> – Katalin POSTA<sup>1</sup> – Erzsébet KISS<sup>1</sup>

1: Szent István University, Faculty of Agricultural and Environmental Sciences, Institute of Genetics, Microbiology and Biotechnology, Páter Károly u. 1., 2100 Gödöllő, Hungary, E-mail: hidvegi.norbert@agr.unideb.hu; kiss.erszebet@mkk.szie.hu

2: University of Debrecen, Institutes for Agricultural Research and Educational Farm, Research Institute of Nyíregyháza, 4400. Nyíregyháza, Westsik Vilmos u. 4-6., Hungary

3: Current contact: P. O. Box 7, Miki-cho post office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan

**Abstract:** This study assessed the function of genes involved in wild strawberry (*Fragaria vesca* L.) fruit development and maturation to better understand the mechanism of non-climacteric fruit ripening. *SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR*) genes of *Fragaria vesca* displayed differential expression between the green and red ripening stages. *SPT*, which encodes a bHLH transcription factor, was characterized in *Arabidopsis thaliana* L. where its recessive mutation caused degenerative carpel and fruit development. The *spt* mutant of *A. thaliana* had shorter, smaller, and wider spatula-shaped siliques than the wild type. *SPT* was expressed throughout the development of marginal and transmission tract tissues, confirming its role in regulating the growth of these tissues. Two *A. thaliana* *SPIRAL* genes, *SPR1* and *SPR2*, are required for directional control of cell elongation. Recessive mutations in either of these genes decreased anisotropic growth of endodermal and cortical root cells and etiolated hypocotyls and caused right-handed helical growth in epidermal cells. The strawberry *SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR*) genes were amplified and *spt* and *spr* mutant *A. thaliana* plants were transformed with *FvSPT::pGWB401*, *FvSPR1-1::pGWB401* and *FvSPR1-2::pGWB401* vector constructs. Silique length and seed number/silique in the *A. thaliana* *spt* mutant were effectively complemented by *FvSPT* whereas *spr* was almost fully complemented by *FvSPR1-2*, but not by *FvSPR1-1*.

**Keywords:** *bHLH* gene; spatula shape-silique; helical root growth; *spr* and *spt* mutants

Received 1 February 2020, Revised 27 March 2020, Accepted 6 April 2020

### Introduction

Fruits are of two ripening types, climacteric, such as in tomato, apple, or banana, or non-climacteric, such as in wild strawberry (*Fragaria vesca* L.), grape, or orange. Climacteric ripening is accompanied by enhanced ethylene production, but this phenomenon cannot be observed in non-climacteric fruit (Chen et al. 2018). However, this categorization is not too stringent, and several studies have reported the regulatory function of ethylene in controlling gene expression during non-climacteric maturation (Li et al. 2016; Megías et al. 2016; Kou and Wu 2018; Tadiello et al. 2018). Investigating and assessing the genes involved in strawberry ripening can contribute to a better understanding of the non-climacteric process in this fruit crop. *FaSPT* (*FaSPATULA*;

GeneBank accession no. *AY679615*) is one of the genes that displayed altered expression during strawberry ripening (Balogh et al. 2005; Tisza et al. 2010).

Recessive mutations of the *SPATULA* (*SPT*) gene in *Arabidopsis thaliana* L. (*spt1* and *spt2*) cause degenerative carpel development and a transmission tract within the style and septum is absent (Alvarez and Smyth 1999). These phenomena are accompanied by inhibited growth and a decrease in the number of ovules. Anatomical gaps caused by rips can mostly be observed in carpel tips and the stigma. In *spt* mutants, the transmission tract and style within the septum bring about an extracellular matrix. Despite this anatomical deformation, fertilization can take place, but at a low frequency. Siliques of *spt* mutants are smaller, broader in the

center and terminus than wild type (WT) siliques, and their shape is spatula-like (Alvarez and Smyth 1998). The *SPT* gene encodes a basic-helix-loop-helix (bHLH) transcription factor that is continuously expressed in the marginal tissues of developing carpels, where it is also likely responsible for their further growth (Bowman and Smyth 1999). Heisler et al. (2001) examined the transcription factors that influenced *SPT* expression in *A. thaliana* and showed that *CRABS CLAW* and *AGAMOUS* genes, which contribute to carpel development (Alvarez and Smyth 2002; Lee et al. 2005), did not impact *SPT* expression, and that *SPT* played a role in flower organogenesis. *SPT* is a homologue of the phytochrome-interacting factor (PIF) which regulates seed dormancy (Josse et al. 2011). Groszmann et al. (2011) found similarity between *SPT* and *ALCATRAZ* (*ALC*) genes, claiming that both were essential in flower and fruit development, and that *A. thaliana alc* mutants could be successfully complemented with *35S::SPT* vectors. Zumajo-Cardona et al. (2017) isolated *paleo*, *SPT* and *ALC* genes from different plants and examined their gene expression and conserved regions, and also performed phylogenetic analyses, noting that these genes may play a role in early floral organ development and specification in *Bocconia frutescens* L. Makkena and Lamb (2013) investigated the role of *SPT* in the regulation of root meristem development in strawberry where its expression increased as fruit ripening progressed, but decreased in response to wounding, auxin and ethylene. In strawberry, RNAi-based gene silencing of *SPT* retarded fruit development (Tisza et al. 2010).

Members of the *SPIRAL* (*SPR*) gene family encode small proteins that regulate the organization of microtubules by affecting cell growth and elongation (Furutani et al. 2000; Nakajima et al. 2004). Members of the *SPR* gene family in *A. thaliana* are classified into two main categories, *spr1* and *spr2*, and five

subgroups of *spr1*, *spr1-1* to *spr1-5* (Nakajima et al. 2006). *A. thaliana* plants harbouring a mutant *SPR* gene develop roots with characteristic helical growth. Epidermal cell rows of roots of *spr* mutants in *A. thaliana* are twined resulting in left-handed helical growth, and cortical cells of etiolated *spr* hypocotyls showed microtubule arrays with irregular orientations (Furutani et al. 2000). The *SPR2* gene codes for a protein that binds to a plant-specific microtubule (Shoji et al. 2004). Mutations in the *SPR2* gene may result in right-handed helical growth in hypocotyls, petioles and petals (Furutani et al. 2000; Buschmann et al. 2004). Using cDNA-AFLP, Balogh et al. (2005) identified the *FaSPR* gene (C11M32M003) from cultivated strawberry (*Fragaria × ananassa* Duch.). Polgári et al. (2010) analysed the cDNA-AFLP fragment and the full-length cDNA (AY695666) of *FaSPR*, showing over 60% homology at the nucleotide level with two gene groups in *A. thaliana* and other plants.

The complementation test is a very efficient tool for functional genomic analysis. In the plant kingdom, the model plant *A. thaliana*, with its well-known genome, has plenty of natural and induced mutants, which are used to prove similar or analogous functions of genes isolated from different organisms (Groszmann et al. 2011).

Our aim was to functionally characterize the *Fragaria vesca* *SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR*) genes. To achieve this, we carried out a complementation analysis using *FvSPT* (XM\_004287975; LOC101290893), *FvSPR1-1* (XM\_004297177; LOC01307108) and *FvSPR1-2* (XM\_004299243; LOC101309836) constructs within the pGWB401 vector and transformed *A. thaliana* Columbia mutants *spt* and *spr*. An understanding of the functionality of *FvSPT* and *FvSPR* genes would allow for their use in transgenic constructs for postharvest applications.

## Materials and Methods

After sowing seeds *ex vitro* in soil, they were incubated at 4°C for 4 days, then placed in the dark. After 4 days, they were put in a 22°C climate chamber (Binder KBWF 240, Tuttlingen, Germany) and grown under an 8-h photoperiod at a photosynthetic photon flux density (PPFD) of 37  $\mu\text{mol m}^{-2} \text{s}^{-1}$  provided by Biolux tubes (Osram L58W, Markham, Canada). When the first flowers appeared (14-16 days after seedling emergence; Smyth et al. 1990), they were cut. Plantlets were then grown under a 16-h photoperiod at a PPFD of 37  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and at 22°C. Plant material was grown at Szent István University.

*FaSPT*, *FaSPRI-1* and *FaSPRI-2* genes (coding sequences), which were identified with cDNA-AFLP (Balogh et al. 2005; Tisza et al. 2010), together with their promoters, were applied in the complementation tests. The homology was analysed with ClustalO (<https://www.ebi.ac.uk/Tools/msa/clustalo/>) between *FvSPRI-1* (XM\_004297177; LOC01307108), *FvSPRI-2* (XM\_004299243; LOC101309836), *AtSPRI-2* (BT024676), *FvSPT* (XM\_004287975 and AY679615) and *AtSPT* (BT026462). For primer design and *in silico* analysis of the promoter regions, we used the “*Fragaria vesca* Whole Genome v2.0a1 assembly & annotation” from GDR (<http://www.rosaceae.org>). Genomic DNA was isolated from 100 mg of fresh plant tissue of in-house *Fragaria vesca* L. cv. Rügen using NucleoSpin Plant II kit (Macherey-Nagel, Düren, Germany) following the manufacturer’s protocol. The *SPT* gene and its promoter (6600 bp), as well as the *SPRI-1* and *SPRI-2* genes and their promoters (9647 bp and 2443 bp, respectively) were amplified with the GoTaq Long PCR Master Mix (Promega, Madison, WI, USA). A total of 100 ng of genomic DNA was used as a template in a 50  $\mu\text{L}$  PCR mix. The PCR

mixture consisted of 25  $\mu\text{L}$  volume of GoTaq Long PCR Master Mix (2 $\times$ ), and 40 pmol of each primer. The PCR conditions were 95°C for 2 min followed by 35 cycles at 95°C for 30 s, 65°C for 7 min. Cycling was followed by a final incubation at 72°C for 10 min. PCR products were separated by electrophoresis on 1.0% agarose gels in 1 $\times$  TAE buffer (Sambrook et al. 1989) and were detected by fluorescence under UV light (302 nm) after staining with 0.1% ethidium bromide. A molecular marker of 1 Kb Plus DNA Ladder (ThermoFisher Scientific, Carlsbad, CA, USA) was used. The PCR products were purified with Wizard® SV Gel and the PCR Clean-Up System (Promega). Purified PCR fragments were ligated into a pDONR221 entry vector (Life Technologies, Carlsbad, CA, USA). The pGWB401 vector (Nakagawa et al. 2007; Tanaka et al. 2011) was used to establish plant transformation constructs containing full-length genomic clones of *FvSPT*, *FvSPRI-1* and *FvSPRI-2* genes (i.e., containing promoters and coding sequences). *A. thaliana spt* and *spr* mutants of Columbia (Col), purchased from the Eurasian Arabidopsis Stock Centre NASC (<http://arabidopsis.info/>), were grown under an 8-h photoperiod at a PPFD of 37  $\mu\text{mol m}^{-2} \text{s}^{-1}$  provided by Biolux tubes (Osram L58W, Markham, Canada), and at 22°C in a climatic chamber (Binder KBWF 240, Tuttlingen, Germany). The *spr1-2* Col mutant (NASC ID: N6547) has defective directional cell elongation, abnormal cortical microtubule function and exhibits right-handed helical growth in roots, which are caused by the *SPRI-2* allele (At1g69230; GenBank: BT26462) mutation by sequence tagged T-DNA insertion line. The *spt* Col mutant (NASC ID: N857133) has a T-DNA insertion in the *SPT* gene (At4g36930; GenBank: BT024676) on chromosome 4, position 17414295 on TAIR10.

Genetic transformation of *spr1-1/spr1-2* and *spt* mutants was carried out when secondary

Table 1. Primer names and sequences applied in RT-qPCR.

Primer name	Position of primer	Sequence (5' – 3')	Amplicon length
<i>FvSPR1</i>	forward	TGCAGATGGCTCAACTCAA	280 bp
	reverse	ACCTGGGAAAGGGTGGAGTA	
<i>FvSPR2</i>	forward	TGTATGAATTACGTAACCAT	178 bp
	reverse	TTCTCTTTTCGACACTCGTC	
<i>FvSPT</i>	forward	ACTATTTAAAATTTAAAAGAA	197 bp
	reverse	ATTAGGAAATCCACTCAGACA	
<i>FvGAPDH</i>	forward	AGGTTGTGCTGGTAATGGAA	218 bp
	reverse	ATTGCAGTGGTGGATACCTT	

flowering (about 1 month after seedling formation) started. In-house *Agrobacterium tumefaciens* GV3101 strain was used for floral dip transformation (Clough and Bent 1998), which was repeated when the tertiary flowers appeared after 1-2 weeks. Seeds were harvested after about 6 weeks then sown in soil. Transformants were selected by treating plants with kanamycin solution (Duchefa, Haarlem, The Netherlands) (Xiang et al. 1999). Specifically, two-leaf *A. thaliana* plantlets were sprayed consecutively with 100, 200 then 400 mg/mL kanamycin for 3 days, 1 week and 2 weeks, respectively after sowing seeds. Plants that survived the third spray were analysed by PCR with a Phire Plant Direct PCR Kit (ThermoFisher Scientific, Carlsbad, CA, USA). The PCR mixture consisted of 10  $\mu$ L of Phire Plant PCR Buffer (2 $\times$ ), 40 pmol of each primer, 0.4  $\mu$ L of Phire Hot Start II DNA Polymerase and 0.5  $\mu$ L of diluted plant tissue. The PCR conditions were 98°C for 5 min followed by 40 cycles at 98°C for 5 s, 60°C for 5 s and 72°C for 20 s. Cycling was followed by a final incubation of 72°C for 1 min. PCR products were separated by electrophoresis based on the same protocol that was used for promoter PCR. The PCR-positive T<sub>1</sub> individuals were grown in a climatic chamber until T<sub>4</sub>. The T<sub>3</sub> and T<sub>4</sub> individuals were analysed with RT-qPCR

for quantification of the transgene expression and determination of transgene copy number (Fletcher et al. 2014). Vector construction and genetic transformation were conducted at Szent István University.

The T<sub>3</sub> and T<sub>4</sub> plants with one transgene copy were examined; we observed and measured their habit, roots and siliques (three biological and technical replicates), and the number of seeds and siliques (three biological and technical replicates) was determined and compared to wild type (WT) and mutant Col plants. Data was analysed statistically in SPSS version 22 (SPSS Inc., IBM Corp., Armonk, NY, USA). Following mean separation by ANOVA using Windows Microsoft Excel (2017), statistical significance was determined using Tukey's multiple range test ( $P < 0.001$ ). Statistical analyses were carried out at the University of Debrecen, IAREF, Research Institute of Nyíregyháza.

To prove the transcription of the *FvSPT*, *FvSPR1-1* and *FvSPR1-2* genes, total RNA was isolated from *F. vesca* (cv. Rügen) and the transformant *A. thaliana* plants. Total RNA was applied to RT-qPCR with primers designed for the exon-exon junction of *FvSPT*, *FvSPR1-1* and *FvSPR1-2*, as well as the *GAPDH* housekeeping gene (primer sequences are listed in Table 1). We calculated the transformation efficiency based on

the number of transformants/number of florets dipped in the transformation (floral dip) solution.

Silique length of T<sub>3</sub> and T<sub>4</sub> progeny (80 siliques per generation, three biological replicates) was measured and seeds were counted under a SMZ-161-BL stereomicroscope (Motic; Hong Kong, China). RT-qPCR and microscopic analyses were carried out at the University of Debrecen, IAREF, Research Institute of Nyíregyháza.

## Results and Discussion

In this study, we isolated the *F. vesca* *FvSPT*, *FvSPRI-1* and *FvSPRI-2* genes (Balogh et al. 2005; Polgári et al. 2010), which showed altered expression in the course of fruit ripening, and introduced them into *A. thaliana* *spt* and *spr1-2* mutants with the objective of trying to complement mutated functions. Using *in silico* analysis for the promoter regions and genes, we determined the *FvSPT*, *FvSPRI-1* and *FvSPRI-2* genes and their promoters based on homology with the At1g69230 (Figure 1) and At4g36930 (Figure 2) genes in *F. vesca* genomic sequences (Shulaev et al. 2011) and GDR data (<http://www.rosaceae.org>). Homology was 84.03%, 69.45% and 74.24% between *FvSPT* (XM\_004287975) and *AtSPT* (At4g36930), *FvSPRI-1* (XM\_004297177) and *AtSPRI-2* (At1g69230), and *FvSPRI-2* (XM\_004299243) and *AtSPRI-2* (At1g69230), respectively. We amplified the *FvSPT* (6600 bp), *FvSPRI-1* (9647 bp) and *FvSPRI-2* (2443 bp) genes, including their promoters. After constructing the *FvSPT::pGWB401*, *FvSPRI-1::pGWB401* and *FvSPRI-2::pGWB401* vector constructs, we confirmed, using colony PCR, that the vectors carried the inserts. The *A. thaliana* *spt* and *spr* mutants (60 plants/line) were transformed with the vector constructs. In

germinated plants that survived three-step kanamycin selection (3 days, one week and two weeks after germination) with 100 mg/mL, 200 mg/mL and 400 mg/mL, respectively, we confirmed that the plants carried the *FvSPT*, *FvSPRI-1* and *FvSPRI-2* genes by applying direct PCR with the specific gene of interest using PCR primers for the putative transformed plants and RT-qPCR. Average transformation efficiency for the three genes was 0.38% with only one transformation at secondary flowering. When redefining the transformation efficiency as the number of transformants/number of seeds set and we used two transformation processes (when secondary and tertiary flowers appeared), then transformation efficiency was much higher (7.6%). After these plants developed until 6 weeks, it was possible to compare the phenotype of the WT Col silique (Figure 3A), the *FvSPT*-complemented plants (Figure 3B), and the *spt* mutant (Figure 3C).

The *spt* mutant plants were significantly shorter (Figure 4C) and had shorter siliques (Figure 5) than WT (Figure 4A). The successfully complemented *FvSPT* transformant was significantly taller than the *spt* mutant (Figure 4D), while its non-malformed silique resembled that of WT (Figure 3), demonstrating that the *FvSPT* gene was able to effectively compensate for the missing silique-related function of *spt*.

When silique length (from an average of 12 siliques/plant) of 80 plants of WT, *spt* and *spr* mutants and complemented Col mutants were compared, the *spt* mutant displayed significantly shortest silique length (3.8 mm), while WT as well as the *FvSPT/FvSPRI-1/FvSPRI-2*-complemented plants had significantly longer siliques (12.8-13.3 mm). Relative to the *spr* mutant, silique length was significantly increased only in the *FvSPRI-1*-complemented plants (Figure 5).

The *spt* mutant produced fewest

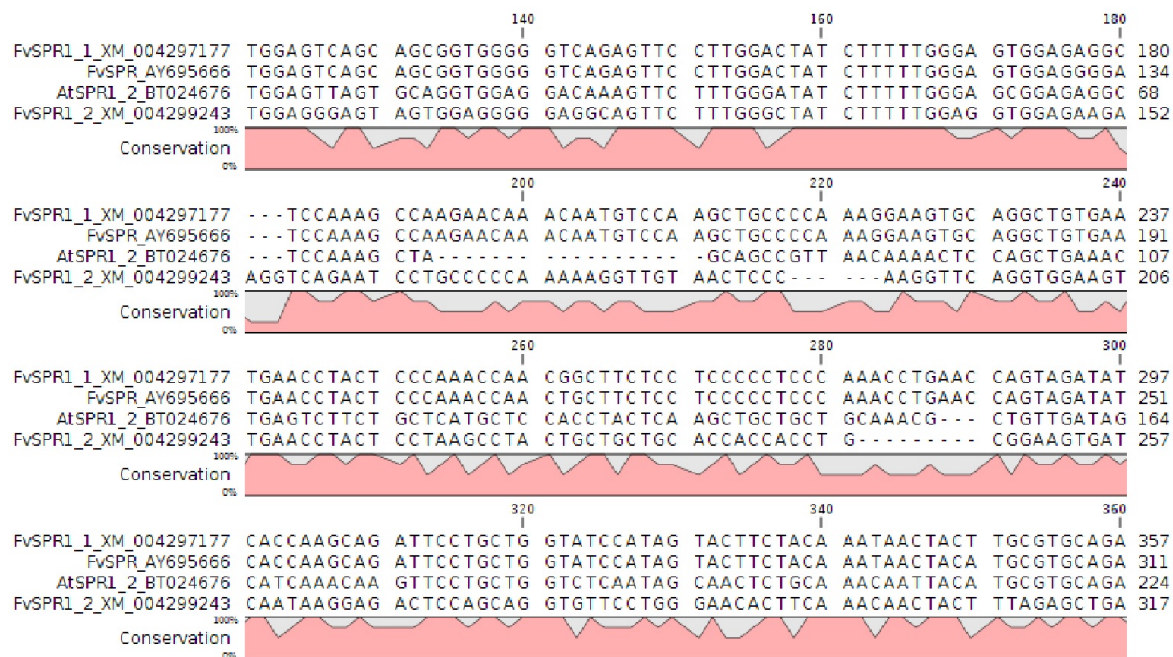


Figure 1. Homology between *FvSPR1-1* (XM\_004297177), *FvSPR* (AY695666), *AtSPR1-2* (BT024676) and *FvSPR1-2* (XM\_004299243).

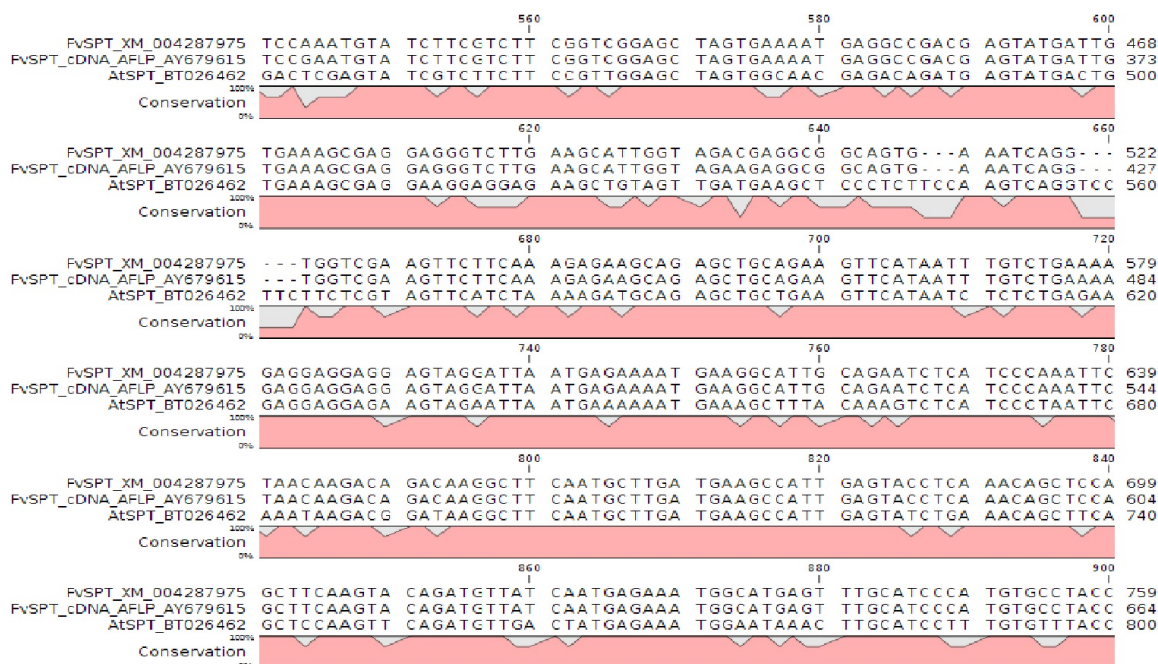


Figure 2. Homology between *FvSPT* (XM\_004287975), *FvSPT* cDNA (AY679615) and *AtSPT* (BT026462).

seeds/silique (7.7), while WT Col produced the most (47.7) (Figure 6). The spr mutant, which had significantly shorter siliques

than the WT (Figure 5), also developed significantly fewer seeds (37.5) than WT (47.7). The number of seeds in *FvSPT-*

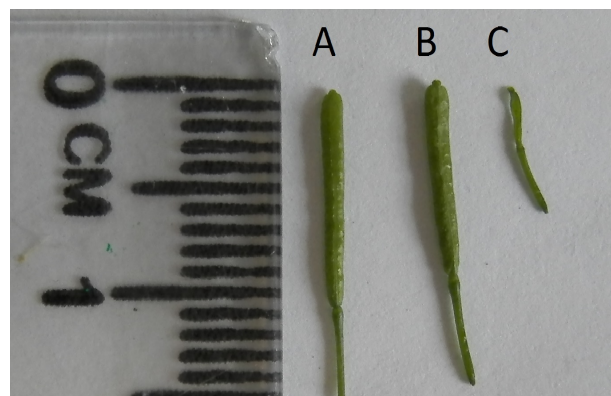


Figure 3. Siliques of *Arabidopsis thaliana* Columbia wild type (A); *FvSPT*-complemented Columbia *spt* mutant (B) and Columbia *spt* mutant (C) from 6-week-old plants.

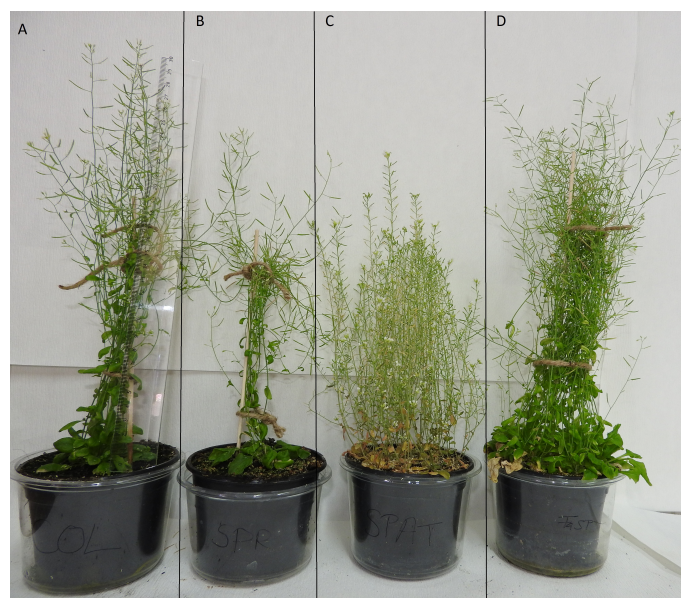


Figure 4. Habit of 8-week-old plants of *Arabidopsis thaliana* Columbia wild type (A), *spiral* mutant, *spr* (B), *spatula* mutant, *spt* (C) and *FvSPT1-2*-complemented (D).

complemented siliques was, as expected, significantly higher than the *spt* mutant, but significantly lower than the WT control (Figure 6). Similarly, the number of seeds in *FvSPRI-1*- and *FvSPRI-2*-complemented siliques was, also as expected, significantly higher than the *spr* mutant, but significantly lower than the WT control (Figure 6). The *FvSPR* complementation was not as pronounced as the *FvSPT* complementation, but the trait (number of seeds/silique)

was still complemented, nonetheless. Despite these differences, *FvSPT*-, *FvSPRI-1*- and *FvSPRI-2*-complemented genotypes displayed the same phenotype as the WT control. As one example, see the comparison between WT and *FvSPT1-2*-complemented plants in Figure 4.

A contrast of the phenotypes of *FvSPRI-1*- and *FvSPRI-2*-complemented, WT and *spr* mutant plants can be seen in Figure 7 and

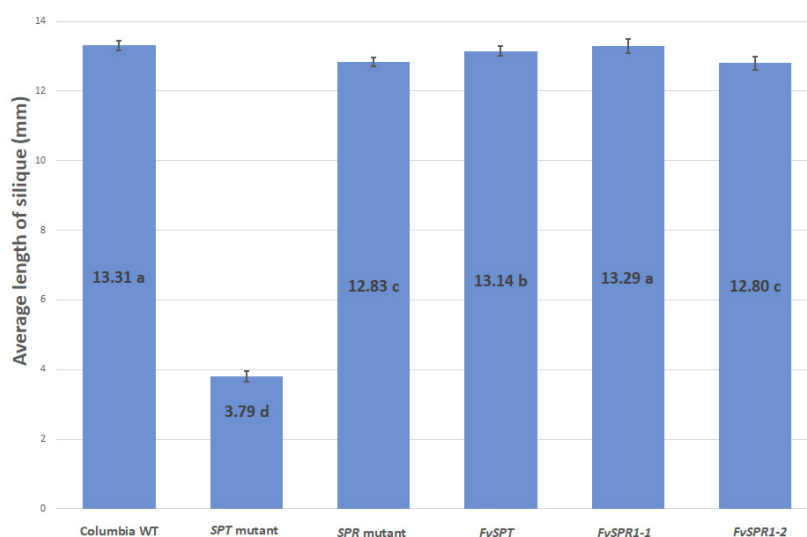


Figure 5. Average length of silique (mm). Col WT: Columbia wild type; *spt* mutant: *spatula* mutant Col; *spr* mutant: *spiral* mutant Col; *FvSPT*: *SPATULA* gene of strawberry (*Fragaria vesca* L.) complemented *A. thaliana* Col; *FvSPR1-1*: *SPIRAL1-1* gene of strawberry complemented *A. thaliana* Col; *FvSPR1-2*: *SPIRAL1-2* gene of strawberry complemented *A. thaliana* Col. Different letters within blue bars indicate significant differences with Col WT based on one-way ANOVA (Tukey's multiple range test;  $P < 0.001$ ); 80 plants/experiment/line and three biological replicates.

Figure 8. The *spr* mutation could only be restored by *FvSPR1-2* (Figure 7). In the case of *FvSPR1-1* plants, similar helical roots developed as in the *spr* mutants. There are three recessive *A. thaliana spr* mutants, *spr1-1*, *spr1-2* and *spr1-3* (Nakajima et al. 2006). We used the *spr1-2* mutant in our experiment, so this could theoretically only be complemented by *FvSPR1-2*, and not by *FvSPR1-1* (*FvSPR1-1*-complemented plants continued to have helical roots, i.e., the mutant phenotype was not corrected), indicating that *FvSPR1-1* does not have the same function.

To show the expression of *FvSPT*, *FvSPR1-1* and *FvSPR1-2* genes, we isolated total RNA from the transformants and confirmed the transcription of these genes by RT-qPCR. The primers designed for exon-exon junctions amplified 146 bp and 265 bp on the cDNA and gDNA, respectively. The *A. thaliana GAPDH* gene was

used as the reference, generating a 130 bp fragment. RT-qPCR results prove that the *FvSPT::pGWB401*, *FvSPR1-1::pGWB401* and *FvSPR1-2::pGWB401* constructs functioned in the *AtSPT*-complemented *A. thaliana* plants.

Our experimental results attested that *FaSPT* and *FaSPR* genes isolated from octoploid *F. × ananassa* by cDNA-AFLP (Balogh et al. 2005), show sequence similarity not only to *A. thaliana AtSPT*, *AtSPR1-1* and *AtSPR1-2*, but also as well as with diploid strawberry (*F. vesca*) *FvSPT*, *FvSPR1-1* and *FvSPR1-2*, but they also have the ability to complement the *A. thaliana* mutant phenotype (*spt* and *spr1-2* mutant Columbia). Similarly to the result of Heisler et al. (2001), in which the WT *AtSPT2* allele complemented the *At-spt2* mutation, *FvSPT* had the same effect, confirming the same functional ability of this strawberry-derived gene. The literature indi-



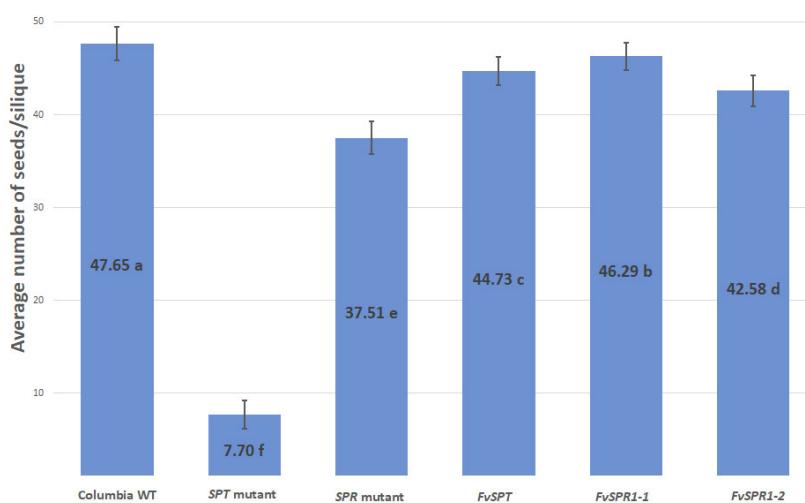


Figure 6. Average number of seeds/silique. Col WT: Columbia wild type; *spt* mutant: *spatula* mutant Col; *spr* mutant: *spiral* mutant Col; *FvSPT*: *SPATULA* gene of *Fragaria vesca* complemented *A. thaliana* Col; *FvSPR1-1*: *SPIRAL1-1* gene of *F. vesca* complemented *A. thaliana* Col; *FvSPR1-2*: *SPIRAL1-2* gene of *F. vesca* complemented *A. thaliana* Col. Different letters within blue bars indicate significant differences with Col WT based on one-way ANOVA (Tukey's multiple range test;  $P < 0.001$ ); 80 plants/experiment/line and three biological replicates.

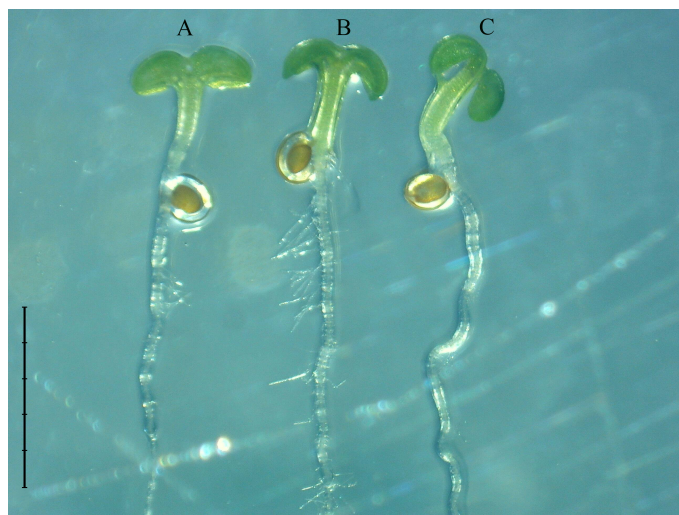


Figure 7. Roots of *FvSPR1-2* complemented *Arabidopsis thaliana* Columbia (A), Columbia wild type (B) and *spiral* mutant (*spr*) of *A. thaliana* Columbia (C) plants after 1 week (Scale bar: 5 mm).

cates that mutant *SPR1-1*, *SPR1-2* and *SPR1-3* genes cause the same abnormal root malformation symptoms in *A. thaliana* (Furutani

et al. 2000). We showed, however, that only *FvSPR1-2* was able to restore the dysfunctional *spr1-2*.



Figure 8. Roots of *spiral* mutant of *Arabidopsis thaliana* Columbia (A), Columbia wild type (B) and *FvSPR1-2*-complemented Columbia (C) plants after 2 weeks (Scale bar: 5 mm).

### Acknowledgements

The research was financed by the Higher Education Institutional Excellence Programme (NKFIH-1150-6/2019) of the Ministry of Innovation and Technology in Hungary, within the framework of the Biotechnology thematic programme of the University of De-

brecen, and that of Szent Istvan University (NKFIH-1159-6/2019) and a grant of the Hungarian Research Fund K 101195 entitled “Functional analysis of genes and their promoters identified during the fruit ripening of strawberry”. The authors thank Dr. Judit Dobránszki (IAREF, University of Debrecen, Hungary) for assistance with statistical analysis.

### References

- Alvarez, J., Smyth, D.R. (1998): Genetic pathways controlling carpel development in *Arabidopsis thaliana*. *Journal of Plant Research* 111: 295-298. <https://doi.org/10.1007/BF02512187>
- Alvarez, J., Smyth, D.R. (1999): *CRABS CLAW* and *SPATULA*, two *Arabidopsis* genes that control carpel development in parallel with *AGAMOUS*. *Development* 126: 2377-2386.
- Alvarez, J., Smyth, D.R. (2002): *CRABS CLAW* and *SPATULA* genes regulate growth and pattern formation during gynoecium development in *Arabidopsis thaliana*. *International Journal of Plant Sciences* 163(1): 17-41. <https://doi.org/10.1086/324178>
- Balogh, A., Koncz, T., Tisza, V., Kiss, E., Heszky, L. (2005): Identification of ripening-related genes in strawberry fruit by cDNA-AFLP. *International Journal of Horticultural Science* 11(4): 33-41. DOI: <https://doi.org/10.31421/IJHS/11/4/602>

Bowman, J.L., Smyth, D.R. (1999): *CRABS CLAW*, a gene that regulates carpel and nectary development in *Arabidopsis*, encodes a novel protein with zinc finger and helix-loop-helix domains. *Development* 126: 2387-2396.

Buschmann, H., Fabri, C.O., Hauptmann, M., Hutzler, P., Laux, T., Lloyd, C.W., Schäffner, A.R. (2004): Helical growth of the *Arabidopsis* mutant *tortifolia1* reveals a plant-specific microtubule-associated protein. *Current Biology* 14: 1515-1521. <https://doi.org/10.1016/j.cub.2004.08.033>

Chen, Y., Grimplet, J., David, K., Castellarin, S.D., Terol, J., Wong, D.C., Gambetta, G.A. (2018): Ethylene receptors and related proteins in climacteric and non-climacteric fruits. *Plant Science* 276: 63-72. <https://doi.org/10.1016/j.plantsci.2018.07.012>

Clough, S.J., Bent, A.F. (1998): Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *The Plant Journal* 16: 735-743. <https://doi.org/10.1046/j.1365-313x.1998.00343.x>

Fletcher, S.J. (2014): qPCR for quantification of transgene expression and determination of transgene copy number. In: Fleury, D., Whitford, R. (ed.) *Crop Breeding* (pp. 213-237). Humana Press, New York, NY. [https://doi.org/10.1007/978-1-4939-0446-4\\_17](https://doi.org/10.1007/978-1-4939-0446-4_17)

Furutani, I., Watanabe, Y., Prieto, R., Masukawa, M., Suzuki, K., Naoi, K., Hashimoto, T. (2000): The *SPIRAL* genes are required for directional control of cell elongation in *Arabidopsis thaliana*. *Development* 127: 4443-4453.

Groszmann, M., Paicu, T., Alvarez, J.P., Swain, S.M., Smyth, D.R. (2011): *SPATULA* and *ALCATRAZ*, are partially redundant, functionally diverging bHLH genes required for *Arabidopsis* gynoeceum and fruit development. *The Plant Journal* 68: 816-829. <https://doi.org/10.1111/j.1365-313X.2011.04732.x>

Heisler, M.G.B., Atkinson, A., Bylstra, Y.H., Walsh, R., Smyth, D.R. (2001): *SPATULA*, a gene that controls development of carpel margin tissues in *Arabidopsis*, encodes a bHLH protein. *Development* 128: 1089-1098.

Josse, E., Gan, Y., Torrent, J., Stewart, K., Gilday, A.D., Jeffree, E.C., Vaistij, F., García, J.F., Nagy, F., Graham, I.A. (2011): A *DELLA* in disguise: *SPATULA* restrains the growth of the developing *Arabidopsis* seedling. *The Plant Cell* 23: 1337-1351. <https://doi.org/10.1105/tpc.110.082594>

Kou, X., Wu, M. (2018): Characterization of climacteric and non-climacteric fruit ripening. In: Guo, Y. (ed.) *Plant Senescence* (pp. 89-102). Humana Press, New York, NY. [https://doi.org/10.1007/978-1-4939-7672-0\\_7](https://doi.org/10.1007/978-1-4939-7672-0_7)

Lee, J.Y., Baum, S.F., Alvarez, J., Patel, A., Chitwood, D.H., Bowman, J.L. (2005): Activation of *CRABS CLAW* in the nectaries and carpels of *Arabidopsis*. *The Plant Cell* 17(1): 25-36. <https://doi.org/10.1105/tpc.104.026666>

Li, L., Lichter, A., Chalupowicz, D., Gamrasni, D., Goldberg, T., Nerya, O., Porat, R. (2016): Effects of the ethylene-action inhibitor 1-methylcyclopropene on postharvest quality of non-climacteric fruit crops. *Postharvest Biology and Technology* 111: 322-329. <https://doi.org/10.1016/j.postharvbio.2015.09.031>

Makkena, S., Lamb, R.S. (2013): The bHLH transcription factor *SPATULA* regulates root growth by controlling the size of the root meristem. *BMC Plant Biology* 13(1): 1. <https://doi.org/10.1186/1471-2229-13-1>

Megías, Z., Martínez, C., Manzano, S., García, A., del Mar Reboloso-Fuentes, M., Valenzuela, J. L. and Jamilena, M. (2016): Ethylene biosynthesis and signaling elements involved in chilling injury and other postharvest quality traits in the non-climacteric fruit of zucchini (*Cucurbita pepo*). *Postharvest Biology and Technology* 113: 48-57. <https://doi.org/10.1016/j.postharvbio.2015.11.001>

Nakagawa, T., Kurose, T., Hino, T., Tanaka, K., Kawamukai, M., Niwa, Y., Toyooka K., Matsuoka, K., Jinbo, T., Kimura, T. (2007): Development of series of gateway binary vectors, pGWBs, for realizing efficient construction of fusion genes for plant transformation. *Journal of Bioscience*

and Bioengineering 104: 34-41. <https://doi.org/10.1263/jbb.104.34>

Nakajima, K., Furutani, I., Tachimoto, H., Matsubara, H., Hashimoto, T. (2004): *SPIRAL1* encodes a plant-specific microtubule-localized protein required for directional control of rapidly expanding *Arabidopsis* cells. *The Plant Cell* 16: 1178-1190. <https://dx.doi.org/10.1105/2Ftpc.017830>

Nakajima, K., Kawamura, T., Hashimoto, T. (2006): Role of the *SPIRAL1* gene family in anisotropic growth of *Arabidopsis thaliana*. *Plant Cell Physiology* 47: 513-522. <https://doi.org/10.1093/pcp/pcj020>

Polgári, D., Kalapos, B., Tisza V., Kovács, L., Kerti, B., Heszky, L., Kiss, E. (2010). *In silico* analysis of a putative *SPIRAL* gene related to strawberry ripening. *Acta Agronomica Hungarica* 58: 267-272. <https://doi.org/10.1556/AAgr.58.2010.3.9>

Sambrook, J., Fritsch, E.F., Maniatis, T. (1989): *Molecular Cloning: A Laboratory Manual* (No. Ed. 2). Cold Spring Harbor Laboratory Press, NY, USA.

Shoji, T., Narita, N.N., Hayashi, K., Asada, J., Hamada, T., Sonobe, S., Hashimoto, T. (2004): Plant-specific microtubule-associated protein *SPIRAL2* is required for anisotropic growth in *Arabidopsis*. *Plant Physiology* 136: 3933-3944. <https://doi.org/10.1104/pp.104.051748>

Shulaev, V., Sargent, D.J., Crowhurst, R.N., Mockler, T.C., Folkerts, O., Delcher, A.L., Jaiswal, P., Mockaitis, K., Liston, A., Mane, S.P., Burns, P., Davis, T.M., Slovin, J.P., Bassil, N., Hellens, R.P., Evans, C., Harkins, T., Kodira, C., Desany, B., Crasta, O.R., Jensen, R.V., Allan, A.C., Michael, T., P., Setubal, J.C., Celton, J.M. (2011): The genome of woodland strawberry (*Fragaria vesca*). *Nature Genetics* 43: 109-116. <https://doi.org/10.1038/ng.740>

Smyth, D.R., Bowman, J.L., Meyerowitz, E.M. (1990): Early flower development in *Arabidopsis*. *The Plant Cell* 2(8): 755-767. <https://doi.org/10.1105/tpc.2.8.755>

Tadiello, A., Busatto, N., Farneti, B., Delledonne, M., Velasco, R., Trainotti, L., Costa, F. (2018): The interference of the ethylene perception machinery leads to a re-programming of the fruit quality-related transcriptome and induces a cross-talk circuit with auxin in apple. *Acta Horticulturae* 1206: 69-74. <https://doi.org/10.17660/ActaHortic.2018.1206.10>

Tanaka, Y., Nakamura, S., Kawamukai, M., Koizumi, N., Nakagawa, T. (2011): Development of a series of gateway binary vectors possessing a tunicamycin resistance gene as a marker for the transformation of *Arabidopsis thaliana*. *Bioscience, Biotechnology and Biochemistry* 75: 804-807. <https://doi.org/10.1271/bbb.110063>

Tisza, V., Kovács, L., Balogh, A., Heszky, L., Kiss, E. (2010): Characterization of *FaSPT*, a *SPATULA* gene encoding a bHLH transcriptional factor from the non-climateric strawberry fruit. *Plant Physiology and Biochemistry* 48: 822-826. <https://doi.org/10.1016/j.plaphy.2010.08.001>

Xiang, C., Han, P., Oliver, D.J. (1999): In solium selection for *Arabidopsis* transformants resistant to kanamycin. *Plant Molecular Biology* 17: 59-65. <https://doi.org/10.1023/A:1007588001296>

Zumajo-Cardona, C., Ambrose, B.A., Pabón-Mora, N. (2017): Evolution of the *SPATULA/ALCATRAZ* gene lineage and expression analyses in the basal eudicot, *Bocconia frutescens* L. (Papaveraceae). *EvoDevo* 8(1): 5. <https://doi.org/10.1186/s13227-017-0068-8>