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

Norbert HIDVÉGI¹,² – Andrea GULYÁS¹,² – Jaime A. TEIXEIRA DA SILVA²,³ – Katalin POSTA¹ – Erzsébet KISS¹

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.erzsebet@mkk.szie.hu
²: University of Debrecen, Institutes for Agricultural Research and Educational Farm, Research Institute of Nyíregyháza, 4400. Nyíregyháza, Westsik Vílmos 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. Siliques of **spt** mutants were transformed with **FvSPR1-1::pGWB401**, **FvSPR1-2::pGWB401** and **FvSPR1-2::pGWB401** vector constructs. Siliques of **spt** mutants were 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; Megfás 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), FvSPR-1 (XM_004297177; LOC101307108) and FvSPR-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 μmol m⁻² 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 μmol m⁻² s⁻¹ and at 22°C. Plant material was grown at Szent István University.

FaSPT, FaSPR1-1 and FaSPR1-2 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 FvSPR1-1 (XM_004297177; LOC01307108), FvSPR1-2 (XM_004299243; LOC101309836), AtSPR1-2 (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 SPR1-1 and SPR1-2 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 μL PCR mix. The PCR mixture consisted of 25 μL volume of GoTaq Long PCR Master Mix (2×), 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× 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, FvSPR1-1 and FvSPR1-2 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 μmol m⁻² 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 SPR1-2 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 |
|-------------|--------------------|--------------------|-----------------|
| FvSPR1      | forward            | TGCAGATGGCTCAACTCAA | 280 bp          |
|             | reverse            | ACCTGGGAAAGGGTGGAGTA |                |
| FvSPR2      | forward            | TGTATGAATTACGTAACCAT | 178 bp          |
|             | reverse            | TTCTCTTTTCGACACTCGTC |                |
| FvSPT       | forward            | ACTATTAAAAATTAAAAGAA | 197 bp          |
|             | reverse            | ATTAGGAAATCCACTCAGACA |               |
| FvGAPDH     | forward            | AGGTTGTGCTGGTAATGGGA | 218 bp          |
|             | reverse            | ATTCAGATGGTGGGATACCTT |               |

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 µL of Phire Plant PCR Buffer (2×), 40 pmol of each primer, 0.4 µL of Phire Hot Start II DNA Polymerase and 0.5 µL 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 T₁ individuals were grown in a climatic chamber until T₄. The T₃ and T₄ 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 T₃ and T₄ 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 T3 and T4 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, FvSPR1-1 and FvSPR1-2 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, FvSPR1-1 and FvSPR1-2 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), FvSPR1-1 (XM_004297177) and AtSPR1-2 (At1g69230), and FvSPR1-2 (XM_004299243) and AtSPR1-2 (At1g69230), respectively. We amplified the FvSPT (6600 bp), FvSPR1-1 (9647 bp) and FvSPR1-2 (2443 bp) genes, including their promoters. After constructing the FvSPT::pGWB401, FvSPR1-1::pGWB401 and FvSPR1-2::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, FvSPR1-1 and FvSPR1-2 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/FvSPR1-1/FvSPR1-2-complemented plants had significantly longer siliques (12.8-13.3 mm). Relative to the spr mutant, silique length was significantly increased only in the FvSPR1-1-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 5). 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-
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 *FvSPR1-1-* and *FvSPR1-2*-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-*, *FvSPR1-1-* and *FvSPR1-2*-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 *FvSPR1-1-* and *FvSPR1-2*-complemented, WT and *spr* mutant plants can be seen in Figure 7 and

**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).
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 Atspt2 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 Debrecen, 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 Dobrászki (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

DOI: 10.18380/SZIE.COLUM.2020.7.1.23
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 tortifolia 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

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 gynoecium 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

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 Rebollos-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

DOI: 10.18380/SZIE.COLUM.2020.7.1.23
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/tpc.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 pcap/cjp020
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-climacteric 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