Transcriptional and hormonal regulation of petal and stamen development by STAMENLESS, the tomato (Solanum lycopersicum L.) orthologue to the B-class APETALA3 gene

Muriel Quinet1,*, Gwennaël Bataille1, Petre I. Dobrev2, Carmen Capel3, Pedro Gómez3,†, Juan Capel3, Stanley Lutts1, Václav Motyka2, Trinidad Angosto3 and Rafael Lozano3

1 Groupe de Recherche en Physiologie végétale, Earth and Life Institute, Université catholique de Louvain, Croix du Sud 4–5 bte L7.07.13, B-1348 Louvain-la-Neuve, Belgium
2 Institute of Experimental Botany, Academy of Sciences of the Czech Republic, Rozvojová 263, Prague 6, 16502, Czech Republic
3 Centro de Investigación en Biotecnología Agroalimentaria (BITAL), Universidad de Almería, 04120 Almería, Spain

* To whom correspondence should be addressed. E-mail: muriel.quinet@uclouvain.be
† Present address: Instituto de Investigación y Formación Agraria y Pesquera, Autovía del Mediterráneo, 04745 La Mojonera, Almería, Spain

Received 2 January 2014; Revised 31 January 2014; Accepted 10 February 2014

Abstract

Four B-class MADS box genes specify petal and stamen organ identities in tomato. Several homeotic mutants affected in petal and stamen development were described in this model species, although the causal mutations have not been identified for most of them. In this study we characterized a strong stamenless mutant in the tomato Primabel cultivar (sl-Pr), which exhibited homeotic conversion of petals into sepals and stamens into carpels and we compared it with the stamenless mutant in the LA0269 accession (sl-LA0269). Genetic complementation analysis proved that both sl mutants were allelic. Sequencing revealed point mutations in the coding sequence of the Tomato APETALA3 (TAP3) gene of the sl-Pr genome, which lead to a truncated protein, whereas a chromosomal rearrangement in the TAP3 promoter was detected in the sl-LA0269 allele. Moreover, the floral phenotype of TAP3 antisense plants exhibited identical homeotic changes to sl mutants. These results demonstrate that SL is the tomato AP3 orthologue and that the mutant phenotype correlated to the SL silencing level. Expression analyses showed that the sl-Pr mutation does not affect the expression of other tomato B-class genes, although SL may repress the A-class gene MACROCALYX. A partial reversion of the sl phenotype by gibberellins, gene expression analysis, and hormone quantification in sl flowers revealed a role of phytohormones in flower development downstream of the SL gene. Together, our results indicated that petal and stamen identity in tomato depends on gene–hormone interactions, as mediated by the SL gene.

Key words: APETALA3, B-class gene, flower morphogenesis, hormone regulation, Solanum lycopersicum, STAMENLESS, tomato.

Introduction

Extensive genetic and molecular studies in several model plant species have led to a broadly accepted model of flower development based on the combined activity of three functions that determine floral organ identity in the so-called ABC model (Coen and Meyerowitz, 1991). The expression of class A genes in the first floral whorl specifies sepal identity, class A and B genes combined in the second whorl specify petal identity, class B and C genes in the third whorl determine stamen identity, and C-class genes in the fourth whorl specify carpel identity. Additional regulatory functions have been added, such as class D genes that are essential for ovule identity (Colombo et al., 1995) and class E genes that...
are necessary for proper floral organ identity in the different whorls (Pelaz et al., 2001). In Arabidopsis, there are two A-class genes, known as APETALA1 (API) and APETALA2 (AP2), two B-class genes known as APETALA3 (AP3) and PISTILLATA (PI), and one C-class gene named AGAMOUS (AG). The E-function genes are SEPALLATA1 (SEPI), 2, 3, and 4 (Pelaz et al., 2001). All of these genes, with the exception of AP2 (and its homologues), are MADS box genes (Theissen et al., 2000), which comprise a broad family of eukaryotic genes that encode transcription factors containing a highly conserved DNA-binding domain (MADS domain). Functional roles of many MADS box genes seem to be conserved among flowering plants, although some homologous genes in different plant species have recruited novel functions through evolutionary mechanisms currently under study (see review of Smaczniak et al., 2012).

Several homologues of Arabidopsis homeotic genes are known in tomato (Solanum lycopersicum L.). MACROCALYX (MC) represents the A class and is involved in the development of sepals in the first whorl and in inflorescence determinacy (Vrebalov et al., 2002). Class B MADS box genes are represented by four genes. In tomato, as in other species belonging to the 'core eudicots' clade, a gene-duplication event in the AP3 gene subfamily led to two paralogous members (Hernandez-Hernandez et al., 2007), namely, the Tomato MADS box gene 6 (TM6) (syn. TDR6; Busi et al., 2003; Pnueli et al., 1991) and the Tomato APETALA3 (TAP3) gene (syn. SIDEF, LeAP3; Kramer et al., 1998; de Martino et al., 2006). A mutation in TAP3 and the silencing of TM6 both resulted in the conversion of stamens into carpels and a more or less severe conversion of petals into sepals (de Martino et al., 2006). Two PI homologues were also identified, namely Tomato PISTILLATA (TPI; de Martino et al., 2006) (syn. SIGL02; Mazzucato et al., 2008) and Solanum lycopersicum GLOBOSA (SIGLO; Mazzucato et al., 2008) (syn. SIGL01, LeP1, TPIB; Leseberg et al., 2008; Geuten and Irish, 2010). Both TPI- and TPIB-silenced plants showed aberrant carpelloid stamens while petals appeared as wild type (Geuten and Irish, 2010). Tomato C-class gene TOMATO AGAMOUS 1 (TAG1) has been identified for its role in the specification of stamen and carpel identities (Pnueli et al., 1994a). In tomato, the two SEP-like genes (E-class) Tomato MADS box gene 5 (TMS) (Pnueli et al., 1994b) and Tomato AGAMOUS-LIKE gene 2 (TAGL2) (syn. TM29; Ampomah-Dwamena et al., 2002; Busi et al., 2003) have been described on the basis of their expression patterns and down-regulated phenotypes. Other MADS box genes expressed during tomato reproductive development have been isolated (Busi et al., 2003). Tomato MADS box gene 4 (TM4) (syn. TDR4; Pnueli et al., 1991; Busi et al., 2003) is homologous to FRUITFULL (FUL) (Lozano et al., 2009). The nucleotide sequences of TAGL1 (syn. ARLEQUIN (ALQ)) and TAGL11 genes show a high similarity to the Arabidopsis D-class genes SHATTERPROOFI (SHPI, AGL1) and SEEDSTICK (STK, AGL11), respectively (Busi et al., 2003; Vrebalov et al., 2009; Giménez et al., 2010), and TAGL2 and TAGL12 share sequence homologies with Arabidopsis AGL2 and AGL12, respectively (Busi et al., 2003).

Several mutants exhibiting partial or complete homeotic transformations in the second and third floral organ whorls have been described in tomato, but the underlying genes have not been identified so far. Hafén and Stevenson (1958) described five stamenless (sl) mutants with more or less severe phenotypes and proposed that they are members of an allelic series. However, two allelic series were represented among sl mutants as analysed by Nash et al. (1985). The most investigated mutants for which petal and stamen identity were affected were sl-2 (Sawhney and Greyson, 1973a, 1973b; Sawhney, 1983), sl (Gómez et al., 1999), and green pistillate (gpi) (syn. pi-2, pistillate 2) (Rasmussen and Green, 1993). The petals are nearly normal in the sl-2 mutant, whereas the stamens are twisted and distorted, bearing naked ovules (Sawhney and Greyson, 1973a). The sl mutant shows sepaloid petals and stamens being replaced by carpels in the third whorl (Bishop, 1954; Gómez et al., 1999). The gpi mutant shows a strong and consistent homeotic transformation of petals into sepals and of stamens into carpels (Rasmussen and Green, 1993). Temperature conditions and plant growth regulators affect the development of sl-2 and sl mutants (Sawhney, 1983; Rastogi and Sawhney, 1990; Singh et al., 1992; Gómez et al., 1999). Low temperatures, as well as gibberellic acid 3 (GA3), result in a reversion of the mutant phenotype in both genotypes. In contrast, sl-2 plants grown at high temperatures or treated with indole-3-acetic acid (IAA) possess carpel-like organs in place of twisted stamens (Sawhney and Greyson, 1973b; Sawhney, 1983). Moreover, the sl-2 flower phenotype was associated with changes in endogenous hormonal contents (Sawhney, 1974; Rastogi and Sawhney, 1990; Singh et al., 1992; Singh and Sawhney, 1998). Despite the physiological characterization of a number of these stamenless mutants, detailed information regarding the cloning and molecular nature of mutations responsible for sl mutants has not been published.

The link between floral homeotic genes and phytohormone pathways was recently addressed (reviewed Chandler, 2011). Genomic studies in Arabidopsis demonstrated that homeotic proteins bind thousands of target sites in the genome and regulate, among other things, the expression of various proteins involved in hormone biosynthesis and signalling (Kaufmann et al., 2009; Ito, 2011). The ways in which hormones contribute to the development of each organ is partly known in Arabidopsis; stamen development is reliant on almost all hormones, petal development is affected by gibberellins (GAs), auxins, and jasmonic acid (JA), and gynoeccium development is predominantly regulated by auxins (Chandler, 2011). Hormones control development by complex interconnected webs of cross-regulation, although examples of hormone crosstalk in floral organ development are currently not extensive (Chandler, 2011).

Our aim in this paper is to increase the otherwise fragmentary knowledge of flower morphogenesis control in tomato by identifying the mutations responsible for the sl phenotypes and investigating how STAMENLESS (SL) interacts with floral meristem identity genes and hormones to specify petal and stamen in tomato. We characterized for the first time the sl mutant identified in the Primabel cultivar (sl-Pr) (Philouze,
1991) showing a strong phenotype and compared it to the previously described sl mutant in the LA0269 accession (sl-LA0269) (Gómez et al., 1999). Recently, the SL locus has been suggested to be the tomato orthologue of the B-function DEFICIENTS (DEF) gene of Antirrhinum majus (Gómez et al., 1999; Mazzucato et al., 2008), although definitive evidence had not been provided to date. We confirm this orthologue’s role by showing that the sl mutations described in Primabel and LA0269 backgrounds correspond to different alleles of the SL locus, which was previously named TAP3 by de Martino et al. (2006). To understand the genetic and hormonal regulation of SL further, we investigated the expression of flower morphogenesis genes and the impact of gibberellins and auxin applications on inflorescence development in sl-Pr. Moreover, we quantified phytohormones in the sl-Pr mutant during flower development to highlight their role in flowers and, particularly, in petal and stamen development.

Materials and methods

Plant material and growth conditions

Seeds from tomato (S. lycopersicum Mill.) cv. Primabel (Pr) and its isogenic stamenless mutant (sl-Pr; Philouze, 1991) were obtained from the French National Institute for Agricultural Research (INRA; Montfavet, France). The seeds of the stamenless (sl-LA0269, LA0269) mutant were kindly provided by the Tomato Genetics Resource Center (University of California, Davis, CA, USA). In Louvain-la-Neuve (Belgium; 50°39′ 95″ N, 4°34′ 30″ E), seeds were germinated at 25 °C in peat compost and seedlings were transplanted to 15 cm pots filled with the same compost, grown in a heated glasshouse with an average temperature of 20 ± 8 °C, and subjected to extra lighting provided by Philips HPLR 400 W bulbs to make a 16-h-long day at a minimum of 150 μmol·m⁻²·s⁻¹ irradiance over a range of 400–700 nm. In Almeria (Spain; 36°50′ 17″ N, 2°27′ 35″ W), seeds were germinated in peat compost and seedlings were directly transplanted to 30 m-long coconut fibre containers and grown under natural plastic greenhouse conditions (average temperature 20 ± 10 °C under approximately 14h natural light). Plants were periodically fertilized with an NPK nutrient solution for which the composition depended on the fertilization requirements for each growing condition.

The sl-Pr and sl-LA0269 mutants were compared in both conditions under different seasons. Histological sections, hormonal treatments, hormonal quantifications, and gene expression analysis were realized on Pr and sl-Pr plants grown in Louvain-la-Neuve under spring conditions while plants used for TAP3 expression analysis (sl-Pr, sl-LA0269, and TAP3-silencing lines) and in situ hybridization (sl-LA0269) were grown in Almería under autumn conditions.

Histological studies

Flower buds of Pr and sl-Pr at different stages of development (from stage 2 to 9; Brukhin et al., 2003) were fixed in 70% ethanol/acetic acid/formaldehyde (18:1:1, by volume; FAA), dehydrated in a graded ethanol series, embedded in paraffin, and sectioned at 5 μm. Serial longitudinal and transversal sections were stained with haematoxylin-fast green and observed with a light microscope.

Pollen viability was estimated according to Alexander (1969). Ten flowers and at least 200 pollen grains per stamen were analysed per treatment.

Hormonal treatments

The apical meristems of 20-day-old Pr and sl-Pr plants (before morphogenesis of the first inflorescence) were treated with 1 mM IAA or 0.5 or 1 mM of GA3 with 0.02% Tween 20. Control plants were either not treated or treated with water and 0.02% Tween 20. A piece of cotton wool was placed on the shoot apex and saturated with 250 μl of solution twice a week for 3 weeks (during morphogenesis of the first inflorescences).

Hormonal quantification

Concentrations of the endogenous polyamines (PAs) and phytohormones including cytokinins (CKs), auxins (IAA), GAs, salicylic acid (SA), JA, brassinosteroids (Br), abscisic acid (ABA), ethylene precursor 1-aminoacyclopropane-1-carboxylic acid (ACC), benzoic acid (BzA), and their metabolites were determined in the inflorescences of Pr and sl-Pr plants at three developmental stages: flower buds <5 mm (stages 4–9; Brukhin et al., 2003), green flowers before anthesis 5–8 mm length (stages 10–13), and flowers at anthesis (stage 20). Phytohormones were extracted with methanol/formic acid/water (15:1:4, by volume) from liquid nitrogen-frozen and homogenized tissues and were subsequently purified by using the dual-mode solid-phase method according to Dobrev and Kaminek (2002). Two phytohormone fractions were obtained; fraction A contained the acidic and neutral hormones (auxins, GAs, SA, JA, Brs, ABA, BzA) and fraction B contained the basic hormones (CKs, ACC). The hormonal analysis and quantification were performed by HPLC (Ultimate 3000, Dionex, Sunnyvale, CA, USA) coupled to a hybrid triple quadrupole/linear ion trap mass spectrometer (3200 Q TRAP; Applied Biosystems, Foster City, CA, USA) using a multilevel calibration graph with H-labelled internal standards (as described in detail by Dobrev and Vankova, 2012; Dijilanov et al., 2013).

Free PAs were extracted twice with 4% HClO₄ (v/v) at 4 °C and derivatized by dansylation as described by Lefèvre et al. (2001). Samples were re-suspended in methanol, filtered (Chromafil PES-45/15, 0.45 μm; Macherey-Nagel, Düren, Germany) and injected onto a Nucleodur C18 Pyramidal column (125×4.6 mm internal diameter, 5 μm particle size; Macherey-Nagel) maintained at 40 °C. Analyses were performed by a Shimadzu HPLC system coupled to a RF-20A fluorescence detector (Shimadzu, s-Hertogenbosch, The Netherlands) with an excitation wavelength of 340 nm and an emission wavelength of 510 nm. The mobile phase consisted of a water/acetonitrile gradient from 40 to 100% acetonitrile and the flow was 1.0 ml min⁻¹.

Molecular identification of sl mutations

For the mutation identification, three independent PCR fragments—corresponding to the complete coding sequence—of TAP3, TM6, TPI, and TP1B amplified from Pr and sl-Pr cDNA were obtained by using the primers listed in Table 1 and then cloned into pCRII-TOPO (TOPO TA Cloning Kit; Invitrogen, Carlsbad, CA, USA) and sequenced. The sl-Pr mutation in the TAP3 coding sequence resulted in the generation of a RsI restriction site and a cleaved amplified polymorphic sequence (CAPS) marker was designed using the PCR conditions and primers SlMutF and R as described in Table 1 [722 bp fragment in wild type (WT), 522 + 200 bp fragments in sl-Pr] to identify heterozygous plants for sl-Pr.

The promoter sequences flanking the TAP3 transcribed sequences in the sl-LA0269 mutant allele were isolated by anchor-PCR as described by Schupp et al. (1999) with minor modifications. Cloning experiments were repeated twice with different sets of gene-specific primers and restriction enzymes to corroborate the specificity of the cloned sequences. Additionally, PCR experiments were performed with specific primers for the sl-LA0269 promoter sequence in combination with primers for the TAP3 coding sequence to confirm the results obtained by anchor-PCR. Heterozygote plants were identified by PCR using Sl-MutF and Sl-both primers (1019 bp fragment corresponding to the sl-LA0269 allele) and the Sl-WTF and Sl-both primers (1185 bp fragment corresponding to the WT allele), respectively (Table 1).
Table 1. List of primers and amplification conditions used for semi-quantitative RT-PCR expression analysis, coding sequence sequencing and cleaved amplified polymorphic sequence (CAPS) marker development

| Gene name | GenBank accession no. | Primer sequences | $T_{m}$ | No. of cycles |
|-----------|----------------------|------------------|---------|--------------|
| ACTIN     | U60480               | actF (ATTCCTCTAGTTTGGTCTGCT) | 55 °C  | 28           |
|           |                      | actR (TCCACAATCCAGGTAATGCTGCT) |         |              |
| TAP3      | DQ674532             | TAP3F (ATGGGCTCTGTGGAAGATCCAG) | 55 °C  | 28           |
|           |                      | TAP3R (ACAGAGCAGGATTCAGGACAAAG) | 55 °C  | 28           |
| TM6       | AY098734             | TM6F (AGAAGGATTTTGAGGATGCAAAAG) | 55 °C  | 28           |
|           |                      | TM6R (TCCAGGTGACGTAGTATGCAAC) | 55 °C  | 28           |
| TPI       | DQ674531             | TPIF (TGGGAAGAGGTTAAATAGAG) | 50 °C  | 28           |
|           |                      | TPIR (AGCAGAGGATTTTGAGGATGCAAAAG) | 50 °C  | 28           |
| TPIB      | XM00424154           | TPIBF (GAATCTCTGTCTACCTTTTG) | 55 °C  | 30           |
|           |                      | TPIBR (TGCTGTCATCTTCAGTGTC) |         |              |
| TAG1      | AY098733             | TAG1F (ACGCGTAAAGGCTTCTTTTG) | 55 °C  | 28           |
|           |                      | TAG1R (ATGAAGACTCTGGGCTGCAAAAG) | 55 °C  | 28           |
| MC        | AF448521             | MCOR (CTCTCTCTGTCTGCTTACCTC) | 55 °C  | 29           |
| TM4       | AY098732             | TM4F (CTGGAAAACGTCGATCTGGTT) | 60 °C  | 29           |
|           |                      | TM4R (CTCTCTCTGTCTGCTTACCTC) | 60 °C  | 29           |
| TM5       | X60758               | TM5F (ACAGGGAATGGAGCCTTCTGG) | 60 °C  | 29           |
|           |                      | TM5R (CTCTCTCTGTCTGCTTACCTC) | 60 °C  | 29           |
| TAGL2     | AY098738             | TAGL2F (GCCCGGAGGACATGGACTGAAG) | 55 °C  | 28           |
|           |                      | TAGL2R (ATGGATCTCAAATTGGCAGAAG) | 55 °C  | 28           |
| TAP3 (CAPS marker) | DQ674532 | TAGL2F (GCCCGGAGGACATGGACTGAAG) | 55 °C  | 28           |
|           |                      | Sl bothR (GGACAGATTCGATGCGGGACT) | 60 °C  |              |
| TAP3 (sl-LA0269) | DQ674532 | Sl bothR (GGACAGATTCGATGCGGGACT) | 60 °C  |              |

Agrobacterium-mediated transformation of tomato plants

Silencing lines were generated by expressing an antisense TAP3 gene construct in tomato cv. Moneymaker plants. For this purpose, a 412 bp cDNA fragment was amplified from the pPG06 plasmid with primers sl5′B forward (AAACCAAAACAAATGGCAAAGTGA) and sl3′B reverse (AGTTTCAATCTGATTGCCAATCACC). This fragment was cloned in an antisense orientation between the BamHII and KpnI restriction sites of a pROK II binary vector (Baulcombe et al., 1986) under the control of a cauliflower mosaic virus 35S promoter (CaMV 35S). The plasmid was subsequently electroporated into Agrobacterium tumefaciens LBA 4404 strain for further use in genetic transformation experiments as described by Ellul et al. (2004). As a consequence, 23 kanamycin-resistant lines were generated from tissue culture. These lines were checked to determine their ploidy levels by flow cytometry (Ellul et al. 2004) and to confirm the presence of the transgene by standard PCR assays. In addition, the TAP3 expression levels in antisense lines were analysed by reverse transcription PCR as described in the next paragraph. A consequence of this analysis was the detection of TAP3 expression in small RNA and cDNA fraction.

Gene expression analyses

Flowers of Pr and sl-Pr plants were sampled at two developmental stages: green flower buds of ≈5 mm length (stages 9–11; Brukhin et al., 2003) and flowers at anthesis (stage 20). Inflorescences with one or two flowers at anthesis of Pr and sl-Pr were also collected after the different hormonal treatments (IAA 1 mM, GA3 1 mM, water). Samples of different plants at stage 4 were pooled. Total RNA was prepared from 150 µg of material using the TRI Reagent Solution (Ambion, Austin, TX, USA) and DNase treatments were realized using RQ1 RNase-free DNase (Promega, Leiden, The Netherlands) according to the manufacturer’s instructions. Reverse transcription was performed with 1 µg of total RNA using the RevertAid H Minus First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany) by following the manufacturer’s instructions. At least three independent PCR amplifications were conducted for each gene using the primer pairs, annealing temperatures, and number of cycles presented in Table 1. Expression differences were analysed by gel densitometry using ImageJ software and expressed as relative values compared to actin expression (peak size of target gene/peak size of actin). Gene expression analyses were repeated twice on two independent cultures and gave similar results.

For in situ hybridization experiments, inflorescences and flower buds of WT and sl-LA0269 at different stages of development (up to stage 8; Brukhin et al., 2003) were sampled. Tissue preparation of sl-LA0269 mutant, sectioning, and transcript detection were performed as described by Lozano et al. (1998). The TAP3 cDNA in the pGEM-T vector was used as a template to synthesize digoxigenin-labeldd sense and antisense RNA probes with T7 and SP6 RNA polymerases, respectively, according to the DIG RNA Labelling Kit (Roche Applied Science) instructions. To produce a negative control, sense RNA probes were hybridized with the same sections and no signals were observed under the given hybridization and detection conditions.

Statistical analysis

Normality tests were performed and no further transformation of the raw data was required. An ANOVA II (SAS 9.2) was performed to evaluate the genotype and floral stage effects on hormonal concentrations and on gene expression. Differences between means were scored for significance according to Tukey’s test. Allelism cross segregations were verified by $\chi^2$ test.
Results

Stamenless mutants exhibit homeotic conversions that affect petals and stamens

At anthesis, WT tomato flowers usually consisted of six green sepals, six yellow petals, and a staminal cone made of six fused yellow stamens surrounding a gynoecium made of six fused green carpels (Fig. 1A, E). The sl-Pr mutant developed flowers with a first whorl having (usually) six green sepals, a second whorl composed of approximately six shorter sepals arising from the homeotic conversion of petals and, because of the homeotic conversion of the third whorl stamens into carpels, a gynoecium (which included the combination of whorls 3 and 4) made from the fusion of 10–12 green carpels (Fig. 1B). Small non-fused carpels and visible ovules were observed in the third whorl of approximately 10% of the sl-Pr mutant flowers (Fig. 1C). The floral phenotype of the sl-LA0269 mutant was weaker than that of sl-Pr as petals showed a normal-like yellow colour and the conversion of stamens into carpels was not always complete, leading to the development of non-fused transformed carpels in the third whorl (Fig. 1E). Depending on the growing conditions, heterozygous plants for sl-Pr and sl-LA0269 were either not distinguishable from the WT or displayed some stamen defects. In the Belgian conditions, most of the heterozygous plants resembled the WT and some plants displaying a fusion of some stamens with the style were observed (Fig. 1D). Under Spanish growing conditions, heterozygous plants showed more stamen defects, mainly for sl-LA0269. Indeed, heterozygous sl-LA0269 plants exhibited a phenotype that was intermediate between the mutant and WT plants and characterized by distorted and short carpelloid stamens (Fig. 1E), which concurred with the description by Gómez et al. (1999). In addition, both mutants developed a variable percentage of parthenocarpic fruits made up of the carpels of whorls 3 and 4, depending on the growing conditions. The transformed organs from whorls 2 and 3 remained attached to the fruits in both mutants (Fig. 1G, H compared to Fig. 1F). Histological sections performed during flower morphogenesis demonstrated that the initiation of the floral organ primordia occurred in a similar fashion to the sl-Pr mutant than in the WT background (Fig. 2A–D) and differences were only visible later during floral organ differentiation and development (Fig. 2E–H), as was described by Gómez et al. (1999) for sl-LA0269. The sl-Pr and sl-LA0269 mutations did not affect vegetative development, flowering time, or inflorescence architecture.

Given the phenotypic similarities between the sl-Pr and sl-LA0269 mutants, allelism tests were performed by crossing heterozygote sl-LA0269 or sl-Pr male parents with sl-Pr or
sl-LA0269 mutant female parents, respectively. As a result, about half of the F1 plants, those carrying the sl-LA0269 and sl-Pr alleles simultaneously, showed a mutant phenotype, which confirmed that both mutations corresponded to SL locus alleles ($\chi^2 = 0.039, P = 0.843$).

**stamenless mutants were affected in the TAP3 gene**

To identify the gene affected by the sl mutation, the coding sequences of known tomato class B genes (TM6, TAP3, TPI, TPIB) were sequenced in Pr and sl-Pr. In comparison to the Pr control background, the TM6, TPI, and TPIB coding sequences of sl-Pr mutant plants were not different (data not shown), and the TAP3 cDNA had two point mutations (Fig. 3A). The first was an A-to-T substitution in position 378 resulting in an aspartic acid instead of a glutamic acid (Fig. 3). The second change, and surely the most important one, was a nucleotide deletion in position 380 of the TAP3 coding sequence of sl-Pr resulting in a frameshift mutation (Fig. 3A). The mutated allele is expected to encode a truncated protein of 161 amino acids because a stop codon in the coding sequence of sl-LA0269, resulting in a frameshift mutation in the WT promoter and three different small sequences of 315, 518, and 270 bp, which were not detected in the WT fragment (Fig. 3A). In the 518 bp sequence we found an EcoRI restriction site that generates the restriction fragment length polymorphism (RFLP) described as being tightly linked to the SL locus (Gómez et al., 1999). Interestingly, these three sequences were homologous to others repeated in different locations of chromosome 4. Given that TAP3 maps within chromosome 4, it is likely that a genomic rearrangement affecting the promoter region of this gene may be responsible for the mutant phenotype observed in the sl-LA0269 mutant.

TAP3 expression was then compared in mutants, heterozygotes, and WT flowers during their development to see if the observed phenotypes were related to a decrease or absence of TAP3 transcript abundance. Whatever the genotype, TAP3 expression was detected in 0.5 mm-long flower buds and in flowers at anthesis (Fig. 4A). Both sl mutations reduced TAP3 expression level compared to WT (Fig. 4A). In sl-Pr, TAP3 transcript level was significantly reduced in both flower buds and flowers at anthesis while the expression decrease was only significant in flower buds in the sl-LA0269 mutant. The heterozygote plants showed an intermediate TAP3 expression level between mutant and WT plants although the difference was not significant. Given that the chromosome rearrangement found in the TAP3 promoter region of sl-LA0269 mutant allele likely encodes a functional SL protein, a feature which was not expected for the truncated protein detected in the sl-Pr allele, we analysed in depth the TAP3 expression profile in the sl-LA0269 mutant by in situ hybridization. The TAP3 gene was expressed in the sympodial and floral meristems, as well as in young flower buds of WT inflorescences (Fig. 4B). During flower morphogenesis, TAP3 transcripts were detected in the centre of the flower bud where the three inner floral organ whorls primordia would be subsequently initiated (Fig. 4C) and then in the organ primordia of the second and
third floral whorls (Fig. 4D). Later, during floral organ differentiation, TAP3 was strongly expressed in the stamens and some expression was observed in the ovule primordia when the carpels just fused (Fig. 4F). This pattern of TAP3 expression in WT flowers is similar to what has been previously reported (de Martino et al., 2006; Mazzucato et al., 2008). In sl-LA0269 inflorescences, TAP3 was weakly expressed in the sympodial and floral meristems, whereas in the young flower buds its transcripts were detected in the meristem domains giving rise to second- and third-whorl primordia (Fig. 4E). In young flowers, TAP3 transcripts were detected at slight levels in transformed stamens (third whorl), and in ovules primordia when the carpels began to fuse (Fig. 4G).

To corroborate that the sl mutants found at the TAP3 gene and the decrease of transcript level were responsible for the mutant phenotypes, sl-Pr and sl-LA0269 mutants were compared with TAP3 antisense lines varying in silencing level. All TAP3 antisense lines showed abnormal phenotypes affecting flower development, most of which resembled those of the sl-LA0269 mutant, whereas the stronger ones were similar to those of the sl-Pr mutant. At anthesis, flowers from TAP3 antisense lines showed a variable degree of homeotic conversion both for petals into sepals in the second whorl and stamens into carpels in the third whorl (Fig. 5A). Interestingly, phenotype features of the antisense line numbers 28 (extreme conversions) and 32 (partial conversions) indicated that severity of homeotic changes were correlated to the silencing level of the TAP3 gene (Fig. 5B). In addition, parthenocarpic fruits to which carpeloid stamens remained attached were observed during antisense fruit ripening (Fig. 5D, E compared to Fig. 5C). Phenotypic alterations affecting the vegetative growth were not found.

Transcriptional regulation of floral organ identity genes in the stamenless mutants

We investigated whether the strong sl-Pr mutation affected other floral organ identity genes by analysing the expression of tomato ABC genes in WT and sl-Pr inflorescences during flower development (Fig. 6). The most significant differences concerned the A-class MC gene. The MC transcript level was significantly higher in mutants than in WT plants in both young flower and flowers at anthesis (Fig. 6). The expression level of the B-class genes TPI, TPIB, and TM6
Fig. 4. Expression of STAMENLESS (SL, TAP3) during flower development in stamenless tomato mutants. (A) Semi-quantitative RT-PCR expression in the WT plants, heterozygote plants (ht) and mutant (sl) plants in green flower buds of ≈5 mm length (fb) and flowers at anthesis (fa) for both sl-Pr and sl-LA0269 mutations. Actin transcripts were used as a PCR control. TAP3 relative expression level compared to actin was analyzed by gel densitometry. Values followed by a same letter (a, b) are not statistically different (P < 0.01). (B–G) Tissue localization of transcripts by means of in situ hybridization (SL antisense probe) in WT and sl-LA0269 mutant. (B) WT young inflorescence carrying flower buds and floral meristem, (C, D) flower buds from WT plants initiating floral organ primordia, (E) sl mutant young inflorescence carrying several flower buds initiating floral organ primordia and a floral meristem. (F), WT flower just after fusion of the carpels, (G) sl mutant flower before fusion of the carpels. 1–4, floral primordia or organs of first, second, third, or fourth floral whorls; F, flower; FM, floral meristem; G, gynoecium; o, ovules; p, petal; s, sepal; st, stamen; sym, sympodial meristem.

Fig. 5. Floral and fruit phenotypes of the WT tomato and TAP3 antisense lines. (A) WT flower (left) and TAP3 antisense flowers (T32 and T28) showing different degrees of conversion for petals into sepals and stamens into carpels. (B) SL (TAP3) expression level in TAP3 antisense lines relative to WT (C). The phenotype of the antisense lines in (A) was correlated to the silencing level of the TAP3 gene in (B) (grey arrows). (C–E) WT fruits with the sepals of whorl 1 remaining attached to the fruit (C) and parthenocarpic fruits of TAP3 antisense lines T32 (D) and T28 (E) with the sepals of whorl 1 and modified organs of whorls 2 and 3 remaining attached to the fruit depending on the phenotype.
was not significantly affected by the sl-Pr mutation during flower development (Fig. 6). The transcript level of the C-class (TAG1) and E-class (TM5 and TAGL2) genes here analysed were also similar in WT and mutant plants (Fig. 6).

Effects of GA and IAA hormone treatments on the stamenless flower phenotype

Because GA$_3$ could play a role in the sl-LA0269 mutant phenotype reversion (Gómez et al., 1999), we tested whether the stronger sl-Pr phenotype could also be reverted by hormonal applications to highlight the link between SL and phytohormones in petal and stamen development. In Pr plants, GA$_3$ and IAA treatments did not modify floral organ identity (Table S1): GA$_3$-treated Pr stamens and carpels (Fig. 7 G, H) were similar to the control ones (Fig. 7 A–C) and the same happened in IAA-treated Pr flowers. In the same way, no floral organ modifications were observed in sl-Pr mutant plants in response to 0.5mM GA$_3$ compared to controls (Table S1). However, a partial reversion of three to six carpels per flower into pseudo-stamens was observed in the third whorl of around half the flowers treated with 1mM GA$_3$ (Fig. 7I, J compared to Fig. 7 D, E). Most reverted flowers produced pollen grains, although pollen viability was lower (52.3%) than it was in WT flowers (91.2%). Moreover, non-fused carpelloid organs bearing external ovules were observed on whorl 3 in around half the 1mM GA$_3$-treated sl-Pr flowers. Mutant plants treated with IAA produced twisted gynoecium and whorl 3 non-fused carpelloid organs with external ovules were observed in a third of the plants (Fig. 7K) but the abnormal third-whorl structures never produced pollen. We also observed ABC gene expression in WT and sl-Pr inflorescences after hormonal treatment, but neither the 1mM IAA nor the 1mM GA$_3$ treatment markedly affected gene expression (Fig. 7L).

Endogenous phytohormone concentrations in stamenless mutant

To further investigate whether the sl mutation affects phytohormone content, we quantified endogenous phytohormone concentrations in the strong sl-Pr mutant during inflorescence development. Most hormone concentrations varied with

---

**Fig. 6.** Semi-quantitative RT-PCR expression analysis of tomato floral organ identity genes in Pr and sl-Pr mutant flowers along with their development: green flower buds of ~5mm length (fb) and flowers at anthesis (fa). Actin transcripts were used as a PCR control. Significant differences between genotypes according to ANOVA are indicated (P < 0.001***). For observed significant differences, relative expression levels in relation to actin are graphically presented. MACROCALYX (MC) relative expression level was analysed by gel densitometry. On the graph, matching letters (a, b) are not statistically different (P < 0.001).

**Fig. 7.** Hormonal treatment impacts on the flowers of the stamenless (sl-Pr) tomato mutant. (A–C) Water-treated (control) Pr flowers with normal (B) gynoecium and (C) stamens. (D–F) sl-Pr mutant flowers treated with water (control) showing (D) sepals in the second floral whorl and (E) gynoecium resulting from the fusion of the carpels of third and fourth floral whorls, (F) some external ovules could be observed on some non-fused carpels. (G, H) Pr flowers treated with 1mM GA$_3$ with normal (G) gynoecium and (H) stamens. Note that similar pictures were obtained for Pr flowers treated with 1mM IAA. (I, J) sl-Pr mutant flowers treated with 1mM GA$_3$ showing a partial reversion of the third floral whorl organs in (J) stamens. (K) sl-Pr mutant flowers treated with 1mM IAA showing a gynoecium with non-fused carpels and external ovules. ci, non-fused carpel; G, gynoecium; o, external ovules; s, stigma; sm, stamen; st, style. (L) Semi-quantitative RT-PCR expression analysis of tomato floral organ identity genes in WT and sl-Pr inflorescences at anthesis treated with water, 1mM IAA, or 1mM GA$_3$. Actin transcripts were used as the PCR control.
inflorescence stage and were reduced in the sl-Pr mutant (Figs 8 and 9). Indeed, the sl-Pr mutation showed decreased GA and auxin concentrations relative to the WT plants (Fig. 8A, B). Both contents tend to decrease after anthesis while CK content showed an overall increase associated with the flower developmental stage (Fig. 8C–F). The effect of the sl-Pr mutation varied with respect to the CK classes; it reduced the contents of bioactive CKs (free bases and ribosides) and CK glucoc conjugates (Fig. 8C–E) and increased the CK phosphate concentrations in the flower at anthesis (Fig. 8F). With respect to the ethylene precursor ACC we observed a significant reduction in sl-Pr floral buds and pre-anthesis flowers compared to the WT blooms but not in the flowers at anthesis (Fig. 8G). The BzA concentration was higher in sl-Pr relative to Pr in inflorescence buds but the difference was no more visible at later flower developmental stages (Fig. 8H). The JA concentration was clearly enhanced by the sl-Pr mutation at all developmental stages (Fig. 9A). The concentrations of both SA (Fig. 9B) and Brs (Fig. 9C) increased with inflorescence development primarily in the Pr; however, the SA level was

| Fig. 8. Impact of the stamenless (sl-Pr) tomato mutation on endogenous phytohormone content. (A) Gibberellins (GA), (B) auxin; (C) bioactive CKs, (D) CK-N-glucosides, (E) CK-O-glucosides, (F) CK phosphates, (G) ethylene precursor ACC, and (H) benzoic acid (BzA) during flower development (developmental stages: flower buds <5mm length, green flowers before anthesis ≈5–8mm length, flowers at anthesis). Bars = SD; ANOVA II results for the genotype (G) and inflorescence stage (I) factors are presented. |
lowered in the mutant flowers at anthesis compared to the WT (Fig. 9B) while the sl-Pr mutation did not significantly affect Brs content (Fig. 9C). The total ABA content was higher in sl-Pr compared to the WT whatever the developmental stage (Fig. 9D). In general, the PA concentration decreased during inflorescence development (Fig. 9E–H). The spermidine, tyramine, and putrescine concentrations were lower in mutant inflorescences than in WT ones even though the difference was not significant for spermidine in flowers at anthesis (Fig. 9E, F, H), but the overall spermine concentration was not affected by the genotype (Fig. 9G).

**Discussion**

**stamenless mutations affect B-class gene TAP3**

Several tomato mutants have been described with various degrees of petal conversion into sepals and stamens...
into carpels (Hafen and Stevenson, 1958; Nash et al., 1985; Philouze, 1991; Rasmussen and Green, 1993; Gómez et al., 1999), but the underlying genes have not yet been formally identified. Four B-class genes are known in tomato, the AP3 homologues TAP3 and TM6 and the PI homologues TPI and TPIB (Pnueli et al., 1991; Busi et al., 2003; de Martino et al., 2006; Mazzucato et al., 2008; Geuten and Irish, 2010). The SL locus has been suggested as the tomato orthologue of B-function DEF gene of Antirrhinum majus and AP3 of Arabidopsis (Gómez et al., 1999; Mazzucato et al., 2008). Both SL and TAP3 map on the long arm of chromosome 4 while the remaining tomato B-class genes map in different chromosomes; that is, TM6 on chromosome 2, TPI on chromosome 6, and TPIB on chromosome 8 (Khush, 1965; Mazzucato et al., 2008; Olimpieri and Mazzucato, 2008). In this study we identified the mutation in the TAP3 promoter region of the sl-LA0269 mutant and described the TAP3 truncated protein encoded by the TAP3 mutant allele of the sl-Pr mutant genome. We have also proved that both mutations were allelic and evidenced that the phenotype showed by homozygous sl mutant plants were identical, or very similar, to those of TAP3 antisense lines. Moreover, the strong phenotype of the sl-Pr mutant was in accordance with a knock-out mutation in the TAP3 gene. TAP3 loss-of-function mutants indeed showed a complete conversion of petals into sepals and of stamens into carpels while TM6, TPI, and TPIB loss-of-function mutations affected mainly or exclusively stamen identity (de Martino et al., 2006; Geuten and Irish, 2010). Together these results provide clear evidence indicating that the SL locus corresponds to the TAP3 gene of tomato.

The intensity of the sl phenotypes could depend on the TAP3 expression level as suggested by the observed TAP3 antisense lines and the sl-LA0269 mutant and could explain the incomplete dominance observed in the heterozygote plants (Figs 4 and 5). However, even if TAP3 is still weakly expressed in the sl-Pr mutant, it exhibits a strong phenotype similar to that of the TAP3 loss-of-function mutant (de Martino et al., 2006), suggesting that the truncated protein may not be functional as the mutation affected a protein region important for the correct specification of petal and stamen identity. The lost of half of the K box domain of the TAP3 protein in sl-Pr mutant plants most likely affect the capacity of this sl-Pr truncated protein to form multimeric MADS complex since interactions between MADS domain proteins are largely achieved via the K domain (Leseberg et al., 2008). The consequence of the loss of the C-terminal domain of TAP3 truncated protein is not clear since its molecular role is not well understood (Geuten and Irish, 2010).

**Regulation of the ABC genes by STAMENLESS**

Phenotypic and genetic analyses performed in this work indicated that stamenless mutations affected the TAP3 gene. In addition, molecular characterization of SL has revealed it is a key regulator of the development of petal and stamen organs, as expected for an AP3 orthologous gene. It is known that TAP3 protein participates in the formation of multimeric MADS complexes regulating petal and stamen development (de Martino et al., 2006; Leseberg et al., 2008; Geuten and Irish, 2010), although the composition of such complexes and their organ specificity require additional research in tomato plants (Smaczniak et al., 2012). In this paper, we have investigated the interactions between TAP3 and the remaining tomato B-class genes involved in flower development.

According to our results, the sl-Pr mutation did not markedly modify the expression of TM6, TPI, and TPIB in flowers indicating that the mutant flower phenotype is not due to a coordinated misregulation of TAP3, TM6, TPI, and TPIB. Most likely, the sl-Pr mutation must be sufficient to prevent the formation of functional complexes determining petal and stamen identity. Previous studies by de Martino et al. (2006) also showed that a tap3 knock-out mutation did not modify the expression of TM6 and TPI in tomato flowers and that TM6 inactivation did not affect TAP3 and TPI expression. However, Geuten and Irish (2010) reported interactions between B-class genes in tomato floral organs and showed that expression of TPI and TPIB was completely absent in second- and third-whorl organs of tap3 knock-out mutants. They also reported that TAP3 expression increased in TPIB RNAi plants and decreased in TPI RNAi flowers compared to WT and that cross-activation of TPI and TPIB took place in the second-whorl organs (Geuten and Irish, 2010). Additional experiments would be required to explain these differences in the regulatory interactions mediated by TAP3 gene. However, the possibility that TAP3 regulates TPI and TPIB expression in a similar way to AP3 is required for the maintenance of PI gene expression during flower morphogenesis in Arabidopsis (Goto and Meyerowitz, 1994; Honma and Goto, 2000) might be studied in detail.

Among the other floral organ identity genes, our results showed that the sl-Pr mutation did not significantly affect the expression of C- and E-class genes, but it increased MC transcript levels, suggesting that TAP3 may be involved in the repression of class A gene MC. Similarly, Sundström et al. (2006) observed increased API expression in the ap3 mutant of Arabidopsis, and their results suggested a direct API regulation by the AP3/PI dimer. However, a putative regulation of MC by complexes containing TAP3 requires further investigation.

**Hormonal regulation of flower development is mediated by STAMENLESS**

It has been shown that low temperatures and/or GA treatment may partly revert the floral phenotype of the weak allelic stamenless mutants sl-LA0269 and sl-2 (Sawhney 1983; Gómez et al. 1999), suggesting a role for phytohormones in the development of tomato floral organs. We investigated whether hormonal applications could also reverse the complete second- and third-whorl organs conversion of the strong sl-Pr mutant. We indeed observed a partial reversion of the third-whorl organs of the sl-Pr mutant in response to 1 mM GA₃, but a higher concentration was required to induce reversion in sl-Pr relative to sl-LA0269 (Gómez et al., 1999). Moreover, according to previous reports (Sawhney, 1983; Sawhney and Greyson, 1973b), IAA strengthens the sl-2 mutant phenotype. Our results showed that IAA affected the flower development
in the sl-Pr mutant but in a different way compared to sl-2 since it increased the development of abnormal carpels in the third floral whorl in sl-Pr. However, according to our results, neither IAA nor GA\textsubscript{3} strongly affected the ABC gene expression in sl-Pr, suggesting that the partial reversion of the mutant does not rely on an upstream regulation of the floral meristem identity genes by hormones and that phytohormones most likely act downstream of the ABC genes at this floral stage in tomato. However, Yu et al. (2004) showed that GA promotes normal floral organ development in Arabidopsis by partly up-regulating the expression of the B- and C-function floral genes, but it did not regulate A-function genes.

The modified phytohormone profile in sl-Pr inflorescences provides further arguments for a phytohormone role in the floral organ development downstream of the SL gene in tomato. We indeed showed that the sl-Pr mutation reduced GA, IAA, most classes of CKs (bioactive and glycosylated forms), ACC, SA, spermine, and tyramine concentrations and increased JA and ABA in flowers. The lower concentrations of GA and IAA were consistent with the fact that an exogenous application of GA\textsubscript{3} and IAA (in a less extent) may partly rescue the flower phenotype of the mutant. Modified phytohormone profiling was also observed in the sl-2 mutant (Sawhney 1974; Rastogi and Sawhney, 1990; Singh et al., 1992; Singh and Sawhney, 1998). A regulation of phytohormones downstream of the floral organ identity genes has been demonstrated in other species. In Arabidopsis, recent genomic studies showed that floral homeotic proteins bind thousands of target genes and that genes involved in the transcriptional control and hormone functions feature prominently among the early and direct targets (Chandler, 2011; Ito, 2011; Sabolowski, 2010). Mutant and gene characterization studies in Arabidopsis have shown that stamen development is reliant on almost all hormones, petal development is affected by GAs, auxins, and JA, and gynoecium development is predominantly regulated by auxins (Chandler, 2011). Our results argue for a similar hormonal regulation of stamens and petals in tomato. We indeed showed a modification in most phytohormones in response to the sl-Pr mutation during flower development and the abnormal petals and stamens in the sl2 mutant was at least partly related to elevated levels of endogenous PAs, IAA, and ABA and to the reduction in GA levels (Sawhney 1974; Rastogi and Sawhney, 1990Singh et al., 1992; Singh and Sawhney, 1998). A decrease in GA levels was often associated with stamen development defects in tomato. GA-deficient tomato mutants gtb-1 and go-2 exhibit abnormal flowers with arrested anther development (Nester and Zeevart, 1988; Jacobsen and Olszewski, 1991). The silencing of GA20-oxidase 1 was also shown to be detrimental for pollen production (Olimpieri et al., 2011). PA involvement in flower development was also reported in the tomato pat mutant, which exhibits aberrations in stamen development and female fertility and showed changes in the different PA contents relative to the WT (Antognonia et al., 2002). How floral organs identity genes affect genes involved in hormone synthesis and perception remains to be investigated in tomato. Notable progress has been made in understanding phytohormone function in floral development, and it is clear that male development in particular is regulated by multiple hormones in concert. However, further investigation is required to understand the complex network between phytohormone pathways, floral organ identity genes, and flower-building genes in different plant species.

**Supplementary material**

Supplementary material is available at JXB online.

**Supplementary Table S1.** Hormonal treatment impact on the number of organs and on the organ size per whorl in WT tomato (Pr) and in the stamenless (sl-Pr) mutant.

**Acknowledgements**

The authors thank Marie Korecká for invaluable technical support in providing phytohormone analyses. This research was supported by the Belgian ‘Fonds de la Recherche Scientifique’ (crédit aux chercheurs I.5.101.08.F), the Spanish Ministry of Economy and Competitiveness (grant BIO2009-11484), and the Czech Science Foundation (grant P506/11/0774). The authors also thank the Campus de Excelencia Internacional Agroalimentario (CeiA3) for supporting this research collaboration.

**References**

Alexander MP. 1969. Differential staining of aborted and non-aborted pollen. Biotechnic and Histochemistry 44, 117–122.

Ampomah-Dwamena C, Morris BA, Sutherland P, Veit B, Yao JL. 2002. Down-regulation of TM29, a tomato SEPELLATA homolog, causes parthenocarpic fruit development and floral reversion. Plant Physiology 130, 605–617.

Antognonia F, Ghetti F, Mazzucato A, Franceschettia M, Bagnia N. 2002. Polyamine pattern during flower development in the parthenocarpic fruit (pat) mutant of tomato. Physiologia Plantarum 116, 539–547.

Baulcombe DC, Saunders GR, Bevan MB, Harrison BD. 1986. Expression of biologically active viral satellite RNA from the nuclear genome of transformed plants. Nature 321, 446–449.

Bishop CJ. 1954. A stamenless male-sterile tomato. American Journal of Botany 4, 540–542.

Brukhin V, Hernould M, Gonzalez N, Chevalier C, Moursas A. 2003. Flower development schedule in tomato Lycopersicon esculentum cv. sweet cherry. Sexual Plant Reproduction 15, 311–320.

Busi MV, Bustamante C, D’Angelo C, Hidalgo-Cuevas M, Boggio SB, Vaile EM, Zabaleta E. 2003. MADS-box genes expressed during tomato seed and fruit development. Plant Molecular Biology 52, 801–815.

Chandler JW. 2011. The hormonal regulation of flower development. Journal of Plant Growth Regulation 30, 242–254.

Coen ES, Meyerowitz EM. 1991. The war of the whorts, genetic interactions controlling flower development. Nature 353, 31–37.

Colombo L, Franken J, Koetje E, van Went J, Dons HJ, Angenent GC, van Tunen AJ. 1995. The petunia MADS box gene FPB1 determines ovule identity. The Plant Cell 7, 1859–1868.

de Martino G, Pana I, Emmanuel E, Levy A, Vivian F, Irish VF. 2006. Functional analyses of two tomato APETALA3 genes demonstrate diversification in their roles in regulating floral development. The Plant Cell 18, 1833–1845.

Djilianov DL, Dobrev PI, Moyankova DP, VANKova R, Georgieva DT, Gadjosova S, Motyka V. 2013. Dynamics of endogenous phytohormones during desiccation and recovery of the resurrection plant species Haberlea rhodopensis. Journal of Plant Growth Regulation 32, 564–574.

Dobrev PI, Kaminek M. 2002. Fast and efficient separation of cytokinins from auxin and abscisic acid and their purification using mixed-mode solid-phase extraction. Journal of Chromatography A 950, 21–29.

Dobrev PI, Vankova R. 2012. Quantification of abscisic acid, cytokinin, and auxin content in salt-stressed plant tissues. In: Shabala S, Ann Cuin T, eds. Plant Salt Tolerance: Methods and Protocols, Methods in Molecular Biology, vol. 913. Berlin: Springer Science + Business Media, pp 251–261.
