



Flower Development in the Solanaceae

Marie Monniaux, Michiel Vandenbussche

► To cite this version:

Marie Monniaux, Michiel Vandenbussche. Flower Development in the Solanaceae. Flower Development, 2686, Springer US, pp.39-58, 2023, Methods in Molecular Biology, 10.1007/978-1-0716-3299-4_2 . hal-04231574

HAL Id: hal-04231574

<https://hal.science/hal-04231574v1>

Submitted on 18 Oct 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Flower development in the Solanaceae

Marie Monniaux and Michiel Vandenbussche

Laboratoire Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB
Lyon 1, CNRS, INRA, F-69342, Lyon, France.

Running head: Molecular mechanisms of flower development in the nightshades (Solanaceae)
family

Keywords: flower / development / Solanaceae / nightshades / ABC model / floral organ identity /
petunia / tomato / Physalis

17 **Introduction**

18 Flower development is the process leading from a reproductive meristem to a mature flower with
19 fully developed floral organs. This multi-step process is complex and involves thousands of genes
20 in intertwined regulatory pathways; navigating through the FLOR-ID website will give an
21 impression of this complexity and of the astonishing amount of work that has been carried on the
22 topic [1]. Our understanding of flower development mostly comes from the model species
23 *Arabidopsis thaliana*, but numerous other studies outside of Brassicaceae have helped apprehend
24 the conservation of these mechanisms in a large evolutionary context [2–4]. Integrating additional
25 species and families to the research on this topic can only advance our understanding of flower
26 development and its evolution.

27 In this chapter, we will review the particular contribution that the Solanaceae family (Fig. 1)
28 has made to the comprehension of flower development. While many of the general features of
29 flower development (i.e. the key molecular players involved in flower meristem identity,
30 inflorescence architecture or floral organ development) are similar to *Arabidopsis*, our main
31 objective in this chapter is to highlight the points of divergence, and emphasize specificities of the
32 Solanaceae. We will not discuss the large topics of flowering time regulation, inflorescence
33 architecture and fruit development, and we will restrict ourselves to the mechanisms included in a
34 time window after the floral transition and before the fertilization. Moreover, this review will not be
35 exhaustive of the large amount of work carried on the topic, and the choices that we made to
36 describe in large details some stories from the literature are based on the soundness of the functional
37 work performed, and surely as well on our own preferences and expertise.

38 First, we will give a brief overview of the Solanaceae family and some of its specificities.
39 Then, our main focus will be on the molecular mechanisms controlling floral organ identity, for
40 which extended functional work in petunia led to substantial revisions to the famous ABC model.
41 Finally, after reviewing some studies on floral organ initiation and growth, we will discuss floral
42 organ maturation, using the examples of the inflated calyx of the Chinese lantern *Physalis* and
43 petunia petal pigmentation.

44 **The Solanaceae family (nightshades): crops and model species**

45 The Solanaceae, also called nightshades, belong to the Asterids clade in the eudicots and gather
46 more than 2,500 species and 115 genera [5]. The family has a world-wide distribution but the
47 greatest diversity in species is found in south and central america, probably reflecting the origin of
48 the family. The Solanaceae include many agronomically important crops, such as tomato (*Solanum*
49 *lycopersicum*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), petunia (*Petunia x*
50 *hybrida*), pepper (*Capsicum annuum*) or eggplant (*Solanum melongena*) (Fig. 1). Several members
51 of the family produce alkaloid compounds with an agronomical interest, such as nicotine in tobacco,
52 or psychoactive or poisonous substances such as those found in belladonna, stramonium or
53 mandrake. Indeed the name Solanaceae might come from the latin *solare*, meaning "to soothe", in
54 reference to the pharmaceutical properties of many members of the family. The Solanaceae display
55 a large variety of inflorescence structures but the family is typified by the characteristic cymose
56 inflorescence, where the terminal flower dies out and new flowers grow from lateral buds. The
57 flowers generally have a type-5 symmetry with 5 petals fused at different degrees and 5 stamens
58 partly fused to the corolla, and the ovary can develop into either a fleshy (e.g. tomato) or dry (e.g.
59 petunia) fruit.

60 The Solanaceae contain model species such as petunia, tomato, potato and tobacco, that can
61 be easily transformed with *Agrobacterium tumefaciens*, and whose genomes have been sequenced
62 (<https://solgenomics.net/>) [6]. These species of agronomical interest have been bred to improve
63 specific traits, and therefore constitute good model systems to understand the genetic processes of
64 domestication [7]. Petunia is a famous bedding plant with high agronomic value, and hundreds of
65 floral varieties selected over the years provide a large repertoire for variation in flower morphology.
66 In particular, petunia has been instrumental to decipher the anthocyanin biosynthesis pathway
67 involved in petal pigmentation [8] and evolution of flower colour in relation to changes in
68 pollinators [9]. Moreover, a large transposon-insertion database allows for reverse genetics to be
69 carried out easily, which distinguishes it from the other model Solanaceae species [10, 11]. In
70 tobacco, the famous Maryland Mammoth short-days flowering mutant plant has revealed the role of
71 photoperiodism on the floral transition [12, 13], but afterwards tobacco has been little used for
72 research on flower development [6]. The same stands true for potato, although this species has
73 revealed the particular parallel between flowering and tuberization, that uses a similar molecular
74 toolkit with day-length-dependent traveling proteins [14]. Tomato was a major model species for
75 classical genetics before the rise of *Arabidopsis* and is still widely used as a model fruit-bearing
76 crop [6, 15]. In the next paragraph, we will review how members of the Solanaceae family, and in
77 particular petunia, have contributed to a better understanding of how flowers are built.

78 **Specification of floral organ identity: revisions to the ABC model**

79 Environmental and endogenous signals inform the plant when to flower, and at that point, the
80 vegetative meristem that produces leaves will turn into an inflorescence meristem that produces
81 flowers. At an early stage, groups of cells in the flower will acquire a specific identity depending on
82 their position, by the apposition of a precise molecular identity. In *Arabidopsis*, this floral organ
83 identity is specified by the combinatorial action of A-, B- and C-class proteins, as summarized in
84 the classical ABC model and in the floral quartet model that derives from it [16–18] (Fig. 2).
85 Although the ABC model is generally considered valid across a wide variety of flowering plants,
86 this often relies on expression data only and the tedious genetic work that is needed to validate the
87 function of ABC genes has only been done in a few species. For this reason, extended work in
88 *petunia*, where forward and reverse genetics can be carried at a large scale [11], has been key to
89 extend the ABC model to species outside of Brassicaceae. In the following paragraphs, we will
90 review this work, together with pieces of evidence from other nightshades, to illustrate how a
91 detailed floral organ identity patterning model, quite distant to the idealized ABC model found in
92 textbooks, has been built over the years (Fig. 2). To help the reader mostly familiar with
93 *Arabidopsis* genes, we have included a phylogeny of the ABC MADS-box transcription factors
94 discussed in this chapter, showing orthologs in *petunia* and *tomato* (Fig. 3).

95

96 *Redefining the A function*

97 In contrast to the B- and C-functions that are generally well conserved in flowering plants, the
98 molecular identity of the players encoding the A-function, and even the existence of the A-function
99 itself, have been debated [19–21]. A-class genes, as formulated in the classical ABC model, have a
100 dual role: on one hand they antagonize the expression of C-class genes in the two first whorls of the
101 flower, and on the other hand they specify alone the identity of sepals, and together with B-class
102 genes the identity of petals [16, 17]. As a result, A-class mutants are expected to develop carpels in
103 the first whorl and stamens in the second whorl of the flower. In *Arabidopsis*, *APETALA1* (*AP1*) and
104 *AP2* are classified as A-class genes. The *ap1* mutant flowers lack petals and have sepals that display
105 bract features [22, 23], whereas the *ap2* mutant sepals are converted into carpel- or leaf-like organs,
106 and its petals are absent or transformed into stamen-like structures [24, 25], suggesting indeed that
107 *AP2* (and *AP1* to some extent) is necessary for sepals and petals to form correctly and to repress C-
108 class gene expression from these organs. However, such expected A-class mutants were never
109 clearly found outside of *Arabidopsis*, suggesting that the A-function as initially defined is not
110 universal [19]. Instead, in *Antirrhinum* only a gain-of-function mutant of the C-class gene *PLE*
111 produces the expected A-class mutant phenotype [26], showing that the wild-type sepal and petal

identity is rather due to the repression of B- and C-class gene expression from the outer whorls of the flower, than by an "added value" of A-class genes [20, 27]. For these reasons, variations to the ABC model have been proposed, and in particular the (A)BC model [16, 20, 28]. In this model, the (A) function merely provides a floral context in which the B- and C-class genes are active to specify petal, stamen and carpel identity, whereas sepals represent the ground floral organ identity. In other words, the A-function plays a cadastral role, setting the outside boundaries of B- and C-genes expression, rather than specifying a precise floral organ identity.

In petunia, the cadastral function of A-class genes on C-class gene expression is clearly apparent in the double mutant for *BLIND* (*BL*) and *BLIND ENHANCER* (*BEN*), in which petals are absent or stamenoid, and sepals are converted into carpel-like structures [29]. *BL* encodes a microRNA from the miR169 family [30] while *BEN* is a member of the *euAP2* lineage [29], to which the Arabidopsis A-class gene *AP2* also belong. Therefore in petunia, the C-repression role of the A-function is fulfilled by the combinatorial action of two distinct molecular players: a microRNA and an AP2-type transcription factor. Although *BEN* encodes the functional equivalent of Arabidopsis *AP2*, they are not orthologs since *BEN* belongs to the *TOE*-type genes of the *euAP2* lineage (Fig. 3), members of which in Arabidopsis redundantly act as floral repressors [31–34]. Combining the *ben* mutant with mutations in the *euAP2*-type genes *ROB1*, *ROB2* and *ROB3* (orthologous to Arabidopsis *AP2*) causes a complete homeotic conversion of sepals into petals, witnessing the full derepression of B-class genes in the first whorl [29]. In addition, mutations in the four members of the *AP1/SQUA* subfamily (*euAP1*, *PFG*, *FLORAL BINDING PROTEIN 26* (*FBP26*) and *FBP29*, see Fig. 3) results in terminal flowers with normal petals and sepals with petaloid sectors, this last feature being strongly enhanced when combined with mutations in *ROBs* genes [35]. This demonstrates the existence of a cadastral function to restrict B-class gene expression to its correct domain, fulfilled by *BEN*, *ROBs* and *AP1/SQUA* genes in petunia. In contrast, in Arabidopsis only indirect evidence for a repressive role of *AP2* on B-class genes had been reported [36]. Therefore, in petunia a combination of different molecular players act to restrict B- and C-class gene expression to their respective domains, and fulfilling altogether the cadastral part of the A-function (Fig. 2).

It is now clear in the Solanaceae family that *AP1*- and *AP2*-like genes are not needed to specify petal identity. In tomato, a knock-out insertion mutant in the gene *MACROCALYX* (*MC*), orthologous to the A-function gene *AP1*, shows no defects in petal identity, with sepals being enlarged like bracts [37, 38]. However, this could be masked by redundancy with other *AP1*-like genes. But as shown previously, in petunia a quadruple mutant of the four *AP1/SQUA* genes leads to terminal flowers with perfectly normal petals, and with enlarged sepals with petaloid sectors, as a

146 result of B-class gene derepression [35]. Similarly, the *rob1 rob2 rob3* mutant (*ROBs* being
147 orthologous to Arabidopsis *AP2*) forms petals, although with some growth and pigmentation defects
148 [29]. To conclude, neither *AP1*-like nor *AP2*-like genes are necessary for basic specification of petal
149 identity in petunia flowers, in sharp contrast to what is known in Arabidopsis and generalized in the
150 textbook ABC model.

151

152 *Variations on the B function: Specializing for a single floral organ*

153 The classical ABC model states that B-genes specify petals and stamens, in combination with A-
154 and C-genes respectively. Indeed, B-class mutants in eudicots usually show a phenotype affecting
155 both the petal and stamen whorls and converting them into sepals and carpels respectively; this is
156 for instance the case in tomato when the *APETALA3* (*AP3*) ortholog *STAMENLESS* is knocked-out
157 [39, 40]. However, the situation is different in petunia: mutant in the B-class gene *PhDEFICIENS*
158 (*PhDEF*), also known as *green petals*, has a full conversion of petals into sepals, but stamens
159 remain unaffected [41]. *PhDEF* is expressed in petal and stamen primordia, suggesting that another
160 gene redundantly controls stamen identity [42]. This gene was found to be its paralog *PhTM6*,
161 which resembles the ancestral *paleoAP3* type of B-class genes, rather than the classical *euAP3* type
162 B-class genes to which *PhDEF* belongs (Fig. 3) [43]. Surprisingly, *PhTM6* appeared to be
163 expressed rather as a C-class gene in whorls 3 and 4 [44]. This unconventional expression pattern
164 for a B-class gene is caused by the fact that C-class genes activate *PhTM6* expression [21]. The
165 *phtm6* mutant has no visible phenotype, while the *phdef phtm6* double mutant displays full
166 homeotic conversion of petals into sepals and stamens into carpels, as would be expected for a B-
167 class mutant [44]. Thus in petunia, B-class genes from the *AP3/DEF* clade have specialized into
168 controlling petal and stamen, or only stamen, identity in a partially redundant fashion (Fig. 4).

169 Furthermore, it was shown that the *AP3/DEF* proteins act in heterocomplexes with
170 *PISTILLATA/GLOBOSA* (*PI/GLO*) proteins, also belonging to the B-class family (Fig. 3), and that
171 this complex activates its own expression [45, 46]. For instance, Arabidopsis *AP3* interacts with *PI*,
172 and Antirrhinum *DEF* interacts with *GLO* [47, 48]. In petunia, there are two *PI/GLO* proteins, both
173 expressed in petals and stamens, and their interaction pattern with the two *AP3/DEF* proteins is
174 logically more complex than in Arabidopsis or Antirrhinum: *PhDEF* interacts with both *PhGLO1*
175 and *PhGLO2*, while *PhTM6* only interacts with *PhGLO2*, which was confirmed genetically in the
176 corresponding mutants [42]. In addition, fusion of the stamen filaments with the inner petal tube is
177 specifically regulated by the *PhDEF/PhGLO1* heterodimer [42]. In summary, the increased
178 complexity in gene number and protein interaction pattern in petunia led to subtle
179 subfunctionalization of these genes in specifying petal and stamen identity and development (Fig.

180 4). But how the different protein complexes divide up tasks to regulate target genes and specify
181 correct organ identity at the molecular level remains to be understood.

182

183 *Multiple functions for C-class genes*

184 The C-function, i.e. the specification of stamen and carpel identity, generally coupled with the
185 control of floral determinacy, is controlled by members of the *AGAMOUS* (*AG*) family. This family
186 is subdivided into the *euAG* and *PLENA* (*PLE*) clades (Fig. 3), whose names come from the rosid
187 species *Arabidopsis* and the asterid species *Antirrhinum*, where *AG* and *PLE* respectively specify
188 the C-function [16, 17]. In contrast, the *Arabidopsis* *PLE*-like genes *SHATTERPROOF1* and 2
189 (*SHP1/2*) play a late role in fruit shattering but are not essential for the C-function [49], while the
190 *Antirrhinum* *euAG*-like gene *FARINELLI* (*FAR*) only has a little contribution to stamen
191 development [50]. These observations led to build a model where, after the gene duplication that
192 generated the *euAG*- and *PLE*-clades, control of the C-function has been randomly allocated to
193 either member of the two clades, with the other member adopting a distinct function after changes
194 in gene expression pattern or protein biochemical properties [50].

195 In Solanaceae, several evidences suggest that the C-function is largely redundantly
196 controlled by both members of the *euAG* and *PLE* clades, which might reflect the ancestral state
197 just after the *euAG/PLE* duplication [50, 51]. Indeed, in petunia single and double knock-out
198 mutants in the *euAG*-like gene *PETUNIA MADS BOX GENE3* (*PMADS3*) and the *PLE*-like gene
199 *FBP6* demonstrate that the two genes redundantly control stamen and carpel identity and floral
200 determinacy [21]. In tomato, different RNAi lines against the *euAG*-like gene *TOMATO*
201 *AGAMOUS1* (*TAG1*) and the *PLE*-like gene *ARLEQUIN/TOMATO AGAMOUS-LIKE1* (*TAGL1*)
202 generated slightly conflicting results, likely due to different degrees of gene down-regulation and
203 possible co-silencing of paralogous genes [52, 53]. Still, these studies suggest a partially redundant
204 function of *TAG1* and *TAGL1* in the control of the C-function [52, 53]. Consistently, the beautiful
205 arlequin tomato (Fig. 1G), a semi-dominant mutant of *TAGL1*, forms carpelloid sepals appearing
206 fleshy and bright red when the fruit is mature [54]. Both genes also appear to play a role in fruit
207 development and ripening [52, 53]. In tobacco, VIGS lines suggest that the *euAG*- and *PLE*-like
208 genes *NbAG* and *NbSHP* redundantly control the C-function, while *NbSHP* has an additional role in
209 fruit dehiscence (Fourquin and Ferrándiz, 2012). Similarly in *Physalis*, downregulation of the
210 *euAG*- and *PLE*-like genes *PFAG1* and *PFAG2* by VIGS revealed their partially redundant role in
211 regulating the C-function [55]. Although work in tomato, tobacco and *Physalis* is not fully clear
212 since gene function was assessed by down-regulation and not by complete knock-out, overall it
213 appears that in Solanaceae control of the C-function is largely shared between *euAG*- and *PLE*-like

214 genes, and that *PLE*-like genes can have an additional role in fruit development or ripening
215 (similarly to the *SHP* genes in Arabidopsis). Together with previous studies in Arabidopsis and
216 Antirrhinum, this supports an evolutionary model with several independent subfunctionalization
217 events between *AG*-family members for the control of the C-function [50, 51]. B-class genes have
218 also been reported to participate in the determination of carpel identity in Solanaceae: as discussed
219 previously, in petunia the *paleoAP3*-type gene *PhTM6* is expressed in the carpel [44], suggesting it
220 plays a role in its establishment, although this has not been formally demonstrated yet. In *Physalis*,
221 it was recently shown that the *GLO*-like gene *DOLL1* is expressed in the carpel whorl where it
222 activates the expression of the ortholog of the carpel regulator *CRABS CLAW* (*CRC*), which ensures
223 proper carpel development and fertility [56, 57].

224 C-class genes can have other roles in addition to the C-function: these genes also trigger
225 nectary development [58]. Interestingly, this newly discovered function is true both in petunia
226 where nectaries are found at the base of the ovary, and in Arabidopsis where nectaries are found at
227 the base of the stamens. In both species, the *AG*-family genes (i.e. *AG* and *SHP1/2* in Arabidopsis,
228 and *FBP6* and *PMADS3* in petunia) redundantly activate expression of the YABBY transcription
229 factor *CRC* (or its orthologs *PhCRC1/2* in petunia), that are essential for nectary development [57,
230 58]. This work revealed that the same *AG-CRC* genetic module is involved in nectary development
231 in two distantly-related species with different nectary positioning, suggesting the possibility of an
232 ancestral mechanism for nectary specification before the asterids-rosids divergence [58].

233 Finally, the petunia C-class genes *FBP6* and *PMADS3* also participate to the D-function,
234 specifying ovule identity [21]. This function has been first identified in petunia, where co-silencing
235 of the D-class genes *FBP7* and *FBP11* converts ovules into carpelloid organs [59]. However, full
236 knock-out lines revealed later that both genes were dispensable for ovule identity (suggesting that
237 other genes had been silenced in the cosuppression lines), but that combining the *fbp7 fbp11*
238 mutations with additional mutation or silencing in *FBP6* or *PMADS3* (therefore creating a partial-
239 C/full-D class mutant) leads to a strong loss of ovule identity [21]. Indeed, this is in accordance
240 with what is observed in Arabidopsis, where the C- and D-class genes *AG*, *SHP1/2* and
241 *SEEDSTICK* also participate in defining ovule identity [60]. In tomato, the D-class genes *Sl-AGL11*
242 and *Sl-MBP3* are expressed both in the carpel, the seeds and the fruit, and overexpression of *Sl-*
243 *AGL11* leads to early fruit ripening together with a dramatic conversion of sepals into fleshy fruit
244 tissue [61]. This suggests that at least one of the D-class genes in tomato has maintained the
245 capacity to specify carpel identity when ectopically expressed, and may have also developed an
246 additional role during fruit development.

247

248 *Uncovering the E function*

249 The Solanaceae flowers brought the first evidence for the existence of an extra floral function in
250 addition to the ABC(D) functions. Indeed, although clearly not gene-specific, co-suppression lines
251 of the petunia *FBP2* or the tomato *TM5* genes (both MADS-box genes orthologous to Arabidopsis
252 *SEP3*) exhibited flowers with homeotic conversion of petals, stamens and carpels into sepaloid
253 organs, together with floral indeterminacy [62, 63]. At that time, this was interpreted as the
254 involvement of *FBP2* and *TM5* in floral meristem identity or in the repression of sepaloid identity
255 [62, 63]. Both of these interpretations still stand true today, although we do not think now of
256 sepaloid identity as being repressed but rather that petal, stamen and carpel identities are activated
257 on a sepaloid background, and that this activation of the B- and C-functions requires genes from the
258 E function, such as petunia *FBP2*, tomato *TM5* or Arabidopsis *SEP3*. Indeed, it was later found in
259 Arabidopsis that petals, stamens and carpels in the *sep1 sep2 sep3* triple mutant were transformed
260 into sepals [64], while all floral organs in a *sep1 sep2 sep3 sep4* mutant developed as leaf-like
261 organs [65], leading to the idea that the *SEP* genes are required for the identity of all floral organs in
262 a largely redundant fashion, thereby incarnating the E function. Molecular support for this
263 additional function came from the identification of "floral quartets", where E-class proteins bridge
264 other MADS-box proteins together in a floral organ specific manner, providing a physical
265 explanation for the implication of *SEP* proteins in the identity of all floral organs [18, 66, 67]. In
266 addition, work in petunia and Arabidopsis further showed that ovule identity also requires *SEP*
267 activity, as illustrated by the homeotic conversions of all ovules into leaf-like organs in the petunia
268 *fbp2 fbp5* mutant [68] and of a part of the ovules into leaf-like or carpel-like organs in the
269 *SEP1/sep1 sep2 sep3* mutant (Favaro et al., 2003). Furthermore, it was later shown that Petunia
270 *AGL6*, a member of the MADS-box *AGL6* subfamily closely related to the *SEP* subfamily (Fig. 3),
271 also performs *SEP-like* functions, redundantly with some of the petunia *SEP* genes [70], adding
272 further genetic complexity to the *SEP* function in these Solanaceous species. More recently, a large
273 genetic study was performed aimed at revealing all individual and redundant functions of Petunia
274 *AGL6* and its six *SEP-like* genes, based on the analysis of single and higher order mutants [35]. This
275 study revealed that the petunia *SEP1/2/3* orthologs (Fig. 3) together with *AGL6* encode the classical
276 *SEP* floral organ identity and floral termination functions, with a master role for the petunia *SEP3*
277 ortholog *FBP2*. Remarkably however, it was found that the *FBP9* subclade members *FBP9* and
278 *FBP23*, for which no clear ortholog is present in Arabidopsis, play a major role in determining
279 floral meristem identity together with *FBP4*, while contributing only very moderately to floral
280 organ identity. Indeed, triple *fbp4 fbp9 fbp23* mutants completely lack flowers and exhibit a highly
281 branched inflorescence structure due to the homeotic transformation of its floral meristems into

282 inflorescence meristems. This shows that in contrast to Arabidopsis, a subset of the Petunia *SEP*
 283 genes (*FBP4*, -9 and -23) have evolved a specific function as floral meristem identity genes rather
 284 than encoding the classical organ identity *SEP* function. This is remarkably similar to the earlier
 285 proposed roles for three orthologous tomato genes *LIN* (*LONG INFLORESCENCE*), *J2*
 286 (*JOINTLESS 2*) and *EJ2* (*ENHANCER OF JOINTLESS 2*) which are responsible for the transition
 287 from the inflorescence to the floral meristem identity [71], suggesting that this is conserved within
 288 the Solanaceae. Interestingly, mutations in *J2* and *EJ2* were individually selected during tomato
 289 breeding as they both caused beneficial traits (elimination of the fruit abscission zone and increased
 290 calyx size), but when combined together affect inflorescence branching and fertility [71]. Finely
 291 controlling the expression levels of *J2* and *EJ2* by genetic engineering allows to generate tomato
 292 varieties with a better combination of beneficial traits [71, 72]. This reveals the wide and complex
 293 roles of the tomato *SEP*-like genes in the control of inflorescence branching, calyx size, flower
 294 abscission and flower fertility. Finally, silencing approaches suggest that the two tomato *SEP1/2/3*
 295 members *TM5* and *TM29* and the tomato *AGL6* gene exhibit classical *SEP* organ identity functions
 296 (Pnueli et al., 1994; Ampomah-Dwamena et al., 2002; Yu et al., 2017) similar to what we found in
 297 petunia.

298 In Arabidopsis, the floral meristem identity function is attributed to members of the
 299 *AP1/SQUA* MADS-box subfamily (*AP1*, *CAL* and *FUL* genes in Arabidopsis) [73, 74] rather than to
 300 a specific subclass of *SEP* genes as described above for petunia and tomato. Interestingly, *AP1*, *SEP*
 301 and *AGL6* genes all form a superclade with shared ancestry (Fig. 3). This suggests that the floral
 302 meristem identity function might have been ancestral to the duplication event that generated those
 303 three different gene families, and has been distributed primarily to *AP1*-like genes in Arabidopsis
 304 and to a subclass of *SEP*-like genes in petunia and tomato.

305

306 *Divergence to the textbook ABC model*

307 Research in the Solanaceae, and in particular in petunia, has led to the construction of a floral organ
 308 identity patterning model quite distinct to the textbook ABC model (Fig. 2). Why so many
 309 differences? Is this a specificity of the Solanaceae? The ABC model is by essence, a simplification
 310 of the reality, and even the founder species of the model, Arabidopsis and Antirrhinum, do not fit
 311 perfectly in the frame. For instance, no complete A-function mutant was ever found in Antirrhinum,
 312 and the A-class gene *AP1* is fully dispensable for petal identity in Arabidopsis [19, 20]. Therefore it
 313 is only normal that, as we discover more and more details about the molecular players of floral
 314 organ identity, the model that was proposed more than 30 years ago becomes less adequate and
 315 needs to be complexified [75]. Moreover, the number of ABC genes within a species necessarily

316 complexifies the model, as each gene copy can evolve new functions (neofunctionalization) or
317 retain part of the ancestral function (subfunctionalization). [76] Therefore, the increased complexity
318 of the model in Fig. 3, as compared to the textbook ABC model, is due to the large number of
319 (A)BC genes in petunia, the high power of functional analysis that can be performed in this species
320 and the large amount of work that was carried on the topic over the years. This only advocates for
321 the need of many more model species where such a fine analysis can be performed, in order to find
322 more general principles and specificities of floral organ identity patterning.

323 **Initiation and fusion of floral organs**

324 While floral organ identity is continuously specified by homeotic genes throughout organ
325 development, other molecular players direct the pattern of floral organ initiation and the hormone
326 auxin appears as a key player in this process (Smyth, 2018 and references therein). The synthetic
327 promoter DR5, containing auxin-responsive elements and fused to a reporter gene like GFP, is often
328 used as a late reporter for auxin signalling [77]. During floral development, *DR5:GFP* expression
329 peaks successively at incipient floral organ primordia, first marking the sepals initiation domain,
330 followed by petals, stamens and carpels [78, 79].

331 So far, Solanaceae have brought relatively little contribution to the understanding of floral
332 organ initiation and growth. Yet, some specific features from the family could strongly enrich the
333 field of research. For instance, flowers have a type-5 symmetry, with 5 sepals, 5 petals and 5
334 stamens initiating successively, and almost jointly within each whorl, in contrast to the type-4
335 symmetry of Arabidopsis flowers. Models, based on periodic auxin accumulation and increasing
336 space in the floral meristem, generate self-organizing patterns of primordia initiation [80, 81].
337 Researchers have attempted to explain the emergence of different floral organ numbers per whorl
338 [82], but these models would strongly benefit from additional data in species with a type-5
339 symmetry, such as the detailed spatio-temporal pattern of DR5 expression that was characterized
340 during tomato flower and fruit development [83].

341 Petal fusion (sympetaly) is a trait of major evolutionary importance, that led to new floral
342 structures and possibly accelerated speciation rates. Some molecular players involved in petal
343 fusion have been found in Arabidopsis, based on mutants with fused petals [84]. Using a model
344 species with fused petals, such as *e.g.* petunia, should allow to identify other actors of petal fusion,
345 and in particular those involved in the evolution of the trait. The petunia mutants *maewest* (*maw*)
346 and *choripetala suzanne* (*chsu*) were isolated in a genetic screen for petal fusion defects [85]. These
347 mutants form partly fused and narrow petals, together with narrow leaves, suggesting general
348 defects in organ laminar lateral growth. Petal fusion is congenital in petunia (i.e. petals are fused by
349 the confluence of their individual primordia), and the defects observed in *maw* or *chsu* appear to be
350 due to defects in petal primordia lateral expansion, suggesting that fusion and lateral growth are
351 inherently coupled during petunia petal development. *MAW* is a WUS-like homeobox (WOX) gene,
352 to which the famous gene *WUSCHEL* belongs, that controls stem cell maintenance in the meristem
353 [86, 87]. In tomato flowers, whose petals are partly fused at the base, mutation in the *MAW* ortholog
354 *UN-FUSED FLOWER* (*UF*) also causes narrow and unfused petals, and narrow leaves [88]. *MAW*
355 and *UF* are homologous to Arabidopsis *WOX1*, whose mutation causes no obvious phenotypic
356 defects in the flower [85]. However, combining mutations in *wox1* and in another WOX gene,

357 *PRESSED FLOWER (PRS)*, results in plants with very narrow leaves and petals, reminiscent of the
358 *petunia maw* phenotype [85, 89]. This indicates that petal lateral growth (and fusion in *petunia*) is
359 controlled by similar classes of genes from the *WOX* subfamily in *Arabidopsis* and *petunia*, but
360 different members have been recruited to fulfill this function in the two species [85, 86]. Although
361 *Arabidopsis WOX1* and *PRS* genes appear to fulfill together the same function as *MAW* in *petunia*,
362 *Arabidopsis* petals are not fused, suggesting that other differences in the regulatory network caused
363 divergence in this trait during evolution. Sympetaly likely has a single origin in asterids [84], hence
364 identifying the key event leading to the evolution of this trait might require comparative work
365 involving a sister group to the asterids, such as *Caryophyllaceae* family where most species form
366 flowers with unfused petals.

367 **Growth and maturation of floral organs : Chinese lanterns and petal colours**

368 The elegant Chinese lanterns from *Physalis* and *Whitania* form an encapsulated fruit after
369 pollination, named the inflated calyx syndrome (ICS), due to late sepal growth (Fig. 1H). Indeed
370 sepals of Chinese lanterns resume growth after pollination, and this trait was shown to be an
371 advantageous morphological novelty, because the inflated calyx has photosynthetic capability and
372 provides a micro-environment improving fruit maturation [90]. It had been proposed that the
373 MADS-box gene *MPF2*, orthologous to Arabidopsis *AGAMOUS-LIKE 24*, and genes from the
374 *MPF2-like-A* family were involved in ICS: down-regulating *MPF2* expression by RNA interference
375 in *Physalis* resulted in small sepals, and ectopic expression of *MPF2* in tomato enlarged its calyx by
376 increasing cell division rates [91, 92]. In tomato, the *MPF2* ortholog *STMADS16* is only expressed
377 in leaves, whereas in *Physalis* *MPF2* is expressed both in leaves and sepals, suggesting that during
378 evolution *MPF2* was recruited in the calyx, by heterotopic expression, to form the Chinese lantern
379 phenotype [91]. However, these results were recently reevaluated, because knocking-out *MPF2* by
380 CRISPR was not sufficient to disrupt the ICS, and neither was the individual knock-out of ten other
381 MADS-box genes from the *AP1*, *SEP4*, *AG* or *DEF/GLO* clades [93]. RNA interference notoriously
382 silences both on- and off-targets with partial efficiencies, which probably explains the inconsistency
383 of phenotypes observed between the RNA interference and CRISPR lines. This suggests that
384 multiple MADS-box genes may play a role in the formation of the inflated calyx, but the exact
385 genetic change that caused the emergence of this novelty has not been pinpointed yet [94].

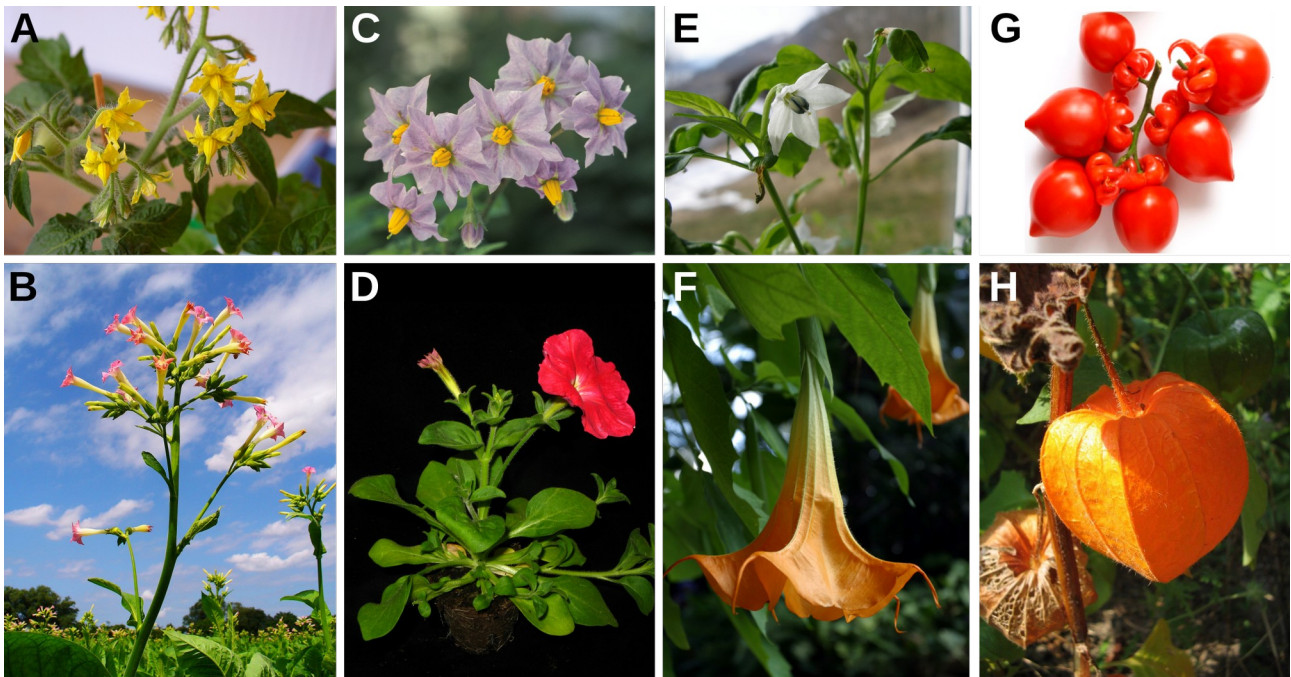
386

387 Mature floral organs acquire a whole set of tissue and cell properties, and in particular petals
388 display a specific pigmentation, crucial for their interaction with pollinators. Since petunia petals
389 are big and showy, and because insertion mutants frequently arise in the line with an active
390 transposase, a mutagenesis screen for the production of anthocyanins (the main pigments
391 accumulating in petunia petals) entails a beautiful and easy phenotyping process. Consequently,
392 petunia has been instrumental in identifying the molecular players of flower colour, with the
393 majority of enzymes and regulators involved in anthocyanin production cloned and characterized in
394 this species [8]. Production of anthocyanins first relies on the production of flavonols, later
395 modified into anthocyanins through the action of specific enzymes and regulators [8]. Interestingly,
396 the mutant phenotypes of these different regulators are distinct: the *an1* mutant loses pigmentation
397 in all tissues, while the *an2* and *an8* mutations only affect pigmentation of the limbs (the upper part
398 of the corolla) and the tube respectively [8, 96, 97]. This suggests that the wild-type petal
399 pigmentation is the result of the global and local action of a combination of regulatory genes. The
400 action mechanism of the regulator AN1 has been elucidated in more details: additional to the

401 regulation of anthocyanin biosynthesis, AN1 regulates the pH of the vacuole of petal epidermal
402 cells, which directly affects the absorption spectra of anthocyanins and hence the resulting petal
403 colour [96]. In particular, AN1 regulates the expression of *PH1* and *PH5*, two vacuolar P-ATPases
404 that pump protons into the vacuole and therefore acidify it [98, 99]. Surprisingly, it was recently
405 found that fruit acidity in lemon and oranges is caused by the same mechanism: the genes *CitPH1*
406 and *CitPH5* acidify the vacuoles in the juice vesicle cells of the fruit and can thereby generate a pH
407 as low as 2 [100]. The regulator of anthocyanin production *AN2* was also found to be involved in
408 the evolution of petal colour between wild petunia species. Indeed, in the white-coloured flowers of
409 *Petunia axillaris*, five independent losses of function in *AN2*, some of them by frame-shift
410 mutations, were found in the wild, affecting flower colour and pollinator preferences [101].
411 Moreover, the surprising "resurrection" of the *AN2* gene after pseudogenization, by a secondary
412 mutation that restored the original reading frame of the gene, has been reported in the purple-
413 coloured petals of *P. secreta* [102]. The study of petal pigmentation has proven to be a rich field of
414 research in the Solanaceae, bridging gaps between the subcellular and the microevolutionary scale.

415 **Conclusion**

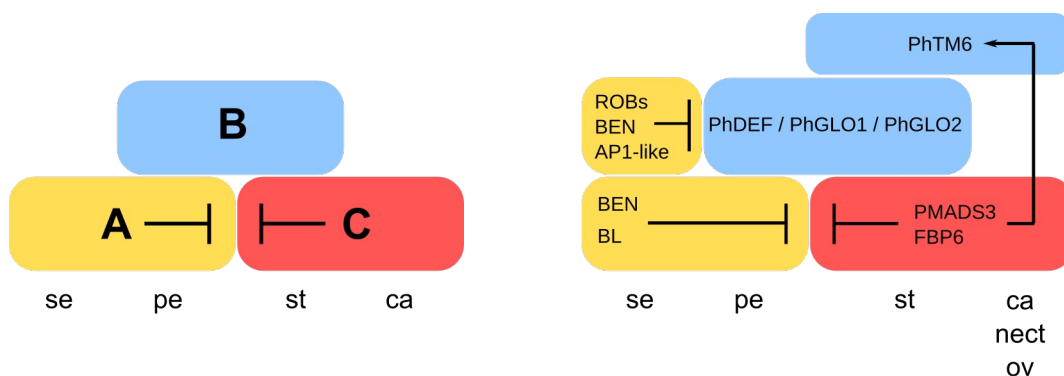
416 Flower development is a massive field of research that can be apprehended under different angles,
417 at different scales and in different species. For the last 30 years, research on *Arabidopsis* has led to
418 the characterization of most key concepts and molecular players of flower development. However,
419 each species has its specificities and *Arabidopsis* is no exception; therefore some of the mechanisms
420 uncovered in *Arabidopsis* are highly divergent in comparison to the common ancestor of eudicots.
421 Moreover, due to the random nature of gene duplication and subfunctionalization in different
422 species, gene functions can appear hidden in redundancy in *Arabidopsis* but be revealed by gene
423 subfunctionalization in other species (or vice versa). Finally, some important features of flower
424 development simply do not exist in *Arabidopsis*, for instance petal fusion or petal pigmentation. For
425 these reasons, including several model species in the research on flower development is now crucial
426 to further improve our understanding of this process [11]. Solanaceae have brought their share of
427 results on this topic and surely this family will continue bringing new insights on flower
428 development, also with potential application for agronomical purposes.



429
 430 **Figure 1.** Flowers and inflorescences from various Solanaceae members: *Solanum lycopersicum*
 431 (tomato, A), *Nicotiana tabacum* (tobacco, B), *Solanum tuberosum* (potato, C), *Petunia x hybrida*
 432 (petunia, D), *Capsicum annuum* (pepper, E), *Brugmansia* sp. (F), fruits from the arlequin tomato
 433 mutant (G), fruit from *Physalis alkegengi* (H). Picture credits respectively, from A to H: Niek
 434 Willems, H. Zell, Keith Weller, Michiel Vandenbussche, Simone Stibbe, Tom Morphy, arlequin
 435 tomato picture reproduced with authorization from Rafael Lozano [54], and Michael Gasperl.
 436 Licences Creative Commons Attribution-Share Alike 2.5 or 3.0 Generic or Unported.

437

438



The textbook ABC model

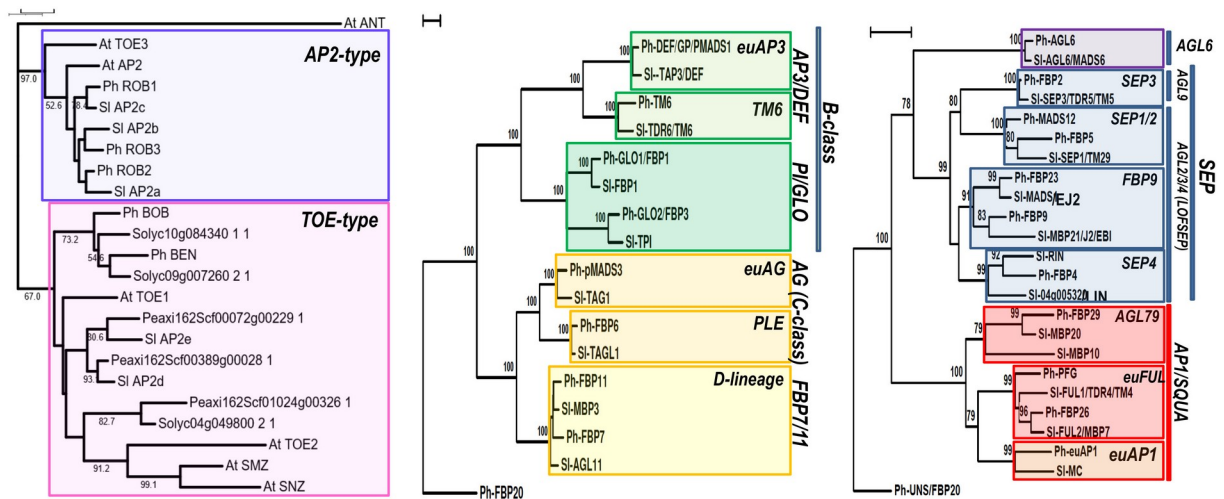
Floral organ identity patterning in petunia

439

440 **Figure 2.** The textbook ABC model and its variation in petunia, as we currently understand it. In the
 441 textbook ABC model, A-class genes and C-class genes mutually repress their expression domains.
 442 A-class genes alone and in combination with B-class genes specify the identities of sepals (se) and
 443 petals (pe) respectively, C-class genes alone and in combination with B-class genes specify the
 444 identities of carpels (ca) and stamens (st) respectively. In the petunia model, the 2 C-class genes
 445 *PMADS3* and *FBP6* redundantly share the C-function and specify carpel identity alone, and stamen
 446 identity together with the 4 B-class genes *PhDEF*, *PhTM6*, *PhGLO1* and *PhGLO2*. These 4 genes
 447 show additional patterns of subfunctionalization detailed in Figure 4. C-class genes additionally
 448 specify nectary (nect) and ovule (ov) development. *PhTM6* is expressed in the stamens and carpels
 449 and its expression is activated by C-class genes; however the function of *PhTM6* in the carpels is
 450 unknown so far. The (A)-function is ensured by the genes *BL*, *BEN*, *ROBs* and *AP1-like*: *BL* and
 451 *BEN* repress C-class genes expression in sepals and petals, and *BEN*, *ROBs* and *AP1-like* genes
 452 repress B-class genes expression in the sepal whorl. In this model, the coloured boxes represent the
 453 region of action of the genes and not necessarily their domain of expression (*BL*, *BEN* and the *ROB*
 454 genes are for instance expressed in all floral organs).

455

456



457
 458 **Figure 3.** Neighbor joining trees of euAP2 transcription factors (left), B/C/D class (middle) and
 459 AP1/SEP/AGL6 superclade (right) MADS-box transcription factors. Prefixes At, Ph and Sl
 460 represent *Arabidopsis thaliana*, *Petunia hybrida* and *Solanum lycopersicum* respectively. The
 461 euAP2 phylogenetic analysis was obtained using the pipeline offered by <https://ngphylogeny.fr>, and
 462 the tree was rooted with Arabidopsis ANT, an AP2 transcription factor not belonging to the euAP2
 463 lineage. Both MADS-box trees were rooted with Ph-UNS/FBP20, a SOC1 subfamily member.
 464 Bootstrap values (based on 1000 replicates) supporting tree branching above 70% are indicated near
 465 the branching points. Scalebars correspond with 0.1 substitutions per site. The MADS trees were
 466 computed with Treecon software [103] using the Tajima & Nei Distance Calculation method and
 467 further default settings.

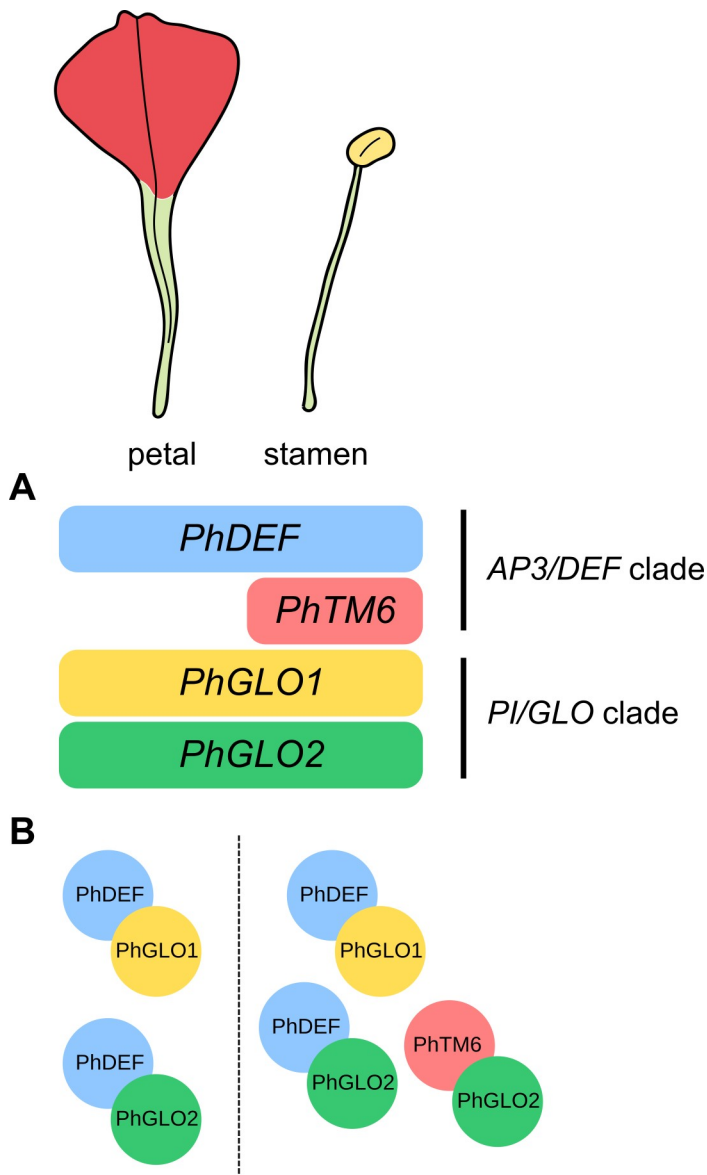


Figure 4. Subfunctionalization of the B-class genes for specification of petal and stamen identity in petunia. **A:** Expression domains of the *PhDEF*, *PhTM6*, *PhGLO1* and *PhGLO2* genes in petal and stamen initiation domains. **B:** Protein complexes formed between AP3/DEF- and PI/GLO-type proteins in each floral organ, based on [42, 44].

1. Bouché F, Lobet G, Tocquin P, Périlleux C (2016) FLOR-ID: an interactive database of flowering-time gene networks in *Arabidopsis thaliana*. *Nucleic Acids Res* 44:D1167–1171. <https://doi.org/10.1093/nar/gkv1054>
2. Moyroud E, Glover BJ (2017) The Evolution of Diverse Floral Morphologies. *Curr Biol* 27:R941–R951. <https://doi.org/10.1016/j.cub.2017.06.053>
3. Smyth DR (2018) Evolution and genetic control of the floral ground plan. *New Phytol* 220:70–86. <https://doi.org/10.1111/nph.15282>
4. Soltis DE, Chanderbali AS, Kim S, et al (2007) The ABC model and its applicability to basal angiosperms. *Ann Bot* 100:155–163. <https://doi.org/10.1093/aob/mcm117>
5. The Plant List (2013) <http://www.theplantlist.org/>
6. Gebhardt C (2016) The historical role of species from the Solanaceae plant family in genetic research. *Theor Appl Genet* 129:2281–2294. <https://doi.org/10.1007/s00122-016-2804-1>
7. Lin T, Zhu G, Zhang J, et al (2014) Genomic analyses provide insights into the history of tomato breeding. *Nat Genet* 46:1220–1226. <https://doi.org/10.1038/ng.3117>
8. Tornielli G, Koes R, Quattrocchio F (2009) The genetics of flower color. In: *Petunia: Evolutionary, Developmental and Physiological Genetics*. Springer International Publishing, pp 269–299
9. Galliot C, Stuurman J, Kuhlemeier C (2006) The genetic dissection of floral pollination syndromes. *Curr Opin Plant Biol* 9:78–82. <https://doi.org/10.1016/j.pbi.2005.11.003>
10. Vandenbussche M, Janssen A, Zethof J, et al (2008) Generation of a 3D indexed *Petunia* insertion database for reverse genetics. *Plant J* 54:1105–1114. <https://doi.org/10.1111/j.1365-313X.2008.03482.x>
11. Vandenbussche M, Chambrier P, Rodrigues Bento S, Morel P (2016) *Petunia*, Your Next Supermodel? *Front Plant Sci* 7:72. <https://doi.org/10.3389/fpls.2016.00072>
12. Amasino RM (2013) My favourite flowering image: Maryland Mammoth tobacco. *J Exp Bot* 64:5817–5818. <https://doi.org/10.1093/jxb/ert083>
13. Parcy F (2019) L’histoire secrète des fleurs. *Humensciences*
14. Abelenda JA, Navarro C, Prat S (2014) Flowering and tuberization: a tale of two nightshades. *Trends Plant Sci* 19:115–122. <https://doi.org/10.1016/j.tplants.2013.09.010>
15. Kimura S, Sinha N (2008) Tomato (*Solanum lycopersicum*): A Model Fruit-Bearing Crop. *CSH Protoc* 2008:pdb.emo105. <https://doi.org/10.1101/pdb.emo105>
16. Schwarz-Sommer Z, Huijser P, Nacken W, et al (1990) Genetic Control of Flower Development by Homeotic Genes in *Antirrhinum majus*. *Science* 250:931–936. <https://doi.org/10.1126/science.250.4983.931>

17. Coen ES, Meyerowitz EM (1991) The war of the whorls: genetic interactions controlling flower development. *Nature* 353:31–37. <https://doi.org/10.1038/353031a0>
18. Theissen G, Saedler H (2001) Plant biology. Floral quartets. *Nature* 409:469–471. <https://doi.org/10.1038/35054172>
19. Litt A (2007) An Evaluation of A-Function: Evidence from the APETALA1 and APETALA2 Gene Lineages. *International Journal of Plant Sciences* 168:73–91. <https://doi.org/10.1086/509662>
20. Causier B, Schwarz-Sommer Z, Davies B (2010) Floral organ identity: 20 years of ABCs. *Semin Cell Dev Biol* 21:73–79. <https://doi.org/10.1016/j.semcdb.2009.10.005>
21. Heijmans K, Ament K, Rijpkema AS, et al (2012) Redefining C and D in the petunia ABC. *Plant Cell* 24:2305–2317. <https://doi.org/10.1105/tpc.112.097030>
22. Irish VF, Sussex IM (1990) Function of the *apetala-1* gene during Arabidopsis floral development. *Plant Cell* 2:741–753. <https://doi.org/10.1105/tpc.2.8.741>
23. Mandel MA, Gustafson-Brown C, Savidge B, Yanofsky MF (1992) Molecular characterization of the Arabidopsis floral homeotic gene APETALA1. *Nature* 360:273–277. <https://doi.org/10.1038/360273a0>
24. Kunst L, Klenz JE, Martinez-Zapater J, Haughn GW (1989) AP2 Gene Determines the Identity of Perianth Organs in Flowers of Arabidopsis thaliana. *Plant Cell* 1:1195–1208. <https://doi.org/10.1105/tpc.1.12.1195>
25. Bowman JL, Smyth DR, Meyerowitz EM (1991) Genetic interactions among floral homeotic genes of Arabidopsis. *Development* 112:1–20
26. Bradley D, Carpenter R, Sommer H, et al (1993) Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the *plena* locus of *Antirrhinum*. *Cell* 72:85–95
27. Monniaux M, Vandenbussche M (2018) How to Evolve a Perianth: A Review of Causal Mechanisms for Perianth Identity. *Front Plant Sci* 9:1573. <https://doi.org/10.3389/fpls.2018.01573>
28. Baum DA, Hileman LC (2006) A Developmental Genetic Model for the Origin of the Flower. In: *Annual Plant Reviews Volume 20: Flowering and its Manipulation*. John Wiley & Sons, Ltd, pp 1–27
29. Morel P, Heijmans K, Rozier F, et al (2017) Divergence of the Floral A-Function between an Asterid and a Rosid Species. *Plant Cell* 29:1605–1621. <https://doi.org/10.1105/tpc.17.00098>
30. Cartolano M, Castillo R, Efremova N, et al (2007) A conserved microRNA module exerts homeotic control over *Petunia hybrida* and *Antirrhinum majus* floral organ identity. *Nat Genet* 39:901–905. <https://doi.org/10.1038/ng2056>
31. Aukerman MJ, Sakai H (2003) Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes. *Plant Cell* 15:2730–2741. <https://doi.org/10.1105/tpc.016238>

32. Jung J-H, Seo Y-H, Seo PJ, et al (2007) The GIGANTEA-regulated microRNA172 mediates photoperiodic flowering independent of CONSTANS in Arabidopsis. *Plant Cell* 19:2736–2748. <https://doi.org/10.1105/tpc.107.054528>
33. Mathieu J, Yant LJ, Mürdter F, et al (2009) Repression of flowering by the miR172 target SMZ. *PLoS Biol* 7:e1000148. <https://doi.org/10.1371/journal.pbio.1000148>
34. Yant L, Mathieu J, Dinh TT, et al (2010) Orchestration of the floral transition and floral development in Arabidopsis by the bifunctional transcription factor APETALA2. *Plant Cell* 22:2156–2170. <https://doi.org/10.1105/tpc.110.075606>
35. Morel P, Chambrier P, Boltz V, et al (2019) Divergent Functional Diversification Patterns in the SEP/AGL6/AP1 MADS-Box Transcription Factor Superclade. *Plant Cell* 31:3033–3056. <https://doi.org/10.1105/tpc.19.00162>
36. Krogan NT, Hogan K, Long JA (2012) APETALA2 negatively regulates multiple floral organ identity genes in Arabidopsis by recruiting the co-repressor TOPLESS and the histone deacetylase HDA19. *Development* 139:4180–4190. <https://doi.org/10.1242/dev.085407>
37. Vrebalov J, Ruezinsky D, Padmanabhan V, et al (2002) A MADS-box gene necessary for fruit ripening at the tomato ripening-inhibitor (rin) locus. *Science* 296:343–346. <https://doi.org/10.1126/science.1068181>
38. Yuste-Lisbona FJ, Quinet M, Fernández-Lozano A, et al (2016) Characterization of vegetative inflorescence (mc-vin) mutant provides new insight into the role of MACROCALYX in regulating inflorescence development of tomato. *Sci Rep* 6:18796. <https://doi.org/10.1038/srep18796>
39. Quinet M, Bataille G, Dobrev PI, et al (2014) Transcriptional and hormonal regulation of petal and stamen development by STAMENLESS, the tomato (*Solanum lycopersicum* L.) orthologue to the B-class APETALA3 gene. *J Exp Bot* 65:2243–2256. <https://doi.org/10.1093/jxb/eru089>
40. de Martino G, Pan I, Emmanuel E, et al (2006) Functional analyses of two tomato APETALA3 genes demonstrate diversification in their roles in regulating floral development. *Plant Cell* 18:1833–1845. <https://doi.org/10.1105/tpc.106.042978>
41. van der Krol AR, Brunelle A, Tsuchimoto S, Chua NH (1993) Functional analysis of petunia floral homeotic MADS box gene pMADS1. *Genes Dev* 7:1214–1228
42. Vandenbussche M, Zethof J, Royaert S, et al (2004) The duplicated B-class heterodimer model: whorl-specific effects and complex genetic interactions in *Petunia hybrida* flower development. *Plant Cell* 16:741–754. <https://doi.org/10.1105/tpc.019166>
43. Vandenbussche M, Theissen G, Van de Peer Y, Gerats T (2003) Structural diversification and neo-functionalization during floral MADS-box gene evolution by C-terminal frameshift mutations. *Nucleic Acids Res* 31:4401–4409. <https://doi.org/10.1093/nar/gkg642>
44. Rijpkema AS, Royaert S, Zethof J, et al (2006) Analysis of the *Petunia* TM6 MADS box gene reveals functional divergence within the DEF/AP3 lineage. *Plant Cell* 18:1819–1832. <https://doi.org/10.1105/tpc.106.042937>

45. Schwarz-Sommer Z, Hue I, Huijser P, et al (1992) Characterization of the Antirrhinum floral homeotic MADS-box gene *deficiens*: evidence for DNA binding and autoregulation of its persistent expression throughout flower development. *EMBO J* 11:251–263
46. McGonigle B, Bouhidel K, Irish VF (1996) Nuclear localization of the Arabidopsis *APETALA3* and *PISTILLATA* homeotic gene products depends on their simultaneous expression. *Genes Dev* 10:1812–1821. <https://doi.org/10.1101/gad.10.14.1812>
47. Tröbner W, Ramirez L, Motte P, et al (1992) *GLOBOSA*: a homeotic gene which interacts with *DEFICIENS* in the control of Antirrhinum floral organogenesis. *EMBO J* 11:4693–4704
48. Goto K, Meyerowitz EM (1994) Function and regulation of the Arabidopsis floral homeotic gene *PISTILLATA*. *Genes Dev* 8:1548–1560. <https://doi.org/10.1101/gad.8.13.1548>
49. Liljegren SJ, Ditta GS, Eshed Y, et al (2000) *SHATTERPROOF* MADS-box genes control seed dispersal in Arabidopsis. *Nature* 404:766–770. <https://doi.org/10.1038/35008089>
50. Causier B, Castillo R, Zhou J, et al (2005) Evolution in action: following function in duplicated floral homeotic genes. *Curr Biol* 15:1508–1512. <https://doi.org/10.1016/j.cub.2005.07.063>
51. Fourquin C, Ferrándiz C (2012) Functional analyses of *AGAMOUS* family members in *Nicotiana benthamiana* clarify the evolution of early and late roles of C-function genes in eudicots. *Plant J* 71:990–1001. <https://doi.org/10.1111/j.1365-3113.2012.05046.x>
52. Pan IL, McQuinn R, Giovannoni JJ, Irish VF (2010) Functional diversification of *AGAMOUS* lineage genes in regulating tomato flower and fruit development. *J Exp Bot* 61:1795–1806. <https://doi.org/10.1093/jxb/erq046>
53. Gimenez E, Castañeda L, Pineda B, et al (2016) *TOMATO AGAMOUS1* and *ARLEQUIN/TOMATO AGAMOUS-LIKE1* MADS-box genes have redundant and divergent functions required for tomato reproductive development. *Plant Mol Biol* 91:513–531. <https://doi.org/10.1007/s11103-016-0485-4>
54. Giménez E, Pineda B, Capel J, et al (2010) Functional analysis of the *Arlequin* mutant corroborates the essential role of the *Arlequin/TAGL1* gene during reproductive development of tomato. *PLoS ONE* 5:e14427. <https://doi.org/10.1371/journal.pone.0014427>
55. Zhao J, Gong P, Liu H, et al (2021) Multiple and integrated functions of floral C-class MADS-box genes in flower and fruit development of *Physalis floridana*. *Plant Mol Biol* 107:101–116. <https://doi.org/10.1007/s11103-021-01182-4>
56. Gong P, Song C, Liu H, et al (2021) *Physalis floridana* *CRABS CLAW* mediates neofunctionalization of *GLOBOSA* genes in carpel development. *J Exp Bot* 72:6882–6903. <https://doi.org/10.1093/jxb/erab309>
57. Bowman JL, Smyth DR (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

58. Morel P, Heijmans K, Ament K, et al (2018) The Floral C-Lineage Genes Trigger Nectary Development in *Petunia* and *Arabidopsis*. *Plant Cell* 30:2020–2037. <https://doi.org/10.1105/tpc.18.00425>
59. Angenent GC, Franken J, Busscher M, et al (1995) A novel class of MADS box genes is involved in ovule development in *petunia*. *Plant Cell* 7:1569–1582. <https://doi.org/10.1105/tpc.7.10.1569>
60. Pinyopich A, Ditta GS, Savidge B, et al (2003) Assessing the redundancy of MADS-box genes during carpel and ovule development. *Nature* 424:85–88. <https://doi.org/10.1038/nature01741>
61. Huang B, Routaboul J-M, Liu M, et al (2017) Overexpression of the class D MADS-box gene *Sl-AGL11* impacts fleshy tissue differentiation and structure in tomato fruits. *J Exp Bot* 68:4869–4884. <https://doi.org/10.1093/jxb/erx303>
62. Angenent GC, Franken J, Busscher M, et al (1994) Co-suppression of the *petunia* homeotic gene *fbp2* affects the identity of the generative meristem. *Plant J* 5:33–44
63. Pnueli L, Hareven D, Broday L, et al (1994) The *TM5* MADS Box Gene Mediates Organ Differentiation in the Three Inner Whorls of Tomato Flowers. *Plant Cell* 6:175–186. <https://doi.org/10.1105/tpc.6.2.175>
64. Pelaz S, Ditta GS, Baumann E, et al (2000) B and C floral organ identity functions require *SEPALLATA* MADS-box genes. *Nature* 405:200–203. <https://doi.org/10.1038/35012103>
65. Ditta G, Pinyopich A, Robles P, et al (2004) The *SEP4* gene of *Arabidopsis thaliana* functions in floral organ and meristem identity. *Curr Biol* 14:1935–1940. <https://doi.org/10.1016/j.cub.2004.10.028>
66. Melzer R, Theissen G (2009) Reconstitution of “floral quartets” in vitro involving class B and class E floral homeotic proteins. *Nucleic Acids Res* 37:2723–2736. <https://doi.org/10.1093/nar/gkp129>
67. Immink RGH, Tonaco IAN, de Folter S, et al (2009) *SEPALLATA3*: the “glue” for MADS box transcription factor complex formation. *Genome Biol* 10:R24. <https://doi.org/10.1186/gb-2009-10-2-r24>
68. Vandenbussche M, Zethof J, Souer E, et al (2003) Toward the analysis of the *petunia* MADS box gene family by reverse and forward transposon insertion mutagenesis approaches: B, C, and D floral organ identity functions require *SEPALLATA*-like MADS box genes in *petunia*. *Plant Cell* 15:2680–2693. <https://doi.org/10.1105/tpc.017376>
69. Favaro R, Pinyopich A, Battaglia R, et al (2003) MADS-box protein complexes control carpel and ovule development in *Arabidopsis*. *Plant Cell* 15:2603–2611. <https://doi.org/10.1105/tpc.015123>
70. Rijpkema AS, Zethof J, Gerats T, Vandenbussche M (2009) The *petunia* *AGL6* gene has a *SEPALLATA*-like function in floral patterning. *Plant J* 60:1–9. <https://doi.org/10.1111/j.1365-313X.2009.03917.x>

71. Soyk S, Lemmon ZH, Oved M, et al (2017) Bypassing Negative Epistasis on Yield in Tomato Imposed by a Domestication Gene. *Cell* 169:1142-1155.e12. <https://doi.org/10.1016/j.cell.2017.04.032>
72. Soyk S, Lemmon ZH, Sedlazeck FJ, et al (2019) Duplication of a domestication locus neutralized a cryptic variant that caused a breeding barrier in tomato. *Nature Plants* 5:471. <https://doi.org/10.1038/s41477-019-0422-z>
73. Bowman JL, Alvarez J, Weigel D, et al (1993) Control of flower development in *Arabidopsis thaliana* by APETALA1 and interacting genes. *Development* 119:721–743. <https://doi.org/10.1242/dev.119.3.721>
74. Kempin SA, Savidge B, Yanofsky MF (1995) Molecular basis of the cauliflower phenotype in *Arabidopsis*. *Science* 267:522–525
75. Bowman JL, Smyth DR, Meyerowitz EM (2012) The ABC model of flower development: then and now. *Development* 139:4095–4098. <https://doi.org/10.1242/dev.083972>
76. Zhang R, Guo C, Zhang W, et al (2013) Disruption of the petal identity gene APETALA3-3 is highly correlated with loss of petals within the buttercup family (Ranunculaceae). *Proc Natl Acad Sci USA* 110:5074–5079. <https://doi.org/10.1073/pnas.1219690110>
77. Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. *Plant Cell* 9:1963–1971. <https://doi.org/10.1105/tpc.9.11.1963>
78. Aloni R, Aloni E, Langhans M, Ullrich CI (2006) Role of auxin in regulating *Arabidopsis* flower development. *Planta* 223:315–328. <https://doi.org/10.1007/s00425-005-0088-9>
79. Lampugnani ER, Kilinc A, Smyth DR (2013) Auxin controls petal initiation in *Arabidopsis*. *Development* 140:185–194. <https://doi.org/10.1242/dev.084582>
80. Smith RS, Guyomarc'h S, Mandel T, et al (2006) A plausible model of phyllotaxis. *Proc Natl Acad Sci USA* 103:1301–1306. <https://doi.org/10.1073/pnas.0510457103>
81. Jönsson H, Heisler MG, Shapiro BE, et al (2006) An auxin-driven polarized transport model for phyllotaxis. *Proc Natl Acad Sci USA* 103:1633–1638. <https://doi.org/10.1073/pnas.0509839103>
82. Kitazawa MS, Fujimoto K (2015) A dynamical phyllotaxis model to determine floral organ number. *PLoS Comput Biol* 11:e1004145. <https://doi.org/10.1371/journal.pcbi.1004145>
83. Goldental-Cohen S, Israeli A, Ori N, Yasuor H (2017) Auxin Response Dynamics During Wild-Type and entire Flower Development in Tomato. *Plant Cell Physiol* 58:1661–1672. <https://doi.org/10.1093/pcp/pcx102>
84. Zhong J, Preston JC (2015) Bridging the gaps: evolution and development of perianth fusion. *New Phytol* 208:330–335. <https://doi.org/10.1111/nph.13517>
85. Vandenbussche M, Horstman A, Zethof J, et al (2009) Differential recruitment of WOX transcription factors for lateral development and organ fusion in *Petunia* and *Arabidopsis*. *Plant Cell* 21:2269–2283. <https://doi.org/10.1105/tpc.109.065862>

86. Costanzo E, Trehin C, Vandenbussche M (2014) The role of WOX genes in flower development. *Ann Bot* 114:1545–1553. <https://doi.org/10.1093/aob/mcu123>
87. Laux T, Mayer KF, Berger J, Jürgens G (1996) The WUSCHEL gene is required for shoot and floral meristem integrity in *Arabidopsis*. *Development* 122:87–96
88. Zhang C, Wang J, Wang X, et al (2020) UF, a WOX gene, regulates a novel phenotype of unfused flower in tomato. *Plant Sci* 297:110523. <https://doi.org/10.1016/j.plantsci.2020.110523>
89. Nakata M, Matsumoto N, Tsugeki R, et al (2012) Roles of the middle domain-specific WUSCHEL-RELATED HOMEBOX genes in early development of leaves in *Arabidopsis*. *Plant Cell* 24:519–535. <https://doi.org/10.1105/tpc.111.092858>
90. Li J, Song C, He C (2019) Chinese lantern in *Physalis* is an advantageous morphological novelty and improves plant fitness. *Sci Rep* 9:596. <https://doi.org/10.1038/s41598-018-36436-7>
91. He C, Saedler H (2005) Heterotopic expression of MPF2 is the key to the evolution of the Chinese lantern of *Physalis*, a morphological novelty in Solanaceae. *Proc Natl Acad Sci USA* 102:5779–5784. <https://doi.org/10.1073/pnas.0501877102>
92. Khan MR, Hu J-Y, Riss S, et al (2009) MPF2-like-a MADS-box genes control the inflated Calyx syndrome in *Withania* (Solanaceae): roles of Darwinian selection. *Mol Biol Evol* 26:2463–2473. <https://doi.org/10.1093/molbev/msp159>
93. He J, Alonge M, Ramakrishnan S, et al (2023) Establishing *Physalis* as a Solanaceae model system enables genetic reevaluation of the inflated calyx syndrome. *Plant Cell* 35:351–368. <https://doi.org/10.1093/plcell/koac305>
94. He C, Saedler H (2007) Hormonal control of the inflated calyx syndrome, a morphological novelty, in *Physalis*. *Plant J* 49:935–946. <https://doi.org/10.1111/j.1365-313X.2006.03008.x>
95. Lemmon ZH, Reem NT, Dalrymple J, et al (2018) Rapid improvement of domestication traits in an orphan crop by genome editing. *Nat Plants* 4:766–770. <https://doi.org/10.1038/s41477-018-0259-x>
96. Spelt C, Quattrocchio F, Mol JN, Koes R (2000) anthocyanin1 of petunia encodes a basic helix-loop-helix protein that directly activates transcription of structural anthocyanin genes. *Plant Cell* 12:1619–1632. <https://doi.org/10.1105/tpc.12.9.1619>
97. Quattrocchio F, Wing J, van der Woude K, et al (1999) Molecular analysis of the anthocyanin2 gene of petunia and its role in the evolution of flower color. *Plant Cell* 11:1433–1444. <https://doi.org/10.1105/tpc.11.8.1433>
98. Verweij W, Spelt C, Di Sansebastiano G-P, et al (2008) An H⁺ P-ATPase on the tonoplast determines vacuolar pH and flower colour. *Nat Cell Biol* 10:1456–1462. <https://doi.org/10.1038/ncb1805>
99. Faraco M, Spelt C, Bliet M, et al (2014) Hyperacidification of vacuoles by the combined action of two different P-ATPases in the tonoplast determines flower color. *Cell Rep* 6:32–43. <https://doi.org/10.1016/j.celrep.2013.12.009>

100. Strazzer P, Spelt CE, Li S, et al (2019) Hyperacidification of Citrus fruits by a vacuolar proton-pumping P-ATPase complex. *Nat Commun* 10:744. <https://doi.org/10.1038/s41467-019-08516-3>
101. Hoballah ME, Gubitz T, Stuurman J, et al (2007) Single gene-mediated shift in pollinator attraction in *Petunia*. *Plant Cell* 19:779–790. <https://doi.org/10.1105/tpc.106.048694>
102. Esfeld K, Berardi AE, Moser M, et al (2018) Pseudogenization and Resurrection of a Speciation Gene. *Curr Biol* 28:3776-3786.e7. <https://doi.org/10.1016/j.cub.2018.10.019>
103. Van de Peer Y, De Wachter R (1994) TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* 10:569–570