Flower Development in the Solanaceae

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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

Introduction

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

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

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

The Solanaceae family (nightshades): crops and model species

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

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

Specification of floral organ identity: revisions to the ABC model

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

Redefining the A function

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

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

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

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

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

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

Multiple functions for C-class genes

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

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

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

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

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

Uncovering the E function

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

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

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

Divergence to the textbook ABC model

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

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

Initiation and fusion of floral organs

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

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

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

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

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

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

Conclusion

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

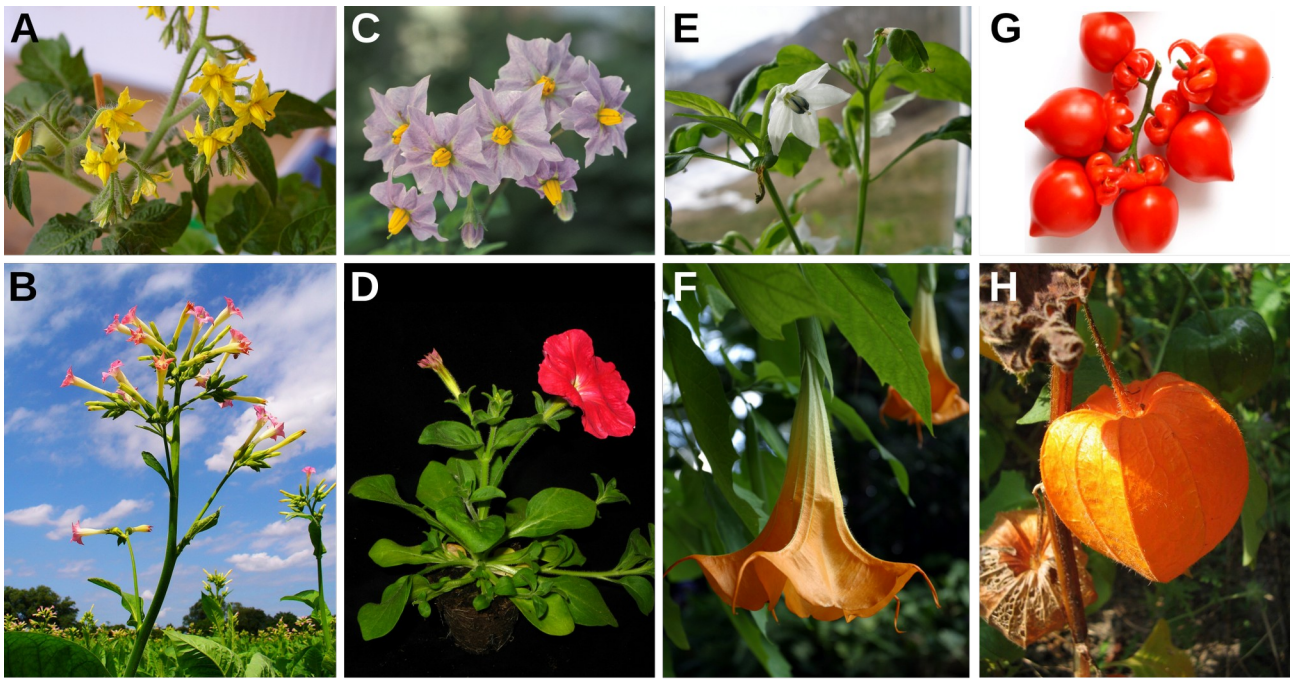

Figure 1. Flowers and inflorescences from various Solanaceae members: *Solanum lycopersicum* (tomato, A), *Nicotiana tabacum* (tobacco, B), *Solanum tuberosum* (potato, C), *Petunia x hybrida* (petunia, D), *Capsicum annuum* (pepper, E), *Brugmansia sp.* (F), fruits from the arlequin tomato mutant (G),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

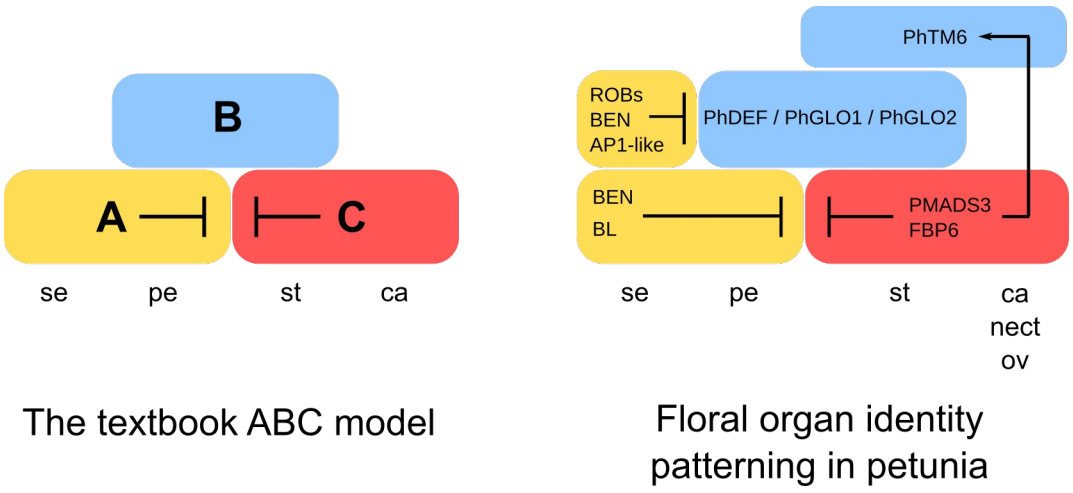

Figure 2. The textbook ABC model and its variation in petunia, as we currently understand it. In the textbook ABC model, A-class genes and C-class genes mutually repress their expression domains. A-class genes alone and in combination with B-class genes specify the identities of sepals (se) and petals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

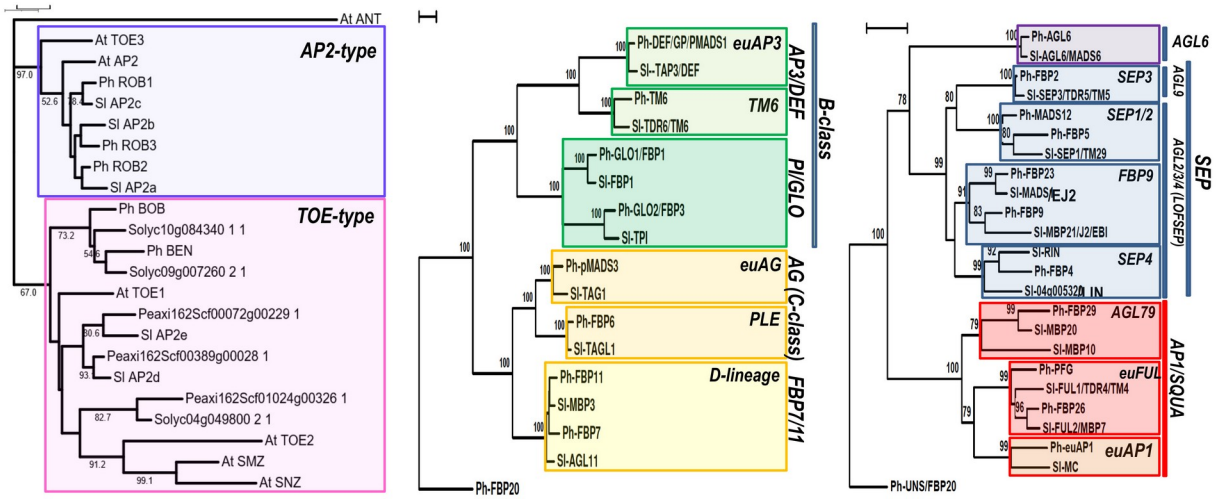

Figure 3. Neighbor joining trees of euAP2 transcription factors (left), B/C/D class (middle) and AP1/SEP/AGL6 superclade (right) MADS-box transcription factors. Prefixes At, Ph and Sl represent *Arabidopsis thaliana*, *Petunia hybrida* and *Solanum lycopersicum* respectively. The euAP2 phylogenetic analysis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

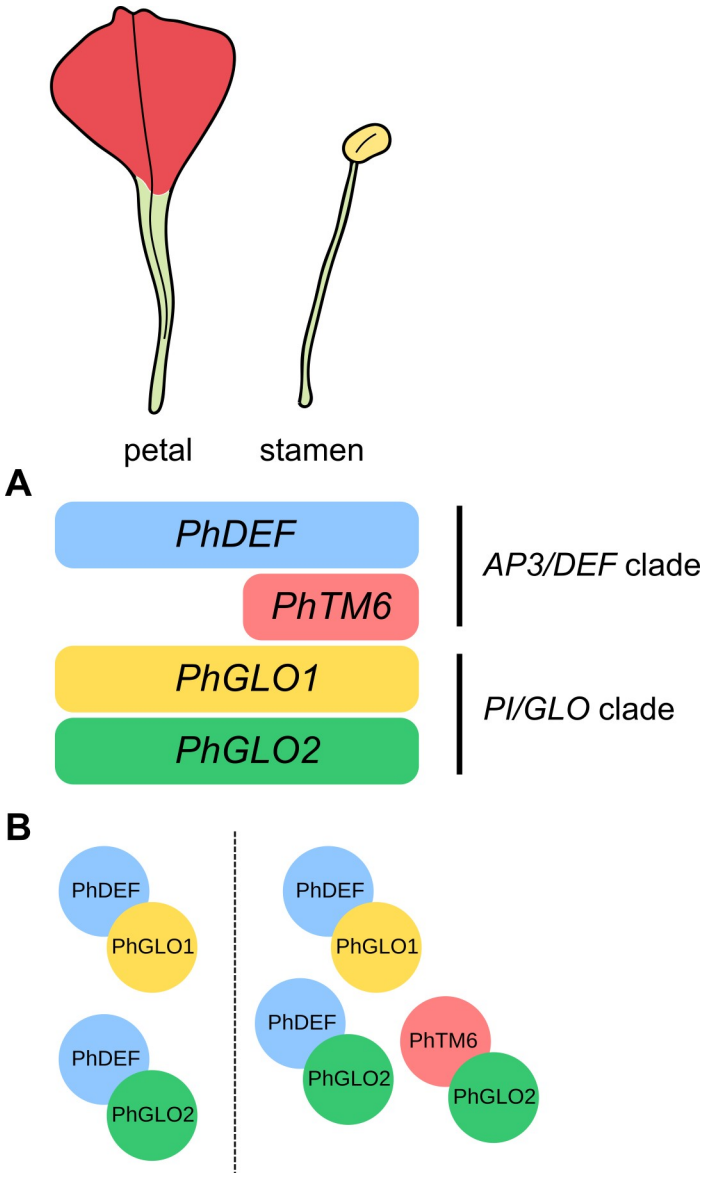

Figure 4. Subfunctionnalization of the B-class genes for specification of petal and stamen identity in petunia. **A**: Expression domains of the *PhDEF*, *PhTM6*, *PhGLO1* and *PhGLO2* genes in petal and stamen initiation domains. **B**: Protein complexes formed between AP3/DEF- and PI/GLO-type proteins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

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