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REVIEW PAPER

Role of the FUL–SHP network in the evolution of fruit morphology and function

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Abstract

***Arabidopsis* research in the last decade has started to unravel the genetic networks directing gynoecium and fruit patterning in this model species. Only recently, the work from several groups has also started to address the conservation of these networks in a wide number of species with very different fruit morphologies, and we are now beginning to understand how they might have evolved. This review summarizes recent advances in this field, focusing mainly on MADS-box genes with a well-known role in dehiscence zone development, while also discussing how these studies may contribute to expand our views on fruit evolution.**

Key words: Fruit dehiscence, fruit evolution, fruit morphology, FRUITFULL, MADS-box, SHATTERPROOF.

Introduction

Genetic networks directing fruit patterning started to be unravelled about a decade ago, with the first identification in *Arabidopsis* of key factors with an impact in carpel and fruit development. Mutations in members of different transcription factor families such as the YABBY gene *CRABS CLAW*, the basic helix-loop-helix (bHLH) gene *SPATULA* or the auxin-response factor *ETTIN* were found to alter carpel morphogenesis (Bowman and Smyth, 1999; Heisler *et al.*, 2001; Sessions *et al.*, 1997). In addition, three members of the MADS-box family, *FRUITFULL* (*FUL*) and *SHATTERPROOF 1* and *2* (*SHP1*/*SHP2*), were shown to be required for correct fruit patterning, regulating lignin deposition, cell expansion, and cell separation processes in the *Arabidopsis* pod (Gu *et al.*, 1998; Liljegren *et al.*, 2000).

The *Arabidopsis* fruit is called a silique, a dehiscent pod derived from two congenitally fused carpels, which at the end of development dries and opens through four longitudinal sutures called dehiscence zones (DZs). DZs are formed at the margin of the valves, the carpel walls, and morphologically they are already distinct at the late stages of gynoecium development, when they are visible as longitudinal furrows (Roeder and Yanofsky, 2005). In mature fruits, the DZs comprise a separation layer composed of small cells defining a fracture plane and an adjacent layer of lignified cells that, together with the lignified endocarp, will create mechanical tensions as the fruit dries, thus facilitating valve detachment (Figs 1 and 2). *shp1 shp2* double mutants fail to develop a functional DZ, which does not lignify or has a defined separation layer, and, as a consequence, the fruits are indehiscent and do not open at the end of development (Fig. 2; Liljegren *et al.*, 2000). *ful* mutants, in contrast, have a very reduced ovary, where the valves do not expand and are composed of small lignified cells, acquiring the characteristics of ectopic DZs (Fig. 2; Gu *et al.*, 1998). Opposite phenotypes are caused by constitutive expression of *FUL* (indehiscent fruits similar to *shp1 shp2* mutants) or *SHP1/2* (small fruits with overlignified valves) (Fig. 2; Ferrándiz *et al.*, 2000b). Further detailed molecular and genetic analyses of these antagonistic roles of *FUL* and *SHP* in DZ formation led to the proposal of a model were *SHP* would be placed at the top of the genetic hierarchy directing DZ formation, while *FUL* would act in the ovary walls to restrict *SHP* expression domain, thus ensuring the correct

Abbreviations: DZ, dehiscence zone; VIGS, virus-induced gene silencing.

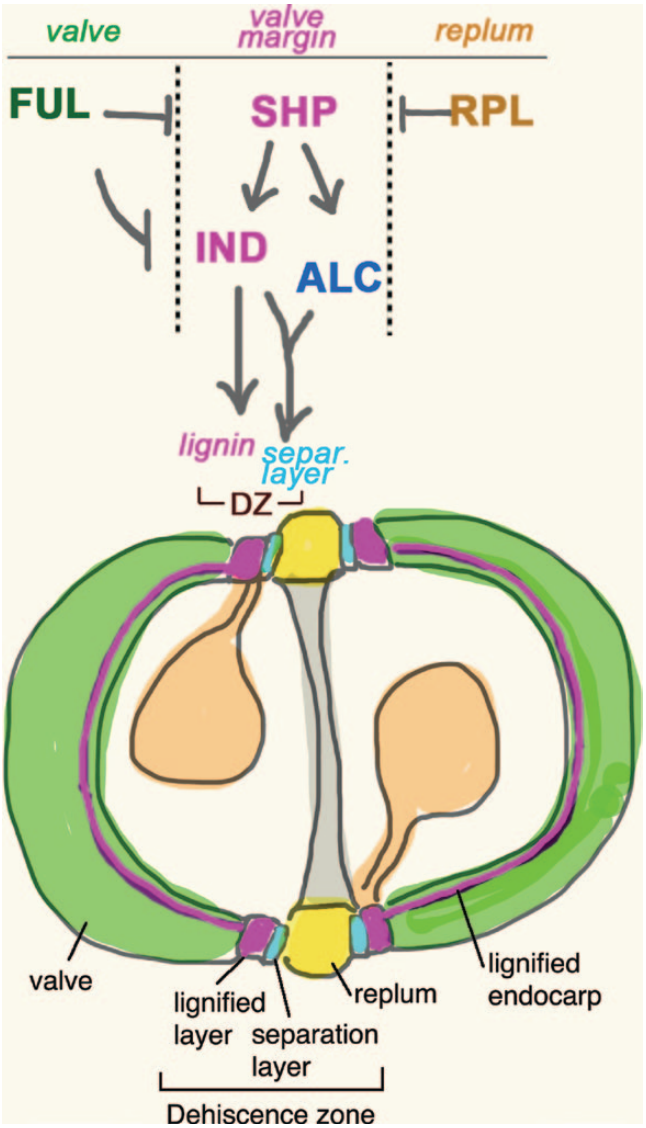

Fig. 1. Simplified genetic model for the development of the DZ in *Arabidopsis*. The cartoon represents a transversal section through the mature ovary, where valves are coloured in green, the lignified layer and the lignified endocarp in pink, the separation layer in blue, and the replum in yellow.

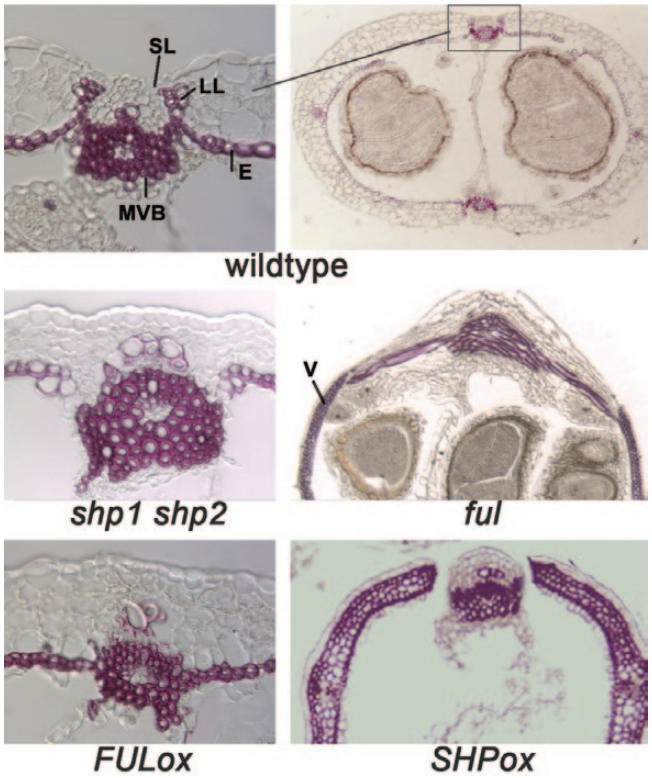

Fig. 2. Fruit lignification patterns of *Arabidopsis* mutants and transgenic lines affected in dehiscence. Top right: transversal section of a mature wild-type ovary. Phloroglucinol staining reveals lignified cells in dark pink. Top left: close up of the DZ. The medial vascular bundle (MVB) appears heavily lignified, together with the endocarp (E) and the lignified layer (LL) of the DZ. The separation layer (SL) appears as parallel rows of small cells. Middle left: *shp1 shp2* double mutant. Note the absence of the lignified and separation layers. Middle right: *ful* mutant. The valves (V) are heavily lignified and composed of small cells. Bottom left: *35S::FUL* line. The lignification pattern is remarkably similar to that of *shp1 shp2* mutants. Bottom right: *35S::SHP1 35S::SHP2* line. The valves are heavily lignified as in *ful* mutants.

spatial positioning of the DZ (Fig. 1; Ferrándiz *et al.*, 2000b; Liljegren *et al.*, 2000). Other genetic functions were identified subsequently that completed the picture, most importantly the bHLH factors INDEHISCENT (IND) and ALCATRAZ (ALC), and the homeodomain factor REPLUMLESS (RPL) (Rajani and Sundaresan, 2001; Roeder *et al.*, 2003; Liljegren *et al.*, 2004), and currently, we understand quite well which are the main elements that ensure the development of this important structure (Fig. 1). The components and the architecture of the network directing DZ formation have been described in detail in several excellent reviews (Dinneny and Yanofsky, 2005; Balanzá *et al.*, 2006; Ostergaard, 2008) and are not the major focus of this paper, so the reader is referred to these other works for more comprehensive information. Briefly, *SHP* genes are expressed at the valve margins from the early stages of gynoecium development, where they activate the expression of *IND*, essential for both separation and lignified layer development, and *ALC*, required only for separation layer formation. *FUL* is expressed at the valves, where it represses *SHP* and *IND* expression, while *RPL* does the same in the replum (the external domain of the septum that divides the ovary in two chambers). Thus, *FUL* and *RPL* keep *SHP*, *ALC*, and *IND* expression restricted to the narrow strip of cells that will differentiate into the DZ at the valve margins.

Although the basic configuration of this genetic network in *Arabidopsis* has been known for some years, our knowledge about the functional conservation of these genes in distantly related species within the angiosperms has been very scarce. Only recently, the increase in available reverse genetic resources and RNA interference or virus-induced gene silencing (VIGS) methodologies in a wide number of species has allowed us to study whether these gene functions have equivalent roles in other species with similar (dry dehiscent) or highly different fruit morphologies (such as fleshy berries).

In this review, we will try to summarize recent progress in this subject, mainly focusing on *FUL* and *SHP*, which in *Arabidopsis* are placed at the top of the regulatory hierarchy directing DZ formation. We will review the latest work

done in several species from a wide range of eudicot families, including other members of the Brassicaceae, other species from the rosid lineage such as peach and legumes, asterids of the Solanaceae family with very different fruit morphologies (such as *Nicotiana*, *Petunia*, and tomato) and in basal eudicots. These studies have permitted us to gain insights into the conservation of these gene functions in eudicots and are expanding our views on how fruits have evolved, opening exciting questions in this field.

The dehiscence network in Brassicaceae

Arabidopsis thaliana belongs to the Brassicaceae family, which contains over 300 genera, including a number of important crops such as *Brassica oleracea* (broccoli, cabbage, cauliflower, etc.), *Brassica rapa* (e.g. turnip, Chinese cabbage), *Brassica napus* (e.g. rapeseed) or *Raphanus sativus* (common radish). The typical fruit of the family is a silique, a two-valved dry capsule, usually dehiscent, but within the family a considerable morphological variability as well as frequent dehiscent–indehiscent transitions are found.

Recently, two comparative gene expression studies of the major gene functions directing DZ formation have been conducted in fruits of different species of Brassicaceae. Muhlhausen *et al.* (2013) used *Lepidium*, a genus closely related to *Arabidopsis*, which contains species with dehiscent and indehiscent siliques, to investigate the expression patterns of *ALC*, *FUL*, *IND*, *RPL*, and *SHP* orthologues in both types of fruits. This work showed that the expression patterns of these orthologues were highly conserved between *Arabidopsis* and *Lepidium campestre* (dehiscent fruits), while in *Lepidium appellianum*, a species with indehiscent fruits, *SHP*, *IND*, and *ALC* expression were absent from valve margins, supporting the suggestion that changes in the genetic pathway identified in *A. thaliana* cause the transition from dehiscent to indehiscent fruits in *L. appellianum*. Subsequent work from Lenser and Theissen (2013) included further functional studies of some of these orthologues that were either downregulated or overexpressed in transgenic *L. campestre* plants. The phenotypes of the transgenic plants were very similar to the corresponding mutants in *Arabidopis*, demonstrating a high degree of conservation of the genetic network and indicating that the dehiscence process remains basically unchanged among species.

A second comparative study on gene expression patterns was carried out on Brassicaceae species of the Brassiceae tribe that possess heteroarthrocarpic fruits (Avino *et al.*, 2012). These fruits are bisected and develop a distal indehiscent segment that may separate as a propagule and a proximal segment that can be dehiscent or indehiscent. This novel fruit type appears to be, at least in part, the consequence of repositioning of valve margins, and thus offers a fantastic opportunity to assess the importance of variations on the basic DZ genetic network to generate morphological diversity. In this work, the expression patterns of the same set of orthologues were examined in two Brassiceae species with heteroarthrocarpic fruits and different dehiscence patterns, *Erucaria erucarioides* and *Cakile lanceolata*. Again, the DZ genes were expressed in the valve margins of dehiscent segments while absent in the indehiscent segments, but did not appear to have a role in the abscission zone that divided the proximal and distal segments (Avino *et al.*, 2012). Although more functional analyses are needed to understand the origin of heteroarthrocarpy, this study also supports the conservation of the dehiscent pathway and the suggestion that modifications in the network may lead to morphological innovations.

Further supporting this idea, a study done in *Brassica* species identified a nucleotide change in a conserved *cis*-element of the 5′ regulatory region of *RPL* at the evolutionary origin of the typical morphology of the replum in this genus, which is very reduced in width compared with that in *Arabidopsis*. This point mutation in the *RPL* promoter was shown to reduce *RPL* expression in fruits and was linked to narrow replum morphology (Arnaud *et al.*, 2011). Interestingly, this same point mutation was identified previously in the sequence of the *RPL* homologue in rice as the likely cause for a similar defect in a different separation process, the reduction of seed abscission selected during rice domestication (Konishi *et al.*, 2006).

Other studies also provide examples of potential biotechnological applications derived from the knowledge gained on this genetic network. Thus, overexpression of the *Arabidopsis FUL* gene in *Brassica juncea* abolished the expression of *SHP* genes in the valve margins and induced the same phenotype of fruit indehiscence observed in *Arabidopsis 35S::FUL* plants (Ostergaard *et al.*, 2006). Similarly, downregulation of *IND* orthologues in *B. oleracea* or loss-of-function *IND* mutants in *B. rapa* showed much reduced fruit shattering (Girin *et al.*, 2010). Such indehiscent phenotypes are desirable traits for crops like canola that rely on seed production, reducing losses due to premature pod shattering, and therefore bear promise of huge biotechnological potential.

The complex story of the SHP and FUL families

While the conservation of the genetic network directing DZ formation in the Brassicaceae has been addressed quite comprehensively, outside this clade comparative studies on the genetic functions of the whole network are still scarce. *IND* and *ALC* orthologues are only present within the Brassicaceae where they have probably diverged recently from a duplication of HECATE-like or SPATULA-like ancestors, respectively, and therefore the reconstruction of their ancestral functions is a complex task, only addressed partially so far (Groszmann *et al.*, 2011; Tani *et al.*, 2011; Kay *et al.*, 2012). *RPL* belongs to the BEL1-type clade of the homeobox family, and, while related genes are found across angiosperms, there are few studies focused on functional characterization of these genes other than those already cited (Konishi *et al.*, 2006; Arnaud *et al.*, 2011). In contrast, *FUL* and *SHP* belong to the much studied MADS-box family and have been the subject of a wealth of different studies, many of them focused

on the phylogenetic relationships of the family in the context of angiosperm evolution but also recently incorporating functional data in different species.

As already mentioned, *FUL* and *SHP* encode MADS-box transcription factors. The MADS-box genes are involved in virtually all aspects of plant development, although initially they were identified by their key roles in flower development. The large MADS-box family has a complex history characterized by many duplication events followed by sequence divergence and neo-functionalization that have played an important role in reproductive innovations during land plant radiation (Alvarez-Buylla *et al.*, 2000; Theissen *et al.*, 2000; Becker and Theissen, 2003; Soltis *et al.*, 2007). Among the MADS-box genes, *FUL* belongs to the APETALA1 (AP1)/FUL clade and *SHP1/2* to the AGAMOUS (AG) clade.

The study of the evolutionary history of gene duplications in the AG subfamily has shown that, early in angiosperm history but after the divergence of angiosperms and gymnosperms, a duplication event generated the C-lineage and D-lineage (Kramer *et al.*, 2004), named after the function of the corresponding genes in stamen and carpel specification (C-function) or in ovule development (D-function). A more recent duplication event in the AG lineage that took place early in the history of the core eudicots, predating the divergence of rosids and asterids, gave rise to the euAG and PLENA/SHP (PLE/SHP) clades, which include *AG* and *SHP1/2*, respectively (Fig. 3A; Kramer *et al.*, 2004; Irish and Litt, 2005).

Genes from the AG lineage have been identified and functionally characterized in various groups of plants, including monocots and basal eudicots, and their involvement in C-function has been broadly demonstrated, suggesting that this role has an ancestral origin. In *Arabidopsis*, *AG* belongs to the euAG clade and *SHP1* and *SHP2* belong to the PLE/SHP clade. *AG* is expressed in developing carpel and stamens from very early stages of development and carries out the canonical C-function, specifying sexual organ identity and floral meristem determinacy (Yanofsky *et al.*, 1990; Bowman *et al.*, 1991). By contrast, *SHP* genes are expressed specifically in carpels at later stages of development, restricted to carpel margins and to ovules, and are involved in fruit lignification and dehiscence (Liljegren *et al.*, 2000). Interestingly, in *Antirrhinum majus*, it was shown that the C-function was carried out by *PLENA* (*PLE*), the *SHP* orthologue, while *FARINELLI* (*FAR*), the *AG* orthologue, was specific to pollen development (Bradley *et al.*, 1993; Davies *et al.*, 1999). Because *Arabidopsis* belongs to the rosid lineage and *Antirrhinum* is an asterid, these studies led to the idea that a functional switch between the euAG and PLE lineages to specify C-function and the subfunctionalization of *SHP* genes to control fruit dehiscence occurred after divergence of the asterids and rosids (Fig. 3A; Causier *et al.*, 2005). However, further work in an increasing number of species has undermined this hypothesis while highlighting the functional plasticity of *euAG* and *PLE/SHP* genes in flower development and the likely ancestral role of *PLE/SHP*

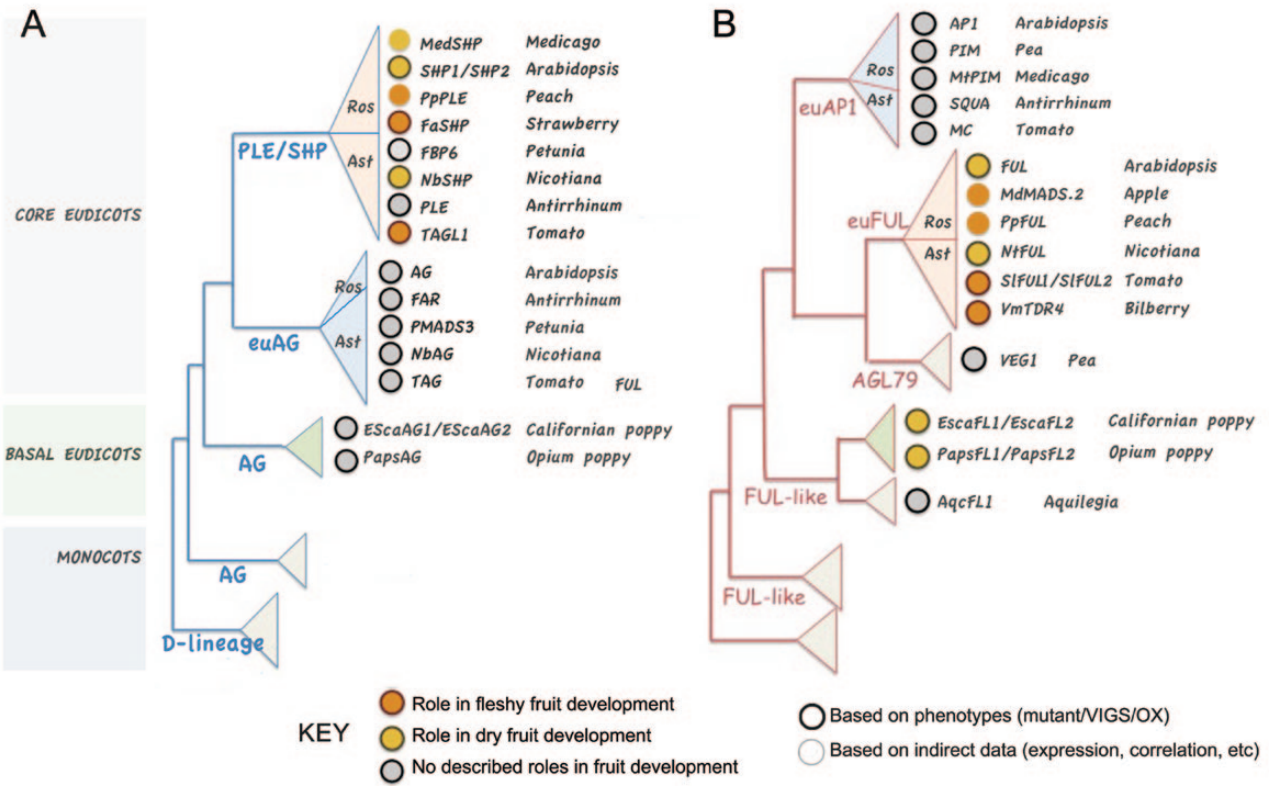

Fig. 3. Simplified phylogenies of AG (A) and AP1/FUL (B) families with the different gene functions discussed in this review mapped onto branches. Trees have been freely adapted from those shown in Kramer *et al.* (2004) and Pabon-Mora *et al.* (2013a). Gene functions related to late fruit development have been colour-coded in yellow (dry fruits) or orange (fleshy fruits). When roles have been inferred from functional data supported by phenotypic effects, circles are highlighted with solid contours. Within the core eudicot clades, species belonging to rosids (Ros) and asterids (Ast) have been indicated.

genes in late fruit development, at least in core eudicots (see below; Airoldi *et al.*, 2010; Fourquin and Ferrandiz, 2012; Heijmans *et al.*, 2012).

Likewise, the same early duplication event in core eudicots was the origin of the euAP1 and FUL lineages, which further underwent a close duplication event before the radiation of core eudicots to generate the euFUL and AGL79 lineages (Fig. 3B; Shan *et al.*, 2007; Pabon-Mora *et al.*, 2013a). *euFUL* genes are most similar in sequence and expression patterns to *FUL*-like genes from basal angiosperms and basal eudicots, their likely functional counterparts, while *euAP1* and *AGL79* genes result from significant changes in sequence and expression patterns that would probably favour their neofunctionalization (Shan *et al.*, 2007; Pabon-Mora *et al.*, 2013a).

In *Arabidopsis*, both AP1 and FUL have roles in floral meristem identity and axillary meristem repression (Bowman *et al.*, 1993; Ferrándiz *et al.*, 2000a), while no function has been assigned to AGL79 yet. *AP1* expression is restricted to floral meristems and later to sepals and petals, while *FUL* is expressed in cauline leaves, stem vasculature, inflorescence meristem, and carpels (Bowman *et al.*, 1993; Mandel and Yanofsky, 1995). In accordance with their different expression patterns, *AP1* and *FUL* also have independent functions. AP1 plays an important role in sepal and petal identity, whereas FUL controls flowering time, cauline leaf morphology, and fruit development (Irish and Sussex, 1990; Bowman *et al.*, 1993; Gu *et al.*, 1998; Ferrándiz *et al.*, 2000a; Melzer *et al.*, 2008). Characterization of *euAP1* genes in other species has demonstrated their conserved role in floral meristem and floral organ identity (Huijser *et al.*, 1992; Berbel *et al.*, 2001; Vrebalov *et al.*, 2002; Benlloch *et al.*, 2006). However, although some studies suggest a broad function of *euFUL* and *AGL79* genes in meristem function, the possible conservation of euFUL fruit-related functions only has been uncovered recently (see below; Fig. 3A; Immink *et al.*, 1999; Bemer *et al.*, 2012; Berbel *et al.*, 2012).

The roles of FUL and SHP in the development of dry fruits from core eudicots

The large Solanaceae family, included in the asterid lineage within the core eudicots, possess fruits derived from a bicarpellate gynoecium with axile placentation that, after fertilization, form predominantly either dry capsules or fleshy berries (Pabon-Mora and Litt, 2011). Detailed functional studies on the role of *euAG* and *PLE*/*SHP* genes have been conducted mainly in two species with dry dehiscent fruits, *Nicotiana benthamiana* and *Petunia hybrida*. Characterization of mutants for *PMADS3* (the *euAG* gene) and *FBP6* (the *PLE*/*SHP* gene) in *Petunia* showed that both genes were required for conferring stamen and carpel identity and for floral meristem determinacy (Heijmans *et al.*, 2012). Similar results were obtained using transitory silencing (by VIGS) in *Nicotiana* (Fourquin and Ferrandiz, 2012), highlighting the largely overlapping roles of the *euAG* and *PLE*/*SHP* genes for the C-function in Solanacecae. These findings contrast with the strong subfunctionalization observed in *Arabidopsis* or *Antirrhinum*, emphasizing the great plasticity in the evolutionary history of the euAG and PLE/SHP lineages.

The study in *Nicotiana* also permitted us to uncover the late function of the *NbSHP* gene in fruit development. *N. benthamiana* fruits are dry capsules that, when mature, open along four DZs that are strongly lignified and structurally similar to those of *Arabidopsis*. In fruits where *NbSHP* expression was reduced, no lignification was observed at the DZs and the fruits failed to dehisce, even long after maturation (Fourquin and Ferrandiz, 2012). Interestingly, the same phenotype was obtained in another species of the genus, *Nicotiana tabacum*, when overexpressing *NtFUL*, the *euFUL* orthologue (Smykal *et al.*, 2007). Altogether, these results indicate that the *PLE*/*SHP* and *euFUL* genes in *Nicotiana* and *Arabidopsis* have equivalent roles in late fruit development, with PLE/SHP promoting lignification and fruit dehiscence, and euFUL inhibiting them, despite the evolutionary distance between these species. It is therefore likely that the role of the FUL–SHP module in fruit lignification and dehiscence was already acquired in the ancestor of asterids and rosids.

Legumes, a large family of the rosids lineage, are characterized by long dry and dehiscent pods derived from a single carpel. In spite of great agronomic importance, the molecular mechanisms involved in fruit dehiscence in legumes are still poorly understood, although a number of works have identified several quantitative trait loci in different species related to pod dehiscence that still remain to be correlated with genetic functions (Weeden *et al.*, 2002; Liu *et al.*, 2007; Isemura *et al.*, 2012). However, the probable conserved role of the FUL–SHP module in fruit development was used to highlight how variations in the network may originate evolutionary innovations through the study of pod morphology in the *Medicago* genus (Fourquin *et al.*, 2013). *Medicago* species display a wide variety of fruit shapes, ranging from the typical legume pod, straight and dehiscent, to spiny spiral pods adapted to epizoochoric dispersal. In this study, coiled pod morphology in the genus was correlated with overlignification of the valve margin and with a small change in the *SHP* coding sequence with the potential to alter SHP affinity for interacting protein partners (Fourquin *et al.*, 2013). Again, this work supports the conservation of FUL–SHP roles in fruit lignification and suggests that modifications in these functions may lead to morphological diversity.

The FUL–SHP network in fleshy fruits

Unlike dry capsules or pods, fleshy fruits have evolved to attract animals for seed dispersal. Fleshy fruit maturation, also called ripening, involves changes in colour, texture, and aroma (Seymour *et al.*, 2008), but not the development of a dehiscence zone, and thus, the role of the FUL–SHP module and other genes in the network are not easy to extrapolate, even if some functional conservation is to be expected.

Fruit ripening has been studied mostly in tomato (*Solanum lycopersicum*), a Solanaceae species and therefore related to *Petunia* and *Nicotiana*. Several independent studies have

addressed the role in tomato of *euAG* and *PLE*/*SHP* orthologues, named *TAG1* and *TAGL1*, respectively (Pnueli *et al.*, 1994; Itkin *et al.*, 2009; Vrebalov *et al.*, 2009; Gimenez *et al.*, 2010; Pan *et al.*, 2010). Interestingly, these studies have shown that *TAGL1* is required for pericarp expansion and climacteric ripening of tomato fruits. Tomato plants with reduced expression of *TAGL1* exhibit yellow-orange fruits with a reduced pericarp and alteration in ethylene and carotenoids levels, suggesting that the role of *PLE*/*SHP* genes in late fruit development is also present in fleshy fruits. In contrast to the *PLE*/*SHP* genes of other Solanaceae, *TAGL1* does not appear to have a role in meristem determinacy or floral organ identity, while downregulation of the *euAG* gene *TAG1* causes stamen-to-petal transformations and loss of floral determinacy, but no significant defects in carpel identity, suggesting that additional gene(s) participate with *TAG1* in this function (Pan *et al.*, 2010). Because the phenotypes of simultaneous downregulation of *TAG1* and *TAGL1* have not been described yet, it is still possible that they are both redundant in carpel specification, much like their counterparts in *Petunia* and *Nicotiana*.

Functional studies on the role of *PLE*/*SHP* genes in other species with fleshy fruits are still scarce. In peach (*Prunus persica*), a species from the rosid lineage and with climacteric fruits, the expression of *PpPLE*, the *PLE*/*SHP* orthologue, increases during fruit ripening (Tadiello *et al.*, 2009) and, interestingly, is more abundant in cultivars prone to split-pit formation (a structure structurally similar to the DZ) and with stronger lignification patterns (Tani *et al.*, 2007). In addition, a recent work by Daminato *et al.* (2013) has been conducted in strawberry (*Fragaria* × *ananassa*, also from the rosid lineage) that describes the phenotypes associated with *FaSHP* transient downregulation during fruit development. Strawberries form false fruits that not derived directly from the ovaries of fertilized gynoecia but from the receptacle of the flower, which grows and ripens in a non-climacteric way, dotted on its surface by the multiple achenes derived from the apocarpous gynoecium. Remarkably, reduced *FaSHP* expression causes a significant delay in ripening in strawberry fruits, a similar phenotype to that observed in tomato, in spite of the profound anatomical and physiological differences between these two fleshy fruits.

Studies on functional conservation of *PLE*/*SHP* genes in fruit development are also being extended to *euFUL* genes. For example, downregulation of *VmTDR4*, the *euFUL* gene from bilberry (the asterid species *Vaccinium myrtillus*) results in reduction of anthocyanin accumulation and altered pigmentation of the mature berry (Jaakola *et al.*, 2010). A detailed study has also addressed the functional characterization of the two *euFUL* genes from tomato, *SlFUL1* (previously called *TDR4* or *TM4*) and *SlFUL2* (previously called *MBP7*). Reducing the expression of both genes greatly affects late fruit development, altering the ethylene-independent ripening processes, including changes in colour, cell-wall composition, cuticle formation, and aroma synthesis (Bemer *et al.*, 2012; Shima *et al.*, 2013). Further indications of functional conservation have also been obtained in apple (*Malus domestica*), where a quantitative trait locus for fruit firmness was associated with one of the *euFUL* genes from this species, *MdMADS2.1* (Cevik *et al.*, 2010).

The phenotypic changes associated with *SlFUL* downregulation in tomato affect aspects of fruit ripening that are different from those caused by decreased expression of *TAGL1* (tomato *SHP*). Together with the dynamics of the corresponding expression patterns during fruit development, these results are compatible with the negative regulation of *TAGL1* by *TDR4*/*SlFUL1* and *MBP7*/*SlFUL2*, a scenario that would support not only the conservation of their roles in late fruit development but also of their regulatory interactions. This idea is further supported by expression studies of *euFUL* and *PLE*/*SHP* genes done in peach, which show opposite trends in expression during fruit ripening, and in cultivars with different lignification patterns and split-pit sensitivity (Tani *et al.*, 2007; Dardick *et al.*, 2010), and strongly parallel the findings described previously for species with dry fruits from both rosid and asterid lineages.

Dehiscent fruits from basal eudicots

The successful use of VIGS technology in the basal eudicots *Eschscholzia californica* (californian poppy) and *Papaver somniferum* (opium poppy) has made these two poppy species good models to study evolution and development (Hileman *et al.*, 2005; Wege *et al.*, 2007). They both possess dry fruits but with very different morphologies. *E. californica* has long siliques derived from two fused carpels that dehisce explosively at maturity, while *P. somniferum* develops indehiscent capsules composed of up to 20 carpels that disperse seeds through small pores opening at the ovary–stigma junction in mature fruits.

Poppy *AG* homologues are sister to the euAG and PLE lineages found in core eudicots. Functional studies both in *E. californica* and *P. somniferum* have shown that *AG* genes carry out typical C-functions, being involved in the specification of stamen and carpel identity and flower determinacy, but have no apparent role in fruit development (Yellina *et al.*, 2010; Hands *et al.*, 2011). These results are consistent with the ancestral role of *AG* genes in sexual identity and flower determinacy, and suggest that the neofunctionalization of *AG*-like genes in fruit development occurred after the duplication that originated the euAG and PLE lineages, specifically in the latter.

Parallel studies have been also carried out to investigate the roles of *FUL*-like genes in basal eudicots. Homologues of *FUL* have been characterized recently in the two poppy model species (Pabon-Mora *et al.*, 2012). VIGS analyses have shown the pleiotropic functions of *FUL*-like genes in flowering time, cauline leaf development, floral meristems, and sepal identity, as well as an important role in fruit development. Downregulation of *FUL*-like genes in *E. californica* and *P. somniferum* caused fruits with ectopic lignification of the pericarp and premature rupture of the ovary walls that strongly resembled the phenotypes of *ful* mutants in *Arabidopsis*. Thus, the *FUL*-like genes in poppies seem to cover all the functions reported for the *euAP1* and *euFUL*

genes in *Arabidopsis*, suggesting that the *AP1*/*FUL* duplication originating the euAP1 and euFUL lineages was followed by the subfunctionalization of the whole set of ancestral FUL-like roles that was then divided between these two lineages (Pabon-Mora *et al.*, 2012). Interestingly, and in contrast to the observations concerning the *AG* genes, the function of the *euFUL* genes in fruit maturation and lignification would appear to have been present before the emergence of the core eudicots.

Two *FUL*-like homologues have also been identified in another basal eudicot, *Aquilegia coerula*, referred to together as *AqcFL1* (Pabon-Mora *et al.*, 2013b). *AqcFL1* silencing via VIGS induced an increase of branching, shorter inflorescences with fewer flowers, and striking modifications of leaf development including a decreased number of leaflets. However, no phenotypes in flowers or fruits were observed in the *AqcFL1*-downregulated plants. While this may argue against the ancestral broad role of *FUL*-like genes in basal eudicots, subsequent phylogenetic studies suggest that, in *Aquilegia*, some *FUL*-like genes may have been lost after a duplication event and this could have caused the loss of FUL-like fruit-related functions (Shan *et al.*, 2007; Pabon-Mora *et al.*, 2013a).

The ancestral role of FUL–SHP in late fruit development

As we have reviewed here, there is increasing evidence linking FUL and SHP function to late fruit development in a growing number of species. Moreover, it appears that SHP function is consistently associated with lignification both in dry and fleshy fruits, at least in core eudicots. In addition, the repression of *SHP* expression by FUL also appears to be conserved, suggesting that this regulatory module (FUL -| SHP → lignin) could be broadly present in eudicots (Fig. 3).

Altogether, this evidence suggests that, following the duplication event at the base of core eudicots, *euFUL* and *PLE*/*SHP* genes may have undergone parallel neofunctionalization or subfunctionalization processes, coevolving to acquire and maintaining their roles in late fruit development. Interestingly, *FUL*-like genes from basal eudicots also appear to have a function in fruits, indicating that this role of FUL may predate the origin of core eudicots and therefore that of the euFUL clade. Because basal eudicots do not possess *PLE*/*SHP* genes, this may suggest the subsequent neofunctionalization of *PLE*/*SHP* genes in core eudicots by which they acquired this late fruit function. However, it is also possible that the *AG* genes in basal eudicots encompassed both C- and late fruit functions, but, as no fruits are formed in the mutants characterized so far, no fruit phenotypes have been observed.

The broad conservation of the FUL–SHP functional module, which might be extended to other components of the DZ network, may have provided an 'evolutionary playground' to generate diversity. In fact, we have discussed several examples where fruit morphological novelties appear to depend on variations on the network, both at *cis* or *trans* levels. In this context, the conserved roles of FUL and SHP in late fruit development both in fleshy and dry fruits also support a second idea, that dehiscence and ripening may share a common origin and are parallel, rather than completely different processes. Future studies should help us to understand better the common themes and connections between genetic networks controlling dehiscence and ripening, and may provide insights on the important question of the evolutionary origin of fleshiness.

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