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 siliqua, 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...
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 Arabidopsis, 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 Brassicaceae tribe that possess heteroarchothecarptic 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 Brassicaceae species with heteroarchothecarptic fruits and different dehiscence patterns, Erucaria erucarioideus 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 heteroarchothecarcy, 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 indehescence 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 APETAL1 (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).

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 neo-functionalization (Shan et al., 2007; Pabon-Mora et al., 2013a).

In Arabidopsis, both API 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. API 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, API and FUL also have independent functions. API 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 euAPI 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 comprising 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 Solanaceae. 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 *PLESHP* orthologues, named *TAGI* 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 *PLESHP* genes in late fruit development is also present in fleshy fruits. In contrast to the *PLESHP* 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 *TAGI* causes stamen-to-petal transformations and loss of floral determinacy, but no significant defects in carpel identity, suggesting that additional gene(s) participate with *TAGI* in this function (Pan et al., 2010). Because the phenotypes of simultaneous downregulation of *TAGI* 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 *PLESHP* 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 *PLESHP* 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 *PLESHP* genes in fruit development are also being extended to *euFUL* genes. For example, downregulation of *VnTDR4*, 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, *SIFUL*1 (previously called *TDR4* or *TM4*) and *SIFUL*2 (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 *SIFUL* 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/SIFUL1* and *MBP7/SIFUL2*, 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 *PLESHP* 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 *euAPI* and *euFUL*
genes in Arabidopsis, suggesting that the API/FUL duplication originating the euAPI 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 maintain 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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