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REVIEW PAPER

Molecular programme of senescence in dry and fleshy fruits

María Dolores Gómez, Francisco Vera-Sirera and Miguel A. Pérez-Amador*

Instituto de Biología Molecular y Celular de Plantas (IBMCP), Universidad Politécnica de Valencia-Consejo Superior de Investigaciones Científicas, Ciudad Politécnica de la Innovación (CPI), Ed. 8E, Ingeniero Fausto Elio s/n, 46022 Valencia, Spain

* To whom correspondence should be addressed. E-mail: mpereza@ibmcp.upv.es

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Abstract

Fruits of angiosperms can be divided into dry and fleshy fruits, depending on their dispersal strategies. Despite their apparently different developmental programmes, researchers have attempted to compare dry and fleshy fruits to establish analogies of the distinct biochemical and physiological processes that occur. But what are the common and specific phenomena in both biological strategies? Is valve dehiscence and senescence of dry fruits comparable to final ripening of fleshy fruits, when seeds become mature and fruits are competent for seed dispersal, or to over-ripening when advanced senescence occurs? We briefly review current knowledge on dry and fleshy fruit development, which has been extensively reported recently, and is the topic of this special issue. We compare the processes taking place in *Arabidopsis* (dry) and tomato (fleshy) fruit during final development steps using transcriptome data to establish possible analogies. Interestingly, the transcriptomic programme of *Arabidopsis* silique shares little similarity in gene number to tomato fruit ripening or over-ripening. In contrast, the biological processes carried out by these common genes from ripening and over-ripening programmes are similar, as most biological processes are shared during both programmes. On the other hand, several biological terms are specific of *Arabidopsis* and tomato ripening, including senescence, but little or no specific processes occur during *Arabidopsis* and tomato over-ripening. These suggest a closer analogy between silique senescence and ripening than over-ripening, but a major common biological programme between *Arabidopsis* silique senescence and the last steps of tomato development, irrespective of its distinction between ripening and over-ripening.

Keywords: *Arabidopsis*, fruit, over-ripening, ripening, silique, tomato, transcriptome.

Introduction

Fruits of angiosperm plants have evolved to allow optimal ovule and seed development in a closed protective structure. Yet at the same time, they have to provide adequate ways to facilitate seed dispersal. The wide variety of fruit types of angiosperms can be roughly divided into dry and fleshy fruits, depending on their dispersal strategies. From simple dry fruits and their 'passive' seed dispersal methods, fleshy fruits have evolved in association with higher animals, mainly mammals and birds, and use them as a dispersal method (Tiffney, 1984, 2004). Therefore, fleshy fruits have developed strategies to become more appealing to their dispersal vectors, like attractiveness through colour and flavour (pigments and volatile compounds), softening (cell wall and cuticle degradation), and palatability and nutritional value for dispersers (aroma, flavour, sugars, antioxidants, vitamins, etc.).

Model systems like *Arabidopsis* and tomato have provided considerable knowledge on the molecular and biochemical phenomena underlying dry and fleshy fruit development, respectively. Several reviews have been published recently that focus on different aspects of fruit development (Gapper *et al.*, 2013; McAtee *et al.*, 2013; Osorio *et al.*, 2013; Ruan *et al.*, 2012; Seymour *et al.*, 2013).

The development and senescence of dry fruits is quite a simple process: the fruit grows after fertilization with little tissue

Abbreviations: ABA, abscisic acid; dab, days after breaker; dpa, days post anthesis; GAs, gibberellins; GO, gene ontology; IAA, indole 3-acetic acid; PCD, programmed cell death; VIGS, virus-induced gene silencing.

differentiation, until it reaches its final length and then enters a senescence programme, similarly to leaf senescence, until final dehiscence/abscission takes place (Fig. 1). This latter process involves a highly regulated differentiation mechanism of the dehiscence zone and progression of cell separation. In contrast, fleshy fruit follows a more complex developmental programme. Firstly, upon ovary fertilization, the fruit grows to reach final fruit size, accompanied by important tissue differentiation, to later enter a complex developmental and biochemical programme—ripening—which peaks when fruits are ready to disperse seeds. Therefore, they have to become appealing and attractive for vectors. Later, fruits continue to develop and ripening leads to the so-called over-ripening processes, during which fruits start to decline and are finally dismantled (Fig. 1).

Researchers have made attempts to compare the apparently quite different developmental programmes of dry and fleshy fruits, and to identify analogies of the different biochemical and physiological processes that occur (Gapper *et al.*, 2013; Seymour *et al.*, 2013). The final stages of fleshy fruit development have been compared to senescence of dry fruits, and show similarities mainly in two characters: change in colour and cell wall modification. In the first case, fleshy fruit loses chlorophyll to allow colour change (carotenoids), while dry fruits lose chlorophyll similarly to leaves during senescence. In the second case, cell wall modifications promote the softening of fleshy fruits and dehiscence of dry fruits. It has also been recently suggested that senescence of dry fruits may correspond to the over-ripening of fleshy ones (Gapper *et al.*, 2013). But are these two processes really analogous? Are there any other biochemical or physiological traits that occur during dry and fleshy fruit development which further support this analogy? Indeed, the specific processes that occur in fleshy fruit have no paralogue in dry fruits, such as the breakdown of carbohydrates into sugars, reduction in acids, and increase in volatiles, which are responsible for flavour and aroma (Klee and Giovannoni, 2011). Activation of new metabolic pathways is unique to fleshy fruit.

When comparing both processes, what are the parallel phenomena in the two biological strategies? Is valve dehiscence of dry fruits comparable to final ripening of fleshy fruits, when seeds become mature and fruits are fully competent for seed dispersal, or to over-ripening, when advanced senescence may take place? Gene expression analysis has been used to characterize pistil/fruit development and senescence in *Arabidopsis* (Wagstaff *et al.*, 2009; Carbonell-Bejerano *et al.*, 2010) and in tomato fruit development (Eriksson *et al.*, 2004; Alba *et al.*, 2005; Lemaire-Chamley *et al.*, 2005; Tiwari and Paliyath, 2011; Zhang *et al.*, 2013). Here the aim was to analyse *Arabidopsis* (dry) and tomato (fleshy) fruit during final development steps using transcriptome data to establish possible analogies. Firstly, a brief review of the current knowledge on dry and fleshy fruit development was compiled as these topics have been extensively reported recently (see above) and in this special issue. Later, a comparative analysis of the transcriptome of *Arabidopsis* silique senescence (Wagstaff *et al.*, 2009) and tomato ripening and over-ripening (Zhang *et al.*, 2013) was carried out. Our data suggest a major transcriptomic programme that is common in both ripening and over-ripening, as well as similarities that suggest a closer analogy between silique senescence and ripening than over-ripening.

Arabidopsis, a model for dry fruit development

For many years, *Arabidopsis* has been the reference plant species used to study nearly all developmental processes, as well as stress responses, and fruit development is no exception (Somerville and Koornneef, 2002). Indeed, a large body

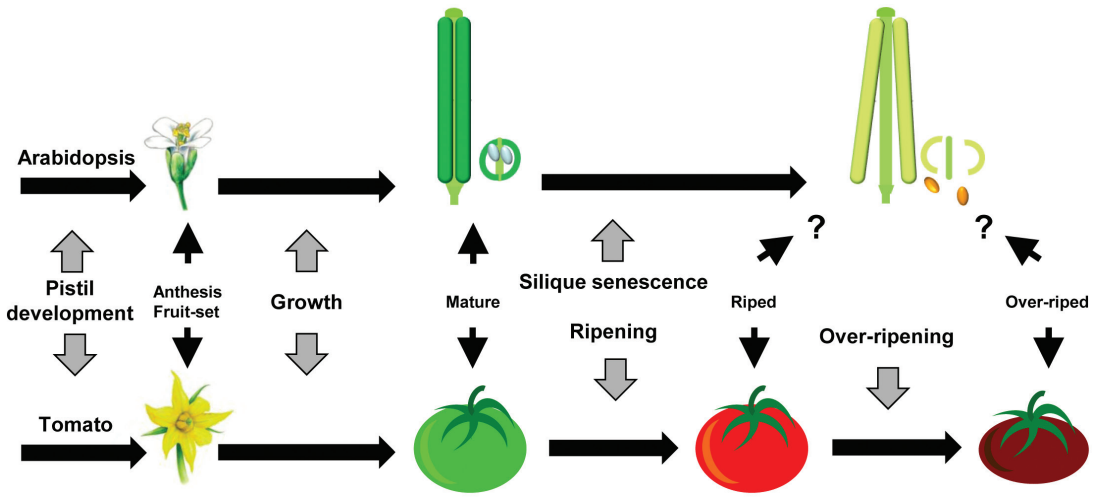

Fig. 1. Scheme of *Arabidopsis* silique development and senescence (upper) and tomato fruit development and ripening and over-ripening (lower). Pistils of *Arabidopsis* and tomato grow and differentiate from anthesis to reach full size at maturity. Subsequently, *Arabidopsis* silique enters a developmentally programmed senescence, which ends with the dehiscence of valves and seed dispersal. In contrast, tomato fruit enters a complex developmental and biochemical programme—ripening—which peaks when fruits are ready to disperse seeds. Tomato fruits continue to develop and ripening leads to over-ripening processes, in which fruits start to decline and are finally dismantled. (This figure is available in colour at *JXB* online.)

of knowledge has been collected in attempts to understand fruit development, and it focuses especially on three key developmental processes: pistil patterning, fruit set, and valve abscission or dehiscence. Fruit morphology or patterning, which is established mainly during pistil ontogeny, has been chiefly studied genetically, and a complex network of genes, which play a role in determining the identity of different tissues in pistils and in hormonal action, have been identified (Martínez-Laborda and Vera, 2009; Roeder and Yanofsky, 2006). Fruit-set, or fertilization, is the process initiated by hormonal signalling upon ovule fertilization, which promotes a switch from the pistil to the fruit development programme once the pistil becomes fully mature and morphologically functional upon anthesis (Dorcey *et al.*, 2009; Ruan *et al.*, 2012). Dehiscence, a specific kind of abscission (Estornell *et al.*, 2013), is the mechanism by which mature dry fruit shatters when seeds complete their development and dispersal is required. To this end, fruits have developed mechanisms to self-disperse seeds by modifying their structure. Among them, many simple fruits, like those of *Arabidopsis*, have developed fruits that eject seeds using mechanical forces which accumulate during maturation. Genetic approaches have helped identify a large number of the genes involved in the differentiation of the tissues and cells that participate in dehiscence layer formation, and also in the programme for the actual separation of valves and seeds. Indeed, silique shattering is perhaps the mostly characterized fruit ripening process in *Arabidopsis*, and it is referenced in detail in a review by Cristina Ferrándiz and Chloé Fourquin in this special issue.

Unlike the large body of knowledge available for these three developmental processes, less attention has been paid to other traits which also occur during *Arabidopsis* fruit development, for instance those relating to programmed cell death (PCD) and senescence. Understanding the morphological changes that occur during fruit development and senescence, or the role that certain plant hormones play in regulating these processes, are questions yet to be fully addressed. A summary of several aspects of *Arabidopsis* silique development and senescence processes follows.

The fruit of *Arabidopsis* is a silique formed by two valves, which are fused to a central replum by specific tissue called valve margin. Tissues that are present in mature fruit are already formed in the gynoecium before fertilization. Yet the tissues of the valve and valve margin region require hormones, mainly gibberellins (GAs) and auxins, which derive from seeds on fertilization (stage 13 and later according to Smyth *et al.*, 1990) to acquire their final differentiated state (Dinneny and Yanofsky, 2005). Early fruit development is characterized by early PCD of specific tissues that undergo senescence. These events can be visualized in a transgenic reporter line where GUS is controlled by the promoter of the bifunctional nuclease *BFN1*, a senescence-related gene (Farage-Barhom *et al.*, 2008; Carbonell-Bejerano *et al.*, 2010). On anthesis, the transmitting tract is already formed by a cell death process that not only disaggregates cells in the septum, which is the structure that divides the silique into two carpels, but also nurtures ovules and seeds along the placental epidermis (Fig. 2A). Later, senescence is detected in the stigma

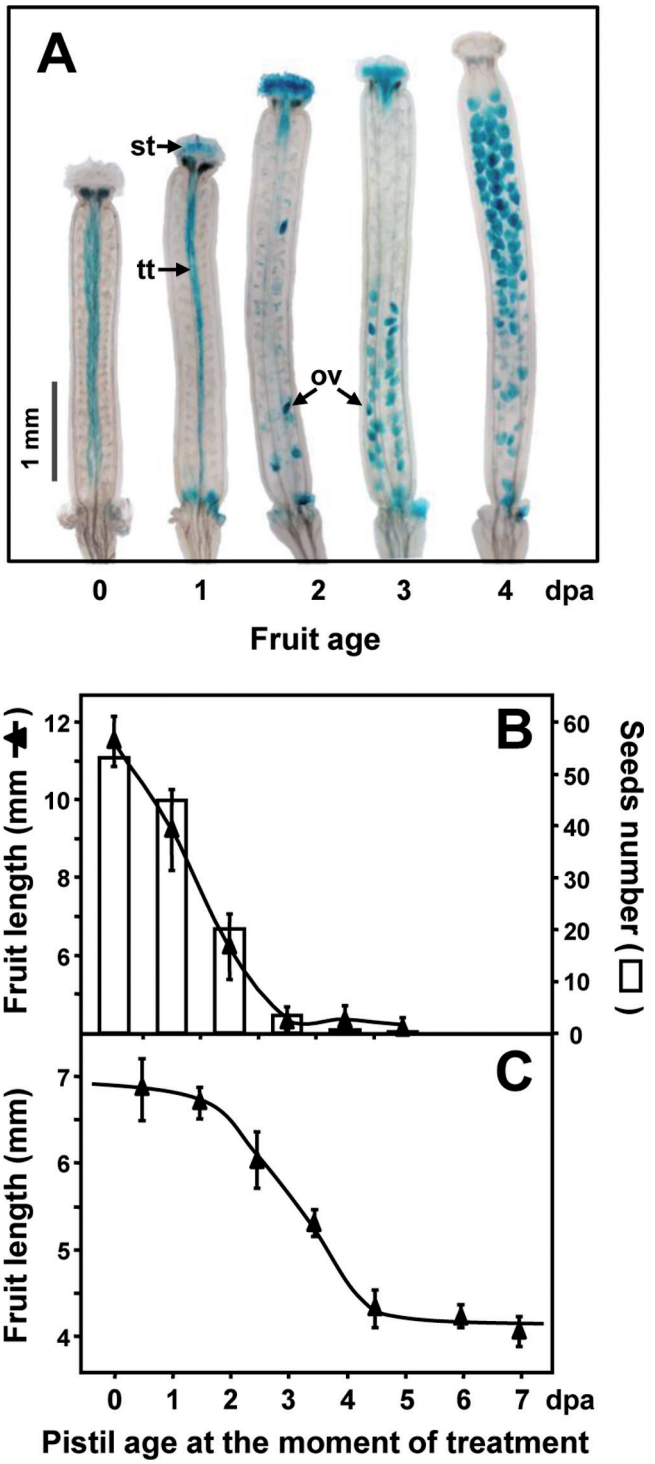

Fig. 2. Senescence of septum, stigma, and unfertilized ovules (A), impaired fruit-set by pollen (B), and GA_3 treatment (C). (A) GUS histochemical assay in pistils of a PromBFN1:GUS line shows transmitting tract, stigma and ovule senescence shortly after anthesis. (B) Response to pollen diminishes after anthesis due to the senescence of stigma at 1–2 dpa. (C) Response to GA_3 is affected by the progressive degradation of unfertilized ovules, between 2 and 4 dpa. ov, ovule; st, stigma; tt, transmitting track. The scale bar in (A) is 1 mm. Adapted from Carbonell-Bejerano P, Urbez C, Carbonell J, Granell A, Perez-Amador MA. 2010. A fertilization-independent developmental program triggers partial fruit development and senescence processes in pistils of *Arabidopsis*. *Plant Physiology* **154,** 163–172 (www.plantphysiol.org), copyright American Society of Plant Biologists. (This figure is available in colour at *JXB* online.)

at 1–2 days post-anthesis (dpa) (Fig. 2A). A third senescence process is observed in unfertilized ovules, starting from those located at the pistil base at 3 days post anthesis (dpa) to later extend to all the ovules at 4 dpa. Interestingly, while the formation of the transmitting tract facilitates proper pollen tube growth and fertilization (Wang *et al.*, 1996), stigma senescence timing correlates with loss of fruit-set responsiveness of the pistil to pollen (Fig. 2B), most probably due to the blockage of germination of the pollen grain on top of degraded stigmal papillae (Carbonell-Bejerano *et al.*, 2010). Furthermore, ovule senescence closely correlates with the cessation of pistil competency to respond to gibberellins (Fig. 2C). In addition, ethylene modulates the GA response in pistils by promoting ovule senescence, and mutants with altered ovule development have impaired GA response in pistils (Carbonell-Bejerano *et al.*, 2011). This evidence suggests that ovules play a critical role in the GA response, and that key GA-signalling mechanism elements can be localized in ovules, but not in valves or in other surrounding tissues. While ovule senescence clearly depends on the absence of fertilization, transmitting tract and stigma senescence occur independently of fertilization.

During this initial phase, *Arabidopsis* fruit rapidly grows in length and width on fertilization of ovules. The outer epidermal cells of the valve elongate and the stomata complete their development. The three mesophyll tissue layers contain photosynthetic cells that expand and form additional vascular strands, and inner epidermis cells (endocarp-a, or ena) expand. Growth is the result of rapid cell division and expansion, which is controlled mainly by GAs and auxin (Vivian-Smith and Koltunow, 1999). Interestingly, increased fruit width on pollination is strictly due to cell expansion in all tissues, while increased silique length is the result of cell expansion and cell division, mainly the mesocarp and endocarp cell layers. Fruits reach their final size at 6–7 dpa (stage 17B, according to Smyth *et al.*, 1990), when siliques are 4- to 5-fold longer than they were at anthesis (Vivian-Smith and Koltunow, 1999).

Later the whole silique undergoes programmed senescence, like the senescence of the leaves of deciduous species. Plant senescence can be defined as the final step of the developmental programme that leads from maturity to the event of cell death (Leopold, 1961; Shahri, 2011; Thomas, 2013). Senescent tissues or organs are degraded, and components are mobilized to other plant parts to facilitate nutrient recovery and recycling. Fruit senescence can be defined as an example of developmentally programmed senescence since it is regulated by biological clocks and depends on changes in gene expression, rather than being regulated by the environment or as a response to stress. Chlorophyll content, a classical marker of senescence, reaches its highest levels at 8–17 dpa (Wagstaff *et al.*, 2009; Carbonell-Bejerano *et al.*, 2010; Kou *et al.*, 2012), and decreases afterwards until approximately 20 dpa when yellowing becomes visible (Wagstaff *et al.*, 2009). Little is known about the molecular mechanism that triggers fruit senescence in *Arabidopsis*. It has been recently reported that silique senescence in *Arabidopsis* is regulated by NAP, an NAC family transcription factor (Kou *et al.*, 2012). The null mutant *nap* shows delayed senescence and a suppressed ethylene-dependent respiration surge. Nevertheless, the role of NAP is not fruit-specific as it also controls leaf senescence (Guo and Gan, 2006). In addition, NAP controls fruit senescence by regulating ethylene (Kou *et al.*, 2012).

Valve dehiscence is a major event that occurs during silique senescence (Fig. 3A). Cells of the inner subepidermal layer (endocarp-b, or enb) develop thickened walls that are later lignified. The valve margin begins to differentiate into narrow strips of cells consisting of a lignification layer (LL) and a separation layer (SL). This specialized structure, the dehiscence zone, facilitates fruit opening and efficient seed release. In stage 18, the ena cells disintegrate and desiccation of the other tissues that form the fruit occurs (Fig. 3B). Interestingly, ena degradation is controlled by GAs; the parthenocarpic fruits generated by treating non-pollinated pistils with GAs or in the quadruple *della* mutant (*gaiT6 rga24 rgl1-1 rgl2-1*), which lacks four of the five GA-repressor DELLA proteins, show advanced ena degradation (Dorcey *et al.*, 2009), although no difference in dehiscence has been reported. In stage 19, pod shatter or dehiscence occurs when valves separate from the replum (Fig. 3C). Dehiscence occurs via a combination of cell wall loosening at the SL and the tension created by the differential mechanical properties between the lignified tissues of the enb layer and the LL in the valve margin, along with the drying of the outer epidermis and mesophyll cells. Later, seed abscission takes place and seeds fall, and only the dried replum and septum remain attached to the pedicel (Ferrándiz, 2002; Dinneny and Yanofsky, 2005; Roeder and Yanofsky, 2006). MADS-box genes *SHATTERPROOF1* (*SHP1*) and SHP2, and the basic helix–loop–helix gene *INDEHISCENT* (*IND*), are major regulators that direct dehiscence zone formation, assisted by GAs and auxin pathways (Arnaud *et al.*, 2010; Sorefan *et al.*, 2009).

But what exactly are the molecular mechanisms behind the fruit developmental programme and senescence in *Arabidopsis*, and which genes participate? Senescence-associated gene expression during *Arabidopsis* fruit development and senescence has been studied by transcriptomic analysis (Wagstaff *et al.*, 2009). This analysis, as well as other similar ones in unfertilized pistils (Carbonell-Bejerano *et al.*, 2010), have indicated that fruits and pistils are photosynthetic organs in early developmental stages after anthesis but, most importantly, that these organs enter a programmed senescence process after maturity before valve dehiscence.

Tomato, a model for fleshy fruit development

Tomato (*Solanum lycopersicum*) has been the reference plant system of choice to dissect development, maturation, ripening, and senescence processes of fleshy fruit (Klee and Giovannoni, 2011). Together with work done on other reference species, such as strawberry, and citrus, grapes or pepper to a lesser extent, major advances have been made in understanding the processes taking place from young fruit

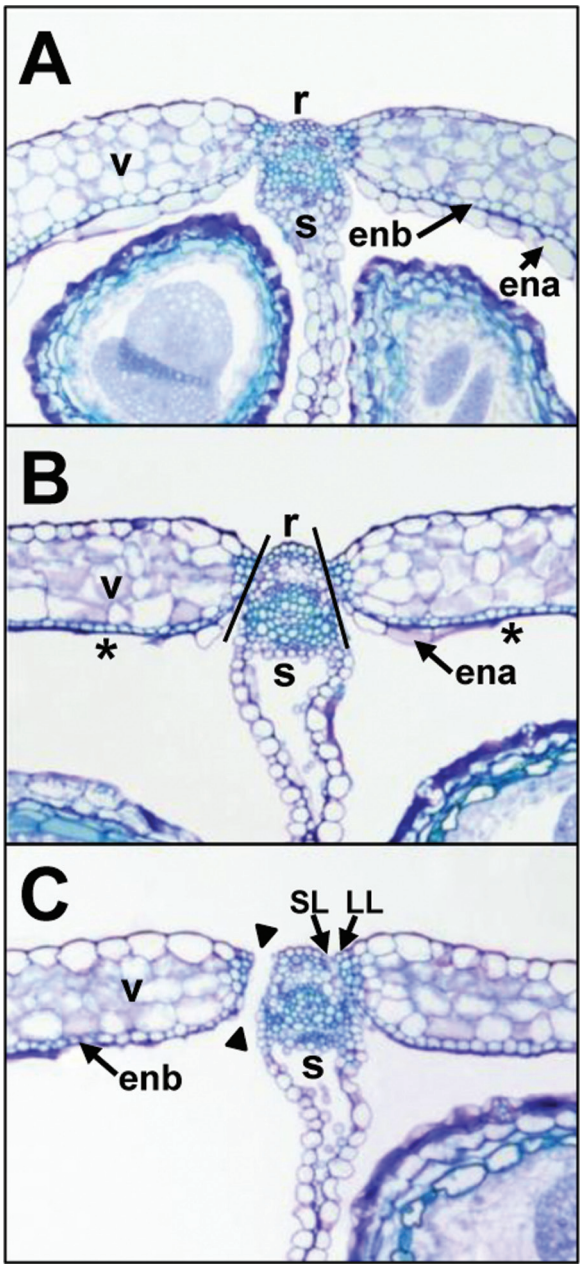

Fig. 3. Dehiscence is the best-known ripening/senescence process of the *Arabidopsis* silique. Transverse cross-sections of siliques at different post-fertilization times are shown. (A) Mature silique reaches its final length and lignification of endocarp-b begins at around 7–8 dpa (stage 17B, according to Smyth *et al.*, 1990). (B) Endocarp-a is completely degraded (asterisk) and endocarp-b is lignified at 9–10 dpa (stage 18). (C) Dehiscence occurs at the last step of fruit senescence, at 14–20 dpa (stage 19). Seed dispersion occurs soon after (stage 20). Timing of events, especially dehiscence, greatly depends on the growth conditions and may vary between experiments. Black lines mark the separation layer in the dehiscence zone; asterisks mark the degradation of endocarp-a; arrowheads denote valve dehiscence at the separation cell layer. ena, endocarp-a; enb, endocarp-b; SL, valve margin separation layer; LL, valve margin lignified layer; v, valve; r, replum; s, septum. Scale bar is 50 µm. (This figure is available in colour at *JXB* online.)

after pollination to maturation, ripening, and over-ripening. Reviews that thoroughly address several aspects of fruit development, especially in tomato, have been published recently (Ruan *et al.*, 2012; Gapper *et al.*, 2013; McAtee *et al.*, 2013; Osorio *et al.*, 2013; Seymour *et al.*, 2013). Therefore, only some aspects of fleshy fruit development are highlighted.

In the tomato cv. Micro-Tom, periclinal and anticlinal cell divisions start at 2 dpa, when the ovary is 1 mm in diameter with 10 cell layers, and they stop by 10–13 dpa. Cell expansion is initiated at 7–8 dpa and progresses until 30 dpa. Final fruit size is due mainly to cell expansion. Unique to fleshy fruit, cell expansion is accompanied by an accumulation of storage products and sugars (McAtee *et al.*, 2013; Seymour *et al.*, 2013). Once seeds are mature, fruit undergoes ripening. During this period, major changes in the relative hormone levels of fruit occur. Auxin, GA, and cytokinin levels decrease, while abscisic acid (ABA) and ethylene levels increase (McAtee *et al.*, 2013). Unlike *Arabidopsis*, ripening of fleshy fruits, like tomato, involves the softening of tissues, changes in colour and texture, and accumulation of sugars, acids and volatile compounds, making fruits more attractive to animals in order to facilitate seed dispersal. The most critical developmental events associated with ripening and regulated by ethylene are (i) modification of colour through chlorophyll degradation with simultaneous carotenoid and flavonoid accumulation; (ii) textural modification by variations in cell turgor and cell wall structure; (iii) changes in sugars, acids, and volatile profiles that affect nutritional quality, flavour, and aroma; and (iv) enhanced susceptibility to opportunistic pathogens due to loss of cell wall integrity (Giovannoni, 2004; Klee and Giovannoni, 2011; Gapper *et al.*, 2013). Fleshy fruit ripening is the developmental process that occurs prior to senescence (Gapper *et al.*, 2013). Therefore, the processes that take place during over-ripening may be considered senescence-associated.

The ripening process has been classically described through the characterization of the physiological changes listed above, for which there is a large amount of literature available.

1. *Colour change.* Colour change is due to a combination of chlorophyll loss and the production of secondary metabolites, such as carotenoids and flavonoids. The production of these metabolites is strongly regulated by ethylene and ABA (Buta and Spaulding, 1994). The photosynthetic capacity of chloroplasts is lost as thylakoid structures begin to disassemble. Chlorophyll loss is associated with chloroplasts being converted into chromoplasts (Egea *et al.*, 2010). Within chromoplasts, lycopene and β-carotene accumulate, and this accumulation provides a visual indication that fruit is mature and suitable for consumption.
2. *Cell wall hydrolysis.* There is extensive literature about modifications of cell walls during ripening (reviewed by Brummell, 2006). More than 50 cell wall structure-related genes show expression variations in ripening tomato fruits, and texture involves complex quantitative trait loci (Seymour *et al.*, 2013).
3. *Sugar, acids, and volatiles.* At the beginning of ripening, the starch that has accumulated throughout development is metabolized to glucose and fructose, the two main sugars in ripe fruit. Organic acids, principally citric and malic acids, also abound. Both sugars and acids are critical for good taste (Centeno *et al.*, 2011; Klee and Giovannoni,

2011; Osorio *et al.*, 2011, 2012). Moreover, volatiles determine fruit flavour (Baldwin *et al.*, 2000). Some 20–30 volatile chemicals have been described to contribute to tomato flavour. They derive from essential amino acids (phenylalanine, leucine, and isoleucine), essential fatty acids (mainly linolenic acid), and carotenoids (β-carotene is the precursor of one of the most important flavour volatiles, β-ionone) (Goff and Klee, 2006).

In tomato, the three major classes of chemicals responsible for flavour, sugar, acids, and volatiles, together with textural and colour changes, participate in creating attractive flesh for seed-dispersal animals.

Comparative analysis of the transcriptome during *Arabidopsis* and tomato fruit development

To determine the degree of analogy between *Arabidopsis* silique senescence and tomato fruit ripening/over-ripening, we analysed the transcriptomic data of *Arabidopsis* and tomato fruit development reported by Wagstaff *et al.* (2009) and Zhang *et al.* (2013), respectively.

In their analysis, Wagstaff *et al.* (2009) used ATH1 Affymetrix GeneChip microarrays to detect changes in the gene expression of siliques at the beginning of visible senescence, at 20 dpa, as compared with those from mature green siliques at 10 dpa. Seeds were removed from both samples, and thus only the gene expression in valves or silique pods was tested. Genes were selected based on a 2-fold difference in the expression levels in both developmental stages. A gene ontology (GO) analysis of upregulated genes has indicated that several relevant categories are associated with final pod development stages (Wagstaff *et al.*, 2009). In addition to the genes encoding seed storage proteins, those genes involved in carbohydrate, amino acid, nitrate, and metal ion transport, as well as the genes of secretory pathways such as exocytosis, protein targeting to the vacuole and vesicle-mediated transport, were upregulated during silique senescence. Furthermore, senescence-related genes were identified, such as *SAG12* and the autophagy genes from the *APG8* and *APG12* families. Finally, ethylene-responsive genes were upregulated, a further indication that ethylene plays a role in silique senescence. Several genes were also identified as being downregulated during silique senescence. These account for loss of vacuolar and chloroplast integrity, including the downregulation of photosystems, cytoskeletal and chromosome organization, and reduction of metabolic processes, such as carbohydrate transport, or secondary metabolites (plastoquinones, flavonoids and anthocyanins).

To analyse tomato fruit development, Zhang *et al.* (2013) used a transgenic line that over-expresses the two genes encoding transcription factors, Delila (Del) and Rosea1 (Ros1), under the control of the fruit-specific E8 promoter. These fruits show enhanced anthocyanin biosynthesis, which results in dark purple fruit. It is noteworthy that purple fruit exhibit an extended shelf-life due to both delayed ripening and susceptibility to pathogen infection. For comparison purposes, the Del/Ros1 transcription factors were transiently silenced by virus-induced gene silencing (VIGS), resulting in purple (non-silenced) and red (silenced) sectors. Red and purple sectors were collected and compared at 8, 30 and 45 days after breaker (dab). A functional classification of differentially expressed genes revealed that ripening-associated genes are more abundant on 8 and 30 dab if compared with 45 dab (Zhang *et al.*, 2013). Therefore, silenced pericarp sectors on 8 and 30 dab were assigned to the early and late ripening processes, respectively. Moreover, silenced sectors at 45 days reflect fruit over-ripening, and showed quicker softening, greater susceptibility to *Botrytis cinerea*, and diminished hydrophilic antioxidant capacity. The advantage of this analysis is that the compared purple and red tissues came from the same fruit. Thus, they are exactly the same age, but at different developmental stages. Therefore, the gene expression data obtained reflect only the changes associated with the developmental process. Other analyses, however, have been done following a classical design, that of comparing tomatoes at different time points and using mature green or a breaker as a control. Therefore, these differences in age also contribute to differences in gene expression (Karlova *et al.*, 2013; Lopez-Gomollon *et al.*, 2012).

A microarray analysis was carried out using the TOM2 oligo array [GEO Platform GPL17060; the Center for Gene Expression Profiles (CGEP), Cornell University, Ithaca, NY, USA]. In all, 241 genes showed minimum 2-fold differences in the expression between the purple and red sectors. Many of these differentially expressed genes have been found to be involved in primary and secondary metabolism, cell wall, oxidative stress, and pathogen resistance (Zhang *et al.*, 2013).

The goal was to attempt to discover just how similar are the biochemical and physiological processes that occur during the senescence of a dry fruit and a fleshy fruit. Transcriptome data from Wagstaff *et al.* (2009) and Zhang *et al.* (2013) for the *Arabidopsis* and tomato transcriptomes were respectively used. The lists of the upregulated and downregulated genes during *Arabidopsis* silique senescence were compared with those from the ripening (8 and 30 dab) and over-ripening (45 dab) tomato fruit.

Firstly, the differentially regulated genes from tomato reported by Zhang *et al.* (2013) were used to identify their corresponding *Arabidopsis* orthologues, and the genes that were upregulated and downregulated during ripening (8 and 30 dab) and over-ripening (45 dab) were grouped. When comparing *Arabidopsis* fruit senescence and tomato fruit ripening, 97 upregulated and 66 downregulated common genes were found (Table 1; Supplementary Tables S1 and S2, respectively, available at *JXB* online). In addition, 57 upregulated and 21 downregulated common genes were detected when comparing *Arabidopsis* fruit senescence and tomato fruit over-ripening (Table 1; Supplementary Tables S3 and S4, respectively, available at *JXB* online). Absolute numbers suggest that *Arabidopsis* silique senescence seems more similar to the ripening stage, but this conclusion cannot be definitively drawn if similar percentages of each subset of genes are considered in the total number of common genes per comparison (Table 1).

Table 1. *Comparison of gene expression during tomato fruit ripening and over-ripening and* Arabidopsis *silique senescence*

The tomato fruit expression data are from Zhang *et al.* (2013). The Arabidopsis silique senescence data are from Wagstaff *et al.* (2009). The percentage of genes commonly regulated in *Arabidopsis* and tomato is indicated in parentheses.

Sample			Tomato fruit ripening (8+30 dab)		Tomato fruit over-ripening (45 dab)	
	Regulation		Up	Down	Up	Down
		# Genes	763	594	380	213
***Arabidopsis* silique**	Up	1238	97 (8–13%)		57 (5–15%)	
senescence (20 dpa)	Down	1482		66 (4–11%)		21 (1–10%)

dab, days after breaker stage of tomato fruit development; dpa, days post-anthesis of *Arabidopsis* silique development.

With a view to unveiling the biological relevance of the processes taking place in *Arabidopsis* and tomato fruit, a GO analysis was carried out using the Singular Enrichment Analysis Tool from AgriGO (Du *et al.*, 2010), and the enriched biological function categories during *Arabidopsis* silique senescence and tomato fruit ripening (Table 2 and Supplementary Table S5, available at *JXB* online) and over-ripening (Table 3 and Supplementary Table S6, available at *JXB* online) were identified. The power of this analysis, unlike the analysis of single genes, is that the GO terms statistically enriched in each comparison highlight the biochemical and physiological processes behind the samples compared (Ashburner *et al.*, 2000; Clark *et al.*, 2005). However, in some cases, GO annotation may be broad and it might not be possible to provide accurate information, especially when a small number of genes is included. In addition, AgriGO focuses especially on the GO terms that describe genes from agricultural species. The ultimate goal of this study was to find out which tomato fruit development stage, if any, is similar to *Arabidopsis* silique senescence. From the list of the 97 common upregulated genes between *Arabidopsis* and tomato ripening (Supplementary Table S1 available at *JXB* online), 144 enriched GO categories were identified, of which 34 were not redundant (Table 2 and Supplementary Table S5 available at *JXB* online). From the list of the 57 common upregulated genes between *Arabidopsis* and tomato over-ripening (Supplementary Table S3 available at *JXB* online), 86 enriched GO categories were detected, of which 20 were non-redundant GO terms (Table 3 and Supplementary Table S6 available at *JXB* online). The downregulated genes were also analysed. From the list of the 66 downregulated common genes in *Arabidopsis* and tomato ripening (Supplementary Table S2 available at *JXB* online), 27 enriched GO categories were detected, but only six were not redundant (Table 4 and Supplementary Table S7, available at *JXB* online). Interestingly, no enriched GO categories were found among the 21 downregulated common genes in *Arabidopsis* and tomato over-ripening (Supplementary Table S4 available at *JXB* online). When comparing the GO categories from the upregulated genes in *Arabidopsis* silique senescence and both ripening and over-ripening (Tables 2 and 3), 14 GO terms were seen to be commonly enriched and four GO terms had common ancestor or child terms (all the 18 GO terms are highlighted in Tables 2 and 3), indicating that these processes take place in both developmental stages and are therefore non-specific for either ripening or over-ripening.

A large set of these common GO terms is related to the *defence response* (GO:0042742, defence response to bacterium; GO:0050832, defence response to fungus; GO:0009595, detection of biotic stimulus; GO:0009867, jasmonic acid-mediated signalling pathway; GO:0010310, regulation of hydrogen peroxide metabolic process; GO:0010363, regulation of the plant-type hypersensitive response; GO:0002679, respiratory burst during defence response; GO:0009862, systemic acquired resistance, salicylic acid-mediated signaling pathway; GO:0009696, salicylic acid metabolic process and its child term GO:0009697, salicylic acid biosynthetic process). In their study, Zhang *et al.* (2013) report that susceptibility to *Botrytis cinerea*, one of the most important post-harvest pathogens, is suppressed by anthocyanin accumulation in the purple tomato, which over-expresses the Del and Ros1 transcription factors regulating anthocyanin biosynthesis. In fact, one of the most striking phenotypes of these purple tomatoes is improved shelf-life compared with red, non-transgenic fruits. Therefore, the enhanced antioxidant capacity of purple fruit likely slows down over-ripening processes (Zhang *et al.*, 2013). In contrast, the silencing of Del and Ros1 by VIGS in purple tomato sectors activates defence responses in not only the late over-ripening stage, but also during ripening. Furthermore, the data suggest that activation of defence responses also occurs during *Arabidopsis* silique senescence.

Other common GO terms can also be related to *response to biotic stress*. GO:0006865 for amino acid transport may be related to increased glutamine levels in tomato fruit, and to reduced glutamate content (Valle *et al.*, 1998), which may prevent or limit microbial infection (Reina-Pinto and Yephremov, 2009). Moreover, GO:0010200 for response to chitin is also commonly co-enriched with terms related to biotic stimulus. In fact, chitin is a good inducer of defence mechanisms in plants as it is the main component of the cell walls of fungi and the exoskeletons of arthropods. Finally, another GO term that is potentially related to pathogen defence is GO:0010167, response to nitrate. One of the genes in this group, *At5g65210* (*TGA1*), is a regulator of pathogenesis-related genes given its interaction with positive regulator NRP1 (Kesarwani *et al.*, 2007; Shearer *et al.*, 2012). Another gene in this category, *At2g43820* (*SGT1*), seems to be involved in the infection response to *Pseudomonas syringae* (Uppalapati *et al.*, 2011).

Table 2. *The non-redundant GO categories among the 97 genes commonly upregulated in* Arabidopsis *silique senescence and tomato ripening*

The GO categories highlighted are common in ripening and over-ripening (Table 2 and Table 3, respectively).

GO term	Description	Genes on the list	Genes in the background	*P*-value[a]	FDR[b]
GO:0006865	Amino acid transport	10/97	85/5782	2.8E-06	7.1E-05
GO:0006820[c]	Anion transport	8/97	116/5782	9.0E-04	1.5E-02
GO:0052542	Callose deposition during defence response	7/97	26/5782	7.7E-07	2.0E-05
GO:0006944	Cellular membrane fusion	8/97	113/5782	7.7E-04	1.3E-02
GO:0071445	Cellular response to protein stimulus	6/97	82/5782	3.1E-03	4.3E-02
GO:0042742	Defence response to bacterium	19/97	175/5782	1.9E-10	1.0E-08
GO:0050832	Defence response to fungus	16/97	139/5782	2.9E-09	1.2E-07
GO:0009595	Detection of biotic stimulus	11/97	47/5782	1.6E-09	7.4E-08
GO:0023034	Intracellular signalling pathway	10/97	185/5782	1.2E-03	1.9E-02
GO:0009867	Jasmonic acid-mediated signalling pathway	13/97	134/5782	6.3E-07	1.7E-05
GO:0000165	MAPKKK cascade	13/97	97/5782	2.0E-08	7.4E-07
GO:0031348	Negative regulation of defence response	14/97	123/5782	3.5E-08	1.3E-06
GO:0043069	Negative regulation of programmed cell death	12/97	77/5782	1.6E-08	6.3E-07
GO:0010260	Organ senescence	5/97	40/5782	7.8E-04	1.3E-02
GO:0031408[c]	Oxylipin biosynthetic process	6/97	79/5782	2.6E-03	3.7E-02
GO:0031325	Positive regulation of cellular metabolic process	11/97	225/5782	1.5E-03	2.4E-02
GO:0009963	Positive regulation of flavonoid biosynthetic process	5/97	44/5782	1.2E-03	1.8E-02
GO:0046777	Protein amino acid autophosphorylation	5/97	59/5782	3.8E-03	4.9E-02
GO:0006612	Protein targeting to membrane	19/97	172/5782	1.5E-10	8.7E-09
GO:0010310	Regulation of hydrogen peroxide metabolic process	11/97	82/5782	2.6E-07	7.7E-06
GO:0043900	Regulation of multi-organism process	9/97	48/5782	2.8E-07	8.1E-06
GO:0010363	Regulation of plant-type hypersensitive response	19/97	162/5782	5.7E-11	3.9E-09
GO:0002831	Regulation of response to biotic stimulus	9/97	45/5782	1.7E-07	5.4E-06
GO:0002679	Respiratory burst during defence response	10/97	53/5782	5.5E-08	1.8E-06
GO:0009737	Response to abscisic acid stimulus	11/97	244/5782	2.9E-03	4.0E-02
GO:0010200	Response to chitin	24/97	170/5782	2.5E-15	4.1E-13
GO:0010583	Response to cyclopentenone	5/97	52/5782	2.3E-03	3.3E-02
GO:0034976	Response to endoplasmic reticulum stress	11/97	148/5782	5.1E-05	1.0E-03
GO:0010167	Response to nitrate	5/97	58/5782	3.5E-03	4.7E-02
GO:0006979	Response to oxidative stress	12/97	267/5782	1.9E-03	2.8E-02
GO:0009414	Response to water deprivation	10/97	159/5782	4.1E-04	7.6E-03
GO:0009611[c]	Response to wounding	10/97	161/5782	4.5E-04	8.1E-03
GO:0009697[c]	Salicylic acid biosynthetic process	13/97	81/5782	2.8E-09	1.2E-07
GO:0009862	Systemic acquired resistance, salicylic acid-mediated signalling pathway	16/97	112/5782	1.6E-10	9.3E-09

[a] Corrected *P*-value.
[b] FDR, false discovery rate.
[c] Common ancestor or child GO term.

The GO term *response to abscisic acid (ABA) stimulus* (GO:0009737) is also enriched in both ripening and over-ripening. Despite the role that ABA plays in fruit development being well known, very little is known about *Arabidopsis* silique development. ABA regulates part of the fruit ripening processes, such as the cell wall genes that control tomato fruit softening (Sun *et al.*, 2012). ABA also controls ripening in other species, such as citrus, grape or strawberry that are considered to be non-climacteric, in cross-talk with ethylene (reviewed in McAtee *et al.*, 2013; Osorio *et al.*, 2013; Seymour *et al.*, 2013). In tomato, a classical climacteric fruit, ABA participates in ripening through ethylene. Enrichment of the GO response to ABA stimulus suggests that, apart from its known function in tomato fruit, ABA also plays a role in *Arabidopsis* silique senescence (Kou *et al.*, 2012).

Finally, the other GO terms that appear in both ripening and over-ripening are also related to functions of defence response genes, such as *MAPKKK cascade* (GO:0000165), or *protein targeting to membrane* (GO:0006612). These terms refer to the molecular functions of those genes that also participate in the above-mentioned processes. For example, the genes termed in the MAPKKK cascade also have descriptors of the jasmonic acid- and salicylic acid-mediated signalling pathway.

Most of these common GO terms that are enriched in both ripening and over-ripening also appear when the GO analysis is carried out with only the 29 genes that are upregulated during *Arabidopsis* silique senescence, and also during tomato fruit ripening and over-ripening (data not shown).

Table 3. *The non-redundant GO categories among the genes commonly upregulated in* Arabidopsis *silique senescence and tomato over-ripening*

The GO categories highlighted are common in ripening and over-ripening (Table 2 and Table 3, respectively).

GO term	Description	Genes on the list	Genes in the background	*P*-value[a]	FDR[b]
GO:0006865	Amino acid transport	5/57	85/5782	1.8E-03	2.6E-02
GO:0042742	Defence response to bacterium	8/57	175/5782	3.6E-04	6.4E-03
GO:0050832	Defence response to fungus	8/57	139/5782	8.0E-05	1.9E-03
GO:0009595	Detection of biotic stimulus	5/57	47/5782	1.4E-04	3.1E-03
GO:0006631[c]	Fatty acid metabolic process	9/57	269/5782	1.4E-03	2.2E-02
GO:0009867	Jasmonic acid-mediated signalling pathway	7/57	134/5782	4.1E-04	6.9E-03
GO:0000165	MAPKKK cascade	5/57	97/5782	3.1E-03	4.2E-02
GO:0015706[c]	Nitrate transport	10/57	60/5782	9.1E-10	1.7E-07
GO:0006612	Protein targeting to membrane	9/57	172/5782	5.6E-05	1.4E-03
GO:0010310	Regulation of hydrogen peroxide metabolic process	5/57	82/5782	1.5E-03	2.3E-02
GO:0010363	Regulation of plant-type hypersensitive response	9/57	162/5782	3.6E-05	9.7E-04
GO:0002679	Respiratory burst during defence response	5/57	53/5782	2.3E-04	4.4E-03
GO:0009737	Response to abscisic acid stimulus	8/57	244/5782	2.9E-03	4.0E-02
GO:0009733	Response to auxin stimulus	6/57	144/5782	3.3E-03	4.4E-02
GO:0010200	Response to chitin	9/57	170/5782	5.1E-05	1.3E-03
GO:0009605[c]	Response to external stimulus	11/57	410/5782	0.0022	3.20E-02
GO:0009723	Response to ethylene stimulus	6/57	146/5782	3.5E-03	4.6E-02
GO:0010167	Response to nitrate	10/57	58/5782	6.8E-10	1.5E-07
GO:0009696[c]	Salicylic acid metabolic process	5/57	85/5782	1.8E-03	2.6E-02
GO:0009862	Systemic acquired resistance, salicylic acid-mediated signalling pathway	8/57	112/5782	1.9E-05	5.7E-04

[a] Corrected *P*-value.
[b] FDR, false discovery rate.
[c] Common ancestor or child GO term.

Table 4. *The non-redundant GO categories among the 66 genes commonly downregulated in* Arabidopsis *silique senescence and tomato ripening*

GO term	Description	Genes on the list	Genes in the background	*P*-value[a]	FDR[b]
GO:0009805	Coumarin biosynthetic process	7/66	30/5782	1.4E-07	7.9E-05
GO:0006598	Polyamine catabolic process	5/66	27/5782	2.6E-05	4.2E-03
GO:0009963	Positive regulation of flavonoid biosynthetic process	5/66	44/5782	2.1E-04	1.8E-02
GO:0010075	Regulation of meristem growth	5/66	56/5782	5.9E-04	3.9E-02
GO:0009605	Response to external stimulus	13/66	410/5782	7.6E-04	4.0E-02
GO:0009411	Response to UV	9/66	108/5782	5.6E-06	1.6E-03

[a] Corrected *P*-value.
[b] FDR, false discovery rate.

After examining the GO terms that are common and specific between *Arabidopsis* silique senescence and tomato fruit ripening (GO terms that were not present when we compared *Arabidopsis* silique senescence and tomato over-ripening), several biological processes can be uncovered that can help establish a correct analogy. The specific GOs for *Arabidopsis* silique senescence and tomato ripening are listed as the non-highlighted entries in Table 2. Several of the GOs that are relevant to this analysis are discussed below.

The GO term *organ senescence* (GO:0010260) is enriched among the genes commonly upregulated in silique senescence and tomato fruit ripening. In our analysis, Senescence-associated gene 21 (*SAG21*, *At4g023809*) and Senescence-related gene 1 (*SRG1*, *At1g17020*) are specifically upregulated during ripening, but not during over-ripening (Supplementary Tables S1 and S3, respectively, available at JXB online). Similarly, *WRKY53* (*At4g23810*), whose over-expression accelerated senescence in leaves (Miao and Zentgraf, 2010), was expressed in tomato ripening, but not during over-ripening.

GO:0043069, *negative regulation of programmed cell death*, is also enriched, but is related to cell death as a result of the defence response. However, PCD occurs in the final stages of senescence during dehiscence, and also in transmitting tract, stigma and ovule senescence during early fruit development, as indicated previously.

Another enriched informative GO term is GO:0009963, *positive regulation of the flavonoid biosynthetic process*. Indeed, flavonoids are synthesized during tomato fruit ripening (Slimestad *et al.*, 2008) and silique senescence in *Arabidopsis* (Stracke *et al.*, 2010; Watanabe *et al.*, 2013). Flavonoids are thought to perform several functions, including protection against UV-B radiation and changing environmental conditions, and defence against pathogen and herbivore attack (Falcone Ferreyra *et al.*, 2012). Moreover in tomato, flavonoids play an important role in controlling water transport across the cuticle and in attracting animal vectors for seed dispersal by adding colour to peel (Mintz-Oron *et al.*, 2008; Adato *et al.*, 2009). It is noteworthy that very little is known about the role of these genes in *Arabidopsis* in spite of them being upregulated during silique senescence.

In contrast to the large number of common GO terms between *Arabidopsis* silique senescence and tomato fruit ripening, fewer terms have been enriched among the common genes involved in *Arabidopsis* and tomato fruit over-ripening. Indeed most of them also appear in the comparison of *Arabidopsis* silique senescence with tomato fruit ripening. Only two GOs are specific to this comparison, and both relate to the hormones auxin and ethylene.

Auxin (*response to auxin stimulus*, GO:0009733) seems to play a fundamental role in fruit maturation. For example, auxin inhibits the ripening process in tomato (Davey and Van Staden, 1978; Rolle and Chism, 1989) and apple (Ireland *et al.*, 2013), or it controls dehiscence in *Arabidopsis*, where low levels are required for seed dehiscence (Sorefan *et al.*, 2009). Several of the genes involved in auxin synthesis and response are on the list of common genes. For example, *GH3.3* (*At2g23170*), encoding indole 3-acetic acid (IAA)-amido synthase, which lowers free levels of IAA and accelerates tomato development (Liu *et al.*, 2005); or *JAZ1*, which is involved in jasmonate signalling and is involved indirectly in auxin homeostasis as jasmonic acid can modulate the expression of *YUCCA8* and *YUCCA9* (Hentrich *et al.*, 2013).

Interestingly, the GO term *response to ethylene stimulus* (GO:0009723) is enriched specifically among the genes upregulated during *Arabidopsis* silique senescence and tomato over-ripening. However, these genes respond to a wide variety of abiotic or biotic stresses, and none is specific of ethylene signalling or response.

Conclusion

Dry and fleshy fruits employ quite different developmental strategies for seed dispersal, hence the many differences found in the physiological, morphological, and biochemical processes taking place during maturation. Notwithstanding, attempts have being made to establish analogies of these apparently different processes. Indeed, a selection of the adequate developmental stages used for comparisons is essential. For the expression data, a caveat in the comparative analysis stems from the limited availability of the proper and complete transcriptomic data of fruit development, which may weaken the outcome of the analysis. Ideally, the independent expression data from the pericarp of *Arabidopsis* mature and senescent siliques, in stages 17B and 18, respectively (Smyth *et al.*, 1990), should be compared with the pericarp of tomato fruits at mature green, ripening and over-ripening.

The analysis carried out in this work reveals that the transcriptome of *Arabidopsis* silique senescence barely shares similarity in gene numbers to either tomato fruit ripening or over-ripening. In contrast, most of the biological terms detected as being enriched between *Arabidopsis* silique senescence and tomato fruit ripening and over-ripening are identical, which strongly suggests a common major biological programme between *Arabidopsis* silique senescence and final tomato development steps, regardless of its distinction between ripening and over-ripening (Fig. 1). These biological functions, which take place in *Arabidopsis* and tomato, are related mainly to the defence and ABA stimulus response. Interestingly, this last GO term reveals a new role for ABA hormone during *Arabidopsis* dehiscence. However, several GO terms are specific of *Arabidopsis* silique senescence and tomato fruit ripening, but very few specific ones appear when comparing *Arabidopsis* silique senescence and tomato fruit over-ripening. This indicates that the biological processes taking place during silique senescence are somewhat similar to those in ripening when compared with over-ripening (Fig. 1). For example, the GO term *senescence* appears to be enriched only in the ripening comparison, but not in over-ripening, which supports the classical view of ripening being analogous to dry fruit senescence. Finally, the auxin response is essentially the only common GO term between silique senescence and the tomato over-ripening process. It is likely that all these described functions were already present in ancestral dry fruits, from which fleshy fruits evolved.

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