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Eprints ID: 7967

To link to this article: DOI:10.1016/j.tplants.2012.06.005
URL: http://dx.doi.org/10.1016/j.tplants.2012.06.005

To cite this version: Ruan, Yong-Ling and Patrick, John William and Bouzayen, Mondher and Osorio, Sonia and Fernie, Alisdair R. ***Molecular regulation of seed and fruit set.*** (2012) Trends in Plant Science, vol. 17 (n° 11). pp. 1360-1385. ISSN 1360-1385

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Molecular regulation of seed and fruit set

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Seed and fruit set are established during and soon after fertilization and determine seed and fruit number, their final size and, hence, yield potential. These processes are highly sensitive to biotic and abiotic stresses, which often lead to seed and fruit abortion. Here, we review the regulation of assimilate partitioning, including the potential roles of recently identified sucrose efflux transporters in seed and fruit set and examine the similarities of sucrose import and hydrolysis for both pollen and ovary sinks, and similar causes of abortion. We also discuss the molecular origins of parthenocarpy and the central roles of auxins and gibberellins in fruit set. The recently completed strawberry (Fragaria vesca) and tomato (Solanum lycopersicum) genomes have added to the existing crop databases, and new models are starting to be used in fruit and seed set studies.

Seed and fruit set: at the heart of food security

Seed and fruit are the key yield components in most crop species. As such, their development has been researched extensively for decades. In broad terms, seed and fruit development can be divided into three stages: set, growth and maturation. Seed and fruit set (see Glossary) are established during and soon after fertilization. This stage features a transition from ovule and ovary to seed and fruit, respectively, and is characterized by extensive cell division and coordinated development of maternal and filial tissues [1,2]. The newly formed fruit and seed then undergo cell expansion and accumulation of storage products, mainly proteins, starch and oils, which are typical features of growth and maturation stages [3].

Research on seed and fruit development has largely focused on the late stages of development. By contrast, much less is known about the mechanisms regulating their early development during the set phase. Understanding this early process is important for several reasons. First, molecular and biochemical pathways responsible for fruit and seed set are likely to have a profound impact on the later stages of development. Second, the set stage determines the fruit and seed number and, to a large degree, their final size through establishing cell numbers and, thus, yield potential. Third, similar to mammals, where early pregnancy is most prone to abortion, the set phase is highly sensitive to internal and external stresses compared with later stages of fruit and seed development [4] or vegetative growth [5,6]. Stresses include insufficient supply of nutrients, drought, heat or cold, which often induce substantial floral, seed and fruit abortion and, hence, irreversible yield losses [4,7,8]. For example, in cereals, water deficit during flowering can reduce yield by up to 60%, largely owing to reductions in grain set [1]. Similarly, heat stress can result in 70% yield loss in tomato (*Solanum lycopersicum*) as a result of flower and fruit abortion [9].

Elucidating the mechanisms underpinning seed and fruit set or those responsible for their abortion is fundamental to our understanding of reproductive biology and is essential for designing approaches to reduce abortion, thereby improving crop yield. Indeed, in the next 50 years, crop yield per unit land area needs to be doubled to meet demand owing to projected global increases in the human population and of living standards, which is likely to be exacerbated by decreases in the availability of arable land [10]. Here, we review major advances in the regulation of seed and fruit set by focusing on: (i) source–sink interactions, (ii) sugar and hormonal signaling and (iii) transcriptional and metabolic pathways. Finally, we outline future directions for furthering our understanding of the molecular mechanisms underlying seed and fruit set and potential applications in alleviating their abortion.

Glossary

Fruit abortion: abscission or stunted growth of fruit.
Fruit set: transition of an ovary to a growing young fruit.
Parthenocarpic fruit: fruit developed without fertilization, resulting in seedless fruit.
Seed abortion: abscission or stunted growth of seed.
Seed set: transition from an ovule to a seed upon fertilization.

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Box 1. Source–sink frameworks are defined by inflorescence phenology and growth patterns

Nutrient sources are determined by inflorescence phenology. Bud burst and early floral development in deciduous woody perennials largely depends upon xylem delivery of nutrients remobilized from stem or root storage pools until emerging leaves from vegetative buds reach photosynthetic competence [115]. Thereafter, fruit and seed set are increasingly supported by current photoassimilates and other nutrients exported from photosynthetic leaves through the phloem (Box 2). By contrast, floral development and fruit and seed set of many herbaceous eudicots (e.g. [12]) and grasses (e.g. [17]) rely exclusively on nutrients delivered through the phloem from source leaves.

Patterns of inflorescence formation influence source–sink relationships. For example, in grasses at floral evocation the shoot apical meristem of each branch (tiller) irreversibly converts to a floral meristem. In order to reach these apically located inflorescences, nutrients are transported through a series of elongating internodes. The developing wheat spike illustrates the competitive disadvantage for floret development within this configuration. Here between 66% and 75% of the uppermost florets within each spikelet atrophy [116]. However, following anthesis, the intense competition for nutrients abates as vegetative growth ceases leaving developing spikelets [17] as the sole growth sink with excess nutrients flowing into stem storage [117]. The grass pattern of inflorescence formation contrasts with one in which the shoot apical meristem remains vegetative whilst inflorescences progressively form from lateral buds in leaf axils. As a result, from floral bud inception onward, competition for nutrients occurs within and between inflorescences as well as with ongoing vegetative growth [12,118].

Do nutrient transport and partitioning constrain fruit and seed set?

Sexual reproductive strategies influence the nature of nutrient partitioning to, and between, reproductive structures (Box 1). Nevertheless, some general principles of resource allocation can be identified. Thus, irrespective of inflorescence phenology and growth pattern (Box 1), manipulating source–sink ratios demonstrates that photoassimilate limitation is a primary driver of flower, fruit and seed abortion in grain [11,12] and fruit [13] crops. Carbon limitation at fruit and seed set [14] applies to other essential nutrients. Of particular significance is the delivery of amino nitrogen compounds to sustain physiological carbon:nitrogen ratios essential for early reproductive development [15]. This scenario is illustrated by an increased seed set arising from enhanced phloem sap concentrations of *S*-methylmethionine in pea (*Pisum sativum*) transformants expressing yeast *S-methylmethionine permease 1* under the control of a phloem-specific promoter [16].

Positive responses of early reproductive development to increased source–sink ratios demonstrate that breeding for higher reproductive potential, through increasing flower numbers per inflorescence [11], has not been matched by absolute increases in nutrient levels reaching these additional reproductive units required to support their development through to seed and fruit set. In general, photosynthetic leaves are the primary nutrient source supporting early reproductive development (Box 1). Thus, factors constraining nutrient supply from a source leaf to a given sink, within an array of competing sinks, could include source leaf photosynthesis, hydraulic conductance of phloem pathways linking source and sink (*Lo*) and sink hydrostatic pressure (P_s) (see Equations I–IV and Figure I in Box 2).

Although flower formation and fruit and seed set are carbon limited (see above), it does not follow that their carbon deficits result from inadequate rates of source leaf photosynthesis. For instance, photoassimilate requirements of flowers, setting fruit and seed are, at a minimum, an order of magnitude less than the subsequent phase of filling fruit and seed, which is sink, not source, limited (e.g. [12,13,17]). Indeed, there are examples of sink-dependent repression of photosynthesis of source leaves supplying photoassimilates fueling seed and fruit set (e.g. [18]). Thus dampening sink repression of photosynthesis can be expected to result in elevated rates of seed and fruit set. For instance, decreasing sugar (glucose) repression of leaf photosynthesis, by compartmenting glucose into mesophyll vacuoles through overexpressing *tonoplast monosaccharide transporter 1* (*TPMT1*), increased photoassimilate export and seed size [19]. Increasing the sink capacity of developing wheat (*Triticum aestivum*) florets by overexpressing ADP-glucose pyrophosphorylase under the control of a grain-specific promoter led to higher rates of seed set supported by increased flag leaf photosynthesis [20]. Together, these findings indicate that photoassimilate limitation of early reproductive development is not imposed by leaf photosynthesis (R_e; see Equation I in Box 2). A plausible explanation for enhanced photosynthesis relieving C limitation during early reproductive development is to elevate hydrostatic pressure in leaf phloem (P_e), which in turn increases leaf–sink hydrostatic pressure differentials to drive higher rates of phloem import into developing reproductive units (see Equations II–IV in Box 2).

There is a growing body of evidence that hydraulic conductances of phloem (sieve tubes) adequately account for the observed rates of phloem transport (e.g. [21,22]), including phloem pathways serving developing reproductive structures [23]. In addition, the buffering capacity of the transport phloem [24] can augment C flows to support seed and fruit set (e.g. [25]). By contrast, vascular pathways within young fruit and seed contain xylem and phloem differentiating from provascular cells [26]. Fluid mechanics predicts that the lowest hydraulic conductances are encountered by flow through plasmodesmata interconnecting provascular cells and non-phloem cells forming symplasmic pathways delivering phloem sap into developing fruitlets and the maternal tissues of seeds (e.g. [27] and Box 2). The relatively low hydraulic conductances of plasmodesmata account for the large differences in hydrostatic pressures (~1 MPa) detected between sieve elements and adjoining vascular parenchyma cells of developing wheat grains and point to where most of the control of phloem transport resides [28] (see Equation II in Box 2). In addition, these large hydrostatic pressure differentials are consistent with bulk flow continuing into the non-phloem symplasmic pathway and, as a result, P_s is likely to reside in non-phloem cells (Box 2). Consistent with this proposition, overexpressing ADP-glucose pyrophosphorylase in developing wheat florets increased grain numbers set per spikelet [20], indicating an enhanced rate of phloem import by lowering P_s (see Equations II–IV in Box 2). Nutrient transfer across the maternal–filial interface is

Box 2. Regulation of nutrient transport and partitioning to developing fruit and seed

Nutrients flow from source leaves to developing fruit or seed sinks in the phloem and specifically in sieve elements organized into longitudinal files called sieve tubes (ST, Figure I). Sugars represent the major osmotica of sieve tube sap and phloem-loading capacities accommodate those of source leaf photosynthesis. Thus rates of sugar export (R_e, mol s^{-1} and see Figure I) from a source leaf correspond to net rates of leaf photosynthesis and collectively determine amounts of sugars available for partitioning (η) between sinks and, hence, their rates of sink import (R_i and see Equation I). A conclusion that equally applies to all phloem-transported nutrients.

$$R_i = R_e \times \eta \quad [I]$$

Depending upon plant species, phloem loading may follow an apoplasmic or symplasmic pathway. Irrespective, nutrients are accumulated to high concentrations in leaf phloem to osmotically create a hydrostatic pressure at the site of export (P_e, MPa; Figure I). Phloem P is dissipated as flow proceeds along ST conduits but primarily at the sink end (P_s) of the transport pathway (Figure I). This generates a hydrostatic pressure difference ($P_e - P_s$) to drive a bulk (volume) flow of phloem sap from source to sink at rates (J, $m^3\ s^{-1}$) modulated by hydraulic conductances (L_o, $m^3 s^{-1}\ MPa^{-1}$) of the transport pathway (Equation II).

$$J = (P_e\text{-}P_s) \times L_o \quad [II]$$

For walls and coats of setting fruits and seeds respectively, unloading from their STs into surrounding non-vascular cells occurs as a bulk flow through interconnecting plasmodesmata (Figure I). Hence P_s is located in non-vascular tissues of these sinks and source to sink L_o is largely determined by plasmodesmal L_o forming the phloem unloading pathway. Rates of import (R_i) of a nutrient into a specified fruit or seed sink depend upon the concentration (C, mol m^{-3}) of the nutrient in the phloem sap and J through phloem termini and unloading pathway (Equation III).

$$R_i = J \times C \quad [III]$$

Given these considerations, partitioning (η) of nutrients between an array (n) of sinks, from a common pool of ST nutrients, is a function of each individual bulk flow rate (R_i) into a specified fruit or seed (j) expressed as a proportion of the combined bulk flow rates into all [(1) to (n)] fruit or seed within an inflorescence (Equation IV).

$$\eta = \frac{R_i(\mathrm{j})}{\Sigma R_i(1) + \cdots\cdots + R_i(\mathrm{n})} \quad [IV]$$

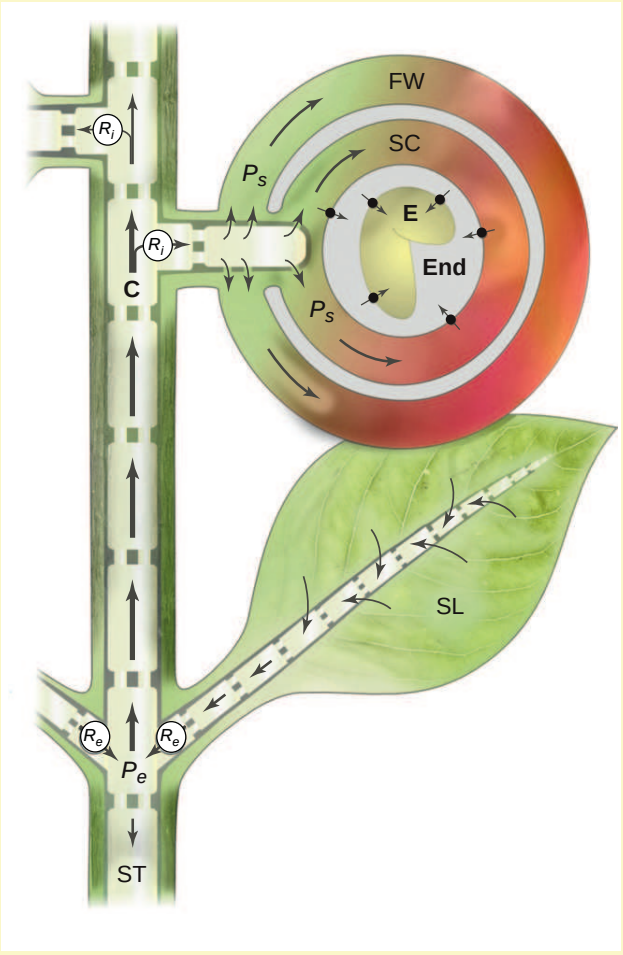

Figure I. Nutrient transport to developing fruit and seed. Following phloem loading (curved arrows) into sieve tubes (ST) of source leaves (SL), nutrients are exported at given rates (R_e) and concentrations (C) by bulk flow (straight arrows) driven by differences in hydrostatic pressures generated by source leaves (P_e) and sinks (P_s). A proportion of ST sap is imported, at given rates (R_i), into walls (FW) and coats (SC) of setting fruit and seed respectively by bulk flow through plasmodesmata (curved arrows through gaps) linking STs with surrounding non-vascular cells. Membrane transporters (circles) facilitate nutrient transport (arrows through circles) from the SC and subsequent uptake into the endosperm (End) and embryo (E) across the symplasmic discontinuity at the maternal–filial interface.

transporter-mediated and turgor-regulated to link with phloem import [29]. Therefore, it is no surprise that seed set depends upon adequate transporter activities at these sites (e.g. [30–32]). These findings highlight opportunities, other than through manipulating photosynthesis, to increase nutrient availability for fruit and seed set. These opportunities include exploiting mechanisms regulating hydraulic conductances of plasmodesmata [33] to amplify rates of phloem import into fruits and enhancing activities of transporters regulating nutrient flows into filial tissues of developing seeds [29].

Regulation of seed and fruit set through sugar metabolism and signaling

As discussed above, plant reproduction depends greatly on an adequate import of photoassimilates, which for most crop species is mainly in the form of sucrose. Efficient utilization of sucrose is crucial for gametophyte development, fertilization and coordinated development of filial and maternal tissues, which collectively determines seed and fruit set.

Regulation of male fertility by sugars

For most flowering plants, pollination is a pre-requisite for seed and fruit set. After germination, pollen tubes elongate through the style and release two sperm into the ovular embryo sac for double fertilization to produce the embryo and endosperm. For pollen to be viable, it must synthesize sufficient starch as an energy source and cellulose and callose to build their internal wall [1,34]. Starch, cellulose and callose are all polymerized from glucose in α- and β-1,4 and β-1,3 linkages, respectively. Glucose in pollen could be derived from: (i) cell wall invertase (CWIN) activity hydrolyzing sucrose into glucose and fructose in the anther or

pollen grain apoplasm; (ii) degradation of starch in anthers by α-amylase [35]; and (iii) sucrose cleavage by sucrose synthase (Sus) to produce fructose and UDP-glucose (fructose is convertible to glucose and UDP-glucose is an immediate substrate for cellulose and callose biosynthesis) [34]. Transgenic suppression of a tapetum- and pollen-specific CWIN gene in tobacco (*Nicotiana tabacum*) results in unviable pollen, characterized by loss of starch and cell wall integrity [36], demonstrating the crucial role of CWIN in pollen development. Consistent with this, male sterility and hence grain or fruit abortion, is attributable to reduction of CWIN and vacuolar invertase (VIN) expression in wheat under drought [37], of CWIN and hexose transporters in rice (*Oryza sativa*) under cold stress [38] and disruption of sucrose metabolism in tomato under heat stress [39]. Indeed, genetic variation in drought tolerance correlates with the expression level of CWIN and starch abundance in the pollen and ovaries in wheat [40]. It is clear that maintaining the sucrose supply and its degradation into hexoses is key to male fertility and seed and fruit set.

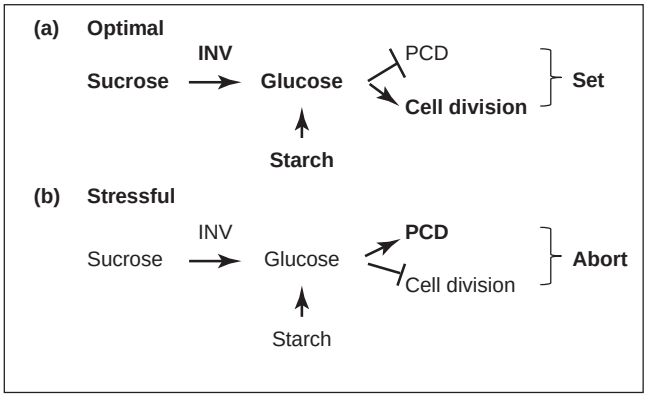

Figure 1. A model for regulation of seed and fruit set through sugar signaling. **(a)** Under optimal conditions, phloem unloaded sucrose is hydrolyzed by invertase (INV) in ovaries and ovules. The resultant glucose functions as a signal to repress programmed cell death (PCD) genes and to promote cell division, which together lead to seed and fruit set. Starch reserve may be remobilized to supplement glucose production, particularly under mild stress conditions. **(b)** Under severe stress, phloem import of sucrose is blocked, which in turn decreases INV activity. This, together with depletion of starch reserve, reduces glucose levels that activate the PCD pathway and inhibit cell division. Consequently, seed and fruit abort.

Invertase modulates seed and fruit set

One of the most important recent findings in reproductive biology research is the similarity between the biochemical and molecular events leading to pollen sterility and ovary abortion [2]. In maize (*Zea mays*), the fertility of the ovaries has a greater influence on kernel number than that of pollen, which is different to the scenario in wheat, rice and barley (*Hordeum vulgare*) where pollen development is more prone to stress than ovaries [1,4].

In maize ovaries, phloem-imported sucrose supplies carbon for starch accumulation in ovary walls and pedicels and to generate high glucose concentrations that flow to embryo sacs following sucrose hydrolysis by CWIN in pedicels and by VIN in nucellar tissues [41]. Upon imposing a water deficit five days before anthesis, sucrose import is blocked owing to leaf photosynthesis being inhibited and ovary wall starch reserves are remobilized, but these soon become depleted if drought persists for several days. Concomitantly, CWIN and VIN activities and glucose concentrations decrease, leading to severe ovary abortion and yield loss [42]. Abortion is triggered by expression of programmed cell death (PCD) genes encoding a ribosome-inactivating protein (RIP2) and phospholipase D (PLD1). Feeding sucrose to water-stressed maize plants reverses the decline of *CWIN* (*Incw2*) and *VIN* (*Ivr2*) expression levels and glucose concentrations as well as blocking expression of PCD genes, *RIP2* and *PLD1*, in ovaries to restore kernel number by about 70% [42]. Remarkably, these are the only sugar-responsive genes among many candidate genes surveyed [4], demonstrating that an invertase-mediated glucose signaling pathway regulating PCD is the primary biochemical and molecular mechanism controlling maize ovary abortion under drought conditions.

Significantly, this mechanism appears to be conserved between monocot and eudicot herbaceous species. For example, RNAi-mediated silencing of a CWIN gene *Lin5* aggravates fruit abortion in tomato [43], whereas elevation of CWIN activity enhances fruit and seed development [44]. Furthermore, in comparison with a heat-susceptible genotype, heat-tolerant tomato exhibits greater sucrose import into, and invertase activities within, young fruit, accompanied by a lower level of expression of a PCD gene, *LePLDα1* [7]. The heat-induced or -enhanced expression of heat-shock proteins (HSPs) is hypothesized to protect CWIN and VIN from misfolding for correct targeting and function [7].

Together, the above analyses support the following model of seed and fruit set regulated by sugars (Figure 1). Phloem-imported sucrose serves as a primary signal sensed by invertase that generates glucose to repress the PCD pathway on the one hand and to promote cell division of filial and fruit tissues on the other (see [2,3]), thereby allowing seed and fruit set to proceed. The model changes under stress where a decreased glucose level activates a PCD pathway leading to seed and fruit abortion. The whole process is conditioned by starch degradation and HSP expression (see above) and probably through interaction with hormones (see below).

Hormonal regulation of fruit and seed set

Upon flower fertilization, fruit and seed undergo concomitant development; however, in contrast to fruit, which can develop in the absence of pollination, seed development is more strictly dependent on successful fertilization. Seed development comprises endosperm proliferation and embryo growth and both processes show multihormonal regulation by auxins, cytokinins, gibberellins (GAs) and brassinolides [45]. Mutations affecting auxin perception [46] or transport [47] resulted in abnormal embryo morphologies whereas mutants altered in components of the cytokinin signaling pathway produced seeds of enlarged size [48]. Likewise, alteration of gibberellic acid response genes [49] results in increased seed size and a low level of active brassinolides induces altered seed shape [50]. Accordingly, defects in the biosynthesis of rice brassinolide or perception genes also result in reduced seed length [51]. However, determining how these hormones

interact to regulate seed set and development has so far remained elusive.

Auxin and gibberellin are the key players in fruit initiation following fertilization [52–55]. This view is supported by the fact that exogenous application or transgenic elevation of these hormones lead to the uncoupling of fruit set from fertilization resulting in the development of parthenocarpic fruit [56,57]. Transcriptional regulators from the ARF (Auxin Response Factors) and Aux/IAA (Auxin/indole acetic acid) type of transcription factor are encoded by two large gene families [58,59] that are known to channel auxin signaling to specific physiological responses [60–63]. A significant advance in our understanding of the auxin-dependent mechanism underlying fruit set came from mutations or transgenic manipulation of specific *ARF* and *Aux/IAA* genes leading to the development of parthenocarpic fruits in both tomato and *Arabidopsis* (*Arabidopsis thaliana*) [61,64,65]. These studies identified auxin signaling as one of the early events in the fruit initiation cascade. Downregulation of the tomato IAA9 and ARF7 results in uncoupling fruit set from pollination and fertilization, giving rise to parthenocarpic fruit, thus suggesting that both genes encode negative regulators of fruit set [61,65]. Although interaction between ARF and Aux/IAA proteins plays a pivotal role in regulating auxin responses [60,66], it is still not known whether IAA9, ARF8 and ARF7 control fruit set through common or distinct pathways. Further investigation should uncover whether heterodimerization between ARF8/ARF7 and IAA9 is part of the control mechanism regulating fruit initiation. Moreover, given that ARF genes can be potentially regulated by siRNAs at both the transcriptional and post-transcriptional levels [67,68], it would be important to know whether the auxin-dependent fruit set is impacted by epigenetic regulation. If proved, this level of regulation may explain the environmentally induced variation in fruit set and parthenocarpy observed in *entire*, a natural tomato mutant impaired in Sl-IAA9 function [61,69], and the initiation of fruit development before and independently of fertilization.

In support of multihormonal control of fruit set, there is strong evidence suggesting that the role of auxin is facilitated by synergistic activity with gibberellins [70,71]. Notably, GA treatment of unpollinated ovaries triggers fruit initiation without impacting the expression of auxin signaling genes [72], whereas auxin-induced fruit development is significantly reduced by simultaneous application of GA biosynthesis inhibitors [70]. Together, these data suggest that auxin may act before GA and that the effect of auxin is mediated at least partly by GA (Figure 2). In line with this hypothesis, GA biosynthesis genes are upregulated after pollination and upon auxin treatment of emasculated flowers [70]. However, in tomato, each hormone seems to play a specific role given that auxin application results in a large number of pericarp cells, whereas GA treatment results in fewer pericarp cells but of a larger size [73]. Interestingly, concomitant treatment with both hormones results in the formation of fruits similar to pollination-induced seeded fruits [73], suggesting that GA and auxin are both required for normal fruit development.

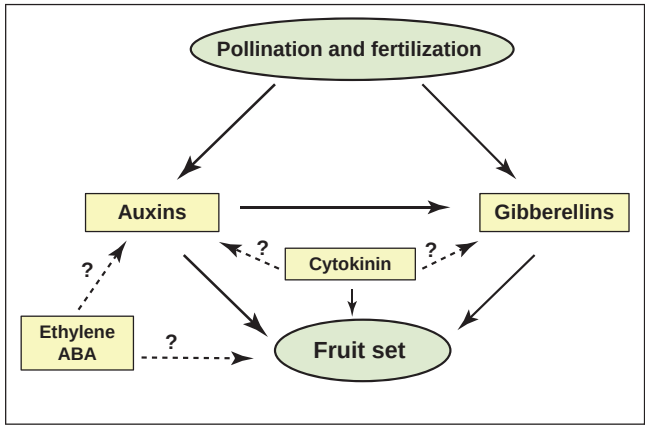

Figure 2. A model for multihormonal regulation of fruit set. Pollination and fertilization result in increased levels of both auxin and gibberellins (GA), which triggers fruit growth through stimulation of cell division and expansion. Auxin can stimulate fruit set either directly or via inducing GA biosynthesis. Each hormone seems to play a specific role given that auxin application results in a high number of pericarp cells, whereas GA treatment results in fewer but larger pericarp cells. Natural fruit set seems to require both hormones given that only parthenocarpic fruits induced by concomitant auxin and GA treatment are similar to pollination-induced seeded fruits. Putative involvement of ethylene and abscisic acid (ABA) in regulating fruit formation has been mainly suggested by transcriptomic studies. In particular, during the transition from anthesis to post-anthesis, ethylene-related genes, along with those related to auxin, account for most of the changes among all phytohormone-related genes, thus indicating that ethylene must play an active, but yet not understood, role in fruit set. Exogenous application of cytokinin can induce parthenocarpic fruit yet the underlying model of action remains unknown. Broken arrows represent effects that are still not sustained by solid and multiple experimental data.

Besides the established role of auxin and GA, an increasing number of studies, building on global transcriptomic profiling (see below), point to the putative involvement of other hormones such as ethylene and abscisic acid (ABA) in regulating fruit formation [72,74,75]. It has been reported that ethylene biosynthesis and ethylene signaling genes decrease after pollination. At the same time, genes related to ABA biosynthesis decreased and the opposite behavior was found for ABA degradation-related genes [76]. These findings suggest that normal fruit development depends on induction of GA and auxin responses, whereas ethylene and ABA responses are attenuated (Figure 2). Nevertheless, the direct contribution of ethylene to fruit set *per se* has remained largely unstudied. By contrast, exogenous application of cytokinin can induce parthenocarpic fruit formation in a range of agricultural species but the mechanism by which these hormones may interact with auxin or GA during fruit set remains to be elucidated.

Transcriptional and metabolic regulation of fruit and seed set

In addition to the more hypothesis-driven studies of fruit and seed set detailed above, recent years have also seen the adoption of broad technologies to assess transcriptional and metabolic programs of fruit development and ripening [77–81], which have revealed both conserved and species-specific changes that underpin both processes. Here we focus on changes during early fruit development – specifically fruit and seed set [82–85] – using tomato as a model because this species has the most pertinent data.

Consistent with analyses in the previous sections, recent studies have identified not only auxin and ethylene

Box 3. Pollination-dependent and -independent fruit set

A detailed comparison of transgenic plants in which IAA9 was antisense inhibited, provoking pollination-independent fruit set, with the wild-type pollination-dependent control at the transcriptomic and primary metabolite levels proved highly revealing [75]. Many genes were commonly regulated in both fruit-set types, with only a small subset being IAA9 dependent. Thus minimal changes in gene expression clearly have dramatic consequences on the developmental fate of the flower. Indeed only at the bud-to-anthesis transition were there major changes in transcripts, with anthesis being the phase at which the most genes were differentially expressed between genotypes, which potentially explains why *AS-IAA9* enters the fruit differentiation process earlier than the wild type and with no requirement for a pollination and fertilization signal [75].

Aside from the comparative aspect of this study, several interesting features of wild-type development were characterized, which expanded upon other early fruit-development studies [119]. Intriguingly, many transcription factors were recruited during this period with the MADS-box genes being particularly strikingly downregulated in young fruit compared with at the ovary stage. When taken together with observations from transgenic experimentation [120–122], these observations strongly implicate *Tomato Agamous1* (*TAG1*), *Tomato Agamous-like6* (*TAGL6*) and *Tomato MADS-box 29* (*TM29*) genes as activators of the fruit set process. Whether IAA9 and/or auxin *per se* act in concert or independently of these MADS-box genes in controlling fruit development remains to be clarified. In addition to these transcription factors, a large number of cell division, protein biosynthesis and cell wall-related genes were upregulated post-anthesis in the wild type (but early during pollination-independent fruit set). However, the links between transcription factor expression and downstream processes remain to be clarified in most instances and are not as well characterized as those orchestrating later stages of fruit development [102].

During pollination-induced fruit set, photosynthesis-related genes were downregulated during the transition from bud to anthesis, whereas they were generally upregulated in the period from anthesis to post-anthesis. By contrast, photosynthesis-related genes were strongly activated in *AS-IAA9* throughout fruit set and notably, all these genes were upregulated in the *AS-IAA9* lines compared with the wild type. In line with the elevation of photosynthesis-related genes, downregulation of *IAA9* was associated with a dramatic upregulation of genes involved in sucrose catabolism, which also suggests that these genes play an important role in the flower-to-fruit transition processes [75].

signaling, but also photosynthesis and sugar metabolism, as major events of the fruit-set program (Box 3) [75]. Transcriptome analyses have revealed that during pollinated fruit set almost 5% of the differentially expressed genes are related to hormone response and metabolism. As mentioned above, *Aux-IAA* and auxin transcription factor (*ARF*) genes show a dramatic shift in their expression levels across natural fruit development, suggesting that these transcriptional regulators play an active role in this developmental process. Moreover, downregulation of *IAA9* results in feedback regulation of several *ARF* and *Aux-IAA* genes [75]. In keeping with the importance of auxin, recent transgenic work has implicated both the auxin receptor homolog TIR and ARF7 in fruit set [86,87]. Moreover, the high number of ethylene-related genes observed to change suggests that, in addition to the well-established role of auxin and GAs [53,73], ethylene is also likely to play an active role in fruit set, albeit one that is antagonistic to that of auxin and GA [72]. Downregulation of the expression of auxin-related negative regulators of fruit-set genes occurs earlier than that of ethylene-related genes, suggesting the existence of a temporal dependence on hormone action for the flower-to-fruit transition to proceed.

Perhaps surprising was the finding that transcriptomic profiling across the flower-to-fruit transition identified many genes that are common to both pollination-induced and pollination-independent fruit set, suggesting that a relatively small number of genes are responsible for the differences in these processes (Box 3). In the same study, gas chromatography–mass spectrometry-based metabolite profiling was applied to extend previous characterization of metabolic shifts occurring during fruit ripening [78] to encompass the early events of the process [75]. There were proportionally far more changes in metabolite than in transcript levels; however, (i) those metabolites and transcripts associated with photoassimilate metabolism, and (ii) those sugars and transcripts associated with both the photosynthetic apparatus and enzymes of the Calvin cycle, revealed common regulation at both levels. In addition, statistical analyses were able to highlight transcriptional regulation of putrescine and spermidine levels in keeping with previous descriptions of the kinetics of their levels during early fruit development [88], and similarly for the regulation of the pathways of ascorbate biosynthesis [78].

Given the widely documented crosstalk between hormone and sugar signaling, it is highly interesting that the *IAA9* antisense plants were also characterized as being upregulated in the Sus pathway of sucrose degradation in young tomato fruit. This pathway is more energy efficient than that mediated by INV [89] and is likely to be the prominent pathway of sucrose degradation in the later stages of development of many heterotrophic tissues, including tomato [90]. Future experiments, examining the roles of a subset the recently cloned SWEET transporters – some of which act as sucrose efflux transporters [91], may further illuminate the fruit-set process regulated by sugars. Returning to the IAA9 transgenics, elevation of the transcripts of this pathway could, at least partially, explain the precocious nature of fruit development. The elevation of sugar levels in the antisense lines could be due to either a more efficient unloading of photoassimilate into fruit, as observed on the introgression of a wild species allele of CWIN [92], or to an increase in photoassimilate production by fruits. Interestingly, increasing auxin sensitivity via downregulation of tomato IAA9 has been reported to promote vascular bundles development [61]. The more abundant vasculature may enhance sink strength and sugar supply to the fruit and hence directly links the effects of auxin and photoassimilates on the initiation of fruit set and early development. Strong activation of photosynthesis-related genes during fruit set in *IAA9*-downregulated lines is a major phenomenon [75]. Strikingly, activation of photosynthesis-related genes is delayed to the post-anthesis stage in the wild type but takes place at the bud stage in *AS-IAA9* [75]. Many recent studies have endorsed the prevailing opinion that fruit growth and metabolism are predominantly supported by photoassimilate supply from source tissues [93,94]. However, it is important to note that the carpel of the fruit is essentially a modified leaf that has folded into a tubular structure enclosing the ovules [95] and that cells in developing fruit contain photosynthetically active chloroplasts and express

both nuclear-encoded and plastid-encoded genes for photosynthetic proteins [96]. Furthermore, a combination of indirect evidence provides support for the hypothesis that during the early stage of fruit development, photosynthesis itself may provide a considerable contribution to both metabolism and growth of the organ [97]. Recent studies have indicated that in tomato pericarp cells, the induction of genes related to photosynthesis and chloroplast biogenesis positively correlate with chloroplast numbers and cell size [98]. However, a more recent direct study of the role of fruit photosynthesis by downregulating chlorophyll biosynthesis in a fruit-specific manner revealed that photosynthesis was unimportant for fruit growth but rather was essential for seed set [99]. Therefore, it would appear that fruit photosynthesis may contribute to the correct spatial distribution of sugars required to trigger the correct hormonal signaling events underlying seed set. That said, unlike the situation described above for fruit set, detailed transcriptomic and metabolomic analysis of seed set is lacking.

Future perspectives

In recent years our level of understanding of the molecular events at the transcriptional, biochemical, hormonal and metabolite levels underlying fruit and seed set has increased considerably. Although to date cereal grains and tomato fruit have predominantly been used as models, there is a growing body of knowledge about other seed and fruit systems. The low hydraulic conductance encountered by plasmodesmata connecting provascular cells with nonvascular cells in fruitlet and seed maternal tissues represents a bottleneck for assimilate import into these young sinks. Thus, enhancing their phloem differentiation and plasmodesmal conductance could enhance rate of phloem import and seed and fruit set. Inv-mediated glucose signaling regulating PCD and IAA-signaling controlling parthenocarpy and vascular development are two new avenues for improving seed and fruit set and, hence, crop yield, and present opportunities to dissect interactions between sugar- and hormone-signaling pathways underpinning seed and fruit set.

To date, most published studies of transcriptional and metabolic regulation are of relatively low resolution at both spatial and temporal levels and are furthermore restricted in coverage of the various cell molecular entities. However, a range of nascent and emerging technologies now allows us to refine our analytical ability further to cope with issues such as subcellular compartmentation and the behavior of different cell types, which should increase our understanding in the years ahead. For example, laser capture microdissection has already been employed to gather tissue-specific transcriptomes in tomato fruit [100] and rice seed [101], and the use of RNAseq technologies and highly sensitive real-time quantitative PCR methods [80] have dramatically enhanced transcriptome coverage. Their application to the floral-to-fruit or floral-to-seed transition should allow us to enhance our understanding of gene regulatory networks to at least the level of those characterized for later stages of fruit development (for a recent review see [102]). Similarly, methods for temporally specific genetic perturbations of fruit genetics using virus-induced gene-silencing protocols have been generated [103] and recently have also been optimized for young fruit [104]. Although changes in the primary metabolism of young fruit are now well documented it will be important to look also at specialized metabolism, particularly in light of the recent report that the absence of certain flavonoids leads to parthenocarpic fruit [105]. Several methods have been established that are capable of this [106–108]. However, although these have been used in the study of fruit development, to date their use has focused on later ripening stages. Along the same lines, methods capable of detecting all major classes of phytohormones have been recently established [109]. The early development of tomato fruit would appear to be a perfect biological situation for their application.

The recent completion of the grape (*Vitis vinifera*) strawberry (*Fragaria vesca*) and tomato genomes [110–112] renders them even better model systems in which to study the fruit set process. It is likely that not only transgenic or mutational studies but also the increasingly widespread adoption of natural genetic diversity [113] will ultimately allow us to take such broad studies from the descriptive to the functional level [114] and may ultimately aid in breeding strategies intent on increasing crop yield and food security.

Acknowledgments

We thank the Australian Research Council (DP110104931; DP120104148) and France's Laboratoire d'Excellence (LABEX) entitled TULIP (ANR-10-LABX-41) for funding support and COST Action FA1106 for providing the networking framework.

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