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REVIEW PAPER

The role of auxin and gibberellin in tomato fruit set

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Abstract

The initiation of tomato fruit growth, fruit set, is very sensitive to environmental conditions. Therefore, an understanding of the mechanisms that regulate this process can facilitate the production of this agriculturally valuable fruit crop. Over the years, it has been well established that tomato fruit set depends on successful pollination and fertilization, which trigger the fruit developmental programme through the activation of the auxin and gibberellin signalling pathways. However, the exact role of each of these two hormones is still poorly understood, probably because only few of the signalling components involved have been identified so far. Recent research on fruit set induced by hormone applications has led to new insights into hormone biosynthesis and signalling. The aim of this review is to consolidate the current knowledge on the role of auxin and gibberellin in tomato fruit set.

Key words: Auxin, fruit set, gibberellin, parthenocarpy, tomato.

Introduction

Fruit set is defined as the transition of a quiescent ovary to a rapidly growing young fruit, which is an important process in the sexual reproduction of flowering plants. The tomato (*Solanum lycopersicum* L.) is one of the most studied fleshy fruits, representing the Solanaceae, a family that contains several other important fruit crops, such as the eggplant (*Solanum melongena* L.) and peppers (*Capsicum* spp.). Tomato fruit set is very sensitive to environmental conditions, in particular, to too low or high temperatures that affect pollen development and anther dehiscence. As a consequence, efficient tomato production is restricted to certain climatic zones. For this reason, tomato seed companies breed at different places in the world to develop cultivars suited for optimal fruit production under the local climate conditions. Nevertheless, even with these optimized lines it is often not possible to grow tomatoes during the summer in warm regions such as the Southern parts of Europe. In the more Northern parts, tomato production is only possible during the warm season, and even then only in modern greenhouses at the expense of a huge amount of energy for heating. So if fruit development could be less dependent on efficient fertilization this would be a big advantage for fruit production in areas that are now unsuitable for efficient fruit set. This requires an understanding of the regulatory mechanisms involved in fruit set. Therefore, tomato mutants that produce seedless fruit (parthenocarpic fruit) without the need for fertilization have been extensively studied.

Fruit set depends on the successful completion of pollination and fertilization (Gillaspy *et al.*, 1993). In tomato, as in most angiosperms, compatible pollen has to germinate on the pistil, and forms a pollen tube. This pollen tube then grows through the style and the ovular micropyle to deliver two sperm cells in the embryo sac. There a double fertilization occurs; one of the two sperm cells fertilizes the egg cell, while the other fuses with two haploid polar nuclei in the central cell. Consequently, both embryo and surrounding tissues may generate signals that stimulate fruit growth. The tomato ovary is composed of two or more carpels, which enclose the locular cavities containing the ovules (Fig. 1A, E). After successful fertilization, the development of the ovary into a fruit starts with a period of cell division, which continues for 10–14 d (Fig. 1B, F). During the following 6–7 weeks, fruit growth mainly

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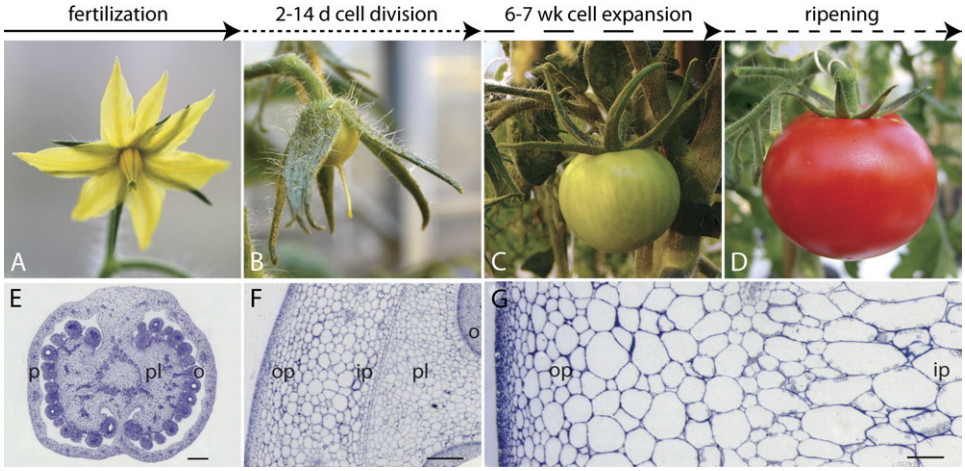

Fig. 1. Overview of tomato fruit development. The first stage is fruit set, the initiation of fruit growth after the flower has been successfully pollinated and fertilized. After fertilization, cell division takes place, which lasts up to 14 d. This period is followed by 6–7 weeks (wk) of mainly cell expansion, during which the volume of the fruit rapidly increases. Once the fruit has reached its final size it starts to ripen. (A, E) Flower and micrograph of an ovary at anthesis, awaiting pollination. Bar=200 μm. (B, F) Fruit of 0.8 mm in diameter, 10 d after pollination, and a micrograph of its pericarp. Bar=200 μm. (C, G) Fruit of 3 cm in diameter, 5 weeks after pollination, and a micrograph of its pericarp. Bar=200 μm. (D) Ripe tomato fruit. P, pericarp; op, outer pericarp; ip, inner pericarp; pl, placenta; o, ovules.

depends on cell expansion (Fig. 1C, G) (Mapelli *et al.*, 1978; Bünger-Kibler and Bangerth, 1983; Gillaspy *et al.*, 1993). The carpel wall develops into the pericarp, and the placenta, to which the ovules are attached, develops into a gel-like substance, consisting of large, thin-walled cells that are highly vacuolated. At the end of the cell-expansion period, the fruit has reached its final size and will start to ripen (Fig. 1D) (Gillaspy *et al.*, 1993). Although the influence of phytohormones, such as auxin and gibberellin, over fruit development was already acknowledged back in the early 20th century (Gustafson, 1937, 1939; Wittwer *et al.*, 1957), the molecular mechanisms that underlie fruit set are still largely unknown and are now starting to be unravelled.

Gustafson (1936) was the first to demonstrate that the application of substances closely related to auxins onto the stigmas of tomato and several other species causes the ovary to develop into a parthenocarpic fruit. The application of pollen extracts to the outside of the ovary showed similar results, which led to the hypothesis that pollen grains contain plant hormones similar to the growth substance auxin. After pollination, the pollen may transfer a sufficient quantity of these hormones to the ovary to trigger fruit growth (Gustafson, 1937). Wittwer *et al.* (1957) showed that a second type of growth substance, gibberellins (GAs), can also stimulate parthenocarpic fruit set. Shortly thereafter, gibberellin-like plant hormones were identified in different families of flowering plants (Phinney *et al.*, 1957), leading to the assumption that these plant hormones are also involved in the fruit developmental programme. This idea was supported by the findings of Sastry and Muir (1963), who studied the effect of gibberellin treatment on diffusible auxin levels in the tomato ovary. They determined the auxin concentrations of the flowers with a classical bioassay, in which the flowers were cut and placed on blocks of agar. Subsequently, the auxin content of these blocks was measured by the standard *Avena* curvature test. At the stage of anthesis, no auxin was present. However, auxin concentrations increased within 28 h after gibberellin treatment, suggesting that it is not auxin, but gibberellin that is transferred from the germinating pollen to the ovary. Subsequently, the gibberellin may induce an increase of the auxin content in the ovary to levels adequate to trigger fruit growth (Sastry and Muir, 1963). Consistently, the concentrations of both growth regulators rapidly increase during the first 10 d after anthesis, probably after pollination and fertilization, which occur between 2 d and 6 d after anthesis (Mapelli *et al.*, 1978). In natural parthenocarpic varieties, these hormones might already have reached a threshold concentration prior to pollination, resulting in the formation of seedless fruit (Gustafson, 1939; Nitsch *et al.*, 1960; Mapelli *et al.*, 1978, 1979). The application of gibberellin can induce an increase in auxin content (Sastry and Muir, 1963), but, in turn, auxin seems to be able to stimulate gibberellin biosynthesis (Koshioka *et al.*, 1994), which indicates that there is a tight regulation between these two hormones during the early stages of fruit development.

Auxin and gibberellin at the cellular level

Although the application of either auxin or gibberellin can trigger tomato fruit development independently of pollination and fertilization, there are several indications that each of these hormones has different effects on cell division and cell expansion. Normally in tomato, the cell division period takes the first 10–14 d of development (Mapelli *et al.*, 1978; Bünger-Kibler and Bangerth, 1983; Gillaspy *et al.*, 1993). However, in fruit induced by the natural auxin indole-3-acetic acid (IAA) this period is shorter, only lasting 10 d, but cell division takes place at a higher rate compared with

that in seeded control fruit, resulting in a fast initial increase in pericarp volume. At the end of the growth period the final number of cells is comparable to that of seeded fruit, but the IAA-induced fruit remain smaller as cell expansion is strongly impaired (Bünger-Kibler and Bangerth, 1983). Treatments with the synthetic auxins 4-chlorophenoxy acetic acid (4-CPA) and 2,4-dichlorophenoxy acetic acid (2,4-D) resulted in tomato fruits that were comparable in size to control fruits, but contained a higher number of pericarp cells (Bünger-Kibler and Bangerth, 1983; Serrani *et al.*, 2007*a*). The stronger effect of the synthetic auxins might be ascribed to their increased stability compared to IAA, which is highly unstable. Alternatively, the IAA level may be regulated by different mechanisms that do not recognize the synthetic auxins. IAA can be conjugated to amino acids by IAA-amido synthetases, while 2,4-D has been shown to be a poor substrate for these enzymes (Staswick *et al.*, 2005).

Gibberellin-induced fruits are generally smaller than seeded fruits. Although the pericarp volume of GA_3-induced fruits is small, the pericarp thickness is comparable to that of seeded fruits. Furthermore, this pericarp contains fewer cells but with a larger volume than the cells of control fruits (Bünger-Kibler and Bangerth, 1983; Serrani *et al.*, 2007*a*). These findings showed that cell expansion might be enhanced by gibberellins. On the other hand, this process might not be directly related to the application of gibberellin, but might be an indirect effect due to the reduced cell division activity. However, a fruit induced by the application of gibberellin together with 2,4-D or 4-CPA looks very similar in size and shape to a fruit induced by pollination (Bünger-Kibler and Bangerth, 1983; Serrani *et al.*, 2007*a*), supporting the hypothesis that cell elongation and cell division activity are co-ordinated by a delicate balance between the two phytohormones. Alternatively, other phytohormones such as cytokinin might be involved (Mapelli, 1981).

Apart from differences in cell elongation and cell division activity, there are several other differences in the morphology of fruits obtained after auxin or GA treatments. 2,4-D treatment leads to an increased number of vascular bundles that are interconnected by transversal tracheids. These tracheids are not present in pollination- or gibberellin-induced fruit and might be necessary for providing nutrients to the high number of pericarp cells in auxin-induced fruit (Serrani *et al.*, 2007*a*). In pollination- or auxin-induced fruit, the locular cavities are filled with jelly, while in GA_3-induced fruit or fruit induced by the application of a very high dosage of auxin, the locular tissue barely developed (Asahira *et al.*, 1968; Serrani *et al.*, 2007*a*). Normally, the jelly develops from the placenta cells, the volume of which increases during fruit development and engulfs the seeds. In contrast, in gibberellin-induced fruit, the ovules degenerate and the jelly does not develop well. In auxin-induced fruit, the ovules do not degenerate, but form pseudoembryos, which are seed-like structures that originate from cell divisions of the inner integument (Asahira *et al.*, 1967; Kataoka *et al.*, 2003; Serrani *et al.*, 2007*a*). So far, their relationship to jelly development and parthenocarpic fruit growth is poorly understood. It has been hypothesized that these pseudoembryos produce hormones and can stimulate fruit growth in a way comparable to seeds (Kataoka *et al.*, 2003).

Auxin and gibberellin at the transcriptome level

Tomato fruit set and early fruit development have also been studied at the transcriptome level. Lemaire-Chamley *et al.* (2005) performed a comparative analysis between developing fruit and other plant organs, and showed that most genes active in the fruit are not exclusively expressed there, underlining the ontogenic relationship between fruit and other tissues (Gillaspy *et al.*, 1993). Apparently, tomato fruit development depends on the regulation of gene activity both in time and intensity (Lemaire-Chamley *et al.*, 2005).

Vriezen *et al.* (2008) compared the transcriptomes from pollinated ovaries and gibberellin-treated ovaries, collected only 3 d after pollination or treatment. The two treatments enabled differentiation between genes induced by pollination and fertilization, and genes involved in fruit growth. As could be expected, pollination triggered genes that were not all triggered after the application of GA_3 and vice versa. Several genes involved in the cell cycle were more rapidly induced by gibberellin application than by pollination. Possibly, this difference can be explained by the time that pollen tubes require in order to reach the ovules, what would suggest that the induction of fruit growth does not take place prior to fertilization (Vriezen *et al.*, 2008), and that the growth substances, which are present in the pollen (Mapelli *et al.*, 1978), are only released after the pollen tubes have reached the ovules and rupture to deliver the nuclei to the embryo sac. Nevertheless, the expression of certain genes, which might be involved in tomato fruit set, was found to be down-regulated after pollination, but before fertilization took place (Olimpieri *et al.*, 2007). These findings suggest that there might be pollen-derived long-range signals as well, which might be necessary to prepare the ovary for fertilization and the subsequent fruit set (O'Neill, 1997).

Pollination appeared to have significant effects on the expression of the auxin signalling genes, such as *Aux/IAA*s or *AUXIN RESPONSE FACTOR*s (*ARF*s) (Kim *et al.*, 1997; Guilfoyle *et al.*, 1998), while these genes were hardly influenced by the gibberellin treatment (Vriezen *et al.*, 2008). In contrast to one of the first models of tomato fruit set, in which gibberellins may induce an increase in the auxin content within the ovary (Sastry and Muir, 1963), these gene expression data indicate that it is auxin that may act prior to gibberellin in the onset of tomato fruit development. Furthermore, the comparative analysis of Vriezen *et al.* (2008) showed that the mRNA levels of several ethylene biosynthesis genes and genes involved in ethylene signalling decreased after pollination. At the same time, transcript levels of abscisic acid (ABA) biosynthesis

genes seemed to decrease and the expression of genes involved in ABA degradation increases (Nitsch *et al.*, 2009). These findings imply that the onset of fruit development depends on the induction of gibberellin and auxin responses, while ethylene and ABA responses are attenuated.

Auxin and gibberellin signalling components

One of the first described auxin signalling components that might be involved in tomato fruit set is *DIAGEOTROPICA* (*DGT*). In tomato, *dgt* mutants, fruit set, fruit weight, number of seeds and locules per fruit were strongly affected (Balbi and Lomax, 2003). These mutants exhibit reduced auxin sensitivity (Kelly and Bradford, 1986; Mito and Bennett, 1995). Therefore, the diminished fruit set might be an effect of reduced auxin responsiveness of the ovary. The *DGT* encodes for a cyclophyllin, a peptidyl-propyl isomerase that catalyses *cis-trans* isomerization of proline residues in peptides. The exact role of DGT in auxin signal transduction is still unknown (Oh *et al.*, 2006). Interestingly, the *dgt* mutation affects a different subset of the auxin responsive *Aux/IAA* genes, depending on the tissue. This suggests that DGT has a differentiated function in the regulation of the early auxin signal in different tissues (Nebenführ *et al.*, 2000; Balbi and Lomax, 2003).

The mechanism of action in auxin signal transduction of the two recently identified *AUCSIA* genes is also unknown, but reduction of *AUCSIA* transcript levels by an RNA interference approach led to a pleiotropic phenotype that could be related to auxin, such as alterations in leaf development and reduced polar auxin transport in the roots. Interestingly, the *AUCSIA*-silenced plants formed parthenocarpic fruit when flowers were emasculated. The total IAA content in these flower buds was 100 times higher than in the wild-type, which seems likely to be the cause of the parthenocarpic fruit growth. However, it is unknown whether the auxin accumulation is the consequence of an increased auxin synthesis, decreased auxin degradation, or altered auxin transport (Molesini *et al.*, 2009).

Another component involved in tomato fruit set is *IAA9*, a member of the tomato *Aux/IAA* gene family of transcriptional regulators that are involved in many aspects of plant responses to auxin (Kim *et al.*, 1997; Ulmasov *et al.*, 1997). The reduction of *IAA9* transcript levels in tomato plants resulted in a pleiotropic phenotype. The transgenic lines formed simple leaves instead of wild-type compound leaves, and fruit development was initiated prior to pollination and fertilization. These phenotypes together with auxin dose–response assays showed that down-regulation of *IAA9* leads to auxin hypersensitivity, suggesting that IAA9 acts as a transcriptional repressor of auxin signalling (Wang *et al.*, 2005). *Aux/IAA* genes encode short-lived nuclear proteins, which can dimerize with ARFs while these are bound to the auxin response elements in the promoters of early auxin response genes (Ulmasov *et al.*, 1997, 1999). The ARF-Aux/IAA heterodimers restrain the transcription of the early auxin response genes, thereby inhibiting the auxin response (Ulmasov *et al.*, 1997; Tiwari *et al.*, 2001). Current models suggest that auxin promotes Aux/IAA protein ubiquitination through the SCF^{TIR1} complex (Gray *et al.*, 2001). As a result, the Aux/IAA protein is degraded by the 26S proteasome and the ARF is released from the repressive effect of the Aux/IAA protein, leading to the activation of the auxin response genes (Dharmasiri *et al.*, 2005; Kepinski and Leyser, 2005). In turn, some of these auxin response genes encode for Aux/IAAs, such as *IAA2* and *IAA14*, the transcription of which, like *IAA9*, is induced by auxin treatment of the unpollinated ovary (Serrani *et al.*, 2008). The mRNAs levels of *IAA2* and *IAA14* were also found to increase in the pollinated ovary, specifically in the placenta and ovular tissues (Vriezen *et al.*, 2008). It seems likely that, in the presence of auxin, either after pollination or auxin application, *de novo* synthesized Aux/IAA proteins are rapidly degraded due to SCF^{TIR1}-mediated ubiquitination. However, despite their rapid turnover, the transcriptional activation of these Aux/IAAs suggests that a minimum level of Aux/IAAs is required in order to create a negative feedback loop in the auxin signal transduction pathway, which enables the plant to fine-tune the strength of the auxin response (Gray *et al.*, 2001).

Recently, a new member of the tomato *ARF* gene family, *SlARF7*, the homologue of *Arabidopsis ARF7*, has been characterized. *SlARF7* mRNA levels are high in the placental tissues of the mature flower, and rapidly decrease after pollination. Decreasing these levels by using an RNAi approach resulted in parthenocarpic fruit development, suggesting that *Sl*ARF7 may act as a negative regulator of fruit set. The parthenocarpic fruits displayed characteristics typically related to high levels of auxin and gibberellin, which indicate that *Sl*ARF7 might be involved in the cross-talk between these two hormones (de Jong *et al.*, 2009). In Arabidopsis siliques, *ARF8/FRUIT WITHOUT FERTILIZATION* (*FWF*) shows a similar expression pattern as *SlARF7* (Goetz *et al.*, 2006), and the mutated *fwf* allele triggers the formation of parthenocarpic siliques (Vivian-Smith *et al.*, 2001). These similarities suggest that *Sl*ARF7 is the functional equivalent of *At*ARF8/FWF. Interestingly, the *fwf* allele contains a mutation in the putative translation initiation codon, but is still transcribed and probably also translated (Goetz *et al.*, 2006), resulting in a truncated protein, which is missing at least part of its DNA binding domain. However, the exact nature of the mutant protein is still unclear (Goetz *et al.*, 2007). Introduction of the *fwf* allele in wild-type plants also induced the formation of parthenocarpic siliques, even though endogenous *At*ARF8 transcript levels were not reduced (Goetz *et al.*, 2007). These findings suggest that the aberrant form of *At*ARF8 may compete with the endogenous *At*ARF8 protein in the formation of protein complexes. Introduction of the *Arabidopsis fwf* mutant allele in tomato also results in parthenocarpic fruit set, indicating that not only *Sl*ARF7, but also the tomato homologue of *At*ARF8/FWF, *Sl*ARF8, plays a role in regulating tomato fruit set (Goetz *et al.*, 2007). This hypothesis is supported by the findings of Gorguet

et al. (2008), who identified *SlARF8* as a candidate gene for two parthenocarpy QTLs. However, instead of being down-regulated after auxin-treatment or pollination, *SlARF8* transcript levels were found to increase after auxin treatment (Serrani *et al.*, 2008), suggesting that although *Sl*ARF8 might have a function in tomato fruit set, it probably functions in a different manner than *Sl*ARF7 or *At*ARF8. The introduction of the aberrant form of *At*ARF8 in tomato may interfere with the protein complex formation of *Sl*ARF7 with other factors that might be involved in tomato fruit set, resulting in parthenocarpic fruit growth. So far, two different models have been postulated that might explain the function of *Sl*ARF7 and *At*ARF8 (Fig. 2). The first model is based on the findings of Goetz *et al.* (2007), who suggests that the ARF8 forms an inhibitory complex together with an Aux/IAA protein, possibly IAA9, repressing the transcription of the auxin response genes and fruit developmental genes. Alternatively, the inhibitory complex may act indirectly by preventing the ARF8 binding to the promoter of the auxin response genes. After pollination and fertilization, auxin causes the degradation of the Aux/IAA, so that the ARF8 together with other signals and activators can stimulate the expression of early auxin response genes, initiating fruit growth. However, the down-regulation of the *AtARF8* mRNA levels after pollination cannot be explained based on this model, unless the activation of the auxin response genes is no longer required once fruit set is initiated. Alternatively, other transcription factors, potentially other ARFs, can take over in stimulating fruit growth. The second model, as put forward by De Jong *et al.* (2009), suggests that in the unpollinated ovary, *Sl*ARF7 acts as a transcriptional activator of auxin response attenuating genes, repressing the auxin and gibberellin signalling pathways that are necessary to initiate tomato fruit development. This model accounts for the down-regulation of the *SlARF7* transcript level after pollination, when repression of fruit development is no longer required. However, mRNA levels are not necessarily in accordance with the protein activity.

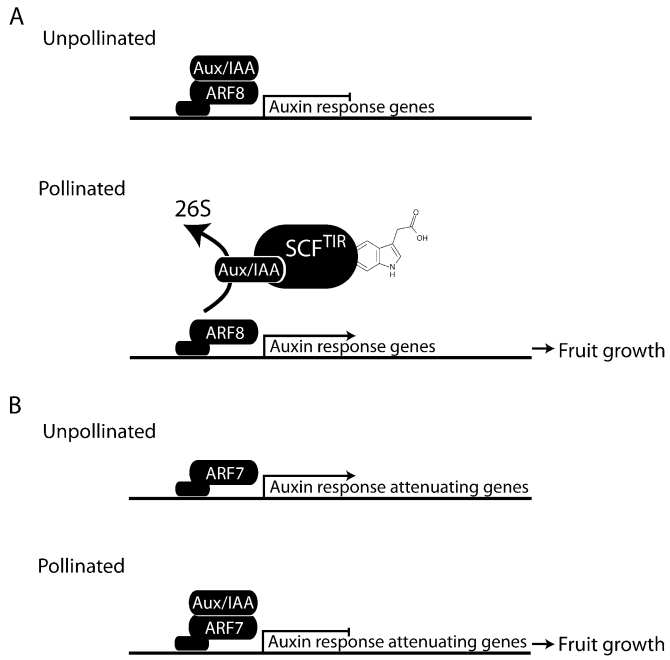

Fig. 2. The two alternative models that represent the putative functions of *At*ARF8 and *Sl*ARF7 in fruit set. (A) The first is a representation of the model that is currently accepted as the way ARFs and Aux/IAA proteins regulate auxin response genes. Before pollination, ARF8 activity is inhibited in a protein complex with Aux/IAAs, resulting in the repression of auxin response genes and fruit developmental genes. Upon pollination, the level of auxin increases and the Aux/IAAs are rapidly ubiquitinated and degraded by the 26S proteasome. This activates ARF8 resulting in the transcription of the auxin response genes and fruit developmental genes. (B) In the second model ARF7 activates auxin response attenuating genes that encode proteins that might repress the auxin response and thus prevent fruit set. After pollination, the inhibitory role of ARF7 is no longer required, the *ARF7* transcript levels decrease and the activity of the ARF7 proteins that are still present might be inhibited by auxin-induced Aux/IAAs, such as IAA9.

The only known gibberellin signalling component that has been shown to be involved in tomato fruit set is *Sl*DELLA (Martí *et al.*, 2007). DELLA proteins restrict cell expansion and proliferation by repressing the gibberellin-response gene activity. GA_3 stimulates the ubiquitination of DELLA proteins and subsequent 26S proteasome-mediated degradation (Dill *et al.*, 2001). Reduction of *SlDELLA* mRNA levels induced the formation of parthenocarpic tomato fruit (Martí *et al.*, 2007). This fruit was smaller and had a more elongated shape than wild-type fruit. The pericarp contained fewer but bigger cells than wild type, which is similar to gibberellin-induced parthenocarpic fruit (Bünger-Kibler and Bangerth, 1983). Thus the reduced *SlDELLA* mRNA levels in the antisense lines may allow the release of repression of downstream proliferating factors involved in the gibberellin signalling pathway, which are normally induced after successful pollination and fertilization (Martí *et al.*, 2007). Thus *Sl*DELLA also appears to be a negative regulator of fruit set by restraining the gibberellin signal and thereby preventing ovary growth prior to pollination and fertilization.

Altogether, these findings show that, although fruit set depends on the positive growth stimuli generated by pollination and fertilization, fruit growth is actively repressed by negative acting factors in the mature ovary. However, some of these factors might not be derived from the ovary itself. Transgenic lines in which *TM29*, a tomato MADS-box gene, is down-regulated also produce parthenocarpic fruit. In addition, the flowers of these transgenic lines have an aberrant flower morphology, as the petals and stamens are partially converted to a sepaloid identity (Ampomah-Dwamena *et al.*, 2002). In flowers of the tomato *parthenocarpic fruit* (*pat*) mutant, the anthers have also lost their organ identity as they are partially transformed into carpel-like structures. This observation led to the hypothesis that the parthenocarpy of the mutant is the secondary effect of a mutation in a gene controlling organ identity and

development (Mazzucato *et al.*, 1998). However, the *pat* mutant is not mutated in the B class MADS box genes (Mazzucato *et al.*, 2008), the class of homeotic genes that specify stamen identity together with class C genes (Coen and Meyerowitz, 1991; Weigel and Meyerowitz, 1994). Nevertheless, fruit set may depend on negative factors derived from inter-organ communication between anthers and ovary (Vivian-Smith *et al.*, 2001), and therefore the right definition of floral organ identity is required.

Most of the auxin and gibberellin signalling components identified, that may have a regulatory role in tomato fruit set, seem to be negative regulators. Apparently, the development of the ovary into a fruit prior to pollination and fertilization is actively arrested in order to prevent parthenocarpic fruit growth, which would be a waste of energy to the plant.

Gibberellin biosynthesis

In higher plants, GA is metabolized through several stages. The early stage, common to all systems that have been studied, is the conversion of *trans*-geranylgeranyl diphosphate (GGPP) to GA_{12}-aldehyde (Hedden and Phillips, 2000). The later stage consists of two parallel pathways. In the non-13-hydroxylation pathway, GA_{12} is converted into GA_9, while in the 13-hydroxylation pathway GA_{12} in converted to GA_{20} by GA 20-oxidases (GA20oxs). Subsequently, GA 3-oxidases (GA3oxs) hydroxylate GA_9 and GA_{20} to the bioactive GA_4 and GA_1. Alternatively, GA_9 and GA_{20} can be converted to GA_7 and GA_3 (Hedden and Phillips, 2000). The young tomato flower bud contains metabolites of both the non-13-hydroxylation and 13-hydroxylation pathways, which levels decrease progressively during ovary development (Fos *et al.*, 2000). After pollination, the total gibberellin content within the ovary increases again, although low levels of the metabolites of the GA_4 biosynthetic pathway are detected (Bohner *et al.*, 1988; Koshioka *et al.*, 1994; Serrani *et al.*, 2007*b*). These findings suggest that the 13-hydroxylation pathway is mostly used in the growing ovary (Fos *et al.*, 2000). The hydroxylation of GA_{20} is likely to be the limiting step in this pathway, since 7–15 d after pollination the levels of GA_{20} were found to be much higher than those of GA_1 (Bohner *et al.*, 1988; Koshioka *et al.*, 1994). In accordance, the transcript levels of two out of the three tomato *GA 20-oxidase* genes, *SlGA20ox1* and *-2*, rapidly increase after pollination, while the transcript levels of the *GA 3-oxidase* genes decrease after anthesis to a similar level in both unpollinated and pollinated ovaries (Rebers *et al.*, 1999; Olimpieri *et al.*, 2007; Serrani *et al.*, 2007*b*). The increase of the gibberellin content after pollination could also result from diminished bioactive gibberellin deactivation by GA 2-oxidases (GA2oxs). In tomato, five *GA2ox* genes have been characterized and they are all expressed in the unpollinated ovary. However, 5 d after pollination their transcript levels are similar to those in unpollinated ovaries, suggesting that, during early fruit development, the increase of gibberellin biosynthesis is mainly caused by the up-regulation of *SlGA20ox1* and *-2* expression, and not by a reduction of gibberellin deactivation (Serrani *et al.*, 2007*b*). However, RNAi lines with reduced transcript levels for *SlGA20ox1*, *-2* or *-3* were still able to set fruit, despite the fact that the gibberellin content in the *SlGA20ox1* and *SlGA20ox2* transgenic lines was significantly reduced as compared to that in the wild type (Xiao *et al.*, 2006). Nevertheless, it is possible that the GA 20-oxidases, especially *Sl*GA20ox1 and -2, act redundantly in the fruit developmental programme, and need to be silenced altogether to have an effect on fruit set.

The natural parthenocarpic tomato mutants *pat* and *pat-2* accumulate high levels of GA_{20} in the unpollinated ovaries (Fos *et al.*, 2000; Olimpieri *et al.*, 2007). Probably, this accumulation leads to the synthesis of bioactive gibberellin, resulting in parthenocarpic fruit growth independent of pollination and fertilization. Accordingly, *SlGA20ox1* was found to be expressed at high levels throughout ovary development and fruit growth in the *pat* mutant (Olimpieri *et al.*, 2007). In the wild type, the transcript level of a negative regulator of the gibberellin response, *SPINDLY* (*SPY*), increases at anthesis and decreases again after pollination. By contrast, in the *pat* mutant this increase did not occur (Olimpieri *et al.*, 2007). The *KNOTTED*-like homeobox (*KNOX*) genes, which might be direct repressors of *GA20ox* expression (Hay *et al.*, 2002), are also highly expressed at anthesis in wild-type plants and transcript levels decrease after pollination. In the *pat* mutant, *KNOX* transcript levels already decrease prior to anthesis (Olimpieri *et al.*, 2007). These findings indicate that *SPY* and members of the *KNOX*-gene family might also act as negative regulators of fruit growth, directly repressing gibberellin signalling and biosynthesis, respectively, in unpollinated ovaries. The parthenocarpic phenotype of the *pat-3*/*pat-4* tomato mutant also depends on gibberellin, but in contrast to the *pat* and *pat-2* mutants, the entire 13-hydroxylation pathway is enhanced, resulting in a high content of GA_1 and GA_3 in the ovary before pollination (Fos *et al.*, 2001). Hence, the *pat, pat-2*, and *pat-3*/*pat-4* gene products seem to interact with different parts of the GA metabolic pathway. However, the nature of these genes and their gene products is still unknown.

Parthenocarpic tomato fruit induced by the synthetic auxin 2,4-D also contains high levels of GA_1 and its precursors, similar to levels in pollinated ovaries. In accordance, expression levels of *GA20oxs* and *SlGA3ox1* were found to be high in the parthenocarpic ovaries as compared to levels in the unpollinated ovaries, whereas transcript levels of *SlGA2ox2* were low (Serrani *et al.*, 2008). Moreover, the induction of parthenocarpic fruit growth by auxin is negated by GA biosynthesis inhibitors (Serrani *et al.*, 2008). These findings indicate that auxin induces fruit set by enhancing gibberellin biosynthesis and diminishing gibberellin inactivation, suggesting that auxin acts prior to gibberellin as the early post-pollination/fertilization signal. However, the conversion of GA_{53} to $GA_{44/19}$ metabolites in the 13-hydroxylation pathway is not induced by auxin application, while it is induced after pollination (Serrani

et al., 2008). Furthermore, the transcript levels of *SlGA3ox1* and *SlGA2ox2* change differently in response to auxin treatment or pollination (Serrani *et al.*, 2008). These differences imply that, although auxin may act as one of the first signals that trigger the fruit developmental programme, the signal transduction pathways induced by pollination and fertilization do not form a single linear cascade via auxin to gibberellin. It is likely that the growth-stimulating signal is partially transduced independently of auxin to stimulate gibberellin biosynthesis (Fig. 3).

Source of auxin

The hypothesis that pollination and fertilization induce fruit growth partially independently of auxin is also supported by the differences in fruit growth between pollinated and auxin-induced fruit, as described earlier. Alternatively, the endogenous hormone levels might be differently affected after pollination or auxin application since both situations have a different source of auxin. So far, it is unclear where the first auxin is produced after pollination, or whether it is transported to other tissues of the ovary.

It is well established that higher plants, such as tomato, use both tryptophan (Trp)-dependent and Trp-independent pathways to synthesize IAA. The IAA biosynthesis via Trp has been studied for a long time, but most genes of the enzymes involved in the Trp-independent route are yet to be identified (Woodward and Bartel, 2005). The auxin biosynthesis pathways are non-redundant, each pathway acts in a tissue-specific and developmental stage-specific manner (Woodward and Bartel, 2005; Zhao, 2008). Tomato plants, homozygous for the *sulfurea* mutation, suffer from auxin deficiency, which is probably due to defects in the Trp-independent pathway. Auxin deficiency was largely confined to the shoot meristems, but also frequently resulted in abnormalities in floral morphology, including missing floral organs, fused organs, and homeotically transformed organs (Ehlert *et al.*, 2008). However, fruit development in the *sulfurea* mutant was unaffected, indicating that auxin synthesis in developing fruits occurs via the Trp-dependent pathway. These findings correspond to the results of Epstein *et al.* (2002), that showed that there is a switch from the Trp-dependent to the Trp-independent auxin production, occurring between the mature green and the red-ripe stages of tomato fruit development.

During fruit development, two peaks of auxin are observed. The first peak reaches its maximum 8 d after pollination at the end of active cell division, and the second peak reaches its maximum at 30 d. The latter was not found in parthenocarpic fruit (Mapelli *et al.*, 1978), suggesting that, at least during the later stages of fruit development, the embryo supplies the auxin necessary for continued fruit growth. The observations that parthenocarpic fruit are generally smaller than wild-type fruit (Mapelli *et al.*, 1978; Sjut and Bangerth, 1983) and that there is a positive correlation between final fruit size and number of seed in the fruit (Varga and Bruinsma, 1976) support this hypothesis.

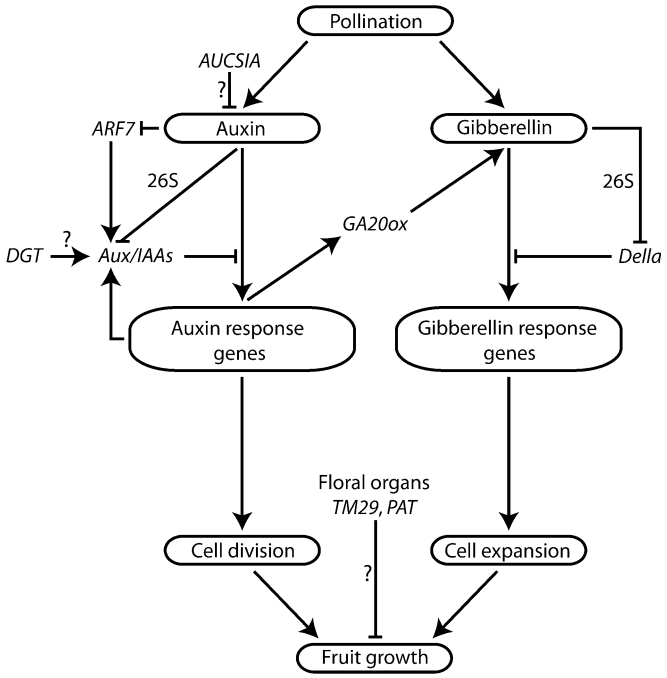

Fig. 3. A model integrating the role of auxin and gibberellin in tomato fruit set. The levels of both hormones increase after pollination, resulting in the activation of auxin and gibberellin response genes, which, in turn, will trigger fruit growth by regulating cell division and cell expansion. The auxin response is tightly regulated in a complex network, although the functions of some of the components in this network, such as AUCSIA and DIAGEOTROPICA (DGT) are not yet clear. Before pollination, the auxin response is inhibited by ARF7 and Aux/IAAs, but upon pollination, these negative regulators are inhibited, or degraded by the 26S proteasome and the auxin response genes are transcribed. Some of these auxin response genes are Aux/IAAs, which create a negative feedback on the auxin signalling response. By contrast, the gibberellin signal pathway is subjected to positive feedback, as gibberellin stimulates the degradation of DELLA, a repressor of gibberellin signalling, through the 26S proteasome. However, gibberellin does not regulate fruit growth independently of auxin, since auxin seems to be able to stimulate gibberellin biosynthesis through the transcriptional activation of *GA 20-oxidases* (*GA20oxs*). Moreover, other factors such as TM29 and PAT, which might be derived from anthers, petals or sepals, also seem to have an important regulatory role in fruit set. The regulatory roles of other hormones, like ethylene, abscisic acid, and cytokinin are not included in this model, but these factors also contribute to the regulatory network that is required for the tight co-ordination, both temporarily and spatially, of fruit growth.

Lemaire-Chamley *et al.* (2005) showed that candidate key genes for auxin biosynthesis, transport, signalling, and responses were already expressed in the locular tissue during the early stages of fruit growth. More detailed analysis of genes differentially expressed between the locular tissue and the outer pericarp, revealed that the expressions of these genes follow a gradient from the central part of the fruit (placenta and locular tissue) to the outer part of the fruit (Lemaire-Chamley *et al.*, 2005). It is possible that, in

response to pollination and fertilization, the auxin is newly synthesized or hydrolysed from its conjugates in the central parts of the tomato fruit, the developing ovule and/or its surrounding tissues, respectively, and subsequently transported to the outer layers. This transport leads to the formation of the auxin gradient in the fruit tissues, which is translated into auxin responses, such as cell division, cell expansion, and into cross-talk with other hormones, such as newly synthesized gibberellins (Lemaire-Chamley *et al.*, 2005).

Conclusions

Auxin and gibberellin are general growth factors involved in many developmental processes throughout the plant. Nevertheless, they play a major role in the onset of fruit development, which is a very specific process. Most of the putative regulators of tomato fruit set that have been identified so far are common signalling components of these hormones, and reduction of their expression often results in pleiotropic effects in plants, including parthenocarpic fruit growth. In order to make a general developmental process, such as growth, subordinate to pollination and fertilization, as in the case of fruit set, a tight network is required to regulate the expression and function of these common signalling components. Figure 3 shows a possible model for such a network of signals that, after pollination, converts an ovary into a fruit. This network includes positive and negative feedback loops in the signal transduction pathways of auxin and gibberellin. Moreover, it comprises cross-talks between growth regulators, in which auxin can promote the biosynthesis of gibberellin, but other growth regulators, such as cytokinin, ethylene, and abscisic acid may also play a role. These hormones together ultimately control the expression of the genes that are actually involved in fruit development, a network which is slowly being unravelled. It is remarkable that several basic questions that seem very obvious have not been answered using the techniques of the last decade. For example, where and when are the first auxin and gibberellin produced after pollination, and are they transported to other tissues of the ovary? The first questions could be answered using the promoters of known tomato auxin response genes and gibberellin biosynthesis genes, to drive marker genes. Likewise, auxin transport can be studied using the tomato orthologues of the auxin efflux carriers, known as PIN proteins in *Arabidopsis*. When the full genome sequence of tomato becomes available in 2009, hopefully, new genes that function in the fruit initiation and developmental pathways of tomato and related species will be identified, and so this complex network will be better understood.

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