Fruit growth-related genes in tomato

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Abstract

Tomato (Solanum lycopersicum Mill.) represents a model species for all fleshy fruits due to its biological cycle and the availability of numerous genetic and molecular resources. Its importance in human nutrition has made it one of the most valuable worldwide commodities. Tomato fruit size results from the combination of cell number and cell size, which are determined by both cell division and expansion. As fruit growth is mainly driven by cell expansion, cells from the (fleshy) pericarp tissue become highly polyploid according to the endoreduplication process, reaching a DNA content rarely encountered in other plant species (between 2C and 512C). Both cell division and cell expansion are under the control of complex interactions between hormone signalling and carbon partitioning, which establish crucial determinants of the quality of ripe fruit, such as the final size, weight, and shape, and organoleptic and nutritional traits. This review describes the genes known to contribute to fruit growth in tomato.

Key words: Cell cycle, cell division, cell expansion, development, endoreduplication, fruit, genetic control, growth, metabolic control, hormones, tomato.

Introduction

The fruit is a plant organ specific to angiosperms that protects the ovule and seed during embryo development and ensures seed dispersal after maturation. At the botanical level, most fruits develop from mature ovaries and, therefore, include carpellar tissues. The physiological function of seed dispersal accounts for a significant part of the adaptive success of angiosperms on Earth. Indeed, a wide diversity of fruit size, form, and composition, as well as a variety of fruit dispersion mechanisms, have emerged as the result of strong environmental selective pressures. For example, the Solanaceae family, which encompasses nearly 10 000 species, has very diverse types of fruits, with capsules, drupes, pyrenes, berries, and several types of dehiscent non-capsular fruits occurring in >90 genera (Knapp, 2002).

In general, the term fruit refers to the fleshy, edible portion that is the major component of fruits, such as grape, banana, apple, citrus, peach, strawberry, melon, and tomato. All of these species are produced worldwide, and continue to rely on breeding schemes for agronomic traits. Particular traits of interest are enhanced yield and quality, increased time of fruit storage and longer shelf-life, optimized cultural practices, and pest and pathogen resistance. The common feature of fleshy fruits is the fine balance between sugars and organic acids, and the accumulation of water, minerals, pigments, aromatic compounds, and vitamins that confer upon the fruit its juiciness and attractiveness. Consequently, fleshy fruits represent a major source of vitamins, fibre, carbohydrates, and phytonutrient compounds essential for human nutrition.
Tomato (Solanum lycopersicum Mill.) fruit, a multicellular berry, has arisen as the model species for fleshy fruits, due to certain advantages for use in both agronomical and fundamental research (Gillaspy et al., 1993; Tanksley, 2004; Klee and Giovannoni, 2011). These advantages include a short life cycle, high multiplication rate, self-pollination, and ease of mechanical crossing. This highly favourable biology has been widely exploited to generate segregating populations such as recombinant inbred lines (RILs) or near isogenic lines (NILs). Tomato has proven amenable for marker-assisted mapping using crosses between small and round, wild tomatoes and domesticated varieties of various sizes and shapes that has enriched our knowledge of the genetic control of fruit development (Grandillo et al., 1999; Causse et al., 2007; Muñoz et al., 2011; Rodriguez et al., 2011). A large spectrum of mutants is now available (Menda et al., 2004; Watanabe et al., 2007; Just et al., 2013) for screening and gene identification by reverse genetic tools, such as the powerful technology of Tilling (Targeting Induced Local Lesions In Genomes; Okabe et al., 2011). Functional analyses of candidate genes are routinely conducted, since tomato is highly suitable to stable transformation via Agrobacterium tumefaciens, and susceptible to transient gene expression via agroinjection (Orzaez et al., 2006). Additionally, the repression of target genes can be obtained by virus-induced gene silencing (VIGS) (Orzaez et al., 2009) or by a genome-editing strategy using the RNA-guided DNA endonuclease system called the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated9 (Cas9) system (Brooks et al., 2014). Finally, the release in 2012 of the full tomato genome sequence represented an extraordinary breakthrough for developmental studies in fleshy fruits (Tomato Genome Consortium, 2012).

The development of plant organs requires mechanisms of differentiation by which tissue identity is imposed on cells as well as mechanisms regulating growth that dictate final organ size. The determination of organ size thus relies on the fine regulation of the number and size of cells that is determined by the cell division and cell expansion processes, respectively. These processes respond to spatial and temporal controls that come under the influence of internal (genotypic) and external (environmental) cues. As a general feature of angiosperms, cell expansion is frequently ascribed to nuclear endopolyploidization in several plant organs, according to the process of endoreduplication (Chevalier et al., 2011, 2014; De Veylder et al., 2011).

This review addresses the current knowledge on fruit development in tomato, in particular describing those genes that are involved in the developmental processes governing fruit growth.

Tomato fruit development and the contribution of endopolyploidization to fruit growth

Fruits typically develop from pre-existing organs, such as carpels inside flowers. In tomato, carpels are formed by ~17–20 rounds of cell divisions that occur during a pre-anthesis period located within the L3 layer of the floral meristem, but which involve virtually no cell expansion (Coome, 1976; Ho, 1992). Obviously, the number of cells formed in the ovary before anthesis is critical for the final size of fruit, and such a positive correlation is frequently observed (Tanksley, 2004). From flower initiation to the double fertilization occurring in ovules (Gillaspy et al., 1993; Brukhin et al., 2003), the morphogenesis and growth of carpels and ovules require the spatial and temporal synthesis and action of auxin, cytokinin, and gibberellins (GAs). In order to keep the ovary in a temporally protected and dormant state, abscisic acid (ABA) and ethylene work to stop growth within the ovary shortly before anthesis when the ovary has reached its mature size (Vriezen et al., 2008). Growth resumes only after successful pollination and fertilization of ovules triggers fruit set—the first phase of fruit development—through the action of ovary-synthesized auxins and GAs (Ruan et al., 2012, and references therein).

Fruit growth is the longest phase of fruit development, as it ranges from 5 to 8 weeks depending on the genotype. Growth proceeds first by a period of intense mitotic activity according to a spatially and temporally organized pattern of cell division. Active cell division within the pericarp is usually restricted to an initial period of 1–2 weeks after fruit set (Fig. 1). Remarkably, cell division occurs within discrete cell layers with well-defined planes of division fulfilling specific purposes. For instance, the two subepidermal layers of the pericarp undergo several rounds of periclinal divisions, thus leading to an increase in the number of pericarp cell layers, whereas the two epidermal cell layers undergo anticlinal divisions in response to the resulting increase in fruit volume (Cheniclet et al., 2005). These various types of cell divisions are differently regulated, because cell divisions promoting cell layer formation occur only within 5–8 days post-anthesis (dpa), whereas randomly oriented cell divisions occur for longer periods up to 10–18 dpa (Cheniclet et al., 2005). Cell divisions in growing fruit account for as much as 80–97% of newly formed cells present after anthesis. How this spatio-temporal pattern of development is related to gene expression, metabolic profiles, and cellular characteristics remains poorly understood (Lemaire-Chamley et al., 2005; Bourdon et al., 2011, 2012), primarily due to the complex nature of fruit in relation to differing cell type.

It has been reported that a second phase of growth related to cell expansion occurs separately after the cell division phase (Gillaspy et al., 1993). In fact, cell expansion starts within very few days after fruit set, concomitantly with cell division (Cheniclet et al., 2005), and lasts for the entire period of fruit growth (Fig. 1). At the end of the cell expansion phase, individual cells in the fleshy part (mesocarp tissue) of the fruit have reached spectacular volumes, exhibiting a >30 000-fold increase from initial cell volume, and can correspond to a >0.5 mm increase in diameter (Cheniclet et al., 2005). This cell enlargement mostly occurs through dramatic increases in the vacuolar compartment and cell vacuolation index. Remarkably, this spectacular cell hypertrophy is closely correlated to an increase in nuclear DNA levels due to endopolyploidization.
Endopolyploidy is the occurrence of different ploidy levels within an organism. In plants, it results mostly from endoreplication that is estimated to occur in >90% of angiosperms (Nagl, 1976; D'Amato, 1984). Endoreduplication occurs in the absence of any obvious DNA condensation and decondensation steps that lead to the production of chromosomes with 2n chromatids or without any change in chromosome number (Joubès and Chevalier, 2000; Edgar and Orr-Weaver, 2001). As a consequence, hypertrophic nuclei arise from successive cycles of DNA replication without segregation of sister chromatids, thereby resulting in the production of polytene chromosomes (Bourdon et al., 2012). The physiological and developmental importance of endoreduplication is still a matter of debate. However, the frequent observation that cell size and ploidy levels are highly and positively correlated in many different plant species, organs, and cell types (Chevalier et al., 2011) suggests a role for endoreduplication in the control of cell size, according to the ‘karyoplasmic ratio theory’. This theory, which was formulated as early as the beginning of the 20th century, states that there is a causal relationship between nuclear and cytoplasmic growth to maintain a constant ratio of nucleus to cell volume (Sugimoto-Shirasu and Roberts, 2003; Chevalier et al., 2014).

In the course of tomato fruit development, endoreduplication produces high levels of nuclear DNA ploidy within the mesocarp and the locular jelly-like tissue (Bergervoet et al., 1996; Joubès et al., 1999; Cheniclet et al., 2005; Bertin et al., 2007). Ploidy levels as high as 512C (where C is the haploid DNA content) have been observed to occur in a large array of tomato genotypes. This extraordinary extent of endoreduplication occurring in tomato fruit is unmatched by any other plant species and makes tomato an excellent model to study the role of endoreduplication as a determinant of fruit size. Cheniclet et al. (2005) demonstrated that the mean ploidy level achieved in pericarp cells correlates not only with the large variation in the fruit weight that can be encountered in the various genotypes, but also with the mean cell size inside the pericarp. The endoreduplication-associated cell expansion in fruit is characterized by profound cellular modifications. It is evident that the formation of polytene chromosomes impacts on nuclear, nucleolar, and chromatin organization within hypertrophied nuclei delimited by a highly invaginated nuclear envelope (Bourdon et al., 2012). These profound nuclear grooves are filled with numerous mitochondria, whose number increases according to nuclear DNA content. Apparently, endoreduplication triggers efficient communication between the nucleus and the cytoplasm, despite the increase in nuclear volume. The cytoplasmic volume of cells also correlates with ploidy levels, which indicates that the maintenance of the nuclear to cytoplasmic ratio to drive cell growth is consistent with the ‘karyoplasmic ratio’ theory. In addition, Bourdon et al. (2012) provided evidence...
for the existence of a strong relationship between endoreduplication and rRNA and mRNA transcription.

As a morphogenetic factor, endoreduplication thus contributes to maintain homeostasis of cytoplasmic components through the establishment of a highly structured cellular system, where multiple physiological functions are integrated to support cell growth during fruit development.

**Modifying fruit growth with cell cycle and endocycle regulatory genes**

Cell division and cell expansion associated with endoreduplication provide, respectively, the building blocks for the fruit organ and the growth-driving force that contribute to establish the final fruit weight/size. Therefore, modifying fruit growth by targeting genes involved in mitosis and endoreduplication has been considered for tomato (Fig. 2).

In Eukaryotes, the cell cycle is composed of four distinct phases: an undifferentiated DNA pre-synthesis phase with a 2C nuclear DNA content, termed the G1 phase; the S phase during which DNA is synthesized, with a nuclear DNA content intermediate between 2C and 4C; a second undifferentiated phase (a DNA post-synthetic phase) with a 4C nuclear DNA content, termed the G2 phase; and the M phase, or mitotic phase. Progression of the cell cycle is ensured by the activity of core regulators consisting of conserved heterodimeric protein complexes. These complexes are formed by a catalytic subunit referred to as cyclin-dependent kinase (CDK) and a regulatory cyclin (CYC) subunit. The presence of the regulatory CYC is essential for the CDK–CYC complex activity as it confers its stability, its localization, and its substrate specificity (Inzé and De Veylder, 2006). Specific CDK–CYC complexes operate at the boundaries between the various phases of the cell cycle in order to phosphorylate target proteins. These post-translational modifications which can be either inhibitory or activating, are essential for the progression through cell cycle boundaries (De Veylder et al., 2007). To allow a subtle regulation of cell cycle progression, the kinase activities of the CDK–CYC complexes are regulated in several ways: (i) CDK activity is finely tuned by phosphorylation and dephosphorylation of the CDK on conserved residues by specific kinases and phosphatases; (ii) the proteolytic destruction of the cyclin subunit by the ubiquitin–proteasome system (UPS) is sufficient to abolish activity of the complex, as CDK alone does not display kinase activity without its cyclin partner; and (iii) the CDK–CYC complexes are inactivated by the specific binding of CDK inhibitors (Churchmann et al., 2006; Torres Acosta et al., 2011).

The endoreduplication cycle (endocycle) consists of successive rounds of DNA synthesis (S phases) in the absence of mitosis, and thus represents a partial and modified cell cycle. Consequently, cell cycle and endocycle progression involves control of CDK–CYC activity levels by common regulatory mechanisms on the molecular level, such as those mentioned above (De Veylder et al., 2011). Central to this regulation is the maintenance of a certain threshold of CDK–CYC activity to allow the commitment to mitosis. When mitotic CDK complexes do not form or their activity is suppressed, this threshold is not exceeded and the level of CDK–CYC activity is insufficient to drive cells into mitosis; endoreduplication can then take place.

![Fig. 2. Genes involved in tomato fruit growth. Mapping of QTLs, cloning of the associated genes, in planta functional analyses, and characterization of mutants have identified genes that impact fruit growth both positively and negatively. These genes are reported according to the respective stages of fruit development in which they are involved: (A) ovary development; (B) fruit set; (C) phase of cell division; and (D) phase of cell expansion.](image-url)
In tomato, several functional analyses have attempted to modulate the CDK–CYC complex activity during fruit development, either through direct targeting of CDK gene expression or through post-translational regulation of the complex (Fig. 2C). Czerednik et al. (2012) generated transgenic plants aimed at down-regulating the canonical CDKA;1, the key player in cell cycle progression, in a fruit-specific manner using an artificial microRNA (amiCDKA) and the TPRP promoter (Fernandez et al., 2009). The amiCDKA plants produced smaller fruits than those of the wild type, and which had a thinner pericarp due to an overall decreased number of cell layers within the exocarp (displaying the smallest cells). In contrast, the mesocarp displayed normal, enlarged cells without any significant difference in ploidy levels. A similar phenotype was obtained with fruit-specific overexpression of either mitosis-associated CDKB1 or CDKB2 (Czerednik et al., 2012). Interestingly, the expression of CDKA;1 was greatly repressed in overexpressing CDKB1 and CDKB2 lines in accordance with the phenotype of the amiCDKA line. More recently, tomato fruits overexpressing CDKA;1 have been reported (Czerednik et al., 2014). CDKA1-overexpressing fruits were visually indistinguishable from those of the wild type, with similar fruit weights and diameters. However, they exhibited a larger placental area, a thicker pericarp, and a reduced number of seeds. Across the pericarp, the number of constituting cell layers was significantly increased, as well as the number of cells per mm², which was also observed in the placenta. Overall, the observations showed that mean cell size was smaller, and was accompanied by a decrease in endoreduplication levels in cells from the mesocarp, placenta, and jelly-like tissues. These data provided another example of the relationship between endoreduplication and final cell size, suggesting a pivotal role for CDKA1 in the regulation of mitotic activities during fruit development. Additionally, CDKA1 overexpression affected the production of seeds in developing fruits that indirectly affected pericarp cell expansion, which is normally driven by seed-produced phytohormones.

Altering CDKA or CDKB1/B2 gene expression in tomato fruits produced phenotypes that highlighted differential effects on cell division, cell expansion, and endoreduplication (Czerednik et al., 2012, 2014). In general, the observed data are difficult to interpret, most probably because they are associated with the specific tissular and developmental pattern of expression of the TPRP promoter, which was used to drive gene misexpression. The TPRP promoter is a fruit-specific promoter whose expression starts early in the ovary and reaches a maximum during the cell expansion phase of fruit development (Fernandez et al., 2009). As a consequence, CDKB1 and CDKB2 were overexpressed outside their natural timing of expression (Joubès et al., 1999, 2001), which may have greatly affected the availability of regulatory cyclins for proper composition of CDK–CYC complexes, and/or created an artificial competition for regulatory cyclins among the misexpressed CDKs. In the absence of measured CDK activity, we speculate that the phenotypes observed by down-regulating CDKA genes, or up-regulating CDKB genes are caused by the lack of endocycle-specific CDKA–CYC complex activity, which is necessary to permit the youngest cells of the outermost layers of pericarp (namely the exocarp) to enter into the phase of endoreduplication-driven cell expansion. In a similar manner, overexpression of CDKA;1 induced mitosis across the pericarp and altered the endoreduplication index (Czerednik et al., 2014), thus supporting the idea that CDKA plays an important part in the onset of endoreduplication and subsequent cell and fruit growth.

The inhibitory effect of phosphorylating CDKA on the Tyr15 residue through the action of the WEE1 kinase represents a good example of a post-translational regulatory mechanism impairing the cell cycle, and potentially triggering the endocyte (Fig. 2D). Gonzalez et al. (2007) generated transgenic tomato plants in which WEE1 was ectopically repressed. Smaller fruits were produced displaying a thinner pericarp composed of smaller cells. There was a strong reduction in endoreduplication as a result of impaired WEE1 kinase activity leading to an enhanced CDK–CYC activity. The WEE1 phosphorylation activity on its CDK targets appears to be an important mode of regulation for the promotion of endoreduplication during fruit development, and contributes to cell size determination, which ultimately influences final fruit size (Chevalier et al., 2011, 2014).

The completion of mitosis and progression from mitosis back into interphase requires the loss of CDK–CYC complex activity, which occurs through proteolytic destruction of the cyclin moiety by the UPS. This process involves a specific E3-type ubiquitin ligase named the anaphase-promoting complex/cyclosome (APC/C), which is activated through its association with CDH1/FZR-type proteins (Heyman and De Veylder, 2012). In plants, the commitment towards endoreduplication involves the selective destruction of mitotic cyclins, thereby preventing the formation of a proper mitotic CDK–CYC complex, and thus impairing the associated kinase activity. This process is achieved by the CCSS2A-mediated activation of the APC/C (Cebolla et al., 1999). The ectopic loss of function of CCSS2A in transgenic tomato plants led to the production of smaller fruits, when compared with the wild type (Mathieu-Rivet et al., 2010) (Fig. 2D). The DNA ploidy levels in these fruits were shifted towards lower levels, which corresponded to a decrease in mean cell size and an increase in cell number. Conversely, the ectopic overexpression of SICSS2A in tomato plants resulted in fruits slightly smaller than those of the wild type. The cytological analyses of these fruits revealed that the overexpression of SICSS2A first slowed down early fruit growth, which then resumed as highly endoreduplicated nuclei, and consequently larger cells, were generated. The resulting accelerated fruit growth (Mathieu-Rivet et al., 2010) was in agreement with the expected involvement of SICSS2A in endoreduplication-driven cell expansion.

The last example in modulating the CDK–CYC complex activity during tomato fruit development refers to the work of Nafati et al. (2011). SIKRPI (Fig. 2D), encoding a tomato CDK-specific inhibitor, was overexpressed in a fruit- and cell expansion-specific manner using the PEPC2 promoter (Fernandez et al. 2009). Increasing SIKRPI significantly reduced the extent of endoreduplication within the mesocarp,
as already described for a strong overexpression of *KRP* genes in other plant tissues or organs (De Veylder *et al.*, 2001; Jasinski *et al.*, 2002; Zhou *et al.*, 2003). However, the same construct was ineffective during the phase of cell expansion and did not alter the final size of fruits, in contrast to previous reports describing plant dwarfism. The mean cell size within the pericarp was unaffected, demonstrating that cell size was, at least partially, uncoupled from DNA ploidy levels. Nevertheless, endoreduplication occurred in these transgenic fruit, but to a far lesser extent than in the wild type. This suggests that DNA ploidy levels were sufficient to support cell growth, which is in agreement with the ‘karyoplasmic ratio theory’ (Schnittger *et al.*, 2003; Chevalier *et al.*, 2014).

**Modifying fruit growth by genes controlling fruit size and shape**

Since its introduction in Europe in the 16th century, the domestication and extensive breeding of tomato led to the creation of cultivated varieties bearing enlarged fruits compared with the wild ancestors (Paran and van der Knaap, 2007). The diversity of fruit size in tomato has been attributed to nearly 30 quantitative trait loci (QTLs; Grandillo *et al.*, 1999).

The Fruit Weight QTL of chromosome 2, number 2 (fw2.2) was the first QTL-associated gene that was identified and cloned (Alpert *et al.*, 1995; Alpert and Tanksley, 1996; Frary *et al.*, 2000). As a major QTL, fw2.2 accounts for as much as a 30% difference in fruit fresh weight between the domesticated tomato and its wild relatives. FW2.2 acts as a negative regulator of cell proliferation (Fig. 2C), thus explaining its major effect on fruit size in tomato. In addition, the role of fw2.2 in the determination of organ size is conserved within the plant kingdom, in both monocotyledonous and dicotyledonous species, such as avocado, maize, soybean, and cherry (Duhan *et al.*, 2010; Guo *et al.*, 2010; Libault *et al.*, 2010; De Franceschi *et al.*, 2013). Unfortunately, no clear biochemical function has been attributed so far to FW2.2 and its orthologues, especially in relation to the regulation of the cell cycle/cell division.

FW2.2 belongs to a multigene family encompassing 17 homologues in tomato (hereafter referred to as FW2.2-Like or FWL genes). FW2.2 and FWL proteins all contain the uncharacterized PLAC8 motif, which was originally identified in proteins from mammalian placenta (Guo *et al.*, 2010). The PLAC8 motif contains two conserved cysteine-rich domains separated by a variable region that are predicted to be transmembrane segments. The original tomato FW2.2 protein possesses these two transmembrane-spanning domains that fix the protein to the plasmalemma (Cong and Tanksley, 2006). From previous reports, it appears that the cysteine-rich domains may be involved in the transport of heavy metals such as cadmium and zinc, as identified in the plant cadmium resistance proteins from *Arabidopsis* (Song *et al.*, 2010). This type of protein may multimerize into a homopentamer to form a transmembrane pore, thereby facilitating metal cation transport (Guo *et al.*, 2010). The association between FW2.2 and its orthologues in relation to ion transport remains to be established, and how this could interfere with the control of cell proliferation is an intriguing question.

fw3.2 is only the second major QTL for fruit size/weight to be fine mapped and cloned in tomato (Chakrabarti *et al.*, 2013) (Fig. 2C). The gene underlying this QTL encodes a P450 enzyme of the CYP78A subfamily, previously identified as KLUH (Anastasiou *et al.*, 2007). The effect of *SIKLUH* is to enlarge fruit volume through an increase in cell number within the pericarp and septum tissues. Repressing *SIKLUH* using an RNA interference (RNAi) strategy led to decreased fruit and seed size and, consequently, plant architecture was modified with a higher number and length of side shoots (Chakrabarti *et al.*, 2013). The cloning of the gene and its function characterization will be an important step towards elucidating the biological function of FW3.2.

fw11.3 is another important locus governing fruit weight in tomato (Van der Knaap and Tanksley, 2003) (Fig. 2C). The fine mapping of fw11.3 demonstrated that it overlaps with *fasciated* (fas) which is a locus governing fruit shape on chromosome 11, but fw11.3 and fas are not allelic (Huang and van der Knaap, 2011). Unlike fw2.2 and fas, the large-fruit allele of fw11.3 is partially dominant. The anticipated cloning of FW11.3 will yield a third example of a cloned QTL regulating fruit weight.

Besides increasing fruit size, the domestication of tomato resulted in a high diversity in fruit shape. From the invariable, round-shaped fruits of wild species, cultivated tomato has diversified into round, flat, rectangular, ellipsoid, obovoid (pear-shaped), heart, oxheart, and long (bell pepper-shaped) fruits (Monforte *et al.*, 2014). This diversity in tomato fruit shape originates from only four mutated genes, *OVATE, SUN, FASCIATED (FAS), and LOCULE NUMBER (LC)* (Rodriguez *et al.*, 2011). Both *OVATE* and *SUN* control fruit elongation, *OVATE* is a negative regulator of growth leading to shorter fruit, whereas *SUN* is a positive regulator of growth resulting in elongated fruit (Fig. 2C). *FAS* and *LC* control the number of fruit locules, which ultimately influences both fruit shape and fruit size (Fig. 2A).

*OVATE* was the first gene associated with fruit shape to be identified by positional cloning (Liu *et al.*, 2002). It belongs to the ovate family protein (OFP) family of as yet unclear function (Liu *et al.*, 2002; Wang *et al.*, 2011). The effects of the *ovate* mutation on fruit shape vary from elongated fruits to pear- or round-shaped fruits depending on the genetic background carrying the *ovate* mutation (Gonzalo and van der Knaap, 2008). This suggests that *OVATE* is not responsible for the observed phenotype and probably interacts with other genes in an epistatic manner. The *ovate* pear-shaped fruit phenotype was complemented both by a genomic DNA fragment covering the *OVATE* gene and by its ectopic overexpression to revert to round-shaped fruits (Liu *et al.*, 2002). As a result, the *ovate* mutation is likely to be a loss-of-function mutation of a negative regulator of plant growth whose function remains to be elucidated. Interestingly, it has been shown in *Arabidopsis* that OVATE-like proteins act as transcriptional repressors that affect, in particular, the expression of AtGA20ox1, a key player in the GA biosynthetic pathway,
thus resulting in reduced cell elongation (Hackbusch et al., 2005; Wang et al., 2007, 2011). The indirect influence of OVATE on fruit shape through regulation of GA biosynthesis in tomato remains to be demonstrated.

The sun mutation causes an elongated-fruit phenotype. A gene duplication event mediated by a retrotransposon placed the SUN gene under the control of the defensin gene DEFL1 promoter, thus leading to high expression in fruit (Xiao et al., 2008; Jiang et al., 2009). The SUN protein belongs to the IQ67 domain-containing plant protein family (Xiao et al., 2008) of unknown function. SUN overexpression results in increased cell number in the longitudinal direction and reduced cell number in the transverse direction of the fruit, thus leading to fruit elongation (Wu et al., 2011).

The number of fruit locules is determined by the number of carpels within the flower. Wild species of tomato produce fruits with 2–4 locules, whereas cultivated varieties can develop >15 locules. As a result, not only can the shape of such fruits be greatly impacted, but this can also account for a tremendous increase in fruit size by as much as 50% (Tanksley, 2004). The QTL fas was identified as a trait governing extreme fruit size that increases the number of locules from two to more than seven, whereas the QTL lc has a weaker effect (Lippman and Tanksley, 2001; Barrero et al., 2006). FAS codes for a YABBY-like transcription factor (Cong et al., 2008), whereas LC has been located in a non-coding region between two putative candidate genes, WUSCHEL, which is a member of the plant-specific WUS homeobox (WOX) transcription factor family, and a gene encoding a WD40 repeat protein (Muños et al., 2011).

The function of most of the WOX genes studied so far can be ascribed to promotion of cell division and/or prevention of premature differentiation (van der Graaff et al., 2009). More specifically, WUS is involved in maintaining stem cell fate and meristem size, and, therefore, it is possible that the function of WUSCHEL is affected in lc. FAS and LC can interact epistatically to produce fruits with an extremely high locule number (Barrero and Tanksley, 2004). Both FAS and LC control floral meristem size and ultimately the development of supernumerary carpels (locules) leading to larger fruits (Cong et al., 2008; Muños et al., 2011). In this context, the link between fruit size, as influenced by FAS and LC, and the activities regulating meristem size and cell division have been clearly established.

Functional analyses using TOMATO AGAMOUS-LIKE1 (TAGLI), which is the orthologue of the duplicated SHATTERPROOF (SHP) MADS box gene of Arabidopsis thaliana, demonstrated the involvement of this transcription factor in the regulation of fruit development (Vrebalov et al., 2009) (Fig. 2A). Tomato plants repressing TAGLI by RNAi produced smaller fruits with a thinner pericarp which displayed fewer cell layers, and altered ripening-related fruit pigmentation, indicating that TAGLI is important for regulating both fruit growth and the ripening process.

**Hormonal regulation of fruit growth**

After successful flower pollination and ovule fertilization, the fruit and seed initiation, a stage that is commonly referred to as fruit set, and subsequent development of both fruit and seeds occur concomitantly according to a precise, genetically controlled process mediated by phytohormones (Gillasp et al., 1993).

Recent reviews have highlighted the role of plant hormones and their interplay in the control of fruit development (Ruan et al., 2012; Kumar et al., 2014). Here, we focus on the most relevant genes, whose functional characterization in tomato revealed an involvement in the growth period of fruit development. Auxin and GA appear to be the predominant hormones required for fruit initiation in response to fertilization, since exogenous applications of both hormones lead to fruit initiation and parthenocarpic development (de Jong et al., 2009). A role for cytokinin, ethylene, and ABA in fruit formation has been demonstrated, but is less well documented thus far (Kumar et al., 2014).

Early fruit development is governed by the allocation of auxin to tissues and cells in order to initiate signal transduction pathways (Fig. 2B). Recent evidence has indicated that the PIN-FORMED (PIN) auxin efflux transport proteins are involved in the processes of fruit set and early fruit development in tomato (Pattison and Catala, 2012). Silencing of the tomato SIPS4 gene, which is predominantly expressed in flower buds and young developing fruit, produced seedless (parthenocarpic) fruits of reduced size exhibiting precocious development (Mounet et al., 2012). The auxin signalling pathway involves an auxin receptor called the TRANSPORT INHIBITOR RESPONSE1 (TIR1) protein. In the presence of auxin, TIR1 recruits the AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) transcriptional repressors and triggers their degradation by the 26S proteasome. The degradation of Aux/IAA repressor proteins releases the Aux/IAA-bound auxin response factors (ARFs), thereby initiating the auxin response through auxin responsive element-mediated gene transcription. The misexpression in tomato of the auxin receptor TIR1 gene, as well as specific members of the Aux/IAA and ARF gene family, alters the normal flower-to-fruit transition and results in uncoupling fruit set from pollination and fertilization and gives rise to parthenocarpic fruits (Wang et al., 2005; de Jong et al., 2009; Ren et al., 2011). Recently, Su et al. (2014) reported that tomato plants silenced for the Aux/IAA transcriptional repressor SIIA17 displayed larger fruits with thicker pericarp tissue (Fig. 2D). This phenotype stemmed from enhanced cell expansion due to high ploidy levels. This suggests an enhancement of the endoreduplication process in the absence of the optimal quantity of SIIA17 during fruit development.

In tomato, fruit set is partly mediated by GAs according to a complex hormonal cross-talk with auxin (Serrani et al., 2008) (Fig. 2B). Auxin synthesized both in the ovary and in the apical shoot prevents unpollinated ovaries from developing by reducing transcript levels of genes encoding GA biosynthetic enzymes, in particular the GA 20-oxidases (Serrani et al., 2007). Pollination then triggers the up-regulation of transcripts for GA 20-oxidases that successively synthesize active GA1 and GA8. The GA 20-oxidase activity thus appears as the limiting factor for active GA biosynthesis and, consequently, for fruit set. Functional analyses in tomato aimed at the constitutive repression of SIGA20ox1.
during fruit initiation and growth resulted in plants severely affected in vegetative development and reduced pollen viability (Olimpieri et al., 2011). Ovaries from these SIGA20ox1-silenced plants remained fertile and developed normally after cross-pollination with wild-type pollen, but remained parthenocarpic. The individual silencing of SIGA20ox1, SIGA20ox2, and SIGA20ox3 genes confirmed the effects on vegetative development but, again, no effects on fruit set were observed (Xiao et al., 2006). Altogether, these data confirmed the pleiotropic developmental role of GAs, but suggested that the expression of more than one GA20ox gene is required to control fruit set in tomato. Interestingly, the heterologous overexpression in tomato of CgGA20ox1 from citrus clearly demonstrated the expected influence of GA and GA20ox activity on fruit set and development (Garcia-Hurtado et al., 2012). The overexpression of CgGA20ox1 resulted in elevated GA content and boosted vegetative development, as shown by longer hypocotyls and roots and increased plant height. In addition, flowers from the transgenic plants exhibited a protruding stigma due to a longer style and fruit displayed parthenocarpic development.

The GA signal transduction pathway requires the recognition of GA by its receptor called GA INSENSITIVE DWARF1 (GID1). The GID1–GA complex interacts with the nuclear repressor DELLA to target it for ubiquitin-dependent proteolytic degradation by the 26S proteasome. The repression of GA-responsive genes is then released to initiate GA signal transduction. Silencing of the SIDELLA1 gene in tomato produced very similar vegetative and reproductive phenotypes to those described for GA20ox1-overexpressing plants (Martí et al., 2007), as fruits were facultative parthenocarpic, smaller in size, and elongated in shape (Fig. 2B).

A number of ABA-deficient mutants have proven valuable toward elucidating the role of ABA in fruit growth. Three ABA biosynthetic mutants have been described: sitiens (sit), flacca (fic), and notabilis (not) that lack, respectively, an ABA-aldehyde oxidase (Harrison et al., 2011), a molybdenum cofactor sulphurase (Sagi et al., 2002), and 9- cis epoxy-carotenoid dioxygenase1 (NCED1) (Burbridge et al., 1999) (Fig. 2D). Although the mutants have been characterized on the molecular level, unfortunately little was reported on the effects of these mutations on fruit growth. Nitsch et al. (2012) reported the phenotypic characterization of notfic double mutant lines. The fruits of these double mutants have considerably reduced ABA levels, and displayed smaller fruit size and cell size, especially within the pericarp. The consequence of increasing ethylene levels, while lowering ABA, suggested that ABA stimulates fruit growth by restricting ethylene levels in normal fruits.

The INHIBITOR OF MERISTEM ACTIVITY (IMA) protein is a mini zinc finger (MIF) protein harbouring an unusual zinc-finger domain that was identified as an important effector of a signalling pathway involving multiple hormones that links cell division, cell differentiation, and hormonal control of development in tomato (Sicard et al., 2008) (Fig. 2A, C). IMA regulates the meristem activity and the processes of flower and ovule development. Carpel primordia within the floral meristem were much smaller in IMA-overexpressing plants, whereas they were enlarged in RNAi-silenced plants. The cell number within the carpel was reduced, leading to a smaller carpel, thus suggesting that IMA encodes an inhibitor of cell division. Consequently, plants overexpressing IMA produced smaller flowers and fruits, whereas RNAi-silenced plants produced fruits composed of supernumerary ovaries. In addition, IMA repressed the expression of WUS which controls the meristem organizing centre and the determinacy of the nucellus during ovule development (Sicard et al., 2008) (Fig. 2A).

**Metabolic control of fruit development**

The early stages of fruit development represent a critical period whereby traits, such as organoleptic composition, are established and ultimately dictate the final quality of the fruit. Water, organic acids (primarily citrate and malate), and minerals accumulate inside the vacuole of expanding cells (Coombe, 1976) while starch accumulates transiently and is converted subsequently to reducing sugars (Wang et al., 1993). Fruit softening, colouring, and sweetening then occur during the ripening phase (Giovannoni, 2004; Gapper et al., 2013). Fruit development and fruit weight are intimately connected to its composition of primary and secondary metabolites (Carrari and Fernie, 2006; Tohge et al., 2014). Therefore, modifying the expression of metabolism-associated genes has been investigated as a means to induce variations in fruit composition and size.

The development of fruit as a sink organ is more dependent upon the allocation of photo-assimilates than on the fruit’s own photosynthetic capacity. Modification of photo-assimilate supply substantially affects fruit development and size through the modulation of cell number and cell size (Bohner and Bangerth, 1988; Bertin et al., 2002). When tomato plants were submitted to extended darkness, fruit growth was severely impaired as the result of a strong repression of cell cycle genes inside fruit tissues (Baldet et al., 2002). Conversely, increasing photo-assimilate availability within the fruit by reducing the number of fruit per truss led to an enhancement in both flower and fruit growth rates. This corresponded to a greater cell number inside the carpel, due to an enhancement of mitotic activities (Baldet et al., 2006). The percentage variation observed in fruit fresh weight resulting from modulating fruit load is even as high as that which can be achieved by genetic modification (Prudent et al., 2009). Hence, the modification of carbon metabolism and photo-assimilate partitioning by the manipulation of key enzymatic activities, such as those involved in primary carbon metabolism and photosynthesis, was expected to have an impact on fruit growth. In order to investigate this, the Arabidopsis Hexokinase 1 (ATHXK1) gene was constitutively overexpressed in tomato plants (Menu et al., 2004) (Fig. 2D). Overexpressing lines exhibited marked phenotypic and biochemical changes in developing fruits, such as reduced fruit size and a decrease in cell expansion. The carbon supply required to support these processes was lower throughout development, most probably due to decreased photosynthesis. Consequently, any sucrose provided to these fruits would be used to fuel cell metabolism.
at the expense of starch storage. Fruit displayed reduced respiratory rates, which were accompanied by lower ATP levels and ATP/ADP ratios in fruit extracts, indicating profound metabolic perturbations.

The role of sucrose synthase (SuSy) in tomato fruit development has been studied by silencing a fruit-specific isoform (D’Aoust et al., 1999) (Fig. 2D). The inhibition of SuSy activity affected fruit set and very early fruit development related to the reduced unloading capacity for sucrose. This led the authors to propose that SuSy participates in the control of sucrose import capacity of young tomato fruit and, consequently, influences fruit set and development. Unfortunately, independent trials using equivalent transgenic plants failed to reproduce these results, and the lack of reports associating SuSy isoforms and QTLs for fruit weight or sugar content raised doubts about the validity of these conclusions (Carrari and Fernie, 2006).

The QTL Lin5 has been identified as a major QTL controlling fruit weight and sugar content (Fridman et al., 2000), and the associated gene was found to code for a cell wall-bound invertase (Fridman et al., 2004). When Lin5 was RNAi silenced, fruit yield was greatly reduced, as well as fruit size, seed size, and seed number (Zanor et al., 2009) (Fig. 2D). In these transgenic plants, metabolic changes were largely confined to sugar metabolism, since sucrose content increased while glucose and fructose contents decreased, as observed at the red ripe stage. Silencing of the vacuolar invertase TIV1 gene by an antisense strategy in tomato gave overall similar results. Production of smaller fruits corresponded to high rates of sucrose accumulation and decreased hexose sugar concentrations during the last stage of development (Klann et al., 1996) (Fig. 2D). Interestingly, changes in the concentration of osmotically active soluble sugars occurred during the phase of cell expansion of fruit growth and affected fruit size. This suggests that the concentration of osmotically active sugars is tied to water influx, which is an important determinant of fruit enlargement.

In order to establish a link between glycolysis, synthesis of hexose phosphates, and their conversion into organic acids, transgenic tomato plants were silenced for the mitochondrial tricarboxylic acid (TCA) cycle-associated malate dehydrogenase (mMDH) gene (Nunes-Nesi et al., 2005) (Fig. 2D). RNAi-mMDH plants showed not only enhanced chloroplastic electron transport rates and photosynthetic activity, but also increased fruit dry matter, indicating that the repression of mMDH improves carbon assimilation. Interestingly, transgenic fruit accumulated the redox-related compound ascorbate and displayed an increased capacity to use l-galactono-lactone which is the immediate precursor of ascorbate biosynthesis, as a respiratory substrate. Correspondingly, silencing of the tomato l-galactono-1,4-lactone dehydrogenase (Gal-LDH), which converts l-galactono-lactone into ascorbate, substantially modified mitochondrial function, including alteration of the ascorbate redox state and the TCA cycle (Alhagdow et al., 2007) (Fig. 2D). Consequently, plant and fruit growth were greatly reduced, since cell expansion was affected. Additionally, fruit from tomato plants silenced for the GDP-d-mannose 3,5-epimerase (GME), which is the central enzyme in ascorbate biosynthesis, exhibited defects in cell expansion and the biosynthesis of non-cellulosic cell wall polysaccharides (Gilbert et al., 2009) (Fig. 2D). Taken together, these findings indicate an ascorbate-mediated link among the energy-generating processes of respiration and photosynthesis, primary metabolism, and developmental processes, all of which are crucial for fruit growth in tomato.

Another example of a gene potentially modifying primary carbon metabolism and photosynthesis in fruit is the chloroplastic isoform of fructose 1,6-bisphosphatase (cp-FBPase), an important enzyme in control of the Calvin cycle. Obiadalla-Ali et al. (2004) generated tomato plants where the activity of this isoform was repressed specifically in fruit (Fig. 2D). Although overall carbohydrate metabolism was only slightly altered, fruit growth and final fruit size were significantly reduced, suggesting that cp-FBPase contributes to fruit photosynthesis in providing carbon for fruit growth.

More recently, the search for genomic regions spanning QTLs connected to yield-associated traits identified nine candidate genes located to tomato chromosome 4 (Bermúdez et al., 2008). Among these genes, a DnaJ chaperone-like encoding gene was isolated and appeared to be associated with changes in primary metabolites across tomato fruit development. The in planta functional analysis aimed at silencing this gene, subsequently named SPA for Sugar Partitioning-Affecting (Fig. 2D), showed that the ripe fruit weight, the number of fruits per plant, and the harvest index were significantly higher in transgenic than in wild-type plants (Bermúdez et al., 2014). SPA as a putative chaperone protein was shown to act through a mechanism involving the regulation of phosphoglucomutase, sugar kinase, and invertase enzyme activities during tomato fruit growth, thus regulating the harvest index by affecting the source–sink carbon distribution.

**Conclusion**

In this review, we focused mainly on developmental and cellular processes that relate to the determination of growth-related traits in tomato fruit, and we described various genetic and functional analyses that provide insight into factors modifying fruit size. Although studies aimed at deciphering the genetic control of fruit size and shape have provided intriguing results, many questions remain unanswered from the available data. Association genetics and functional genomics approaches have allowed the localization of major QTLs, and the genes controlling these traits have subsequently been identified. Since we are still far from understanding the influence of genes, such as FW2.2, FW3.2, FW11.3, or OIVATE, SUN, and LC, on the process of fruit development, such as cell proliferation, in-depth functional analyses are required to unravel the molecular bases of their respective regulatory properties.

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