Shaping a fruit: Developmental pathways that impact growth patterns

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Angiosperms produce seeds as their progeny enclosed in maternally-derived structures called fruits. Evolutionarily, fruits have contributed enormously to the success of the Angiosperms phylum by providing protection and nutrition to the developing seeds, while ensuring the efficient dispersal upon maturity. Fruits vary massively in both size and shape and certain species have been targeted for domestication due to their nutritional value and delicious taste. Among the vast array of 3D fruit shapes that exist in nature, the mechanism by which growth is oriented and coordinated to generate this diversity of forms is unclear. In this review, we discuss the latest results in identifying components that control fruit morphology and their effect on isotropic and anisotropic growth. Moreover, we will compare the current knowledge on the mechanisms that control fruit growth, size and shape between the domesticated Solanaceae species, tomato and members of the large family of Brassicaceae.

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1. Introduction

The Angiosperm phylum of flowering plants is the most successful plant phylum comprising >90% of all plants on Earth. They evolved during the Cretaceous Period 100–125 million years ago and their subsequent fast diversification in evolutionary terms remains an enigma and one, which Darwin famously referred to as ‘an abominable mystery’. Flowering plants encase their seeds in a fruit or a vessel and from which the phylum name is derived (Greek: Angio = vessel; sperm = seed). Formation of flowers and fruits is indeed considered crucial for their success allowing attraction of pollinators for efficient fertilisation of the ovules and protection and nurturing of the developing seeds. The huge variety of fruit morphologies have led to ingenious ways for efficient seed dispersal. In addition to shape, size also varies significantly among fruits from different or even within species, ranging from the smallest known fruits from Wolffia angusta that are no larger than a grain of table salt to the giant pumpkins (Cucurbita maxima) that through intensive selection and breeding including highly specialised growth conditions can exceed 1000 kg. The latter case also highlights the result of domestication, a process that Darwin convincingly used to demonstrate evolution by natural selection [1,2]. Domestication of vegetable and fruit crops has often led to dramatic changes in fruit size and created a diversity of fruit shapes within the same species [3–5]. However, in the context of plant development, morphological diversity in crop plants is often underexplored.

Growth and shape of natural structures have been of interest to scientists at least since ancient Greece. From the early days of genetics, it was recognised that features controlling organ size and shape are inherited through generations [6–12]. In tomato, one of the earliest studies into the genetic inheritance of fruit morphology is that of elongated fruit and locule number, traits with a strong genetic inheritance [11,9]. Initially, the locus for elongated and pear-shaped fruit was called pyriform (pr) [11] but was renamed to ovate after pr was found to co-segregate with oblong and oval fruit shape [13]. Fruit cell number was the initial term for two related traits, namely fasciated (for fas) and locule number (lc) reflecting flat-shaped tomatoes with many carpels as opposed to the wild-type carpel number of two [9,12]. The loci fas and lc control the same trait, the number of carpel primordia, but with a different degree of severity on the trait [14]. Linkage mapping placed several of the fruit shape loci together with other morphological traits and created one of the first linkage maps in plants [15,16]. In the Brassicaceae, George Harrison Shull crossed the tetraploid Capsella bursa-pastoris (heart-shaped fruits) with a natural variant of C. bursa-pastoris, named “heegeri”, which has cylindrical fruits. Shull found a 15:1 segregation in the F₂ generation of heart-to-cylinder [17] leading him to suggest that two genetic loci contribute to the trait. This observation is in agreement with observations reported by the botanist Edmund W. Sinnott two decades later. Sinnott used pumpkin as a model system and his data suggested that it is possible to differentiate between gene activities that regulate shape and those that only affect size [18].

The quantitative nature of fruit weight initially hampered the discovery of genes controlling size traits in crop plants. However, with the advent of molecular genetic linkage maps and improvement in quantitative mapping tools, a large number of loci underlying quantitative traits have been identified including those for fruit and grain size [19–21,3]. This has led to supporting evidence from modern day’s developmental genetics and the identification of key factors involved in determining shape and weight in domesticated vegetables and fruits [5,22]. In this review, we provide an overview of the current knowledge in fruit growth with a particular emphasis on examples from fleshy fruits (tomato) and dry dehiscent fruits (Brassicaceae).

2. Shape classifications in tomato and Brassicaceae species

Tomato varieties have traditionally been classified based on fruit morphology into shape categories described by the International Union for the Protection of New Varieties of Plants (UPOV) and the International Plant Genetic Resources Institute [23,24]. A revised tomato classification scheme was developed based on fruit shape analyses that were conducted on a large collection of cultivated accessions that included many heirloom varieties featuring the most diverse fruit shapes [14] (Fig. 1). Independent classification using contour morphometric data from scanned tomato fruit images in conjunction with elliptic Fourier shape modeling and Bayesian classification techniques led to similar results [25]. The latter classification is especially helpful to growers and breeders as it allows also an unbiased evaluation of uniformity of fruit shapes within a certain tomato variety [25]. In fruit and vegetable crops, shape and size uniformity is critical to the industry whereas for molecular geneticists uniformity suggests a strong genetic basis for the trait. Moreover, consumers recognize use type in tomatoes based on their morphology: from the small oval-shaped grape tomato for salads to the blocky and squared Roma tomato for soups and stews to the large and flat beefsteak tomato for slicing. The model plant Arabidopsis thaliana belongs to the large Brassicaceae family, which also contains the Brassica genus including important crop plants such as oilseed rape (B. napus) and broccoli (B. oleracea). Members of the Brassicaceae family exhibit an extraordinary diversity in fruit shape with different basic shape structures such as cylindrical, disc-formed, spherical as well as more complex structures including heart-shaped fruits or fruits that develop outgrowths [26] (Fig. 2). This wide variation among closely related and even within species provides an excellent resource for studying organ-shape formation. In many cases, it is not immediately evident what advantages the different shapes provide for fitness and dispersal. It is also unclear how such variation in form can evolve when coordination of tissue growth and specification is of pivotal importance for timely development and seed release.

3. General developmental principles of the female reproductive structure

Fruits represent the final stage of the life cycle of a plant. Whereas plant growth ensues through auxillary shoot development or indeterminate above ground stems, tomato and Brassicaceae fruits classically represent the final growth stage of a terminating floral meristem. Fruits develop from carpels which originate from the fourth and final whorl in the floral meristem. Carpels comprise the female reproductive tissue that produce the female gametophytes. At anthesis, pollen grains land and germinate at the apical stigma and pollen tubes grow through the style and ovary to deliver the male gametophyte and fertilise the ovules. Fertilisation marks the beginning of fruit development and is in most species required for fruits to develop [5,27,28]. In the first phase after fertilisation, fruit growth occurs mainly via cell division, but subsequently enters a second phase of cell expansion, which continues until the fruit has reached its final size [28–32]. There are examples, however, such as avocado, where cells continue to divide throughout fruit development up until ripening [33]. Fruit development is finalized with the ripening and seed dispersal stage. The latter stage as well as fruit set following fertilisation are critical for proper fruit development. Despite the impact of poor fertilisation on shape and size of the affected fruits, this process is not associated with regulating the morphology of the organ in a consistent manner and will therefore not be discussed in this review. The processes of ripening and seed dispersal will also not be discussed because that stage in general does not affect fruit morphology. Readers interested in these
Fig 1. Tomato fruit shape variation. The effect of the fruit shape genes SUN, OVATE, SOV1, LC and FAS on tomato shapes. Size bar, 1 cm. The name of the varieties are shown in the graph. Image composition by Carmen Kraus, University of Georgia.
two topics are referred to the following comprehensive reviews [34–39].

By comparing growth of different fruit forms, it may be possible to obtain information of overall principles in growth control without identifying the specific genetic components underlying them. This was recently done by combining sector analysis and mathematical modelling in Capsella (heart-shaped fruit) and Arabidopsis (cylindrical fruit). This work demonstrated that the different shapes of fruits from Arabidopsis and Capsella arise through different patterns of anisotropic growth during three distinct phases [30]. These experimental data can be accounted for by tissue-level models in which specified growth rates vary in space and time and are oriented by a proximo-distal polarity field, which deforms as the tissue grows. By switching certain parameters of the model in specific tissues, it was possible to change overall structure of the fruit model. Interestingly, fruits with these new model fruit shapes exist among members of the Brassicaceae family thus showing that modulations of the model are able to at least broadly account for the growth dynamics of shapes of other Brassicaceae species. Such basic differences in growth patterns may therefore explain the multiple incidents of evolutionary switches between different shapes of fruits in the Brassicaceae [40].

In tomato, modelling fruits from a mutagenized population showed that weight was controlled by two growth processes: i) isotropic and proportional growth of all fruit tissues or ii) anisotropic growth of the pericarp only [41]. In another study, modelling of fruits during the cell expansion stage of growth (see below) showed that these later stages led to a differential patterning of vasculature and concomitant changes in water distribution in fruits that featured different shapes [42]. While these studies modelled growth at a relatively late stage in development (after anthesis) and may not address all growth patternings, they describe the consequences of overall fruit morphology on functioning of the tissues as well as their impact on fruit quality [42].

4. Principles of organ growth at the cellular level

The rate, duration and plane of cell division as well as the direction and degree of cell expansion are key parameters that contrib-ute profoundly to the overall final size and shape of the fruit. These processes start as early as in the meristem by regulating the architecture and organization of this tissue and continue until the later stages of fruit development prior to ripening [28]. The initial processes are under tight control by meristem organization, organ identity, positioning and boundary genes [43–46]. Also critical are regulators of the cell cycle [47–51] and hormone dynamics [52], cell wall biosynthesis and modification [53–55] and turgor pressure [56,57]. Moreover, cell size is affected by endoreduplication, a process that is common in higher plants. Maturing cells often side-step the mitosis stage several times resulting in larger cells with higher nuclear ploidy levels, bypassing chromatid segregation and cytokinesis [58–60]. Endoreduplication is proposed to occur in many multicellular organisms as a mechanism for cell enlargement and differentiation, and is critical for the full development of tomato fruits [61]. However, cloning of natural tomato mutants that underlie domestication of fruit size and shape characters have so far not revealed any genes known to be directly involved in cell-cycle control, endoreduplication, or hormone, cell wall and turgor pressure pathways. In contrast, modifiers affecting fruit morphology have been identified in abundance [62–67] and most of these genes will be discussed later in this review. However, it is important to note that in line with previous theories [18], common principles are likely to underlie shape determination of all above-ground plant organs [68]. Tissue-level models of leaf and petal growth have led to the suggestion that shape depends on patterns of anisotropic growth oriented by a polarity field [69–71]. These studies have recently been extended by demonstrating that such models also can account for the growth patterns and diversity of three-dimensional fruit shapes [30].

4.1. Early growth and differentiation in angiosperm flowers - termination of meristematic activities in the centre of flowers

The earliest stages of fruit development commence when the remaining stem cells in the floral system turn into carpel primordia, effectively terminating growth of the floral meristem. Thus, the size and organization within each floral meristem provides the foundation of the fruit that will arise after several stages of development including pollination and fertilization. The CLAVATA and WUSCHEL signaling pathway functions in parallel with other pathways to control meristem organization. The CLV-WUS pathway is highly conserved in the higher plant kingdom with a central role played by WUS [72]. The CLV receptor–ligand system comprises a receptor-like kinase (CLV1), an LRR receptor (CLV2) and a ligand peptide (CLV3). The role of the CLV-WUS pathway is to control the boundary between the central (meristematic stem cells) and peripheral (towards organ initiation) zones in the meristem [73,74]. WUS encodes a homeodomain transcription factor and it enhances stem cell activity while repressing differentiation. It directly increases the expression of CLV3. CLV3, on the other hand, encodes a small peptide that is required to limit cell division in the central zone. Interaction of CLV3 with its receptor CLV1 restricts the domain of the central zone and indirectly limits WUS expression [75]. These two genes function in a negative feedback loop mechanism regulating meristem size and floral organ number [76,77]. In Arabidopsis, loss-of-function mutations in any of the CLV genes leads to enlarged meristems due to excess undifferentiated cells in the central zone [78]. In the shoot apical meristem, this results in a fasciated stem, whereas the larger floral meristem leads to an increased number of carpels and hence wider fruits with more locules [79–81]. In tomato, two natural mutations named fasciated (fas) and locale number (lc) exhibit a synergistic effect on meristem size and the number of locules, the seed compartments in the tomato fruit [82,83] (Fig. 1). Mutations in these genes greatly increases locule number from two in wild progenitors and small cultivated toma-
toes up to 10–15 in the very large fruited types [14]. Initially, the gene underlying *fas* was thought to be *Yabby2*, a transcription factor belonging to the family that is known to regulate abaxial–adaxial development of lateral organs [84]. Later studies discovered that the underlying cause of the mutation was a 296-kb inversion at the *fas* locus, possibly impacting the promoter of *SICL3* [85]. This structural variant mutation was strongly correlated to locule number and flat fruit shape, implying that it could be the cause of the mutation at *fas* [14,46]. Transformations using a genomic 10 kb construct carrying 6 kb of the wild type *CLV3* promoter rescued the high locule phenotype in mutant tomatoes, effectively demonstrating that *SICL3* underlies *fas* [45]. Expression of *SICL3* is likely reduced as a result of the promoter mutation leading to increased locule number [45]. The effect of reduced expression of *SICL3* on floral organ number is similar to the defect observed in *clv3* mutants in Arabidopsis demonstrating the conserved nature of this gene in regulating meristem architecture in different plant species. *Yabby2* is abolished as a result of the 296-kb inversion; however, its role in controlling locule number in tomato is predicted to be minor if any.

The other natural mutation in tomato that affects locule number is at the *lc* locus [82]. The locus was fine-mapped to a 1.6 kb region 3’ of the likely tomato ortholog of *WUS* [46]. Association mapping led to the identification of two SNPs that are 1 kb downstream of *WUS* [46]. Closer inspection of these two mutations showed that they were located in a putative CARG box known to regulate WUS expression in Arabidopsis [22]. *WUS* expression is required to maintain stem cell identity and restriction of cellular differentiation which is needed for organogenesis. As cells move out of the central zone into the peripheral zone to initiate organ formation, the expression of *WUS* would need to be reduced in the differentiating cells. In Arabidopsis, *WUS* expression is reduced by the transcriptional repressor KNUCKLES (KNU) [86]. In parallel, *WUS* expression is directly repressed by the floral identity protein AGAMOUS (AG), a MADS box transcription factor critical for carpel identity and development [87]. AG has been shown to bind to the WUS CARG box that is 3’ of the gene [88]. The role of AG’s binding to the CARG box is to recruit Polycromb Group proteins leading to the represive histone H3 Lys-27 methylation at the locus [89]. In tomato, it has been hypothesized that the two SNP mutations in *lc* lead to reduced or abolished binding of AG to the WUS 3’ regulatory region, effectively reducing the ability of AG to turn off WUS expression [22]. Delayed downregulation of *WUS* would lead to an expansion of the *SICL3* expression zone. However, since *SICL3* and *CLV1* together result in downregulation of *WUS* expression, the phenotypic consequences of delayed termination of *WUS* expression is expected to be small. Indeed, in tomato the *lc* mutation alone has a minor effect on increasing locule number [14,82]. On the other hand, when the delayed downregulation of *SIWUS* is coupled with the reduced expression of *SICL3* leading to the weaker control of the *WUS* expression zone, the synergistic effect of both mutations on locule number is relatively high. Indeed, tomato varieties with the highest number of locules are nearly always carrying mutations in both *lc* and *fas* [46,14].

The tomato gene, *FASCICATED AND BRANCHED (FAB)*, is the ortholog of the Arabidopsis *CLV1* gene with *fab* mutants having very similar developmental defects [45] indicating that other components of the CLV-WUS pathway are functionally conserved as well between tomato and Arabidopsis. Recently, other key components of the CLV-WUS pathway were identified in tomato, including *FASCICATED INFLORESCENCE (FIN)*, *FAB2* and *SIRRA3a* which encode different enzymes regulating the arabinosylation of *SICL3* and other CLE peptides [45]. The arabinosyl modifications are essential for the biological activity of the CLEs and application of a chemically produced glycosylated CLV3 peptide will rescue *clv3* mutant meristems [90].

### 4.2. Organ initiation and patterning

The floral meristem terminates with the initiation of the female reproductive structure, the gynoecium. AG encodes a MADS box transcription factor that is critically required for the specification of cells in the terminating floral meristem to form the gynoecium primordium [87]. As described above, AG initiates gynoecium development by recruiting PcG proteins to the *WUS* promoter resulting in downregulation of its expression [88]. This in part determines the termination of stem cell activity in the central zone as these cells become subsumed by the emerging gynoecium. Brassicaceae gynoecia are roughly cylindrical in shape (Fig. 2B) where the lateral parts develop into valves (seed pod walls) that are fused to a medial replum (Fig. 2A). Between the valves and the replum, narrow strips of tissue made up of a few cell files form the valve margins where fruit dehiscence will take place to allow the seeds to be released upon maturity [44,39]. Differences in gynoecia morphology are apparent within the Brassicaceae family. For example, whereas the style in Arabidopsis fruits is relatively short (Fig. 2A,B), it can span more than a third of the *Brassica* fruit length (Fig. 2B). Also, while Arabidopsis has a replum of 8–10 cell rows, the replum of *Brassica* fruits generally only have one row of cells [91].

For Brassicaceae species, the gynoecia develop similarly and the mechanism for its formation may therefore be highly conserved. In Arabidopsis, detailed knowledge of the hormonal and genetic interactions required for proper gynoecium development is emerging [92,93]. It is known that the plant hormone auxin plays a pivotal part both in terms of biosynthesis and distribution. For example, basal-to-apical transport of auxin is important to allow growth along the proximo-distal axis, whereas biosynthesis at the apical region as well as lateral auxin transport is required to mediate formation of a radial style that will facilitate efficient fertilisation [94–96]. In the medial region of the gynoecium, another hormone, cytokinin, interacts with auxin dynamics to ensure meristematic activity and formation of the ovules [97]. Some of the key regulators of gynoecium patterning in *Arabidopsis* have been identified in other Brassicaceae species and genetic interactions between them established. *FRUITFULL (FUL)* and *REPLUMLESS (RPL)* genes specify valve and replum formation, respectively, and they do so at least partly by restricting the expression of valve-margin identity genes, such as *SHATTERPROOF1 (SHP1)*, *SHP2*, *INDEHISCENT (IND)*, and *CALCITRAZ (ALC)* [44,98–103]. Whereas *Brassica* *FUL* and *IND* genes have conserved functions in fruit tissue development [104,105], *Brassica* *RPL* genes have lost their ability to specify replum tissue [91].

The dynamic activities of auxin and cytokinin are tightly regulated via intricate transcription factor networks [106,97]. A major regulator of gynoecium patterning and polarity is the auxin response factor, ETTIN (ETT) [107,108]. ETT interacts directly with a basic helix-loop-helix (bHLH) protein, INDEHISCENT (IND) to regulate auxin distribution. Their activity is feedback-regulated such that the interaction between ETT and IND is sensitive to auxin itself [106]. IND interacts with another bHLH protein, SPATULA (SPT) and this interaction is required to mediate formation of auxin maxima in the medial region of the apex allowing the apex to undergo a bilateral–to-radial symmetry change [94]. SPT also promotes formation of the medial region by facilitating cytokinin signalling necessary for the formation of placenta and ovules [97].

In tomato, the role of many orthologs of the Arabidopsis genes that are involved in gynoecium patterning are not well understood. The most likely tomato ortholog of AG is *TAG1*. RNAi suppression of *TAG1* leads to defects in carpel development and determinacy by producing fruits within fruits [109–111]. However, carpel identity
was not changed similarly as in the eg mutant in Arabidopsis. On the other hand, different genes that modify the morphology of the fruit have been found to function during the earliest stages of gynoecium development. For example, a knock-out of ENHANCER OF J2 (EJ2) leads to a slightly elongated shape of the fruit in addition to several pleiotropic effects such as jointless fruit stems and enhanced inflorescence branching [112]. EJ2 encodes a MADS box protein that is a paralog of SEP4 in Arabidopsis. Another gene with a large effect on fruit shape is OVA1. Initially identified as the founding member of the Ovate Family Proteins (OPF) class in plants [62,113] and described as early as 1908 [9], the ova1 mutation only controls fruit shape with no apparent changes in leaf and other floral organ shapes or any other developmental defects [114] (Fig. 1). OVA1 is expressed in floral meristems and initiating floral primordia [66]. The null mutation leads to changes in gynoecium shapes due to changes in cell division patterns in the proximal part of the fruit. Compared to wild type, the number of cells in the proximo-distal direction is increased whereas the number of cells in the mediolateral direction are decreased in ova1 mutants [66]. This effect is particularly enhanced in the presence of a locus that modifies the effect of ova1 [115] (Fig. 1). Overexpression of Arabidopsis OPF members often lead to reduced organ length such as siliques, leaves and cotyledons [116,117,113]. Moreover, in pepper, a paralog of ova1 has also been implicated in regulating fruit shape suggesting that this family may control organ shape in other plants as well [118]. The molecular functions of OVA1 are not well understood. However, it has been shown to interact with proteins associated with microtubules [22] suggesting a role in cell division patterns by its interaction with the cytoskeleton.

4.3. Fruit development after successful fertilisation of the ovules

Induction of post-fertilization fruit growth depends on the hormonal activities of both auxin, cytokinin and gibberellin [28]. Fertilization-induced fruit growth follows an auxin-signal produced in the fertilized ovules, which induces gibberellin biosynthesis required to degrade the growth-repressing DELLA proteins, thereby facilitating growth [119,120]. How or if these activities regulate fruit shape is unknown, we describe the effects of auxin dynamics in tomato during fruit growth below.

Immediately following fertilisation, cell division reinitiates in the growing fruit tissues. This initial phase of cell division is followed by the prolonged phase of cell enlargement. Changes in division rates and direction could profoundly impact fruit morphology after fertilisation has occurred. Whereas gynoecium development appears highly conserved among the different Brassicaceae species, morphology establishment which occurs after fertilisation may be controlled in very different ways [30,26]. For example, the valves of mature Capsella fruits are extended and flattened at the distal end resulting in a heart-shaped appearance of the organ (Fig. 2D). The role of Arabidopsis patterning genes has been investigated in other Brassicaceae species as well. When the FUL gene encoding a MADS box protein is knocked out in species that develop highly diverse fruit shapes, such as Arabidopsis, Capsella and Lepidium, fruits fail to elongate after fertilisation and the valves become dramatically reduced [44,26]. The similarity in the ful mutant phenotype in these species suggests that the diversity of shapes originates primarily from the differential activities of factors during post-fertilisation growth. The phenotype of the Capsella ful mutant and indeed the Arabidopsis ful mutant is intriguingly similar to the heegeri mutant described by Shull [17]. The wild-type background of the heegeri variant is the tetraploid Capsella bursa-pastoris. The genome sequence for C. bursa-pastoris was recently published revealing two copies of the FUL gene [121] and it is therefore possible that mutations in these two FUL genes are responsible for the heegeri fruit phenotype. Unfortunately, heegeri seeds are no longer available so we may never be able to assess if this is indeed the case. However, with respect to the function of FUL, it will be interesting to study whether the Capsella FUL gene is sufficient to create the shoulders of the Capsella fruit. This could be achieved either by a difference in expression pattern in comparison to Arabidopsis through changes in cis-regulatory elements or by changes to the amino acid sequence potentially leading to alternative downstream targets. In this context, it is interesting to note that FUL in Arabidopsis has recently been found to influence differential growth in stem and inflorescence branches through direct control of the SAUR10 gene [122]. An alternative possibility is that FUL does not control shape formation per se, but acts upstream of species-specific shape programs to simply promote growth. This latter scenario would agree with Sinnott’s hypothesis in which FUL would promote tissue growth whereas other factors are responsible for determining the species-specific fruit shape [18].

The role of FUL homologs, SIFUL1 and SIFUL2 in tomato development are to control the fruit ripening process via its interactions with RIN, encoding another MADS box protein that controls fruit ripening [123–125,38]. So far, a role for FUL in fruit patterning and expansion has not been found in tomato. Instead, the fruit shape gene SUN impacts fruit shape most noticeably immediately following fertilisation in tomato [126,31] (Fig. 1). The shape change mediated by SUN coincides with the cell division stage and is completed 7–10 days post pollination. This change is due to increased cell division along the proximo-distal axis as well as increased cell elongation [127]. SUN encodes a protein that is a member of the IQ67-domain (IQD) family [65] and members of this family have been shown to bind calmodulin in a calcium-dependent manner [128]. Mechanistically, how SUN mediates cell division patterns is not known. A role for auxin has been implied from overexpression studies that showed a dramatic auxin-like effect on fruit and leaf shape as well as nearly abolished seed development in tomato [127]. Moreover, promoters of several Arabidopsis members of the IQD family are targets of the Auxin Response Factor 5 (ARF5 also known as MONOPTEROS) [129], implying that a role for auxin signalling underlies the changes in tomato shape mediated by SUN. Insights into the function of members of the IQD family have come from studies in Arabidopsis. AtIQD1 interacts with a kinesin-light chain-related protein and proposed to be involved in cellular trafficking by its association with microtubules [130]. More recently, the entire IQD family has been shown to localize in distinct subcellular compartments and are most often associated with microtubules. Overexpression of several IQD genes indeed showed changes in cell shape and organ morphology that is associated with altered microtubule structure in the cells [131]. These results start shedding important insights into how SUN and other IQD family proteins may alter cell division patterns and cell shape in tomato and other plants. Downstream effects of SUN expression in tomato were identified in gene expression studies which showed that the central role is to control calcium-regulated processes [132]. Moreover, the co-differential expression studies showed that SUN results in profound shifts of gene expression in the different parts of elongating fruit tissues compared to wild type control samples. These shifts occur in genes that are involved in key processes such as cell division, cell wall, and patterning [132]. Supporting a role for SUN-like genes in other crops, a homolog of SUN in cucumber may also underlie shape variation in this vegetable [133].

The gene KLUH/PLASTOCHRON1 regulates organ size and initiation in Arabidopsis and rice respectively [134,135]. In tomato, this gene was found to underlie the fw3.2 QTL [64,136]. Notably, not only fruit size was increased but also seed size whereas fruit ripening time was delayed. The gene encodes a CYP450 of the 78A class and is highly expressed in meristems and young tissues [22,64]. The most likely mutation is a single SNP in the promoter leading to increased gene expression and larger fruit. Even though SIKLUH
is differentially expressed in young flower buds, the increase in fruit weight in the mutant becomes only apparent after fertilisation. The increase in fruit weight results from increased number of cell layers in the pericarp of the developing fruit after anthesis. The delay in ripening that is associated with the larger fruit weights has been hypothesised to be the result of the extension of the cell proliferation stage [64]. KLHU has been found to regulate seed size in rice, wheat and soybean [137–139] as well as biomass in maize [140]. Moreover, CaKLHU is associated with fruit weight in pepper suggesting that domestication-driven increases in fruit weight are in part through the KLHU signalling pathway [64]. It has been hypothesised that KLHU generates a mobile growth promoting signal different from the known phytohormones. However, the exact molecular and biochemical nature of the “mobile” signal remains elusive and the substrate for this subfamily of P450 enzymes is also yet to be deciphered [141,134].

The increase in size from a 1–2-mm wide gynoecium to a 5–10-cm wide tomato fruit is predominantly the result of a dramatic increase in cell size in the pericarp [28,31]. In the developing gynoecium and fruit, the transition to and maintenance of cell enlargement in pericarps has been described [142], but is not well understood at the molecular genetic level. The tomato gene Cell Size Regulator (CSR) controls cell size in the pericarp and underlies the fwl1.3 QTL [67,85]. CSR encodes a protein of unknown function that is homologous to the FAD-like protein in Arabidopsis [143]. Its expression is undetectable in meristems, young floral buds, anthesis-stage flowers and gynoecia or very young developing fruit. Only in fruits starting about 5 days post pollination, CSR is expressed to detectable levels peaking at 25–33 days post pollination and declining rapidly at the onset of ripening [67]. In addition to increasing cell size, coexpression analyses suggest a role in shoot development and phloem/xylem histogenesis. Moreover, these studies also suggested a potential link between auxin and cytokinin signalling that is critical for the transition of the cell division to cell expansion phase fruit growth [67].

5. How knowledge on fruit growth can lead to crop improvement

The largest challenge facing agriculture today is the secure supply of food for an ever-increasing human population. This challenge is exacerbated with the reduction in land suitable for growing crops due to urbanization and the effects of climate change. It is therefore more important than ever to develop innovative ways for improving crop performance. The final shape and size of produce are critical aspects for all vegetable and fruit crops. Therefore, the knowledge of the genes and the developmental pathways that impact morphology will provide crucial insights into processes of economic importance. Whereas shape and weight of a vegetable like tomato dictate the market class and its culinary purpose, Brassicaceae fruits provide different functions. For example, oilseed rape (Brassica napus) is the third largest source of vegetable oil for human consumption. In addition, the residues from oil extraction provide the second largest source of protein meal for animal fodder. Pod (i.e. fruit) architecture holds great potential as a trait that could lead to significant yield gain for oilseed rape. Several crop physiological studies have demonstrated that seed filling in oilseed rape critically depends on photosynthesis in the pod walls and observations indicate that photosynthesis in the pods contributes >50% of the assimilates for seed filling, most of which is derived from photosynthesis [144]. Thus, strategies based on knowledge of fruit formation in closely related model species such as Arabidopsis and Capsella may allow increasing the photosynthetically active pod surface and lead to more assimilates being available for pod filling, thereby increasing seed size and yield.

6. Concluding remarks

Multicellular organisms develop organs with specialized functions that are vital for survival. The morphology of these organs adopt is shaped through evolution and selection to facilitate their optimal performance. Fruits from members of the angiosperm phyllum exhibit enormous variation in size and shape and therefore provide excellent systems to understand the genetic control of the mechanisms underlying organ shape establishment. Although our focus has been on the fruit of specific model systems such as tomato, Arabidopsis and Capsella, it is likely that principles such as those that guide anisotropic growth are conserved in other organs in all plant species. Given the tight control that must be in place to govern organ growth in all multicellular organisms, such principles are expected to go beyond fruit growth and understanding the mechanisms underlying fruit-shape determination therefore has implication for highly diverse disciplines ranging from medicine to crop improvement.

Developmental genetics and mathematical modelling have been pivotal disciplines in allowing the recent advance in our understanding of fruit formation and the resulting shape and size of the organ. Although, we are only beginning to identify the specific genetic components involved in the process and the interactions between them, it is now possible to identify precise and subtle genetic changes that can be used to predictively modify fruit development and facilitate increased crop production. Increasing yield through intelligent crop design will enable more efficient agricultural practices, thus reducing inputs and land use with obvious benefits for the environment.

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