Explaining Tomato Fruit Growth by a Multi-Scale Model on Regulation of Cell Division, Cell Growth and Carbohydrate Dynamics

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Abstract

A multi-scale approach to model tomato fruit growth is proposed, in order to account for the interaction between gene functioning and growth conditions, and, ultimately, to explain the fruit phenotype of various genotypes in diverse growth environments. There is particular focus on: (I) cell division regulated by cell cycle genes, (II) cell expansion as influenced by polyploidy resulting from endoreduplication and carbohydrate and water dynamics. The growth processes at gene, cell and tissue, fruit and plant scale have been identified and included in the model. Sub-populations of cells differing in age are considered to act as sinks competing for carbohydrates. The key cell cycle genes of tomato were incorporated into an existing model of the gene regulatory network of the cell cycle. This model was modified to simulate endoreduplication. Moreover, the modelled cell cycle process was made sensitive to temperature and assimilate supply. The multi-scale approach required that a simulation could only proceed if a calculation task at a neighbouring scale had been performed. Preliminary model results indicate that cell number and ploidy level were very important in determining fruit growth. Subsequently, in the cell expansion phase, growth rate was limited by assimilate supply which in the end determined the realized fruit size. Observations at gene, cell and tissue scale are in progress in order to calibrate and validate the model, to enable reliable prediction of cell division and expansion of cells in tomato fruit tissues at contrasting conditions of temperature and carbohydrate supply.

INTRODUCTION

Until now, most fruit growth models used experimentally determined, empirical relationships between environment and growth. Only a few models explain fruit growth as a result of processes occurring at lower hierarchical scales. For tomato, Liu et al. (2007) explained fruit growth by water and sucrose uptake and for turgor driven expansion regarding the fruit as one big cell. Their model described most lower scale processes empirically. The model of Bertin et al. (2007) explained fruit growth on basis of cell dynamics. This model did not explain cell divisions from biological, underlying processes but merely described these by an empirical function, fitted to the data. Creating a more realistic growth model requires incorporating basic insight into processes related to cell division, cell expansion, endoreduplication and cell fate. These cell dynamics follow from processes that occur across several organizational levels, starting at the gene level and probably ending at the fruit level. Incorporating these organizational levels would facilitate the simulation and prediction of gene-environment interactions explicitly, in a more mechanistic way than the G×E approach that uses Quantitative Trait Loci (QTL) but treats underlying genetic processes as a black box (Quilot et al., 2005; Yin et

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al., 2004). Such a multi-scale model that considers several organizational levels could tackle questions as to what extent tomato fruit sink strength is determined by the developmental, genetic programme of cell and tissue formation, and how much control is exerted by environmental factors during growth. Grosso modo, many of the mechanisms explaining tomato fruit growth can be subdivided into two categories:

- Up-scaling of sub cellular gene related processes to the organ level to unravel the basis for differences in fruit yield among genotypes.
- Exploring possible mechanisms of temperature and carbohydrate effects on fruit growth by including effects on cell division and cell growth.

Although decades of research on tomato have generated much knowledge, only a (small) part of the relevant processes is quantitatively known, potentially hampering model development. Yet, a provisional model could show to what extent the current advances in genetic and physiological knowledge can already predict fruit growth with a bottom-up approach, and identify the gaps in knowledge. A mechanistic modelling approach is proposed, which captures the integrated behaviour of cell division, cell and tissue expansion, endoreduplication, as well as transport and metabolism of carbohydrates. After the initial model is built with the aid of literature data, a further development should be accompanied by parameterisation and validation using experimental data.

METHODS

A model prototype is constructed that in the near future should be followed by experimental verification and possible model improvement, as is often done in systems biology (Kitano, 2002). The modelling and experimental methodology are described below. The experimental methodology shall consist of measurements of fruit growth, cyclin gene expression, cell division, cell expansion and endoreduplication of fruit pericarp tissue at different light, fruit load and temperature levels, for different fruit size genotypes. These results will be reported in a following paper.

The modelling consists of models at different aggregation levels (Fig. 1), described in Sections A-E below, and a platform that exchanges information between models.

Modelling

1. Gene Regulatory Network of the Cell Cycle. An ordinary differential equation (ODE) model on the gene regulated cell cycle was used to derive cell division events. A division was initiated when the checkpoint of the end of the so-called gap phase 2 was reached. For this, the existing generic model of the eukaryotic cell cycle (Tyson and Novak, 2001) was adapted for tomato using 11 cycle genes as observed for tomato (see, e.g., Czerednik et al., 2012) (Fig. 2). The model was also extended with a module on endoreduplication using the mechanisms of the endocycle in Arabidopsis (Magyar et al., 2012). The switch to endoreduplication was determined by passing a threshold of cyclin built-up. The cell mass affecting cyclin concentrations and thus exerting its influence on the checkpoints in the cell cycle, was itself again affected by processes at other scales and therefore, was an important variable in interactions between models.

2. Cell. In the model, fruit cells were initiated at the moment of anthesis, i.e. directly after pollination, and all existing cells obtained a specific identity. The mechanistic model only considered two cell types: undifferentiated proliferating cells and specialized parenchyma cells that expand. The gene regulatory network determined the number of cycles during the cell division period, and the onset and duration of the endoreduplication phase. Endoreduplication was limited to parenchyma cells in pericarp and jelly tissue. Only essential chemical processes for cell growth could be dealt with in our model, given the extreme complexity of the cell's biochemistry. Sucrose was assumed to be the sole form of imported sugar taken up symplastically by cells prior to hydrolysis into hexoses (fructose and glucose) by sucrose synthase (SuSy). The hexoses are an energy and carbon source for growth of cell organelles, growth and maintenance respiration. Hexoses are

also transported into the vacuole where they act as precursors during starch formation (see e.g. Ho, 1996). Most of the hexoses are subsequently transformed into starch (Schaffer and Petreikov, 1997). Starting at around 20 days after anthesis (DAA), starch is degraded to form soluble sugars that support growth since the symplastic supply route for sucrose slowly shuts down (see e.g. Ruan and Patrick, 1995). Shutdown of the symplastic supply route was modelled by an empirical, logistic decrease of vascular tissue conductivity. We applied a simple approximation of these processes by keeping enzyme concentrations constant and explained sugar availability in tissues solely by symplastic sucrose import and starch breakdown. Enzyme reactions were calculated with Michaelis-Menten kinetics. Temperature influence on modelled processes was incorporated by an Arrhenius equation. Ultimately, sugar and temperature driven cell size increase resulted in a new mitosis or endocycle if a specific threshold size was attained (Fig. 3), and under the condition that the checkpoint for end of gap phase (level A) had been reached. No water dynamics were incorporated.

3. Tissues. The model could mechanistically calculate the size, i.e. volume, of two tissues (jelly and pericarp) only, using for each tissue models of level A and B, while the size of other tissues (columella, seed, vascular bundles, endocarp, exocarp) was estimated on the basis of the size of these two tissues. For the mesocarp and pericarp tissue, at anthesis a number of cell classes on the basis of their age in thermal time was assumed. The model kept track of cell numbers and size within each age class. Thus, for each age class a calculation at gene level (A) and cell level (B) that was representative of all cells in the age class was performed. The populations of cells of different ages each formed a single sink for sugars supplied to the fruit. Sugars were allocated according to relative sink strength of the ensemble of cell age classes of all tissues. Tissue growth limitations were apparent at the fruit scale because of the supply of water and sugars from the peduncle. Constraints with respect to growing space for different tissues, physical pressure of surrounding tissue and rigidness of the skin will be simulated in a future 3D model version. The import of sugar and water is directed through vascular bundles, situated in the middle of the pericarp. Sugar and water conductivity of these bundles was derived from their surface area in cross sections of the pericarp, as measured in fruits of different development stages.

4. Fruit. At a truss usually 5 fruits are held which differ in age. From level C for each fruit a certain sink strength per time step was calculated from the total sink of different tissues. Different fruits were assumed to compete for assimilates arriving from the phloem in the supporting truss rachis. Moreover, all trusses on the plant compete for sugars residing in one central pool, and receive sugars in proportion to their sink strength relative to total fruit sink strength (relative sink strength approach following Marcelis et al., 1998). For now, phloem sugar supply is assumed constant within the day and calculated from sugar supply at plant level on a daily basis (see level E).

5. Plant. A mechanistic tomato plant model, based on Marcelis et al. (2008), was used to calculate assimilation and dissimilation of carbohydrates (CH_2O). The plant was described in terms of biomass in the fractions leaves, stems, fruits and roots. The growing leaf biomass was translated into area according to a seasonally changing specific leaf area. Photosynthetically active radiation (PAR) was captured by the leaf canopy quantified by the leaf canopy quantified by LAI and following a Lambert-Beer type negative exponential decay with depth. Absorbed PAR was converted into CH_2O using the photosynthesis equations from Farquhar et al. (1980). The produced assimilates were for 30% allocated to leaf, stem and root growth, and the remaining 70% formed the sugar supply for fruit growth and maintenance.

Platform

The platform managed the tasks of the different models and invoked a computation at a specific aggregation level if a modelling process needed the outcome to proceed. The platform and all the models at different aggregation levels were programmed in Matlab.

RESULTS AND DISCUSSION

Stable oscillations were realized with the cell cycle model. The output consisted of time series of cell division and endocycle events (Fig. 2). This output was generated in advance of the full model calculations, by running a fixed number of scenarios with the cell cycle model. This procedure realized a considerable decrease of computation time of the full model.

At unconstrained sugar and water supply, the logistic growth of cells in the cell division phase generated cells of 0.08×10^{-3} µg and in the expansion phase 0.9 µg maximally. For an endoreduplicating cell, a threshold of 65% of the supposed maximum cytoplasm/DNA ratio should be reached to initiate a new endocycle (Fig. 3). At the default fruit load (5 fruits per truss, and 8 trusses per plant) the unconstrained growth was reduced by 50% on average, using climate conditions of an average year, which is close to estimates for source/sink ratios in tomato (Marcelis et al., 1998).

In the current model set-up the cell clearly has a pivotal role in fruit growth. The different cell types create the upper threshold of the fruit's potential size, because (1) the number of vascular cells determine the supply capacity of sugars and water, (2) the number of pericarp cells together with (3) the cell's maximum size determine the fruit's sink size, (4) the competition between cells for sugars determine the slope of the sugar concentration gradient and the size of the adjacent cells. Yet, such cell related constraints again are a result of internal cell physiological mechanisms, e.g. activity of enzymes that control sink strength. If such internal cell properties are not constant, as we assume here for enzymes, and as was reported for e.g. starch synthesis by N'tchobo et al. (1999), many of the reported mechanisms might not be so strict anymore.

CONCLUSIONS

A fruit model containing five hierarchical scales is shown to contain the most relevant processes that are reported in literature to drive fruit growth. The simulated cell and fruit mass are realistic. The modelled cell dynamics play the most important role in determining the final fruit size.

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Literature Cited

Bertin, N., Lecomte, A., Brunel, B., Fishman, S. and Génard, M. 2007. A model describing cell polyploidization in tissues of growing fruit as related to cessation of cell proliferation. Journal of Experimental Botany 58:1903-1913.

Czerednik, A., Busscher, M., Bielen, B.A.M., Wolters-Arts, M., de Maagd, R.A. and Angenent, G.C. 2012. Regulation of tomato fruit pericarp development by an interplay between CDKB and CDKA1 cell cycle genes. Journal of Experimental Botany 63:2605-2617.

Farquhar, G.D., von Caemmerer, S. and Berry, J.A. 1980. A biochemical-model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78-90.

Ho, L.C. 1996. The mechanism of assimilate partitioning and carbohydrate compartmentation in fruit in relation to the quality and yield of tomato. Journal of Experimental Botany 47:1239-1243.

Kitano, H. 2002. Systems biology: a brief overview. Science 295:1662-1664.

Liu, H.-F., Génard, M., Guichard, S. and Bertin, N. 2007. Model-assisted analysis of tomato fruit growth in relation to carbon and water fluxes. Journal of Experimental Botany 58:3567-3580.

Magyar, Z., Horváth, B., Khan, S., Mohammed, B., Henriques, R., De Veylder, L., Bakó, L., Scheres, B. and Bögre, L. 2012. *Arabidopsis* E2FA stimulates proliferating and endocycle separately through RBR-bound and RBR-free complexes. The EMBO Journal 31:1480-1493.

Marcelis, L.F.M., Elings, A., de Visser, P.H.B. and Heuvelink, E. 2008. Simulating growth and development of tomato crop. Acta Hort. 821:101-110.
Marcelis, L.F.M., Heuvelink, E. and Goudriaan, J. 1998. Modelling biomass production and yield of horticultural crops: a review. Scientia Horticulturae 74:83-111.
N'tchobo, H., Dali, N., Nguyen-Quoc, B., Foyer, C.H. and Yelle, S. 1999. Starch synthesis in tomato remains constant throughout fruit development and is dependent on sucrose supply and sucrose synthase activity. Journal of Experimental Botany 50:1457-1463.
Quilot, B., Génard, M., Lescourret, F. and Kervella, J. 2005. Simulating genotypic variation of fruit quality in an advanced peach x *Prunus davidiana* cross. Journal of Experimental Botany 56:3071-3081.
Ruan, Y.L. and Patrick, J.W. 1995. The cellular pathway of post-phloem sugar transport in developing tomato fruit. Planta 196:434-444.
Schaffer, A.A. and Petreikov, M. 1997. Sucrose-to-starch metabolism in tomato fruit undergoing transient starch accumulation. Plant Physiology 113:739-746.
Tyson, J.J. and Novak, B. 2001. Regulation of the eukaryotic cell cycle: molecular antagonism, hysteris and irreversible transitions. Journal of Theoretical Biology 210:249-263.
Yin, X., Struik, P.C. and Kropff, M.J. 2004. Role of crop physiology in predicting gene-to-phenotype relationships. Trends in Plant Science 9:426-432.

Figures

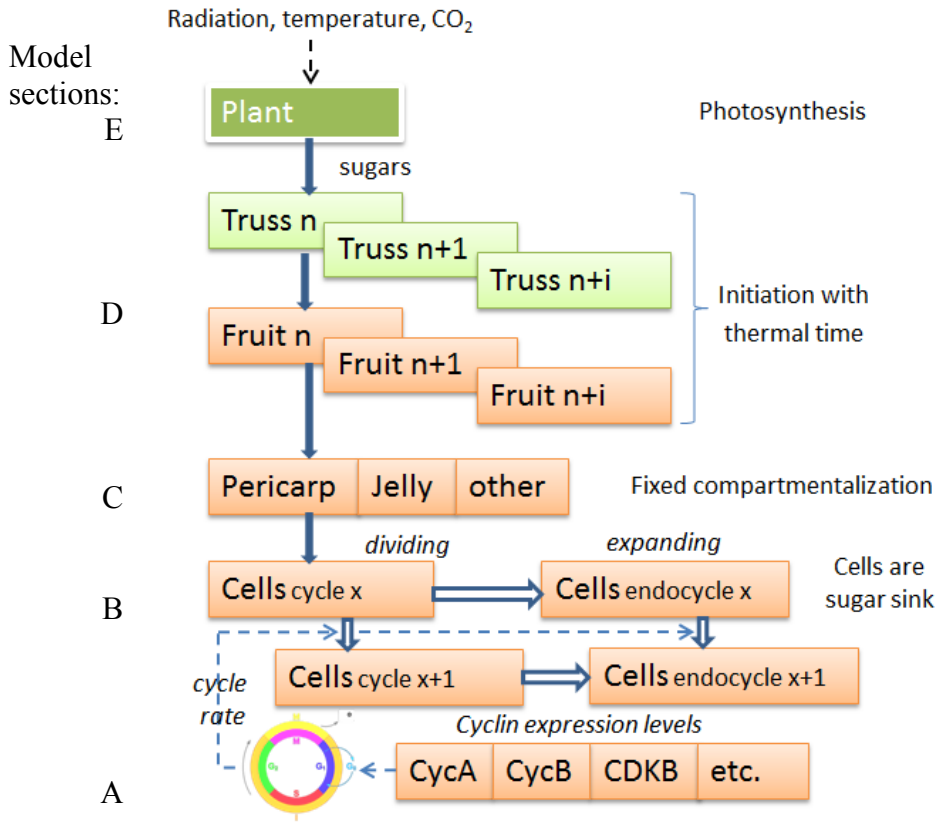

Fig. 1. State variables at the different aggregation levels, dealt with in model sections A to E (see main text). The thick solid arrows between levels indicate sugar flow. The open arrows indicate shift from cell division to expansion phase. The dividing and endoreduplicating cells are the sink for sugars from photosynthesis.

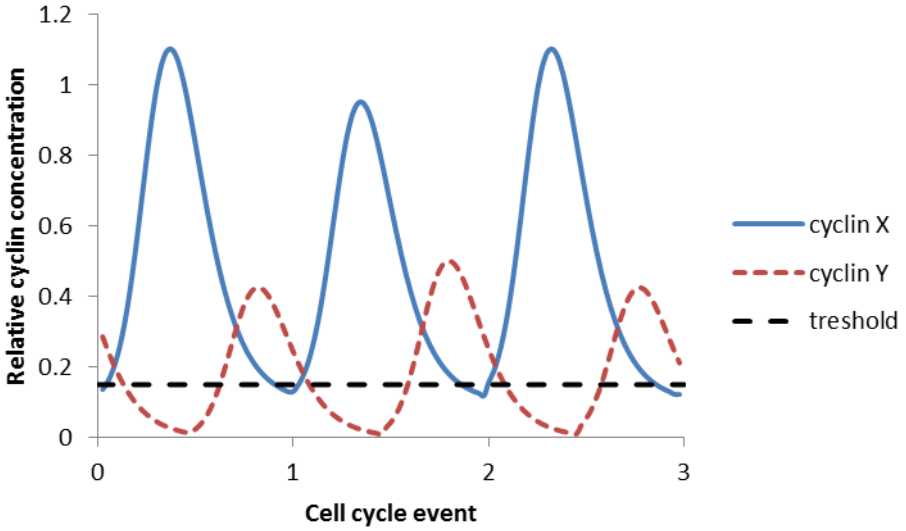

Fig. 2. Illustration of the oscillations of cyclin concentrations in the cell cycle model. A cell cycle event is initiated when cyclin X drops below a given threshold concentration level. Thus, the time period between two divisions depends on cyclin dynamics.

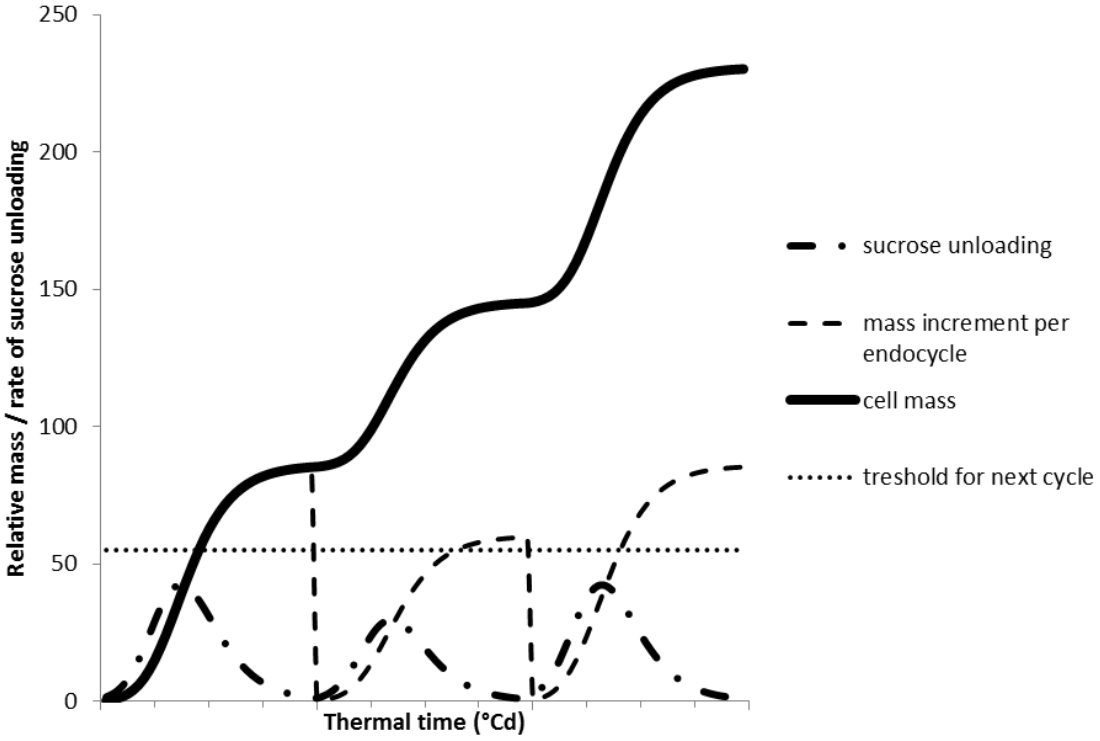

Fig. 3. Simulated development of mass of an endoreduplicating cell and the flux of imported sucrose (arbitrary units). The threshold indicates the minimum ratio of cytoplasm versus DNA required for a new endocycle to occur.