The expression of cell proliferation-related genes in early developing flowers is affected by a fruit load reduction in tomato plants

Pierre Baldet*, Michel Hernould, Frédéric Laporte, Fabien Mounet, Daniel Just, Armand Mouras, Christian Chevalier and Christophe Rothan

UMR 619 Physiologie et Biotechnologie Végétales, Institut National de la Recherche Agronomique, Universités Bordeaux 1 et 2, Centre de Recherche de Bordeaux, BP 81, F-33883 Villenave d’Omon Cedex, France

Received 29 June 2005; Accepted 4 December 2005

Abstract

Changes in photoassimilate partitioning between source and sink organs significantly affect fruit development and size. In this study, a comparison was made of tomato plants (Solanum lycopersicum L.) grown under a low fruit load (one fruit per truss, L1 plants) and under a standard fruit load (five fruits per truss, L5 plants), at morphological, biochemical, and molecular levels. Fruit load reduction resulted in increased photoassimilate availability in the plant and in increased growth rates in all plant organs analysed (root, stem, leaf, flower, and fruit). Larger flower and fruit size in L1 plants were correlated with higher cell number in the pre-anthesis ovary. This was probably due to the acceleration of the flower growth rate since other flower developmental parameters (schedule and time-course) remained otherwise unaffected. Using RT-PCR, it was shown that the transcript levels of CYCB2;1 (cyclin) and CDKB2;1 (cyclin-dependent kinase), two mitosis-specific genes, strongly increased early in developing flower buds. Remarkably, the transcript abundance of CYCD3;1, a D-type cyclin potentially involved in cell cycle regulation in response to mitogenic signals, also increased by more than 5-fold at very early stages of L1 flower development. By contrast, transcripts from fw2.2, a putative negative regulator of cell division in tomato fruit, strongly decreased in developing flower bud, as confirmed by in situ hybridization studies. Taken together, these results suggest that changes in carbohydrate partitioning could control fruit size through the regulation of cell proliferation-related genes at very early stages of flower development.

Key words: Cell cycle genes, flower development, fruit load, Solanum lycopersicum.

Introduction

Plant growth and development depend largely upon the partitioning of assimilated carbon between photosynthetic sources such as mature leaves, and photosynthetically less active or inactive sink tissues such as flowers, roots, and fruit (Farrar et al., 2000). Competition of sink organs for a common pool of carbohydrates is known to influence plant growth (Veliath and Ferguson, 1972; Fisher, 1977; Bohner and Bangerth, 1988). In tomato cultivars with an indeterminate type of growth, the continuous production of fruits generates a collection of strong sinks in the plant. Reduction of sink number by flower pruning enhances plant and fruit growth by modulating the competition between leaves and fruits for assimilates (Hurd et al., 1979; Heuvelink and Buiskool, 1995; Gautier et al., 2001; Bertin et al., 2002). The sink strength of a developing fruit, i.e. its capacity to attract photoassimilates, depends both on its sink activity and size. The young growing fruit, which constitutes a strong utilization sink with high sugar transport and metabolic activity, has a high sink activity. Its sink size, which is influenced by fruit position on the truss and efficiency of photoassimilate transport in the plant (Ho, 1988), depends on both the number and size of the fruit cells. Cell number is determined by the mitotic activity of the pre-anthesis ovary in the developing flower, and, later, by the mitotic activity of the early developing fruit originating from successful fertilization of the ovules (Bohner and Bangerth, 1988; Gillaspy et al., 1993; Joubès...
et al., 1999; Cong et al., 2002). Fruit cell size is mostly determined during the subsequent phase of cell expansion that precedes the onset of ripening. However, how the plant modulates fruit development according to the size of the carbohydrate pool available remains largely unknown (Bertin, 2005).

One possible mechanism for carbohydrate control of fruit size is the regulation of mitotic activity in pre- and post-anthesis ovaries. Early developmental stages of fruit development require the action of key regulators of the cell cycle machinery, such as the cyclin-dependent kinases (CDKs) complexed to their cyclin regulatory subunits (Joubès et al., 1999, 2001). In plants, as in animals, the determination of organ size also requires the co-ordination of organ growth with cell division, a process mediated through D-type cyclins which associate with CDKs to control the entry into the cell cycle (Mizukami, 2001; Dewitte and Murray, 2003). Recently, fw2.2, a major quantitative trait locus (QTL) accounting for as much as 30% difference in fruit fresh weight between the domesticated tomato and its wild relatives, has been cloned and characterized (Frary et al., 2000). fw2.2 is postulated to function as a negative regulator of cell division in pre-anthesis ovaries and fruit (Frary et al., 2000; Nesbitt and Tanksley, 2001), although its precise role and level of action remain unknown (Tanksley, 2004). Indeed, in nearly isogenic tomato lines containing large- and small-fruit alleles at the fw2.2 locus, the mitotic activity of the developing fruit is inversely correlated with the timing of expression of the fw2.2 alleles (Cong et al., 2002). Furthermore, through analysis of an artificial fw2.2 gene dosage series, Liu et al. (2003) clearly demonstrated that fruit mass is negatively correlated with fw2.2 transcript level. These results reinforce the hypothesis that fw2.2 controls the mitotic activity of the developing fruit and, thus, modulates final fruit size. The main effect of the genetically-induced variations in fruit size (and thus, in fruit sink strength) found in the fw2.2 gene dosage series is the change in inflorescence number, which can be related to the change in the amount of photoassimilates available for flower initiation and growth (Liu et al., 2003). Conversely, whether imposed modifications of photoassimilate distribution in the plant, for example, those triggered by variations in inflorescence number, could affect fruit size by affecting key cell cycle regulators, including fw2.2, in the flower and fruit, remains an open question.

To address this question, a fruit-load reduction experiment was conducted with tomato plants. First, the short-term response of vegetative and reproductive organs to a strong perturbation of the photoassimilate supply was analysed at the whole plant level. Plants differing in fruit number per truss (one versus five fruits) were compared for a range of physiological and biochemical traits. Second, the expression of major cell cycle genes during flower development was investigated, and the impact of such a fruit load change on their expression was analysed. The relationship between these cell cycle genes and the effect of photoassimilate availability on fruit size is discussed relative to their role in ovary development.

Materials and methods

Plant materials and growth conditions

The plants used in this study were domesticated tomato (Solanum lycopersicum L., cv. Palmiro; De Ruiter Seeds, Bergschenhoek, The Netherlands) grown in a greenhouse at Sainte-Livrade/Lot during the years 2002 and 2003. Seeds were sown during the first week of November, and two months after sowing, 280 seedlings were transplanted into stone wool slabs at four plantlets per 2 m loaf (Saint-Gobain Cultilène, Bleiswijk, The Netherlands) with a density of 2.5 plants m⁻². Plants were watered daily with a solution containing 5.1 mM KNO₃, 0.5 mM K₂SO₄, 1.5 mM KH₂PO₄, 3.1 mM Ca(NO₃)₂, 1.5 mM MgSO₄ (pH 5.8), and cultivated with temperature control within the ranges of 24–26/18–20 °C day/night, respectively. From the appearance of the first inflorescence, the plants were pruned to five flowers per truss and pollination was carried out by bees. For developmental studies, flowers were tagged at anthesis and harvested at specific time points during development. The rate of plant growth was determined by measuring the appearance of inflorescences during the time-course of cultivation.

Fruit removal experiment

Four months after sowing, half of the 280 plants were randomly selected to be included in the fruit removal experiment. At this time, on the first ten inflorescences of each plant, the second fruit of the truss was kept while the other fruits of the inflorescence were pruned; these plants represented the load of one fruit per truss (L1 plants). From these L1 plants and for the following month, on each new inflorescence, once it had been ensured that the anthesis of the second flower of the truss had occurred and that fruit would develop, the first fruit and the remaining flowers were removed. The other half of the crop was maintained at five fruits per truss (L5 plants) as control plants. It should be noted that each plant was used only once during the time-course of the experiment. On the first day of the change of fruit load from five to one fruit per truss (referred to as T0) and then at 12 and 30 d after that change (referred to as T12 and T30), flower buds at several developmental stages up to fully-opened flower, and expanding fruits at 25 d post anthesis (dpa) were harvested from six L1 and six L5 plants. Bud length, fruit diameter as well as bud and fruit fresh weights were measured. Dry weights were measured after drying the samples in an oven at 80 °C for one week.

Soluble sugars and starch extraction and assay

The carbohydrate content was measured in fully-opened flowers. Soluble sugars and starch were extracted and assayed as previously described by Baldet et al. (2002).

Extraction of total RNA and estimation of relative transcript levels with RT-PCR

Total RNA from flower buds at several developmental stages was extracted with the NucleoSpin® RNA plant kit (Macherey-Nagel, Hoerdt, France). For RT-PCR experiments, first-strand cDNA was synthesized using SuperScript II RNase H⁻ Reverse Transcription Kit (Invitrogen, Carlsbad, CA, USA) and then PCR was performed as previously described by Baldet et al. (2002), using the set of primers defined in Table 1. Following gel electrophoresis, transfer onto nylon membranes and detection with radiolabelled cDNA probes, the
Effect of fruit load reduction on cell division in flowers

Table 1. Sets of PCR primers used to amplify gene-specific regions and corresponding size of the amplified product

<table>
<thead>
<tr>
<th>cDNA</th>
<th>Primer sequence (5’→3’)</th>
<th>Location</th>
<th>Size (bp)</th>
<th>Accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slactin1</td>
<td>Sense TGG CAT CAT ACT TTC TAC AAT G</td>
<td>323–344</td>
<td>615</td>
<td>U60480</td>
</tr>
<tr>
<td></td>
<td>Antisense CTA ATA TCC ACG TCA CAT TTC AT</td>
<td>915–938</td>
<td></td>
<td></td>
</tr>
<tr>
<td>fw2.2</td>
<td>Sense ATG TAT CAA ACG GTA GGA TA</td>
<td>56–75</td>
<td>438</td>
<td>AF261774</td>
</tr>
<tr>
<td></td>
<td>Antisense CTA TCC ATA TTA GCT TGC C</td>
<td>656–674</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LeCDKB2;1</td>
<td>Sense GGA GGC TGC TGA AAA TGC TG</td>
<td>46–65</td>
<td>521</td>
<td>AJ297917</td>
</tr>
<tr>
<td></td>
<td>Antisense GTA TAA GCT CTG CCA AGT CC</td>
<td>548–567</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LeCycB2;1</td>
<td>Sense GAG GAG GTT TGT GCT CCA CTG GTG G</td>
<td>1192–1216</td>
<td>541</td>
<td>AJ243455</td>
</tr>
<tr>
<td></td>
<td>Antisense AAG TTA GTA ACT GTA CAT GCC C</td>
<td>1709–1733</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LeCycD3;1</td>
<td>Sense TTA TCT TTC ATT GAT CAT ATG AGG</td>
<td>772–798</td>
<td>525</td>
<td>AJ243415</td>
</tr>
<tr>
<td></td>
<td>Antisense CTA GGT AAT CTA GAG AAC AAG ATA TCG</td>
<td>1271–1297</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LeKRP1</td>
<td>Sense CAA CAT TCA GAC CCC TGG TTC C</td>
<td>584–604</td>
<td>296</td>
<td>AJ441249</td>
</tr>
<tr>
<td></td>
<td>Antisense GTC TAG AGA TGA AAC GAG GC</td>
<td>861–880</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Hybridization signal was quantified using a Phosphor imager and Quantity One software (Bio-Rad, Hercules, CA, USA). For each flower stage, six repeats, including two RNA extractions and three independent RT-PCR reactions for each RNA sample, were analysed.

Histological analysis and in situ hybridization

For histological analysis, 8 μm thick sections of flower buds of 0.3–15 mm in length were prepared from 20 flowers harvested on ten individual L1 and L5 plants, as described by Bereretide et al. (2002). In parallel, 1 mm thick sections of the carpel wall of the equatorial region of fruits were also prepared from 20 fruits. For the analysis of the number of cell layers and tissue surface, flower buds at stage 18 (beginning of the corolla limb aperture) and fruits at breaker stage were harvested at the indicated times. On the digital images of these stained sections the number of cell layers of the carpel wall was counted by using Image-Pro Plus 5.0 software (Media Cybernetics, Silver Spring, MD, USA). The surface of the cortex, including the placenta and the columella, but not the ovules, was measured using the same procedure.

For in situ hybridization, flower buds were sampled and processed as previously described (Bereretide et al. 2002). In order to use fw2.2 as a probe, a set of primers spanning nucleotides 56–75 and 656–674 was designed from fw2.2 (accession number AF261774) to PCR amplify tomato genomic DNA. The sense and antisense digoxigenin-labelled riboprobes were generated by run-off transcription using T7 and SP6 RNA polymerases according to the manufacturer’s protocol (Roche Diagnostics, Meylan, France).

Results

Effect of a fruit load reduction on flower development

The effect of a fruit load reduction on plant growth was characterized for all tomato plant organs. In agreement with previous studies (Heuvelink and Buiskool, 1995; Gautier et al., 2001; Bertin et al. 2002), the reduction of the fruit load from five fruits (L5 plants) to one fruit per truss (L1 plants) resulted in the increase of the dry weight, the length, and the surface area of all plant organs (data not shown). As shown in Fig. 1A, both dry weight, length of fully-opened flowers, and diameter of fruit at 25 dpa were affected by fruit load reduction. Interestingly, the growth rate of the plant, defined by measuring the appearance rate of the inflorescence (0.8±0.1 truss per week) remained unchanged, whatever the growth condition (data not shown). The soluble sugar content of fully-opened flowers and fruit at 25 dpa was similar in L1 and in L5 plants, whereas starch content was significantly reduced in flowers and increased in fruit (Table 2). Stem and root accumulated high levels of starch, like fruit, while the root was the only organ to display a 2-fold increase in soluble sugar content (data not shown).

Since the growth of the flower bud was stimulated (Fig. 1A), the effects of a low fruit load on the entire flower development in L1 plants (L1) relative to L5 plants were investigated in detail. In this study, flower growth was measured from the 1 mm length bud stage through the fully-opened stage. Although the flowers of L1 plants were larger (Fig. 1B), the complete development of tomato flowers took 20 d, whatever the fruit load (Fig. 1C). The discrepancy in bud length appeared as early as 14 d before anthesis and became even more pronounced later on. Interestingly, the basis of such a difference between the two growth curves did not lie in the chronological appearance of the different flower developmental stages, as defined by Brukhin et al. (2003). For instance, megaspore meiosis (stage 12) occurred 8 d before the fully-opened flower stage in both L5 and L1 plants and corresponds to 8 mm buds in L5 plants but to 10 mm buds in L1 plants (Fig. 1C). To what extent an increase in ovary size could be related to the increase in flower size observed in Fig. 1B was investigated next. Histological analysis of tomato flowers from L1 and L5 plants (Fig. 2) clearly indicate that, in L1 plants at the beginning of the corolla limb aperture, the number of cell layers in the carpel wall and the surface of the placental cortex increased 25% and 60%, respectively. In ripening fruit (breaker stage), the same relative difference in number of cell layers was observed in the carpel wall, whereas cortex surface increased by 2.5-fold (Fig. 2). These data suggest that the increase in fruit size induced by low fruit load is most likely triggered very early in carpel development in the developing flower, but also results from later cell proliferation events affecting mostly the cortex area.
Expression of cell cycle genes and of fw2.2 is affected in flower buds from plants cultured under low fruit load

Since the fruit size increase in L1 plants could be due to a significant increase in the ovary cell number before anthesis, it was determined whether the change in fruit load (L1 versus L5 plants) could affect the expression of key components of the cell cycle machinery and regulation. Therefore, the expression profiles were investigated in L5 and L1 flower buds of the mitosis-specific genes CDKB2;1 and CYCB2;1, which encode proteins involved in the G2-M transition of the cell cycle (Joubès et al., 1999, 2001; Dewitte and Murray, 2003), of the D-type cyclin CYCD3;1, a gene positively regulated by sugars (Dewitte and Murray, 2003) whose product drives the entrance into the cell cycle, and of the CDK inhibitor KRP1 (Bisbis et al., 2006), which encodes a putative interactor of the CDK/CycD3 kinase complexes (De Veylder et al., 2001). In L5 flower buds, the expression patterns of CDKB2;1 and CYCB2;1...
were similar, with high transcript levels in flower buds at stage 3 declining gradually throughout flower development (Fig. 3), which is consistent with high mitotic activity at the very early stages of flower development. By comparison, CYCD3;1 displayed much less variation of expression during flower development and KRP1 expression appeared almost constitutive. For L1 flower buds, only data obtained after 30 d are presented since the expression of all the genes measured earlier in time after the fruit load reduction displayed comparable patterns (data not shown). The most striking differences in gene expression were observed between L1 and L5 flower buds for the mitotic activity markers CDKB2;1 and CycB2;1 and, particularly, for CYCD3;1. When compared with L5 flower buds, CDKB2;1

Table 2. Effects of a reduction of the fruit load on the carbohydrate content of tomato flower and fruit

Changes in sugar content, including total soluble sugars and starch, were measured in intact fully-opened flowers (stage 18) and fruit at 25 dpa harvested as described in Fig. 1. Each measurement of sugar content is the mean of three extractions performed on flowers and fruit from six L1 and L5 plants, ± 95% confidence intervals.

<table>
<thead>
<tr>
<th>Time</th>
<th>Fruit load</th>
<th>Flowers at stage 18</th>
<th>Fruit at 25 dpa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total soluble sugars (nmol eq. Glc mg⁻¹ DW)</td>
<td>Starch (nmol eq. Glc mg⁻¹ DW)</td>
</tr>
<tr>
<td>0 d</td>
<td>L5</td>
<td>287±34</td>
<td>6.9±1.3</td>
</tr>
<tr>
<td>30 d</td>
<td>L5</td>
<td>297±53</td>
<td>8.1±1.9</td>
</tr>
<tr>
<td></td>
<td>L1</td>
<td>294±41</td>
<td>5.3±0.9</td>
</tr>
</tbody>
</table>

Fig. 2. Histological analysis of flowers from L1 and L5 plants. Median transverse sections of flowers at the beginning of the corolla limb aperture (stage 18 according to Brukhin et al., 2003) and fruit at breaker stage were taken 20 d and 30 d after the beginning of the fruit load change. Staining the section with toluidine blue allowed the measurement of the number of cell layers of the carpel wall and the surface of the cortex area that includes the placental cortical area and the columella. Data for L5 and L1 plants, illustrated by empty and black bars, respectively, represent the mean (±SD) of 20 flowers and fruits from six plants cultured in the two fruit load conditions. Student’s tests show that the means of L1 and L5 plants are significantly different (P < 0.05) in surface and number of cell layers. Bar represents 1 mm for flower and 1 cm for fruit.
and CYCB2;1 transcript levels were higher in very early stages of flower development (stages 3–9) in L1 plants and remained elevated until stages 17–20 (Fig. 3). An even more dramatic effect was observed for CYCD3;1, which displayed a 5–6-fold increase in transcript levels at stages 3–9 of flower development and only dropped to a low basal level in flowers close to anthesis (stages 17–20). Finally, it is also worth noting that although not significantly different, KRP1 transcript levels were found to be systematically lower in L1 flowers, throughout the entire developmental period.

In addition, RT-PCR analysis revealed that the transcript levels of fw2.2 in developing flowers were significantly different in L1 plants versus L5 plants (Fig. 4). In L5 plants, fw2.2 transcripts were very abundant early during flower development (stage 3), declined gradually up to the beginning of corolla limb aperture (stage 17) and resumed at anthesis (stage 20). The same trend was observed in L1 plants, except that the levels of the fw2.2 transcripts were significantly lower at stage 3 and higher at stage 20 in L1 flower buds. In order to confirm RT-PCR results and to detail the spatial distribution of fw2.2 transcripts in developing flowers further, flower buds (stages 1–14) from

![Fig. 4. Expression analysis of fw2.2 during flower development. The fw2.2 mRNA relative abundance normalized to ACTIN1 was determined throughout flower development as described in Fig. 3. Star symbols above bars indicate that Student’s tests show that the means in mRNA relative abundance are significantly different ($P < 0.05$) in L1 and L5 plants.](https://academic.oup.com/jxb/article-abstract/57/4/961/558894)}
L1 and from L5 plants were compared by in situ hybridization (Fig. 5). In L5 plants, during the early stages of flower development (stages 1 and 3), transcripts were detected in the whole flower meristem and particularly in emerging primordia, with the exception of sepals. From stages 7–10, which are characterized by the appearance of archesporial and microsporogenesis, transcripts were present at a high level in stamens and carpels (stage 9). Later on (stage 14), transcripts were essentially detected in ovules. By contrast, in L1 flowers, fw2.2 hybridization signal of flower buds at stages 1, 3 and 9 was fainter than that observed in L5 flowers, thus confirming RT-PCR data (Fig. 4). In particular, the weaker labelling of stage 9 flowers, which is consistent with the 40% reduction in fw2.2 mRNA abundance previously observed in L1 flower buds (Fig. 4), appeared mostly to affect the stamens and the carpel.

Fig. 5. Detection of fw2.2 in developing flowers by in situ hybridization. Longitudinal sections of flower buds at stage 1, 3, 9, and 14 as defined according to Brukhin et al. (2003), harvested on L1 and L5 plants 20 d after the beginning of the fruit removal experiment, were prepared and analysed as described in the Materials and methods. Hybridization signal appears as dark staining. Compared to L1 flowers, L5 flowers show a heavy labelling in the carpel (ca) and stamens (st) at stage 9 and in ovules (ov) at stage 14 and a lighter signal in sepals (se) and petals (pe).
Discussion

Fruit removal from the plant increases ovary/fruit cell number and organ size through enhanced flower growth rate

It has long been known that alterations of assimilate partitioning induced by modifications of leaf/fruit ratio and CO₂ enrichment (Bertin et al., 2002) or flower pruning (Gautier et al., 2001) affect the development of tomato plants and fruit. Indeed, in this experiment, the reduction of the tomato fruit load from 5 fruits per truss (L5 tomato plants) to 1 fruit per truss (L1 tomato plants) effectively resulted in a strong increase in the growth rate of all the plant organs analysed, including flowers and fruit (Fig. 1A), as well as leaves, stem, and roots (data not shown). In particular, 30 d after fruit removal, flower bud length and fruit diameter were increased by 38% and 28%, respectively, in L1 plants compared with L5 plants (Fig. 1A). Enhanced growth was not accompanied by spectacular changes in carbohydrate content in L1 plants, except in roots and stem (Table 2 and data not shown). Soluble sugar and starch contents of the flower and fruit were not or hardly affected (Table 2). This strongly suggests that the carbon excess resulting from the reduction in sink number in L1 plants was redistributed throughout the plant and invested in carbohydrate storage (e.g. roots) and/or growth (e.g. flower and fruit) in sink organs. Modifications of the growth rate of L1 flowers and fruits could also arise from the indirect effects of fruit removal on the hormonal status of the plant, for example, distribution and levels of cytokinins in the flower and fruit, since hormones participate in the regulation of early fruit development (Bohner and Bangerth, 1988; Gillaspy et al., 1993).

Higher flower growth rate is associated with increased mitotic activity at early stages of flower development

Larger fruit size in L1 plants was probably linked to the larger number of cells in the pre-anthesis ovary (Fig. 2). Analysis of carpel wall from L1 fruit at breaker stage, i.e. when cell number and cell size are almost fixed in the fruit (Gillaspy et al., 1993; Joubé et al., 1999), indicated that, in ripening flower, the relative increase in cell number was the same as that observed in pre-anthesis ovary and that fruit cell sizes were not different in L1 and L5 fruits (Fig. 2). These results exclude a strong effect of fruit removal on the early stages of fruit development, during the cell division or cell expansion phases (Gillaspy et al., 1993). Conversely, although this is not an absolute rule (Tanksley, 2004), a strong link between fruit size and pre-anthesis ovary size is often observed in a variety of conditions affecting fruit size, ranging from fruit position on the truss, modifications in environmental conditions (Bertin et al., 2002; Bohner and Bangerth, 1988) or genetic origin of the plant (Coombe, 1976). Remarkably, in the present study, the total time-course and schedule of flower development, from flower initiation to fully-opened flower, were not affected by fruit removal (Fig. 1C). As shown in Fig. 1C, the main change observed between L1 and L5 flower bud development was the increased growth rate of L1 flower buds, which occurred very early during flower development. The most likely explanation for these results is an early increase in mitotic activity of L1 flower buds since, at similar stages of flower development in Arabidopsis, the growth of the flower is mostly driven by intense cell proliferation, first in the flower meristem (stages 2–5), then, from stage 7 onwards, in the gynoecia (Breuil-Broyer et al., 2004).

Indeed, through the comparative analysis of the expression of cell-cycle gene markers in L1 and L5 plants, strong indications were obtained that fruit load reduction can affect the mitotic activity of developing tomato flowers (Fig. 3). Several classes of genes can be considered to be markers of the cell-cycle progression in the plant, among them the plant specific cyclin-dependent kinases CDKB and the B-type cyclins CYCB (de Jager et al., 2005). Both are found only in proliferating tissues, show strong cell-cycle regulation of expression and have been shown to be involved in the G2–M transition of the cell cycle (Dewitte and Murray, 2003; de Jager et al., 2005). In particular, the mitotic activity of the developing tomato fruit undergoing cell division is related to the differential expression of the mitosis-specific genes CDKB2;1 and CYCB2;1 (Joubé et al., 2000, 2001). The repression of CDKB2;1 and CYCB2;1 in response to sugar depletion in tomato cell suspension cultures and excised roots (Joubé et al., 2000, 2001) has been described previously. Similarly, it has been demonstrated that the reduction in tomato fruit growth as a consequence of the decline in photoassimilate supply in tomato plants subjected to extended darkness was related to a strong repression of CDKB2;1 and CYCB2;1 genes inside fruit tissues (Baldet et al., 2002). On the contrary, in this study, it is shown that fruit load reduction in L1 plants led to a strong increase in the expression of both CDKB2;1 and CYCB2;1 genes in young flower buds (stages 3 and 9) (Fig. 3). It is thus tempting to associate the acceleration of the growth rate during the early stages of flower development in response to increased availability of photoassimilate supply with the enhancement of mitotic activities inside flower tissues.

Is fw2.2 involved in the relation between sugar supply and ovary cell proliferation in tomatoes?

Cell proliferation is known to be controlled by stimulatory signals such as sugars and hormones through the activation of D-type cyclins (Dewitte and Murray, 2003). Steady-state CYCD3;1 transcript levels can give an approximation of CYCD3;1-associated kinase activity since CYCD3;1-associated kinase activity and protein abundance mirror expression during the cell cycle (de Jager et al., 2005). Thus, the remarkable increase in CYCD3;1 mRNA (5–6-fold) at the early stages of flower development (stages 3–14) in L1 plants (Fig. 3) strongly suggests that the effects

Discussion

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It has long been known that alterations of assimilate partitioning induced by modifications of leaf/fruit ratio and CO₂ enrichment (Bertin et al., 2002) or flower pruning (Gautier et al., 2001) affect the development of tomato plants and fruit. Indeed, in this experiment, the reduction of the tomato fruit load from 5 fruits per truss (L5 tomato plants) to 1 fruit per truss (L1 tomato plants) effectively resulted in a strong increase in the growth rate of all the plant organs analysed, including flowers and fruit (Fig. 1A), as well as leaves, stem, and roots (data not shown). In particular, 30 d after fruit removal, flower bud length and fruit diameter were increased by 38% and 28%, respectively, in L1 plants compared with L5 plants (Fig. 1A). Enhanced growth was not accompanied by spectacular changes in carbohydrate content in L1 plants, except in roots and stem (Table 2 and data not shown). Soluble sugar and starch contents of the flower and fruit were not or hardly affected (Table 2). This strongly suggests that the carbon excess resulting from the reduction in sink number in L1 plants was redistributed throughout the plant and invested in carbohydrate storage (e.g. roots) and/or growth (e.g. flower and fruit) in sink organs. Modifications of the growth rate of L1 flowers and fruits could also arise from the indirect effects of fruit removal on the hormonal status of the plant, for example, distribution and levels of cytokinins in the flower and fruit, since hormones participate in the regulation of early fruit development (Bohner and Bangerth, 1988; Gillaspy et al., 1993).

Higher flower growth rate is associated with increased mitotic activity at early stages of flower development

Larger fruit size in L1 plants was probably linked to the larger number of cells in the pre-anthesis ovary (Fig. 2). Analysis of carpel wall from L1 fruit at breaker stage, i.e. when cell number and cell size are almost fixed in the fruit (Gillaspy et al., 1993; Joubé et al., 1999), indicated that, in ripening flower, the relative increase in cell number was the same as that observed in pre-anthesis ovary and that fruit cell sizes were not different in L1 and L5 fruits (Fig. 2). These results exclude a strong effect of fruit removal on the early stages of fruit development, during the cell division or cell expansion phases (Gillaspy et al., 1993). Conversely, although this is not an absolute rule (Tanksley, 2004), a strong link between fruit size and pre-anthesis ovary size is often observed in a variety of conditions affecting fruit size, ranging from fruit position on the truss, modifications in environmental conditions (Bertin et al., 2002; Bohner and Bangerth, 1988) or genetic origin of the plant (Coombe, 1976). Remarkably, in the present study, the total time-course and schedule of flower development, from flower initiation to fully-opened flower, were not affected by fruit removal (Fig. 1C). As shown in Fig. 1C, the main change observed between L1 and L5 flower bud development was the increased growth rate of L1 flower buds, which occurred very early during flower development. The most likely explanation for these results is an early increase in mitotic activity of L1 flower buds since, at similar stages of flower development in Arabidopsis, the growth of the flower is mostly driven by intense cell proliferation, first in the flower meristem (stages 2–5), then, from stage 7 onwards, in the gynoecia (Breuil-Broyer et al., 2004).

Indeed, through the comparative analysis of the expression of cell-cycle gene markers in L1 and L5 plants, strong indications were obtained that fruit load reduction can affect the mitotic activity of developing tomato flowers (Fig. 3). Several classes of genes can be considered to be markers of the cell-cycle progression in the plant, among them the plant specific cyclin-dependent kinases CDKB and the B-type cyclins CYCB (de Jager et al., 2005). Both are found only in proliferating tissues, show strong cell-cycle regulation of expression and have been shown to be involved in the G2–M transition of the cell cycle (Dewitte and Murray, 2003; de Jager et al., 2005). In particular, the mitotic activity of the developing tomato fruit undergoing cell division is related to the differential expression of the mitosis-specific genes CDKB2;1 and CYCB2;1 (Joubé et al., 2000, 2001). The repression of CDKB2;1 and CYCB2;1 in response to sugar depletion in tomato cell suspension cultures and excised roots (Joubé et al., 2000, 2001) has been described previously. Similarly, it has been demonstrated that the reduction in tomato fruit growth as a consequence of the decline in photoassimilate supply in tomato plants subjected to extended darkness was related to a strong repression of CDKB2;1 and CYCB2;1 genes inside fruit tissues (Baldet et al., 2002). On the contrary, in this study, it is shown that fruit load reduction in L1 plants led to a strong increase in the expression of both CDKB2;1 and CYCB2;1 genes in young flower buds (stages 3 and 9) (Fig. 3). It is thus tempting to associate the acceleration of the growth rate during the early stages of flower development in response to increased availability of photoassimilate supply with the enhancement of mitotic activities inside flower tissues.

Is fw2.2 involved in the relation between sugar supply and ovary cell proliferation in tomatoes?

Cell proliferation is known to be controlled by stimulatory signals such as sugars and hormones through the activation of D-type cyclins (Dewitte and Murray, 2003). Steady-state CYCD3;1 transcript levels can give an approximation of CYCD3;1-associated kinase activity since CYCD3;1-associated kinase activity and protein abundance mirror expression during the cell cycle (de Jager et al., 2005). Thus, the remarkable increase in CYCD3;1 mRNA (5–6-fold) at the early stages of flower development (stages 3–14) in L1 plants (Fig. 3) strongly suggests that the effects
on cell number observed in the ovary are the result of changes in CYCD3;1 levels in the young flower buds, still largely undifferentiated (Breuil-Broyer et al., 2004). In planta, functional analyses revealed that CYCD3;1 may antagonize KRP, a CDK inhibitor (De Veylder et al., 2001), through strong protein–protein interactions (Jasinski et al., 2002; Zhou et al., 2003). KRPs are thought to integrate inhibitory signals that regulate mitotic activity of plant cells under unfavourable growth conditions (De Veylder et al., 2001) and their expression, at least at the transcriptional level, is indeed regulated by growth conditions (Menges and Murray, 2002). Interestingly the expression of KRP1, encoding a tomato inhibitor of CDK activity (Bisbis et al., 2006), seemed to display a slight and progressive decrease in developing flowers upon the reduction of fruit load in L1 plants. These findings support the hypothesis that the carbohydrate supply to the developing flower may control the aptitude of the cell to progress within the cell cycle by adjusting the expression of key genes such as CYCD3;1 and KRP1 at very early stages of flower development.

In tomato, the major fruit size QTL is controlled by fw2.2 (Frary et al., 2000; Tanksley, 2004). Recently, Nesbitt and Tanksley (2001) proposed a model to explain the role of fw2.2 in the direct control of fruit size and, as a consequence, in sink–source relationships at the whole plant level. According to this model, the changes in timing and intensity of fw2.2 expression in early developing fruit due to the large fruit fw2.2 allele, result in increased fruit size and cell number (Cong et al., 2002) and, hence in increased fruit sink strength and in decreased photoassimilate availability for other plant organs. As a consequence, fw2.2 affects inflorescence number by modulation of photosynthetic partitioning in the plant. By contrast, these results indicate that increasing photosynthetic availability in the plant leads to a decrease in fw2.2 transcript levels early during flower development (Fig. 4) and in ovary carpel (Fig. 5). These changes in fw2.2 transcript levels are inversely correlated with flower bud growth rate (Fig. 1C) and with CYCD3;1, CDKB2;1 and CYCB2;1 expression (Fig. 3). Since fw2.2 acts as a negative regulator of cell division in tomato fruit (Frary et al., 2000), the present results reinforce the early hypothesis of Nesbitt and Tanksley (2001) that fw2.2 may link sugar supply with ovary cell proliferation in tomato flowers. Considering that the fw2.2 gene may encode a Ras-like G-protein (Frary et al., 2000) localized in the membrane (Tanksley, 2004), fw2.2 may function upstream of CYCD3;1 and KRP in a signalling pathway linking the modulation of cell cycle machinery in the developing flowers with hormonal or sugar signals. It is hypothesized that the primary role of fw2.2 in tomato is to adjust flower/fruit growth to the prevailing plant conditions, for example, to the level of photoassimilate supply available for the development of the reproductive organs.

Acknowledgements

We thank the engineers, C Favé, G Talon, F Fraye and all the staff from the AIREL (Association Inter-régionale de Recherche et d’Expérimentation Légumière) at Ste Livrade/Lot, who helped us in managing the cultures. We wish to thank Drs B Ricard and P Scott for language corrections. This work was supported by the Région Aquitaine, the Fonds Européen de Développement Régional (FEDER), and the Groupement d’Intérêt Économique (GIE) ‘Fruits et Légumes’.

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