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ORIGINAL ARTICLES

Effect of Gibberellin and Auxin on Parthenocarpic Fruit Growth Induction in the cv Micro-Tom of Tomato

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Abstract The effect of applied gibberellin (GA) and auxin on fruit-set and growth has been investigated in tomato (*Solanum lycopersicum* L.) cv Micro-Tom. It was found that to prevent competition between developing fruits only one fruit per truss should be left on the plant. Unpollinated ovaries responded to GA_3 and to different auxins [indol-3-acetic acid, naphthaleneacetic acid, and 2,4-dichlorophenoxyacetic acid (2,4-D)], 2,4-D being the most efficient. GA_3- and 2,4-D-induced fruits had different internal morphology, with poor locular tissue development in the case of GA, and pseudoembryos development in the case of 2,4-D. Also, GA_3 produced larger cells in the internal region of the mesocarp (IM) associated with higher mean C values, whereas 2,4-D produced more cell layers in the pericarp than pollinated fruits. The smaller size of GA_3-compared with 2,4-D-induced fruits was due to them having fewer cells, only partially compensated by the larger size of IM cells. Simultaneous application of GA_3 and 2,4-D produced parthenocarpic fruits similar to pollinated fruits, but for the absence of seeds, suggesting that both kinds of hormones are involved in the induction of fruit development upon pollination. It is concluded that Micro-Tom constitutes a convenient model system, compared to tall cultivars, to investigate the hormonal regulation of fruit development in tomato.

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Introduction

Fruit development occurs normally after fruit-set (defined as the changeover from the static condition of the flower ovary to the rapidly growing condition of the young fruit) following ovary fertilization. In the case of tomato (*Solanum lycopersicum* L.), one of the most studied fleshy fruits, fruit growth takes place in two consecutive phases: an active division phase, lasting about 7–10 days postanthesis, and a cell expansion phase (Gillaspy and others 1993). During the growth process the ovary wall develops into a pericarp composed of exocarp, mesocarp, and endocarp, while the placental parenchyma, supported by the columella, grows by division and expansion, enclosing the developing seeds and filling the locular cavities with a jelly-like homogenous tissue (locular tissue) (Ho and Hewitt 1986; Gillaspy and others 1993).

Fruit-set can also be induced by application of plant growth substances to unpollinated ovaries; the role of hormones on tomato fruit growth has been the subject of recent reviews (Gorquet and others 2005; Srivastava and Handa 2005). Application experiments of gibberellins (GAs) to unpollinated ovaries (Sjut and Bangerth 1982/83; Alabadí and Carbonell 1998; Fos and others 2000, 2001) and of inhibitors of GA biosynthesis to pollinated ovaries (Fos and others 2000, 2001) suggest that fruit-set in tomato depends on GAs. Overexpression of genes of IAA biosynthesis (Pandolfini and others 2002) and auxin application (review of Abad and Monteiro 1989; Koshioka and others 1994) also induce fruit-set and growth,

generally more efficiently that GAs (Asahira and others 1967; Bünger-Kibler and Bangerth 1982/83; Sjut and Bangerth 1982/83; Alabadí and others 1996; Alabadí and Carbonell 1998). Transcriptome analysis of expanding locular cells from pollinated fruits showed preferential expression of genes involved in synthesis, transport, and response to auxins in this tissue (Lemaire-Chamley and others 2005). Transgenic lines displaying downregulation of *SlIAA9*, an *Aux/IAA* gene, display parthenocarpic fruit development capability (Wang and others 2005). These observations, together with the increase in the content of auxin-like substances (Mapelli and others 1978) and IAA (Sjut and Bangerth 1981) after anthesis, indicate that these hormones are also involved in tomato fruit-set and development. There is some information indicating that GA and auxin application induces different histologic development of tissue ovaries. For instance, Asahira and others (1968) reported that although auxin induced cell enlargement, GA-treated fruits had poor cell division and enlargement. In contrast, Bünger-Kibler and Bangerth (1982/83) found that IAA application resulted in fruits with smaller cells, whereas GA_3-induced fruits had considerably less but larger cells. Also, development of pseudoembryos (embryo-like or embryoid structures, originated from division of the innermost integument cells and formed in the ovule cavity) with different morphology in auxin- and GA-induced fruits has been described (Kataoka and others 2003).

The tomato cultivar Micro-Tom (Scott and Harbaugh 1989) has been proposed as a convenient model system to perform research on the hormonal regulation of berry fruit development because of its small size, rapid growth, and easy transformation (Meissner and others 1997; Eyal and Levy 2002; Dan and others 2006). It has been shown that the phenotype of this cultivar is the result of point mutations in the genes *Dwarf* (*D*) (encoding 6-deoxocatasterone dehydrogenase, of the brassinosteroid biosynthesis pathway, which leads to mis-splicing), *Self-Pruning* (*SP*) (which controls the determinate/indeterminate phenotype), and *Internode length reduction* (*Ilr*) (probably similar to *Miniature, Mnt*, still uncharacterized) (Martí and others 2006). The dwarf phenotype of Micro-Tom is not the result of GA deficiency (Martí and others 2006). The Micro-Tom cultivar also carries several *Cf* (*Cladosporium fulvum*) resistance genes (Martí and others 2006). Although pollinated ovaries of Micro-Tom develop into normal fruits, it has been suggested this cultivar should be used with caution due to the above-mentioned mutations. In particular, it is not known whether Micro-Tom ovaries respond to hormones shown to be capable of inducing fruit-set in wild-type cultivars.

In this work we have carried out a comparative characterization, at histologic levels, of the response of unpollinated Micro-Tom ovaries to GAs and auxins, and have shown that this cultivar constitutes a good and advantageous system, compared to tall cultivars, to investigate hormonal regulation of fruit development. Parthenocarpic fruits induced by GAs and auxin show different morphologic and histologic development. It is suggested that both kinds of hormones are necessary for normal fruit development after pollination.

Materials and Methods

Plant Material and Growth Conditions

Tomato plants (*Solanum lycopersicum* L.) cv Micro-Tom (seeds obtained originally from Dr A. Levy) were used in the experiments. Plants (one per pot) were grown in 1-L pots with a mixture of peat:vermiculite (1:1), cultured in a greenhouse under 24°C (day)/20°C (night) conditions, and irrigated daily with Hoagland's solution. Natural light was supplemented with Osram lamps (Powerstar HQI-BT, 400W) to get a 16-h light photoperiod.

Only one flower per truss and the first two trusses were left per plant for the experiments, unless otherwise stated. All nonselected flowers were removed 2 days before anthesis.

Plant Hormone Applications

Application of gibberellic acid (GA_3) (Duchefa, Haarlem, The Netherlands), indol-3-acetic acid (IAA) (Duchefa), naphthaleneacetic acid (NAA) (Duchefa), and 2,4-dichlorophenoxyacetic acid (2,4-D) (Duchefa) was carried out to emasculated ovaries the day equivalent to anthesis (day 0) in 10 µl of 5% ethanol and 0.1% Tween 80 (Sigma-Aldrich, St. Louis, MO, USA) solution, and their weight determined 20 days later (cell expansion stage). Control ovaries were treated with the same volume of solvent solution. Flower emasculation was carried out two days before anthesis (day -2) to prevent self-pollination. Due to the low fruit weight variability of the experimental system, only eight ovaries (from four plants) were used per treatment.

Histology and Determination of Cellular Parameters

Ovary and fruit tissue sections were fixed in 4% paraformaldehyde-0.1 M sodium phosphate buffer pH 7.2 by applying a vacuum for 3 min and then for 8 h at 4°C. After dehydration in ethanol (15, 30, 50, 70, 85, and 100% series), the samples were embedded in paraffin [immersed in

Histo-clear (National Diagnostics, Atlanta, GA, USA)/ethanol series (2:1, 1:1, 1:2), followed by Histo-clear/paraffin series (2:1, 1:1, 1:2), and finally paraffin (Paraplast Plus, Sigma-Aldrich].

Sections (8 μm thick) were cut with a microtome (model HM 330, Microm, Walldorf, Germany) and stained with toluidine blue. Sections of 0, 5, and 10 days postanthesis (dpa) were viewed on a microscope (eclipse E600, ACT-1 software, Nikon, Tokyo, Japan) and photographed with a spot digital camera (DMX1200F, Nikon). Tissue fragments from 20, 30, and 45 dpa were observed on a stereomicroscope (SMZ200, Nikon) because of their large size, and images were acquired with a digital camera (Colorview 12 CCD, Olympus, Tokyo, Japan). All measurements were performed in six to eight independent sections, using analySIS® (Soft Imaging System, Münster, Germany) software.

For cell size and shape determinations, squares between 0.5 and 1.5 mm^2 (depending on fruit age) were delimited on sections of external mesocarp (EM), located between the central ring of vascular bundles and epidermis, and on sections of internal mesocarp (IM), between the ring of vessels and endocarp, and their surface area and the numbers of cells inside the squares calculated. Mean cell area was estimated as the ratio square cell area/cell number.

Number of cell layers was estimated by counting the number of cells along a line across the pericarp perpendicular to the epidermis and endocarp (avoiding vascular vessels).

Cell sections were circular (C) or more or less elliptical, and in the latter case their major axis was perpendicular or parallel to the epidermis and endocarp. For cell shape classification we determined the lengths of the major axes perpendicular (A) and parallel (B) to the epidermis in 50–60 cells per fruit region (EM and IM) and section. When $A > B$ the cells were elliptical perpendicular to the epidermis (Pe), and when $A < B$ they were elliptical parallel to the epidermis (Pa). Cell eccentricity ($0 \leq e \leq 1$) is a parameter that determines how much a cell section deviates from being circular (e = 0 for a perfect circle):

$$e^2 = (1 - m^2/M^2),$$

where m = minor axis and M = maximum axis. For classification purposes, cells with $e \leq 0.5$ were considered circular, and those with $e > 0.5$ elliptical. For $e = 0.5$, $m = 0.866M$. This means that we considered a cell as circular when $0.866M \leq m \leq M$, and elliptical when $m < 0.866M$. Therefore, we established that cells with $0.866A \leq B \leq 1.134A$ were C, cells with $B < 0.866A$ were Pe, and cells with $B > 1.134A$ were Pa.

Ploidy Determination

Ploidy was determined in entire 0- and 5-dpa ovaries and in exocarp (peel removed with a razor blade including several layers of cells in addition to the actual epidermis), mesocarp, and locular gel of pollinated and hormone-induced fruits from 10 dpa. Two hundred to 400 mg of fresh tissue was sliced with a razor blade into thin strips smaller than 0.5 mm in a glass Petri dish containing 0.4 ml of nuclei isolation buffer (high-resolution DNA kit, solution A: nuclei isolation, Partec GmbH, Münster, Germany). The nuclei extract was mixed with 1 ml of staining buffer (high-resolution DNA kit, solution B: DAPI staining; Partec GmbH, Münster, Germany), shaken for 1 min, and filtered through 100-μm nylon mesh (Nyblot). The filtrates (more than 5000 nuclei per extract) were analyzed using a Partec PA-II flow cytometer (Partec GmbH). Data were plotted on a semilogarithmic scale. Calibration of C values was performed using young leaf extract.

Peak areas were used to determine nuclei numbers with different ploidy. Ploidy distribution represented nuclei percentages corresponding to all the peaks. Mean C values (MCV) correspond to the sum of the number of nuclei of each ploidy level multiplied by its endoreduplication cycle, divided by the total number of nuclei (Barow and Meister 2003).

Results

Effect of Number and Position of Ovaries per Truss on Fruit Growth

With the aim of establishing a system without competition between growing fruits in Micro-Tom, we induced fruit-set by GA_3 application to different numbers of unpollinated ovaries (1-3) in the first three trusses. GA_3 was chosen because there was previous evidence that GAs are necessary for fruit-set in tomato (Fos and others 2001). All the GA_3-treated ovaries set and developed to mature fruits (Figure 1). The mean weight of the total GA_3-induced fruits was similar in the three trusses, indicating that there was no competition between trusses. However, despite GA_3 being applied simultaneously to the three ovaries of a truss (synchronized treatment), the weight of fruits within a truss depended on fruit position, the fruit coming from the older flower being larger than the fruit coming from the youngest one (Figure 1). In the case of trusses where only one or two unpollinated ovaries were treated with GA_3, some of the untreated ovaries of the same truss also developed parthenocarpically (Figure 1). This means that GA_3 can be transported acropetally and alter the growth of other ovaries within the truss. As a result, only one ovary/

fruit per truss and a maximum of three trusses per plant were used in the experiments.

Response of Unpollinated Ovaries to Auxins and Gibberellin

Three different kinds of auxins were applied to unpollinated ovaries for fruit growth-inducing activity in Micro-Tom: IAA, NAA, and 2,4-D. 2,4-D was active at all the doses assayed (20-2000 ng per ovary), but NAA only at 200 and 2000 ng per ovary, and IAA at 2000 ng per ovary (Figure 2). Unpollinated ovaries treated with solvent solution (control) did not grow (< 5 mg at day 20 compared to about 1 mg at day 0) but did not abscise. Due to the much higher activity of 2,4-D, this auxin was used in all the subsequent experiments. This kind of auxin has also been used as a substitute for IAA by many authors in diverse application experiments.

Weight comparisons of 20-day-old parthenocarpic fruits induced by GA_3 and 2,4-D showed that the maximum response to GA_3 was attained with 2000 ng per ovary, whereas the maximum to 2,4-D was obtained with a dose about ten times lower (200 ng per ovary), and fruits of similar size to pollinated fruits even with just 20 ng per ovary (Figure 3A). Parthenocarpic fruits larger than pollinated fruits could be obtained with high doses of 2,4-D, although the weight of GA_3-induced fruits was always lower than pollinated fruits (Figure 3A).

Although fruits were different sizes, the external morphology of GA_3- and 2,4-D-induced fruits was quite similar to pollinated fruits at optimal doses. Sometimes fruits induced with 2,4-D were elongated at the stylar end (results not presented). At higher doses of 2,4-D (> 200 ng per ovary), the fruits were more flattened (results not shown) and the external surface had a small number of protuberances produced by mesocarp cell proliferation but not connected to the vascular system (Figure 3C). In contrast, the internal morphology of GA_3 and 2,4-D fruits was quite different. Jelly tissue development was almost absent in GA_3-induced fruits at all doses, so these fruits had empty locular cavities (Figure 3B). A similar effect, though less severe, could be observed at supraoptimal doses of 2,4-D (Figure 3B). Also, 2,4-D produced several morphologic effects absent in GA_3-induced fruits: (1) development of pseudoembryos, which remained engulfed into the locular tissues at advanced fruit growth stages (Figure 4C, F). In GA_3-induced ovaries some ovules had limited growth, but most of them degenerated (Figure 4B, E); (2) an increase in the number of vascular bundles (Figure 5C); and (3) development of tracheids connecting transversally the

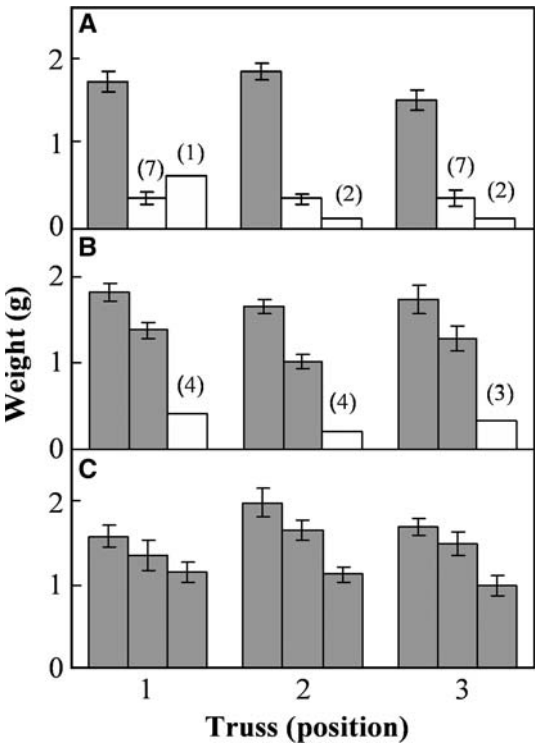

Fig. 1 Effect of number of unpollinatated tomato ovaries per truss treated with GA_3 (2000 ng per ovary) on parthenocarpic growth. **A** One ovary per truss. **B** Two ovaries per truss. **C** Three ovaries per truss. In all cases plants with the first three trusses and three unpollinated ovaries per truss were used, and both treated and untreated ovaries were left on the truss. Fruits were collected 20 days after treatment. Values are means of eight replicates ± SE. All GA_3-treated ovaries developed. In the case of untreated ovaries, figures on histograms indicate the number of ovaries grown (when < 8). At each truss, left bar corresponds to the lower ovary, middle bar to middle ovary, and right bar to upper ovary. Gray bars correspond to GA_3-treated ovaries and white bars to nontreated ovaries. Truss position: 1, lower; 2, medium; 3, upper

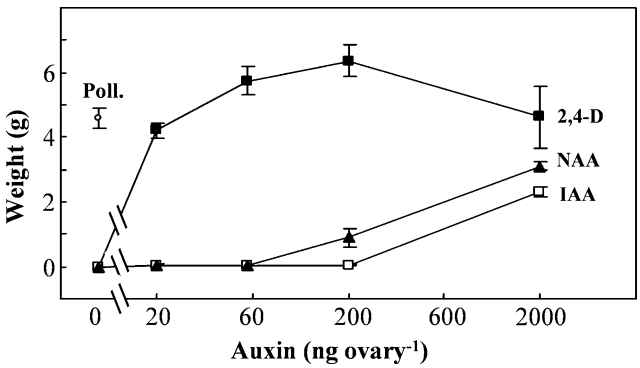

Fig. 2 Dose response of unpollinated tomato ovaries to 2, 4-dichlorophenoxyacetic acid (2,4-D), indole-3-acetic acid (IAA), and naphthaleneacetic acid (NAA) treatment. Fruits were collected 20 days after hormone application, and values are means of eight fruits ± SE. Values of pollinated ovaries are also included as control. Poll., pollinated

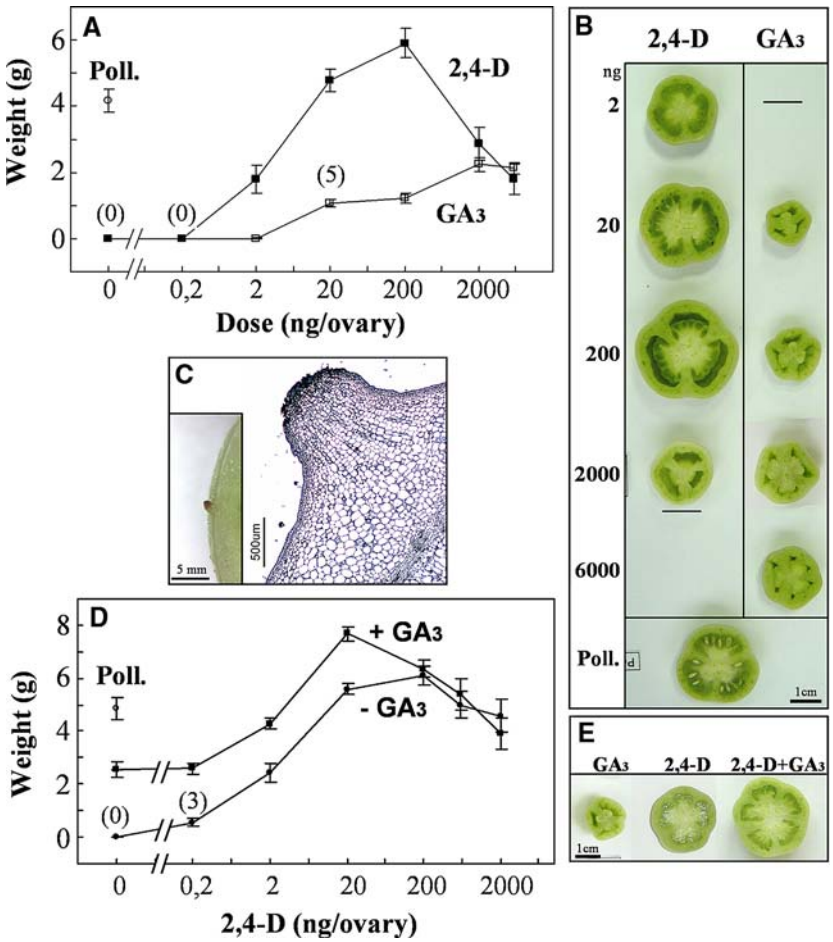

Fig. 3 Response of unpollinated ovaries to GA_3 and 2,4-D treatments. **A** Dose response to GA_3 and 2,4-D (0-6000 ng per ovary). **B** Pictures of transverse sections of representative pollinated, GA_3-, and 2,4-D-induced fruits with different amounts of hormones. **C** Representative protuberance on the epidermis of 2,4-D-induced fruits. **D** Effect of GA_3 (2000 ng per ovary) on the response to 2,4-D. **E** Pictures of transverse sections of representative fruits induced by single and joint application of GA_3 (2000 ng) and 2,4-D (20 ng). Fruits were collected 20 days after treatment. Values are means of eight fruits ± SE. Figures in brackets indicate the numbers of fruits developed when less than eight. Values of pollinated ovaries are also included as control. Poll., pollinated

longitudinal vascular bundles in the cambium region of the pericarp (Figure 5C, D).

The application of GA_3 (2000 ng per ovary) simultaneously with up to 20 ng of 2,4-D produced an additive growth effect. At supraoptimal doses of 2,4-D (200 ng and more), GA_3 could not revert the negative effect of 2,4-D (Figure 3D). Interestingly, at low doses of 2,4-D (2 and 20 ng), simultaneous addition of GA_3 produced fruits of similar size and shape (including development of locular tissue) to pollinated fruits (Figure 3D, E).

Histology of Pollinated and GA_3- and Auxin-Induced Fruits

The different pericarp parameters measured during fruit development are depicted in Figure 6A, which shows a transverse section of a representative 20-day-old pollinated fruit. Because gradient differences between epidermis and endocarp were apparent, two different regions were considered for cell size and shape determinations: external mesocarp (EM), located between the central ring of vascular bundles and epidermis, and internal mesocarp (IM), between the ring of vascular vessels and endocarp, as described in Materials and Methods. Representative micrographs of transverse pericarp sections from GA_3-induced (2000 ng per ovary) and 2,4-D-induced (200 ng per ovary) 20-day-old fruits are presented in Figure 5B and C. Figure 5D shows a mesocarp region containing more bundles of tracheids in 2,4-D-treated fruits (as mentioned before).

Fruits induced by GA_3 and 2,4-D had thicker pericarp than pollinated fruits ($P = 0.008$) throughout development, more with 2,4-D than with GA_3 ($P = 0.001$) (Figure 6A). The number of pericarp cell layers increased between day 0 and day 10 (cell division period) and remained relatively constant afterward (expansion period). Some increase in the number of layers occurred in pollinated and GA_3-induced fruits after day 20. The number of layers produced in 2,4-D-induced fruits was higher ($P = 0.006$) and in GA_3-induced fruits the number was lower ($P = 0.0015$) than in pollinated fruits (Figure 6B). The combined application of GA_3 and 2,4-D had an intermediate effect on pericarp thickness and number of layers compared to separate hormone application (data not presented). No apparent differences in mean cell size (estimated as transversal

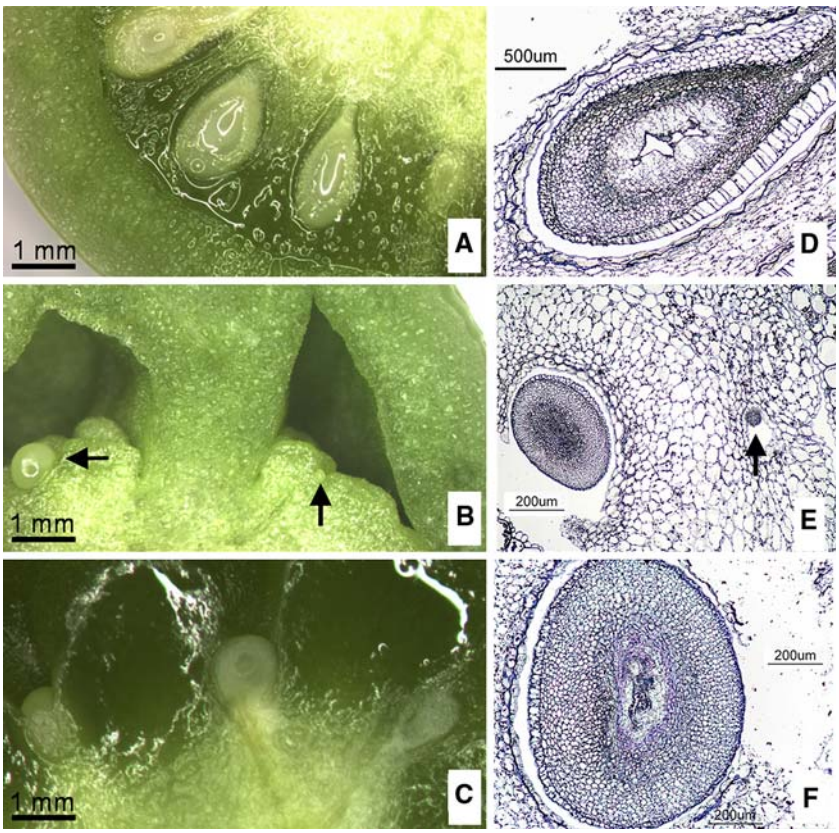

Fig. 4 Images of developing seeds in pollinated fruits (**A, D**), poor developing and degenerated ovules in GA_3-induced fruits (**B, E**), and pseudoembryos in 2,4-D-induced fruits (**C, F**). Cross-sections (D, E, F) were obtained from paraffin-embedded tissues. Fruits were collected 20 days after pollination or hormone treatment. Arrows in C indicate ovule position in GA_3-treated ovaries

section areas) between treatments were found in the EM region of the mesocarp at different developmental stages (Figure 6C). In contrast, significantly larger cells were present in the IM region of GA_3-induced fruits compared to pollinated and 2,4-D-induced fruits ($P = 0.007$) (Figure 6D).

At day 0 similar percentages (about 33%) of circular (C) and elliptic cells with their major axis oriented parallel (Pa) and perpendicular (Pe) to the epidermis were found in transverse sections of the entire pericarp (Figure 7). These percentages changed dramatically during fruit development, mainly during cell expansion, though differently in the external (EM) and internal mesocarp (IM). Compared with day 0, at day 5 (cell division stage) changes in cell shape were found in GA_3-induced ovaries (with higher % of Pe cells in both EM and IM; $P = 0.004$) and in pollinated ovaries (with an increase of Pa cells in EM; $P = 0.008$). In 20-day-old fruits (cell expansion stage) no apparent differences in cell orientation (estimated as % of cell number) between pollinated and GA_3- and 2,4-D-induced fruits were found. In this case most of the cells in EM were Pa (60–70%), whereas in IM they were Pe (more than 80%) (Figure 7). However, in IM the Pe cells were more elliptical in GA_3-induced fruits ($e = 0.78 \pm 0.03$) than in pollinated ($e = 0.68 \pm 0.04$; $P = 0.0001$) and 2,4-D-induced fruits ($e = 0.70 \pm 0.04$; $P = 0.002$). No significant differences of eccentricity were found between fruits in EM cells.

Ploidy of Pollinated and GA_3- and Auxin-Induced Fruits

Ploidy of cells from day 0 ovaries was essentially similar to that of very young leaves, which were used as a reference pattern. This ploidy level increased progressively during fruit development in all fruit tissues (exocarp, mesocarp, and locular gel plus placenta), where cells with C values up to 256 were found in 45-day-old fruits (data not presented). The distribution of C values showed some variation between tissues, but no large differences between pollinated and parthenocarpic fruits were found, particularly for exocarp and locular plus placenta (see Figure 8A as an example for tissues of 20-day-old fruits).

The time course of mean C values (MCV) showed a continuous increase in the exocarp, mesocarp, and locular gel plus placenta during fruit growth. No significant differences of MCV were found between pollinated and GA_3- and 2,4-D-induced fruits in the exocarp (Figure 8B). In contrast, MCVs of mesocarp cells in GA_3-induced fruits were higher than those in pollinated ($P = 0.0003$) and 2,4-D-induced fruits ($P = 0.002$) from 5 days after anthesis

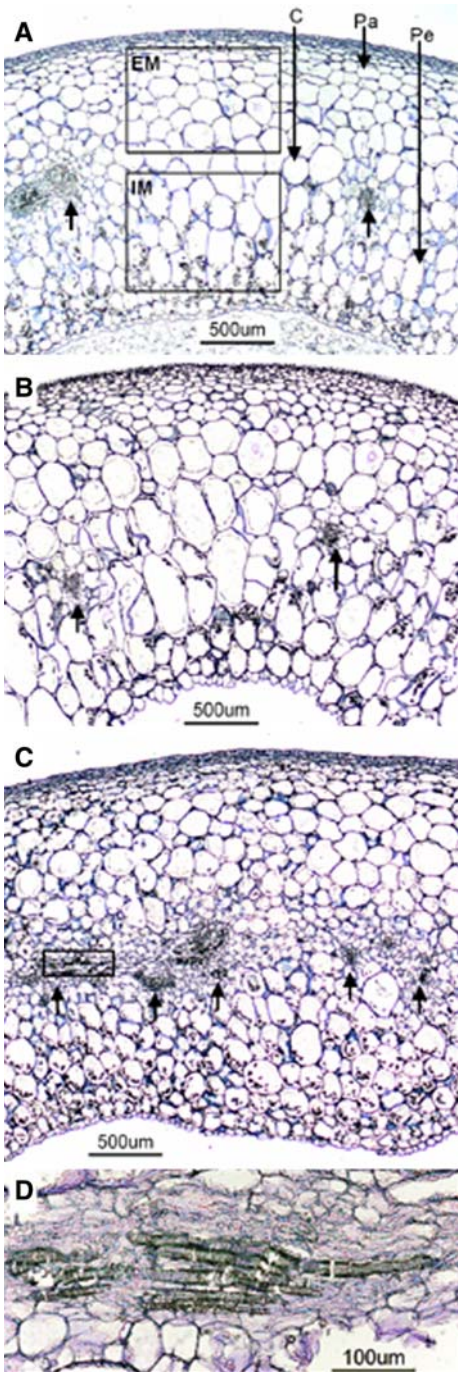

Fig. 5 Effect of GA_3, 2,4-D, and pollination on pericarp histology. Representative pictures of transverse pericarp sections of 20-day-old fruits. **A** Pollinated. **B** Parthenocarpic fruit induced with GA_3 (2000 ng per ovary). **C** Parthenocarpic fruit induced with 2,4-D (200 ng per ovary). **D** Enlarged square from C showing representative tracheid bundles developed in 2,4-D-induced fruits

(Figure 8B and inset). In locular gel plus placenta, MCVs of GA_3-induced fruits were also higher than those of 2,4-D-induced fruits ($P = 0.001$) from 15 days after anthesis, but smaller than pollinated fruits ($P = 0.001$) (Figure 8B).

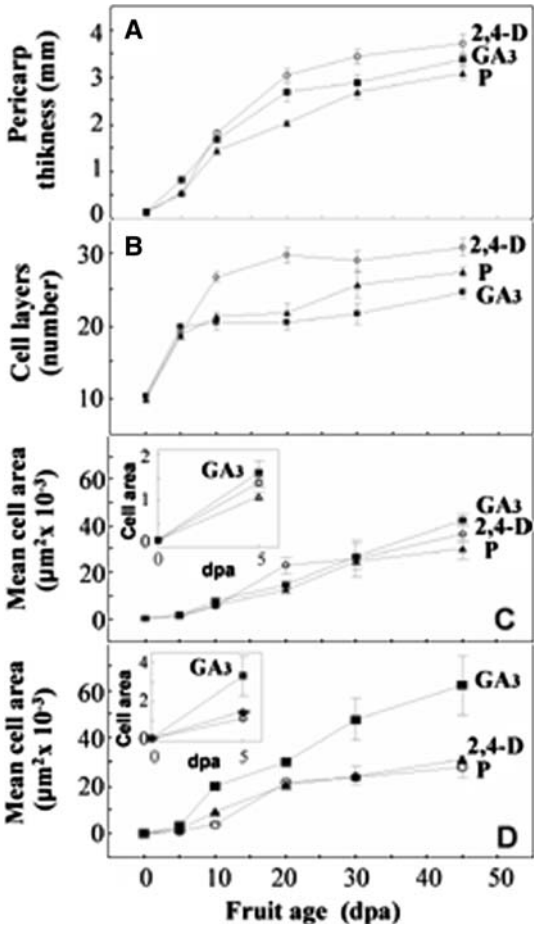

Fig. 6 Effect of GA_3, 2,4-D, and pollination on pericarp development. **A** Pericarp thickness. **B** Number of cell layers. **C** Mean cell area of external mesocarp. **D** Mean cell area of internal mesocarp. Values are means of eight sections, one section per fruit, and eight fruits. The parameters determined are indicated in the transversal pericarp section of pollinated ovary (Figure 5A). EM, external mesocarp; IM, internal mesocarp; C, circular cell; Pa, elliptic cell parallel to the surface of the pericarp; Pe, elliptic cell perpendicular to the epidermis. Arrows indicate the position of longitudinal vascular bundles

Discussion

Micro-Tom as a Model System to Investigate the Hormonal Regulation of Fruit-Set and Growth

We have found that unpollinated ovaries of tomato cv Micro-Tom grow parthenocarpically in response to GA and auxin application (Figures 2, 3). This means that in spite of carrying the mutations *d*, *sp*, and *ilr*/*mnt* that produce a very dwarf habit and small fruits (Martí and others 2006), the response of this cultivar to GAs and auxins was similar to that observed previously in diverse wild-type cultivars of tomato (Asahira and others 1967; Sjut and Bangerth 1982/83; Koshioka and others 1994; Alabadí and others 1996; Alabadí and Carbonell 1998; Fos and others 2000, 2001).

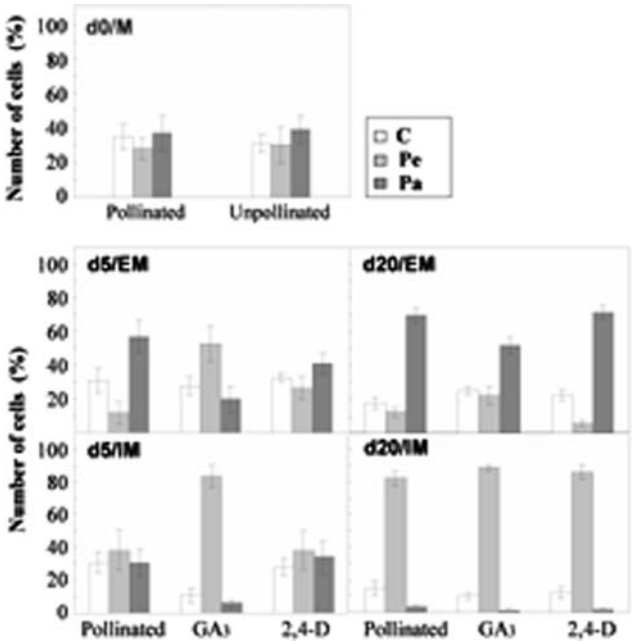

Fig. 7 Effect of GA_3 (2000 ng per ovary), 2,4-D (200 ng per ovary), and pollination on mesocarp cell shape. White bars correspond to circular cells (C), light-gray bars to elliptic cells perpendicular to the epidermis (Pe), and dark-gray bars to elliptic cells parallel to the epidermis (Pa). See Materials and Methods for definitions of C, Pa, and Pe cells. Determinations were carried out at day 0 (equivalent to anthesis), at day 5 (representative cell division stage), and at day 20 (representative cell expansion stage). External (EM) and internal (IM) mesocarp regions were analyzed separately at days 5 and 20. Values are means of 50-60 cells per region and fruit, from six fruits ± SE

Therefore, our results support the proposed idea that Micro-Tom may be a good system to investigate the hormonal regulation of berry fruit development (Meissner and others 1997). Moreover, compared to tall cultivars, Micro-Tom has the advantage of having a reduced stature, which allows many plants to be cultivated in a relatively small space and thus a large number of experimental replicates and a shorter fruit development cycle (less than 3 months from seeding compared to more than 4 months in tall cultivars). Interestingly, although Micro-Tom is a brassinosteroid mutant (Martí and others 2006), pollinated fruits grow normally and parthenocarpic fruit-set could not be induced nor enhanced by brassinolide application (data not presented), indicating that brassinosteroids are not involved in fruit-set and growth in tomato. However, the observation that brassinosteroid synthesis seems to be localized in developing tomato fruit, associated with the locular jelly and seeds (Montoya and others 2005), may deserve further investigation. It is common that people use several ovaries per truss in hormone application experiments (Alabadí and others 1996; Fos and others 2000; Kataoka and others 2003). We have shown that to prevent growth competition between fruits in Micro-Tom, only one ovary per truss should be used because hormone transport between ovaries of a truss occurs, at least in the case of applied GAs (Figure 1). The reduced number of fruits that have to be left per plant to prevent competition may be a disadvantage compared to tall cultivars showing weaker or no competition between fruits.

Gibberellins and Auxins Have Different Effects at Morphologic and Histologic Levels

Parthenocarpic fruits induced by optimal doses of GA_3 and 2,4-D had external shapes quite similar to pollinated fruits. However, at high doses of 2,4-D some malformations were apparent (similar to those described for overexpression of IAA-biosynthesis genes; Pandolfini and others 2002) and small epidermal protuberances were produced that were never found in pollinated and GA_3-induced fruits

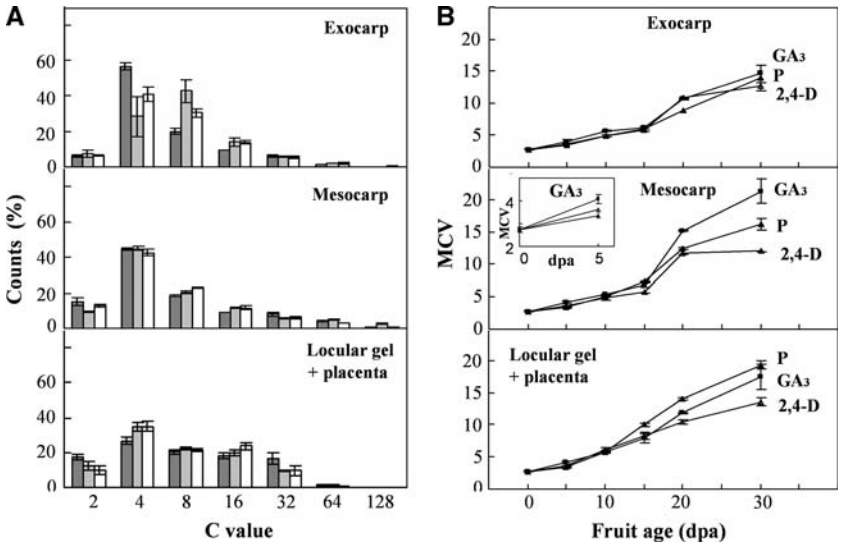

Fig. 8 Effect of GA_3 (2000 ng per ovary), 2,4-D (200 ng per ovary), and pollination on fruit cell endoreduplication. **A** Percentages of nuclei with different C values in exocarp, mesocarp, and locular and placenta tissues from 20-day-old fruits. **B** Time course of mean C values (MCV) in exocarp, mesocarp, and locular and placenta tissues. Dark bars, pollinated fruits; gray bars, GA_3-treated ovaries; white bars, 2,4-D-treated ovaries

(Figure 3C). In contrast, GA_3- and 2,4-D-induced fruits were quite different internally in that locular tissue of GA_3-induced fruits barely developed, resulting in almost empty locular cavities, whereas 2,4-D-induced fruits had filled locules similar to those of pollinated fruits (except at very high doses of hormone) (Figure 3B). Puffiness following GA_3 and 2-hydroxymethyl-4-chlorophenoxyacetic acid (another synthetic auxin) application has been described previously (Asahira and others 1968); in the latter case, however, it was probably the result of an overoptimum amount of applied auxin, as occurred with 2,4-D (Figure 3B), although no dose effect was investigated. Another interesting difference between GA_3- and 2,4-D-induced fruits was that most of the unfertilized ovules degenerated in the former case, whereas they developed to some extent in the case of auxin application (due to proliferation of the outer integuments and the formation of pseudoembryos from division of the innermost integument cells in the internal ovule cavity) (Figure 4). Induction of pseudoembryos by auxin application has been reported earlier (Asahira and others 1967; Kataoka and others 2003) but their possible relation to parthenocarpic fruit growth is unclear.

Both GA_3- and 2,4-D-induced fruits had thicker pericarp compared with that of pollinated fruits (Figure 6A). However, at the cellular level this was the result of different processes: increase of cell divisions (cell layers) in the case of 2,4-D (Figure 6B), and increase of cell size in the IM in the case of GA_3 (Figure 6D). In addition, 2,4-D (at doses of 20 ng or more per ovary) stimulated vascular development, characterized by an increase in the number of vascular bundles (see arrows in Figure 5C), and multiple transversal bundles of tracheids apparently connecting the former ones (Figure 5D), which were never present in pollinated or GA-induced fruits. This probably facilitates the arrival of nutrients which, together with the higher number of cells, produces the greater growth of auxin-induced fruits. Interestingly, the addition of a small quantity of 2,4-D (2-20 ng) to the optimal dose of GA_3 (2000 ng) produced fruits essentially identical in size and shape to pollinated ones, including normal development of locular tissue and vascular bundles (Figure 3D, E). These observations suggest that there is an interaction between these hormones during tomato fruit development.

Gibberellins and Auxins Have a Different Effect at the Cellular Level

In both pollinated and parthenocarpic grown fruits most of the cells had an elliptical shape, although their orientation was not homogeneous throughout the mesocarp. For instance, most of the cells in the EM were parallel to the epidermis (Pa), whereas in the IM they were perpendicular (Pe) (Figure 7). Interestingly, although there was no qualitative difference in the distribution (percentages) of Pa and Pe cells between pollinated and 2,4-D- and GA-induced fruits, absolute eccentricity values of IM cells in GA_3-treated fruits were significantly higher than those of pollinated and 2,4-D-induced fruits. This effect of GA on polar cell elongation is common in stem-elongating tissues (Cowling and Harberd 1999). It is in contrast, however, to the effect on mesocarp cell expansion induced by GA_3 in other species like pea (Vercher and Carbonell 1991), where multidirectional expansion was observed. The increase of Pe cells in the IM may be relevant to the mode of action of GAs on fruit-set in tomato because this effect could be detected in ovaries 5 days after treatment but not in pollinated or 2,4-D-induced fruits of the same age.

Ploidy in different fruit tissues (exocarp, mesocarp, and locular gel plus placenta) increased progressively during growth due to endoreduplication up to 256C. This agrees with ploidy data reported from different tomato cultivars (Bergervoet and others 1996; Joubès and others 1999; Cheniclet and others 2005). Mature IM cells of fruits induced with GA_3 were larger (Figure 6D) and had higher MCV (Figure 8B) than 2,4-D-induced and pollinated fruits. In the case of 2,4-D lower ploidy compared with pollinated fruits, it was not associated with reduced mesocarp cell size. This suggests that GAs may regulate the size of mesocarp cells by altering ploidy levels. This is in agreement with the strong positive correlation between mean cell size and ploidy observed in 20 tomato lines displaying a large fruit weight range (Cheniclet and others 2005). GA_3-induced fruits also had fewer mesocarp cells (Figure 6B). This raises the possibility that the increase of cell size in GA_3-induced fruits is not direct but due to compensation induced by reduction of cell divisions (a similar effect to that described in relation to leaf morphogenesis; Tsukaya 2006), and that this compensation is mediated by ploidy level alteration. Thus, the smaller size of GA_3- compared to 2,4-D-induced fruits (about 3 times at optimal doses; Figure 3A) would be due to reduced cell divisions in the former, only partially compensated by cell size increase. The effect of GA_3 on ploidy is rapid because it was observed as early as 5 days after hormone application, associated with an increase of IM cells. The importance of ovary cell number at anthesis to final fruit size has been previously reported in pollinated *Lycopersicon pimpinellifollium* fruits using near-isogenic mutants (Bohner and Bangerth 1988). Also, Bertin and others (2003) suggested that the large variation of fruit size between competing fruits from two isogenic lines bearing cells of similar size was due to genes controlling cell division. According to these authors, the fruit size difference disappears in the absence of fruit competition, probably as a result of hormonal imbalance within the truss.

Conclusions

Parthenocarpic fruit-set and growth in response to GA and auxin application in the dwarf cultivar Micro-Tom were similar to those described previously in diverse tall cultivars. Therefore, the results presented show that Micro-Tom, due to its small size and reduced reproductive cycle, is a convenient cultivar of tomato for use as a model in research on the hormonal regulation of fruit-set and growth. It was also found that GAs and auxins seem to interact to regulate fruit growth through cell division and expansion.

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