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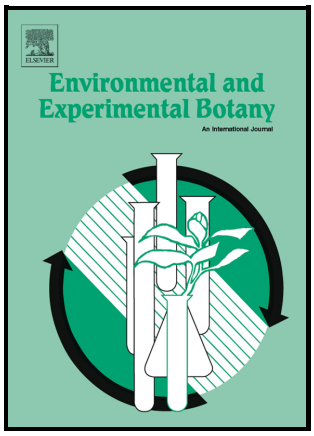

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Evidence of the very early effects of water deficit on cell division and expansion processes in tomato ovaries

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***Keywords*:** Cell division, Cell enlargement, Cell layer, Fruit growth, Tomato ovary, Water deficit.

Abstract

Water deficit (WD) is known to reduce the final mass of tomato fruit. Despite the recorded positive relationship between final fruit mass and cell number, the latter being determined in the pre-anthesis period of ovary development, very few studies have investigated the effects of WD on ovary growth before anthesis. In this study, cell division and expansion in tomato ovaries (cv. H1311) were studied under two irrigation regimes: a control regime that maintained soil water content at field capacity and a WD regime with a 50% reduction in water supply compared to the control. The ovaries were sampled at 6 and 2 days before anthesis, at anthesis, at 2 and 5 days after anthesis, and at breaker stage. Effects of WD were already significant in the pre-anthesis period and the mesophyll layers contributed 96% of the final loss of fruit mass under WD. Overall, the results suggest that cell expansion was the predominant contributor to the loss of fruit mass, although cell number decreased at fruit scale. This was due to the relative contributions of the different layers to fruit cell number and mean cell volume, and to the specific patterns of development and response to WD occurring in each layer. This study provides important information on the regulation of fruit growth processes under WD and will help better predict the impact of it on fruit yield.

***Keywords*:** Cell division, Cell enlargement, Cell layer, Fruit growth, Tomato ovary, Water deficit.

Abbreviations: DAA, days after anthesis, DBA, days before anthesis; E1, outer epidermis layer; E2 (E3), outer sub-epidermal layer; I1, inner epidermis layer; I2, inner sub-epidermal layer; M, mesocarp

layers; M′, new mesocarp cell layers formed after anthesis; LSD, least significant difference; WD, water deficit.

1.1. Introduction

In horticultural crops, the final size and mass of the fruit are critically important in determining yield and are key market criteria. In the context of climate change, these criteria are greatly threatened by environmental fluctuations, being affected in particular by drought and high temperature events, both classed among the highest-impact abiotic stress factors (Ma et al., 2020). In tomato, the final mass of the fruit results from a given number of cell divisions, the number of fertilizations that have taken place in the ovary, and the extent of cell expansion before ripening (Bohner and Bangerth, 1988b; Gillaspy et al., 1993). The processes of cell division and expansion are regulated at several time points in the course of ovary and fruit development, i.e. before and after fertilization (Van der Knaap et al., 2014; Mu et al., 2017). The processes of tomato fruit development and growth after pollination and fertilization of the ovules are well described. Cell division continues 10-20 days after fertilization depending on genotype (Bertin et al., 2009) and both the duration and intensity of division are important factors in determining the final number of cells in the fruit (Zhang et al., 2006; Xiao et al., 2009; Van der Knaap et al., 2014). Concomitant to cell division, the cells expand to reach a 30,000-fold increase compared with initial cell volume (Edgar and Orr-Weaver, 2001; Tanksley, 2004; Chevalier et al., 2014) and cell expansion continues until ripening (Jong et al., 2015).

Many studies have described genetic and environmental variations in tomato fruit mass in relation to fluctuations in pericarp cell number and size (e.g Bertin et al., 2003; Fanwoua et al., 2012). Bohner and Bangerth (1988a) concluded that differences in the final fruit size and cell number between proximal and distal fruits of the same tomato truss result from the different numbers of ovary cells before fertilization. These authors also showed that the notable difference in the size of mature fruits between a wild-type tomato and a semi-isogenic mutant was due to the number of ovary cells at anthesis (Bohner and Bangerth, 1988b). There is, however, little information available on the plasticity of the early stages of fruit cell establishment, i.e. before anthesis and fertilization. Natural mutation in tomato is known to increase the number of cell layers in the ovary, leading to a positive effect on final fruit size (Frary et al., 2000). However, observation of 20 tomato lines displaying a wide range of fruit masses by Cheniclet et al. (2005) revealed a notable lack of change in cell layer number and cell size at anthesis, whereas wide variations in growth occurred after anthesis. Studies of cell numbers for fleshy fruits in other species have shown that cell doubling (mitosis) in apple and grape fruits before anthesis was respectively, 4 and 11 times higher than that following anthesis, leading to the conclusion that there is vast scope for variation in cell number to occur before anthesis (Coombe, 1976).

The impact of water deficit (WD) on cell wall rheology and cell expansion where several mechanisms are activated, has been well-documented (Tardieu et al., 2011; Avramova et al., 2017). By contrast, only a few studies have addressed the effects of WD on cell division in fruit tissues and

there have been differences in the results reported depending on species and genotype (Ripoll et al. 2014; 2016). Reduced cyclin-dependent kinase activity and the ensuing blocked G1-to-S transition under water deficit conditions reduce cell division (Skirycz and Inzé, 2010) in different plant organs such as leaves (Pereyra-Irujo et al., 2008; Avramova et al., 2015, 2017; Koch et al., 2019), roots (Sacks et al., 1997), fruits (Gucci et al., 2009; Pećinar et al., 2020) and seeds (Setter and Flannigan, 2001; Abid et al., 2017). Although information concerning the effects of WD on cell proliferation and cell size in fruit tissue is limited, several studies have shown that tomato plants under WD produce smaller fruits with higher dry matter concentration (Candido et al., 2015; Ripoll et al., 2016; Lovelli et al., 2017; Chakma et al., 2021). Based on Wolf and Rudich's (1988) study, the shorter growth period may account for the reduction in the mass of individual fruits subjected to WD. On the other hand, the effects of high temperature on cell number and size in tomato fruit have been attributed to opposing variations in the duration of cell division and expansion that produce compensatory effects (Bertin, 2005). In addition, cell number and size have been reported to affect fruit quality traits, including dry matter content and texture (Nardozza et al., 2011; Aurand et al., 2012). Fruits with high dry matter content were associated with smaller cells, smaller intercellular spaces and more cell structures, possibly resulting in greater tissue firmness (Reeve, 1970; Aurand et al., 2012). Many studies describe the effects of WD on fruit growth or yield, more rarely considering the relation of final fruit mass with initial ovary growth at the cell level. In the present work we tested the hypothesis that the impact of WD on tomato fruit yield and growth results from specific effects on the kinetics of cell proliferation and expansion processes from the pre-anthesis period and we expect that a better understanding of these effects would allow to propose more efficient irrigation strategies in the context of decreasing water resources. To this end, we investigated the effects of WD on cellular processes in the pre- and post-anthesis periods of ovary development in tomato, and related these effects to differences in final fruit mass. The study was performed on an industrial-type cultivar with a large pericarp which constitutes on average 82% of the total fresh fruit mass at maturity.

1.2. Materials and methods

1.2.1. Plant materials, treatments and sample preparation

The study was carried out from August to December 2021, in a glasshouse located in Avignon, France. Seeds of the determinate tomato cultivar, ‘H1311’, were sown in plug trays and seedlings were transplanted at the 7-leaf stage into 7.5 L pots filled with peat moss (Klasmann Potgrond h70 047). The plants were divided into two groups of 10 plants and each group was subjected to contrasting irrigation treatments from before the occurrence of first anthesis until the end of the experiment: the first group was irrigated so as to maintain soil humidity at field capacity (control), and the second group was irrigated with a 50% reduction in water supply compared to the control (water deficit: WD),

as described in Koch et al. (2019). Photosynthetically-active radiation (PAR) and temperature were measured hourly in the glasshouse. The mean PAR was 565.5 µmol m^2 s^{-1}, and the average day and night air temperatures were 26.2 and 18.0 ºC, respectively over the experimental period.

The developmental stages of the ovaries before anthesis were determined on the basis of the morphological features proposed by Brukhin et al. (2003), by means of daily visual observation, and the non-destructive measurement of the length of the flower buds until the latter reached anthesis. Once anthesis occurred, flowers were tagged to determine ovary age measured in days after anthesis (DAA). The ovaries were sampled from different plants at the first or second proximal points (close to the stalk) in flowering trusses at 6 and 2 days before anthesis (DBA; corresponding to Brukhin et al.'s floral stages 11 and 18 (2003)), at anthesis (0-DBA), at 2 and 5 days after anthesis (DAA; Fig. 1), and at breaker stage (onset of maturation with chlorophyll breakdown). All samplings, including three to five replicates per treatment and per ovarian developmental stage, were carried out between 9 and 10 AM. The samples were fixed immediately after harvest in a fixing solution [90% (v/v) ethanol, 1% (v/v) acetic acid] for 72 h at 4°C, rinsed twice in 70% ethanol, and embedded in 5% agarose gel. Semi-thin (20 µm-thick) equatorial sections of each ovary (4 sections for each ovary) were cut with a vibratome (Leica Biosystems VT1000 S). The cell walls were stained for 1 min in a 10 µg mL^{-1} Calcofluor White M2R solution in 50 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol, 150 mM NaCl (pH 7) (TBS), and the sections were washed in TBS (Brukhin et al., 2013). All sections were observed under an epifluorescence microscope (BX-61; Olympus) at 200x magnification and digital images were taken using the Orca Flash 4.0 LT camera (Hamamatsu). The images were analyzed using ImageJ software (Wayne Rasband) (Schneider et al., 2012).

1.2.2. Cytological analysis of ovary and fruit sections

To estimate the number of cell layers and mean cell size of the mesocarp of the ovary or young fruit, Schneiklet et al. (2005) analyzed microscopic images of pericarp fragments. Renaudin et al. (2017) followed this method and, assuming the mean cell size to be the same in all fruit positions and ignoring the vascular bundles, they introduced the analysis of microscopic images, allowing the cell number per layer and the total cell number of the ovary to be estimated. Accordingly, assuming the ovary shape to be spherical from 6 DBA to 5 DAA, each sample's diameter (d) was calculated from the largest equatorial area measured under a microscope. At the breaker stage, the tomato shape was considered to be a deformed sphere and the fruit's diameter and height (h) were measured with a digital caliper. Pericarp thickness and the number of cell layers were measured in three pericarp segments containing no vascular bundles, using four slices from each of the three ovary replicates (12 regions per developmental stage and treatment). The pericarp was divided into six groups of cell layers consisting of the outer epidermis (E1), outer sub-epidermal (E2), new mesocarp cell layers formed after anthesis (M′), mesocarp (M), inner sub-epidermal (I2), and inner epidermis (I1; Fig. 3). In the three pericarp segments from each sample, the periclinal and anticlinal diameter of cells from layers

E1, E2, I2, and I1 were first measured. The mean periclinal diameter (w_i) and anticlinal diameter (h_i) of each cell layer (i) were then calculated following the method described in Renaudin et al. (2017). The measurement provided for each ovary is thus the average of 12 sets of cytological measurements (4 slices per ovary and 3 pericarp regions per slice).

1.2.3. Statistical analysis

The effects of irrigation treatments on cell characteristics were analyzed in interaction with ovary age using two-way ANOVA (R Software). The least significant difference (LSD) test was used to separate the means ± SE at a probability level of $P \leq 0.05$.

1.3. Results

1.3.1. Effect of water deficit on dry mass, pericarp cell layers and cell number in the pre- and post-anthesis periods of ovary development

Figure 2 shows the evolution of the ovary dry mass, pericarp thickness, cell layers and cell number from 6 DBA to 5 DAA. All sampled ovaries had been initiated and developed during a period when the irrigation treatments were well established. In the control plants, ovary dry mass and pericarp thickness increased by, respectively, 85 and 54% from 6 DBA to anthesis (Fig. 2A and B). More than 60% of this increase occurred between 6 and 2 DBA, while no significant growth occurred between 2 and 0 DBA. Growth resumed after anthesis, with a large increase in ovary dry mass, from 2 DAA (0.65 mg) to 5 DAA (3.63 mg). Over this period, ovary fresh mass increased from 3.5 to 20 mg (not shown). In WD plants, trends were similar, but the increase in dry mass and pericarp thickness prior to anthesis was, respectively, 9 and 41% lower than in the control ovaries. From anthesis to 5 DAA, ovary dry mass increased by a factor of 4 and 2.6 respectively in the control and WD plants. Thus, at 5 DAA, the dry mass and pericarp thickness of WD ovaries were significantly reduced by 40 and 34% respectively compared to the control ovaries.

At 6 DBA, the number of pericarp cell layers was about 8 layers in both the control and WD plants (Fig. 2C). On average, two layers were then added before anthesis in the control ovaries and one layer in the WD ovaries. From anthesis until 5 DAA, the cell layer number increased rapidly under both treatments (+106 and +67% in the control and WD ovaries respectively). The overall cell layer generation rates under the WD and control treatments were, respectively, 0.64 and 1.18 layer(s) per day from 6 DBA to 5 DAA. The rate under WD was 50 and 45% lower than in the control samples prior to and after anthesis respectively.

A continuous increase in the total pericarp cell number occurred during the stages studied. In the control ovaries, the total cell number increased by 37% from 6 DBA to anthesis, was relatively stable between anthesis and 2 DAA, and then increased rapidly by 52% between 2 and 5 DAA. In the WD plants, the trend was similar, but the increases in ovary cell numbers were 42 and 22% lower than in the control plants before and after anthesis respectively. Last, at 5 DAA, the number of ovary cells was

reduced by 17% in the WD plants compared to the control plants, despite similar numbers at 6 DBA (Fig. 2D).

1.3.2. Effects of water deficit on the patterns of cell division and expansion in individual cell layers during the early stages of tomato ovary development

The evolution of ovary cell traits in each cell layer from 6 DBA to 5 DAA is shown in Fig. 3, and the structure of the tomato ovary along with the range of variations in cell volume due to treatment and stage of development are illustrated in Fig. 4. The mean cell number and cell volume in the E1 cell layer of the control ovaries increased by 7 and 41% respectively between 6 DBA and anthesis. After anthesis, and especially from 2 to 5 DAA, cell number in this layer dramatically increased (+68%), while the mean cell volume remained stable (Fig. 3A). In the E2 layer of the control ovaries, cell number and size increased by 4 and 39% before anthesis respectively. Both cell division and expansion continued in the E2 cell layers until 5 DAA (Fig. 3D). Trends were similar in the control and WD ovaries, but the size and number of cells were observed to be significantly reduced under WD. At anthesis, the mean cell volumes of the E1 and E2 layers under the WD treatment were 5.9 and 4.5% lower than under control conditions respectively, and at 5 DAA both were 4% lower than in the control plants. These differences were due to those already observed at 6 DBA, which were maintained, although the rate of cell expansion before anthesis seemed to be slightly higher in WD ovaries than in control ovaries. The relative effects of WD on cell proliferation in these two layers were more severe than those on cell size; in E2, the cell number was 10.4% lower in the WD ovaries than in the control ovaries at anthesis, and in E1 and E2 they were respectively 9.3 and 12.2% lower at 5 DAA (Fig. 3A and B). These differences were due to the smaller number of cells in the WD ovaries at 6 DBA and to a slightly lower division rate after anthesis, especially in the E1 layer.

The M cell volume increased between 6 DBA and 2 DBA and then remained stable for about four days before increasing rapidly after fertilization at 2 DAA. Similar trends were observed in the control and WD ovaries, but the increase in cell volume after 2 DAA was significantly lower in the WD ovaries than in the control ovaries (Fig. 3A). At 5 DAA, the first M′ layers appeared and at this stage, the cell volumes of M and M′ layers were, respectively, 25 and 19% lower in the WD ovaries than in the control ovaries. The number of cells in the M layer increased rapidly during the period of development studied. This increase slowed slightly between anthesis and 2 DAA under both treatments (Fig. 3C). The M cell number at anthesis was 24% lower in the WD ovaries than in the control ovaries, although the numbers were similar at 6 DBA (Fig. 3C). At 5 DAA, the cell numbers of M and M′ layers were, respectively, 12 and 58% lower in the WD ovaries than in the control ovaries (Fig. 3C and D).

In the control plants, the cell volume of the inner sub-epidermal (I2) and epidermis (I1) layers increased by 24 and 48% from 6 DBA to 2 DAA, respectively, and by 79 and 60% from 2 to 5 DAA, respectively. As with other layers, cell expansion slowed down around anthesis and then resumed (Fig.

3E and F). The number of cells in these two layers gradually increased over the observed period and similar trends were observed in the control and WD ovaries. WD significantly reduced the size and number of cells in these two layers, the differences being visible and maintained throughout ovary development from 6 DBA onwards. At anthesis the mean cell size and cell number of the I2 layer were reduced by, respectively, 3.6 and 10.1% under the WD treatment and in the I1 layer they were reduced by, respectively, 11.7 and 1.6% compared to the control (Fig. 3E and F). The heatmaps in Fig. 4 show that the reduction in cell volume due to WD was at its greatest at 5 DAA and in the M layer, although significant differences were observed between treatments at 6 DBA in all layers, but with a smaller variation range.

1.3.3. Contribution of the different cell layers to fruit traits in response to WD depending on developmental stage

At the breaker stage, the average fresh mass of the fruit was 72 g for the control and 44 g for the WD samples. At this stage, the number of cell layers was 37 and 33, respectively, in the control and WD ovaries. WD significantly decreased the cell number in all layers (Fig. 5A) so that the total pericarp cell number was decreased by 38% under WD compared to control fruits, while this relative difference was 15% at anthesis (Fig. 2D). Indeed, from anthesis to breaker stage, the total number of pericarp cells increased 10-fold and 13-fold under WD and control treatments, respectively. This increase was mostly due to cell division in the external layers (+32.8% of cells on average in E1, E2, E3 in the control ovaries and + 22.4% for the same layers in the WD ovaries). In the M+M′ layers, the number of cells increased 7-fold and 5-fold from anthesis to breaker stage under control and WD conditions, respectively, while in the internal layers 2-fold and 1.6-fold increases were observed on average in the control and WD ovaries respectively.

At the breaker stage, the mean cell volume in the M+M′, I2 and I1 layers was significantly higher in the control ovaries than in the WD ovaries, while no differences were observed in the E1 and E2+E3 layers (Fig. 5C). In line with the differences in evolution of cell numbers between anthesis and breaker stage, the expansion was much more important in the M+M′ and I2 layers (with 2723-fold and 2275-fold increases on average in the control and WD ovaries respectively) over this period. In contrast, cells hardly grew at all in the external layers (less than 10-fold under both treatments in E1, E2, E3).

As a consequence, the contributions of the different layers to the whole pericarp were quite different at anthesis and breaker stages (Fig. 5B and D). At anthesis the M layers made the greatest contribution to total pericarp cell number and volume. WD decreased the contribution of the M layer to the number of cells but increased its contribution to pericarp volume. At the breaker stage, the M and M′ layers made the greatest contribution to pericarp volume, but the E1+E2 layers accounted for more than 50% of the total cell number. At this stage, WD increased the contribution of E1 to cell number and the contribution of M+M′ to pericarp volume. Overall, the M layer's decrease in volume

represented 74 and 96% of fruit volume loss due to WD at the anthesis and breaker stages respectively. At anthesis, E1 and E2+E3 accounted for 16% of fruit volume loss and the two inner layers for 10%.

1.4. Discussion

Water deficit is known to decrease final tomato fruit mass and strongly reduce its water content (Mitchell et al., 1991; Ripoll et al., 2014; Cui et al., 2020) as observed in the present study. The extend of these effects depends on the intensity of water deficit and the plant developmental stage at which the period of deficit occurs (Ripoll et al., 2016). In most studies, WD is applied post-anthesis, while in the field it may cover the whole period of fruit development including pre-anthesis stages. In this context, the present study aimed to better understand the impact of WD on the growth processes determining early growth potential of some kind and provide new insights to mitigate the adverse effects of water scarcity on the final yield and quality of horticultural crops.

1.4.1. Water deficit affects the generation of cell layers early during tomato ovary development

The early development of tomato fruit includes several successive stages, i.e. flower bud initiation (10-15 days before macroscopic appearance), flower bud development and pollination (at anthesis), fertilization (from anthesis to 2 DAA), and fruit set (designating fruit growth inception), which is then followed by normal fruit growth (Bertin 1995). All these phases are highly sensitive to temperature and light, but also to competition for carbon-assimilates and the hormonal regulation involved (Atherton and Harris 1986; Kinet et al., 1985; Bertin 1995, 2005). In the present study, ovary dry mass and pericarp thickness were significantly reduced by WD before anthesis, during pollination and fertilization (from 2 DBA to 2 DAA), and after fruit set (from 2 to 5 DAA). The patterns were similar under both treatments, with an arrest in growth around anthesis at the time of fertilization and a subsequent rapid increase, as reported in the literature (Renaudin et al., 2017; Shen et al., 2019). Interestingly the 40% decrease in fruit fresh mass observed at the breaker stage was of the same order of magnitude as the decrease in ovary dry mass at 5 DAA (-40%) and 13% of this difference related to the growth of the ovary before fertilization (Fig. 2A). The increase in pericarp thickness in the pre- and post-anthesis periods was reduced under WD (Fig. 2B), associated with the negative effect of WD on the generation of new cell layers (Fig. 2C). The ovary pericarp contains five epidermal and sub-epidermal cell layers consisting of the outer epidermis (E1), two outer sub-epidermal (E2, E3), inner sub-epidermal (I2), and inner epidermis (I1), along with mesocarp cell layers that are separated in two groups: M layers contain mesocarp cells located around vascular bundles that have been produced before anthesis. M′ layers are produced after anthesis from cells originally in the E2, E3, and, on rare occasions, I2 cell layers (Renaudin et al., 2017). The number of mesocarp cell layers, which mostly results from periclinal and oblique cell divisions, has been reported to be between 4 and 7 layers depending on tomato genotype (Mazzucato et al., 1998; Cong et al., 2002; Fanwoua et al., 2012), and

most are formed during the first few days after anthesis (Xiao et al., 2009; Renaudin et al., 2017). In agreement with these findings, the number of cell layers in the present study doubled from anthesis to 5 DAA, but 6 fewer layers were measured at 5 DAA under the WD treatment, corresponding to a 45% reduction in cell-layer generation rate during this period.

The total cell number increased throughout the stages studied (Fig. 2D), and it was notably reduced by WD at the beginning of flower bud development. The relative difference between treatments increased afterwards, suggesting that no compensation occurred through, for instance, changes in the length of the division period. Indeed, 40% of the relative difference in cell number observed between treatments at the breaker stage had already been recorded at anthesis. This result suggests that cell division in the pre-anthesis period makes a significant contribution to the reduction of final fruit mass under WD when treatment is applied during flower bud development. There are no reports in the literature of the effect of WD on ovary cell division before fertilization. However, the negative effects of WD on fruit cell division and expansion after fertilization has been reported for olive (Gucci et al., 2009) and tomato (Ojeda et al. 2001; Rapoport and Costagli, 2004; Pećinar et al., 2020), with moderate effects when WD was applied at anthesis or after (Ripoll et al., 2016). Besides the negative effect of WD on cyclin-dependent kinase activity (Skirycz and Inzé, 2010), it is also possible that carbon starvation is induced by WD and this could also contribute to a decrease in cell proliferation in fruit tissue (Bertin, 2005; Baldet et al., 2006).

1.4.2. The WD impact on division and expansion is specific to the different cell layers during the pre- and post-anthesis period

As described in the literature for cherry tomato grown under unstressed conditions (Chevalier et al., 2014; Renaudin et al., 2017), each cell layer has an individual pattern of division and expansion. The present study confirms these patterns for a large pericarp tomato cultivar. In addition, it reveals a layer-dependent response to WD. Cell expansion predominated in E1 before anthesis whereas division increased dramatically after anthesis (Fig. 3A). This probably resulted from the increase in the rate of anticlinal cell division (perpendicular to the fruit surface) in the E1 cell layer after fertilization, which was itself necessitated by the increase in ovary volume caused by the growing inner layers. Similar trends were observed in E2, except that the intensity of cell division was much lower after fertilization and that cell enlargement continued until 5 DAA (Fig. 3B). The fact that the concomitant progression of division and expansion seen in E2 was not observed in E1 suggests the co-presence before anthesis of dividing cells and growing-endoreduplicating cells in E2. This is consistent with the ploidy mapping of tomato fruit, which has reported ploidy levels up to 32C in the external cell layers, but not in the outer epidermis (Boudon et al., 2011) and with the rapid increase of ploidy in tomato ovaries during the first days after anthesis (Bertin et al., 2007; Renaudin et al., 2017). WD reduced the cell number and cell volume in the epidermal and sub-epidermal layers, with the difference between treatments remaining stable from 6 DBA to 5 DAA. The inner sub-epidermal (I2) and epidermis (I1)

cells exhibited very different growth patterns from those of the outer layers, but a comparable response to WD. The cell size of these two layers showed a slight increase before fertilization and a dramatic increase after it, while cell division almost ceased after anthesis (Fig. 3E and F). It has already been reported that, after anthesis, cell divisions occurred more frequently in the outer pericarp (Tanksley, 2004; Xiao et al., 2009; Pabón-Mora and Litt, 2011). In the M layers, the number of cells increased rapidly during the period of development studied, cell division ceased during fertilization (0 to 2 DAA) and cell expansion ceased from 2 DBA to 2 DAA (Fig. 3C). While in cherry tomato, the mean cell number in each M layer is constant from 6 DBA (stage 11) to 36 DAA (Renaudin et al., 2017), the present results for a larger fruit cultivar (mean fresh mass of 70 g at maturity) showed an increase in mean cell number in the M layers from 6 DBA to 5 DAA. By contrast with the outer cell layers, WD effects on mesophyll cell numbers increased during the pre-anthesis period and decreased after 2 DAA, whereas mean cell volume was not affected in the pre-anthesis period and greatly reduced after 2 DAA. Similar trends were observed in M′ with a shift in the time of appearance.

1.4.3. Each cell layer has a specific contribution to the loss of tomato fruit mass under WD

Whether at anthesis or at the breaker stage, the mesophyll layers make by far the greatest contribution to loss of fruit mass under WD. This is unsurprising given that the M and M′ layers contribute to more than 90% of fruit volume and these layers were the most affected by WD, especially after anthesis. It can be assumed that, as the cell population enlarges in the mesophyll layers, internal competition for resources increases (Génard et al., 2022), especially since the large vessels are mostly found in the M and M′ layers. In the I2 layer, considerable expansion of cells was also observed between anthesis and the breaker phase, but, here, division ceased at an earlier point and the cell number was relatively low, explaining the small contribution to loss of fruit mass (2.3%). The external layers (E1, E2, E3) accounted for more than 80% of the final cell number and their final mean cell volume was not affected by WD (Fig. 5), meaning that their contribution to loss of fruit mass was minimal (less than 1%). In the external layers, the relative differences in mean cell volume between treatments were at their greatest at 6 DBA. Similarly, differences in cell number were again due to the reduction observed at 6 DBA rather than to a lower division rate except in the M layers, where the rate of cell proliferation in the pre-anthesis period was reduced by WD. This initial difference was probably caused by an extremely early effect of WD on the size of the floral meristem (containing three cell layers) in relation to phytohormone regulation (Filippis et al., 2013).

Overall, the results suggest that it is cell expansion rather than cell division that contributed to the loss of fruit mass under WD, although cell number was reduced at the fruit level. This was due to the relative contribution of the different layers to fruit cell number and mean cell volume, and to the patterns of development and response to WD specific to each cell layer. Because the external layers accounted for most of the cell number in the mature fruit and for the least of the fruit volume, the

contribution of cell division to the loss of fruit mass under WD seems to be limited at whole fruit scale.

1.5. Conclusions

Our results provide new insights to understand the regulation of fruit growth by WD and highlight the need to better consider the different phases of development affected. They also suggest integrating the specific contribution and responses to WD of the different cell layers. To this end, predictive models which equate the fruit with its cell population (e.g. Baldazzi et al., 2019) may be useful. In the future, these models should consider cell subpopulations with specific developmental patterns to improve the prediction of fruit mass plasticity.

Author contributions

NB and ALF proposed, organized, and planned the experiment. LA and TB carried out the experiment. LA produced the draft manuscript, with improvements from NB. All authors commented on and contributed to the preparation of the final manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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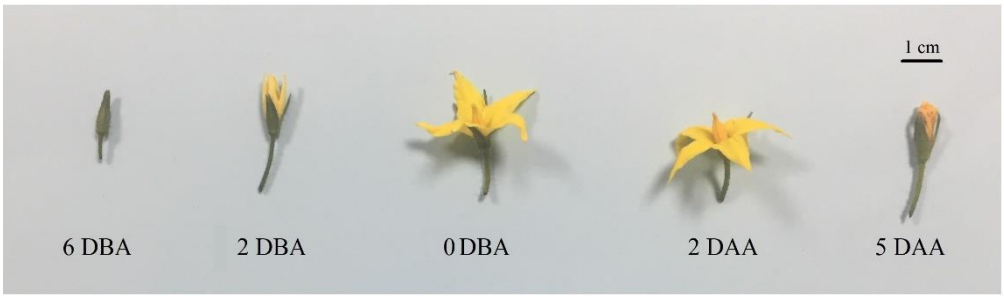

Fig. 1. Illustration of the five developmental stages of tomato flowers and ovaries studied. Age is given in days before (DBA) or after (DAA) anthesis (0 DBA).

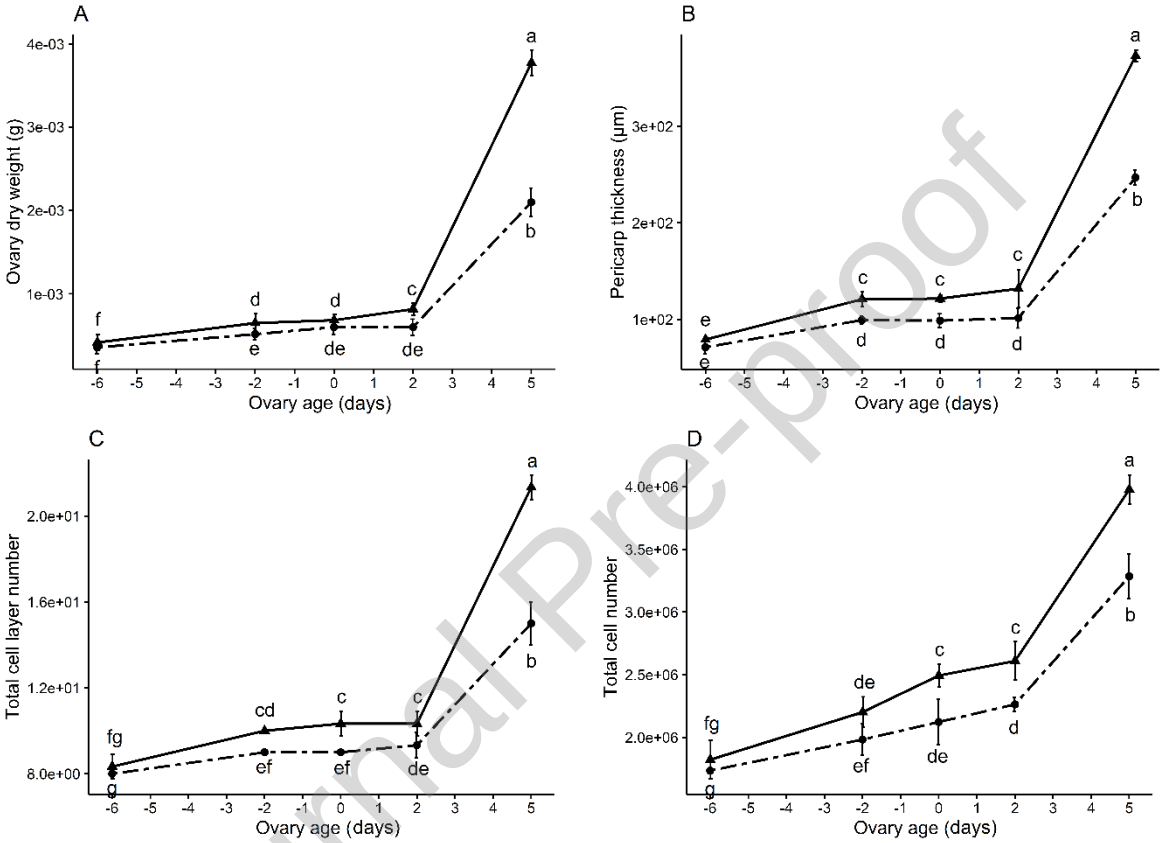

Fig. 2. Time plots of ovary dry weight (A), pericarp thickness (B), total cell layers (C), and total cell number (D) of tomato (cv H1311) in the pre- and post-anthesis periods under two water regimes. Each point is the mean (± SE) of a minimum of three to five ovary replicates under water deficit (⊸) or control (⊸) treatments. Different letters indicate significant differences at $P \leq 0.05$ calculated by the least significant difference test.

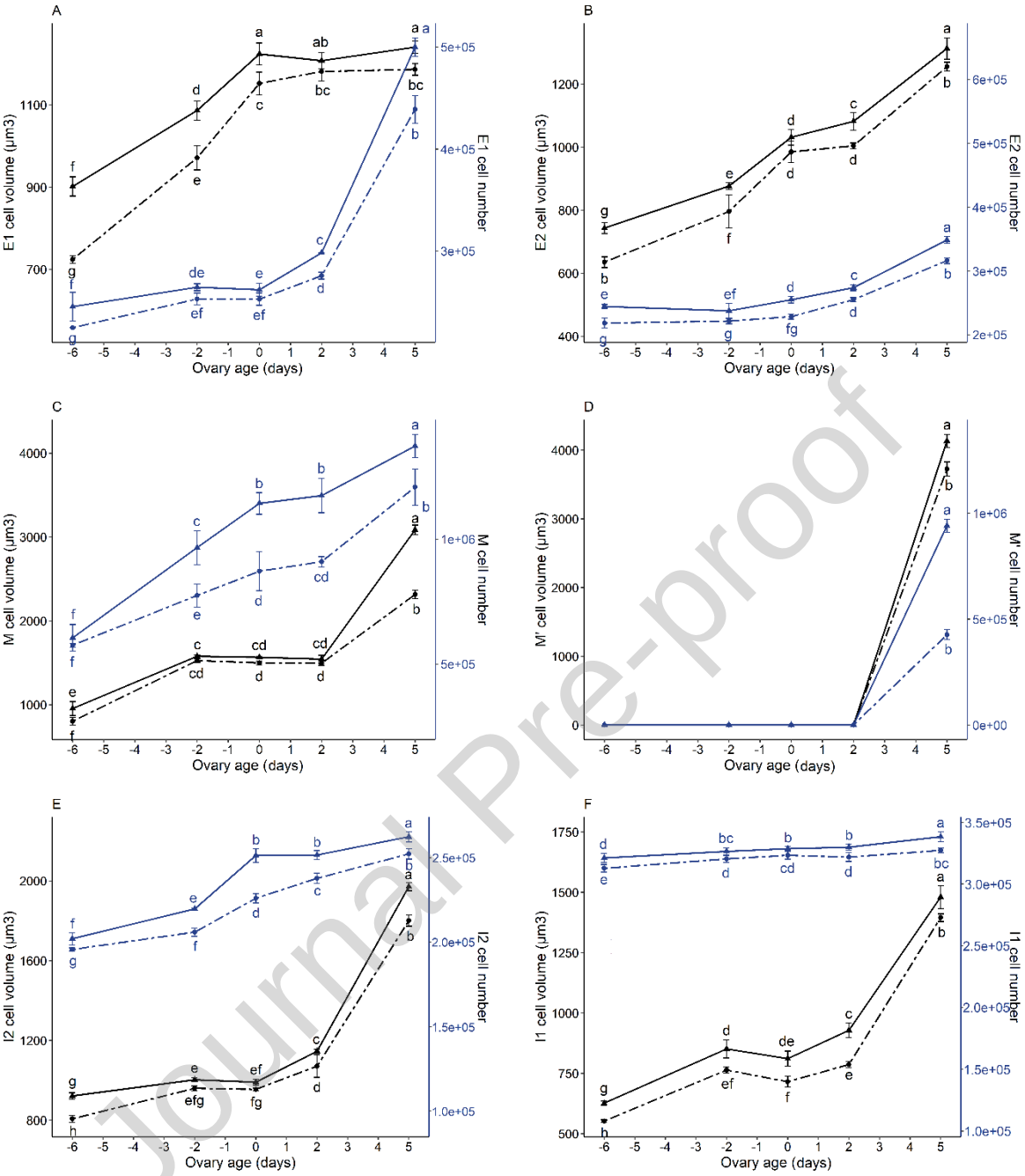

Fig. 3. Time plot of mean cell volume (black, left axis) and mean cell number (blue, right axis) in cell layers E1 (A), E2 (B), M (C), M′ (D), I2 (E), and I1(F) of the pericarp of tomato (cv H1311) ovaries during the pre- and post-anthesis periods under two water regimes. E1, E2, M′, M, I2, and I1 represent, respectively, the outer epidermis, outer sub-epidermal, new mesocarp formed after anthesis, mesocarp, inner sub-epidermal, and inner epidermis layers. Layer E3 is not shown and is similar to E2 since it is the periclinal division of E2 cells at the early stage of ovary development that generates the E3 layer (Renaudin et al., 2017). Each point represents the mean (± SD) of three ovary replicates under water deficit (-•-) and control (-▲-) treatments (each ovary is the average of 4 pericarp slices and 3 regions per slice). Different letters indicate significant differences at $P \leq 0.05$ calculated by the least significant difference test.

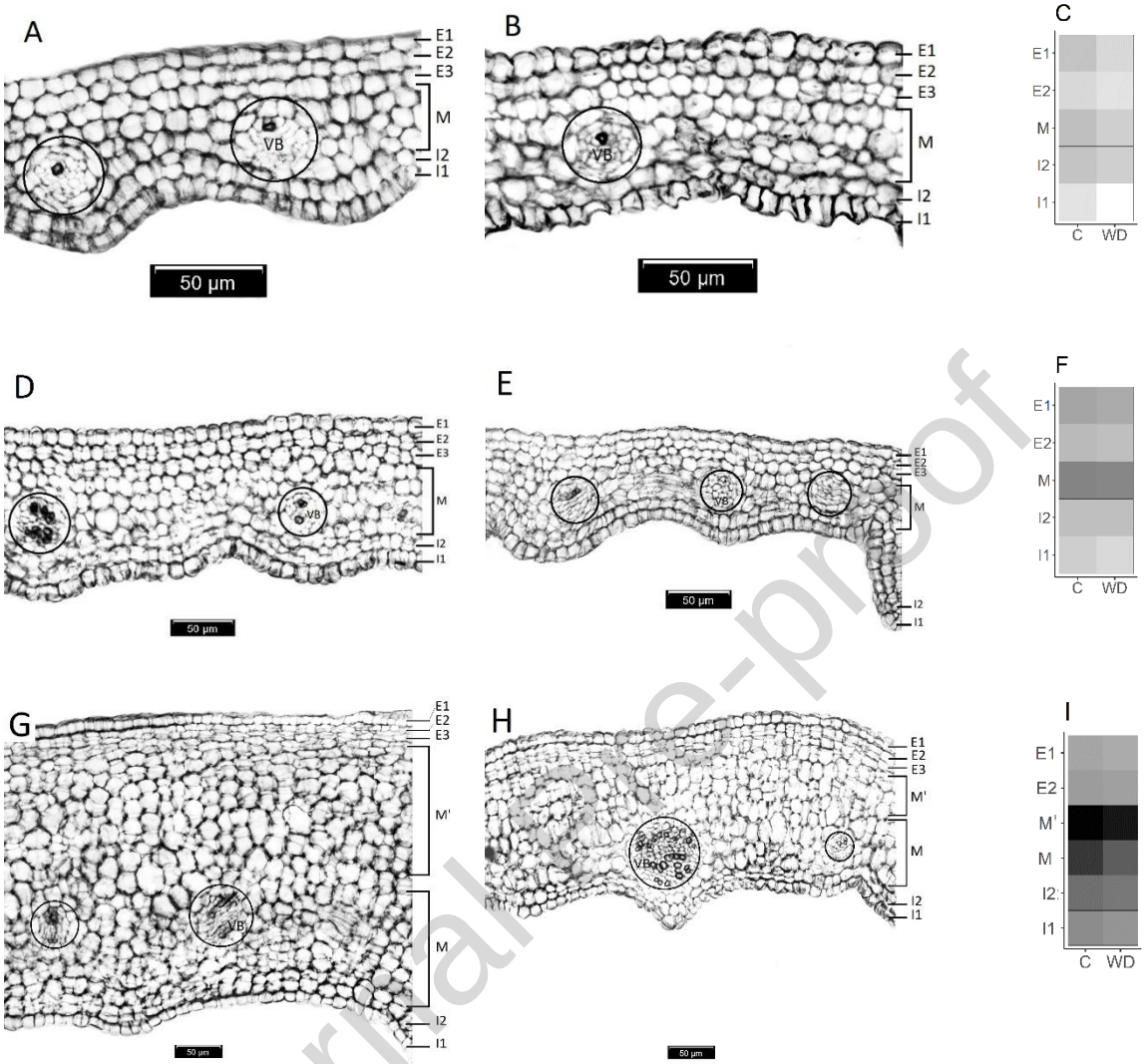

Fig. 4. Illustration of the structure of tomato (cv H1311) pericarp at 6 DBA under control (A) and water deficit (B) treatments, at anthesis under control (D) and water deficit (E) treatments, and at 5 DAA under control (G) and water deficit (H) treatments. Heat maps (C, F, and I for 6 DBA, anthesis and 5 DAA, respectively) illustrate the range of variations in cell volume across the cell layers resulting from the different treatments (water deficit: WD and control: C) and developmental stages. Values correspond to the means of 3 ovaries per stage and treatment (value for each ovary is the average of 4 pericarp slices and 3 regions per slice). Tonal values range from white for low cell volumes to black for high cell volumes. VB, E1, E2 (E3), M′, M, I2, and I1 represent the vascular bundles, outer epidermis, outer sub-epidermal, new mesocarp formed after anthesis, mesocarp, inner sub-epidermal, and inner epidermis layers, respectively. Nomenclature is taken from Renaudin et al. (2017).

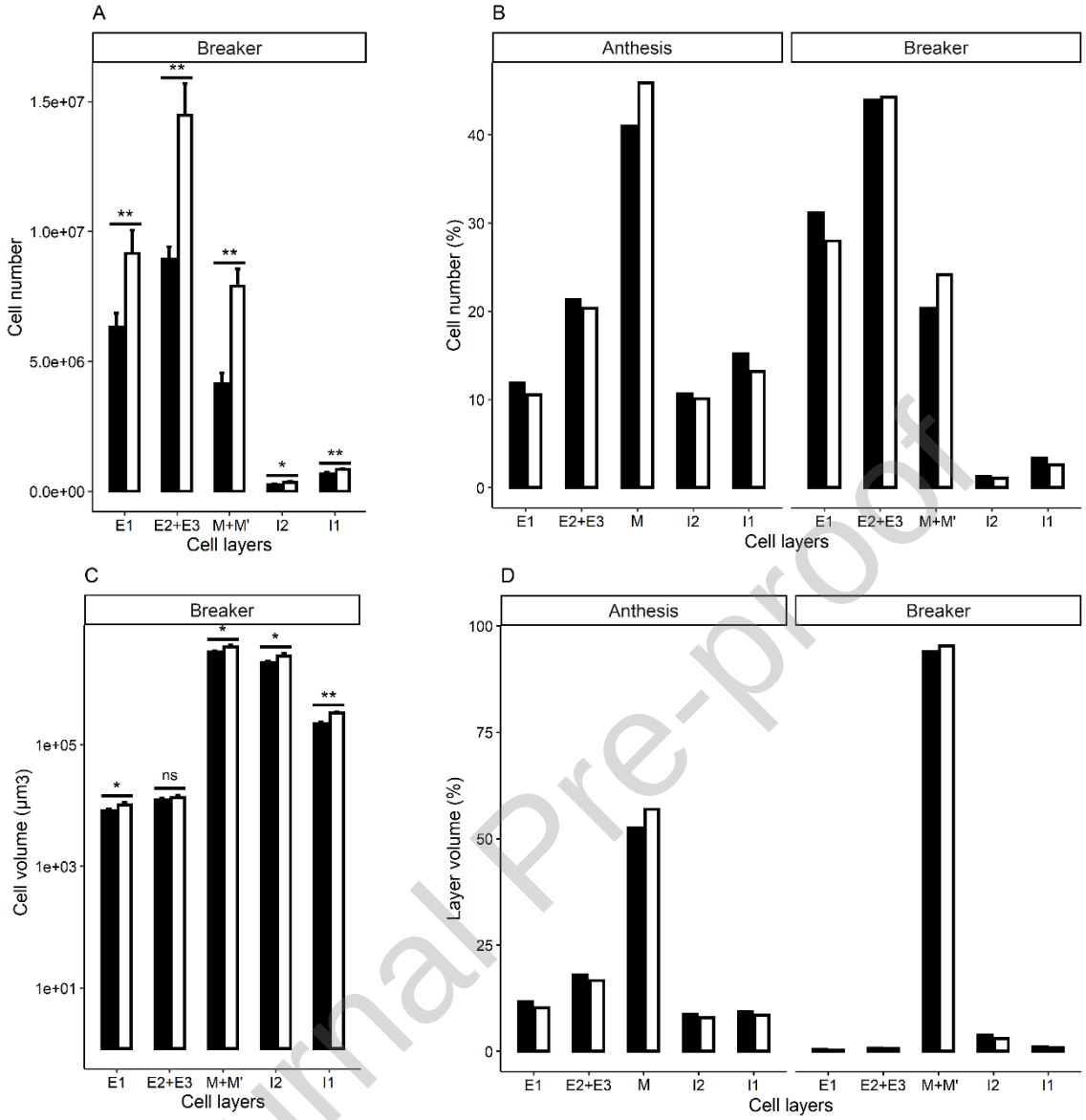

Fig. 5. Comparison of mean cell traits in each cell layer at the breaker stage (A, C) and their contribution to the whole pericarp at anthesis and breaker stages (B, D) under water deficit (black) and control (white) treatments. (A) Mean cell number in each group of cell layers (mean ± SE, n=3), (B) relative cell-number values for each group of cell layers with reference to total pericarp cell number, (C) Mean cell volume in each group of cell layers (mean ± SD, n=3), and (D) relative cell volume in each group of cell layers with reference to total pericarp volume. E1, E2+E3, M, M′, I2, and I1 represent outer epidermal, outer sub-epidermal, mesocarp, new mesocarp, inner sub-epidermal, and inner epidermal layers, respectively. **, *, and ns indicate significant differences at $P \leq 0.01$, $P \leq 0.05$, and no significant differences, respectively, calculated by the least significant difference test.

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CRediT authorship contribution statement

NB and ALF proposed, organized, and planned the experiment. LA and TB carried out the experiment. LA produced the draft manuscript, with improvements from NB. All authors commented on and contributed to the preparation of the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Highlight

- Water deficit affects the generation of cell layers early during tomato ovary development
- Water deficit impact on division and expansion is specific to the different cell layers
- Each cell layer has a specific contribution to the loss of tomato fruit mass under water deficit