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IMPLICATION OF CELLULAR TRAITS IN THE RESPONSE OF TOMATO UNDER WATER DEFICIT*

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SUMMARY: The effects of reduced irrigation treatments (PRD or DI) on the reduction of pericarp cell size are the most expressed during the fruit expansion phase. At maturity, PRD and DI have differ in effects on the outer layers: under PRD cells in most cell layers reached a similar final size as in FI treatment, while in DI cell grew slower and reached smaller cell size than in FI.

***Key words**: pericarp, cell size, drought*

INTRODUCTION

The ability to transport and accumulate water and assimilates in the economically important organs such as fruit is very important for the productivity of crops and depends on the resistance and adaptation of plants to water reduction. Final tomato fruit size depends on fruit growth, especially on the pericarp cells growth. According to Ho and Hewitt (1986), the development of pericarp tissue determines more than two-thirds of the potential fruit weight. Tomato fruit development consists of three phases, including cell division, cell expansion, and ripening (Gillaspy et al., 1993). Both division and expansion activities in pericarp tissue, as determinants of tomato growth, depend on some environmental conditions, such as temperature and light (Bertin, 2005, Fanwoua et al., 2012), and water supply (Granier et al., 2000). It is well known that water deficit affects differentiation of reproductive tissue in citrus fruit (Syvertsen, 1990) through cell size effect (Đaković and Jovanović, 2003). It is well

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known that DI restricts fruit growth rate and final tomato fruit size (Pulupol et al., 1996, Davies et al., 2000, Savić et al., 2008, Pervez et al., 2009). However, the effect of PRD on cell division and cell expansion of tomato fruit size or tomato pericarp are not clearly understood. Some studies have demonstrated beneficial effects of PRD on tomato fruit yield, without reduction in fresh weight (Zegbe et al., 2006). The objective of the present study was to determine the effect of different irrigation treatments (FI, PRD and DI) on tomato fruit histology during fruit development in tomato wild type.

MATERIAL AND METHOD

Tomato plant (*Solanum lycopersicum* L.), cv. Ailsa Craig was grown from seed and at the fifth leaf stage repotted into pots (one plant per pot) filled with 11 kg of commercial compost (Potground H, Klasmann-Deilmann, Germany) and grown in a chamber (photoperiod 14h; light intensity at plant level 300 $\mu molm^{-2}s^{-1}$, day/night temperature 25/18°C and relative humidity 70%) at the Faculty of Agriculture, University of Belgrade. Pots (height 65 cm, diameter 20 cm, volume $20dm^3$) were specially designed for PRD experiments in such a way that they were vertically separated into two equally sized compartments (Fig. 1A and B). The root of each fifth leaf old plant was divided into approximate halves and repotted into these two hydraulically separated pot compartments. Compartments were classified as PRD-L (left side) and PRD-R (right side) (Fig. 1B). Ten days after repotting, 15 plants per genotype were subjected to the three following irrigation treatments: full irrigation (FI), in which the whole root system was irrigated daily to reach field capacity around 35%; partial root-zone drying (PRD), where the amount of water in one half was kept to 35% (wet side) while the other half was allowed to dry (dry side); the irrigation from wet to dry side was shifted when volumetric soil water content of the dry side had decreased to 15%-20%, and so alternating until the end of the experiment, and deficit irrigation (DI), in which water was evenly applied to the whole root system to reach 15-20% soil water content. The volumetric soil water content of both compartments of each pot was measured daily using TDR probes (time domain reflectometer, TRASE, Soil Moisture Equipment Corp., USA) at 20 cm depth. In total PRD and DI, plants received about 30-40% of the water that was applied for irrigation of the FI plants, respectively for PRD and DI.

For histological analyses, the first five fruits of the third truss were used. Tomato fruits were harvested at 12 daa (indicated on late cell division) and 44daa (indicated on cell expansion). Pericarp was divided into two equatorial halves, and on one half was used for histological analysis. Slides for light microscopy were made according to a standard paraffin procedure (Ruzin, 1999). Pericarp sections were observed with an Eclipse E800, Nikon stereomicroscope and images were acquired by a Photometric cool snap HQ^2 digital camera.

Mean and individual cell size measurements were estimated using the "analyze particles" tool of Image J (Rasband, 1997-2009, http://rsbweb.nih.gov/ij), after manual adjustment of the segmentation threshold. Cell size was estimated on three to four independent sections devoid vascular bundles. Coloration on pericarp cross section was obtained by the ROI color coder plug-in of Image J software using sixteen color classes.

Figure 1. Tomato plants under climatic chamber conditions (A) and tomato plant in irrigation treatment (B).

For the analysis of the effects of PRD and DI treatments on cell growth kinetics within the pericarp, we identified the different cell layers on cross sections in relation to the position of vascular bundles. The cell layers located between the vascular bundles and the exterior pericarp were named: E1 (outer epidermis), E2 (outer sub-epidermis), E3 and E4 and the cell layers distributed between vascular bundles and internal pericarp were named: I1 (inner epidermis), I2 (inner sub-epidermis) and I3. During cell division new cell layers generated by E2 and I2 were named E2a, E2b, E2c, etc. and I2a, I2b, I2, etc. respectively as illustrated on Fig. 2.

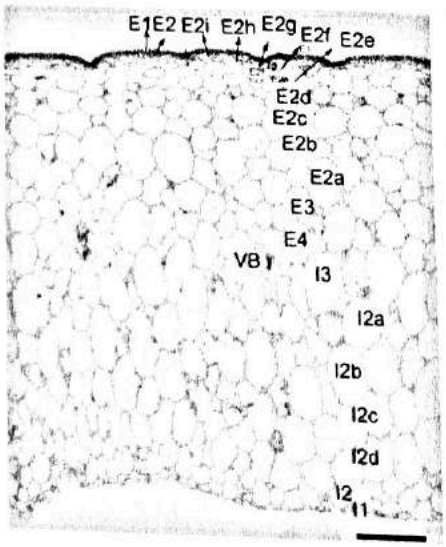

Figure 2. Tomato pericarp cross section with marked cell layers at 44 daa. The scale bars represent 500 µm. VB: vascular bundle.

To get more precise insight on effects on cell area, differences between treatments observed at 12 daa and 44 daa, were analyzed separately by Unequal HSD test. The distribution of cell sizes was calculated with the mean and standard deviation of at least 1000 measurements per treatment. Modeled cell size distribution was plotted

using square root transformed data. The statistical analysis was carried out with the statistical package STATISTICA (Statsoft, USA).

RESULTS

The analysis of cell size combined with the identification of cell layers allowed us to visualize the spatial distribution of cell sizes (mean cell size per cell layer) according to their position in the pericarp at 12 daa to 44 daa, indicated late cell division and cell expansion phases, respectively. At 12 daa we identified about 23 layers for FI, four of them in the external part: E1 (outer epidermis), E2, E3, E4, and three of them in the inner part: I1 (inner epidermis), I2, I3, relative to the position of vascular, and cell layers were generated, mainly from periclinal divisions of the outer sub-epidemal layer (E2 successively giving E2a, E2b, E2c, etc.) and to a lesser extent from periclinal cell divisions of the inner sub-epidermis (I2 generating I2a, I2b, etc). In the course of pericarp development these newly formed layers were therefore pushed away towards the central part of the mesocarp. At 12 daa in wild type (Fig. 3A), significant differences were observed among treatments: fewer cell layers were generated in PRD conditions (21) compared to FI (23) and even fewer in DI conditions (19). These differences were maintained, to a lesser extent, until the 44 daa (Fig. 3B).

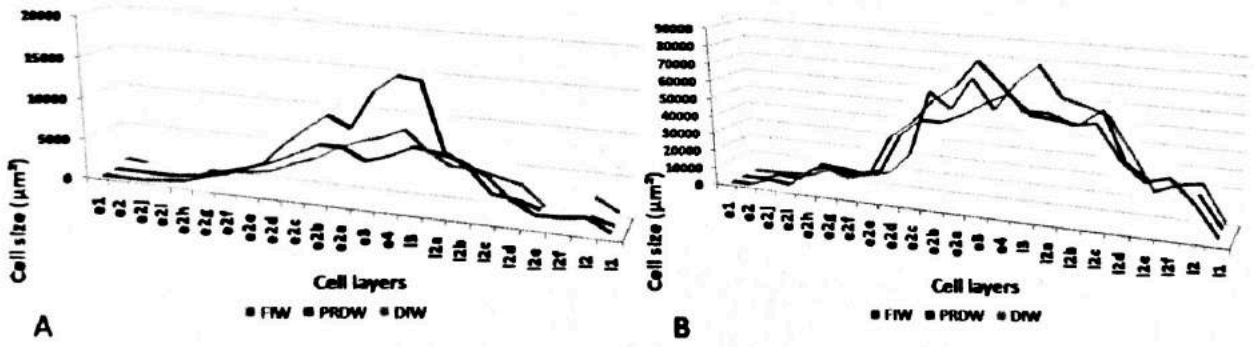

Figure 3. Kinetics of mean cell area per pericarp layer in full irrigation treatment (FI), partial root drying (PRD), deficit irrigation (DI) at 12 daa (A) and 44 daa (B). Each point is the mean of up to 100 cells per cell layer. Cell areas are given in μm^2. Identification of cell layers refers to Figure 2. Breaks in the lines indicate that the respective layers were not yet formed at this stage.

Mean cell size in pericarp cell layers is shown for wild type at 12 daa (Fig. 3a) and 44 daa (Fig. 3b). At 12 daa cell size was heterogeneous in all cell layers and treatments, ranging from 105 to 15.206 μm^2. The lowest cell size was observed in cell layers which were formed later, originating from E2. In FI and PRD, the lowest cell size was in E2j, while in DI it was E2h, as in DI cell layers to the E2j it had not formed. The highest mean cell size values were noted for PRD and DI in E4 (values 15206 μm^2 and 8156 μm^2) and for FI in I3 and I2a (value 7773 μm^2). Sizes of E4, I3, I2a cell layers give the largest contribution to the overall pericarp size in three treatments at 12 daa, without significant differences between treatments in mentioned cell layers.

Cell sizes were still heterogeneous at 44 daa, when cells of the most central cell layers (which were present at anthesis) underwent rapid expansion, while the outer and inner epidermis cells remained small (Fig. 3b). At 44 daa the smallest cells were

noted in E1 layer, with significant differences ($p<0.001$) between PRD and DI on cell size, value from 229 μm^2 (DI) to 339 μm^2 (FI). The largest contribution to whole pericarp size at 44 daa was provided by the central pre-existing cell layers (generated before anthesis), eg. for FI, E3 cell layer (value 83165 μm^2), for PRD E4 cell layer (value 64000 μm^2) and for DI I2a cell layers (value 78256 μm^2). In WT, the negative effects of reduced irrigation (PRD or DI) on cell size appeared late in the development and were maximum for most outer cell layers (e.g. E3, E2a, E2b, E2c), whereas the inner layers generated after anthesis (I2a, I2b, I2c) were weakly affected or not affected at all. Under PRD at 44 daa, cells reached the size similar to those in FI treatment in most cell layers. In DI, all cell layers grew slower and reached slightly smaller cell size than in FI. Differences between treatments were significant ($p<0.001$) at 44 daa.

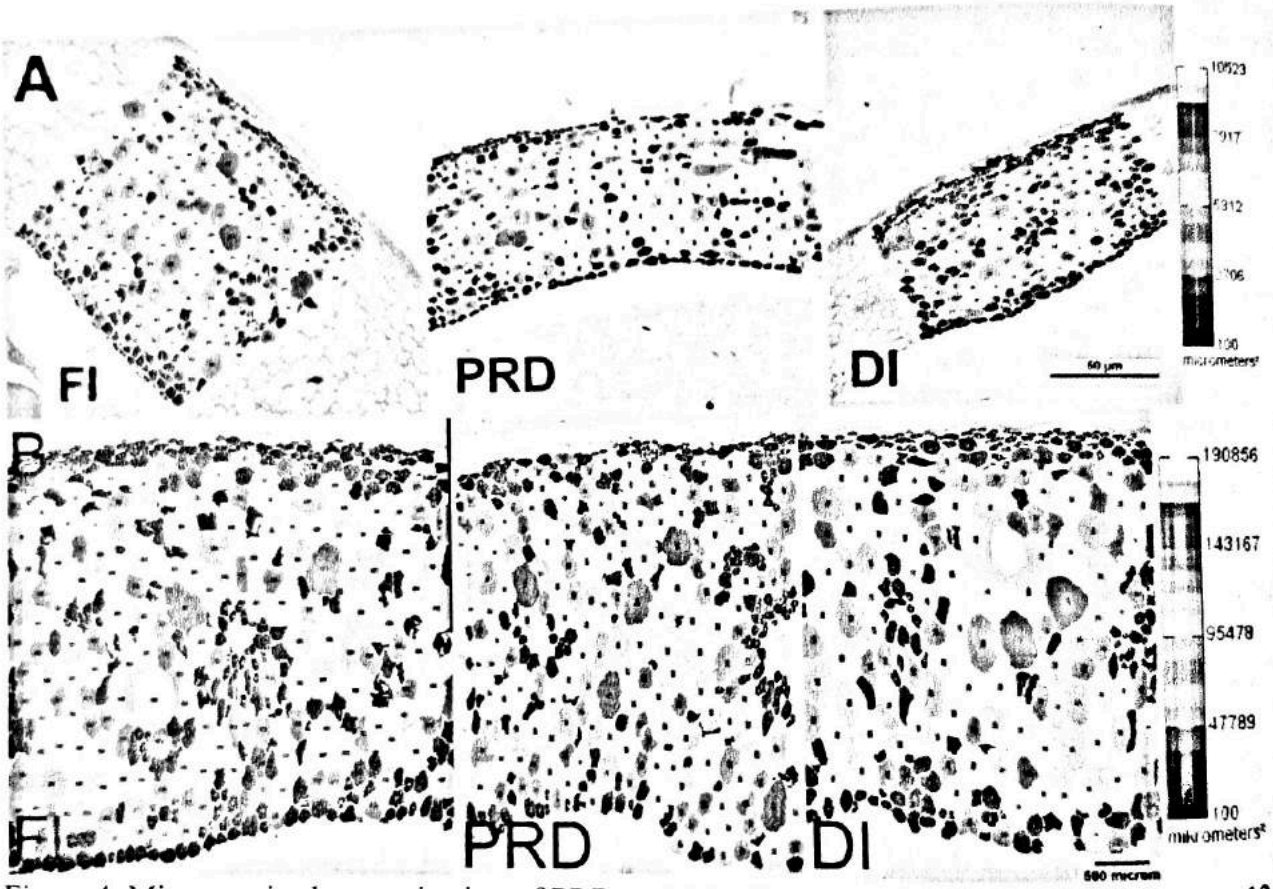

Figure 4. Microscopic characterization of PRD and DI effects on WT tomato fruit pericarp at 12 daa (A) and 44 daa (B). Identification of cell layers refer to Figure 2. Cells have been colored according to their class of section area, except for outer and inner epidermis, and first layers of very small cells below the outer epidermis, and vascular bundles. The scale bars represent 50 µm (A) and 500 µm (B).

Fig. 4. illustrates the effects of the irrigation treatments on the spatial distribution of cell sizes (colored according to their class of section area) in cross section of a wild type fruit pericarp at 12 daa and 44 daa. At 12 daa in PRD treatment cell size was smaller than in FI in most of the cell layers, especially in the outer pericarp. The figure clearly shows the strong negative effect of DI on number of cell layers and on cell size in outer and inner pericarp parts. At 44 daa, according to cell size colouring, under PRD similar cell sizes were recorded inside cell layers compared with FI, as well as a negative impact on cell size, under DI treatment, in most of cell layers which gives great impact on whole pericarp size, eg. cell layers I2a, E2b, I3.

Figure 5 shows the distribution of cell areas measured on isolated cells at 12 daa and 44 daa. At 12 daa, the range of cell areas was similar for PRD and DI (no significant difference between treatments) comparing with FI. At 44 daa, comparing with 12 daa, differences between three irrigation treatments were clearly visible, and cell sizes were larger and more heterogeneous at 44 daa than at 12 daa.

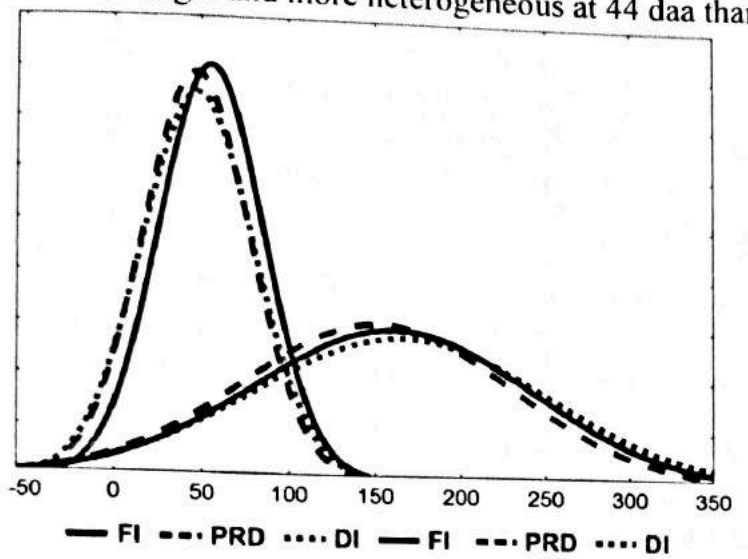

Figure 5. Frequency distributions of cell areas at 12 daa (red lines) and 44 daa (black lines), measured from anatomical sections. Each curve represents one irrigation treatment: FI (full line), PRD (dotted lines) or DI (dashed line).

Treatment effects varied during fruit development. At 12 daa, wild type pericarp cells were smaller and less heterogeneous in PRD and DI than in FI conditions, and differences were significant between FI and PRD, DI ($p<0.01$) (Table 1). Cell areas measured at 12 daa were significantly lower ($p<0.01$) in PRD and DI than in FI and the ranges were very narrow, indicating small and homogeneous cell size in the whole pericarp under DI or PRD. At 44 daa, cell size distributions were very similar in all treatments in wild type fruits, without treatment effects (Table 1).

Table 1. Unequal HSD test for pericarp cell size distribution at 12 and 44 daa under different irrigation treatments.

		12 daa			44 daa	
treatment	mean	Unequal HSD ($p < 0.01$)	Unequal HSD ($p < 0.05$)	mean	Unequal HSD ($p < 0.01$)	Unequal HSD ($p < 0.05$)
FI	3988,21	a	a	32508,83	ab	a
PRD	3155,09	**b**	b	29126,88	b	b
DI	3329,24	**b**	b	34931,23	a	a

DISCUSSION

The lower number of cell layers and reduction of cell size under DI show that this method of irrigation affected both the process of cell division and the process of cell expansion. In most outer pericarp cell layers, the negative impact of DI was mainly at 44 daa, where the cell expansion probably slowed down earlier than in FI, suggesting that at this late stage, water deficit represented a limiting factor for cell expansion. Water deficit in the inner pericarp affected cell expansion only indirectly by slowing down the rate of cell division, delaying the generation of new cells and therefore their expansion. Ojeda et al. (2001) indicated that pericarp cells in grapevines could reduce after early DI application, and that this change is irreversible. This result supported the hypothesis (Boyer, 1988) that early water deficit leads to cell wall structural changes due to cellulose sensitivity to the water deficit (Iraq and al., 1989), reducing the cell growth during cell expansion (Ojeda et al., 2001). Cell size reduction could be also explained by cell competition inside tomato pericarp, because pericarp cells are considered as competitive sinks in peach fruit (Quilot i Genard, 2008) or in tomato fruit (Bertin, 2005) probably as consequence of source limitation or limitation of phloem transport towards fruit under DI treatment (Rančić, 2011). In this sudy, we recognised PRD treatment as an irrigation method without a negative effect on cell division and cell expansion, which is indicated through the absence of reduction in pericarp cell size during final fruit development.

CONCLUSION

This study showed that tomato plants under PRD (70% of FI) could produce fruit with similarly sized pericarp cells as in control plants' fruits at 44 daa, eg. the phase near mature fruit stage. However, DI (60% of FI) negatively affected the whole pericarp. The study of water deficit effects on fruit histology and cytology are in progress and could bring new data which could help understanding the effects of water deficit on fruit pericarp in different tomato genotypes. Future investigations are needed to analyze phases of fruit development precisely and help us understand the effects of PRD not only on fruit growth, but also on fruit quality.

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