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Comparative effects of regulated deficit irrigation (RDI) and partial root-zonedrying (PRD) on growth and cell wall peroxidase activity in tomato fruits

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Partial root drying (PRD) Regulated deficit irrigation (RDI) Peroxidase activity

Abstract
The effects of regulated deficit irrigation (RDI) and partial root-zone drying (PRD) on tomato fruit growth and cell wall peroxidase activity in tomato exocarp were investigated in growth chamber conditions. The RDI treatment was 50% of water given to fully irrigated (FI) plants and the PRD treatment was 50% of water of FI plants applied to one half of the root system while the other half dried down, with irrigation shifted when soil water content of the dry side decreased 15–20%. RDI significantly reduced fruit diameter, though PRD reduced fresh weight while having no significant effect on fruit diameter. The activity of peroxidase was significantly higher in RDI and PRD treated plants compared to those of FI. Differences between RDI and PRD were expressed on temporal basis. In the fruits of RDI treated plants peroxidase activity began to increase in the phase when fruit growth started to decline with the peak of enzyme activity of 6.1 HRPEU g FW reached in the phase of mature green fruits when fruit growth rate was minimal. Increase of peroxidase activity in PRD fruits coincided with the ripening phase and the peak of enzyme activity (5.3 HRPEU g FW) was measured at the end of fruit ripening. These data potentially identified contrasting and different roles of tomato exocarp cell wall peroxidase in RDI and PRD treated plants. In RDI treated plants peroxidase may have a role in restricting fruit growth rate, although the increase in enzyme activity during ripening of PRD treated fruit pointed out that peroxidase may also control fruit maturation by inducing more rapid process.

Introduction
Tomato is one of the most widely grown vegetables in the world because of special nutritive value of its fruit (reach source of minerals, vitamins, organic acids, essential amino acid, antioxidants, etc.). Therefore, any factor influencing tomato yield has been attracted considerable interest. Among environmental factors drought is a major limiting factor of tomato growth and productivity thus the successful production of tomato requires irrigation (Benton Jones, 1999; Jonson et al., 1992; Grange and Andrews, 1994). However, water resources in many parts of the world are limited and thus there is an urgent need to apply effective irrigation strategy to operate under the condition of water scarcity (Fereres and Soriano, 2007).

Investigations over last two decades showed that new deficit irrigation methods (RDI and PRD) can aid in coping with situation where water supply is restricted (FAO, 2002). Regulated deficit irrigation (RDI) is deficit irrigation technique where crops
are irrigated with lower amounts of water and the minor stress that develops has minimal effects on the yield (English and Raja, 1996). RDI has been assessed for tomato giving different results. Pulupol et al. (1996) observed a significant reduction in dry mass yield for a glasshouse cultivar using RDI, while Mitchell et al. (1991) and Zegbe-Domínguez et al. (2006) reported no reduction of yield for a field-grown processing cultivar. However, although the effects on yield may be different, many of the obtained results have shown that RDI saves substantial amounts of irrigation water and increases water use efficiency (Kirda et al., 2004; Topcu et al., 2006).

Partial root drying (PRD) is a further development of the deficit irrigation technique and with this technique half of the root zone is irrigated while the other half is allowed to dry out. The treatment is then reversed, allowing the previously well-watered side of the root system to dry down while fully irrigating the previously dry side (Dry et al., 1996; Loveys et al., 2000; Stoll et al., 2000; Topcu et al., 2006). PRD technique was developed on the basis of knowledge of root-to-shoot chemical signalling in drying soil and, therefore, understanding of this process is essential for successful application of the PRD technique (Dodd et al., 1996; Davies et al., 2002). Recent results showed that PRD has the potential to increase water use efficiency, decrease tomato plant growth, and maintain yield and quality of yield when compared with classical irrigation methods (Davies et al., 2000; Stikic- et al., 2003; Tahi et al., 2007). Tomato fruit size and quality of the yield depends on the rate and duration of fruit growth. According to Gillaspy et al. (1993) tomato fruit growth and development can be divided into three phases. The first phase involves: ovary development, fertilization and fruit set. During the second phase of fruit growth cell division takes place. The third phase is phase of cell expansion and during this phase the rapid growth of fruit takes place. The final size of fruit depends on the rate of cell division and expansion and also on duration of fruit growth (Ho, 1992; de Koning, 1994). After reaching its final size, fruit development continues by ripening. This phase is followed by many metabolic changes including biosynthesis of carotenoids and change of fruit color from green to orange and finally red (Gillaspy et al., 1993; Carrari and Fernie, 2006). However, fruit growth and ripening is very complex process because the interaction between different biochemical and physiological processes as well as environmental factors may significantly change fruit growth and obtained yield (Thompson et al., 1998, 1999; Frary et al., 2000; Carrari and Fernie, 2006).

Biochemical investigations of plant cell growth mechanism showed that cell growth in different plant tissues is regulated by the activity of three groups of enzymes. These enzymes are: xyloglucan endotransglycosylase (XET), expansins and cell wall- associated peroxidase. Xyloglucan endotransglycosylase (XET) is an enzyme capable of cleaving and rejoining xyloglucan chain (Palmer and Davies, 1996), and expansins catalyze the irreversible extension of cell wall (McQueen-Mason and Cosgrove, 1995). Peroxidases (EC.1.11.1.7), as heme enzymes, catalyze many different reactions in plants. These reactions also include cross-linking in cell walls as a result of the formation of diferuloyl bridges between pectin residues, and isodityrosine bridges between hydroxyproline-rich extensin molecules (Fry, 1986; Hatfield et al., 1999). In this way the role of cell wall peroxidases in cell growth have been implicated in “locking” together cellulose microfibriles by formation of phenolic cross-linkages between cell wall components and, this way, decreases the ability of cell wall to expand (Fry, 1986; MacAdam et al., 1992a; Passardi et al., 2004). The results of Thompson et al. (1998) showed that growth of tomato fruit is controlled by the fruit epidermis. The enzymes from this tissue regulate the growth of fruit cells. Thompson et al. (1998) and Rose et al. (1997) also demonstrated that the activity of XET and expansins is correlated with the fruit growth. Many results
showed the increase in peroxidase activity or its different isoforms during maturation or cessation of tomato fruit growth (Thompson et al., 1998; Andrews et al., 2000, 2001). However, similarly to leaf growth analyses (Bacon et al., 1997; Jovanovic´ et al., 2004), there is a limited number of reports about the involvement of cell wall enzymes in the inhibition of fruit growth during environmental stresses that may significantly reduce crop yield.

The aim of this study was to investigate and compare the effects of partial root-zone drying (PRD), RDI and full irrigation (FI) on tomato fruit growth and development. Measurements of cell wall ionically bound peroxidase were done with aim to determine its potential role in the regulation of tomato fruit growth and development in plants exposed to different irrigation treatments.

Materials and methods

Plant material and growing conditions

Tomato plants (Lycopersicon esculentum L., cv. Sunpak) were raised from seed in compost (Potground H, Klasmann-Deilmann, Germany) filled seed trays in a growth chamber (photoperiod was 14 h; light intensity at plant level 300 mmol m\(^{-2}\) s\(^{-1}\), temperature 25/18 8C and relative humidity 70%). When fifth leaf appeared, washed roots were divided into approximately two equal halves and repotted into two separate plastic bags (volume 10 dm\(^{-3}\) each) containing the same compost. The bags were joined by plastic tape and placed together into a big pot, thereby the root system of each plant was split into two hydraulically separate compartments. After transplanting all plants were fully irrigated. The volumetric soil water content of both compartments of each pot was measured daily by using theta probe-type ML2X (Delta-T Device Ltd., UK) with the length of 20 cm. Ten days after transplanting, plants were subjected to three irrigation treatments:

FI in which the whole root system was irrigated to reach value of field capacity around 35%;
RDI in which 50% water of FI was evenly applied to the whole root system;
partial root drying (PRD) where 50% water of FI was applied to one half of the root while the other half was allowed to dry, and the irrigation was shifted when soil water content of the dry side had decreased to 15–20% (Fig. 1). Compartments were classified as PRD-L (left side) and PRD-R (right side).

Fruits were harvested from each of the first five flower trusses of a single plant. The harvested fruits had been 15th, 22th, 29th, 36th and 43th day old from fruits appearing. All of the fruits were still green except the oldest, which was just starting to change color.
Individual fruits were first weighted and then fruit equatorial diameter was measured with a digital caliper ruler. Maturation of fruits was followed by visual daily estimation.

Isolation of cell wall ionically bound fraction

Pieces of the exocarp from the equatorial region of each fruit were taken for experiments according to procedure of Thompson et al. (1998). Samples (0.5 g) were homogenized in 10 volumes of in the ice-cold 50 mM sodium phosphate buffer, pH 7.5 The homogenate was centrifuged at 4 8C for 5 min at 2500 \(\times\) g, and the supernatant
was discarded. The pellet, washed by resuspension in 10 ml of 50 mM sodium phosphate buffer, pH 7.5, containing 1% (w/v) Triton X-100, and five times in 10 ml of the same medium without Triton (centrifuged as previously), was considered to be a purified cell wall fraction. In order to obtain ionically bound enzymes, the cell wall fraction was incubated in the same buffer.

Fig. 1. Changes in volumetric soil water content (%) for full irrigation (FI, &), partial root-zone drying (PRD-L, ~ and PRD-R, ~), and regulated deficit irrigation (RDI, *) treatments of tomato plants. Vertical arrows represent time of watering of plants with 1 M NaCl added, for 30 min with continuous stirring at 4 °C.

Table 1
Effects of PRD, RDI and FI treatments on fruit weight, diameter and duration of maturations (days)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>RDI</th>
<th>PRD</th>
<th>FI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fruit diameter (mm)</td>
<td>59.6</td>
<td>2.3*</td>
<td>72.5 0.9</td>
<td>77.4 2.5</td>
</tr>
<tr>
<td>Fruit FW (g)</td>
<td>79.9</td>
<td>3.6***</td>
<td>125.0 8.1**</td>
<td>185.0 2.9</td>
</tr>
<tr>
<td>Yield per plant (g)</td>
<td>190.0</td>
<td>16.2<em><strong>8</strong></em></td>
<td>323.0 17.5*</td>
<td>422.0 16.5</td>
</tr>
<tr>
<td>Duration of fruit ripening (days)</td>
<td>36</td>
<td></td>
<td>43</td>
<td>50</td>
</tr>
</tbody>
</table>

Supernatant obtained after centrifugation at 1000 × g for 10 min was collected for peroxidase assay (Bacon et al., 1997).

Determination of peroxidase activity

Peroxidase (EC 1.11.1.) activity was determined by a guaiacol test, detailed by Chance and Maehly (1955). Assay was done by adding 10 ml of the supernatant, to 2 ml of 20 mM sodium phosphate buffer pH 5.5 containing 0.56% (v/v) of guaiacol. The reaction was started by adding 0.4 ml of 0.03% of hydrogen peroxide. After incubation at the 25 °C for 10 min the absorbance at 470 nm was measured (SPECTRO UV–vis RS, 1166, Lambomed, Inc., USA). Peroxidase activity was expressed in horseradish peroxidase equivalence units (HRPEU) per gram of fresh weight. One unit of horseradish peroxidase activity is capable of forming 1.0 mg purpurogallin from pyrogallol in 20 s at pH 6.0 at 20 °C in the pyrogallol test.
The absorbance change (DA$_{470 \text{ min} - 1}$) was converted into HRPEU by estimating the time for horseradish peroxidase solution (Sigma, Poole, Dorset, UK) of similar activity (0.004 units) to reach the same absorbance as cell wall peroxidase during 10 min.

Statistical analysis
The measured traits in both treatments have been analyzed for statistically significant differences by Student’s unpaired t-tests (Sigma Plot 6.0 for Windows-SPW 6.0, Jandel Scientific, Erckhart, Germany).

Results and discussion

Soil water status
Changes of soil water content in FI, PRD and RDI treatments during the experimental period are shown in Fig. 1. Soil water content values in FI treatment were kept close to the field capacity (35%). In RDI treatment soil water content decreased during the experimental period and last 15 days was kept between 15% and 20%. The difference in soil water content between the PRD wet and dry sides was significant during the whole experimental period. However, soil water content of the PRD wet side was maintained similar to that of FI only in the beginning the treatments and after the second shifting the soil water content of wetted side was lower than FI by 2–10%. These results provided evidence that the extraction of water by the roots from watered PRD compartment was greater comparing to full irrigation.

Similar pattern of soil water dynamics has also been observed in PRD treated tomato and other crops (Kirda et al., 2004; Zegbe-Domínguez et al., 2006; Liu et al., 2007). However, some other authors showed that soil water content of PRD wet side of tomato plants was maintained mostly during the whole treatment period (Sobeih et al., 2004). There are several possible explanations for these discrepancies in soil water response to PRD treatment. One explanation may be that there are genotypic differences in the response of tomato plants to PRD in such a way that roots of some genotypes (as our variety Sunpak) after several shifting periods adapt to PRD treatment by extracting much more water from the wet side. Higher water uptake may be the result of the increase in root growth or root hydraulic conductivity. The results of Laing et al. (1996) showed that exposure of roots to soil drying and soil re-watering increases root growth, which may contribute to the increased root biomass (Kang et al., 1998). Accordingly, the increase in root growth in PRD treated grape and tomato was found (Dry et al., 2000; Mingo et al., 2004).

Means S.E. for at least five measurements are given (*, ** and *** indicate differences between FI and RDI/PRD samples significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively).

Also, the results of Martre et al. (2002) showed that waterchannels—aquaporins play a major role in water uptake during episodes of soil water deficit and re-watering. In presented study the mechanisms regulating root growth or water uptake in PRD plants were not investigated but the obtained results pointed out that future work should include their assessment.

Fruit growth
The obtained results showed that fruit diameter, fruit biomass, yield per plant and
duration of ripening differed between investigated treatments (Table 1). Fruits of RDI treated plants were the smallest (with the most reduced fresh weight). Although we did not measure any parameters of plant water regime, the fruit fresh weight and soil water content (Fig. 1) pointed out that RDI plants were exposed to significant drought stress. Similar effects of drought on tomato fruit growth was reported by Jonson et al. (1992) and Grange and Andrews (1994).

PRD and FI treated plants showed no significant differences in fruit diameter, but significant differences ($P < 0.01$, <0.05) were found in fruit fresh weight and yield per plant. In PRD treated plants fruit fresh weight and yield per plant were reduced for 32.5% and 23.5% compared with fully irrigated plants.

In most commercially grown tomato cultivars, fruit development takes about 7 weeks, depending on temperature (Gillaspy et al., 1993). Over this period tomato fruit diameter increases from few mm to 35–70 mm and fresh weight increases from 1.4 mg to 23–80 g (Andrews, 2003). Although our results are in accordance with these data, they also showed that duration of fruit ripening depends on treatment. Fruits of RDI treated plants were the first to reach maturation (the 30th day of experiment) comparing to other treatments (the 43rd and 50th day for PRD and FI treatments, respectively).

Time course of changes in fruit growth rate (FGR), fruit diameter and cell wall ionically bound peroxidase activity in tomato fruit exocarp of plants exposed to investigated treatments are presented in Fig. 2. Fruit growth rate profiles for all treatments were obtained by fitting the changes in FGR by the third order regression line (Fig. 2A). The obtained profiles were symmetrical, bell-shaped curves similar to that obtained by Monselise et al. (1978). The initial short lag phase of growth is followed by a phase of rapid fruit expansion, after which FGR declined and finally as fruit ripened, FGR reached the near zero values. Comparison of the curves for different treatments showed the similar maximal fruit growth rate for RDI and FI treatments (ca. 2.8 mm day$^{-1}$ and 3.0 mm day$^{-1}$, respectively), with lower maximal value for PRD treatment (ca. 2.5 mm day$^{-1}$). Also, curves showed that maximal fruit growth rate of RDI treated plants was accomplished after 15 days of fruit appearing, and after 22 days in PRD and FI treatments.

In another presentation of fruit growth, tomato fruits diameter were fitted by second order of regression (Fig. 2B). The obtained curves followed a sigmoidal pattern of growth, similar to that reported by Monselise et al. (1978). Such presentation of fruit growth, similarly to Fig. 2A, showed that exposure of tomato plant to drought in RDI treatment resulted in the earlier termination of rapid fruit growth phase comparing to PRD or FI treatment. In RDI treated plants the rapid growth started when fruits passed the age of 10 days and lasted for another additional 10 days. After this period, when RDI fruits were in mature green phase (according to Gillaspy et al., 1993), they reached final fruit diameter of 59.6 mm. This diameter did not change significantly until fruit reached its red-ripe phase. PRD and FI treatments prolonged the period of fruit rapid growth for about two additional weeks and as a result the final fruit size was significantly bigger (77.4 mm and 72.5 mm for FI and PRD, respectively).

Final size of the fruit is determined by the rate and duration of cell enlargement (Ho, 1992). Similarity in fruit diameter curves for PRD and FI treatments pointed out that in presented experiment duration of fruit growth was more important for obtaining the final size of PRD and FI fruits than maximal FGR values.
The effects of different irrigation treatments (FI (+), PRD (~) and RDI (•)) on fruit growth rate (A), fruit diameter (B) and cell wall peroxidase activity in exocarp (C) during tomato fruit development.

The potential size of tomato fruit depends also on the rate of water accumulation because water may account for 95% of the total fresh weight (Ho, 1992). Although PRD and FI fruits had a similar diameter, the fresh weight of PRD fruits was reduced (Table 1). One can suppose that higher fresh weight of FI fruits was the result of longer ripening period that allowed higher accumulation of water in these fruits comparing to PRD fruits. According to Zegbe-Domínguez et al. (2006) lower water content in tomato fruit could be preferential for processing industry.

Measurements of cell wall ionically bound peroxidase activity were started at 15th day after fruit appearance because before this period the preliminary results did not show any significant differences in enzyme activity between the investigated treatments (data not shown). The activity of peroxidase was significantly higher in RDI and PRD treated plants compared to those of FI (Fig. 2C). The comparison of the results on temporal scale, as well as the fruit growth data, showed differences between RDI and PRD treatments. In the fruits of RDI plants, peroxidase activity began to increase from the 15th day and this phase coincided with the phase when fruit growth started to decline (Fig. 2A). The peak of enzyme activity of 6.1 HRPEU g⁻¹ FW was reached in the phase of mature green fruits (36th day) when FGR rate was minimal. Contrarily, the results for PRD treated plants showed that a rapid increase in peroxidase activity occurred after 36th day and close to the period when FGR stopped. The peak of enzyme activity (5.3 HRPEU g⁻¹ FW) was at 43rd day that also presents the end of fruit ripening in PRD treated plants. However, during measuring period (from 15th to 43rd day after fruit appearance) the obtained results did not show any significant differences in peroxidase activity in fruits of FI plants, because fruits of these plants continued to grow during whole experimental period.

The obtained results of peroxidase activity in the fruits of PRD and RDI treated plants rise the important question: can the increase of enzyme activity be linked with fruit growth cessation or ripening? It is difficult to answer this question because of limited publish data concerning the effects of environmental factors on cell wall peroxidase activity.
activity in fruits or leaves. Bacon et al. (1997) found that inhibition of leaves growth in *Lolium temulentum* exposed to drought was connected with the increase in peroxidase activity and Jovanovic’ et al. (2004) found similar effect in maize leaves exposed to drought.

One of the mechanisms of controlling cell wall peroxidase activity might involve plant hormone abscisic acid (ABA) since results of Lee and Lin (1996) and Lin and Kao (2001) demonstrated that ABA led to the increased activity of this enzyme in the roots of rice. Another possibility is drought or PRD induced increase in apoplastic pH value that in turn may increase peroxidase activity and reduce fruit growth (Bacon et al., 1998; Mingo, 2003). If we assume that ABA accumulation stimulates cell wall peroxidase activity (Bacon, 1999), differences in ABA accumulation in plants exposed to RDI and PRD treatments might provide a possible explanation for observed differences in enzyme activity. Our unpublished results showed that in RDI treated tomato plants xylem ABA started to increase (from 34.8 pmol ml$^{-1}$ to 180.0 pmol ml$^{-1}$) 2 days before the increase in peroxidase activity.

In the same experiment similar increase in xylem ABA (from 34.8 pmol ml$^{-1}$ to 155.8 pmol ml$^{-1}$) was measured later in PRD plants in the phase of fruit growth that corresponds to the start of fruit ripening. However, the causal significance of xylem ABA or fruit ABA accumulation for cell wall peroxidase activity in investigated fruits, has yet to be established.

During fruit ripening cell wall metabolism as well as mechanical properties change (Vicente et al., 2007) and these changes may induce formation of new isoenzymes in the cell wall of tomato exocarp (Andrews et al., 2000). Andrews et al. (2002) also showed that peroxidase isoenzymes located within the outer fruit exocarp may have a dual role in restricting tomato fruit expansion and producing protective barriers against pathogen attack in the epidermis. The increase in peroxidase activity might be attributed to the synthesis of specific isoform expressing higher enzymatic activity. Also, there is a possibility that the increase in peroxidase activity during ripening of PRD treated fruits was induced by ethylene. Tomato fruits being climacteric, increase ethylene production as a part of metabolic changes (Gillaspy et al., 1993), that in turn may also induce peroxidase activity (Andrews, 1995).

In conclusion, presented data potentially identified contrasting and different roles of tomato exocarp cell wall peroxidase in RDI and PRD treated plants. In RDI treated plants, peroxidase may have a role in restricting fruit growth rate. Increased enzyme activity of fruit pericarp, that mediates cross-linking of cell wall phenolics and therefore reduces the cell expansion, consequently causes the reduction of fruit diameter. On the other hand, the increase in enzyme activity during ripening of PRD treated fruit pointed out that peroxidase may also control fruit maturation by inducing more rapid process.

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