Biochemical mechanisms of fruit growth regulation in draught stressed tomato plants

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The effects of partial root-zone drying (PRD) and full irrigation (FI) techniques on tomato fruit growth and cell wall peroxidase activity in tomato exocarp were investigated in growth chamber conditions. The PRD treatment was 50% of water given to 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% to 20%. PRD treatment reduced fresh weight while having no significant effect on fruit dry weight. The activity of peroxidase was significantly higher in PRD treated plants compared to those of FI. Differences between FI and PRD were expressed on temporal basis. In the fruits of FI treated plants peroxidase activity began to increase shortly before the phase when intensive fruit growth started to decline with the peak of enzyme activity of 3.3 HRPEU g^{-1}FW. The highest increase of peroxidase activity in PRD fruits coincided with the ripening phase and the peak of enzyme activity (5.95 HRPEU g^{-1}FW) was measured at the end of fruit ripening. These data potentially identified different roles of tomato exocarp cell wall peroxidase in PRD treated plants. In FI treated plants a role of peroxidase in restricting fruit growth rate was confirmed, but the increase in enzyme activity during ripening of PRD treated fruit pointed out that cell wall peroxidase may also control fruit maturation by inducing more rapid process.

Key words: tomato fruits, exocarp, partial root drying (PRD), peroxidase activity

Tomato fruit size and quality depends on the rate and duration of fruit growth and therefore, the understanding of tomato growth mechanism and factors that may influence it are of significant importance. The growth mechanism of tomato fruit, as in most plant cells and organs, is biochemically regulated by activities of 3 groups of enzymes including: xyloglucan endotransglycosylase (XET), expansins and cell wall-associated peroxidase. These enzymes are concentrated in the skin or exocarp (the outer skin) of the fruits. Xyloglucan endotransglycosylase (XET) is an enzyme capable of cleaving and rejoining xyloglucan chain[1], and expansins catalyze the irreversible extension of cell wall[2]. 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[3,4]. 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, thus decreasing the ability of cell wall to expand[3,5,6].

Correlations between peroxidase activity and the cessation of growth have been reported for several plant species[7,8]. The appearance of different peroxidase isozymes and increases in peroxidase activity within the tomato fruit exocarp during maturation have been associated with cell wall stiffening and the cessation of growth[9,10,11]. However, there is limited data of the involvement of cell wall peroxidase in inhibition of tomato fruit growth during environmental stresses, especially drought.

The aim of presented paper was to investigate the role of cell wall peroxidase in regulation of fruit growth in tomato plants grown in the conditions of optimal water regime and under partial root drying (PRD). Partial root drying (PRD) is a new irrigation strategy which applies alternating regimes of irrigation to half the root system while the other half dries 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. The PRD technique was developed on the basis of knowledge of plant reaction on drought[12]. Recent results showed that application of this technique may save water for irrigation without sacrificing yield and fruit quality in many crops, including tomato[13,14,15,16].

EXPERIMENTAL

Plant material and growing conditions

Tomato plants (*Lycopersicon esculentum L., cv.* Astona F_1) were raised from seeds and transplanted into pots filled with commercial compost (Potground H, Klasmann-Deilmann, Germany) in a growth chamber with controlled temperature and light conditions. The pots were specially designed for PRD experiments in such a way that roots of investigated plants were separated into two equally sized hydraulically isolated plastic compartments. Ten days after transplanting, plants were subjected to two irrigation treatments: 1) full irrigation (FI) in which the whole root system was irrigated daily to reach value of field capacity around 35% and 2) 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% to 20%. 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). Fruits were harvested from each of the first five flower trusses of a single plant. The harvested fruits had been 15^{th}, 22^{nd}, 29^{th}, 36^{th} and 43^{rd} day old from fruits appearing. Individual fruits were first weighted and than fruit equatorial diameter was measured with a digital caliper ruler. Maturation of fruits was followed by visual daily estimation. At the end of the experiment on the basis of fruit dry weight (DW) and the amounts of water used for irrigation the parameter crop water use efficiency ($WUE_{c)}$ was calculated and expressed as g DW L^{-3} H_2O.

Isolation of cell wall ionically bound fraction

Pieces of the exocarp from the equatorial region of each fruit were taken for experiments according to the procedure of Thompson *et al.*[9]. Samples (0.5 g) were homogenized in 10 volumes of the ice-cold 50 mM sodium phosphate buffer, pH=7.5 The homogenate was centrifuged at 4 ^{o}C for 5 min at 2500 x 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 X-100 (centrifuged as previously), was considered to be a purified cell wall fraction. In order to obtain ionically bound enzyme, the cell wall fraction was incubated in the same buffer with 1 M NaCl added, for 30 min with continuous stirring at 4 ^{o}C. Supernatant obtained after centrifugation at 1000 x g for 10 min

was collected for peroxidase assay[7].

Determination of peroxidase activity

Peroxidase (EC 1.11.1.) activity was determined by the guaiacol test, detailed by Chance and Maehly[17] and modified by Bacon *et al.*[7]. Assay was done by adding 10 μL 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 minutes 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 g of fresh weight. One unit of horseradish peroxidase activity is capable of forming 1.0 mg purpurogallin from pyrogallol in 20s at pH 6.0 at 20°C in the pyrogallol test[17]. The absorbance change (ΔA_{470} 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.

Fig. 1

The calibration curve (Fig.1) was used to convert assayed extracted activity into horseradish peroxidase equivalence units (HRPEU). Each value is the mean of tree determinations of activity. The equation and consequent calibration for conversion into HRPEU is $y = 22.38x+2.05$, where y = time taken for horseradish peroxidase to reach absorbance x. This value for x is divided by the time taken for horseradish peroxidase to reach equivalent absorbance as the extracted activity during 10 minutes and multiplied by the amount of horseradish peroxidase used in the assay (0.004 units), to calculate the equivalence of the extracted activity with units of horseradish peroxidase activity.

Statistical analysis

The measured traits have been analyzed for statistically significant differences by Student's unpaired *t*-tests (Sigma Plot for Windows 4.0 package - SPW 4.0, Jandel Scientific, Erckhart, Germany).

RESULTS AND DISCUSSION

The results of tomato fruit growth rate and fruit diameter showed different effects of applied FI and PRD treatments (Table I). PRD treatment resulted in smaller and lighter fruits (52.5±2.3 mm and 314.0± 27.3 g) than FI (61.5±2.9 mm and 419.0±29.9 g). Although fruit dry weight was smaller in PRD than in FI treated plants (24.1±0.7 and 28.0±1.9g, respectively) no statistically significant differences were found between compared watering regimes.

Table I

Although PRD treated tomato plants received only half the amount of water that was used for growing FI plants (Table II) the result for water use efficiency of PRD treated tomato plants was two-fold higher than of FI treated plants (1.0±0.03 and 0.5±0.01, respectively) due to the modest effect of the applied treatment on reduction of fruit dry weight. These results confirmed the high potential of partial root drying as a water saving method for tomato growing.

Table II

Tomato fruit growth and development can be divided into three phases[18]. The first phase involves: ovary development, fertilization and fruit set. The second phase of fruit growth is the phase of cell division. The third one is the phase of cell expansion and during this phase the rapid growth of fruit takes place. The effects of applied treatments were analyzed on the basis of the time course of changes in fruit growth rate (FGR), fruit diameter and cell wall ionically bound peroxidase activity in tomato fruit exocarp. Fruit growth rate profiles for both treatments were obtained by fitting the changes in fruit growth rate (FGR) by the third order regression line (Fig. 2A). The fruit growth rate profiles were symmetrical, bell-shaped curves similar to the curve obtained by Monselise *et al.*[19]. 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 similar maximal fruit growth rates for PRD and FI treatments (ca. 2.10mm day^{-1} and 2.24 mm day^{-1}, respectively), although period of maximal growth rate was ca. 5 days shorter for PRD than for FI treated plants.

Another presentation of fruit growth was done in such a way that the results of tomato fruits diameter were fitted by second order of regression (Fig.2B). Such presentation of fruit growth, similarly to Fig**.** 2A, showed that exposure of tomato plant to drought in PRD treatment resulted in the earlier termination of rapid fruit growth phase comparing to FI treatment. In PRD treated plants the rapid growth rate started when fruits passed the age of 10 days and lasted for another

additional 10 days. After this period and, when PRD fruits were according to Gilespy *et al.*[18] in mature green phase, they reached the final diameter of 52.5±2.3 mm. This diameter did not change significantly until fruit reached its red-ripe phase. FI treatment prolonged the period of fruit rapid growth for about 5 additional days and as a result the final fruit size was bigger (61.5±2.9 mm).

Cell wall bound peroxidase activity in fruits of both FI and PRD treated plants was assayed from 15th day from fruits appearing (Fig.2C). In PRD treated plants peroxidase activity of 0.94 HRPU increased dramatically reaching its maximum at the 43rd day from fruits appearing and was 5.95 HRPEU g^{-1}FW. Peroxidase activity in FI treated plants showed fewer changes during the experimental period. After the initial significant increase in peroxidase activity it reached its maximum at the 30th day when it began to drop expressing values ranging from 1.1 to 3.3 HRPEUg^{-1}FW over the investigated period.

Comparing PRD and FI treated plants significant statistical differences in peroxidase activity were at the 29th and 43rd day from fruits appearing ($P<0.05$ and $P<0.001$, respectively).

Temporal changes of peroxidase activity were similar in both treatments for the 15th, 22nd and 29th day from fruits appearing, in both treatments showing increasing trend. This increase in peroxidase activity was significant between 15th and 29th day from fruits appearing, at the same time as fruits expansion generally ceased. After that period which coincided with termination of intensive fruit growth cell wall bound peroxidase activity in fruits of FI treated plants got the decreasing trend but the peroxidase activity of PRD treated plants continued to grow indicating possibly different role of cell wall peroxidase in drought stressed plants. As it is known that during fruit ripening changes in the cell wall may induce formation of new peroxidase isoenzymes[10], the increase in peroxidase activity might be attributed to the synthesis of specific isoform expressing higher enzymatic activity. Also, this increase in peroxidase activity during ripening of drought stressed PRD treated plants might be caused by other factors which are known to induce peroxydase activity such as the synthesis of plant hormones ethylene[20] and abscisic acid[21], or the increase of apoplastic pH value[22].

CONCLUSION

Although PRD treated tomato plants received only half the amount of water that was used

for growing FI plants it affected only tomato fruit diameter and fresh weight which were both similar, but somewhat smaller in drought stressed plants. The effect on tomato fruit dry weight was not significant. One can suppose that due to the shorter period of intensive fruit growth and faster ripening tomato fruits accumulate less water in PRD treated plants. These results might be of interest for growers and processing industry.

The observation that there is a good temporal correlation between epidermal peroxidase activity and growth termination confirms the results and the hypothesis proposed by Thompson *et al.*[9] that the tomato epidermis has a special role in regulation of tomato fruit growth. The increased enzyme activity that mediates cross-linking of cell wall phenolics and therefore reduces the cell expansion consequently causes the reduction of the fruit diameter. Furthermore, our results of the high increase in fruit exocarpic cell wall peroxidase activity after termination of fruit growth during its ripening in plants stressed by PRD treatment point out that cell wall peroxidase might influence fruit maturation by inducing more rapid process.

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IZVOD

Biohemijski mehanizmi regulacije rastenja ploda paradajza u biljkama izloženim stresu suše

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U ovom radu ispitivan je efekat različitih tehnika navodnjavanja na rastenje ploda i na aktivnost peroksidaze ćelijskog zida u egzokarpu ploda paradajza. Ispitivane su tehnike delimičnog sušenja korenovog sistema (PRD) i optimalnog navodnjavanja (FI) u uslovima stakleničkog gajenja paradajza. Pri primeni PRD tehnike supstrat u polovini korenovog sistema zalivan je sa 50% vode upotrebljene za za zalivanje biljaka tretmanom optimalnog navodnjavanja, dok je druga polovina korenovog sistema isušivana sve dok se sadržaj vode u supstratu nije spustio na 15% do 20%, kada je izvršena inverzija zalivanja. PRD tretman je izazvao samnjenje sveže mase ploda, dok je suva masa ploda ploda ostala nepromenjena. Aktivnost peroksidaze je bila značajno veća kod biljaka gajenih PRD tretmanom nego kod optimalno navodnjavanih biljaka. U egzokarpu plodova optimalno navodnjavanih biljaka aktivnost peroksidaze je rasla neposredno pre faze smanjenja intenzivnog rastenja dostižući maksimalnu vrednost od 3,3 HRPU. Kod PRD tretmana najintenzivniji porast peroksidazne aktivnosti u egzokarpu se vremenski poklapao sa fazom zrenja ploda. Maksimalna enzimska aktivnost od 5,95 HRPU je izmerena na kraju faze zrenja. Ovi podaci ukazuju na različite uloge peroksidaze ćelijskog zida u egzokarpu ploda kod PRD tretiranih biljaka. Kod optimalno navodnjavanih biljaka potvrdjena je uloga peroksidaze ćelijskog zida u restrikciji rastenja plodova, a porast enzimske aktivnosti tokom faze zrenja u egzokarpu ploda PRD tretiranih biljaka ukazuje na mogućnost da peroksidaza ćelijskog zida indukujući brži proces učestvuje u kontroli sazrevanja ploda.

Table I Effects of PRD and FI treatments on fruit diameter, fresh weight and dry weight

	Treatments	
Parameter	**PRD**	**FI**
Fruit diameter (mm)	52.5±2.3*	61.5±2.9*
Fruit FW per plant (g)	314.0±27.3*	419.0±29.9*
Fruit DW per plant (g)	24.1±0.7	28.0±1.9

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

Table II The influence of PRD and FI treatments on amount of used water and water use

efficiency (WUE_c).

	Treatmens	
Parameter	**PRD**	**FI**
Amount of water used per plant (L)	25.0±0.0***	50.0±0.0
WUE_c (g DW L^{-3} H_2O)	1.0±0.03***	0.5±0.01

*, ** and *** indicate differences between PRD and FI samples significant at P< 0.05, P<0.01 and P<0.001, respectively

Figure 1. Calibration curve for peroxidase assay

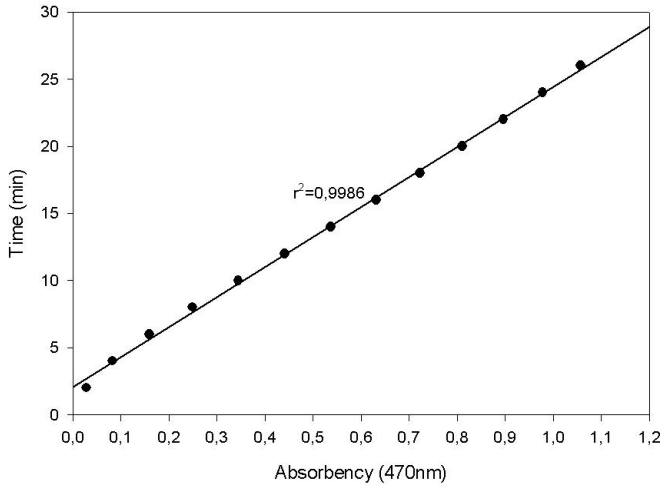
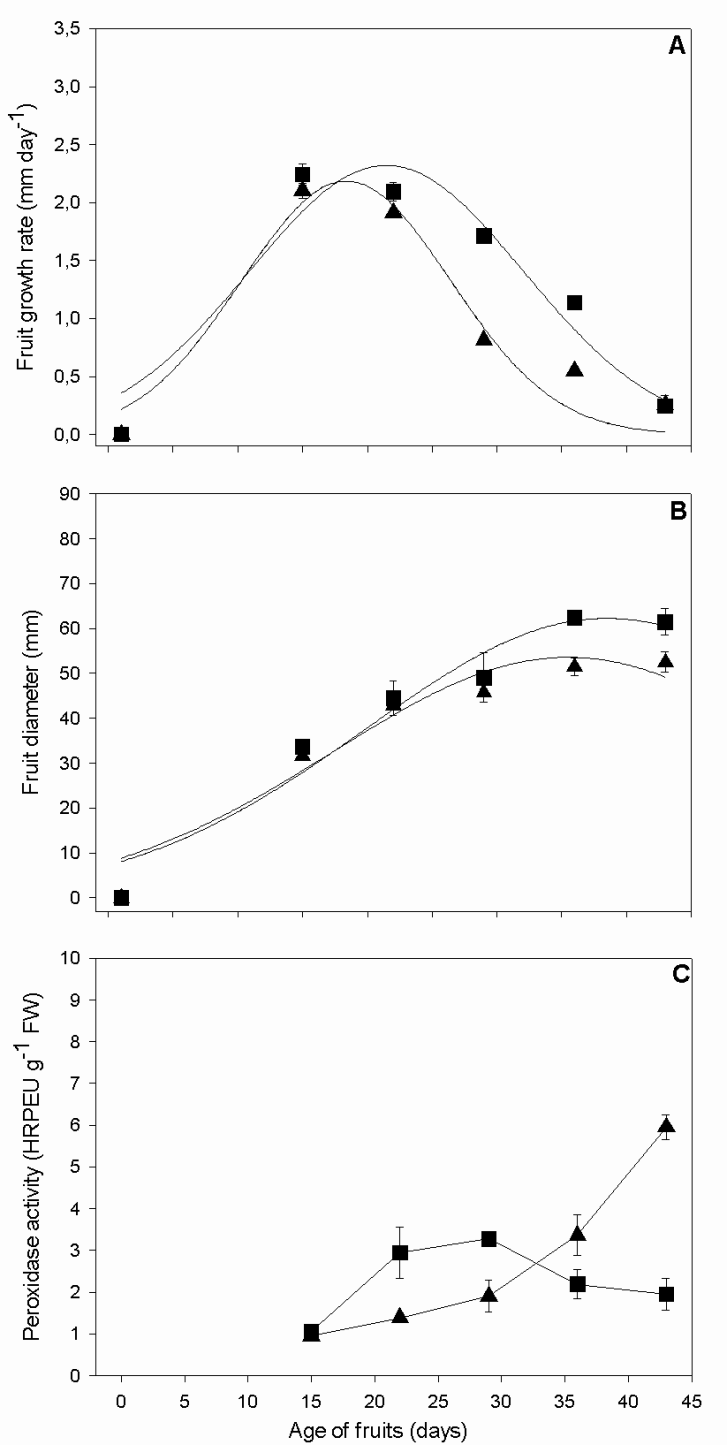


Fig 2. The effects of different irrigation treatments (FI-■ and PRD-▲) on fruit growth rate (A), fruit diameter (B) and cell wall peroxidase activity in exocarp (C) during tomato fruit development.


A
Fruit growth rate (mm day-1)
3,5
3,0
2,5
2,0
1,5
1,0
0,5
0,0
B
Fruit diameter (mm)
90
80
70
60
50
40
30
20
10
0
C
Peroxidase activity (HRPEU g-1 FW)
10
9
8
7
6
5
4
3
2
1
0
0
5
10
15
20
25
30
35
40
45
Age of fruits (days)