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THE ROLE OF CELL WALL PEROXIDASE IN THE INHIBITION OF LEAF AND FRUIT GROWTH

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Summary. The aim of the work presented was to investigate the role of cell wall bound peroxidase in the inhibition of leaf and fruit growth during unfavorable environmental conditions. In the first experiment, plants of two maize genotypes, differing in drought resistance and growth dynamics, were subjected to drought in order to observe leaf growth parameters (LER and SER) in parallel with bulk and segmental peroxidase activity in the growth zone. The results obtained indicated that drought significantly reduced LER, length of elongation zone and local growth rates within it in both genotypes. Bulk peroxidase activity was increased in both genotypes to different degrees (143% in low-LER and susceptible genotype and 238% in high-LER and resistant genotype) while segmental peroxidase activity also increased in almost every segment of the elongation zone with notable peaks in some regions.
In the second experiment the same maize genotypes were subjected to nitrogen deficiency, where growth parameters and peroxidase activity were observed in the division and elongation zone separately. Nitrogen deficiency also caused a reduction of LER, cell division and elongation rate and shortening of the growth zone. This was followed by a significant increase in peroxidase activity in both regions of growth zones: in the low LER genotype this increase was expressed most in division zone, while in all other regions investigated the values were almost the same.
The third experiment includes the effect of a partial root drying (PRD) treatment on tomato fruit growth and peroxidase activity in cell walls of fruit epidermis. Results obtained in all experiments allowed us to confirm that under stress conditions cell wall associated peroxidase plays an important role in biochemical inhibition of leaf and fruit growth.

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Abbreviations: LER – leaf elongation rate, SER – segmental elongation rate

Introduction

Several studies have confirmed the role of cell wall bound peroxidase (EC 1.11.1.7) in biochemical regulation of leaf growth (MacAdam et al., 1992, Bacon et al., 1997, deSouza and MacAdam, 1998 etc). Recent results indicate that reduction in leaf growth rate and cessation of cell expansion under drought conditions may be associated with changes in cell wall bound peroxidase activity (Bacon et al, 1997) while no results about biochemical regulation by cell-wall peroxidase activity under nitrogen deficiency have been reported as yet.

According to current investigations, several cell wall enzymes, including peroxidase, mediate cell expansion of fruit in the same manner as in other plant organs. The role of cell-wall peroxidase in the biochemical regulation of tomato fruit growth was investigated by Thompson et al. (1998) during development, while Andrews et al. (2000) investigated peroxidase isoforms during the same period. They were concentrated on fruit epidermis since this tissue is considered as the growth controlling part. Our work was also focused on the activity in this tissue during fruit development and during partial root drying in order to investigate the role of this enzyme in biochemical control of tomato fruit growth in unfavorable growth conditions.

Materials and Methods

Plant material and growth conditions

Two maize (*Zea mays* L.) genotypes, provided by Maize Research Institute "Zemun Polje" (Belgrade, Yugoslavia) and differing in growth dynamics and drought susceptibility were chosen for experiments. Line F-2 was characterized as a slow growing (low-LER) and drought susceptible genotype and hybrid ZPSC-677 as a fast growing (high-LER) and drought resistant genotype. After seed germination, plants were potted in a mixture made of soil, peat and sand and grown in a phytotrone chamber (t = 23/18 ºC, RH = 70 %, photoperiod 14 h, PPFD of 200 $\mu mol.m^{-2}.s^{-1}$). Plants were irrigated daily until the appearance of the third leaf when randomly selected plants were exposed to drought by withholding water through the whole growth period of the experimental leaf.

Plants for the nitrogen deficiency experiment were grown in perlite as a substrate and were irrigated with two types of nutrient solutions: 100 % NO_3-N and 0 % NO_3-N (Hewitt, 1966).

Seeds of tomato (*Lycopersicon esculentum* Mill.) were germinated in commercial compost in a growth cabinet until the appearance of the fifth leaf. The plants were then removed from their pots and the root system of each plant was divided and repotted into two separate 3.0 l. plastic bags containing the same compost. These two separate compartments were irrigated alternately for 10 days to the end of experiment.

Determination of growth parameters

Leaf elongation rate – the length of the third leaf was measured daily through the whole growth period and according to these data leaf elongation rate (LER) was calculated in $mm.h^{-1}$.

Segmental elongation rate (SER) – on the day of leaf maximal elongation rate (LER_{max}) in the elongation zone of the third leaf the spatial distribution of local growth rates (SER) was determined and calculated in $mm\ mm^{-1}.h^{-1}$ (Schnyder et al., 1987).

In the nitrogen deficiency experiment measurements of epidermal cell length along the growth zone were performed and, on the basis of these data, lengths of the division and the elongation zone were determined.

Dynamics of fruit growth. This parameter was determined by measuring fruit diameter every five days from the immature phase until the end of ripening.

Peroxidase assay

Peroxidase activity in maize leaves in both experiments was determined during the day of maximal growth rate (LER_{max}). In the drought experiment it was determined both in the whole elongation zone (bulk peroxidase activity) and in 2 mm-long sections of the growth zone (segmental peroxidase activity). In the nitrogen deficiency experiment, the growth zone was divided into division and elongation zone so activity was measured in those parts separately. Samples were taken by excising the whole growth zone of the third leaf (bulk peroxidase activity), 2 mm long segments of the zone growth zone (segmental peroxidase activity) or division and elongation zone separately using razor blades.

Samples of tomato epidermis were taken from fruits by peeling frozen fruits collected at different periods of PRD treatment: 55 days at the stage of developing fruits (early green stage), 80 days from mature but non-ripened fruits (mature green stage) and 95 days from ripened fruits. Samples were taken from fruits of five randomly selected plants in both PRD and control treatment. The strips of epidermis were cut and peeled from the equatorial region using razor blades.

All samples were collected on ice and, after determination of fresh weight, were stored in the freezer at –20 ºC. The method for cell wall extraction and isolation of peroxidase activity is precisely described by Bacon et al. (1997).

Peroxidase activity was determined sprectrophotometrically at a wavelength of 470 nm in reaction mixture containing 10 µl of enzyme extract, 1 ml of guiacol solution (276 µl of guiacol per 50 ml of 20 mmol phosphate buffer, pH 5.5) and 0.06% hydrogen peroxide. Activity in maize leaves was expressed in horseradish peroxidase units (HRPEU) per mg of leaf fresh weight while activity in tomato fruit was expressed in nmol per hour and mg of fresh weight.

Results

I Drought experiment

LER_{max} and bulk peroxidase activity

According to results from table 1 maximal leaf elongation rate (LER_{max}) and cell wall bound peroxidase activity of the genotypes investigated significantly differed in both experimental conditions: LER_{max} was higher in ZPSC-677 (high-LER genotype) and was followed by lower peroxidase activity compared to F-2 (low-LER genotype).

SER and segmental peroxidase activity

The profiles of segmental elongation rates (SER) (Fig. 1.A) showed both distribution of growth rates through the zone and length of the elongation zone of the third leaf. Profile of local growth rates is showing differences between lengths of the growth zone and maximal values at the middle of elongation zone in both experimental conditions. Drought affects reductions in SER values and shortening of the elongation zone in both genotypes with more expressed influence in low-LER and drought susceptible genotype.

The profile of peroxidase activity in the elongation zone (segmental activity) of the genotypes observed, presented in figure 1B, indicates different activities through the leaf elongation zone and pointed to the appearance of peaks in some regions of the elongation zone. Drought caused an increase in activity in almost all segments of the elongation zone.

II Nitrogen deficiency experiment

Results for maximal leaf elongation rate (LER_{max}) presented in Table 2 show that nitrogen deficiency caused a reduction in this parameter being, more expressed in the high-LER genotye. Data from Fig. 2 also confirmed different lengths of the growth zone in the genotypes investigated and the effect of nitrogen deficiency, which affected the division zone more than the elongation zone.

Peroxidase activity didn't differ significantly between genotypes but nitrogen deficiency caused a significant increase in both regions of the growth zone (Fig. 3). It is also notable that peroxidase activity reached almost the same values in division and elongation zones.

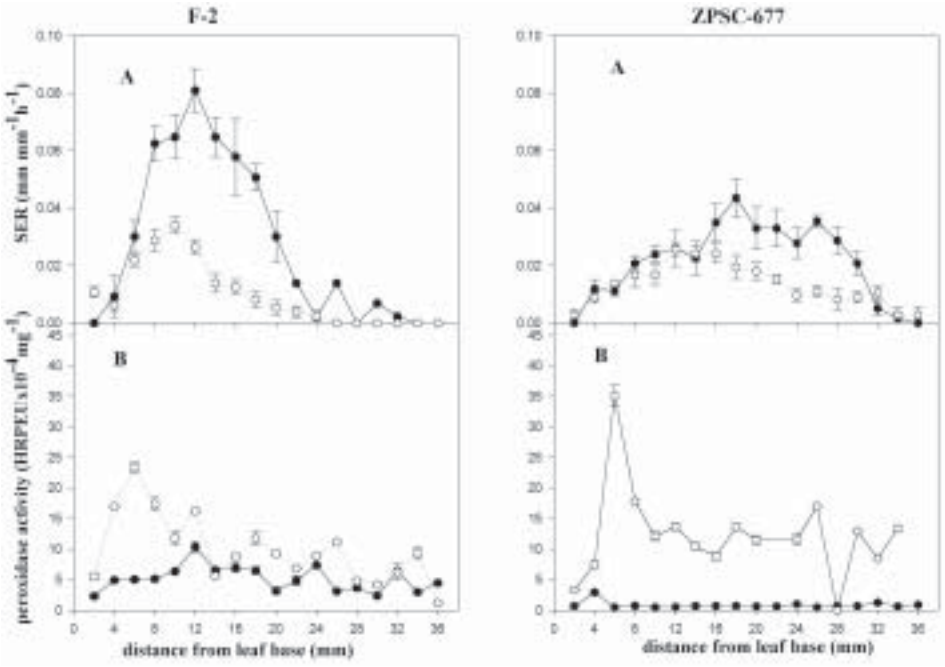

Fig. 1. The spatial distribution of SER (A) and cell wall peroxidase activity (B) through the growing zone of the 3rd leaf in control (closed symbols) and drought conditions (open symbols). Each point is the mean of 10 plants ± S.E. of the 3rd leaf.

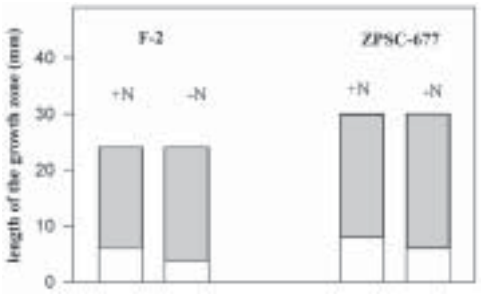

Fig. 2. The length of the division zone (white bars) and elongation zone (gray bars) of plants well supplied with nitrogen and subjected to nitrogen deficiency.

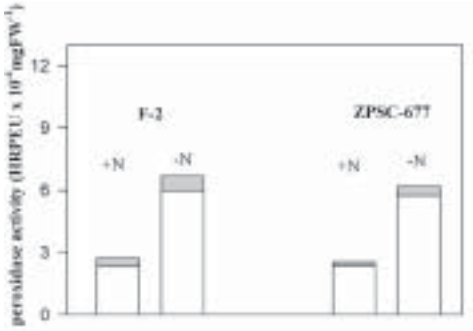

Fig. 3. Peroxidase activity of the division zone (white bars) and elongation zone (gray bars) of plants well supplied with nitrogen or subjected to nitrogen deficiency.

III Dynamics of fruit growth and peroxidase activity at different developmental stages

Changes of tomato fruit diameter and peroxidase activity at different developmental stages in both experimental conditions are presented in Fig. 4. The profile of changes

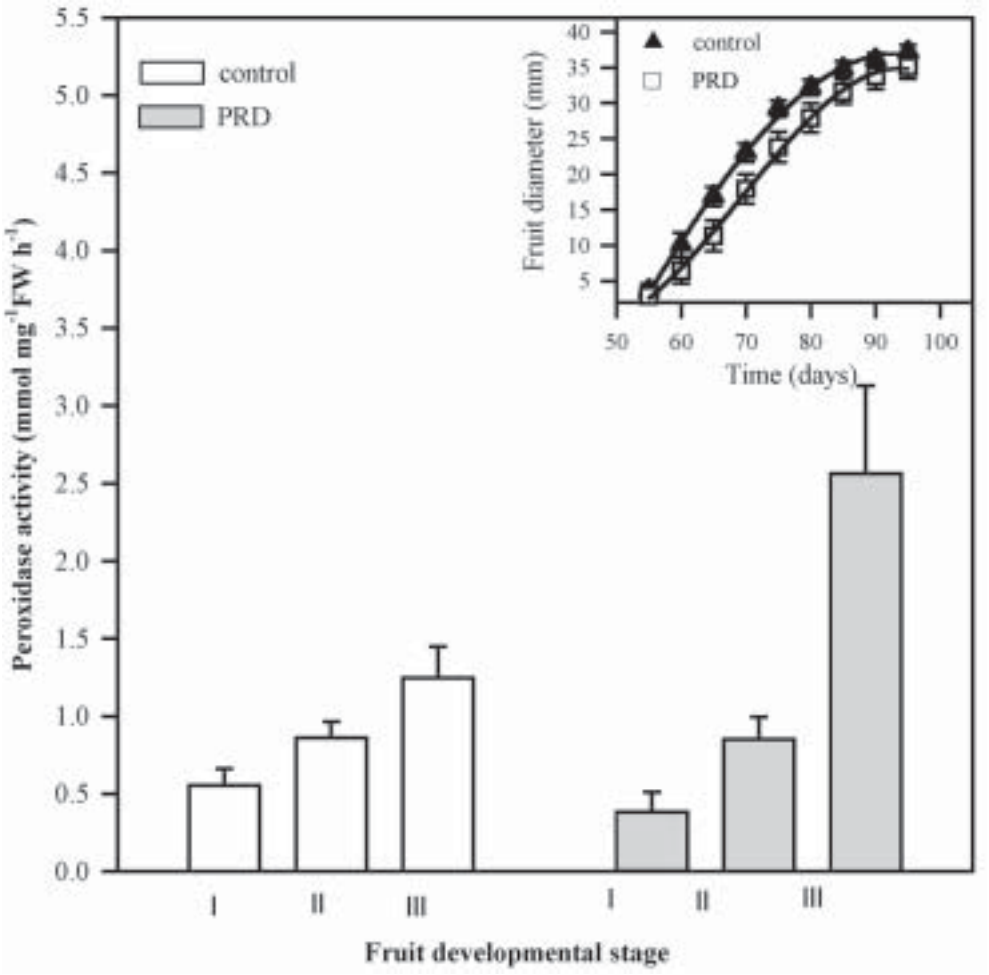

Fig. 4. Peroxidase activity in fruit epidermis at different developmental stages. Inset: fruit diameter during investigated period.

in fruit diameter (inset in Fig. 4) showed a characteristic sigmoid shape with maximal velocities in immature developmental stages and decreasing rates towards maturity. PRD treatment, as indicated, didn't significantly affect growth dynamics or final size of fruit due to slightly higher growth rates although fruit growth stopped earlier than in controls.

Peroxidase activity was rose during fruit development showing significant changes at the cessation of growth (between the immature and green mature phases) in both treatments, while a significant change was also recorded in treated plants between green and complete maturity. Treatment caused significant changes in peroxidase activity only at the stage of complete maturity.

Discussion

Effect of drought on leaf growth and peroxidase activity

Data from Table 1 shows that higher values for LER_{max} in ZPSC-677 are followed by lower peroxidase activity in both experimental conditions. Drought caused significant and almost the same reduction in maximal leaf growth rate of the third leaf in both genotypes while the increase in peroxidase activity was recorded in both genotypes but with different ranges (Table 1) which may point to the differential responses of susceptible and tolerant genotypes to drought.

Table 1. Maximal growth rates (LER_{max}) and bulk peroxidase activity under controlled and drought conditions. *Indicates significance at the 0.1% level.

genotype	LER_{max} (mm/mm/h)			bulk peroxidase activity (HRPEU x 10^{-4} $mg^{-1}FW$)		
	control	drought	Reduction (% of control)	control	drought	Increase in activity (% of control)
F-2	1.17±0.06	0.60±0.03*	49	4.9648±0.4839	12.0470±1.4743*	143
ZPSC-677	2.60±0.13	1.38±0.06*	47	0.6634±0.0776	2.2436±0.3298*	238

The profile of segmental elongation rates (SER) in the growth zone showed that drought shortened the elongation zone and also reduced local growth rates while peroxidase activity showed several peaks of activity in the zone. The first peak was noticed in the division zone, the second in the region of maximal growth rates, while the third was recorded at the end of the elongation zone. This effect was also recorded by Bacon et al (1997). According to these authors, the physiological role of the first peak of activity is not yet understood but may be explained by the high density of cell walls and very fragile tissue; the second peak may be responsible for growth deceleration while cessation of growth can be ascribed to the third peak at the end of the elongation zone. Our results suggest that reduction of leaf growth rate in drought conditions is a consequence of both reduced cell division and cell elongation rate. Since cell-wall peroxidase activity takes part in cross-linking of cell-wall polymers

Table 2. Maximal leaf elongation rate (LER_{max}) on the day of maximal growth rate. *Indicates significance at the 0.1% level.

genotype	LER_{max} (mm/h)		Reduction (% of control)
	+N	-N	
F-2	0.8917 ± 0.0299	0.6833 ± 0.0293*	23
ZPSC-677	1.2625 ± 0.0847	0.9333 ± 0.0374*	26

(Fry, 1986) making it less extensible, it may be supposed that the increase in activity of this enzyme is responsible for maize leaf growth reduction under drought stress condition.

Effect of nitrogen deficiency on leaf growth and peroxidase activity

Nitrogen deficiency is another limiting factor for leaf growth. Volonec and Nelson (1983) have found that optimal quantities of nitrogen increased the number of adult cells per day for 90% in *Tall Fescue* leaves. MacAdam et al. (1989) in *Tall Fescue* leaf blades and Palmer et al. (1996) in maize leaves obtained similar results. Our results showed that nitrogen deficiency affected shortening of the division zone in both genotypes, while the elongation zone remains the same. Peroxidase activity rose significantly in both regions of the elongation zone but there were no genotypic differences. This increase in the division zone, as in the drought experiment, has no valid physiological explanation yet, while the increase in the elongation zone may cause a reduction of cell elongation rates. This led us to the conclusion that cell wall ionically bound peroxidase activity is also taking part in the biochemical inhibition of leaf growth subjected to these unfavorable environmental conditions.

Fruit growth and peroxidase activity during development and partial root drying

Recent investigations have shown that cell wall peroxidase activity changes during fruit development reaching maximal values at the time of growth cessation (Thompson et al., 1998). Our data confirmed the previous study and revealed that significant changes in the activity in well-watered plants are only correlated with growth cessation and not with ripening. However, in treated plants we have recorded significant changes between all stages investigated, which may point to a special physiological role for this activity under stress conditions irrespective of fruit growth. We didn't record significant changes in activity between treatments during fruit ontogeny, as was expected due to lack of growth reduction, confirming the role of this enzyme in growth control. Changes in activity in mature fruits may be due to the appearance of new isoforms and further investigations in this field may provide some explanations.

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