EFFECT OF HEAT STRESS ON SUBCELLULAR LOCALIZATION OF Ca^{2+} IN TOMATO FRUITS

GRAŻYNA GARBACZEWSKA¹, DANUTA CHOŁUJ² AND ZOFIA STARCK²

¹Department of Botany, ²Department of Plant Physiology

Warsaw Agricultural University

02-528 Warsaw, Rakowiecka 26/30, Poland

(Received: December 30, 1997. Accepted: September 21, 1998)

ABSTRACT

The aim of this paper was to compare the fruit cell ultrastructure and subcellular localization of Ca^{2+} after heat stress with the use of the potassium antimonate method (Slocum and Roux 1982, Tretyn et al. 1992).

The tomato plants Robin cv., relatively tolerant to heat stress, were grown under uncontrolled greenhouse conditions to the stage of fruiting. The plants were placed for 20h in two temperature regimes: 23°C (optimal temperature) or 40°C (heat stress) in darkness, under water vapour saturated atmosphere. Immediately after heat stress the fruits were harvested to estimate water soluble and insoluble calcium contents and subcellular localization of Ca^{2+} . After heating the concentration of calcium in tomato fruits increased about twice. In both temperature treatments the water soluble fractions were lower than insoluble ones at smaller differences between insoluble and soluble fractions after heat stress.

The shapes and localization of Ca^{2+} detected with the use of potassium antimonate method show that in fruits of control plants the precipitates were numerous, small and of oval shape. They were dispersed in cytosol or adjoined to endoplasmic reticulum or to external membrane of chloroplast. In the fruit of heated plants the precipitates were irregular in shape, amorphous and singly dispersed in the cytosol. We observed also some cytological changes in the structure of membranes and organelles of the plants of both experimental treatments.

The heat induced increase of calcium content and the changes in subcellular localization of Ca^{2+} under heat stress suggest that calcium ions may be involved in avoiding heat injury. The problem requires more detailed further investigations.

KEY WORDS: calcium localization, cell ultrastructure, heat stress, potassium antimonate method, tomato fruit.

INTRODUCTION

Diverse physiological functions of calcium including a role of Ca^{2+} as structural component of cell wall and membranes, affecting their integrity are recently very often reported (Cramer et al. 1985, Palta 1996). The soft fruits, such as apple (Povaiah 1993) or tomato (Adams and Ho 1993) are especially sensitive to low Ca^{2+} content. Deficit of calcium causes physiological disorders. Therefore, an uninterrupted, continuously and precisely controlled flux of Ca^{2+} to tomato fruits must take place.

In our previous paper (Starck et al 1994), it was demonstrated that during heat stress in tomato cv. Robin, relatively more tolerant to high temperature, the Ca^{2+} import into tomato fruits (estimated as ^{45}Ca) increased, in comparison to control plants contrast in response to high temperature of more sensitive cv. Roma. On the basis of these observations we suggested, that perhaps calcium is involved in the mechanism of fruit protection against heat injury.

In Chołuj's opinion (1996) calcium content in tomato plant tissue, as well as Ca/K ratio are involved in avoiding some heating injuries. Also Tawfik and Palta (1996) postulated the calcium to be able to protect plant from heat stress. Effect of Ca^{2+} on the viability of cell suspension cultures of sugar beet was presented by Biyasheva et al. (1993). The membranes of

potato leaf tissue showed a significantly higher thermostability if the plants were supplied with Ca and N before supraoptimal temperature (Tawfik and Palta 1992). Beet root treated with calcium effectively prevented the leakage of betacyanin induced by heat, (Cooke et al. 1986, Toproven and Glinka 1976). Increase of soluble Ca^{2+} fraction in the cytosol probably caused by loss of membrane associated Ca^{2+} concentration was reported by Williams (1994). It is also known, that various environmental stimuli can alter the cellular Ca^{2+} distribution (Busk 1995). Nevertheless, the question remains still open whether there are some changes in ultrastructural Ca^{2+} localization in tomato fruit cells during the heat stress.

Under optimal conditions the highest amount of Ca^{2+} is located in the cell wall (Bush and McColl 1987, Cleland et al. 1990), and in the central vacuole, while much less in organelles. Calcium concentration in the cytosol is three to four order of magnitude lower than in the above mentioned compartments (Clarkson and Handson 1980, Gilroy et al. 1993). Endoplasmic reticulum is a Ca^{2+} store like the vacuole (Bush 1995). In some cases Ca is sequestered in high amount in vacuole as an insoluble Ca oxalate (Borchert 1990), as well as salt of phosphoric acids (Gilroy et al. 1993).

Present investigations are the continuation of our previous experiments, indicating increased translocation of Ca^{2+} into heated tomato fruits. The aim of this paper is to explain

whether the heat did affect the Ca^{2+} partitioning into water soluble fractions in tomato fruits. Special attention is paid to its subcellular localization in fruit cell under optimal conditions and after heating.

MATERIAL AND METHODS

Tomato plants cv.Robin (relatively tolerant to heat stress) were grown to the stage of fruiting under uncontrolled greenhouse conditions on peat substratum with complex fertilization. It was allowed to develop one cluster with 3-4 fruits only (each 7-10 g F.W.). The tomato plants were transferred into two temperature regimes: 23°C (control temperature) or 40°C (heat stress) in darkness, under water vapour – saturated atmosphere for 20 hrs. Immediately after heat stress the fruits were harvested to estimate the water soluble and insoluble calcium content and to determine subcellular localization of Ca^{2+} .

To estimate Ca fractions the fruit samples (about 1 g F.W.) were homogenized in 5 cm³ of deionized H₂O adjusted to pH 7; then the extraction of soluble Ca in cool water (0°C) took place over 1 hr. The homogenates were centrifuged at 12 000 rpm for 15 min. and supernatant was collected as a water soluble Ca fraction. The residue was washed with deionized water. After washing the samples were dissolved in 20% HCl. Calcium content was estimated in water fraction and in residue – by the atomic absorption spectrometry. The average values given in the table were calculated for 8 fruits harvested from 2 plants, 4 fruits from each. Statistical evaluation of the results was performed by "ANOVA" analysis using the Tukey test.

Cytochemical localization of Ca^{2+}

Small slides cut from the apical part of tomato fruits were fixed using an antimonate-fixation procedure to localize Ca^{2+} in the tissue as described by Slocum and Roux (1982) and Tretyn et al. (1992). Immediately after heat stress the fruit tissue fragments were placed in 2% (v/v) solution of glutaraldehyde in 0.01 M K-phosphate buffer, pH 7.4. Fixation was carried out at 40°C in darkness for 1 h. After rinsing three times with the buffer, the material was placed in 1% (v/v) solution of potassium antimonate, which had earlier been powdered in a mortar and dissolved in 0.1 M K₂HPO₄. The mixture was heated until complete potassium antimonate dissolution, next it was cooled. The pH of the solution was stabilized using K₂HPO₄ at pH 7.6 and K-phosphate buffer was added to obtain a 1% (v/v) solution of potassium antimonate in 0.1 M K-phosphate buffer. The prefixed plant tissues were placed in the antimonate solution and incubated for 3 h at 25°C. The samples were then washed in the buffer and trans-

ferred to 1% OsO₄ in 0.1 M K-phosphate buffer pH 7.2 and fixed for 2 h. When the fixation process was completed, the fruit sections were washed in the buffer and distilled water, then dehydrated in ethanol and acetone. After dehydration the samples were embedded in Epon 812. Ultrathin sections were cut with a Richert Jung Ultracut (Leica) ultramicrotome. The sections were poststained in uranyl acetate and lead citrate. Electron microscope examinations were conducted with JEM 100C (Laboratory of Electron Microscopy of Warsaw Agricultural University).

RESULTS AND DISCUSSION

Immediately after heat treatment of whole plants, the concentration of Ca^{2+} in tomato fruits increased more than twice (Table 1) similarly to our earlier experiments (Starck and Witek-Czupryńska 1993).

After both the temperature treatments, but more in control plants, the water soluble fraction was lower than the insoluble one. Therefore the proportion between Ca-insoluble to Ca-soluble decreased during heat treatment. Similar relations were described also in tomato fruits by Minamide and Ho (1993). Increase of Ca^{2+} in fruits caused by high temperature treatment was similar in both the Ca-fractions.

Cytological localization of Ca^{2+} precipitates in fruits cells

It was presented by Jian et al. (1997) that the antimonate precipitation is a reliable technique for studying subcellular localization of calcium. Few Ca^{2+} deposits were found in the cell walls and the entrance of plasmodesmata of poplar apical bud cells and also in the cytosol and nucleus. On the other hand it is known whether the antimonate precipitates derived from free Ca^{2+} , bound Ca^{2+} or both (Jian et al. 1997). After both temperature treatments the calcium precipitates were observed in parenchyma cells of the tomato fruits. They were located both in the symplast and apoplast. Stress heat induced differences in the sizes, shapes and numbers of calcium precipitates and their localization. In the control plants we observed the numerous small and oval shaped precipitates of Ca^{2+} in the parenchyma cells (Fig. 1). They were located in cytosol, near the cisternae of endoplasmic reticulum and on the surface of chloroplast external membrane (Fig. 2). Some of the precipitates are localized in the stroma of chloroplast (Figs 1 and 2).

Similar subcellular calcium precipitation with potassium antimonate was observed in sweet clover columella cells, as punctate with vacuoles, mitochondria and euchromatine in nucleus (Hilaire et al. 1995). Antimonate precipitates were also seen in the cell wall and inside the plasmodesmata (Fig. 1), in middle lamella (Fig. 4) and in central vacuole (Fig. 1). Roy et al. (1994b) observed that the calcium ions were located in the apple as a constituent of the cell wall. Calcium plays an important role in interacting with pectin acid polymers to form cross bridges that prevent cell separation. Also in tomato fruits the calcium was observed by Roy et al. (1994a) as a constituent of the cell wall, forming cross-bridges preventing cell separation. In our experiments the calcium deposits were sporadic in cell wall of the parenchyma cells in contrast to epidermis (Fig. 3) where numerous calcium precipitates were shown.

Localization of Ca^{2+} precipitates in the fruit cells after heat stress

We observed that the membranes like tonoplast and plasma membrane have many gaps and they were strongly osmophil-

TABLE 1. Calcium content in tomato fruits in water soluble and insoluble fraction $\mu\text{mol. (g DM)}^{-1}$

Numbers in parentheses – percentage of total Ca^{2+} content.				
Treatments	Total	Water soluble	Water insoluble	Insol./sol.
Control	41.8	16.2 (39)	25.6 (61)	1.58
Heat	91.3	40.2 (44)	51.1 (56)	1.27
Increase during stress	49.5	24.0 (48)	25.5 (52)	1.06

LSD_{0.05} = 10.8

Figs 1-4. Fruits of control plants from the optimal temperature 23°C.

Fig. 1. The parenchyma cells of tomato fruit cv. Robin. The small, oval precipitates of Ca^{2+} are localized in the cytoplasm: near the cisternae of endoplasmic cisternae (ER), on the surface of the cell wall (CW) and in the channels of plasmodesmata (PL), and in the vacuole (Va). Magn. 40 000 x

Fig. 2. In the parenchyma cell of tomato fruit small deposits of Ca^{2+} localized on the external surface of chloroplast membrane (CHL) are visible (arrow) and in the stroma of these organelles. Other oval precipitates of calcium are dispersed in the cytosol and also near the tonoplast. Calcium deposits are located sporadically in cell wall. Magn. 40 000 x.

Fig. 3. Fragment of epiderma cell of tomato fruit. The numerous, small deposits of calcium are localized in the thickened external cell wall (CW) and near the cuticula of epiderma. Some of oval Ca^{2+} precipitates are also localized in the cytosol, but large deposit of calcium can be seen on the surface of tonoplast. Magn. 40 000 x

Fig. 4. Fragment of parenchyma cells of tomato fruit. Note the numerous precipitates of calcium in the middle lamella (MD). Magn. 40 000 x.

Figs 5-6. Fruits after heat stress, temperature 40°C.

Fig. 5. After heat stress in the cytosol of parenchyma cell of tomato fruit the biggest, irregular, amorphous Ca^{2+} aggregates are localized. Some of them are connected with cisternae of endoplasmic reticulum. Magn. 65 000 x

Fig. 6. In the parenchyma cell of tomato fruit after heat stress irregular Ca^{2+} precipitates (thin arrows) and microtubule - like structures (thick arrow) are visible in the cytoplasm. One end of microtubular-like structures contacts the tonoplast while the other, the plasma membrane. Deposits of calcium are present also in the chloroplast. Magn. 40 000 x

lic contrasted as compared to fruit cells treated with control temperature. In the cells of parenchyma we noted particularly numerous microtubule-like structures (Fig. 5) which seem to be involved in intercellular and intracellular transport. They were very strongly contrasted and in many cases they were connected with cisternae of endoplasmic reticulum by one end and with plasma membrane by the other. In the stroma of chloroplast irregular and large precipitates of calcium were located (Fig. 5).

In the apoplast of the fruit cells treated with heat stress the calcium precipitates were found sporadically, as well as in the cell wall and in the central vacuole.

In the parenchyma cells of tomato fruits treated with heat stress of amorphous, single, big Ca^{2+} aggregates irregular shapes were dispersed in the cytosol (Fig. 6).

Concluding remarks

In tomato fruits treated with optimal or high temperature the subcellular localization of Ca^{2+} antimonate precipitates were observed in the symplast and apoplast of parenchyma cells, but the heat stress caused some differences in shapes, sizes and localization of deposits. Heat stress may cause the release of Ca^{2+} associated with cell wall and membranes to the cytosol, where calcium was precipitated. It may protect the cell against toxicity of high, soluble Ca – concentration in the cytosol.

According to the experiments presented in this paper, after plant heating the Ca was deposited as amorphous, relatively big aggregates. On the other hand its total content in heated fruits increased, as our earlier experiments have shown (Starck and Witek-Czupryńska, 1993, Starck et al. 1994), and these facts coincided with lower heat stress injury of the fruits e.g. lower inhibition of photosynthates imported to them. Therefore, the precipitation of Ca^{2+} in heated fruit cells may prevent fruits against toxicity of high, soluble Ca concentration in cytosol. It is rather difficult to conclude on physiological importance of the changes in subcellular Ca localization in fruit cells affected by heat stress. Nevertheless the subcellular Ca^{2+} distribution after heating treatment was drastically changed.

On the basis of the presented preliminary results it would be too early to speculate upon the nature, and even more, the functions of various calcium precipitates observed after heat stress. The problem needs further, more detailed studies.

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ODDZIAŁYWANIE PONADOPTYMALNEJ TEMPERATURY NA SUBKOMÓRKOWE ROZMIESZCZENIE WAPNIA W OWOCACH POMIDORA

STRESZCZENIE

Celem podjętych badań było porównanie ultrastruktury komórek owocu pomidora w warunkach optymalnej temperatury i po przegrzaniu roślin oraz subkomórkowa lokalizacja wapnia z zastosowaniem antymonianu potasu (Slocum and Roux, 1982, Tretyn et al. 1992).

Rośliny pomidora cv. Robin bardziej tolerancyjne na stres przegrzania niż inne odmiany rosły w niekontrolowanych warunkach szklarniowych do stadium owocowania. Rośliny były umieszczone na okres 20 godzin w dwóch warunkach termicznych: 23°C – optymalna temperatura i w 40°C w ciemności, w atmosferze nasycenia parą wodną.

Pomiary zawartości wapnia rozpuszczalnego i nierozpuszczalnego oraz wycinki tkanek do badań cytologicznych wykonywano bezpośrednio po przegrzaniu roślin. Stężenie wapnia w owocach pomidora wzrastało po przegrzaniu około dwukrotnie. Rozpuszczalna frakcja wapnia była niższa od nierozpuszczalnej w tkankach owoców roślin traktowanych różnymi temperaturami.

Struktura i lokalizacja precypitatów wapnia w roślinach kontrolnych była inna niż w roślinach po przegrzaniu po zastosowaniu metody cytochemicznej z antymonianem potasu. Precypitaty były liczne, małe, miały owalne kształty. W większości znajdowały się w cytosolu lub stykały się z błonami endoplazmatycznego retikulum bądź chloroplastów. W owocach pomidora w warunkach przegrzania precypitaty były rozmieszczone pojedynczo i miały większe wymiary i nieregularne kształty. Obserwowano również zmiany w strukturze błon i organelli obu eksperymentalnych grup roślin.

Wzrost zawartości Ca^{2+} i zmiany w jego subkomórkowym rozmieszczeniu po przegrzaniu roślin sugerują, że jony te mogą być związane z przeciwdziałaniem negatywnym skutkom przegrzania roślin mającym swój wyraz w uszkodzeniach struktury komórkowej. Powyższe przypuszczenie wymaga dalszych badań.

SŁOWA KLUCZOWE: lokalizacja wapnia, ultrastruktura komórki, stres przegrzania, metoda z antymonianem potasu, owoce pomidora.