Effect of a brassinosteroid analogue and high temperature stress on leaf ultrastructure of *Lycopersicon esculentum*

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Abstract

The ultrastructure of tomato leaf disks treated with a biostimulator (0.01 mg dm^{-3} BB6, brassinosteroid analogue from Cuba), and subjected to high temperature (40 °C for 1.5 h) was studied. High temperature stress caused the appearance of granules in the nucleus, nucleolus and cytoplasm. In chloroplasts and in mitochondria the internal membrane system was disorganised and in chloroplasts some starch granules were detected. These symptoms were more marked in the cells treated with BB6. The influence of BB6 on the ultrastructure of leaf cells was apparent also before being subjected to heat stress.

Additional key words: biostimulator, chloroplast, tomato, ultrastructure.

Introduction

Plant species vary in the sensibility and response to environmental stresses. Stress usually inhibits growth, cell division, and affects morphology and ultrastructure (Smertenko *et al.* 1997). In tomato plants high temperatures (> 35 °C) have a negative effect on cell metabolic activity, growth and photosynthesis (Bar-Tsur *et al.* 1985). At present, diminishing the adverse effects of environmental stresses by using some of the natural or synthetic products with biological activity is possible (Clouse *et al.* 1996, Creelman and Mullet 1997).

Brassinosteroids are widely distributed throughout the plant kingdom and have often growth-promoting activity when applied exogenously (Fujioka and Sakurai 1997). Li and Chori (1999) reported that these compounds can induce stem elongation, pollen tube growth, leaf bending and epinasty, root growth inhibition, induction of ethylene biosynthesis, proton pump activation, changes in xylem differentiation, and regulate gene expression. Kulaeva *et al.* (1991) reported the increased synthesis of heat shock proteins after application of two brassinosteroids in wheat leaves subjected to high temperature stress. This facts makes brassinosteroids and their analogues to become interesting products for application in agriculture. The present work was carried out with the aim of studying the leaf ultrastructure of tomato plants treated with a Cuban synthetic brassinosteroid (BB6), and subjected to high temperatures.

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Materials and methods

Tomato (*Lycopersicon esculentum* Mill. cv. Campbell-28) seeds were sown (22nd December 1998) into pots under field conditions (day/night temperature 27/17 °C, relative humidity (RH) 80 - 90 %, maximum photosynthetically active radiation (PAR) 1600 μmol s^{-1} m^{-2}).

Fifteen days after germination (11th January 1999), leaf disks of 0.5 cm diameter were taken from the central lobule of the last completely expanded leaf (the first true leaf from the bottom) of five plants. The disks were placed in two flasks in distilled water and two in water plus 0.01 mg dm^{-3} BB6 (formulation produced by Natural Products Laboratory from University of La Habana, which contains a brassinosteroid analogue with spirostanic structure as active ingredient), and incubated for 24 h under laboratory conditions (temperature 23/18 °C, RH, 70 - 75 %, maximum irradiance 400 μmol s^{-1} m^{-2}); then, one of the flasks of each variant was subjected to temperature 40 °C for 1.5 h. Samples of 1 mm^2 thick were fixed in 5 % glutaraldehyde with phosphate buffer, pH 7.2, during the night at 4 °C. After postfixation with osmium tetroxide 1 % in phosphate buffer pH 7.2 for 1 h, the samples were dehydrated in graded series of ethanol, acetone and embedded into Spurr's medium (Spurr 1968). Sections of 500 mm were cut with a diamond knife in a *LKB Nova* (Uppsala, Sweden) ultramicrotome, mounted in copper grids and post-stained with uranyl acetate and lead citrate. Samples were photographed in a *JEOL JEM 2000EX* (Tokyo, Japan) electron microscope.

Results and discussion

In a tomato leaf cell neither submitted to high temperature nor treated with the BB6, the nucleolus has fine granulation and small clear zones, and the nucleus a fine granulation and zones with a more dense material distributed near the periphery (Fig. 1). In the nucleus of a cell subjected to high temperature stress (40 °C for 1.5 h; Fig. 2) zones of high density are observed in the nucleolus, and clear zones which were bigger than in the untreated cell. However, there were no abundant granules like those found in radical meristematic cells in *Allium*

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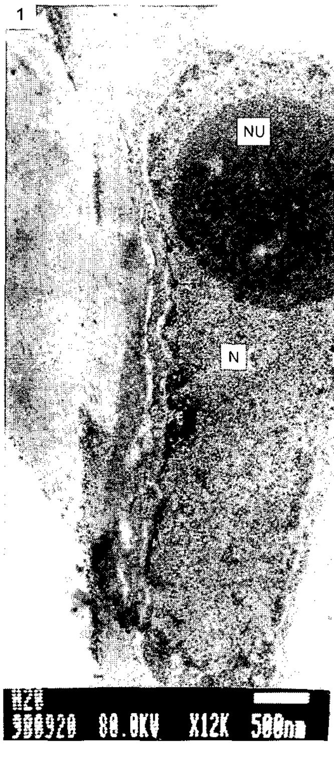

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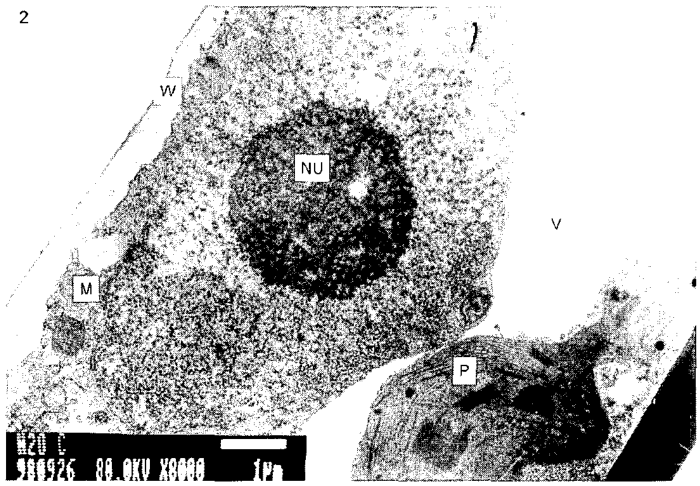


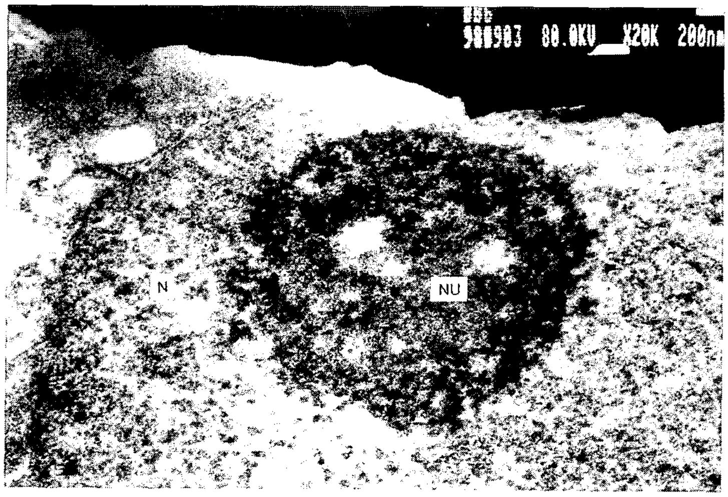
Fig. 1. Nucleus ultrastructure of mesophyll cell of control tomato leaf (no heat-treatment, without BB6). N - nucleus, NU - nucleolus.

Fig. 2. Ultrastructure of mesophyll cell of tomato leaf incubated in water without BB6 and submitted to heat shock (40 °C, 1.5 h). NU - nucleolus, M - mitochondrion, P - plastid, V - vacuole, W - cell wall.

cepa under heat shock (Risueño *et al.* 1973), which surrounded the fibrous part of the nucleolus.

After 24-h incubation in BB6 and without being subjected to heat shock (Fig. 3), the nucleolus presented some clear zones and a granulation greater than those found in the nucleus of the cells subjected to heat shock without BB6 (Fig. 2). That was a characteristic effect of brassinosteroids without stress conditions, as a form of preparing the cells to face the stress. The leaf cells subjected to high temperature and treated with BB6 (Fig. 4) showed segregation of the nuclear and nucleolar elements similar to that shown in Fig. 3, but with larger clear zones and more granulation in some parts of the nucleus. This can be considered a characteristic heat shock effect, which was increased by the effect of the BB6. Differences were also found between nucleoli from the BB6 treated and non-treated cells.

As Kulaeva *et al.* (1991) has pointed out, the most typical ultrastructural change caused by heat shock is the formation of cytoplasmic granules. These structures have been observed under heat shock in many plant cells. They contain mRNA and proteins of low molecular mass. The heat shock proteins protect mRNA from stress-induced degradation. The brassinosteroids activate the synthesis of a number of polypeptides with a mass similar to that of the proteins induced by heat shock (Kulaeva *et al.* 1991).



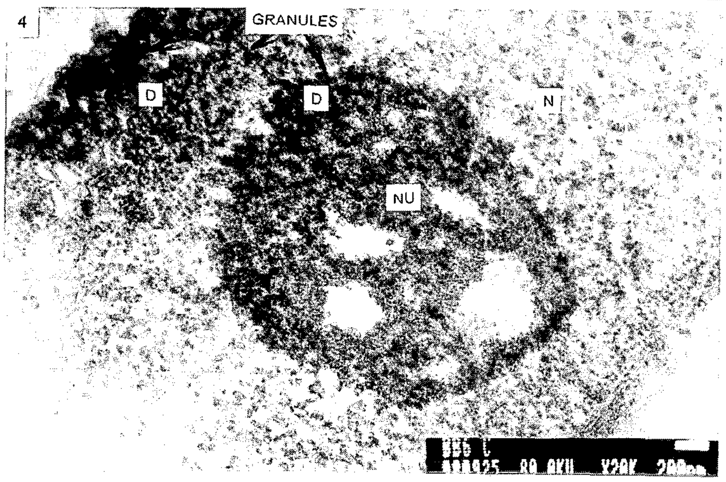
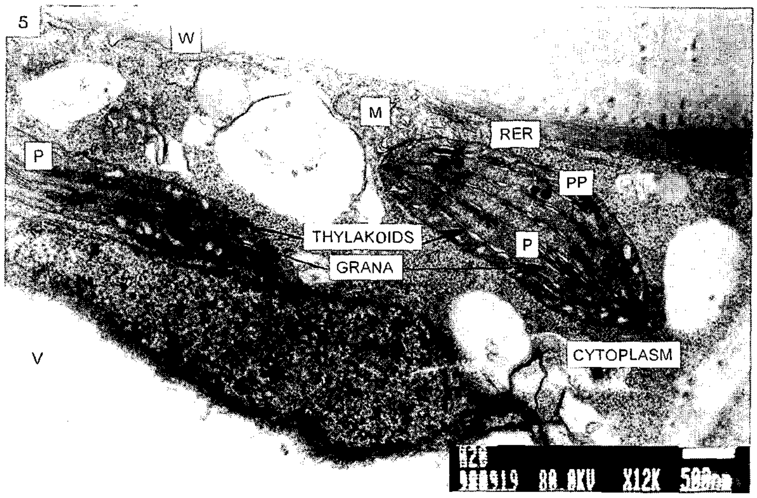


Fig. 3. Nucleus ultrastructure of mesophyll cell of tomato leaf incubated in 0.01 mg dm^{-3} BB6. N - nucleus, NU - nucleolus.

Fig. 4. Nucleus ultrastructure of mesophyll cell of tomato leaf incubated in 0.01 mg dm^{-3} BB6 and submitted to heat shock (40 °C, 1.5 h). D - zones of more density, N - nucleus, NU - nucleolus.

For mesophyll cells from control plants, a dense cytoplasm, mitochondria with non-dilated crest and a rough endoplasmic reticulum were typical (Fig. 5). In contrast, zones of cytoplasmic granulation as well as vesiculation, in cells under heat stress without BB6 were found (Fig. 6). A rough endoplasmic reticulum was observed, but it was neither so abundant nor so well-developed as in mesophyll cells from BB6-treated leaves (Fig. 7). This structure might be an index of the active synthesis of proteins in mesophyll cells from BB6-treated leaves, where granule groups were seen in the cytoplasm in plants treated by BB6 without heat stress. This is a very interesting aspect, because brassinosteroids caused changes similar to those produced by stress, but before the stress happens. In the mesophyll cell treated with BB6 and heat shock changes in chloroplasts and mitochondria are evident (Fig. 8).

It is known that heat shock promotes protein synthesis in cells in response to temperature stress. Specific proteins of low molecular mass are involved in the thermotolerance of oxidative phosphorylation (Downs and Heckathorin 1998).



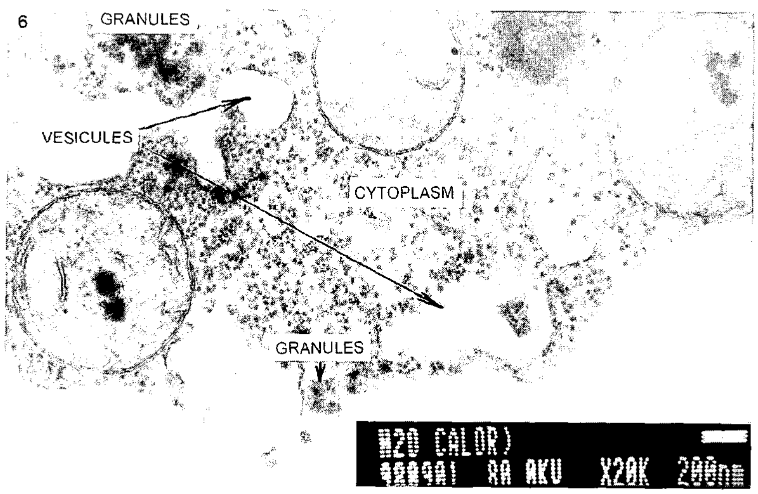


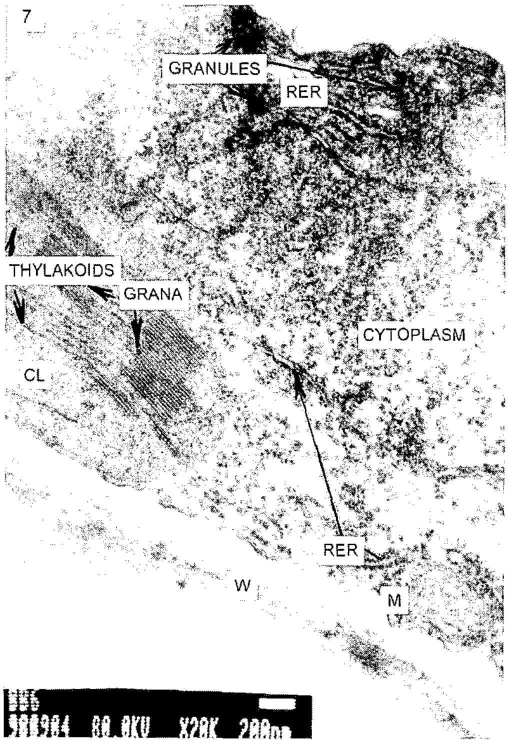
Fig. 5. Ultrastructure of mesophyll cell of control tomato leaf. M - mitochondrion, P - plastids, PP - plastoglobules, RER - rough endoplasmic reticulum, V - vacuole, W - cell wall.

Fig. 6. Ultrastructure of mesophyll cell of tomato leaf incubated in water without BB6 and submitted to heat shock (40 °C, 1.5 h).

In control plants, chloroplasts with plastoglobules, dense stroma and well-organised membrane systems were observed (Fig. 5). However, after heat shock (Fig. 9) chloroplasts showed large internal vesiculation, which have been described in plant cells under other types of stress (López-Carbonell *et al.* 1994a,b, Holopainen 1996, Pastor *et al.* 1999).

In some cases, leaf cell death as a result of heat shock was observed. In these cells both chloroplasts and other cellular components were disorganised with a total break down of internal membranes, provoking starch granules to release (Figs. 10 *a*, *b*). Similar results were described by Gross and Parthier (1994) and (Pääkkönen *et al.* 1998). In cells treated with BB6 more starch granules can be seen (Fig. 10*a*). Heat stressed mitochondria of BB6-treated and non-treated cells (Figs. 6, 8), showed membrane system disorganisation, crest swelling, but they maintained the double membrane, in contrast with most of plastids that lost it (Figs. 10*a*,*b*).

In conclusion, the symptoms provoked by heat shock were more marked in the cells treated with BB6. The influence of BB6 on the ultrastructure of leaf cells was apparent also before being subjected to heat stress



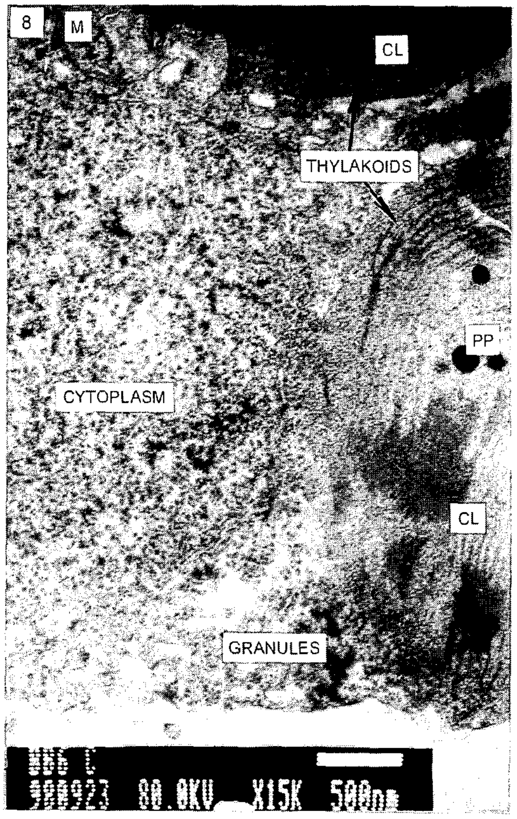


Fig. 7. Ultrastructure of mesophyll cell of tomato leaf incubated in BB6. CL - chloroplasts, M - mitochondrion, RER - rough endoplasmic reticulum, W - cell wall.

Fig. 8. Ultrastructure of mesophyll cell of tomato leaf incubated in BB6 and submitted to heat shock (40 °C, 1.5 h). CL - chloroplasts, M - mitochondrion, PP - plastoglobules.

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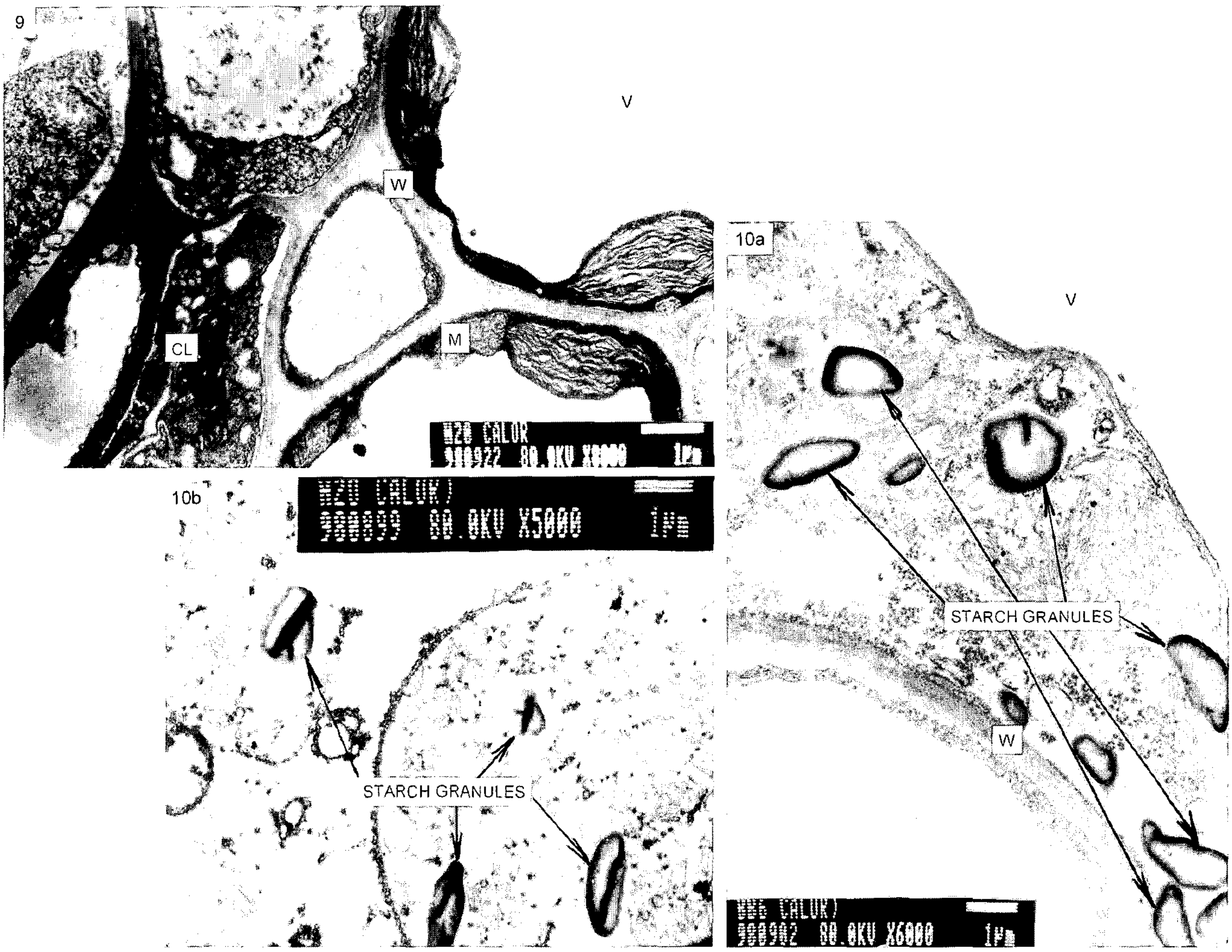

Fig. 9. Ultrastructure of mesophyll cells of tomato leaf incubated without BB6 and submitted to heat shock. CL - chloroplasts, M - mitochondrion, V - vacuole, W - cell wall.

Fig. 10*a*. Starch granules in mesophyll cell of tomato leaf incubated in BB6 and submitted to heat shock. V - vacuole, W - cell wall.

Fig. 10*b*. Starch granules in mesophyll cell of tomato leaf incubated without BB6 and submitted to heat shock.

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