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Effects of chilling on the root cell ultrastructure of two soybean cultivars

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Abstract

Two soybean [*Glycine max* (L.) Merr.] cultivars: Aldana (more resistant) and Essor (less resistant to low temperature) were subjected to chilling at 5 °C for 24 h, and then the ultrastructure of the root meristem cells was investigated. The ultrastructure of control root cells of the tested cultivars differed in the number of condensed mitochondria, plastids with phytoferritin, deformed vacuoles, as well as multivesicular bodies (MB) in cytoplasm and vacuoles. Chilling induced concentric endoplasmic reticulum (ER) arrangement in both soybean cultivars, while the circular Golgi apparatus (GA) occurred only in cv. Essor and MB in the cytoplasm of cv. Aldana cells. Additionally, in cv. Aldana chilling increased the number of condensed mitochondria, MB in vacuoles and multilamellar structures (MS) in cytoplasm whereas in cv. Essor it enlarged the population of plastids with phytoferritin and the number of MB in cytoplasm. After chilling treatment the population of deformed vacuoles with phenolic compounds in the form of electron dense granules increased but the number of multilamellar structures (MS) in the vacuoles of both cultivars decreased. The ultrastructural changes induced by the chilling stress were not lethal but rather adaptive, especially in more resistant cv. Aldana.

Additional key words: *Glycine max,* low temperature, transmission electron microscopy.

Introduction

It is well documented that abiotic stresses like salinity (Pareek *et al*. 1997, Gupta 2007), acid rain (Gabara *et al.* 2003) and extreme temperatures (Pareek *et al.* 1997, Kratsch and Wise 2000, Sowiński *et al.* 2005) induce changes in the cell ultrastructure. Many ultrastructural studies focused on aerial parts of chilled plants demonstrated that chloroplasts were the earliest injured organelles showing swelling, rearrangement and distortion of thylakoid membranes, grana disintegration, starch depletion and sometimes disruption of chloroplast envelope (Musser *et al.* 1984, Kratsch and Wise 2000, Garstka *et al.* 2005). Moreover, in chilling-sensitive plants mitochondria swelling, enlargement of Golgi apparatus (GA) vesicles and dilation of endoplasmic reticulum (ER) cisternae were also described (Kratsch and Wise 2000).

It is known that roots are very chilling sensitive plant organs since in cucumber (*Cucumis sativus*) marked changes in the ultrastructure of cortical cells appeared within only 15 min of exposure to low temperature (Lee *et al.* 2002). However, only a few papers concerning chilling effects on root cell ultrastructure were published (Podbielkowska and Kacperska-Palacz 1971, Podbielkowska *et al.* 1975, Čiamporová and Mistrík 1993, Čiamporová and Trginová 1996, Lee *et al.* 2002, Helliot *et al.* 2003, Stępiński and Kwiatkowska 2003, Szafrańska *et al.* 2005, Abdrakhimowa *et al.* 2006).

The most common cold-induced ultrastructural changes in root cells include: vacuolization, distended ER, higher number of GA cisternae, enlarged plastids deprived of starch, swollen mitochondria with reduced number of cristae, lost integrity of nuclei envelope, higher chromatin condensation and granular component disappearance from a nucleolus (Crèvecoeur *et al.* 1983, Gašparíková *et al.* 1996, Wanke *et al.* 1998, Lee *et al.* 2002).

Although the cell ultrastructure in cold treated plants has been studied for a long time (Kratsch and Wise 2000), there are only a few papers comprising this type of stress in sensitive and tolerant plant species (Wise and

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Abbreviations: AA - ascorbic acid, CW - cell wall; ER - endoplasmic reticulum; FeSOD - Fe superoxide dismutase; GA - Golgi apparatus; M - mitochondrium; MB - multivesicular body; MF - myeline figure; MS - multilamellar structure; P - plastid; POX - peroxidase ; ROS - reactive oxygen species; S - starch grain; V - vacuole.

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Naylor 1987) or different lines of the same species (Sowiński *et al.* 2005), especially carried on roots (Gašparikowá *et al.* 1996, Čiamporová and Trginová 1996, Abdrakhimowa *et al.* 2006).

Therefore, the aim of the present study was to analyse the chilling effect on the ultrastructure of root meristem in two soybean cultivars characterized by different resistance to cold.

Materials and methods

Plants: Two soybean [*Glycine max* (L.) Merr.] cultivars Aldana (more resistant) and Essor (less resistant to chilling) were used in these studies. The soybean seeds of cv. Aldana were obtained from the Institute of Breeding and Acclimatization of Plants (Radzików, Poland) while those of cv. Essor - from Rustica Program Genetique Lavour, France. After surface sterilization in the fungicide (*Thiuram*, *Organica-Sarzyna*, *Sarzyna*, Poland) the seeds were germinated on the cotton wool wetted with distilled water in plastic boxes, at 25 °C. The 3-d-old seedlings were transferred to a growth chamber at 5 °C for 24 h. Plants growing at 25 °C were the control. All experiments were carried out in darkness.

Electron microscopy: For the electron microscopic observations 5 root tips of the control and chilled roots of each cultivar were fixed in 2.5 % glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 0 - 4 °C for 2 h. After washing in the buffer and postfixation with 2 % osmium tetroxide they were dehydrated in the ethanol series, subsequently infiltrated with the mixture of Epon-Spurr and propylene oxide and embedded in Epon-Spurr resin. The ultrathin sections after staining with uranyl acetate and lead citrate (Reynolds 1963) were examined in a *Jeol 1010* (Tokyo, Japan) transmission electron microscope at 80 kV.

The ultrastructure of meristematic cells both of the control and chilled plants was observed and the number of altered plastids, mitochondria, Golgi apparatus and vacuoles (expressed as the percentage of the total number of those organelles in the analyzed microphotographs) as well the frequency of circular ER, multivesicular bodies, myelin-like figures and multilamellar structures in the cytoplasm and vacuoles (expressed as the percentage of the analysed cell profiles in which those structures occurred) were determined on 50 micrographs from each series.

Statistical analyses: Data are shown as means with the standard error (SE). The significance of differences between mean values of the control and chilled material and between the tested cultivars was determined by two-proportion *z*-test. Differences at $P < 0.05$ were considered significant.

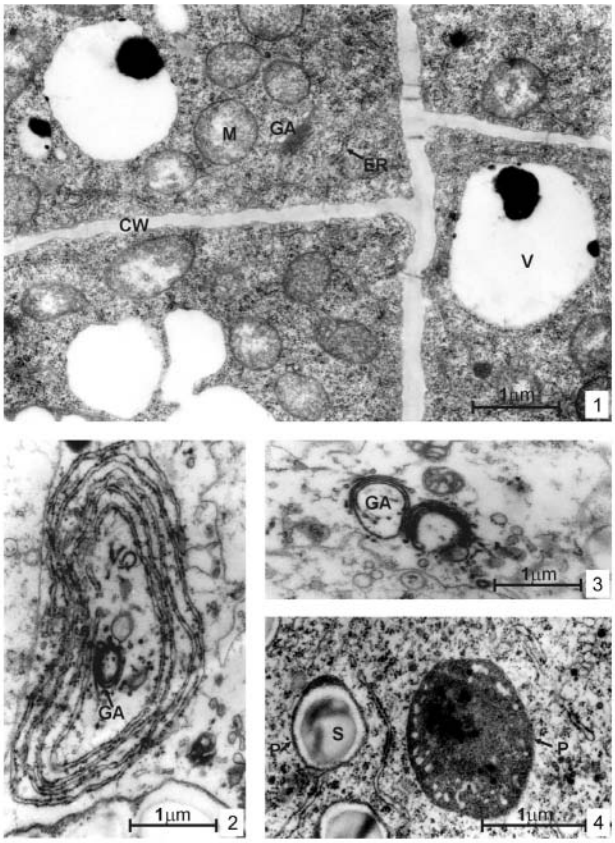
Results

In meristematic cells of the control roots of cv. Aldana (Fig. 1) as well as cv. Essor the ER cisternae evenly distributed in the cytoplasm and GA composed of typical dictyosomes with numerous vesicles were visible (Table 1). Additionally two types of mitochondria: *1*) oval in shape with matrix of electron-density similar to cytoplasm (Fig. 1) and *2*) various in shape with condensed matrix and swollen cristae were present in the cells of both cultivars. The second type was quite numerous in cv. Essor (18.84 %), while rare (1.62 %) in

Table 1. Effect of chilling on the ultrastructure of meristematic cells in *Glycine max* cvs. Aldana and Essor roots [% of cell area]. Means ± SE, $n = 250$. Letters denote significant differences between control and chilling in Aldana (a), control and chilling in Essor (b), Aldana and Essor at 25 °C (c) and at 5 ° C (d) at $P < 0.05$.

Structures	Aldana		Essor	
	25 °C	5 °C	25 °C	5 °C
ER circular	0.00a	3.10 ± 0.18a	0.00b	4.10 ± 0.01b
GA circular	0.00	0.00d	0.00b	5.10 ± 0.01bd
Condensed mitochondria	1.62 ± 0.13ac	75.38 ± 0.89ad	18.84 ± 0.44c	17.72 ± 0.43d
Plastids with phytoferritin	17.47 ± 0.43ac	11.66 ± 0.35ad	6.08 ± 0.25bc	23.53 ± 0.50bd
Deformed vaculoles	23.63 ± 0.49ac	57.23 ± 0.77ad	16.50 ± 0.41bc	60.68 ± 0.80bd
Multilamellar structures (cytoplasm)	2.07 ± 0.14a	8.66 ± 0.30ad	2.73 ± 0.17	4.70 ± 0.22d
Multilamellar structures (vacuoles)	2.26 ± 0.15a	0.50 ± 0.07a	2.44 ± 0.16b	1.02 ± 0.10b
Multivesicular bodies (cytoplasm)	0.00ac	4.33 ± 0.21ad	6.01 ± 0.25bc	11.17 ± 0.34bd
Multivesicular bodies (vacuoles)	1.94 ± 0.14ac	4.60 ± 0.21ad	5.65 ± 0.24c	6.83 ± 0.26d
Myeline figures (cytoplasm)	0.50 ± 0.07	2.36 ± 0.15	1.09 ± 0.10	2.35 ± 0.15
Myeline figures (vacuoles)	0.26 ± 0.05	0.50 ± 0.07	0.25 ± 0.05	0.34 ± 0.06

cv. Aldana (Table 1). Moreover, two types of plastids were seen: *1*) filled with starch grains (Fig. 4) and *2*) additionally containing phytoferritin (Fig. 4). Although the former type of plastids dominated in both tested cultivars, 17.47 % of the latter were noticed in cv. Aldana



Figs. 1 - 4. Fragments of soybean root meristem from the control (1, 4) and chilled roots (2, 3). 1 - Fragment of the control cv. Aldana root meristematic cell, the ER cisternae evenly distributed in the cytoplasm, GA composed of typical dictiosomes with numerous vesicles around, the mitochondria round in shape, matrix of electron-density similar to cytoplasm; the vacuoles with electron dense deposits of phenolic compounds. 2 - ER cisternae arranged circularly in chilled soybean root meristematic cell of cv. Aldana. 3 - Concentric GA in chilled soybean root meristematic cell of cv. Essor. 4 - Plastids with phytoferritin in the control soybean root meristematic cell of cv. Essor.

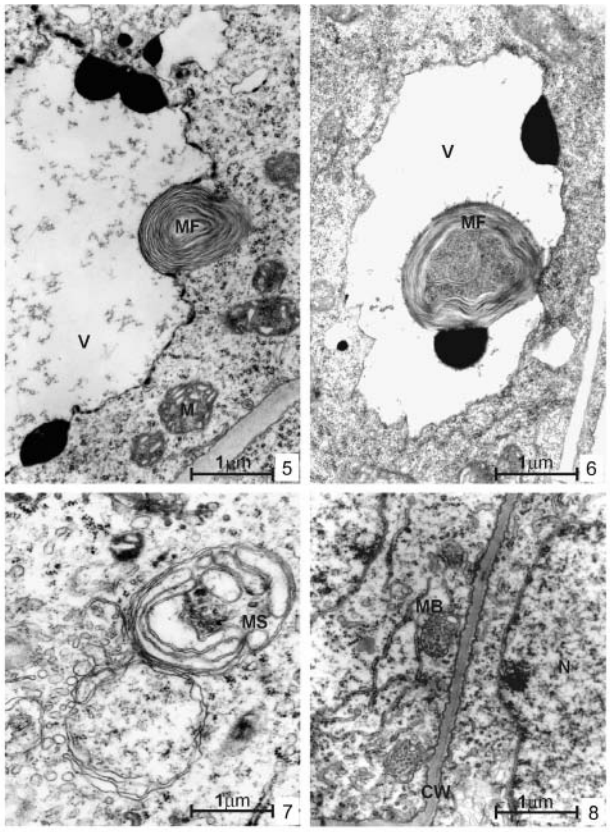
while only 6.08 % in cv. Essor (Table 1). The root meristematic cells of both cultivars had also two types of vacuoles: *1*) more or less oval in shape (Fig. 1) and *2*) deformed. While in cv. Aldana deformation in shape comprised 23.63 % of vacuoles, in cv. Essor it concerned only 16.50 % of these organelles (Table 1). In the control roots of cv. Essor the myeline figures (MF), multilamellar structures (MS) and the multivesicular bodies (MB) were observed in the cytoplasm and vacuoles of meristematic cells. In cv. Aldana the situation was similar, the only exception being lack of MB in cytoplasm and the higher number of those structures in vacuoles (Table 1).

The chilling stress caused appearance of cup-shaped or circular dictyosomes almost deprived of vesicles in cv. Essor (Fig. 3) and MB in the cytoplasm of cv. Aldana cells (Table 1). Moreover, it induced disturbances in the organisation of ER in the root meristem of both tested soybean cultivars *i.e.* 3.10 and 4.10 % of ER cisternae were arranged circularly in cv. Aldana and cv. Essor, respectively (Table 1). In the cytoplasm bordered with such circular ER mitochondria, GA and small vesicles were often visible (Fig. 2).

The other ultrastructural changes induced by chilling stress were only quantitative as compare to the control. In cv. Essor 4-fold enhancement in the number of plastids with phytoferritin was noticed while in cv. Aldana their number was lowered by 6.21 % (Table 1).

In cv. Aldana the number of mitochondria with swollen cristae and condensed matrix (Figs. 5, 10) increased by 73.76 % and the higher numbers of MS in cytoplasme and MB in vacuoles were observed. In cv. Essor the number of these structures remained unchanged but the number of MB in the cytoplasm (Fig. 8) increased after chilling stress (Table 1).

In both tested soybean cultivars the percentage of the



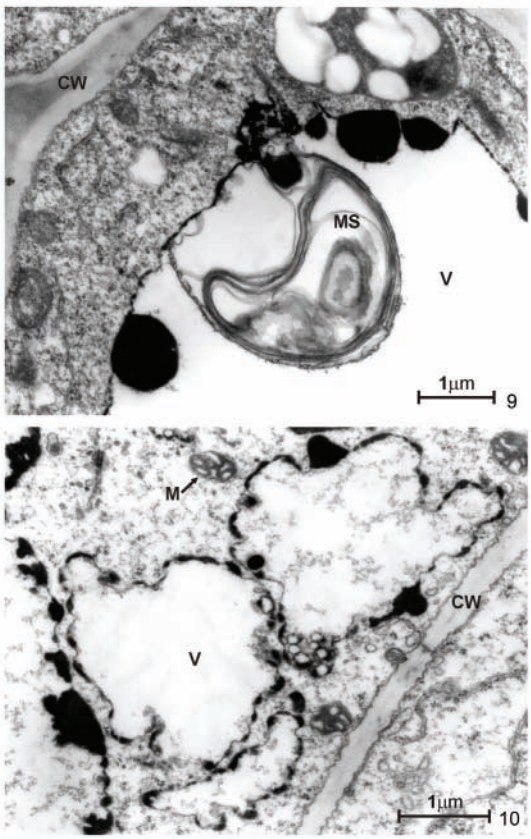
Figs. 5 - 8. Fragments of the chilled soybean root meristematic cells of cv. Aldana (5, 7) and cv. Essor (6, 8). 5 - The condensed mitochondria and myeline figures in the cytoplasm. 6 - The myeline figures in the vacuoles. 7 - The multilamellar structures in cytoplasme. 8 - The multi-vesicular bodies in the cytoplasm.

deformed vacuoles was enhanced (Table 1). These vacuoles contained phenolic compounds in the form of numerous electron dense deposits (Fig. 10). On the other hand, in both cultivars, chilling treatment reduced the number of MS in vacuoles (Fig. 9, Table 1).

Moreover, chilling increased the frequency MF in the vacuoles (Fig. 6) and cytoplasm (Fig. 5) of both soybean cultivars cells but those changes were not statistically significant (Table 1).

Discussion

It is well documented that chloroplasts are the earliest and the most strongly cold affected organelles (Murphy and Wilson 1981, Musser *et al.* 1984, Wise and Naylor 1987, Garstka *et al.* 2005, Sowiński *et al.* 2005). Plastids in root cells seem to be less sensitive to cold than chloroplasts but some ultrastructural changes in those organelles were also observed, *e.g.* in young plastids of maize root cortical cells lower number of starch grains and osmophilic globules appearance were noticed (Crèvecoeur *et al.* 1983). On the contrary, Čiamporová and Trginová (1996) reported accumulation of starch grains only in the cold-sensitive maize line while in the cold-tolerant one they observed higher frequency of thylakoids and their dilation due to low temperature. Moreover, swollen plastids with damaged envelopes were observed in cold treated root cells of cucumber (Lee *et al.* 2002).



Figs. 9 - 10. Fragments of the chilled soybean cv. Aldana root meristematic cells. 9 - Multilamellar structures in the vacuoles. Phenolic compounds in the form of electron dense deposits present in the vacuoles. 10 - The condensed mitochondria and deformed vacuoles with deposits of phenolic compounds.

Contrary to the above described hypersensitive plants in chilled soybean roots the deformed plastids appeared neither in cv. Essor nor in cv. Aldana. Instead of that, significantly higher number of plastids with phytoferritin deposits was seen in cv. Essor while lower in cv. Aldana. The role of phytoferritin is controversial *i.e.* this protein seemed to be either a pro-oxidant or a protective molecule during oxidative stress (Lipiński and Drapier 1997). Intracellular iron can react with hydrogen peroxide generating hydroxyl radical (Briat and Lebrun 1993) and finally, through the Haber-Weiss reaction, other ROS that promote the oxidative damages. Thus, it cannot be excluded that phytoferritin located in plastids lowered the level of iron in cytoplasm and in this way protected the root meristem of cv. Essor against the oxidative stress. On the other hand, the slight decrease in the number of plastids with phytoferritin deposits in cv. Aldana after chilling could be connected with higher requirement of the soybean plants for iron-containing proteins such as FeSOD, an antioxidant enzyme.

Only in the plants hypersensitive to cold (*e.g. Episcia reptans*) mitochondria are malformed by low temperature (Kratsch and Wise 2000), but in the leaves of chilling-resistant pea as well as chilling-sensitive cucumber they remained unaffected in spite of cold treatment at 5 °C for 12 h (Wise and Naylor 1987). On the other hand, in the root cells of cucumber mitochondria reacted as early as 15 min after chilling exposure and after 16 h of the treatment they became swollen, translucent and they showed reduced number of cristae and ribosomes (Lee *et al.* 2002).

Although no disturbances in mitochondrial ultrastructure in the root cells of both examined soybean cultivars after chilling stress appeared, a significantly higher number of condensed mitochondria with the electron-dense matrix and swollen cristae were observed in cv. Aldana. Such ultrastructure is typical of mitochondria with higher level of ADP than ATP due to inhibited oxidative phosphorylation. In mitochondria 1 - 2 % of reduced oxygen is constitutively converted to superoxide through the initiation of one-electron reductions of O_2 by the electron transport chain (Richter *et al.* 1995, Skulachev 1998). Since these organelles are a major source of superoxide in plants subjected to cold (Purvis *et al.* 1995) we suppose that the increase in the number of condensed mitochondria in cv. Aldana up to 75 % probably resulted from the limitation of their activity in order to lower superoxide production.

It was proved that GA was also sensitive to chilling

stress because the dictyosomes with higher number of cisternae were observed in root meristem of *Zea mays* of both chilling-sensitive and chilling-tolerant genotypes after the exposure to 6 °C (Gašparíková *et al.* 1996). Such Golgi reaction was not observed in the soybean tested in the present experiment. Instead of that chilling stress induced appearance of a few circular or cup-shaped forms of GA but only in less resistant cv. Essor. Circular GA was also induced by selenium treatment (Glińska and Gabara 2000). Circular and cup-shaped dictyosomes appeared also after treatment with *Brefeldin A*, an inhibitor of secretion (Robinson *et al.* 1997) therefore, it cannot be excluded that their appearance in the chilled soybean plants might be attributed to disturbances in this process.

ER is also sensitive to chilling stress. In the wheat root cells ER cisternae were more frequent in the tolerant cultivar than in the sensitive one (Abdrakhimova *et al.* 2006). On the other hand, in maize the increase in the number of ER cisternae and their elongation were noticed in both sensitive and tolerant genotypes (Gašparíková *et al.* 1996). Similarly, we observed chilling induced changes of ER ultrastructure in both examined soybean cultivars, but they concerned cisternae rearrangement into circular forms.

ER reorganization could be connected with the synthesis of proteins (Čiamporová and Mistrík 1993). Kosová *et al*. (2007) widely discussed the expression and accumulation of dehydrins as important component of plant protection against cold stress, especially in meristematic tissue. The alternations of ER ultrastructure could also result from increased synthesis of phenolic compounds (Kuraś *et al.* 1999, Stefanowska *et al.* 2003) as the enzymes necessary for their biosynthesis are present on ER membranes (Wagner and Hrazdina 1984).

On the other hand, concentric ER cisternae observed in *A. cepa* root cells treated with respiration inhibitors (Podbielkowska *et al.* 1975) suggest that such reorganization of ER in the cold treated soybean may result from limited metabolic activity. The lowered metabolic activity explained also changes in the number of condensed mitochondria observed in the tested roots.

In the cytoplasm enclosed by circular ER, different organelles were noticed in the root meristems of both tested soybean cultivars. Such structures were defined by Belyavskaya (2004) as cytosegresomes and their partial degradation leads to the formation of myelin-like bodies. The enhanced number of MS and MB was also seen in the cytoplasm of the chilled soybean root cells. Multivesicular bodies or myelin-like structures, in addition to numerous invaginations of the plasma membrane were observed after the chilling stress as well as in the *Brassica napus* var. *oleifera* mesophyll cells (Stefanowska *et al.* 2002). These membranous structures appeared also in the cytoplasm of cucumber root cells as soon as 15 min after exposure to chilling stress (Lee *et al.* 2002). The number of MB increased also in the vacuoles of chilled cv. Aldana root cells. Additionally, chilling stress induced significant increase in the number of deformed vacuoles in both examined soybean cultivars. It cannot be excluded that the vacuoles containing membranous structures represented secondary lysosomes involved in digestion processes. In the chilling sensitive cucumber roots after 16 h of exposure to low temperature some cells showed signs of the final stages of disintegration. The cytoplasm in these cells was sparsely granular and contained scattered vacuoles of varying sizes (Lee *et al.* 2002). On the contrary, neither of the tested soybean cultivar root cells has shown such symptoms of autolysis.

Our investigations revealed the presence of phenolic compounds in the vacuoles of both cultivars. Additionally Szafrańska *et al.* (2005) demonstrated the higher amount of soluble phenolic compounds in response to chilling in the roots of cv. Aldana. Phenolics can directly scavenge ROS acting in phenolics/AA/POX system which can operate in vacuoles and apoplast (Takahama and Oniki 2000, Michalak 2006).

In conclusion, our results showed chilling-induced changes in the ultrastructure of root meristematic cells of two examined soybean cultivars which were not lethal but rather adaptive, especially in more resistant cv. Aldana. The higher number of condensed mitochondria in that cultivar after chilling probably lowered ROS production and together with the higher level of phenolic compounds, protected cell structures against oxidative damage. On the other hand, in less resistant cv. Essor the higher population of plastids with phytoferritin suggested that these organelles were probably engaged in blockade of oxidative burst. Further biochemical analyses should be made to resolve this problem.

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