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Differential ultrastructural changes in tomato hormonal mutants exposed to cadmium

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ABSTRACT

Cadmium (Cd) is a toxic heavy metal, which can cause severe damage to plant development. The aim of this work was to characterize ultrastructural changes induced by Cd in miniature tomato cultivar Micro-Tom (MT) mutants and their wild-type counterpart. Leaves of *diageotropica* (*dgt*) and *Never ripe* (*Nr*) tomato hormonal mutants and wild-type MT were analysed by light, scanning and transmission electron microscopy in order to characterize the structural changes caused by the exposure to 1 mM $CdCl_2$. The effect of Cd on leaf ultrastructure was observed most noticeably in the chloroplasts, which exhibited changes in organelle shape and internal organization, of the thylakoid membranes and stroma. Cd caused an increase in the intercellular spaces in *Nr* leaves, but a decrease in the intercellular spaces in *dgt* leaves, as well as a decrease in the size of mesophyll cells in the mutants. Roots of the tomato hormonal mutants, when analysed by light microscopy, exhibited alterations in root diameter and disintegration of the epidermis and the external layers of the cortex. A comparative analysis has allowed the identification of specific Cd-induced ultrastructural changes in wild-type tomato, the pattern of which was not always exhibited by the mutants.

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1. Introduction

Contamination of the soil by toxic elements such as heavy metals is a major environmental concern (Gratão et al., 2005; Teklić et al., 2008; Paiva et al., 2009). Cadmium (Cd) is probably one of the most toxic heavy metals, particularly at high concentrations, inhibiting plant growth and development, whereas at low concentrations Cd may also stimulate growth depending on the plant species (Gratão et al., 2005, 2008a; Mobin and Khan, 2007; Wahid and Ghani, 2008). Cd can also negatively interfere with important plant processes such as water transport, oxidative phosphorylation in mitochondria, photosynthesis and chlorophyll content (Djebali et al., 2005; Vitória et al., 2006; Tukaj et al., 2007; Lage-Pinto et al., 2008), which is dependent upon metal concentration, plant species, organ/tissue and duration of exposure (Benavides et al., 2005). Moreover, Cd is capable of inducing oxidative, stress which in turn can result in a variety of antioxidant responses (Ferreira et al., 2002; Fornazier et al., 2002; Gomes-Junior et al., 2006; Lindberg et al., 2007; Gratão et al., 2008b,c; Smeets et al., 2008; Tamas et al., 2008).

The biochemical responses of plants to Cd (see Benavides et al., 2005 for a review) and the effect of Cd on the ultrastructure of plant organs (Vitória et al., 2003, 2006) have been investigated in some detail. However, as far as we are aware, there is no specific information available as to how hormonal mutations causing a loss of sensitivity to auxin or ethylene can modify anatomical structure and the response of plants to Cd.

Some of the damage reported is related to leaf structure disorganization, reduced intercellular air spaces, drastic structural thylakoid alterations in the chloroplast (Aravind and Prasad, 2005; Djebali et al., 2005), stomatal closure, softening of cell wall thickening (Vitória et al., 2003) and decrease in chlorophyll content and efficiency of Rubisco activity (Vassilev et al., 2003). In addition, Cd has also been reported to cause disruption of the nuclear envelope, plasmalemma and mitochondrial membranes, severe plasmolysis and high chromatin condensation (Stoyanova and Tchakalova, 1997; Liu and Kottke, 2004).

The use of several model systems has been a successful approach to study the complex network of factors influencing plant responses to the environment and their modulation by hormones. The minia-

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ture tomato cultivar Micro-Tom (MT) has been proposed as a genetic model based on its small size, growth at high density and production of viable fruits and seeds in pots with 50–100 mL of substrate, all within a reduced life cycle ranging between 70 and 100 d (Meissner et al., 1997). Moreover, MT plants harbouring natural genetic variations and hormonal mutations have been produced (Lima et al., 2004; Zsögön et al., 2008), such as the *diageotropica* (*dgt*) mutation that causes a loss of sensitivity to auxin (Kelly and Bradford, 1986; Oh et al., 2006), and the *Never ripe* (*Nr*) mutation, which blocks ethylene perception in tomato leading to incomplete fruit ripening (Wilkinson et al., 1995; Lin et al., 2008). The aim of this work was to characterize the ultrastructural changes induced by Cd in these two mutants and their wild-type Micro-Tom counterpart.

2. Materials and methods

2.1. Plant material

Seeds of *Lycopersicon esculentum* cv. MT and the mutants *dgt* and *Nr* near isogenic to MT (Zsögön et al., 2008) were germinated in boxes containing a mixture of 1:1 (v/v) commercial pot mix (Plantmax HT Eucatex, Brazil) and vermiculite, supplemented with a mixture of 1 g L^{-1} NPK 10:10:10 and 4 g L^{-1} lime. After the first true leaves appeared, seedlings were transplanted to 1 L Leonard pots (Vincent, 1975) (2 seedlings per pot) filled with sand and polystyrene (4:3) and Hoagland's nutrient solution (Hoagland and Arnon, 1950). After 30 d, the growth of the plants was continued in the same Leonard pots filled with a mixture of sand and polystyrene, half of the pots were maintained on Hoagland's solution (control) and half were treated with Hoagland's solution in the presence of 1 mM $CdCl_2$ (Gratão et al., 2008a). The solutions were changed weekly and the total volume was maintained at a constant level by using distilled water. The experiments were carried out in a glasshouse under natural daylight (May–August 2006 and 2007) with temperatures in the range of 20–30 °C. After a period of 85 d post germination, corresponding to 40 d of exposure to $CdCl_2$, samples were collected during the morning period (9:00–10:00 a.m.) from the median third of the 3rd or 4th leaf from the apex, rinsed and used for analyses.

2.2. Scanning electron microscopy (SEM)

Leaf samples were collected and immediately fixed in 2% glutaraldehyde in 0.05 M sodium cacodylate buffer, at pH 7.2. Post-fixation was carried out in 1.0% osmium tetroxide in 0.1 M sodium cacodylate buffer, pH 7.2, for 1 h. The samples were then rinsed 3 times in distilled water and dehydrated through an ethanol series (30, 50, 70, 80%), followed by 3 washes in 100% ethanol. The samples were finally critical point dried through liquid carbon dioxide. The dried samples were mounted in metal stubs, sputter coated with 20 nm gold and examined under a scanning electron microscope (LEO 435 VP, Cambridge, England) at 20 kV, and the images digitalized. The number of totally closed stomata in the abaxial epidermis of the leaves of the MT wild-type and *dgt* and *Nr* mutants were expressed as a percentage derived from an average of 12 observations of leaf micrographs (about 1 cm^2 each).

2.3. Light (LM) and transmission electron microscopy (TEM)

Leaf and root fragments of tomato plants were fixed in a modified Karnovsky solution with 2% glutaraldehyde and 2.5% paraformaldehyde in 0.05 M cacodylate buffer (pH 7.2) for 48 h. Subsequently, the samples were rinsed in cacodylate buffer (0.1 M) and fixed for 1 h at room temperature with 1.0% osmium tetroxide with the same buffer. The post-fixed samples were dehydrated in a graded acetone series and embedded in Epon resin for 48 h. Semithin sections (120–200 nm) were collected in glass slides, stained with toluidine blue (2% in water), for 5 min, rinsed in distilled water and air-dried. The sections were permanently mounted in Entellan resin, observed and documented using an upright light microscope (AxioPlan, Zeiss, Jena, Germany). Ultra-thin sections (60–90 nm) of leaves were collected on copper grids (300 mesh) and stained with 2.5% uranyl acetate followed by 0.1% lead citrate (Reynolds, 1963). Sections were observed at 50 kV using a transmission electron microscope (Em 900, Zeiss, West Germany) and the images digitalized.

Table 1
Effect of Cd on closure of stomatal pores in abaxial epidermis of Micro-Tom (MT), *diageotropica* (*dgt*) and *Never ripe* (*Nr*) leaves.

Cultivar	Treatment	%Closed stomata
Micro-Tom (MT)	0 mM of $CdCl_2$	31.4 ± 1.14
Micro-Tom (MT)	1 mM of $CdCl_2$	77.0 ± 2.6
diageotropica (*dgt*)	0 mM of $CdCl_2$	31.2 ± 1.58
diageotropica (*dgt*)	1 mM of $CdCl_2$	27.7 ± 0.83
Never ripe (*Nr*)	0 mM of $CdCl_2$	36.4 ± 1.64
Never ripe (*Nr*)	1 mM of $CdCl_2$	28.3 ± 1.8

Results are expressed as mean ± s.e. Each value is the average of 12 observations of leaves.

3. Results

The majority of the stomata on the abaxial epidermis of MT, *Nr* and *dgt* tomato leaves were open in the control plants not subjected to Cd treatment (Table 1, Fig. 1A, C and E). However, in the Cd-treated wild-type MT plants, the majority of the stomata were closed (Table 1, Fig. 1B), whilst in the *Nr* and *dgt* mutants, most of the stomata appeared to remain open in the presence of Cd (Table 1, Fig. 1D and F).

Histological analyses under light microscopy of representative leaves of *Nr* and *dgt* exposed to 1 mM $CdCl_2$ for 40 d, revealed important anatomical alterations. These could be seen as a thinner leaf blade resulting from smaller palisade parenchyma cells (especially in the second layer) and a decrease in the number and size of the spongy parenchyma cells (Fig. 2E and H). Cd treatment induced an increase in the area of intercellular spaces in *Nr* leaves (Fig. 2E), but a decrease in the area in *dgt* leaves (Fig. 2H). No differences were observed in the MT leaves in relation to the intercellular spaces or size of the palisade parenchyma cells, when the control plants (Fig. 2A) were compared with Cd-exposed plants (Fig. 2B).

A disorganization of the chloroplasts in the mesophyll cells of both untreated mutant controls, *Nr* (Fig. 2F) and *dgt* (Fig. 2I) was noticeable, when compared to the MT control (Fig. 2C).

Cross-sections of the roots of MT, *Nr* and *dgt* controls (zero $CdCl_2$) (Fig. 3A, C and E) exhibited a well-defined epidermis, a cortex composed of several layers of turgid highly vacuolated cells and the vascular cylinder. Roots of MT (Fig. 3B) and the *Nr* mutant (Fig. 3D) exposed to Cd, exhibited considerable anatomical differences when compared to the controls. MT plants exposed to Cd exhibited a decrease in root diameter, disintegration of the epidermis and the more external cortical cells layers and more conspicuous intercellular air spaces (Fig. 3B), when compared to the control plants (Fig. 3A). In roots of *Nr* (Fig. 3D) exposed to Cd, a greatly reduced diameter was the most important alteration observed. These effects following Cd exposure in MT and *Nr*, were not observed with the same intensity in *dgt* roots (Fig. 3F).

Distinct differences in chloroplast ultrastructure were observed among MT, *Nr* and *dgt* control plants grown in the absence of Cd. In MT leaves, chloroplasts were elongated with an ellipsoidal morphology and a typical arrangement of the grana and stroma (Fig. 4A

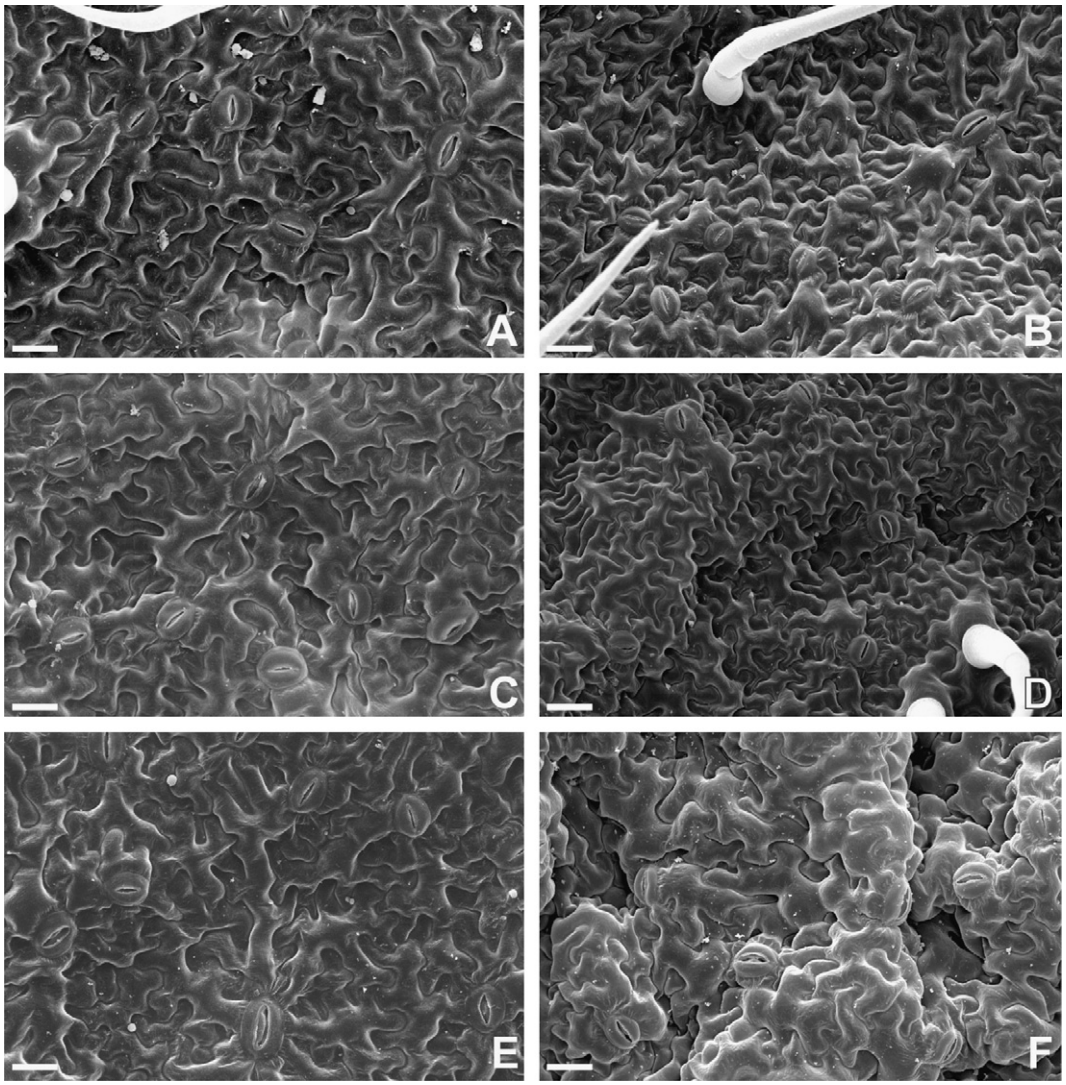

Fig. 1. Leaf epidermis of Micro-Tom, *Nr* and *dgt* mutants grown in nutrient solution with and without 1 mM $CdCl_2$, observed by scanning electron microscopy. (A, C and E) Leaves of Micro-Tom, *Nr* and *dgt* plants, respectively, grown in nutrient solution in absence of $CdCl_2$ (control); (B, D and F) leaves of Micro-Tom, *Nr* and *dgt* plants, respectively, grown in nutrient solution supplemented with 1 mM $CdCl_2$. Bars = 20 μm.

and B). However, *Nr* control leaves exhibited more rounded chloroplasts with abundant starch grains when compared to the wild-type (Fig. 4A and E), whereas the chloroplasts in the mesophyll cells of *dgt* control leaves appeared to be disorganised (Fig. 4I and J). Ultrastructural observations of the leaves of MT, *Nr* and *dgt* plants grown in 1 mM $CdCl_2$, indicated a moderate to severe disorganization of the thylakoid system and stroma (Fig. 4C, D, G, H, K and L), which was not observed in the control plants of MT and *Nr* (Fig. 4A, B, E and F). The number of starch grains in the chloroplasts of Cd-treated MT (Fig. 4C and D) and *Nr* leaves (Fig. 4G and H) was reduced when compared to the untreated controls (Fig. 4A, B, E and F). Moreover, in leaves of all three plant types exposed to Cd, dense spots of osmiophilic material (plastoglobuli) located adjacent to the grana stacks, were observed in the chloroplasts (Fig. 4C, D, G and K).

The main alteration observed in the leaves of MT, *Nr* and *dgt* plants exposed to Cd was the distortion of the chloroplast membrane, disorganization of the grana and disarrangement of the thylakoids (Fig. 4C, D, G, H, K and L). Ultrastructural analysis of plants grown in 1 mM $CdCl_2$ revealed an increased number of peroxisomes in Cd-treated leaves of MT plants (Fig. 4C and D) and of mitochondria in Cd-treated leaves of *dgt* plants (Fig. 4L), but such changes in organelles were not observed for *Nr*.

4. Discussion

The results of SEM revealed variations in stomata closure between the MT and the mutant lines of tomato plants exposed to Cd (Table 1, Fig. 1B, D and F). In response to Cd-induced stress, plants have previously been shown to induce water loss prevention mechanisms (Baryla et al., 2001), that include stomatal closure (Vitória et al., 2003) and a reduction in stomatal length (Shi and Cai, 2008). Stomatal closure is caused by a decrease in guard cell turgor induced by ABA and the efflux of K^+ and associated anions, such as Cl^- and/or malate, which are triggered by an increase in cytoplasmic Ca^{2+} concentration (Tanaka et al., 2005). However, the stomatal closure could be caused by a direct interaction of toxic Cd on guard cells. The stomatal closure and decrease in leaf conductance in MT would limit the diffusion of CO_2 to the site of carboxylation and thus reduce photosynthetic CO_2 uptake (Perfus-Barbeoch et al., 2002). In contrast to the leaves of the MT plants, the majority of the stomata in *Nr* and *dgt* mutants grown in the presence of Cd were open (Fig. 1D and F). The *Nr* locus gene encodes a protein with homology to the *Arabidopsis thaliana* ethylene receptor ETR1 (Wilkinson et al., 1995). The *Nr* mutant has lost the capacity to respond to either endogenously generated or exogenously applied ethylene (Lanahan

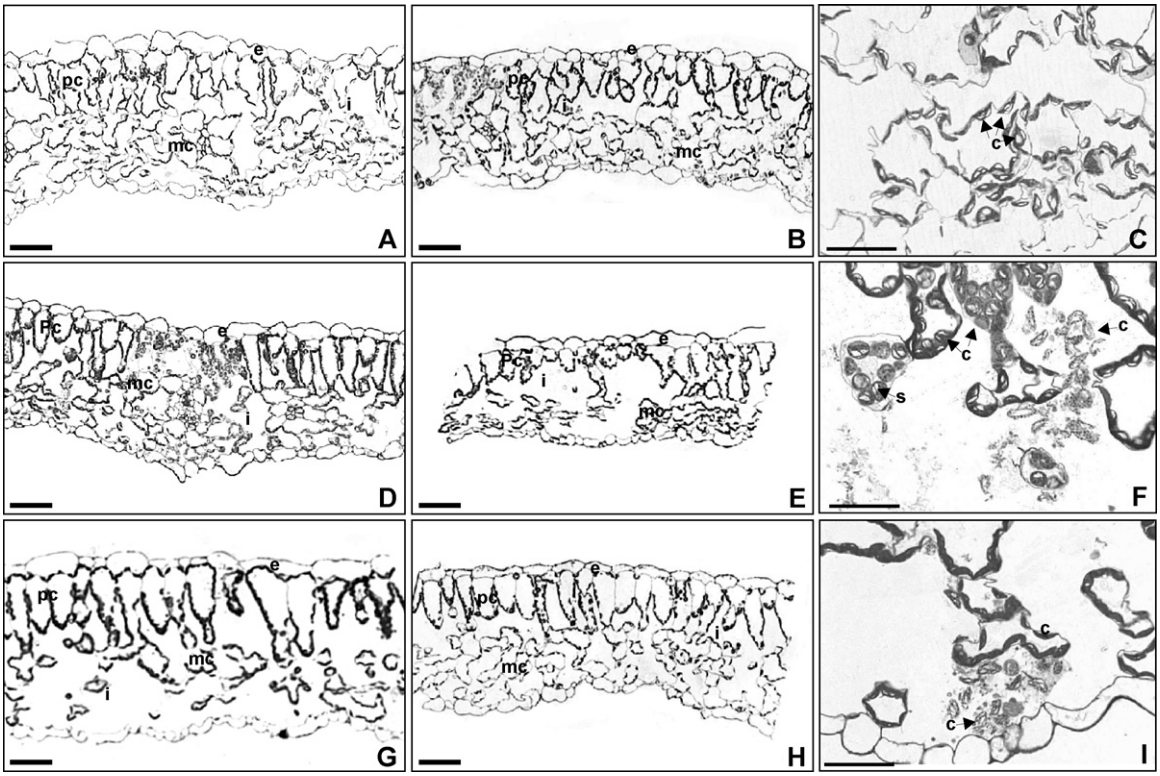

Fig. 2. Leaf cross-sections of Micro-Tom, *Nr* and *dgt* mutants grown in nutrient solution with and without 1 mM $CdCl_2$, observed by light microscopy. Leaves of Micro-Tom (A and C), *Nr* (D and F) and *dgt* (G and I) grown in nutrient solution in absence of $CdCl_2$ (control); (B, E and H) leaves of Micro-Tom, *Nr* and *dgt* plants, respectively, grown in nutrient solution supplemented with 1 mM $CdCl_2$; e = epidermal cells; pc = palisade cells; mc = mesophyll cells; i = intercellular spaces; c = chloroplast; s = starch grain. Bars = 50 μm (A, B, D, E, G and H) or 20 μm (C, F and I).

et al., 1994). Ethylene can also cause an increase in cytosolic Ca^{2+} by activation of Ca^{2+}-permeable channels in the plasma membranes of guard cells (Desikan et al., 2006). The stomata of the *etr1-1* and *etr1-3* ethylene receptor mutants of *A. thaliana* are insensitive to ethylene and do not produce hydrogen peroxide indicating that ETR1 mediates ethylene-induced stomatal closure (Desikan et al., 2005, 2006). This insensitivity to ethylene may explain the opened stomata in *Nr* mutants grown in the presence of Cd (Fig. 1D).

The *dgt* tomato mutant is a spontaneous single gene recessive mutant of the wild-type (Daniel et al., 1989). In *dgt* tomato plants, some auxin responses and transport appeared normal (Fujino et al., 1988; Rice and Lomax, 2000), however, the *dgt* mutation prevents auxin-induced growth and renders growth insensitive to the hormone (Christian et al., 2003). It has been proposed that the *dgt* gene encodes an integral component of a specific auxin signal transduction pathway, a cyclophilin (CYP), Le-CYP1 (Oh et al., 2006). The increase in cytoplasmic Ca^{2+} concentrations related to stomatal movements is a common component of the guard cell ABA-turgor and ABA-nuclear signalling pathways (Webb et al., 2001; Hetherington and Brownlee, 2004). However, this Ca^{2+}-dependent signalling pathway is linked to two calcineurin B-like calcium sensors and a target kinase, which can be related to phosphorylation and activate the potassium channel (Oh et al., 2008). Thus, this calcineurin complex in a specific auxin signal transduction pathway may also help to explain the fact that the majority of the stomata in *dgt* tomato mutant appeared to remain open in response to Cd treatment (Fig. 1F).

Based on the analysis of epidermal cells by SEM, the inhibitory effect of Cd on stomatal opening in MT leaves (Fig. 1B) may be partially explained by the possibility that Cd can directly interfere with Ca signalling and calmodulin regulation (Perfus-Barbeoch et al., 2002). Cheverry et al. (1988) also indicated that the Cd-generated perturbation of Ca levels could stimulate stress ethylene production.

According to the results obtained by light microscopy (LM), an increase in the area of intercellular spaces in *Nr* leaves (Fig. 2E), as well as the decrease in the size of palisade parenchyma cells in *Nr* (Fig. 2E) and *dgt* (Fig. 2H) leaves exposed to Cd, may be related to a reduction of cell volume (Behboodi and Samadi, 2004). However, the reduced cell size and small intercellular spaces observed in the leaves of the *dgt* mutant in the presence of Cd (Fig. 2H), could also indicate that Cd can inhibit leaf expansion, resulting in a decrease in turgor potential and cell plasticity (Barceló et al., 1988). No differences were observed in the size of the intercellular spaces or mesophyll cells in MT leaves grown in the presence of Cd (Fig. 2B). In pea plants exposed to Cd, the ultrastructural analysis of leaves showed an increase in mesophyll cell size and a reduction of intercellular spaces, as well as severe disturbances in chloroplast structure (Sandalio et al., 2001). However, the disorganization in leaf structure of tomato plants induced by Cd reported by Djebali et al. (2005) was essentially marked by a reduced mesophyll cell size and reduced intercellular spaces.

In leaves of *dgt* mutant (Fig. 2G), the palisade mesophyll cells are even bigger in the mutant than in the wild-type. This phenomenon could be related to a compensatory cell enlargement caused by some mutations or transgenes, in which a reduction in cell number is accompanied by an increase in cell size (Krizek, 2009; Micol, 2009).

The growth of roots is controlled by the coordinated action of several phytohormones such as abscisic acid, auxin and ethylene (Davies et al., 2005; Albacete et al., 2008). The most obvious effect exhibited by *Nr* mutant roots (Fig. 3D) was the reduction in diameter, which can be related to the increased sensitivity of the *etr1-1* mutant to severe stress (Wang et al., 2008). This aspect was

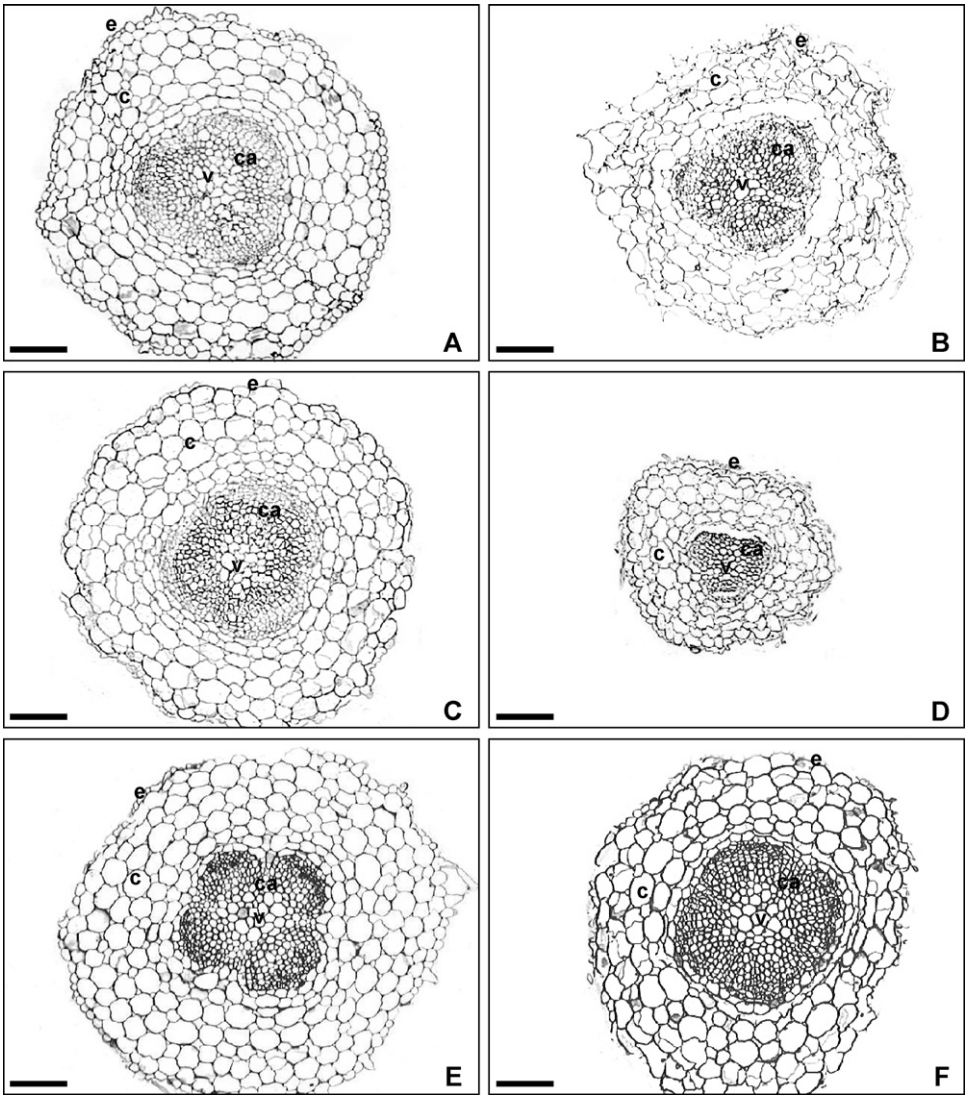

Fig. 3. Root cross-sections of Micro-Tom, *Nr* and *dgt* mutants grown in nutrient solution with and without 1 mM $CdCl_2$, observed by light microscopy. (A, C and E) Roots of Micro-Tom, *Nr* and *dgt* plants, respectively, grown in nutrient solution in absence of $CdCl_2$ (control); (B, D and F) roots of Micro-Tom, *Nr* and *dgt* plants, respectively, grown in nutrient solution supplemented with 1 mM $CdCl_2$. e = epidermis; c = cortex; ca = cambium; v = vascular cylinder. Bars = 100 μm.

demonstrated through the study of *etr1-1* mutants, which showed increased sensitivity to osmotic and salt stress, providing physiological and genetic evidence that the ethylene receptor ETR1 can modulate the response of plants to abiotic stresses (Wang et al., 2008). It is known that *dgt* roots are poorly developed and lack lateral root initiation (Ivanchenko et al., 2006). However, no alterations were observed in *dgt* roots following Cd exposure.

The formation of secondary tissues is not very common in herbaceous plants such as tomato. However, it is also known that secondary tissue formation can be correlated to the large number of stressful conditions (Reinhardt and Rost, 1995; Kawasaki et al., 2008; Fernandez-Garcia et al., 2009). Equivalent data is not available for Cd and it is therefore difficult to make a more definitive comment about it and further investigation is still needed. There has been widespread speculation about the types of Cd complexes that might exist in the phloem, although the mobility of Cd in the phloem has not previously been investigated in detail (Reid et al., 2003). Moreover, the formation of the apoplastic barrier (Casparian bands) in the endodermis started closer to the root apex in Cd treated plants, which can precede xylem element formation (Lux et al., 2004). In addition, the deposition of suberin lamellae (second stage of endodermal development) can be accelerated after Cd treatment and the Casparian band can also be formed in the exodermis as a reaction to environmental stress (Lux et al., 2004).

The alterations observed in the ultrastructure of the chloroplasts in leaves of plants exposed to Cd might be due to an increase in the production of reactive oxygen species (ROS) which in high concentration in the cellular environment can cause oxidative damage to cellular structure and function (Choudhury and Panda, 2005). In this study, the effect of Cd on the ultrastructure of chloroplasts involved disorganization of the thylakoid system and stroma (Fig. 4C, D, G, H, K and L). These changes are similar to the disorganization of the thylakoid membranes observed in *Nicotiana tabacum* cells by Vijaranakul et al. (2001). Structural alterations in chloroplasts can occur when high concentrations of Cd decrease photosynthetic activity (Rascio et al., 1993; Carginale et al., 2004). In addition, Cd can also induce thylakoid distortions and an increase in the number and size of plastoglobuli and peripherical vesicles (Hakmaoui et al., 2007), as observed in this study (Fig. 4C, D, G, H, K and L). The number and size of plastoglobuli can be increased in chloroplasts following exposure to stress, acting in the synthesis and recycling of lipophilic compounds produced during oxidative metabolism (Olmos et al., 2006). Moreover, senescence can also be characterized by a disintegration of organelle structures and

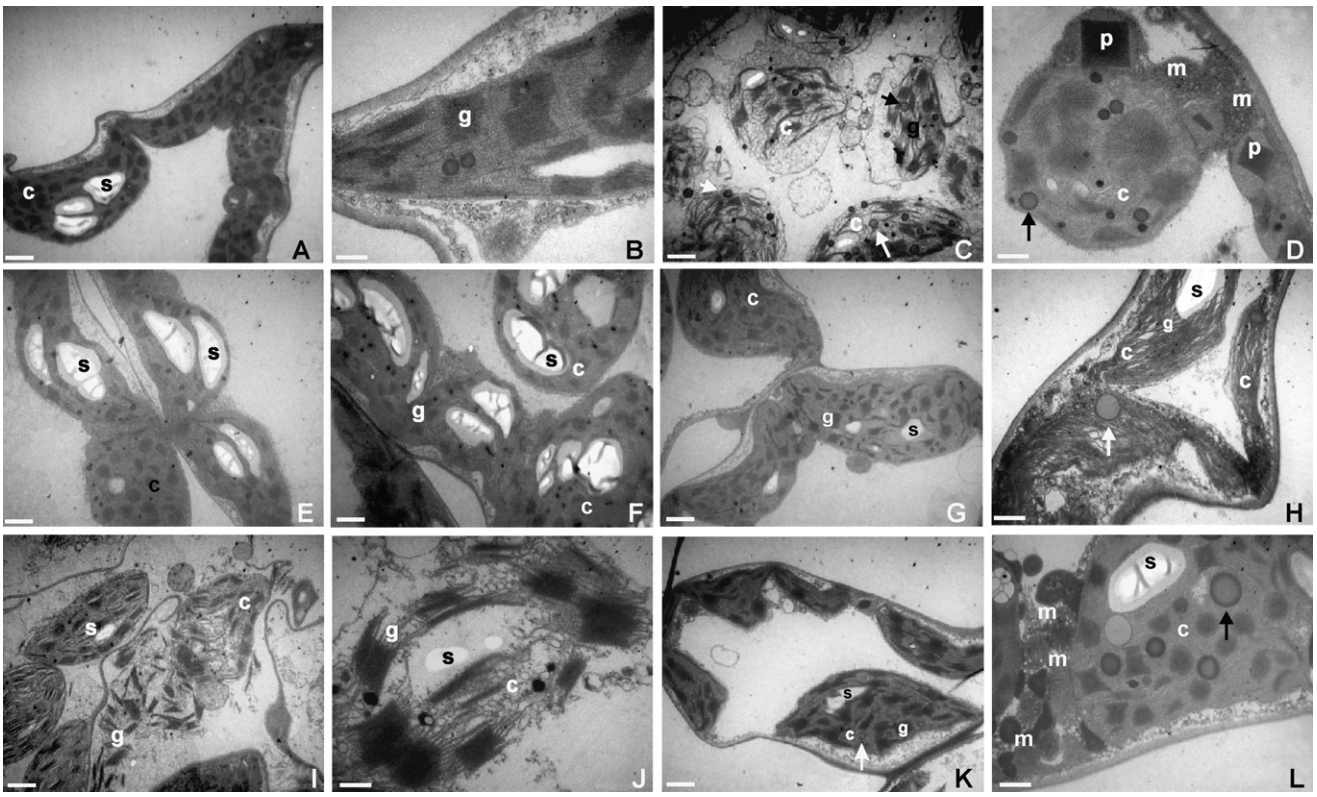

Fig. 4. Organelle ultrastructure in leaf mesophyll cells of Micro-Tom, *Nr* and *dgt* mutants grown in nutrient solution with and without 1 mM $CdCl_2$, observed by transmission electron microscopy. Plants of Micro-Tom (A and B), *Nr* (E and F) and *dgt* (I and J) grown in nutrient solution in absence of $CdCl_2$ (control) and plants of Micro-Tom (C and D), *Nr* (G and H) and *dgt* (K and L) grown in nutrient solution with 1 mM $CdCl_2$. c = chloroplast; g = grana; s = starch grain; m = mitochondria; p = peroxisome. Arrows indicate plastoglobuli. Bars = 2 μm (A, C, E, G, I and K) or 1 μm (B, D, F, H, J and L).

increase in number and size of plastoglobuli (Sandalio et al., 2001). It was suggested that these ultrastructural changes induced premature senescence in wheat (Ouzounidou et al., 1997).

The chloroplasts in the control *Nr* mutant (Fig. 4E and F) contained numerous starch grains. The starch accumulation in *Nr* chloroplasts is consistent with the role of ethylene in starch degradation during fruit ripening (Rice and Lomax, 2000). However, in this work we did not analyze fruit ultrastructure.

The size and number of starch grains in the chloroplasts of Cd-treated MT (Fig. 4C and D) and *Nr* leaves (Fig. 4G and H) were shown to be reduced when compared to the control untreated leaves (Fig. 4A, B, E and F). The decrease in water uptake, which has been shown to occur in plants submitted to Cd stress (Rascio et al., 2008), is one of the most important environmental factors inhibiting photosynthesis (Lee et al., 2008). The absence or decrease in number of starch grains in the leaf chloroplast stroma may be related to photosynthesis inhibition by Cd (Djebali et al., 2005). It has been demonstrated in different plant species that Cd can decrease carbon assimilation (Perfus-Barbeoch et al., 2002), thus the low starch content of the stressed plants is probably due to the reduced carbon fixation (Devi et al., 2007). No significant alterations in starch grain number were observed in *dgt* leaves following Cd exposure (Fig. 4I, J, K and L).

We also noticed some interesting chloroplast disorganization in the control, *Nr* and *dgt* leaves that had not been exposed to Cd. This apparent chloroplast disorganization was more pronounced in *dgt* leaves (Figs. 2I and 4I and J), but was also visible in *Nr* (Fig. 2F). Chloroplast disorganization contrasts with the dark green phenotype of *dgt* leaves (Coenen et al., 2003), however, the dark green phenotype, which is also visible in GA deficient mutants, is mainly due to a reduction in cell size which causes an increased concentration of chloroplasts per leaf area (Koornneef et al., 1990). Thus, this disorganization observed could be counterbalanced by chloroplast concentration per leaf area. However, further investigation is necessary.

The information available in this work is an important step towards obtaining a better understanding of the structural changes caused by Cd and its effects on metabolic processes. This work is perhaps the first one to integrate a study of hormonal mutants with a heavy metal and ultrastructural changes. Although the hormonal interaction has been studied intensively, the molecular and cellular mechanisms underlying this interplay are unknown.

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