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BRIEF COMMUNICATION

Cd-induced system of defence in the garlic root meristematic cells

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Abstract

Studies on cadmium effects in the root meristematic cells of *Allium sativum* L. were carried out using electron microscopy in order to explain the possible mechanisms of garlic seedlings' tolerance to Cd stress. Seedlings were treated with 0.01, 0.10 and 1.00 mM $CdCl_2$ solutions for 0.5, 1, 2, 4, 8, 10, 12, 24 and 48 h, respectively. The results indicated that cell walls, plasma membrane and main organelles actively participated in Cd detoxification and tolerance at low Cd concentrations. Once excessive Cd ions entered the cytosol, a defence mechanism becomes activated, protecting the cells against cadmium toxicity. However, under high Cd content in cells, the cell structure was damaged, even leading to cells death.

Additional key words: *Allium sativum* L., dictyosomes, endoplasmic reticulum, mitochondria, nucleus, ultrastructure, vesicles.

Cadmium is one of the most mobile heavy metals. It can be readily taken up by roots and translocated to shoots where it can accumulate to high level. Also, it can easily enter the food chain (Yu *et al.* 2006). At low concentrations Cd is not toxic to plants, but at higher concentrations it inhibits root growth and cell division in many plants including onion (Liu *et al.* 1992) and garlic (Liu *et al.* 2003). Besides, it can inhibit photosynthesis by impairing chlorophyll synthesis (Sanità di Toppi *et al.* 2005a, Tukaj *et al.* 2007) and affect cell metabolism by altering the behaviour of key enzymes in important pathways (Verma and Dubey 2001, Sanità di Toppi *et al.* 2005b, Shamsi *et al.* 2008).

Plants have a range of potential mechanisms at the cellular level that might be involved in the detoxification of heavy metals (Hall 2002). It has been reported that heavy metal tolerance in higher plants is the result of different processes which prevent excess of heavy metals in the cytoplasm and organelles. Although the Cd toxicity and plant tolerance mechanisms were widely discussed (*e.g.* Zenk 1996, Sanità di Toppi *et al.* 2007), Cd tolerance strategies on subcellular level has not been fully explained yet. In the present study, we have tried, by means of electron microscopy, to explain the possible mechanisms of Cd tolerance of garlic seedlings.

Healthy and equal-sized garlic cloves were chosen from bulbs that had not started the formation of green leaves or root growth. The bases of bulbs remained submerged in the tap water at 20 - 24 °C. When roots reached about 1.5 cm in length, they were immersed into 0.01, 0.10 and 1.00 mM $CdCl_2$ solutions for 0.5, 1, 2, 4, 8, 10, 12, 24 and 48 h. Tap water was used as the control. The methods for preparation of ultrastructural sections and observations were described previously (Liu and Kottke 2003). Briefly, terminal roots (1 - 3 mm) were fixed in 2 % formaldehyde and 2.5 % glutaraldehyde, post-fixed by 2 % osmium tetroxide, dehydrated and embedded in *ERL* resin. Ultrathin sections (75 nm) were examined with transmission electron microscopy (*JEM-1230*, *Jeol*, Tokyo, Japan).

A typical ultrastructure was observed in control cells. Plasma membrane was unfolded with a uniform shape in all parts. Large amounts of endoplasmic reticulum (ER), dictyosomes, mitochondria and ribosomes were immerged in dense cytoplasm. The nucleus with well-stained nucleoplasm and distinct nucleolus was located in the center of cells (Fig. 1*a*).

The effects of Cd on garlic root meristematic cells were concentration- and time-dependent. At concentrations 0.01 and 0.10 mM Cd activated a cellular defence. The root cells exposed to 0.01 mM Cd for 1 h and 0.10 mM Cd for 0.5 h did not show any visible changes of the ultrastructure. A series of changes appeared in

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Abbreviations: ER - endoplasmic reticulum; rER - rough endoplasmic reticulum; TEM - transition electron microscopy.

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these concentration but longer treatments. Cell walls were dark and some electron dense granules were precipitated in the cell walls, especially between the cell walls and plasma membrane at 0.01 mM Cd after 4 h and 0.10 mM Cd after 2 h (Fig. 1*b*). The invaginations into the vesicles occurred actively in plasma membrane, and pinocytotic vesicles containing some electron dense granules were often seen (Fig. 1*b*). Dictyosomes were sensitive to Cd stress and secreted vesicles increased obviously and were mainly distributed nearby plasma membrane (Fig. 1*c*). They were gradually disintegrated with increasing Cd concentration and prolonging of treatment duration. At the same time, parallel arrays of rough endoplasmic reticulum (rER) with regularly extended cisternae appeared markedly in cytoplasm and vesiculated rER could be observed in some cells (Fig. 1*d*). These vesicles from membranes of ER might carry some polysaccharides and proteins for Cd detoxification. One or more vesicles wrapped by ER could be seen in some cells (Fig. 1*e*). Mitochondria had an oval shape and densely packed well-developed cristae. The structural alternations of mitochondria were found after the long Cd treatments (0.10 mM Cd for 24 h and 1.00 mM Cd for 12 h). At first, a spherical shape was exhibited (Fig. 1*f*) and then a progressive disappearance of cristae was observed with increasing Cd concentration and exposure. Some electron dense granules were revealed in mitochondrial cristae and matrix (Fig. 1*f*). Electron dense granules wrapped by elongated mitochondria were also observed.

Another important change was the formation of diverse vesicles/vacuoles within the cytoplasm after Cd exposure. Usually, several vesicles gradually fused together producing a bigger cytoplasmic vacuole (Fig. 1*g*), or they were repeatedly wrapped and formed "multivesicular

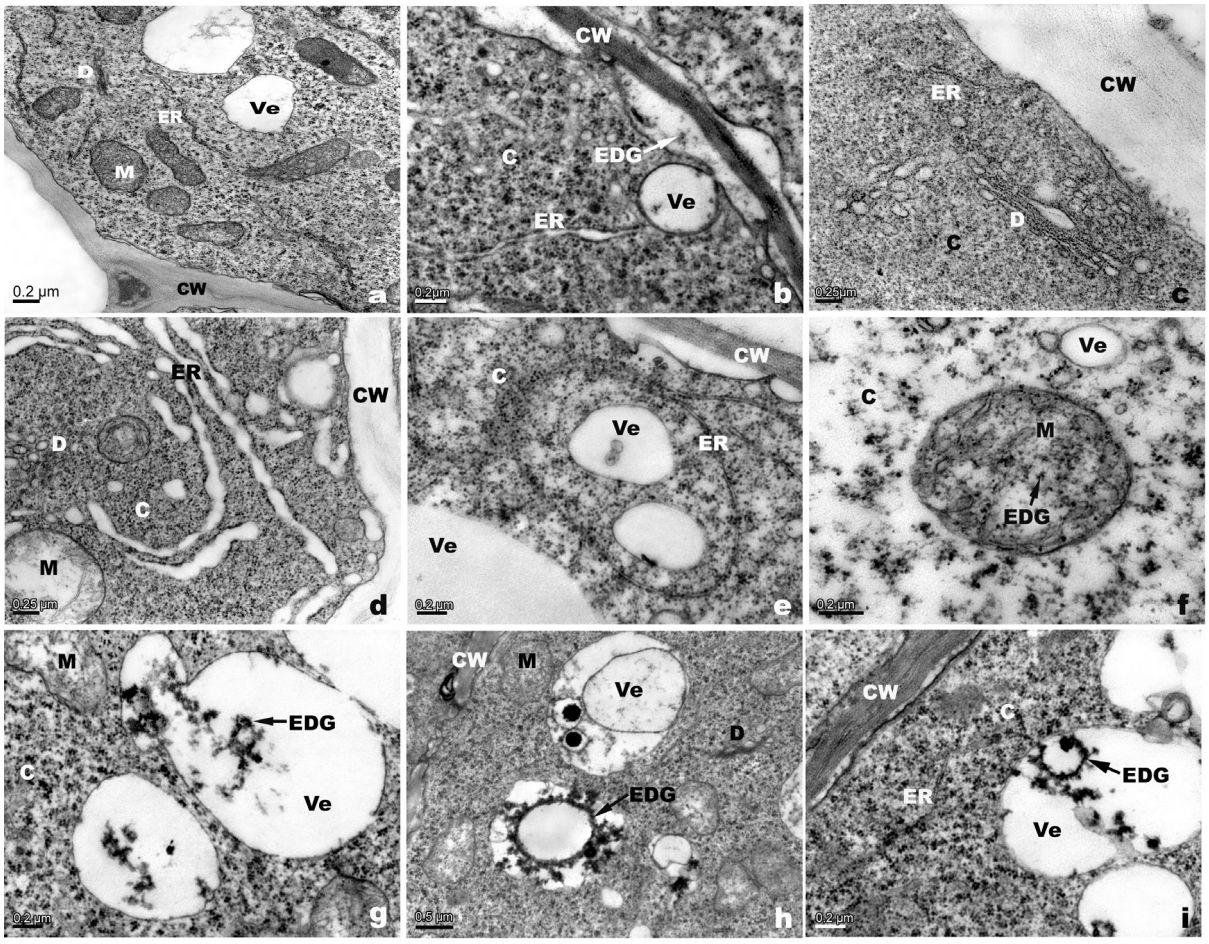

Fig. 1. TEM micrographs showing toxic effects of Cd on ultrastructure of the root meristematic cells of *A. sativum*: *a* - control cells showing well developed root tip cells; *b* - electron dense granules (*arrows*) precipitated in the cell walls and between the cell walls and plasma membrane, and invaginations and pinocytotic vesicles from plasma membrane (0.10 mM Cd, 1 h); *c* - dictyosome vesicles mainly distributed nearby plasma membrane (0.10 mM Cd, 2 h); *d* - vesiculated rER and small vesicles from ER distributed near cell wall (0.10 mM Cd, 2 h); *e* - vesicles wrapped by ER in some cells (0.10 mM Cd, 24 h); *f* - mitochondria of spherical shape and some electron dense granules in cristae and matrix (1.00 mM Cd, 8 h); *g* - vesicles gradually fused together producing a big cytoplasmic vacuole (0.01 mM Cd, 8 h); *h* - vesicles repeatedly wrapped and formed "multivesicular bodies" in cytoplasm and electron dense granules surrounded by vesicular systems (0.10 mM Cd, 4 h); *i* - electron dense granules precipitated in small vesicles (0.01 mM Cd, 12 h). C - cytoplasm, CW - cell wall, D - dictyosome, ER - endoplasmic reticulum, EDG - electron dense granules, M - mitochondria, Ve - vesicle.

bodies" (Fig. 1*h*) in cytoplasm, which became clearly vacuolated. Electron dense granules were always surrounded by vesicular systems.

Presence of electron dense granules in Cd treated cells was another important characteristic. As mentioned above, they firstly appeared between the cell walls and plasma membrane at 0.01 mM Cd for 2 h, and increased in number at longer exposure. Finally electron dense granules were mainly precipitated in cell wall (Fig. 1*b*), in small vesicles in cytoplasm (Fig. 1*g*) and in degenerated mitochondria (Fig. 1*f*). At higher Cd concentrations (0.01 - 0.10 mM Cd for 24 h), electron dense granules were aggregated and formed larger precipitates of circular or amorphic shape and they were encircled by the membrane.

The toxic effects of Cd became progressively more evident as the cells were subjected to increasing Cd concentration and longer exposure (0.10 - 1.00 mM Cd for 48 h). The typical were disintegration of dictyosomes and ER, decrease in mitochondrial cristae, losing vesicles membrane function and release of some electron dense granules from vesicles and vacuoles into cytoplasm. In this way, Cd led to disintegration and death in some cells.

The target of toxic chemicals is always at the molecular level and the effects are reflected in the structure of cell and its organelles. We suppose that once excessive Cd ions entered the cytosol, a defence mechanism becomes activated, protecting the cells against Cd toxicity. The cell wall is the first barrier against Cd, where Cd ion can be immobilized by binding to pectins or proteins (Leita *et al.* 1996). The chemical analysis of Cd in fractions of *Allium cepa* epidermal cells performed by inductively coupled plasma mass spectrometry (ICP-MS) indicated that 56 % of total Cd was located in cell walls (Wierzbicka *et al.* 2007). Similarly, the result of our former study using energy dispersive X-ray analyses (EDXA) revealed presence of Cd ions in walls of root cells of *A. cepa* (Liu *et al.* 2007). Cytochemical evidence also confirms that cysteine-rich proteins, commonly referred to as phytochelatins, were localized in electron dense granules in root cell walls of *Allium cepa* (Liu and Kottke 2004), which seemed to contribute substantially to Cd detoxification. Also the plasmalemma is thought to play a critical role in metal tolerance of plants (Quartacci *et al.* 2001).

It is known that dictyosomes and ER were sensitive organelles to heavy metals. Under the influence of low Cd concentration (0.01 mM Cd for 2 h), the numerous ER and dictysosme vesicles appeared in the cells. The vesicles carried polysaccharide or proteins and migrated to the plasma membrane to repair it. In the cytoplasm, vesicles arising from the ER and dictyosomes also contained Cd deposits. Usually, several vesicles gradually fused together producing a bigger cytoplasmic vacuole. The increased vacuolation in Cd-exposed cells can prevent the circulation of free Cd ions in the cytosol and forces them into a limited area. These observations confirmed that root cells had a rapid and effective defence system against Cd toxicity involving ER and dictyosomes, however, the role of the ER in plant cells contaminated with heavy metals has not yet been unequivocally determined (Wierzbicha *et al.* 2007).

Previous research by electro energy loss (EEL) spectra in our group gave clear indications that Cd was localized in form of electron dense granules deposited in various cell regions under Cd stress (Liu and Kottke 2003, 2004). These granules as an index of Cd stress were also confirmed in this experiment. These precipitate enclosed in vesicles were often seen. The plant vacuoles are the final destination for practically all toxic substances (Clemens 2006). Therefore, these granules containing Cd can be thought to be a mechanism for detoxification of Cd. The previous observation (Liu and Kottke 2003) indicated that the vesicles contained phytochelatins (PC) and form stable PC/Cd complexes sequestered in the plant vacuoles (Saxena *et al.* 1999, Clemens 2006).

Mitochondrial degeneration or abscission is remarkable characteristic of *A. sativum* root meristematic cells exposed to Cd at high concentration and/or long treatment time. The alteration does not occur when the Cd concentration was low. Cd also induced the formation of reactive oxygen species (ROS), which caused oxidative damage to DNA, proteins and lipids (Pinot *et al.* 2000).

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Frowine, S.A.: Moth Orchid. The Complete Guide to *Phalaenopsis*. -Timber Press, Portland - London 2008. 204 pp. ISBN-13: 978-0-88192-870-9.

S.A. Frowine, author of this monograph is a professional horticulturist and graduated active garden writer and speaker. His long year interest and specialization are orchids, especially moth orchids, where are the topics of this book.

Why are the moth orchids so interesting? These decorative plants created about 75 % of all purchased orchids. Plants are easy to grow; the plants on the market have the reasonable price. There is the wide scale of flower colours and some of them emit delicious perfumes. They are successfully cultivated in home conditions not only near windows, but under artificial light. Plants from this orchid group are best choice for home orchid growers.

Common name moth orchid is used for plants of genus *Phalaenopsis* and closely related orchid genus *Doritaenopsis*. The say true, mostly these names is only taxonomical synonyms.

The first chapter of the book describes morphological features of moth orchids, basic principles of cultivation and hybrid creation, and elucidates the rules of giving names to orchid plant genera, grex, hybrids and cultivars. Second chapter deals with botanical species of genus *Phalaenopsis*. For each described genus is given native areal, the year of first description, morphological characteristics and culture possibility and contribution of this genus to cultivated hybrids. Next five chapters introduce contemporary cultivated plants and hybrids categorized according “base” colour of the flower. In spite of the fact, that even author of the book supposes this category may be subjective, for general orientation within the cultivars such categories are very suitable. Plants are divided to: white and pink, yellow and orange, red and purple, harlequins. The last of these chapters covers novelties, multifloras and miniatures. Next chapter describes cultivation principles for growing moth orchids in greenhouses and in home conditions. Plants are not extremely demanding for cultivation skills and as well for environmental conditions. In this chapter are given optimal range of irradiance, temperature, air humidity, air ventilation, as well as suitable soil mixtures and fertilization needs. Growing seedling, insects and disease control are also describes. The last chapter: Selecting and Buying Moth Orchids give us some advices for choice of suitable plants for home decoration. At the end of the book the useful lists are added: List of plant sources, List of fragrant *Phalaenopsis* and List of intergeneric hybrids. Index of all mentioned plants is also included.

The book is rich illustrated by photographs (mostly moth orchid cultivars) and by drawing (mostly morphological features and instructional picture). The list of illustrated hybrids is organized by registration year and by originator of the cultivar. This book is the hansom handbook for everyone who cultivates the orchid and suits to bookshelf of all plant lovers.

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