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Short note

Cadmium Induced Ultrastructural Changes in Chloroplasts of Elodea nuttallii (Planch). H. St. John Leaves

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Abstract. The effect of cadmium (Cd) on the ultrastructure of plastid apparatus in leaves of *Elodea nuttallii* (Planch). H. St. John was investigated in experimental conditions using transmission electron microscopy (TEM). The experimental plants were cultivated in a green house environment after preliminary adaptation. The plants were exposed to Cd during 5 days period with concentrations 1 mg/l and 3 mg/l, consistent with previous studies. Cultivated plants under condition of the experiment without Cd were used as the controls. The results showed that at concentration 1 mg/l Cd chloroplasts have a well organized internal membrane system relative to the control, but fragmentation and weak swollen thylakoids were observed. Increasing of cadmium concentration at 3 mg/l Cd show hardly affected plastid ultrastructure, as swollen thylakoid membrane and reduction of grana stacks. The established structural changes of photosynthetic apparatus of *E. nuttallii* are analyzed and compared to previous researches of cadmium toxicity on *E. canadensis* under the same conditions.

Key words: cadmium, chloroplast ultrastructure, thylakoid membranes, *Elodea nuttallii*.

Introduction

The problem of water contamination with heavy metals is important in ecological aspect. Aquatic plants actively accumulate heavy metals in polluted freshwater basins through their roots, stems and leaves (Jackson, 1998). Many techniques have been developed and applied in the removal of pollutants from the aquatic environment especially phytoremediation with the use of aquatic macrophytes (Rai, 2009). *Elodea canadensis* Michx. and *Elodea nuttallii* (Planch). H. St. John, a cosmopolitan aquatic macrophytes with an important role in the ecology of many littoral zones, have high sensitivity to a wide range of contaminants. Therefore, it was interesting to investigate the accumulation of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn in this species (Cegłowska et al., 2016). Cadmium (Cd) is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water (Pinto et al., 2004). Cd appears to cause more marked ultrastructural changes in *E. canadensis* such as destruction of the protoplast (Stoyanova & Tschakalova, 1993; 1999). The most frequently observed ultrastructural effect to this toxic metal is damage to chloroplasts and decreased photosynthetic activity. Ultrastructural investigations indicated that Cd, applied at toxic concentrations, disorganizes the chloroplast membrane system, leading to changes in the ratios of the main structural components of thylakoid membranes and their lipid composition (Dalla Vecchia et al.,

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2005). During investigation after toxic metal accumulation in *E. canadensis* and *E. nuttallii*, Thiébaut et al. (2010) established higher accumulated amounts of Cd for *E. nuttallii*. From this point of view, it is of interest to compare the structural reaction to cadmium relative to both species. A study under the same experimental conditions would make it possible to evaluate *E. nuttallii* as phytoremediator.

The aim of the present study is to establish the ultrastructure changes in the organization of photosynthetic apparatus of *E. nuttallii* after dose concentration of Cd and make comparative analysis of earlier data for *E. canadensis* under the same experimental conditions.

Material and Methods

Methodical settings were compliant with previous results for structural organizations of plastid apparatus of *E. canadensis* under the influence of stated cadmium concentrations (Stoyanova & Tchakalova, 1999). The experimental plants were cultivated in a green house environment after preliminary adaptation. Cultivated plants under condition of the experiment without Cd were used as controls. The heavy metal was incorporated into the aquatic environment as $CdSO_4$ in concentrations of 1 and 3 mg/l. After 5 days treatment with Cd *E. nuttallii* leaf samples were taken for transmission electron microscopy. For transmission electron microscopy (TEM) specimens were fixed in 3% glutaraldehyde (pH 7.4) in 0.1 M sodium phosphate buffer (pH 7.4) for 12 h at 4°C temperature and post-fixed with 2% $KMnO_4$ for 4 h, dehydrated with ethanol and acetone and embedded in Durcopan (Fluka, Switzerland). Ultra-thin sections were cut by ultramicrotome Reichert-Jung and stained lead citrate (Reynolds, 1963). The observations were examined with a JEOL1200 EX transmission electron microscope.

Results and Discussion

The plastid apparatus in the leaves of the control plants is composed of chloroplasts with a typical organization of the internal membrane system that consists of granal and stromal thylakoids. The granas are structured by 6-17 thylakoids and their height varies. Stromal thylakoids are long and numerous. Osmophilic plastoglobules and no presence of starch grains were established in the stroma (Fig. 1A).

After five days treatment of plants with a low concentration of Cd 1mg/l changes in the structural organization of the internal membrane system were observed. (Fig.1B). The inner membrane system with granal and stromal thylakoids was normally structured but partially swelled. Single larger plastoglobules were found in the stroma that do not generally disturb the shape of the plastids.

In plants exposed to a higher concentration of Cd 3 mg/l the observed changes are more strongly expressed. Impaired orientation of the inner membrane system was observed in comparison with the control plants. In some of the chloroplasts significantly higher and wider grana with strongly swelled thylakoids were observed (Fig. 1C-D). The shape of these chloroplasts was more rounded and their internal volume was larger than those of the control plants. An increase in the number of plastoglobules was observed in the stroma. The outer chloroplast membrane was significantly fragmented.

The structural analysis of the changes of the plastid apparatus registered under conditions of low concentrations of Cd in leaves of *E. nuttallii* shows that they are similar to those observed in *E. canadensis* by Stoyanova (1998) and Dalla Vecchia et al. (2005). Both species have a close resistance range. The most commonly observed change in the structure of chloroplasts is fragmentation and mild swelling of the thylakoid membranes and this transformation is not associated with thylakoid destruction. Similar changes of the plastid apparatus of *E. canadensis* have been reviewed by Esposito et al. (2007).

In previous studies on the effect of higher concentrations of cadmium on changes in the ultrastructure of chloroplasts in *E. canadensis* serious destructions has been reported (Stoyanova & Cshakalova, 1990). The authors observed a strong swelling of the stromal and granal thylakoid membranes that made them difficult to differentiate. Most likely this is due to the destructive effect of cadmium on the lipids structuring the inner membrane system. Deformed and dilated thylakoids have been observed at high cadmium concentrations (Esposito et al., 2007). A similar thylakoid degradation has been established in chloroplasts in the leaves of aquatic plants treated with other heavy metals such as Pb, Cu, Zn, Ni (Stoyanova & Tschakalova 1997; Stoyanova 1999; Stoyanova & Balgjiev, 2002; Nyquist & Greger, 2007; Stoyanova-Koleva & Tchakalova 2008; Chandra & Yadav, 2011; Ceglowska et al., 2016).

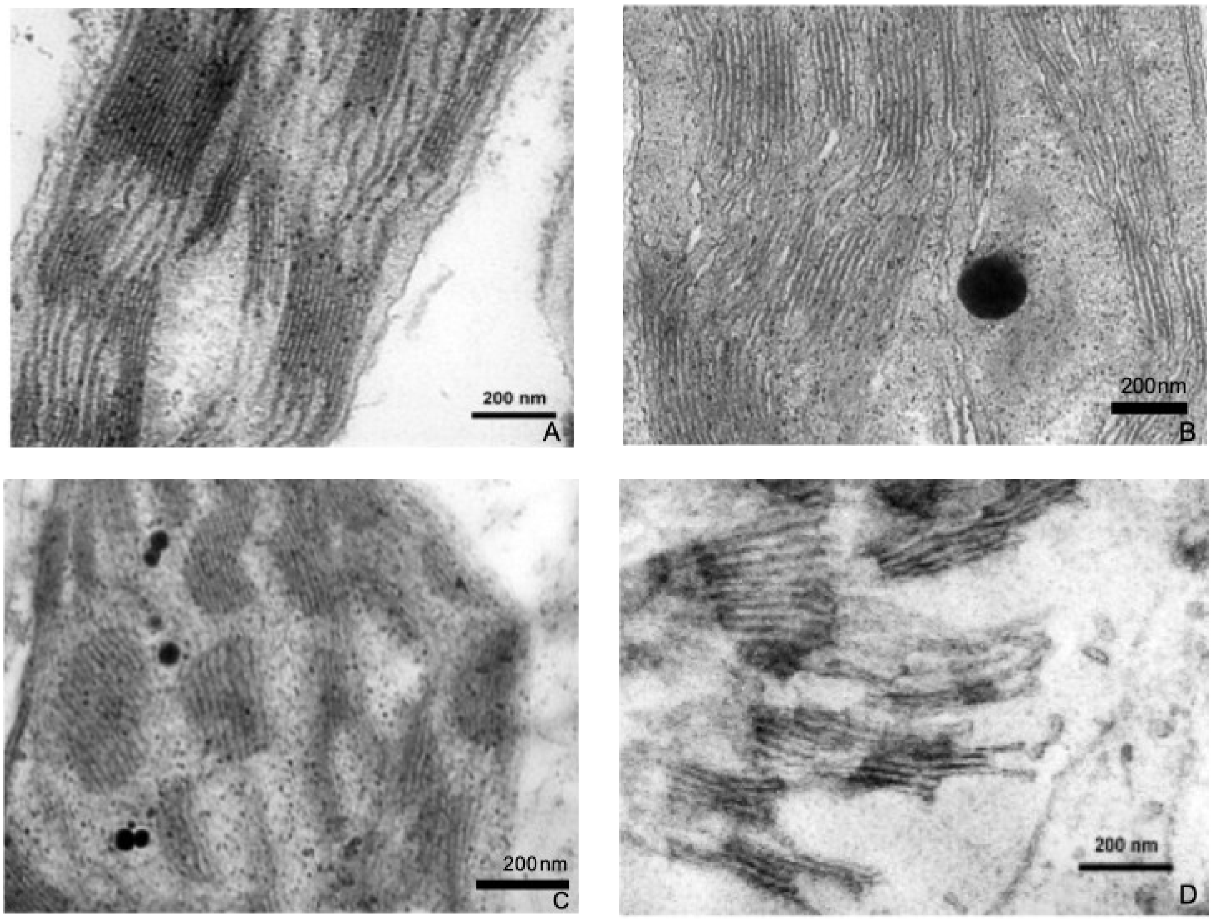

Fig. 1. A: TEM micrograph of chloroplast in a cell of *E. nuttallii* control plants. **B:** TEM micrograph of chloroplast in a cell of *E. nuttallii* treated with Cd 1mg/l. **C-D:** TEM micrograph of chloroplast in a cell of *E. nuttallii* treated with Cd 3 mg/l.

In the stroma of the studied plastids at higher concentrations of Cd there was an increase in the number of plastoglobules. This is related to the destructive effect of heavy metal on the lipid components of thylakoid membranes (Ouzounidou et al., 1997). Our observations are consistent with the results of a study by Stoyanova & Tchakalova (1999), where the authors reported the presence of multiple plastoglobules in the chloroplasts of *E. canadensis* leaves exposed to high concentrations of lead. It has been shown that their size and number increase under conditions of stress, simultaneously with the destruction of thylakoid membranes.

Conclusions

In the present study of the structural organization of chloroplasts in *E. nuttallii* in response to exposure to Cd 1 mg/l, observed changes in thylakoids are associated with less noticeable swelling of granal thylakoids and preservation of stromal functional integrity and activity. The higher concentration of Cd 3 mg/l induces destructions of the photosynthetic apparatus of *E. canadensis* that leads to loss of their functional ability. In contrast the changes in the structure of thylakoid membranes caused by the same Cd concentration in *E. nuttallii* do not affect their function so hard and the chloroplasts retained their functionality close to the control plants. Based on a comparison of the structural organization of chloroplasts it can be said that *E. nuttallii* has a greater capacity to accumulate heavy metal and will therefore be a more efficient species in the biological treatment of water contaminated with cadmium.

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