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Impact of long-term cadmium exposure on mineral content of *Solanum lycopersicum* plants: Consequences on fruit production

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ABSTRACT

In young tomato plants, modifications in mineral composition by short-term cadmium (Cd) treatments have been extensively examined. However, long-term Cd treatments have been fewly investigated, and little information about Cd-stress in fruiting plants is available. In the present work, we examined the changes in mineral nutrients of roots, stems, leaves, flowers, seeds and fruit pericarp of tomato plants submitted to a long-term Cd stress. After a 90-day culture period in hydroponic contaminated environment (0, 20 and 100 μM $CdCl_2$), fruit production was affected by increasing external Cd levels, with the absence of fruit set at 100 μM Cd. Meanwhile, Cd altered the plant mineral contents with an element- and organ-dependent response. At 20 μM, Cd triggered a significant increase in Ca content in roots, mature leaves, flowers and developing fruits. However, at 100 μM Cd, Ca content was reduced in shoots, and enhanced in roots. Cd stress reduced Zn and Cu contents in shoots and increased them in roots. High Cd level led to a significant decrease in K and Mg content in all plant organs. Furthermore, Fe concentration was reduced in roots, stems and leaves but increased in flowers, seeds and red ripe fruits. Our results suggest that tomato plants acclimatize during long-term exposure to 20 μM Cd, while 100 μM Cd results in drastic nutritional perturbations leading to fruit set abortion.

1. Introduction

Tomato, one of the most important horticultural crops in the world, constitutes a considerable source of minerals, vitamins, and antioxidants. It is also an important component of the traditional Mediterranean diet, which has been found to be associated with a reduced risk of various cancers and heart diseases (Galgano et al., 2007; Nguyen and Schwartz, 1999). Unfortunately, a large part of this crop is grown in greenhouses, using fertilizers for improving the nutrient supply in soils and pesticides for disease control and crop protection (Mench, 1998; Moral et al., 2002). Consequently, these agricultural practices amplify the risk of elevating soil contamination by heavy metals and of altering the quality of agricultural products (Mench, 1998).

Among heavy metals, cadmium (Cd) is one of the most dangerous elements to plants, since elevated levels in the soil solution cause numerous harmful effects. It has been shown that Cd may cause root damages, inhibition of photosynthesis and respiration, reduction of chlorophyll content and alteration of the key enzyme activities of various metabolic pathways, leading to lower yield (Dong et al., 2006; Gratao et al., 2012; Irfan et al., 2013; Wu et al., 2007). However, the severity of the above-mentioned disturbances is related to many factors, such as the species (Wu et al., 2007), Cd concentrations (Djebali et al., 2010) and exposure time (Singh et al., 2004).

Physiological disorders caused by Cd, including plant biomass reduction, can be an indirect consequence of nutrient deficiencies. Mineral nutrition disturbances arise from deleterious Cd effects on the metabolism of essential elements, including calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) (Carvalho Bertoli et al., 2012). In crop plants, Cd could interfere synergistically or antagonistically with nutrient uptake (Bachir et al., 2004; Clemens, 2006; Wu et al., 2007). However, there were significant differences among species and varieties, and inconsistencies exist between the results of the experiments. In fact, the mechanism by which Cd inhibits the uptake and utilization of mineral elements is not completely clear to date. It is assumed that Cd may interfere with nutrient uptake by affecting the permeability of plasma membrane (Obata and Umebayashi, 1997) and modify the activity of the nutrient transporters

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(Clemens, 2006), leading to changes in nutrient concentration and composition.

Cd-induced changes in mineral composition have been examined extensively in seedlings using short-term treatments (Boulila-Zoghlami et al., 2006; López-Millán et al., 2009). However, few investigations have been done on the effect of chronic-Cd-stress on plants producing fruit (Carvalho Bertoli et al., 2012). Since fruits are sink organs that import mineral and photo-assimilates from roots and leaves, their growth and development may be impacted by Cd stress as well (Carvalho Bertoli et al., 2012; Gratao et al., 2012; Moral et al., 1994). In a previous study (Hediji et al., 2010), tomato plant exposure to high Cd concentration (i.e. 100 μM) led to significant disturbances in leaf metabolic profiles, which induced plant growth reduction and fruit set inhibition. When treated with a lower Cd concentration (i.e. 20 μM), tomato plants produced fruits and presented only moderate changes of their leaf metabolic profiles, suggesting their relative tolerance to long-term Cd treatment, although they showed vegetative growth reduction.

The present study aims at determining the influence of long-term Cd stress on the accumulation of mineral nutrients (i) in all parts of tomato plants and (ii) in fruit pericarp at different developing stages. Consequences on fruit production were also analyzed.

2. Materials and methods

2.1. Plant material and harvest

Tomato seed (*Solanum lycopersicum* L., cv Thomas) germination was performed on moistened filter paper for six days at 25 °C in the dark. The seedlings were selected for uniformity and transplanted to a 6 L plastic beaker (one plant per beaker) filled with continuously aerated nutrient solution containing: 3.8 mM KNO_3, 0.2 mM NaCl, 3.1 mM $Ca(NO_3)_2$, 2.0 mM NH_4NO_3, 0.8 mM KH_2PO_4, 0.3 mM K_2HPO_4, 0.75 mM $MgSO_4$, 10 μM $MnSO_4$, 1.0 μM $CuSO_4$, 1.0 μM $ZnSO_4$, 30 μM H_3BO_3, 0.04 μM $(NH_4)_6Mo_7O_{24}$, and 90 μM EDTA-Fe-K (Saglio and Pradet, 1980). Twenty days after transplanting, $CdCl_2$ was added to a fresh nutrient solution at 20 or 100 μM. Culture without Cd was used as a control. Experiments performed on six plants each, were run in duplicate. Plants were grown in a growth chamber with a 16-h-day (25 °C)/8-h-night (18 °C) cycle, an irradiance of about 300 μmol photons m^{-2} s^{-1} and 70–80% humidity. Flowers were tagged at anthesis (flower opening) and the number of fruits per plant was monitored. Flower maturity was determined as fully opened flowers (anthesis). Fruits were harvested at six stages expressed in days post-anthesis (DPA): expansion phase (25 DPA), mature green stage (MG, 43 ± 5 DPA), breaker (B, 46 ± 5 DPA), turning (T, 47 ± 5 DPA), orange (O, 48 ± 6 DPA) and red ripe (RR, 51 ± 5 DPA). For each treatment and developmental stage, 6 fruits from different plants were harvested. The equatorial pericarp of fruits was hand dissected. After 116 days of growth, corresponding to 90 days of exposure to $CdCd_2$, the different plant organs were collected and used for further determination of dry weight (DW) or mineral elements as described below.

2.2. Determination of cadmium and mineral nutrient contents

Plant materials (roots, leaves, stems, flowers, seeds and fruit pericarp) were dried at 70 °C until constant weight, weighed and ground to a fine powder. Cadmium and mineral nutrients were analyzed by digestion of dried samples with an acid mixture (HNO_3/$HClO_4$ 3:1, v/v) as described by Van Assche et al. (1988). Metal ion concentrations were determined by atomic absorption spectrometry (*Analyst* 300, Perkin-Elmer) using an air-acetylene flame.

2.3. Data analyses

Data presented are the mean values of three biological replicates for mineral element contents, and of six biological replicates for physiological analyses. Mean comparison between treatments and control was done using Student's *t*-test ($P < 0.05$).

3. Results

3.1. Effect of Cd on fruit production

The effects of Cd on flower and fruit number and fruit fresh weight at different development stages (expansion phase (25 DPA), mature green (MG, 43 DPA), breaker (B, 46 DPA), turning (T, 47 DPA), orange (O, 48 DPA) and red ripe (RR, 51 DPA)) are reported in Figs. 1 and 2. Cd exposure had a significant effect on mature flower number (Fig. 1a), which influences fruit production. At 20 μM Cd, flowers reached maturity but they were 56% lower than in the control after 54 days of Cd treatment (corresponding to 80 days of plant development). Concomitantly, fruit number per plant was 72% lower than in the control after 66 days of Cd treatment (corresponding to 92 days of plant development) (Fig. 1b). This decrease was due to the reduction of developing flower number and the increase of aborted flower number. This reduction in fruit number was accompanied by a significant decrease in fruit fresh weight (a 54% and 33% reduction compared to the control at 25 DPA and RR stages, respectively) (Fig. 2). By contrast, long-term exposure to 100 μM Cd led to the abortion of all plant flowers at the immature flower bud stage (Fig. 1a).

3.2. Cadmium accumulation in the fruit pericarp

Under 20 μM Cd treatment, changes in Cd content in the pericarp of fruit at different development stages (25 DPA, MG, B, T, O and RR) revealed that Cd accumulation increased significantly between the early

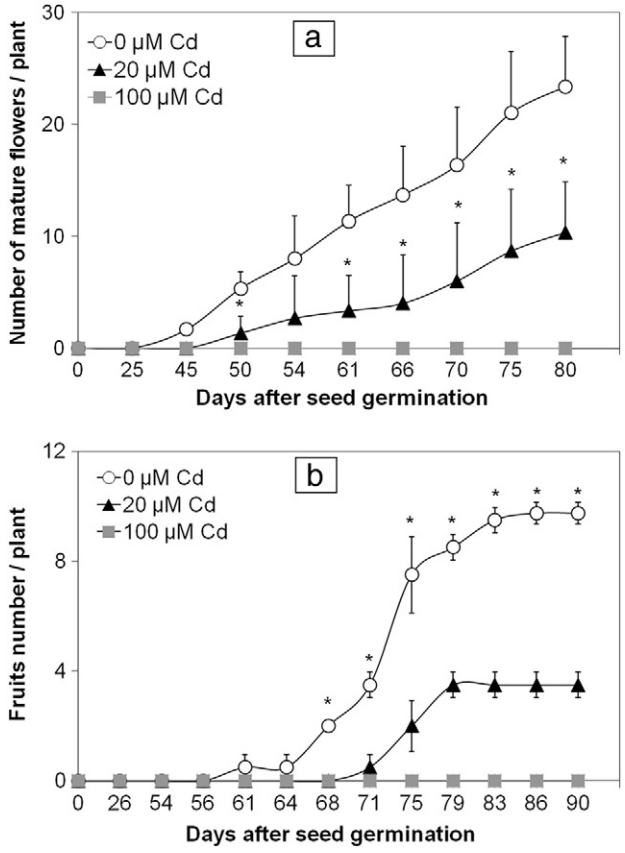

Fig. 1. Effect of cadmium on mature flower number (a) and fruit formation (b) of tomato plants submitted to different Cd concentrations. Mean of 6 replicates ± SD. Star (*) indicates significant differences between Cd treatment and control (0) at $P < 0.05$ level (Student's *t*-test).