Variation among Solanaceae crops in cadmium tolerance and accumulation

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Research article

Variation among *Solanaceae* crops in cadmium tolerance and accumulation

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Abstract – Plantlets of cape gooseberry (*Physalis peruviana*), pepino (*Solanum muricatum*), pepper (*Capsicum annuum*), tobacco (*Nicotiana tabacum*) and tomato (*Lycopersicon esculentum*) were watered with several nutrient solutions containing cadmium (Cd) at doses ranging from 0 to 500 mg·L^{-1} (cape gooseberry) or 0 to 200 mg·L^{-1} (rest of the crops). Cadmium depressed the growth of roots, stems and leaves and caused a reduction in dry matter and chlorophyll concentration, especially at the higher doses. Crops most affected by Cd were tobacco, tomato and pepper. Significant differences among crops for Cd accumulation in the leaves were detected. Thus, Cd concentrations in cape gooseberry leaves were much lower than in the other crops. Cape gooseberry and pepino grow relatively well under high Cd concentrations, though they accumulate low (cape gooseberry) and high (pepino) Cd concentrations.

accumulation / Cd / heavy metals / *Solanaceae* / tolerance

1. INTRODUCTION

Cadmium (Cd) is a non-essential element for plants, animals and humans. In plants, Cd induces growth retardation, interferes with the absorption and movement of nutrients, inhibits enzymes, reduces photosynthesis and generates free radicals (Moral et al., 1994; Prasad, 1995; van Assche, 1990). In addition, in humans, intake of food with high Cd levels can lead to the development of the "Itai-Itai" disease, kidney dysfunction (proteinuria), lung injury (emphysema), rhinitis and chronic bronchitis (Nogawa and Kido, 1996). Because of this, the increase in Cd concentration in soils and crops during the last few decades (Jones et al., 1987) causes great concern.

The increase in Cd levels in soils is mainly the result of extensive use of fertilizers that contain Cd as an impurity, such as phosphate fertilizers, sewage biosolids (sludges), composted solid wastes, and ashes from combustion of coal (Alloway and Steinnes, 1999). Lands dedicated to high-value crops are subjected to intensive fertilization and, therefore, are prone to increases in the Cd levels in soil.

There are considerable differences among crops in accumulation of and tolerance to Cd (Bingham et al., 1975; Chizzola, 1994; Kuboi et al., 1986; He and Singh, 1994; Römer et al., 2002); consequently, identifying crops "tolerant" (as opposed to "sensitive") to Cd (i.e., those in which an increase in one unit of Cd concentration in the soil leads to a lower reduction in growth and development when compared with other crops) and that accumulate low levels of this heavy metal is of interest, especially for cultivation in soils prone to Cd accumulation. In this respect, many solanaceous crops are exacting plants grown in areas of intensive agriculture with high inputs of mineral and organic fertilizers. In addition, *Solanaceae*, together with *Chenopodiaceae*, *Cruciferae* and *Asteraceae*, are considered heavy accumulators of Cd (Kuboi et al., 1986).

In this work, we studied the accumulation of and tolerance to Cd in five *Solanaceae* crops belonging to different genera. The crops studied include two widely grown vegetable crops, pepper (*Capsicum annuum*) and tomato (*Lycopersicon esculentum*), two lesser-known crops that are being introduced in several regions as an alternative for vegetable crop diversification, cape gooseberry (*Physalis peruviana*) and pepino (*Solanum muricatum*), and an industrial crop, tobacco (*Nicotiana tabacum*). The objective was to study the variation in accumulation of and tolerance to Cd within this family and to identify crops suited for soils with high levels of Cd.

2. MATERIALS AND METHODS

2.1. Plant material and growing conditions

Plantlets of cape gooseberry (line 'ECU-238'), pepino (clone 'Sweet Long'), pepper (cultivar 'Negral'), tobacco (cultivar

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'Samsun') and tomato (line NE-1) were used for the experiment. Seeds of each crop (except for pepino) were germinated in Petri dishes in a growing chamber. As soon as the radicle emerged, they were transplanted to seedling trays filled with a commercial substrate and transferred to a greenhouse with temperature control. Explants of pepino were micropropagated in vitro at different dates so that plants at a similar stage of development to plants grown from seeds could be selected. After the explants had rooted, they were transferred to the same type of seedling tray as the rest of species and henceforth subjected to the same conditions as the rest of crops. Four weeks after the transplanting of the seed-grown crops, plantlets of all the crops, selected for uniformity and with three to four developed leaves, were removed from the seedling trays and the roots were carefully cleaned with water. Immediately, they were transferred to pots (6 × 6 × 8 cm) filled with vermiculite. The plants were watered every two days with Hoagland solution (5 mM $Ca(NO_3)_2$, 5 mM KNO_3, 1 mM $MgSO_4$, 1 mM KH_2PO_4, 50 µM H_3BO_3, 10 µM FeEDTA, 4.5 µM $MnCl_2$, 3.8 µM $ZnSO_4$, 0.3 µM $CuSO_4$, and 0.1 µM $(NH_4)_6Mo_7O_{24}$) supplemented with Cd (as $CdCl_2$) at 0, 100, 200 or 500 $mg \cdot L^{-1}$ for cape gooseberry and 0, 15, 50, 100 or 200 $mg \cdot L^{-1}$ for the other crops. The pH of the solutions was adjusted to 5.5. An excess of solution was applied to avoid build-up of Cd and other salts in the vermiculite. Higher doses of Cd were used for cape gooseberry, as preliminary experiments showed that it had a higher tolerance to Cd (unpublished data).

2.2. Growth parameters and chlorophyll analysis

Fourteen days after Cd treatments were initiated, the plants were harvested and plant tissues separated into three parts: roots, stem and leaves, and the maximum length (mm) of roots, stems and leaves was measured. For plants irrigated with Cd concentrations of 0 and 200 $mg \cdot L^{-1}$, the dry weight after dessication at 60 °C was measured. For chlorophyll concentration analysis, 0.5 g of fresh leaves were ground with 4 mL of pure acetone. After that, 20 mL of acetone were added and the mixture was vacuum-filtered. In order to extract the chlorophyll that remained in the leaf paste, 10 mL of acetone were added to the paste, which was subjected again to vacuum filtration. This process was repeated if necessary, so that in the end the leaf paste had a white color. The extract was brought to 50 mL with acetone. Subsequently, 12.5 mL of distilled water (20% v/v) were added to each extraction. The optical density (D) of the acetonic extract was measured at 645 and 663 nm wavelengths (with an 8-nm bandwidth and a resolution of 1 nm), using a Jenway 6305 UV/V spectrophotometer (Jenway, Dunmow, UK). Chlorophyll concentration ($mg \cdot g^{-1}$ of fresh weigth) was determined using the Arnon equation (Arnon, 1949). Absolute values for chlorophyll concentration obtained in this way are lower than real ones, because the bandwidth of the spectrophotometer is 8 nm and the wider the bandwidth, the lower the absorbance obtained. Nonetheless, the main objective here is not to determine absolute concentrations of chlorophyll, but to compare relative values among different species and cadmium concentrations. Therefore, the values obtained must be used for comparison within this experiment but are not for comparing the absolute concentrations with other experiments in which measurements have been made at other bandwidths.

2.3. Cadmium analysis

Cadmium concentrations were measured in roots and leaves. Other research works (Mench et al., 1989; Sawert et al., 1987) found that concentrations of Cd in stems and leaves of several solanaceous crops (pepper, potato, tobacco and tomato)subjected to treatments with Cd are similar. Therefore, for practical reasons, and because chlorophyll content is also measured in leaves, Cd levels in the aerial part were only determined in leaves. Cadmium concentration was analyzed on dried material (60 °C), which was milled and calcined at 450 °C for 3 h or more, until the ashes presented a light color. The ashes were moistened with 2 mL of water and 2 mL of concentrated HCl. The mixture was heated until the first vapors appeared and immediately distilled water (2–3 mL) was added. Subsequently, the mixture was filtered and the extract was brought to 100 mL with distilled water. Cadmium concentration was determined with a ThermoElemental atomic absorption spectrometer (SOLAAR AA Spectrometers, Cambridge, UK). The results are expressed in mg of Cd per kg of fresh weight.

2.4. Statistical analysis

For each combination of factors (crop and Cd treatment), five plants were used. Each plant was considered as an experimental unit. Bartlett's tests for homogeneity of variance (Snedecor and Cochran, 1989) indicated that it could be assumed that variances among combinations of treatments were homogeneous for the growth parameters and chlorophyll concentration ($P > 0.05$), but were heterogeneous for Cd concentrations in roots and leaves ($P < 0.01$). The logarithmic transformation of data for Cd concentrations did not result in homogeneity of variances among combinations of treatments ($P < 0.01$). Therefore, a different treatment was given to each set of data (Little and Hills, 1978). For the length of organs and chlorophyll content, an analysis of variance (ANOVA) was performed and the least significant difference (LSD) statistic among any combination of factors was calculated from the ANOVA. For Cd concentrations in leaves and roots, homogeneity of variances within each Cd dose could be assumed ($P > 0.05$), and, therefore, an ANOVA and a LSD was calculated for each Cd dose (Little and Hills, 1978).

3. RESULTS AND DISCUSSION

3.1. Growth parameters

In general, cadmium caused a reduction in the growth of the part of the plant studied (Fig. 1). With the exception of cape gooseberry, where no significant differences were found among treatments ($P > 0.05$), in the other crops there was a considerable reduction in the length of roots as a consequence of increasing Cd concentrations (Fig. 1). Thus, for pepino, pepper, tobacco and tomato, at a dose of 200 $mg \cdot L^{-1}$ in the nutrient solution, there was a reduction ($P < 0.01$ in all cases) of more than 25% in the root length when compared with the control.

Cadmium also induced a reduction in stem length, especially at the highest concentrations of Cd in the nutrient solution (Fig. 1). The species most affected by Cd treatments were tobacco and tomato, with a reduction at the 200 $mg \cdot L^{-1}$ dose

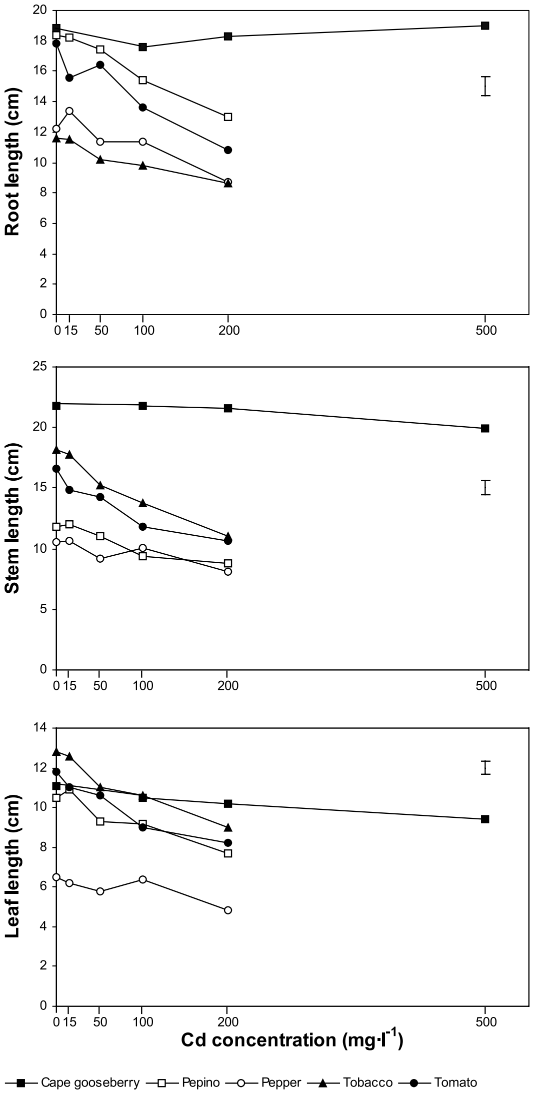

Figure 1. Variation in the length of roots (above), stems (center) and leaves (below) in five crops of the *Solanaceae* family watered with different doses of cadmium (mg·L^{-1}) for 14 days. Values for each combination of crop and cadmium dose are based on the mean of five plants. Bar represents the least significant difference (LSD; P = 0.05) for comparisons of means between any combination of crop and cadmium dose.

of Cd of 36.1% and 39.6%, respectively, when compared with the control (P < 0.01). Cape gooseberry only showed a statistically significant stem length reduction (P < 0.05) at the highest dose (500 mg·L^{-1}), but even in this case, the difference was much smaller than those of the other species at much lower Cd concentrations (Fig. 1). Regarding the leaves, they suffered overall variations similar to those of roots and stem, so that the higher the Cd concentration, the greater the reduction in leaf length. As occurred with the other parts of the plant, tomato and tobacco were the crops where Cd had the greatest effect, with a decrease in leaf length of more than 30% (P < 0.01). Similarly, cape gooseberry was much less affected by Cd than the other crops (Fig. 1).

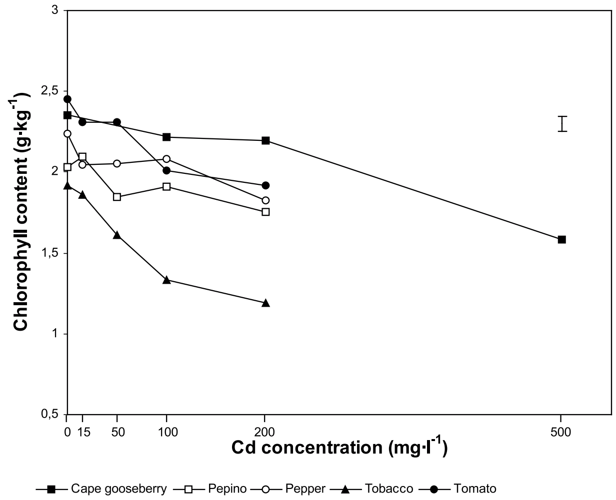

Figure 2. Chlorophyll concentrations (g·kg^{-1}) in leaves of five crops of the *Solanaceae* family watered with different doses of cadmium (mg·L^{-1}) for 14 days. Values for each combination of crop and cadmium dose are based on the mean of five plants. Bar represents the least significant difference (LSD; P = 0.05) for comparisons of means between any combination of crop and cadmium dose.

When the reduction in dry weight of the whole plant at 0 and 200 mg·L^{-1} is studied (not shown), four statistically significant groups (P < 0.05) can be separated, viz. (1) pepino (20.8%), (2) cape gooseberry (32.0%), (3) pepper (51.2%), and (4) tomato and tobacco (59.5% and 65.5%, respectively). It is remarkable that variation in dry weight is much greater than variation in the length of the parts of the plant, except for the pepino. Thus, while in cape gooseberry there is a smaller reduction than in pepino for the length of the parts of the plant as a consequence of treatments with Cd, the contrary occurs for dry weight.

3.2. Chlorophyll content

As a mean, increasing Cd concentrations in the nutrient solution resulted in a reduction of chlorophyll content (Fig. 2). However, there were differences among crops in the reduction of chlorophyll content caused by Cd. Thus, at doses of 200 mg·L^{-1} of Cd in the nutrient solution, the greatest decrease in chlorophyll content was detected in tobacco, with a reduction of close to 40% (P < 0.01), and the lowest in cape gooseberry with a decrease of below 10% (non-significant). Nonetheless, at a Cd dose of 500 mg·L^{-1}, chlorophyll content in cape gooseberry was greatly affected, with a reduction of around 33% (P < 0.01).

3.3. Cadmium accumulation

Increasing concentrations of Cd in the watering solutions resulted in an increase in the concentration of Cd in roots and leaves in all the species tested, although there were significant differences in the Cd concentration between roots and leaves and among crops (Fig. 3). For a given concentration of Cd in the watering solution, Cd concentrations in leaves are much lower than in roots (Fig. 3). Significant differences (P < 0.05) among crops were found for all Cd concentrations (except for the control solution) (Fig. 3).

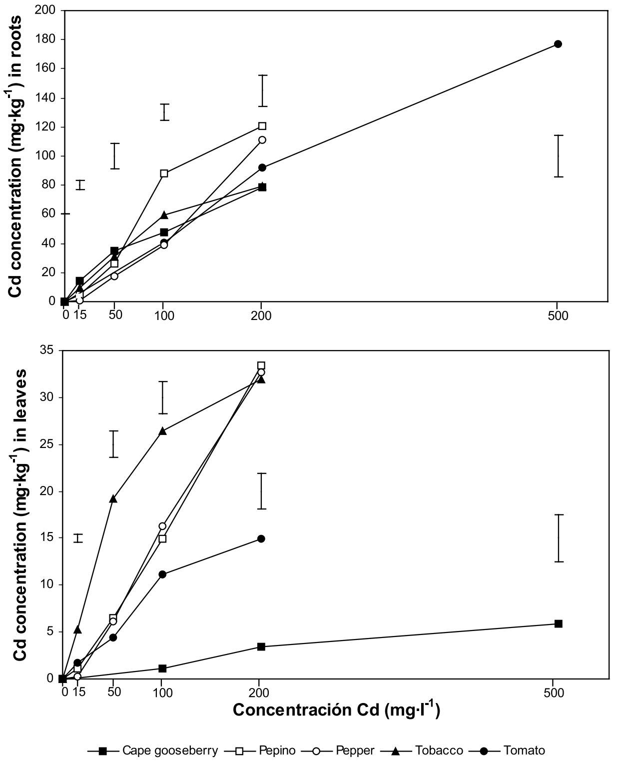

Figure 3. Cadmium concentrations (mg·kg^{-1}) in roots (above) and leaves (below) in five crops of the *Solanaceae* family watered with different doses of cadmium (mg·L^{-1}) for 14 days. Values for each combination of crop and cadmium dose are based on the mean of five plants. Bars represent the least significant difference (LSD; $P = 0.05$) for comparisons of means within each dose of cadmium.

Differences among crops for Cd concentrations in leaves are more apparent than those in roots (Fig. 3). The crop with the greatest levels of Cd accumulation in the range 15–100 mg·L^{-1} is tobacco. Within this range Cd concentrations in tobacco leaves are significantly greater ($P < 0.01$) than those of the other crops. Remarkably, cape gooseberry accumulates much lower Cd in the leaves than the other crops ($P < 0.01$). For example, at doses of 100 mg·L^{-1} of Cd, cape gooseberry has a Cd concentration in the leaves of 1.14 mg·kg^{-1}, between 13 and 23 times lower than the other crops ($P < 0.01$). Concentrations of Cd of 200 mg·L^{-1} induce a further increase in Cd concentration in leaves, especially in pepino and pepper (Fig. 1). As a consequence, at this dose, three significantly different groups ($P < 0.01$) can be established for Cd concentration in leaves: one of them comprises tobacco, pepino and pepper, which present similar Cd concentrations in leaves, with values between 30 and 35 mg·kg^{-1}; another one corresponds to tomato, which does not reach 15 mg·kg^{-1}, and the last one to cape gooseberry with a Cd concentration in the leaves of below 5 mg·kg^{-1}. Cadmium concentration in cape gooseberry leaves at 500 mg·L^{-1} did not reach 6 mg·kg^{-1} (Fig. 3).

When the ratio Cd concentration in leaves/roots is studied, ample differences are found among the crops. Thus, the average ratio is much lower in cape gooseberry (0.033) ($P < 0.01$) than in the other crops (0.155 in tomato, 0.226 in pepino, 0.350 in pepper and 0.490 in tobacco). Regarding the relationship between the Cd concentration in leaves to chlorophyll content, in those crops with a higher Cd content in the leaves, such as pepino, pepper or tobacco, an increase in a unit of Cd concentration causes smaller reductions in chlorophyll content than in the crops with a smaller content, such as tomato, or, notably, cape gooseberry (Fig. 4).

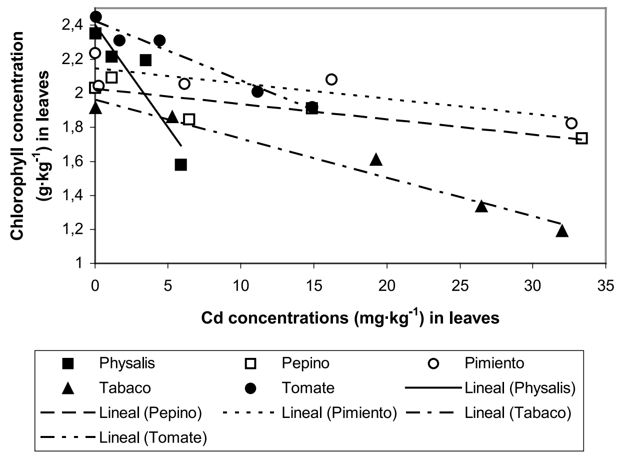

Figure 4. Relationship between the concentration of cadmium in leaves and total chlorophyll concentration in five crops of the *Solanaceae* family. Values for each combination of crop and cadmium dose are based on the mean of five plants.

3.4. Cd Tolerance and accumulation

The existence of differences in the tolerance and accumulation of Cd among crops belonging to different families has been demonstrated in several works (Bingham et al., 1975; Chizzola, 1994; Kuboi et al., 1986; He and Singh, 1994; Römer et al., 2002). Here, we found that an increase in Cd concentration in the nutrient solution produces negative effects, consisting of a reduction of size (Fig. 1), dry weight and chlorophyll content (Fig. 2) in all the crops studied, although there are considerable differences among them in Cd tolerance and accumulation. Cape gooseberry and pepino can be considered tolerant to Cd because they suffer a smaller reduction than the rest of the crops in growth parameters, especially in the case of cape gooseberry (Fig. 1), and in dry matter content. Furthermore, cape gooseberry accumulates much less Cd in the leaves than the other crops (Fig. 3).

Differences among crops in Cd accumulation are important when a solanaceous crop has to be included in a crop rotation in a soil with high levels of cadmium. However, other aspects also have to be considered in recommending a crop for soils with high Cd concentration, i.e. if it is preferable to recommend a species that does not accumulate Cd in its organs, or a species that accumulates it although its growth is not disturbed. This depends of course on the plant organ harvested, and on its use (food, cigarettes, extraction of specific molecules, products for the chemical industry, phytoremediation, etc.). In any case, the selection of the crop for cultivation in this type of soil should be made considering that the final products obtained do not contain Cd concentrations above the thresholds authorized by the legislation. This would require additional experiments specific for each crop and produce. For example, it is especially relevant that among all the species studied, tobacco is by far the crop that accumulates the most Cd in the leaves, the part for which it is used. Furthermore, 50% of Cd inhaled with smoke is absorbed in the lungs compared with around 5% of Cd present in food that is absorbed in the digestive system (Sharma et al., 1983). It is because of the higher accumulation and bioavailability of Cd in tobacco that this crop should only be grown in soils with low Cd content.

As with other crops (Cieslinski et al., 1996; Marchiol et al., 1996), the greatest Cd accumulation in the crops studied occurs in roots, with concentrations much higher than those of leaves (Fig. 1), which suggests that an important proportion of the Cd taken up by roots remains sequestered in the root system (Prasad, 1995; Welch and Norvell, 1999), especially in cape gooseberry. The fact that most of the Cd in this species remains retained in the roots (Fig. 3) suggests that cape gooseberry might have physiological differences that make the Cd concentration in the leaves much lower than in the other crops studied. In fact, when compared with the other crops, small increases in Cd concentration in the leaves in cape gooseberry result in a greater decrease in chlorophyll concentration in this crop (Fig. 4). Reduction of chlorophyll content by Cd results in a lower efficiency of the photosynthesis process, and therefore negatively influences growth and development (Larsson et al., 1998).

4. CONCLUSIONS

This study shows that within the *Solanaceae*, a family considered as a heavy accumulator of Cd (Kuboi et al., 1986), significant differences among crops exist in accumulation of Cd in leaves, dry matter and chlorophyll contents and tolerance to Cd when Cd concentration in the nutrient solution increases. These differences can be used to recommend crops of this family in soils with high levels of Cd. Thus, cape gooseberry poorly accumulates Cd in the leaves, and keeps a relatively good growth. Therefore, this crop seems to be suitable for cultivation in soils rich in Cd. Pepino is also suitable, since it also maintains a good dry matter content, although it accumulates Cd in its leaves. Further experiments are needed in both fruit vegetables to estimate the Cd fruit content when they are cultivated with high levels of Cd, and to compare it with the toxicity threshold. For both species, the investigation of possible varietal differences in the behavior towards Cd should also be investigated. On the contrary, tobacco, pepper and tomato are not suitable for cultivation with high levels of cadmium because they accumulate Cd in their leaves and their growth is greatly reduced.

Differences in Cd accumulation among different crops, as well as the likely presence of different mechanisms of tolerance in pepino and cape gooseberry could be of interest in the study of the physiology of transport and accumulation of, and tolerance to Cd. For further studies of Cd tolerance, cape gooseberry and pepino seem to be good models, since both grow relatively well under high Cd concentrations, though they accumulate low (cape gooseberry) and high (pepino) Cd concentrations in leaves.

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