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ARTIGO

Tolerance to cadmium in the germination and development of jack beans

Cádmio no desenvolvimento de plantas de feijão-de-porco

Sarita Cordeiro Rossi[1]
Ana Maria Magalhães Andrade Lagoa[1*]
Marlene Aparecida Schiavinato[1]

[1]Universidade Estadual de Campinas – UNICAMP, Rua Roxo Moreira, s/n, 13083-970, Campinas, SP, Brasil

Autor Correspondente:
*E-mail: alagoa@iac.sp.gov.br

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ABSTRACT: Cadmium (Cd) is a metal that contaminates the environment mainly through agroindustrial, metallurgical and chemical industrial activities. It can affect mineral absorption by plants, the stomatal aperture, photosynthesis and water balance by reducing the crop yield. Aiming at the discovery of new species able to tolerate Cd, this study evaluated the germination and development of jackbean plants in the presence of Cd to determine whether there is tolerance to this metal. Seeds and seedlings of this species were grown in plastic pots containing sand with Cd concentrations of 0, 500 and 2000 µmol L^{-1} applied as $CdCl_2.2.5H_2O$. The experiments were conducted in a greenhouse under natural photoperiod conditions. Percent germination was close to 100% 13 days after sowing. Seedlings accumulated much of the applied Cd in the shoot and produced high biomass. No alterations to the photosynthetic apparatus were observed at the lower concentrations. This indicates that jack bean plants exhibit characteristics of tolerance to Cd and that further studies should be performed to report if this species can be used as phytoremediator.

RESUMO: *O cádmio (Cd) é um metal que contamina o ambiente principalmente por meio das atividades agroindustriais, metalúrgicas e químicas. Ele pode afetar a absorção de minerais pelas plantas, a abertura estomática, a fotossíntese e o balanço hídrico diminuindo a produção vegetal. Visando à descoberta de novas espécies capazes de tolerar o Cd, o presente estudo avaliou a germinação e o desenvolvimento de plantas de feijão-de-porco na presença de Cd para averiguar se há tolerância a este metal. Sementes e plântulas desta espécie foram cultivadas em vasos plásticos contendo areia com concentrações de Cd de 0, 500 e 2000 µmol L^{-1} aplicadas como $CdCl_2.2,5H_2O$. Os experimentos foram conduzidos em casa de vegetação, sob condições fotoperiódicas naturais. As porcentagens de germinação ficaram próximas de 100% aos 13 dias após a semeadura. As plântulas acumularam grande parte do Cd aplicado na parte aérea e obtiveram elevada produção de biomassa. Não apresentaram ainda alterações significativas no aparelho fotossintético nas menores concentrações. Isso indica que as plantas de feijão-de-porco apresentam características de tolerância ao Cd e que novos estudos devem ser realizados para relatar se esta espécie possui indicativos de fitorremediadora.*

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1 Introduction

There has been given special attention to the study of phytoremediation for the recovery of degraded soils (SHEN et al., 2002; LASAT, 2002). It consists of procedures which involve the use of plants and microbiota associated to its roots to remove, immobilize or render the contaminants harmless to the ecosystem (SUSARLA; MEDINA; McCUTCHEON, 2002). It is a technique of low cost that maintains the fertility of the soil and is environmentally acceptable, since it uses sunlight as an energy source (ROBINSON et al., 2003). Many species of plants are able to absorb heavy metals (HMs) through their roots and can accumulate them in plant tissues (ANDRADE; JORGE; SILVEIRA, 2005). Therefore, there is the need to study new species to verify tolerance to HMs and if, beyond that, these could be possible candidates to phytoremediation (ZANCHETA et al., 2011).

The cadmium occupies the seventh place in the top 20 most toxic compounds existing in nature (AL-KEDHAIRY; AL-ROKAYAR; AL-MISNED, 2001). Its density is of 8,6 g cm^{-3} and the anthropogenic activities which release the most worrying quantities into the environment are: mining, metallurgical activities, fossil fuel burning, waste incineration, pesticides, phosphate fertilizers, sewage sludge and battery waste (WAGNER, 1993; LAGRIFFOUL et al., 1998). It is a divalent cation (Cd^{+2}) readily absorbed causing phytotoxicity (CHEN et al., 2011). Furthermore, due to its having high mobility from soil to plants, it easily enters the food chain (GUIMARÃES et al., 2008).

The plants, when submitted to Cd stress, may present nutritional and physiological disorders (PAIVA; CARVALHO; SIQUEIRA, 2002). This includes senescence symptoms, low chlorophyll concentrations, disorders in water balance and/or nutrition, interference with stomatal opening, photosynthesis and transpiration, directly or indirectly affect the germination and growth of plants (BENAVIDES; GALLEGO; TOMARO, 2005; ZHOU; PHILIPPE; QIU, 2006; WANG et al., 2008). Changes in photosynthesis and respiration can also interfere in plant growth, resulting in a low agricultural production (KÄRENLAMPI et al., 2000).

The species *Canavalia ensiformis* L. is a leguminous used in green manure crops of fast growth and which has considerable aerial green mass essential to the phytoremediation. Recent works have shown that this species has the phytoremediation potential for lead (ALMEIDA et al., 2008; ROMEIRO et al., 2007) and for copper (ZANCHETA et al., 2011). Based on these characteristics, the aim of this study was to evaluate in the germination and development of these species seedlings in a nutritious solution if there is tolerance to Cd.

2 Material and Methods

The experiments were conducted in the greenhouse of the Plant Physiology Department, IB (Biology Institute), of the State University of Campinas (UNICAMP), Campinas, SP, during the months of April to May of 2006, under natural photoperiodic conditions.

In the first experiment administered to study the germination of jack beans plants in the presence of Cd, seeds were sown in plastic vessels with 500 mL capacity (5 seeds per vessel), containing washed sand and cadmium chloride ($CdCl_2.2,5H_2O$) in concentrations of 0, 500 and 2000 µmol L^{-1} of Cd. The counting of germinated seeds was conducted during 13 days.

The experimental outline was entirely randomized, with 7 repetitions per treatment. To the obtained data, analysis of variance was applied, followed by the calculation of minimal difference by the Duncan test at 5% of probability. For the germination data, the sine arc $\sqrt{p}$ was used, where p is the ratio of germinated seeds. Such data transformation was applied in order to reach a normal distribution of the data.

In the second experiment, which consisted in the evaluation of the development of seedlings exposed to Cd, seeds of jack beans were put to germinate in plastic trays with washed sand. A week after the sowing (after issuing of the primary leaf), it was transplanted a seedling per vessel with the capacity of 2 L, containing washed water contaminated with $CdCl_2.2,5H_2O$ in concentrations of 0, 500 and 2000 µmol L^{-1} of Cd. The plants were watered once a week with 100 mL of a complete nutritious solution of Hoagland & Arnon (HOAGLAND; ARNON, 1938) and they stayed in the vessels until the moment of final reaping.

In the final reaping, the plants were separated in roots, stems and leaves, the roots being washed in running water to remove the sand excess. Following, they were conditioned in paper bags for the drying process at 60 °C during 72 hours for subsequent milling and weighing to determine the total of Cd and N.

The height, leaf number, leaf area and the dry mass of the roots, stems and leaves were determined after 35 days from transplantation. The leaf area was measured by means of the equipment Model L.I 3100 – Area Meter- SR n° LAM 1018, Licor- USA.

The gas exchanges were determined in the central leaflet of the first trifoliate completely expanded, from the base to the apex of the plants, in the morning period, starting at 8:00 AM. These measures were taken in a growth chamber with controlled temperature, humidity and luminosity, with a portable analyser of gases by infrared (IRGA- Infra Red Gas Analyser, Li 6200, Licor). The same leaves of the gas exchange evaluation were used for pigment extraction, according to Hiscox and Israelstam (1979). The water potential of the leaves was measured by means of a pressure chamber (Plant Moisture Stress, PMS Instruments).

The determination of total nitrogen (N) in the tissue was conducted by the Kjeldahl method (BREMNER, 1965) where samples of dry and ground plant material were digested by a digestive mixture, sulfuric acid and hydrogen peroxide. After slowly heating up to 360 °C, a greenish extract was obtained, which was cooled to room temperature and after the addition of 5 mL of water MilliQ and the contents of each tube was used in the dosage according to the Kjeldahl method.

The concentrations of Cd in the roots and aerial parts (stems and leaves) were conducted by the dry route method, according to Abreu (1997). The dry route method consisted in incinerating the material in muffle at 500 °C for two and a half hours. Hydrochloric acid and nitric acid were added and, after filtering, the volumes were completed to 25 mL with heated deionized water. Concentrations of Cd in the extracts were measured by spectrometry of plasma induced flame (ICP- AES- Jobin Yvon).

The ability of the species to translocate Cd from the roots to the aerial part was calculated by the translocation index (IT) suggested by Abichequer and Bohnen (1998) using the following Equation 1:

$$TI\% = \frac{Cd\ content\ of\ shoot\ (leaves + stem)(mg/plant)}{Cd\ content\ of\ plant\ (mg/plant)} \times 100 \quad (1)$$

The influence of the metal in the production of dry mass in all parts of the plant was calculated by the relative production index using the following Equation 2:

$$RP\% = \frac{dry\ mass\ production\ Cd\ absent(g)}{dry\ mass\ production\ Cd\ present(g)} \times 100 \quad (2)$$

The findings were submitted to analysis of variance and means were compared by the Duncan test ($p < 0.05$).

3 Results and Discussions

There was a delay in the velocity of germination of jack bean seeds at the highest concentration of Cd (2000 µmol L^{-1}) (Figure 1). However, from the tenth day on, at all concentrations, the percentage of germination was close to 100%. Munzuroglu and Geckil (2002) observed that the percentage of germination of cucumber and wheat seeds diminished with the increase of Cd concentrations from 50 to 80 µmol L^{-1}. Commonly, the stadium of germination inside the life cycle of the plant is very well protected against various stresses. Nevertheless, after the inhibition of subsequent plant development processes, the plants become, generally, sensible to the stress by Cd (LI et al., 2005). In this study, there were no possible damages in the activities of β and α amylases, what did not compromise the respiration and did not inhibit the growth of the embryonic axis and the seed radicles (GUIMARÃES et al., 2008), thus

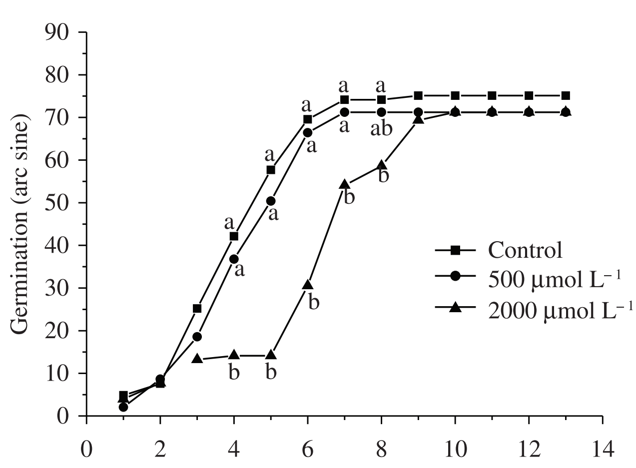

Figure 1. Germination of jack bean seeds in function of time after the sowing submitted to different concentrations of cadmium (µmol L^{-1}) under natural conditions of temperature and photoperiod. Averages with the same letter did not have significant differences by the Duncan test $p < 0.05$.

there were no damages in germination. This would be a clear effect of the toxicity to Cd if it were to occur.

The delay in germination may have occurred so that the plants could adjust their mechanisms of tolerance and be able to germinate even in the presence of Cd (HALL, 2002). In some species, depending on the Cd concentrations, besides diminishing the germination, some seeds die even before reaching the end of the experiment. This was reported by Peralta et al. (2001) where there was a decrease of 50% in germination of alfalfa seeds in treatment with 40 mg L^{-1} of Cd and the germinated seeds perished.

In the second experiment, the number of leaves and the dry mass of the roots did not show differences among the measures of growth. With the increase in concentrations of Cd, the height, leaf area and dry matter mass of stems and leaves decreased (Tables 1 and 2). The growth of eucalyptus species was also reduced with the increase in concentrations of Cd (SOARES et al., 2005) and barley plants exposed to this metal suffered a decrease in growth (GUO et al., 2004). It is known that Cd can reduce the stem growth due to the suppression of cell elongation (SANITÀ DI TOPPI; GABRIELLI, 1999). Andrade, Jorge and Silveira (2005) verified that with the addition of 0,554 mg L^{-1} of Cd in nutrient solution, the jack bean plant growth was not affected and chlorosis in the leaves was not observed. In this study, the plants exposed to the concentration of 2000 µmol L^{-1} presented with subtle chlorosis, in new leaves as well as old, and the roots did not get darkened, typical signs of toxicity to metal. The mild chlorosis observed could have been triggered by the competition of Cd with Fe for the absorption sites on the plasma membrane (SIEDLECKA; KRUPA, 1999).

Relative to the gas exchange, there was a significant decrease in CO_2 absorption rates in treatments with Cd (Table 2). The same was observed by El-Shintinawy (1999) in soybean plants, in the presence of Cd at concentrations of 0.1; 0.2 and 0.3 mM. This may have occurred due to alterations caused in the functions of the stomata, the electron transportation or in the Calvin cycle (BARCELÓ; VÁZQUES; POSCHENRIEDER, 1998). Being highly toxic, Cd causes major disorders in transpiration (ZHOU; PHILIPPE; QIU, 2006). In spite of that, in this study, the transpiration was not affected by the presence of Cd, an uncommon fact given the exposure to this metal. Taking into account these outcomes, it is possible to infer that the Cd action on gas exchange in jack beans may have been in the electron transportation or in the Calvin cycle. The total chlorophyll level of plants presented significant differences, which corroborated what was prior described and also with Vitória et al. (2003) and Barceló, Vázques and Poschenrieder (1998) and Kupper et al. (2007) who asserted that one of the effects of Cd in plants is the modification of chlorophyll biosynthesis, decreasing its production.

The water potential of plants became more negative as Cd exposure was increased (Table 2). According to Prasad (1995), water absorption in bean plants also decreased due to the addition of Cd at concentrations of 3 µM. As the growth of higher plants in substratum containing Cd affects the hydric/water balance, there is a decrease of elasticity of the cell wall and, consequently, of the cell expansion. Therefore, once more, the decrease in biomass is a direct effect of all these

Table 1. The dry matter mass, the total N, the concentration of Cd in parts of the jack bean plants and the indices of translocation and relative production.

Cd treatment ($\mu mol\ L^{-1}$)	Root	Steam	Leaf
		Dry mass (g)	
0	0.55 ± 0.05NS	1.16 ± 0.08a	2.16 ± 0.14a
500	0.51 ± 0.04NS	1.21 ± 0.06a	2.06 ± 0.15ab
2000	0.41 ± 0.06NS	0.77 ± 0.50b	1.66 ± 0.09b
		Total N ($g\ kg^{-1}$)	
0	16.79 ± 0.71NS	11.12 ± 0.73a	16.67 ± 0.81a
500	19.23 ± 0.69NS	17.52 ± 0.91b	19.54 ± 0.56b
2000	18.22 ± 0.95NS	19.99 ± 0.77b	23.87 ± 0.87c
		Cd content ($mg\ kg^{-1}$)	
0	0.82 ± 0.02a	0.88 ± 0.38a	1.22 ± 0.05a
500	7.79 ± 1.56b	11.38 ± 1.78b	7.60 ± 0.58b
2000	20.32 ± 1.96c	7.48 ± 1.78b	12.61 ± 0.86c
TI			
500	68,9%		
2000	48,0%		
RP			
500	98,5%		
2000	74,9%		

Averages with the same letter do not present significant differences by the Duncan test at $p < 0.05$ (average ± SE, n = 7).

Table 2. Effect of different concentrations of Cd in the photosynthesis, transpiration, water potential, chlorophyll content, height, leaf number and leaf area in jack bean plants.

	Cd ($\mu mol\ L^{-1}$)		
	0	500	2000
Photosynthesis ($\mu M\ m^{-2}\ s^{-1}$)	1.31 ± 0.05a	0.89 ± 0.19ab	0.552 ± 0.08b
Transpiration ($mmol\ m^{-2}\ s^{-1}$)	0.406 ± 0.04NS	0.276 ± 0.35NS	0.34 ± 0.05NS
Water potencial (MPa)	–4.0 ± 0.29a	–7.17 ± 0.44b	-10. 23 ± 0.43c
Total chlorophyll($mg\ g^{-1}$ MS)	9.32 ± 0.07a	5.99 ± 0.77b	6.13 ± 0.34b
Height (cm)	24.93 ± 3.06a	25.36 ± 2.15b	11.24 ± 0.81b
Number of leaves	3.43 ± 0.20NS	3.43 ± 0.20NS	2.86 ± 0.14NS
Leaf Area (cm^2)	535.04 ± 40.67a	545.78 ± 31.01a	372.5 ± 23.70b

Averages with the same letter do not present significant differences by the Duncan test $p < 0.05$ (average ± SE, n = 7).

transformations suffered by the plants when exposed to Cd (POSCHENRIEDER et al., 1989).

The total N concentration of the stems and leaves (Table 1) suffered an increase with the accretion of Cd concentrations. Wahid et al. (2007) observed that varieties of *Vigna radiata* (L.) sensitive to Cd have had difficulty in assimilating N in growing doses of this HM, due to the decrease of nitrate reductase enzyme activity in sprouts. However, the resistant varieties appeared to be sustaining the enzyme activity, obtaining thus, a better assimilation of N. It was what apparently happened to the jack bean plants in this study, indicating resistance to Cd.

The RP at the concentration of 500 $\mu mol\ L^{-1}$ was of 98.5% and at the highest concentration it was 74.9%. The Cd slightly affected the dry matter production of jack bean plants, inducing an inhibition of 27% in the concentration of 2000 $\mu mol\ L^{-1}$. As the green mass produced by this species is very high, even with the decrease observed it was possible to conclude that there was no major impact of Cd in the development of the plants.

The Cd levels in the roots, stems and leaves of the jack bean plants increased with the increase of concentration of Cd in the solution (Table 1). The IT of Cd was of 68.9% to the concentration of 500 $\mu mol\ L^{-1}$ and of 48% to the concentration of 2000 $\mu mol\ L^{-1}$. Such result demonstrates that at the lowest concentration, the Cd is easily absorbed by the root system and translocated, via the xylem, to the aerial part, through transpiration than at the highest concentration. Contrary to what occurred with Chen et al. (2003), where carrot and radish plants, absorbed larger quantities of Cd at the root, the jack bean plants accumulated a large part of the Cd in the aerial part and produced high biomass at different concentrations of Cd. This shows that this species has tolerance to Cd in these experimental conditions. Nonetheless, these results should be confirmed in experimentations in contaminated soils, with the imposition of real conditions, aiming to obtain indicatives of phytoremediator plants of this HM.

4 Conclusions

Given the fact that the Cd did not greatly affect the germination and development of jack bean plants, this species presented important characteristics of tolerance to Cd.

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