Ciência Rural, Santa Maria, v.54:02, e20210823, 2024 http://doi.org/10.1590/0103-8478cr20210823
ISSNe 1678-4596
BIOLOGY

In vitro sensitivity of potato plants to cadmium exposure

Luana da Silva Cadore[1] Dilson Antônio Bisognin[1*] Francielly Baroni Mendes[1] Janaina de Fatima Spanevello[1] Jacson Ricardo Librelotto[1] Thaíse da Silva Tonetto[1]

[1]Departamento de Fitotecnia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brasil. E-mail: dilson.bisognin@ufsm.br. [*]Corresponding author.

ABSTRACT: Cadmium (Cd) is a heavy metal that is extremely dangerous to human health and can be found naturally in soils, deposited through industrial waste or phosphate fertilization. In this study, we evaluated the morphological responses of *in vitro* grown plants of potato in the presence of Cd, and define a procedure for assessing Cd sensitivity of different clones. The potato clone SMIJ461-1 was cultured in the presence of Cd at concentrations of 0, 20, 40, 60, 80, 90, 110, 120, 140, and 160 µM. Survival, rooting, number and height of shoots, leaf and root counts were evaluated at 7, 14, 21, and 28 days of cultivation. Adverse effects of Cd on the growth of the potato plants, exerted in a concentration-dependent manner, were observed for shoot height, leaf and root counts. Results of this study indicated that Cd concentrations of 0, 45, 90, and 135 µM can be used for future *in vitro* evaluation of potato clone sensitivity to Cd exposure.
Key words: *Solanum tuberosum* L., heavy metal, growth, plant micropropagation.

Sensibilidade *in vitro* de plantas de batata expostas ao cádmio

RESUMO: O cádmio (Cd) é um metal pesado extremamente perigoso à saúde humana, podendo ser encontrado naturalmente nos solos, depositado através de resíduos industriais ou da adubação fosfatada. Este trabalho teve por objetivo avaliar as respostas morfológicas de plantas de batata cultivadas *in vitro* na presença de Cd e definir uma metodologia para inferir sobre a sensibilidade de diferentes clones. Foi estudado o clone SMIJ461-1 de batata, nas concentrações de 0, 20, 40, 60, 80, 90, 110, 120, 140, 160 µM de Cd adicionadas ao meio de cultura. A sobrevivência, o enraizamento, o número de brotações, a altura da parte aérea e o número de folhas e raízes foram avaliados aos 7, 14, 21 e 28 dias de cultivo. Foram observados efeitos adversos de concentrações de Cd sobre o crescimento *in vitro* das plantas de batata do clone SMIJ461-1 para a altura da parte aérea e o número de folhas e raízes, sendo estes efeitos dependentes das concentrações de Cd adicionadas ao meio de cultura. Os resultados deste estudo indicam que as concentrações de 0, 45, 90 e 135 µM de Cd podem ser utilizadas para a avaliação *in vitro* da sensibilidade de clones de batata a presença de Cd.
Palavras-chave: *Solanum tuberosum* L., metal pesado, crescimento, micropropagação de plantas.

Potato (S*olanum tuberosum* L.) is the third most important food crop worldwide and is grown in more than 160 countries (FAO, 2018). According to the Brazilian Potato Association, potato agribusiness in Brazil involves approximately 5.000 producers, with an area of approximately 130.000 hectares and an annual production of 3.5 million of tons of potato. A large part of this production is destined for the market, with 18% used by the potato chip processing industry (PEREIRA & SILVA, 2019). Potatoes are rich in carbohydrates, proteins, B vitamins, iron, potassium, calcium, phosphorus, and starch. Their nutritional value, combined with ease of preparation and gastronomic versatility, makes potato a part of the human staple diet (YE et al., 2020).

Cadmium (Cd), a heavy metal, is a soil contaminant with no known essential biological function, but is toxic to plants and animals even at low levels. Contamination of agricultural soils by Cd and other heavy metals is the main source of these elements in plants, which introduces them into the food chain. Cd is naturally present in the soil and its concentration depends on the origin, composition, and development of the soil. Besides that, Cd is a component of phosphate fertilizers, in the form of Cd sulfate, and its application contribute to Cd accumulation in the soil (ZHANG&REYNOLDS, 2019). Sources and brands of phosphate fertilizers differ in Cd content. Among nineteen samples, six had higher than 12 mg Kg^{-1}, one had higher than 43

Received 11.17.21 Approved 05.12.23 Returned by the author 07.14.23
CR-2021-0823.R1
Editors: Leandro Souza da Silva Tanja Mimmo

mg Kg^{-1}, and the rest have less than 3 mg Kg^{-1} of fertilizer (BIZZARRO et al., 2006).

The risks to human health linked to the consumption of potatoes contaminated by toxic metals, including Cd, are a cause for concern worldwide (PENG et al., 2018). Moreover, since potato is an important and staple food for humans, it carries a risk of introducing Cd in the human diet. Thus, identification of potato clones tolerant to Cd accumulation is essential for human health and improving food security. The highly controlled and well-defined conditions associated with fast results of *in vitro* cultivation should facilitate the assessment of the sensitivity of potato plants to increasing concentrations of Cd. The objective of this study was to evaluate the morphological responses of *in vitro* grown plants of potato in the presence of Cd, and define a procedure for assessing Cd sensitivity of different clones.

The study was carried out at the Tissue Culture Laboratory of the Plant Breeding and Propagation Center (MPVP), Department of Plant Science of the Federal University of Santa Maria, Rio Grande do Sul, Brazil. Nodal segments of plants of potato clone SMJ461-1 were examined after 25 days of *in vitro* cultivation. Cadmium nitrate [$Cd(NO_3)_2.4H_2O$] in stock solution was administered at concentrations of 0 (control), 20, 40, 60, 80, 90, 110, 120, 140, and 160 µM. Approximately 10 mL of culture medium for each treatment was added to the test tubes, sealed, and autoclaved for 20 min at 120 °C and 1 atm pressure for sterilization. For micropropagation, a nodal segment (approximately 1.0 cm in length) was placed in each test tube. The explants were cultivated for 28 days in a climatized room at a temperature of 25 ±1 °C with a photoperiod of 16 h and light intensity of 35 µM m^{-2} s^{-1}, obtained by cold fluorescent lamps. Survival, rooting, number and height of shoots, leaf and root counts were evaluated at 7, 14, 21, and 28 days of cultivation. The experiment was a completely randomized design, with five replicates of four plants. Data were subjected to variance analysis, and the significant F test of means was compared by polynomial regression analysis at 5% probability of error using R Studio software.

The variance analysis did not show significant differences ($P > 0.5$) for percentage of survival and rooting and number of shoots for any evaluation (Table 1). Significant differences ($P < 0.01$) were found for shoot height, leaf and root counts in potato plants of clone SMIJ461-1 subjected to varying Cd exposure for all time periods. Despite presenting significant differences at seven days of cultivation, the variation coefficients observed at 14 days were similar to those obtained at 21 and 28 days of cultivation, indicating adequate experimental precision for all evaluated traits.

Cadmium concentration was inversely associated to shoot height, leaf and root counts of plants at 7, 14, 21, and 28 days of cultivation (Figure 1). Growth reduction of plants was observed from a certain concentration of Cd in the culture medium, depending upon each trait and day of evaluation. Potato plants of SMIJ461-1 clone showed reductions in shoot height, leaf and root

Table 1 – Summary of analysis of variance for percentage of survival and rooting, number and height (cm) of shoots, leaf and root counts of potato plants of clone SMIJ461-1 cultivated *in vitro* at different cadmium (Cd) concentrations at 7, 14, 21 and 28 days.

	--Survival (%)--		----Rooting (%)---		-Shoot number-		---Shoot height---		-----Leaf count----		-Root count-	
	SM	Fc	SM	Fc	SM	Fc	SM	Fc	SM	Fc	SM	Fc
At 7 days of *in vitro* cultivation												
[Cd]	0	0^{ns}	1246.29	8.16^{ns}	0.11	7.59^{ns}	0.81	11.39^{*}	2.96	13.58^{*}	3.25	5.44^{*}
Average	100		86.33		0.88		0.76		0.91		2.29	
CV (%)	0		14.32		13.81		34.97		51.12		33.8	
At 14 days of *in vitro* cultivation												
[Cd]	56.94	0.91^{ns}	337.04	3.79^{ns}	0.09	2.89^{ns}	5.90	20.73^{*}	6.95	17.22^{*}	2.75	4.71^{*}
Average	98.5		94.33		0.95		3.02		4.20		3.92	
CV (%)	8.03		9.99		12.24		17.65		15.11		19.47	
At 21 days of *in vitro* cultivation												
[Cd]	114.35	1.79^{ns}	255.58	2.88^{ns}	0.28	1.59^{ns}	8.86	14.39^{*}	6.17	12.56^{*}	2.79	9.26^{*}
Average	97.83		95.33		0.97		4.57		5.96		4.25	
CV (%)	8.17		9.89		13.79		17.18		11.76		12.91	
At 28 days of *in vitro* cultivation												
[Cd]	265.95	3.47^{ns}	255.250	2.87^{ns}	0.02	1.10^{ns}	10.44	13.19^{*}	10.65	10.13^{*}	4.38	9.79^{*}
Average	95.82		95.32		0.96		5.71		7.32		4.69	
CV (%)	9.13		9.90		15.68		15.56		14.00		14.25	

SM = square mean, Fc = calculated F, and ^{ns} = no significant and * = significant at a 0.001 level of error probability.

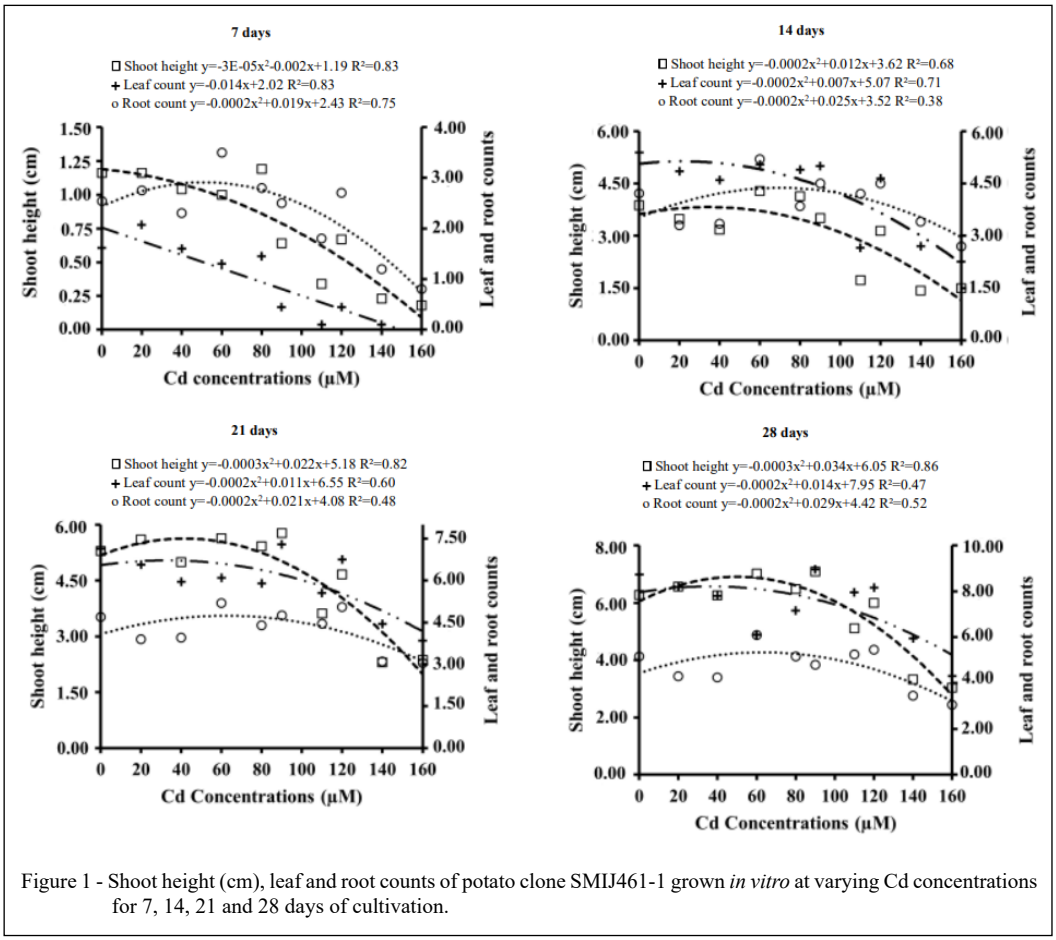

Figure 1 - Shoot height (cm), leaf and root counts of potato clone SMIJ461-1 grown *in vitro* at varying Cd concentrations for 7, 14, 21 and 28 days of cultivation.

counts in the presence of Cd at 14 days of cultivation, which were dependent on the concentration of Cd in the culture medium. Thus, future studies evaluating the *in vitro* sensitivity of potato plants to Cd should be carried out based on data from 14 days of *in vitro* cultivation, mainly when the goal is to evaluate a high number of clones. Moreover, evaluating at 14 days of cultivation is important, because it facilitates and accelerates the process of *in vitro* identification of potato clones for Cd sensitivity.

Considering the variation coefficient and the advantages of evaluating the plants at 14 days of cultivation, we can define the Cd concentration in the culture medium based on the maximum technical efficiency, which is the concentration that plants have already presented some level of sensitivity (STORCK et al., 2000). For the clone SMIJ461-1, leaf count was first affected at 16.5 µM, shoot height at 29.5 µM, and root count at 74 µM of Cd in the culture medium at 14 days of cultivation, which concentration can be used for evaluating other potato clones in the future.

A similar study in *Jatropha curcas* L. reported that a 40 mg dm^{-3} Cd concentration caused a reduction in plant height, stem diameter, leaf count, and leaf area, which can collectively restrict the photosynthesis rate (CHAVES et al., 2014). In addition, height reduction, chlorosis, necrosis, and desiccation of leaves were identified as common symptoms of Cd exposure in most plant species (HE et al., 2017). Cd affects the absorption of mineral nutrients and reduces plant growth by inhibiting stomatal opening, photosynthesis, and carbohydrate metabolism (NAZAR et al., 2012). Plants showed varied responses to Cd exposure, but results similar to the present study were observed by KHAN et al. (2020) in *Brassica rapa* ssp. *Chinensis*. They found that 50 µM of Cd resulted in a reduction in shoot height and leaf count. In addition, GONÇALVES et al. (2009) reported that Cd exposure increased the number of adventitious roots in Asterix and Macaca cultivars of potato, suggesting a defense mechanism of plants to combat the adverse effects of this heavy metal. The negative effects of Cd on plant growth can be explained by considering various cellular interactions, nutritional deficiency, and phytotoxicity (GONÇALVES et al., 2009). The

mechanisms underlying Cd tolerance in plants are not yet fully understood (TIRYAKIOGLU et al., 2006), but studies on certain species and cultivars have indicated plant adaptation to its presence.

In this study, potato plants of the clone SMIJ461-1 showed that the morphological effects depended on the evaluated trait and the Cd concentration in the culture medium. In addition to revealing morphological effects of Cd exposure, the results suggested that Cd concentrations of 45, 90, and 135 µM should be used in future experiments to evaluate other potato clones based on shoot height, leaf and root counts of plants at 14 of *in vitro* cultivation. The addition of 45 µM of Cd to the culture medium reduced shoot height and leaf count, while 90 µM of Cd reduced root count, and 135 µM of Cd drastically affected plant growth (Figure 1) and showed chlorosis and necrosis at 14 days of *in vitro* cultivation. Root darkening and thickening were observed at Cd concentrations over 90 µM after 28 days of cultivation (data not shown). As we do not know the *in vitro* sensitivity to Cd exposure of other potato clones, it is worse to include the concentration of 135 µM, even resulting in drastic morphological effects as observed for SMIJ461-1. Evaluation of other clones will improve and expand our knowledge about potato plant behavior in terms of Cd absorption and translocation, and may suggest possible mechanisms of tolerance and/or accumulation sites in response to Cd exposure.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This study was partially funded by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES).

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

REFERENCES

CHAVES, L. H. G.; SOUZA, R. S. Growth, distribution and contente of cádmium in Jatropha curcas plants **Ciências Agrárias**, v.37, n.3, p.286-291, 2014. Available from: <http://www.scielo.mec.pt/pdf/rca/v37n3/v37n3a04.pdf>. Accessed: Dec. 13, 2020.

FAO. **Food and Agriculture Organization of the United Nations**. Rome: FAO, 2018. Available from: <http://faostat.fao.org/site/567/DesktopDefault.aspx?pageID=567#ancor>. Accessed: Dec. 02, 2020.

GONÇALVES, J. F. et al. *In vitro* growth of potato plantlets in different doses of cádmium. **Ciência Rural**, v.39, n.9, p.2625-2628, 2009. Available from: <https://www.scielo.br/pdf/cr/v39n9/a38v39n9.pdf>. Accessed: Dec. 02, 2020. doi: 10.1590/S0103-84782009000900038.

HE, S. et al. Morphological and physiological responses of plants to cadmium toxicity: A Review. **Pedosphere**, v.27, n.3, p.421-438, 2017. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S1002016017603394>. Accessed: Dec. 02, 2020. doi: 10.1016/S1002-0160(17)60339-4.

KHAN, K. Y. et al. Study amino acid contents, plant growth variables and cell ultrastructural changes induced by cadmium stress between two contrasting cadmium accumulating cultivars of *Brassica rapa* ssp. *chinessis* L. (pak choi). **Ecotoxicology and Environmental Safety**, v.200, p.110-748, 2020. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S014765132030587X?via%3Dihub>. Accessed: Dec. 02, 2020. doi: 10.1016/j.ecoenv.2020.110748.

MAR, S. S.; et al. The influence of phosphate fertilizer application levels and cultivars on cadmium uptake by Komatsuna (*Brassica rapa* L. var. *perviridis*). **Soil Science and Plant Nutrition**, v.58, p.49-502, 2012. Available from: <https://www.tandfonline.com/doi/pdf/10.1080/00380768.2012.704394?needAccessed=true>. Accessed: Sept. 08, 2021. doi: 10.1080/00380768.2012.704394.

NAZAR, R. et al. Cadmium toxicity in plants and role of mineral nutrients in its alleviation. **American Journal of Plant Sciences**, v.3, p.1476-1489, 2012. Available from: <https://www.scirp.org/journal/paperinformation.aspx?paperid=24162>. Accessed: Dec. 02, 2020. doi: 10.4236/ajps.2012.310178.

PENG, Y. et al. Risk assessment for potentially toxic metal (loid) s in potatoes in the indigenous zinc smelting area of northwestern Guizhou Province, China. **Food and Chemical Toxicology**, n.120, p.328-339, 2018. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S027869151830471X?via%3Dihub>. Accessed: Dec. 02, 2020. doi: 10.1016/j.fct.2018.07.026.

PEREIRA, A. S.; SILVA, G. O. Potato, evolution in the offer of brazilian cultivars and in the production of seed tubers. **SEEDnews**, n.2, p.35-39, 2019. Available from: <https://seednews.com.br/artigos/2941-batata-edicao-marco-2019>. Accessed: Dec. 02, 2020.

STORCK, L. et al. **Experimentação vegetal**. Santa Maria: Ed. UFSM, 2000. 198p.

TIRYAKIOGLU, M. et al. Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance. **Journal of Trace Elements in Medicine and Biology**, n.20, p.181-189, 2006. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0946672X06000745?via%3Dihub>. Accessed: Dec. 02, 2020. doi: 10.1016/j.jtemb.2005.12.004.

YE, Y. et al. Cultivar diversity and organ differences of cadmium accumulation in potato (*Solanum tuberosum* L.) allow the potential for Cd-safe staple food production on contaminated soils. **Science of the Total Environment**, n.711, p.134-534, 2020. Available from: <https://www.sciencedirect.com/science/article/abs/pii/S0048969719345255>. Accessed: Dec. 02, 2020. doi: 10.1016/j.scitotenv.2019.134534.