JPMB | Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2025 | Volume 13 | Issue 1

Effects of cadmium stress on *in vitro* growth of grass pea (*Lathyrus sativus* L.) genotypes

Zahra Salami[1], Alireza Pourmohammad*[1], Ali-Asghar Aliloo[1], Farhad Behtash[2]

[1.] Department of Plant Production and Genetics, Faculty of Agriculture, University of Maragheh, Maragheh, Iran
[2.] Department of Horticultural Sciences, Faculty of Agriculture, University of Maragheh, Maragheh, Iran

Original paper

OPEN ACCESS

Edited by
Prof. Ahmad Arzani,
Isfahan University of Technology, Iran

Date
Received: 12 May 2025
Accepted: 12 June 2025
Published: 16 May 2026

Correspondence
Prof. Alireza Pourmohammad
pourmohammad@maragheh.ac.ir
pourmohammad@ymail.com

Citation
Salami, Z., Pourmohammad, A.R., Aliloo, A.A. and Behtash, B. (2025). Effects of cadmium stress on *in vitro* growth of grass pea (*Lathyrus sativus* L.) genotypes. *J Plant Mol Breed. 13* (1): 94-105.
doi: 10.22058/jpmb.2025.2060346.1340.

Copyright: © 2025 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution License (CC BY-NC 4.0).

Abstract: Grass pea is a legume with great potential for use in both human food and animal feed. This study was performed to determine the tolerance of ten grass pea genotypes in cadmium stress condition, based on dry matter accumulation at callus and seedling stages under *in vitro* conditions. The calli and seedlings were exposed to various cadmium concentrations (0, 12.5, 25, and 50 µM). Callus cultures were grown on MS medium supplemented with 2,4-D (2 mg/L), and the seedlings were grown with BAP (1.5 mg/L). Both calli and seedlings survived at 50 µM cadmium, while 25 µM had no significant effect on their growth. At 25 µM cadmium, callus and seedling root growth were reduced by 50.41% and 35.27%, respectively. Hence, callus was less tolerant to cadmium than seedlings, likely due to its higher cadmium accumulation The present study suggests that grass pea exhibits high tolerance to cadmium, and can also surviving low to moderate concentrations of cadmium at seedling and callus stages. Tolerant callus lines may serve as valuable sources for regenerating cadmium-tolerant plants, which should be further evaluated for their detoxification potential at the adult growth stages.

Keywords: Callus; biomass; heavy metal tolerance; seedling.

Introduction

Among grain legumes, grass pea (*Lathyrus sativus* L.) has noted for its tolerance to dry conditions and adaptability to difficult environments (Arzani, 2006; Tripathi et al., 2022; Dogrusoz et al., 2024). Grass pea has adapted to diverse environmental condition across the globe ((Mekonen et al., 2022; Goncalves et al., 2024). Grass pea is a cool-season pulse grown on the Indian subcontinent, southern Europe, and northern Africa for forage and grain production (Campbell, 1997). Grass pea is a climate-elastic, nutrient-rich legume crop grown mainly in fragile agro-ecosystems (Kumar et al., 2013; Sanches et al., 2024). In recent years, grass pea has appeared for cultivation in challenging environments (Sarkar et al., 2019). The grass pea potential has recently gained recognition notwithstanding the presence of a plant toxin called β-ODAP (Lambein et al., 2019; Barpete et al., 2022; Boulfekhar et al., 2023).

Cadmium is among the most hazardous metals due to its high mobility and toxicity even at low concentration, posing a serious threat to both plants and humans. Cadmium is a non-essentialan unnecessary element that adversely impacts plant growth and development due to its high toxicity (Pinto et al., 2004). Therefore, plant cells activate multiple defense mechanisms to neutralize the toxic action of cadmium (Di Toppi and Gabbrielli, 1999). Cadmium is highly phytotoxic and causes growth inhibition, which eventually leads to plant death. One possible mechanism of damage to plant tissues is the induction of oxidative stress by enhanced generation of free radicals(Zhang et al., 2005).

The understanding of grass pea's stress tolerance mechanisms is the first step for a more efficient exploitation of it for multiple abiotic stress resistance studies such as cadmium stress and opens doors for further progresses on crop breeding efforts (Sanches et al., 2024). Tissue culture techniquies provides a tool i) to develop new germplasm via somaclonal variation and *in vitro*-induced mutagenesis, enabling better adaptation to changing demands and ii) to screen tolerant genotypes (Singh and Chand, 2003; Arzani, 2008) . *In vitro* culture of plant cells in the presence of high concentrations of metals develops a useful tool to study the adaptive mechanisms of plants living in harmful environments (Lasat, 2002). Species and cultivars differ in tolerance to heavy metals, and related genotypes can be worth tools in investigating the toxicity or tolerance mechanisms (Foy et al., 1978).

Plants regenerated from callus or plantlet are prone to generating somaclonal variants that may exhibit altered responses biotic and abiotic stresses (Arzani, 2008). The present study was carried out to investigate the role of genotypes and cadmium concentrations in cadmium tolerance. Explants from mesocotyl of ten grass pea genotypes were taken, and their callus and organogenesis responses were studied. Efforts have also been directed toward identifying the genotype with the highest tolerance, aiming to establish an *in vitro* regeneration protocol for grass pea as a crucial initial step in biotechnology research.

Materials and Methods

Plant materials

This research was conducted at the Research Laboratory of Plant Production and Breeding Department, Faculty of Agriculture, University of Maragheh, Maragheh, East Azerbaijan Province, Iran. The genotypes were procured from the International Center for Agricultural Research in the Dry Areas (ICARDA). Table 1 shows the origin of grass pea genotypes used in this experiment. Initially, seeds were sterilized using 70% ethanol for 30 seconds followed by an immersion in 1.5% sodium hypochlorite for 15 minutes. After washing three times with sterilized distilled water, the seeds were cultured on MS medium supplemented with 0.8% agar and 30 g/l sucrose. Then the cultures were incubated in the culture room at 25±1° C under a 16/8 h light/darkphotoperiod illuminated by fluorescent lamps.

The study was carried out as a factorial experiment based on a completely randomized design (CRD) with ten genotypes, five levels of cadmium (0, 12.5, 25, 50, mM), two replications, and five samples. Cadmium stress levels were determined by Probit analysis. In such a way that, after a preliminary experiment and analyzing the results related to the cadmium stress threshold using probit analysis, 0, 12.5, 25, and 50 µM concentrations were determined as the suitable treatment for screening genotypes (data not shown). The main levels of cadmium were

arranged in 12.5 to 125 µM given that higher concentrations resulted in the death of the plantlet. After determining the cadmium stress levels, experiments were performed under *in vitro* conditions by inducing cadmium stress to the seedlings and callus obtained from the mesocotyl explant.

Seedling traits

Germinated seeds were transferred to MS medium containing various concentrations of cadmium to evaluate their tolerance. The cultures were transferred to a growth chamber with similar conditions to the seed germination stage. After 30 days of culture, the seedling traits were recorded as follows: number of plumules, length of rootlet, length of plumule, length of seedling, fresh and dry (after 24 hours of oven-drying at 70°C) weight of rootlet, plumule, and seedling.

Table 1. The grass pea genotypes used in cadmium tolerance experiment.

Genotype No.	Origin
1	Local check (Iran)
2	Bangladesh
3	Canada
4	Ethiopia
5	Hungary
6	Greece-I
7	Greece-II
8	Greece-III
9	Greece-IV
10	Greece-V

Callus traits

Callus cultures were induced using mesocotyl explants of *L. sativus* cultured on modified MS medium (8.34 mg/L FeSO4 and 30 mg/L AgNO3), supplemented with 1.5 mg/L BAP, and 2 mg/L 2,4-D. Green callus of grass pea genotypes was sub-cultured on MS medium supplemented with 2,4-D (2 mg/1), kinetin (0.3 mg/1), and thiamine (0.4 mg/1) at 27±1°C.

After 30 days, the callus traits were recorded as follows: callus fresh weight, callus dry weight (24 hours, 70°C), and callus relative growth rate (RGR) (Arzani and Mirodjagh, 1999). Thus, after carefully removing the callus from the culture vessel, accumulated water was blotted out gently without squeezing the tissue. Then, the tissue was transferred onto preweighed aluminum foil, and the weight was determined on a single-pan digital balance. After the fresh weight was recorded, the tissues were oven dried at 70°C to a constant weight on the same foils for determination of their dry weight.

Plant growth parameter

Growth was measured from the fresh weight of callus and seedlings. Plant tolerance index was computed from fresh weight (FW) by using the formula: (FW treated/FW control) × 100, and expressed as percent tolerance (Wilkins, 1978).

Cadmium stress tolerance indices

Thirty days after culturing, cadmium stress tolerance indices for seedling and callus dry weight were calculated to select the most tolerant genotypes as follows: Tolerance index (TOL) Rosielle and Hamblin (1981), mean productivity (MP) Rosielle and Hamblin (1981), stress tolerance index (STI) Fernandez (1993), geometric mean productivity (GMP) Fernandez (1993), harmonic mean (HARM) Fernandez (1993), stress susceptibility index (SSI) Fischer and Maurer (1978), relative decrease in yield (RDI) Bidinger et al.

(1987), stress susceptibility percentage index (SSPI) Mousavi et al. (2008), stress and non- stress production index (SNPI) (Mousavi et al., 2008). Formulas of cadmium stress tolerance indices are shown in Table 2.

Data analysis

Before data analysis, a normality test was performed using Kolmogorov-Smirnov test. Data were investigated by one-way analysis of variance (ANOVA) using SPSS (ver. 20) and were represented as mean plus/minus standard error (SE). Duncan's multiple range test was used for displaying differences between the treatments (P<0.05). Cluster analysis based on the measured traits was performed to classify genotypes. Principal components analysis was used to confirm the grouping of cluster analysis. NTSYS software applied for Cluster analysis, PCA analysis, and a three-dimensional scatterplot of grass pea genotypes.

Table 2. Cadmium stress tolerance indices used in evaluating of grass peas genotypes (Lathyrus sativus) to cadmium stress.

Cadmium stress tolerance indices	Reference
$TOL=Y_P-Y_S$	(Rosielle and Hamblin, 1981)
$MP=(Y_P+Y_S)/2$	(Rosielle and Hamblin, 1981)
$STI=(Y_P\times Y_S)/(\bar{Y}_P\times\bar{Y}_s)$	(Fernandez, 1993)
$GMP=\sqrt{Y_P\times Y_S}$	(Fernandez, 1993)
$HARM=(Y_P\times Y_S)/(Y_P+Y_S)$	(Fernandez, 1993)
$SSI=[1-\frac{Y_S}{Y_P}]\Big/[1-\frac{\bar{Y}_S}{\bar{Y}_P}]$	(Fischer and Maurer, 1978)
$RDI=(\frac{Y_S}{Y_P})\Big/(\frac{\bar{Y}_S}{\bar{Y}_P})$	(Bidinger et al., 1987)
$SSPI=[(Y_P-Y_S)/2\bar{Y}_P]\times100$	(Mousavi et al., 2008)
$SNPI=\sqrt[3]{(Y_P+Y_S)/(Y_P-Y_S)}\times\sqrt[3]{(Y_P\times Y_S\times Y_P)}$	(Mousavi et al., 2008)

Ys and YP: Yield (dry weight) of genotypes under stress and non-stress conditions, respectively.

$\bar{Y}_P$ and $\bar{Y}_s$: Mean yield of genotypes under stress and non-stress conditions, respectively.

Results and Discussion

Seedling

Genotypes significantly differed for fresh and dry weight of seedlings. Cadmium treatments significantly affected these traits as well. With increasing the concentration of cadmium, the fresh weight of seedlings decreased. In addition, the interaction genotype × cadmium was significant for the number of plumules (Table 3). The cadmium is well known to reduce biomass and inhibit plant growth (Verma et al. (2008). Ferreira et al. (2002) reported the inhibitory effects of cadmium on seedling growth. Recently, Goncalves et al. (2024) reported a significant genotypic variation for the agronomic traits of grass pea. In another study, (Dogrusoz et al., 2024) reported that there were no differences between the two grass pea varieties. The variation among genotypes can be used in grass pea improvement programs to develop new cultivars (Arslan et al., 2022).

Mean comparison of seedling fresh weight and dry weight of grass pea genotypes by Duncan's multiple range test showed Iranaian local genotype

had lowest values in both traits (Table 4). The highest value for these traits belonged to the Ethiopian genotype. Based on agronomic data (not shown), the genotype of Ethiopia has higher forage yield and yield components might have a positive relationship with it. One of the most important growth indices is the dry weight of seedlings which indicates the accumulation of dry matter in the plant.

Table 3. Analysis of variance for the seedling traits of grass pea genotypes under in vitro cadmium conditions.

Source of Variation	df	MS		
		Seedling fresh weight	Seedling dry weight	number of plumules
Genotype (G)	9	0.064*	0.048*	0.204 ns
Cadmium (Cd)	3	0.123*	0.070 ns	0.252 ns
G × Cd	27	0.021ns	0.017 ns	0.642**
Error	40	0.027	0.020	0.138

The reduction percentage of dry weight of seedlings, showed that the concentration of 50 mg/l had the highest reduction for root/shoot length ratio (57.4%), length root (23.8%), and length of seedling (48.8%) (Table 5). Piwowarczyk et al. (2016) reported that salinity stress significantly reduced length of grass pea seedling organs (root and shoot) in tissue culture conditions. Cadore et al. (2023) in a study on *in vitro* sensitivity of potato plants to cadmium exposure reported adverse effects of Cd on the growth of the potato plants for shoot height, leaf and root counts. They 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. Gulec sen et al. (2023) reported that the effect of salt stress, genotype and their interactions were significant on the examined traits of grass pea. Tokarz et al. (2020) reported that grass pea seedlings on the media with osmotic stress were much smaller than those growing in the presence of chemical stress, but had a significantly higher content percentage of dry weight.

Table 4. Mean comparisons of genotypes for the effects of cadmium on growth-related traits of grass pea seedling *in vitro* using Duncan's multiple range test.

Genotype	Seedling fresh weight	Seedling dry weight
Local check (Iran)	0.202c	0.135c
Bangladesh	0.439abc	0.380abc
Canada	0.505ab	0.420ab
Ethiopia	0.618a	0.544a
Hungary	0.358abc	0.297abc
Greece-I	0.492ab	0.436ab
Greece-II	0.547ab	0.482ab
Greece-III	0.531ab	0.465ab
Greece-IV	0.298bc	0.256bc
Greece-V	0.396abc	0.343abc

Table 5. Mean comparisonsof different cadmium stress treatments for seedling traits of grass pea genotypes *in vitro* using Duncan's multiple range test.

Cadmium treatment (µg/l)	Length of root (cm)	Length of seedling (cm)	Length of root/ shoot	Fresh weight of root (g)	Dry weight of root (g)	Fresh weight of shoot (g)	Fresh weight of seedling (g)	Dry weight of seedling (g)	Dry weight root/shoot
0	2.42a	3.7575a	1.8531a	0.2360a	0.0347a	0.1945ab	0.4305a	0.0627a	1.2132a
12.5	2.26ab	3.9150a	1.3678ab	0.2245a	0.0285ab	0.2235ab	0.4480a	0.0567a	1.0041ab
25	2.005b	3.7500a	1.1996bc	0.2380a	0.0259b	0.2265ab	0.4845a	0.5455a	0.9953ab
50	1.24c	2.8650a	0.7897c	0.2105a	0.0245b	0.2725a	0.4830a	0.5545a	0.7964b

Didwania et al. (2019) investigated the phytotoxic effects of cadmium on seedling growth of *Allium cepa* and showed the inhibitory effects on root and shoot length of *Allium* at 80 ppm and 100 ppm. One of the reasons for the decrease in root length and seedling length may be the influx of cadmium ions into the roots, which subsequently affects the seedling's growth. The roots absorb nutrients from the environment in the form of ions (John et al., 2009).

Table 6. Cadmium stress tolerance indices calculated for seedling dry weight in *in vitro* conditions.

Genotype	TOL	MP	STI	GMP	HARM	SSI	RDI	SSPI	SNPI
Local	-0.0795	0.1352	0.0020	0.1222	0.0041	-4.6462	2.9026	-9.2689	0.0945
Bangladesh	-0.1535	0.3802	0.0192	0.3724	0.1055	-2.0589	1.9955	-17.8967	-0.5931
Canada	-0.0185	0.4207	0.0278	0.4170	0.2121	-0.8779	1.6111	-2.1569	0.5387
Ethiopia	0.2378	0.5442	0.0392	0.5225	0.3238	1.4644	0.8488	27.6903	1.0956
Hungary	0.157	0.297	0.0088	0.2438	0.0442	-1.7168	1.8841	18.3047	0.1324
Greece-I	0.105	0.436	0.0261	0.4332	0.1665	0.8813	1.0386	12.2420	0.9186
Greece-II	0.1755	0.4827	0.0298	0.4604	0.2103	1.0666	0.9782	20.4616	-0.439
Greece-III	0.141	0.4655	0.0317	0.4300	0.2760	1.2795	0.9090	16.4393	-0.03265
Greece-IV	0.1645	0.2567	0.0082	0.2431	0.0305	1.9661	0.6855	19.1792	0.3979
Greece-V	0.324	0.343	0.0127	0.3023	0.0640	2.6148	0.4744	37.7754	0.4334

Tolerance index (TOL), Mean productivity (MP), Stress tolerance index (STI), Geometric mean productivity (GMP), Harmonic mean (HARM), Stress susceptibility index (SSI), relative decrease in yield (RDI), Stress susceptibility percentage index (SSPI), Stress non- stress production index (SNPI).

Table 7. Analysis of varaince of callus traits of grass pea genotypes exposed to cadmium stress in *in vitro* conditions.

Source of variation	df	Mean square (MS)			
		Callus fresh weight	Callus dry weight	Callus relative growth rate	Callogenesis index
Genotype	9	0.018^{ns}	0.016*	0.021^{ns}	0.487^{ns}
Cadmium	3	0.136**	0.123**	3.912**	23.05**
Genotype × Cadmium	27	0.015^{ns}	0.013^{ns}	0.036^{ns}	0.100^{ns}
Error	40	0.009	0.008	0.037	0.952

It has also been reported that the reduction in shoot length due to the adverse effect of cadmium originates from reduced cell division and reduced growth of meristem cells. The exposure of cadmium to roots reduces root growth through the reduction of root cell division, destruction of the cell wall, cadmium competition with the absorption of micronutrients and decreased root respiration (Qian et al., 2011). Cadmium toxicity has been observed in various forms, including reduced shoot and root used to assess plant resistance to varying concentrations of a metal is the rate of root growth. This is because roots are particularly sensitive to the presence of toxic metals and are the first organ to be exposed to toxicity. Root length is used as the most important criterion for the effect of metal toxicity on plants (Baker and Proctor, 1990).

Cadmium stress tolerance indices for seedling dry weight

Most stress tolerance indices selected Ethiopiaian genotype as a tolerant genotype and genotype Greece-V as a susceptible genotype (Table 6).

Callus traits

The studied genotypes varied significant only for callus dry weight. Cadmium had a significant effect on callus fresh weight, callus dry weight, callus RGR and callogenesis index (Figure 1).

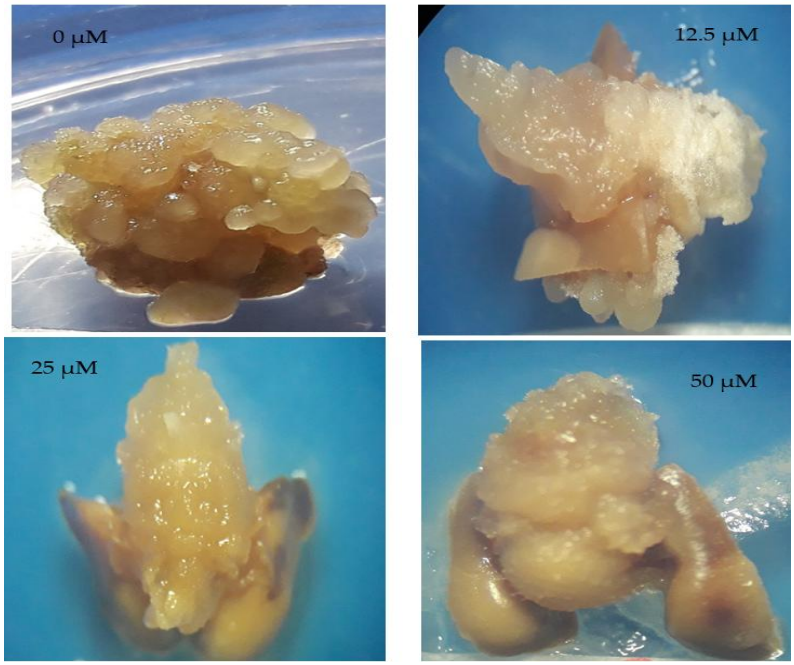


Figure 1. Images showing calli of grass pea genotypes subjected to cadmium stress under in vitro conditions.

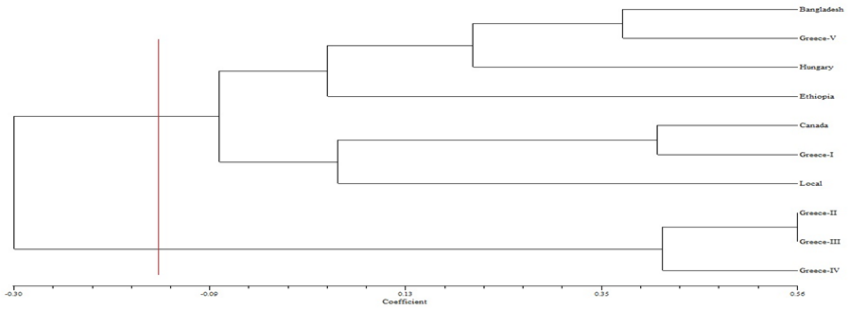


Figure 2. Dendrogram of the grass pea genotypes exposed to cadmium stress under *in vitro* conditions**.**

Also, genotype × cadmium was insignificant for all evaluated traits (Table 7). Callus growth inhibition by cadmium has also been recorded by (Fornazier et al., 2002).

Grouping of grass pea genotypes by cluster analysis and principal components analysis (PCA)

Cluster analysis by the UPGMA algorithm and Euclidean measure distance based on all measured traits, divided grass pea genotypes exposed to cadmium stress into two groups (Figure 2). The first group consisted of genotypes Greece-II, Greece-III, and Greece IV. This grouping matched with the origin of genotypes. The second group involved other genotypes. In a study by Mekonen et al. (2022) cluster analyses grouped the 25 grass pea accessions into two distinct subgroups.

In principal components analysis, the first four components explained 81.69% of the total variation. Also, principal components analysis confirmed the results of cluster analysis and genotypes again located in two groups (Figure 3). This fact shows the accuracy of grouping by cluster analysis.

Plant growth parameter

The callus and seedlings of *Lathyrus sativus* which have been exposed to different concentrations of cadmium, displayed a reduction in growth rate. A continuous reduction in fresh weight and dry weight was observed in callus grown on ascending concentrations of cadmium (Table 7).

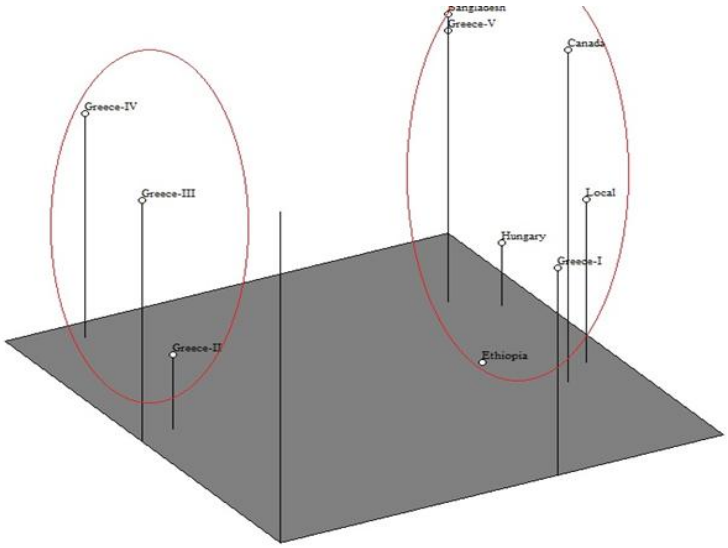


Figure 3. The PCA scatterplot of grass pea genotypes subjected to cadmium stress *in vitro.*

At 50 µM cadmium, seedlings and callus showed 72.932% and 68.129% reduction in tolerance, respectively (Table 9). As cadmium levels increased, traits root length, shoot length, seedling length, number of root and number of shoots decreased Piwowarczyk et al. (2017) reported that grass pea dry matter increased under osmotic stress and the highest dose of PEG caused a reduction in seedling growth in cultivars *in vitro* culture. Shekhawat et al. (2010) studied cadmium tolerance *of in vitro* grown callus and seedlings of *Brassica juncea* and concluded that that callus and seedlings showed 73.61% and 74.76% reduction in tolerance, in terms of fresh weight and dry weight, respectively. (Wajda et al., 1989)studied the toxicity of cadmium in callus cultures of *Catharanthus roseus* and *Haplopappus gracilis* . Concentrations of cadmium causing a 50% decrease in growth rate were experimentally determined.

Conclusion

In vitro culture of seedlings showed a grass pea genotype from Ethiopia as a tolerant genotype to cadmium. However, the results of callus culture were not consistent with the *in vitro* culture of seedlings. In other words, genotypes Hungary, Greece-I, Greece-III, and Greece-V were tolerant genotypes under callus induction medium. Initially, we observed that genotypes responded to metal toxicity, usually by growth inhibition, particularly in terms of biomass reduction. In this study, *L. sativus* callus and seedlings were treated with different concentrations of cadmium and showed a moderate decrease in growth at a lower concentration. Genetic manipulation and selection of favorable somaclonal variants from callus or seedling culture are supplementary tools for plant breeding.

Supplementary materials

No supplementary material is available for this article.

Author contributions

Conceptualization, Z.S., A.P.; methodology, A.A.; software, Z.S.; validation, Z.S., A.P. and A.A.; formal analysis, Z.S.; investigation, Z.S.; resources, F.B.; data curation, A.A.; writing—original draft preparation, A.P.; writing—review and editing, A.P.; visualization, F.B.; supervision, A.P.; project administration, A.A. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflict of interest

The authors declare no conflict of interest.

References

Arslan, M., Yol, E., and Türk, M. (2022). Disentangling the genetic diversity of grass pea germplasm grown under lowland and highland conditions. *Agronomy* 12(10): 2426.

Baker, A., and Proctor, J. (1990). The influence of cadmium, copper, lead, and zinc on the distribution and evolution of metallophytes in the British Isles. *Plant Syst. Evol.* 173: 91-108.

Arslan, M., Yol, E., and Türk, M. (2022). Disentangling the genetic diversity of grass pea germplasm grown under lowland and highland conditions. *Agronomy* 12(10): 2426.

Arzani, A. (2006). Karyotype study in some *Lathyrus* L. accessions of Iran. *Iran. J. Sci. Technol. Trans. A: Sci.* 30(A1): 9–17.

Arzani, A. (2008). Improving salinity tolerance in crop plants: a biotechnological view. *In Vitro Cell. Dev. Biol. Plant* 44(5): 373–383.

Arzani, A., and Mirodjagh, S.-S. (1999). Response of durum wheat cultivars to immature embryo culture, callus induction and in vitro salt stress. *Plant Cell Tissue Organ. Cult.* 58(1): 67–72.

Baker, A., and Proctor, J. (1990). The influence of cadmium, copper, lead, and zinc on the distribution and evolution of metallophytes in the British Isles. *Plant Syst. Evol.* 173: 91–108.

Barpete, S., Gupta, P., Sen Gupta, D., Kumar, J., Bhowmik, A., and Kumar, S. (2022). Neurotoxin (N-Oxalyl-L-α, β-Diamino Propionic Acid) Content in Different Plant Parts of Grass Pea (*Lathyrus sativus* L.) Spanning Seedling to Maturity Stage: Does It Increase over Time? *Molecules* 27(12): 3683.

Bidinger, F., Mahalakshmi, V., and Rao, G.D.P. (1987). Assessment of drought resistance in pearl millet [*Pennisetum americanum* (L.) Leeke]. I. Factors affecting yields under stress. *Aust. J. Agric. Res.* 38(1): 37–48.

Boulfekhar, R., Ohlund, L., Kumaresan, K.M., Megoura, M., Warkentin, T.D., Ispas-Szabo, P., Sleno, L., and Mateescu, M.A. (2023). Diamine oxidase as a therapeutic enzyme: study of germination from vegetal sources and investigation of the presence of β-N-oxalyl-L-α, β-diaminopropionic acid (β-ODAP) using LC-MS/MS. *Int. J. Mol. Sci.* 24(5): 4625.

Cadore, L.d.S., Bisognin, D.A., Mendes, F.B., Spanevello, J.d.F., Librelotto, J.R., and Tonetto, T.d.S. (2023). In vitro sensitivity of potato plants to cadmium exposure. *Ciencia Rural* 54(2): e20210823.

Campbell, C.G. (1997). *Grass pea, Lathyrus sativus L.* Bioversity international.

Di Toppi, L.S., and Gabbrielli, R. (1999). Response to cadmium in higher plants. *Environ. Exp. Bot.* 41(2): 105–130.

Didwania, N., Jain, S., and Sadana, D. (2019). In-vitro phytotoxic effects of cadmium on morphological parameters of *Allium cepa*. *Biological* 12(1): 137.

Dogrusoz, M.c., Mut, H., Basaran, U., and Gulumser, E. (2024). Enhancing drought resistance in grass pea: The positive regulatory role of plant-smoke solution. *S. Afr. J. Bot.* 166: 483–491.

Fernandez, G.C. (1993). "Effective selection criteria for assessing plant stress tolerance", in: *Adaptation of food crops to temperature and water stress: proceedings of an international symposium, Taiwan, 13-18 August 1992.*).

Ferreira, R.R., Fornazier, R.F., Vitória, A.P., Lea, P.J., and Azevedo, R.A. (2002). Changes in antioxidant enzyme activities in soybean under cadmium stress. *J. Plant Nutr.* 25(2): 327–342.

Fischer, R., and Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust. J. Agric. Res.* 29(5): 897–912.

Fornazier, R.F., Ferreira, R.R., Vitoria, A., Molina, S., Lea, P., and Azevedo, R. (2002). Effects of cadmium on antioxidant enzyme activities in sugar cane. *Biol. Plant.* 45: 91–97.

Foy, C., Chaney, R.t., and White, M. (1978). The physiology of metal toxicity in plants. *Annu. Rev. Plant Physiol.* 29(1): 511–566.

Goncalves, L., Rubiales, D., Lourenco, M., and Patto, M.C.V. (2024). Exploring grass pea (*Lathyrus sativus* L.) genetic diversity in Mediterranean changing climate conditions. *Eur. J. Agron.* 156: 127142.

Gulec sen, k., basaran, u., copur dogrusoz, M., Gulumser, E., and Mut, H. (2023). Growth and Biochemical Responses of Grass Pea (*Lathyrus sativus* L.) Genotypes Under Salt (NaCl) Stress Generated by Irrigation Water, and Changes in Soil pH and EC. *Gesunde Pflanz.* 75(3): 667–675.

John, R., Ahmad, P., Gadgil, K., and Sharma, S. (2009). Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L. *International Journal of Plant Production,* (3): 1735–8043.

Kumar, S., Gupta, P., Barpete, S., Sarker, A., Amri, A., Mathur, P., and Baum, M. (2013). Grass pea. *Genetic and genomic resources of grain legume improvement*: 269–292.

Lambein, F., Travella, S., Kuo, Y.-H., Van Montagu, M., and Heijde, M. (2019). Grass pea (*Lathyrus sativus* L.): orphan crop, nutraceutical or just plain food? *Planta* 250: 821–838.

Lasat, M.M. (2002). Phytoextraction of toxic metals: a review of biological mechanisms. *J. Environ. Qual.* 31(1): 109–120.

Mekonen, D.A., Abraham, A., Oselebe, H., Afiukwa, C., Ilesanmi, O., and Abebe, T.D. (2022). Genetic diversity and population structure analysis of Grass pea (*Lathyrus sativus* L.) accessions collected from North-Western Ethiopia using SSR markers. *Genet. Resour. Crop Evol.* 69(3): 1247–1260.

Mousavi, S., Yazdi, S.B., Naghavi, M., Zali, A., Dashti, H., and Pourshahbazi, A. (2008). Introduction of new indices to identify relative drought tolerance and resistance in wheat genotypes. *Desert* 12(2165-178).

Pinto, A., Mota, A.d., De Varennes, A., and Pinto, F. (2004). Influence of organic matter on the uptake of cadmium, zinc, copper and iron by sorghum plants. *Sci. Total Environ.* 326(1-3): 239–247.

Piwowarczyk, B., Tokarz, K., and Kamińska, I. (2016). Responses of grass pea seedlings to salinity stress in in vitro culture conditions. *Plant Cell Tissue Organ. Cult.* 124(2): 227–240.

Piwowarczyk, B., Tokarz, K., Makowski, W., and Łukasiewicz, A. (2017). Different acclimatization mechanisms of two grass pea cultivars to osmotic stress in in vitro culture. *Acta Physiol. Plant.* 39(4): 96.

Qian, H., Li, J., Pan, X., Sun, L., Lu, T., Ran, H., and Fu, Z. (2011). Combined effect of copper and cadmium on heavy metal ion bioaccumulation and antioxidant enzymes induction in *Chlorella vulgaris*. *Bull. Environ. Contam. Toxicol.* 87(5): 512–516.

Rosielle, A., and Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non-stress environment 1. *Crop Sci.* 21(6): 943–946.

Sanches, M., Sampaio, A.M., Araújo, S., van Eeuwijk, F., Van Breusegem, F., and Patto, M.C.V. (2024). Grass pea (*Lathyrus sativus*) interesting panoply of mechanisms to cope with contrasting water stress conditions–a controlled study of sub populational differences in a worldwide collection of accessions. *Agric. Water Manag.* 292: 108664.

Sarkar, A., Emmrich, P.M., Sarker, A., Zong, X., Martin, C., and Wang, T.L. (2019). *Grass Pea: remodeling an ancient insurance crop for climate resilience.*

Shekhawat, G.S., Verma, K., Jana, S., Singh, K., Teotia, P., and Prasad, A. (2010). In vitro biochemical evaluation of cadmium tolerance mechanism in callus and seedlings of *Brassica juncea*. *Protoplasma* 239(1): 31–38.

Singh, A.K., and Chand, S. (2003). Somatic embryogenesis and plant regeneration from cotyledon explants of a timber-yielding leguminous tree, *Dalbergia sissoo* Roxb. *J. Plant Physiol.* 160(4): 415–421.

Tokarz, B., Wójtowicz, T., Makowski, W., Jędrzejczyk, R.J., and Tokarz, K.M. (2020). What is the difference between the response of grass pea (*Lathyrus sativus* L.) to salinity and drought stress?—A physiological study. *Agronomy* 10(6): 833.

Tripathi, K., Gore, P.G., Ramya, K., and Sarker, A. (2022). "Grass pea an inherent abiotic stress-tolerant legume: current status and future scope under changing environment," in *Developing climate resilient grain and forage legumes*. Springer), 125–139.

Verma, K., Shekhawat, G., Sharma, A., Mehta, S., and Sharma, V. (2008). Cadmium induced oxidative stress and changes in soluble and ionically bound cell wall peroxidase activities in roots of seedling and 3–4 leaf stage plants of *Brassica juncea* (L.) czern. *Plant Cell Rep.* 27: 1261–1269.

Wajda, L., Kuternozinska, W., and Pilipowicz, M. (1989). Cadmium toxicity to plant callus culture in vitro—I. Modulation by zinc and dependence on plant species and callus line. *Environ. Exp. Bot.* 29(3): 301–305.

Zhang, H., Jiang, Y., He, Z., and Ma, M. (2005). Cadmium accumulation and oxidative burst in garlic (*Allium sativum*). *J. Plant Physiol.* 162(9): 977–984.

Disclaimer/Publisher's Note: The statements, opinions, and data found in all publications are the sole responsibility of the respective individual author(s) and contributor(s) and do not represent the views of JPMB and/or its editor(s). JPMB and/or its editor(s) disclaim any responsibility for any harm to individuals or property arising from the ideas, methods, instructions, or products referenced within the content.

JPMB
Journal of Plant Molecular Breeding
Published by Genetics and Agricultural Biotechnology Institute of Tabarestan
2025 | Volume 13 | Issue 1

مقاله پژوهشی

اثر تنش کادمیوم بر رشد درون شیشه‌ای ژنوتیپ‌های خلر (*Lathyrus sativus*)

زهرا سلامی[۱]، علیرضا پورمحمد[۱]، علی اصغر علیلو[۱]، فرهاد بهتاش[۲]

[۱] گروه مهندسی تولید و ژنتیک گیاهی، دانشکده کشاورزی، دانشگاه مراغه، مراغه، ایران
[۲] گروه مهندسی علوم باغبانی، دانشکده کشاورزی، دانشگاه مراغه، مراغه، ایران

چکیده: خلر (*Lathyrus sativus*) لگومی است که پتانسیل بالایی برای استفاده در غذای انسان و دام دارد. این مطالعه به منظور تعیین تحمل ژنوتیپ های خلر از نظر ماده خشک به تنش کادمیوم در مراحل کالوس و گیاهچه در شرایط درون شیشه‌ای انجام شد. برای این منظور، کالوس و گیاهچه‌های ده ژنوتیپ به طور جداگانه در معرض غلظت‌های مختلف کادمیوم (۰، ۱۲.۵، ۲۵، ۵۰ میکرومولار) قرار گرفتند. کالوس روی محیط کشت موراشیگ و اسکوگ حاوی۲،۴-D (۲ میلی‌گرم در لیتر) و گیاهچه با BAP (۱.۵ میلی‌گرم در لیتر) کشت شدند. نتایج نشان داد که کالوس و گیاهچه خلر قادر به زنده ماندن در غلظت ۵۰ میکرومولار کادمیوم بودند. با این حال، رشد بهینه کالوس و گیاهچه در غلظت ۲۵ میکرومولار کادمیوم مشاهده شد. از سوی دیگر، سم‌زدایی سلولی به کادمیوم در خلر با تجزیه و تحلیل رشد از نظر وزن تر و وزن خشک کالوس و گیاهچه مورد بررسی قرار گرفت. در غلظت ۲۵ میکرومولار کادمیوم، کالوس و گیاهچه به ترتیب ۵۰/۴۱ و ۳۵/۲۷ درصد کاهش تحمل داشتند. از این رو، کالوس در مقایسه با گیاهچه‌هایی که در معرض تنش کادمیوم قرار داشتند، کم-تحمل بود. بنابراین، نتیجه گیری می‌شود که گیاهچه‌ها تحمل بیشتری داشتند. مطالعه حاضر نشان داد که خلر نسبت به کادمیوم بسیار متحمل است که می‌تواند در مقادیر کم تا متوسط کادمیوم در مراحل گیاهچه و کالوس نیز زنده بماند.

کلمات کلیدی: تحمل به فلز سنگین، زیست توده،کالوس، گیاهچه.

ویراستار علمی
دکتر احمد ارزانی،
دانشگاه صنعتی اصفهان، ایران

تاریخ
دریافت: ۲۲ اردیبهشت ۱۴۰۴
پذیرش: ۲۱تیر ۱۴۰۴
چاپ: ۲۶ اردیبهشت ۱۴۰۵

نویسنده مسئول
دکتر علیرضا پورمحمد،
pourmohammad@maragheh.ac.ir
pourmohammad@ymail.com

ارجاع به این مقاله
Salami, Z., Pourmohammad, A.R., Aliloo, A.A. and Behtash, B. (2025). Effects of cadmium stress on *in vitro* growth of grass pea (*Lathyrus sativus* L.) genotypes. *J Plant Mol Breed. 13* (1): 94-105.
doi: 10.22058/jpmb.2025.2060346.1340.