

Effect of exogenous application of proline on antioxidant enzyme activities and lipoperoxidation content of faba bean (*Vicia faba* L.) Under cadmium stress

Efeito da aplicação exógena de prolina nas atividades de enzimas antioxidantes e no conteúdo de lipoperoxidação em fava (*Vicia faba* L.) sob estresse por cádmio

Efecto de la aplicación exógena de prolina en las actividades de enzimas antioxidantes y en el contenido de lipoperoxidación en haba (*Vicia faba* L.) bajo estrés por cadmio

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ABSTRACT

Field experiment we attempted to clarify the mechanism that might be involved in the ameliorative effects of proline on faba bean plants grown under different Cd levels. The effect of proline on MDA, glutathione contents, antioxidant enzyme activities and membrane stability. These were studied in plants grown in a nutrient solution supplemented with different concentrations of Cd (0 or 150 $\mu\text{mol/l}$) and sprayed with proline with different concentrations (0, 25 or 50 mg / l). Heavy metal stress caused an increase in malondialdehyde (MDA) content and the activity of certain enzymes, but glutathione were strongly depressed at relatively high Cd levels. Application of proline caused a significant increase in ascorbate peroxidase (APX) and catalase (CAT) activity in the plant compared with untreated plants. Antioxidant activities are enhanced, helping to reduce oxidative damage and develop tolerance to heavy metal stress in proline-treated bean plants.

This proline induced increase in tolerance to heavy metal stress. The results show that treatment with α -tocopherol reduced the adverse effects of Cd stress on bean plants and may play a key role in stress tolerance.

Keywords: antioxidant enzymes, cadmium, lipid peroxidation, proline and *Vicia faba*.

RESUMO

Em um experimento de campo, buscamos esclarecer o mecanismo envolvido nos efeitos benéficos da prolina em plantas de fava (*Vicia faba*) cultivadas sob diferentes níveis de cádmio (Cd). Foi estudado o efeito da prolina sobre o teor de malondialdeído (MDA), glutatona, as atividades de enzimas antioxidantes e a estabilidade da membrana em plantas cultivadas em solução nutritiva com diferentes concentrações de Cd (0 ou 150 $\mu\text{mol/l}$) e tratadas com pulverizações de prolina em diferentes concentrações (0, 25 ou 50 mg/l). O estresse por metais pesados causou um aumento no teor de MDA e na atividade de certas enzimas, enquanto os níveis de glutatona diminuíram significativamente em concentrações altas de Cd. A aplicação de prolina aumentou significativamente a atividade da ascorbato peroxidase (APX) e da catalase (CAT) em comparação com plantas não tratadas. As atividades antioxidantes melhoraram, ajudando a reduzir os danos oxidativos e a desenvolver tolerância ao estresse por metais pesados em plantas de fava tratadas com prolina. Este aumento de tolerância induzido pela prolina destaca sua importância frente ao estresse por Cd. Os resultados também indicam que o tratamento com α -tocoferol reduziu os efeitos adversos do estresse por Cd em plantas de fava, sugerindo que pode desempenhar um papel fundamental na tolerância ao estresse.

Palavras-chave: enzimas antioxidantes, cádmio, peroxidação lipídica, prolina, *Vicia faba*.

RESUMEN

En un experimento de campo, intentamos aclarar el mecanismo involucrado en los efectos beneficiosos de la prolina sobre las plantas de haba (*Vicia faba*) cultivadas bajo diferentes niveles de cadmio (Cd). Se estudió el efecto de la prolina sobre el contenido de malondialdehído (MDA), glutatión, las actividades de enzimas antioxidantes y la estabilidad de la membrana en plantas cultivadas en una solución nutritiva con diferentes concentraciones de Cd (0 o 150 $\mu\text{mol/l}$) y tratadas con pulverizaciones de prolina a diferentes concentraciones (0, 25 o 50 mg/l). El estrés por metales pesados provocó un aumento en el contenido de MDA y en la actividad de ciertas enzimas, mientras que los niveles de glutatión disminuyeron significativamente a concentraciones altas de Cd. La aplicación de prolina incrementó notablemente la actividad de la ascorbato peroxidasa (APX) y de la catalasa (CAT) en comparación con plantas no tratadas. Las actividades antioxidantes mejoraron, ayudando a reducir el daño oxidativo y a desarrollar tolerancia al estrés por metales pesados en las plantas de haba tratadas con prolina. Este aumento de tolerancia inducido por prolina muestra su importancia frente al estrés por Cd. Los resultados también indican que el tratamiento con α -tocoferol redujo los efectos adversos del estrés por Cd en las plantas de haba, lo que sugiere que podría desempeñar un papel clave en la tolerancia al estrés.

Palabras clave: enzimas antioxidantes, cadmio, peroxidación lipídica, prolina, *Vicia faba*.

1 INTRODUCTION

The heavy metals like cadmium (Cd) and other pollutants easily absorbed by soil and accumulated in different plant parts such as root, stem and leaf. Cd induces clastogenic and genotoxic disturbance in plants and inhibits root growth and cell division in different plants (Shikha and Singh, 2021 ; Fojtova and Kovarik, 2000 ; García et al.,2022). Available reports confirm that Cd induces oxidative burst via the generation of reactive oxygen species (ROS), such as H_2O_2 , $\text{O}_2\cdot$ -radicals and OH radicals as well as disturbance to antioxidative systems (Romero-Puertas et al., 2004). Agroecosystem contamination by cadmium (Cd), a persistent pollutant is a grave problem as it can remain in soils for a long time (Mohammed et al.,2024., Jiménez et al., 2021., Rashmi et

al.,2020). Cadmium being highly soluble and readily available is promptly taken up by plants and introduced into food chains becoming toxic for all organisms including plants, animals and even humans (Dar et al., 2022 ; Naikoo et al., 2021).

Bean seeds (*Vicia faba*) are also a rich source of mineral salts, phosphorus, magnesium and iron as well as group B vitamins. Cultivated green-podded string beans are distinguished by a higher vitamin content and therefore have a higher biological value than yellow podded varieties (Crépon et al., 2010). Faba bean is cultivated in sustainable cropping systems due to its ability of providing nitrogen by biological fixation and consequent reduction of chemical inputs consumption in plant production (Ghanbari et al., 2010; Stoltz and Nadeau, 2014).

Plants respond to drought stress at the physiological, cellular and molecular levels, but the extent of damage depends on the age and stage of plant growth. To fight against abiotic stress, plant has developed mechanisms to minimize the potential heavy metal damages. Within the cell is detoxified preferentially by binding to S-containing ligands such as metallothioneins (MTs), glutathione (GSH) and phytochelatins (PCs). Further the ligand Cd complexes are most likely removed by amino acid (Kuznetsov and Shevyakova, 1999; Matysik et al., 2002; Szabados and Savouré, 2009; Dong et al., 2022). Accumulation are not yet completely understood (Szabados and Savouré, 2009). Moreover, proline accumulation in plants could be only useful as a possible drought injury sensor instead of its role in stress tolerance mechanism (Jahari et al., 2010). In addition, Vendruscolo et al., (2007) reported that proline is involved in tolerance mechanisms against oxidative stress and this is the main strategy of plants to avoid detrimental effects of stress. Proline plays an important role as a sink for energy to regulate redox potentials (Gupta et al., 2016), and protects plants from free radical that induced damage by quenching of singlet oxygen (Matysik et al., 2002). Therefore, the present study was carried out to examine the effect of proline treat (0, 25 and 50 mg/l) on some physiological and biochemical attributes of *Vicia faba* bean plants under Cd treatment (150 µM/l).

2 METHODOLOGY

2.1 PLANT MATERIAL AND GROWTH CONDITIONS

Seeds of *Vicia faba*. L were germinated in the dark at 25°C for 7 days. After germination, plants were transferred to separated containers for hydroponics. Three-liter pots were used, containing ten plants each. Plants were grown in a controlled climate room at 24±2 °C and 50 % relative humidity, with a photoperiod of 16 h and a light intensity of 150µmol m⁻² s⁻¹. The hydroponic medium consisted of Hoagland nutrient solution (Hoagland and Arnon, 1959). The medium was continuously aerated (100 ml min⁻¹) and replaced every 3 days. At three-leave stage,

The 14-day-old *Vicia faba* seedlings were treated with two heavy metals (cadmium) added separately to the nutrient solution in the form of CdCl_2 , at a constant concentration of $150\mu\text{mol/l}$ combined with different concentrations of proline (0, 25 and 50 mg/l)). The seedlings were harvested after 14 days of treatment, and split into two parts : roots and stems.

2.2 ANALYTICAL METHODS

2.2.1 Lipidperoxidation measurement

The level of lipid peroxidation was measured by estimating malondialdehyde (MDA, a product of lipid peroxidation) depending on the method of Heath and Packer (1968). Leaf samples (0.5 g) were homogenized in 3mL 5% (w/v) trichloroacetic acid (TCA) and the homogenate was centrifuged at $10000\times g$ for 10min. Then, the supernatant (1mL) was mixed with 4mL thiobarbituric acid (TBA) reagent (0.5% of TBA in 20% TCA), heated in a water bath at 95°C for 30min and quickly cooled by transferring in ice bath. After that, MDA content was measured by observing the difference in absorbance at 532nm using an extinction coefficient of $155\text{mM}^{-1}\text{cm}^{-1}$ and expressed as nmol of MDA g^{-1}FW .

2.2.2 Determination of membrane stability index

Membrane stability indices (MSI) were estimated, in 9 samples for each treatment, using duplicate 0.2 g samples of fully-expanded leaf tissue (Rady, 2016). One sample of each duplicate was placed in test-tube containing 10 ml of double-distilled water and heated at 40°C in a water bath for 30 min. The electrical conductivity (C_1) of the solution was recorded using a conductivity bridge. The second sample was boiled at 100°C for 10 min, and the conductivity was also measured (C_2). The formula: $\text{MSI} (\%) = [1 - (C_1 \div C_2)] \times 100$ was applied for calculating the MSI.

2.2.3 Determination of glutathione

Glutathione is determined according to the method of Weck Berker and Cory (1988), which is based on measuring the absorbance of 2-nitro 5 mercapturic acid resulting from the reduction of 5-5 acid 'thiol-bis-2-nitrobenzoic (DTNB). Enzymes extraction and activities determination Leaves (0.5g, fresh weight) were homogenized in 50 Mm potassium phosphate buffer pH 7.6. Then, the homogenized samples were centrifuged at $12000\times g$ for 20 min and the supernatant crude extract was used for the estimation of both catalase (CAT) and ascorbate peroxidase (APX) activities (Loggini et al., 1999). CAT activity was determined as a decrease in absorbance at 240 nm for 3 min following decomposition of H_2O_2 (Cakmak and Horst, 1991). The reaction mixture 3 ml contained 50mM phosphate buffer pH 7.2, 15 mM H_2O_2 and 100 μl of crude enzyme extract.

Enzyme was quantified using the extinction coefficient 39400 M⁻¹cm⁻¹. Ascorbate peroxidase (APX) activity was determined following the decrease of ascorbate and measuring the change in absorbance at 290 nm for 3 min in 3 ml of a reaction mixture containing 50 Mm potassium phosphate buffer pH 7.2, 0.5 Mm ascorbic acid, H₂O₂ and 100 µl of crude enzyme extract (Nakano and Asada, 1981). Enzyme was quantified using the extinction coefficient 2800M⁻¹cm⁻¹.

2.2.4 Determination of free proline content

The free proline was estimated according to Bates et al., (1973). The color intensity was determined spectrophotometrically at spectrophotometer.

2.3 STATISTICAL ANALYSIS

Results were expressed as mean ± SD of three separate experiments. For each experiment, statistical analysis was performed using one-way ANOVA and the multiple comparison method of Tukey, with the aid of R 3.4.3 computer program.

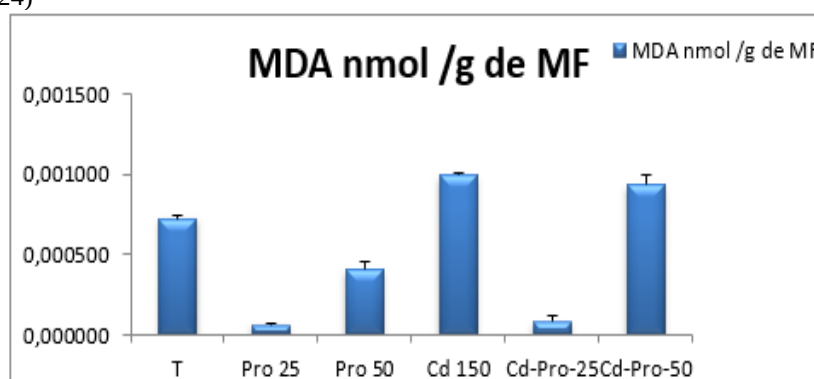
3 RESULTS AND DISCUSSIONS

3.1 RESULTS

3.1.1 Lipidperoxidation (MDA)

The results of MDA activity under the effect of cadmium, proline, and the Cd/Pro combination are shown in Figure 1.

Figure 1. Variation in MDA activity under the effect of cadmium, proline, and the Cd/Pro combination in *Vicia faba*. (Amri et al., 2024)

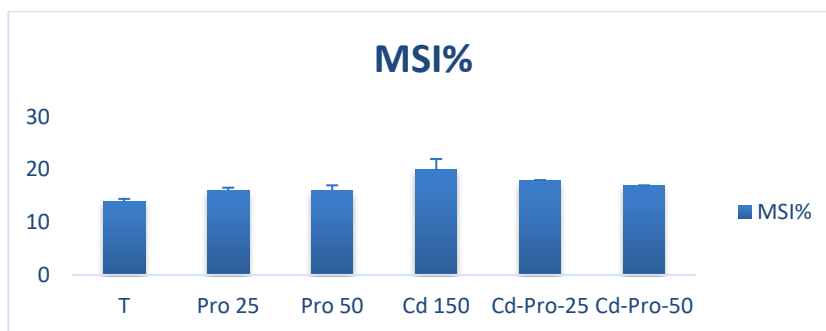


3.1.2 Membrane Stability Index (MSI)

According to the results presented in Figure 2. statistical analysis revealed a highly significant difference in the membrane stability index of leaves (p=0.004746).

An increase in stability was recorded in the Cd-stressed group without proline treatment and the group treated with proline. Groups exposed to stress showed that proline treatment improved stability compared to the control.

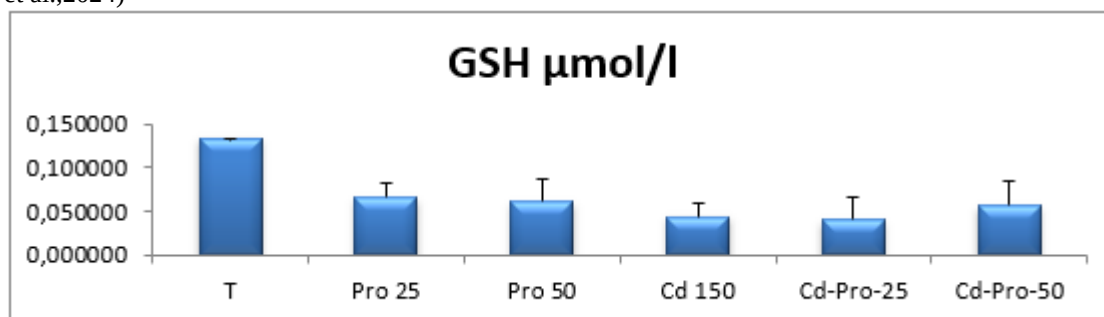
Figure 2. Effects of proline on the membrane stability index in *Vicia faba* leaves under or without metal stress (Cd). (Amri et al.,2024)



3.1.3 Glutathione

The results are summarized in Figure 3. showing variations in GSH levels under the influence of cadmium, proline, and the Cd/Pro combination.

Figure 3. Variation in GSH activity under the effect of cadmium, proline, and the Cd/Pro combination in *Vicia faba*. (Amri et al.,2024)



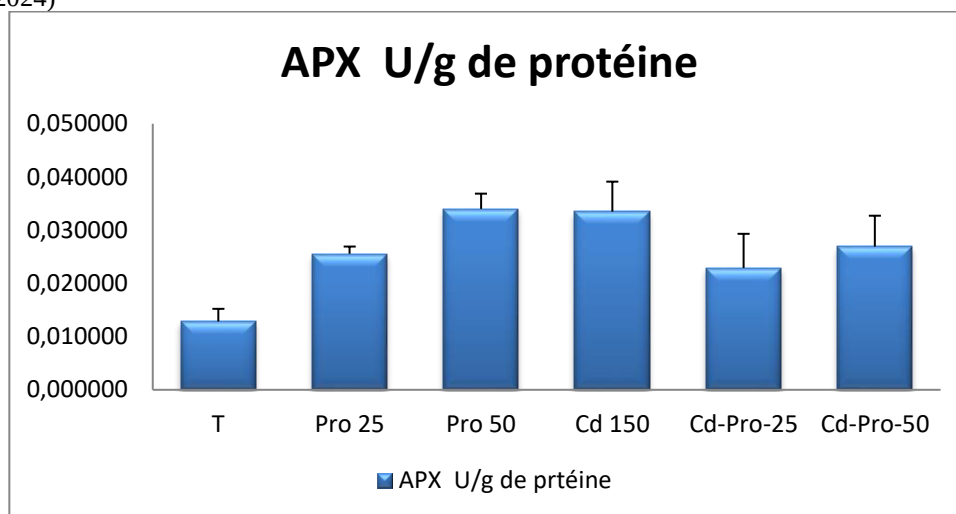
The analysis reveals a decrease in GSH content in plants treated with cadmium, proline, and the Cd/Pro combination compared to the control. Variance analysis revealed significant differences between seedlings treated with cadmium and proline compared to those treated with the Cd/Pro interaction.

3.1.4 Biomarkers

3.1.4.1 APX

Figure.4 shows that treatment with cadmium, proline, and the Cd/Pro combination tends to stimulate APX enzymatic activity (approximately 2-fold). This was confirmed by variance analysis, which revealed highly significant differences ($p = 0.009$).

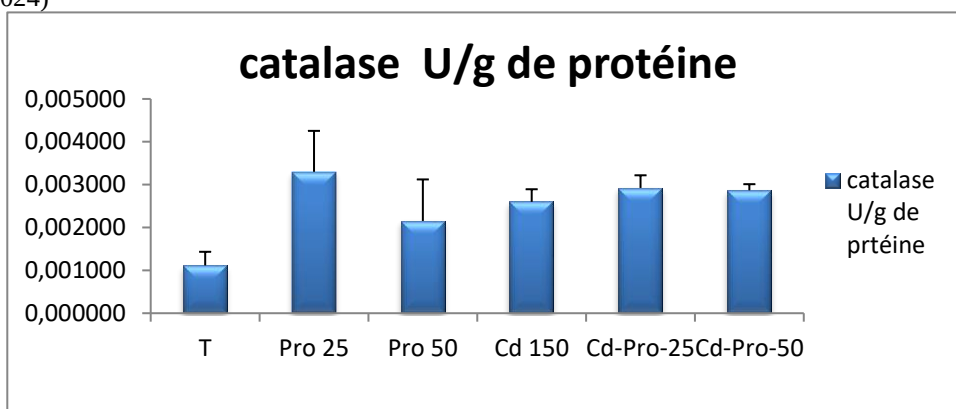
Figure 4. Variation in APX activity under the effect of cadmium, proline, and the Cd/Pro combination in *Vicia faba*. (Amri et al., 2024)



3.1.4.2 CAT

The results of CAT activity under the influence of cadmium, proline, and the Cd/Pro combination are presented in Figure.5.

Figure 5. Variation in CAT activity under the effect of cadmium, proline, and the Cd/Pro combination in *Vicia faba*. (Amri et al., 2024)

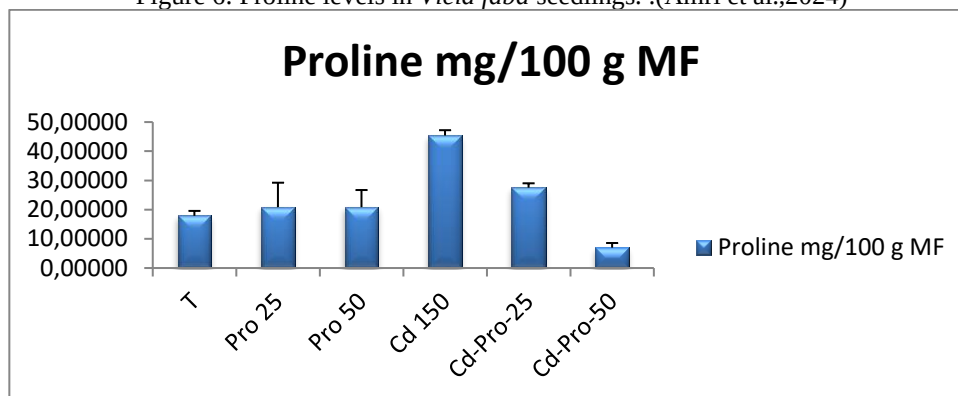


The analysis of catalase activity, as shown in Figure 5, reveals a slight increase compared to the control. Variance analysis detected significant differences in CAT activity between seedlings treated with cadmium and proline compared to those treated with the Cd/Pro interaction.

3.1.5 Proline

The effects of the three types of treatments are represented in Figure 6.

Figure 6. Proline levels in *Vicia faba* seedlings. (Amri et al.,2024)



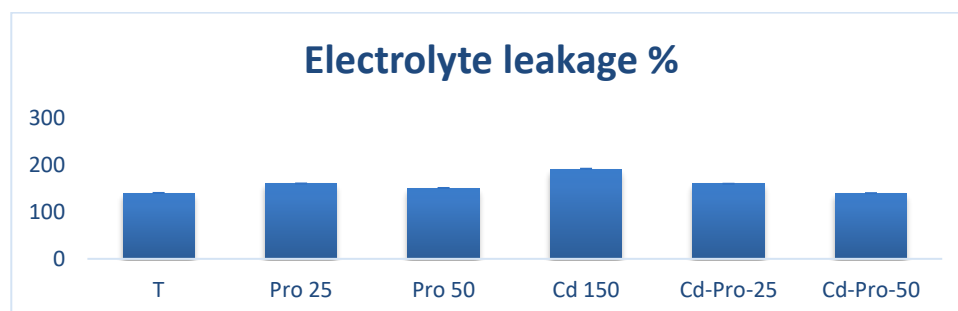
The results indicate an increase in proline content compared to the control. However, a decrease was observed in seedlings treated with the Cd/Pro-50 combination. Variance analysis revealed significant differences ($p \leq 0.05$) between proline levels in plants depending on the treatments applied.

3.1.6 Electrolyte leakage

Electrolyte leakage was measured using a conductivity meter from the leaves of *Vicia faba*.

The results (Figure 7) clearly show that metal stress induced by Cd applied for 15 days caused an increase in membrane electrolyte leakage in leaves compared to the control. The addition of proline to the nutrient solution reduced the electrolyte leakage from the leaves. However, groups treated with Cd and proline recorded higher increases compared to groups treated with proline alone. Statistical analysis revealed a highly significant difference between the groups ($p=0.0001852$).

Figure 7. Effects of proline on electrolyte leakage in *Vicia faba* leaves under or without metal stress (Cd). (Amri et al.,2024)



3.2 DUSCUSSIONS

It is well-established that heavy metal stress generates excessive reactive oxygen species (ROS), leading to cellular toxicity, membrane dysfunction, and cell death (Shahid et al., 2014). Plants develop enzymatic and non-enzymatic mechanisms to eliminate ROS (Hassanein et al., 2012).

Among ROS, superoxide is converted by SOD into H₂O₂, which is further scavenged by CAT and APX. Overexpression of the APX gene in plants has shown improved protection against oxidative stress (Yasar et al., 2008). In our study, metal stress activated antioxidant enzymes, such as CAT and APX, consistent with findings by Gill (2014). The increased activity of these antioxidants is considered a mechanism of tolerance to metal stress (Nadgórska-Socha et al., 2013; Gill, 2014).

The application of proline, alone or in combination, significantly reduces the inhibitory effects of metal stress by improving both photosynthetic pigments and enzymatic antioxidants (CAT and APX) (Al-Whaibi et al., 2012).

Our results show proline accumulation in *Vicia faba* plants, consistent with findings by John et al. (2008) and Abdelhaleim et al., (2022). These studies reported that proline accumulation could be a protective response, not only due to its osmoprotective role in preventing water deficit under heavy metal stress but also due to its radical-scavenging properties and protein stabilization (Ben Ahmed et al., 2010).

Furthermore, proline accumulation has been reported to serve as a nitrogen storage compound, thereby protecting cell structure (Ben Ahmed et al., 2010).

In our study, proline accumulation in response to Cd was higher in untreated plants compared to proline-treated plants. This might be due to the decreased activation of the enzyme responsible for proline degradation under stress, leading to increased proline levels (Al Khateeb and Al-Qwasemeh, 2014). Our results suggest that proline content decreases with proline application, highlighting its beneficial role in mitigating the harmful effects of metal stress. However, further studies are needed to elucidate the physiological and biochemical roles of proline in higher plants.

Electrolyte leakage (EL), lipid peroxidation (LP), and the membrane stability index (MSI) in *Vicia faba* plants showed varying responses when exposed to Cd and proline treatments. The application of Cd significantly increased EL and LP, represented by the elevated levels of malondialdehyde (MDA), compared to the control plants. The results showed a marked increase in MDA levels and ion leakage in response to 150 mM of Cd compared to the control.

However, treating stressed plants with proline significantly reduced MDA levels compared to the reference controls. In contrast, exposure to Cd alone caused an increase in MDA levels. Proline demonstrated significant effects in mitigating the damage in *Vicia faba* plants. Membrane damage can be indirectly assessed by measuring solute leakage (electrolyte leakage) and MSI (Ali et al., 2008).

The stimulation of the stress effect on MDA levels could be attributed to damage to the plasma membrane. Heavy metals are capable of inducing overproduction of ROS, such as hydrogen

peroxide, hydroxyl radicals, and superoxide anion, which in turn leads to lipid peroxidation and oxidative stress (Zhang et al., 2009). Oxidative damage caused by heavy metals was demonstrated by Vantová et al. (2013) and Zhang et al. (2022).

4 CONCLUSION

From the above mentioned results, it could be concluded that, Cd stress had inhibitory effects on the growth and some physiological and antioxidants parameters of *Vicia faba*. Plant treated with different concentration of proline could be contribute to a reduction of the injurious effect of heavy metal stress on growth and some related physiological and antioxidant responses of broad bean (*Vicia faba*) plants. The results of this experiment have clearly shown proline that could be used effectively to protect *Vicia faba* seedlings from the destructive effects of Cd stress. The effectiveness of proline in inducing Cd stress tolerance depends up on the concentration of proline applied. Finally to complete this research in the futur, several other tests including cytogenetic testing.

REFERENCES

- Abdelhaleim, M. S., Rahimi, M., & Okasha, S. A. (2022). Assessment of drought tolerance indices in faba bean genotypes under different irrigation regimes. *Open Life Sciences*, 17(1), 1462–1472. <https://doi.org/10.1515/biol-2022-0187>
- Al Khateeb, W., & Al-Qwasemeh, H. (2014). Cadmium, copper, and zinc toxicity effects on growth, proline content, and genetic stability of *Solanum nigrum* L., a crop wild relative for tomato; comparative study. *Physiology and Molecular Biology of Plants*, 20, 31–39. <https://doi.org/10.1007/s12298-014-0237-9>
- Ali, B., Hayat, S., Fariduddin, Q., & Ahmad, A. (2008). 24-Epibrassinolide protects against the stress generated by salinity and nickel in *Brassica juncea*. *Chemosphere*, 72(9), 1387–1392. <https://doi.org/10.1016/j.chemosphere.2008.04.026>
- Al-Whaibi, M. H., Siddiqui, M. H., & Basalah, M. O. (2012). Salicylic acid and calcium-induced protection of wheat against salinity. *Protoplasma*, 249, 769–778. <https://doi.org/10.1007/s00709-011-0313-2>
- Bates, L. S., et al. (1973). Rapid determination of free proline for water-stress studies. DOI: 10.1007/BF00018060.
- Ben Ahmed, C., Ben Rouina, B., Sensoy, S., Boukhriss, M., & Ben Abdullah, F. (2010). Exogenous proline effects on photosynthetic performance and antioxidant defense system of young olive tree. *Journal of Agricultural and Food Chemistry*, 58(7), 4216–4222. <https://doi.org/10.1021/jf902912h>
- Cakmak, I., & Horst, W. J. (1991). Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). DOI: 10.1111/j.1399-3054.1991.tb01893.x.
- Crépon, K., et al. (2010). Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. DOI: 10.1016/j.fcr.2009.12.021.
- Dar, O. I., et al. (2022). Source, bioaccumulation, degradability, and toxicity of triclosan in aquatic environments: A review. DOI: 10.1016/j.eti.2021.102122.
- Dong, Y., Kong, X., Luo, X., & Wang, H. (2022). Adsorptive removal of heavy metal anions from water by layered double hydroxide: A review. DOI: 10.1016/j.chemosphere.2022.134685.
- Fojtová, M., & Kovařík, A. (2000).** Genotoxic effect of cadmium is associated with apoptotic changes in tobacco cells. DOI : 10.1046/j.1365-3040.2000.00560.x.
- Ghanbari, A., et al. (2010). Effect of maize (*Zea mays* L.)-cowpea (*Vigna unguiculata* L.) intercropping on light distribution, soil temperature, and soil moisture in arid environment. DOI : Indisponible.
- Gill, C., Weisburd, D., Telep, C. W., Vitter, Z., & Bennett, T. (2014). Community-oriented policing to reduce crime, disorder, and fear and increase satisfaction and legitimacy among citizens: A systematic review. *Journal of Experimental Criminology*, 10, 399–428. <https://doi.org/10.1007/s11292-014-9206-9>

Hassanein, M., et al. (2012). The state of molecular biomarkers for the early detection of lung cancer. DOI: 10.1158/1940-6207.CAPR-12-0133.

Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: II. Role of electron transfer. *Archives of Biochemistry and Biophysics*, 125(3), 850–85. [https://doi.org/10.1016/0003-9861\(68\)90188-9](https://doi.org/10.1016/0003-9861(68)90188-9)

Hoagland, D. R., & Arnon, D. J. (1959). The water-culture method of growing plants without soil. *California Agricultural Experiment Station Circular*, 347, 26–29.

Jiménez, C. G. V., Manzano, N. P. B., Falcón, P. M. V., García, M. A. P., & Bernat-Rodríguez, J. (2021). Metales pesados en agua y sedimentos de dos lagunas costeras de Tabasco, México / Heavy metals in water and sediments of two coastal lagoons of Tabasco, Mexico. *Brazilian Journal of Animal and Environmental Research*, 4(4), 5993–6002. <https://doi.org/10.34188/bjaerv4n4-092>

Johari-Pireivatlou, M. (2010). Effect of soil water stress on yield and proline content of four wheat lines. DOI: Indisponible.

John, O. P., Naumann, L. P., & Soto, C. J. (2008). Paradigm shift to the integrative big five trait taxonomy. *Handbook of Personality: Theory and Research*, 3(2), 114–158. <https://doi.org/10.1002/9780470561119.ch7>

Kuznetsov, V. V., & Shevyakova, N. I. (1999). Proline under stress: Biological role, metabolism, and regulation. DOI: Indisponible.

Loggini, B., et al. (1999). Antioxidative defense system, pigment composition, and photosynthetic efficiency in two wheat cultivars subjected to drought. DOI: 10.1104/pp.119.3.1091.

Matysik, J., et al. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. DOI: Indisponible.

Mohammed, O. E., et al. (2024). The impacts of cadmium, an environmental pollutant, on honeybees (*Apis Mellifera*): A review. DOI : En attente de publication.

Nadgórska-Socha, A., Ptasiński, B., & Kita, A. (2013). Heavy metal bioaccumulation and antioxidative responses in *Cardaminopsis arenosa* and *Plantago lanceolata* leaves from metalliferous and non-metalliferous sites: A field study. *Ecotoxicology*, 22, 1422–1434. <https://doi.org/10.1007/s10646-018-0224-2>

Naikoo, G. A., et al. (2021). Bioinspired and green synthesis of nanoparticles from plant extracts with antiviral and antimicrobial properties: A critical review. DOI: 10.1016/j.jscs.2021.101304.

Nakano, Y., & Asada, K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. DOI: 10.1093/oxfordjournals.pcp.a076232.

Rady, M. M., et al. (2016). The effect of compost on growth and yield of *Phaseolus vulgaris* plants grown under saline soil. DOI: 10.1007/s40093-016-0138-9.

Rashmi, H. B., & Negi, P. S. (2020). Phenolic acids from vegetables: A review on processing stability and health benefits. DOI : 10.1016/j.foodres.2020.109298.

Romero-Puertas, M. C., et al. (2004). Cadmium-induced subcellular accumulation of $O_2^{\cdot-}$ and H_2O_2 in pea leaves. DOI : 10.1111/j.1365-3040.2004.01206.x.

Shahid, M., et al. (2014). Heavy-metal-induced reactive oxygen species: phytotoxicity and physicochemical changes in plants. DOI: 10.1007/978-1-4614-6435-8_232.

Shikha, D., & Singh, P. K. (2021). In situ phytoremediation of heavy metal-contaminated soil and groundwater: a green inventive approach. DOI : 10.1016/j.chemosphere.2021.130556 .

Stoltz, E., & Nadeau, E. (2014). Effects of intercropping on yield, weed incidence, forage quality, and soil residual N in organically grown forage maize (*Zea mays* L.) and faba bean (*Vicia faba* L.). DOI: 10.1016/j.fcr.2014.08.003.

Szabados, L., & Savouré, A. (2010). Proline: A multifunctional amino acid. DOI: 10.1016/j.tplants.2009.11.009.

Vantová, I., Bačkor, M., Klejdus, B., Bačkorová, M., & Kováčik, J. (2013). Copper uptake and copper-induced physiological changes in the epiphytic lichen *Evernia prunastri*. *Plant Growth Regulation*, 69, 1–9. <https://doi.org/10.1007/s10725-012-9792-3>

Vendruscolo, E. C. G., et al. (2007). Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. DOI: 10.1016/j.jplph.2006.10.004.

Weckbecker, G., & Cory, J. G. (1988). Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells in vitro. DOI: 10.1016/0304-3835(88)90099-5.

Yasar, F., et al. (2008). Effect of salt stress on antioxidant defense systems, lipid peroxidation, and chlorophyll content in green bean. DOI: 10.1134/S1021443708050132.

Zhang, X., Jiang, L., Geng, C., Cao, J., & Zhong, L. (2009). The role of oxidative stress in deoxynivalenol-induced DNA damage in HepG2 cells. *Toxicon*, 54(4), 513–518. <https://doi.org/10.1016/j.toxicon.2009.04.006>.

Zhang, Y. M., Lin, C. Y., Li, B. Z., Cheng, Y. X., Xu, W. B., Xiao, Y., ... & Shu, M. A. (2022). The health risk for consumers under heavy metal scenarios : Reduce bioaccumulation of Cd in estuary mud crab (*Scylla paramamosain*) through the antagonism of Se. *Science of The Total Environment*, 844, 157149. <https://doi.org/10.1016/j.scitotenv.2022.157149>.