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The role of arbuscular mycorrhizal fungi in enhancing chromium tolerance of common beans

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ABSTRACT

Chromium (Cr) is a heavy metal harmful to health and food crop development, impacting food security. Common bean (*Phaseolus vulgaris*), a crop of national importance, can form symbiotic relationships with arbuscular mycorrhizal fungi (AMF) and adapt to various environmental conditions. This study evaluated the influence of two AMF species on bean plant development in chromium-contaminated soils. The experiment involved growing plants in three-liter pots filled with soil and inoculating them with *Rhizophagus clarus* or *Claroideoglomus etunicatum*, with or without chromium. Conducted in a controlled growth chamber, the fourth vegetative stage measured gas exchange, electron transport rate, biochemical analysis, chromium content, mycorrhizal colonization rate, and plant growth. Results showed that plants inoculated with *R. clarus* had higher CO_2 assimilation rate (Pn) and stomatal conductance (Gs). Plants without chromium had higher superoxide dismutase activity, while those with *C. etunicatum* had lower catalase and peroxidase activity. Chromium increased malondialdehyde in *R. clarus*-inoculated plants, decreased peroxide in control plants, and reduced proline in *C. etunicatum*-inoculated plants. Final analysis indicated that *C. etunicatum*-inoculated plants had higher soil chromium content and lower pod chromium levels. This study highlights the potential of specific AMF species to improve bean development in chromium-contaminated soils and provides insights into plant-microorganism interactions and physiological responses to chromium tolerance.

Keywords: Heavy metal; *Rhizophagus clarus; Claroideoglomus etunicatum*; *Phaseolus vulgaris*; Mycorrhizal colonization.

1 INTRODUCTION

The contamination of food crops by heavy metals has led to the exploration of soil remediation techniques, focusing on the stabilization or mobilization of these elements (MARQUES; RANGEL; CASTRO, 2008; LOZANO-REÁTEGUI et al., 2023; ETSESAMI, 2018). Present in industrial effluents, heavy metals are highly toxic and pose significant health risks (Leandro-SILVA et al., 2020). Soils retain heavy metals, but when this capacity is exceeded, metals enter the food chain. Excessive heavy metals in the body can disrupt the antioxidant system (BARBOSA et al., 2019).

Arbuscular mycorrhizal fungi (AMF) form symbiotic and mutualistic associations with plant roots, enhancing nutrient and water absorption, promoting phytostabilization, tolerance to environmental stresses, and acting as bioremediation agents (FONSECA et al., 2020; NUNES et al., 2019; WANG; LIN; YIN, 2005; WU et al., 2016). Bioremediation uses microorganisms to detoxify pollutants in the soil, decomposing or transforming hazardous substances into less harmful forms, reducing their environmental impact (FARIA et al., 2017). *R. clarus* is an AMF species that produces large spores, the largest of the *Rhizophagus* genus (KOBAYASHI et al., 2018). It survives and reproduces in semi-arid ecosystems, even with low water availability, and is studied for the development of inoculants for forest seedlings (TOMAZELLI et al., 2022; OLIVEIRA et al., 2022). *Claroideoglomus etunicatum*, with a global distribution, protects plants against oxidative damage during drought by increasing antioxidants (NACOON et al., 2021). Both fungi stimulate the production of essential oils in plants exposed to heavy metals, increasing the concentration of main constituents (URCOVICHE et al., 2015; MERLIN et al., 2020).

Leather tanning generates significant environmental impact through the production of chromium-rich effluents. Inadequate disposal contaminates soil and water (GOMES et al., 2017; MAURYA et al., 2022; ŚWIETLIK; TROJANOWSKA, 2016). Presidente Prudente, SP, is the fifth largest leather producer in the state (SEADE, 2019). Due to intense leather treatment activities, there is growing concern about chromium contamination in agricultural soils used for food crops.

Chromium (Cr) is a heavy metal that can exist in various oxidation states, with Cr^{3+} and Cr^{6+} being the most common and used in leather tanning (NASCIMENTO, 1983). Cr^{3+} interacts complexly with organic matter in soil and aquatic environments, forming organic complexes, adsorbing onto organic surfaces, and incorporating into organic structures (SINHA; PAKSHIRAJAN; CHATURVEDI, 2018). Cr^{3+} is considered more stable and generally less harmful at low concentrations (GOMES; ROGERO; TIRAPEGUI, 2005; LAL SINGH, 2019). Under alkaline conditions, Cr^{3+} can oxidize to form Cr^{6+} (hexavalent chromium), which is highly soluble and toxic. Cr^{6+} is carcinogenic and a potent oxidizing agent, posing risks to human health and the environment, with its high solubility increasing water body contamination (CASTILHOS; TEDESCO; VIDOR, 2002; SANTOS; ALMEIDA, 2020). Studies show that Cr^{6+} affects chloroplast structure, altering size, shape, and internal structure (MAJHI; PANDA, 2010; RODRIGUEZ et al., 2012; QUADRO et al., 2019), chlorophyll content (TIWARI et al., 2009), chlorophyll a fluorescence, and gas exchange (LIU et al., 2008), as well as antioxidant enzymes (SAMANTARY, 2002).

Many plant species can accumulate heavy metals in their tissues. Agricultural plants, such as beans, are sensitive to physiological changes, especially in the early vegetative stages, and have adaptive mechanisms to respond to environmental stressors, including heavy metals (PORTUONDO FARIAS et al., 2020). The common bean (*Phaseolus vulgaris* L.), from the Fabaceae family (SILVA; DUARTE, 2020), has high nutritional demand and a short cycle (Almeida et al., 2022). The IAC Imperador cultivar is a high-yield variety in organic farming, with a determinate type I growth habit, early maturation, and a 75-day cycle (TEIXEIRA et al., 2017). Resistant to pathogens and highly productive, the common bean is crucial for food security due to its high protein content and adaptability (SILVA; FERREIRA; NASCENTE, 2021). Our study evaluated the bioremediation potential of the mycorrhizal fungi *Rhizophagus clarus* and *Claroideoglomus etunicatum* in the tolerance of *Phaseolus vulgaris* L. in chromium-contaminated soils.

2 MATERIALS AND METHODS

The experiment was carried out at the Center for Studies in Plant Ecophysiology of West Paulista – CEVOP (University of West Paulista – UNOESTE). A total of 30 pots were used. Half of the pots received 10 mg/kg^{-1} of chromium (Cr^{6+}) from the solution prepared with chromium salt ($K_2Cr_2O_7$) before planting. The soil in the planting pots underwent a stabilization period that lasted approximately 15 days. Cr^{6+} concentration was chosen according to the limits determined in an industrial scenario by CETESB (CETESB, 2017).

Phaseolus vulgaris L. seeds cultivar IAC Imperador was treated previously with a 10% hydrogen peroxide solution and then washed with deionized water. Sowing took place in May 2021, using 6 seeds in each planting pot. After 20 days of the emergence of all plants thinning was carried out, leaving 2 plants per pot. When they reached the fourth vegetative stage, one plant from each pot was used to evaluate the parameters related to photosynthesis and biochemicals and then removed thus, only one plant per pot completed the cycle. Water supplied amount to the plants was done manually so that the soil was always moist. Plants were kept in a Fitotron-type acclimatized chamber (Eletrolab, model EL 011) with a controlled temperature of 30° C during the day and 20° C at night. The planting pots contained 400 spores of mycorrhizal inoculant (about 200 g of inoculant) of *Rhizophagus clarus* (=*Glomus clarum*) (AMF 1) or *Claroideoglomus etunicatum* (=*Glomus etunicatum*) (AMF 2) and, 3 Kg of vegetable soil sterilized by high temperature. The spores were donated from UNIPAR's Glomales bank. Each pot received only 3 kg of sterilized topsoil for the control treatment. The experimental design was completely randomized, consisting of a 3 x 2 factorial scheme, with colonization by two AMF species and a control (without inoculum), combined with two Chromium treatments (0 and 10 $mg.kg^{-1}$ of Cr^{6+}) using 5 repetitions for each treatment.

Instantaneous measurements of gas exchange were performed by analyzing the CO_2 concentration by infrared (Li-6400XTR, LiCor, USA) with the modulated light fluorometer (LI-6400-40). CO_2 assimilation (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration (E) were quantified. Chlorophyll *a* fluorescence measurements were performed simultaneously with gas exchange measurements, an optical fluorescence meter was used. (FluorPen FP100, Photo Systems Instruments) according to Kotakis (2013) with modifications (ETR = DFFF *ΔF/Fm'*0,5* 0,84) according to Bilguer *et al.* (1995).

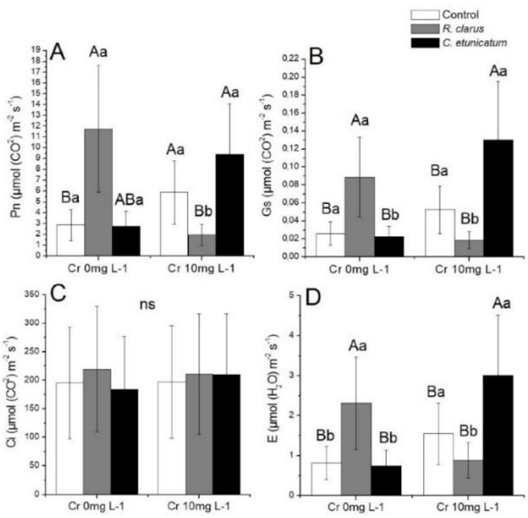
Protein content was determined according to Bradford's methodology (1976). Enzyme superoxide dismutase activity (SOD) was determined following the method of Giannopolitis and Ries (1977). Enzyme catalase activity (CAT) and enzyme peroxidase activity (POD) were estimated according to Peixoto et al. (1999). Lipid peroxidation was performed by quantification of malondialdehyde (MDA) concentration using the method of Li et al. (2010). Hydrogen peroxide (H2O2) content was determined using the method by Velikova et al. (2000). Proline content was extracted in sulfosalicylic acid, and its concentration was estimated according to the methodology described by Bates et al. (1973). Chromium content was determined in the roots, stems, leaves, and pods evaluated in the soil following the methodology of Davies *et al.* (2001). Chromium phytoextraction coefficients were obtained for roots, shoots, and grains, and were calculated according to Kumar et al. (1995).

Fresh roots were washed under running water and cut into segments to determine the rate of mycorrhizal colonization using the root segment observation method of Phillips and Hayman (1970). Colonized segments were evaluated with a stereoscopic microscope (40–100×) (GIOVANETTI; MOSSE 1980). The fresh and dry mass (g) of the aerial parts (leaves and stems), roots, and pods were weighed on a bench scale to obtain the average values. Other biometric measurements conducted included stem length, diameter, number of nodes, number of leaves, and number of pods.

3 RESULTS

The results indicated that the applied doses of Chromium did not cause significant responses in the intercellular concentration of CO_2 (Ci) (Figure 1–C). However, *R. clarus* inoculum showed a higher average for the rate of CO_2 assimilation (Pn) and stomatal conductance (Gs) (Figure 1–A and B) at dose 0 compared to the other treatments. In the chromium-enriched treatment, there was a decrease in both parameters, but in Pn, the control treatment showed a decrease.

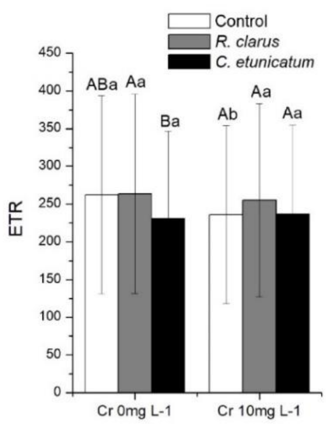
Figure 1 - Physiological effects caused in bean plants inoculated and subjected to chromium doses.



Different uppercase letters indicate statistical differences (P<0.05) among the doses; different lowercase letters indicate statistical differences (P<0.05) within the treatment. Source: The Author (2023)

The control treatment showed a decrease in the electron transport rate (ETR) of 10% between the dose of 0 Cr (mg L^{-1}) and the dose of 10 Cr (mg L^{-1}). Among the treatments with dose 0 Cr (mg L^{-1}), *C. etunicatum* had the lowest average, with a reduction of 11% compared to the control and 12.4% compared to *R. clarus* (Figure 2).

Figure 2 - Electron transport rate in bean plants inoculated and subjected to chromium doses.



Different uppercase letters indicate statistical differences (P<0.05) among the doses; different lowercase letters indicate statistical differences (P<0.05) within the treatment. Source: The Author (2023).

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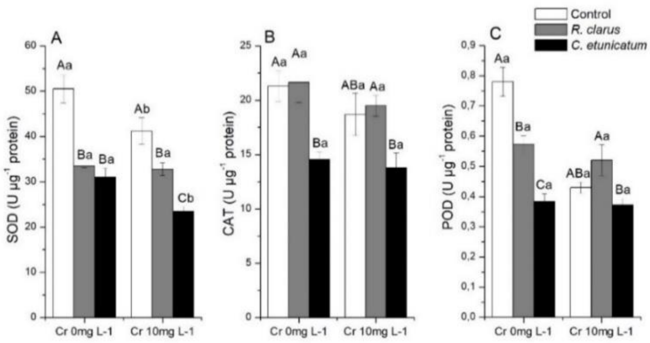
Regarding the analysis of antioxidant enzymes, the superoxide dismutase (SOD) enzyme, at the dose of 0 Cr (mg L^{-1}), *R. clarus* showed a decrease of 33.8%, and *C. etunicatum* showed a decrease of 38.6% compared to the control treatment (Figure 3 – A). Comparing the doses 10 Cr (mg L^{-1}) and 0 Cr (mg L^{-1}) within each treatment, there was a decrease of 18.4% in the control treatment and 24.5% in *C. etunicatum*. The response of catalase (CAT) showed that *C. etunicatum* had the lowest averages compared to the control treatment, with a decrease of 31.7% at the dose of 0 Cr (mg L^{-1}) and 26.23% at the dose of 10 Cr (mg L^{-1}) (Figure 3 – B). The peroxidase enzyme (POD) activity showed that among the treatments with the dose of 0 Cr (mg L^{-1}), the one inoculated with *C. etunicatum* presented a decrease of 50.7% compared to the control. At the dose of 10 Cr (mg L-1), the decrease was 13.3% (Figure 3 – C).

Figure 3 - Activity of antioxidant enzymes in bean plants inoculated and subjected to chromium doses.



Different uppercase letters indicate statistical differences (P<0.05) among the doses; different lowercase letters indicate statistical differences (P<0.05) within the treatment. Source: The Author (2023).

In the control treatment, we quantified the levels of malondialdehyde (MDA) across different doses, and our findings revealed no significant differences among them. When comparing the levels of MDA among different doses of Chromium in plants that were inoculated with *R. clarus*, we observed a significant 49% increase in MDA levels in the plants treated with a dose of 10 (mg L^{-1}) compared to the control dose (Figure 4-A). In bean plants inoculated with *C. etunicatum*, we observed the lowest mean malondialdehyde (MDA) content among the treatments at a dose of 10 (mg L^{-1}), showing a significant decrease of 34.2% compared to the control.

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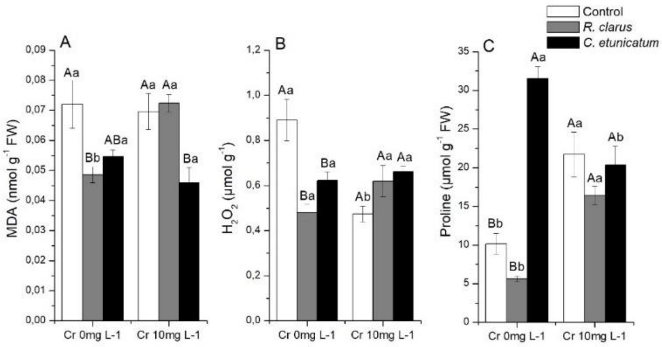
Figure 4 - Malondialdehyde, hydrogen peroxide, and proline content in bean plants inoculated and subjected to chromium doses.

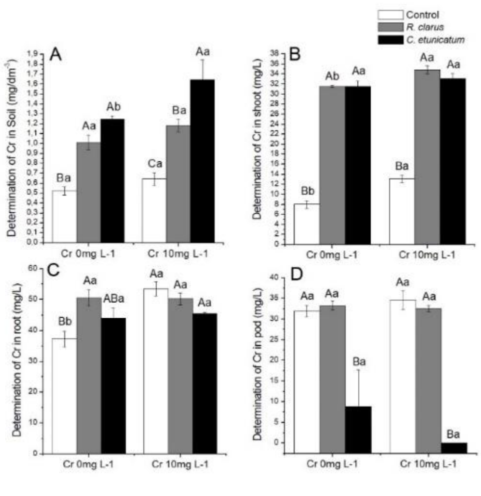


Different uppercase letters indicate statistical differences (P<0.05) among the doses; different lowercase letters indicate statistical differences (P<0.05) within the treatment. Source: The Author (2023).

Significant differences were observed in the hydrogen peroxide content between the doses of 0 and 10 (mg L^{-1}) (Figure 4 - B). When analyzing the treatments that did not receive Cr, those inoculated with *R. clarus* exhibited the lowest average of these levels, displaying a notable reduction of 46% compared to the control.

Cr application at a dose of 10 (mg L^{-1}) led to an increase in the proline content in both the control plants and those inoculated with *R. clarus* (Figure 4 - C), We observed a decrease in proline content specifically in the plants inoculated with *C. etunicatum* at the dose of 10 (mg L^{-1}). This reduction was not observed in the other treatments (Figure 4 - C). When comparing all the treatments, it is evident that the plants inoculated with *C. etunicatum*, without chromium application, exhibited a significant increase in proline content.

Figure 5 – Determination of Chromium found in the soil and the plant after completion of the entire growth cycle of common bean plants, with or without inoculation with AMF (Arbuscular Mycorrhizal fungi).



Mean values of chromium content in the soil (mg dm^{-3}) (A), above-ground parts (B), roots (C), and pods (D) of *Phaseolus vulgaris L*. plants treated with 10mg Cr L^{-1} H2O, determined by atomic absorption. Different uppercase

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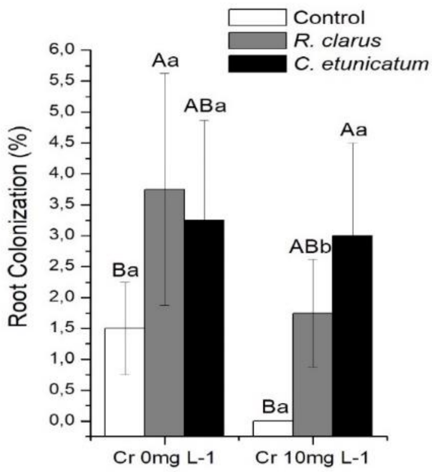
letters indicate statistical differences (P<0.05) among the doses, while different lowercase letters indicate statistical differences (P<0.05) within the treatment. Source: The Author (2023).

Chromium determination in the soil revealed that among the treatments containing chromium, the one inoculated with *C. etunicatum* exhibited the highest concentration of the metal in the soil, showing a significant increase of 156% compared to the non-inoculated treatment (Figure 5 - A). The chromium content in the above-ground part of the plant indicates that *C. etunicatum* did not exhibit statistical differences among the doses of chromium. However, in the non-inoculated treatment, there was a lower amount of chromium in the above-ground part, while in the roots, there was an increase of 43% in chromium content (Figure 5 - B, C). Chromium determination in plants inoculated with *C. etunicatum*, compared to the control treatment, revealed a decrease of 72.3% in the dose of 0 (mg L^{-1}) and a decrease of 100% in the dose of 10 (mg L^{-1}). When compared to plants inoculated with *R. clarus*, this reduction was 73.3% in the dose of 0 (mg L^{-1}) and 100% in the dose of 10 (mg L^{-1}) (Figure 5 - D).

Root mycorrhizal colonization percentage revealed that the *R. clarus*-inoculated treatment, when exposed to increasing levels of chromium, exhibited a significant decrease in mycorrhizal colonization of 53.3% (Figure 6).

Figure 6 – Percentage of root colonization by AMF in common bean plants subjected to different doses of Chromium.



Mean values of the percentage (%) of root colonization by AMF in Phaseolus vulgaris L. colonized with the arbuscular mycorrhizal fungi, *Rhizophagus clarus*, and *Claroideoglomus etunicatum*, and subjected to chromium treatment (10mg Cr L^{-1} H_2O) are presented. Different uppercase letters indicate statistical differences (P<0.05) among the doses, while different lowercase letters indicate statistical differences (P<0.05) within the treatment. Source: The Author (2023).

Comparing each dose, the non-inoculated treatments exhibited lower colonization, indicating possible external contamination that occurred during the experiment. However, it was observed that this colonization occurred in very low amounts, especially in the plants that received the heavy metal.

Regarding the effects on the dry and fresh weights of the above-ground part, no significant differences were observed (Table 1). Similarly, there were no differences found in the

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length and diameter (Table 2). Inoculated common bean plants with C. etunicatum showed a significant decrease of 46.6% in the dry weight of the pods at the dose of 10 (mg L^{-1}) compared to the dose of 0 (mg L^{-1}). Interestingly, there was a simultaneous increase of 23.6% in the number of pods (Table 2). On the other hand, common bean plants inoculated with R. clarus and exposed to chromium increment exhibited a significant reduction of 25.4% in the dry weight of the roots.

Table 1 - Chromium effects on the fresh weight and dry weight of inoculated common bean plants.

	Cr (mg L^{-1})	FWS	FWP	FWR	DWS	DWP	DWR
Control	0	44.4^{Aa}±7.2	36.0^{Ab}±6.4	42.4^{Aa}±19.6	6.3^{Ab}±0.9	1.3 ^{Aa}±0.2	5.3 ^{Aa} ±1.1
	10	54.4^{Aa}±7.9	48.7^{Aa}±10.5	23.4^{ABb}±3.3	8.0^{Aa}±1.5	1.0 ^{Aa}±0.2	5.2 ^{Aa} ±0.9
Rhizophagus clarus	0	48.5^{Aa}±2.9	47.3^{Aa}±8.3	35.8^{Aa} ±7.5	7.4 ^{Aa}±0.6	1.2^{Aa}±0.3	7.0 ^{Aa}±1.4
	10	43.6^{Aa}±7.6	36.5^{Ab}±5.3	35.6^{Aa} ±7.9	6.5^{Aa} ±0.9	0.9 ^{Aa}±0.1	5.2^{Ab}±1.2
Claroideoglomus etunicatum	0	41.9^{Aa}±7.2	42.6^{Aa}±3.5	29.7^{Aa} ±9.5	6.2^{Aa}±0.5	1.3^{Aa}±0.3	6.4 ^{Aa}±0.4
	10	45.7^{Aa}±5.4	47.4^{Aa}±2.9	18.4^{Ba} ±4.6	7.1^{Aa}±0.5	0.7^{Ab}±0.12	6.4 ^{Aa}±0.4

Mean ± standard deviation values of fresh weight of shoots (FWs), pod fresh weight (FWp), root fresh weight (FWr), shoot dry weight (DWs), pod dry weight (DWp), and root dry weight (DWr) of Phaseolus vulgaris L. plants submitted to chromium dose (10mg Cr L^{-1} H_2O). Different uppercase letters indicate statistical differences (P<0.05) among the doses, while different lowercase letters indicate statistical differences (P<0.05) within the treatment. Source: The Author (2023).

Table 2 – Chromium effects on the above-ground development of inoculated common bean plants.

	Cr (mg L^{-1})	LN	PN	NN	SL	D
Control	0	56.25^{Aa} ±3.5	20.75^{Aa}±2.9	4.75^{Ba} ±0.5	79.52^{Aa}±8.5	3.48^{Aa}±0.3
	10	60.75^{Aa}±6.3	23.5^{ABa}±1.7	5.25^{Aa} ±0.5	93.25^{Aa}±14.4	3.61^{Aa}±0.1
Rhizophagus clarus	0	52.25^{Aa} ±2.8	23.0^{Aa}±1.6	5.5^{ABa} ±0.5	88.5^{Aa}±0.9	3.65^{Aa}±0.02
	10	55.0^{ABa} ±1.2	21.5^{Ba}±5.0	5.25^{Aa}±0.9	78.75^{Aa}±16.7	3.86^{Aa}±0.3
Claroideoglomus etunicatum	0	57.75^{Aa}±0.5	22.25^{Ab}±1.5	6.0^{Aa} ±0.5	81.75^{Aa}±4.7	3.72^{Aa}±0.1
	10	51.75^{Bb}±4.7	27.5^{Aa}±4.1	5.5^{Aa}±0.5	82.75^{Aa}±13.4	3.81^{Aa}±0.4

Mean ± standard deviation values of leaf number (L_N), pods number (P_N), nodes number (N_N), shoot length (S_L - cm), and diameter (D- cm) of *Phaseolus vulgaris L*. plants submitted to chromium dose (10mg Cr L^{-1} H_2O). Different uppercase letters indicate statistical differences (P<0.05) among the doses, while different lowercase letters indicate statistical differences (P<0.05) within the treatment. Source: The Author (2023).

4 DISCUSSION

Microorganism-based technologies are an alternative for bioremediation. Despite the toxicity of Cr to many microorganisms, several resistant bacterial species have been identified (JAMIL et al., 2022). Cr has a toxic effect on plant growth (DIACONU et al., 2020). Our studies showed that strains of *C. etunicatum* have greater tolerance to Cr, mitigating its harmful effects.

Photosynthetic activity ensures the biological production of plants. Stressful conditions cause imbalances in protection systems and cellular metabolism, reducing photosynthesis and impacting overall productivity. This provides valuable information on plant adaptation and stability, helping to understand their environmental responses. Reduced growth often results from a decline in photosynthetic activity. Factors such as substrate composition and cultivation location are significant in photosynthetic limitations. Evaluating gas exchange parameters improves understanding of plants' adaptive capacity and their ability to thrive under various conditions (PEREIRA et al., 2019).

In this study, the vegetative stage during which gas exchange and electron transport rate were measured is crucial for plant growth. Adverse factors at this stage significantly impact bean production. Physiological processes such as gas exchange and electron transport directly influence plant growth and development. Disturbances at this stage affect yield and productivity. Understanding and monitoring these processes allow for the identification of growth-impeding factors and the implementation of measures to mitigate their effects, ensuring optimal bean production (KRON; SOUZA; RIBEIRO, 2008).

No differences were found in the values of intercellular CO2 concentration (Ci) and electron transport rate (ETR) among the treatments and concentrations used in this study. These parameters reflect the efficiency of photosynthesis and electron transport, which are crucial for energy production and carbon assimilation. The absence of differences suggests that the treatments and concentrations did not significantly impact these processes. However, it is essential to consider other physiological and biochemical parameters, in addition to overall plant performance and growth, for a comprehensive understanding of the effects of the treatments on plant physiology.

A study on cowpea under different fertilization showed that physiological variables such as instantaneous carboxylation efficiency, stomatal conductance, and water use efficiency did not differ significantly between the vegetative and pre-flowering periods. This suggests that the availability of light and nutrients was adequate for optimal photosynthetic performance. The plants maintained a consistent physiological state during these phases, with no apparent limitations in light and nutrients. Costa (2020) highlights the importance of considering these physiological parameters in understanding plant responses to different fertilization strategies and their impact on photosynthetic efficiency.

In this study, comparing two AMF species, *R. clarus* showed a reduction in photosynthesis and transpiration with increased chromium, while *C. etunicatum* exhibited higher averages under these conditions and increased stomatal conductance. These results suggest that *C. etunicatum* improves plant performance and stress tolerance in the presence of chromium. Further research is needed to understand the underlying mechanisms and explore the applications of *C. etunicatum* in chromium-contaminated environments. According to Maia (2019), in his research on the morphophysiological responses of sabiá (*Mimosa caesalpiniaefolia* Benth.) associated with AMFs and rhizobia in manganese mining soil, mycorrhizal treatments showed higher values of net photosynthesis, transpiration, and stomatal conductance. This highlights the role of fungi in promoting plant resilience and stress tolerance. The findings suggest that the symbiotic association between plants and AMFs can enhance physiological processes and help plants adapt to challenging environments.

Soil contamination by heavy metals is a significant environmental issue due to the extensive use of these elements in industry and agriculture. Heavy metals are released into the soil through industrial waste, mining, and agrochemicals, posing risks to ecosystems and human health by persisting in the environment and accumulating in the food chain (SALA et al., 2021). Like other abiotic stress factors, heavy metals can cause direct or indirect damage to plant cells through the high production of reactive oxygen species (ROS). These reactive species can lead to oxidative stress in cells, damaging lipids, proteins, and nucleic acids (MARQUES et al., 2019). The resulting oxidative stress can cause cellular dysfunction, DNA damage, lipid peroxidation, and compromised enzymatic activity. Heavy metals can also directly interfere with vital cellular processes, such as enzymatic inhibition and alteration of cell membrane permeability, compromising plant growth and metabolism (SOUZA, 2018). A study on cowpea under water restriction and foliar silicon application showed that enzymes like ascorbate peroxidase and superoxide dismutase are crucial for stress tolerance by eliminating ROS and promoting stress defense (SILVA et al., 2019).

The first line of plant defense involves anatomical and physiological adaptations in the photosynthetic apparatus. The second strategy includes the activation of antioxidant defenses, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), as well as non-enzymatic components (Foyer et al., 1994; Halliwell et al., 1989). Enzymes like SOD and CAT detoxify reactive oxygen species (ROS). Peroxidases (POD) use H_2O_2 as an oxidant and phenolic compounds as electron donors, eliminating H_2O_2 formed by SOD (Freitas et al., 2008; Barbosa et al., 2014). Our study showed that treatments with arbuscular mycorrhizal fungi reduced enzymatic activity, even under stress. A study with *Aspergillus* sp. in the presence of cadmium (Cd) also showed reduced enzymatic activity, intensified in the control concentration of the fungal strain (GUELFI et al., 2003). This supports the idea that fungi can alleviate stress, reducing the need for enzymatic activation, even in the presence of chromium.

It is worth noting that the soil in the treatment without the addition of Cr contained a small natural presence of Cr (Figure 5), helping to understand the biochemical responses of plants with or without fungal agents. Plants inoculated with R. clarus, without Cr stress, showed reduced activity of antioxidant enzymes such as SOD, CAT, and POD, resulting in lower MDA levels and decreased lipid peroxidation. The reduction in enzymatic activity is attributed to the neutralization of ROS by the fungal inoculant. The research indicates that the application of microorganisms for Cr bioremediation increases the antioxidant capacity of plants (VISHNUPRADEEP et al., 2022; JAMIL et al., 2022).

In the same condition, the proline content increased. Proline is one of the most studied amino acids due to its sensitivity to adverse conditions; it contributes to the elimination of ROS and the stabilization of subcellular structures (BHAGYAWANT et al., 2019). In addition to acting as an important osmoregulator and scavenger of ROS, proline also plays a role in reducing the toxicity of heavy metals by inducing the formation of phytochelatins that bind to heavy metals (HAYAT et al., 2012). In a study utilizing *Claroideoglomus etunicatum* inoculum in basil plants, a significant accumulation of proline was observed in the inoculated leaves under stressful conditions. This finding highlights the beneficial remedial role of proline as an osmolyte for plants, demonstrating its capacity to support plant resilience in various environments (BONACINA et al., 2020).

Plants inoculated with *C. etunicatum* showed a significant decrease in MDA content in chromium treatments, indicating lower levels of lipid peroxidation (Figure 4). This suggests that inoculation with *C. etunicatum* reduced the oxidative damage caused by chromium, as evidenced by the lower MDA levels. Additionally, there was a decrease in the activity of SOD, CAT, and POD enzymes in plants inoculated with *C. etunicatum* under chromium stress. This result can be attributed to the action of arbuscular mycorrhizal fungi in neutralizing reactive oxygen species (ROS), reducing membrane degradation and MDA levels. These effects can benefit the plants, indicating an active role of *C. etunicatum* in water stress tolerance. Islam et al. (2016) and Vishnupradeep et al. (2022) observed similar results in maize plants inoculated with bacteria exposed to Cr stress, reporting that the bacteria reduced metal toxicity and oxidative stress, decreasing the accumulation of antioxidant enzymes.

Heavy metal contamination has emerged as a significant global environmental risk in recent decades, raising new concerns about its remediation (SHARMA et al., 2022). Due to its global presence in the environment and food sources (NESHAT et al., 2022), chromium (Cr) poses an increased risk of various types of cancer and is recognized as a neurotoxic agent (WISE et al., 2022). Chromium has no essential function in plants and, therefore, no specific mechanism for chromium uptake exists in plants (OLIVEIRA, 2012; SHARMA et al., 2020). On the other hand, Cr uptake and translocation in plants are limited to the roots when evaluated in a compartmentalized manner (MANGABEIRA et al., 2011). The deposition of Cr in the aerial parts of plants depends on its chemical form. After undergoing chemical modifications, it can bind to the plant cell wall (SHARMA et al., 2020; CARY et al., 1977).

Determining chromium levels in the soil reveals that metals can be retained through various mechanisms. The chemical properties of the soil are crucial, as metals are adsorbed by electrostatic forces or form specific bonds. Organic compounds can also metabolize these metals, influencing their retention in the soil (MATOS et al., 1996). Evaluating the response of soil and plants to metallic chromium includes determining its levels in the aerial parts, grains, and soil of the plant, providing insights into the system's ability to handle chromium contamination. Soil analysis indicated the natural presence of chromium (Cr), evidenced by the small amounts found in pots that did not receive the metal-containing solution. Comparing the average values of all treatments, control plants showed lower Cr concentration in the soil, suggesting metal metabolism by the plants. In contrast, higher metal detection was found in inoculated pots, indicating a potential role of arbuscular mycorrhizal fungi in increasing Cr absorption or retention (Fig. 5 – B, C, and D).

Regarding Cr absorption, the leaves were the plant organs that accumulated the highest amount of the compound. Similar results were described by Andrade, Jorge, and Silveira (2005) in a bean species cultivated in cadmium-contaminated soils. The plants were inoculated with *Glomus etunicatum* (or *Claroideoglomus etunicatum), G. intraradices*, and *G. macrocarpum*, and mycorrhization increased the metal concentrations in the aerial parts, with *G. etunicatum* being the most promising species for the phytoremediation of bean species. On the other hand, concerning the chromium levels detected in the roots and pods (final commercial product) of plants from treatments inoculated with *C. etunicatum*, we observed a

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decrease in Cr absorption. The occurrence of soil contamination by heavy metals, including Cr, has been reported in maize seeds (*Zea mays* L.) by Onyejekwe et al., 2019.

A study that evaluated the productivity of wheat, lettuce, and radish crops in soil treated with tannery waste and hexavalent chromium found that the sludge altered the physicochemical properties of the soil. Cr^{6+} had a toxic effect and reduced the productivity percentage of the plants, especially corn, while its total levels in the soil were reduced (CASTILHOS; TEDESCO; VIDOR, 2002). Our findings are highly relevant, as pods and their seeds are final commercial products that can be consumed by humans or animals, and Cr has the capacity to be retained and accumulated in biological tissues. However, treatment with *C. etunicatum* can reduce its contamination.

R. clarus showed greater sensitivity to increased Cr, with higher colonization in the absence of stress. In contrast, *C. etunicatum* maintained its colonization in the presence of Cr, suggesting increased tolerance of beans to the metal. This was supported by a decrease in MDA content, indicating reduced oxidative damage, and the maintenance of photosynthetic activities and biomass. Additionally, *C. etunicatum* contributed to lower Cr accumulation in the pods and exhibited higher colonization under Cr contamination. These findings highlight the potential of *C. etunicatum* in mitigating the adverse effects of chromium contamination in beans.

5 CONCLUSION

In summary, the results of this study provide evidence that the inoculation of AMF, especially *C. etunicatum*, can be a promising strategy to improve the tolerance of bean plants to Cr contamination, reducing the negative effects on plant physiology and facilitating the remediation of soils contaminated with heavy metals. These findings contribute to the advancement of knowledge in the field of bioremediation and highlight the potential of plant-AMF interactions in mitigating the environmental impacts caused by heavy metal contamination.

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