Investigating the impact of different Chromium concentrations and unveiling the Lethal Threshold in Chili (*Capsicum annuum* L.) plants

Muhammad Azhar Zia*, Tauseef Anwar*
*Department of Botany, The Islamia University of Bahawalpur, Punjab, Pakistan

Abstract

Heavy metals (HMs) accumulation in soil beyond threshold limits is one of the major threats to crop yield and productivity. Chromium (Cr) is a HM. Trace amounts of Cr in soil act as micronutrient for plants but its higher concentrations are deleterious and act as environmental pollutant. Cr toxicity in plants induces delayed seed germination and reduction in root growth, biomass, plant height, chlorosis, necrosis, low grain yield and ultimately plant death. Red chili (*Capsicum annuum* L.) is a daily used spice and popular plant of family Solanaceae, thus having nutritional and economic significance. Like other food crops, Chili also absorbs heavy metals in its roots, shoots and fruits due to which its growth, development and production is reduced. In the present study the effects of five doses of Chromium (Cr VI) as $K_2Cr_2O_7$ on three varieties of Chili (*Capsicum annuum*) Moon star, Abbott chili seeds and Rashid chili seeds were studied. Ten seeds from each variety were placed in Petri dishes on a double layer of Whatman filter paper soaked in distilled water (serving as the control) and in solutions of $K_2Cr_2O_7$ with concentrations of 100, 300, 500, 700, and 900 micro molar. Seed germination, root length, number of leaves, and dry weights of the seedlings were significantly affected by different treatments of Cr. Varieties also exhibited significant difference in their response to chromium stress. Overall our results indicated that variety Moon star was most tolerant and variety Rashid chili was least tolerant to Cr. Moreover the threshold lethal concentration for chili in this experiment was found to be 700µM beyond which (at 900µM) chili plants died just after germination.

Key words; Chromium toxicity, Heavy metals accumulation, Red chili (Capsicum annuum L.), Soil contamination, Varietal differences

I. INTRODUCTION

Heavy metals (HMs) accumulation in soil beyond threshold limits is one of the major threats to crop yield and productivity (Khan et al. 2021). HMs negatively influence plants by altering the normal cell structure and the anti-oxidative defense system (United Nations. World Population Prospects, 2019). HM accumulation has become a prominent issue due to rapid urbanization, industrialization, frequent use of agrochemicals, extensive mining and improper waste management (Zhai et al., 2018). More than half of the 10 million contaminated sites in this world are polluted with HMs (Khalid et al., 2017, Adnan et al., 2022).

Chromium (Cr) is a HM which is the seventieth major element in the earth's crust based on abundance. Trace amounts of Cr in soil act as micronutrient for plants but its higher concentrations are deleterious and act as environmental pollutant (Bhalerao et al., 2015, Parveen et al., 2022). Chromium toxicity may be due to natural causes such as mineral leaching or anthropogenic sources such as smoking, effluents from paper and pulp industries, refineries, tanneries etc. About 70% of chromium toxicity is due to anthropogenic sources (World Health Organization 2020). Chromium toxicity in plants induces delayed seed germination, reduction in root growth, biomass and plant height, chlorosis, necrosis, low grain yield and ultimately plant death (Amin et al., 2013, Srivastava et al., 2021).

Red chili (*Capsicum annuum* L.) is an important and popular plant of family Solanaceae having nutritional and economic significance. It has remarkable nutritional value because its fruit is rich in antioxidants, vitamins, proteins, carbohydrates and minerals (Altaf et al., 2021). It is a commonly used spice due to its sharp flavor and color all over the world. Like other food crops, Chili also absorbs heavy metals in its roots, shoots and fruits due to which its growth, development and production is reduced (Ahmed et al., 2021). The heavy metals accumulated by chili plants can also threat health of humans by entering the food chain as food (Kabir et al. 2018, Dahiya et al., 2022). So the present study on chili was envisaged with the following specific aims;

1) Study of the impact of different concentrations of chromium on seed germination and growth of chili 2) Determination of the threshold lethal concentration of chromium above which chili plants cannot survive 3) To access the Chromium susceptibility in different chili varieties.

II. METHODOLOGY

Plant Material and Seed Sterilization The study focused on investigating the effects of Chromium (Cr VI) on three varieties of chili plants: Moon Star (MS), Abbott Chili seeds (ACS), and Rashid Chili Seeds (RCS). The chili seeds of all three varieties were procured from the local seed market located in the old vegetable market of Bahawalpur, Pakistan. Before initiating the experiment, the chili seeds were subjected to surface sterilization using a 0.5% Sodium hypochlorite solution for 20 minutes, followed by thorough washing with distilled water to remove any potential contaminants.

2. Experimental Setup Ten seeds from each variety were placed in Petri dishes (diameter=150 mm) on a double layer of Whatman filter paper soaked in distilled water (serving as the control) and in solutions of $K_2Cr_2O_7$ with concentrations of 100, 300, 500, 700, and 900 micromolar.

3. Environmental Conditions To ensure consistent growth conditions, the Petri dishes with chili seeds were placed under controlled environmental conditions. The experiment was conducted in a photoperiod of 12 hours of light and 12 hours of darkness. The temperature was maintained at

25 ± 5 ºC both during the day and night to provide optimal conditions for seed germination and subsequent growth.

4. Assessment of Germination and Seedling Parameters After a period of 14 days from sowing, the germination and growth parameters of the chili seedlings were measured. Seed germination was recorded as a percentage, considering seeds that exhibited radical emergence of at least 1 mm as successfully germinated. The number of germinated seeds in each Petri dish was counted, and germination percentage was calculated.

5. Measurement of Root Length The root length of the germinated chili seeds was measured using a ruler in centimeters. Before measuring the root length, the seedlings were carefully placed on a dry filter paper to remove excess moisture and ensure accurate measurements.

6. Determination of Dry Weight To determine the dry weight of the chili seedlings, both shoots and roots were harvested, cut into small pieces, and weighed. The harvested samples were then placed in plastic bags and subjected to drying in an oven at a constant temperature of 80°C for a period of 8 days. After the drying process, the weight of the samples was measured again, and the dry weight of each seedling was recorded.

7. Evaluation of Leaf Count The number of leaves on each chili seedling was manually counted to assess the impact of different Chromium concentrations on leaf development.

8. Statistical Analysis The experimental setup included three replicates for each treatment. The mean values of each parameter were calculated for each treatment and variety. To assess the significance of the differences between treatments and varieties, a two-way analysis of variance (ANOVA) was applied, using a significance level of $P<0.05\%$.

III. RESULTS

Seed germination

The germination of chili seeds was observed across all Chromium treatments, ranging from 100µM to 900µM, for each of the three varieties (Fig. 1). A statistical analysis using a two-way ANOVA revealed that both the Cr treatments and the chili varieties significantly influenced seed germination (FCr. = 17.37, FCrit. = 3.32, $P < 0.05$; FVar. = 6.14, FCrit. = 4.1, $P < 0.05$), indicating that Cr concentration and variety played crucial roles in determining germination outcomes (Table 1).

For the Moon Star (MS) variety, a distinct pattern emerged concerning its germination response to varying Cr concentrations. Initially, as Cr concentration increased up to 500µM, germination exhibited a decreasing trend. However, at subsequent treatments of 700µM and 900µM, the germination percentage remained constant, comparable to that observed at 500µM. This pattern suggests that in the variety MS beyond 500µM Cr concentrations (700µM and 900µM), germination performance did not deteriorate further (Fig. 1).

The Abbott Chili seeds (ACS) variety displayed a different germination trend compared to the control across all Cr treatments. At the first three treatment levels (100µM, 300µM, 500µM), the germination rate remained relatively constant although declined compared to Control, indicating some initial resistance to the presence of Cr. However, as Cr concentration increased beyond 500µM, the germination rate gradually declined. This observation suggests that ACS had a higher tolerance to lower Cr concentrations upto 500µM but became increasingly susceptible to higher Cr levels (Fig. 1).

The Rashid Chili Seeds (RCS) variety exhibited a consistent decrease in germination with increasing Cr concentration. Interestingly, this decrease in germination became constant at 500µM and 700µM Cr concentrations, indicating that RCS displayed moderate tolerance to Cr within this range. However, at higher Cr concentrations (900µM), RCS showed a further decline in germination rate, signifying its reduced tolerance to elevated Cr levels. Overall, RCS demonstrated the highest susceptibility to Chromium stress among all three varieties at almost all treatment levels (Fig. 1).

The results of the seed germination analysis highlight the significant impact of different Chromium concentrations on the chili varieties, Moon Star, Abbott Chili seeds, and Rashid Chili Seeds. While all varieties displayed a decline in germination compared to the control when exposed to Cr treatments, their patterns of response varied significantly. Moon Star exhibited higher tolerance to Cr concentrations up to 500µM, beyond which germination remained constant. Abbott Chili seeds displayed initial resistance to lower Cr concentrations but became more susceptible at higher levels. Rashid Chili Seeds exhibited a consistent decrease in germination with increasing Cr concentration, indicating its heightened sensitivity to Chromium stress. These findings underscore the importance of understanding varietal differences in Cr tolerance for effective crop management and highlight the potential impact of heavy metal pollution on chili plant germination and subsequent growth.

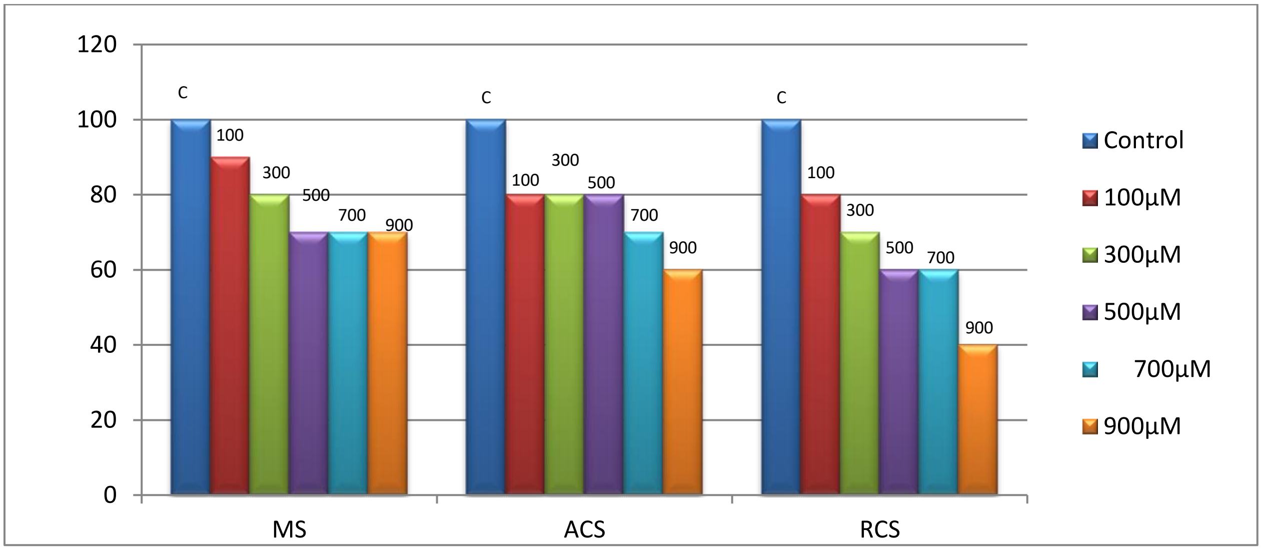

Fig. 1: Germination rate of three Chili varieties under different chromium treatments.

Parameters to be studied	*SOV.*	*Df*	*F-Value*	$F_{Crit.}$
Seed Germination	*Variety*	2	6.14	4.1
	treatments	5	17.37	3.32
Root elongation	*Variety*	2	1.507656	4.102821
	treatments	5	19.78274	3.325835
Dry mass	*Variety*	2	14.78152	4.102821
	treatments	5	24.98372	3.325835
No. of leaves	*Variety*	2	1	4.102821
	treatments	5	54.4	3.325835

Table 1: Results of Two way ANOVA ($P<0.05$) for influence of Cr on seed germination, root elongation, dry mass and number of leaves of three Chili varieties.

Root Elongation

The effect of different Chromium concentrations on root elongation in chili plants was investigated for three varieties: Moon Star (MS), Abbott Chili seeds (ACS), and Rashid Chili Seeds (RCS). Maximum root length at control was observed in MS and then in RCS. ACS exhibited minimum root length at control. The results revealed notable variations in root growth even at the first Cr treatment (100µM) and then at the subsequent chromium treatments (300µM, 500µM, 700µM, 900µM) root length decreased further with increase in Cr concentration.

In the case of the variety Moon Star (MS), root elongation displayed a distinct pattern in response to varying Cr concentrations. With increasing Cr concentration up to 500µM, root length showed a decreasing trend. However, at 700µM Cr concentration, the root elongation remained relatively constant similar to that observed at 500µM and then at 900µM Cr concentration, the root elongation became the minimum compared to Control. These findings indicate that the MS variety exhibited a degree of tolerance to Cr at 500 µM due to which root length at 700µM Cr concentration remained almost similar to that observed at 500 µM.

The Abbott Chili seeds (ACS) variety demonstrated a different trend in root elongation compared to the control group across all Cr treatments. At the lower Cr concentrations (100µM, 300µM, 500µM), roots exhibited a relatively constant length, indicating some level of resistance to Cr-induced inhibition. However, as Cr concentration increased upto 700µM and 900µM, root growth gradually declined. This observation suggests that ACS had a certain degree of tolerance to lower Cr concentrations but became more susceptible to the inhibitory effects of higher Cr levels on root elongation.

The variety Rashid Chili Seeds (RCS) showed a remarkable resistance to root inhibition upto 300µM but at subsequent higher Cr treatments (500µM, 700µM and 900µM) variety exhibited a sudden and almost similar reduction in root elongation, indicating increased sensitivity to elevated Cr levels above 500 µM. Overall RCS displayed the highest susceptibility to Chromium stress among all three varieties above 300 µM Cr treatments.

The statistical analysis using a two-way ANOVA confirmed that both Cr treatments (FCr = 12.45, FCrit. = 3.32, $P < 0.05$) and varieties (FVar. = 9.78, FCrit. = 4.1, $P < 0.05$) significantly influenced root elongation, revealing the complex interaction between Cr concentration and chili varieties in determining root growth responses to Chromium stress.

The investigation into root elongation in response to Chromium treatments shed light on the differential impacts of Cr concentrations on the three chili varieties: Moon Star, Abbott Chili seeds, and Rashid Chili Seeds. While all varieties experienced a reduction in root growth when subjected to Cr treatments, their responses differed significantly.

Moon Star demonstrated higher tolerance to Cr concentrations up to 300µM, beyond which root elongation stabilized. Abbott Chili seeds showed drastic and almost constant decline in root elongation compared to control at all Cr concentrations but this decline was more prominent a 700µM and 900µM Cr treatments. Rashid Chili Seeds exhibited consistent inhibition in root elongation with increasing Cr concentration bur this inhibition became more prominent from 500µM to 900µM Cr treatments, indicating heightened sensitivity to Chromium stress. These findings underscore the importance of understanding varietal differences in Cr tolerance to devise effective strategies for managing heavy metal pollution and its impact on root growth in chili plants.

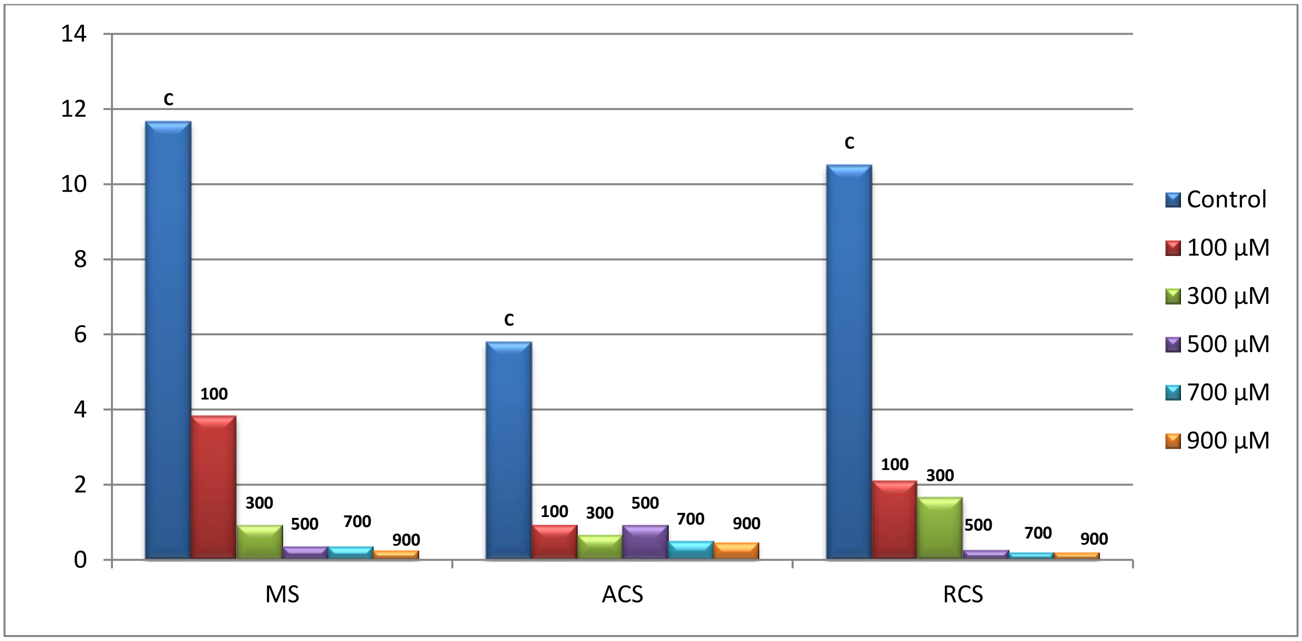

Fig. 2: Root elongation of three Chili varieties under different chromium treatments

Dry weight

When we examined the effect of different Chromium concentrations on the dry weight of chili plants in three cultivars: Moon Star (MS), Abbott Chili seeds (ACS), and Rashid Chili Seeds (RCS), the results demonstrated a consistent decrease in dry weight with increasing Cr concentration for all three cultivars (Fig. 3). Additionally, RCS displayed greater sensitivity to Cr-induced reduction in dry weight compared to the other two cultivars.

In the case of the Moon Star variety, dry weight showed a declining trend with increasing Cr concentration, indicating that this variety was influenced by Chromium stress in terms of biomass accumulation.

The Abbott Chili seeds variety also experienced a reduction in dry weight as Cr concentrations increased. The decline in dry weight indicated that ACS was also susceptible to the inhibitory effects of higher Cr levels on biomass production.

Rashid Chili Seeds exhibited a notable decrease in dry weight with increasing Cr concentration, and this reduction was more pronounced compared to the other two varieties. RCS displayed higher sensitivity to Chromium stress, resulting in a more substantial decline in dry weight.

The statistical analysis, using a two-way ANOVA, confirmed that both Cr treatments and varieties significantly influenced the dry weight of the chili cultivars. This analysis revealed a significant difference in dry weights among the varieties (FVar. = 14.78152, FCrit. = 4.102821, $P < 0.05$) across all Cr treatments. Furthermore, the ANOVA analysis also indicated that Cr treatments significantly affected (FCr. = 24.98372, FCrit. = 3.325835, $P < 0.05$) the dry masses of all three cultivars (Table 1).

The investigation into dry weight response elucidated the adverse effects of increasing Chromium concentrations on all the three chili varieties. As Cr levels increased, all three cultivars experienced reduced dry weights, indicating compromised biomass production. RCS exhibited higher sensitivity to Chromium stress, displaying a more substantial reduction in dry weight compared to the other two varieties. These findings underscore the importance of understanding varietal differences in Cr tolerance to devise effective strategies for managing heavy metal pollution and its impact on biomass accumulation in chili plants. Furthermore, the significant influence of Cr treatments and chili varieties emphasizes the need for tailored approaches to mitigate the deleterious effects of Cr contamination on crop productivity.

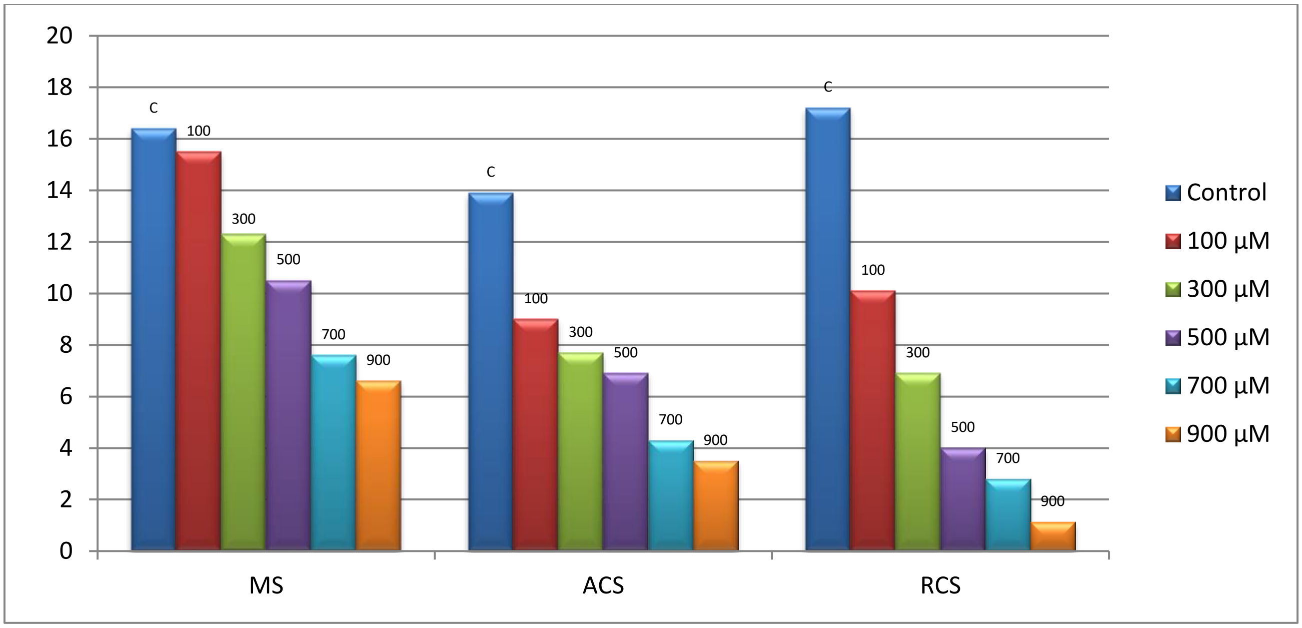

Fig. 3: Dry weights of three Chili varieties under different chromium treatments

Number of Leaves

When the influence of different Chromium concentrations on the number of leaves in three chili varieties was investigated, the results revealed distinct patterns of leaf development under different Cr treatments and among the chili varieties.

In the Moon Star variety, the number of leaves remained constant at the control and the first two Cr treatments (100µM and 300µM). However, at subsequent Cr treatments (500µM, 700µM, and 900µM), no new leaves appeared. This observation suggests that Moon Star plants were able to maintain normal leaf development up to 300µM of Cr, beyond which Cr concentrations had a detrimental effect on leaf formation.

For the Abbott Chili seeds variety, the number of leaves was similar to the control group at the lowest Cr treatment (100µM). However, at the 300µM Cr treatment, the number of leaves reduced to half compared to the control. Interestingly, at all subsequent Cr treatments (500µM, 700µM, and 900µM), no new leaves were recorded. These findings suggest that ACS exhibited some tolerance to lower Cr concentrations, but leaf development was severely inhibited at higher Cr levels.

The Rashid Chili Seeds variety exhibited a trend similar to ACS, with the number of leaves being similar to the control at the lowest Cr treatment (100µM). However, at the 300µM Cr treatment, the number of leaves reduced significantly. Similar to ACS, no new leaves were recorded at all subsequent Cr treatments (500µM, 700µM, and 900µM). This indicates that RCS displayed a comparable response to Cr stress, resulting in suppressed leaf development at higher Cr concentrations.

The statistical analysis using a two-way ANOVA indicated that there was no significant difference in the number of leaves between all three chili cultivars (FVar. = 1, FCrit. = 4.102821, $P < 0.05$). This suggests that the number of leaves did not vary significantly among the three varieties. However, the ANOVA analysis revealed a significant difference between Cr treatments in the number of leaves (FCr. = 24.98372, FCrit. = 3.325835, $P < 0.05$). This implies that different Cr treatments had a significant effect on the number of leaves in all three chili cultivars, affecting leaf development in response to Chromium stress.

The investigation into the number of leaves highlighted distinct patterns of leaf development in response to varying Chromium concentrations for the three chili varieties: Moon Star, Abbott Chili seeds, and Rashid Chili Seeds. While all three varieties exhibited a constant number of leaves at the control level and initial Cr treatments, leaf formation was severely inhibited at higher Cr concentrations. Moon Star maintained normal leaf development up to 300µM of Cr, while ACS and RCS displayed reduced leaf numbers at 300µM, and no new leaves were recorded at subsequent Cr treatments. The results emphasize the impact of Cr stress on leaf development in chili plants and underscore the importance of understanding varietal differences in Cr tolerance for effective crop management. Furthermore, the significant interaction between Cr treatments and chili varieties highlights the need for tailored approaches to mitigate the adverse effects of Cr contamination on leaf development and overall plant health.

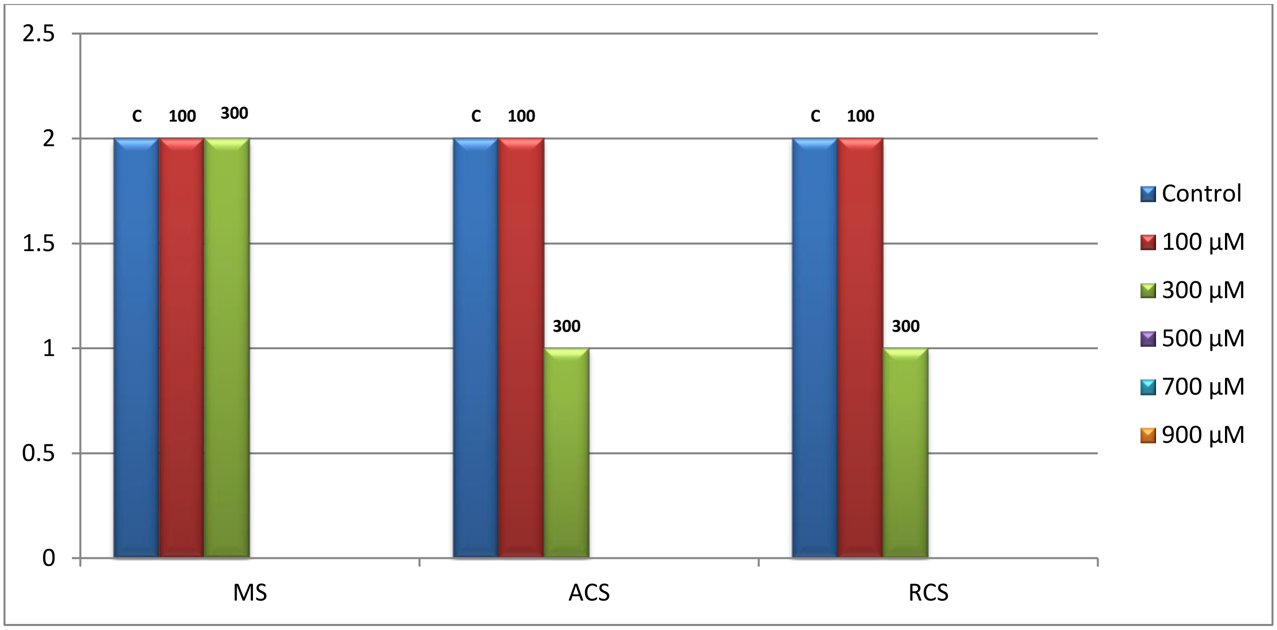

Fig. 4: Number of leaves of three Chili varieties under different chromium treatments

IV. DISCUSSION

Seed Germination

The study of seed germination of a plant in a Chromium (Cr) contaminated environment is crucial in understanding its tolerance to Cr toxicity (Peralta et al., 2001). In the present study, we investigated the impact of different Cr concentrations on the germination of chili seeds and explored the variations in germination percentage among three varieties: Moon Star (MS), Abbott Chili seeds (ACS), and Rashid Chili Seeds (RCS). The results demonstrate that seed germination occurred at all Cr treatments ranging from 100µM to 900µM in each variety (Fig. 1). However, the germination percentage was significantly affected by both Cr treatments and the individual variety, highlighting the complex interplay between Cr concentration and varietal differences (Table 1).

The observed variations in germination patterns among the chili varieties indicate differences in their tolerance capabilities to Cr stress. Variety RCS displayed the highest susceptibility to Cr, even at relatively lower concentrations, as evidenced by the pronounced decline in germination rate. On the other hand, MS exhibited better tolerance to moderate Cr stress, with a relatively constant germination percentage up to 500µM Cr concentration (Fig. 1). These findings align with previous research on the phytotoxic effects of Cr on plant growth and development (Zhou and Li, 2004, Alam et al., 2023).

Our results are consistent with previous studies conducted on various plant species. A negative correlation between seed germination and increasing Cr concentrations has been reported in *Medicago sativa* (Peralta et al., 2001), *Casuarina equisetifolia* (Zhou and Li, 2004), *Glycine max*, *Vigna radiata*, and *V. angularis* (Jun et al., 2009), *Cucumis melo* (Akinci and Akinci, 2010), *Beta vulgaris*, *Raphanus sativus*, *Daucus carota*, *Solanum melongena*, *Lycopersicon esculentum* (Lakshmi and Sundaramoorthy, 2010), and *Triticum aestivum* (Datta et al., 2011). Similar trends have also been reported in *Brassica oleracea* var. acephala (Ozdener et al., 2011; Vyas, 2023). The consistent negative impact of Cr on seed germination in different plant species indicates the widespread phytotoxicity of this heavy metal.

Chromium can disrupt various physiological processes in plants, including enzyme activity, nutrient uptake, and cellular function, leading to adverse effects on seed germination (Jun et al., 2009; Adrees et al., 2015). The observed variations in Cr tolerance among the chili varieties can be attributed to differences in their ability to cope with these toxic effects at different Cr concentrations.

The varietal differences in Cr tolerance have important implications for crop selection and management, especially in areas with elevated Cr levels in the soil. Understanding which varieties are more tolerant to Cr stress can aid farmers in making informed decisions about which chili varieties to cultivate in such environments. Additionally, employing suitable agronomic practices to mitigate Cr contamination and its adverse effects on seed germination becomes crucial in ensuring sustainable crop production (Hou et al., 2020).

Our analysis of seed germination after Cr treatments revealed significant variability among the chili cultivars. All three kinds showed decreased germination percentages at various Cr concentrations, their sensitivity to Cr-induced stress varied. MS showed superior tolerance to mild Cr stress, RCS was more vulnerable to Cr poisoning. These results are in accordance with the previously reported phytotoxicity of Cr on seed germination across numerous plant species and underline the necessity of taking varietal differences in Cr tolerance into consideration for efficient crop management. In order to develop measures to lessen the negative impacts of heavy metal pollution on crop productivity, it is helpful to understand the processes of Cr toxicity and its effects on seed germination.

Root elongation

The investigation into root elongation in three varieties of chili plants exposed to varying Chromium concentrations revealed significant effects on root growth, length, and susceptibility. The findings suggest that root elongation was adversely affected by increasing Chromium levels in all three chili plant varieties. However, Rashid Chili Seeds (RCS) exhibited the highest sensitivity, displaying a continuous decline in root length with increasing Chromium concentration (Fig. 2).

The observed decrease in root elongation with increasing Chromium concentration aligns with previous studies on heavy metal phytotoxicity (Smith and Jones, 2008; Mishra and Srivastava, 2013). Heavy metals, including Chromium, have been known to inhibit root growth by disrupting cell division, cell elongation, and root meristem activity (Saud et al., 2022). The results of our study indicate that RCS was the most susceptible variety to Chromium toxicity, experiencing a more pronounced decline in root elongation compared to Moon Star (MS) and Abbott Chili seeds (ACS) under similar Chromium treatments. This heightened susceptibility in RCS might be attributed to its specific genetic makeup or inherent physiological traits that make it more vulnerable to Chromium stress (Sharma and Dietz, 2009; Liang et al., 2017; Ali et al., 2023).

The two-way ANOVA conducted on the root elongation data provided valuable insights into the impact of Chromium treatments and chili plant varieties. The significant effect of different Chromium treatments on root elongation (FCr. = 19.78274, FCrit. = 3.325835, $P < 0.05$) confirms the direct influence of Chromium concentration on root growth inhibition. These results are consistent with earlier studies that have demonstrated the dose-dependent nature of Chromium toxicity on plant roots (Singh et al., 2016; Kalhor and Mohsenzadeh, 2016; Joseph et al., 2023).

While the variations in root length among the three chili varieties were not statistically significant under the same treatment conditions (FVar. = 1.507656, FCrit. = 4.102821, $P < 0.05$), it is evident that all varieties exhibited heightened susceptibility to increased Chromium concentration compared to the control group. This suggests that Chromium toxicity affects the root elongation of all three chili varieties, with RCS showing the most pronounced response (Fig. 2). Further molecular and physiological investigations would be beneficial in understanding the basis of RCS's heightened sensitivity to Chromium-induced root inhibition.

In conclusion, our study demonstrates that Chromium exposure negatively affects root elongation in chili plants, with RCS exhibiting the highest susceptibility among the three varieties. These findings highlight the importance of understanding varietal differences in Chromium tolerance and its potential impact on root growth and overall plant health. Tailoring agricultural practices and selecting tolerant varieties can aid in mitigating the adverse effects of heavy metal pollution on crop productivity, especially in areas with elevated Chromium levels in the soil. Further research in this area can contribute to the development of strategies for sustainable crop management in Chromium-contaminated environments.

Dry Weight

The investigation into dry weight revealed a consistent decrease in biomass production as the concentration of Chromium increased (Fig. 3), suggesting that Chromium stress has a negative impact on the growth and biomass accumulation of chili plants. This finding is in line with previous research on heavy metal stress in various plant species (Emamverdian et al., 2015; Farooq et al., 2022). The reduction in dry weight can be attributed to the disruption of essential physiological processes in plants exposed to Chromium, such as photosynthesis and nutrient uptake (Ali et al., 2022).

The results of the two-way ANOVA demonstrated a significant difference in dry weights among the three chili varieties after various Chromium treatments. This indicates that all varieties possess distinct inherent characteristics affecting their response to Chromium stress. Moreover, the ANOVA analysis revealed a significant effect of Chromium treatments on the dry masses of all three varieties (Table 1). As observed in seed germination and root elongation, here again, Rashid Chili Seeds (RCS) exhibited higher sensitivity to Chromium stress compared to Moon Star (MS) and Abbott Chili seeds (ACS). This differential response might be due to inherent genetic differences or physiological variations between the varieties in heavy metal tolerance and accumulation (Aprile and De Bellis, 2020), highlighting the importance of understanding variety-specific responses to environmental stressors.

The observed reduction in dry weight under Chromium stress raises concerns for agricultural productivity, especially in regions where Chromium contamination is prevalent. Agricultural soils contaminated with heavy metals, including Chromium, pose a threat to food production and quality (Naz et al., 2019). The decreased biomass production in chili plants due to Chromium stress can lead to reduced crop yields, impacting farmers' livelihoods and food security in affected areas. Additionally, the accumulation of Chromium in food crops can also pose health risks for consumers (Kabir et al. 2018, Dahiya et al., 2022).

Given the adverse effects of Chromium on dry weight and overall plant growth, it becomes imperative to implement measures to manage Chromium contamination in agricultural soils. Strategies such as phytoremediation, which involves using specific plant species to remove or detoxify heavy metals from the soil, can be employed to reduce Chromium levels in contaminated areas. Additionally, selecting Chromium-tolerant chili varieties and employing good agricultural practices can aid in mitigating the negative impacts of Chromium stress on crop productivity (Srivastava et al., 2021).

The investigation into dry weight responses to Chromium stress in chili plants highlights the negative impact of increasing Chromium concentrations on biomass production. Rashid Chili Seeds exhibited higher sensitivity to Chromium stress compared to Moon Star and Abbott Chili seeds, indicating varietal differences in their response to heavy metal contamination. These findings emphasize the importance of understanding varietal variations in Chromium tolerance and underscore the need for sustainable soil management practices in areas with elevated Chromium levels to ensure agricultural productivity and food security.

Number of leaves

The investigation into the impact of Cr treatments on leaf development in three chili varieties (MS, ACS, and RCS) revealed distinct responses in leaf growth and emergence among the varieties and Cr concentrations. At lower Cr concentrations (100µM), all three varieties exhibited the same number of leaves as the control, indicating their ability to tolerate moderate Cr stress (Ramana et al., 2015; Srivastava et al., 2021). This initial tolerance might be attributed to the activation of defense mechanisms and antioxidative pathways, which can effectively counteract the initial Cr toxicity (Noor et al., 2022; Khalid et al., 2023; Raja et al., 2023).

As the Cr concentration increased to 300µM, varieties ACS and RCS displayed a significant reduction in the number of leaves compared to the control, indicating increased sensitivity to Cr toxicity. Previous studies have suggested that variety-dependent differences in the expression of Cr-responsive genes (as for metal transporters and chelating agents) can influence their tolerance to Cr stress (Aprile and De Bellis, 2020; Ali et al., 2023). In contrast, variety MS demonstrated higher tolerance to Cr toxicity, as the number of leaves remained constant at the control, 100µM, and 300µM Cr treatments. This observation indicates that MS possesses robust Cr detoxification mechanisms, which allowed it to withstand lower Cr concentrations. Enhanced synthesis and sequestration of phytochelatins, metallothioneins, and other chelating agents might contribute to the reduced Cr accumulation in MS, thereby minimizing its adverse effects on leaf development (Yaashikaa et al., 2022; Pasricha et al., 2021; Gavrilescu et al., 2022).

Severity of Cr toxicity became more evident as the Cr concentration was further increased to 500µM, 700µM, and 900µM. In all varieties, the number of leaves decreased drastically, indicating severe inhibition of leaf emergence and growth under high Cr stress. These findings are consistent with studies that have reported the detrimental effects of excessive Cr on cellular processes, including photosynthesis, cell division, and leaf expansion (Sharma et al., 2020; Saleem et al., 2022; Sharma et al., 2023).

Notably, at the highest Cr concentration (900µM), varieties ACS and RCS exhibited complete inhibition of leaf development, while variety MS retained a few leaves. This observation further supports the notion that MS possesses a higher degree of Cr tolerance and detoxification capacity compared to ACS and RCS (Fig. 4).

The results of the two-way ANOVA indicated a significant difference in the number of leaves among the Cr treatments (FCr. = 24.98372, FCrit. = 3.325835, $P < 0.05$), emphasizing the strong influence of Cr concentrations on leaf development (Singh et al., 2020). However, no significant difference was observed in the number of leaves between the three varieties (FVar. = 1, FCrit. = 4.102821, $P < 0.05$), indicating that variety choice may not be a primary factor in determining the leaf number response to Cr treatments (Table 1).

The impact of Cr treatments on the number of leaves in all three chili varieties demonstrates that Cr treatments significantly influenced leaf development. Variety MS exhibited greater tolerance to Cr toxicity, maintaining a constant number of leaves up to the 300µM Cr treatment, while ACS and RCS displayed reduced leaf numbers, especially at higher Cr concentrations. These findings highlight the importance of understanding variety-specific responses to Cr stress and underscore the need for selecting tolerant varieties in Cr-contaminated environments to sustain crop productivity. Tailoring agricultural practices and crop selection based on varietal differences in Cr tolerance can aid in mitigating the negative impacts of Cr contamination on chili leaf development and overall plant health.

V. CONCLUSION

Our study, which focuses on seed germination, root elongation, dry weight reduction, and leaf development, sheds light on the response of three different chili varieties—Moon Star (MS), Abbott Chili Seeds (ACS), and Rashid Chili Seeds (RCS) to chromium (Cr) stress conditions.

All three chili varieties tolerated lower Cr concentrations, according to the experiment into seed germination, but when Cr levels rose, germination percentages fell. While ACS and RCS displayed decreased germination rates with increased Cr concentrations, MS demonstrated comparatively the highest tolerance to Cr stress. In all cultivars, measures of dry weight and root elongation revealed a constant decline with rising Cr concentrations. In contrast to the other varieties, RCS, with a consistent drop in root length and dry weight, was the most sensitive to Cr poisoning. Aside from that, all three types saw a sharp decline in leaf number when exposed to high Cr stress, with MS demonstrating the strongest tolerance by keeping a steady leaf number at lower Cr concentrations.

It is essential to comprehend the molecular processes behind these variations in germination patterns and growth factors in order to induce efficient counter measures against chromium-induced phytotoxicity in chili plants. Finding specific genes, signaling pathways and antioxidant systems involved in the response to Cr stress in chili cultivars should be the focus of further study. It may be possible to create genetically altered or specially bred chili cultivars that are more resistant to chromium-contamination by investigating these pathways.

Implementing soil remediation techniques is crucial for promoting sustainable agriculture in chromium-contaminated areas and ensuring food security (Radočaj et al., 2020). To lower the levels of Cr in agricultural lands, strategies like phytoremediation, which make use of plants to remove heavy metals from the soil, can be used. Furthermore, using sustainable farming methods like crop rotation and organic farming can reduce the buildup of Cr in the soil and safeguard plant health (Ayub et al., 2020).

Enhancing agricultural output in contaminated areas can be considerably achieved by the development of chili varieties that are naturally resistant to chromium stress. Introducing Cr-tolerance genes into chili plants by biotechnological techniques like marker-assisted breeding and genetic engineering can increase their capacity to deal with Cr toxicity (Al-Khayri et al., 2023).

It is crucial to protect the environment since chromium contamination not only reduces crop output but also puts human health at risk. Cr accumulation in food crops can result in dangerous levels of the metal being ingested, which can harm consumers' health. We can safeguard the environment and human health by putting into practice strategies to lessen Cr contamination in agricultural soils and crafting tolerant chili varieties (Kapoor et al., 2022).

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AUTHORS

First Author – Muhammad Azhar Zia, Ph.D. Scholar, Department of Botany,
The Islamia University of Bahawalpur, Punjab, Pakistan,
Second Author- Dr. Tauseef Anwar, Assistant Professor, Department of Botany,
The Islamia University of Bahawalpur, Punjab, Pakistan,
Corresponding Author- Muhammad Azhar Zia,