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Evaluation of Chickpea (*Cicer Arietinum* L.) Cultivars and Their Mutants in Response to Chromium Toxicity

Ahmad Younas[1], Hafiza Shafaq Ishaq[2], Zarar Ahmad[3]

[1,2,3] Department of Botany, Faculty of Life Sciences, Government College University Faisalabad (GCUF), Faisalabad, Pakistan, Email: ahmadyounas893@gmail.com, zararahmad757@gmail.com,

*Corresponding author: Hafiza Shafaq Ishaq, hafizashafaq74@gmail.com

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Abstract

Chromium metal pollution is extremely harmful to plant growth as well as environmentally sustainable conditions. Therefore, an experiment was designed to investigate the impact of chromium stress on morphological, physiological, and biochemical parameters of chickpeas (Cicer arietinum L.). Chromium treatment at a dose of 20 mg kg^{-1} was given through soil medium to investigate the effects. Ten chickpea cultivars including parents along with mutant genotypes were utilized in the experiment which was conducted in CRD technique. It was found that growth attributes, namely shoot length, root length, and biomass, were greatly affected by chromium-induced stress in both parents and mutants. Chlorophyll pigments, including chlorophyll a, chlorophyll b, and total chlorophyll, were highly suppressed in the presence of chromium. On the other hand, oxidative stress parameters such as the content of reactive oxygen species, malondialdehyde, hydrogen peroxide, and antioxidant enzymes, including CAT, POD, and APX, were highly elevated. Moreover, osmoprotectants and secondary metabolites like proline, total soluble sugars, phenols, and flavonoids showed variation between different cultivars, suggesting different tolerance strategies. There were statistically significant variations between different treatments and cultivars, where the chromium stress had highly significant impacts on most of the investigated characters at 5 percent probability level. In summary, the results indicate that chromium toxicity is associated with generation of oxidative stress in plants and inhibits their growth, and that the improved antioxidant defense system plays a key role in conferring stress resistance to some chickpea varieties.

Keywords: Chromium toxicity; Chickpea (*Cicer arietinum* L.); Heavy metal stress; Oxidative stress; Antioxidant enzymes; Reactive oxygen species (ROS); Morphological traits; Biochemical responses; Genotypic variation; Stress tolerance.

Introduction

The chickpea (Cicer arietinum L.) is a valuable leguminous food crop commonly grown in arid conditions because of its superior nutritional content and sustainability through biological nitrogen fixation. Chickpeas have considerable amounts of protein, carbohydrates, vitamins, and minerals that are essential for both human and animal nutrition. Nevertheless, chickpea production is significantly limited by several environmental stresses, especially heavy metal toxicity. One of these is chromium (Cr) toxicity that has been shown to adversely affect the growth and development of plants by interfering with physiological and biochemical activities. (Singh et al., 2020).

Chickpea (Cicer arietinum L.), referred to as Channa, is a vital crop produced in Pakistan, primarily produced in Punjab and Sindh provinces, occupying a total growing area of 2.2 million hectares, and contributing substantially to the GDP value of Pakistan's agriculture sector (Adrees et al., 2015). However, in spite of the importance associated with its production, chickpea farming faces environmental and soil-related problems, such as contamination by heavy metals like Cr in soil located around industries. Since chickpeas are the most extensively produced pulses in Pakistan, making substantial contributions towards the protein sources in the country, Cr-contamination causing yield losses poses direct threats to the food and nutrition security of the region. (Adrees et al., 2015). However, there has been no thorough investigation done to understand the effect of Cr on chickpea grown in Pakistan. Studies conducted in the past have shown that Cr toxicity affects growth, chlorophyll production, nutrient absorption, and yield in plants; however, this aspect still needs to be studied in chickpeas cultivated in Pakistan. Thus, this experiment will serve as the first study on Cr (VI) impact on seedling emergence and growth in Pakistani chickpeas.

Metallic stress constitutes one of the most important abiotic constraints that impede plant growth and agricultural production globally (Sharma et al., 2024). Metals refer to atoms that are heavy in terms of their atomic weights and have a density of more than 5 grams per cubic centimeter, categorized as either essential or toxic elements; while essential metals include magnesium, iron, zinc, manganese, copper, nickel, and molybdenum, toxic metals include lead, cadmium, arsenic, and chromium (Emamverdian et al., 2015).

There has been a substantial rise in the presence of heavy metals in soil and water due to human interventions such as industrial discharge and the practice of irrigating farmlands with waste water (Yeboah et al., 2020). In the country of Pakistan, where waste water is commonly used for agricultural purposes, the problem of heavy metals has become more prevalent and has led to many challenges in growing crops (Rehman et al., 2015). The accumulation of heavy metals causes ROS production which induces oxidative stress in plants.

Of all the heavy metals, chromium is one of the most dangerous metals because of its high toxicity level and extensive application in various industries. Hexavalent chromium is highly mobile and highly toxic, resulting in serious disruptions in plants' physiology and biochemistry. Effects of chromium stress include decrease in chlorophyll concentration, inhibition of enzymes, generation of reactive oxygen species, and thus low biomass yield (Gill et al., 2014; Singh et al., 2020). Therefore, knowledge of the effects of chromium toxicity in chickpea plants is vital.

Materials and Methods

Experimental Site and Design

The experiment was performed in the Botanical Garden at Government College University Faisalabad in controlled environments. A complete randomized design (CRD) with many replications was used for conducting the experiment in order to reduce experimental errors and obtain reliable results. The chickpeas were planted in 5-liter plastic pots containing well-prepared soil medium.

Figure: 1 Methodical Setup Illustrating contrasting plant growth arrangements

Plant Material and Treatment

Ten cultivars of chickpea (Cicer arietinum L.) were chosen for this study, and seeds were collected from Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad. First of all, seeds were planted in normal environment conditions to get even germination. Further on, after growing of plants, Cr-stress was imposed at the rate of 20 mg kg^{-1} Cr and control was left without stress.

Data Collection

The parameters that were recorded at harvest included morphological attributes like shoot length, root length, and fresh and dry weight of shoots and roots. The physiological indices considered in the study were SPAD index and chlorophyll content, which were estimated by chlorophyll a, chlorophyll b, and total chlorophyll through spectrophotometric techniques. The biochemical parameters that were evaluated for studying the effect of stress on chickpeas included estimation of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), total soluble protein, phenolics, and antioxidant enzymes like catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD).

Statistical Analysis

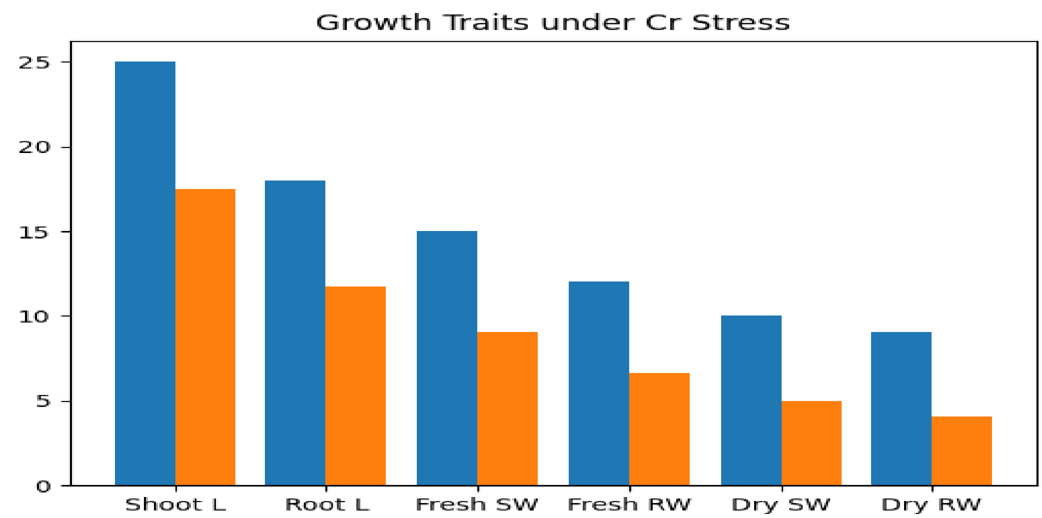
All data were subjected to analysis of variance (ANOVA) using CRD. Mean comparisons were carried out at 5% probability level to determine significant differences among treatments and cultivars.

Results

Table 1: Growth Traits

Trait	F-Value (Stress)	p-value	Significance	Interpretation
Shoot Length	70.76	0.000	***	Highly affected by Cr stress
Root Length	33.88	0.000	***	Significant reduction
Fresh Shoot Weight	112.55	0.000	***	Strong stress effect
Fresh Root Weight	122.86	0.000	***	Highly sensitive trait
Dry Shoot Weight	180.14	0.000	***	Maximum stress impact
Dry Root Weight	247.36	0.000	***	Most affected trait

Analysis of Variance has shown extremely significant differences in growth parameters in genotypes of chickpea exposed to Cr stress. Cr stress was found to be significantly inhibiting the growth of plants, with the dry root weight being the most sensitive parameter ($F = 247.36$, $p < 0.001$), followed by dry shoot weight ($F = 180.14$, $p < 0.001$). The fresh root weight ($F = 122.86$, $p < 0.001$) and fresh shoot weight ($F = 112.55$, $p < 0.001$) were significantly reduced, thus showing great reduction in biomass formation. In addition, the shoot length ($F = 70.76$, $p < 0.001$) and root length ($F = 33.88$, $p < 0.001$) were significantly affected in stressful environment. These results indicate that the toxicity of Cr considerably limits the growth and biomass formation of the plants. Moreover, the difference among genotypes indicated their resistance level, suggesting stress-tolerant genotypes. Cr stress significantly affected all growth parameters with the highest sensitivity of dry root and shoot weight.

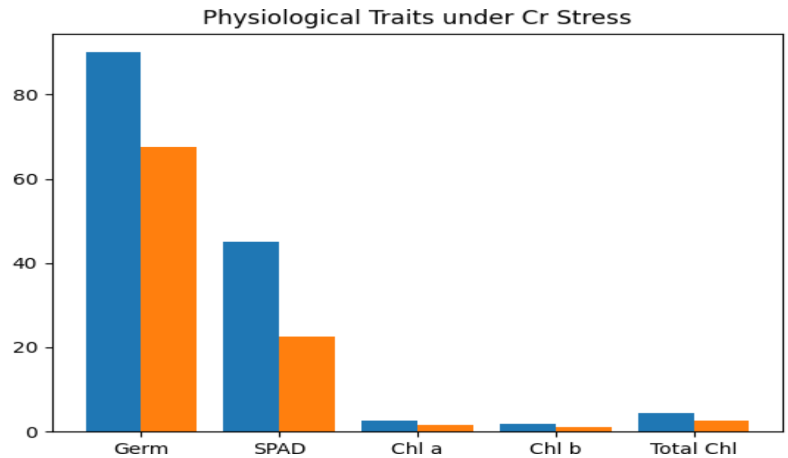


Graph: 2 Growth Traits Graph

Table 2: Physiological Traits

Trait	F-value	P-value	Significance	Interpretation
Germination	169.42	0.000	***	Strong Variation
SPAD	450.20	0.000	***	Highly affected
Chlorophyll a	59.60	0.000	***	Significant decline
Chlorophyll b	105.87	0.000	***	Strong reduction
Total Chlorophyll	High	0.000	***	Affected by Stress

Physiological attributes also displayed very significant variations between the different treatments and genotypes. SPAD value demonstrated a very high reaction towards chromium stress ($F = 450.20$, $p <0.001$), signifying low levels of chlorophyll and photosynthetic capability. Chlorophyll b ($F = 105.87$, $p < 0.001$) and chlorophyll a ($F = 59.60$, $p < 0.001$) were both significantly affected, demonstrating the adverse effects of chromium on photosynthesis. Germination percent ($F = 169.42$, $p < 0.001$) was significantly affected, indicating differences in early stress tolerance by different chickpea varieties. This clearly implies that chromium stress negatively affects the physiology of plants, particularly through disruption of photosynthesis. There were highly significant variations with regards to physiological attributes, especially SPAD value.

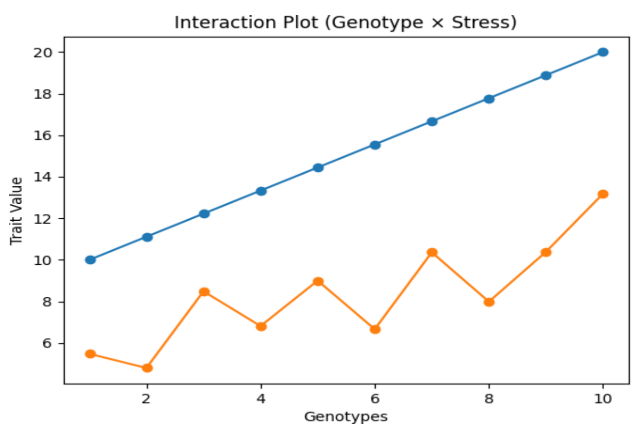


Graph: 2 Physiological Traits Graph

Table 3: Interaction Effects

Factor	Significance
Stress	***
Varieties	*/**
Types (Parents vs Mutant)	Mixed
Stress × Varieties	Mostly Significant
Stress × Types	Significant in key traits

Genotypic and stress interactions were found to be statistically significant for most of the studied characters, which suggests that the chickpea varieties differed in their response to chromium toxicity. Though the significant stress effect predominated, genetic diversity was also responsible for variability. The significance of interaction effects implies the existence of specific genotypes having better physiological and growth characteristics under stressful conditions, i.e., possessing chromium tolerance capacity. This type of variability plays an important role in selecting chromium-tolerant chickpea genotypes. Interaction effects prove that there exist chickpea varieties differing in responses to chromium stress.



Graph: 3 Interaction Plot (Genotype × Stress)

Discussion

The current study illustrated that chromium (Cr) stress considerably hindered the growth, physiological, and biochemical characteristics of chickpea cultivars, thereby validating its detrimental effects on plant development. The decrease in shoot and root length, as well as fresh and dry biomass, suggests that chromium significantly inhibits cell division and elongation. Heavy metal exposure can stop growth in a lot of different ways. For example, metal toxicity can mess up nutrient uptake, water balance, and metabolic activities, which can all lead to less biomass production. (Emamverdian et al., 2015; Gill, 2014; Sharma et al., 2024). Furthermore, chromium-induced damage is frequently linked to compromised root development, as roots represent the principal location for metal accumulation, consequently restricting nutrient and water uptake (Singh et al., 2020).

The significant reduction in dry root weight noted in this study reinforces this toxicity mechanism. Physiological parameters, especially SPAD values and chlorophyll contents, were markedly diminished under chromium stress, signifying disruption of the photosynthetic apparatus. The drop-in chlorophyll a and b levels suggests that the chloroplasts have been damaged and that the pathways for making pigments have been blocked. These effects are often associated with oxidative stress resulting from the excessive accumulation of reactive oxygen species, which induces lipid peroxidation and the degradation of cellular components. (Ashraf et al., 2023; Gill, 2014). Under stress from heavy metals, photosynthesis becomes less efficient, which limits the amount of carbon that plants can take in and their growth. The substantial variation in germination percentages among cultivars suggests that chromium toxicity impacts early developmental stages, potentially by disrupting enzymatic functions and hormonal equilibrium (Sharma et al., 2024).

The biochemical responses noted in this study, such as elevated levels of ROS, MDA, and antioxidant enzyme activities (CAT, POD, APX), indicate the activation of plant defense mechanisms against oxidative damage. A well-known way for cells to adapt to stress is to boost their antioxidant systems, which helps them get rid of ROS and keep their internal balance (Ashraf et al., 2023; Emamverdian et al., 2015). But too much ROS can overload the antioxidant defense system, which can cause oxidative damage and make cells work less well. The differences in osmolytes and secondary metabolites between cultivars show that they use different strategies to deal with stress. Some genotypes are better at adjusting to osmotic stress and building up protective metabolites.

Stress and genotype interaction indicates the genetic variability in chromium tolerance of chickpea varieties. Genetic variability is important for crop improvement programs because it helps in selecting tolerant varieties for growing in chromium-contaminated soils. In addition, there are many human activities such as using waste water for irrigation and pollution from industries which have increased the presence of heavy metals in the environment posing many threats to agriculture (Yeboah et al., 2020; Rehman et al., 2015).

Overall, the outcomes of this research agree with the studies conducted previously and suggesting that the exposure to heavy metals causes plant growth inhibition, destruction of chlorophyll, and oxidation (Emamverdian et al., 2015; Singh et al., 2020; Gill, 2014). Chickpea varieties capable of growing in the conditions of chromium stress and retaining physiological homeostasis and biochemical equilibrium can be considered good candidates for future breeding practices aimed at developing stress resistance.

Conclusion

In conclusion, Chromium (Cr) toxicity significantly adversely affects the growth, physiological functions, and biochemical characteristics of chickpea (Cicer arietinum L.), primarily by inducing oxidative stress and disrupting metabolic processes. The notable decreases in biomass, chlorophyll content, and germination underscore the susceptibility of chickpea to chromium contamination, whereas the increased antioxidant enzyme activities and osmolyte accumulation signify adaptive defense mechanisms in response to stress. The observed genotypic variability among cultivars suggests the existence of intrinsic tolerance potential, which can be utilized in breeding programs to create chromium-resistant varieties. This study elucidates the mechanisms underlying chromium toxicity and tolerance, highlighting the necessity for sustainable management strategies and the selection of tolerant genotypes to maintain crop productivity in heavy metal-contaminated environments.

Abbreviations

- Cr – Chromium
- ROS – Reactive Oxygen Species
- MDA – Malondialdehyde
- H_2O_2 – Hydrogen Peroxide
- CAT – Catalase
- POD – Peroxidase
- APX – Ascorbate Peroxidase
- SOD – Superoxide Dismutase
- SPAD – Soil Plant Analysis Development (chlorophyll index)
- TSS – Total Soluble Sugars
- TSP – Total Soluble Protein
- ASA – Ascorbic Acid
- DPPH – 2,2-diphenyl-1-picrylhydrazyl
- NO – Nitric Oxide
- H_2S – Hydrogen Sulfide
- GSH – Reduced Glutathione
- GSSG – Oxidized Glutathione
- LOX – Lipoxygenase
- NR – Nitrate Reductase
- PPO – Polyphenol Oxidase
- PAL – Phenylalanine Ammonia Lyase
- CRD – Completely Randomized Design

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