

Phytoremediation Potential and Differential Chromium (Cr) Accumulation and Gene Expression in Brassica juncea Varieties for Contaminated Soils in Turkey

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Abstract

Several agricultural areas in the Anatolian Plateau of Turkey are contaminated with chromium due to mining and leather tanning activities, posing a serious threat to both safe farming and human health. Cleaning polluted soils for reuse in agriculture is a vital and primary goal of this research. This study aimed to identify suitable *Brassica juncea* varieties for phytoremediation of metal-contaminated soils in Turkey, with the goal of promoting safe farming practices. Six varieties—Early Raya, Sindh Raya, S-9, Ganj Sarhen, JS-13, and THB-8—developed by the Agricultural Institute of Pakistan for drought and salt tolerance, were tested for metal tolerance at different Cr levels (0–1000 μM). Physiological parameters were recorded, and the contents of Cr, Ca, Fe, Mg, and K in plant tissues were quantified using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Additionally, the gene expression of several enzymes induced by ROS was examined using RT-qPCR. Early raya and Sindh raya under Cr stress showed different expression patterns of these genes, highlighting significant genotypic differences in chromium uptake, nutrient balance, and molecular responses. Our results classified the varieties into three different groups: Early raya and THB-81 as accumulators, Sindh raya, and S-9 as excluders, and Ganj sarhen, and JS13, as intermediate Cr accumulators. This study offers important insights into genotype-specific detoxification strategies. It lays the fundamental groundwork for future breeding programs and phytoremediation research aimed not only at reducing Cr contamination and promoting safe agriculture in contaminated soils in Turkey and other countries facing similar pollution issues.

Introduction

In recent years, there has been significant development activity involving industrialization and rapid urbanization across nearly all of Turkey. The leather and textile industries are among the sectors that have experienced considerable growth. In many parts of Turkey, the environment has reached or exceeded its carrying capacity regarding soil and water pollutants such as lime ($\text{Ca}(\text{OH})_2$), sodium sulfide (Na_2S), sodium chloride (NaCl), various dyes, and toxic heavy metals, especially chromium (Cr), released by these industrial sectors (Harmancioglu et al. 2008).

Chromium (Cr) is one of the toxic heavy metals that can harm soil, groundwater, and ecosystems due to its widespread and unconscious use in industrial processes (Ertani et al. 2017; Adnan et al. 2024). In Turkey, several regions, especially Anatolia, have significant mining and leather tanning activities, most of which generate waste effluent that is a major source of soil and river water pollution (Akçay et al. 2003; Dundar et al. 2012; Tokatli et al. 2021). These activities negatively impact agriculture, animal husbandry, and the quality of drinking water by contaminating soil and rivers. Turkey's most polluted rivers include the Euphrates, Tigris, Gediz, and Menderes; although they are vital for irrigation, they pose serious risks to public health and food safety (Aydin and Kucuksezgin 2012; Şentürk and Yıldız 2015). The increasing industrial activity continues to elevate environmental pollution, with long-lasting effects on regional ecosystems (Saxena 2025). As industrial use of chromium grows, its presence as an environmental contaminant has increased significantly (Coetzee et al. 2020).

Chromium lacks any essential function in plant metabolism; however, it exerts widespread phytotoxic effects that impair nearly all biological functions in plants (Shahid et al. 2017). Cr can interfere with plants' ability to absorb and maintain the balance of vital mineral elements. Competition with other elements interferes with physiological and metabolic functions, leading to growth suppression, oxidative damage, chlorosis, root impairment, and nutritional imbalance, which can ultimately result in plant mortality (Anjum et al. 2016; Singh and Malaviya 2019). One of the most crucial consequences of Cr stress is the impairment of antioxidant defense systems and the disturbance of the equilibrium between the generation of reactive oxygen species (ROS), which cause cellular damage (Samantary 2002; Saud et al. 2022).

Chromium contamination poses a serious threat to global food security by impairing the growth and yield of essential crops, including vegetables, cereals, and legumes (Vasilachi et al. 2023). To reduce its harmful effects, it is crucial to investigate how chromium behaves in soil environments, assess its toxic impact on plant growth and metabolism, and develop effective, long-term remediation strategies. Among the benefits these activities can provide are improving soil quality and encouraging environmental sustainability (Xia et al. 2019). Recent research has focused on how crop plants absorb and accumulate chromium and how this process may affect human health through the food chain (del Real et al. 2013; Zulfiqar et al. 2023). Phytoremediation, the use of plants to extract, sequester, or detoxify pollutants, stands out as a sustainable and efficient method to clean up environments contaminated with heavy metals (Memon 2016; Doğru et al. 2021). Enhancing the phytoremediation capacity of plants requires a thorough understanding of the physiological and molecular processes that control the uptake of metals, their interaction with essential nutrients, and the mechanisms involved in detoxification (Memon and Schröder 2009). Additionally, identifying and utilizing promising plant genotypes that can either accumulate or exclude the target metal is crucial for successful phytoremediation.

Grown for both agricultural and industrial purposes worldwide, *Brassica juncea* L. (Brown mustard), is an important edible oilseed crop with significant nutritional and economic value. It is also recognized for its strong ability and significant potential in phytoremediation, especially in areas affected by environmental pollution such as heavy metal-contaminated soils (Anjum et al. 2012). Due to its reported tolerance to several heavy metals, *B. juncea* is considered a sustainable and environmentally friendly option for soil remediation (Singh et al. 2024). Finding and selecting genotypes with natural tolerance to heavy metals is an effective way to develop improved varieties suitable for growing in areas contaminated with heavy metals (del Real et al. 2013). However, a notable gap remains in understanding *B. juncea* genotypes that can be adapted to Turkey's specific climate conditions and provide efficient Cr control under field-related stress levels.

This study was conducted to investigate the capacity of six *Brassica juncea* varieties, Early raya, JS-13, Sindh raya, S-9, Ganj sarhen, and THB-81, to accumulate and tolerate chromium (Cr) at different concentrations (0, 10, 50, 100, 200, 500, and 1000 μM). The study also explores the potential roles of key elements like Ca, Fe, K, and Mg in reducing Cr toxicity. To understand the molecular mechanisms that boost antioxidant defenses under chromium stress, the transcription levels of ROS-related genes—

ascorbate peroxidase (APX), respiratory burst oxidase homolog (RBOH), and iron superoxide dismutase (FeSOD)—were assessed via RT-qPCR in the root tissues of the Cr-accumulator variety (Early raya) and the Cr-excluder variety (Sindh raya).

Our work offers an important and practical, comprehensive, two-pronged approach for addressing contaminated sites polluted with Cr. One approach utilizes accumulator varieties for active soil remediation, whereas the other employs excluder varieties such as Sindh raya for sustainable and safe farming. This innovative, multi-varietal comparison of chromium phytoremediation provides a more thorough and practical approach to managing soil contamination, addressing cleanup, food safety, and developing effective and safe strategies for metal-contaminated soils.

MATERIALS AND METHODS

Plant material and experimental design

Seeds of *Brassica juncea* (JS-13, Early raya, Sindh raya, S-9, Ganj sarhen, and THB-81) were obtained from the Agricultural Research Institute, Tandojam, Pakistan. Seeds were surface-sterilized with 5% sodium hypochlorite and Tween-20 for 15 min, then rinsed with distilled water. The plant growth conditions and cultivation methods are the same as described previously (Meraklı et al. 2022). Seeds (100 per pot) were sown in vermiculite and germinated under controlled conditions ($25 \pm 2^\circ\text{C}$ day/ $20 \pm 2^\circ\text{C}$ night, 16 h light/8 h dark, $150 \mu\text{mol}/\text{m}^2/\text{s}$ light intensity, $60 \pm 5\%$ humidity). On day 5, germinated seeds (radicle ≥ 2 mm) were counted, and the germination rate (%) was calculated according to (Akinci and Akinci 2010). Seedlings were treated with $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ at 0, 10, 50, 100, 200, 500, and 1000 μM in 1/4-strength Hoagland solution (Hoagland and Arnon 1938). Control plants received only the nutrient solution. After two weeks, seedlings were harvested, and roots were rinsed with sterile deionized water. The experiment was performed in triplicate.

Growth parameters

Plants were harvested after 15 days (two weeks), carefully removed from the pots, washed to remove vermiculite residue, and separated into leaves, stems, and roots. Root and plant lengths were measured, and the fresh weight (FW) of roots and shoots was recorded after harvesting. Plant and root lengths were measured using a meter scale. The samples were oven-dried at 72°C for approximately 48 hours.

Metal analysis

Dried plant samples (0.5 g) were digested in a mixture of nitric and perchloric acid (HNO_3 : HClO_4 , 5:2 v/v) on a hot plate, then filtered through Whatman No. 42 ($0.25 \mu\text{m}$) and diluted to 10 ml with Milli-Q water (Memon et al. 1979). Metal concentrations were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES; SPECTRO ARCOS) (Meraklı et al. 2022). Chromium standards (Sigma-Aldrich, Germany) and blank samples were prepared according to the method described by (Meraklı et al. 2022).

Gene expression analysis

Total RNA was extracted from the roots of control and Cr-treated plants using the RNeasy Plant Kit (Thermo Fisher Scientific) following the manufacturer's protocol. To eliminate genomic DNA contamination, the RNA samples were treated with RNase-Free DNase. RNA concentrations were measured using a NanoDrop spectrophotometer. cDNA synthesis was performed using the Bio-Rad iScript cDNA Synthesis Kit. The reaction mixture contained iScript reaction mix, reverse transcriptase, the RNA template, and nuclease-free water. Quantitative real-time PCR (qRT-PCR) was performed using the LightCycler 480 system (Roche, Germany) with SYBR Green Master Mix. The reaction included gene-specific primers for APX, FeSOD, RBOH, and ubiquitin. The qRT-PCR procedure followed standard protocols (Meraklı and Memon 2023). Gene-specific primers (see Table S1) were designed based on mRNA sequences obtained from the GenBank and EMBL databases. The ubiquitin gene served as the reference gene. Gene expression quantification was performed using the ΔC_t method, where ΔC_t values are inversely proportional to the initial template amount (Applied Biosystems). Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

Statistical analysis

Data (mean $\pm$ SD) were analyzed using SPSS (24.0) and Microsoft Excel. Tukey's test was used to evaluate differences in plant growth, and simple correlation coefficients were used to assess the significance of metal concentrations. Data normality was assessed using the Shapiro-Wilk test, and variance homogeneity was examined using Levene's test. A three-way ANOVA was used to investigate the effects of the factors and their interactions. Post hoc comparisons were performed using Duncan's test. Principal Component Analysis (PCA) was performed to identify patterns of Cr accumulation and better understand the relationships among the studied variables. The principal components that explained most of the total variance were extracted, and the distribution of samples was assessed based on Cr accumulation levels. The positioning of the different factors was visualized using biplot graphs, and the loadings of the principal components were interpreted to elucidate their contribution to the observed variation. R (version 4.2.2), Python (version 3.10.0), and SPSS (version 24.0) were used for the analyses, and $p < 0.05$ was considered significant.

RESULTS

Effects of Chromium (Cr) on growth in *Brassica juncea* varieties

Bar charts in Fig. 1 illustrate how Cr influences various growth metrics across six *Brassica juncea* varieties: Early raya, Ganj sarhen, JS-13, Sindh raya, S-9, and THB-81 grown at different chromium concentrations (0, 10, 50, 100, 500, and 1000 μM). These metrics include seed germination, plant height, root length, root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight (Fig. S1; Fig. 1).

Across all varieties and parameters, a clear dose-dependent response to increasing chromium levels is evident. Low Cr concentrations (10, 50, 100 μM) generally have a stimulating or neutral effect on plant

growth compared to the control (0 μM). However, as Cr levels rise to 200, 500, and especially 1000 μM , all growth measurements significantly decrease across all varieties, indicating chromium toxicity. The varieties show different levels of tolerance or sensitivity to chromium stress (Fig. S1; Figs. 1a-g). The average germination rates were obtained from *Brassica juncea* varieties grown under different Cr concentrations. Early raya had the highest germination rate among the tested varieties, reaching 90.33% even at 1000 μM Cr. In contrast, Sindh raya showed the most noticeable reduction, with germination dropping to 56.66% (Figure 1a). The lowest germination was recorded in Sindh Raya and S-9. Based on all growth traits (root, shoot length, and root and shoot fresh and dry weight), these varieties were also most adversely affected, showing marked declines in seedling growth and biomass production (Figs. 1a-g). Furthermore, THB-81 experienced a notable increase in biomass, suggesting that moderate Cr levels (200 μM Cr) could trigger a beneficial physiological response in this variety. The data shown in Fig. 1 indicate that chromium concentrations above 200 μM are toxic to some *Brassica juncea* varieties, causing significant decreases in root and shoot development, as well as reduced overall plant height. Conversely, some varieties, notably JS-13, Early raya, and THB-81, display some tolerance or even slight benefits at very low Cr levels (10-100 μM). On the other hand, Sindh raya and S-9 are among the most sensitive, exhibiting less vigorous growth and a faster decline in performance as Cr levels increase (Figs. 1a-g). Overall, the data in Fig. 1 highlight the genetic differences in how plants respond to heavy metal stress, suggesting that varieties like Early raya and THB-81 should be studied more closely for phytoremediation, particularly in regions with low to moderate chromium pollution.

Cr accumulation in *Brassica juncea* varieties

Cr was mostly accumulated in plant roots, with restricted translocation to aboveground tissues (Fig. 2). In particular, at 1000 μM Cr, THB-81 and Early raya accumulated the maximum amount of Cr in their roots, containing 552.28 $\mu\text{g g}^{-1}$ DW and 429 $\mu\text{g g}^{-1}$ DW, respectively (Fig. 2a). The accumulation of Cr differed among varieties, but the highest amount of Cr accumulation in the stem was observed in Early raya (88.01 $\mu\text{g g}^{-1}$ DW) (Fig. 2b). Early raya also accumulated relatively high metal levels far above the toxic concentration in the shoots. Moreover, the lowest Cr uptake and the least amount of translocation to aboveground parts were observed in Sindh raya and S-9, suggesting an exclusion strategy in these varieties (Fig. 2b). The Cr content in leaves differed in varieties, supporting the existence of variety-specific mechanisms for Cr translocation and sequestration (Fig. 2c).

Accumulation patterns of Fe, Ca, K, and Mg in different *Brassica juncea* varieties

The results showed that metal accumulation differed among varieties. The overall accumulation patterns varied among varieties, with increasing Cr concentrations. The accumulation of Fe, Ca, Mg, and K in plant tissues changed dramatically when exposed to Cr stress (Figs. 3a-m and Table S2).

Figure 3 shows the increase in accumulation of major essential elements (Ca, Mg, K, and Fe) in the plant tissues with a concomitant increase in Cr levels in the media. This aligns with known responses to heavy metal stress, where plants modify their nutrient uptake and transport. The patterns of accumulation differ notably between roots, stems, and leaves, illustrating how various tissues respond to and manage

metal stress. Roots typically show the highest metal accumulation, particularly at elevated Cr levels, as they serve as the initial point of contact. As illustrated in Figures 1 and 2, different *Brassica juncea* varieties respond uniquely—some are better at accumulating or regulating certain metals, which likely accounts for their different levels of Cr tolerance (Fig. 3). For all varieties, leaf Fe content increases with increasing Cr concentration. Ganj sarhen and Sindh raya exhibit particularly high Fe accumulation in their leaves at the maximum Cr level (1000 μ M). In contrast, S-9 and THB-81 have the lowest overall Fe levels in their leaves, indicating they may be less effective at transporting Fe to the shoots or that Fe uptake is more restricted (Fig. 3i). Across all varieties, root Fe content significantly surpasses leaf Fe, a common plant strategy to prevent heavy metal toxicity in photosynthetic tissues (Fig. 3g). The highest root Fe accumulation occurs in JS-13, Early raya, and Ganj sarhen at 1000 μ M Cr. S-9 shows the lowest root Fe, possibly related to its overall slower growth and greater sensitivity. Although the Fe content in the stem tissues is lower than in roots and leaves, it still increases with high Cr (Fig. 3h). Similarly, Ca content in the leaves increases with an increase in Cr concentrations across most varieties (Figs. 3a, b, c). Interestingly, Ganj sarhen has exceptionally high Ca content in its leaves, peaking at 1000 μ M Cr in the media. In contrast, S-9 and Sindh raya show the lowest leaf Ca content, which may reflect a general stress response or less effective nutrient management (Fig. 3c). Root Ca content also increases as Cr levels rise (Fig. 3a). Ganj sarhen and THB-81 display the highest Ca accumulation in their roots, suggesting a strong capacity for Ca uptake and storage at the root level. At the same time, the Ca content in these varieties also increases in stems, indicating efficient translocation from roots to shoots (Fig. 3b). Magnesium (Mg) content in leaves generally rises with increasing Cr levels (Fig. f). Ganj sarhen displays a distinct pattern, maintaining consistently low leaf Mg across all concentrations, unlike other varieties that show increases. This may suggest a different homeostatic regulation. Early raya, JS-13, and THB-81 have the highest leaf Mg accumulation. Stem and root Mg levels also increase with Cr concentration (Figs. 3d, e) in these varieties. In all varieties, root K levels positively correlate with increasing Cr concentration (Figs. 3k, l, m). JS-13 and THB-81 showed the highest K accumulation in roots, indicating that they may absorb and sequester K more effectively in roots as a defense against Cr toxicity. S-9 and Sindh raya varieties accumulated the lowest amount of K in roots across all treatments, suggesting that these varieties are possibly more affected by Cr stress compared to other varieties, indicating their high sensitivity to Cr toxicity (Fig. 3). Leaves generally contain the highest K concentration compared to roots and stems, and K levels increase with rising Cr in all varieties (Fig. 3m). Early raya and JS-13 have the highest leaf K and Ganj sarhen and S-9 show the lowest K in leaves with increase in Cr concentration. Cr may compete with other metal transporters in these varieties, causing shifts in uptake patterns. Cr-tolerant plant varieties might actively increase the absorption of other metals, such as Ca, Mg, and K, either as a stress response or to mitigate Cr toxicity. For example, calcium (Ca) acts as a secondary messenger in stress signaling, while increased iron (Fe) uptake might counteract Cr-induced oxidative stress. Some varieties, such as Ganj sarhen (for Ca) and JS-13 (for Fe and K), show notably high metal accumulation in specific tissues. This buildup, mostly in roots and stems, could prevent Cr from reaching sensitive photosynthetic tissues such as leaves. On one hand, JS-13, Early raya, and THB-81 accumulate potassium (K) significantly, especially under high Cr levels, supporting their tolerance to Cr stress. On the other hand, the S-9 and Ganj sarhen show low potassium

accumulation in their leaves. Overall, the data suggests that JS-13, Early raya, and THB-81 are more resilient, likely due to their ability to regulate and store essential metals more effectively under Cr stress, thereby boosting their tolerance. In contrast, S-9 and Sindh raya both seem to be sensitive and are less capable of handling this stress, as shown by their lower metal accumulation.

Correlation between Cr and Fe, Ca, K, and Mg uptake

Plants grown in metal-enriched media absorb metal ions to varying amounts depending on their uptake capacity and tolerance mechanisms. For this purpose, the accumulator plant, Early raya, and the excluder plant, Sindh raya, were selected to investigate the relationships between Cr and Fe, Mg, K, and Ca (Figs. 4a and b). A regression analysis was conducted between Cr and other elements (Ca, Mg, K, Fe) in the roots of the accumulator, Early raya, and the excluder, Sindh raya. Fe ($R^2 = 0.9614$), Mg ($R^2 = 0.9711$), and Ca ($R^2 = 0.9154$) exhibited the highest positive correlations with Cr accumulation in Early raya. At the same time, K showed a relatively weaker correlation ($R^2 = 0.7371$) compared to the other elements (Fig. 4a). Cr accumulation exhibited relatively weak associations with Fe ($R^2 = 0.7994$), Mg ($R^2 = 0.8293$), K ($R^2 = 0.5740$), and Ca ($R^2 = 0.4627$) in Sindh raya, implying a lower efficiency in nutrient regulation under Cr stress compared to Early raya (Fig. 4b). These results indicate that the nutrient homeostasis in response to Cr stress in Early raya is more robust than Sindh raya, highlighting varietal differences in Cr affinity.

PCA analysis of six *Brassica juncea* varieties grown on different chromium concentrations

Based on chromium accumulation in their tissues, the PCA analysis reveals the distribution of six *Brassica juncea* varieties (Early raya, JS-13, Sindh raya, S-9, Ganj sarhen, and THB-81) (Fig. 5). The PCA biplot was generated to evaluate the accumulation and tolerance potential of *Brassica juncea* varieties exposed to different Cr concentrations. In the PCA analysis, the two components, PC axis 1 and PC axis 2, accounted for 83.1% and 16.5% of the total variance, respectively, resulting in a total cumulative variance of 99.6%. PCA results showed that the varieties fall into three groups according to their Cr accumulation. Here, we aimed to assess the variety-specific selectivity for Cr accumulation. As shown in Figure 5, the biplot displays the distinct Cr accumulation status of six *Brassica juncea* varieties.

Accordingly, a clear distinction is observed between the different Cr treatments across the six studied varieties. As a result, the *B. juncea* varieties were positioned on the right, middle, and left of the PCA biplot. One group, in green, includes Cr accumulators (Early raya and THB-81), the second, in gray, represents intermediate types (JS 13, Ganj sarhen), and the third, in red, comprises Cr excluders (S-9 and Sindh raya). The separation along PCA1 (which accounts for 83.1% of the variance) particularly emphasizes the contrasting patterns of metal accumulation (Fig. 5).

Gene expression in *Brassica juncea* varieties— Early raya and Sindh raya

Primers were designed using the mRNA sequences from the GenBank and EMBL databases for the three ROS-related genes Ascorbate Peroxidase (APX), Iron-Superoxide Dismutase (FeSOD), and Respiratory Burst Oxidase Homolog (RBOH) (Table S1), with the ubiquitin gene serving as a reference. Gene

expression patterns for enzymes involved in oxidative stress differed between the accumulator Early raya and the excluder Sindh raya. APX, FSD, and RBOH were expressed in Early raya, with their expression levels increasing concomitantly with an increase in Cr. On the other hand, only one gene, APX, was expressed in Sindh raya, with increasing Cr level. A substantial expression of Ascorbate peroxidase (APX) was observed in Sindh raya at 1000 μM Cr (a 13.74-fold increase relative to the control), indicating a Cr concentration-dependent response. The expression of FeSOD and RBOH was slightly altered with increasing Cr levels in the media (see Fig. 6).

DISCUSSION

This study aimed to find a solution for Cr pollution using environmentally friendly and cost-effective methods, such as phytoremediation, an emerging technology for sustainable solutions to metal-polluted soils (Han et al. 2023; Lee et al. 2025). In this context, we obtained six *Brassica juncea* varieties developed by a geneticist in the oilseed department at the agricultural institute in Pakistan, which were chosen for their tolerance to drought and salt stress. We used this genetic resource to identify suitable metal-resistant varieties to address the Cr pollution problem in Turkey. We examined various physiological and molecular parameters to understand how different varieties respond to Cr stress. Our results show that the toxic effects of Cr were partly reduced by the accumulation of specific macro- and micronutrients, and that different genotypes employ distinct strategies for Cr detoxification. Additionally, we identified Cr-tolerant, accumulator, and excluder varieties of *Brassica juncea* to combat Cr pollution both in Turkey and worldwide.

Growth responses and Cr accumulation in different varieties of *Brassica juncea*

Concomitant increases in Cr levels (0–1000 μM) in media negatively affected germination and growth, with Early raya preserving a high germination rate (90.33% at 1000 μM), while Sindh raya showed a significant decline (56.83%). Growth inhibition was most pronounced in Sindh raya and S-9. Furthermore, the Early raya and THB-81 varieties exhibited enhanced tolerance, possibly attributable to efficient detoxification strategies such as chromium sequestration and compartmentalization into metabolically low-active tissues, including the cell wall, leaf trichomes, and vacuoles, as well as high expression of ROS-related genes and enzymes (Wakeel et al. 2020; Han et al. 2023).

Cr has no particular biological role in plant cells and is not necessary for plant growth and development (Oliveira 2012; Sharma et al. 2020). Several plant species have developed different strategies to overcome Cr toxicity. Data in the present study show that THB-81 sequestered up to 552.28 $\mu\text{g g}^{-1}$ DW Cr in roots with no toxicity effect. This is likely due to the vacuolar compartmentalization of Cr or high cell wall binding (Shahandeh and Hossner 2000; Pulford et al. 2001; Han et al. 2023; Shourie et al. 2024). On the contrary, the S-9 and Sindh raya varieties accumulated very low Cr in their roots and shoots, indicating divergent varietal strategies for metal stress response.

The significant Cr accumulation in stems of Early raya (88.01 $\mu\text{g g}^{-1}$ DW) indicates that Cr is possibly translocated via xylem loading, potentially through non-selective cation channels or sulfur transporters,

which are known to facilitate Cr(III) uptake (Prado et al. 2010; Gill et al. 2015; de Oliveira et al. 2016; Zhang et al. 2018). On the other hand, Sindh raya showed minimal Cr uptake ($7.83 \mu\text{g g}^{-1}$ DW in roots), probably due to active efflux, restricted metal influx, and limited translocation to the shoots, which may be due to lignified casparian strips in the root endodermis acting as a barrier to apoplastic movement of Cr. (Yamaji et al. 2013) have shown similar results with Zn accumulation in rice. The different varietal responses to Cr exclusion or accumulation are presumably due to the downregulation or upregulation of metal transporters, e.g., HMA4, NRAMP3, or increased chelation of Cr in the rhizosphere caused by high expression of exudate biosynthesis genes rendering Cr in an innocuous form (Clemens 2006; Shourie et al. 2024).

Cr-nutrient interactions

Strong correlations were observed between Cr and essential metals, such as Fe, Ca, Mg, and K, in the Cr-tolerant varieties (Early raya and THB-81), suggesting competitive metal uptake and a stress response to cope with Cr stress (Vert et al. 2002). Notably, THB-81 had higher Ca levels in its roots ($811.36 \mu\text{g g}^{-1}$ DW), which could help reduce Cr toxicity by stabilizing membrane integrity and reducing Cr-induced damage (Hirschi 2004). In contrast, Cr-sensitive varieties (S9 and Sindh raya) exhibited weaker correlations, indicating that they primarily rely on passive exclusion of Cr.

Principal component analysis (PCA) and genetic implications

Principal Component Analysis (PCA) was employed to investigate genotype-specific responses to Cr stress and its interaction with other nutrients (Dolezalova et al. 2015). In the present study, PCA distinguished *Brassica juncea* varieties based on their physiological responses to Cr stress, categorizing them into several distinct groups. This classification highlights a variety-specific management of Cr stress, with some accumulating Cr and others excluding it. These varietal differences may reflect different strategies in metal transporter expression, detoxification capacity, or intracellular compartmentalization (Shanker et al. 2005).

The close relationship between THB-81 and Early raya in the accumulator group indicates that these varieties may share genetic traits that enhance Cr uptake and sequestration, such as duplicated metal transporter genes or improved root cortical aerenchyma (DalCorso et al. 2019). Conversely, excluder varieties like S-9 and Sindh raya may carry allelic variations that provide resistance by limiting metal translocation into the xylem. This could occur through thicker exodermal layers or increased activity of FRD3-like efflux transporters (Durrett et al. 2007).

The hypothesis that these varieties use different strategies for chromium uptake, exclusion, and detoxification is supported by the varying spatial distribution of varieties in the PCA analysis. These findings have important practical implications in the phytoremediation of heavy metals. Accumulator varieties show promise for phytoremediation of Cr-contaminated soils, whereas excluder genotypes might be better suited for safe cultivation in polluted areas. This PCA-based classification provides a

useful framework for breeding efforts aimed at enhancing Cr tolerance or reducing metal accumulation in edible plant parts.

Distinctive gene expression patterns in *Brassica juncea* varieties under Cr stress

Sindh raya and Early raya showed varied reactions to the gene expression of oxidative-related enzymes when subjected to different levels of Cr. This pronounced varietal response is possibly due to their ability to cope differently with oxidative stress. Exposure of Sindh raya to 1000 μM Cr increased APX (ascorbate peroxidase) expression to 13.74-fold compared to controls. Meanwhile, the levels of FeSOD (Fe-superoxide dismutase) and RBOH (respiratory burst oxidase homolog) remained unchanged despite Cr exposure, suggesting that Sindh raya primarily depends on the APX system for oxidative stress defense. Similar findings were reported by (Jung et al. 2020) in oilseed rape seedlings exposed to Cd stress. These findings indicate that the two varieties employ different mechanisms to manage ROS generated by metal toxicity (see Fig. 6a). In contrast, Early raya displays a more active and cohesive antioxidant defense system. Unlike Sindh raya, all three genes (Fig. 6b) showed strong induction, particularly at elevated Cr levels. This synchronized response in Early raya suggests a highly flexible strategy against oxidative stress. FeSOD and RBOH expression are crucial in the plant's response system, which is activated during oxidative stress. For example, (Regon et al. 2022) demonstrated that these genes are crucial for aromatic Keteki Joha rice to adapt to oxidative stress caused by ferrous iron (Fe^{2+}). The activation of these ROS-related genes by heavy metals or other abiotic stresses suggests that Early raya has developed a more comprehensive mechanism to handle metal stress (Wang et al. 2008; Małecka et al. 2019).

Practical implications for phytoremediation strategies

Our data in Table 1 provides a clear, evidence-based framework for selecting the most suitable plant variety to achieve a specific environmental goal, rather than relying on a generic approach (del Real et al. 2013). Here, Early raya and THB-81 are grouped as Cr accumulators, which tolerate Cr toxicity through Mg accumulation, while S-9 and Sindh raya were shown to be strong excluders of Cr.

Table 1

The *Brassica juncea* varieties are tentatively classified into six groups, considering the influence of other essential elements on Cr uptake and translocation

Early raya	Strong accumulator	Co-accumulates Mg, maybe stress-tolerant via internal detoxification
THB-81	Accumulator (esp. at high Cr)	Possibly tolerates via moderate Mg accumulation
JS-13	Moderate accumulator	Fe uptake seems unrelated to Cr
S-9	Strong excluder	Cr-resistant, may avoid uptake or sequester in roots
Sindh raya	Strong excluder	Cr-resistant, similar to S-9
Ganj sarhen	Weak Cr association, high Ca	Likely Ca-mediated defense strategy

The different metal strategies studied here can guide future plant breeding and genetic engineering efforts. As shown in Table 1, pinpointing specific mechanisms—such as Ca-mediated defense, Mg-mediated tolerance, or Cr-opposed uptake—lays a foundation for creating new plant varieties optimized for phytoremediation or food safety. This is a significant and unique finding regarding how different varieties respond to Cr and how it interacts with other essential elements in plants. These varieties could enable safe cultivation in areas contaminated with metal pollutants.

CONCLUSION

Given that Turkey is one of the world's leading chromium exporters and that various regions, particularly regions (i.e., Usak, Manisa, İzmir, Diyarbakir) of Anatolia, suffer from extensive Cr contamination due to leather tanning and mining activities. The findings of this study hold substantial environmental and agricultural significance for safe agriculture and food, not only for Turkey but also for other countries facing similar challenges. This research will be important in addressing global environmental issues. In this context, we explored solutions for alleviating the Cr pollution problem by using suitable plant varieties for remediation or safe cultivation in metal-polluted soils. Our results grouped the *Brassica juncea* varieties into three categories: the first group, Cr accumulators (Early raya and THB-81); the second group, intermediate types (JS-13 and Ganj sarhen); and the third group, excluders (S-9 and Sindh raya). We propose that Early raya and THB-81 varieties could be used for phytoremediation of Cr-contaminated sites. In contrast, Sindh raya and S-9 (excluders) could be cultivated for safe agriculture in polluted areas, thereby reducing Cr uptake into the food supply. These variations in Cr uptake, translocation, and accumulation across varieties offer important insights to local authorities, agronomists, and breeders in Turkey and beyond. This helps in developing customized, sustainable approaches to tackle chromium pollution in agriculture.

Declarations

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Author Contributions

NM performed most of the experiments, while ARM and HA assisted with some of them. NM and ARM wrote the manuscript, with contributions from all authors. NM, ARM, HA, BK, LAB, and AK interpreted the data. ARM designed and supervised the project. All authors have read and agreed to the published version of the manuscript.

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Data Availability

All data generated or analyzed during this study are included in this article and its supplementary information files. This study ensures complete transparency and accessibility of all data produced or examined.

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Figures

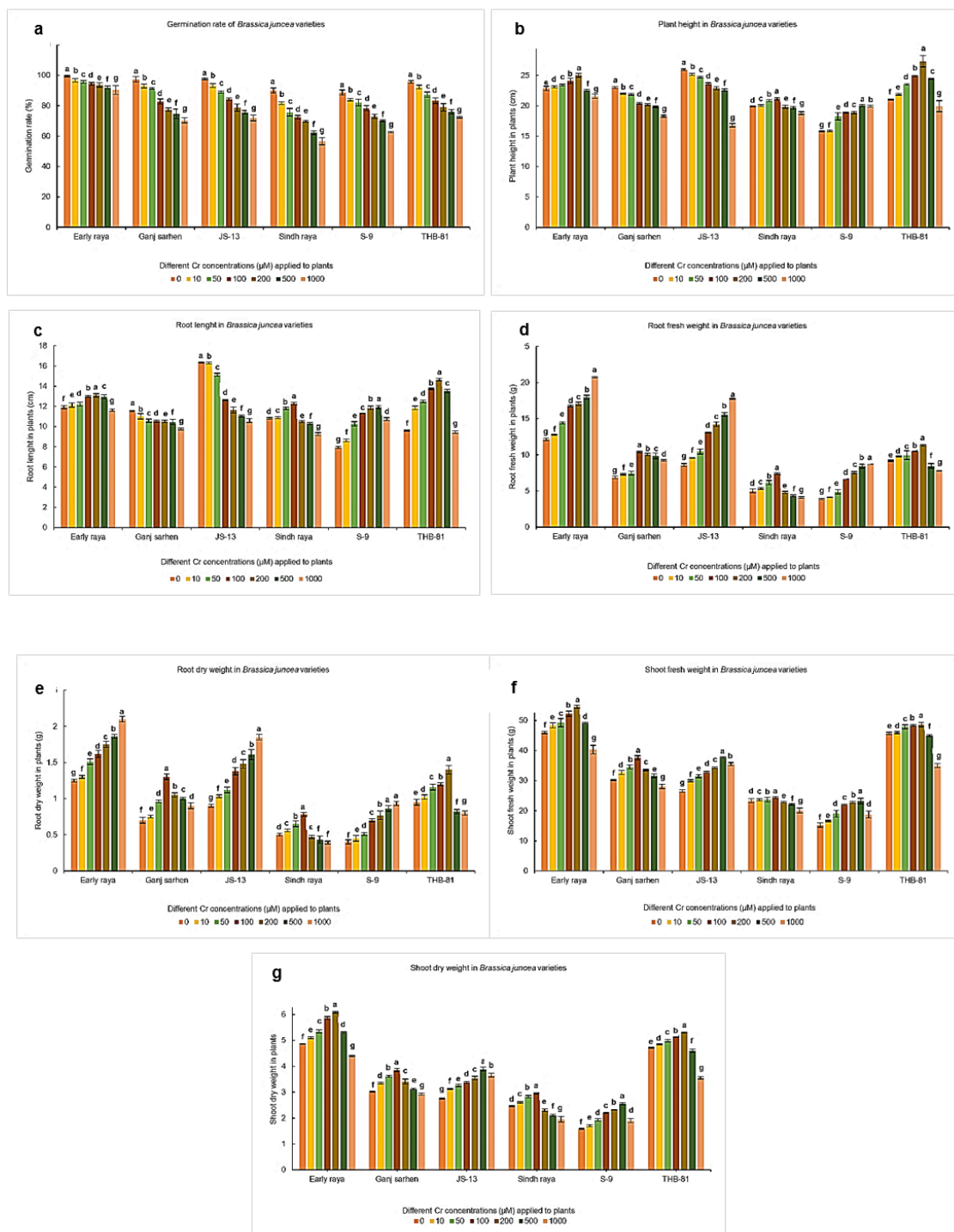


Figure 1

shows germination rate (a), plant height (b), root length (c), root fresh weight (d), root dry weight (e), shoot fresh weight (f), and shoot dry weight (g) of *Brassica juncea* varieties- Early raya, Ganj sarhen, JS-13, Sindh raya, S-9, and THB-81 grown at different Cr concentrations (0-1000 μM). Data are shown as $\pm$ standard deviations (SD) (n = 3). Grouping (a-g) was performed using Tukey's test. Different letters indicate significant differences, highlighting varietal responses to Cr.

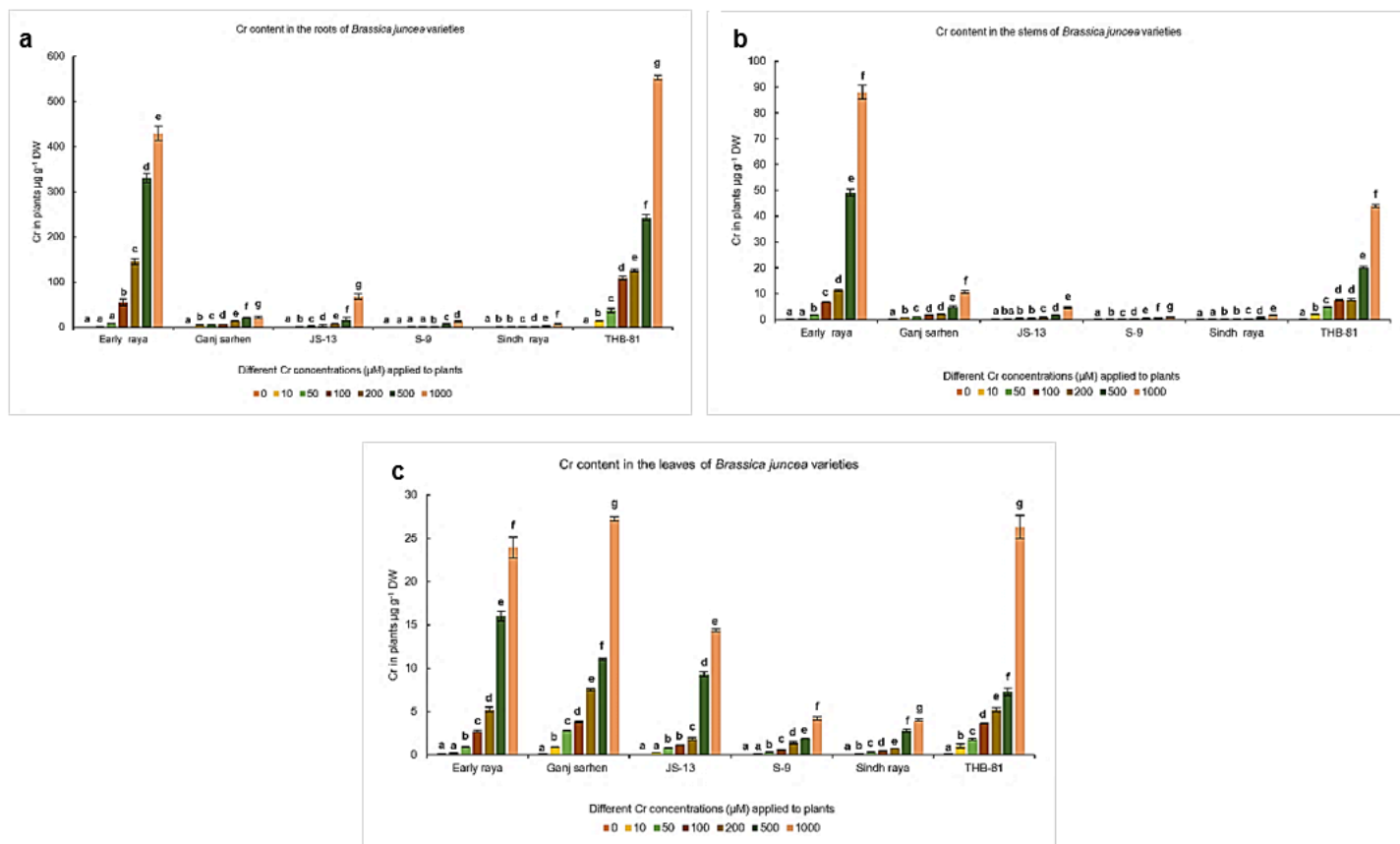


Figure 2

Chromium in different tissues of *Brassica juncea* varieties ($\mu\text{g g}^{-1}$ DW). The accumulation of Cr ($\mu\text{g g}^{-1}$ DW) in the roots (a), stems (b), and leaves (c) of *Brassica juncea* varieties- JS-13, Early raya, Sindh raya, S-9, Ganj sarhen, and THB-81 treated with different concentrations (0, 10, 50, 100, 200, 500, and 1000 μM) of CrCl_3 for two weeks (15 days). Data are means $\pm$ standard deviations (SD) ($n = 3$), with values indicating significant differences from the control at $p < 0.05$. Different letters indicate significant differences, highlighting varietal responses to Cr.

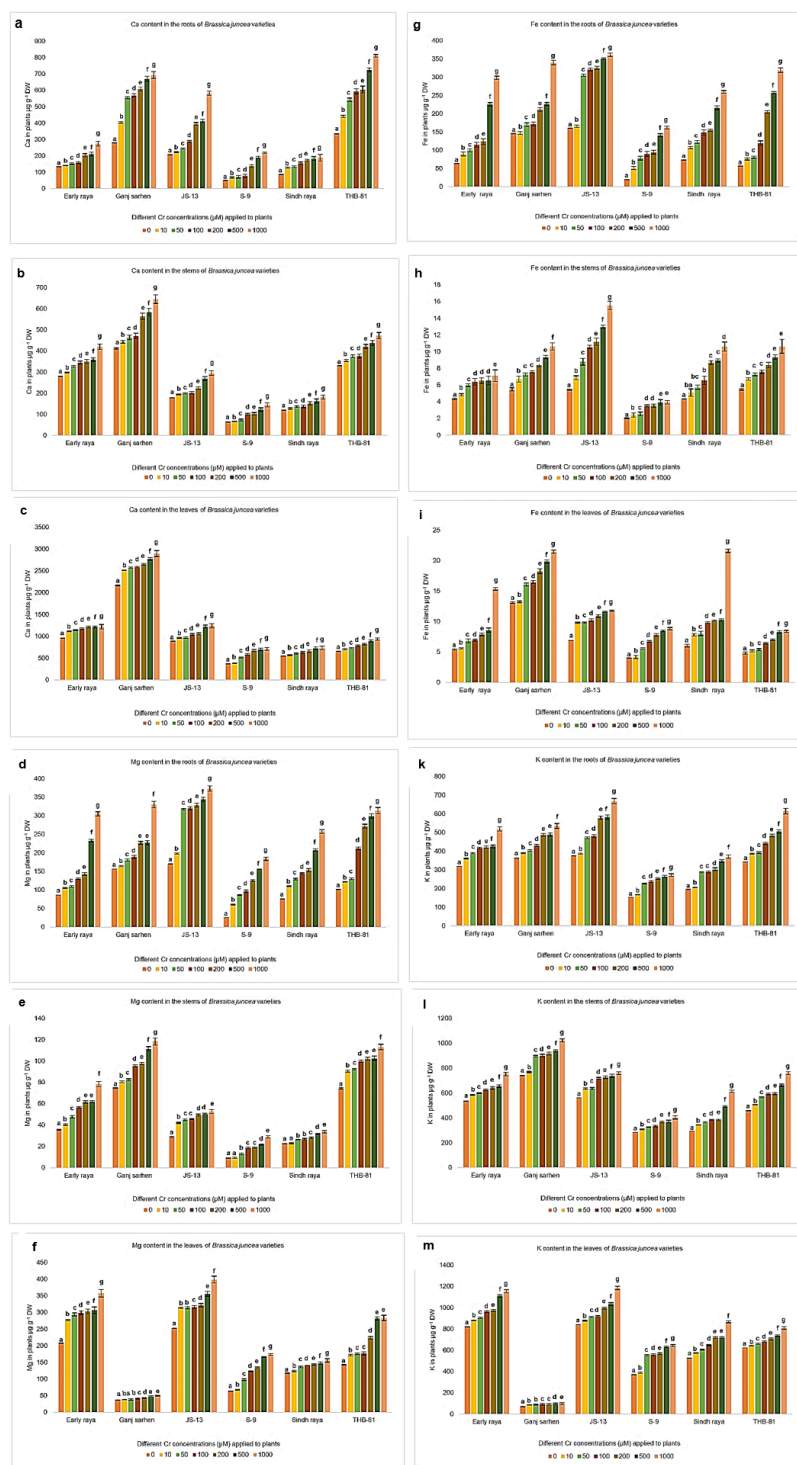


Figure 3

Ca (a, b, c), Mg (d, e, f), Fe (g, h, i), and K (k, l, m) distribution in different tissues of *Brassica juncea* varieties. The accumulation of metals ($\mu\text{g g}^{-1}$ DW) in roots, stems, and leaves of *Brassica juncea* varieties- JS-13, Early raya, Sindh raya, S-9, Ganj sarhen, and THB-81 plants, treated with CrCl_3 at different concentrations for two weeks (15 days). Data are means $\pm$ standard deviations (SD) ($n = 3$), with values

indicating significant differences from the control at $p < 0.001$. Different letters indicate significant differences, highlighting varietal responses to Cr.

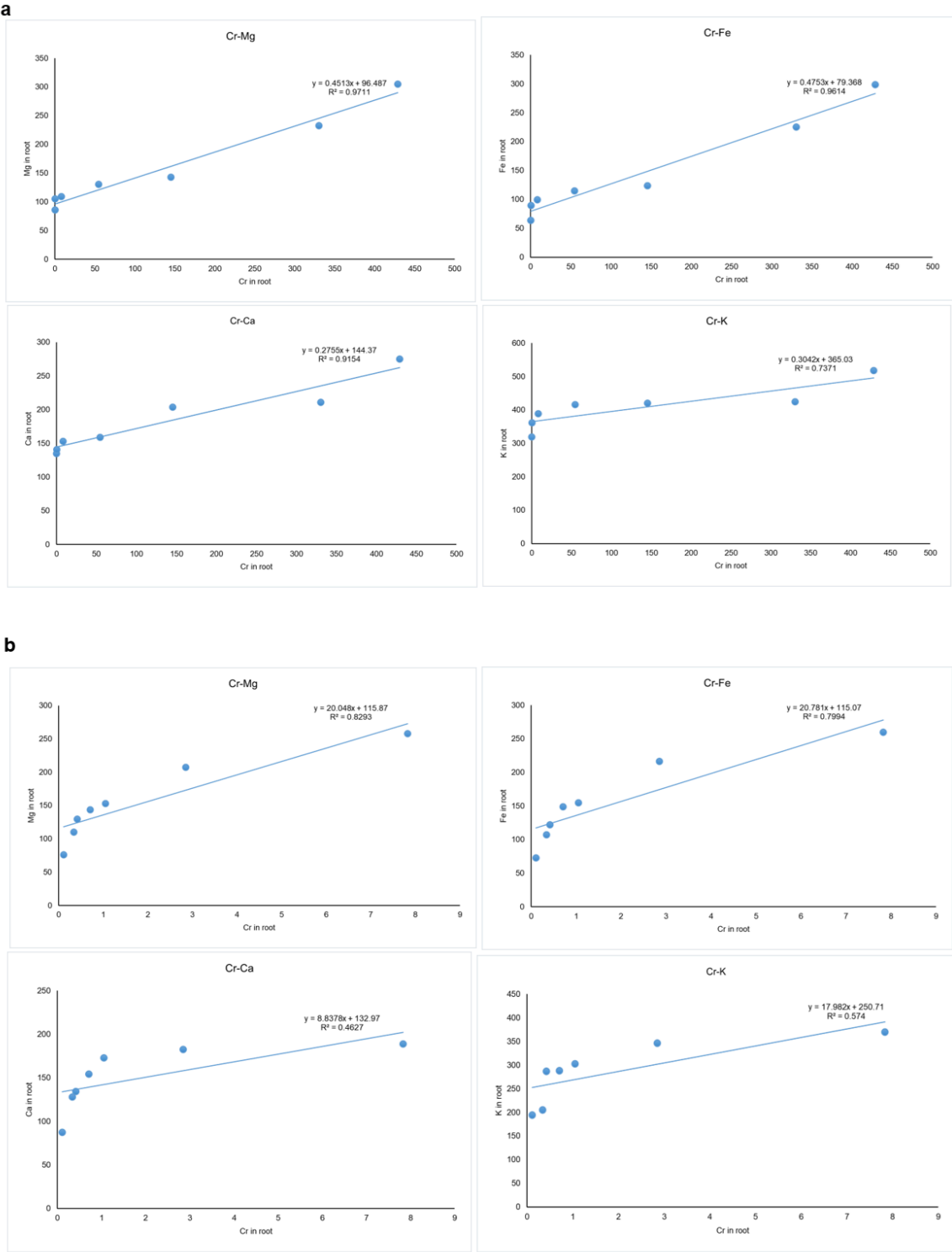


Figure 4

Regression analysis between Cr and Ca, Fe, Mg, and K contents ($\mu\text{g g}^{-1}$ DW) in the roots of *Brassica juncea* Early raya (a) and Sindh raya (b) under chromium stress.

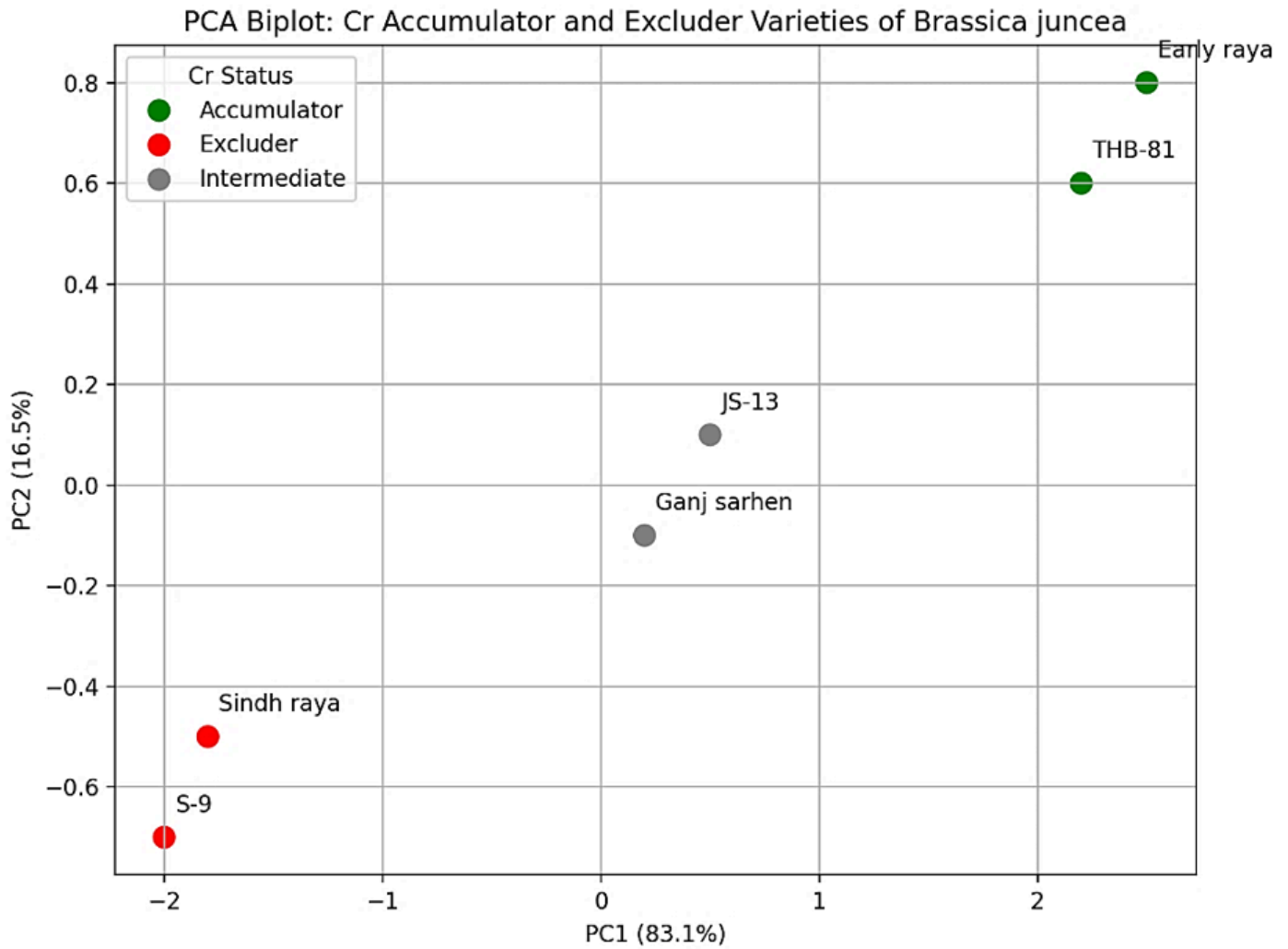


Figure 5

A PCA biplot showing Cr accumulator and excluder varieties of *Brassica juncea*. The total variance explained by PC1 and PC2 is 99.6%, indicating strong differentiation between varieties.

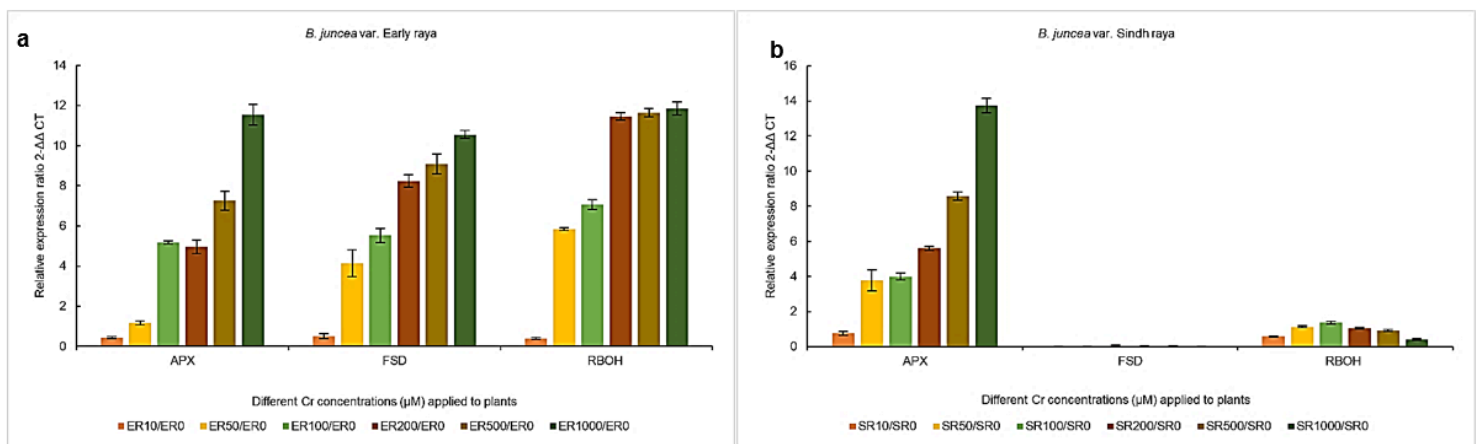


Figure 6

Expression of Ascorbate Peroxidase (APX), Iron-Superoxide Dismutase (FeSOD), and Respiratory Burst Oxidase Homolog (RBOH) in *Brassica juncea* var. Early raya (a) and Sindh raya (b) grew at different levels of Cr.

Supplementary Files

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