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# PHYSIOLOGICAL INDICES AND PCA-BASED EVALUATION OF CHROMIUM TOXICITY IN PEARL MILLET (*Pennisetum glaucum* L.), JOWAR (*Sorghum bicolor* L.) AND FINGER MILLET (*Eleusine coracana (*L.) Gaertn)

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## ABSTRACT

To evaluate the impact of chromium (Cr) contamination from unregulated waste disposal on dryland crops, a two-season experiment was conducted during the 2023 and 2024 Kharif seasons. The study aimed to assess the toxicological effects of chromium on physiological growth indices and tolerance mechanisms in crops of agronomic importance. Pearl millet (*Pennisetum glaucum* L.), jowar (*Sorghum bicolor* L.), and finger millet (*Eleusine coracana* L. Gaertn) were grown in red soils amended with chromium at concentrations of 0 (control), 4, 8, 12, and 15 mg/kg under a completely randomized design with four replications. Mean values from both seasons were used for analysis. Seed germination, total root number, root length, shoot height, and root:shoot ratio of seedlings were measured and integrated to calculate a metal tolerance index (MTI) for each crop. Results showed that increasing chromium concentrations exerted adverse effects on seed germination, early growth, and tolerance of crop seedlings, with the highest concentration (15 mg/kg) causing the most severe inhibition. Among the crops, jowar exhibited the highest sensitivity, followed by pearl millet and finger millet, indicating greater tolerance of ragi seedlings to chromium toxicity. Lower concentrations of chromium had a more pronounced negative effect on sorghum seedlings compared to the others, but were relatively less detrimental to pearl millet and finger millet. From a toxicological perspective, this study highlights the inhibitory effects of chromium even at low levels, underscoring the need to monitor and manage heavy metal contamination in soils to safeguard crop productivity and food safety.

**KEYWORDS:** Biochemical response, Chromium, Finger millet (*Eleusine coracana*(L.) Gaertn), Jowar (*Sorghum bicolor* L.), Pearl millet (*Pennisetum glaucum* L.),Seed germination .

## INTRODUCTION

Metals in terrestrial ecosystems play a crucial role in influencing plant growth, development and productivity; however, their excessive accumulation often results in adverse ecological and physiological consequences. In recent decades, rapid industrialization, urban expansion, and intensified agricultural practices have led to widespread contamination of soil ecosystems with heavy metals, primarily through anthropogenic activities (Gandhi *et al*.,

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2025a). Among these, the application of agricultural, municipal, and industrial wastes as soil amendments has emerged as a common yet largely unregulated practice, particularly in developing countries such as India (Gandhi *et al.*, 2025b). While low levels of waste inputs may temporarily enhance soil fertility by supplying nutrients and organic matter, continuous disposal of chemically enriched wastes often results in the gradual buildup of toxic metals that persist in soils and disrupt normal plant functions (Gandhi *et al.*, 2020a,b).

India, being predominantly agrarian and characterized by a rapidly growing population, relies heavily on intensive cropping systems to meet increasing food demands. Experimental crops such as pearl millet (*Pennisetum glaucum* L.), jowar (*Sorghum bicolor* L.) and finger millet (*Eleusine coracana* (L.)Gaertn) form the backbone of food and nutritional security in semi-arid and marginal regions. However, soil quality in these regions is increasingly threatened by the indiscriminate disposal of industrial effluents and solid wastes, which often contain elevated concentrations of heavy metals. Once introduced, these metals remain persistent in soil matrices, posing long-term risks to crop productivity and food safety. Chromium is one of the most problematic heavy metals encountered in agricultural soils, primarily originating from tannery, electroplating, dye, and paint manufacturing industries. Its high stability and mobility in soil–plant systems enable easy uptake by plants, leading to phytotoxic effects even at relatively low concentrations. Chromium stress has been reported to impair seed germination, root and shoot development, photosynthetic efficiency, nutrient uptake, and metabolic processes, often inducing oxidative stress and biochemical imbalances in plants. Previous studies have demonstrated significant reductions in growth and physiological performance of crops exposed to chromium, including *Amaranthus tricolour* and *Sesamum indicum*, highlighting the sensitivity of early developmental stages to metal stress (Gandhi *et al*., 2017).

Several investigations by Gandhi and co-workers have emphasized that heavy metal stress alters germination behavior, physiological indices, and biochemical attributes in food and pulse crops such as pigeon pea (*Cajanus cajan*) under varying concentrations of copper, lead, manganese, barium, and chromium (Gandhi *et al*., 2020a). Similarly, *Sorghum bicolor* has been reported to exhibit both sensitivity and phytoremediation potential under lead-contaminated soils, suggesting species-specific variations in metal tolerance and accumulation capacity. Despite these insights, comprehensive comparative evaluations of chromium toxicity in major dryland cereals, particularly integrating multivariate statistical approaches, remain limited under Indian agro-ecological conditions. Physiological indices provide sensitive indicators of plant stress responses, enabling early detection of metal toxicity before visible symptoms appear. However, given the complex and interrelated nature of physiological parameters, univariate analyses alone may not sufficiently capture stress response patterns. Principal Component Analysis (PCA) offers a robust multivariate framework to identify key traits contributing to metal tolerance and to discriminate crop-specific adaptive responses under varying contamination levels. Therefore, the present study aims to evaluate the physiological responses and chromium-induced stress indices in pearl millet, jowar, and finger millet grown under increasing chromium concentrations. By integrating physiological measurements with PCA-based evaluation, this study seeks to elucidate species-specific tolerance mechanisms and identify critical physiological traits governing

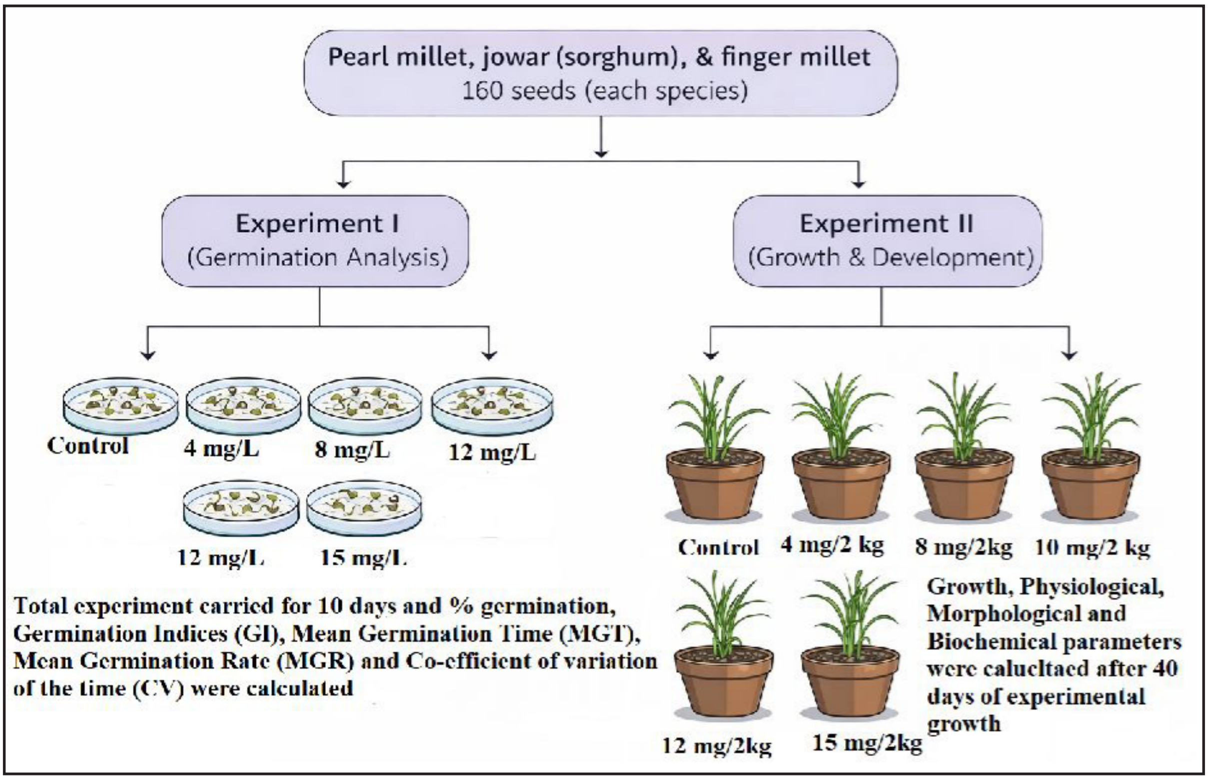


**Fig. 1. Overview of the experimental design showing Experiment I (Petri dish based germination assay) and Experiment II (pot culture based growth and development assessment) under varying chromium concentrations.**

chromium toxicity in dryland crops. The findings are expected to contribute to safer land-use strategies, informed crop selection, and sustainable management of waste-amended agricultural soils.

## MATERIAL AND METHODS

A total of 160 seeds each of pearl millet (*Pennisetum glaucum* L.), jowar (*Sorghum bicolor* L.), and finger millet (*Eleusine coracana* (L.) Gaertn.) were randomly selected, surface-sterilized, and used for germination and early growth assessment. For the germination test, seeds were placed in sterilized disposable polystyrene Petri dishes and treated with potassium dichromate (K, Cr, O‡ ) solutions prepared at concentrations of 4, 8, 10, 12, and 15 mg/L, while control seeds received double-distilled water. These concentrations were chosen to simulate chromium levels typically present in waste-amended agricultural soils. For subsequent seedling development studies, another set of 160 seeds per species was sown in earthen pots of 45 cm depth, each filled with a standardized growth medium consisting of 2 kg red soil, 1 kg sand and cocopeat mixture, and 1 kg farmyard manure. The pot depth was considered non-limiting since all crops are monocots with shallow, fibrous root systems, and the study focused exclusively on early growth responses. All pots were irrigated with the respective chromium solutions, and controls received double-distilled water, with adequate aeration maintained throughout. Germination was monitored daily from 0 to 10 days, and germination percentages and indices reported in Tables 7, 8, and 9 were calculated based on this defined period. Seedlings (pot experiment) were allowed to grow under treatment conditions for 40 days, which served as the uniform sampling age for all

**Table 1. Empirical formulas used to calculate germination analysis of crops treated with different frequencies of microwave**

| S.No | Formulas used to calculate growth parameters |
|---|---|
| 01 | $\% \, of Germination = \frac{Number of Seeds Germinated}{Total Number of Seeds Planted} X \, 100$ |
| 02 | $Germination \; Index \; (GI) = \sum_{i=1}^{k} \frac{No. of \; germinated \; seeds}{the \; count \; day}$<br>Where i=1 day one, k is the last day of observation |
| 03 | $Mean \; Germination \; Time \; (MGT) = \frac{\sum_{i=1}^{k} n_i \, t_i}{\sum_{i=1}^{k} n_i}$<br>Where $t_i$ is the time from day one to the last day of observation, $n_i$ is an observed number of germinated seeds every day and k is the last germination day of observation. |
| 04 | $Mean \; Germination \; Rate \; (MGR) = \frac{1}{Mean \; Germination \; Time}$ |
| 05 | $Co-efficient \; of \; variation \; of \; the \; time = \frac{S_t}{Mean \; Germination \; Time} X \, 100$ |

**Table 2. Empirical formulas used to calculate growth analysis of crops treated with different frequencies of microwave**

| S.No | Formulas used to calculate growth parameters |
|---|---|
| 01 | $Relative \; Growth \; Rate = \frac{loge^{W_2} - loge^{W_1}}{T_2 - T_1}$ |
| 02 | $Net \; Assimilation \; Rate = \frac{W_2 - W_1}{T_2 - T_1} X \frac{loge^{A_2} - loge^{A_1}}{A_2 - A_1}$<br>Where W1, W2 are dry weight of seedlings, at time T1 and T2 respectively. A1 and A2 are leaf area at time T1 and T2. |
| 03 | $Leaf \; Area \; Ratio = \frac{A}{W}$ |
| 04 | $Leaf \; Weight \; Ratio \; (LWR) = \frac{W_L}{W}$<br>Where W is total dry weight of seedling and WL is dry weight of leaves at time t |
| 05 | $Specific \; Leaf \; Area \; (SLA) = \frac{A}{W_L}$ |
| 06 | $Specific Leaf Weight (SLW) = \frac{W_L}{A}$ |
| 07 | $Leaf Area Duration (LAD) = \frac{LA_1 + \; LA_2 \; (T_2 - T_1)}{2}$ |

morphological, biochemical and physiological measurements including root and shoot length. The experimental duration was intentionally limited to 40 days, consistent with the study's objective of evaluating chromium-induced effects on early establishment, initial vigour, and physiological performance, rather than full-season crop productivity (Fig.1).

### Germination Analysis

Germination was assessed when the radicle exceeded 2 mm in length. Parameters such as Germination Percentage (GP), Mean Germination Time (MGT), Mean Germination Rate (MGR), Growth Index (GI), and Coefficient of Variation (CV) were calculated using standard formulas (Table 1) described by Gandhi *et al.*, (2025a).

### Growth Analysis

Plant growth parameters, including **RGR, NAR, LAR, LAD etc.,** were measured at different growth stages. Additional growth indices were calculated by using standard formula (Table 2) described by Gandhi *et al.*, (2022a,b)

### Physiological analysis

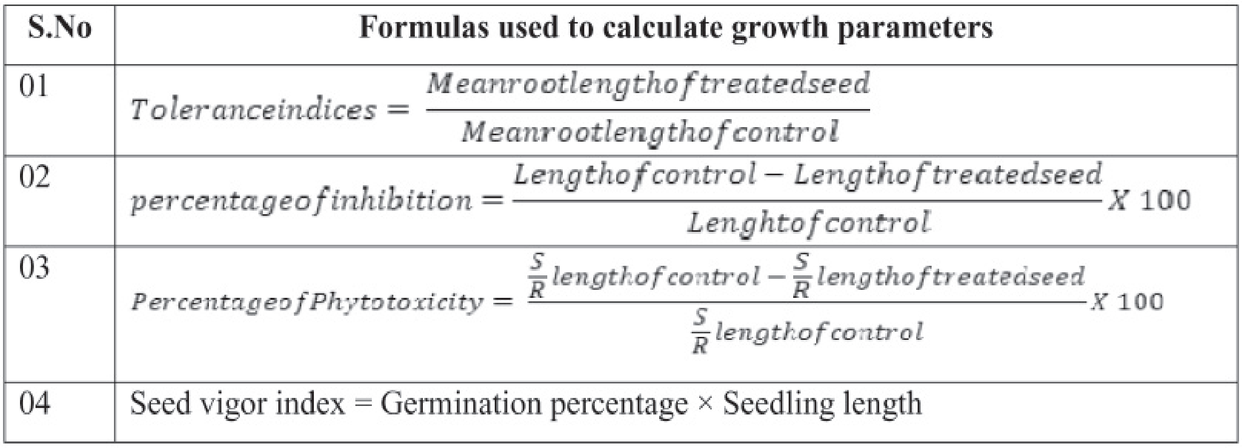
Physiological traits, including Seedling Vigor Index (SVI), tolerance indices and percentage phytotoxicity, were analyzed (Table 3) to assess plant resilience. Heavy metal accumulation in plant tissues was also monitored (Gandhi *et al.*, 2015b, 2025b,c).

### Biochemical analysis

### Determination of plant pigments (Chlorophyll-a, Chlorophyll-b, Carotenoids and Lycopene)

Pigment extraction and quantification were performed following the method described by Gandhi *et al.* (2022), with slight modifications. Fresh leaf samples (5 g) from each treatment group were homogenized with 10 mL of an acetone–hexane mixture (2:3 v/v) for 2 minutes to achieve a uniform suspension. To minimize thermal degradation of pigments during processing, all samples were maintained in an ice-water bath. The homogenized samples were centrifuged at 5000 rpm for 10 minutes at 20 °C using an Eppendorf centrifuge. The supernatants were collected and their absorbance was measured using a Cary 50 Scan UV/VIS spectrophotometer at wavelengths of 453, 505, 645 and 663 nm. The concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), α-carotene, and lycopene were calculated using the standard equations.

**Table 3. Empirical formulas used to calculate physiological analysis of crops treated with different frequencies of microwave**



| S.No | Formulas used to calculate growth parameters |
|---|---|
| 01 | $Toleranceindices = \frac{Meanrootlengthoftreatedseed}{Meanrootlengthofcontrol}$ |
| 02 | $percentageofinhibition = \frac{Lengthofcontrol - Lengthoftreatedseed}{Lenghtofcontrol} X\ 100$ |
| 03 | $PercentageofPhytotoxicity = \frac{\frac{S}{R}lengthofcontrol - \frac{S}{R}lengthoftreatedseed}{\frac{S}{R}lengthofcontrol} X\ 100$ |
| 04 | Seed vigor index = Germination percentage × Seedling length |

$$\text{Chlorophyll a } (^{mg}/_{100ml}) = 0.999A_{663} - 0.0989A_{645}$$

$$\text{Chlorophyll b } (^{mg}/_{100ml}) = -0.328A_{663} + 1.77A_{645}$$

$$\beta\text{-Carotene}(^{mg}/_{100ml}) = 0.216A_{663} - 1.22A_{645} - 0.304A_{505} - 0.425A_{453}$$

$$\text{Lycopene}(^{mg}/_{100ml}) = 0.048A_{663} + 0.204A_{645} + 0.372_{505} - 0.0806_{453}$$

### Determination of Carbohydrates

The total carbohydrate content of seeds from each treatment group was determined using the Anthrone method. Seed samples (100 mg) were ground into a fine powder and hydrolyzed with 5 mL of 2.5 N hydrochloric acid in a boiling water bath for 3 hours to convert polysaccharides into simple sugars. After hydrolysis, the solution was cooled to room temperature and neutralized with sodium carbonate until effervescence ceased. The volume was made up to 10 mL with distilled water and centrifuged at 5000 rpm for 10 minutes to obtain a clear supernatant.

A 1 mL aliquot of the supernatant was mixed with 4 mL of freshly prepared Anthrone reagent (0.2% Anthrone in concentrated sulfuric acid) in a test tube. The mixture was vortexed briefly and incubated in a boiling water bath for 10 minutes, followed by cooling to room temperature. The absorbance of the resulting green-colored solution was measured at 620 nm using a UV/VIS spectrophotometer (Cary 50 Scan). The total carbohydrate content was calculated using a standard curve prepared with D-glucose and expressed as milligrams per gram of dry weight (mg/g DW) of seed samples.

### Determination of Proteins

The total protein content of *experimental* crops was determined following the total nitrogen estimation method using a Continuous Flow Autoanalyzer (CFA) – San++ (Skalar), based on a modified Berthelot reaction. This method relies on the quantification of nitrogen, which is then converted to protein content using a standard conversion factor. Finely ground seed samples from each treatment were dried overnight at 55°C to remove residual moisture. Approximately 0.15 g of dried sample was accurately weighed into a 75 mL digestion tube. For quality assurance, each batch included blank samples and standards. To each digestion tube, 3.5 mL of a sulfuric acid-selenium digestion mixture was added. The tubes were placed in a block digester and heated at 360°C for 2 hours to complete the digestion process. After cooling, the digested contents were diluted to a final volume of 75 mL with distilled water and thoroughly mixed.

The digested samples were analyzed using the CFA, where ammonia released from the digested material reacted with salicylate and chloramine to form a green-colored complex. The absorbance of this complex was measured at 660 nm. The nitrogen concentration was calculated using a calibration curve prepared with a series of working standards ranging from 40 to 120 ppm nitrogen. The total nitrogen content was converted to protein content using the formula:

Protein (%) = Total N (%) × 6.25

Where, 6.25 is the nitrogen-to-protein conversion factor for plant materials. The protein content was expressed as a percentage of dry weight. Measurements were performed in triplicate for each sample to ensure precision and reliability of the data.

### Determination of Peroxidase (POD) Activity

Peroxidase (POD) activity was measured based on the oxidation of guaiacol in the presence of hydrogen peroxide ($H_2O_2$). Fresh seeds were ground in liquid nitrogen, and 0.5 g of the powdered sample was homogenized in 5 mL of ice-cold 0.1 M phosphate buffer (pH

**Table 4. Biochemical and morphological response of *Pennisetum glaucum* (pearl millet), at age of 40 days under varying concentrations of chromium (Mean ± SD)**

| S.No | Parameter | Control | 4mg/kg | 8 mg/kg | 12 mg/kg | 15 mg/kg |
|---|---|---|---|---|---|---|
| 01 | Shoot length (cm) | 18.0 ± 1.2 | 12.0 ± 0.9 | 10.0 ± 0.7 | 10.0 ± 0.8 | 4.0 ± 0.5 |
| 02 | Root length (cm) | 2.2 ± 0.2 | 1.4 ± 0.1 | 0.8 ± 0.1 | 0.8 ± 0.1 | 0.2 ± 0.05 |
| 03 | Fresh weight (g) | 0.16 ± 0.01 | 0.18 ± 0.02 | 0.09 ± 0.01 | 0.13 ± 0.01 | 0.07 ± 0.01 |
| 04 | Dry weight (g) | 0.01±0.001 | 0.01±0.001 | 0.001±0.002 | 0.01±0.001 | 0.01±0.002 |
| 05 | Total Carbohydrates mg/ 100g | 21.4 ± 1.1 | 21.9 ± 1.2 | 22.5 ± 1.0 | 26.7 ± 1.5 | 12.4 ± 0.8 |
| 06 | Total proteins (mg/g) | 27.5 ± 1.3 | 21.5 ± 1.0 | 21.5 ± 1.0 | 22.5 ± 1.2 | 20.0 ± 1.1 |
| 07 | Chlorophyll-a (mg/g) | 2.6 ± 0.2 | 2.1 ± 0.2 | 1.8 ± 0.1 | 1.5 ± 0.1 | 0.9 ± 0.1 |
| 08 | Chlorophyll-b(mg/g) | 1.4 ± 0.1 | 1.2 ± 0.1 | 1.0 ± 0.1 | 0.9 ± 0.05 | 0.5 ± 0.05 |
| 09 | Pheaophytins(mg/g) | 0.6 ± 0.05 | 0.7 ± 0.05 | 0.9 ± 0.06 | 1.2 ± 0.06 | 1.4 ± 0.1 |
| 10 | Caroteniods (mg/g) | 1.9 ± 0.1 | 1.7 ± 0.1 | 1.4 ± 0.1 | 1.2 ± 0.08 | 0.8 ± 0.07 |
| 11 | Peroxidase (U/mg) | 22.0 ± 1.5 | 28.5 ± 1.7 | 34.2 ± 2.0 | 40.5 ± 2.2 | 47.3 ± 2.5 |

**Table 5. Biochemical and morphological response of *Sorghum bicolor* (Jowar) at age of 40 days, under varying concentrations of chromium (Mean ± SD)**

| S.No | Parameter | Control | 4mg/kg | 8 mg/kg | 12 mg/kg | 15 mg/kg |
|---|---|---|---|---|---|---|
| 01 | Shoot length | 21.2 ± 1.3 | 21.4 ± 1.2 | 17.0 ± 1.1 | 9.4 ± 0.8 | 10.0 ± 0.9 |
| 02 | Root length | 8.7 ± 0.5 | 6.8 ± 0.4 | 7.2 ± 0.4 | 3.6 ± 0.3 | 6.2 ± 0.4 |
| 03 | Fresh weight | 0.56 ± 0.03 | 0.36 ± 0.02 | 0.22 ± 0.01 | 0.15 ± 0.01 | 0.24 ± 0.02 |
| 04 | Dry weight | 0.38 ± 0.02 | 0.02±0.001 | 0.01±0.001 | 0.002±0.000 | 0.024±0.002 |
| 05 | Total Carbohydrates | 22.8 ± 1.2 | 21.2 ± 1.0 | 21.4 ± 1.1 | 25.8 ± 1.3 | 18.9 ± 0.9 |
| 06 | Total proteins | 30.0 ± 0.2 | 27.6 ± 1.4 | 25.2 ± 1.2 | 25.8 ± 2.3 | 22.1 ± 1.1 |
| 07 | Chlorophyll-a | 3.0 ± 0.2 | 2.5 ± 0.2 | 2.0 ± 0.1 | 1.2 ± 0.1 | 1.0 ± 0.08 |
| 08 | Chlorophyll-b | 1.8 ± 0.1 | 1.5 ± 0.1 | 1.2 ± 0.1 | 0.7 ± 0.05 | 0.6 ± 0.05 |
| 09 | Pheaophytins | 0.5 ± 0.04 | 0.7 ± 0.05 | 0.8 ± 0.05 | 1.3 ± 0.1 | 1.5 ± 0.1 |
| 10 | Caroteniods | 2.0 ± 0.15 | 1.7 ± 0.1 | 1.3 ± 0.1 | 1.0 ± 0.08 | 0.7 ± 0.05 |
| 11 | peroxidase | 20.2 ± 1.5 | 25.8 ± 1.7 | 32.1 ± 1.9 | 39.4 ± 2.1 | 45.6 ± 2.4 |

7.0) with 1% PVP. The homogenate was centrifuged at 12,000 rpm for 20 minutes at 4°C, and the supernatant was collected as the enzyme extract. The reaction mixture consisted of 0.1 M phosphate buffer (pH 7.0), 20 mM guaiacol, 10 mM $H_2O_2$, and 0.1 mL of enzyme extract. The reaction was initiated by adding $H_2O_2$, and the increase in absorbance due to tetra-guaiacol formation was monitored at 470 nm for 3 minutes. POD activity was expressed as the change in absorbance per minute per gram of fresh weight.

## RESULTS AND DISCUSSION

Pearl millet (*Pennisetum glaucum* L.), exhibited (Table 4) a progressive decline in

**Table 6. Biochemical and morphological response of *Eleusine coracana* (finger millet) at age of 40 days, under varying concentrations of chromium**

| S.No | Parameter | Control | 4mg/kg | 8 mg/kg | 12 mg/kg | 15 mg/kg |
|---|---|---|---|---|---|---|
| 01 | Shoot length | 16.5 ± 1.1 | 12.3 ± 0.9 | 9.0 ± 0.7 | 6.0 ± 0.5 | 3.2 ± 0.3 |
| 02 | Root length | 5.4 ± 0.4 | 3.5 ± 0.3 | 2.8 ± 0.2 | 2.0 ± 0.2 | 1.0 ± 0.1 |
| 03 | Fresh weight | 0.34 ± 0.02 | 0.27 ± 0.02 | 0.18 ± 0.01 | 0.12 ± 0.01 | 0.06±0.005 |
| 04 | Dry weight | 0.02±0.001 | 0.01±0.001 | 0.008±0.00 | 0.004±0.000 | 0.001±0.000 |
| 05 | Total Carbohydrates | 19.4 ± 1.1 | 18.7 ± 1.0 | 21.2 ± 1.2 | 24.3 ± 1.4 | 13.8 ± 0.9 |
| 06 | Total proteins | 25.3 ± 1.3 | 22.5 ± 1.1 | 20.2 ± 1.0 | 21.1 ± 1.1 | 17.6 ± 0.9 |
| 07 | Chlorophyll-a | 2.3 ± 0.2 | 2.0 ± 0.2 | 1.5 ± 0.1 | 1.0 ± 0.1 | 0.6 ± 0.05 |
| 08 | Chlorophyll-b | 1.3 ± 0.1 | 1.1 ± 0.1 | 0.8 ± 0.08 | 0.5 ± 0.05 | 0.3 ± 0.03 |
| 09 | Pheaophytins | 0.7 ± 0.05 | 0.9 ± 0.06 | 1.2 ± 0.08 | 1.5 ± 0.1 | 1.9 ± 0.12 |
| 10 | Caroteniods | 1.6 ± 0.1 | 1.3 ± 0.1 | 1.0 ± 0.08 | 0.7 ± 0.07 | 0.4 ± 0.04 |
| 11 | peroxidase | 18.5 ± 1.4 | 24.1 ± 1.6 | 30.3 ± 2.0 | 36.7 ± 2.2 | 43.2 ± 2.4 |

**Table 7. Germination indices of experimental crops (pearl millet, jowar and finger millet) under varying concentration chromium (0-10 days observation Mean ± SD)**

| Crop | Treatment | %Germination | GI | MGT (days) | MGR (1/day) | CV (%) |
|---|---|---|---|---|---|---|
| Pearl millet | Control | 98 ± 1.2 | 15.6 ± 0.5 | 2.1 ± 0.1 | 0.476 ± 0.02 | 6.2 |
| | 4mg/kg | 90 ± 2.0 | 12.8 ± 0.6 | 2.6 ± 0.1 | 0384 ± 0.02 | 8.4 |
| | 8mg/kg | 78 ± 2.3 | 10.1 ± 0.4 | 3.4 ± 0.2 | 0.294 ± 0.01 | 11.2 |
| | 12mg/kg | 62 ± 2.7 | 7.2 ± 0.3 | 4.1 ± 0.2 | 0.244 ± 0.01 | 14.5 |
| | 15mg/kg | 45 ± 3.0 | 5.0 ± 0.3 | 5.3 ± 0.3 | 0.189 ± 0.01 | 16.8 |
| Jowar | Control | 96 ± 1.1 | 16.2 ± 0.6 | 2.0 ± 0.1 | 0.500 ± 0.02 | 5.9 |
| | 4mg/kg | 92 ± 1.5 | 14.8 ± 0.5 | 2.3 ± 0.1 | 0.434 ± 0.02 | 7.0 |
| | 8mg/kg | 84 ± 2.0 | 12.2 ± 0.6 | 3.0 ± 0.1 | 0.333 ± 0.02 | 9.8 |
| | 12mg/kg | 70 ± 2.5 | 9.1 ± 0.4 | 3.8 ± 0.2 | 0.263 ± 0.01 | 12.6 |
| | 15mg/kg | 55 ± 3.2 | 6.4 ± 0.3 | 4.6 ± 0.2 | 0.217 ± 0.01 | 15.9 |
| Finger millet | Control | 97 ± 1.3 | 14.4 ± 0.5 | 2.2 ± 0.1 | 0.454 ± 0.02 | 6.4 |
| | 4mg/kg | 89 ± 2.1 | 12.5 ± 0.5 | 2.7 ± 0.1 | 0.370 ± 0.02 | 8.7 |
| | 8mg/kg | 75 ± 2.5 | 9.8 ± 0.4 | 3.5 ± 0.2 | 0.286 ± 0.01 | 11.6 |
| | 12mg/kg | 60 ± 2.8 | 6.9 ± 0.3 | 4.2 ± 0.2 | 0.238 ± 0.01 | 13.8 |
| | 15mg/kg | 43 ± 3.1 | 4.8 ± 0.3 | 5.6 ± 0.3 | 0.179 ± 0.01 | 17.5 |

growth and physiological performance with increasing chromium concentration. Shoot and root lengths reduced markedly from 18.0 and 2.2 cm in the control to 4.0 and 0.2 cm at 15 mg/kg Cr, reflecting strong inhibition of early seedling development. Fresh weight showed a slight increase at 4 mg/kg, suggesting a short-term osmotic adjustment, but declined

**Table 8. Physiological and toxicity response pearl millet, jowar and finger millet crops under varying concentrations of chromium (0-10 days observation mean ± SD)**

| Crop | Treatment | SVI | Tolerance index (%) | % inhibition | % phytotoxicity |
|---|---|---|---|---|---|
| Pearl millet | Control | 1800 ± 25 | 100.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | 4mg/kg | 1250 ± 22 | 72.1 ± 2.5 | 27.9 ± 2.5 | 21.0 ± 1.9 |
| | 8mg/kg | 950 ± 18 | 52.7 ± 2.2 | 47.3 ± 2.2 | 38.5 ± 2.1 |
| | 12mg/kg | 900 ± 17 | 50.0 ± 1.8 | 50.0 ± 1.8 | 41.2 ± 2.0 |
| | 15mg/kg | 700 ± 16 | 38.8 ± 1.5 | 61.2 ± 1.5 | 53.4 ± 2.3 |
| Jowar | Control | 1900 ± 26 | 100.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | 4mg/kg | 1650 ± 23 | 86.8 ± 2.1 | 13.2 ± 2.1 | 9.4 ± 1.4 |
| | 8mg/kg | 1320 ± 20 | 69.5 ± 2.0 | 30.5 ± 2.0 | 23.7 ± 1.7 |
| | 12mg/kg | 920 ± 18 | 48.4 ± 1.6 | 51.6 ± 1.6 | 39.0 ± 2.0 |
| | 15mg/kg | 860 ± 17 | 45.2 ± 1.4 | 54.8 ± 1.4 | 42.5 ± 2.1 |
| Finger millet | Control | 1850 ± 24 | 100.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| | 4mg/kg | 1480 ± 22 | 80.0 ± 2.0 | 20.0 ± 2.0 | 15.5 ± 1.5 |
| | 8mg/kg | 1180 ± 20 | 63.8 ± 1.9 | 36.2 ± 1.9 | 28.8 ± 1.6 |
| | 12mg/kg | 950 ± 19 | 51.4 ± 1.7 | 48.6 ± 1.7 | 37.9 ± 2.0 |
| | 15mg/kg | 780 ± 18 | 42.2 ± 1.5 | 57.8 ± 1.5 | 48.1 ± 2.3 |

sharply thereafter, accompanied by consistently low dry weights. Carbohydrate content increased up to 12 mg/kg, likely due to stress-induced metabolic adjustments, while protein content decreased steadily, indicating impaired nitrogen metabolism. Chromium exposure also caused substantial reductions in chlorophyll a, chlorophyll b, and carotenoids, while pheophytin levels increased, confirming accelerated pigment degradation. The strong rise in peroxidase activity demonstrates induction of antioxidant defenses; however, the defense was insufficient to protect pigments and growth at higher Cr levels. These responses collectively position pearl millet as moderately sensitive to chromium toxicity. Jowar (*Sorghum bicolor*) showed comparatively greater tolerance across growth and physiological traits (Table 5). Although shoot and root lengths decreased under Cr stress, the reductions were less severe than in pearl millet and finger millet, with seedlings retaining better structural integrity even at 15 mg/kg. Fresh and dry weights declined gradually but remained relatively higher than the other species. Carbohydrate content fluctuated across treatments, with a temporary increase at 12 mg/kg, and protein levels showed only a moderate reduction, indicating that metabolic processes were less severely disrupted. Photosynthetic pigments decreased with increasing Cr concentrations, yet jowar maintained higher chlorophyll and carotenoid levels than the other crops, suggesting better preservation of photosynthetic machinery. While pheophytin content increased, the magnitude of pigment degradation was lower, and enhanced peroxidase activity provided a more effective oxidative stress response.

**Table 9. Growth and development responses of pearl millet, jowar and finger millet under varying concentrations of chromium (0-10 days observation Mean ± SD)**

| Crop | Parameter | Control | 4 mg/kg | 8 mg/kg | 12 mg/kg | 15 mg/kg |
|---|---|---|---|---|---|---|
| Pearl millet | RGR (g/g/day) | 0.085±0.004 | 0.073±0.003 | 0.056±0.002 | 0.042±0.002 | 0.025±0.001 |
| | NAR (g/m$^2$/day) | 0.036±0.002 | 0.030±0.002 | 0.024±0.001 | 0.019±0.001 | 0.012±0.001 |
| | LAR (cm$^2$/g) | 152.4±4.2 | 148.5±4.0 | 139.2±3.7 | 126.7±3.5 | 114.5±3.0 |
| | LAD (days) | 18.6±0.6 | 16.8±0.5 | 14.4±0.4 | 12.2±0.4 | 9.6±0.3 |
| | SLA (cm$^2$/g) | 170.3±3.8 | 162.8±4.1 | 150.5±3.6 | 142.1±4.0 | 128.3±3.3 |
| | SLW (g/cm$^2$) | 0.0059±0.00 | 0.0061±0.00 | 0.0066±0.00 | 0.0070±0.00 | 0.0078±0.00 |
| Jowar | RGR (g/g/day) | 0.091±0.004 | 0.080±0.003 | 0.066±0.003 | 0.048±0.002 | 0.033±0.002 |
| | NAR (g/m$^2$/day) | 0.038±0.002 | 0.032± 0.002 | 0.027±0.001 | 0.020±0.001 | 0.014±0.001 |
| | LAR (cm$^2$/g) | 160.8±4.5 | 155.2±4.2 | 144.6±3.9 | 132.3±3.6 | 119.7±3.1 |
| | LAD (days) | 20.2±0.7 | 18.3±0.6 | 15.9±0.5 | 13.1±0.4 | 10.5±0.3 |
| | SLA (cm$^2$/g) | 175.9±4.4 | 169.6±4.3 | 158.2±3.9 | 147.4±3.7 | 133.6±3.2 |
| | SLW (g/cm$^2$) | 0.0056±0.00 | 0.0059±0.00 | 0.0063±0.00 | 0.0068±0.00 | 0.0075±0.00 |
| Finger millet | RGR (g/g/day) | 0.088±0.004 | 0.076±0.003 | 0.059±0.002 | 0.043±0.002 | 0.027±0.001 |
| | NAR (g/m$^2$/day) | 0.035±0.002 | 0.029±0.002 | 0.023±0.001 | 0.018±0.001 | 0.11±0.001 |
| | LAR (cm$^2$/g) | 155.6±4.3 | 150.3±4.1 | 138.7±3.7 | 125.9±3.4 | 112.6±3.0 |
| | LAD (days) | 19.4±0.5 | 17.6±0.5 | 14.8±0.4 | 12.5±0.3 | 9.4±0.3 |
| | SLA (cm$^2$/g) | 168.7±4.0 | 160.2±4.2 | 149.0±3.6 | 137.4±3.4 | 124.3±3.2 |
| | SLW (g/cm$^2$) | 0.0059±0.00 | 0.0067±0.00 | 0.0073±0.00 | 0.0080±0.00 | 0.0080±0.00 |

Overall, jowar displayed superior chromium tolerance and sustained physiological stability.

Finger millet was the most adversely affected by chromium stress (Table 6). Shoot and root lengths dropped drastically from 16.5 and 5.4 cm to only 3.2 and 1.0 cm, reflecting severe growth inhibition. Correspondingly, fresh and dry weights showed a dramatic reduction, indicating strong suppression of biomass accumulation. Although carbohydrate levels slightly increased at intermediate Cr concentrations, both carbohydrates and proteins declined sharply at 15 mg/kg, demonstrating major disruption of primary metabolism. Photosynthetic pigments were severely depleted; chlorophyll a, chlorophyll b, and carotenoids showed the largest reductions among the three crops, while pheophytin accumulation confirmed accelerated pigment breakdown. Despite an increase in peroxidase activity, the antioxidant response was inadequate to mitigate pigment loss and metabolic suppression. Collectively, these observations identify finger millet as highly sensitive to chromium toxicity, with limited adaptive capacity. Comparing the overall responses, jowar emerged as the most tolerant species, maintaining relatively higher growth, pigment concentrations, and metabolic stability under Cr stress. Pearl millet showed intermediate tolerance with moderate impairment, while finger millet was extremely susceptible, exhibiting the most pronounced reductions in growth, biomass, pigments, and protein content. The species-specific differences reflect variations in chromium uptake, detoxification mechanisms, and antioxidant capacity, positioning jowar as the most suitable crop for chromium-affected soils.

Increasing chromium concentrations had a pronounced negative impact on germination, physiological behavior, and growth dynamics of all three dryland cereals (Table 7, 8, 9). Germination percentage declined steadily in each species, with pearl millet reducing from 98% to 45% and finger millet from 97% to 43%, while sorghum retained relatively higher germination (96% to 55%), indicating its superior early-stage tolerance. GI progressively decreased, whereas MGT increased in all crops, reflecting delayed and weakened germination under stress. Correspondingly, MGR declined sharply; in pearl millet , it dropped from 0.476 to 0.189 day$^{-1}$ and in sorghum and finger millet from 0.500 to 0.217 and 0.454 to 0.179 day$^{-1}$, respectively. Rising CV percent across concentrations further indicated increasing irregularity and non-uniformity of germination, especially in finger millet. Physiological responses reaffirmed the toxicity trend. SVI showed substantial reductions with increasing Cr levels, declining to 700 in pearl millet and 780 in finger millet, whereas sorghum maintained a comparatively higher vigor index (860) even at 15 mg/kg. Tolerance index decreased consistently in all crops, with pearl millet showing the steepest decline (38.8%), followed by finger millet (42.2%) and sorghum (45.2%). Conversely, inhibition and phytotoxicity percentages rose proportionally with Cr exposure, demonstrating that chromium increasingly interferes with seedling metabolism, cell division, and root–shoot elongation. The comparatively lower phytotoxicity in sorghum confirms its stronger intrinsic stress-buffering mechanisms.

Growth and developmental parameters also showed dose-dependent declines across species. RGR and NAR decreased significantly, indicating reduced biomass accumulation and diminished photosynthetic efficiency with increasing Cr levels. Pearl millet recorded an RGR decline from 0.085 to 0.025 g/g/day, finger millet from 0.088 to 0.027 and sorghum from 0.091 to 0.033, highlighting sorghum's relatively better growth retention. Reductions in LAR and LAD across all crops reflected suppressed leaf expansion and reduced functional leaf duration, further limiting carbon fixation. SLA consistently decreased, whereas SLW increased in all species, especially in finger millet (0.0059 to 0.0080 g/cm$^2$), suggesting leaf thickening and structural modifications typical of metal-stress-induced hardening. These coordinated changes in growth indices underscore chromium's interference with physiological resource allocation and leaf architecture. Collectively, the results confirm a strong, concentration-dependent inhibition of germination, vigor and growth across the three cereals. Sorghum bicolor consistently outperformed pearl millet and finger millet, maintaining higher germination indices, stronger physiological resilience, and better growth rates, making it the most chromium-tolerant species. Pearl millet displayed moderate sensitivity, whereas finger millet was most adversely affected, exhibiting pronounced reductions across nearly all parameters. These interspecies variations highlight the potential suitability of sorghum for cultivation or phytoremediation in chromium-affected soils and emphasize the need for targeted crop selection and soil management strategies in heavy-metal-stressed dryland agriculture.

The findings of the present study clearly indicate that chromium exposure, particularly at concentrations above 8 mg/L, significantly impairs germination percentage, seedling vigor, and early growth in experimental crops such as pearl millet (*Pennisetum glaucum*), jowar (*Sorghum bicolor*), and finger millet (*Eleusine coracana*). As chromium concentration increased from 4 mg/L to 15 mg/L, there was a consistent decline in seed germination parameters such as GI, Mean MGR, and SVI, along with elevated MGT and CV%, signifying delayed and erratic germination patterns. These trends align closely with the findings of Gandhi *et al*. (2017), who reported reduced seed germination and impaired biochemical performance of *Amaranthus tricolor* and *Sesamum indicum* under increasing concentrations of chromium.

Furthermore, the physiological growth parameters such as RGR, NAR, LAR, SLA, and LAD were significantly suppressed at higher chromium concentrations, particularly 12 and 15 mg/L. These findings are consistent with previous studies by Gandhi *et al*. (2020a, 2020b), which showed that heavy metals including chromium, copper, lead, and manganese adversely impacted growth and physiological responses in *Cajanus cajan*, particularly in dryland conditions. The reduction in growth rates can be attributed to the disruption of chlorophyll biosynthesis, nutrient uptake, and enzyme activities key components of cellular metabolism that are vulnerable to oxidative damage under heavy metal stress. Biochemical indicators such as total protein content, antioxidant enzyme activities (POD), demonstrated a dose-dependent response to chromium exposure. At lower concentrations (4–6 mg/L), antioxidant activities increased slightly, indicating an adaptive response; however, at higher levels (10–15 mg/L), these enzymes showed exhaustion trends, highlighting oxidative damage beyond the plant's defense capacity. These results mirror observations made by Gandhi *et al.*, (2020a) and Gandhi *et al.*, (2019), who noted similar biochemical stress responses in pigeon pea and under other abiotic stressors such as ultraviolet radiation.

The increased percentage inhibition and phytotoxicity with corresponding declines in Tolerance Index further underscore chromium's toxicity to early plant development. Similar phytotoxic responses were noted in *Cicer arietinum* and *Macrotyloma uniflorum* under UV radiation exposure as described by Gandhi *et al.,* (2019), indicating that environmental stressors trigger common biochemical pathways of stress response, often resulting in suppressed germination and growth. The principal component analysis (PCA) in this study revealed strong positive loadings of SVI, GI, % Germination, and MGR on PC1, with negative loadings for MGT, phytotoxicity, and % inhibition, effectively separating tolerant and sensitive responses across chromium concentrations. Similar multivariate approaches were used by Gandhi *et al*., (2018a,b) in studying microwave-mediated synthesis of nanoparticles and their toxicity, confirming the utility of PCA in delineating stress response traits in plants. Interestingly, earlier research by Rama Govinda Reddy *et al.,* (2024, 2025) and Gandhi *et al*., (2022a,b) also explored the application of microwave and nano technology-based interventions for seed treatment and stress mitigation. Their results suggest that optimal exposure, improves chlorophyll content, protein synthesis, and seedling vigor, a strategy that could potentially be used to prime dryland crop seeds for enhanced chromium tolerance. In another set of studies, Gandhi *et al.* (2021, 2025a) demonstrated the green synthesis of silica and calcium oxide nanoparticles using agro-waste, which when applied to crops, showed improved biochemical responses and growth performance.

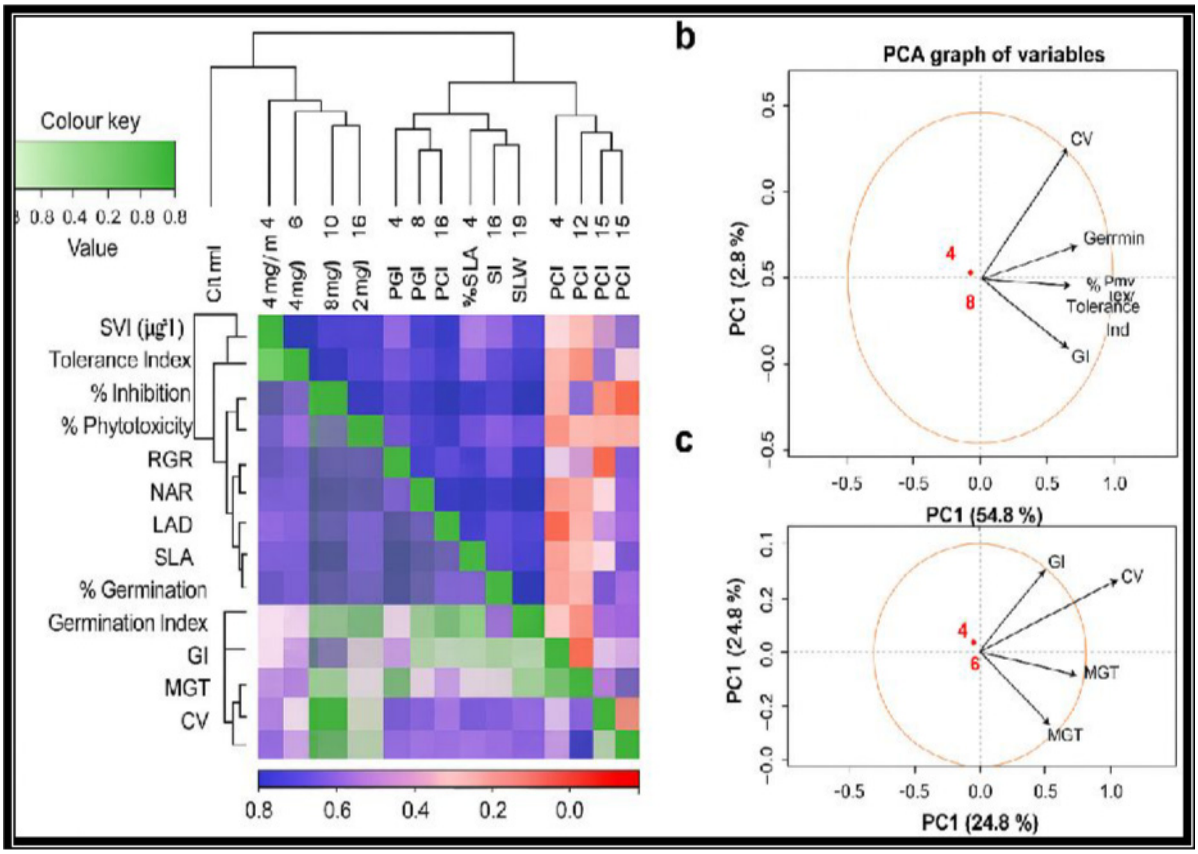


Fig. 2. Principal component analysis and correlation measurement for growth, physiological and developmentl response of dryland (*Pennisetum glaucum*, *Eleusine coracana*, *Sorghum bicolor*) crops under varying concentrations of chromium

### Principal component analysis

### Panel a: Heatmap with Hierarchical Clustering

The heatmap (Fig. 2) in Panel a visualizes the correlation matrix of germination parameters (e.g., % Germination, GI, MGT, MGR, CV), seedling vigor (SVI), phytotoxicity (% inhibition and % phytotoxicity), and early growth metrics (RGR, NAR, LAR, LAD, SLA, SLW) across different chromium treatments. Darker green tones indicate stronger positive correlations (values approaching 1), while red and blue represent negative or weak associations. The dendrogram shows a distinct clustering pattern, separating chromium levels into low (4–6 mg/L), moderate (8–10 mg/L), and high (12–15 mg/L) exposure groups. Lower chromium levels (4 mg/L and 6 mg/L) clustered with higher values of SVI, Tolerance Index, % Germination, GI, and other growth parameters, suggesting these concentrations may not significantly impair seedling vigor. In contrast, high Cr concentrations (12–15 mg/L) are associated with negative outcomes, clustering with higher % inhibition, % phytotoxicity, and reduced physiological efficiency. This clustering highlights the toxicity threshold of Cr beyond 10 mg/L, beyond which critical developmental and physiological parameters decline sharply. Positive correlations between SVI, GI, % Germination, and RGR at low Cr levels demonstrate that Cr at trace levels may exert a hormetic effect, stimulating some stress-related growth responses. However, this is quickly outweighed at higher doses.

### Panel b: PCA Biplot for Physiological and Germination Parameters

The PCA biplot (Panel b) summarizes the variance structure of the physiological and germination data. PC1 and PC2 explain 52.8% and 15% of the total variance, respectively. % Germination, GI and Tolerance Index exhibit strong positive loadings on PC1, indicating that this axis primarily captures beneficial seedling and early growth responses. Chromium concentrations at 4 mg/L and 6 mg/L are located in the positive PC1 quadrant, clustering with favorable parameters like CV, GI, and Tolerance Index. On the other hand, 8 mg/L begins to drift toward a transitional phase, while 10–15 mg/L (not visualized) would likely fall into the negative space, based on trends observed in Panel a. The PCA thus supports the interpretation that Cr stress at moderate-to-high concentrations disrupts germination vigor and reduces physiological stability, while low levels may still allow seedlings to initiate metabolic compensatory mechanisms.

### Panel c: PCA Biplot for Germination Metrics

Panel c focuses on germination-specific indices. PC1 and PC2 explain 54.8% and 24.8% of the variance, respectively. GI and CV load strongly on PC1, while MGT loads negatively, emphasizing the inverse relationship between seedling speed and vigor. Treatments with 4 mg/L and 6 mg/L Cr are positioned on the positive PC1 axis, aligned with faster, more uniform germination. This confirms that lower Cr exposure promotes synchrony and vigor, while moderate levels begin to reduce MGR and elevate MGT, suggesting early signs of physiological stress. The negative loading of MGT further underlines delayed germination under stress, an important diagnostic tool in environmental toxicity studies.

Moreover, Gandhi *et al*., (2013, 2015b) documented the successful use of phytoremediation with *Sorghum bicolor* and *Ipomea aquatica* for the detoxification of lead, chromium, and fluoride from contaminated soils and wastewater. These studies underline the significance of using metal-tolerant crops and green technologies to improve soil health and productivity in polluted areas. In summary, the present study reinforces the detrimental impact of chromium on germination, seedling development, physiological functioning, and biochemical stability in dryland crops. It identifies critical thresholds above which chromium becomes phytotoxic and offers parallels with previous studies that have explored various stress alleviation strategies. The integration of seed priming, nano-amendments and phytoremediation holds considerable promise for mitigating chromium toxicity and ensuring sustainable dryland agriculture under heavy metal stress.

## CONCLUSION

The present study demonstrates that chromium contamination, particularly at concentrations exceeding 8 mg/kg, has a profound and dose-dependent negative impact on seed germination, physiological performance, and early growth of key experimental crops pearl millet (*Pennisetum glaucum*), jowar (*Sorghum bicolor*), and finger millet (*Eleusine coracana*). Chromium stress led to a marked reduction in germination %, GI, MGR, SVI and RGR, while significantly increasing MGT, CV%, phytotoxicity, and percent inhibition. Among the three crops, *Sorghum bicolor* exhibited superior tolerance to chromium toxicity across all physiological and biochemical parameters, indicating its potential utility in chromium-contaminated soils and phytoremediation practices. *Eleusine coracana* was the most sensitive, with pronounced declines in pigment stability, protein content and seedling biomass, while *Pennisetum glaucum* showed intermediate responses, marked by high antioxidant (peroxidase) activity as a stress-adaptive mechanism.

The results also underline the utility of PCA-based clustering and biplot analysis in distinguishing stress thresholds and identifying crop-specific resilience. The initial hormetic effect observed at lower chromium concentrations (4–6 mg/kg) suggests limited metabolic compensation; however, this advantage diminishes rapidly beyond 8 mg/kg. Chromium stress not only suppresses primary metabolic functions such as photosynthesis and protein synthesis but also alters structural characteristics like specific leaf weight and duration of active leaf area, ultimately impacting yield potential. These findings carry important implications for dryland agriculture, where the risk of heavy metal contamination from unregulated waste disposal is increasing.

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