CHROMIUM AND SALT INDUCED PERTURBATION IN GROWTH, PHYSIO-BIOCHEMICAL AND ENZYMATIC ATTRIBUTES IN *Vigna radiata* (L.) WILCZEK

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Abstract. Chromium (Cr) is a highly toxic, heavy, non-essential metal and the World Health Organization (WHO) recommends a total chromium concentration of 0.05 mg/L. One way to remove heavy metals (HM) from contaminated sites is to use plants that can accumulate HM and are relatively tolerant of it. Mungbean *Vigna radiata* (L.) Wilczek may be used as a bioindicator to assess Cr toxicity due to its response to Cr stress. The purpose of this study is to evaluate the impact of chromium and sodium chloride salt stress on mungbean growth, relative water content, amino acid, soluble sugar, proline, phenol, chloroplastic pigment, and antioxidant response. In combination with salt treatment, 15 µM metal plus 25000 µM salt, 25 µM metal plus 25000 µM salt, and 35 µM metal plus 25000 µM salt were applied to "Gujarat Anand Mungbean-5" mung bean seedlings. A hydroponic growth medium was used in this experiment to test the tolerance mechanism of *V. radiata* to single and combined stresses of Cr and NaCl. Under salt and Cr stress conditions, plant growth parameters decreased, indicating that plants required more energy for absorbing nutrients, maintaining water, and synthesizing and transporting organic compounds for osmotic balance. *V. radiata* is further able to tolerate salt by reducing the uptake of Cr, as well as avoiding Cr toxicity. It appears that the Gujarat Anand Mungbean-5 variety of mung beans has the pivotal salt and chromium tolerant capacity.

Keywords: chromium; mung bean; metal toxicity; salt; *Vigna radiata.*

INTRODUCTION

In Indian culture, mungbean crop was adapted from India since prehistoric times and is an important legume. Mungbeans are also referred to as chickasano peas, golden gram, green gram, chiroko, and commonly as mung [2]. Additionally, it is thought to be derived from var. Sublobata, grows in Burma and India and is spread throughout northern and southern America, southern and eastern Africa, the Caribbean, and Australia. *Vigna radiata* was formerly called *Phaseolus aureus* Roxb, and in turn, it was subdivided into three subgroups, Radisca, Glabra, and Sublobata [15]. Furthermore, those roots contain nodules that fix atmospheric nitrogen symbiotically with rhizobium bacteria [12]. This product contains between 1% and 1.5% fat, 4.5 and 5.5% ash, 3.5, and 4.5% fiber, and 25 to 28% protein. The nutritive value of this protein-rich legume depends on the amino acids that it contains, notably lysine and cysteine, but it has limited amounts of methionine and cysteine. Rather than sodium, iron, and zinc, the seeds contain high levels of potassium, calcium, and magnesium. Additionally, the seeds are a great source of carbohydrates and vitamins. Furthermore, sprouting can enhance vitamin C levels, niacin content, and phytosterol content [15].

Over the last century, industrialization, urbanization, and water disposal have adversely affected the biosphere by polluting it and contaminating the soil. The existence of some heavy metals such as copper, zinc, cobalt, iron, manganese, and molybdenum, in trace amounts, is beneficial, but in excessive amounts will be toxic to nature [14]. Chromium (Cr) is the toxic metal that has the most devastating effects on human growth and development because it is non-essential, highly toxic, and heavy. Plant parts are easily contaminated with heavy metals (HM), yet one strategy for removing HM from contaminated sites involves the use of plants that can accumulate HM and are relatively tolerant to it. The strategy is described as phytoremediation and is considered to be more effective and cost-effective than physical remediation. Moreover, this HM decreases the uptake of some other essential nutrients and affects the metabolic system of the plant. As well as decreasing the leaf area, shoot and root growth, and increasing water stress, it disturbs the stomal regulation, the water content of leaves, nitrogen-fixing ability, as well as abscisic acid levels [20]. In response to Cr stress, mungbean shows changes in growth and physio-biochemical attributes that can be used as a bioindicator to assess Cr toxicity [18]. Cultivation of mungbean plants can be achieved using a hydroponic medium. Hydroponic technology is growing the plant in a nutrient solution without soil. The controlled conditions facilitate nutrient availability for plant growth and can be used for research, as well as studies on nutrient uptake and its interactions with stress factors [27].

We aim to evaluate the impact of metal stress and salt stress on growth, relative water content, amino acid, soluble sugar, proline, phenol, chloroplastic pigment, and antioxidant response of mungbean [*Vigna radiata* (L.) Wilczek]. The AAU website states that GAM 5 is a semi-rectangular plant with a green stem, dark green leaves with a purple vein, and high resistance to the yellow mosaic virus. The reported average yield of GAM 5 is 1811 kg/ha. It has 5.1 g of 100 seed weight. Moreover, this grain contains 17.62% soluble sugar and 24.33% carbohydrates (http://www.aau.in/crop-varieties). This experiment assessed the tolerance mechanism of *V. radiata* by exposing the plant to single or combined stresses of Cr and NaCl in different concentrations under a hydroponic medium.

MATERIALS AND METHODS

Seed characteristics and collection

Experimental materials included seeds of the local variety GAM-5 (Gujarat Anand Mungbean-5) that were obtained from the Anand Agriculture University (AAU), Gujarat. They were rinsed with distilled water to avoid contamination from surface contaminants. The 100 sterilized seeds of GAM 5 were germinated in sterile petriplates without any salt or metal treatments.

Growth medium and treatment

6 days old germinated seedlings were acclimatized for 7 days under the hydroponic solution of Hoagland's nutrient medium in a plant tissue culture lab. Design of hydroponic system: As preparations, we used two glasses of different sizes, a small one and a large one. In the small one, we drilled holes at both sides of the glass edge and also drilled a hole at the center of the glass. As for the large glass, the same procedure was followed except for the hole below. Additionally, it was supplemented with half-strength Hoagland's nutrient medium. Finally, it was tied with thread inside the large glass. In a small glass bottom, there was a hole pierced in the middle where the germinated seed was planted. The seedling's main root was then immersed into Hoagland's liquid nourishment and poured into a large glass.

Afterward, metal and salt were treated with different concentrations of chromium chloride (15 µM, 25 µM, 35 µM), and salt with sodium chloride (15000 µM, 25000 µM, 35000 µM). The combination of treatment of metal and salt was provided to seedlings as 15 µM metal plus 25000 µM salt, 25 µM metal plus 25000 µM salt, and 35 µM metal plus 25000 µM salt. A plant growth room that has adequate lighting was used for proper photosynthesis with all the petriplates and designed glasses placed in it. After the plant growth room ceased treatment, plants were harvested to analyze their growth, physio-biochemical, and enzymatic attributes.

Growth parameters

Leaf relative water content

Leaf weights were obtained both immediately after sampling and after soaking in distilled water for 8 h at room temperature. Leaf turgid weights were obtained after drying. The leaves were then dried in an oven at 80°C for 48 h to determine their leaf water content [23]. RWC = [(FW-DW) / (TW-DW)] × 100

Determination of mineral ion contents

For the determination of the ion contents, dried plant samples (leaf, stem, and root) were dried at 80°C for 48 h in the oven using the method described by Panda et al. [21]. After that, homogenized weighted samples of about 0.5 gm were kept in volumetric flasks of 25 mL each. In the fume hood, a mixture of acidic HNO_3 and $HClO_4$ was applied to the sample in the flask. The liquid was heated at a high temperature until the liquid became colourless and red NO_2 fumes stopped escaping. In an attempt to create a volume of 25 mL out of the evaporated contents, 20 mL of distilled water was added after cooling to room temperature. Following this filtration, aliquots of the solution were used to measure the amounts of some intermediate elements, including calcium, sodium, potassium, magnesium, iron, zinc, and copper, by ICP mass spectrometry (Thermofisher, USA).

Total free Amino Acid

In a mortar and pestle, we homogenized a leaf sample weighing 1 g in 5 mL of 80% acetone after being extracted for free amino acids according to Moore et al. [18]. After centrifugation at 4000-5000 rpm for 10 minutes, the supernatants were taken. Two to three extractions with 5 mL of ethanol were performed and then the ethanolic extracts were combined for a total volume of 20 mL. In the final volume of 2 mL, distilled water was added to an aliquot of 0.2 M acetate buffer and 4% ninhydrin in methyl cellosolve. An appropriate volume of ninhydrin solution was mixed with the aliquot. For each tube, 15 min of boiling water bath was followed by 1.5 mL of n-propanol. The tubes were then cooled at room temperature, and the intensity in each tube was read at 570 nm against a blank in a spectrophotometer. The amount of total free amino acids was estimated by preparing a standard plot against leucine (10-100 g).

Proline estimation

After homogenizing the leaf sample (0.5 g) with 10mL of 3% aqueous sulphosalicylic acid and centrifuging it at 10000 rpm for 10 min, leaf samples were extracted from control, Cr, and salt-treated plants. Using Whatman No.2 filter paper, the supernatant was collected and filtered. A test tube was filled with 1 mL of the filtrate and 2 mL of acid-ninhydrin and glacial acetic acid. Using a boiling water bath, 4 mL of the reaction mixture was heated for 20 min. Afterward, the reaction mixture was terminated in an ice bath, after which toluene was added and stirred for 20-30 seconds. After the mixture settled down, biphase emerged. As a result, the toluene layer separated and warmed at room temperature. As a result, absorbance was measured at 520 nm against a blank. In order to calculate proline content, the standard curve was plotted against L-Proline [6].

Proline can be calculated using the formula below: [µmoles of proline / g tissue = (µg proline per mL × mL toluene)/115.5 × (5 /g of sample)]; Where 115.5 is the molecular mass of proline.

Total Soluble Sugar estimation

The amount of soluble sugar was estimated by Hedge et al. [12] and Sadasivam and Manickam [25] using 1 g of leaf samples homogenized in 5 mL 80% acetone for a minute. As a second extraction, 5 mL of ethanol was added to the residue twice, and each time the ethanol was boiled at 80°C. After the supernatants from these two extractions were combined, a total of 20 mL of ethanol was collected. It was divided into aliquots and mixed with 2 mL of Anthrone reagent before being diluted with distilled water and heated for 8 min in a water bath. Afterward, it was cooled down to room temperature and measured using a

spectrophotometer at 630 nm. The total amount of soluble sugar was assessed by using a standard curve constructed against glucose (0-500 m µg).

Phenol estimation

According to Swain and Hillis [32], the total phenol content of 0.5 to 1 g of the leaf sample was determined by homogenizing it in 10 mL of 80% ethanol and centrifuging it for 20 min at 10,000 rpm. A repeat extraction was performed with 5 mL of 80% ethanol and allowed to evaporate at room temperature before dilution with 5 mL of distilled water. The reaction mixture was divided into diverse aliquots and the total volume equaled 3 mL. 0.2 mL of FCR reagent was poured into each tube and the reaction was incubated for 3 min at room temperature. Following this, two mL of saturated 20% sodium carbonate was added to each and the samples were heated for one minute in a boiling water bath. Readings were made using a UV-visible spectrophotometer at 560 nm. Here, phenol concentrations were determined by comparing phenol standards prepared against catechol.

Catalase activity

To measure catalase activity [8], a smooth pulp of leaves was prepared in a mortar and pestle with 0.1 M phosphate buffer (pH 7.0) in a prechilled state. The sample was centrifuged for 30 min at 15,000 rpm for enzyme production. The supernatant was collected and used as a source of enzymes. One mL of enzyme extract was added to 2 mL of hydrogen peroxide and 3 mL of phosphate buffer in a test tube. The mixtures were mixed well, and the tube was incubated at 20^0C for 1 min. As a final step, 10mL of 0.7N H_2SO_4 was added to end the reaction. Finally, the solution was titrated with 0.01N $KMnO_4$ until it has a faded purple color for at least 15 sec.

Biostatistical analysis

To determine different parameters, each metal and salt-treated sample was performed in triplicate. The data were interpreted by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparisons ($p < 0.05$) [10]. based on SPSS software (SPSS for Windows 20.0, SPSS Inc., USA). A principal component analysis (PCA) was also performed by MetaboAnalystR5.0 software to detect the effects of chromium and salt on the root, stem, and leaf, as detailed by Pang et al. [22].

RESULTS

Effects of Cr and salt stress on plant growth

All Cr, salt, and combinations of salt and Cr treatments were applied to *V. radiata* species to assess the effects on the plant's height, fresh and dry weight of root, stem, and leaves. Following a 7-day treatment with various concentrations of salts (such as NaCl and Cr), they were examined in hydroponic culture conditions.

Plant height

In contrast to the control plants, which measured 25 cm in height, the 15000 µM NaCl treated plants grew 24.33 cm in height. The plant height, however, decreased in all of the treatments compared to the control plants. In the case of high Cr treatment (35 M) and high Cr and NaCl treatment (30 µM + 25000 µM) respectively, a significant reduction in plant height was observed i.e., 18.66 ± 1.15cm and 19 ± 1.73cm respectively (Fig. 1 & 2A). However, the combination of Cr and NaCl had negligible effects on the mung plants after treatment with salt and metals.

Fresh weight of leaf, stem, and roots

Similar to the plant height, the maximum fresh weight in the untreated mung leaves was 0.086 ± 0.001 g, as compared to the treated mung plants. The next highest fresh weight of leaves was observed in 15000 µM NaCl (0.081± 0.001g) followed by the combination of 15 µM Cr + 25000 µM NaCl (0.078± 0.001g), and 25,000 µM NaCl (0.075± 0.001g). The rest treatments were showing a downtrend in the fresh weight of leaves (Fig. 2B). In the case of the fresh weight of the stem, the same behavior was shown by the control mung bean stem with the highest value of 0.388± 0.008g, wherein the salt-treated plants showed a better stem height of 0.361± 0.004g, 0.358± 0.007g, and 0.328± 0.005g in the respective 15000, 25000 and 35000 µM NaCl treatments (Fig. 2C). Mung bean seedlings without treatment presented the highest value of fresh root weight, namely 0.107 ±0.0015g. Then, 15000 µM NaCl, a combination of 15 µM Cr and 25000 µM NaCl were applied to seedlings of mung bean. The fresh weights were 0.104± 0.001g and 0.102± 0.002g respectively. Yet, all the seedlings treated with Cr showed the least fresh weight of roots (Fig. 2D).

Dry weight of leaf, stem, and roots

After the removal of water content from the leaf, the measured dry weight of the leaf was observed to be better again in the control seedlings with a weight of 0.0096 ± 0.0003g. This parameter was showing a better trend in the salt-treated categories i.e., in 25,000 µM and 35,000 µM NaCl that attained a dry weight of 0.0095 ± 0.0002g and 0.0091± 0.0001g respectively. The initial combination of 15 µM Cr and 25,000 µM NaCl was also observed to possess a good amount of dry weight of root i.e., 0.0087± 0.0002g (Fig. 3). Like that of the dry weight of the leaf, the dry weight of the stem followed the same pattern. The decreasing order of dry weight of stem in control, then salt treatments and followed by the combinatorial treatments (Fig. 3). A similar trend was also observed in this parameter (Fig. 3).

Effects of Cr and salt stress on relative water content

At a high metal concentration (35 µM), the relative water content (RWC%) of the leaf was significantly decreased by Cr treatment with 68.67±2.92% (Fig. 3). Under 15000 µM NaCl treated plants, the water content of the sample was less affected than in the control. Furthermore, solitary Cr treatments and combinations of Cr and salt did not significantly alter the relative water content. Overall, our data showed

that RWC% of the leaf was decreased with increasing concentrations of Cr, salinity, and combined treatments of Cr and salt.

Effects of Cr and salt stress on total amino acid

Comparing the results of the experiments with a control group, it was discovered that a high concentration of Cr and sodium chloride caused more amino acid accumulation. It was significantly elevated to 88.14 ± 1.404 g and 63.14 ± 1.301 g in respective solitary treatments of Cr (35 µM) and NaCl (35000 µM). With increasing in respective 15 µM, 25 µM, and 35 µM concentrations of Cr, total amino acid increased to 63.01±1.89g, 73.25 ± 2.04g, and 88.14 ± 1.20g, respectively. Nonetheless, the level of total amino acids decreased with increasing concentrations of salt and combined Cr treatment. At moderate salinity

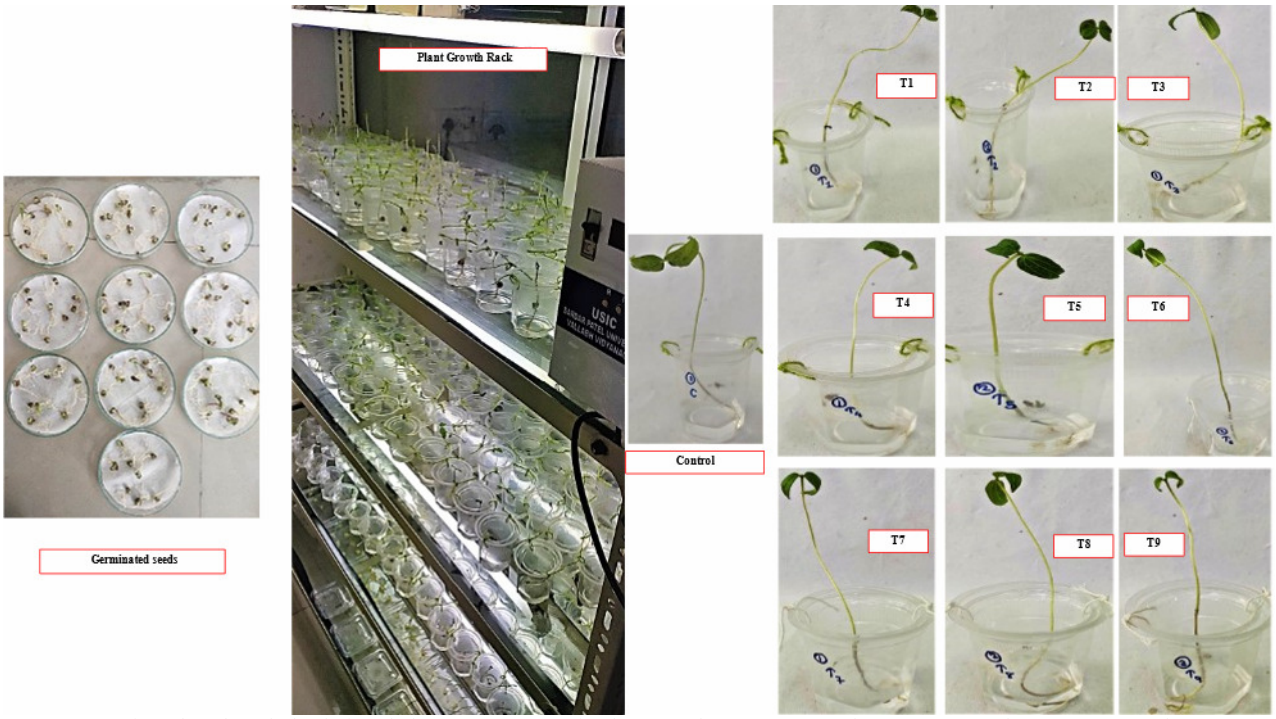


Figure 1. Germinated seeds and The designed glasses arrangement in plant growth room: C-Control, T1- 15 µM $CrCl_3$, T2- 25 µM $CrCl_3$, T3- 35 µM $CrCl_3$, T4- 15,000 µM NaCl, T5- 25,000 µM NaCl, T6- 35,000 µM NaCl, T7- combination of 15 µM $CrCl_3$+ 25,000 µM NaCl, T8- combination of 25 µM $CrCl_3$+ 25,000 µM NaCl and T9- combination of 35 µM $CrCl_3$+ 25,000 µM NaCl.

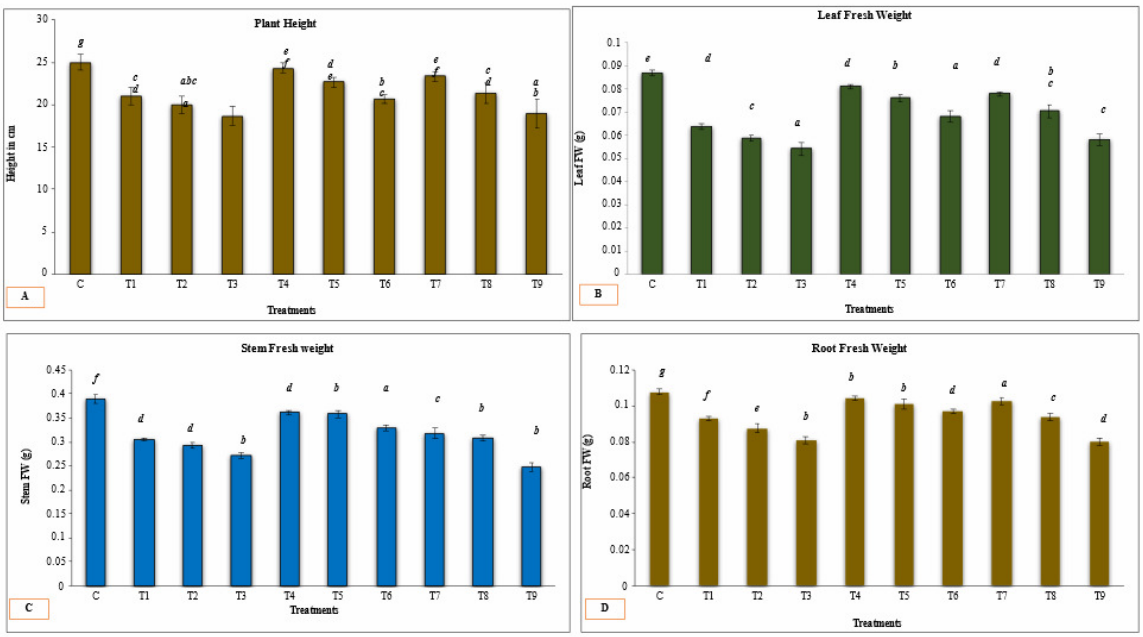


Figure 2. The plant heights, leaf fresh weight, stem fresh weight, root fresh weight (mean ± standard deviation, n=3) of mung plants after the various treatments of C-Control, T1- 15 µM $CrCl_3$, T2- 25 µM $CrCl_3$, T3- 35 µM $CrCl_3$, T4- 15,000 µM NaCl, T5- 25,000 µM NaCl, T6- 35,000 µM NaCl, T7- combination of 15 µM $CrCl_3$ + 25,000 µM NaCl, T8- combination of 25 µM $CrCl_3$ + 25,000 µM NaCl and T9- combination of 35 µM $CrCl_3$ + 25,000 µM NaCl. The columns with different letters are significant at $P< 0.05$ based on one-way analysis of variance followed by Duncan's multiple range test [10].

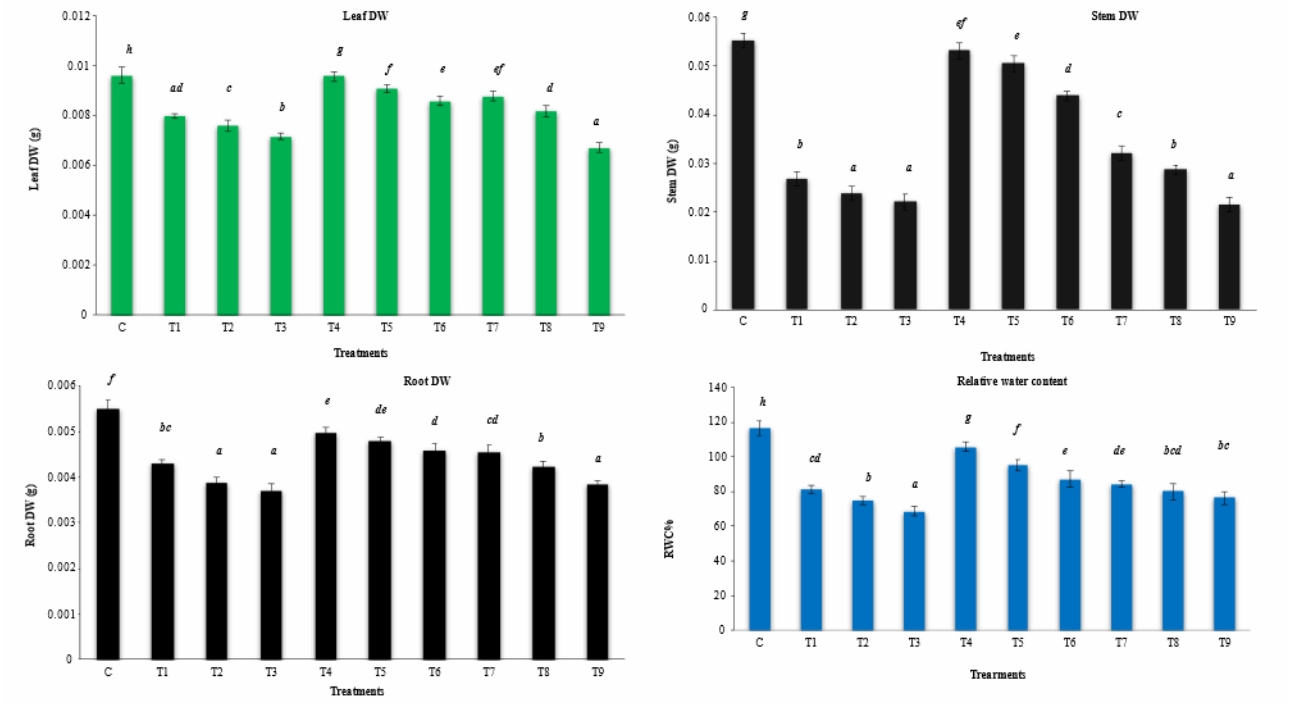


Figure 3. The dry weight (mean ± standard deviation, n=3) of leaf, stem, and roots along with the relative water content of leaves. C-Control, T1- 15 µM $CrCl_3$, T2- 25 µM $CrCl_3$, T3- 35 µM $CrCl_3$, T4- 15,000 µM NaCl, T5- 25,000 µM NaCl, T6- 35,000 µM NaCl, T7- combination of 15 µM $CrCl_3$ + 25,000 µM NaCl, T8- combination of 25 µM $CrCl_3$ + 25,000 µM NaCl and T9- combination of 35 µM $CrCl_3$ + 25,000 µM NaCl. The columns with different letters are significant at P< 0.05 based on one-way analysis of variance followed by Duncan's multiple range test [10].

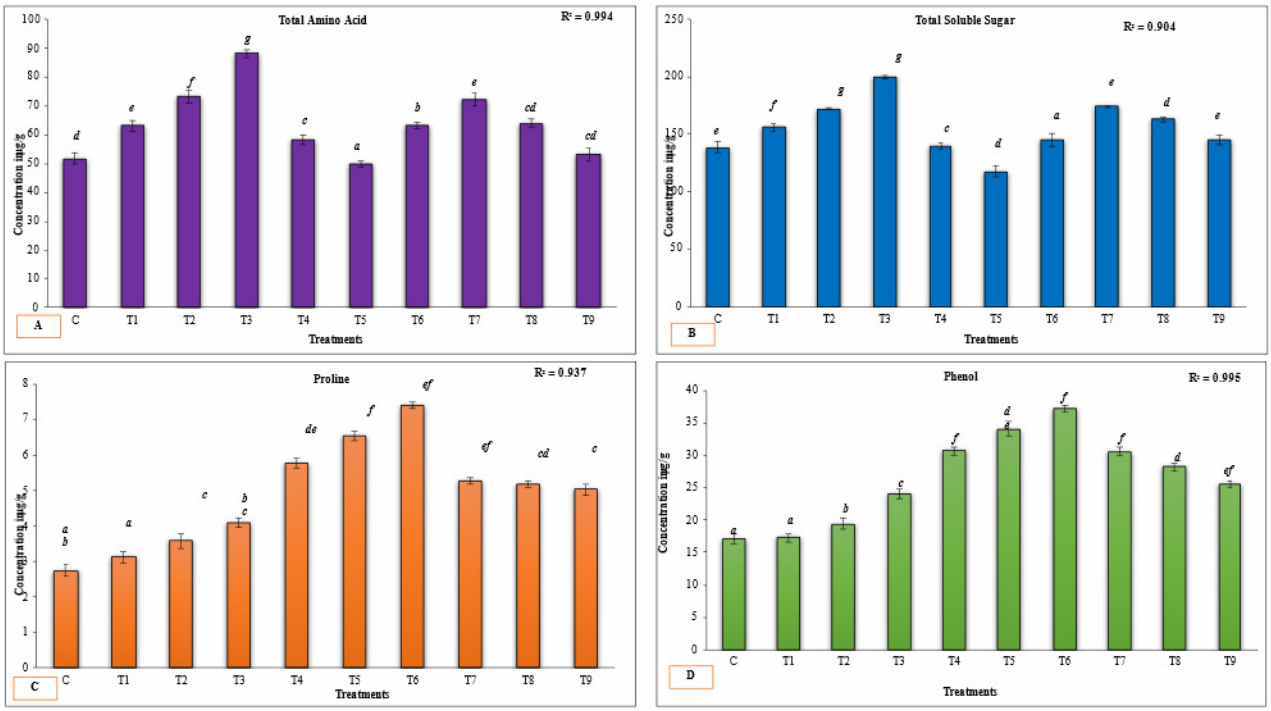


Figure 4. Estimation of total amino acids (A); Total soluble sugar (B); Proline (C) and Phenol (D) (mean ± standard deviation, n=3). C-Control, T1- 15 µM $CrCl_3$, T2- 25 µM $CrCl_3$, T3- 35 µM $CrCl_3$, T4- 15,000 µM NaCl, T5- 25,000 µM NaCl, T6- 35,000 µM NaCl, T7- combination of 15 µM $CrCl_3$ + 25,000 µM NaCl, T8- combination of 25 µM $CrCl_3$ + 25,000 µM NaCl and T9- combination of 35 µM $CrCl_3$ + 25,000 µM NaCl. The columns with different letters are significant at P< 0.05 based on one-way analysis of variance followed by Duncan's multiple range test [10].

(25000 µM), the plant showed a reduced level of an amino acid (49.92 ± 1.249µg) compared to the plant exposed to combined Cr + NaCl and Cr alone (Fig. 4A). One-way ANOVA confirmed the level of significance at P<0.05.

Effects of Cr and salt stress on total soluble sugar

As a comparison with the control, there was no significant change in total soluble sugar content at low (15000 µM) and high (35000 µM). However, at moderate salinity (25000 µM), it was slightly less i.e., 117.39 ± 5.06µg than the control (138.62 ± 5.17 µg). When the soluble sugar content of seedlings was increased with increasing levels of Cr treatment and decreased with increasing levels of Cr and NaCl treatment, this pattern was observed. The maximum content of TSS was 199.53 ± 1.33µg recorded in plants treated with a high concentration of Cr (35 µM) (Fig. 4B). The total soluble sugar content of individual treatments was concurrent with one-way ANOVA at P<0.05.

Effects of Cr and salt stress on proline content

Increasing the concentration of Cr and NaCl alone significantly increased the proline content in *V. radiata*, but no significant changes were observed when both solutions were used together. There was an increase in proline content in all plants treated compared to controls. At high salinity (35000 µM), the proline was raised maximum to 7.38 ± 0.1µg. Among all treatments, the lowest value of proline was 3.12 ± 0.152µg recorded in plants treated with Cr (15 µM) (Fig. 4C). The proline content of seedlings was significantly justified as P<0.05 operated in one-way ANOVA.

Effects of Cr and salt stress on phenol content

On this *V. radiata* seedling, phenolic content was increased with solitary treatment of salt and Cr. In contrast, it decreased in plants that received combined Cr and NaCl treatments. Based on our data, each of the treated plants contained more phenol than the control plant. Fortunately, the phenol content remained the same or decreased (17.33 ± 0.597 µg) at low Cr (15 µM) compared to the control (17.08 ± 0.822 µg). Salinity (35000 µM) induced an accumulation of total phenol to 37.14 ± 0.523 µg. Comparatively, Cr-treated plants had a lower concentration of phenol than salt-treated plants (Fig. 4D). According to Duncan's [10] test, these values were significant at p<0.05 while calculated as one-way ANOVA with Duncan's posthoc test [10].

Effects of Cr and salt stress on catalase activity

The current study showed that catalase activity increased with increasing concentrations of both Cr and NaCl, and a combination of both Cr and salt. Maximum catalase activity of 2.59 ± 0.055 units/min/ g was detected in *V. radiata* seedlings subjected to high salinity (35000 µM). Furthermore, salt-treated plants had a higher and better catalase activity than control plants. No significant alteration was observed at low (15 µM) and moderate (25 µM) Cr treated and Cr + NaCl (15 µM + 25000 µM) treated plants with respective values of 0.508 ± 0.007 units/min/µg, 0.525 ± 0.014 units/min/µg and 0.607 ± 0.034 units/min/µg as compared to control (0.486 ± 0.024 units/min/µg) (Fig. 5).

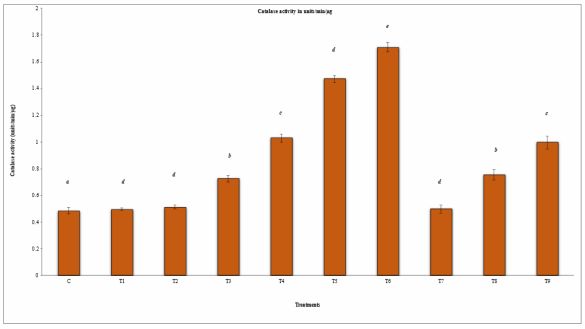


Figure 5. Catalase activity of mung seedlings with different treatments (mean ± standard deviation, n=3). C-Control, T1- 15 µM $CrCl_3$, T2- 25 µM $CrCl_3$, T3- 35 µM $CrCl_3$, T4- 15,000 µM NaCl, T5- 25,000 µM NaCl, T6- 35,000 µM NaCl, T7- combination of 15 µM $CrCl_3$ + 25,000 µM NaCl, T8- combination of 25 µM $CrCl_3$ + 25,000 µM NaCl and T9- combination of 35 µM $CrCl_3$ + 25,000 µM NaCl. The columns with different letters are significant at P< 0.05 based on one-way analysis of variance followed by Duncan's multiple range test [10].

Effects of Cr and salt stress on Mineral Ion content

Results showed that Ca^{2+}, K^{+}, Mg^{+}, B, Mn^{2+}, Fe^{2+} and Zn^{2+} contents of seedlings treated with Cr (15 µM) were higher than those grown under control and declined with increasing Cr concentration. Under solitary treatments of salinity, ions content was not different except for Na^{+}, Fe^{2+}, Zn^{2+}, and Cu^{2+}. Na^{+} content increased with salinity but was depleted both in combined Cr and salt treatments and in Cr alone treatments. The plant Fe^{2+} content was comparatively high under salt with 25000 µM concentration than other salt-treated and combined Cr with salt-treated samples. Zn^{2+} and Cu^{2+} contents were increased and present in more amounts with high salinity (35000 µM) compared to control and other solitary and combined treatments of salt and Cr except for the 15 µM Cr treated plant. The level of mineral ions excluding K^{+} and Mg^{2+} were dropped in combined Cr and NaCl treated plants (Fig. 6A-I).

PCA analysis

This PCA method has been employed to examine high dimensional data that has been perturbed by chromium and salt-induced factors in terms of morphological, biochemical, and pigment attributes. As a dimension reduction tool, PCA falls under multiple variables or multiple observations of a variable and it is used when data is correlated and there is a high degree of correlation between variables. Moreover, the PCA tool shows the maximum variance in the dataset as described below.

PCA analysis for morphological attributes

Three-dimensional and biplot PC plots were applied to a simple dataset consisting of 10 samples, including control. A total of two principal components PC1 and PC2 were derived that explained 99.3% and 0.7% of the variance in 30 variables of a dataset (Fig.

7A-B). As indicated by the spot, the T4 value does appear to match the control value. According to this study, at least T4 and T3 samples were significantly affected by NaCl (25000 µM), salt stress (15000 µM), and a combination of these stress factors (15000 µM + 25000 µM), compared to the control. According to this loading plot, heavy metals can decrease the height and weight of plants and eventually affect plant growth.

PCA analysis for biochemical attributes

In addition, PCA analysis shows evidence of biochemical attributes morphologically as well as the difference between samples in terms of TAA, proline, phenol, and total soluble sugar levels. Similar variables clustered together. Of the three principal components, PC1 had the most clusters and accounted for 90.7% of the variance (Fig. 8A-B). For the control variables, there is no matching experimental value.

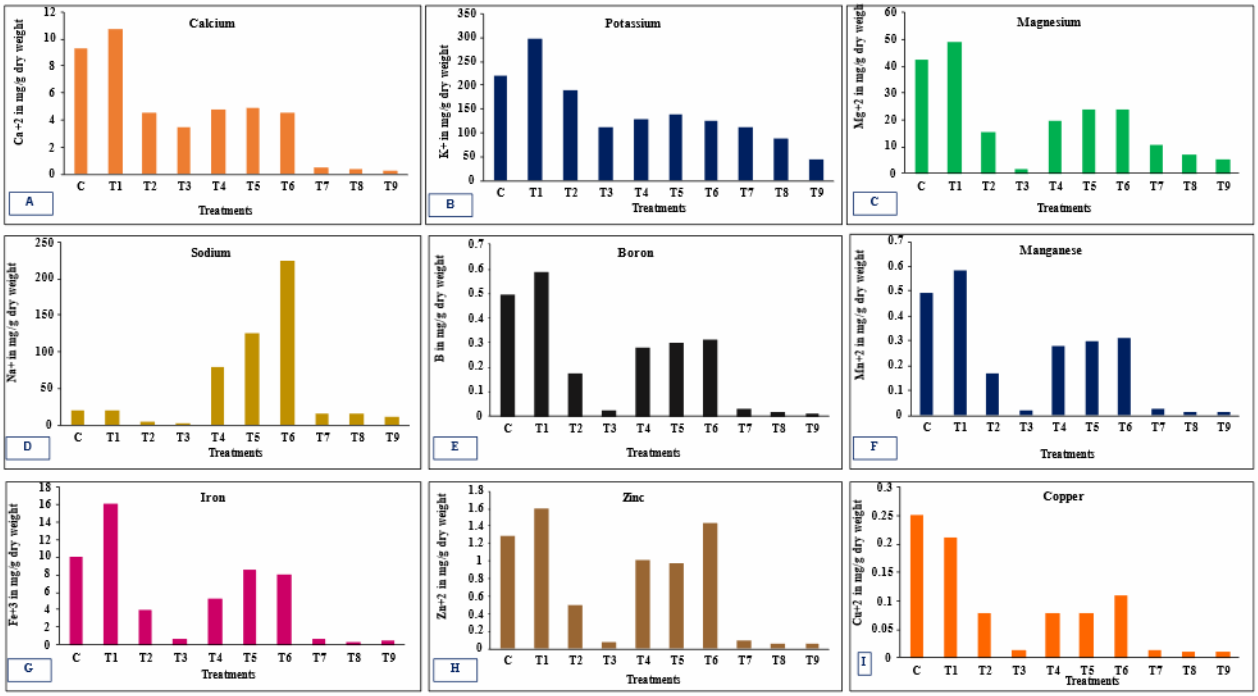
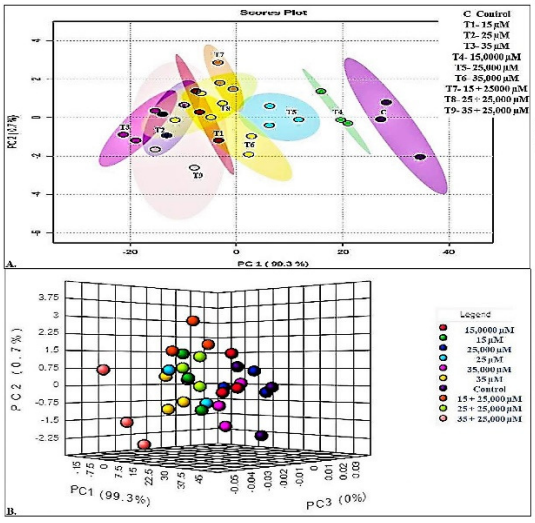


Figure 6. Mineral content (A-Ca; B- K, C-Mg; D- Na; E- B; F-Mn; G- Fe; H-Zn; I-Cu) in the leaves of different treated mung seedlings. C-Control, T1- 15 µM $CrCl_3$, T2- 25 µM $CrCl_3$, T3- 35 µM $CrCl_3$, T4- 15,000 µM NaCl, T5- 25,000 µM NaCl, T6- 35,000 µM NaCl, T7- combination of 15 µM $CrCl_3$ + 25,000 µM NaCl, T8- combination of 25 µM $CrCl_3$ + 25,000 µM NaCl and T9- combination of 35 µM $CrCl_3$ + 25,000 µM NaCl.



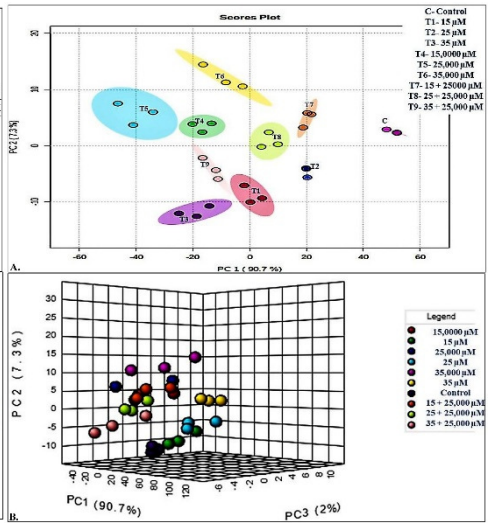


Figure 7. (Left) Two Dimensional (A) and Three dimensional (B) PCA analysis for morphological attributes.
Figure 8. (Right) Two Dimensional (A) and Three dimensional (B) PCA analysis for biochemical attributes.

DISCUSSION

Overall, in these morphological growth parameters combined treatment of Cr and salinity (15 µM + 25,000 µM) caused an increased growth compared to Cr alone in all growth parameters. All these values were well supported by the one-way ANOVA at a $P<0.05$ level of significance. In *V. radiata*, the dry weight of stem and leaf under salinity treatment was increased as compared to fresh weight. In contrast, the dry weight of stem and root declined significantly in Cr-treated plants compared to fresh. There was no significant change between the fresh and dry weight of the combined treatment of Cr and salt.

In the current study, results showed that all assessed growth parameters decreased in *V. radiata* seedlings exposed to Cr and NaCl. Our findings are well corroborated with the previous reports of NaCl-induced growth inhibition in mung bean [1;13;26]. The reduced level of growth in seedlings of *V. radiata* under salt and Cr could be attributed to the use of more photosynthetic energy for various organic osmolytes. However, the plant height, fresh and dry biomass of leaf, stem, and root found to be less or unaffected under both solitary treatment of low salinity (15000 µM) and combined treatment with chromium (15 µM Cr + 25000 µM NaCl) may be attributed to the minimum effects of low concentration of NaCl on nutrient metabolism. Observations were made which indicate the growth was reduced when exposed to high levels of Cr (35 µM) alone and when combined with a salt (35 µM + 25000 µM). Consequently, a high concentration of Cr can cause nutritional imbalance and hinder plant growth.

It is crucial for a plant's development and growth that the water level is kept constant [19]. The results of our experiment confirmed that RWC% remains stable at low salinities (15000 µM). Furthermore, there were no visible signs of leaf injury at such low concentrations of salt. It suggests that low salinity hardly affected seedlings of *V. radiata*, since these plants were developed to grow in an environment with little salt through water uptake. It also helps to minimize the toxic level of Cr in combined treatment as shown in Figure 3. In contrast, as salt and Cr concentrations increased, leaf turgor decreased progressively. So, it causes leaves and flowers to wilt due to water loss of plant cells.

In the present study, more free amino acids were accumulated (88.14 ± 1.40g) under the high Cr (35 µM) treated plants than in the control plants (51.64 ±1.833g). It is possible that the presence of metal-responsive amino acids such as histidine, asparagine, glutamine, and proline, in plants is the cause of the elevated levels [28]. In addition, it goes well when Cr and salt are combined. However, the free amino acid level declined when the combined concentration of Cr and salt was increased. According to this study, salt may counteract the effects of metal Cr on plants. Unlike Tejera et al. [34], common bean (*P. vulgaris* cv. Contender) treated with a 25 mM dose of salt did not experience an increase in the content of total free amino acids. The increase in amino acid concentration at low and high salt might be due to protein degradation or it could be related to nodule osmotic adjustment during solitary salt concentrations. In addition to these amino acids, a few other amino acids, such as ornithine and citrulline, do not remain in proteins [4,17].

Earlier studies suggest that several nonstructural carbohydrates accumulate in different plants, depending on the stress conditions. These include hexose, sucrose, sugar alcohol, etc. TSS was found to be gradually increased with the increase in metal concentration by itself. A strong correlation has been reported in previous research between sugar accumulation and osmotic stress tolerance in soybean plants, *Arabidopsis thaliana*, and *Craterostigma plantagineum* [31,33,5]. According to the hypothesis [16], sugar acts as an osmoticum and regulates osmotic pressure, as well as protecting some macromolecules. At both low and high levels of salt alone and when both Cr and NaCl were combined, the accumulation of sugar was unaffected, suggesting that the TSS concentration is adequate to conserve the osmotic regulation while adapting to stress conditions.

Amorphous proline, an imino group-containing amino acid widely distributed in the cell, plays vital roles in osmotic adjustment, preserving plasma membrane integrity, acting as a radical scavenger, and providing nitrogen and carbon to the cell [16]. Shiva Prakash [30] demonstrated that proline and sodium concentrations were positively correlated, and enhancing the concentration of salt and Cr alone in cultivars significantly increased proline content. Moreover, if both Cr and NaCl are used in combination, there are no significant changes observed with increasing concentration.

The phenolic compound plays a key role in the scavenging of harmful ROS in plants under stress conditions, thus reducing the peroxidation of cellular membranes. This results in increased membrane stability and faster recovery from oxidative damage. The phenol level in *V. radiata* increased with salt concentration, as evidenced by our results. According to the study, the plant was highly affected by salt stress and was able to stimulate the phenylpropanoid biosynthesis pathway, resulting in several phenolic compounds with high antioxidative activity [28]. Furthermore, phenol content remained unaffected by low and moderate Cr concentrations, indicating that it is maintained to a level sufficient to minimize oxidative damage. Following the records for Cr and NaCl combined treatments, phenolic content slowly decreases when the supply of Cr is high with the supply of NaCl, suggesting that Cr may counteract salt stress.

Manchanda and Garg [5] showed that salt and metal stress in plants produced reactive oxygen species, including hydrogen peroxide (H_2O_2),

superoxide radicals, and hydroxyl radicals. Catalase is a cellular enzyme that converts hydrogen peroxide into molecular oxygen and water as well as preventing AOS from affecting various cellular components. We found that CAT activity increases under high salt concentrations (35000 µM). Additionally, our results suggested that CAT activity increases with a higher level of salt concentration. We also found that CAT activity is higher under salt and Cr combined stress than Cr alone. This result suggests that salt-induced H_2O_2 scavenging of oxygen by CAT will be unaffected or little affected by Cr stress, while Cr with NaCl will not influence the level of CAT activity.

It was observed that Cr and salt toxicity is a result of excessive chromium and salt uptake causing an osmotic imbalance and ionic imbalance. Among the minerals inhibited in the presence of Cr are magnesium, calcium, potassium, iron, and manganese [24]. In the case of salinity, Ca^{2+}, Na^{+}, and Mg^{2+} usually contribute to salinity, and K^{+} may or may not contribute as well [3]. In excessive amounts, Na^{+}, Cl^{-}, and B can be toxic to plants, resulting in the deterioration of the seedlings of *V. radiata*. Increasing NaCl concentration leads to an increasing accumulation of Na^{+} ions, which reduces the obligatory intake of K^{+} required to sustain plant metabolism [23]. It has been suggested that preferential accumulation of Na^{+} or Cl^{-} and NaCl is metabolically important because it takes less energy to absorb inorganic ions than to synthesize organic solutes [35]. It was stated earlier that plant tissue absorbs Na^{+} at the cost of calcium and potassium under salinity. Rather, Na^{+} was the main contributor to osmotic potential and supported ion homeostasis. In contrast, under solitary treatment with low chromium (15 µM), all mineral ions were measured in far higher amounts than in other treatments and controls. Furthermore, the Mg^{2+} content of total tissue decreased when chromium and salt were added together versus just chromium and salt. As Shaul [28] suggested, magnesium is critical for photosynthesis, and its fluctuation can be used to predict enzyme activity in the chloroplast. In a low-chromium environment, a seedling's magnesium content indicates that its photosynthetic system may have been unaffected. Increased salt concentration demonstrated stimulatory effects on Mg^{2+} and B concentrations, as well as Mn^{2+}, Fe^{2+}, and Cu^{2+}. As opposed to this, high Na^{+} content hinders Ca^{2+} uptake, as Na^{+} penetrates root membranes, displaces Ca^{2+}, and makes it inactive, which results in Ca imbalance [3].

The present study concluded that *V. radiata* can tolerate high concentrations of Cr and NaCl as long as ion homeostasis and osmotic balance are maintained. However, Cr and salt stress remarkably reduced plant biomass, growth, and photosynthetic pigments and generated severe oxidative stress in *V. radiata*. Under salt and Cr stressed conditions, plant growth parameters showed a reduction suggesting that plants required more energy to absorb nutrients, maintain water, and synthesize and transport organic compounds for osmotic balance. Furthermore, to combat oxidative stress caused by ROS, antioxidant enzyme activity (CAT) increases. This suggests a strategic mechanism of defense for *V. radiata*. Salinity accelerated higher accumulation of organic metabolites including amino acid, phenol, and soluble sugar demonstrates their role in ROS scavenging, osmotic regulation, pH maintenance, nitrogen storage, and protection of several cellular macromolecules in *V. radiata* under enhanced salinity conditions. Salt further enhances the tolerance potential of *V. radiata* by reducing the uptake of Cr and by reducing Cr toxicity, a phenomenon known as cross-tolerance. This causes the plant to cope both with salt stress and Cr. All these parameters can be used as a selection criterion to screen *V. radiata* cultivars for the potential to accumulate Cr and to withstand salt stress. *V. radiata* can also be recommended for phytoremediation because it acquires Cr by oxidation. To fully understand the mechanisms of tolerance of Cr and salt toxicity, genetic interactions among the above parameters should be evaluated comprehensively. A major challenge facing agriculture is the administration of salt and heavy metal-induced toxicity in crops.

Abbreviations. CAT- Catalase; Cr- Chromium; GAM-5 (Gujarat Anand Mungbean-5); GPx- Guaiacol Peroxidase HM-Heavy metals

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Abbasi, H., Robab, M.I., Akhtar, A., Sharf, R., (2012): Chromium toxicity in mung bean, *Vigna radiata* and bioremediation by *Pseudomonas fluorescens*. International Journal of Plant, Animal and Environmental Sciences, 2(1): 99-101.

[2] Akpapunam, M., (1996): Mung bean (*Vigna radiata* (L.) Wilczek). Food and Feed from Legumes and Oilseeds. Springer, Boston, MA 209–215. doi:10.1007/978-1-4613-0433-3_23.

[3] Alam, S.M., (1999): Nutrient uptake by plants under stress conditions. Handbook of Plant and Crop Stress, 2: 285-313.

[4] Ashraf, M., Waheed, A., (1993): Responses of some local/exotic accessions of lentil (*Lens culinaris* Medic.) to salt stress. Journal of Agronomy and Crop Science, 170(2): 103-112. doi.org/10.1111/j.1439-037X.1993.tb01063.x

[5] Bartels, D., Sunkar, R., (2005): Drought and salt tolerance in plants. Critical Reviews in Plant Sciences, 24(1): 23-58.

[6] Bates, L.S., Waldren, R.P., Teare, I.D., (1973): Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205–207.

[7] Benito, B., Haro, R., Amtmann, A., Cuin, T.A., Dreyer, I., (2014): The twins K^{+} and Na^{+} in plants. Journal of Plant Physiology, 171(9): 723-731. doi.org/10.1016/j.jplph.2013.10.014

[8] Braber, J.M., (1980): Catalase and peroxidase in primary bean leaves during development and senescence. Zeitschrift für pflanzenphysiologie, 97(2): 135–144.

[9] Diwan, H., Khan, I., Ahmad, A., Iqbal, M., (2010): Induction of phytochelatins and antioxidant defence

system in *Brassica juncea* and *Vigna radiata* in response to chromium treatments. Plant Growth Regulation, 61(1): 97-107. doi:10.1007/s10725-010-9454-0.

[10] Duncan, D.B., (1955): Multiple range and multiple F test. Biometrics, 11: 1-42.

[11] Gupta, S., Pratap, A., (2016): Mungbean-Summer cultivation in India (Pocket Guide), AICRP on MULLaRP, Indian Institute of Pulses Research, Kanpur-208024. Extension bulletin, pp: 55-80.

[12] Hedge, J.E., Hofreiter, B.T., Whistler, R.L., (1962): Carbohydrate chemistry. Academic Press, New York, pp. 371-380.

[13] Jabeen, N., Abbas, Z., Iqbal, M., Rizwan, M, Jabbar, A, Farid, M, Ali, S., Ibrahim, M., Abbas, F., (2015): Glycine betaine mediates chromium tolerance in mung bean through lowering of Cr uptake and improved antioxidant system. Archives of Agronomy and Soil Science, 62(5): 648–662.

[14] Keeling, S.M., Stewart, R.B., Anderson, C.W.N., Robinson, B.H., (2003): Nickel and cobalt phytoextraction by the hyperaccumulator *Berkheya coddii*: implications for polymetallic phytomining and phytoremediation. International Journal of Phytoremediation, 5: 235-244.

[15] Lambrides, C.J., Godwin, I.D., (2007): Mungbean. pp. 69-90. In Kole C. (eds) Pulses, Sugar and Tuber Crops. Genome Mapping and Molecular Breeding in Plants, vol 3. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-34516-9_4.

[16] Manchanda, G., Garg, N., (2008): Salinity and its effects on the functional biology of legumes. Acta Physiologiae Plantarum, 30(5): 595-618. doi.org/10.1007/s11738-008-0173-3

[17] Mansour, M.M.F., (2000): Nitrogen containing compounds and adaptation of plants to salinity stress. Biologia Plantarum, 43(4): 491-500. doi.org/10.1023/A:1002873531707

[18] Moore, S., Stein, W.H., (1948): Photometric ninhydrin method for use in the chromatography of amino acids. Journal of Biological Chemistry, 176: 367-388.

[19] Munns, R., (2002): Comparative physiology of salt and water stress. Plant, Cell & Environment, 25(2): 239-250.

[20] Munzuroğlu, Ö., Zengin, F.K., Yahyagil, Z., (2008): The abscisic acid levels of wheat (*Triticum aestivum* L. cv. Çakmak 79) seeds that were germinated under heavy metal (Hg^{++}, Cd^{++}, Cu^{++}) stress. GU Journal of Science, 21(1):1-7.

[21] Panda, A., Rangani, J., Kumari, A., Parida, A.K., (2017): Efficient regulation of arsenic translocation to shoot tissue and modulation of phytochelatin levels and antioxidative defense system confers salinity and arsenic tolerance in the halophyte *Suaeda maritima*. Environmental and Experimental Botany, 143: 149-171.

[22] Pang, Z., Chong, J., Li, S., Xia, J., (2020): MetaboAnalystR 3.0: Toward an Optimized Workflow for Global Metabolomics. Metabolites, 10(5): 186.

[23] Parida, A.K., Jha, B., (2013): Inductive responses of some organic metabolites for osmotic homeostasis in peanut (*Arachis hypogaea* L.) seedlings during salt stress. Acta Physiologiae Plantarum, 35(9): 2821-2832.

[24] Peralta-Videa, J.R., Lopez, M.L., Narayan, M., Saupe, G., Gardea-Torresdey, J., (2009): The biochemistry of environmental heavy metal uptake by plants: Implications for the food chain. The International Journal of Biochemistry & Cell Biology, 41(8-9): 1665–1677.doi:10.1016/j.biocel.2009.03.005

[25] Sadasivam, S., Manickam, A., (1992): Biochemical methods for agricultural sciences. New Age International. Wiley Eastern Limited, New Delhi, pp. 187-188.

[26] Saha, P., Mukherjee, A., Biswas, A.K., (2015): Modulation of NaCl induced DNA damage and oxidative stress in mungbean by pretreatment with sublethal dose. Biologia Plantarum, 1: 139-146. doi:10.1007/s10535-014-0460-3

[27] Samreen, T., Shah, H.U., Ullah, S., Javid, M., (2017): Zinc effect on growth rate, chlorophyll, protein and mineral contents of hydroponically grown mungbeans plant (*Vigna radiata*). Arabian Journal of Chemistry, 10: S1802–S1807. doi:10.1016/j.arabjc.2013.07.005

[28] Sharma, S.S., Dietz, K.J., (2006): The significance of amino acids and amino acid-derived molecules in plant responses and adaptation to heavy metal stress. Journal of Experimental Botany, 57(4): 711-726. doi.org/10.1093/jxb/erj073

[29] Shaul, O., (2002): Magnesium transport and function in plants: the tip of the iceberg. Biometals, 15(3): 307-321.

[30] Shiva Prakash, N., (1993): Isolation of salt tolerant cell lines of *Cajanus cajan* and comparative study of their physiological responses with intact plants under salt stress. Plant Physiology and Biochemistry, New Delhi, 20: 11-15.

[31] Streeter, J.G., Lohnes, D.G., Fioritto, R.J., (2001): Patterns of pinitol accumulation in soybean plants and relationships to drought tolerance. Plant Cell & Environment, 24(4): 429-438. doi.org/10.1046/j.1365-3040.2001.00690.x

[32] Swain, T., Hillis, W.E., (1959): The phenolic constituents of *Prunus domestica* I. - The quantitative analysis of phenolic constituents. Journal of the Science of Food and Agriculture, 10(1): 63-68. doi.org/10.1002/jsfa.2740100110

[33] Taji, T., Ohsumi, C., Iuchi, S., Seki, M., Kasuga, M., Kobayashi, M., Yamaguchi-Shinozaki, K., Shinozaki, K., (2002): Important roles of drought-and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana.* The Plant Journal, 29(4): 417-426. doi.org/10.1046/j.0960-7412.2001.01227.x

[34] Tejera, N.A., Campos, R., Sanjuan, J., Iluch, C., (2005): Effect of sodium chloride on growth, nutrient accumulation, and nitrogen fixation of common bean plants in symbiosis with isogenic strains. Journal of Plant Nutrition, 28(11): 1907-1921. doi.org/10.1080/01904160500306458

[35] Zeng, Y., Li, L., Yang, R., Yi, X., Zhang, B., (2015): Contribution and distribution of inorganic ions and organic compounds to the osmotic adjustment in *Halostachys caspica* response to salt stress. Scientific Reports, 5(1): 1-12. doi.org/10.1038/srep13639

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