Physiological and Biochemical Attributes of Chromium Detoxification are Regulated by Root synthesized Organic Acids in Rice Varieties

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ABSTRACT

To know effective physiological response against chromium toxicity, 25 rice varieties were cultivated on the hydroponic solution treated with 100µM chromium and physiological as well as biochemical features were evaluated compared with non-treated control plants. In this investigation, it was found that the concentration of citric acid synthesized and secreted by roots influences the mitigation of chromium toxicity. In varieties BR-58, BR-63 and BR-68 chromium uptake were significantly higher than the control plant but their translocation to shoot was restricted indicating elevated Cr retention in roots. This retention was facilitated by root secreted citric acid which was assured by significant rhizospheric pH reduction (15%, 18.5% and 20.9% respectively) under chromium stress. Furthermore, BR-73 showed an efficient exclusion mechanism keeping down metal uptake by citric acid ensured by 15% rhizosphere pH reduction. In contrast, varieties of rhizospheres with a pH reduction of less than 10% were unable to withstand chromium toxicity. The findings indicate that a reduction of 15% or more in rhizospheric pH serves as the benchmark for the necessary level of organic acid secretion required for chromium tolerance. Moreover, the strategies employed for tolerance differ based on genotypes rather than species. Furthermore, it offers an efficient screening technique for metal tolerant rice plants.

Key word: rhizospheric pH, chromium tolerant, organic acids, adsorption, *Oryza sativa*

INTRODUCTION

Chromium (Cr), a hazardous heavy component found in the outer layers of the Earth, has adverse effects on the environment. It is thoroughly used in leather tanning, electroplating process, steel production, metal finishing, catalyst uses and pigmentation. The relevant sources of Chromium (Cr) exposure in the environment are industrial discharges and domestic sewage (Nath et al. 2008;). In plants, accumulated Cr inhibits growth by limiting the absorption of nutrients (Ullah et al., 2023; Shanker et al., 2005). It also causes chlorosis in young plants, lowers pigment content, alters enzymatic functions, damages root cells, and induce ultrastructural changes to the cell membrane and chloroplast (Panda and Choudhury 2008). Cr also significantly impairs the development of stems and leaves during the early growth stage of the plant, as well as the formation of dry matter in seedlings (Dey et al., 2023; Nematshahi et al., 2012). The toxicity of Cr is dependent on the metal species that determine Cr's absorption, transport, and accumulation. Numerous investigations on the chemistry of chromium in soil and its uptake by plants have indicated that Cr is harmful for plant growth (Arun et al. 2005).

In order to withstand the toxicity of heavy metals, plants have an intricate and interrelated system of defense mechanisms. Plant's physical barriers, which include cell walls, physiologically active tissues like trichomes, and morphological features like thick cuticle, are their first line of protection against

metals (Al-Khayri et.al., 2023, Wong et.al., 2004, Harada et.al., 2010). To counteract and lessen the negative effects of HMs, plants activate several cellular defense mechanisms when the metal ions penetrate biophysical barriers and enter tissues and cells. Tolerating or neutralizing metal toxicity mostly involves the biogenesis of several cellular macromolecules, including asnicotianamine, putrescine, spermine, mugineic acids, organic acids, phytochelatins, metallothioneins, cellular exudates, heat shock proteins, certain amino acids, and hormones (Viehweger 2014, Dalvi and Bhalerao 2013, Sharma and Dietz 2006). Ineffectiveness of the aforementioned approaches in plants increases the production of ROS (Mourato et.al. 2012). In order to eliminate the free radicals, plants then increase their enzymatic antioxidants, which includes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reductase (GR), as well as their nonenzymatic antioxidants, such as ascorbate (AsA), glutathione (GSH), carotenoids, alkaloids, tocopherols, proline, and phenolic compounds (Sharma et.al., 2012, Rastgoo et.al., 2011). However, genetic potential of plant species as well as the metal type are the key determinant of metal tolerance level (Solanki and Dhankhar 2011).

Root excreted low molecular weight organic acid (LMWOA) also known to regulate different stress especially oxidative stress (Airaki et al., 2012). Synthesized LMWOA released to the rhizosphere through increased efflux (De La Fuente et al., 1997; López-Bucio et al., 2000a) but not authenticated for every plant (RRRRR). Root excretion organic acid and its impact on physiology was studied enough in previous. Most of this OA detoxify heavy metal in two ways. First one in the internal chelating of heavy metals both in photosynthetic and non-photosynthetic tissues (Fernando et al., 2010). Second one is the insoluble complex formation of organic acid with heavy metal and release phosphorus ion (Pi) from the bound complex. Here positively charged cation of heavy metal react with negatively charged carboxylic group of organic acids (OAs) to form insoluble complexes that not absorbed by plant (Zhang et al., 2018).

However, these LMWOAs are synthesized in mitochondria and enzymes participate in glycolysis, TCA and glyoxylate cycles are also responsible for the synthesis (Igamberdiev and Eprintsev, 2016). Stress condition enhanced LMWOA synthesis by hampering the standard pathway of these cycles. Furthermore, anaplerotic reactions (chemical reactions that form inter- mediates of the TCA cycle) rat enhance OAs synthesis to cope with stress (Dong et al., 2004). Thus, higher level of transcription of genes encoding TCA cycle enzymes as well as their enhanced enzymatic activities upregulate the LMWOA synthesis and help plants to cope with metal toxicity (Zhou et al., 2018, Uhde-Stone et al., 2003a).

In japonica rice, OsFRDL4 and OsFRDL2 are upregulated under Aluminum stress to secrete citric acid from roots (Yokosho et al., 2011). But types and amount of root secreted organic acids are the lack of clear evidence though it is assumed that surrounding environment may responsible for this specificity (RRRRRR).

Consumption of carbon sources for production and efflux of OAs under stress utilize significant proportion of carbon imposing an energy cost to plants which is economically important for plants especially fast-growing annual crops (Koyama et al., 2000; Herz et al., 2018). Plants optimize its carbon loss through tissue-specific or location specific exudation of OAs from the roots firstly. Secondly, it limits the amount of OAs release by negative regulators of OA exudation like GABA (Ramesh et al., 2015). But belowground rhizosphere deposition was not even considered in any models for carbon allocation (Reyes et al., 2020). So, it demands future studies on this significant issue. Moreover, the regulatory mechanisms regarding types and amount of root secreted organic acids remain largely elusive.

However, effective physiological responses against toxic chromium in rice plants remain poorly understood. These types of responses against metal toxicity are classified as either internal or external (Kochian et al., 2004). Local rice variety, Pokkali develop tolerant against chromium by limiting its Fe reductase and Fe transporter activities (Kabir, 2016). Furthermore, Zeng Fanrong et.al., (2008) reported that rice plants released oxalic, malic, and citric acid at the rhizosphere and enhanced Cr accumulation. But there are no reports on how organic acids work or if they have any effective role against chromium

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toxicity in rice. Moreover, external mechanisms, such as root exudation-mediated heavy metal avoidance or tolerance, remain ill-defined. Therefore, different high-yielding rice varieties were exposed to Cr stress and evaluated the physiological and biochemical features to gain new insights into metal tolerance in rice.

MATERIALS AND METHODS

Plant cultivation

In this study, seeds from 25 authentic rice varieties (Bangladesh Rice Research Institute variety BRRI 50 to 73 and 22) were initially collected at the germplasm center of the Bangladesh Rice Research Institute. After being sterilized with 95% (v/v) ethanol, water-washed seeds were allowed to germinate in wet filter paper Petri dishes for two to three days at room temperature in the dark. The plants that germinated uniformly were transferred to the Hoagland and Arnon (1950) hydroponic solution (100 ml). For rice seedlings under chromium stress, a hydroponic solution containing 100 µM K2Cr2O7 (a source of the heavy metal chromium) was used, while the solution for the control plant did not contain any chromium addition. The hydroponic solution's pH was adjusted to 6.0. the chromium-treated and untreated control seedlings were cultured in a nutrient solution in a growth chamber (Temp. 260-280 , Humidity 70%-80%) with 10 hours of light and 14 hours of darkness (550–560 mmol s-1 per mA). The nutritional solution was guaranteed to be continuously aerated. After seven days of culture, seedlings treated with and without chromium were collected, and distinct roots and shoots were used for each experiment.

Evaluation of morphological characteristics

Seven-day-old plant's root length and shoot height were measured in centimeters. The roots and shoots were then dried in an oven set to 80o C for two days in order to calculate their dry weight.

Measurement of electrolyte leakage

Electrolyte leakage (EL) was measured using the Lutts et al. (1996) methodology.

Determination of chlorophyll concentration:

A pre-chilled mortar and pestle was used to homogenize 100 mg of fresh leaf tissue with 5–10 mL of 90% (v/v) acetone and then centrifuged for five to ten minutes at 3000 rpm. To perform a spectrophotometric analysis, the clear supernatant was collected. The concentration of chlorophyll in leaves was measured with 90% (v/v) acetone based on the Lichtenthaler and Wellburn (1985) procedure.

Determination of Cr and Fe by AAS (atomic absorption spectroscopy)

Roots and shoots were first cleaned with CaSO4 and deionized water and then dried in an oven at 80o C for three days. The samples were digested for 15 minutes at 100º C using mixes of HNO3 and H2O2 (3:1), and atomic absorption spectroscopy was used for investigation.

Determination of total soluble proteins

Fresh plant tissue was homogenized in 1-2 milliliters of ice-cold 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged for 15 minutes at 4°C at 12,000 rpm and Quantification of proteins was done using the supernatant. 100 µL of the sample and 1.0 mL of Bradford reagent were mixed and allowed to sit at room temperature for 5 to 10 minutes. The absorbance was measured with a UV-Visible spectrophotometer at 595 nm. Absorbance measurements were plotted against known BSA concentrations to create a standard calibration curve. Each sample protein concentration was determined by extrapolating the absorbance values from the standard curve.

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Estimation of lipid peroxidation

The roots and shoots were homogenized with 5% (w/v) trichloroacetic acid (TCA) and centrifuged at 11,500×g for 15 minutes. Following centrifugation, thiobarbituric acid (TBA) was added to the separated supernatant, then mixture was heated in a water bath for 30 minutes at 95 °C. As the mixture cooled, absorbance was measured at 532 nm. Malondialdehyde was measured and reported as nmol of MDA mg^{-1} FW using an extinction value of 155 mM^{-1} cm^{-1} (Heath and Packer 1968).

Determination of rhizospheric pH change

A digital pH meter was employed to measure the media's pH both before and after the seedlings were cultured. Using these data, the pH reduction was calculated and expressed as a percentage.

Silver nitrate precipitation test:

First, 0.1 M silver nitrate ($AgNO_3$) was prepared. Then a few drops of silver nitrate were added to each tube holding the culture fluid. The organic acid indication, white precipitate, was seen.

Thin-layer chromatography (TLC)

Rice plant root exudates (BR-58, BR-63, BR-68, and BR-73), treated and untreated with Cr, were analyzed using thin-layer chromatography (TLC). The mobile phase was made up of ethanol, NH4OH, and H2O in the ratio 75.5:12.5:12.5. The TLC plate was then placed in the TLC chamber after the placement of the samples and standards of known organic acids on it. The TLC plate is removed from the chamber and allowed to dry when the solvent has travelled a sufficient distance. The TLC plate was then examined under a UV light, and the spots were noted. The sample's organic acids were determined by comparing them with known benchmarks.

Enzymatic analysis

The enzymes CAT (EC. 1.11.1.6), POD (EC. 1.11.1.7), SOD (EC. 1.15.1.1), and GR (EC. 1.6.4.2) were extracted from one-week-old plants using a modified version of Goud and Kachole's (2012) approach. After being crushed in 100 mM phosphate buffer, the roots and shoots tissues were centrifuged for 10 minutes at 13000×g. For the Catalase (EC. 1.11.1.6) analysis, the reaction mixture (2 ml) consisted of 400 µL of 6% (v/v) H2O2, 100 µL of root extract and 100 mM potassium phosphate buffer (pH 7.0). A UV spectrophotometer was used to measure the absorbance at 240 nm (extinction coefficient of 0.036 mM-1 cm-1) at 30-second to one-minute intervals after the addition of root or shoot extract. The unit of measurement for CAT activity is mmol of H2O2 oxidized min-1 (mg protein 1).

Similarly, two ml reaction mixture was prepared to measure peroxidase (EC 1.11.1.7) activity. Here 100 mM potassium phosphate buffer (pH 6.5), 1ml of 50 mM pyrogallol, 400 µL of 200 mM H_2O_2, and 100 µL of root extract as the enzyme source were all included in the combination. In the spectrophotometer, the absorbance variations were measured from 30 seconds to 1.5 minutes at 430 nm (extinction coefficient 12 mM-1 cm-1). The concentration of peroxidase is measured in mmol pyrogallol oxidized min-1 (mg protein-1). Additionally, 50 mM sodium carbonate/bicarbonate buffer (pH 9.8), 0.1 mM EDTA, 0.6 mM epinephrine, and enzyme made up the SOD (EC. 1.15.1.1) assay combination. Then adrenochrome formation was observed at 475 nm using a UV-Vis spectrophotometer. The quantity of enzyme required for 50% inhibition of epinephrine oxidation is established as per unit SOD activity. In order to perform glutathione reductase (EC. 1.6.4.2) analysis, 100 µL of root extract was added to the reaction mixture, which also contained 1 mL of 0.2 M phosphate buffer (pH 7.0), 1 mM EDTA, 0.75 ml of distilled water, 0.1 mL of 20 mM oxidized glutathione (GSSG), and 0.1 mL of 2 mM NADPH. At 340 nm, the oxidation of NADPH by GR was then measured. The extinction coefficient of 6.12 mM-1 cm-1 was then used to calculate the rate of GR activity (nmol min-1).

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Determination of hydrogen peroxide and superoxide (O2-)

Tissues were centrifuged at 10,000×g for 15 minutes, after being pulverized in 0.1% (v/w) trichloroacetic acid (TCA). Before reading the absorbance at 390 nm, the reaction mixture was made by mixing potassium iodide (M) and phosphate buffer (10mM, pH7.0) with the supernatant. It was left in the dark for an hour (Alexieva et al., 2001). A standard calibration curve with known H2O2 levels was used for quantification.
For superoxide (O2-) measurement, plant samples were centrifuged at 5000 rpm for 7 minutes at 4 0C after they had been crushed in 1 milliliter of 65 mM potassium phosphate buffer (pH 7.8). 50µl of 10 mM hydroxylamine hydrochloride, 0.5 ml of supernatant, and 450µl of 65 mM potassium phosphate buffer (pH 7.8) were used to create the reaction mixture, which was then incubated for 30 minutes at 25 °C. After that, 125µl of 7mM alpha-napthyl amine and 10mM sulfanilamide were added to the mixture, and then incubated for 20 minutes at 25 °C. The absorbance at 530 nm was measured using spectrophotometry. Superoxide (O2-) levels were determined using a standard curve built with known NO values.

Estimation of metabolites (Glutathione, phytochelatin and proline) content:

The method developed by Anderson et al. (1992) was used to extract glutathione. In order to determine total glutathione, GSSG was first converted to GSH by mixing the leaf extract with 130 mM sodium phosphate buffer (pH 7.4) and one unit of glutathione reductase. The mixture was then kept at 30°C for 10 minutes. The final reaction mixture was then made by adding 50 mM of NADPH and 7 mM of sodium phosphate buffer (pH 6.8) that contained 6 mM of DTNB. Absorbance was measured at 412 nm after this reaction mixture was held at 30°C for 10 minutes. To estimate glutathione, a standard curve of known quantities of GSH was employed (Griffith 1980). Phytochelatin content was determined using the previously described procedure by Mahmud et al. (2018).

To measure the amount of proline samples of leaves and roots were centrifuged at 11,500×g for 12 minutes after being homogenized in 3% (v/w) sulfosalicylic acid. Next, the 100 µL plant extract supernatant was mixed with 200 µL glacial acetic acid, 100 µL of 3% (v/w) sulfosalicylic acid, and 200 µL acidic ninhydrin. The mixture was then heated for 60 minutes at 96◦C and immediately chilled on ice. Spectrophotometer was used to take a reading at 520 nm (Bates et al., 1973). Calculations were performed using a standard curve with known proline concentrations.

Statistical analysis

Completely randomized block design with four independent replications was adopted in each experiment.t-test at 0.05% significance level with the help of Microsoft Excel 2007 was performed as statistical analysis. Moreover, Graph Pad Prism was applied to prepare graphical presentations.

RESULT

Morpho-physiological parameters:

Seedlings grown on the hydroponic solution containing Cr showed retardation of growth in most of the rice varieties except BR-58, BR-63, BRRI-68, and BR-73. No significant shoot and root length as well as root and shoot dry weight reduction were observed in these four varieties for chromium toxicity compared with the control (table. 1). Moreover, chlorophyll concentrations in the shoot of these four varieties remained unchanged under chromium stress, whereas in other varieties, it decreased meaningfully under chromium stress.

Furthermore, no meaningful differences between control and chromium treated plant was observed in these four varieties (BR-58, BR-63, BRRI-68, and BR-73) in case of electrolyte leakage. But in

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remaining varieties electrolyte leakage was found to be increased significantly under chromium stress equated to control. Considering the above-mentioned parameters BRRI-58, BRRI-63, BRRI-68 and BRRI-73 found to cope with chromium toxicity.

Table 1. Morpho-physiological features of 25 high yielding rice varieties grown in absence or presence of Chromium on hydroponic solution. Different letters indicate significance difference between means of treatments (number of replications is 4) followed by t-test. Data were from one-week plats.

Variety	Treatment	Root length (cm)	Root dry wt (mg)	Shoot length (cm)	Shoot dry wt (mg)	Total Chlorophyll (µgm/mg)	Electrolyte leakage in root (µs cm^{-1} g^{-1})
BRRI-22	Control	4.83±0.29^a	2.67±0.58^a	7.5±.5^a	7.33±0.58^a	130.58±5.56^a	0.955±0.08^a
	Treatment	3.5±0.5^b	0.77±0.25^b	4.83±0.29^b	5.33±0.58^b	79.27±5.77^b	1.750±0.23^b
BRRI-50	Control	6±0.87^a	2±0^a	10.5±0.87^a	5.33±0.58^a	255.18±47.22^a	0.915±0.60^a
	Treatment	2.17±0.58^b	0.36±0.13^b	3.17±0.76^b	2.33±0.58^b	162.30±20.14^b	1.962±0.86^b
BRRI-51	Control	7.83±1.44^a	2±0^a	8.5±0.5^a	6.83±0.29^a	290.57±8.92^a	1.263±0.15^a
	Treatment	3.67±1.15^b	0.83±0.29^b	7.17±0.29^b	5.5±0.5^b	268.09±20.79^b	2.568±0.81^b
BRRI-52	Control	9.67±0.58^a	4.33±0.58^a	11.33±0.58^a	12.67±0.58^a	200.24±11.85^a	0.531±0.41^a
	Treatment	5.83±0.29^b	1.77±0.87^b	8.17±0.76^b	10.67±0.58^b	180.93±3.28^b	1.502±0.58^b
BRRI-53	Control	8±1^a	3.33±0.58^a	11±0.5^a	9.33±0.58^a	309.21±3.32^a	0.789±0.18^a
	Treatment	6.17±0.29^b	2±0^b	9±0.5^b	7.67±0.58^b	288.94±3.17^b	1.669±0.59^b
BRRI-54	Control	5±0.87^a	3.83±0.29^a	9±0.5^a	8.67±1.15^a	424.33±42.49^a	0.869±0.29^a
	Treatment	3.5±0^b	3±0^b	9±0^a	8.83±1.04^a	220.44±78.13^b	1.718±0.307^b
BRRI-55	Control	7.33±0.29^a	3.83±0.29^a	13.83±0.76^a	10.17±0.29^a	227.89±31.12^a	0.955±0.21^a
	Treatment	6.33±0.58^b	2.67±0.29^b	11.33±0.58^b	8.66±0.58^b	216.90±20.56^a	1.483±0.48^b
BRRI-56	Control	6.67±0.58^a	4±0.5^a	10.67±0.58^a	8.67±0.29^a	416.20±170.96^a	1.203±0.08^a
	Treatment	5.33±0.29^b	3±0^b	9.33±0.58^b	7.33±0.29^b	387.21±48.55^a	1.758±0.19^b
BRRI-57	Control	8.33±0.58^a	1.83±0.29^a	9.83±0.58^a	7.83±0.29^a	264.99±44.99^a	1.083±0.14^a
	Treatment	6.5±0.5^b	0.93±0.12^b	8±0.87^b	6.67±0.58^b	232.14±58.09^a	2.145±0.74^b
BRRI-58	Control	9.17±2.02^a	3.33±0.58^a	10.83±0.76^a	8.5±0.5^a	262.39±78.84^a	1.735±0.23^a
	Treatment	7.33±2.31^a	2.67±1.15^a	10.5±0.5^a	8.33±0.58^a	300.94±42.66^a	1.902±0.81^a
BRRI-59	Control	8.17±0.76^a	2.83±0.29^a	8±1^a	8.67±0.29^a	454.60±54.07^a	1.259±0.35^a
	Treatment	5.67±0.58^b	2±0^b	6±1^b	7.5±0.5^b	211.31±52.26^b	2.185±0.16^b
BRRI-60	Control	9±0^a	2.33±0.58^a	13.83±0.29^a	10.33±0.58^a	347.63±8.61^a	1.865±0.56^a
	Treatment	7.67±0.58^b	1.67±0.58^b	11.5±0.87^b	9.17±1.15^b	316.30±40.03^a	2.026±0.46^a
BRRI-61	Control	6.67±0.58^a	3.67±0.58^a	9±0^a	8±0^a	274.89±6.00^a	0.997±0.58^a
	Treatment	4±1.73^b	2.33±0.58^b	7.67±0.58^b	6.67±0.58^b	165.69±57.69^b	1.941±0.49^b
BR-62	Control	6.33±2.25^a	2.67±0.58^a	9±2.783^a	6.67±2.52^a	363.50±40.25^a	0.819±0.18^a
	Treatment	6.33±3.21^a	3±1^a	9.5±2.179^a	6.66±2.08^a	282.39±38.00^b	0.925±0.44^a

BRRI-63	Control	3.33±0.28[a]	3.33±0.58[a]	10.83±0.58[a]	8±1[a]	310.99±88.42[a]	1.932±0.913[a]
	Treatment	4.5±0.87[a]	2.67±0.58[a]	10.17±1.89[a]	7.33±0.58[a]	306.85±26.59[a]	1.944±0.48[a]
BRRI-64	Control	9.33±0.58[a]	4±0[a]	10.67±0.76[a]	10.33±0.58[a]	465.75±10.17[a]	1±0[a]
	Treatment	6.67±0.58[b]	3.17±0.29[b]	9.33±0.29[b]	9.17±0.29[b]	382.09±15.69[b]	1.825±0.72[b]
BRRI-65	Control	3.33±1.15[a]	4.17±1.26[a]	11.5±1.80[a]	10±0[a]	336.40±30.52[a]	1.189±0.27[a]
	Treatment	5.5±1.80[a]	3.67±0.58[a]	13.67±1.61[a]	9.33±0.58[a]	173.67±44.59[b]	1.653±0.74[a]
BRRI-66	Control	8.33±1.53[a]	3.67±0.58[a]	9±1[a]	7±0[a]	414.54±38.93[a]	0.766±0.12[a]
	Treatment	4.67±0.58[b]	2.5±0.5[b]	7.17±0.29[b]	5.67±0.58[b]	263.36±20.61[b]	1.516±0.41[b]
BRRI-67	Control	5.67±0.58[a]	4±1.73[a]	11±0.5[a]	9.33±0.58[a]	512.35±43.78[a]	0.976±0.24[a]
	Treatment	4.13±0.55[b]	0.93±0.12[b]	9.17±0.29[b]	8.33±0.29[b]	209.97±89.81[b]	2.222±0.48[b]
BRRI-68	Control	4.33±1.53[a]	2.33±0.58[a]	7.17±2.84[a]	9.67±1.53[a]	251.98±22.95[a]	0.869±0.10[a]
	Treatment	4.83±1.89[a]	2.83±0.29[a]	7.5±3.04[a]	9.33±0.58[a]	268.57±76.08[a]	0.942±0.64[a]
BRRI-69	Control	5.17±1.04[a]	3.33±0.58[a]	9.5±0[a]	7.33±0.58[a]	430.47±53.04[a]	1.136±0.460[a]
	Treatment	6.17±2.57[a]	4.33±3.21[a]	8.83±2.08[a]	7.33±2.08[a]	262.21±42.71[b]	1.031±0.540[a]
BRRI-70	Control	4.33±2.57[a]	1.67±0.29[a]	10.67±1.15[a]	6.83±0.58[a]	829.50±93.83[a]	1.018±0.57[a]
	Treatment	3±0[b]	0.87±0.12[b]	6.5±0.5[b]	4.33±0.58[b]	424.15±79.69[b]	1.361±0.591[a]
BRRI-71	Control	7.5±0.5[a]	3.17±0.76[a]	10±0.87[a]	9.33±0.58[a]	459.82±48.06[a]	0.892±0.11[a]
	Treatment	6.17±0.29[b]	2.17±0.29[b]	7±0.87[b]	7.5±0.5[b]	326.48±75.24[b]	1.761±0.540[b]
BRRI-72	Control	11.33±0.58[a]	3.5±0.5[a]	9.17±0.76[a]	9±1[a]	393.12±112.24[a]	1.253 ±0.60[a]
	Treatment	7.33±1.15[b]	2.17±0.29[b]	7.33±0.29[b]	5.33±0.58[b]	375.90±68.46[a]	2.142±0.618[b]
BRRI-73	Control	8.33±0.29[a]	3.5±0.5[a]	9.33±1.53[a]	8±1[a]	352.32±87.64[a]	0.989±0.23[a]
	Treatment	7±0[b]	2.17±0.29[b]	11±1[a]	9±1[a]	232.54±28.91[b]	1.538±0.3[a]

Chromium and Iron content:

As the initial investigation indicates that BR-58, BR-63, BRRI-68, and BR-73 varieties owned the detoxification mechanism, analysis of chromium concentration in root and shoot was conducted in these four varieties. Root's chromium content of rice varieties BR-58, BR-63, and BR-68 was significantly higher under chromium stress compared with the control plant. But in the shoot, no meaningful differences in chromium content between control and chromium-stressed plants of the varieties were observed (Fig. 1). This analysis concludes that chromium translocation from root to shoot was inhibited in varieties BR-58, BR-63, and BR-68 under the stressed condition.

Furthermore, significant iron content compared with the control plant was found only in the root of varieties BR-58 and BR-63 and in the shoot of BR-58 among the varieties grown under chromium stress.

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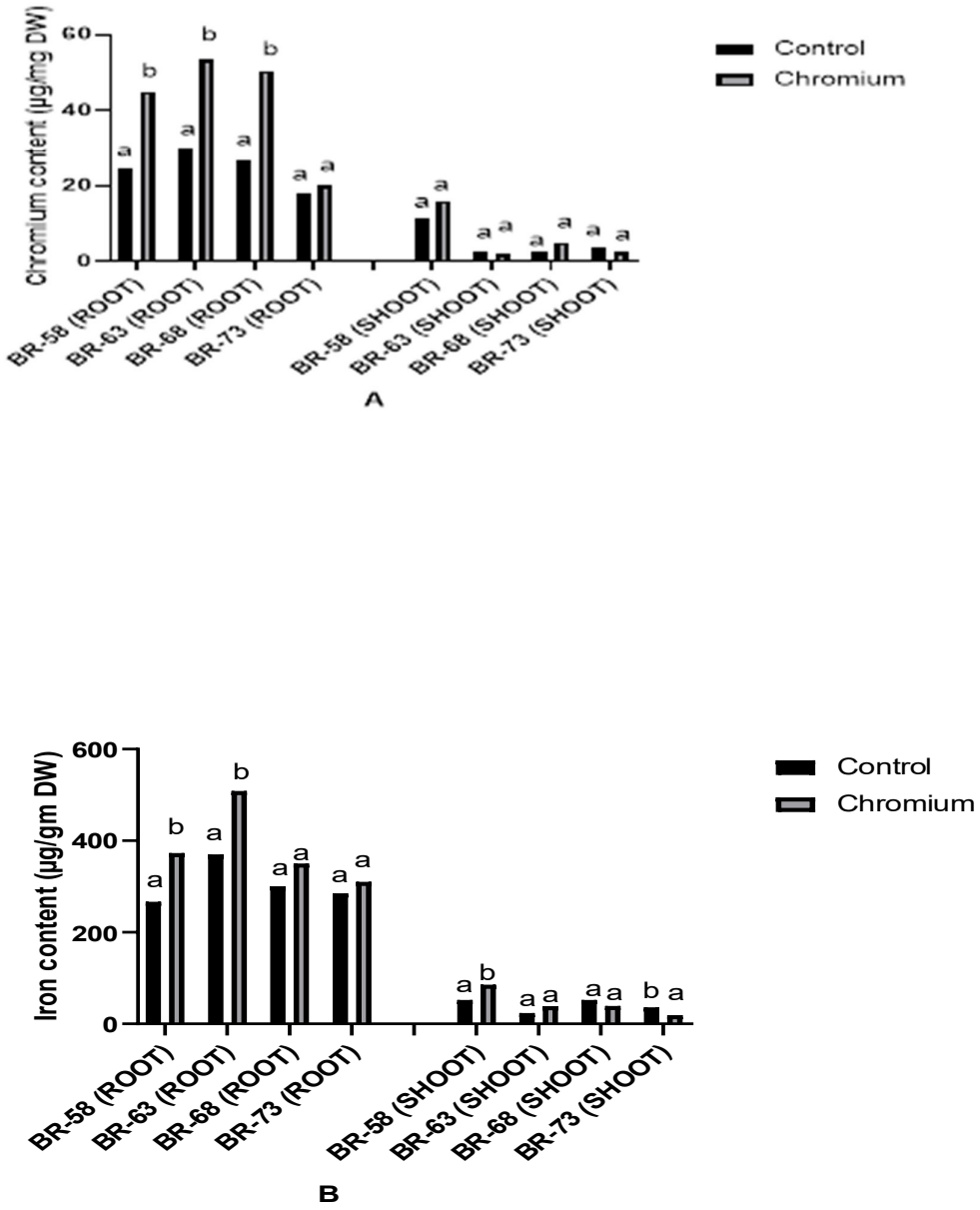

Figure 1. Chromium (A) and Iron (B) concentrations in rice seedlings grown under chromium stress and non-stress condition. Significant deviations among the treatments were denoted by different letters followed by t-test at 5% ($P<0.05$) significance level (number of replications is 4)

Lipid peroxidation and total soluble protein:

Malondialdehyde (MDA) content both in root and shoot of varieties BR-58, BR-63, BR-68 and BR-73 showed no significant differences between non treated control and treatments indicating the tolerance of these varieties against chromium induced oxidative damage (Fig. 2).

Moreover, no significant difference of total soluble protein content between control and chromium stressed plants except in the root of variety BR-63.

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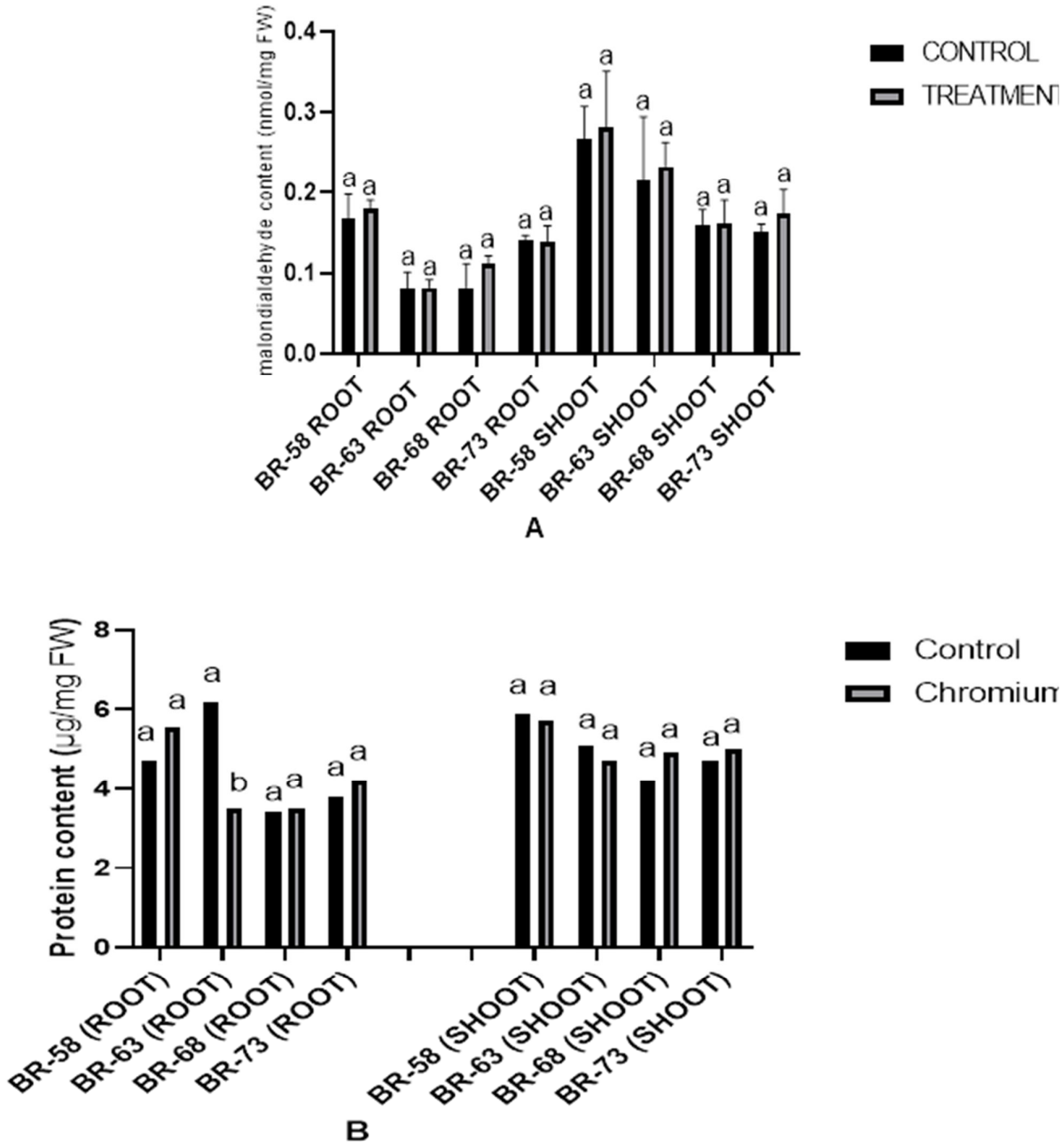

Figure 2: Malondialdehyde (A) and total soluble protein content (B) in the roots and shoots of 7 days old rice plants (BR-58,63,68,73) grown under Chromium stress and without chromium stress. Different letters in each column indicate significant differences between means of treatments followed by t-test at 5% significance level (number of replications is 4).

Determination of root secreted organic acids and rhizospherep Hreduction rate:

Rhizosphere pH decrease after culture (due to low molecular weight organic acids released by rice roots) on the Hongland solution was mentioned in all rice varieties, including the chromium-sensitive variety BR-51, in which it was less than 10% in both stress and non-stress conditions (fig. 3). But in BR-58, BR-63, BR-68 and BR-73 (treated as chromium tolerant) this reduction rate was over 15% under chromium stress conditions. The required amount of low molecular weight organic acids (LMWOA) release is assured by more than 15% rhizospheric pH reduction to avoid chromium toxicity.

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Furthermore, rhizospheres with less than 10% pH reduction could not release a sufficient amount of LMWOA to cope with chromium toxicity.

However, a white precipitate in the silver nitrate test supports that the root of rice seedlings releases organic acid. Furthermore, thin-layer chromatographic analysis confirmed that both the Cr-treated and non-treated rice seedlings secreted citric acid.

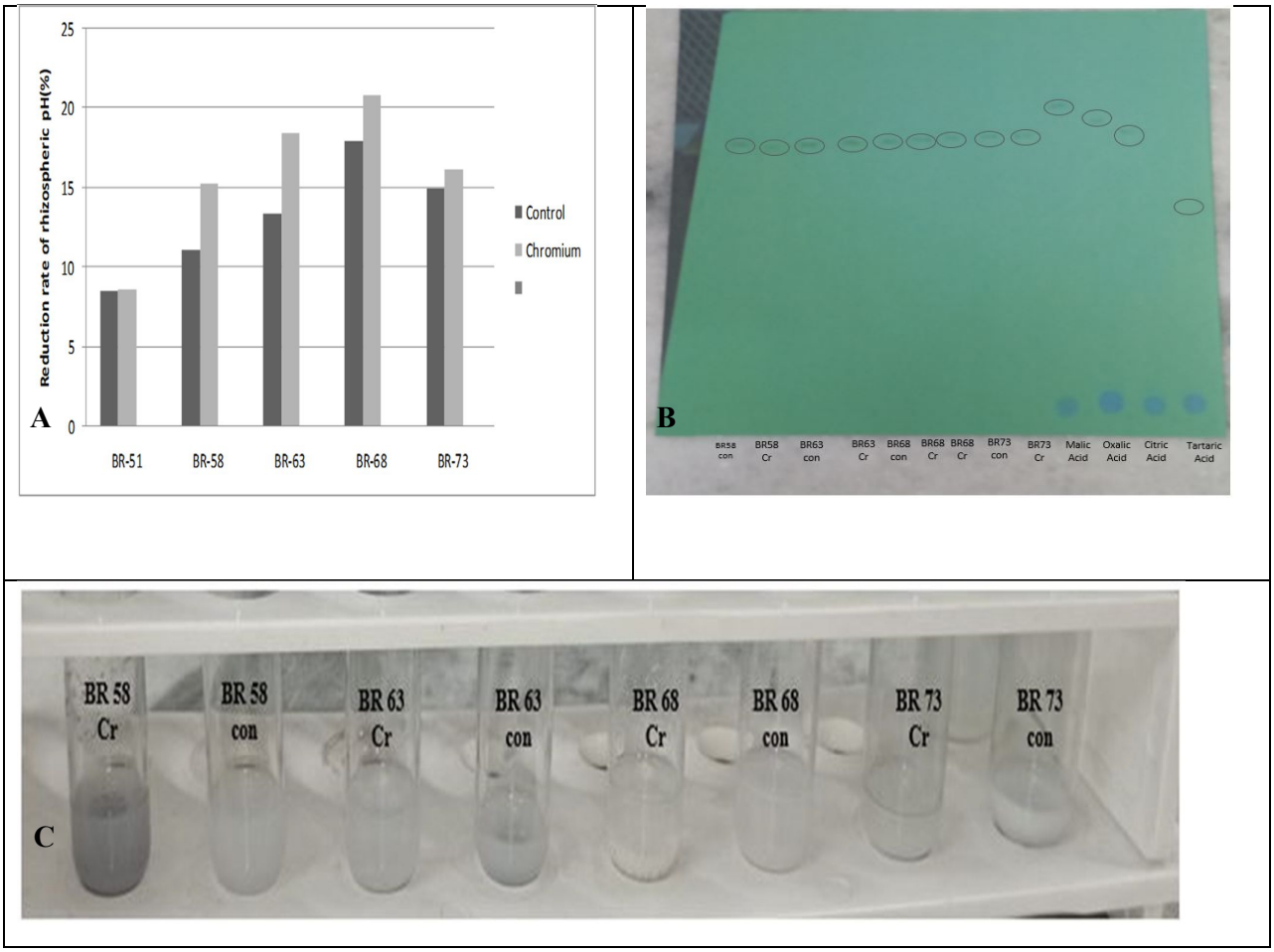

Figure-3: A) Rhizosphoric pH reduction rate (%) under Chromium stress and without chromium stress. B) TLC paper with spots of organic acid C) precipitation of organic acids in $AgNO_3$ test

Enzymatic activity:

CAT and POD activities in the roots of the varieties BR-58, BR-63 and BR-68 grown under chromium stress were significantly increased when compared with the control plant (Table. 2). GR activities in the roots of varieties BR-58 and BR-68 were also increased due to chromium. Moreover, enhancement of SOD activities was observed only in the root of variety BR-68 for toxic chromium. Moreover, no mentionable changes of enzymatic activities (CAT, POD, SOD and GR) were observed in roots between control and treated plants of variety BR-73.

Furthermore, no mentionable enhancement of CAT, POD, and SOD activities was followed in the shoots of varieties BR-58, BR-63, BR-68, and BR-73 under chromium stress. But GR activity in the shoot of varieties BR-58, BR-68 and BR-73 grown under chromium stress was found to be increased significantly compared with the control plant.

Furthermore, reactive oxygen species (ROS) especially superoxide (O_2^-) and hydrogen peroxide (H_2O_2) were also estimated. H_2O_2 content in the root of varieties BR-58, 63 and 68 was enhanced meaningfully

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than that of control plant but in the shoot of these varieties, no meaningful changes were observed. One the other hand no significant differences of superoxide (O_2^-) concentration was discovered except in the root of BR-68.

Table 2 : Changes in enzyme activity in roots and shoots of rice varieties grown in the presence or absence of chromium. Different letters indicate significant differences between mean (including standard deviation) of treatments (number of replication is 4) followed by t-test. Data were from one week old plants.

			BR-58	BR-63	BR-68	BR-73
R O O T	CAT (min-1protein-1)	Cr-	4.27±1.01^a	4.29±1.37^a	1.02±0.87^a	0.64±0.53^a
		Cr+	7.08±0.51^b	7.80±0.35^b	2.34±0.38^b	0.62±0.48^a
	POD (min-1protein-1)	Cr-	4.01±0.02^a	1.36±0.79^a	1.45±0.22^a	0.94±0.57^a
		Cr+	7.90±0.75^b	3.80±0.54^b	2.51±0.44^b	0.64±0.35^a
	SOD (min-1protein-1)	Cr-	8.0x10^{-2}±1.0x10^{-2}a	6.0x10^{-2}±7.0x10^{-2}a	1.0x10^{-2}±1.0x10^{-2a}	4.0x10^{-2}±1.0x10^{-2a}
		Cr+	8.0x10^{-2}±0^a	3.0x10^{-2}±1.0x10^{-2a}	5.0x10^{-2}±1.0x10^{-2b}	2.0x10^{-2}±0^a
	GR (nmol.NADH. min-1gm protein-1)	Cr-	2.0x10^{-2}±1.0x10^{-2a}	8.0x10^{-2}±2.0x10^{-2a}	4.0x10^{-2}±0^a	5.0x10^{-2}±2.0x10^{-2}a
		Cr+	3.0x10^{-2}±1.0x10^{-2b}	6.0x10^{-2}±1.0x10^{-2a}	5.0x10^{-2}±1.0x10^{-2b}	5.0x10^{-2}±1.0x10^{-2a}
	Super Oxide(O2-) (µg/mg)	Cr-	0.526±o.24^a	1.011±0.32^a	0.706±0.12^a	0.528±0.09^a
		Cr+	0.606±0.16^a	1.469±0.32^a	0.476±0.03^b	0.569±0.08^a
	H2O2 (µg/mg)	Cr-	1.2x10^{-2}±0.3x10^{-2a}	2.7x10^{-2}±1.0x10^{-2a}	2.9x10^{-2}±1.0x10^{-2a}	8.1x10^{-2}±1.0x10^{-2a}
		Cr+	4.3x10^{-2}±0.8x10^{-2b}	79.8x10^{-2}±3.0x10^{-2b}	7.4x10^{-2}±1.0x10^{-2b}	10.2x10^{-2}±2.0x10^{-2a}
S H O O T	CAT (min-1protein-1)	Cr-	0.55±0.27^a	2.66±0.39^a	0.88±0.68^a	0.56±0.34^a
		Cr+	0.79±0.31^a	2.81±1.98^a	0.95±0.05^a	0.82±0.19^a
	POD (min-1protein-1)	Cr-	1.31±0.18^a	1.04±0.16^a	1.86±0.21^a	0.46±0.06^a
		Cr+	1.79±0.38^a	1.59±0.35^a	2.02±0.12^a	0.61±0.09^a
	SOD (min-1protein-1)	Cr-	2.0x10^{-2}±1.0x10^{-2a}	2.0x10^{-2}±0^a	2.0x10^{-2}±1.0x10^{-2a}	2.0x10^{-2}±1.0x10^{-2a}
		Cr+	3.0x10^{-2}±0^a	2.0x10^{-2}±1.0x10^{-2a}	2.0x10^{-2}±0^a	3.0x10^{-2}±0^a
	GR (nmol.NADH. min-1gm protein-1)	Cr-	2.0x10^{-2}±0^a	2.0x10^{-2}±1.0x10^{-2a}	2.0x10^{-2}±0^a	2.0x10^{-2}±0^a
		Cr+	3.0x10^{-2}±0^b	2.0x10^{-2}±1.0x10^{-2a}	3.0x10^{-2}±0^b	3.0x10^{-2}±1.0x10^{-2b}
	Super Oxide(O2-) (µg/mg)	Cr-	0.542±0.08^a	0.810±0.19^a	0.152±0.04^a	0.271±0.15^a
		Cr+	0.579±0.17^a	0.585±0.09^a	0.237±0.04^a	0.342±0.14^a
	H2O2 (µg/mg)	Cr-	2.7±2.0x10^{-2a}	1.5x10^{-2}±1.0x10^{-2a}	1.5x10^{-2}±1.0x10^{-2a}	2.4x10^{-2}±1.0x10^{-2a}
		Cr+	2.3x10^{-2}±2.0x10^{-2}a	2.0x10^{-2}±1.0x10^{-2a}	1.4x10^{-2}±1.0x10^{-2a}	2.9±1.0x10^{-2a}

Metabolites (glutathione, proline and phytochelatin)

Amount of glutathione in the root of variety BR-58 and BR-68 as well as in shoot of varieties BR-58, BR-68 and BR-73 were significantly raised under chromium stress compared with non -treated control plant (Table 3). However, no significant change of glutathione content was followed both in root and shoot of variety BR-63.

Moreover, secondary metabolite proline was significantly increased in roots of varieties BR-58, BR-63 and BR-68 under chromium stress compared with control plants. But a meaningful difference of proline content in the shoot of these varieties was not observed.

Furthermore, phytochelatin content in the root of BR-58 was significantly increased when grown on hydroponic solution supplemented with chromium compared with non-treated control plants. But in the shoot of all varieties, it was not increased significantly.

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Table 3: Metabolites in roots and shoots of rice varieties grown in the presence or absence of chromium. Different letters indicate significant differences between mean (including standard deviation) of treatments followed by t-test (number of replications is 4). Data were from one week old plants.

			Glutathione (µgm/mg FW)	Proline (µgm/mg FW)	Phytochelatine (µgm/mg FW)
R O O T	BR-58	Cr-	1.6x10-2±0.1x10 -2a	12.4x10-2±1.2x10	3.4x10-2±0.7x10 -2a
		Cr+	2.0x10-2±0.7x10 -3b	18.2x10-2±2.8x10 -2b	4.9x10-2±0.7x10 -2b
	BR-63	Cr-	1.8x10-2±0.4x10 -2a	14.7x10-2±1.7x10 -2a	5.2x10-2±1.5x10 -2a
		Cr+	1.9x10-2±0.3x10 -2a	24.9x10-2±1.8x10 -2b	6.7x10-2±2.1x10 -2a
	Br-68	Cr-	1.2x10 2±0.1x10 -2a	8.2x10-2±2.1x10 -2a	3.7x10-2±0.4x10 -2a
		Cr+	1.8x10-2±0.3x10 -3b	1.1x10-2±2.2x10 -2b	4.2x10-2±1.2x10 -2a
	BR-73	Cr-	1.6x10-2±0.2x10 -2a	11.3x10-2±1.3x10 -2a	6.8x10-2±0.3x10 -2a
		Cr+	1.6x10-2±0.2x10 -2a	15.0x10-2±5.2x10 -2a	6.9x10-2±0.7x10 -2a
S H O O T	BR-58	Cr-	1.3x10-2±0.46x10 -2a	15.1x10-2±6.1x10 -2a	4.9x10-2±0.2x10 -2a
		Cr+	2.8x10-2±0.3x10 -2b	17.9x10-2±3.9x10 -2a	4.9x10-2±0.8x10 -2a
	BR-63	Cr-	1.7x10-2±0.4x10 -2a	32.2x10-2±8.6x10 -2a	4.6x10-2±0.5x10 -2a
		Cr+	1.7x10-2±0.4x10 -2a	31.1x10-2±6.9x10 -2a	5.2x10-2±0.9x10 -2a
	Br-68	Cr-	1.5x10-2±0.2x10 -3a	16.5x10-2±6.5x10 -2a	4.1x10-2±0.5x10 -3a
		Cr+	1.7x10-2±0.6x10 -3b	14.6x10-2±2.9x10 -22a	4.9x10-2±0.7x10 -2a
	BR-73	Cr-	1.9x10-2±0.2x10 -2a	12.4x10-2±2.8x10 -2a	7.1x10-2±0.6x10 -2a
		Cr+	2.4x10-2±0.2x10 2b	9.7x10-2±1.4x10 -2a	7.0x10-2±1.4x10 -2a

DISCUSSION

Heavy metal stress significantly affects the physiology of plants (Rehman, et, al., 2021, Rolf et.al, 2004) and crop production is reduced as a consequence (Jewell et al., 2010). On the other hand, plants also cope with heavy metal toxicity through the mechanism of avoidance and tolerance (Yu et, al., 2019). Metal-tolerant rice varieties are proving to be a boon for the farmers. Plant breeders employ selection method on a large number of plants cultivated under metal stress to assess heavy metal-tolerant varieties. However, assessment of tolerance level is very crucial for selecting a tolerant variety as it varies among the different species or varieties. In this investigation, twenty-five authentic high-yielding rice varieties were developed in the chromium treated hydroponic solution as treatment and without chromium as control. The result of chromium toxicity on different morphological and physiological parameters such as root and shoot length, total chlorophyll content, and electrolyte leakage in rice plants was identified by comparing with control plants, as these parameters are significantly hampered in rice plant for chromium (Riaz et, al., 2024, Khatun, et. al, 2019). Fargasova (2001) also mentioned that photosynthetic pigments as well as photosynthetic processes are damaged for toxic metals. However, in this study, varieties BR-58, BR-63, BR-68, and BR-73 were proved chromium tolerant based on morpho-physiological features. Moreover, constant level of total protein and MDA content under chromium stress compared with control plants, indicates resistance against chromium as higher lipid peroxidation, cut down protein level, is well documented in rice against chromium toxicity (Khatun, et. al., 2019, Mukta, et.al, 2019)

Analysis of chromium content in root and shoot exhibits that chromium translocation to shoot is inhibited compared with the control plant in variety BR-58, BR-63 and BR-68. Vacuolar sequestration can restrict heavy metal chromium in root cells vacuoles (Huda et al., 2017) with the help of thiol (SH)-containing molecule Phytochelatin and helps plants to survive under stress (Huda et al., 2017). In the present investigation, in variety BR-58 and BR-68, a significant increase of phytochelatin content and its precursor glutathione equated with control plants ensure the chromium sequestration in roots. However, in variety BR-63 no enhanced phyochelatine ensure that low molecular weight citric acid play

vital role on vacuolar sequestration. Because citrate function as counterions being stored in the plant cell vacuole (Martinoia, et.al,1994 and Meyer et.al., 2011). A similar result was also found in rice under Cd stress due to exogenous silicon application (Bari, et.al, 2020).

Plant roots absorb metal ions stored in the xylem, form a complex with the chelator, and then transport them to the shoot. The molecules that function as chelators inside the cell to sequester the heavy metals in the cell vacuole are organic acids, amino acids, and phosphate derivatives (Rauser 1999). In rice, exogenous application of organic acids enhances heavy metal uptake and transport to the aerial portion through the xylem (Khatun et. al., 2019) as well as sequesters in cell vacuoles (Huda et. al., 2016). On the other hand, secreted organic acids by plant roots form non-toxic compounds with heavy metals by their carboxyl groups and prevent their entrance to the plant (Guan, et. al., 2024, Yu G et al 2019). Previous investigations indicate that plants release significant amounts of low molecular organic acids (LMWOAs) in response to heavy metal toxicity, which is an exclusion mechanism rendering metal uptake and also unique to each species (Guan, et, al., 2024, Montiel-Rozas et. al., 2016). Lowering pH significantly influenced plant metal uptake that enhance phytoextraction strategy (Wang, et. al., 2006). However, the release of organic acid was well documented by a white precipitate in the silver nitrate test in this study. Moreover, thin-layer chromatographic analysis confirmed that both the Cr-treated and non-treated rice seedlings secrete citric acid. In this investigation, reduction of rhizospheric pH (more than 15%), as well as decreased translocation of Cr to shoots in varieties BR-58, BR-63 and BR-68 indicates secreted LMWOAs confer metal tolerance by sequester mechanism. Moreover, in variety BR-73, reduced rhizospheric pH (more than 15%) and reduced chromium uptake propose an effective exclusion mechanism. Zeng Fanrong et.al., (2008) reported that rice plants released oxalic, malic, and citric acid at the rhizosphere and enhanced Cr accumulation. But this report could not provide any evidence regarding the amount of low molecular organic acids (LMWOAs) secretion to confer chromium tolerance as well as any other mechanism of chromium detoxification rather than accumulation, as the investigation was limited to two rice genotypes.

However, stability constant of OA-metal complexes specifies the detoxification capacity of organic acids. Chelating complex between Al and citrate in carrot form at the ratio 1:1 where oxalate form complex with Al at the ratio of 1:3 (Kyoma et.al., 1990) indicating that stability constant is variable among different ratio. These two organic acids ensure detoxification mechanism by preventing Al to bind ATP or other ligands. Moreover, in aquatic solution, higher concentration of malate is required to alleviate Al rhizotoxicity through chelation (Thomas et.al., 2005). Aluminum-OA complex is precipitated in apoplast and excrete more malate in Al-tolerant wheat than the Al-sensitive wheat variety (Ryan et.al., 1995).

Furthermore, Consumption of carbon sources for production and efflux of Organic acids (OA) under stress consumed significant proportion of carbon imposing an energy cost to plants which is economically important for fast-growing annual crops like rice (Koyama et al., 2000; Herz et al., 2018). Furthermore, plants optimize its carbon loss by limiting the amount of OAs release by negative regulators of OA exudation like GABA (Ramesh et al., 2015).

But in our investigation, it was evident that the required amount of citric acid secretion to cope with chromium toxicity was assured by more than 15% rhizospheric pH reduction, whereas sensitive rice plants reduce less than 10% rhizospheric pH.

It is the first report that provides rice varieties (BR-58, 63, 68 and 73) ignore its carbon optimization process to produce citric acids for coping with heavy metal chromium. Moreover, rice plant release citric acid to such a height that can neutralize chromium and it measured by its 15% rhizospheric pH reduction. Our investigation also concludes that 15% and above rhizospheric pH reduction in rice plant is the benchmark for required amount of citric acid secretion to be chromium tolerant. This investigation also discovered that under chromium stress, root secreted LMWOAs adopted two mechanisms such as vacuolar sequestration through chelation, and metal exclusion to avoid chromium toxicity in rice varieties

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However, limited activity of Fe transporters can reduce Fe uptake, which can enhance the tolerance level of rice plants against Cr toxicity (Kabir 2016). But in the present study, Fe concentration in the root and shoot of these four varieties was not followed when compared with the control plant, indicating that regulation of Fe transporter was not involved with chromium tolerance.

Oxidative stress generated from ROS is pronounced in the plant cells during metal toxicity, and activation of antioxidant mechanisms is initiated (Ghori, 2019). Reduce protein content, lipids peroxidation, inefficient enzyme activities, DNA damage and abnormal constituents of cells are the result of excessive accumulations of reactive oxygen species (ROS) in plant. ROS also interacts with hormones and epigenetic modifiers to regulate developmental processes and stress responses of plant (Kong et al., 2018). In this present investigation, under chromium stress, ROS was found to be increased in the root of these varieties without any significant lipid peroxidation compared with the control plant. Two types of mechanisms are present in the plant to scavenge the ROS, and the first one is enzymatic and the other one is non-enzymatic. Hydrogen peroxide (H2O2) generated first from oxidized metabolic and then convert into water by means of enzymatic activities while termination of free radical chain reactions by the help of non-enzymatic antioxidants (Moussa Ziad 2019). Neutralization of ROS by through enzymatic process enhance the plant tolerance level against diverse biotic and abiotic stresses (Kanto et al. 2015). In the present study, GR activity was enhanced significantly in the root of varieties BR-58 and BR-68 and the shoot of varieties BR-58, BR-68, and BR-73 under chromium stress. Furthermore, , SOD activity was found to be increased only in the root of variety BR-68 under same stress. Enhanced GR and SOD activity reported in rice under Cd stress also (Bari, et.al., 2020)

Glutathione, a non-enzymatic antioxidant, is an efficient scavenger of O2, H2O2 and OH (Gill and Tuteja, 2010). In our study, the amount of glutathione in the roots of varieties BR-58 and BR-68 as well as in the shoots of varieties BR-58, BR-68 and BR-73 was significantly raised under chromium stress. However, our investigation again notices that citric acid up regulate the antioxidant defense system as a secondary function along with chromium avoidance mechanisms to keep down ROS levels.

Proline (Pro) an amino, responsible to mitigate biotic and abiotic stress in plant (Ágneset.al., 2018). It is also reported as metals chelator (Gill and Tuteja, 2010). In the present investigation, proline content was found to be enhanced significantly in the roots of BR-58, BR-63, and BR-68 to enhance the tolerance against Cr toxicity. The findings of this study hold practical significance for both crop improvement and environmental remediation.

CONCLUSION

The exudation of citric acid has been shown to be the key physiological response against chromium toxicity in rice plant. But the concentration of citric acid is deviated according to the genotypes of rice plants. The assessment of the amount of citric acid exudates followed by rhizosphere pH reduction rate has allowed knowing the potentiality of rice varieties to cope with chromium toxicity. Furthermore, rice genotypes that can reduce their rhizosphere pH by 15% or more by releasing organic acid are chromium tolerant. However, the findings offer an efficient screening technique for metal tolerant rice plants.

DECLARATION

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Conflicts of interest/Competing interests: The authors declare that they have no conflict of interest.

Ethics approval: Not applicable

Consent to participate: All participants are in the list of authors and have full consent

Consent for publication: All participants are in the list of authors and have full consent

Availability of data and material: The datasets are available from the corresponding author on reasonable request.

Generative AI: Not applicable

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REFERENCE

1. Ágnes Szepesi,Réka Szőllősi (2018). Mechanism of Proline Biosynthesis and Role of Proline Metabolism Enzymes Under Environmental Stress in Plants, in:Parvaiz Ahmad, Mohammad AbassAhanger, Mohammed Nasser Alyemeni (Eds), Plant Metabolites and Regulation Under Environmental Stress. Academic press.pp 337-353. https://doi.org/10.1016/B978-0-12-812689-9.00017-0
2. Airaki M, Leterrier M, Mateos RM, Valderrama R, Chaki M, Barroso JB, Del Río LA, Palma JM, Corpas FJ. (2012). Metabolism of reactive oxygen species and reactive nitrogen species in pepper (Capsicum annuum L.) plants under low temperature stress. Plant, Cell & Environment 35: 281–29
3. Al Mahmud, J., Hasanuzzaman, M., Nahar, K., Bhuyan, M. B., & Fujita, M. (2018). Insights into citric acid-induced cadmium tolerance and phytoremediation in Brassica juncea L.: Coordinated functions of metal chelation, antioxidant defense and glyoxalase systems. *Ecotoxicology and environmental safety*, *147*, 990-1001.https://doi.org/10.1016/j.ecoenv.2017.09.045.
4. Alexieva, V., Sergiev, I., Mapelli, S., & Karanov, E. (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell & Environment*, *24*(12), 1337-1344.https://doi.org/10.1046/j.1365-3040.2001.00778.x.
5. Al-Khayri, J. M., Rashmi, R., Toppo, V., Chole, P. B., Banadka, A., Sudheer, W. N., ... & Rezk, A. A. S. (2023). Plant secondary metabolites: The weapons for biotic stress management. *Metabolites*, *13*(6), 716. https://doi.org/10.3390/metabo13060716.
6. Anderson, J. S., Lall, S. P., Anderson, D. M., &Chandrasoma, J. (1992). Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (Salmo salar) reared in sea water. *Aquaculture*, *108*(1-2), 111-124.https://doi.org/10.1016/0044-8486(92)90322-C.
7. Bari, M. A., Prity, S. A., Das, U., Akther, M. S., Sajib, S. A., Reza, M. A., & Kabir, A. H. (2020). Silicon induces phytochelatin and ROS scavengers facilitating cadmium detoxification in rice. *Plant Biology*, *22*(3), 472-479.https://doi.org/10.1111/plb.13090.
8. Bates, L. S., Waldren, R. P. A., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*, 205-207.https://doi.org/10.1007/BF00018060.
9. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3.
10. Dey, S. R., Sharma, M., & Kumar, P. (2023). Effects and responses of chromium on plants. In *Chromium in Plants and Environment* (pp. 385-427). Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-44029-8_14.

11. Dong D, Peng X, Yan X. (2004). Organic acid exudation induced by phosphorus deficiency and/or aluminium toxicity in two contrasting soybean genotypes. Physiologia Plantarum 122: 190–199
12. Fargašová, A. (2001). Phytotoxic effects of Cd, Zn, Pb, Cu and Fe on Sinapis alba L. seedlings and their accumulation in roots and shoots. *Biologia plantarum*, *44*, 471-473. https://doi.org/10.1023/A:1012456507827.
13. Fernando DR, Mizuno T, Woodrow IE, Baker AJ, Collins RN (2010). Characterization of foliar manganese (Mn) in Mn (hyper)accumulators using X-ray absorption spectroscopy. New Phytologist 188:1014–1027.
14. Ghori, N. H., Ghori, T., Hayat, M. Q., Imadi, S. R., Gul, A., Altay, V., & Ozturk, M. (2019). Heavy metal stress and responses in plants. *International journal of environmental science and technology*, *16*, 1807-1828. https://doi.org/10.1007/s13762-019-02215-8.
15. Gill, S. S., & Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry*, *48*(12), 909-930. http://dx.doi.org/10.1016/j.plaphy.2010.08.016.
16. Goud, P. B., &Kachole, M. S. (2012). Antioxidant enzyme changes in neem, pigeonpea and mulberry leaves in two stages of maturity. *Plant signaling & behavior*, *7*(10), 1258-1262. https://doi.org/10.4161/psb.21584.
17. Griffith, O. W. (1980). Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Analytical biochemistry*, *106*(1), 207-212. https://doi.org/10.1016/0003-2697(80)90139-6.
18. Guan, J., Zhang, Y., Li, D., Shan, Q., Hu, Z., Chai, T., ... & Qiao, K. (2024). Synergistic role of phenylpropanoid biosynthesis and citrate cycle pathways in heavy metal detoxification through secretion of organic acids. *Journal of Hazardous Materials*, *476*, 135106. https://doi.org/10.1016/j.jhazmat.2024.135106
19. Harada, E., Kim, J. A., Meyer, A. J., Hell, R., Clemens, S., & Choi, Y. E. (2010). Expression profiling of tobacco leaf trichomes identifies genes for biotic and abiotic stresses. *Plant and cell physiology*, *51*(10), 1627-1637.https://doi.org/10.1093/pcp/pcq118
20. Heath, R. L., & Packer, L. (1968). Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of biochemistry and biophysics*, *125*(1), 189-198.https://doi.org/10.1016/0003-9861(68)90654-1.
21. Herz K, Dietz S, Gorzolka K, Haider S, Jandt U, Scheel D, Bruelheide H. (2018). Linking root exudates to functional plant traits. PLoS One 13: e0204128.
22. Huda, A. N., Haque, M. A., Zaman, R., Swaraz, A. M., & Kabir, A. H. (2017). Silicon ameliorates chromium toxicity through phytochelatin-mediated vacuolar sequestration in the roots of Oryza sativa (L.). *International journal of phytoremediation*, *19*(3), 246-253.https://doi.org/10.1080/15226514.2016.1211986.
23. Huda, A. N., Swaraz, A. M., Reza, M. A., Haque, M. A., & Kabir, A. H. (2016). Remediation of chromium toxicity through exogenous salicylic acid in rice (Oryza sativa L.). *Water, Air, & Soil Pollution*, *227*, 1-11.https://doi.org/10.1007/s11270-016-2985-x.
24. Igamberdiev AU, Eprintsev AT. (2016). Organic acids: the pools of fixed carbon involved in redox regulation and energy balance in higher plants. Frontiers in Plant Science 7: 1042.
25. Jewell, M. C., Campbell, B. C., & Godwin, I. D. (2010). Transgenic plants for abiotic stress resistance. *Transgenic crop plants*, 67-132.https://doi.org/10.1007/978-3-642-04812-8_2.
26. Kabir, A. H. (2016). Biochemical and molecular changes in rice seedlings (Oryza sativa L.) to cope with chromium stress. *Plant Biology*, *18*(4), 710-719. https://doi.org/10.1111/plb.12436
27. Kanto, U., Jutamanee, K., Osotsapar, Y., Chai-arree, W., &Jattupornpong, S. (2015). Promotive effect of priming with 5-aminolevulinic acid on seed germination capacity, seedling growth and antioxidant enzyme activity in rice subjected to accelerated ageing treatment. *Plant Production Science*, *18*(4), 443-454. https://doi.org/10.1626/pps.18.443.
28. Khatun, M. R., Mukta, R. H., Islam, M. A., & Huda, A. N. (2019). Insight into citric acid-induced chromium detoxification in rice (Oryza sativa. L). *International Journal of Phytoremediation*, *21*(12), 1234-1240. https://doi.org/10.1080/15226514.2019.161962.

29. Kochian, L. V., Hoekenga, O. A., & Pineros, M. A. (2004). How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency. *Annu. Rev. Plant Biol.*, *55*(1), 459-493.https://doi.org/10.1146/annurev.arplant.55.031903.141655.
30. Kong, X., Tian, H., Yu, Q., Zhang, F., Wang, R., Gao, S., ... & Ding, Z. (2018). PHB3 maintains root stem cell niche identity through ROS-responsive AP2/ERF transcription factors in Arabidopsis. *Cell Reports*, *22*(5), 1350-1363.doi: https://10.1016/j.celrep.2017.12.105.
31. Koyama H., Ojima K., Yamaya T (1990). Utilization of anhydrous aluminum phosphate as a sole source of phosphorus by selected carrot cell line. Plant physiol. 31:173-177.
32. Lichtenthaler, H. K., & Wellburn, A. R. (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. https://doi.org/10.1042/bst0110591.
33. Liujie Wu., Yuriko Kobayashi, Jun Wasaki, and Hiroyuki Koyama (2018). Organic acid excretion from root: a plant mechanism for enhancing phosphorus acquisition, enhancing aluminum tolerance, and recruiting beneficial rhizobacteria. Soil science and plant nutrition. 64(6): 697-704.
34. López-Bucio J, De la Vega OM, Guevara-García A, Herrera-Estrella L. (2000). Enhanced phosphorus uptake in transgenic tobacco plants that overproduce citrate. Nature Biotechnology 18: 450–453.
35. Lutts, S., Kinet, J. M., &Bouharmont, J. (1996). NaCl-induced senescence in leaves of rice (Oryza sativaL.) cultivars differing in salinity resistance. *Annals of botany*, *78*(3), 389-398.https://doi.org/10.1006/anbo.1996.0134.
36. Mahmud JA, Hasanuzzaman M, Nahar K, Borhannuddin Bhuyan MH, Fujita Masayuki(2018) Insights into citric acid-induced cadmium tolerance and phytoremediation in Brassica juncea L.: Coordinated functions of metal chelation, antioxidant defense and glyoxalase systems. Ecotoxicology and Environmental Safety 147:990–1001. https://doi.org/10.1016/j.ecoenv.2017.09.045
37. Martinoia, E.; Rentsch, D. Malate (**1994**) Compartmentation—Responses to a Complex Metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol*, *45*: 447–467.
38. Meyer, S.; Scholz-Starke, J.; De Angeli, A.; Kovermann, P.; Burla, B.; Gambale, F.; Martinoia, E. Malate (**2011**). Transport by the Vacuolar AtALMT6 Channel in Guard Cells Is Subject to Multiple Regulation. *Plant J. 67*: 247–257.
39. Montiel-Rozas, M. D. M., Madejón, E., &Madejón, P. (2016). Effect of heavy metals and organic matter on root exudates (low molecular weight organic acids) of herbaceous species: An assessment in sand and soil conditions under different levels of contamination. *Environmental Pollution*, *216*, 273-281.https://doi.org/10.1016/j.envpol.2016.05.080.
40. Mourato M, Reis R, Martins LL(2012) "Characterization of plant antioxidative system in response to abiotic stresses: a focus on heavy metal toxicity," in *Advances in Selected Plant Physiology Aspects*, G. Montanaro and B. Dichio, Eds., pp. 23–44, InTech, Vienna, Austria, http://hdl.handle.net/10400.5/4410.
41. Mourato, M., Reis, R., & Martins, L. L. (2012). Characterization of plant antioxidative system in response to abiotic stresses: a focus on heavy metal toxicity. *Advances in selected plant physiology aspects*, *12*, 1-17.
42. Moussa, Z., Judeh, Z. M., & Ahmed, S. A. (2019). Nonenzymatic exogenous and endogenous antioxidants. *Free radical medicine and biology*, *1*, 11-22. https://doi.org/10.5772/intechopen.87778.
43. Mukta, R. H., Khatun, M. R., & Nazmul Huda, A. K. M. (2019). Calcium induces phytochelatin accumulation to cope with chromium toxicity in rice (Oryza sativa L.). *Journal of Plant Interactions*, *14*(1), 295-302.https://doi.org/10.1080/17429145.2019.1629034.
44. Mukti, Gill. (2014). Heavy metal stress in plants: a review. *Int J Adv Res*, *2*(6), 1043-1055.
45. Nath, K., Singh, D., Shyam, S., & Sharma, Y. K. (2008). Effect of chromium and tannery effluent toxicity on metabolism and growth in cowpea (Vigna sinensis L. SaviexHassk) seedling. *Research in Environment and Life Sciences*, *1*(3), 91-94.

46. Nematshahi, N., Lahouti, M., &Ganjeali, A. (2012). Accumulation of chromium and its effect on growth of (Allium cepa cv. Hybrid). *European Journal of Experimental Biology*, *2*(4), 969-974.
47. Panda, S. K., & Choudhury, S. (2005). Chromium stress in plants. *Brazilian journal of plant physiology*, *17*, 95-102.https://doi.org/10.1590/S1677-04202005000100008.
48. Poonam Panchal, Anthony J. Miller, and Jitender Gir (2021).Organic acids: versatile stress-response roles in plants.Journal of Experimental Botany. 72(11): 4038–4052.
49. Ramesh SA, Tyerman SD, Xu B, et al. (2015). GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters. Nature Communications 6: 7879.
50. Rastgoo, L., Alemzadeh, A., &Afsharifar, A. (2011). Isolation of two novel isoforms encoding zinc-and copper-transporting P1B-ATPase from Gouan (Aeluropus littoralis). *Plant Omics J*, *4*(7), 377-383.
51. Rauser, W. E. (1999). Structure and function of metal chelators produced by plants: the case for organic acids, amino acids, phytin, and metallothioneins. *Cell biochemistry and biophysics*, *31*, 19-48. https://doi.org/10.1007/BF02738153.
52. Reyes F, Pallas B, Pradal C, Vaggi F, Zanotelli D, Tagliavini M, Gianelle D, Costes E. (2020). MuSCA: a multi-scale source-sink carbon allocation model to explore carbon allocation in plants. An application to static apple tree structures. Annals of Botany 126: 571–585.
53. Riaz, A., Qin, Y., Zheng, Q., Chen, X., Jiang, W., Riaz, B., ... & Zeng, F. (2024). Cr (VI) behaves differently than Cr (III) in the uptake, translocation and detoxification in rice roots. *Science of The Total Environment*, *948*, 174736.https://doi.org/10.1016/j.scitotenv.2024.174736.
54. Rolf D.V. inebrooke, Kathryn L, Cottingham, Jon Norberg, Marten Scheffer, Stanley I. Dodson,Stephen C,Maberly, Ulrich Sommer (2004 Impacts of multiple stressors on biodiversity and ecosystem functioning: The role of species co-tolerance. *Oikos*, *104*(3), 451-457. https://doi:10.1111/j.0030-1299.2004.13255.x
55. Shanker, A. K., Cervantes, C., Loza-Tavera, H., &Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment international*, *31*(5), 739-753.https://doi.org/10.1016/j.envint.2005.02.003.
56. Shanker, A. K., Cervantes, C., Loza-Tavera, H., &Avudainayagam, S. (2005). Chromium toxicity in plants. *Environment international*, *31*(5), 739-753.https://doi.org/10.1016/j.envint.2005.02.003.
57. Sharma, P., Jha, A. B., Dubey, R. S., &Pessarakli, M. (2012). Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *Journal of botany*, *2012*(1), 217037.https://doi.org/10.1155/2012/217037.
58. 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.https://doi.org/10.1093/jxb/erj073.
59. Solanki, R., & Dhankhar, R. (2011). Biochemical changes and adaptive strategies of plants under heavy metal stress. *Biologia*, *66*(2), 195-204.https://doi.org/10.2478/s11756-011-0005-6.
60. Szepesi, Á., & Szőllősi, R. (2018). Mechanism of proline biosynthesis and role of proline metabolism enzymes under environmental stress in plants. In *Plant metabolites and regulation under environmental stress* (pp. 337-353). Academic Press.https://doi.org/10.1016/B978-0-12-812689-9.00017-0.
61. Thomas BK., David RP., Richard WZ. (2005). Organic acid secretion as a mechanism of aluminum resistance: a model incorporating the cortex, epidermis, the external unstirred layer. J. Exp. Bot. 56: 1853-1865.
62. Ullah, S., Liu, Q., Wang, S., Jan, A. U., Sharif, H. M. A., Ditta, A., ... & Cheng, H. (2023). Sources, impacts, factors affecting Cr uptake in plants, and mechanisms behind phytoremediation of Cr-contaminated soils. *Science of the Total Environment*, 165726. https://doi.org/10.1016/j.scitotenv.2023.165726.
63. Viehweger, K. (2014). How plants cope with heavy metals. *Botanical Studies*, *55*, 1-12. https://doi.org/10.1186/1999-3110-55-35

64. Wang, A. S., Angle, J. S., Chaney, R. L., Delorme, T. A., & Reeves, R. D. (2006). Soil pH effects on uptake of Cd and Zn by Thlaspi caerulescens. *Plant and soil*, *281*, 325-337.https://doi.org/10.1007/s11104-005-4642-9.
65. Weidinger, A., & Kozlov, A. V. (2015). Biological activities of reactive oxygen and nitrogen species: oxidative stress versus signal transduction. *Biomolecules*, *5*(2), 472-484.https://doi.org/10.3390/biom5020472
66. Wong, H. L., Sakamoto, T., Kawasaki, T., Umemura, K., & Shimamoto, K. (2004). Down-regulation of metallothionein, a reactive oxygen scavenger, by the small GTPase OsRac1 in rice. *Plant physiology*, *135*(3), 1447-1456.https://doi.org/10.1104/pp.103.036384.
67. Yokosho K, Yamaji N, Ma JF. 2011. An Al-inducible MATE gene is involved in external detoxification of Al in rice. The Plant Journal 68:1061–1069.
68. Yu, G., Ma, J., Jiang, P., Li, J., Gao, J., Qiao, S., & Zhao, Z. (2019, August). The mechanism of plant resistance to heavy metal. In *IOP conference series: earth and environmental science* (Vol. 310, No. 5, p. 052004). IOP Publishing.https://doi:10.1088/1755-1315/310/5/052004.
69. Zeng, F., Chen, S., Miao, Y., Wu, F., & Zhang, G. (2008). Changes of organic acid exudation and rhizosphere pH in rice plants under chromium stress. *Environmental Pollution*, *155*(2), 284-289. https://doi.org/10.1016/j.envpol.2007.11.019
70. Zhang Y, Chen FS, Wu XQ, Luan FG, Zhang LP, Fang XM, Wan SZ, Hu XF, Ye JR. (2018). Isolation and characterization of two phosphate-solubilizing fungi from rhizosphere soil of moso bamboo and their functional capacities when exposed to different phosphorus sources and pH environments. PLoS One 13: 1–14.
71. Zhou Y, Yang Z, Xu Y, Sun H, Sun Z, Lin B, Sun W, You J. (2018). Soybean NADP-malic enzyme functions in malate and citrate metabolism and contributes to their efflux under Al stress. Frontiers in Plant Science 8: 1–11
72. Ziad Moussa, Zaher MA, Judeh, Saleh A. Ahmed(2019) Nonenzymatic Exogenous and Endogenous Antioxidants, In Rizwan Ahmad (Edited), Free Radical Medicine andBiology. Intech Open.pp-1-22. DOI: 10.5772/intechopen.87778