INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2026, Vol. 5, NO.1, 141-153
doi: https://doi.org/10.56612/ijaaeb.v5i1.178
ARK: https://n2t.net/ark:/71142/IJAaEB.v5i1.178
https://eminentscientists.com/

Mitigating chromium toxicity in oat (*Avena sativa* L.) with exogenous salicylic acid

Hafiz Muhammad Mohsin Hassan[1*], Warda Abbas[1], Syeda Sadaf Zehra[1], Muhammad Uzair Zafar[1], Anam Shehzadi[1]

[1]Department of Botany, The Islamia University of Bahawalpur, Punjab, Pakistan.

Abstract

This study investigated the protective role of exogenous salicylic acid (SA) in mitigating chromium (Cr) toxicity in oat (*Avena sativa* L.). A pot experiment was conducted in a shade house using a factorial design with six treatment groups: Control (No Cr, No SA), $CrCl_3$ 2 mM, SA 0.5 mM, SA 2 mM, $CrCl_3$ 2 mM + SA 0.5 mM, $CrCl_3$ 2 mM + SA 2.0 mM. Chromium stress significantly reduced plant growth, yield, and anatomical parameters. Chromium treatment significantly decreased the root-shoot ratio, while both concentrations of SA improved growth considerably. The SA (2.0 mM) treatment proved to be most effective in enhancing overall plant growth and grain yield of oat. Anatomical analysis revealed that SA application mitigated the damage induced by Cr. Specifically, the thickness and area of stem sclerenchyma were significantly increased by 1.3- and 1.5-fold, respectively, with the 2.0 mM SA treatment compared to those in the control. Furthermore, SA improved the thickness of the leaf midrib, metaxylem, and phloem, as well as the root epidermis, endodermis, and phloem, particularly in the Cr + SA treatment. These anatomical changes are crucial for water conservation and overall plant survival under heavy metal stress. Our findings suggest that exogenous application of SA can effectively alleviate Cr-induced stress, promoting the healthy development of oat seedlings.

ARTICLE TYPE
Research Paper (RP)

SECTION
Plant Biology (PB)

HANDLING EDITOR
Ozyigit, I.I. (PB)

ARTICLE HISTORY
Received: 12 Jul, 2025
Accepted: 29 Sep, 2025
Online: 7 Oct, 2025
Published: 01 Jan, 2026

KEYWORDS
Anatomy;
Cereal crop;
Growth regulator;
Heavy metal stress;
Stress alleviation

Introduction

Heavy metal exposure to the environment has grown dramatically during the last century due to industrial operations, home wastewater discharge, agricultural fertilizers, and improper home solid waste disposal (Budi et al*.,* 2022). Some heavy metals (HMs), such as arsenic and mercury, among others, can selectively target proteins and enzymes to alter their activities and impede cellular metabolism, which further affects the general development and yield of plants (Riyazuddin et al*.,* 2021). Higher level of heavy metals causes stunted growth, chlorosis, nutrient imbalance, and alterations in the defense mechanisms of plants (Varma et al., 2021). The poisonous heavy metals can have a major negative impact on a plant's physiological and metabolic processes, which can ultimately cause health problems for humans and animals by entering the food chain (Amanullah et al., 2023).

Of several heavy metals known in nature, lead, chromium, and cadmium are the most common ones that cause toxicity (Balali-Mood et al*.,* 2021). Plants' ability to eliminate or safely collect metal

***CONTACT** Hafiz Muhammad Mohsin Hassan, mohsinkhan74248@gmail.com, Department of Botany, The Islamia University of Bahawalpur, Punjab, Pakistan.

CITATION (APA): Hassan, H.M.M., Abbas, W., Zehra, S.S., Zafar, M.U., Shehzadi, A. (2026). Mitigating chromium toxicity in oat (*Avena sativa* L.) with exogenous salicylic acid. *International Journal of Applied and Experimental Biology*, 5(1):141-153. https://doi.org/10.56612/ijaaeb.v5i1.178

cations determines how they react to and tolerate heavy metals (HMs) (Thakur et al., 2022). It is well known that the hazardous element chromium may seriously harm plants (Sharma et al., 2020). Chromium is a dangerous element that adversely affects plant metabolic processes, obstructing crop growth and yield, and lowering the quality of vegetables and grains (Wakeel et al., 2020). Plant metabolism of antioxidants can be impacted by chromium. Plants that are exposed to chromium-induced oxidative stress are driven to undergo lipid peroxidation, which severely damages the cell membrane (Iftikhar et al., 2025). Chromium can cause genotoxicity in many different plant species (Wakeel et al., 2020). In agriculture, various techniques are employed to help plants adapt to biotic and abiotic stressors, including those associated with metals. In recent years, consideration has been given to seed priming and exogenous foliar application of suitable solutes such as sugar polyols, amino acids, proline, and salicylic acid (Ellouzi et al., 2023). Salicylic acid (SA) is a member of a broad class of plant phenolics from a chemical perspective, and it may be extracted from plants in both free and conjugated forms (Maruri-López et al., 2019).

Oat (*Avena sativa*) is a crop used as cereal fodder and is a biennial member of the Poaceae family (Kim et al., 2021). It resembles barley in shape and has different varieties, such as black, red, yellow, and white oats (Martín-Diana et al., 2021). Oats are grown on 3.52 thousand hectares of land in Pakistan, mostly as a feed crop, yielding 264 tons of output annually, with 2.03 million hectares contributed by the Punjab province, so more than 35% of the total area is used for fodder farming (Ibrahim et al., 2020). Among grains produced worldwide, oats rank sixth, behind wheat, corn, rice, barley, and sorghum (Mert, 2020). They are high in protein, fiber, vitamins, and minerals, and are typically eaten as oatmeal (Paudel et al., 2021). Specifically, oats are considered high-value crops because they have balanced levels of important amino acids like lysine, high levels of proteins and lipids, and 2-6% of β-glucan (Nogala-Kałucka et al., 2020).

Based on the earlier-mentioned reports, it was hypothesized that salicylic acid mitigates the Cr-induced stress in *A. sativa* by altering anatomical structures and key physiological processes. Thus, the current study was conducted with a primary objective that salicylic acid acts as a plant growth regulator in alleviating Cr-induced stress in *A. sativa* by modulating structural features and physiological processes.

Materials and Methods

Plant seeds and soil preparation

Certified *A. sativa* seeds were purchased from the Ayub Agricultural Research Institute, Faisalabad, Pakistan. The soil was prepared by mixing sand, clay, and manure in a 1:2:1 ratio, respectively, to form a fertile soil mixture. The soil was filled in 6 kg polythene bags and watered for two days to keep fully moist. Small holes were pierced in the sides of polythene bags to let excess water out and to keep the soil aerated. The current study was conducted at the Botanical Garden of Islamia University, Bahawalpur, Pakistan.

Experimental design and procedure

The experiment was laid out in a completely randomized design. A total of 120 polythene bags (pots) filled with the soil mixture were arranged in six groups. Each group contained 20 pots for each treatment. The seeds of *A. sativa* were sown in each pot to a depth of 2.5 cm to 5 cm. The pots were covered with plastic sheets to avoid frost at night and the winds. The pots were properly and regularly watered until the seeds germinated. Seedlings started emerging at 7 days after sowing. After that, the thinning of plants was done to maintain 10 plants per pot. All treatments were applied in three turns at an equal interval of 10 days. The first treatments were given to plants after 10 days of germination. The oat seedlings were subjected to six different treatments (Control (No Cr, No SA), $CrCl_3$ 2 mM, SA 0.5 mM, SA 2 mM, $CrCl_3$ 2 mM + SA 0.5 mM, $CrCl_3$ 2 mM + SA 2.0 mM). Chromium salt was applied through the soil, whereas SA was applied as a foliar spray. However, the control group was treated with water without chromium. Each treatment was applied in each pot in equal quantity, i.e., Cr treatment as 50 mL in soil in each pot and salicylic acid solution as 10 mL as a foliar spray.

Growth parameters and yield

Plants were sampled 10 days after the completion of the third treatment to record morphological parameters. The soil in the pots was softened, and one plant from each replicate was carefully uprooted without causing damage to the roots, and then washed with tap water to remove soil particles. Different traits, such as root length (cm) and shoot length (cm), were measured, and

the number of leaves was counted. Fresh and dry weights of root and shoot (FWS, FWR, DWS, and DWR) were also measured properly. At 130 DAS, 10 plants were selected from each treatment, and their yield (number of seeds and seed weight) attributes were measured. Grain yield was recorded on a 100-seed weight basis (i.e., 100 grains of *A. sativa* were randomly selected and weighed). The number of seeds was measured by taking seeds from 10 plants of each treatment.

Anatomical features

Plant samples were selected randomly from each treatment. The preservative solution (FAA) containing 50% ethanol, 5% acetic acid, 10% formalin, and 35% distilled water was prepared. Freehand sectioning methods were employed for sectioning the samples, and double stains (safranin and fat green) and different grades of ethyl alcohol were used for dehydration (Ruzin, 1999). The sample slides were examined (4X, 10X, and 40X) with a compound microscope. Snaps were taken using a smartphone camera. Different anatomical parameters (i.e., stem area, lamina thickness, vascular bundle area, number of vascular bundles, sclerenchyma thickness, parenchyma cell area, phloem thickness, metaxylem area, epidermis thickness, epidermal cell area, etc.) of *A. sativa* were appraised under a microscope and measured with the help of Microsoft PowerPoint (Koehler et al., 2020).

Statistical analysis of data

The data for growth, yield, and anatomical attributes with three replications were subjected to statistical analysis using the R (version 4.4.1) package agricolae. A one-way ANOVA was executed, followed by Tukey's HSD test ($\alpha = 0.05$), the ggplot2 package in the R environment, and Microsoft Excel was used to prepare the bar graphs for data representation. Principal component analysis (PCA) was performed to differentiate the various attributes according to the different treatments by the FactoMineR package, and PCA results visualization was done by the factoextra package.

Results

Growth analysis

Chromium stress resistance of *A. sativa* was assessed by analyzing the impact of foliar applications of salicylic acid (SA) on the growth and development of *A. sativa*. The findings of the vegetative growth attributes revealed that significant ($P < 0.05$) variations were observed between the control group and all other treatment groups. A significant decrease in root length and shoot length was recorded under Cr stress as compared to the control (**Figure 1**). The number of leaves (**Figure 1**) was also significantly decreased due to Cr application compared with the control group. However, exogenous applications of salicylic acid (SA) were found to be effective in improving root length, shoot length, and the number of leaves. The maximum shoot length was recorded at SA 0.5 mM. The positive effect of salicylic acid was also observed on root length. The shoot length of the oat plant was significantly increased when salicylic acid was applied at a maximum concentration of 2.0 mM as compared to its lower concentration, i,e. 0.5 mM. The combined treatment of Cr and SA caused a significant improvement in shoot length (**Figure 1**).

Yield parameters

The impact of Cr toxicity on yield and yield-related components of *A. sativa* was examined. Plants showed a negative response to Cr treatments, and a decrease in seed output of plants was recorded. The current study shows that Cr harmed plant grains, grain weight, and overall biomass. The number of grains and the weight of grains were significantly ($P < 0.05$) reduced at Cr as compared to the control group. The highest quantity of grains was observed at SA 2.0 mM as compared to all other respective treatments. The number of grains was significantly increased when applying combined treatments (Cr + SA) as compared to Cr alone. The weight of grains was also increased significantly by exogenous application of salicylic acid, applied alone as well as in combination with Cr. The greatest weight of grains was observed at Cr 2 mM + SA 2.0 mM, and the lowest weight of grains was observed at 2.0 mM Cr stress (**Figure 2**).

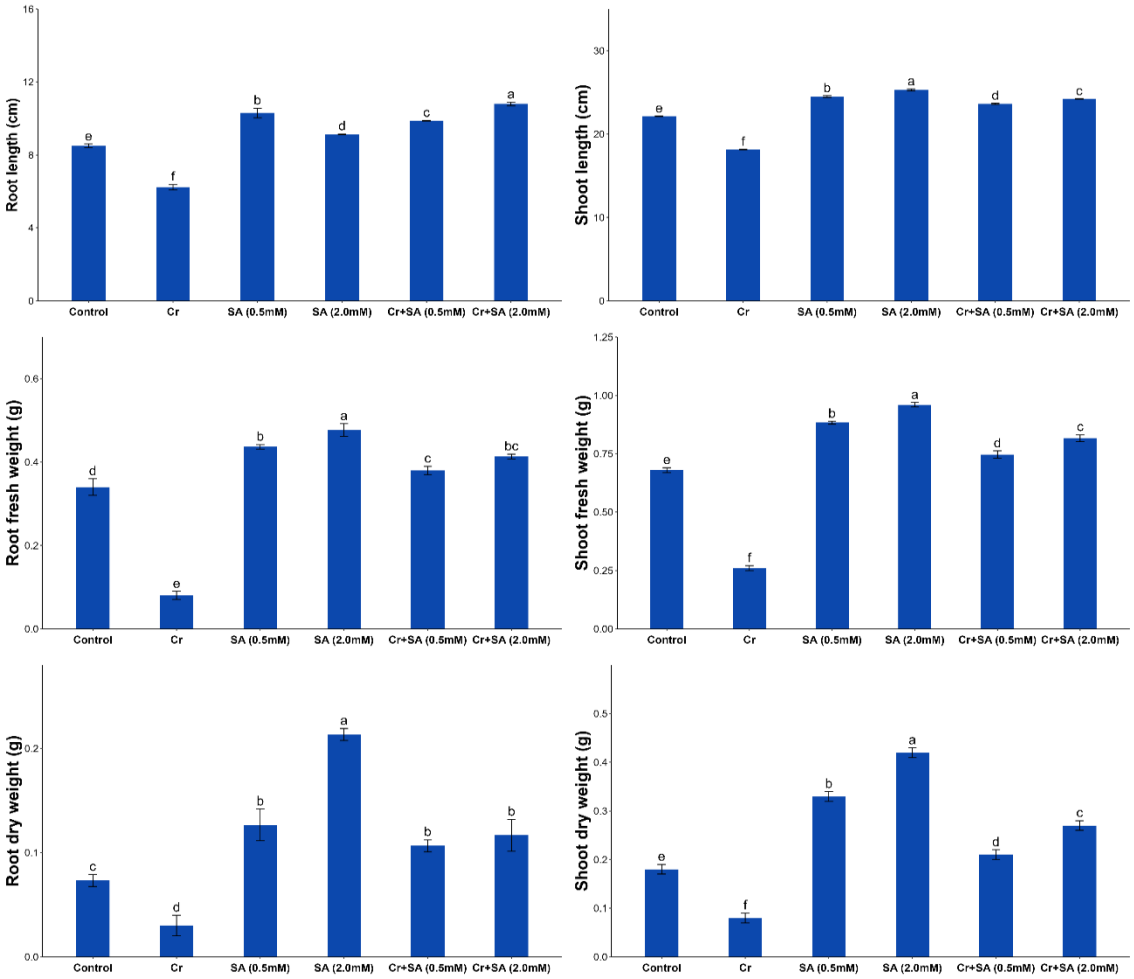

Figure 1: Root length, shoot length, and fresh and dry weights of the root and shoot of *Avena sativa* at different treatments. Each bar in the graph represents the mean of three replications (±SD). Mean values with the same alphabetic letters were not significantly different at $P > 0.05$, but different alphabets (a, b, c, d, e, and f) were considered as significantly different at $P < 0.05$.

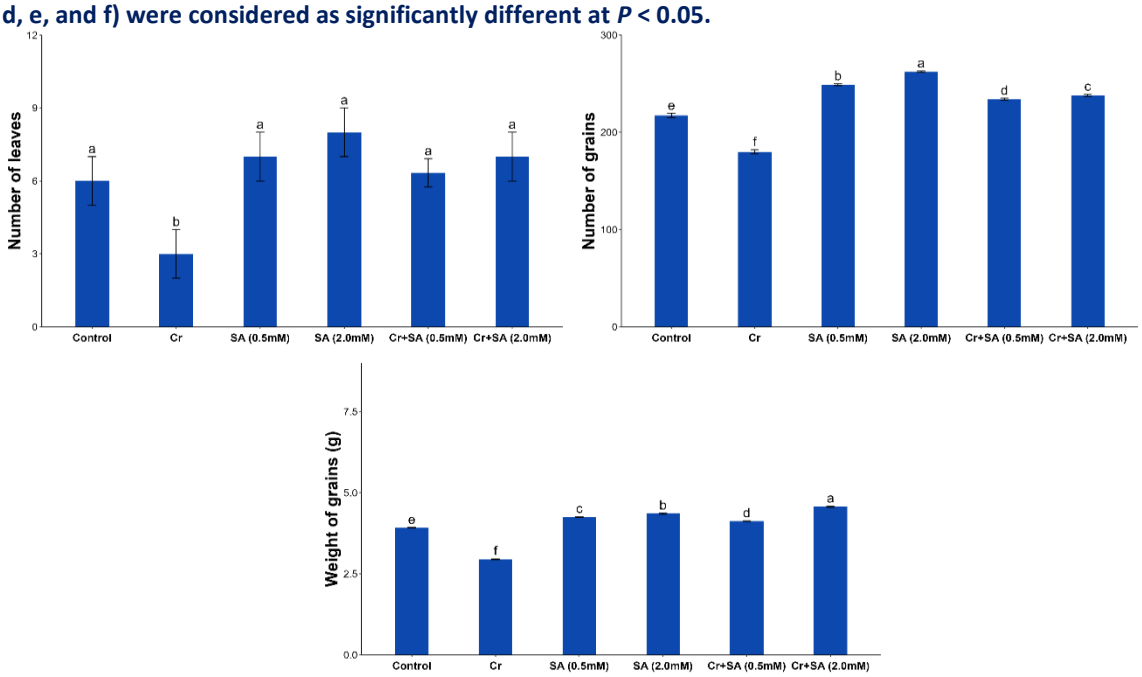

Figure 2: Number of leaves/plant, number of grains/plant, and weight of 100 grains at different concentrations of Cr and salicylic acid. Each bar in the graph represents the mean of three replicates (±SD). Means with distinct letters (a, b, c, d, e, and f) are significant at $P < 0.05$, but nonsignificant with the same letters (ab, b, b, ac, etc).

Anatomical parameters of the stem

Stem area, number of vascular bundles, and sclerenchyma thickness were significantly ($P < 0.05$) increased by applying salicylic acid (2.0 mM) and Cr in combination. The maximum thickness of sclerenchyma and stem area were observed at Cr 2 mM + SA 2.0 mM. Both parameters were increased by 1.3- and 1.5-fold, respectively, as compared to the respective control group (**Table 1**). A nonsignificant difference was observed in sclerenchyma thickness between the (Cr 2 mM + SA 0.5 mM) and (SA 2.0 mM) groups. Metaxylem area and phloem thickness were significantly increased by increasing SA concentration in the collective form (Cr + SA). At high concentrations (Cr 2 mM + SA 2.0 mM), both parameters were increased by 1.8- and 1.29-fold as compared to the respective control group. SA 0.2 mM and Cr 2 mM + SA 0.5 mM showed a nonsignificant difference in phloem thickness, but a significant difference was detected from the control group. A nonsignificant difference was observed in the parenchyma cell area and epidermal cell area between the control group and the SA 0.5 mM. The number of vascular bundles shows a nonsignificant difference between SA 0.5 mM, control, and SA 2.0 mM. The Cr 2 mM + SA 0.5 mM and Cr 2 mM + SA 2.0 mM showed significant differences from each other. Epidermis thickness and cell area were significantly increased by applying SA in combination (Cr + SA) as compared to the control group (**Figure 3**).

Table 1: Effects of different treatments on different anatomical parameters (stem area, vascular bundle area, number of vascular bundles, sclerenchyma thickness, parenchyma cell area, phloem thickness, metaxylem area, epidermis thickness, epidermal cell area) of the stem. All columns of the table characterize the three replicates' mean (±SD). Means with different alphabet letters (a, b, c, d, e, f) were considered significant at $P < 0.05$, and means with the same letters (ab, b, b., etc) were considered non-significant.

	Control	Cr (2 mM)	SA1 (0.5 mM)	SA2 (2.0 mM)	Cr + SA1	Cr + SA2
SA	18.02^{e}±0.015	11.40^{f}±0.100	19.30^{d}±0.100	20.0^{c}±0.011	22.23^{b}±0.057	24.14^{a}±0.010
VBA	1.203^{e}±0.015	0.120^{f}±0.010	1.543^{b}±0.015	1.426^{c}±0.011	1.35^{d}±0.010	1.626^{a}±0.005
NVB	11.00^{de}±1.0	8.666^{e}±0.577	15.00^{c}±1.00	12.666^{cd}±0.577	17.666^{b}±0.577	20.333^{a}±1.527
SCT	1.08^{d}±0.01	0.30^{e}±0.10	1.26^{c}±0.010	1.516^{b}±0.015	1.426^{c}±0.015	1.666^{a}±0.011
PCA	3.226^{c}±0.015	1.540^{e}±0.01	3.120^{cd}±0.01	3.350^{b}±0.010	3.040^{d}±0.01	4.200^{a}±0.10
PTH	1.040^{d}±0.020	0.210^{e}±0.010	1.106^{c}±0.023	1.320^{a}±0.017	1.233^{b}±0.015	1.350^{a}±0.026
MXA	0.233^{d}±0.020	0.166^{e}±0.015	0.296^{c}±0.015	0.343^{bc}±0.020	0.373^{b}±0.020	0.426^{a}±0.011
ETH	0.320^{c}±0.020	0.043^{e}±0.011	0.126^{d}±0.020	0.153^{d}±0.020	0.446^{a}±0.015	0.396^{b}±0.005
ECA	0.0243^{c}±0.005	0.007^{e}±0.001	0.016^{d}±0.005	0.023^{cd}±0.005	0.035^{b}±0.002	0.111^{a}±0.001

SA: Stem area (μm^2); VBA: Vascular bundle area (μm^2); NVB: No. of vascular bundles; SCT: Sclerenchyma thickness (µm); PCA: Parenchyma cell area (μm^2); PTH: Phloem thickness (µm); MXA: Metaxylem area (μm^2); ETH: Epidermis thickness (µm); ECA: Epidermal cell area (μm^2)

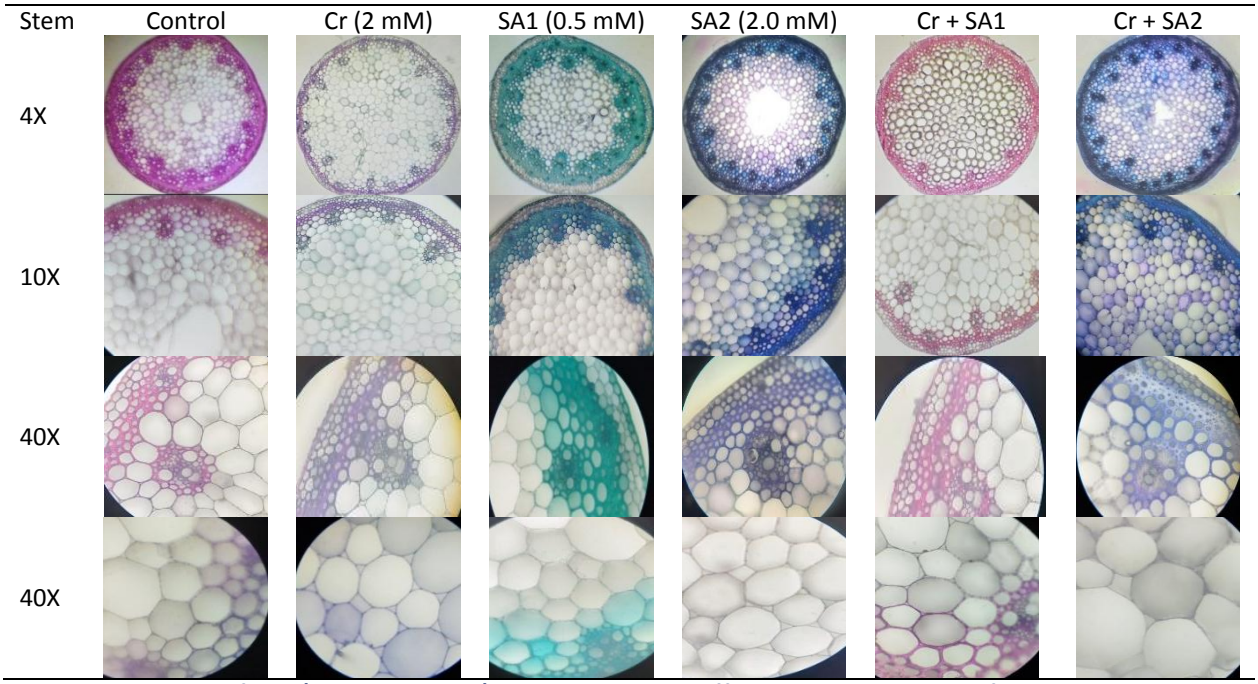

Figure 3: Images of oat (*Avena sativa* L.) stem illustrating different anatomical modifications subjected to metal stress and SA

Anatomical parameters of the leaf

A significant increase was recorded in midrib thickness, metaxylem area, phloem thickness, and bulliform cell area by applying different treatments of salicylic acid and Cr + SA in combined form as compared to the control group (**Figures 4, & 5**).

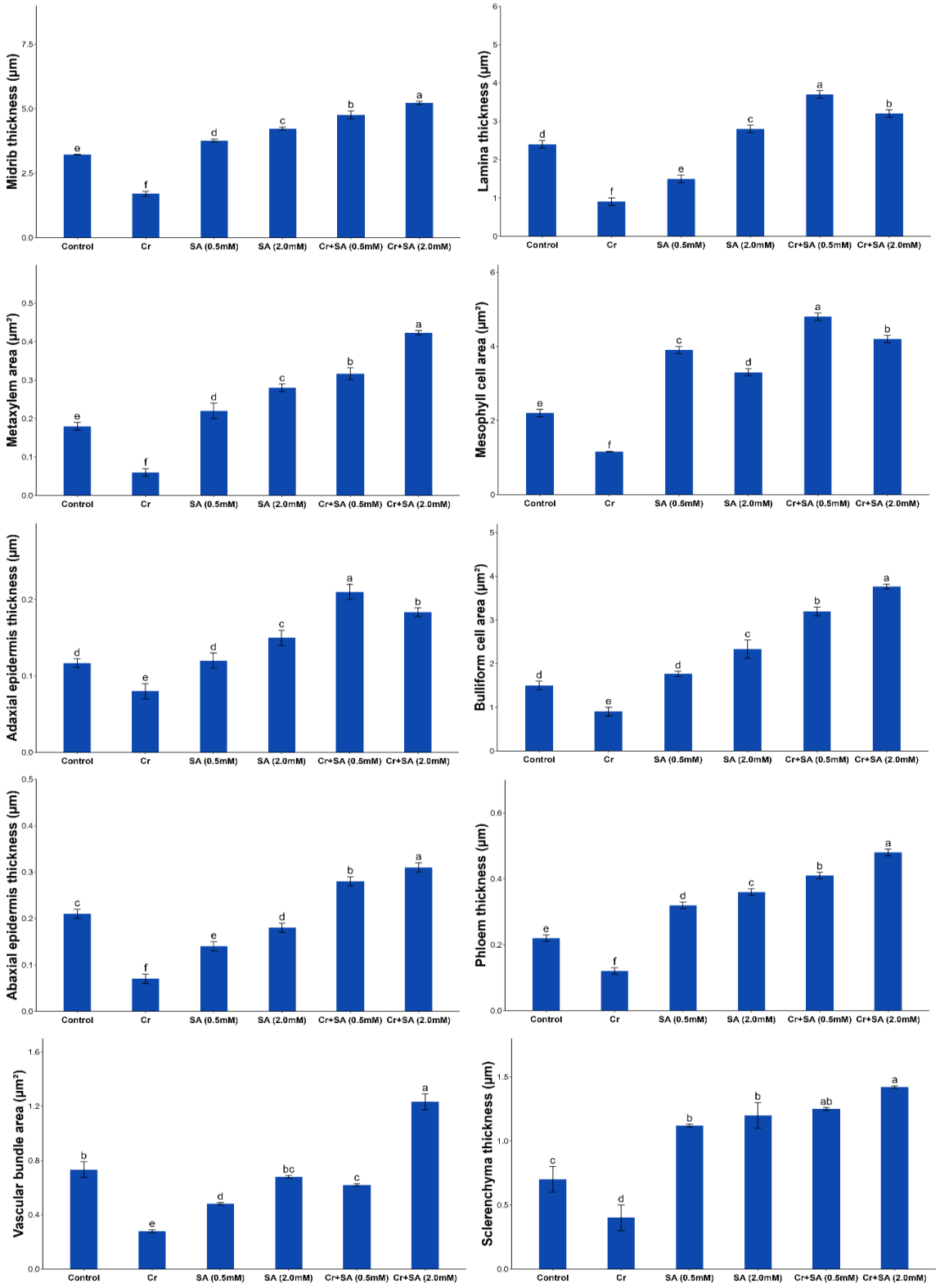

Figure 4: Different anatomical parameters (midrib thickness, lamina thickness, metaxylem area, mesophyll cell area, adaxial epidermis thickness, bulliform cell area, abaxial epidermis thickness, phloem thickness, vascular bundle area, sclerenchyma thickness) of *A. sativa* leaf. Each bar in the graph represents the mean of three replicates (±SD). Means with the same alphabets (a, ab, b, a, cd, etc.) were considered as nonsignificant at $P > 0.05$; results with different alphabets (a, b, c, d, e, f) were considered as significant at $P < 0.05$ and $P < 0.01$.

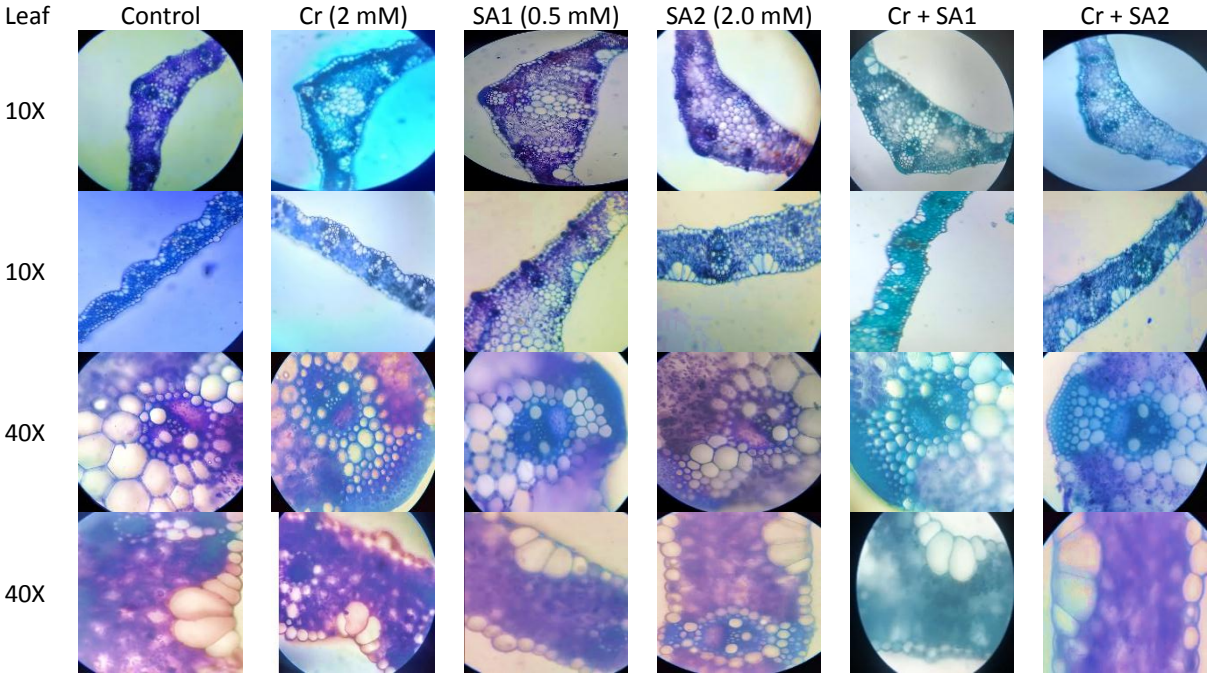

Figure 5: Different anatomical parameters of *A. sativa* leaf subjected to different treatments of Cr and SA

In the SA (0.5 mM) and control groups, a nonsignificant difference was observed while studying the bulliform cell area. Lamina thickness also showed a significant difference between the respective groups. The highest lamina thickness was recorded at Cr 2 mM + SA 0.5 mM as compared to the control (**Figures 4 & 5**). But there was a significant decrease in SA 0.5 mM as compared to the control. A nonsignificant difference was detected in adaxial epidermis thickness between the control group and the SA 0.5 mM. The greatest thickness was recorded in Cr 2 mM + SA 0.5 mM, and the lowest thickness was recorded at Cr alone. Mesophyll cell area was significantly increased by the foliar applications of salicylic acid and the combined treatment Cr + SA, as compared to the control group. By analyzing sclerenchyma thickness, a nonsignificant difference was recorded between Cr 2 mM + SA 0.5 mM and Cr 2 mM + SA 2.0 mM, and a significant difference was found as compared to the control group. A nonsignificant difference in vascular bundle area was found between the SA 2.0 mM and the control group. Moreover, there was a decline in vascular bundle area at SA 0.5 mM and Cr as compared to the control group (**Figures 4 & 5**).

Anatomical parameters of the root

The root area of the oat plant was significantly ($P < 0.05$) increased by applying salicylic acid in the Cr + SA groups as compared to the control (**Table 2**). Non-significant results were observed between the root area of Cr 2 mM + SA 0.5 mM and SA (2.0 mM), control, and SA (0.5 mM) groups. The highest epidermis thickness, endodermis thickness, and phloem thickness were detected in the Cr + SA groups as compared to the control group. Xylem thickness was decreased significantly $P < 0.05$) in the Cr group as compared to the control and all other respective groups. The results also indicated that the cortex thickness was increased significantly by SA in the Cr + SA groups as compared to the control. Still, there was no significant difference between Cr 2 mM + SA 2.0 mM and Cr 2 mM + SA 0.5 mM. The lowest cortex thickness was observed in the Cr group, and the highest thickness was detected in Cr 2 mM + SA 2.0 mM (**Table 2**, **Figure 6**).

Table 2: Effects of different treatments on different anatomical parameters (root area, epidermis thickness, phloem thickness, xylem thickness, endodermis thickness, cortex cell area, and cortex thickness) of the root. Each column in the table presents the mean of three replicates (±SD). Means with different alphabet letters (a, b, c, d, e, and f) were considered significant at $P < 0.05$ and $P < 0.01$, and means with the same letters (ab, b, b., etc) were considered as non-significant at $P > 0.05$.

	Control	Cr (2 mM)	SA1 (0.5 mM)	SA2 (2 mM)	Cr + SA1	Cr + SA2
RA	2.60^{c}±0.173	1.566^{d}±0.152	2.76^{c}±0.152	3.33^{b}±0.152	3.60^{b}±0.20	4.23^{a}±0.057
ET	0.326^{d}±0.015	0.176^{e}±0.005	0.356^{c}±0.005	0.546^{b}±0.005	0.643^{a}±0.015	0.616^{a}±0.005
PT	0.423^{c}±0.005	0.250^{d}±0.010	0.423^{c}±0.005	0.470^{c}±0.010	1.80^{a}±0.10	1.266^{b}±0.115
XT	0.60^{a}±0.057	0.116^{d}±0.015	0.270^{c}±0.010	0.316^{c}±0.015	0.463^{b}±0.015	0.546^{a}±0.020
EnT	0.083^{c}±0.005	0.033^{d}±0.015	0.120^{c}±0.020	0.180^{b}±0.017	0.216^{b}±0.015	0.276^{a}±0.015
CCA	0.116^{c}±0.015	0.066^{c}±0.011	0.306^{b}±0.100	0.360^{b}±0.020	0.520^{a}±0.010	0.623^{a}±0.025
CT	0.233^{c}±0.057	0.083^{d}±0.005	0.216^{c}±0.015	0.276^{bc}±0.015	0.316^{ab}±0.015	0.383^{a}±0.05

RA: Root area (μm^2); ET: Epidermis thickness (µm); PT: Phloem thickness (µm); XT: Xylem thickness (µm); EnT: Endodermis thickness (µm); CCA: Cortex cell area (μm^2); CT: Cortex thickness (µm)

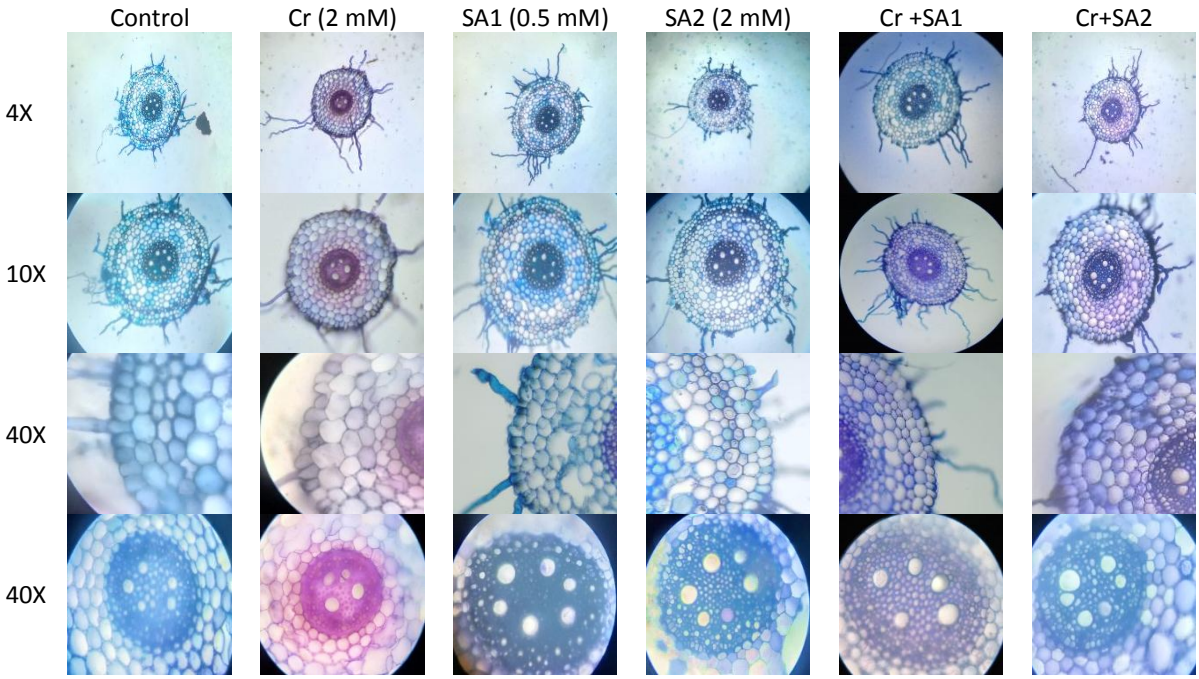

Figure 6: Different anatomical parameters of *A. sativa* root exposed to different treatments of Cr and SA

Principal component analysis (PCA)

PCA was performed to determine the variation between the different attributes of oat under different treatments. We selected the first two principal components, accounting for about 92.7% of the total variance (PC1: 78.5% and PC2: 14.2%). The scatterplot of PCA demonstrated (**Figure 7A**) the distribution of different attributes of oat plants according to PCA1 and PCA2. PCA1 is strongly correlated with RFW, SFW, and SL in the positive part. MCA and LT or PT and MT showed a strong correlation with PCA2 in a positive direction. Four distinct cluster groups were seen while studying the treatment groups (**Figure 7B**). The clusters close together show similar physiological responses according to the treatments. The Cr separated from PCA1 represents the significant impact of chromium stress.

Discussion

As a heavy metal contaminant, Cr has a considerable harmful effect on plants (Kundu et al., 2018). In plants, Cr toxicity harms a variety of physiological, biochemical, and molecular properties, slowing growth and lowering total yield. According to Dotaniya et al. (2014), increased Cr deposition in plants reduces seed germination and decreases root and shoot development rates, affecting total biomass and yield. Research suggests that high levels of Cr in plants can reduce chlorophyll concentration and inhibit photosynthesis (Sharma et al., 2020; Qin et al., 2024). Previous research has demonstrated that excessive Cr deposition in plant tissues disrupts the cellular cycle, water and mineral balance, enzyme function, nitrogen absorption, the antioxidant system, and other critical metabolic functions (Ugwu and Agunwamba, 2020; Ali et al., 2023). In the current study, we

investigated the effects of Cr toxicity on the growth, yield, and anatomical attributes of *A. sativa*. All growth and yield attributes in *A. sativa* were significantly reduced due to the exogenous application of Cr.

Many other previous researchers have found similar results, as Cr treatments have been shown to lower root and shoot lengths, as well as fresh and dry weights, in various plant species, including *Oryza sativa* (Ma et al., 2016), *Brassica napus* (Gill et al., 2015), and Lacy Phacelia (*Phacelia tanacetifolia*) (Inci, 2025). Likewise, shoot development in *Allium cepa* was inhibited when Cr (III) in varying concentrations was applied (Nematshahi et al., 2012; Naseem et al., 2024).

Our findings suggest that SA is involved in the improvement of parameters linked to the development of *A. sativa* seedlings under Cr stress. Our results were similar to those of earlier-published studies in which SA application significantly improved the growth of mung bean (Imran et al., 2021) and rice (Wang et al., 2021) under cadmium stress. This suggests that SA can lower the toxicity caused by heavy metal stress during the vegetative growth phase by enhancing photosynthesis, water relations, and nutrient absorption through detoxification processes (Zhang et al., 2022).

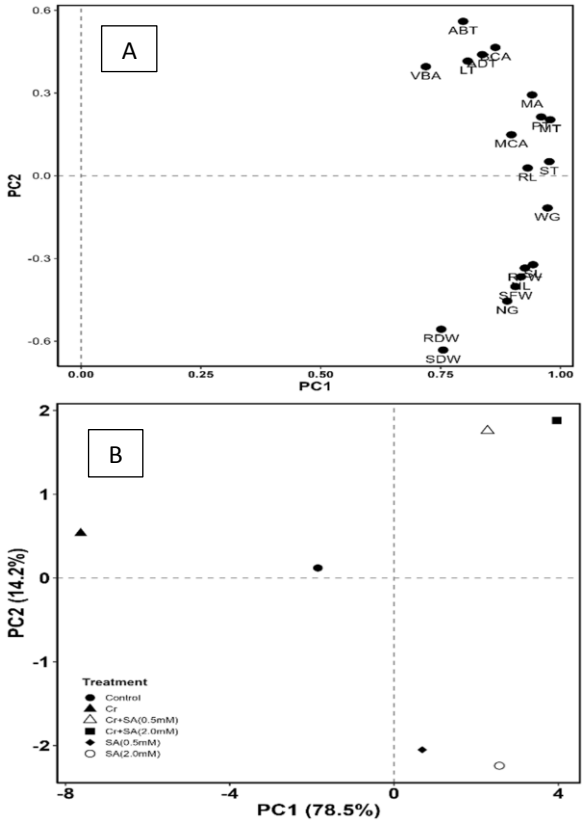

Figure 7: 2D scatterplot (A) represents the distribution of growth, yield, and leaf anatomical parameters (i.e. RDW = root dry weight, SDW = shoot dry weight, NG = number of grains, RFW = root fresh weight, SFW = shoot fresh weight, NL = number of leaves, WG = weight of grains, RL= root length, SL= shoot length, MCA = mesophyll cell area, ST = sclerenchyma thickness, PT = phloem thickness, MA = metaxylem area, VBA = vascular bundle area, ABT = abaxial epidermis thickness, ADT = adaxial epidermis thickness, LT = lamina thickness, BCA = bulliform cell area, and MT = midrib thickness), and (B) represent treatments according to the two main principal components.

Of the different organs of the plant, roots play a significant role in resisting all types of soil-borne stresses, including those of metals. Moreover, as the first organ to come into contact with the soil, the root is in charge of absorbing and moving ions and water. In our study, the root anatomical features of *A. sativa* were adversely affected by the Cr stress. It has been observed that in *Vigna unguiculata* (Fontenele et al., 2017) and *O. sativa* (Ashraf and Tang, 2017) structural changes in their roots occurred due to metal-induced oxidative stress, which may reduce their resilience. The most prevalent modifications are cell wall modifications in exodermis, which are in direct contact with contaminants (Yadav et al., 2021). Plants under severe mental stress generally have smaller roots due to decreased vascular bundle size, cell division, and cell size (Batool et al., 2015; Gao et al., 2022). However, exogenous supplementations of salicylic acid demonstrated that increased vascular tissues, specifically the metaxylem area, in *A. sativa* under stress conditions can improve water and nutrient conduction and reduce resistance (Horie et al., 2012; Strock et al., 2021). Root area, cortex thickness, and cortex cell area of *A. sativa* were also declined by heavy metal stress (Cr), but foliar applications of SA significantly enhanced their size, particularly at Cr + SA 2.0 mM. Likewise, exposure to Cd (250-1000 µM) reduced the root width of chickpea plants by reducing the number of cortical cell layers (from 12-14 in control plants to 8-10 in Cd-treated plants) (Liza et al., 2020); chickpea plant roots treated with Cd (250-1000 µM) showed decreased diameter of metaxylem vessels. Heavy metals reach the stem by the root system through vascular tissues, causing structural changes mostly in the xylem and adjacent tissues (Yadav et al., 2021). According to Liza et al. (2020), chickpea plants exposed to Cd (250–1000 µM) exhibited a decrease in stem diameter, primarily due to smaller cells and reduced vascular components. Similar results were recorded in the current investigation (**Figure 3**, **Table 1**). Sclerification, which occurs outside of the cortex and epidermis, is one of the most

significant structural changes caused by increasing stress levels. Sclerification strengthens plant tissues (Lo et al*.,* 2008; Sarwar et al., 2022), controls water loss, and avoids desiccation (Wasim et al., 2022). In our investigation, sclerification outside of the vascular bundles and cortical area significantly declined under Cr stress, but significantly increased at Cr + SA 2.0 mM in *A. sativa* stem (**Figure 3**), which is a critical anatomical response to stress situations that shields the stem from injury and provides mechanical strength. Sclerification promotes species survival in stressful circumstances (Naz et al*.,* 2018). In the current experiment, salicylic acid spraying mitigated the heavy metal stress by enhancing the vascular bundle area and sclerenchyma thickness significantly in *A. sativa* stem.

Only very small amounts of metals are translocated to leaves; even small amounts can result in significant structural changes, and a reduction in the size of the leaf's cells and vascular bundles, which may have an impact on stomatal parameters and pigment production (Batool et al*.,* 2015; Tóth et al., 2024). The midrib is made up of cortical cells as well as specialized tissues such as phloem and xylem, which are necessary for the flow of water and minerals in leaves (Lechthaler *et al.,* 2019). A significant increase was observed in metaxylem area, phloem thickness, and vascular bundle area in *A. sativa* as a sign of adaptation in stress conditions by foliar application of SA. Also, a considerable increase in the number of bulliform cells was observed in leaf blades, demonstrating their significance in leaf rolling (Matschi et al., 2020).

The current investigation concluded that yield and growth parameters (root-shoot length and fresh and dry weight) of *A. sativa* were seriously affected by the Cr stress. Anatomical parameters of *A. sativa* were also altered by the Cr stress. The results of our study suggest that foliar applications of salicylic acid in appropriate concentrations increased yield and growth attributes of *A. sativa* under Cr stress. Chromium significantly impacts crop growth, yield, and grain quality. It is necessary to investigate rapid, efficient, and cost-effective methods for removing Cr from soil and other environmental sites.

Author(s), Editor(s) and Publisher's declarations

Acknowledgement
All authors acknowledge the Department of Botany, the Islamia University, Bahawalpur, for its laboratory facilities. Special thanks to Dr. Syeda Sadaf Zehra for her support during the whole study period.

Source of funding
None declared.

Contribution of authors
Research supervision: SSZ. Conceptualization and designing the study: HMMH, WA. Conduction of experiment: HMMH, WA. Data collection, visualization, and interpretation: HMMH, WA. Formal statistical analysis: HMMH. Preparation of initial draft: HMMH. Review of initial draft: SSZ. Revision and proofreading: SSZ, MUZ, AS.

Permissions and ethical compliance
This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material
No supplementary material is included with this manuscript.

Conflict of interest
The authors declare no conflict of interest.

Availability of primary data and materials

As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent

All authors have critically read this manuscript and agreed to publish in IJAaEB.

Disclaimer/editors'/publisher's declaration

All claims/results/prototypes included in this manuscript are exclusively those of the authors and do not necessarily express those of their affiliated organizations/enterprises, or those of the publisher/journal management, or the editors/reviewers. Any product mentioned in this manuscript, or claim rendered by its manufacturer, is not certified by the publisher/Journal management. The journal management disowns responsibility for any injury to organisms, including humans, animals, and plants, or property resulting from any ideas/opinions, protocols/methods, guidelines, or products included in the publication. The IJAaEB publisher/Management stays impartial/neutral pertaining to institutional affiliations and jurisdictional claims in maps included in the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process

It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

References

Ali, S., Mir, R.A., Tyagi, A., Manzar, N., Kashyap, A.S. et al. (2023). Chromium toxicity in plants: Signaling, mitigation, and future perspectives. *Plants (Basel, Switzerland)* 12(7):1502. https://doi.org/10.3390/plants12071502

Amanullah, F., Ahmed, H.A.I., Hou, P. (2023). *Avena sativa* under toxic elements. *In* "Medicinal Plant Responses to Stressful Conditions" (A.A.H. Abdel Latef, ed.). First Edition, pp. 81-93. CRC Press. https://doi.org/10.1201/9781003242

Anjum, S.A., Ashraf, U., Imran, K., Tanveer, M., Shahid, M. et al. (2017). Phyto-toxicity of chromium in maize: oxidative damage, osmolyte accumulation, anti-oxidative defense and chromium uptake. *Pedosphere* 27(2):262-273.

Ashraf, U., Tang, X. (2017). Yield and quality responses, plant metabolism and metal distribution pattern in aromatic rice under lead (Pb) toxicity. *Chemosphere* 176:141-155.

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M.R., Sadeghi, M. (2021). Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. *Frontiers in Pharmacology* 12:643972.

Batool, R., Hameed, M., Ashraf, M., Ahmad, M.S.A., Fatima, S. (2015). Physio-anatomical responses of plants to heavy metals. *In* "Phytoremediation for Green Energy". (M. Öztürk, M. Ashraf, A. Aksoy, M. Ahmad, eds.). pp. 79-96. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-7887-0_5

Budi, H.S., Catalan Opulencia, M.J., Afra, A., Abdelbasset, W.K., Abdullaev, D. et al. (2024). Source, toxicity and carcinogenic health risk assessment of heavy metals. *Reviews on Environmental Health* 39(1):77-90.

Dotaniya, M.L., Thakur, J.K., Meena, V.D., Jajoria, D.K., Rathor, G. (2014). Chromium pollution: a threat to environment-a review. *Agricultural Reviews* 35(2):153-157.

Ellouzi, H., Zorrig, W., Amraoui, S., Oueslati, S., Abdelly, C. et al. (2023). Seed priming with salicylic acid alleviates salt stress toxicity in barley by suppressing ROS accumulation and improving antioxidant defense systems, compared to halo- and gibberellin priming. *Antioxidants* (*Basel, Switzerland*) 12(9):1779. https://doi.org/10.3390/antiox12091779

Fontenele, N.M.B., Otoch, M.D.L.O., Gomes-Rochette, N.F., de Menezes Sobreira, A.C., Barreto, A.A.G.C. et al. (2017). Effect of lead on physiological and antioxidant responses in two Vigna unguiculata cultivars differing in Pb-accumulation. *Chemosphere* 176:397-404.

Gao, T., Wang, H., Li, C., Zuo, M., Wang, X. et al. (2022). Effects of heavy metal stress on physiology, hydraulics, and anatomy of three desert plants in the Jinchang Mining Area, China. *International Journal of Environmental Research and Public Health* 19 (23):15873.

Gill, R.A., Zang, L., Ali, B., Farooq, M.A., Cui, P. et al. (2015). Chromium-induced physio-chemical and ultrastructural changes in four cultivars of *Brassica napus* L. *Chemosphere* 120:154-164.

Horie, T., Karahara, I., Katsuhara, M. (2012). Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. *Rice* 5:1-18.

Iftikhar, F., Zulfiqar, A., Kamran, A., Saleem, A., Arshed, M.Z. et al. (2025). Antioxidant responses in chromium-stressed maize as influenced by foliar and root applications of fulvic acid. *Scientific Reports* 15(1):1289. https://doi.org/10.1038/s41598-024-84803-4

Imran, K.H.A.N., Seleiman, M.F., Chattha, M.U., Jalal, R.S., Mahmood, F. et al. (2021). Enhancing antioxidant defense system of mung bean with a salicylic acid exogenous application to mitigate cadmium toxicity. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* 49(2):12303-12303.

Inci, H.S. (2025). Chromium (VI) accumulation in different plant organs of Lacy Phacelia (*Phacelia tanacetifolia* Benth.): Implications for phytoremediation. *BMC Plant Biology* 25(1):414. https://doi.org/10.1186/s12870-025-06471-4Koehler,

Lechthaler, S., Colangeli, P., Gazzabin, M., Anfodillo, T. (2019). Axial anatomy of the leaf midrib provides new insights into the hydraulic architecture and cavitation patterns of *Acer pseudoplatanus* leaves. *Journal of Experimental Botany* 70(21):6195-6201.

Liza, S.J., Shethi, K.J., Rashid, P. (2020). Effects of cadmium on the anatomical structures of vegetative organs of chickpea (*Cicer arietinum* L.). *Dhaka University Journal of Biological Sciences* 29(1):45-52.

Lo, T.Y.., Cui, H.Z., Tang, P.W.C., Leung, H.C. (2008). Strength analysis of bamboo by microscopic investigation of bamboo fibre. *Construction and Building Materials* 22(7):1532-1535.

Ma, J., Lv, C., Xu, M., Chen, G., Lv, C. et al. (2016). Photosynthesis performance, antioxidant enzymes, and ultrastructural analyses of rice seedlings under chromium stress. *Environmental Science and Pollution Research* 23:1768-1778.

Martín-Diana, A.B., García-Casas, M.J., Martínez-Villaluenga, C., Frías, J., Peñas, E. et al. (2021). Wheat and oat brans as sources of polyphenol compounds for development of antioxidant nutraceutical ingredients. *Foods* 10(1):115.

Maruri-López, I., Aviles-Baltazar, N.Y., Buchala, A., Serrano, M. (2019). Intra and extracellular journey of the phytohormone salicylic acid. *Frontiers in Plant Science* 10:423.

Matschi, S., Vasquez, M.F., Bourgault, R., Steinbach, P., Chamness, J. et al. (2020). Structure-function analysis of the maize bulliform cell cuticle and its potential role in dehydration and leaf rolling. *Plant Direct* 4(10):e00282. https://doi.org/10.1002/pld3.282

Mert, I.D. (2020). The applications of microfluidization in cereals and cereal-based products: An overview. *Critical Reviews in Food Science and Nutrition* 60(6):1007-1024.

Naz, N., Fatima, S., Hameed, M., Ashraf, M., Naseer, M. et al. (2018). Structural and functional aspects of salt tolerance in differently adapted ecotypes of *Aeluropus lagopoides* from saline desert habitats. *International Journal of Agriculture and Biology* 20:41–51.

Naseem, Z., Naveed, M., Asif, M., Alamri, S., Nawaz, S. et al. (2024). Enhancing chromium resistance and bulb quality in onion (*Allium cepa* L.) through copper nanoparticles and possible health risk. *BMC Plant Biology* 24(1):777. https://doi.org/10.1186/s12870-024-05460-3

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.

Nogala-Kałucka, M., Kawka, A., Dwiecki, K., Siger, A. (2020). Evaluation of bioactive compounds in cereals. Study of wheat, barley, oat and selected grain products. *Acta Scientiarum Polonorum Technologia Alimentaria* 19(4):405-423.

Paudel, D., Dhungana, B., Caffe, M., Krishnan, P. (2021). A review of health-beneficial properties of oats. *Foods* 10 (11):2591. https://doi.org/10.3390/foods10112591

Qin, C., Lian, H., Alqahtani, F.M., Ahanger, M.A. (2024). Chromium mediated damaging effects on growth, nitrogen metabolism and chlorophyll synthesis in tomato can be alleviated by foliar application of melatonin and jasmonic acid priming. *Scientia Horticulturae* 323:112494. https://doi.org/10.1016/j.scienta.2023.112494.

Riyazuddin, R., Nisha, N., Ejaz, B., Khan, M.I.R., Kumar, M. et al. (2021). A comprehensive review on the heavy metal toxicity and sequestration in plants. *Biomolecules* 12(1):43. https://doi.org/10.3390/biom12010043.

Ruzin, S.E. (1999). "Plant Microtechnique and Microscopy". Oxford University Press, Oxford.

Sarwar, Y., Asghar, A., Hameed, M., Fatima, S., Ahmad, F. et al. (2022). Structural responses of differentially adapted *Cenchrus setigerus* Vahl ecotypes to water deficit. *Environmental and Experimental Botany* 194:104746. https://doi.org/10.1016/j.envexpbot.2021.104746.

Sharma, A., Kapoor, D., Wang, J., Shahzad, B., Kumar, V. et al. (2020). Chromium bioaccumulation and its impacts on plants: an overview. *Plants* 9(1):100.

Strock, C.F., Burridge, J.D., Niemiec, M.D., Brown, K.M., Lynch, J.P. (2021). Root metaxylem and architecture phenotypes integrate to regulate water use under drought stress. Plant, Cell & Environment 44:49–67.

Thakur, M., Praveen, S., Divte, P.R., Mitra, R., Kumar, M. et al. (2022). Metal tolerance in plants: Molecular and physicochemical interface determines the "not so heavy effect" of heavy metals. *Chemosphere* 287: 131957.

Tóth, C., Simon, L., Tóth, B. (2024). Microanatomical changes in the leaves of *Arundo donax* (L.) caused by potentially toxic elements from municipal sewage sediment. *Plants (Basel, Switzerland)* 13(5):740. https://doi.org/10.3390/plants13050740

Ugwu, E.I., Agunwamba, J.C. (2020). A review on the applicability of activated carbon derived from plant biomass in adsorption of chromium, copper, and zinc from industrial wastewater. *Environmental Monitoring and Assessment* 192(4):240.

Varma, S., Jangra, M. (2021). Heavy metals stress and defense strategies in plants: An overview. *Journal of Pharmacognosy and Phytochemistry* 10(1):608-614.

Wakeel, A., Xu, M., Gan, Y. (2020). Chromium-induced reactive oxygen species accumulation by altering the enzymatic antioxidant system and associated cytotoxic, genotoxic, ultrastructural, and photosynthetic changes in plants. *International Journal of Molecular Sciences* 21(3):728

Wasim, M.A., Naz, N., Zehra, S.S. (2022). Anatomical characteristic, ionic contents and nutritional potential of Buffel grass (*Cenchrus ciliaris* L.) under high salinity. *South African Journal of Botany* 144:471-479.

Wang, F., Tan, H., Huang, L., Cai, C., Ding, Y. et al. (2021). Application of exogenous salicylic acid reduces Cd toxicity and Cd accumulation in rice. *Ecotoxicology and Environmental Safety* 207:111198.

Yadav, V., Arif, N., Kováč, J., Singh, V.P., Tripathi, D.K. et al. (2021). Structural modifications of plant organs and tissues by metals and metalloids in the environment: A review. *Plant Physiology and Biochemistry* 159:100-112.

Zhang, Z.W., Deng, Z.L., Tao, Q., Peng, H.Q., Wu, F. et al. (2022). Salicylate and glutamate mediate different Cd accumulation and tolerance between *Brassica napus* and *B. juncea*. *Chemosphere* 292:133466.