



# Rare earth element scandium mitigates the chromium toxicity in *Lemna minor* by regulating photosynthetic performance, hormonal balance and antioxidant machinery

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## ABSTRACT

Chromium (Cr) toxicity is a serious problem that threatens the health of living organisms and especially agricultural production. The presence of excess Cr leads to biomass loss by causing the imbalance of biochemical metabolism and inhibiting photosynthetic activity. A new critical approach to cope with Cr toxicity is the use of the rare earth elements (REEs) as an antioxidant defence system enhancer in plants. However, the effect of scandium (Sc), which is one of the REEs, is not clear enough in *Lemna minor* exposed to Cr toxicity. For this purpose, the photosynthetic and biochemical effects of scandium (50  $\mu$ M and 200  $\mu$ M Sc) treatments were investigated in *Lemna minor* under Cr stress (100  $\mu$ M, 200  $\mu$ M and 500  $\mu$ M Cr). Parameters related to photosynthesis ( $F_v/F_m$ ,  $F_v/F_o$ ) were suppressed under Cr stress. Stress altered antioxidant enzymes activities and hormone contents. Sc applications against stress increased the activities of superoxide dismutase (SOD), NADPH oxidase (NOX), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and glutathione S-transferase (GST). In addition to the antioxidant system, the contents of indole-3-acetic acid (IAA), abscisic acid (ABA) and jasmonic acid (JA) were also rearranged. However, in all treatment groups, with the provision of ascorbate (AsA) regeneration and effective hormone signaling, reactive oxygen species (ROS) retention which result in high hydrogen peroxide ( $H_2O_2$ ) content and lipid peroxidation (TBARS) were effectively removed. Sc promoted the maintenance of cellular redox state by regulating antioxidant pathways included in the AsA-GSH cycle. Our results showed that Sc has great potential to confer tolerance to duckweed by reducing Cr induced oxidative damage, protecting the biochemical reactions of photosynthesis, and improving hormone signaling.

## 1. Introduction

Heavy metal pollution has become a serious environmental problem worldwide in the last several decades because of its concentrations exceeding tolerable levels. Chromium (Cr) is a naturally occurring, highly active heavy metal which can easily convert from one oxidation state to another due to its high redox potential and is the 17th most abundant element on earth (Bhalerao and Sharma, 2015; Prado et al., 2016; Shahid et al., 2017). While Cr is required in trace amounts by plants and animals for carbohydrate metabolism and growth stimulation

(Samantaray et al., 1998), it becomes a significant pollutant for the environment at high concentrations. Natural resources and various anthropogenic activities are responsible for the release of globally polluting Cr into soil, air and water. As a result of contamination of cultivated areas and drinking system, Cr easily gets in the food chain and directly or indirectly affects the health of all organisms (WHO, 2020).

Cr toxicity results in the production of reactive oxygen species (ROS) via Fenton and Haber-Weiss reactions in plants (Costa et al., 2010). Oxidative stress caused by ROS affects plant processes that induce loss of cellular homeostasis, which negatively affects growth and development

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stages such as delayed seed germination, decreased biomass, less plant height, photosynthetic deterioration, membrane damage, leaf chlorosis, necrosis and low grain yield (Amin et al., 2013; Srivastava et al., 2021). Stress avoidance mechanisms of plants are limited and need flexible means of adaptation to change. One of the common features for combating stress factors is the synchronized function of antioxidant systems that help alleviate cellular damage by scavenging reactive oxygen species. Defending against oxidative stress is provided by the production of enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), while glutathione, carotenoids and ascorbate represent non-enzymatic components (Bhaduri and Fulekar, 2012). Simultaneously with this system, plant hormones such as abscisic acid (ABA), indole acetic acid (IAA) and salicylic acid (SA) have important functions that control and regulate plant metabolism and development through varied biochemical and physiological processes. Depending on environmental stimuli, these hormones can have effects near or far from their location. Therefore, hormones are vital in avoiding abiotic stress by escaping or trying to survive under stress condition. Accordingly, Cr stress often alters growth production and signal delivery or transmission besides hormones that can promote specific protective mechanisms (Pál et al., 2018).

*Lemnaceae* is the world's smallest and fastest growing angiosperms with large amounts of biomass. *Lemna* species have great economic potential and many practical applications in biotechnological and ecological fields. Their morphological and physiological properties enable to valuable bioassays under limited conditions in a short time, therefore they represent model laboratory organisms. *Lemna* species are used as bioindicators for *in vivo* and *in vitro* ecotoxicological tests (Basigliani et al., 2018). Decreased photosynthetic pigments and mineral nutrients, increased cell death, elevated ROS levels and disruption of redox homeostasis, regulation of total and isozymatic antioxidant enzyme activities, and increased lipid peroxidation have been reported in *Lemna minor*, which was previously exposed to heavy metal stress (Lu et al., 2018). Likewise, the contribution of changes in hormone contents such as increased ABA and SA to protection has been noted in a few plants exposed to Cr stress (Khanna et al., 2016; Miura and Tada, 2014). Although *Lemna minor* can be used in phytoremediation applications considering its tolerance, accumulation capacity and biomass productivity, it has been determined that heavy metals have toxic effects in various metabolic pathways after a certain threshold value (Delimi et al., 2022).

Rare earth elements (REEs) are Group IIIA elements consisting of the lanthanides, a series of 15 metallic chemical elements with atomic numbers from 57 to 71, scandium (atomic number 21) and yttrium (atomic number 39). They share some physical and chemical properties with common transition elements. Scandium (Sc) is one of the rare earth elements prevalent in areas inhabited by microorganisms and biotopes such as the world's oceans, lakes and rivers, soils, wastewater, industrial areas and mining areas. It is also considered a promising element in the development of new technologies in the pharmaceutical, diagnostic and medical industries (Syrvatka et al., 2022). Sc is an ingredient of almost all plants and the average scandium content is between 0.008 and a few tenths of a ppm, but the biological roles of Sc in the development and functioning of plants has not been described in detail until now. In the present study, we aimed to evaluate the effects of Sc on chlorophyll fluorescence, ROS accumulation, antioxidant capacity, glutathione redox state, lipid peroxidation and endogenous phytohormone contents in the amelioration of Cr stress in *Lemna minor*.

## 2. Material and methods

### 2.1. Experimental design and treatments

Duckweed (*Lemna minor* L.) cultures were hydroponically grown in Hoagland solution under controlled conditions (16/8 h light/dark regime at 24 °C, 70% relative humidity and 350  $\mu\text{mol m}^{-2} \text{s}^{-1}$

photosynthetic photon flux density) and the solutions were refreshed every three days. For stress and scandium applications, chromium (Cr) stress (100  $\mu\text{M}$  (Cr1), 200  $\mu\text{M}$  (Cr2) and 500  $\mu\text{M}$  (Cr3) Chromium (VI) oxide, respectively) and scandium (50  $\mu\text{M}$  (Sc1) and 200  $\mu\text{M}$  (Sc2)  $\text{Sc}_2\text{O}_3$ , respectively) were applied for 7 days. Chromium concentrations were chosen from available data on Cr(VI) concentrations typically occurring in both surface and ground polluted waters (Prado et al., 2016). For determination of Sc application, in the preliminary experiment, 50  $\mu\text{M}$ , 100  $\mu\text{M}$  and 200  $\mu\text{M}$  Sc were applied to *L. minor* plants.

The experiment was designed as twelve groups and was listed in Supplementary Table S1. Plants were harvested after one-week treatment period and rinsed with deionized water.

### 2.2. Determination of relative growth rate

The relative growth rate (RGR) values were calculated according to the formula suggested by Hunt et al. (2002).

$$\text{RGR} = [\ln(\text{DW}_2) - \ln(\text{DW}_1)] / (t_2 - t_1)$$

where  $\text{DW}_1$  = dry weight (g) at  $t_1$ ,  $\text{DW}_2$  = dry weight (g) at  $t_2$ ,  $t_1$  is initial harvest, and  $t_2$  is final harvest.

### 2.3. Photosystem II efficiency and OJIP analysis

The changes in the photochemistry of PSII were detected by Handy PEA (Plant Efficiency Analyser, Hansatech Instruments Ltd.). Supplementary Table S2 included the descriptions for the calculated parameters. The average parameter values of treatment groups in *Lemna minor* plants were represented in the radar plots.

### 2.4. Determination of $\text{H}_2\text{O}_2$ , TBARS and proline contents

Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) content of the leaf samples was determined as Velikova et al. (2000) reported, lipid peroxidation level (Thiobarbituric acid reactive substances (TBARS) content) was calculated according to Rao and Sresty (2000), and Pro content was measured according to Bates et al. (1973).

### 2.5. Determination of enzymatic/non-enzymatic antioxidants

For protein and enzyme extractions, 0.5 g of each leaf sample was homogenized in 50 mM Tris-HCl (pH 7.8) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2% Triton X-100, 1 mM phenylmethylsulfonyl fluoride and 2 mM dithiothreitol (DTT). The total soluble protein content of the enzyme extracts was determined (Bradford, 1976). Superoxide dismutase (SOD) isozyme/enzyme activity was defined (Beauchamp and Fridovich, 1971; Laemmli, 1970). The activity of catalase (CAT) isozyme/enzyme was determined using the procedure suggested by Woodbury et al. (1971) and Bergmeyer (1970). The isozymes/enzyme capacity of peroxidase (POX) was measured according to the method suggested by Seevers et al. (1971) and Herzog and Fahimi (1973). The enzyme/isozyme activities of glutathione S-transferase (GST) and glutathione peroxidase (GPX) were determined (Hossain et al., 2006; Ricci et al., 1984). The isoforms and total NADPH oxidase (NOX) activity were calculated (Jiang and Zhang, 2002; Sagi and Fluhr, 2001).

Ascorbate peroxidase (APX) and glutathione reductase (GR) were spectrophotometrically and electrophoretically carried out (Mittler and Zilinskas, 1993; Nakano and Asada, 1981). The contents of ascorbate (AsA) and oxidized ascorbate (DHA) were estimated (Dutilleul et al., 2003). The procedure for monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) was performed (Dutilleul et al., 2003). Glutathione (GSH) was assayed according to Paradiso et al. (2008), utilizing aliquots of supernatant neutralized with 0.5 M K-P buffer. Based on enzymatic recycling, glutathione is oxidized by DTNB

and reduced by NADPH in the presence of GR, and glutathione content is evaluated by the rate of absorption changes at 412 nm. Oxidized glutathione (GSSG) was determined after removal of GSH by 2-vinylpyridine derivatization. Standard curves with known concentrations of GSH and GSSG were used for the quantification. GSH redox status was obtained (Shi et al., 2013).

The Gel Doc XR + System was used to photograph stained gels and was subsequently evaluated using Image Lab software v4.0.1 (Bio-Rad, California, USA). Enzyme standards are used in gels for normalization.

## 2.6. Gas chromatography-mass spectrometry (GC-MS) analysis of hormone contents

The hormone contents were analyzed according to the protocol detailed by Turan et al. (2014) using 1 g of fresh leaves collected from each group.

## 2.7. Determination of ion concentrations

The contents of Sc and Cr were analyzed by ICP-AES (Varian-Vista) (Nyomora et al., 1997).

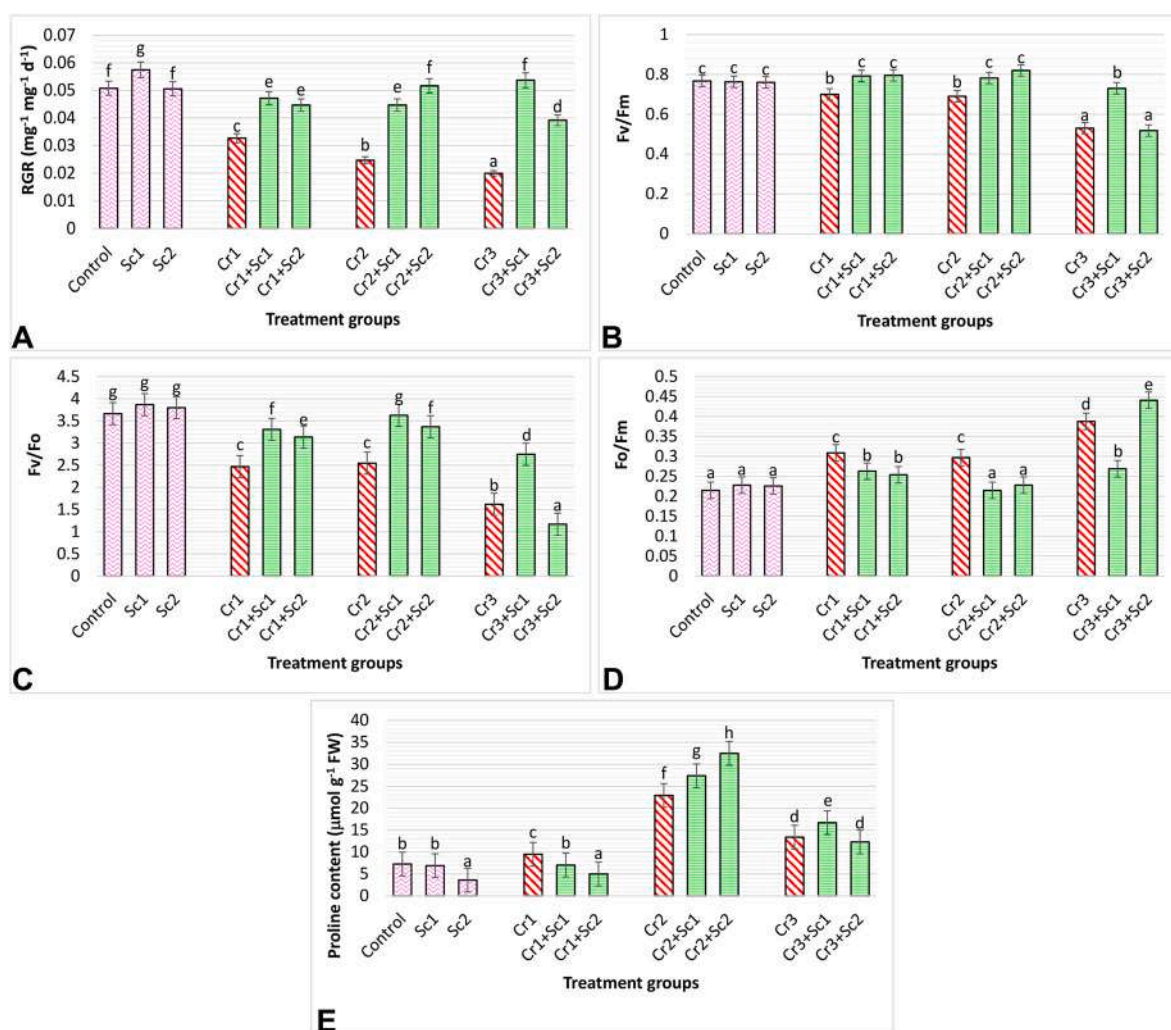
## 2.8. Statistical analysis

The experiments were repeated thrice independently and each data point was the mean of six replicates. All data obtained were subjected to a one-way analysis of variance (ANOVA). Statistical analysis of the values was performed by using SPSS 20.0. Tukey's post-test was used to compare the treatment groups. Comparisons with  $p < 0.05$  were considered significantly different. In all figures, the error bars represent standard errors of the means. The variation from low levels to high ones in the parameters obtained from the OJIP analysis compared to the control group was depicted on the color scale from blue to red.

## 3. Results

### 3.1. Effects of Sc on relative growth rate, photosynthetic yield and proline content in Cr-stressed *Lemma minor*

As shown in Fig. 1A, reductions in relative growth rate (RGR) of 35.6%, 51.3% and 60.6% were seen in response to Cr stress treatments, respectively. However, Sc treatments in addition to *L. minor* exposed to Cr stress resulted in significant RGR induction in all groups. Similarly, due to Sc1 treatment under control conditions, an increase in the RGR of *L. minor* was detected and no significant change in Sc2 concentration was observed. In *L. minor* exposed to Sc under control conditions, the



**Fig. 1.** The relative growth rate (RGR, A), maximal quantum yield of PSII photochemistry ( $F_v/F_m$ , B), potential photochemical efficiency ( $F_v/F_o$ , C), physiological state of the photosynthetic apparatus ( $F_o/F_m$ , D) and proline content (E) in *L. minor* under scandium (Sc1, 50  $\mu\text{M}$ ; Sc2, 200  $\mu\text{M}$ ) with/without chromium stress (Cr1, 100  $\mu\text{M}$ ; Cr2, 200  $\mu\text{M}$ ; Cr3, 500  $\mu\text{M}$ ).

ratios of  $F_v/F_m$  (Fig. 1B),  $F_v/F_o$  (Fig. 1C), and  $F_o/F_m$  (Fig. 1D) were similar to control levels at both concentrations (Sc1 and Sc2). As observed in Fig. 1B, decreases of approximately 9% under Cr1 and Cr2 and 10% under Cr3 were detected in the  $F_v/F_m$  of *L. minor* stressed alone. Likewise,  $F_v/F_o$  ratios decreased at all doses when exposed to stress (Fig. 1C). Both Sc concentrations applied with stress increased  $F_v/F_m$  and  $F_v/F_o$  ratios in all groups except Cr3+Sc2. On the other hand, while  $F_o/F_m$  values under stress increased in line with the data at each dose, they decreased in all groups except Cr3+Sc2 with Sc applications (Fig. 1D). As seen in Fig. 1E in Sc applications alone, there was no significant change in proline (Pro) content in Sc1 compared to the control group, while it caused a 50% reduction in Sc2. However, after exposure

to stress, Pro increased 1.3, 3.1 and 1.8 times, respectively, in *L. minor* compared to the control group. In Sc applications with stress, Pro content decreased in Cr1+Sc groups compared to stress alone. No reduction was achieved under Cr2+Sc and Cr3+Sc applications.

### 3.2. Effects of Sc on photosynthetic machinery in Cr-stressed *Lemna minor*

The radar graph showing the JIP test parameters calculated from the treatment groups was given in Fig. 2. Applications of Sc under control conditions did not significantly change the  $ABS/RC$ ,  $ET_o/RC$ ,  $TR_o/RC$ ,  $\Psi E_o/(1-\Psi E_o)$ ,  $\Phi P_o/(1-\Phi P_o)$ ,  $\gamma RC/(1-\gamma RC)$ ,  $DI_o/RC$ ,  $PI_{ABS}$  and  $PI_{total}$

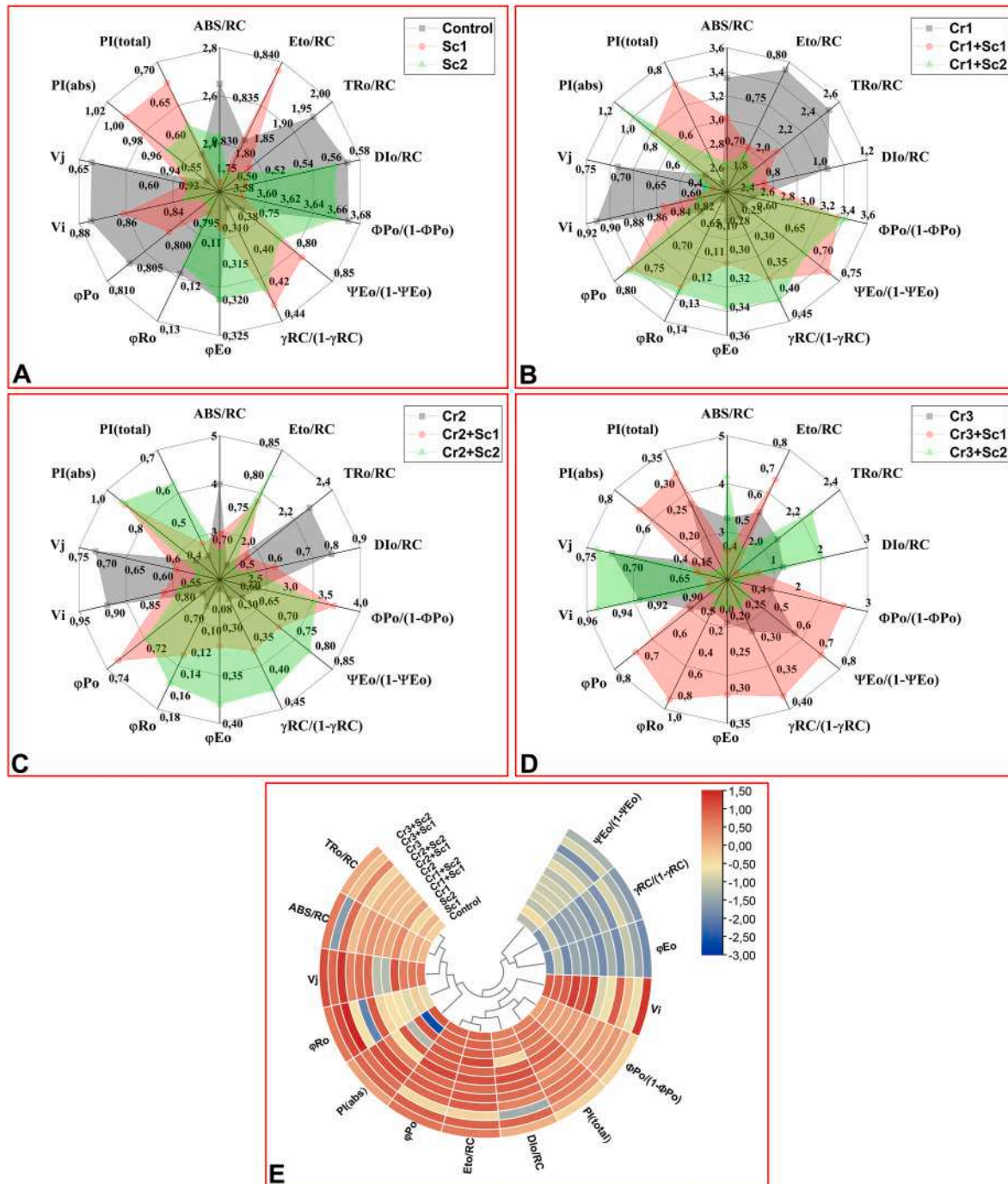


Fig. 2. The effects of scandium (Sc1, 50  $\mu$ M; Sc2, 200  $\mu$ M) and chromium (Cr1, 100  $\mu$ M; Cr2, 200  $\mu$ M; Cr3, 500  $\mu$ M) applications on quantum efficiencies, structural indicators, and performance indices. The parameters derived from the OJIP transient and their definitions were given in Supplementary Table 2.

values. In *Lemna minor* treated with chromium stress (Cr1, Cr2 and Cr3), the efficiency of the light reaction  $\Phi P_o/(1 - \Phi P_o)$  and the performance index ( $PI_{total}$ ) in the energy absorption pathway were decreased. On the other hand, absorption flux per active reaction centre (ABS/RC) and  $TR_o/RC$  increased with chromium treatments. Cr-induced modifications on these parameters were reversed by the Sc treatments. In addition, Sc applications prevented the increase in energy loss ( $DI_o/RC$ ) after stress treatments applied to *L. minor*.

3.3. Effects of Sc on H<sub>2</sub>O<sub>2</sub> content, lipid peroxidation, Cr and Sc content in Cr-stressed *Lemna minor*

As seen in Fig. 3A, no increase in H<sub>2</sub>O<sub>2</sub> accumulation was calculated over the experimental period after *L. minor* exposure to Sc. In contrast, the stress treatments (Cr1, Cr2 and Cr3) had 3.4, 4.9 and 6.2 times higher H<sub>2</sub>O<sub>2</sub> content, respectively. This effect was reversibly attenuated by both Sc treatments, except Cr3+Sc2, in *L. minor* under stress conditions. Similar to the H<sub>2</sub>O<sub>2</sub> content results as shown in Fig. 3B, the TBARS content was not changed in *L. minor* treated with scandium alone. Cr1, Cr2 and Cr3-stressed plants showed increases in TBARS content compared to control. However, these increases in TBARS content under stress were blocked by all Sc treatments, except for Cr1+Sc2 and Cr3+Sc2. As given in Fig. 3C, the same amount and very low Cr contents were detected in the control conditions and in Sc alone applications. In Cr alone groups, increasing Cr contents were observed in parallel with increasing concentrations. On the other hand, Cr + Sc treatments had dose-related decreased Cr contents compared to stress alone. The very low Sc content was detected in the control conditions and there were dose-related increased Sc contents under Sc alone applications (Fig. 3D). Also at Cr alone groups, the very low Sc contents were observed. On the other hand, Cr + Sc treatments had dose-related increased Sc contents liken to stress alone.

3.4. Effects of Sc on antioxidant system-related enzyme and isoenzyme activities in Cr-stressed *Lemna minor*

Fig. 4A showed that six superoxide dismutase isozymes (Mn-SOD1-2-

3 and Fe-SOD1-2-3) emerged in the determination of isozymes in *L. minor*. As evident from the all isozymes densities, *L. minor* treated with Sc alone showed increasing SOD activity in comparison with the control group (Fig. 4B). Like control plants, Cr1 and Cr2 stresses decreased SOD activity, while Cr3 stress did not cause a significant change (Fig. 4B). Sc treatments with stress increased SOD activities compared to stress alone, except for Cr3+Sc2. Overall, band intensities of all isozymes supported these changes in SOD activity (Fig. 4A).

Fig. 4C presented that two catalase isozymes (CAT1-2) appeared in the determination of isozymes in *Lemna minor*. Duckweed treated with Sc alone showed increases CAT activity in comparison with the control group (Fig. 4D). Like control plants, Cr1 stress decreased CAT activity, while Cr2 stress increased. No change observed under Cr3 alone (Fig. 4D). Sc treatments with stress decreased CAT activities compared to stress alone in all groups.

As it was seen in Fig. 5A, three bands for POX isozymes (POX1-2-3) were identified in *L. minor*. Examining the effects of applying Sc to plant under control conditions, both doses resulted in increases in POX activity (Fig. 5B). All POX isozymes supported the induced levels of POX activity (Fig. 5A). All doses applied chromium stress increased POX activity compared to control. These increments were not maintained with Cr + Sc applications, except for Cr2+Sc1. Analysis of NOX isoenzymes revealed five isoforms (NOX1-2-3-4-5) (Fig. 5C). Sc1 and Sc2 treatments under control conditions caused remarkable increases in NOX activity of *L. minor* (Fig. 5D). In particular, NOX2-3 isozymes were responsible for these changes in Sc treatments alone (Fig. 5C). While no significant changes were observed in NOX activity of *L. minor* under Cr1 and Cr2 alone treatment, a decrease (30%) was detected in Cr3 treatment. In Cr + Sc applications, a decrement detected in Cr3+Sc2 group compared to stress alone, while NOX activity was increased in all other groups (Fig. 5D).

Fig. 6A showed the presence of four isozymes (GST1-2-3-4) for GST in *L. minor*. As observed in Fig. 6B, increases in GST activity emerged under Sc alone. Band intensities in the all isoforms were responsible for these changes (Fig. 6A). However, in GST activities under stress alone, an increase of 19.5% under Cr1 was detected compared to the control group, while no changes observed at Cr2 and Cr3. As against stress alone

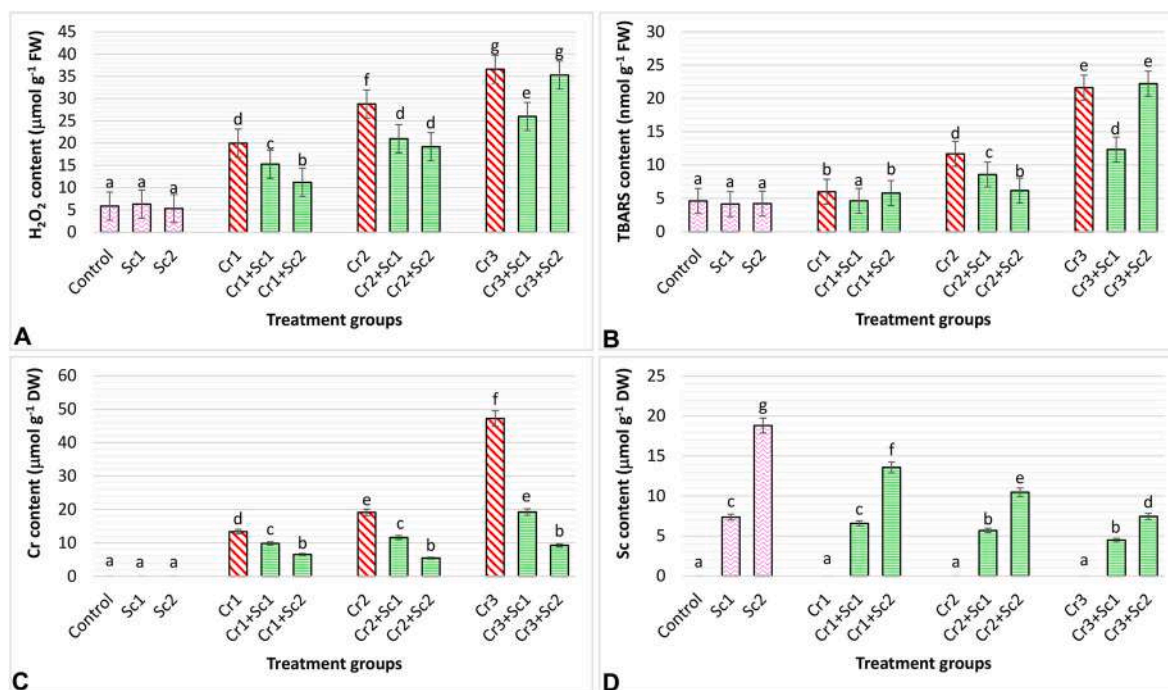


Fig. 3. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, A), lipid peroxidation (TBARS, B), chromium (Cr, C) and scandium (Sc, D) contents in *L. minor* under scandium (Sc1, 50 μM; Sc2, 200 μM) with/without chromium stress (Cr1, 100 μM; Cr2, 200 μM; Cr3, 500 μM).

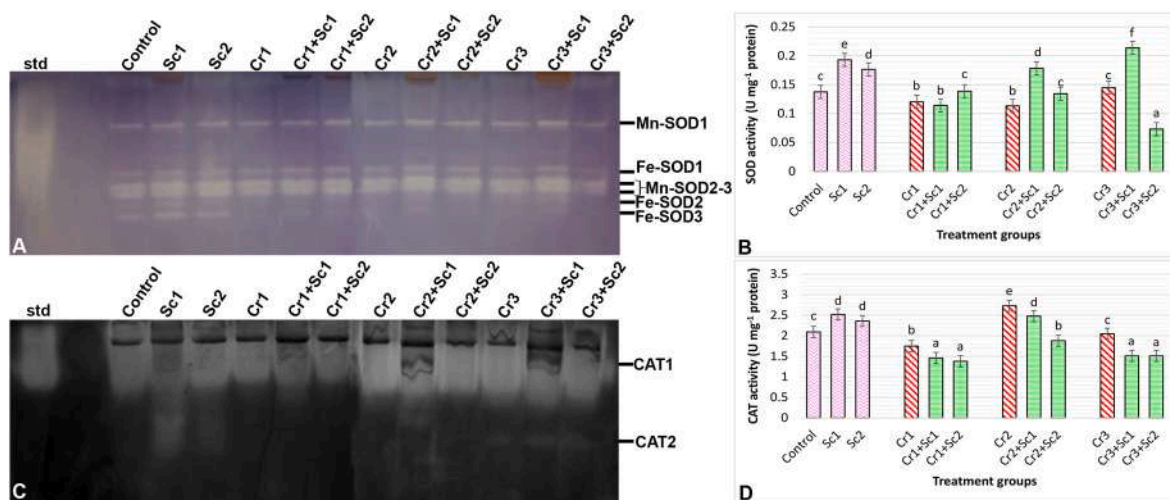


Fig. 4. Relative band intensity of different types of superoxide dismutase isoenzymes (SOD, A) and SOD activity (B), relative band intensity of different types of catalase isoenzymes (CAT, C) and CAT activity (D) under scandium (Sc1, 50 µM; Sc2, 200 µM) with/without chromium stress (Cr1, 100 µM; Cr2, 200 µM; Cr3, 500 µM).

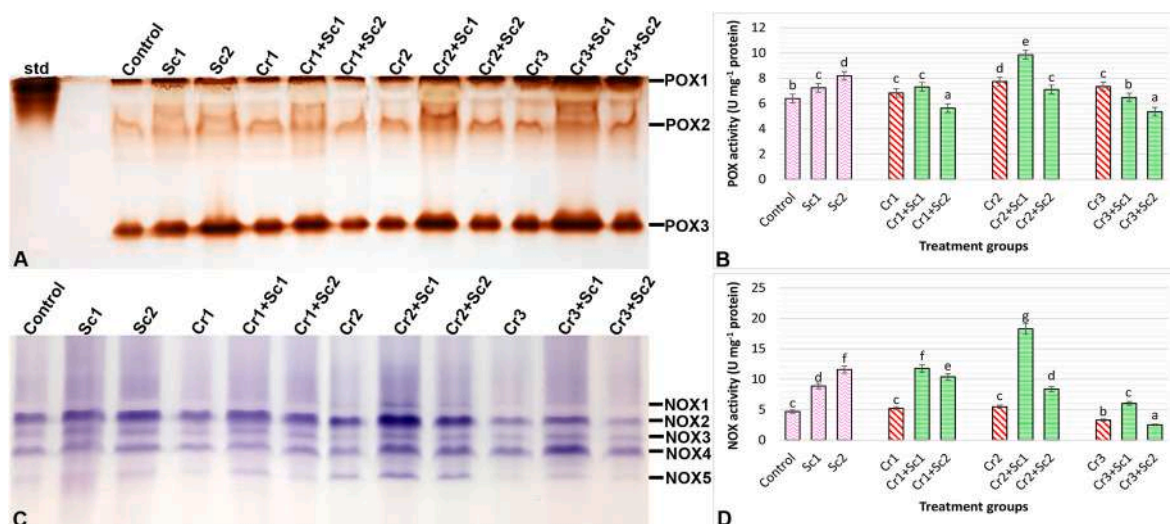


Fig. 5. Relative band intensity of different types of peroxidase isoenzymes (POX, A) and POX activity (B), relative band intensity of different types of NADPH oxidase isoenzymes (NOX, C) and NOX activity (D) under scandium (Sc1, 50 µM; Sc2, 200 µM) with/without chromium stress (Cr1, 100 µM; Cr2, 200 µM; Cr3, 500 µM).

groups, there were noticeable increases in Cr + Sc applications in all groups, except for Cr1+Sc2. As it was seen in Fig. 6C, there are four isozymes (GPX1-2-3-4) appeared for GPX in *L. minor*. As observed in Fig. 6D, increases in GPX activity shown under Sc alone. Band intensities in the GPX3-4 isoforms were responsible for these changes (Fig. 6C). On the other hand, in GST activities under stress alone, a decrease of 34.2% in Cr2 was detected compared to the control group, while no changes observed in Cr1 and Cr3. Likened to stress alone groups, there were noticeable increases in Cr + Sc applications in all groups, except for Cr3+Sc2.

### 3.5. Effect of Sc on enzyme and non-enzyme activities associated with the AsA-GSH cycle in Cr-stressed *Lemna minor*

In this study, four APX isoenzymes (APX1-2-3-4) were observed throughout the experiment (Fig. 7A). Applications of Sc1 and Sc2 under control conditions resulted in approximately the 1.5 and 1.2 times increases at both doses, respectively. The increments were observed when Cr2 and Cr3 stress were applied (Fig. 7B). In the Cr1 alone group, on the contrary, a decrease occurred. Sc, applied with stress, led to increases in

APX activities in all groups, except for Cr3+Sc2. Overall, varying intensities of the APX1-2-3 bands were effective in APX activity (Fig. 7A). As seen in Fig. 7C, four isozymes (GR1-2-3-4) were detected to evaluate GR isozymes. Similar to APX activity results, Sc treatments alone increased GR activities in *L. minor*. However, Cr1 and Cr3 alone decreased GR activity, while Cr2 alone treatment did not lead to significant changes in GR activity compared to control plants. All Cr + Sc treatments increased GR activities, except for Cr3+Sc2 (Fig. 7D).

Sc2 alone caused an increase in MDHAR activity, while Sc1 alone was not (Fig. 8A). Cr1 and Cr2 stresses decreased MDHAR activity but Cr3 alone did not affect. These results were broken and increases were seen at all Sc concentrations treated with Cr. Compared to the stress alone treatment, the highest increase was detected in the Cr2+Sc1 group with 4.35-fold. As given in Fig. 8B, Sc1 treatments under control conditions increased DHAR activity in *L. minor*, while Sc2 did not. In stress alone treatments, all doses caused increments. Significant increases in DHAR activities were observed in Sc groups applied with Cr1 and Cr2 compared to stressed plants. On the contrary, Sc treatments with Cr3 led to decreases at both doses. In comparison with control groups, *L. minor* treated with Sc alone did not change the AsA content at low dose (Sc1)

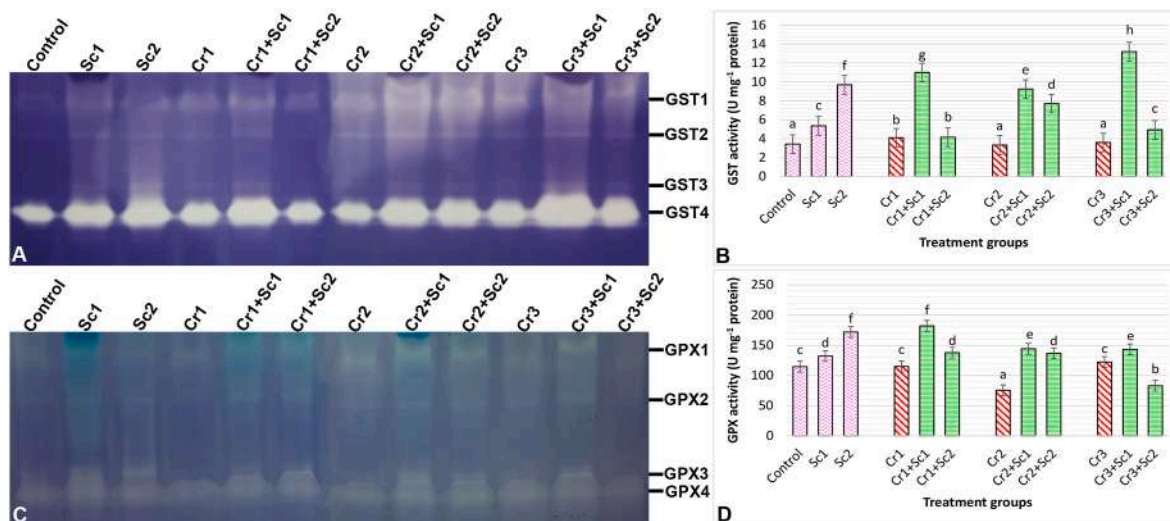


Fig. 6. Relative band intensity of glutathione S-transferase isoforms (GST, A), GST activity (B), relative band intensity of different types of glutathione peroxidase isoenzymes (GPX, C) and GPX activity (D) under scandium (Sc1, 50 µM; Sc2, 200 µM) with/without chromium stress (Cr1, 100 µM; Cr2, 200 µM; Cr3, 500 µM).

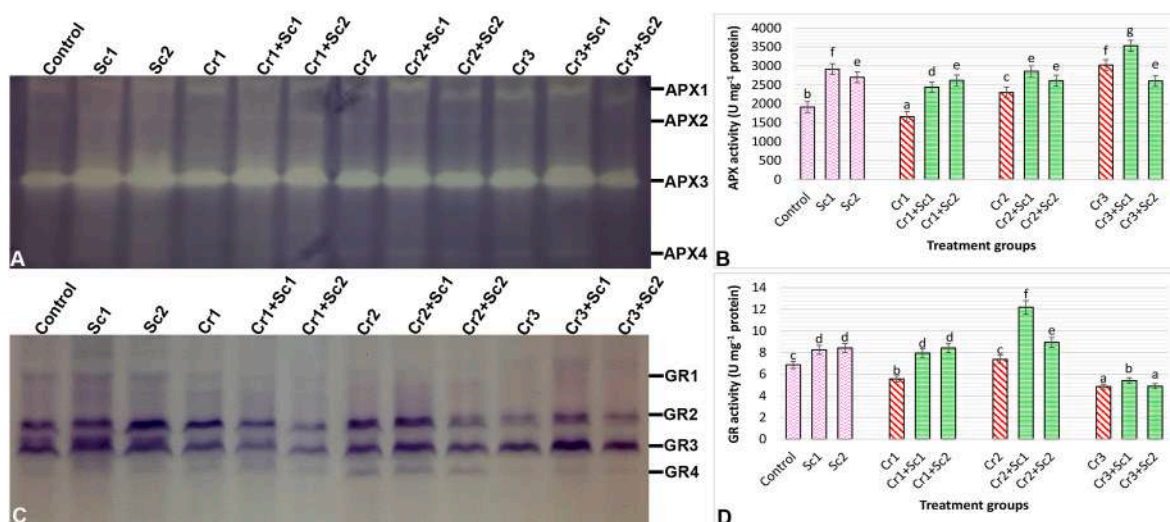


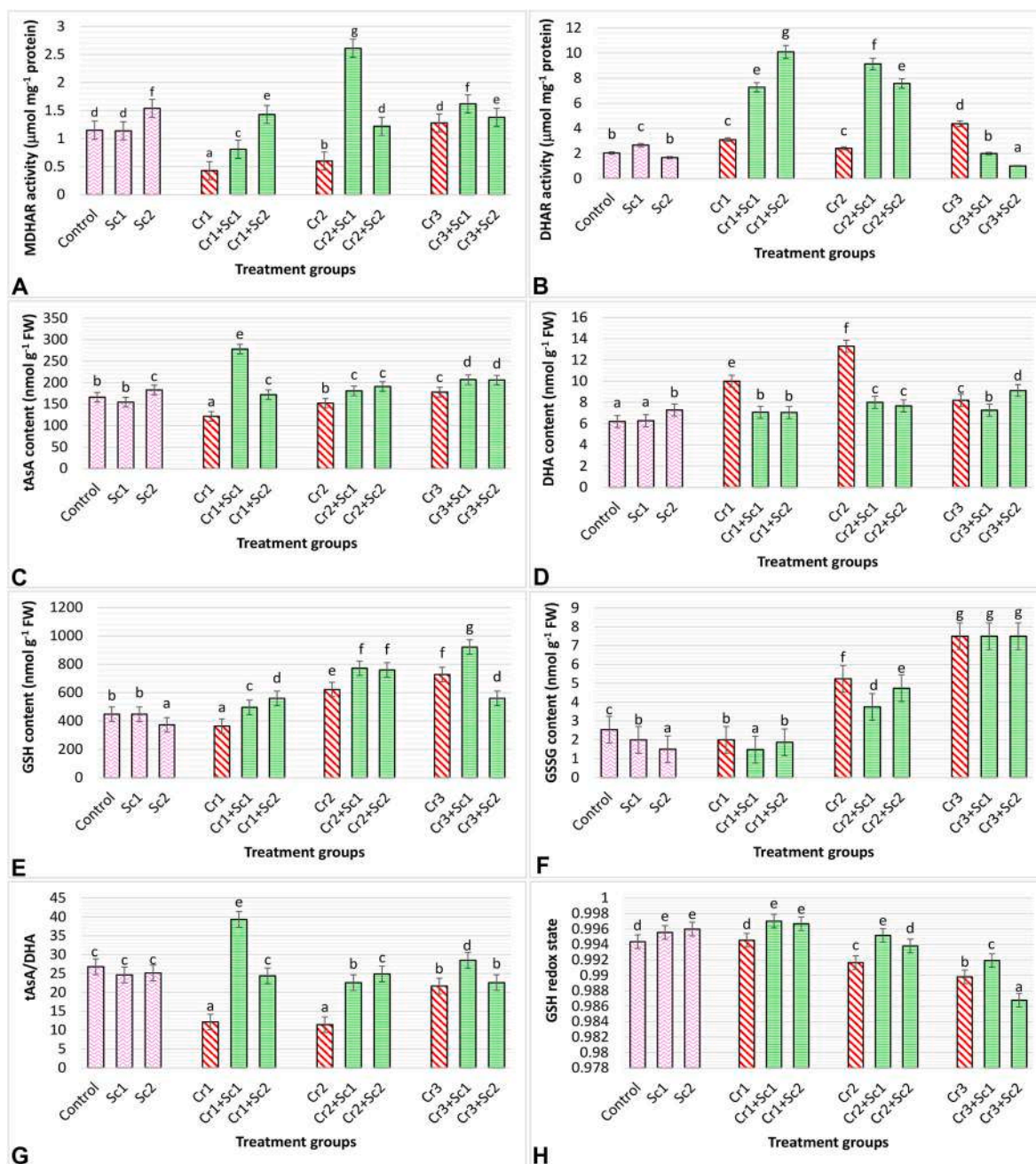
Fig. 7. Relative band intensity of ascorbate peroxidase (APX, A), APX activity (B), relative band intensity of glutathione reductase isoenzymes (GR, C) and GR activity (D) under scandium (Sc1, 50 µM; Sc2, 200 µM) with/without chromium stress (Cr1, 100 µM; Cr2, 200 µM; Cr3, 500 µM).

but increased it at high dose (Sc2) (Fig. 8C). The reduction rate in AsA content caused by Cr1 was 26.9%. Conversely, Cr3 caused an increment, while Cr2 did not effect. On the other hand, the AsA levels were increased with all Sc treatments compared to stress alone. In *Lemna minor* exposed to Sc1 alone, there was any differ in DHA contents and Sc2 alone exhibited an increase (Fig. 8D). DHA content was approximately 1.6, 2.1 and 1.3 times higher than control under Cr1, Cr2 and Cr3 stresses, respectively. Sc treatments with stress significantly reduced their increased DHA content, except for Cr3+Sc2. Based on the results of AsA and DHA contents, there were reductions in AsA/DHA in stressed *Lemna minor* (Fig. 8G). These rates increased in Cr + Sc treated groups. As shown in Fig. 8E, Sc treatments could not induce GSH content under control conditions. There were increases in GSH content in Cr2 and Cr3 stress groups. On the contrary, there was a decrease in Cr1. Sc applications against stress have succeeded in increasing GSH levels in all groups, except for Cr3+Sc2. The results presented in Fig. 8F revealed that both treatments of Sc decreased the GSSG content in *L. minor* compared to the control group. Likewise, Cr1 stress caused reduction in GSSG content. Conversely, Cr2 and Cr3 treatments induced GSSG contents. However, GSSG content could not be reversed by Sc treatments. As a

result of these data, the stress-induced reductions in GSH redox state (Fig. 8H) could be abrogated by Sc treatments, except Cr3+Sc2.

### 3.6. Effects of Sc on hormone content in Cr-stressed *Lemna minor*

As shown in Fig. 9A, the increased IAA contents were detected in *L. minor* exposure to Sc alone. The stress treatments (Cr1, Cr2 and Cr3) had lower IAA content. This effect was reversibly attenuated by both Sc treatments, except Cr3+Sc2, in *L. minor* under stress conditions. As detected in Fig. 9B, the ABA contents were not changed in *L. minor* treated with scandium alone. Cr1, Cr2 and Cr3 stressed plants showed increases in ABA content compared to control. However, these increases in ABA content under stress were blocked by all Sc treatments. The results presented in Fig. 9C revealed that both treatments of Sc increased the GA content in *L. minor* compared to the control group. Conversely, Cr1, Cr2 and Cr3 treatments reduced GA contents. The decreased GA content could be induced by Sc treatments. *Lemna minor* exposed to Sc alone exhibited increases in SA content (Fig. 9D). SA contents were lower than control under Cr stress at all doses. Sc treatments with stress induced their decreased SA content. As seen in Fig. 9E, the increased CK



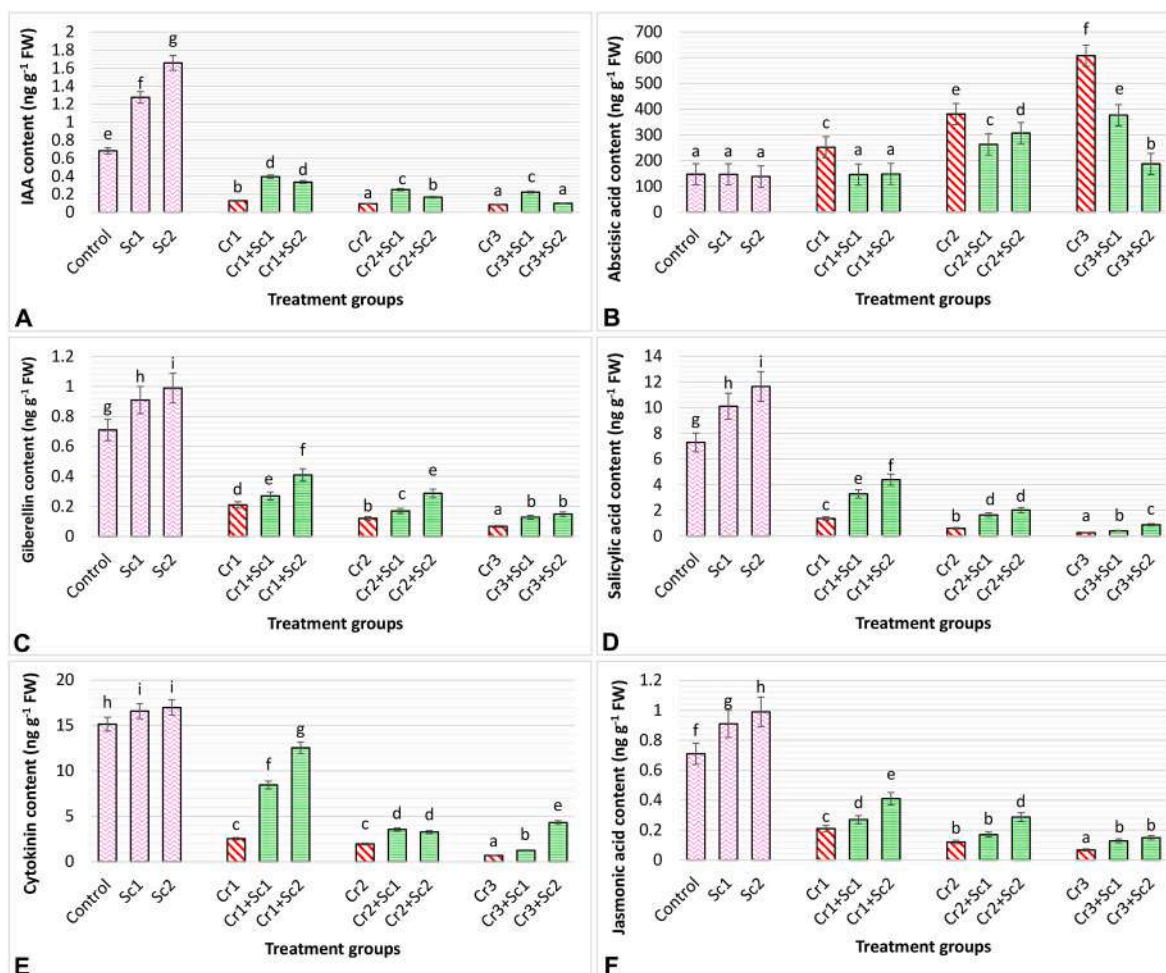
**Fig. 8.** The monodehydroascorbate reductase activity (MDHAR, A), dehydroascorbate reductase activity (DHAR, B), ascorbate content (tAsA, C), dehydroascorbate content (DHA, D), glutathione content (GSH, E), oxidized glutathione content (GSSG, F), tAsA/DHA (G) and GSH redox state (H) in *L. minor* under scandium (Sc1, 50 µM; Sc2, 200 µM) with/without chromium stress (Cr1, 100 µM; Cr2, 200 µM; Cr3, 500 µM).

contents were calculated in *L. minor* under Sc alone. Cr stress decreased CK contents in all groups. This effect was reversed by both Sc treatments in *L. minor* under stress conditions. Similar to the CK content results, as shown in Fig. 9F, JA contents were increased in *L. minor* treated with scandium alone. Cr1, Cr2 and Cr3 stressed plants showed decreases in JA content compared to the control group. However, these decreases in TBARS content under stress were blocked by all Sc treatments.

**4. Discussion**

Heavy metals may cause metabolic disorders and growth inhibition in plants. Morphological symptoms such as growth inhibition and loss of biomass are particularly evident at high metal concentrations. Consistent with the results of the present study, previous researches found a

significant negative linear correlation between the relative growth rate of plants and the Cr concentration in solution (Zhang et al., 2022). Previous studies show that appropriate amounts of REE promote seed germination and plant growth (Zicari et al., 2018). In the present study, RGR reductions caused by Cr were prevented by Sc applications. Similarly, La<sup>3+</sup> induced rapid growth responses in *Avena* coleoptile segments, producing a prolonged stimulatory effect on the elongation rate. A possible reason for this result may be that at low concentrations REEs act similarly to micronutrients. In addition, REEs can stimulate plants to produce isoenzymes for fat decomposition in order to supply the demand for fatty acid, lecithin, increase plant vigor and promote seed germination (Hu et al., 2004). It has been found that Cr affects the chloroplast mainly in the form of weak lamellae, less grana and an underdeveloped thylakoid lumen. Chromium ions replace the Ca<sup>2+</sup>



**Fig. 9.** The contents of phytohormone IAA (A), Abscisic acid (B), Gibberellin (C), Salicylic acid (D), Cytokinin (E), Jasmonic acid (F) in the leaves of *Lemna minor* under scandium (Sc1, 50  $\mu$ M; Sc2, 200  $\mu$ M) with/without chromium stress (Cr1, 100  $\mu$ M; Cr2, 200  $\mu$ M; Cr3, 500  $\mu$ M).

cofactor, which is known to be very important for water splitting in photosystem II (PSII), therefore changes the structure and function of the oxygen evolving complex. Also, in addition to the oxygen evolving complex, Cr interacts with a lot of fundamental electron acceptor proteins such as  $Q_B$  involved in electron transport of photosystem II (Oves et al., 2016). Cr also affects the efficiency of photosystem I (PSI) by interacting with monomeric and multimeric subunits, thus disrupting the overall electron pathway in the electron transport chain and eventually reducing the energy conversion efficiency of PSII (Ayyaz et al., 2020). In this study, in chlorophyll fluorescence analyzes,  $F_v/F_m$ , which expresses the maximum primary photochemistry efficiency of PSII, decreased under all Cr stress concentrations, and the decreasing and an increasing trend, respectively, in the  $F_v/F_o$  and  $F_o/F_m$  values, which are considered the most sensitive parameters of plant photosynthetic capacity, indicated a decrease in photosynthetic capacity by inactivating the performance of reaction centers in PSII. Similarly, Mathur et al. (2016) reported that Cr toxicity in wheat leads to a decrease in the electron transport rate and PSII heterogeneity of PSII in a number of active reaction centers, as well as having extensive effects on the light harvesting complex of PSII. In the current study, Cr-triggered harmful effects were blocked by Sc applications. The higher  $F_v/F_m$  and  $F_v/F_o$  in *Lemna minor* treated with Cr + Sc showed that Sc was able to reverse the inhibition of the active site of PSII. Similar to the results in this study, Cheng et al. (2021) reported a dose-dependent increase in chlorophyll content and photosynthetic quantum yield with respect to rare earth elements. Since the chloroplasts are the sites of photosynthetic energy conversion, element balance in chloroplasts is important for sustaining

photosynthesis. The reason why Sc treatments improved photosynthetic activity impaired by Cr stress may be that low REE concentrations affected the membrane potential and proton transmembrane gradient by increasing transmembrane transport of ions and metabolites to chloroplasts, and promoting absorption and transport of functional elements for chloroplasts, thereby promoting photosynthesis (Nouet et al., 2011; Wen et al., 2011).

Chlorophyll fluorescence is often used to predict crop yield under various stress conditions. Also, it can be used to analyze detailed information about energy transfer in photosynthesis. A descriptive tool to provide information about the status of PSII, the JIP test relies on rapid kinetic analysis of chlorophyll fluorescence and signals that provide detailed information about the structure and function of the photosynthetic apparatus (Rastogi et al., 2020). To assess the damages induced by Cr stress in PSII, changes in the reduction in electron transport, various phenomenological fluxes (ABS/RC,  $TR_o/RC$ ,  $ET_o/RC$  and  $DI_o/RC$ ) were measured. In the current study, Cr increased energy absorption (ABS/RC) compared to control. On the other hand,  $DI_o/RC$  increased as  $ET/RC$  decreased beyond Quinone A ( $Q_A$ ) in the Cr-stressed plants. Such effects may be due to damage to PSII. Quantum efficiency, energy flows for absorption and capture, electron transport and energy dissipation flows showed that the energy capture, transport and termination efficiency of *Lemna minor* is low. This means that energy transfer and utilization through light collection, light energy capture, electron transport decreases with increasing Cr stress. Our findings is similar to report that published by Ayyaz et al. (2021) on *Brassica napus* exposed to Cr stress. On the other hand, Sc increased the total energy flow reaching the

reaction centers of PSII by decreasing the ABS/RC values. At the same time, excess Cr increased TR<sub>o</sub>/RC, followed by a decrease in Q<sub>A</sub>. In addition to inducing re-oxidation of the reduced Q<sub>A</sub>, Sc transferred electrons to Q<sub>B</sub> with high capacitance. This indicated that the scandium maintained the PQ size in the Cr-stressed plant. Cr stress effectively reduced the transport between Q<sub>A</sub> and Q<sub>B</sub>, but eliminated this reduction by lowering ET<sub>o</sub>/RC. However, Sc treatments to stressed *L. minor* stimulated the photon capture capacity of active reaction centers and increased the number of captured photons with a marked decrease in DI<sub>o</sub>/RC levels. Interestingly, Sc2 did not maintain the improved photosynthetic capacity against the highest Cr treatment.

The application of Cr stress to *Lemna minor*, increased the accumulation of ROS and hence H<sub>2</sub>O<sub>2</sub> content as detected by Varga et al. (2013). Among the antioxidant enzymes in charge of minimizing oxidative damage caused by stress, SOD and CAT constitute the primary line of defense. SOD catalyzes the conversion of superoxide anions to H<sub>2</sub>O<sub>2</sub> and oxygen, while CAT converts H<sub>2</sub>O<sub>2</sub> to water and molecular oxygen. Conversely, in this study, SOD activity was decreased in *L. minor* exposed to Cr stress. Failure to scavenge O<sub>2</sub><sup>-</sup> radicals by SOD resulted in lipid peroxidation and thus high TBARS content. Studies in some plants exposed to heavy metal stress have shown that this trend is related to the heavy metal threshold and may cause a decrease in SOD enzyme activity depending on the increasing concentration (Demirevska-Kepova et al., 2004; Xiao et al., 2008). This indicates that exposure to Cr has a strong effect on duckweed. In addition, the activity of NADPH oxidase (NOX) is evaluated as an important source of superoxide radicals and hydrogen peroxide in plants (Chu Puga et al., 2019). Despite the decreased SOD and NOX activity, the source of the accumulated H<sub>2</sub>O<sub>2</sub> may be its diffusion from other cellular fractions or the cytoplasm to the chloroplast or disruptions in the electron transport chains in the chloroplast and mitochondria (Cheeseman, 2007). It is known that increasing SOD activity is associated with increased tolerance of plants to environmental stress (Berwal and Ram, 2018). On the other hand, Dridi et al. (2022) found that REE applications increased SOD activity in *Helianthus annuus*. Similarly, in this study, Sc treatments induced SOD activities in both control and stress conditions.

Another enzyme for detoxification is POX (Apel and Hirt, 2004). Our results showed that although POX activity increased under Cr stress, H<sub>2</sub>O<sub>2</sub> toxicity could not be eliminated due to insufficient CAT and GPX activities in general, and H<sub>2</sub>O<sub>2</sub> and TBARS levels increased with increasing Cr application. Similar results showing increased TBARS content with Cr accumulation in the plant reported by Wakeel et al. (2019). In the AsA-GSH cycle, the coordinated functions of APX, MDHAR, DHAR and GR along with AsA and GSH split H<sub>2</sub>O<sub>2</sub> into water and oxygen and further recycle AsA and GSH (Mishra et al., 2022). Several studies have shown that maintaining a high AsA/DHA and/or GSH/GSSG, maintained by increased AsA and GSH or reduced DHA and GSSG, may be the main strategy for defense against abiotic stress-induced ROS accumulation (Fotopoulos et al., 2010). However, in this study, due to decreased MDHAR and significantly increased DHAR activity in Cr-stressed *L. minor*, the total amount of AsA decreased and there was a decrease in AsA/DHA. Likewise, although there was no significantly increased GPX and GST activities, there was a decrease in GSH/GSSG due to decreased GR and increased DHAR activities. Looking at Cr + Sc applications, APX, GST, GR, GPX, MDHAR and DHAR activities increased. In addition, AsA/DHA and GSH/GSSG were induced in parallel with the AsA-GSH content in stressed duckweed treated with Sc. Apparently, scandium's ability to induce enzymes of the AsA-GSH cycle had a direct effect on the regeneration of ascorbate and glutathione and reorganized the AsA pool in *L. minor* under excess Cr. Our findings are consistent with the fact that REEs applied to *L. minor* (Ippolito et al., 2010) and tomato (Ippolito et al., 2011) exposed to abiotic stress have similar effects on the antioxidant system. With all these above-mentioned observations, Sc treatments yielded lower levels of H<sub>2</sub>O<sub>2</sub> and TBARS under Cr stress. These results show that Sc can regulate the damage caused by Cr accumulation in *L. minor* and maintain the GSH

redox status by increasing the antioxidant content in the AsA-GSH cycle.

Acting as chemical messengers with highly complex regulation, plant hormones allow plants to maintain their growth plasticity during development and are collectively key tools in plants' defense against environmental stress conditions (Bücker Neto et al., 2017). Auxin (indole-3-acetic acid, IAA) is synthesized from indole via tryptophan metabolic pathways (Zhao, 2010). These pathways can interact effectively with biotic and abiotic stresses. In addition, oxidative stress arising from heavy metal may be efficient in stabilizing auxin against oxidizing IAA (Ma et al., 2018). Difference in pH levels in the apoplast and cytoplasm during auxin transport direct the regulation of this flow, and a decrease in the pH level of the apoplast has a significant effect on the auxin signaling mechanisms achieved by the acidity of auxins (Robert and Friml, 2009). In addition, the role of auxin under heavy metal stress can be attributed to the auxin-ROS linkage induced by several genes that play a role in the regulation and stimulation of antioxidant enzymes in plants and the regulation of H<sub>2</sub>O<sub>2</sub> levels, which generated through the action of specific mechanisms involved in auxin homeostasis (Emamverdian et al., 2020). In the light of this information, in parallel with the above-mentioned antioxidant system regulations, decreasing IAA contents with Cr stress was associated with increasing H<sub>2</sub>O<sub>2</sub> and thus changing pH. The reason why Sc can alleviate Cr damage in the plant may be that it effectively scavenges the superoxide anion radical. It attenuates the degradation of IAA induced by the increased free radical, or it succeeds in protecting the plant from Cr stress by inhibiting acetic acid oxidase (IAAO) activity (Peng and Zhou, 2009). Abscisic acid (ABA) is a hormone associated with tolerance to adverse environmental conditions, and the ABA signaling pathway is a central regulator of the abiotic stress response in plants. Oxidative stress triggered by heavy metal accumulation causes an increase in ABA concentration in the plants, which plays a significant role in stimulating antioxidants and enhancing their protective capacity (Bücker Neto et al., 2017). Furthermore, ABA can mitigate conditions caused by heavy metal-induced dehydration in plants. ABA can maintain water balance by regulating stomata and improve the drought stress intensity resulting from metal toxicity in the plants (Saradadevi et al., 2017). In the current study, Cr stress triggered significant increases in ABA content. Likewise, ABA concentration in plant tissues is known to increase after heavy metal exposure, suggesting that this phytohormone plays a role in inducing protective mechanisms against heavy metal toxicity (Hollenbach et al., 1997). The ABA content in plants treated with Cr + Sc was lower than Cr stress treatment. The reason was partly related to lesser free-radical injury chloroplast membrane, decreasing damage to photosynthetic apparatus, maintaining normal osmotic pressure, and weakening root-sourced signal by Sc. Gibberellic acid (GA) provides defense for plants against environmental stresses by modulating antioxidant enzyme activities as well as decreasing the amount of excessive ROS under stressful conditions (Iftikhar et al., 2019). GAs especially in the embryonic cell increases substantially seed germination and then passes through starchy endosperm and, finally, transfers to cells containing starch, thereby inducing  $\alpha$ -amylase and proteinase. It has been pointed out that REEs promoted germination because the amylase activity and respiratory rate increased, and REEs make GAs induce the synthesis of  $\alpha$ -amylase (Fashui et al., 2003). These could be the reason for the GA contents regulated with Sc applications in the current study. Salicylic acid (SA), an endogenous plant hormone, can increase the resistance of plants under stress through mechanisms such as the production of some stress proteins (Song et al., 2014). SA has four main metabolic pathways including metal chelating compounds, antioxidant defense systems, osmolytes and secondary metabolites to enable the plants to cope with heavy metal stress (Ashraf and Foolad, 2007). When it comes to antioxidant pathways, SA as a small signaling molecule may play an important role in systemic acquired resistance (Hernández et al., 2017). Our results showed that SA and GA contents decreased with Cr stress. This may be due to the down-regulation of genes that are effective in the regulation of GA and SA under heavy metal stress (Colebrook et al., 2014) and to promote

plant growth (Kang et al., 2017). Cytokinins (CK) are regulatory biomolecules that can improve plant tolerance to heavy metals through various signaling pathways and they have been defined as factors that trigger cell division in the presence of auxin. One of the mechanisms of CK under heavy metal stress conditions is to completely increase plant productivity by reducing the amount of CK to cope with stress (Kohli et al., 2013). In our current study, it was determined that CK contents decreased with Cr stress. The reason behind the decreased CK content may be the increased accumulation of ABA under stress condition, which can activate specific enzymes involved in CK degradation and inhibit the expression of genes responsible for the control of CK biosynthesis (Veselov et al., 2017). Another reason is that may be increased heavy metal concentrations leading to an increase in CK methylation patterns (Erturk et al., 2015). Besides, the efficacy of CK in interacting with ROS is concentration dependent and may ameliorate oxidative damage by decreasing ROS accumulation (Pilarska et al., 2017). Meanwhile, CK, IAA and GA can improve the structural and functional attributes of photosynthetic apparatus, i.e., pigment contents and photosynthetic activity (Shao et al., 2010). In the present study, it has been found that application of Sc treatments increased the contents of CK in *L. minor*. This indicated that Sc would promote the photosynthetic process and productivity (Cui et al., 2019). Jasmonic acid (JA) regulates the expression of numerous genes and specific protective mechanisms in response to heavy metal stress (Li et al., 2018). In this study, decreases in JA content occurred with increasing Cr toxicity. This may be due to the rapid decline in growth processes and intensification of aging in plant after prolonged exposure to heavy metals. Although jasmonates are signaling molecules of increased resistance to stress, their activity is also due to the slowing of the activity of the photosynthetic apparatus (Maksymiec, 2007). On the other hand, the Sc concentrations applied under control conditions and together with Cr stress rearranged the phytohormone contents, resulting in alleviation of the harmful effects caused by stress, as understood from the decreased H<sub>2</sub>O<sub>2</sub> and TBARS levels, together with the induced antioxidant system activity. Similarly, Fashui et al. (2003) reported REE and antioxidant-phytohormone relationships. Our results are related to the fact that the production of phytohormones is affected by the presence of Sc, and this effect is especially the synergistic effect of REEs with hormones (Hu et al., 2004; Ramos et al., 2016).

## 5. Conclusion

Cr toxicity resulted a decline in photosynthetic efficiency and chlorophyll fluorescence of *Lemna minor*. Sc improved these parameters and eliminated the negative effects on photosystems, but 200 µM Sc did not remove the adverse effects under the highest Cr concentration. Sc applications caused the low levels of endogenous Cr contents in *L. minor*. Due to insufficient antioxidant capacity and hormone contents, Cr stress triggered the H<sub>2</sub>O<sub>2</sub> accumulation and then induced the lipid peroxidation. However, Sc treatments decreased the contents of H<sub>2</sub>O<sub>2</sub> and TBARS by activating GST, GPX and the components of the AsA-GSH cycle. The regeneration of AsA and GSH contributed to the alleviation of Cr-induced oxidative damage. On the other hand, 200 µM Sc did not sustain this effect under the highest Cr concentration. Sc might be supplying the protection against Cr stress by inducing hormone contents.

## Credit author statement

E.Y. and C.O.K. designed experiments; F.N.A., B.A., E.Y. and R.E. carried out data analysis; M.T., phytohormone contents; F.A., B.A., C.O.K. and E.Y. interpreted the results and wrote up the first draft of the manuscript; C.O.K. and E.Y. critically edited the whole manuscript. All authors read and approved the final manuscript.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

The authors do not have permission to share data.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2022.120636>.

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